

“GOOD” BUFFER-BASED CATIONIC LIPIDS**CROSS-REFERENCE TO RELATED APPLICATIONS**

[001] The present application claims benefit of U.S. Provisional Application No. 63/175,429, filed April 15, 2021, and U.S. Provisional Application No. 63/313,578, filed February 24, 2022, each of which is incorporated by reference in its entirety.

BACKGROUND

[002] Delivery of nucleic acids has been explored extensively as a potential therapeutic option for certain disease states. In particular, messenger RNA (mRNA) therapy has become an increasingly important option for treatment of various diseases, including for those associated with deficiency of one or more proteins.

[003] Efficient delivery of liposome-encapsulated nucleic acids remains an active area of research. The cationic lipid component plays an important role in facilitating effective encapsulation of the nucleic acid during the loading of liposomes. In addition, cationic lipids may play an important role in the efficient release of the nucleic acid cargo from the liposome into the cytoplasm of a target cell. Various cationic lipids suitable for *in vivo* use have been discovered. However, there remains a need to identify lipids that can be synthesized efficiently and cheaply without the formation of potentially toxic by-products.

[004] “Good” buffers (or Good’s buffers) are buffering agents for biochemical and biological research that were first selected and described by Norman Good and his colleagues (Good, N.E., et al. (1966) Hydrogen Ion Buffers for Biological Research. *Biochemistry* 5(2), 467-477). Most biological reactions take place near-neutral pH between 6 and 8. Good therefore reasoned that an ideal buffer for biochemical or biological applications would have a pKa value in this region to provide maximum buffering capacity. Additional selection criteria included high solubility, lack of toxicity, limited interference with biochemical reactions, very low absorbance between 240 nm and 700 nm, enzymatic and hydrolytic stability, minimal changes due to temperature and concentration, limited effects due to ionic or salt composition of the solution, limited interaction with mineral cations, and limited permeability of biological membranes.

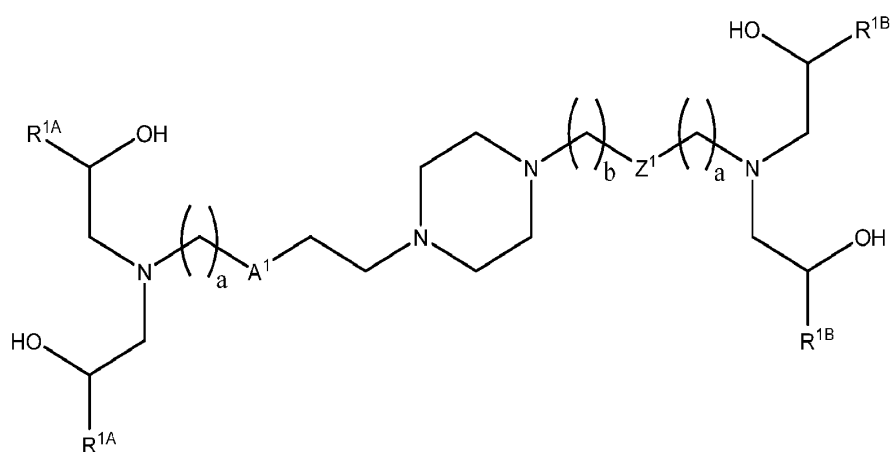
[005] The foregoing characteristics make “Good” buffers exceptionally good starting points for the synthesis of cationic lipids for use in *in vivo* settings. Many “Good” buffers remain crucial tools in modern biochemistry and biology laboratories and are therefore readily available at low cost.

SUMMARY

[006] The present invention provides, among other things, a novel class of cationic lipid compounds for *in vivo* delivery of therapeutic agents, such as nucleic acids. It is contemplated that these compounds are capable of highly effective *in vivo* delivery while maintaining a favorable toxicity profile.

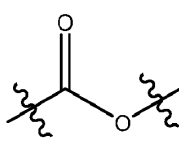
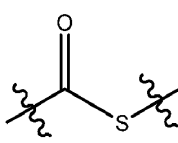
[007] The cationic lipids of the present invention can be synthesized from readily available starting reagents, such as “Good’s” buffers (see Table 1). The cationic lipids of the present invention also have unexpectedly high encapsulation efficiencies. The cationic lipids of the present invention also comprise cleavable groups (e.g., esters and disulphides) that are contemplated to improve biodegradability and thus contribute to their favorable toxicity profile.

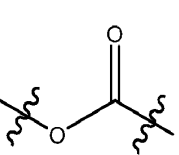
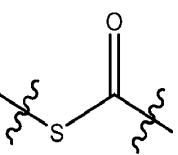
[008] In an aspect, provided herein are cationic lipids having a structure according to Formula (I):



or a pharmaceutically acceptable salt thereof,

wherein:

A¹ is selected from ,  and -S-S-, wherein the left hand side of each depicted structure is bound to the -(CH₂)_a-;

Z¹ is selected from ,  and -S-S-, wherein the right hand side of each depicted structure is bound to the -(CH₂)_a-;

R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted acyl and $-W^1-X^1-Y^1$; each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

each X^1 is independently selected from $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,

each Y^1 is independently selected from hydrogen, $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;

b is 1, 2, 3, 4 or 5; and

each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[009] In an aspect, provided herein are cationic lipids that are pharmaceutically acceptable salts of Formula (I).

[010] In an aspect, provided herein are compositions comprising the cationic lipid of the present invention or a pharmaceutically acceptable salt thereof, one or more non-cationic lipids, one or more cholesterol-based lipids and one or more PEG-modified lipid. In an aspect, the composition is a lipid nanoparticle, optionally a liposome.

[011] In an aspect, the compositions comprising the cationic lipids of the present invention may be used in therapy.

[012] In embodiments, the mRNA encodes a peptide or protein for use in vaccine.

[013] In embodiments, the mRNA encodes an antigen.

BRIEF DESCRIPTION OF DRAWINGS

[014] Figure 1 shows that lipid nanoparticles comprising the lipids described herein are highly effective in delivering hEPO mRNA and show high levels of hEPO protein expression at 6 hours post-IM injection dose.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

Definitions

[015] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification. The publications and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.

[016] *Amino acid*: As used herein, the term “amino acid,” in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain. In some embodiments, an amino acid has the general structure $\text{H}_2\text{N}-\text{C}(\text{H})(\text{R})-\text{COOH}$. In some embodiments, an amino acid is a naturally occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a d-amino acid; in some embodiments, an amino acid is an l-amino acid. “Standard amino acid” refers to any of the twenty standard l-amino acids commonly found in naturally occurring peptides. “Nonstandard amino acid” refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. As used herein, “synthetic amino acid” encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and/or substitutions. Amino acids, including carboxy- and/or amino-terminal amino acids in peptides, can be modified by methylation, amidation, acetylation, protecting groups, and/or substitution with other chemical groups that can change the peptide’s circulating half-life without adversely affecting their activity. Amino acids may participate in a disulfide bond. Amino acids may comprise one or posttranslational modifications, such as association with one or more chemical entities (e.g., methyl groups, acetate groups, acetyl groups, phosphate groups, formyl moieties, isoprenoid groups, sulfate groups, polyethylene glycol moieties, lipid moieties, carbohydrate moieties, biotin moieties, etc.). The term “amino acid” is used interchangeably with “amino acid residue,” and may refer to a free amino acid and/or to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide.

[017] *Animal*: As used herein, the term “animal” refers to any member of the animal kingdom. In some embodiments, “animal” refers to humans, at any stage of development. In some embodiments, “animal” refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, a bovine, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians,

fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

- [018] *Approximately or about:* As used herein, the term “approximately” or “about,” as applied to one or more values of interest, *refers* to a value that is similar to a stated reference value. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).
- [019] *Biologically active:* As used herein, the term “biologically active” refers to a characteristic of any agent that has activity in a *biological* system, and particularly in an organism. For instance, an agent that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active.
- [020] *Delivery:* As used herein, the term “delivery” encompasses both local and systemic delivery. For example, delivery of mRNA *encompasses* situations in which an mRNA is delivered to a target tissue and the encoded protein is expressed and retained within the target tissue (also referred to as “local distribution” or “local delivery”), and situations in which an mRNA is delivered to a target tissue and the encoded protein is expressed and secreted into patient’s circulation system (*e.g.*, serum) and systematically distributed and taken up by other tissues (also referred to as “systemic distribution” or “systemic delivery”).
- [021] *Expression:* As used herein, “expression” of a nucleic acid sequence refers to translation of an mRNA into a polypeptide, assemble multiple polypeptides into an intact protein (*e.g.*, enzyme) and/or post-translational modification of a polypeptide or fully assembled protein (*e.g.*, enzyme). In this application, the terms “expression” and “production,” and grammatical equivalents thereof, are used interchangeably.
- [022] *Functional:* As used *herein*, a “functional” biological molecule is a biological molecule in a form in which it exhibits a property and/or activity by which it is characterized.
- [023] *Half-life:* As used herein, the term “half-life” is the time required for a quantity such as nucleic acid or protein *concentration* or activity to fall to half of its value as measured at the beginning of a time period.
- [024] *Helper lipid:* The term “helper lipid” as used herein refers to any neutral or zwitterionic lipid material including cholesterol. Without wishing to be held to a particular theory, helper lipids may add stability, rigidity, and/or fluidity within lipid bilayers/nanoparticles.

- [025] *Improve, increase, or reduce*: As used herein, the terms “improve,” “increase,” or “reduce,” or grammatical equivalents, *indicate* values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control subject (or multiple control subject) in the absence of the treatment described herein. A “control subject” is a subject afflicted with the same form of disease as the subject being treated, who is about the same age as the subject being treated.
- [026] *In Vitro*: As used *herein*, the term “*in vitro*” refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, *etc.*, rather than within a multi-cellular organism.
- [027] *In Vivo*: As used herein, the term “*in vivo*” refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, *in vitro* systems).
- [028] *Isolated*: As used *herein*, the term “isolated” refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) produced, prepared, and/or manufactured by the hand of man. isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is “pure” if it is substantially free of other components. As used herein, calculation of percent purity of isolated substances and/or entities should not include excipients (*e.g.*, buffer, solvent, water, *etc.*).
- [029] *Liposome*: As used *herein*, the term “liposome” refers to any lamellar, multilamellar, or solid nanoparticle vesicle. Typically, a liposome as used herein can be formed by mixing one or more lipids or by mixing one or more lipids and polymer(s). In some embodiments, a liposome suitable for the present invention contains a cationic lipids(s) and optionally non-cationic lipid(s), optionally cholesterol-based lipid(s), and/or optionally PEG-modified lipid(s).
- [030] *messenger RNA (mRNA)*: As used herein, the term “messenger RNA (mRNA)” or “mRNA” refers to a polynucleotide that encodes at least one polypeptide. mRNA as used

herein encompasses both modified and unmodified RNA. The term “modified mRNA” related to mRNA comprising at least one chemically modified *nucleotide*. mRNA may contain one or more coding and non-coding regions. mRNA can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, *etc.* Where appropriate, *e.g.*, in the case of chemically synthesized molecules, mRNA can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, backbone modifications, *etc.* An mRNA sequence is presented in the 5′ to 3′ direction unless otherwise indicated. In some embodiments, an mRNA is or comprises natural nucleosides (*e.g.*, adenosine, guanosine, cytidine, uridine); nucleoside analogs (*e.g.*, 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (*e.g.*, methylated bases); intercalated bases; modified sugars (*e.g.*, 2′-fluororibose, ribose, 2′-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (*e.g.*, phosphorothioates and 5′-N-phosphoramidite linkages).

[031] *Nucleic acid*: As used herein, the term “nucleic acid,” in its broadest sense, refers to any compound and/or substance that is or can be incorporated into a polynucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into a polynucleotide chain via a phosphodiester linkage. In some embodiments, “nucleic acid” refers to individual nucleic acid residues (*e.g.*, nucleotides and/or nucleosides). In some embodiments, “nucleic acid” refers to a polynucleotide chain comprising individual nucleic acid residues. In some embodiments, “nucleic acid” encompasses RNA as well as single and/or double-stranded DNA and/or cDNA. In some embodiments, “nucleic acid” encompasses ribonucleic acids (RNA), including but not limited to any one or more of interference RNAs (RNAi), small interfering RNA (siRNA), short hairpin RNA (shRNA), antisense RNA (aRNA), messenger RNA (mRNA), modified messenger RNA (mmRNA), long non-coding RNA (lncRNA), micro-RNA (miRNA) multimeric coding nucleic acid (MCNA), polymeric coding nucleic acid (PCNA), guide RNA (gRNA) and CRISPR RNA (crRNA). In some embodiments, “nucleic acid” encompasses deoxyribonucleic acid (DNA), including but not limited to any one or more of single-stranded DNA (ssDNA), double-stranded DNA (dsDNA) and complementary DNA (cDNA). In some embodiments, “nucleic acid” encompasses both RNA and DNA. In embodiments, DNA may be in the form of antisense

DNA, plasmid DNA, parts of a plasmid DNA, pre-condensed DNA, a product of a polymerase chain reaction (PCR), vectors (*e.g.*, P1, PAC, BAC, YAC, artificial chromosomes), expression cassettes, chimeric sequences, chromosomal DNA, or derivatives of these groups. In embodiments, RNA may be in the form of messenger RNA (mRNA), ribosomal RNA (rRNA), signal recognition particle RNA (7 SL RNA or SRP RNA), transfer RNA (tRNA), transfer-messenger RNA (tmRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), SmY RNA, small Cajal body-specific RNA (scaRNA), guide RNA (gRNA), ribonuclease P (RNase P), Y RNA, telomerase RNA component (TERC), spliced leader RNA (SL RNA), antisense RNA (aRNA or asRNA), cis-natural antisense transcript (cis-NAT), CRISPR RNA (crRNA), long noncoding RNA (lncRNA), micro-RNA (miRNA), piwi-interacting RNA (piRNA), small interfering RNA (siRNA), transacting siRNA (tasiRNA), repeat associated siRNA (rasiRNA), 73K RNA, retrotransposons, a viral genome, a viroid, satellite RNA, or derivatives of these groups. In some embodiments, a nucleic acid is a mRNA encoding a protein such as an enzyme.

[032] *Patient:* As used herein, the term “patient” or “subject” refers to any organism to which a provided composition may be *administered*, *e.g.*, for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some embodiments, a patient is a human. A human includes pre- and post-natal forms.

[033] *Pharmaceutically acceptable:* The term “pharmaceutically acceptable,” as used herein, refers to substances that, within the *scope* of sound medical judgment, are suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[034] *Pharmaceutically acceptable salt:* Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge *et al.*, describes pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences* (1977) 66:1-19. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and *organic* acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid, or malonic acid, or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate,

digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4} \text{ alkyl})_4$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, sulfonate, and aryl sulfonate. Further pharmaceutically acceptable salts include salts formed from the quaternization of an amine using an appropriate electrophile, *e.g.*, an alkyl halide, to form a quaternized alkylated amino salt.

[035] *Systemic distribution or delivery:* As used herein, the terms “systemic distribution” or “systemic delivery,” or grammatical equivalents thereof, refer to a delivery or distribution mechanism or approach that affect the entire body or an entire organism. Typically, systemic distribution or delivery is accomplished via body’s circulation system, *e.g.*, blood stream. Compared to the definition of “local distribution or delivery.”

[036] *Subject:* As used herein, the term “subject” refers to a human or any non-human animal (*e.g.*, mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for *diagnosis* or treatment of a disease. The term “subject” is used herein interchangeably with “individual” or “patient.” A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[037] *Substantially:* As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will *understand* that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

- [038] *Target tissues:* As used herein, the term “target tissues” refers to any tissue that is affected by a disease to be treated. In some *embodiments*, target tissues include those tissues that display disease-associated pathology, symptom, or feature.
- [039] *Therapeutically effective amount:* As used herein, the term “therapeutically effective amount” of a therapeutic agent means an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the symptom(s) of the disease, disorder, and/or condition. It will be appreciated by those of ordinary skill in the art that a therapeutically effective amount is typically administered via a dosing regimen comprising at least one unit dose.
- [040] *Treating:* As used herein, the term “treat,” “treatment,” or “treating” refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may *be* administered to a subject who does not exhibit signs of a disease and/or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

Chemical definitions

- [041] *Acyl:* As used herein, the term “acyl” refers to $R^2-(C=O)-$, wherein R^2 is, for example, any alkyl, alkenyl, alkynyl, heteroalkyl or heteroalkylene.
- [042] *Aliphatic:* As used herein, the term aliphatic refers to C_1-C_{40} hydrocarbons and includes both saturated and unsaturated hydrocarbons. An aliphatic may be linear, branched, or cyclic. For example, C_1-C_{20} aliphatics can include C_1-C_{20} alkyls (*e.g.*, linear or branched C_1-C_{20} saturated alkyls), C_2-C_{20} alkenyls (*e.g.*, linear or branched C_4-C_{20} dienyls, linear or branched C_6-C_{20} trienyls, and the like), and C_2-C_{20} alkynyls (*e.g.*, linear or branched C_2-C_{20} alkynyls). C_1-C_{20} aliphatics can include C_3-C_{20} cyclic aliphatics (*e.g.*, C_3-C_{20} cycloalkyls, C_4-C_{20} cycloalkenyls, or C_8-C_{20} cycloalkynyls). In certain embodiments, the aliphatic may comprise one or more cyclic aliphatic and/or one or more heteroatoms such as oxygen, nitrogen, or sulfur and may optionally be substituted with one or more substituents such as alkyl, halo, alkoxy, hydroxy, amino, aryl, ether, ester or amide. An aliphatic group is unsubstituted or substituted with one or more substituent groups as described herein. For example, an aliphatic may be substituted with one or more (*e.g.*, 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, $-COR''$, $-CO_2H$, $-CO_2R''$, $-CN$, $-OH$, $-OR''$, $-OCOR'$, $-OCO_2R''$, $-NH_2$, $-NHR''$, $-N(R'')_2$, $-SR''$ or $-SO_2R''$, wherein each instance of R'' independently is C_1-C_{20} aliphatic

(*e.g.*, C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, **R''** independently is an unsubstituted alkyl (*e.g.*, unsubstituted C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, **R''** independently is unsubstituted C₁-C₃ alkyl. In embodiments, the aliphatic is unsubstituted. In embodiments, the aliphatic does not include any heteroatoms.

Alkyl: As used herein, the term "alkyl" means acyclic linear and branched hydrocarbon groups, *e.g.* "C₁-C₃₀ alkyl" refers to alkyl groups having 1-30 carbons. An alkyl group may be linear or branched. Examples of alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, tert-pentyl, hexyl, isohexyl, *etc.* The term "lower alkyl" means an alkyl group straight chain or branched alkyl having 1 to 6 carbon atoms. Other alkyl groups will be readily apparent to those of skill in the art given the benefit of the present disclosure. An alkyl group may be unsubstituted or substituted with one or more substituent groups as described herein. For example, an alkyl group may be substituted with one or more (*e.g.*, 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, -COR'', -CO₂H, -CO₂R'', -CN, -OH, -OR'', -OCOR', -OCO₂R'', -NH₂, -NHR'', -N(R'')₂, -SR'' or -SO₂R'', wherein each instance of **R''** independently is C₁-C₂₀ aliphatic (*e.g.*, C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, **R''** independently is an unsubstituted alkyl (*e.g.*, unsubstituted C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, **R''** independently is unsubstituted C₁-C₃ alkyl. In embodiments, the alkyl is substituted (*e.g.*, with 1, 2, 3, 4, 5, or 6 substituent groups as described herein). In embodiments, an alkyl group is substituted with a -OH group and may also be referred to herein as a "hydroxyalkyl" group, where the prefix denotes the -OH group and "alkyl" is as described herein.

[043] As used herein, "alkyl" also refers to a radical of a straight-chain or branched saturated hydrocarbon group having from 1 to 50 carbon atoms ("C₁-C₅₀ alkyl"). In some embodiments, an alkyl group has 1 to 40 carbon atoms ("C₁-C₄₀ alkyl"). In some embodiments, an alkyl group has 1 to 30 carbon atoms ("C₁-C₃₀ alkyl"). In some embodiments, an alkyl group has 1 to 20 carbon atoms ("C₁-C₂₀ alkyl"). In some embodiments, an alkyl group has 1 to 10 carbon atoms ("C₁-C₁₀ alkyl"). In some embodiments, an alkyl group has 1 to 9 carbon atoms ("C₁-C₉ alkyl"). In some embodiments, an alkyl group has 1 to 8 carbon atoms ("C₁-C₈ alkyl"). In some embodiments, an alkyl group has 1 to 7 carbon atoms ("C₁-C₇ alkyl"). In some embodiments, an alkyl group has 1 to 6 carbon atoms ("C₁-C₆ alkyl"). In some embodiments, an alkyl group has 1 to 5 carbon atoms ("C₁-C₅ alkyl"). In some embodiments, an alkyl group has 1 to 4 carbon atoms ("C₁-C₄ alkyl"). In some embodiments, an alkyl group has 1 to 3 carbon atoms ("C₁-C₃ alkyl"). In some

embodiments, an alkyl group has 1 to 2 carbon atoms (“C₁-C₂ alkyl”). In some embodiments, an alkyl group has 1 carbon atom (“C₁ alkyl”). In some embodiments, an alkyl group has 2 to 6 carbon atoms (“C₂-C₆ alkyl”). Examples of C₁-C₆ alkyl groups include, without limitation, methyl (C₁), ethyl (C₂), n-propyl (C₃), isopropyl (C₃), n-butyl (C₄), tert-butyl (C₄), sec-butyl (C₄), iso-butyl (C₄), n-pentyl (C₅), 3-pentanyl (C₅), amyl (C₅), neopentyl (C₅), 3-methyl-2-butanyl (C₅), tertiary amyl (C₅), and n-hexyl (C₆). Additional examples of alkyl groups include n-heptyl (C₇), n-octyl (C₈) and the like. Unless otherwise specified, each instance of an alkyl group is independently unsubstituted (an “unsubstituted alkyl”) or substituted (a “substituted alkyl”) with one or more substituents. In certain embodiments, the alkyl group is an unsubstituted C₁-C₅₀ alkyl. In certain embodiments, the alkyl group is a substituted C₁-C₅₀ alkyl.

[044] Affixing the suffix “-ene” to a group indicates the group is a divalent moiety, *e.g.*, arylene is the divalent moiety of aryl, and heteroarylene is the divalent moiety of heteroaryl.

[045] *Alkylene:* The term “alkylene,” as used herein, represents a saturated divalent straight or branched chain hydrocarbon group and is exemplified by methylene, ethylene, isopropylene and the like. Likewise, the term “alkenylene” as used herein represents an unsaturated divalent straight or branched chain hydrocarbon group having one or more unsaturated carbon-carbon double bonds that may occur in any stable point along the chain, and the term “alkynylene” herein represents an unsaturated divalent straight or branched chain hydrocarbon group having one or more unsaturated carbon-carbon triple bonds that may occur in any stable point along the chain. In certain embodiments, an alkylene, alkenylene, or alkynylene group may comprise one or more cyclic aliphatic and/or one or more heteroatoms such as oxygen, nitrogen, or sulfur and may optionally be substituted with one or more substituents such as alkyl, halo, alkoxyl, hydroxy, amino, aryl, ether, ester or amide. For example, an alkylene, alkenylene, or alkynylene may be substituted with one or more (*e.g.*, 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, -COR”, -CO₂H, -CO₂R”, -CN, -OH, -OR”, -OCOR”, -OCO₂R”, -NH₂, -NHR”, -N(R’’)₂, -SR” or -SO₂R”, wherein each instance of R” independently is C₁-C₂₀ aliphatic (*e.g.*, C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, R” independently is an unsubstituted alkyl (*e.g.*, unsubstituted C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, R” independently is unsubstituted C₁-C₃ alkyl. In certain embodiments, an alkylene, alkenylene, or alkynylene is unsubstituted. In certain embodiments, an alkylene, alkenylene, or alkynylene does not include any heteroatoms. *Alkenyl:* As used herein, “alkenyl” means any linear or branched hydrocarbon chains having one or more unsaturated carbon-carbon double bonds that may occur in any stable point along the chain, *e.g.* “C₂-C₃₀ alkenyl” refers to an alkenyl group

having 2-30 carbons. For example, an alkenyl group includes prop-2-enyl, but-2-enyl, but-3-enyl, 2-methylprop-2-enyl, hex-2-enyl, hex-5-enyl, 2,3-dimethylbut-2-enyl, and the like. In embodiments, the alkenyl comprises 1, 2, or 3 carbon-carbon double bond. In embodiments, the alkenyl comprises a single carbon-carbon double bond. In embodiments, multiple double bonds (*e.g.*, 2 or 3) are conjugated. An alkenyl group may be unsubstituted or substituted with one or more substituent groups as described herein. For example, an alkenyl group may be substituted with one or more (*e.g.*, 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, -COR'', -CO₂H, -CO₂R'', -CN, -OH, -OR'', -OCOR'', -OCO₂R'', -NH₂, -NHR'', -N(R'')₂, -SR'' or -SO₂R'', wherein each instance of R'' independently is C₁-C₂₀ aliphatic (*e.g.*, C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, R'' independently is an unsubstituted alkyl (*e.g.*, unsubstituted C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, R'' independently is unsubstituted C₁-C₃ alkyl. In embodiments, the alkenyl is unsubstituted. In embodiments, the alkenyl is substituted (*e.g.*, with 1, 2, 3, 4, 5, or 6 substituent groups as described herein). In embodiments, an alkenyl group is substituted with a-OH group and may also be referred to herein as a "hydroxyalkenyl" group, where the prefix denotes the -OH group and "alkenyl" is as described herein.

[046] As used herein, "alkenyl" also refers to a radical of a straight-chain or branched hydrocarbon group having from 2 to 50 carbon atoms and one or more carbon-carbon double bonds (*e.g.*, 1, 2, 3, or 4 double bonds) ("C₂-C₅₀ alkenyl"). In some embodiments, an alkenyl group has 2 to 40 carbon atoms ("C₂-C₄₀ alkenyl"). In some embodiments, an alkenyl group has 2 to 30 carbon atoms ("C₂-C₃₀ alkenyl"). In some embodiments, an alkenyl group has 2 to 20 carbon atoms ("C₂-C₂₀ alkenyl"). In some embodiments, an alkenyl group has 2 to 10 carbon atoms ("C₂-C₁₀ alkenyl"). In some embodiments, an alkenyl group has 2 to 9 carbon atoms ("C₂-C₉ alkenyl"). In some embodiments, an alkenyl group has 2 to 8 carbon atoms ("C₂-C₈ alkenyl"). In some embodiments, an alkenyl group has 2 to 7 carbon atoms ("C₂-C₇ alkenyl"). In some embodiments, an alkenyl group has 2 to 6 carbon atoms ("C₂-C₆ alkenyl"). In some embodiments, an alkenyl group has 2 to 5 carbon atoms ("C₂-C₅ alkenyl"). In some embodiments, an alkenyl group has 2 to 4 carbon atoms ("C₂-C₄ alkenyl"). In some embodiments, an alkenyl group has 2 to 3 carbon atoms ("C₂-C₃ alkenyl"). In some embodiments, an alkenyl group has 2 carbon atoms ("C₂ alkenyl"). The one or more carbon-carbon double bonds can be internal (such as in 2-butenyl) or terminal (such as in 1-butenyl). Examples of C₂-C₄ alkenyl groups include, without limitation, ethenyl (C₂), 1-propenyl (C₃), 2-propenyl (C₃), 1-butenyl (C₄), 2-butenyl (C₄), butadienyl (C₄), and the like. Examples of C₂-C₆ alkenyl groups include the aforementioned C₂-C₄ alkenyl groups as well as pentenyl (C₅),

pentadienyl (C₅), hexenyl (C₆), and the like. Additional examples of alkenyl include heptenyl (C₇), octenyl (C₈), octatrienyl (C₈), and the like. Unless otherwise specified, each instance of an alkenyl group is independently unsubstituted (an “unsubstituted alkenyl”) or substituted (a “substituted alkenyl”) with one or more substituents. In certain embodiments, the alkenyl group is an unsubstituted C₂-C₅₀ alkenyl. In certain embodiments, the alkenyl group is a substituted C₂-C₅₀ alkenyl.

[047] *Alkynyl:* As used herein, “alkynyl” means any hydrocarbon chain of either linear or branched configuration, having one or more carbon-carbon triple bonds occurring in any stable point along the chain, *e.g.*, “C₂-C₃₀ alkynyl”, refers to an alkynyl group having 2-30 carbons. Examples of an alkynyl group include prop-2-ynyl, but-2-ynyl, but-3-ynyl, pent-2-ynyl, 3-methylpent-4-ynyl, hex-2-ynyl, hex-5-ynyl, *etc.* In embodiments, an alkynyl comprises one carbon-carbon triple bond. An alkynyl group may be unsubstituted or substituted with one or more substituent groups as described herein. For example, an alkynyl group may be substituted with one or more (*e.g.*, 1, 2, 3, 4, 5, or 6 independently selected substituents) of halogen, -COR”, -CO₂H, -CO₂R”, -CN, -OH, -OR”, -OCOR”, -OCO₂R”, -NH₂, -NHR”, -N(R”)₂, -SR” or -SO₂R”, wherein each instance of R” independently is C₁-C₂₀ aliphatic (*e.g.*, C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, R” independently is an unsubstituted alkyl (*e.g.*, unsubstituted C₁-C₂₀ alkyl, C₁-C₁₅ alkyl, C₁-C₁₀ alkyl, or C₁-C₃ alkyl). In embodiments, R” independently is unsubstituted C₁-C₃ alkyl. In embodiments, the alkynyl is unsubstituted. In embodiments, the alkynyl is substituted (*e.g.*, with 1, 2, 3, 4, 5, or 6 substituent groups as described herein).

[048] As used herein, “alkynyl” also refers to a radical of a straight-chain or branched hydrocarbon group having from 2 to 50 carbon atoms and one or more carbon-carbon triple bonds (*e.g.*, 1, 2, 3, or 4 triple bonds) and optionally one or more double bonds (*e.g.*, 1, 2, 3, or 4 double bonds) (“C₂-C₅₀ alkynyl”). An alkynyl group that has one or more triple bonds and one or more double bonds is also referred to as an “ene-yne”. In some embodiments, an alkynyl group has 2 to 40 carbon atoms (“C₂-C₄₀ alkynyl”). In some embodiments, an alkynyl group has 2 to 30 carbon atoms (“C₂-C₃₀ alkynyl”). In some embodiments, an alkynyl group has 2 to 20 carbon atoms (“C₂-C₂₀ alkynyl”). In some embodiments, an alkynyl group has 2 to 10 carbon atoms (“C₂-C₁₀ alkynyl”). In some embodiments, an alkynyl group has 2 to 9 carbon atoms (“C₂-C₉ alkynyl”). In some embodiments, an alkynyl group has 2 to 8 carbon atoms (“C₂-C₈ alkynyl”). In some embodiments, an alkynyl group has 2 to 7 carbon atoms (“C₂-C₇ alkynyl”). In some embodiments, an alkynyl group has 2 to 6 carbon atoms (“C₂-C₆ alkynyl”). In some embodiments, an alkynyl group has 2 to 5 carbon atoms (“C₂-C₅ alkynyl”). In some

embodiments, an alkynyl group has 2 to 4 carbon atoms ("C₂-C₄ alkynyl"). In some embodiments, an alkynyl group has 2 to 3 carbon atoms ("C₂-C₃ alkynyl"). In some embodiments, an alkynyl group has 2 carbon atoms ("C₂ alkynyl"). The one or more carbon-carbon triple bonds can be internal (such as in 2-butyne) or terminal (such as in 1-butyne). Examples of C₂-C₄ alkynyl groups include, without limitation, ethynyl (C₂), 1-propynyl (C₃), 2-propynyl (C₃), 1-butyne (C₄), 2-butyne (C₄), and the like. Examples of C₂-C₆ alkenyl groups include the aforementioned C₂-C₄ alkynyl groups as well as pentynyl (C₅), hexynyl (C₆), and the like. Additional examples of alkynyl include heptynyl (C₇), octynyl (C₈), and the like. Unless otherwise specified, each instance of an alkynyl group is independently unsubstituted (an "unsubstituted alkynyl") or substituted (a "substituted alkynyl") with one or more substituents. In certain embodiments, the alkynyl group is an unsubstituted C₂-C₅₀ alkynyl. In certain embodiments, the alkynyl group is a substituted C₂-C₅₀ alkynyl.

[049] Aryl: The term "aryl" used alone or as part of a larger moiety as in "aralkyl," refers to a monocyclic, bicyclic, or tricyclic carbocyclic ring system having a total of six to fourteen ring members, wherein said ring system has a single point of attachment to the rest of the molecule, at least one ring in the system is aromatic and wherein each ring in the system contains 4 to 7 ring members. In embodiments, an aryl group has 6 ring carbon atoms ("C₆ aryl," e.g., phenyl). In some embodiments, an aryl group has 10 ring carbon atoms ("C₁₀ aryl," e.g., naphthyl such as 1-naphthyl and 2-naphthyl). In some embodiments, an aryl group has 14 ring carbon atoms ("C₁₄ aryl," e.g., anthracyl). "Aryl" also includes ring systems wherein the aryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the radical or point of attachment is on the aryl ring, and in such instances, the number of carbon atoms continue to designate the number of carbon atoms in the aryl ring system. Exemplary aryls include phenyl, naphthyl, and anthracene.

[050] As used herein, "aryl" also refers to a radical of a monocyclic or polycyclic (e.g., bicyclic or tricyclic) 4n+2 aromatic ring system (e.g., having 6, 10, or 14 π electrons shared in a cyclic array) having 6-14 ring carbon atoms and zero heteroatoms provided in the aromatic ring system ("C₆-C₁₄ aryl"). In some embodiments, an aryl group has 6 ring carbon atoms ("C₆ aryl"; e.g., phenyl). In some embodiments, an aryl group has 10 ring carbon atoms ("C₁₀ aryl"; e.g., naphthyl such as 1-naphthyl and 2-naphthyl). In some embodiments, an aryl group has 14 ring carbon atoms ("C₁₄ aryl"; e.g., anthracyl). "Aryl" also includes ring systems wherein the aryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the radical or point of attachment is on the aryl ring, and in such instances, the number of carbon atoms continue to designate the number of carbon atoms in the aryl ring

system. Unless otherwise specified, each instance of an aryl group is independently unsubstituted (an “unsubstituted aryl”) or substituted (a “substituted aryl”) with one or more substituents. In certain embodiments, the aryl group is an unsubstituted C₆-C₁₄ aryl. In certain embodiments, the aryl group is a substituted C₆-C₁₄ aryl.

[051] *Arylene:* The term “arylene” as used herein refers to an aryl group that is divalent (that is, having two points of attachment to the molecule). Exemplary arylenes include phenylene (e.g., unsubstituted phenylene or substituted phenylene).

[052] *Carbocyclyl:* As used herein, “carbocyclyl” or “carbocyclic” refers to a radical of a non-aromatic cyclic hydrocarbon group having from 3 to 10 ring carbon atoms (“C₃-C₁₀ carbocyclyl”) and zero heteroatoms in the non-aromatic ring system. In some embodiments, a carbocyclyl group has 3 to 8 ring carbon atoms (“C₃-C₈ carbocyclyl”). In some embodiments, a carbocyclyl group has 3 to 7 ring carbon atoms (“C₃-C₇ carbocyclyl”). In some embodiments, a carbocyclyl group has 3 to 6 ring carbon atoms (“C₃-C₆ carbocyclyl”). In some embodiments, a carbocyclyl group has 4 to 6 ring carbon atoms (“C₄-C₆ carbocyclyl”). In some embodiments, a carbocyclyl group has 5 to 6 ring carbon atoms (“C₅-C₆ carbocyclyl”). In some embodiments, a carbocyclyl group has 5 to 10 ring carbon atoms (“C₅-C₁₀ carbocyclyl”). Exemplary C₃-C₆ carbocyclyl groups include, without limitation, cyclopropyl (C₃), cyclopropenyl (C₃), cyclobutyl (C₄), cyclobutenyl (C₄), cyclopentyl (C₅), cyclopentenyl (C₅), cyclohexyl (C₆), cyclohexenyl (C₆), cyclohexadienyl (C₆), and the like. Exemplary C₃-C₈ carbocyclyl groups include, without limitation, the aforementioned C₃-C₆ carbocyclyl groups as well as cycloheptyl (C₇), cycloheptenyl (C₇), cycloheptadienyl (C₇), cycloheptatrienyl (C₇), cyclooctyl (C₈), cyclooctenyl (C₈), bicyclo[2.2.1]heptanyl (C₇), bicyclo[2.2.2]octanyl (C₈), and the like. Exemplary C₃-C₁₀ carbocyclyl groups include, without limitation, the aforementioned C₃-C₈ carbocyclyl groups as well as cyclononyl (C₉), cyclononenyl (C₉), cyclodecyl (C₁₀), cyclodecenyl (C₁₀), octahydro-1H-indenyl (C₉), decahydronaphthalenyl (C₁₀), spiro[4.5]decanyl (C₁₀), and the like. As the foregoing examples illustrate, in certain embodiments, the carbocyclyl group is either monocyclic (“monocyclic carbocyclyl”) or polycyclic (e.g., containing a fused, bridged or spiro ring system such as a bicyclic system (“bicyclic carbocyclyl”) or tricyclic system (“tricyclic carbocyclyl”)) and can be saturated or can contain one or more carbon-carbon double or triple bonds. “Carbocyclyl” also includes ring systems wherein the carbocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups wherein the point of attachment is on the carbocyclyl ring, and in such instances, the number of carbons continue to designate the number of carbons in the carbocyclic ring system. Unless

otherwise specified, each instance of a carbocyclyl group is independently unsubstituted (an “unsubstituted carbocyclyl”) or substituted (a “substituted carbocyclyl”) with one or more substituents. In certain embodiments, the carbocyclyl group is an unsubstituted C₃-C₁₀ carbocyclyl. In certain embodiments, the carbocyclyl group is a substituted C₃-C₁₀ carbocyclyl.

[053] In some embodiments, “carbocyclyl” or “carbocyclic” is referred to as a “cycloalkyl”, i.e., a monocyclic, saturated carbocyclyl group having from 3 to 10 ring carbon atoms (“C₃-C₁₀ cycloalkyl”). In some embodiments, a cycloalkyl group has 3 to 8 ring carbon atoms (“C₃-C₈ cycloalkyl”). In some embodiments, a cycloalkyl group has 3 to 6 ring carbon atoms (“C₃-C₆ cycloalkyl”). In some embodiments, a cycloalkyl group has 4 to 6 ring carbon atoms (“C₄-C₆ cycloalkyl”). In some embodiments, a cycloalkyl group has 5 to 6 ring carbon atoms (“C₅-C₆ cycloalkyl”). In some embodiments, a cycloalkyl group has 5 to 10 ring carbon atoms (“C₅-C₁₀ cycloalkyl”). Examples of C₅-C₆ cycloalkyl groups include cyclopentyl (C₅) and cyclohexyl (C₆). Examples of C₃-C₆ cycloalkyl groups include the aforementioned C₅-C₆ cycloalkyl groups as well as cyclopropyl (C₃) and cyclobutyl (C₄). Examples of C₃-C₈ cycloalkyl groups include the aforementioned C₃-C₆ cycloalkyl groups as well as cycloheptyl (C₇) and cyclooctyl (C₈). Unless otherwise specified, each instance of a cycloalkyl group is independently unsubstituted (an “unsubstituted cycloalkyl”) or substituted (a “substituted cycloalkyl”) with one or more substituents. In certain embodiments, the cycloalkyl group is an unsubstituted C₃-C₁₀ cycloalkyl. In certain embodiments, the cycloalkyl group is a substituted C₃-C₁₀ cycloalkyl.

[054] *Halogen:* As used herein, the term “halogen” means fluorine, chlorine, bromine, or iodine.

[055] *Heteroalkyl:* The term “heteroalkyl” is meant a branched or unbranched alkyl, alkenyl, or alkynyl group having from 1 to 14 carbon atoms in addition to 1, 2, 3 or 4 heteroatoms independently selected from the group consisting of N, O, S, and P. Heteroalkyls include tertiary amines, secondary amines, ethers, thioethers, amides, thioamides, carbamates, thiocarbamates, hydrazones, imines, phosphodiesteres, phosphoramidates, sulfonamides, and disulfides. A heteroalkyl group may optionally include monocyclic, bicyclic, or tricyclic rings, in which each ring desirably has three to six members. Examples of heteroalkyls include polyethers, such as methoxymethyl and ethoxyethyl.

[056] *Heteroalkylene:* The term “heteroalkylene,” as used herein, represents a divalent form of a heteroalkyl group as described herein.

- [057] *Heteroaryl*: The term “heteroaryl,” as used herein, is fully unsaturated heteroatom-containing ring wherein at least one ring atom is a heteroatom such as, but not limited to, nitrogen and oxygen.
- [058] As used herein, “heteroaryl” also refers to a radical of a 5-14 membered monocyclic or polycyclic (e.g., bicyclic or tricyclic) $4n+2$ aromatic ring system (e.g., having 6, 10, or 14 π electrons shared in a cyclic array) having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4 ring heteroatoms) ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus (“5-14 membered heteroaryl”). In heteroaryl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. Heteroaryl polycyclic ring systems can include one or more heteroatoms in one or both rings. “Heteroaryl” includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the point of attachment is on the heteroaryl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heteroaryl ring system. “Heteroaryl” also includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more aryl groups wherein the point of attachment is either on the aryl or heteroaryl ring, and in such instances, the number of ring members designates the number of ring members in the fused polycyclic (aryl/heteroaryl) ring system. Polycyclic heteroaryl groups wherein one ring does not contain a heteroatom (e.g., indolyl, quinolinyl, carbazolyl, and the like) the point of attachment can be on either ring, i.e., either the ring bearing a heteroatom (e.g., 2-indolyl) or the ring that does not contain a heteroatom (e.g., 5-indolyl).
- [059] In some embodiments, a heteroaryl group is a 5-10 membered aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus (“5-10 membered heteroaryl”). In some embodiments, a heteroaryl group is a 5-8 membered aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus (“5-8 membered heteroaryl”). In some embodiments, a heteroaryl group is a 5-6 membered aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus (“5-6 membered heteroaryl”). In some embodiments, the 5-6 membered

heteroaryl has 1 or more (e.g., 1, 2, or 3) ring heteroatoms selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus. In some embodiments, the 5-6 membered heteroaryl has 1 or 2 ring heteroatoms selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus. In some embodiments, the 5-6 membered heteroaryl has 1 ring heteroatom selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus. Unless otherwise specified, each instance of a heteroaryl group is independently unsubstituted (an “unsubstituted heteroaryl”) or substituted (a “substituted heteroaryl”) with one or more substituents. In certain embodiments, the heteroaryl group is an unsubstituted 5-14 membered heteroaryl. In certain embodiments, the heteroaryl group is a substituted 5-14 membered heteroaryl.

[060] Exemplary 5-membered heteroaryl groups containing 1 heteroatom include, without limitation, pyrrolyl, furanyl and thiophenyl. Exemplary 5-membered heteroaryl groups containing 2 heteroatoms include, without limitation, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, and isothiazolyl. Exemplary 5-membered heteroaryl groups containing 3 heteroatoms include, without limitation, triazolyl, oxadiazolyl, and thiadiazolyl. Exemplary 5-membered heteroaryl groups containing 4 heteroatoms include, without limitation, tetrazolyl. Exemplary 6-membered heteroaryl groups containing 1 heteroatom include, without limitation, pyridinyl. Exemplary 6-membered heteroaryl groups containing 2 heteroatoms include, without limitation, pyridazinyl, pyrimidinyl, and pyrazinyl. Exemplary 6-membered heteroaryl groups containing 3 or 4 heteroatoms include, without limitation, triazinyl and tetrazinyl, respectively. Exemplary 7-membered heteroaryl groups containing 1 heteroatom include, without limitation, azepinyl, oxepinyl, and thiepinyl. Exemplary 5,6-bicyclic heteroaryl groups include, without limitation, indolyl, isoindolyl, indazolyl, benzotriazolyl, benzothiophenyl, isobenzothiophenyl, benzofuranyl, benzoisofuranyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzoxadiazolyl, benzthiazolyl, benzisothiazolyl, benzthiadiazolyl, indolizinyl, and purinyl. Exemplary 6,6-bicyclic heteroaryl groups include, without limitation, naphthyridinyl, pteridinyl, quinolinyl, isoquinolinyl, cinnolinyl, quinoxalinyl, phthalazinyl, and quinazolinyl. Exemplary tricyclic heteroaryl groups include, without limitation, phenanthridinyl, dibenzofuranyl, carbazolyl, acridinyl, phenothiazinyl, phenoxazinyl and phenazinyl.

[061] As used herein, “heterocyclyl” or “heterocyclic” refers to a radical of a 3- to 14-membered non-aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus (“3-14 membered heterocyclyl”). In

heterocyclyl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. A heterocyclyl group can either be monocyclic ("monocyclic heterocyclyl") or polycyclic (e.g., a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic heterocyclyl") or tricyclic system ("tricyclic heterocyclyl")), and can be saturated or can contain one or more carbon-carbon double or triple bonds. Heterocyclyl polycyclic ring systems can include one or more heteroatoms in one or both rings.

"Heterocyclyl" also includes ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more carbocyclyl groups wherein the point of attachment is either on the carbocyclyl or heterocyclyl ring, or ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups, wherein the point of attachment is on the heterocyclyl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heterocyclyl ring system. Unless otherwise specified, each instance of heterocyclyl is independently unsubstituted (an "unsubstituted heterocyclyl") or substituted (a "substituted heterocyclyl") with one or more substituents. In certain embodiments, the heterocyclyl group is an unsubstituted 3-14 membered heterocyclyl. In certain embodiments, the heterocyclyl group is a substituted 3-14 membered heterocyclyl.

[062] In some embodiments, a heterocyclyl group is a 5-10 membered non-aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus ("5-10 membered heterocyclyl"). In some embodiments, a heterocyclyl group is a 5-8 membered non-aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus ("5-8 membered heterocyclyl"). In some embodiments, a heterocyclyl group is a 5-6 membered non-aromatic ring system having ring carbon atoms and 1 or more (e.g., 1, 2, 3, or 4) ring heteroatoms, wherein each heteroatom is independently selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus ("5-6 membered heterocyclyl"). In some embodiments, the 5-6 membered heterocyclyl has 1 or more (e.g., 1, 2, or 3) ring heteroatoms selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus. In some embodiments, the 5-6 membered heterocyclyl has 1 or 2 ring heteroatoms selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus. In some embodiments, the 5-6 membered heterocyclyl has 1 ring heteroatom selected from oxygen, sulfur, nitrogen, boron, silicon, and phosphorus.

[063] Exemplary 3-membered heterocyclyl groups containing 1 heteroatom include, without limitation, azirdinyl, oxiranyl, thiorenlyl. Exemplary 4-membered heterocyclyl groups containing 1 heteroatom include, without limitation, azetidiny, oxetanyl and thietanyl. Exemplary 5-membered heterocyclyl groups containing 1 heteroatom include, without limitation, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothiophenyl, dihydrothiophenyl, pyrrolidinyl, dihydropyrrolyl and pyrrolyl-2,5-dione. Exemplary 5-membered heterocyclyl groups containing 2 heteroatoms include, without limitation, dioxolanyl, oxathiolanyl and dithiolanyl. Exemplary 5-membered heterocyclyl groups containing 3 heteroatoms include, without limitation, triazoliny, oxadiazoliny, and thiadiazoliny. Exemplary 6-membered heterocyclyl groups containing 1 heteroatom include, without limitation, piperidinyl, tetrahydropyranyl, dihydropyridiny, and thianyl. Exemplary 6-membered heterocyclyl groups containing 2 heteroatoms include, without limitation, piperazinyl, morpholinyl, dithianyl, dioxanyl. Exemplary 6-membered heterocyclyl groups containing 2 heteroatoms include, without limitation, triazinanyl. Exemplary 7-membered heterocyclyl groups containing 1 heteroatom include, without limitation, azepanyl, oxepanyl and thiepanyl. Exemplary 8-membered heterocyclyl groups containing 1 heteroatom include, without limitation, azocanyl, oxecanyl and thiocanyl. Exemplary bicyclic heterocyclyl groups include, without limitation, indolinyl, isoindolinyl, dihydrobenzofuranyl, dihydrobenzothienyl, tetrahydrobenzothienyl, tetrahydrobenzofuranyl, tetrahydroindolyl, tetrahydroquinoliny, tetrahydroisoquinoliny, decahydroquinoliny, decahydroisoquinoliny, octahydrochromenyl, octahydroisochromenyl, decahydronaphthyridiny, decahydro-1,8-naphthyridiny, octahydropyrrolo[3,2-b]pyrrole, indolinyl, phthalimidyl, naphthalimidyl, chromanyl, chromenyl, 1H-benzo[e][1,4]diazepiny, 1,4,5,7-tetrahydropyrano[3,4-b] pyrrolyl, 5,6-dihydro-4H-furo[3,2-b]pyrrolyl, 6,7-dihydro-5H-furo[3,2-b]pyranyl, 5,7-dihydro-4H-thieno[2,3-c]pyranyl, 2,3-dihydro-1H-pyrrolo[2,3-b]pyridiny, 2,3-dihydrofuro[2,3-b]pyridiny, 4,5,6,7-tetrahydro-1H-pyrrolo-[2,3-b]pyridiny, 4,5,6,7-tetrahydrofuro[3,2-c]pyridiny, 4,5,6,7-tetrahydrothieno [3,2-b]pyridiny, 1,2,3,4-tetrahydro-1,6-naphthyridiny, and the like.

[064] *Heterocycloalkyl*: The term “heterocycloalkyl,” as used herein, is a non-aromatic ring wherein at least one atom is a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus, and the remaining atoms are carbon. The heterocycloalkyl group can be substituted or unsubstituted.

[065] As understood from the above, alkyl, alkenyl, alkynyl, acyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl groups, as defined herein, are, in certain embodiments, optionally substituted. Optionally substituted refers to a group which may be substituted or

unsubstituted (e.g., “substituted” or “unsubstituted” alkyl, “substituted” or “unsubstituted” alkenyl, “substituted” or “unsubstituted” alkynyl, “substituted” or “unsubstituted” heteroalkyl, “substituted” or “unsubstituted” heteroalkenyl, “substituted” or “unsubstituted” heteroalkynyl, “substituted” or “unsubstituted” carbocyclyl, “substituted” or “unsubstituted” heterocyclyl, “substituted” or “unsubstituted” aryl or “substituted” or “unsubstituted” heteroaryl group. In general, the term “substituted” means that at least one hydrogen present on a group is replaced with a permissible substituent, e.g., a substituent which upon substitution results in a stable compound, e.g., a compound which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction. Unless otherwise indicated, a “substituted” group has a substituent at one or more substitutable positions of the group, and when more than one position in any given structure is substituted, the substituent is either the same or different at each position. The term “substituted” is contemplated to include substitution with all permissible substituents of organic compounds, any of the substituents described herein that results in the formation of a stable compound. The present invention contemplates any and all such combinations in order to arrive at a stable compound. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any suitable substituent as described herein which satisfy the valencies of the heteroatoms and results in the formation of a stable moiety.

[066] Exemplary carbon atom substituents include, but are not limited to, halogen, -CN, -NO₂, -N₃, -SO₂, -SO₃H, -OH, -OR^{aa}, -ON(R^{bb})₂, -N(R^{bb})₂, -N(R^{bb})₃X⁻, -N(OR^{cc})R^{bb}, -SeH, -SeR^{aa}, -SH, -SR^{aa}, -SSR^{cc}, -C(=O)R^{aa}, -CO₂H, -CHO, -C(OR^{cc})₂, -CO₂R^{aa}, -OC(=O)R^{aa}, -OCO₂R^{aa}, -C(=O)N(R^{bb})₂, -OC(=O)N(R^{bb})₂, -NR^{bb}C(=O)R^{aa}, -NR^{bb}CO₂R^{aa}, -NR^{bb}C(=O)N(R^{bb})₂, -C(=NR^{bb})R^{aa}, -C(=NR^{bb})OR^{aa}, -OC(=NR^{bb})R^{aa}, -OC(=NR^{bb})OR^{aa}, -C(=NR^{bb})N(R^{bb})₂, -OC(=NR^{bb})N(R^{bb})₂, -NR^{bb}C(=NR^{bb})N(R^{bb})₂, -C(=O)NR^{bb}SO₂R^{aa}, -NR^{bb}SO₂R^{aa}, -SO₂N(R^{bb})₂, -SO₂R^{aa}, -SO₂OR^{aa}, -OSO₂R^{aa}, -S(=O)R^{aa}, -OS(=O)R^{aa}, -Si(R^{aa})₃, -OSi(R^{aa})₃, -C(=S)N(R^{bb})₂, -C(=O)SR^{aa}, -C(=S)SR^{aa}, -SC(=S)SR^{aa}, -SC(=O)SR^{aa}, -OC(=O)SR^{aa}, -SC(=O)OR^{aa}, -SC(=O)R^{aa}, -P(=O)₂R^{aa}, -OP(=O)₂R^{aa}, -P(=O)(R^{aa})₂, -OP(=O)(R^{aa})₂, -OP(=O)(OR^{cc})₂, -P(=O)₂N(R^{bb})₂, -OP(=O)₂N(R^{bb})₂, -P(=O)(NR^{bb})₂, -OP(=O)(NR^{bb})₂, -NR^{bb}P(=O)(OR^{cc})₂, -NR^{bb}P(=O)(NR^{bb})₂, -P(R^{cc})₂, -P(R^{cc})₃, -OP(R^{cc})₂, -OP(R^{cc})₃, -B(R^{aa})₂, -B(OR^{cc})₂, -BR^{aa}(OR^{cc}), C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₄ carbocyclyl, 3-14 membered heterocyclyl, C₆-C₁₄ aryl, and 5-14 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

or two geminal hydrogens on a carbon atom are replaced with the group =O, =S, =NN(R^{bb})₂, =NNR^{bb}C(=O)R^{aa}, =NNR^{bb}C(=O)OR^{aa}, =NNR^{bb}S(=O)₂R^{aa}, =NR^{bb}, or =NOR^{cc};

[067] each instance of R^{aa} is, independently, selected from C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, 3-14 membered heterocyclyl, C₆-C₁₄ aryl, and 5-14 membered heteroaryl, or two R^{aa} groups are joined to form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

[068] each instance of R^{bb} is, independently, selected from hydrogen, -OH, -OR^{aa}, -N(R^{cc})₂, -CN, -C(=O)R^{aa}, -C(=O)N(R^{cc})₂, -CO₂R^{aa}, -SO₂R^{aa}, -C(=NR^{cc})OR^{aa}, -C(=NR^{cc})N(R^{cc})₂, -SO₂N(R^{cc})₂, -SO₂R^{cc}, -SO₂OR^{cc}, -SOR^{aa}, -C(=S)N(R^{cc})₂, -C(=O)SR^{cc}, -C(=S)SR^{cc}, -P(=O)₂R^{aa}, -P(=O)(R^{aa})₂, -P(=O)₂N(R^{cc})₂, -P(=O)(R^{cc})₂, C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, 3-14 membered heterocyclyl, C₆-C₁₄ aryl, and 5-14 membered heteroaryl, or two R^{bb} groups, together with the heteroatom to which they are attached, form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

[069] each instance of R^{cc} is, independently, selected from hydrogen, C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, 3-14 membered heterocyclyl, C₆-C₁₄ aryl, and 5-14 membered heteroaryl, or two R^{cc} groups, together with the heteroatom to which they are attached, form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups;

[070] each instance of R^{dd} is, independently, selected from halogen, -CN, -NO₂, -N₃, -SO₂H, -SO₃H, -OH, -OR^{ee}, -ON(R^{ff})₂, -N(R^{ff})₂, -N(R^{ff})₃+X, -N(OR^{ee})R^{ff}, -SH, -SR^{ee}, -SSR^{ee}, -C(=O)R^{ee}, -CO₂H, -CO₂R^{ee}, -OC(=O)R^{ee}, -OCO₂R^{ee}, -C(=O)N(R^{ff})₂, -OC(=O)N(R^{ff})₂, -NR^{ff}C(=O)R^{ee}, -NR^{ff}CO₂R^{ee}, -NR^{ff}C(=O)N(R^{ff})₂, -C(=NR^{ff})OR^{ee}, -OC(=NR^{ff})R^{ee}, -OC(=NR^{ff})OR^{ee}, -C(=NR^{ff})N(R^{ff})₂, -OC(=NR^{ff})N(R^{ff})₂, -NR^{ff}C(=NR^{ff})N(R^{ff})₂, -NR^{ff}SO₂R^{ee}, -SO₂N(R^{ff})₂, -SO₂R^{ee}, -SO₂OR^{ee}, -OSO₂R^{ee}, -S(=O)R^{ee}, -Si(R^{ee})₃, -OSi(R^{ee})₃, -C(=S)N(R^{ff})₂, -C(=O)SR^{ee}, -C(=S)SR^{ee}, -SC(=S)SR^{ee}, -P(=O)₂R^{ee}, -P(=O)(R^{ee})₂, -OP(=O)(R^{ee})₂, -OP(=O)(OR^{ee})₂, C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, 3-10 membered heterocyclyl, C₆-C₁₀ aryl, 5-10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{gg} groups, or two geminal R^{dd} substituents can be joined to form =O or =S;

- [071] each instance of R^{ee} is, independently, selected from C_1 - C_{50} alkyl, C_2 - C_{50} alkenyl, C_2 - C_{50} alkynyl, C_3 - C_{10} carbocyclyl, C_6 - C_{10} aryl, 3-10 membered heterocyclyl, and 3-10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{eg} groups;
- [072] each instance of R^{ff} is, independently, selected from hydrogen, C_1 - C_{50} alkyl, C_2 - C_{50} alkenyl, C_2 - C_{50} alkynyl, C_3 - C_{10} carbocyclyl, 3-10 membered heterocyclyl, C_6 - C_{10} aryl and 5-10 membered heteroaryl, or two R^{ff} groups, together with the heteroatom to which they are attached, form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{eg} groups; and
- [073] each instance of R^{gg} is, independently, halogen, -CN, -NO₂, -N₃, -SO₂H, -SO₃H, -OH, -OC₁-C₅₀ alkyl, -ON(C₁-C₅₀ alkyl)₂, -N(C₁-C₅₀ alkyl)₂, -N(C₁-C₅₀ alkyl)₃+X⁻, -NH(C₁-C₅₀ alkyl)₂+X⁻, -NH₂(C₁-C₅₀ alkyl)+X⁻, -NH₃+X⁻, -N(OC₁-C₅₀ alkyl)(C₁-C₅₀ alkyl), -N(OH)(C₁-C₅₀ alkyl), -NH(OH), -SH, -SC₁-C₅₀ alkyl, -SS(C₁-C₅₀ alkyl), -C(=O)(C₁-C₅₀ alkyl), -CO₂H, -CO₂(C₁-C₅₀ alkyl), -OC(=O)(C₁-C₅₀ alkyl), -OCO₂(C₁-C₅₀ alkyl), -C(=O)NH₂, -C(=O)N(C₁-C₅₀ alkyl)₂, -OC(=O)NH(C₁-C₅₀ alkyl), -NHC(=O)(C₁-C₅₀ alkyl), -N(C₁-C₅₀ alkyl)C(=O)(C₁-C₅₀ alkyl), -NHCO₂(C₁-C₅₀ alkyl), -NHC(=O)N(C₁-C₅₀ alkyl)₂, -NHC(=O)NH(C₁-C₅₀ alkyl), -NHC(=O)NH₂, -C(=NH)O(C₁-C₅₀ alkyl), -OC(=NH)(C₁-C₅₀ alkyl), -OC(=NH)OC₁-C₅₀ alkyl, -C(=NH)N(C₁-C₅₀ alkyl)₂, -C(=NH)NH(C₁-C₅₀ alkyl), -C(=NH)NH₂, -OC(=NH)N(C₁-C₅₀ alkyl)₂, -OC(NH)NH(C₁-C₅₀ alkyl), -OC(NH)NH₂, -NHC(NH)N(C₁-C₅₀ alkyl)₂, -NHC(=NH)NH₂, -NHSO₂(C₁-C₅₀ alkyl), -SO₂N(C₁-C₅₀ alkyl)₂, -SO₂NH(C₁-C₅₀ alkyl), -SO₂NH₂, -SO₂(C₁-C₅₀ alkyl), -SO₂O(C₁-C₅₀ alkyl), -OSO₂(C₁-C₆ alkyl), -SO(C₁-C₆ alkyl), -Si(C₁-C₅₀ alkyl)₃, -OSi(C₁-C₆ alkyl)₃, -C(=S)N(C₁-C₅₀ alkyl)₂, C(=S)NH(C₁-C₅₀ alkyl), C(=S)NH₂, -C(=O)S(C₁-C₆ alkyl), -C(=S)S(C₁-C₆ alkyl), -SC(=S)S(C₁-C₆ alkyl), -P(=O)₂(C₁-C₅₀ alkyl), -P(=O)(C₁-C₅₀ alkyl)₂, -OP(=O)(C₁-C₅₀ alkyl)₂, -OP(=O)(OC₁-C₅₀ alkyl)₂, C_1 - C_{50} alkyl, C_2 - C_{50} alkenyl, C_2 - C_{50} alkynyl, C_3 - C_{10} carbocyclyl, C_6 - C_{10} aryl, 3-10 membered heterocyclyl, 5-10 membered heteroaryl; or two geminal R^{gg} substituents can be joined to form =O or =S; wherein X⁻ is a counterion.
- [074] As used herein, the term "halo" or "halogen" refers to fluorine (fluoro, -F), chlorine (chloro, -Cl), bromine (bromo, -Br), or iodine (iodo, -I).
- [075] As used herein, a "counterion" is a negatively charged group associated with a positively charged quarternary amine in order to maintain electronic neutrality. Exemplary counterions include halide ions (e.g., F⁻, Cl⁻, Br⁻, I⁻), NO₃⁻, ClO₄⁻, OH⁻, H₂PO₄⁻, HSO₄⁻, sulfonate ions (e.g., methanesulfonate, trifluoromethanesulfonate, p-toluenesulfonate, benzenesulfonate, 10-camphor sulfonate, naphthalene-2-sulfonate, naphthalene-1-sulfonic

acid-5-sulfonate, ethan-1-sulfonic acid-2-sulfonate, and the like), and carboxylate ions (e.g., acetate, ethanoate, propanoate, benzoate, glycerate, lactate, tartrate, glycolate, and the like).

[076] Nitrogen atoms can be substituted or unsubstituted as valency permits, and include primary, secondary, tertiary, and quarternary nitrogen atoms. Exemplary nitrogen atom substituents include, but are not limited to, hydrogen, -OH, -OR^{aa}, -N(R^{cc})₂, -CN, -C(=O)R^{aa}, -C(=O)N(R^{cc})₂, -CO₂R^{aa}, -SO₂R^{aa}, -C(=NR^{bb})R^{aa}, -C(=NR^{cc})OR^{aa}, -C(=NR^{cc})N(R^{cc})₂, -SO₂N(R^{cc})₂, -SO₂R^{cc}, -SO₂OR^{cc}, -SOR^{aa}, -C(=S)N(R^{cc})₂, -C(=O)SR^{cc}, -C(=S)SR^{cc}, -P(=O)₂R^{aa}, -P(=O)(R^{aa})₂, -P(=O)₂N(R^{cc})₂, -P(=O)(NR^{cc})₂, C₁-C₅₀ alkyl, C₂-C₅₀ alkenyl, C₂-C₅₀ alkynyl, C₃-C₁₀ carbocyclyl, 3-14 membered heterocyclyl, C₆-C₁₄ aryl, and 5-14 membered heteroaryl, or two R^{cc} groups, together with the N atom to which they are attached, form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 R^{dd} groups, and wherein R^{aa}, R^{bb}, R^{cc} and R^{dd} are as defined above.

[077] In certain embodiments, the substituent present on a nitrogen atom is a nitrogen protecting group (also referred to as an amino protecting group). Nitrogen protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.

[078] For example, nitrogen protecting groups such as amide groups (e.g., -C(=O)R^{aa}) include, but are not limited to, formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3-phenylpropanamide, picolinamide, 3-pyridylcarboxamide, N-benzoylphenylalanyl derivative, benzamide, p-phenylbenzamide, o-nitrophenylacetamide, o-nitrophenoxycetamide, acetoacetamide, (N'-dithiobenzyloxyacylamino)acetamide, 3-(p-hydroxyphenyl)propanamide, 3-(o-nitrophenyl)propanamide, 2-methyl-2-(o-nitrophenoxy)propanamide, 2-methyl-2-(o-phenylazophenoxy)propanamide, 4-chlorobutanamide, 3-methyl-3-nitrobutanamide, o-nitrocinnamide, N-acetylmethionine derivative, o-nitrobenzamide and o-(benzyloxymethyl)benzamide.

[079] Nitrogen protecting groups such as carbamate groups (e.g., -C(=O)OR^{aa}) include, but are not limited to, methyl carbamate, ethyl carbamate, 9-fluorenylmethyl carbamate (Fmoc), 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluorenylmethyl carbamate, 2,7-di-t-butyl-[9-(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl carbamate (DBD-Tmoc), 4-methoxyphenacyl carbamate (Phenoc), 2,2,2-trichloroethyl carbamate (Troc), 2-

trimethylsilylethyl carbamate (Teoc), 2-phenylethyl carbamate (hZ), 1-(1-adamanty1)-1-methylethyl carbamate (Adpoc), 1,1-dimethyl-2-haloethyl carbamate, 1,1-dimethyl-2,2-dibromoethyl carbamate (DB-t-BOC), 1,1-dimethyl-2,2,2-trichloroethyl carbamate (TCBOC), 1-methyl-1-(4-biphenyl)ethyl carbamate (Bpoc), 1-(3,5-di-t-butylphenyl)-1-methylethyl carbamate (t-Bumeoc), 2-(2'-and 4'-pyridyl)ethyl carbamate (Pyoc), 2-(N,N-dicyclohexylcarboxamido)ethyl carbamate, t-butyl carbamate (BOC), 1-adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1-isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4-nitrocinnamyl carbamate (Noc), 8-quinolyl carbamate, N-hydroxypiperidiny carbamate, alkylidithio carbamate, benzyl carbamate (Cbz), p-methoxybenzyl carbamate (Moz), p-nitobenzyl carbamate, p-bromobenzyl carbamate, p-chlorobenzyl carbamate, 2,4-dichlorobenzyl carbamate, 4-methylsulfinylbenzyl carbamate (MsZ), 9-anthrylmethyl carbamate, diphenylmethyl carbamate, 2-methylthioethyl carbamate, 2-methylsulfonylethyl carbamate, 2-(p-toluenesulfonyl)ethyl carbamate, [2-(1,3-dithianyl)]methyl carbamate (Dmoc), 4-methylthiophenyl carbamate (Mtpc), 2,4-dimethylthiophenyl carbamate (Bmpc), 2-phosphonioethyl carbamate (Peoc), 2-triphenylphosphonioisopropyl carbamate (Ppoc), 1,1-dimethyl-2-cyanoethyl carbamate, m-chloro-p-acyloxybenzyl carbamate, p-(dihydroxyboryl)benzyl carbamate, 5-benzisoxazolymethyl carbamate, 2-(trifluoromethyl)-6-chromonylmethyl carbamate (Tcroc), m-nitrophenyl carbamate, 3,5-dimethoxybenzyl carbamate, o-nitrobenzyl carbamate, 3,4-dimethoxy-6-nitrobenzyl carbamate, phenyl(o-nitrophenyl)methyl carbamate, t-amyl carbamate, S-benzyl thiocarbamate, p-cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, p-decyloxybenzyl carbamate, 2,2-dimethoxyacetylvinyl carbamate, o-(N,N-dimethylcarboxamido)benzyl carbamate, 1,1-dimethyl-3-(N,N-dimethylcarboxamido)propyl carbamate, 1,1-dimethylpropynyl carbamate, di(2-pyridyl)methyl carbamate, 2-furanylmethyl carbamate, 2-iodoethyl carbamate, isoborynl carbamate, isobutyl carbamate, isonicotinyl carbamate, p-(p'-methoxyphenylazo)benzyl carbamate, 1-methylcyclobutyl carbamate, 1-methylcyclohexyl carbamate, 1-methyl-1-cyclopropylmethyl carbamate, 1-methyl-1(3,5-dimethoxyphenyl)ethyl carbamate, 1-methyl-1-(p-phenylazophenyl)ethyl carbamate, 1-methyl-1-phenylethyl carbamate, 1-methyl-1-(4-pyridyl)ethyl carbamate, phenyl carbamate, p-(phenylazo)benzyl carbamate, 2,4,6-tri-t-butylphenyl carbamate, 4-(trimethylammonium)benzyl carbamate, and 2,4,6-trimethylbenzyl carbamate.

[080] Nitrogen protecting groups such as sulfonamide groups (e.g., $-S(=O)_2R^{aa}$) include, but are not limited to, p-toluenesulfonamide (Ts), benzenesulfonamide, 2,3,6-trimethyl-4-

methoxybenzenesulfonamide (Mtr), 2,4,6-trimethoxybenzenesulfonamide (Mtb), 2,6-dimethyl-4-methoxybenzenesulfonamide (Pme), 2,3,5,6-tetramethyl-4-methoxybenzenesulfonamide (Mte), 4-methoxybenzenesulfonamide (Mbs), 2,4,6-trimethylbenzenesulfonamide (Mts), 2,6-dimethoxy-4-methylbenzenesulfonamide (iMds), 2,2,5,7,8-pentamethylchroman-6-sulfonamide (Pmc), methanesulfonamide (Ms), β -trimethylsilylethanesulfonamide (SES), 9-anthracenesulfonamide, 4-(4',8'-dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzyisulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamide.

[081] Other nitrogen protecting groups include, but are not limited to, phenothiazinyl-(10)-acyl derivative, N'-p-toluenesulfonylaminoacyl derivative, N'-phenylaminothioacyl derivative, N-benzoylphenylalanyl derivative, N-acetylmethionine derivative, 4,5-diphenyl-3-oxazolin-2-one, N-phthalimide, N-dithiasuccinimide (Dts), N-2,3-diphenylmaleimide, N-2,5-dimethylpyrrole, N-1,1,4,4-tetramethyldisilylazacyclopentane adduct (STABASE), 5-substituted 1,3-dimethyl-1,3,5-triazacyclohexan-2-one, 5-substituted 1,3-dibenzyl-1,3,5-triazacyclohexan-2-one, 1-substituted 3,5-dinitro-4-pyridone, N-methylamine, N-allylamine, N-[2-(trimethylsilyl)ethoxy]methylamine (SEM), N-3-acetoxypyrrolamine, N-(1-isopropyl-4-nitro-2-oxo-3-pyrroline-3-yl)amine, quaternary ammonium salts, N-benzylamine, N-di(4-methoxyphenyl)methylamine, N-5-dibenzosuberylamine, N-triphenylmethylamine (Tr), N-[(4-methoxyphenyl)diphenylmethyl]amine (MMTr), N-9-phenylfluorenylamine (PhF), N-2,7-dichloro-9-fluorenylmethyleneamine, N-ferrocenylmethylamino (Fcm), N-2-picolylamino N'-oxide, N-1,1-dimethylthiomethyleneamine, N-benzylideneamine, N-p-methoxybenzylideneamine, N-diphenylmethyleneamine, N-[(2-pyridyl)mesityl]methyleneamine, N-(N',N'-dimethylaminomethylene)amine, N,N'-isopropylidenediamine, N-p-nitrobenzylideneamine, N-salicylideneamine, N-5-chlorosalicylideneamine, N-(5-chloro-2-hydroxyphenyl)phenylmethyleneamine, N-cyclohexylideneamine, N-(5,5-dimethyl-3-oxo-1-cyclohexenyl)amine, N-borane derivative, N-diphenylborinic acid derivative, N-[phenyl(pentaacylchromium- or tungsten)acyl]amine, N-copper chelate, N-zinc chelate, N-nitroamine, N-nitrosoamine, amine N-oxide, diphenylphosphinamide (Dpp), dimethylthiophosphinamide (Mpt), diphenylthiophosphinamide (Ppt), dialkyl phosphoramidates, dibenzyl phosphoramidate, diphenyl phosphoramidate, benzenesulfenamide, o-nitrobenzenesulfenamide (Nps), 2,4-dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2-nitro-4-methoxybenzenesulfenamide, triphenylmethylsulfenamide, and 3-nitropyridinesulfenamide (Npys).

- [082] In certain embodiments, the substituent present on an oxygen atom is an oxygen protecting group (also referred to as a hydroxyl protecting group). Oxygen protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.
- [083] Exemplary oxygen protecting groups include, but are not limited to, methyl, methoxymethyl (MOM), methylthiomethyl (MTM), t-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), p-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (p-AOM), guaiacolmethyl (GUM), t-butoxymethyl, 4-pentenylloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, t-butyl, allyl, p-chlorophenyl, p-methoxyphenyl, 2,4-dinitrophenyl, benzyl (Bn), p-methoxybenzyl, 3,4-dimethoxybenzyl, o-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, p-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl N-oxido, diphenylmethyl, p,p'-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, α -naphthylidiphenylmethyl, p-methoxyphenyldiphenylmethyl, di(p-methoxyphenyl)phenylmethyl, tri(p-methoxyphenyl)methyl, 4-(4'-bromophenacyloxyphenyl)diphenylmethyl, 4,4',4''-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4''-tris(levulinoyloxyphenyl)methyl, 4,4',4''-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4',4''-dimethoxyphenyl)methyl, 1,1-bis(4-methoxyphenyl)-1'-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodisulfuran-2-yl, benzisothiazolyl S,S-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylhexylsilyl, t-butyl dimethylsilyl (TBDMS), t-butyl diphenylsilyl (TBDPS), tribenzylsilyl, tri-p-xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), t-butylmethoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate,

triphenylmethoxyacetate, phenoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantate, crotonate, 4-methoxycrotonate, benzoate, p-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl)ethyl carbonate (Psec), 2-(triphenylphosphonio)ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate, alkyl allyl carbonate, alkyl p-nitrophenyl carbonate, alkyl benzyl carbonate, alkyl p-methoxybenzyl carbonate, alkyl 3,4-dimethoxybenzyl carbonate, alkyl o-nitrobenzyl carbonate, alkyl p-nitrobenzyl carbonate, alkyl S-benzyl thiocarbonate, 4-ethoxy-1-naphthyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, o-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (E)-2-methyl-2-butenate, o-(methoxyacyl)benzoate, α -naphthoate, nitrate, alkyl N,N,N',N'-tetramethylphosphorodiamidate, alkyl N-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-dinitrophenylsulfonate, sulfate, methanesulfonate (mesylate), benzylsulfonate, and tosylate (Ts).

[084] In certain embodiments, the substituent present on a sulfur atom is a sulfur protecting group (also referred to as a thiol protecting group). Sulfur protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.

[085] Exemplary sulfur protecting groups include, but are not limited to, alkyl, benzyl, p-methoxybenzyl, 2,4,6-trimethylbenzyl, 2,4,6-trimethoxybenzyl, o-hydroxybenzyl, p-hydroxybenzyl, o-acetoxybenzyl, p-acetoxybenzyl, p-nitrobenzyl, 4-picolyl, 2-quinolinylmethyl, 2-picolyl N-oxido, 9-anthrylmethyl, 9-fluorenylmethyl, xanthenyl, ferrocenylmethyl, diphenylmethyl, bis(4-methoxyphenyl)methyl, 5-dibenzosuberyl, triphenylmethyl, diphenyl-4-pyridylmethyl, phenyl, 2,4-dinitrophenyl, t-butyl, 1-adamantyl, methoxymethyl (MOM), isobutoxymethyl, benzyloxymethyl, 2-tetrahydropyranyl, benzylthiomethyl, phenylthiomethyl, thiazolidino, acetamidomethyl, trimethylacetamidomethyl, benzamidomethyl, allyloxycarbonylaminomethyl, phenylacetamidomethyl, phthalimidomethyl, acetylmethyl, carboxymethyl, cyanomethyl, (2-

nitro-1-phenyl)ethyl, 2-(2,4-dinitrophenyl)ethyl, 2-cyanoethyl, 2-(Trimethylsilyl)ethyl, 2,2-bis(carboethoxy)ethyl, (1-m-nitrophenyl-2-benzoyl)ethyl, 2-phenylsulfonyl, 2-(4-methylphenylsulfonyl)-2-methylprop-2-yl, acetyl, benzoyl, trifluoroacetyl, N-[[[p-biphenyl]isopropoxy]carbonyl]-N-methyl]-γ-aminothiobutyrate, 2,2,2-trichloroethoxycarbonyl, t-butoxycarbonyl, benzyloxycarbonyl, p-methoxybenzyloxycarbonyl, N-ethyl, N-methoxymethyl, sulfonate, sulphenylthiocarbonate, 3-nitro-2-pyridinesulfonyl sulfide, oxathiolone.

Compounds of the Invention

[086] Liposomal-based vehicles are considered an attractive carrier for therapeutic agents and remain subject to continued development efforts. While liposomal-based vehicles that comprise certain lipid components have shown promising results with regard to encapsulation, stability and site localization, there remains a great need for improvement of liposomal-based delivery systems. For example, a significant drawback of liposomal delivery systems relates to the construction of liposomes that have sufficient cell culture or *in vivo* stability to reach desired target cells and/or intracellular compartments, and the ability of such liposomal delivery systems to efficiently release their encapsulated materials to such target cells.

[087] In particular, there remains a need for improved lipids compounds that demonstrate improved pharmacokinetic properties and which are capable of delivering macromolecules, such as nucleic acids, to a wide variety cell types and tissues with enhanced efficiency. Importantly, there also remains a particular need for novel lipid compounds that are characterized as having reduced toxicity and are capable of efficiently delivering encapsulated nucleic acids and polynucleotides to targeted cells, tissues and organs.

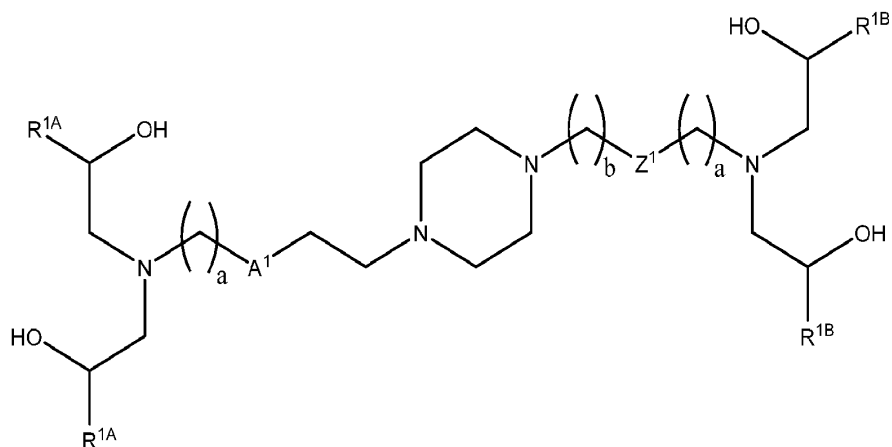
[088] Described herein a novel class of cationic lipid compounds for improved *in vivo* delivery of therapeutic agents, such as nucleic acids. In particular, a cationic lipid described herein may be used, optionally with other lipids, to formulate a lipid-based nanoparticle (*e.g.*, liposome) for encapsulating therapeutic agents, such as nucleic acids (*e.g.*, DNA, siRNA, mRNA, microRNA) for therapeutic use.

[089] In embodiments, compounds of the invention as described herein can provide one or more desired characteristics or properties. That is, in certain embodiments, compounds of the invention as described herein can be characterized as having one or more properties that afford such compounds advantages relative to other similarly classified lipids. For example, compounds disclosed herein can allow for the control and tailoring of the properties of

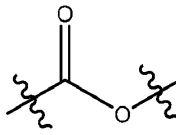
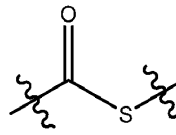
liposomal compositions (*e.g.*, lipid nanoparticles) of which they are a component. In particular, compounds disclosed herein can be characterized by enhanced transfection efficiencies and their ability to provoke specific biological outcomes. Such outcomes can include, for example enhanced cellular uptake, endosomal/lysosomal disruption capabilities and/or promoting the release of encapsulated materials (*e.g.*, polynucleotides) intracellularly. Additionally, the compounds disclosed herein have advantageous pharmacokinetic properties, biodistribution, and efficiency (*e.g.*, due to the different disassociate rates of the polymer group used).

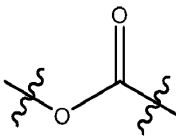
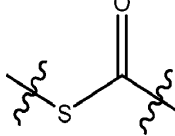
[090] The present application demonstrates that not only are the cationic lipids of the present invention synthetically tractable from readily available starting materials, but they also have unexpectedly high encapsulation efficiencies. Additionally, the cationic lipids of the present invention have cleavable groups such as ester groups and disulphides. These cleavable groups (*e.g.* esters and disulphides) are contemplated to improve biodegradability and thus contribute to their favorable toxicity profile.

[091] Provided herein are compounds which are cationic lipids and pharmaceutically acceptable salts thereof. For example, the cationic lipids of the present invention include compounds having a structure according to Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

A¹ is selected from ,  and -S-S-, wherein the left hand side of each depicted structure is bound to the -(CH₂)_a-;

Z^1 is selected from , , and -S-S-, wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$;

R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted acyl and $-W^1-X^1-Y^1$;

each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

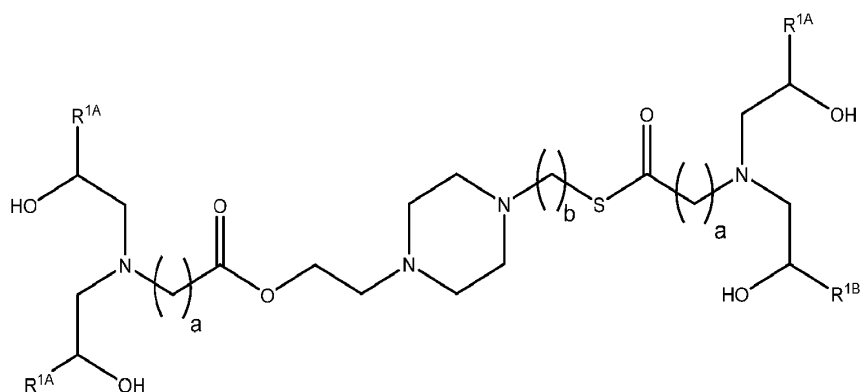
each X^1 is independently selected from $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,

each Y^1 is independently selected from hydrogen, $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;

b is 1, 2, 3, 4 or 5; and

each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[092] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (li):



or a pharmaceutically acceptable salt thereof, wherein:

R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted acyl and $-W^1-X^1-Y^1$;

each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

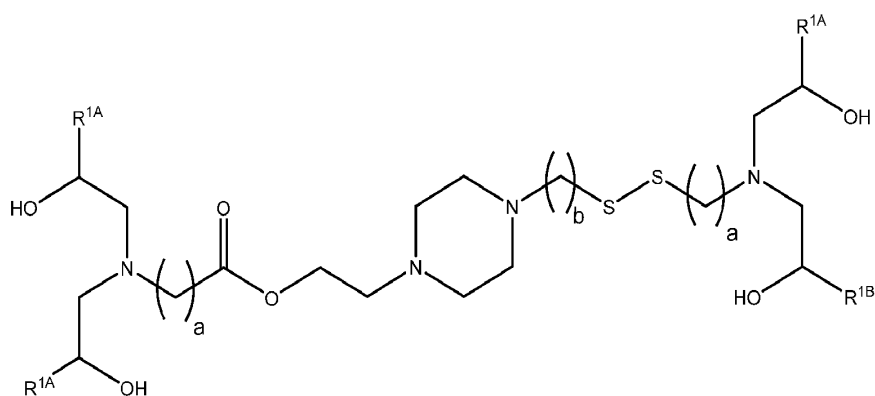
each X^1 is independently selected from $-^*O-(C=O)$ -optionally substituted alkyl, $-(^*C=O)-O$ -optionally substituted alkyl, $-^*O-(C=O)$ -optionally substituted alkenyl, and $-(^*C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,

each Y^1 is independently selected from hydrogen, $-^*O-(C=O)$ -optionally substituted alkyl, $-(^*C=O)-O$ -optionally substituted alkyl, $-^*O-(C=O)$ -optionally substituted alkenyl, and $-(^*C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;

b is 1, 2, 3, 4 or 5; and

each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[093] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (lii):



or a pharmaceutically acceptable salt thereof, wherein:

R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted acyl and $-W^1-X^1-Y^1$;

each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

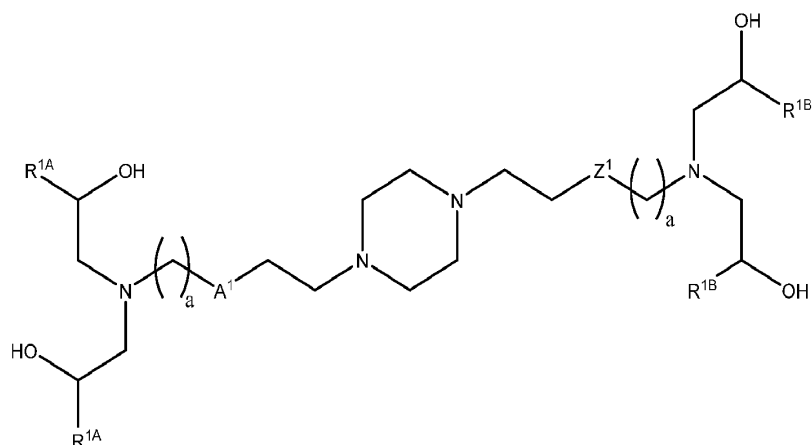
each X^1 is independently selected from $-^*O-(C=O)$ -optionally substituted alkyl, $-(^*C=O)-O$ -optionally substituted alkyl, $-^*O-(C=O)$ -optionally substituted alkenyl, and $-(^*C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,

each Y^1 is independently selected from hydrogen, $-^*O-(C=O)$ -optionally substituted alkyl, $-(^*C=O)-O$ -optionally substituted alkyl, $-^*O-(C=O)$ -optionally substituted alkenyl, and $-(^*C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;

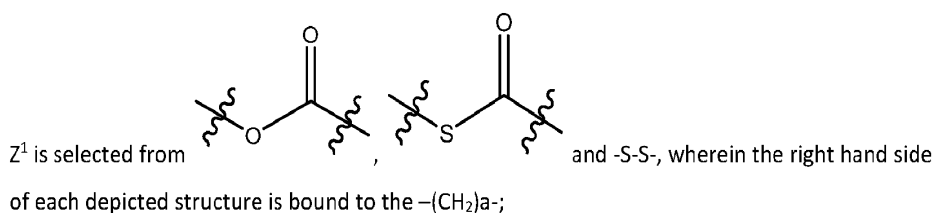
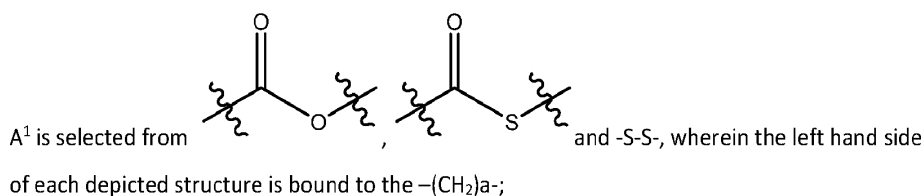
b is 1, 2, 3, 4 or 5; and

each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[094] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (Ia):



or a pharmaceutically acceptable salt thereof wherein:



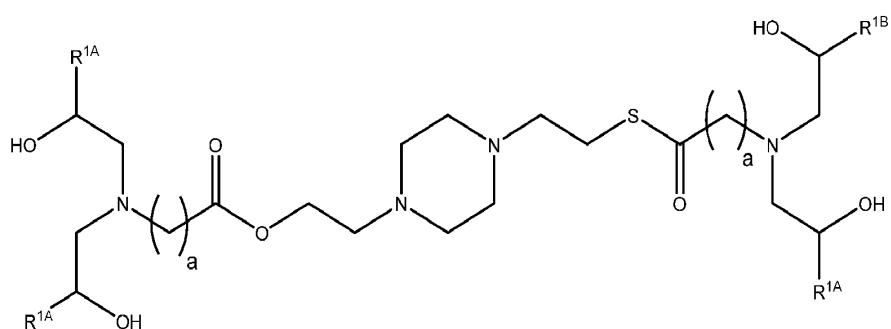
R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, and optionally substituted alkynyl, optionally substituted acyl and -W¹-X¹-Y¹; each W¹ is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

each X¹ is independently selected from -*O-(C=O)-optionally substituted alkyl, -(C=O)-O-optionally substituted alkyl, -*O-(C=O)-optionally substituted alkenyl, and -(C=O)-O-optionally substituted alkenyl, wherein the atom marked with a * is connected to W¹,

each Y¹ is independently selected from hydrogen, -*O-(C=O)-optionally substituted alkyl, -(C=O)-O-optionally substituted alkyl, -*O-(C=O)-optionally substituted alkenyl, and -(C=O)-O-optionally substituted alkenyl, wherein the atom marked with a * is connected to X¹;

each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[095] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (Ib):



or a pharmaceutically acceptable salt thereof wherein:

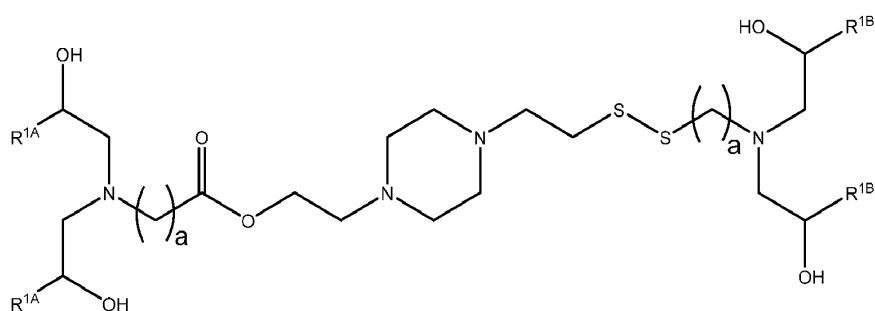
R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted acyl and $-W^1-X^1-Y^1$; each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

each X^1 is independently selected from $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,

each Y^1 is independently selected from hydrogen, $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;

each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[096] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (Ic):

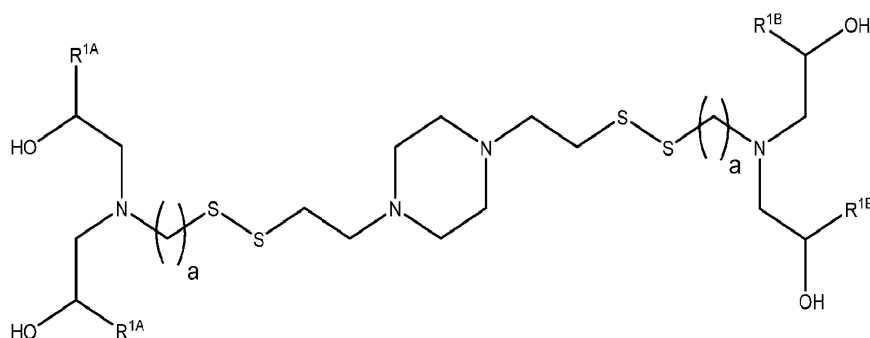


or a pharmaceutically acceptable salt thereof wherein:

R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl optionally substituted acyl and $-W^1-X^1-Y^1$; each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

each X^1 is independently selected from $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,
 each Y^1 is independently selected from hydrogen, $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;
 each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

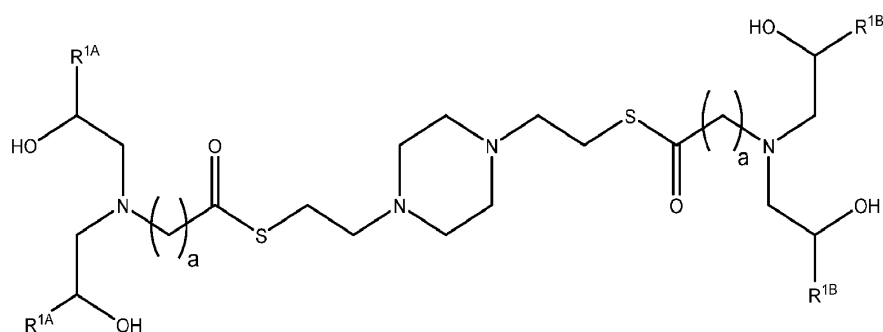
[097] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (Id):



or a pharmaceutically acceptable salt thereof wherein:

R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl optionally substituted acyl and $-W^1-X^1-Y^1$;
 each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,
 each X^1 is independently selected from $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,
 each Y^1 is independently selected from hydrogen, $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;
 each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[098] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (Ie):



or a pharmaceutically acceptable salt thereof wherein:

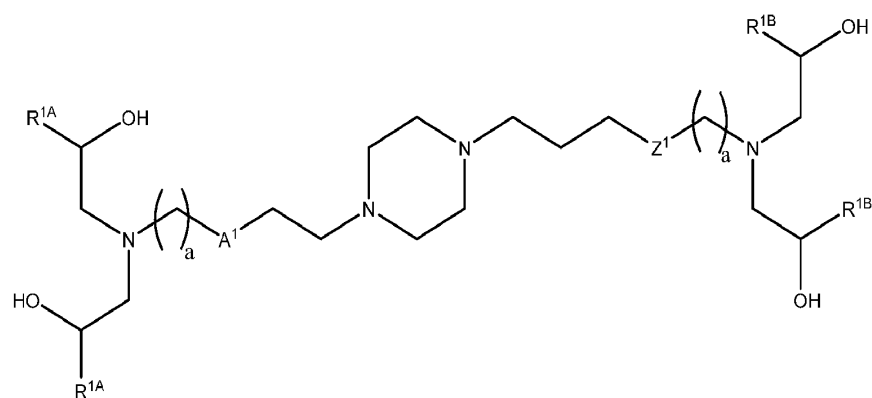
R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl optionally substituted acyl and $-\text{W}^1-\text{X}^1-\text{Y}^1$; each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

each X^1 is independently selected from $-\text{*O}-(\text{C}=\text{O})$ -optionally substituted alkyl, $-(\text{*C}=\text{O})$ -O-optionally substituted alkyl, $-\text{*O}-(\text{C}=\text{O})$ -optionally substituted alkenyl, and $-(\text{*C}=\text{O})$ -O-optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,

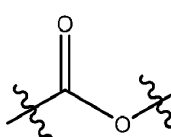
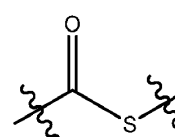
each Y^1 is independently selected from hydrogen, $-\text{*O}-(\text{C}=\text{O})$ -optionally substituted alkyl, $-(\text{*C}=\text{O})$ -O-optionally substituted alkyl, $-\text{*O}-(\text{C}=\text{O})$ -optionally substituted alkenyl, and $-(\text{*C}=\text{O})$ -O-optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;

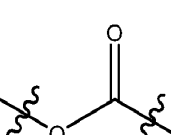
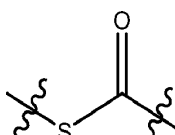
each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[099] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (IIa):



or a pharmaceutically acceptable salt thereof wherein:

A^1 is selected from ,  and -S-S-, wherein the left hand side of each depicted structure is bound to the $-(CH_2)_a-$;

Z^1 is selected from ,  and -S-S-, wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$;

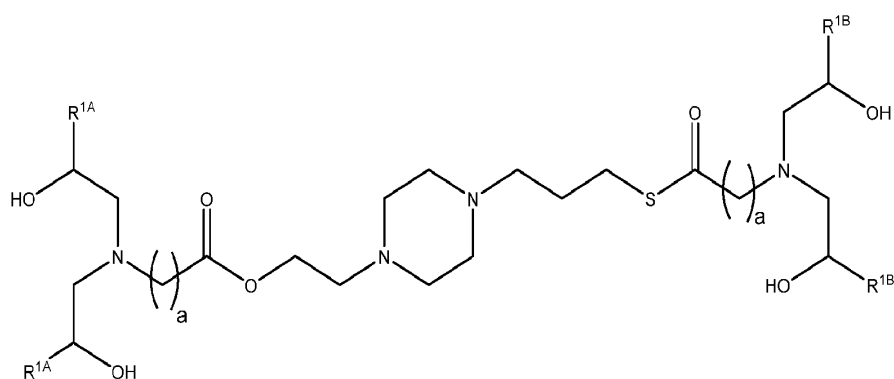
R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, and optionally substituted alkynyl, optionally substituted acyl and $-W^1-X^1-Y^1$; each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

each X^1 is independently selected from $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,

each Y^1 is independently selected from hydrogen, $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;

each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[0100] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (IIb):



or a pharmaceutically acceptable salt thereof wherein:

R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted acyl and $-W^1-X^1-Y^1$;

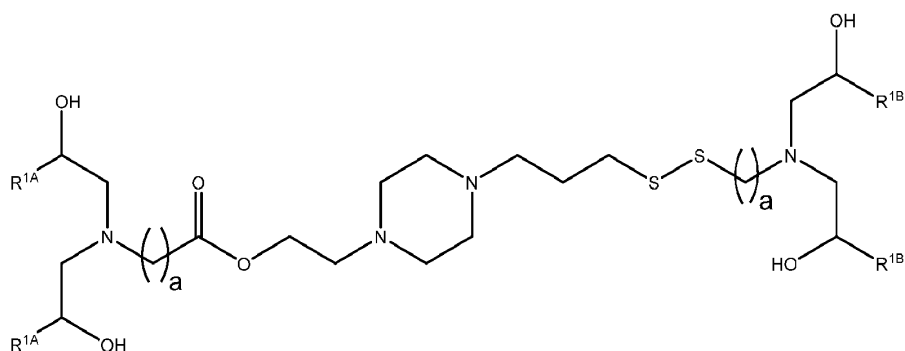
each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

each X^1 is independently selected from $-O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,

each Y^1 is independently selected from hydrogen, $-O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;

each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[0101] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (IIc):



or a pharmaceutically acceptable salt thereof wherein:

R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl optionally substituted acyl and $-W^1-X^1-Y^1$;

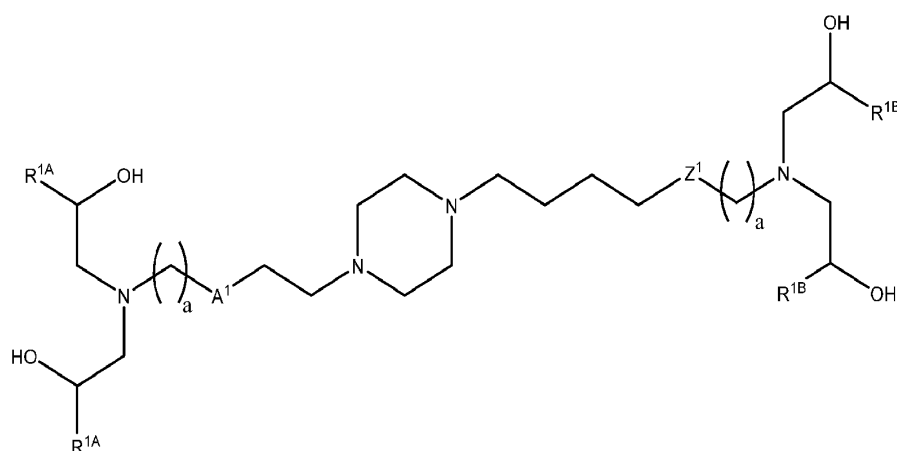
each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

each X^1 is independently selected from $-O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,

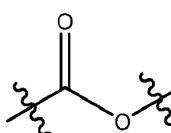
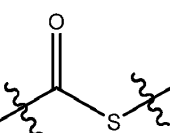
each Y^1 is independently selected from hydrogen, $-O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;

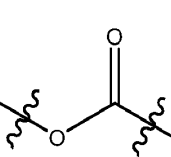
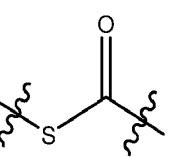
each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[0102] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (IIIa):



or a pharmaceutically acceptable salt thereof wherein:

A^1 is selected from ,  and -S-S-, wherein the left hand side of each depicted structure is bound to the $-(CH_2)_a-$;

Z^1 is selected from ,  and -S-S-, wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$;

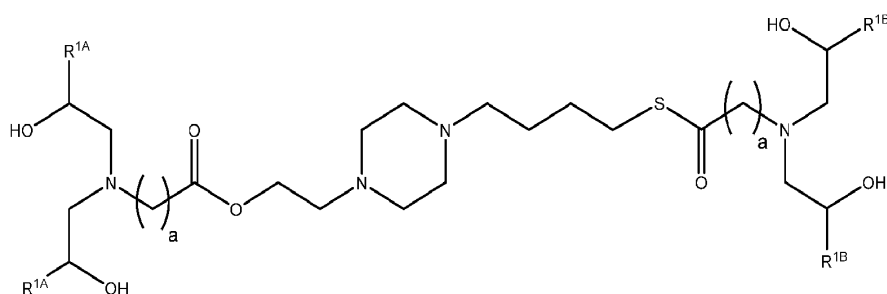
R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, and optionally substituted alkynyl, optionally substituted acyl and $-W^1-X^1-Y^1$; each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

each X^1 is independently selected from $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,

each Y^1 is independently selected from hydrogen, $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;

each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

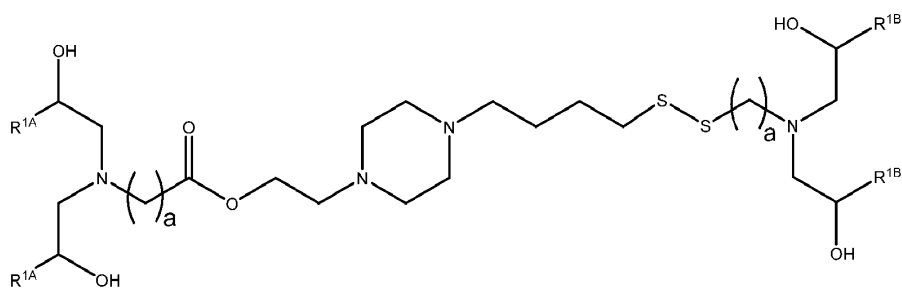
[0103] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (IIIb):



or a pharmaceutically acceptable salt thereof wherein:

R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted acyl and $-W^1-X^1-Y^1$; each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl, each X^1 is independently selected from $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 , each Y^1 is independently selected from hydrogen, $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ; each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

[0104] In embodiments, the cationic lipids of the present invention include compounds having a structure according to Formula (IIIc):



or a pharmaceutically acceptable salt thereof wherein:

R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl optionally substituted acyl and $-W^1-X^1-Y^1$; each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl,

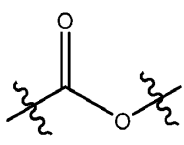
each X^1 is independently selected from $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O-$ optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O-$ optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 ,
 each Y^1 is independently selected from hydrogen, $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O-$ optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O-$ optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;
 each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

Linkers of the invention

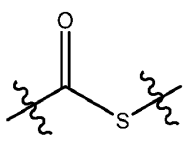
[0105] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or a pharmaceutically acceptable salt thereof), A^1 and Z^1 are the same.

[0106] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or a pharmaceutically acceptable salt thereof), A^1 and Z^1 are different.

[0107] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or

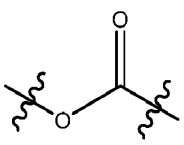
a pharmaceutically acceptable salt thereof), A^1 is , wherein the left hand side of each depicted structure is bound to the $-(CH_2)_a-$.

[0108] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or

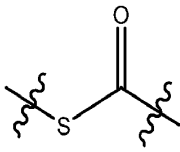
a pharmaceutically acceptable salt thereof), A^1 is , wherein the left hand side of each depicted structure is bound to the $-(CH_2)_a-$.

[0109] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or a pharmaceutically acceptable salt thereof), A^1 is $-S-S-$, wherein the left hand side of each depicted structure is bound to the $-(CH_2)_a-$.

[0110] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or

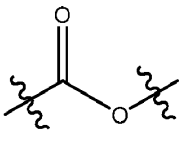
a pharmaceutically acceptable salt thereof), Z^1 is , wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

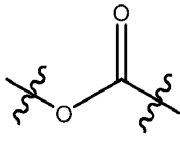
[0111] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or

a pharmaceutically acceptable salt thereof), Z^1 is,  wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

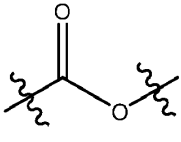
[0112] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or a pharmaceutically acceptable salt thereof), Z^1 is, $-S-S-$, wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

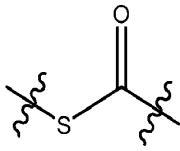
[0113] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or

a pharmaceutically acceptable salt thereof) A^1 is , wherein the left hand

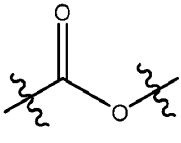
side of each depicted structure is bound to the $-(CH_2)_a-$ and Z^1 is , wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

[0114] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or

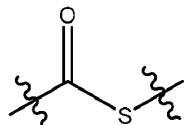
a pharmaceutically acceptable salt thereof) A^1 is , wherein the left hand

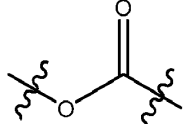
side of each depicted structure is bound to the $-(CH_2)_a-$ and Z^1 is , wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

[0115] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or

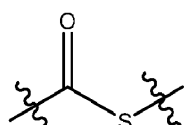
a pharmaceutically acceptable salt thereof) A^1 is , wherein the left hand side of each depicted structure is bound to the $-(CH_2)_a-$ and Z^1 is $-S-S-$, wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

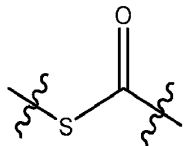
[0116] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or

a pharmaceutically acceptable salt thereof) A^1 is , wherein the left hand

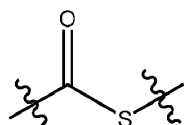
side of each depicted structure is bound to the $-(CH_2)_a-$ and Z^1 is , wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

[0117] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or

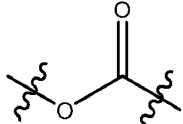
a pharmaceutically acceptable salt thereof) A^1 is , wherein the left hand

side of each depicted structure is bound to the $-(CH_2)_a-$ and Z^1 is , wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

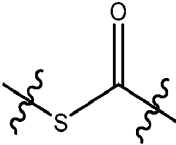
[0118] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or

a pharmaceutically acceptable salt thereof) A^1 is , wherein the left hand side of each depicted structure is bound to the $-(CH_2)_a-$ and Z^1 is $-S-S-$, wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

[0119] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or a pharmaceutically acceptable salt thereof) A^1 is $-S-S-$, wherein the left hand side of each

depicted structure is bound to the $-(CH_2)_a-$ and Z^1 is , wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

[0120] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or a pharmaceutically acceptable salt thereof) A^1 is $-S-S-$, wherein the left hand side of each

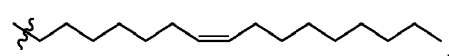
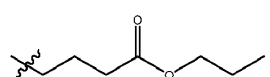
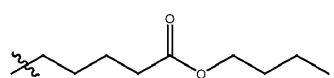
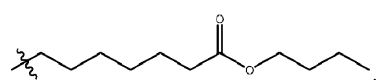
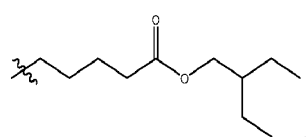
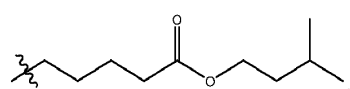
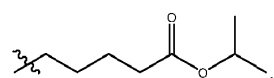
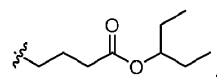
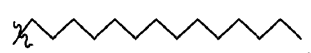
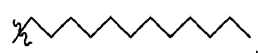
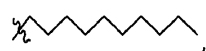
depicted structure is bound to the $-(CH_2)_a-$ and Z^1 is , wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

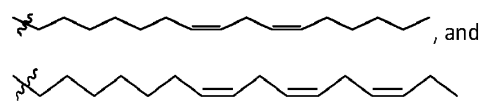
[0121] In any of the above embodiments (e.g. a compound of Formula (I), (Ia), (IIa), (IIIa) or a pharmaceutically acceptable salt thereof) A^1 is $-S-S-$, wherein the left hand side of each depicted structure is bound to the $-(CH_2)_a-$ and Z^1 is $-S-S-$, wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

Side chains of the invention

[0122] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIC) or a pharmaceutically acceptable salt thereof),

R^{1A} and R^{1B} may each independently be selected from: ,





[0123] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), each a is preferably independently selected from 2, 3 and 4.

[0124] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), each a is the same.

[0125] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), each a is different.

[0126] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R^{1A} and R^{1B} are preferably each independently selected from optionally substituted alkyl and optionally substituted alkenyl.

[0127] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), $W^1-X^1-Y^1$ is defined as follows:

each W^1 is independently selected from optionally substituted C_{1-20} alkyl and optionally substituted C_{2-20} alkenyl,

each X^1 is independently selected from $-*O-(C=O)$ -optionally substituted C_{1-20} alkyl, $-(C=O)-O$ -optionally substituted C_{1-20} alkyl, $-*O-(C=O)$ -optionally substituted C_{2-20} alkenyl, and $-(C=O)-O$ -optionally substituted C_{2-20} alkenyl, wherein the atom marked with a * is connected to W^1 ,

each Y^1 is independently selected from hydrogen, $-*O-(C=O)$ -optionally substituted C_{1-20} alkyl, $-(C=O)-O$ -optionally substituted C_{1-20} alkyl, $-*O-(C=O)$ -optionally substituted C_{2-20} alkenyl, and $-(C=O)-O$ -optionally substituted C_{2-20} alkenyl, wherein the atom marked with a * is connected to X^1 .

[0128] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), $W^1-X^1-Y^1$ is defined as follows:

each W^1 is independently selected from optionally substituted C_{A-B} alkyl and optionally substituted C_{C-D} alkenyl,

each X^1 is independently selected from $-O-(C=O)$ -optionally substituted C_{A-B} alkyl, $-(C=O)-O$ -optionally substituted C_{A-B} alkyl, $-O-(C=O)$ -optionally substituted C_{C-D} alkenyl, and $-(C=O)-O$ -optionally substituted C_{C-D} alkenyl, wherein the atom marked with a * is connected to W^1 ,

each Y^1 is independently selected from hydrogen, $-O-(C=O)$ -optionally substituted C_{A-B} alkyl, $-(C=O)-O$ -optionally substituted C_{A-B} alkyl, $-O-(C=O)$ -optionally substituted C_{C-D} alkenyl, and $-(C=O)-O$ -optionally substituted C_{C-D} alkenyl, wherein the atom marked with a * is connected to X^1 .

In some embodiments C_{A-B} is C_{1-20} and C_{C-D} is C_{2-20} . In some embodiments C_{A-B} is C_{1-15} and C_{C-D} is C_{2-15} . In some embodiments C_{A-B} is C_{1-10} and C_{C-D} is C_{2-10} . In some embodiments C_{A-B} is C_{3-15} and C_{C-D} is C_{3-15} . In some embodiments C_{A-B} is C_{3-10} and C_{C-D} is C_{3-10} . In some embodiments C_{A-B} is C_{3-8} and C_{C-D} is C_{3-8} .

[0129] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R_{1A} and R_{1B} are each independently selected from optionally substituted C_{5-50} alkyl, optionally substituted C_{5-50} alkenyl, optionally substituted C_{5-50} alkynyl, optionally substituted C_{5-50} acyl, and $-W^1-X^1-Y^1$, wherein $-W^1-X^1-Y^1$ is as defined herein and C_{A-B} is C_{1-20} and C_{C-D} is C_{2-20} .

[0130] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R_{1A} and R_{1B} are each independently selected from optionally substituted C_{5-50} alkyl, optionally substituted C_{5-50} alkenyl, optionally substituted C_{5-50} alkynyl, and optionally substituted C_{5-50} acyl.

[0131] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R_{1A} and R_{1B} are each independently selected from optionally substituted C_{5-40} alkyl, optionally substituted C_{5-40} alkenyl, optionally substituted C_{5-40} alkynyl, optionally substituted C_{5-40} acyl, and $-W^1-X^1-Y^1$, wherein $-W^1-X^1-Y^1$ is as defined herein and C_{A-B} is C_{1-15} and C_{C-D} is C_{2-15} .

[0132] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R_{1A} and R_{1B} are each independently selected from optionally substituted C_{5-40} alkyl, optionally substituted C_{5-40} alkenyl, optionally substituted C_{5-40} alkynyl, and optionally substituted C_{5-40} acyl.

- [0133]** In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₃₀ alkyl, optionally substituted C₅₋₃₀ alkenyl, optionally substituted C₅₋₃₀ alkynyl, optionally substituted C₅₋₃₀ acyl and -W¹-X¹-Y¹, wherein -W¹-X¹-Y¹ is as defined herein and C_{A-B} is C₁₋₁₀ and C_{C-D} is C₂₋₁₀.
- [0134]** In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₃₀ alkyl, optionally substituted C₅₋₃₀ alkenyl, optionally substituted C₅₋₃₀ alkynyl, and optionally substituted C₅₋₃₀ acyl.
- [0135]** In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₂₅ alkyl, optionally substituted C₅₋₂₅ alkenyl, optionally substituted C₅₋₂₅ alkynyl, optionally substituted C₅₋₂₅ acyl, and -W¹-X¹-Y¹, wherein -W¹-X¹-Y¹ is as defined herein and C_{A-B} is C₃₋₁₅ and C_{C-D} is C₃₋₁₅.
- [0136]** In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₂₅ alkyl, optionally substituted C₅₋₂₅ alkenyl, optionally substituted C₅₋₂₅ alkynyl, and optionally substituted C₅₋₂₅ acyl.
- [0137]** In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R^{1A} and R^{1B} are each: independently selected from optionally substituted C₅₋₂₀ alkyl, optionally substituted C₅₋₂₀ alkenyl, optionally substituted C₅₋₂₀ alkynyl, optionally substituted C₅₋₂₀ acyl, and -W¹-X¹-Y¹, wherein -W¹-X¹-Y¹ is as defined herein and C_{A-B} is C₃₋₁₀ and C_{C-D} is C₃₋₁₀.
- [0138]** In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₂₀ alkyl, optionally substituted C₅₋₂₀ alkenyl, optionally substituted C₅₋₂₀ alkynyl, optionally substituted C₅₋₂₀ acyl, and -W¹-X¹-Y¹, wherein -W¹-X¹-Y¹ is as defined herein and C_{A-B} is C₃₋₈ and C_{C-D} is C₃₋₈.

- [0139]** In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R1A and R1B are each: independently selected from optionally substituted C5-20 alkyl, optionally substituted C5-20 alkenyl, optionally substituted C5-20 alkynyl, and optionally substituted C5-20 acyl.
- [0140]** In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R1A and R1B are optionally substituted alkyl. In embodiments, R1A and R1B are each independently selected from optionally substituted C5-50 alkyl. In embodiments, R1A and R1B are each independently selected from optionally substituted C₅₋₄₀ alkyl. In embodiments, R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₃₀ alkyl. In embodiments, R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₂₅ alkyl. In more preferred embodiments, R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₂₀ alkyl.
- [0141]** In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R1A and R1B are optionally substituted alkenyl. In embodiments, R1A and R1B are each independently selected from optionally substituted C5-50 alkenyl. In embodiments, R1A and R1B are each independently selected from optionally substituted C5-40 alkenyl. In embodiments, R1A and R1B are each independently selected from optionally substituted C5-30 alkenyl. In embodiments, R1A and R1B are each independently selected from optionally substituted C5-25 alkenyl. In embodiments, R1A and R1B are each independently selected from optionally substituted C5-20 alkenyl.
- [0142]** In any of the above embodiments (e.g. a compound of Formula(I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R1A and R1B are optionally substituted alkynyl. In embodiments, R1A and R1B are each independently selected from optionally substituted C5-50 alkynyl. In embodiments, R1A and R1B are each independently selected from optionally substituted C5-40 alkynyl. In embodiments, R1A and R1B are each independently selected from optionally substituted C5-30 alkynyl. In embodiments, R1A and R1B are each independently selected from optionally substituted C5-25 alkynyl. In more preferred embodiments, R1A and R1B are each independently selected from optionally substituted C5-20 alkynyl.
- [0143]** In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof),

R^{1A} and R^{1B} are optionally substituted acyl. In embodiments, R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₅₀ acyl. In embodiments, R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₄₀ acyl. In embodiments, R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₃₀ acyl. In embodiments, R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₂₅ acyl. In more preferred embodiments, R^{1A} and R^{1B} are each independently selected from optionally substituted C₅₋₂₀ acyl.

[0144] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R^{1A} and R^{1B} are -W¹-X¹-Y¹ wherein -W¹-X¹-Y¹ is as defined herein. In embodiments, R^{1A} and R^{1B} are each independently selected from -W¹-X¹-Y¹, wherein -W¹-X¹-Y¹ is as defined herein and CA-B is C1-20 and CC-D is C2-20. In embodiments, R^{1A} and R^{1B} are each independently selected from -W¹-X¹-Y¹, wherein -W¹-X¹-Y¹ is as defined herein and CA-B is C1-15 and CC-D is C2-15. In embodiments, R^{1A} and R^{1B} are each independently selected from -W¹-X¹-Y¹, wherein -W¹-X¹-Y¹ is as defined herein and CA-B is C3-15 and CC-D is C3-15. In preferred embodiments, R^{1A} and R^{1B} are each independently selected from -W¹-X¹-Y¹, wherein -W¹-X¹-Y¹ is as defined herein and CA-B is C3-10 and CC-D is C3-10. In more preferred embodiments, R^{1A} and R^{1B} are each independently selected from -W¹-X¹-Y¹, wherein -W¹-X¹-Y¹ is as defined herein and CA-B is C3-8 and CC-D is C3-8.

[0145] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R^{1A} and R^{1B} are not optionally substituted.

[0146] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), each R^{1A} is the same.

[0147] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), each R^{1A} is different.

[0148] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), each R^{1B} is the same.

[0149] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), each R^{1B} is different.

- [0150] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R^{1A} and R^{1B} are the same.
- [0151] In any of the above embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), R^{1A} and R^{1B} are different.
- [0152] In embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), the cationic lipids of the present invention include compounds selected from those depicted in Tables A-F.
- [0153] In embodiments (e.g. a compound of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof), the cationic lipids of the present invention include compounds selected from those depicted in Tables A-F and compounds of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof of the invention described by examples 1-13.
- [0154] In embodiments, a composition comprising the cationic lipid of any one of the preceding embodiments (e.g. a compound of Formula (I), (Ia), (Ib), (Ic), (Id), (Ie), or a pharmaceutically acceptable salt thereof), one or more non-cationic lipids, one or more cholesterol-based lipids and one or more PEG-modified lipid is provided. In embodiments, this composition is a lipid nanoparticle. In embodiments, the one or more cationic lipid(s) constitute(s) about 30 mol %-60 mol % of the lipid nanoparticle. In embodiments, the one or more non-cationic lipid(s) constitute(s) 10 mol %-50 mol % of the lipid nanoparticle. In embodiments, the one or more PEG-modified lipid(s) constitute(s) 1 mol %-10 mol % of the lipid nanoparticle. In embodiments, the cholesterol-based lipid constitutes 10 mol %-50 mol % of the lipid nanoparticle. In embodiments, the lipid nanoparticle encapsulates a nucleic acid, optionally an mRNA encoding a peptide or protein. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 70%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 75%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 80%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 85%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 90%. In embodiments, the lipid nanoparticles have an encapsulation percentage for mRNA of at least 95%.

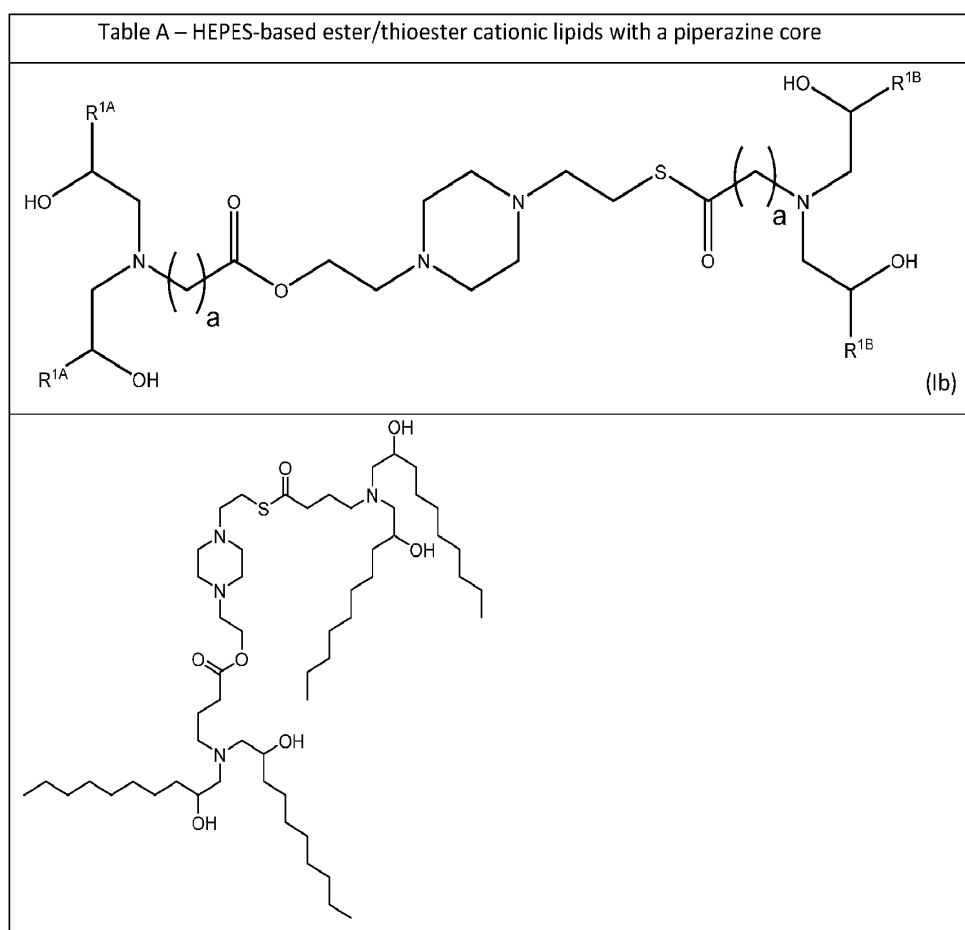
[0155] In embodiments, the composition of any one of the preceding embodiments is for use in therapy.

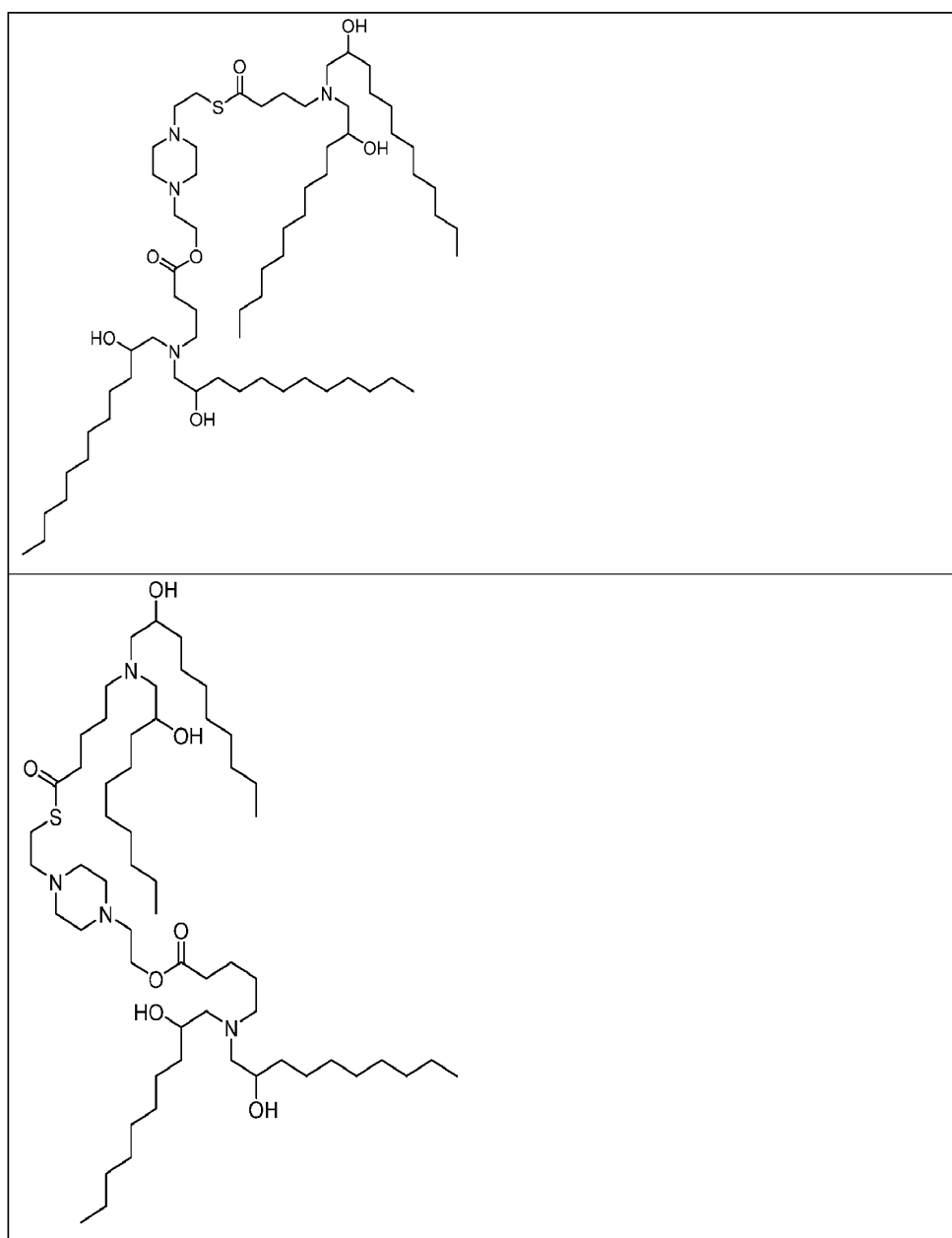
[0156] In embodiments, the composition of any one of the preceding embodiments is for use in a method of treating or preventing a disease amenable to treatment or prevention by the peptide or protein encoded by the mRNA, optionally wherein the disease is (a) a protein deficiency, optionally wherein the protein deficiency affects the liver, lung, brain or muscle, (b) an autoimmune disease, (c) an infectious disease, or (d) cancer.

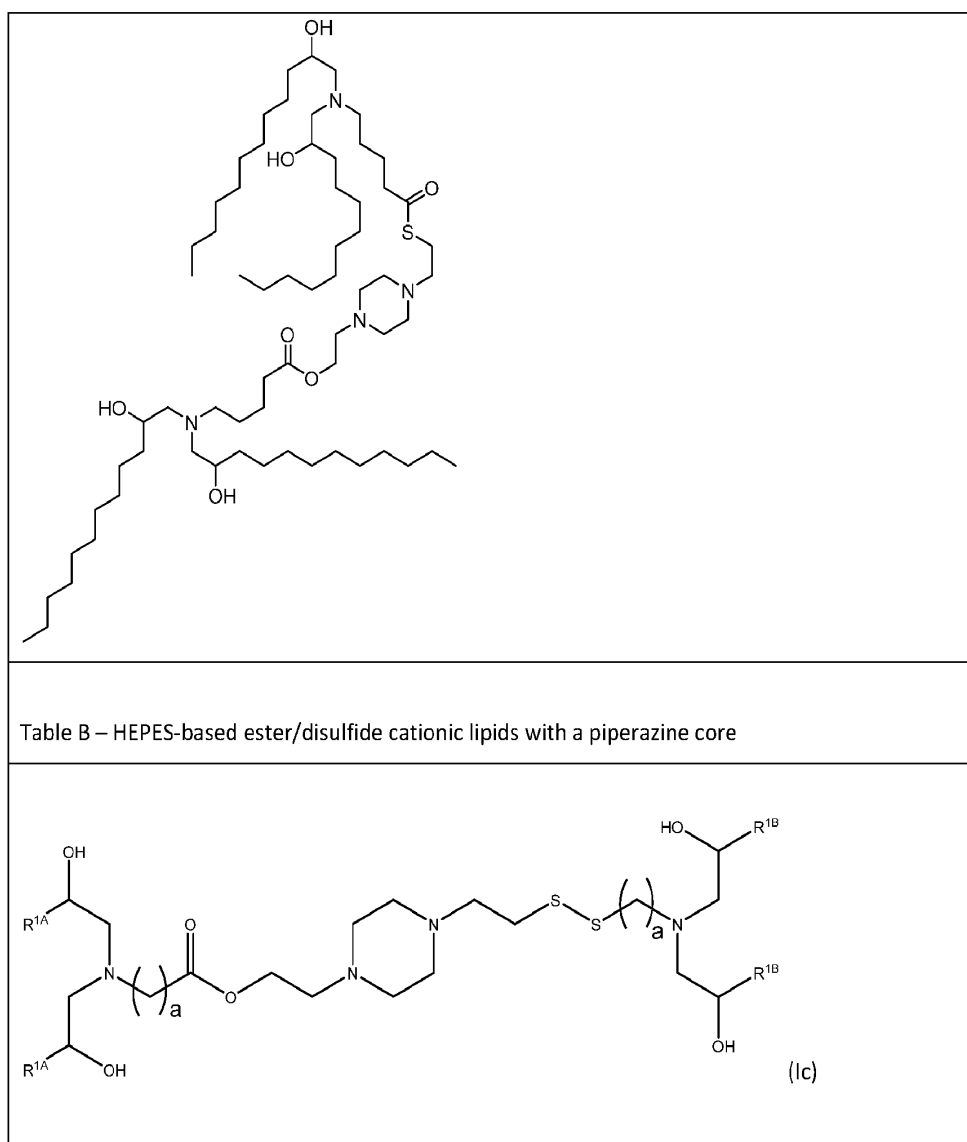
[0157] In embodiments, the composition is administered intravenously, intrathecally or intramuscularly, or by pulmonary delivery, optionally through nebulization.

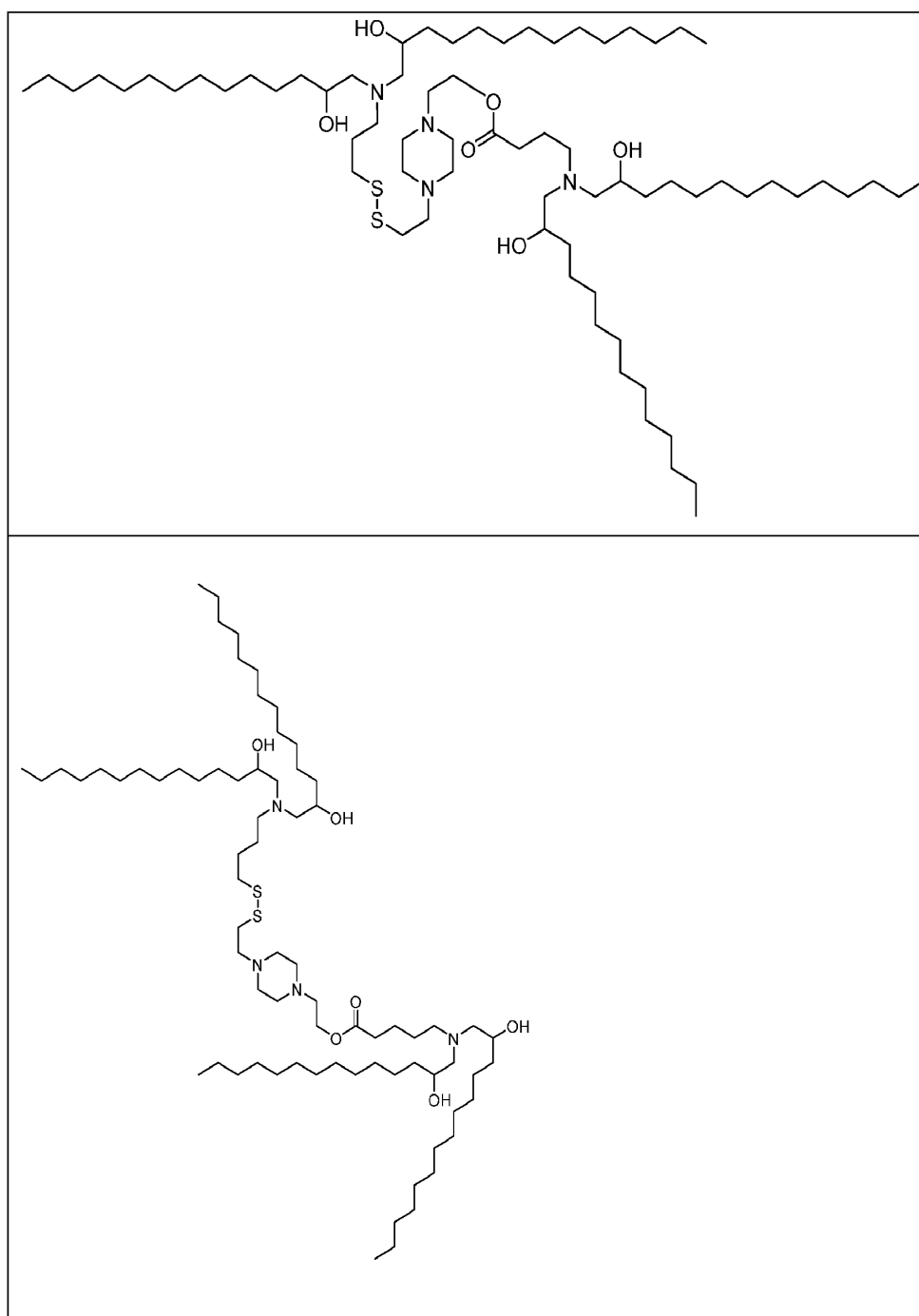
Exemplary Compounds

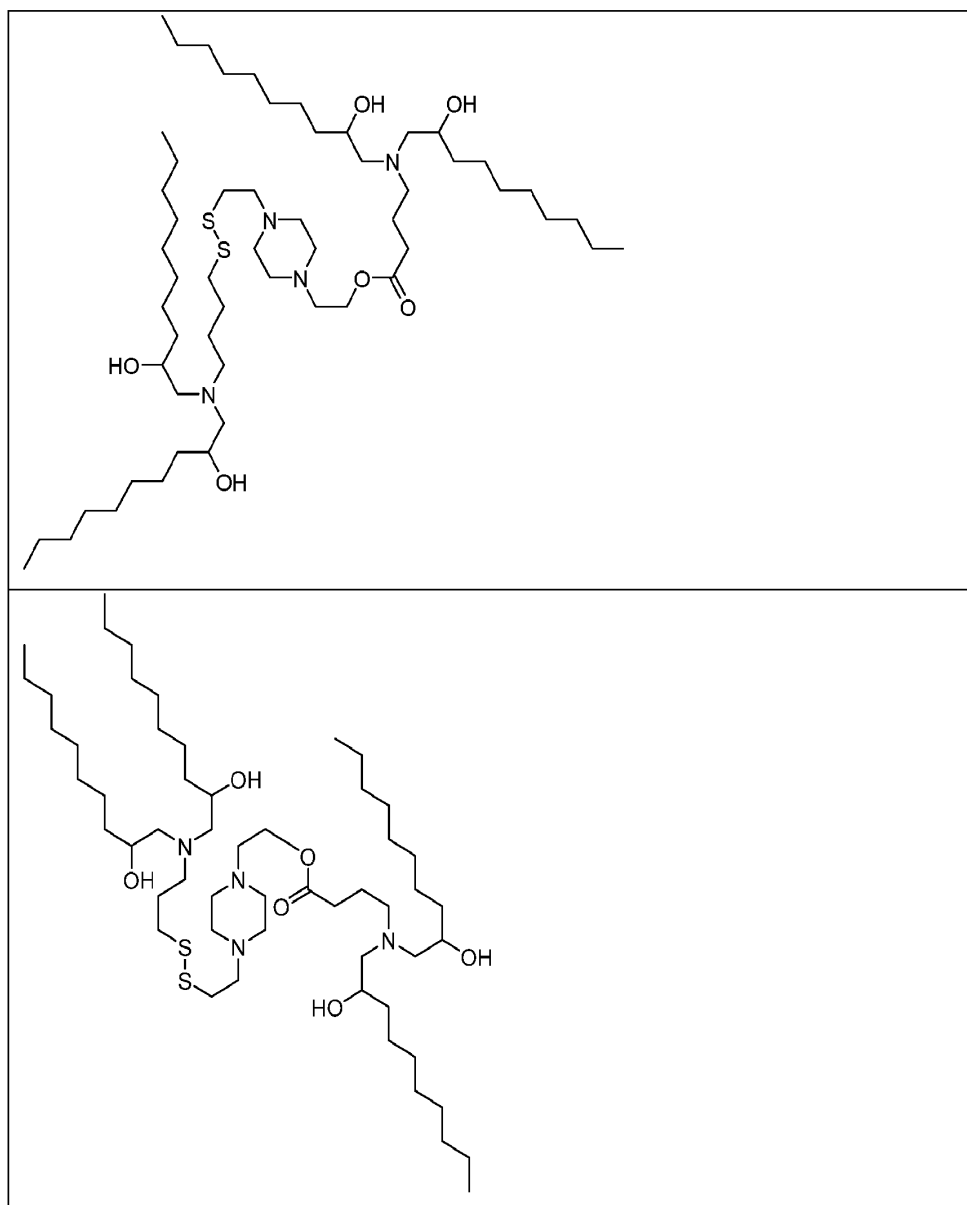
[0158] Exemplary compounds include those described in Tables A-F.

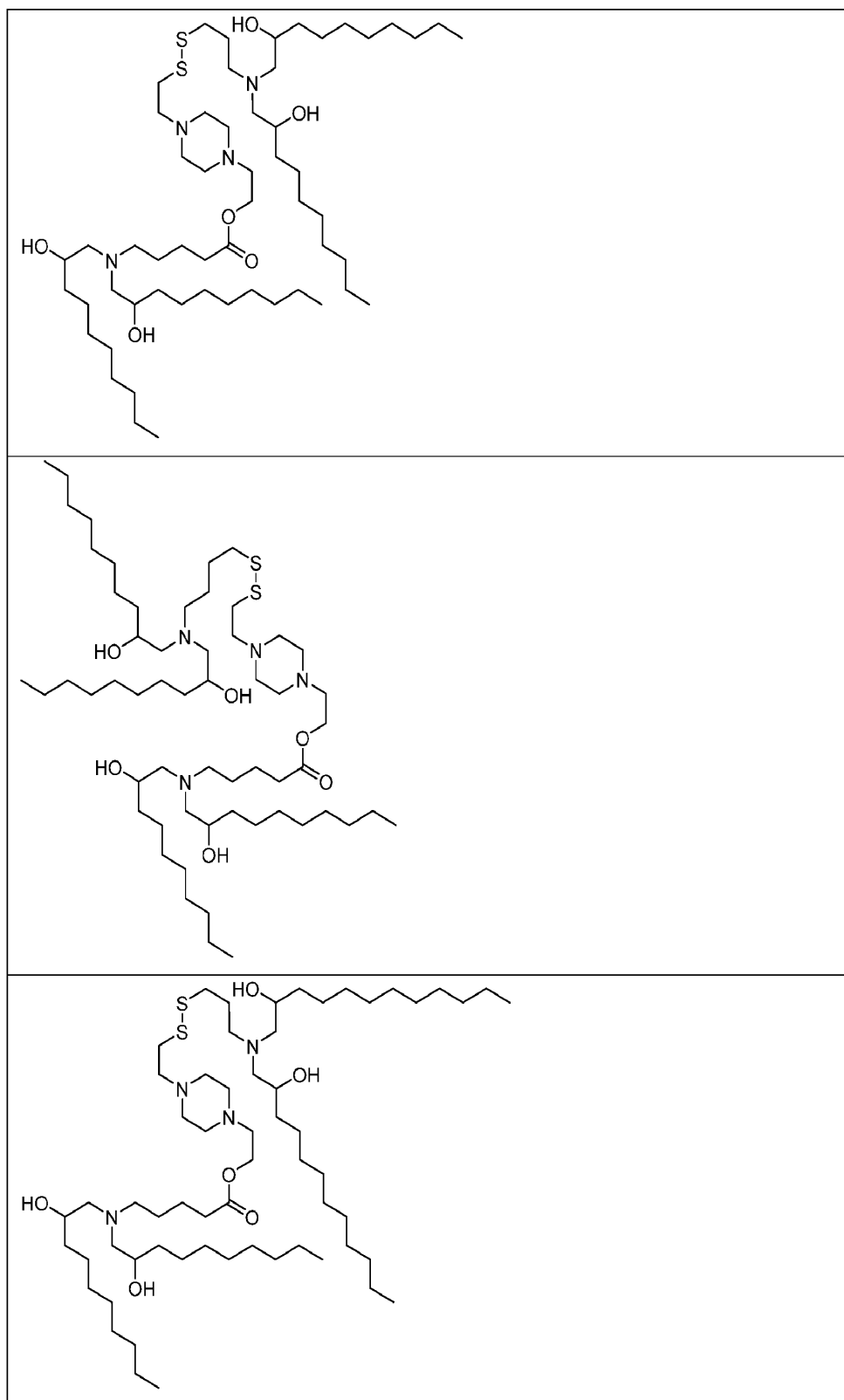


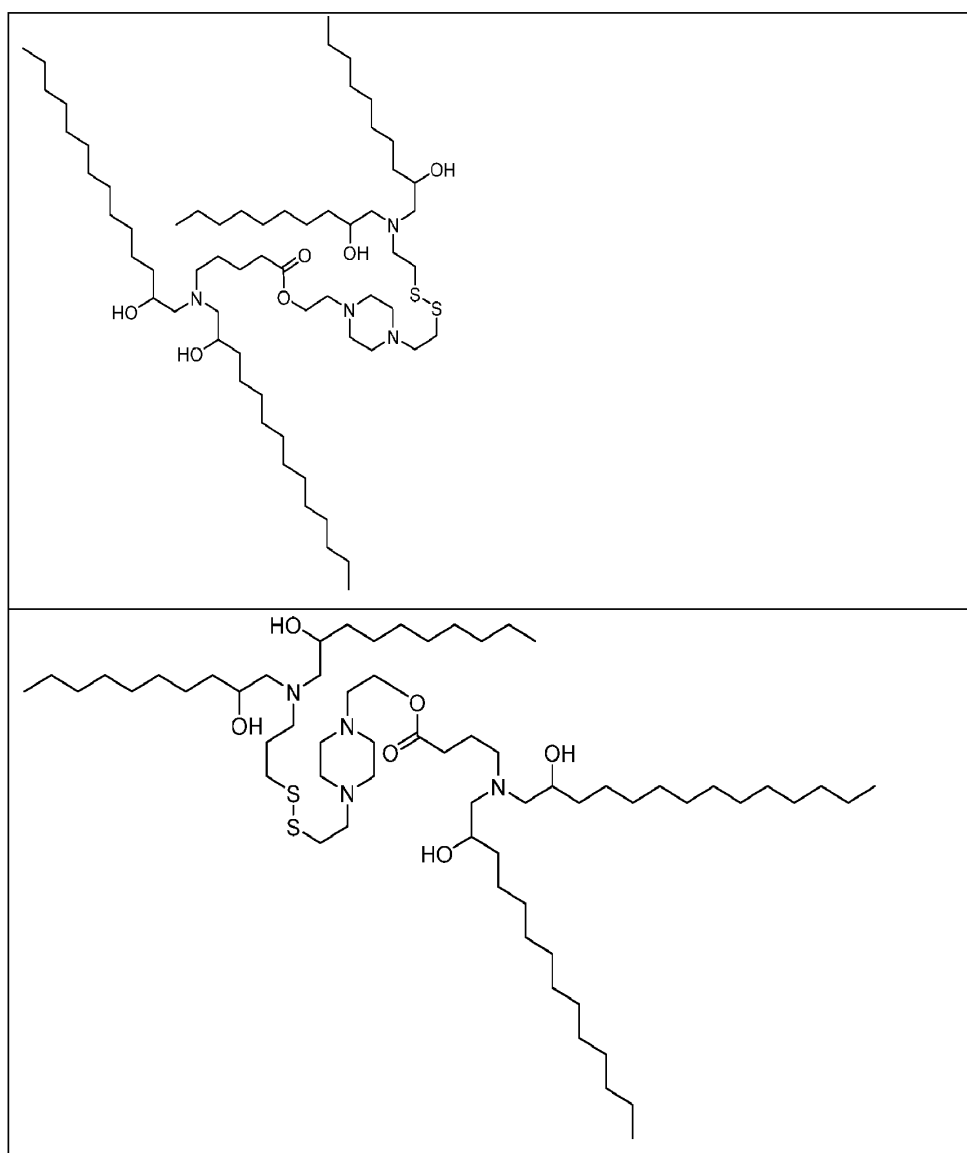


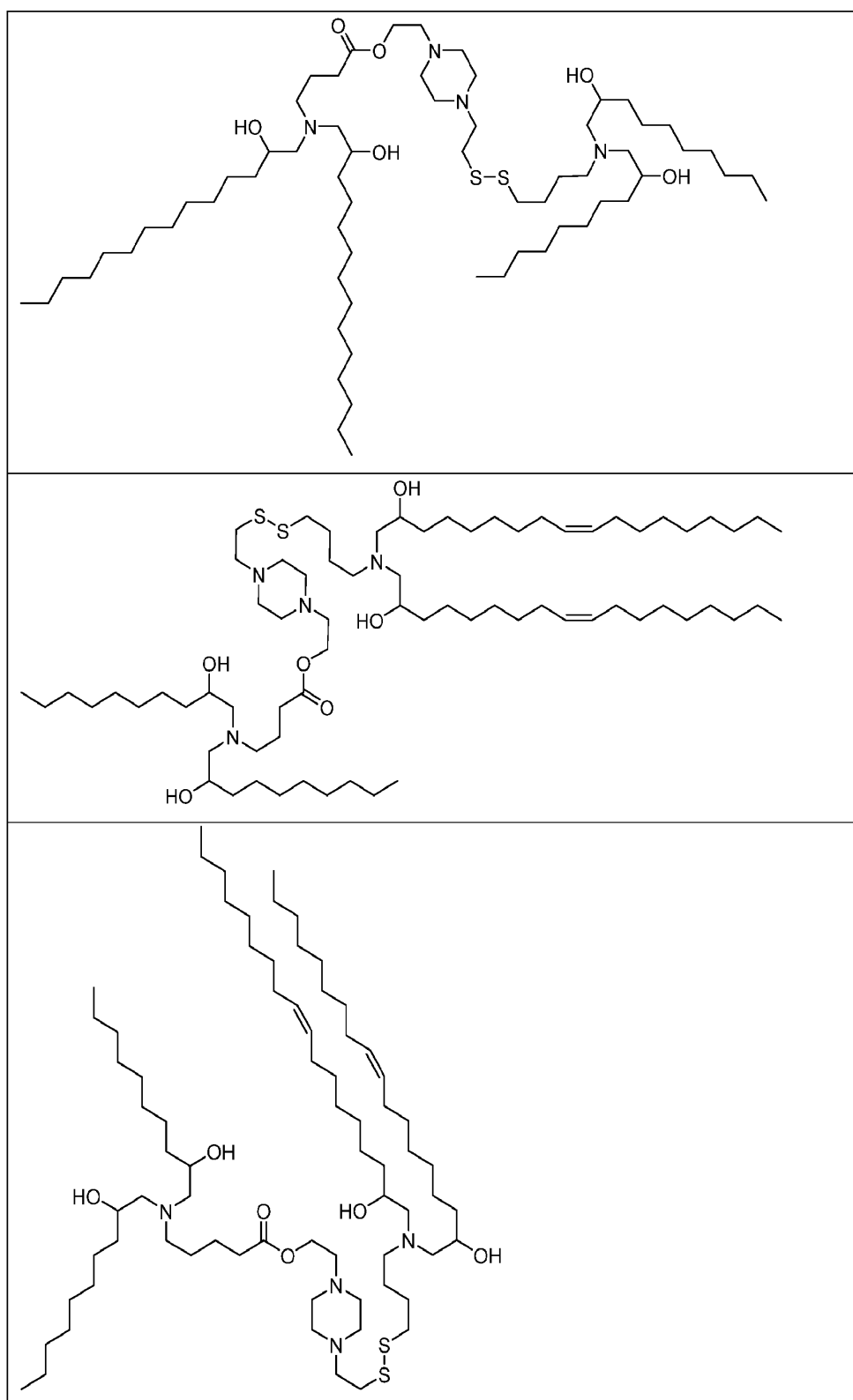


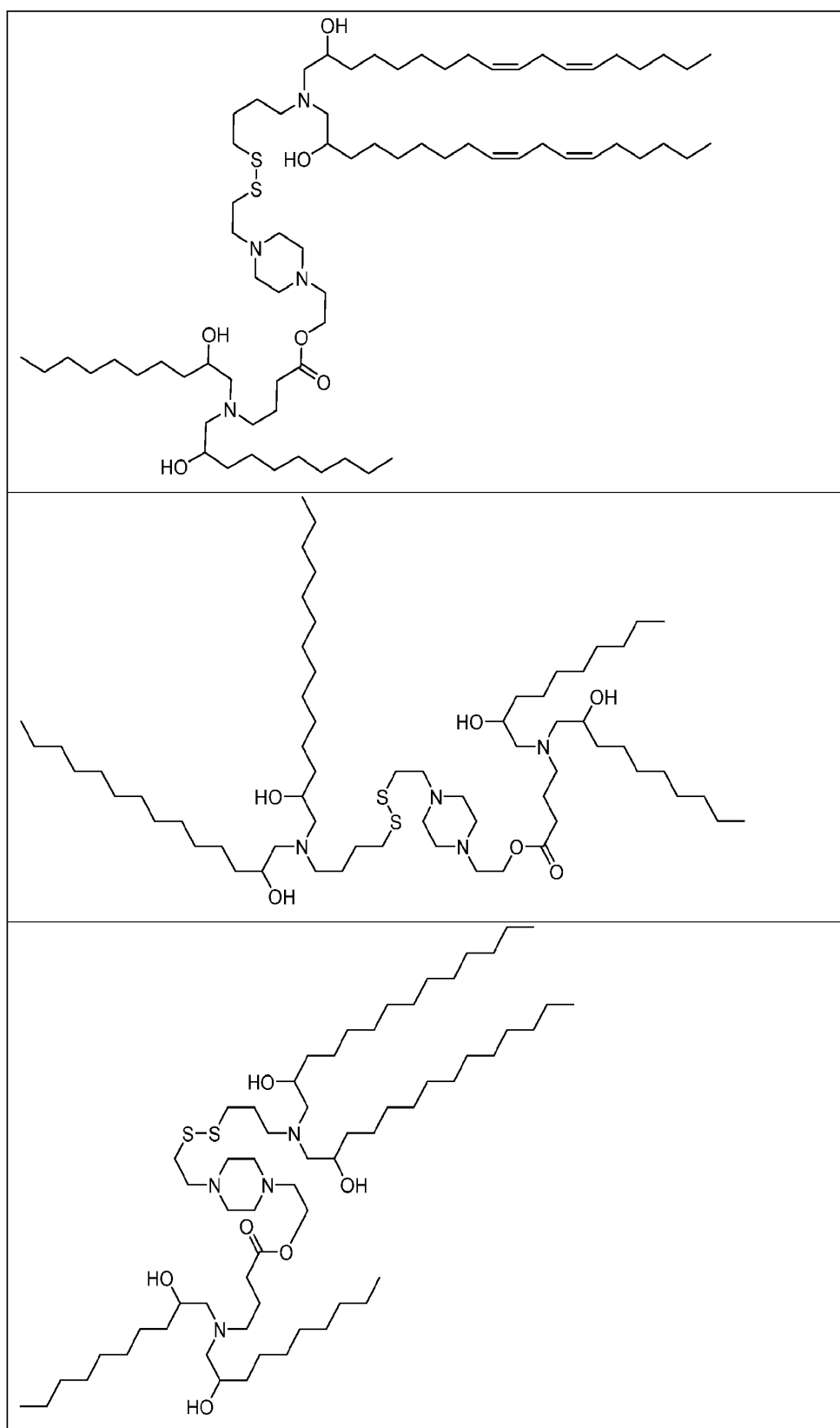


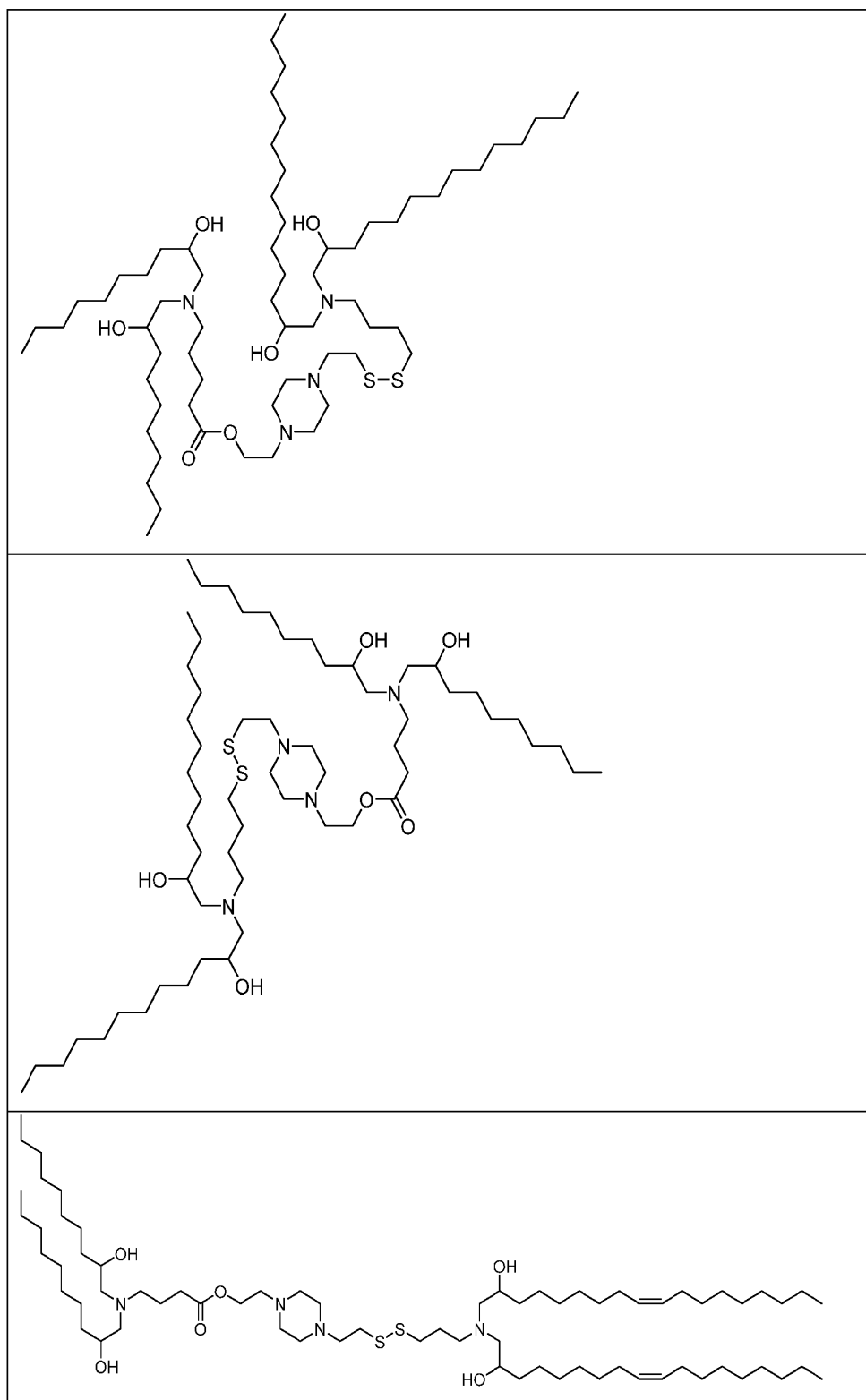


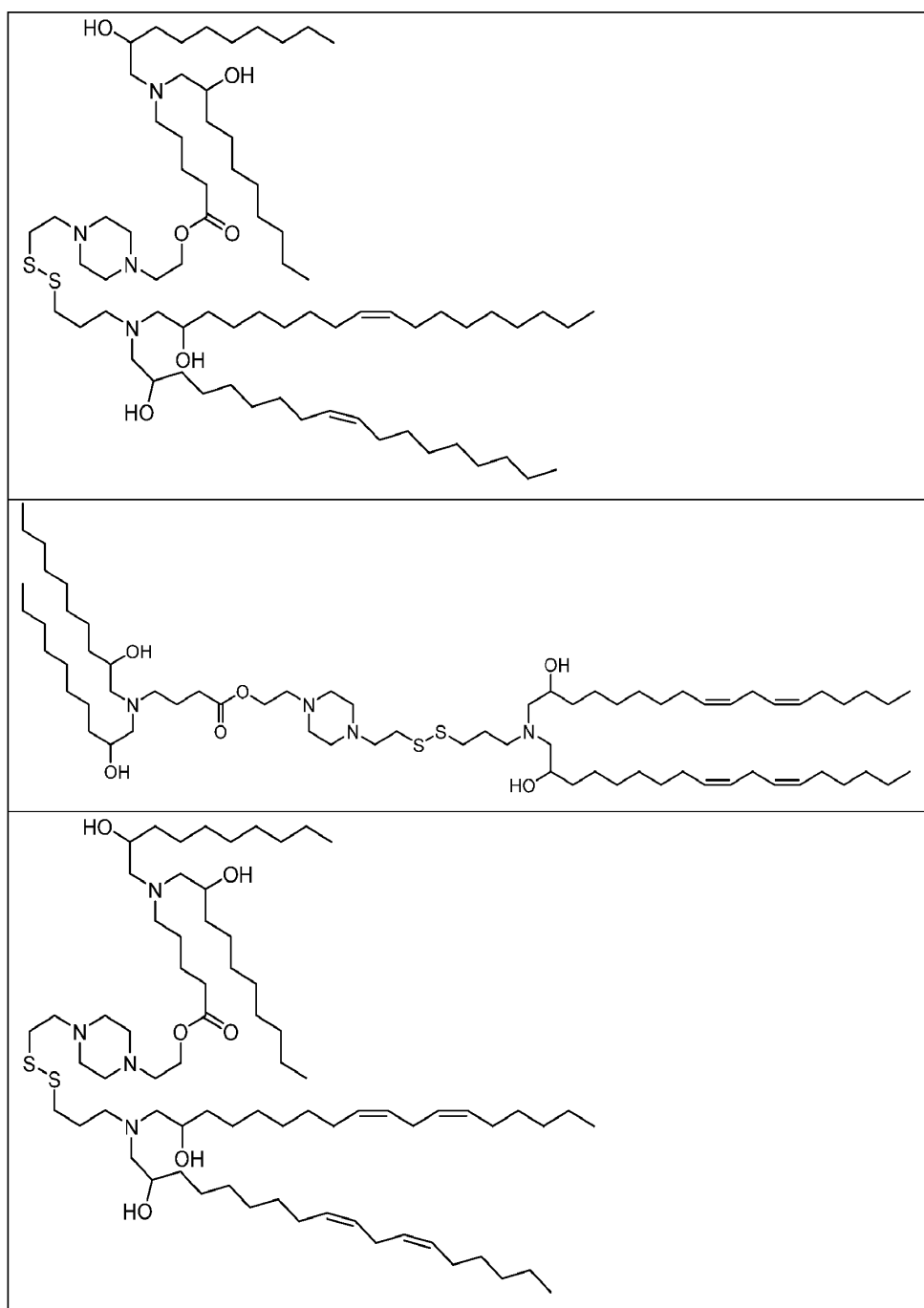


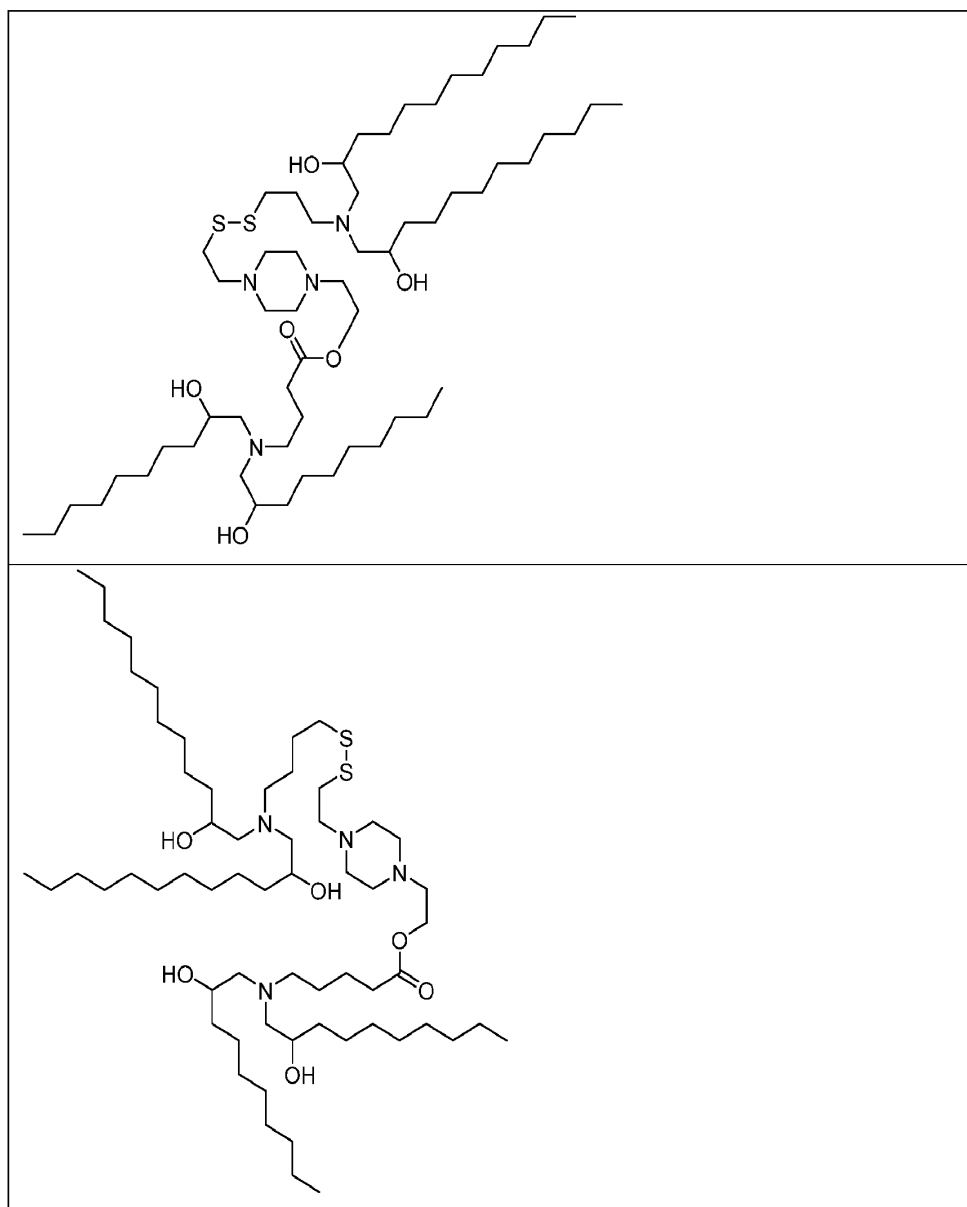


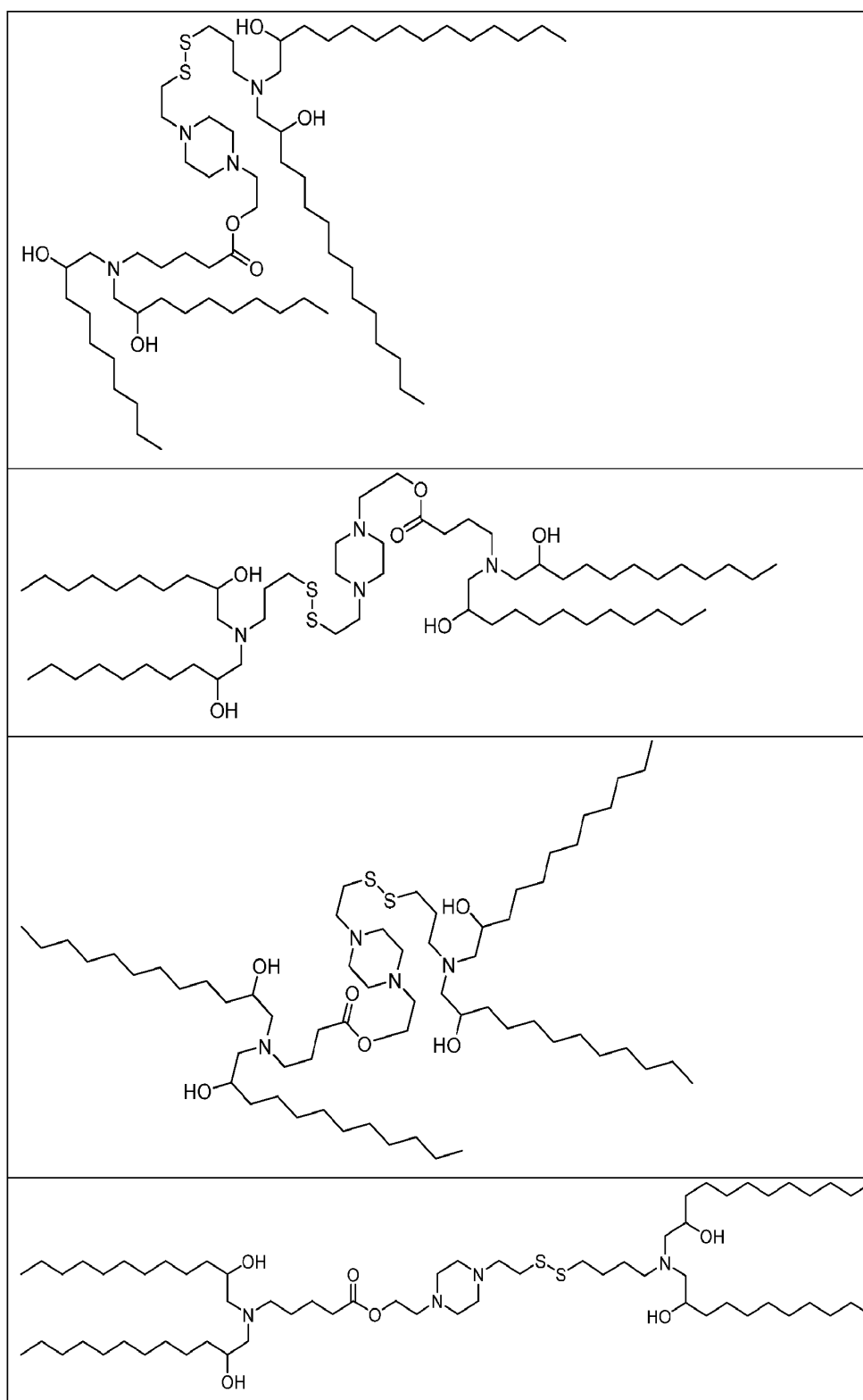


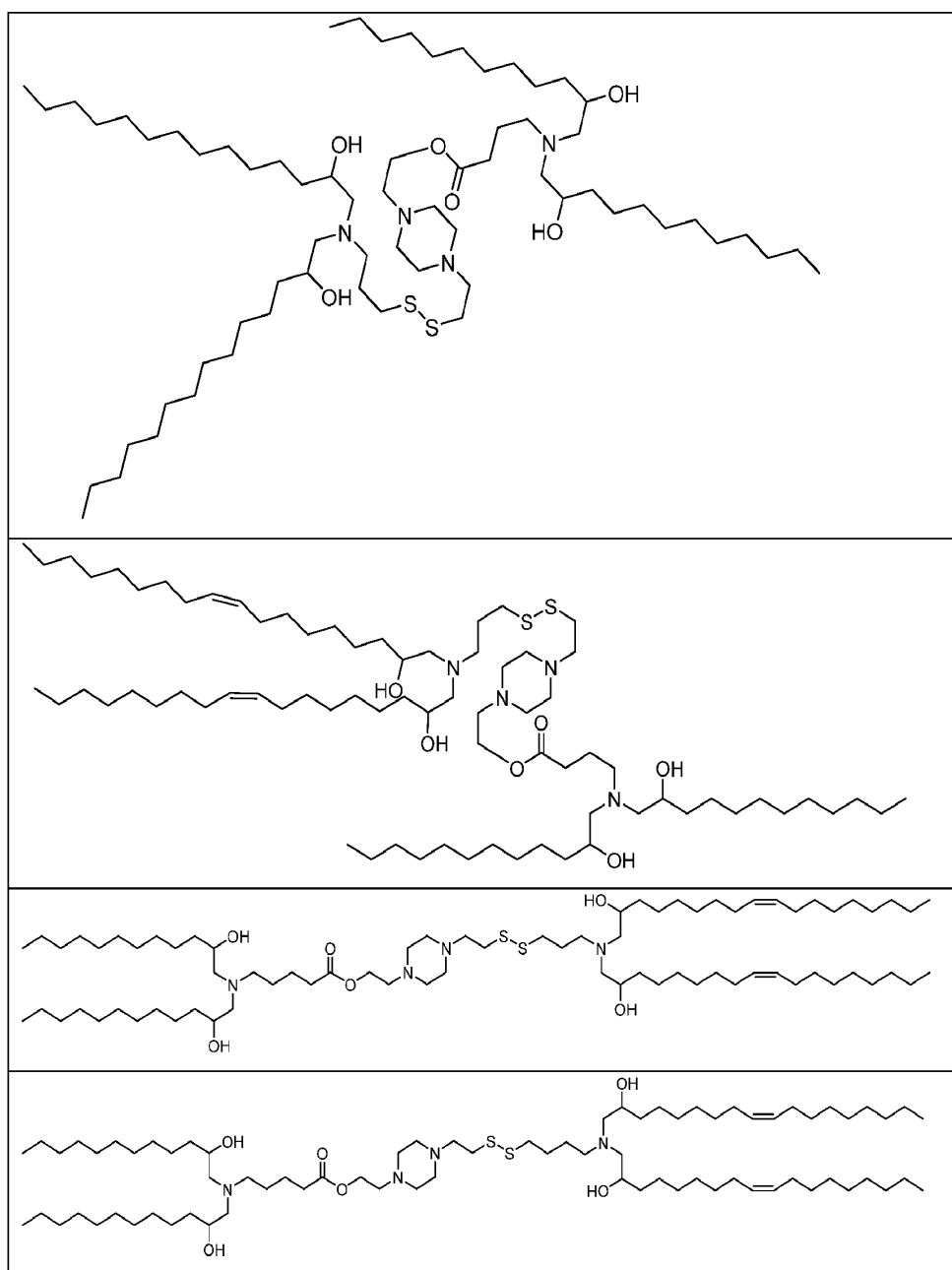


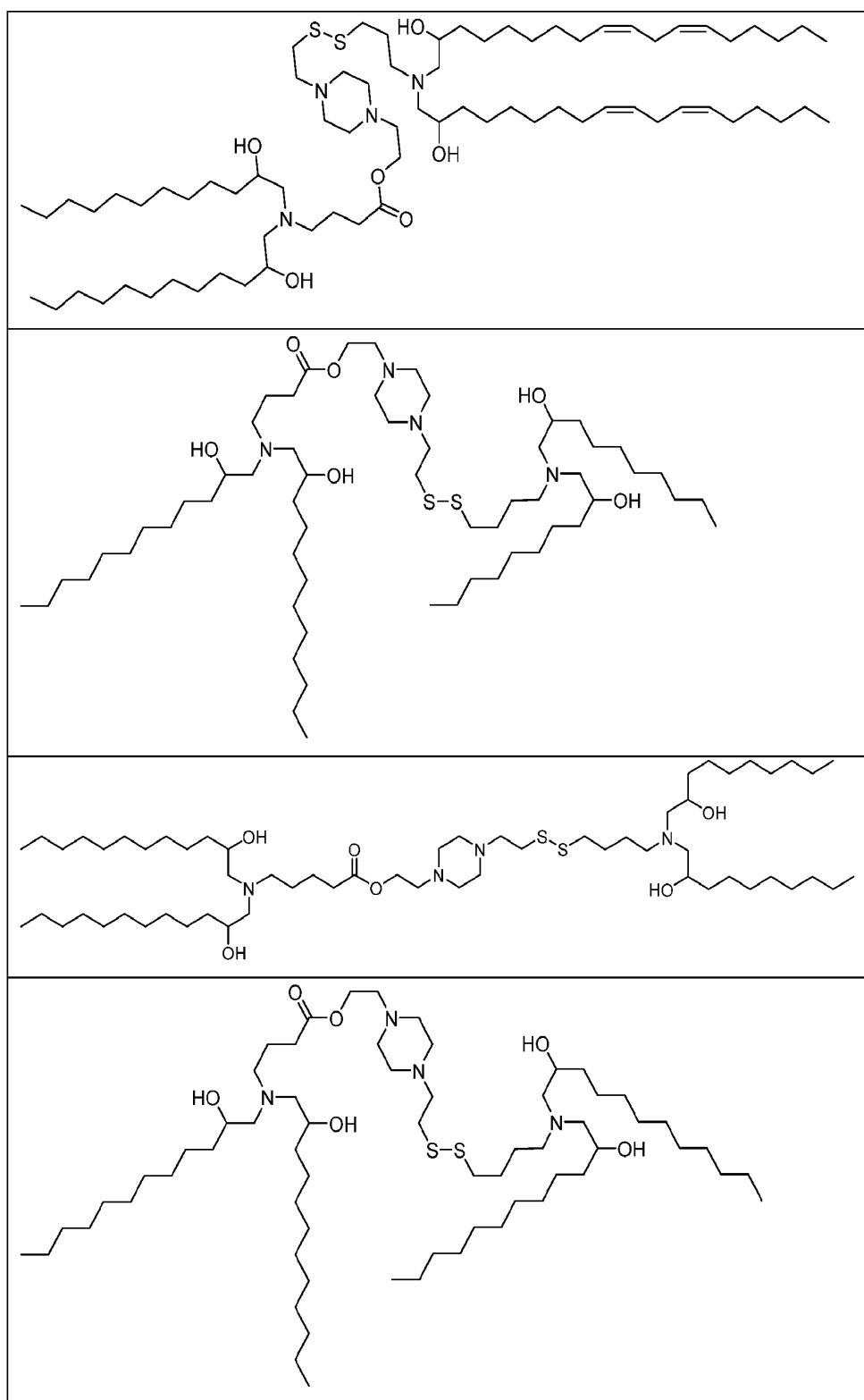


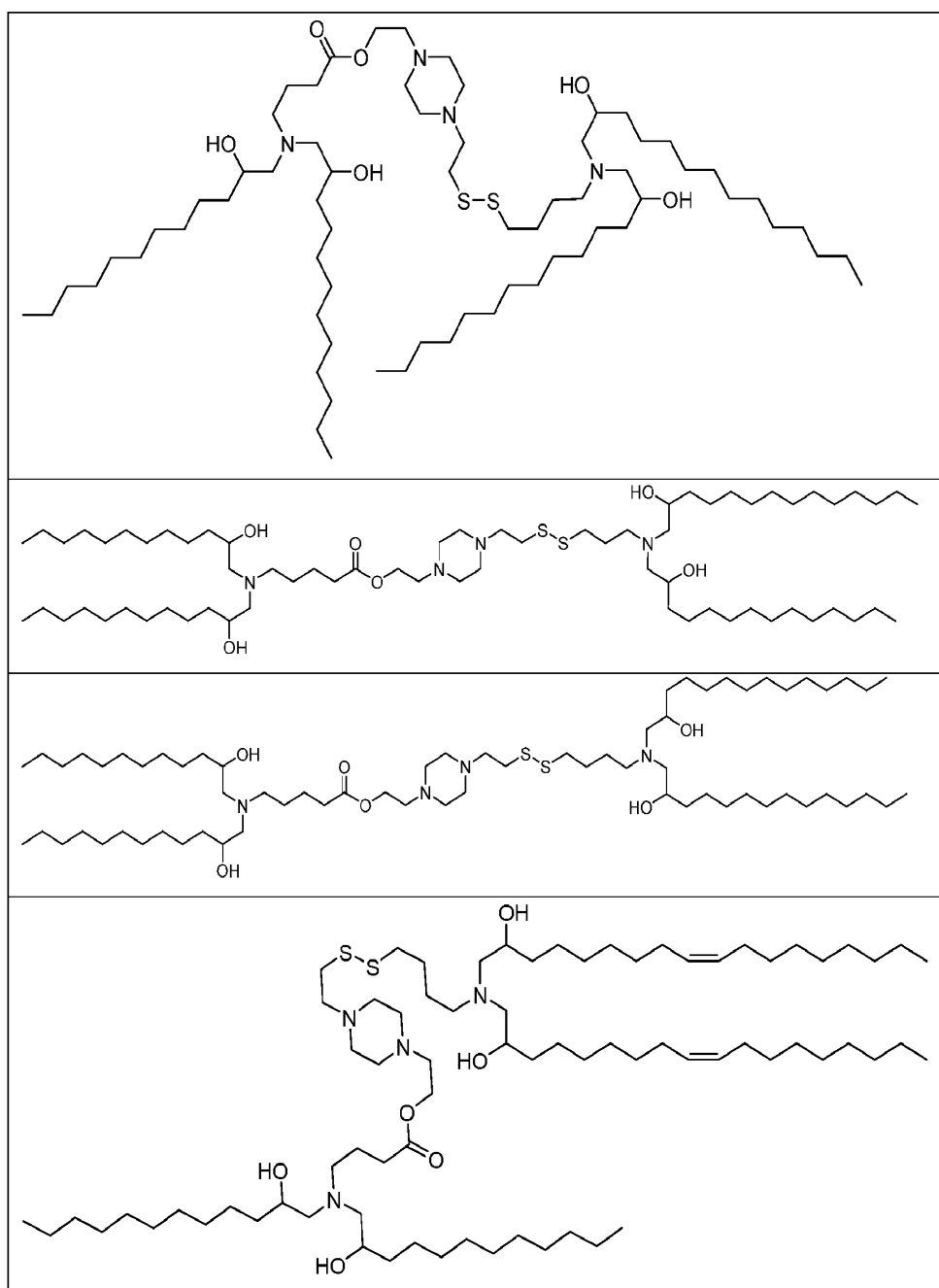


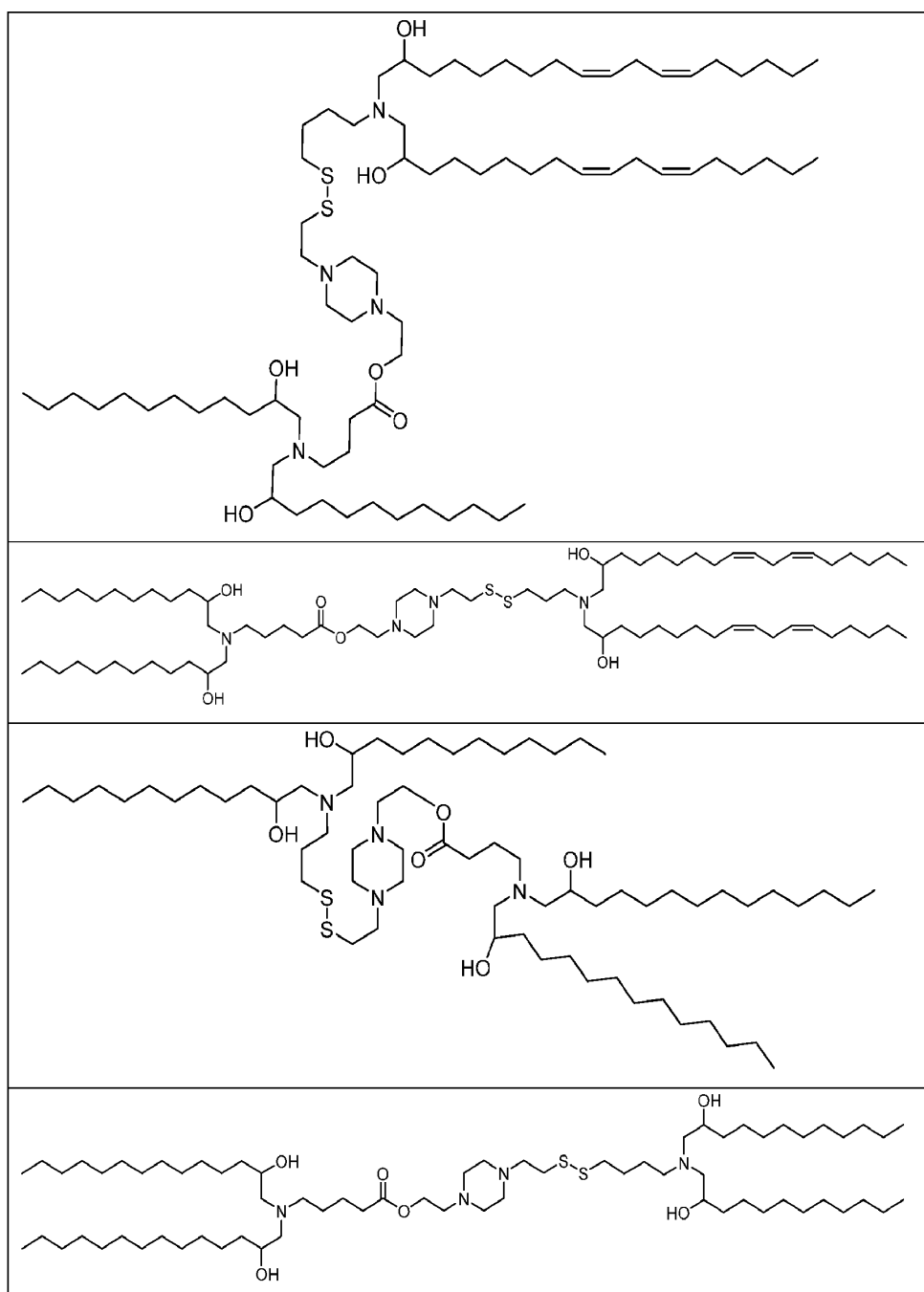


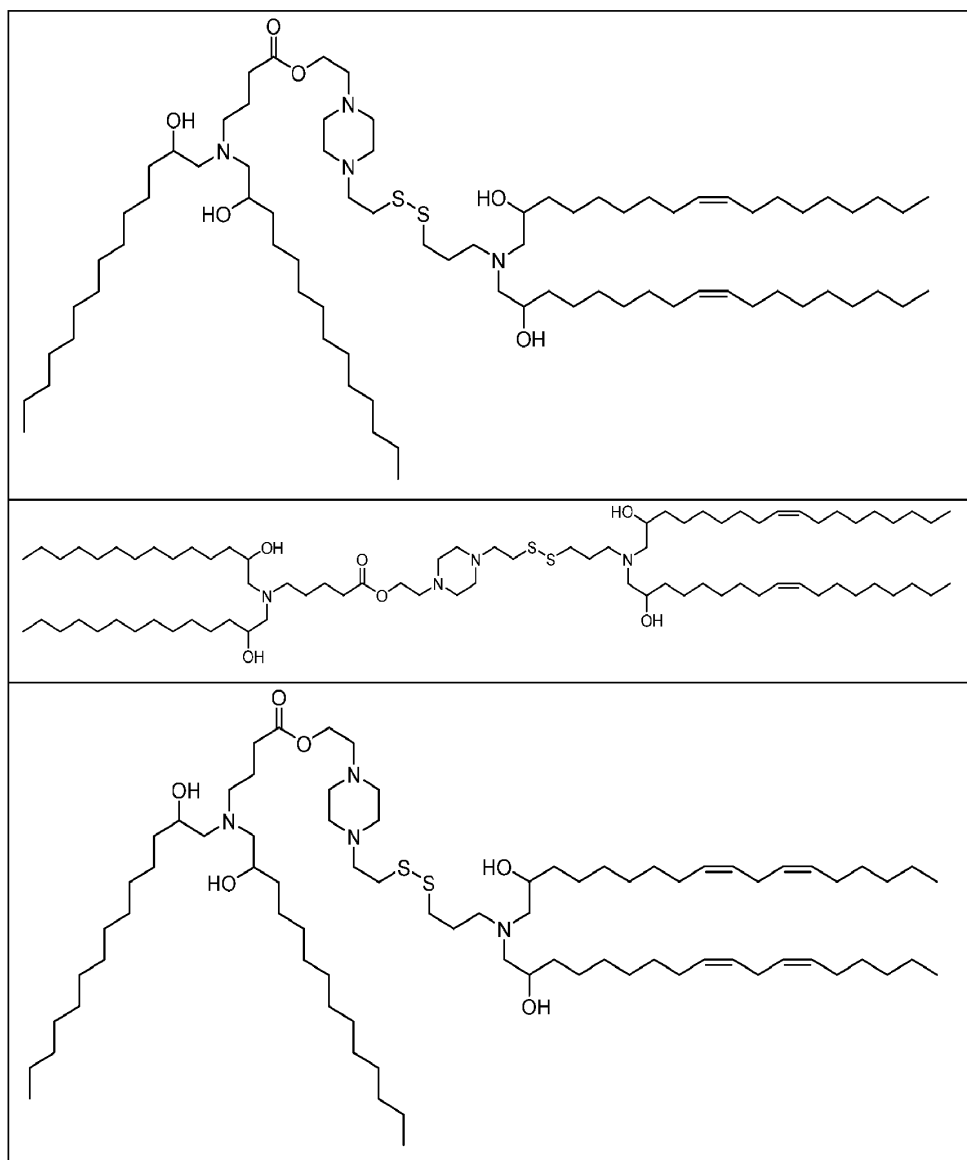


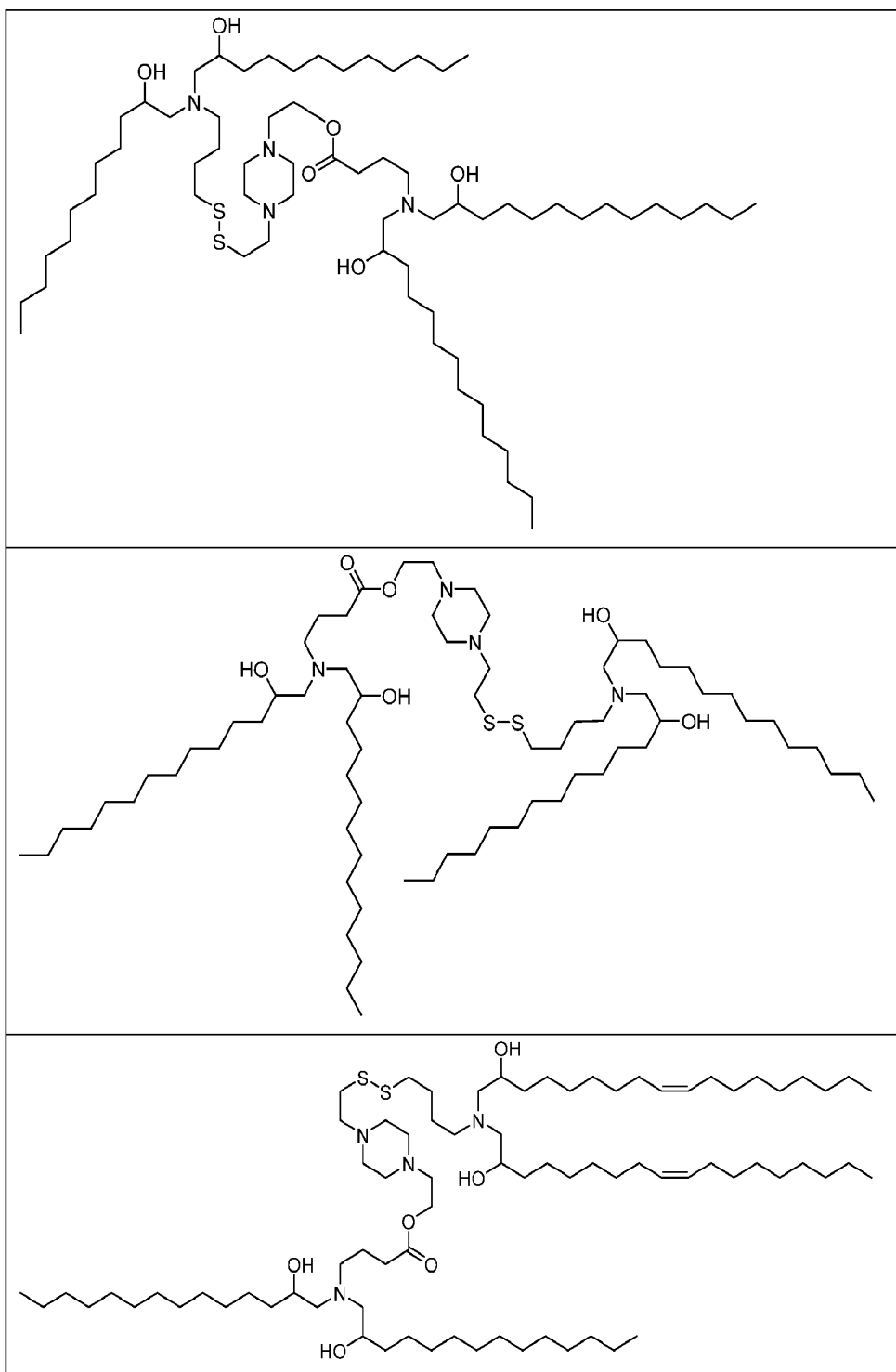


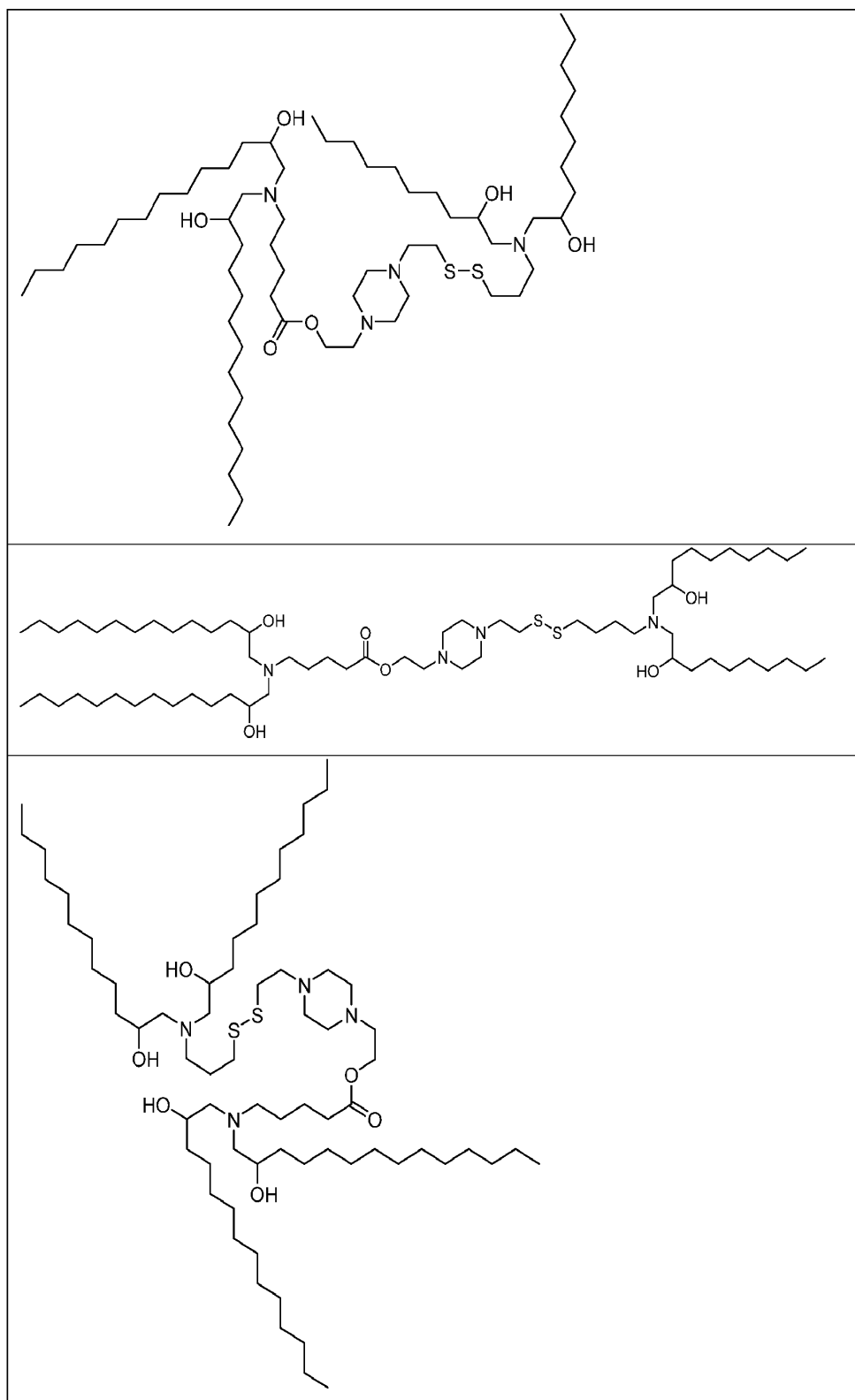


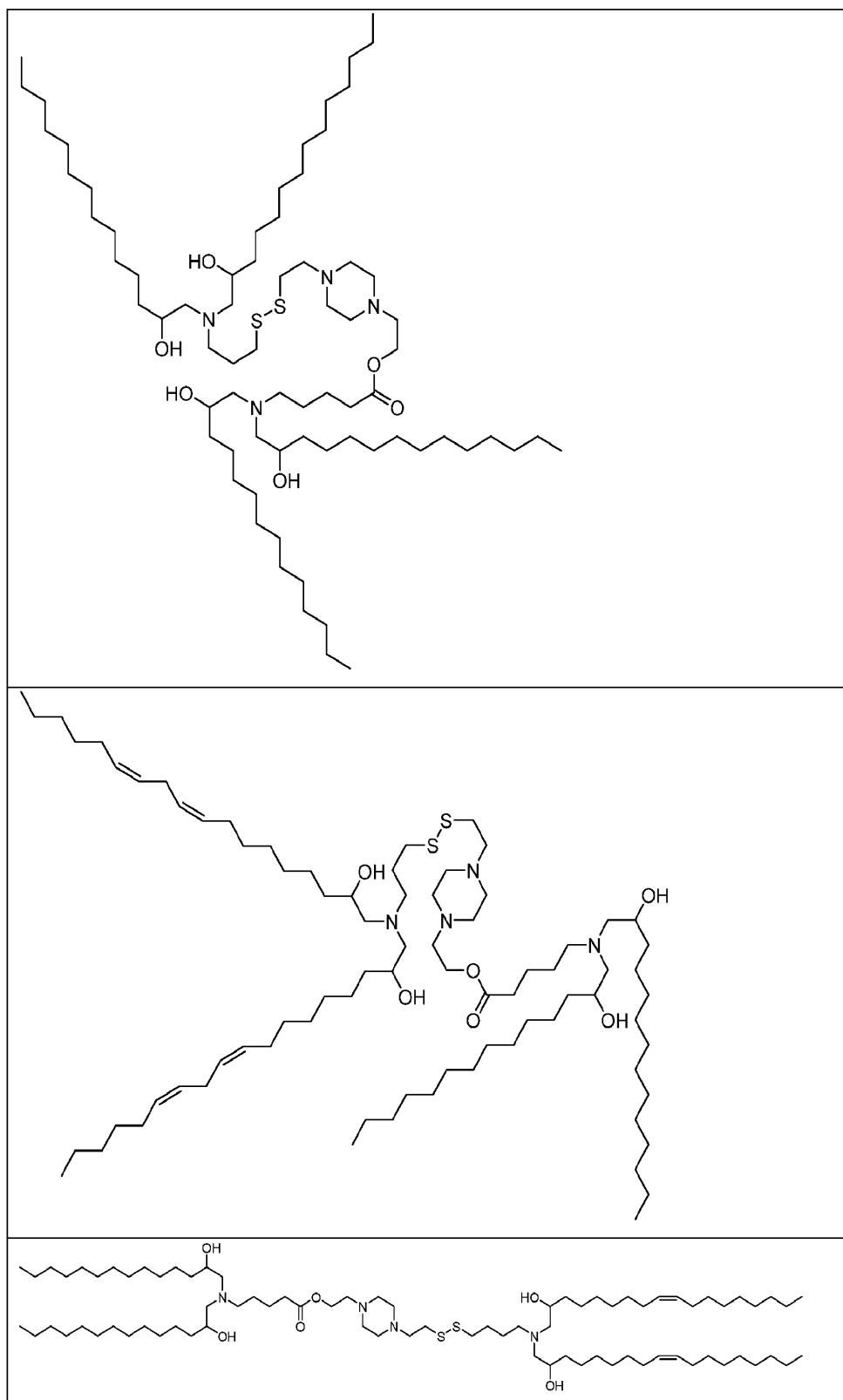


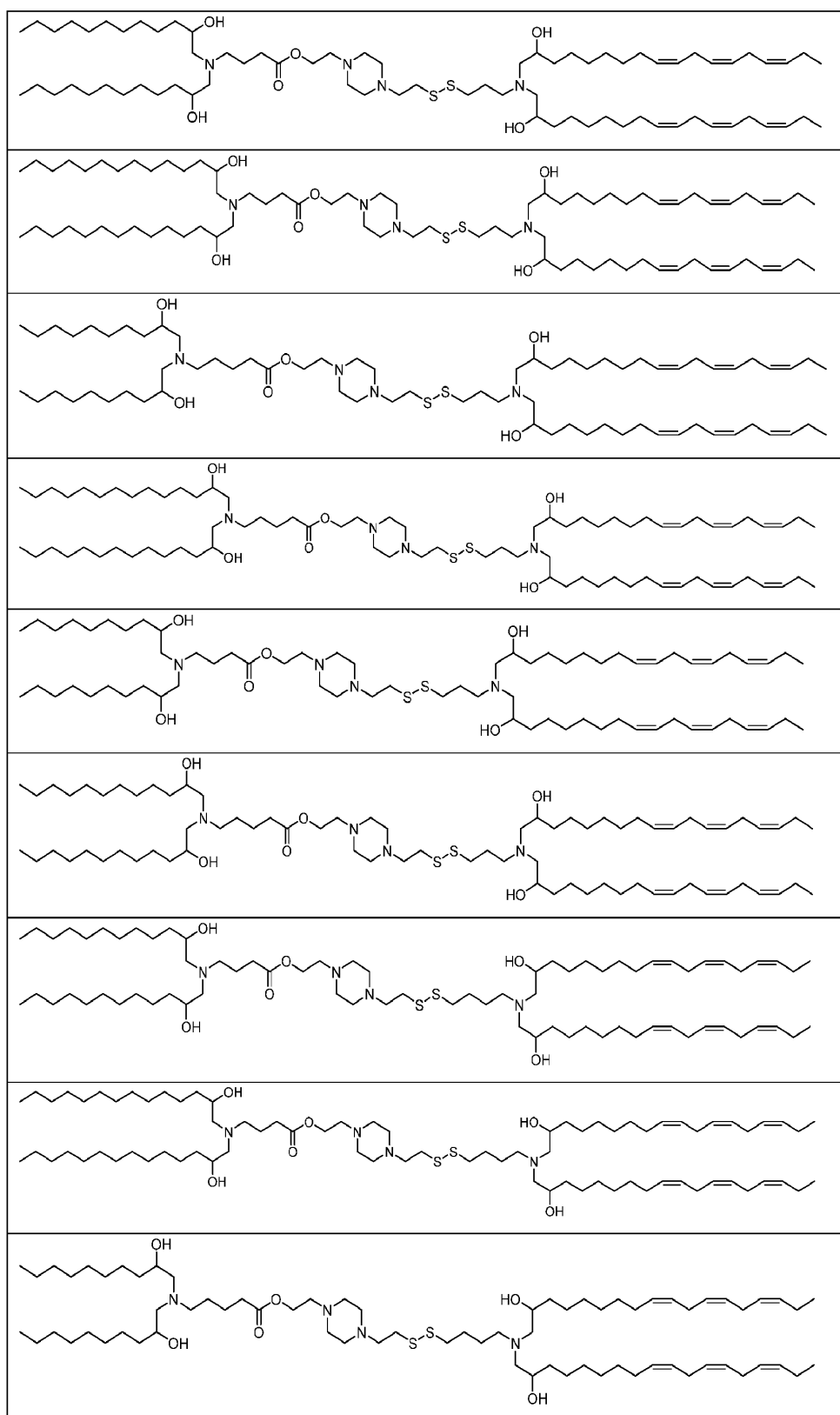


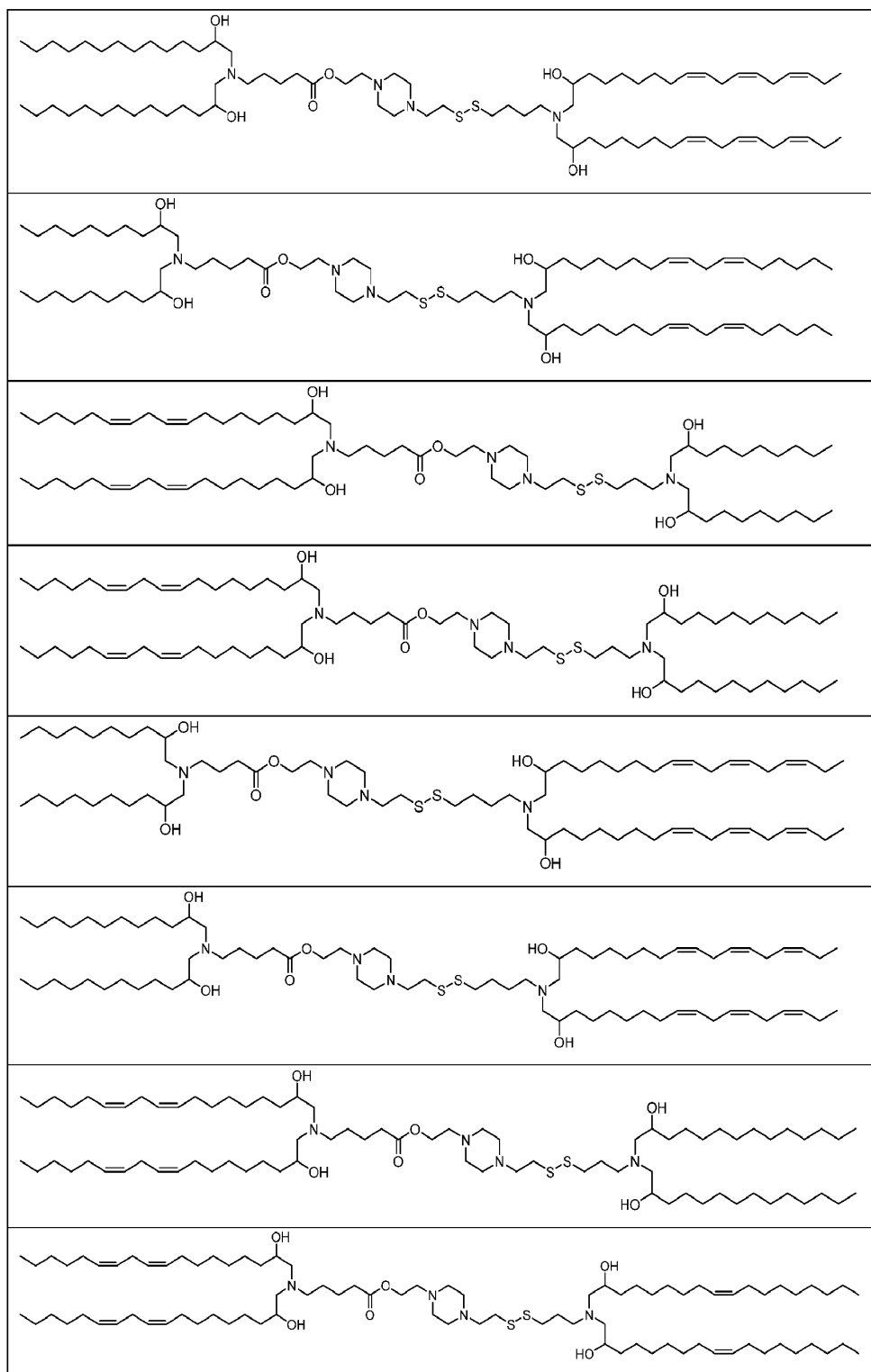


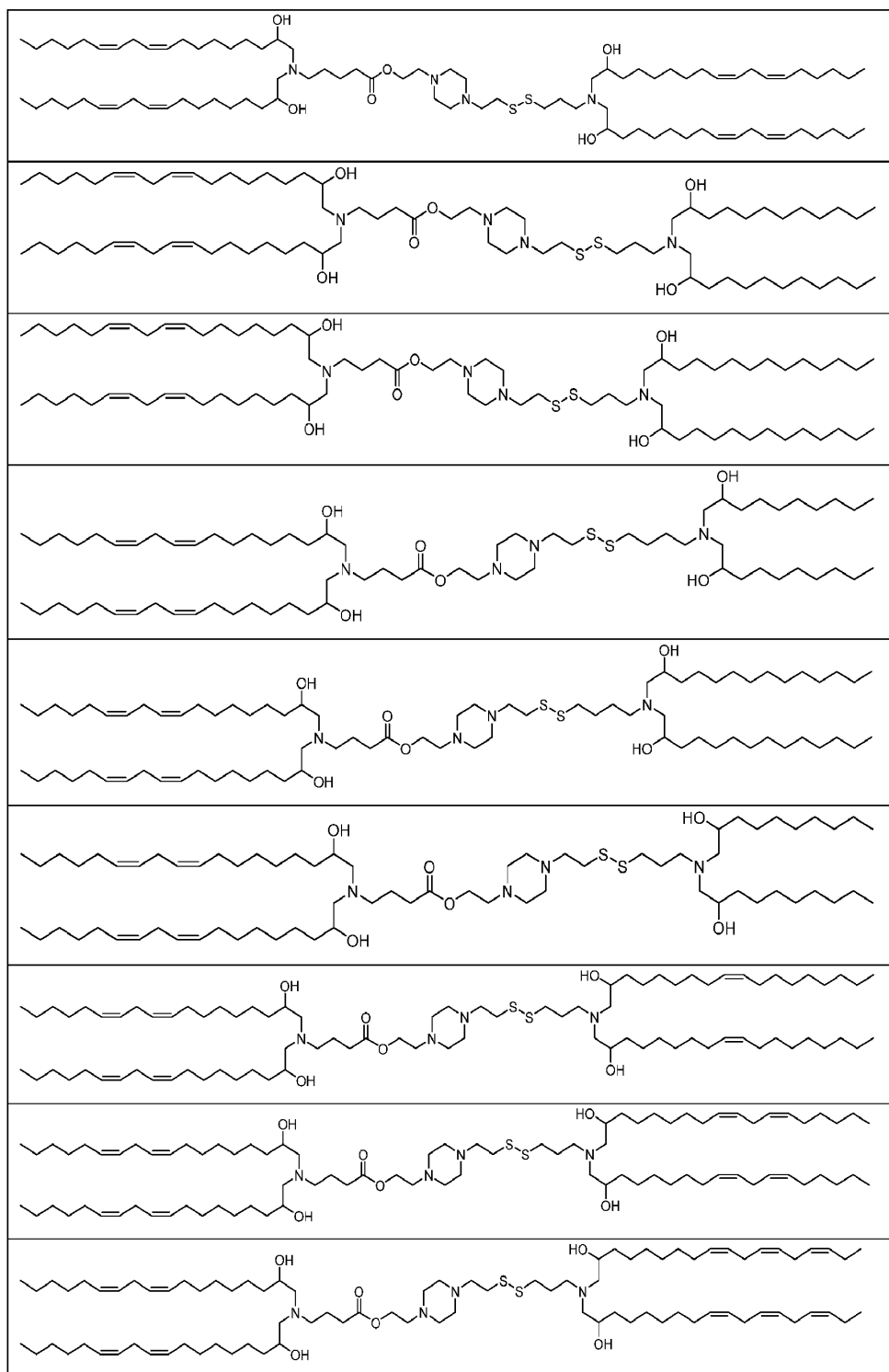


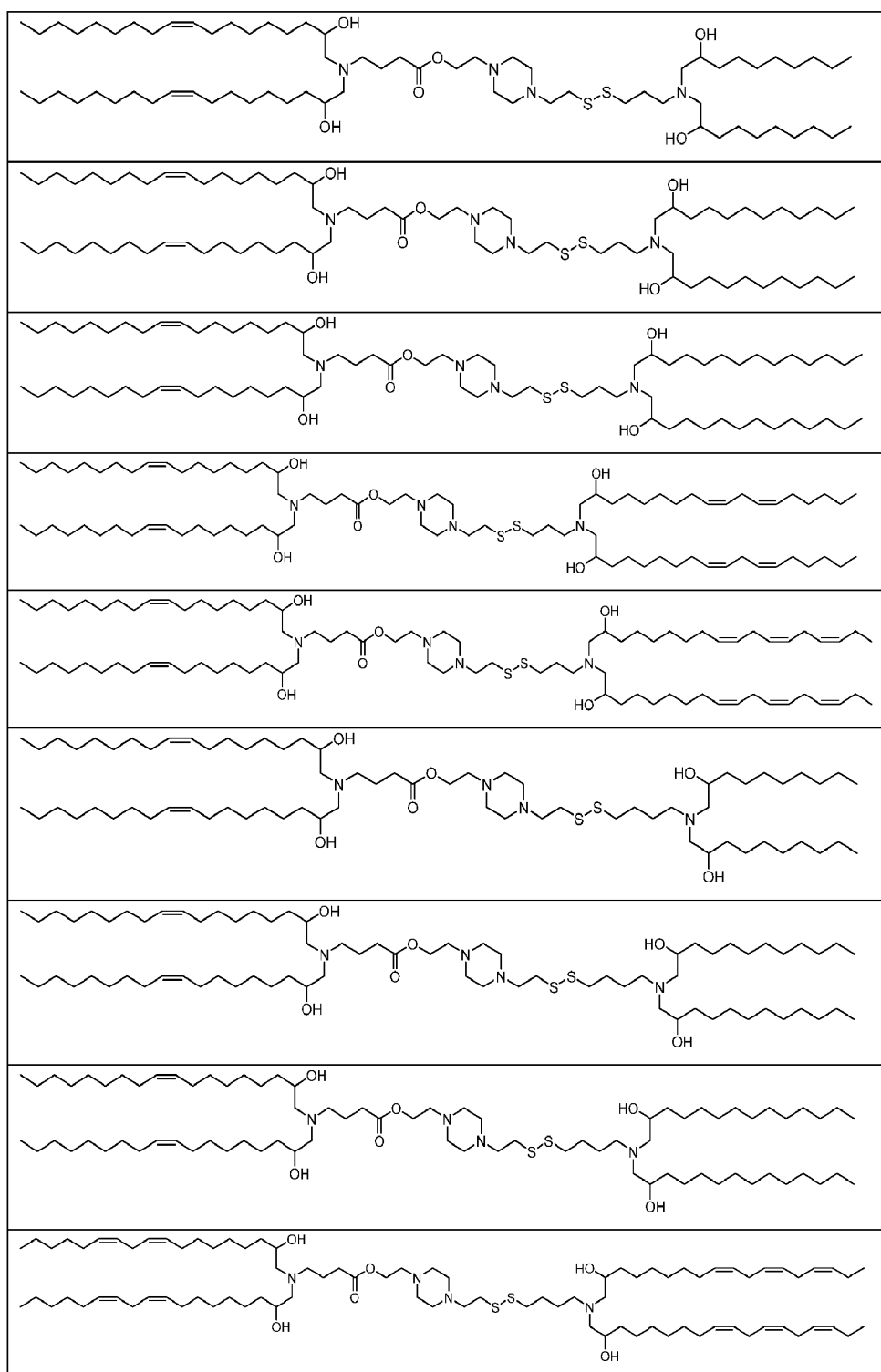


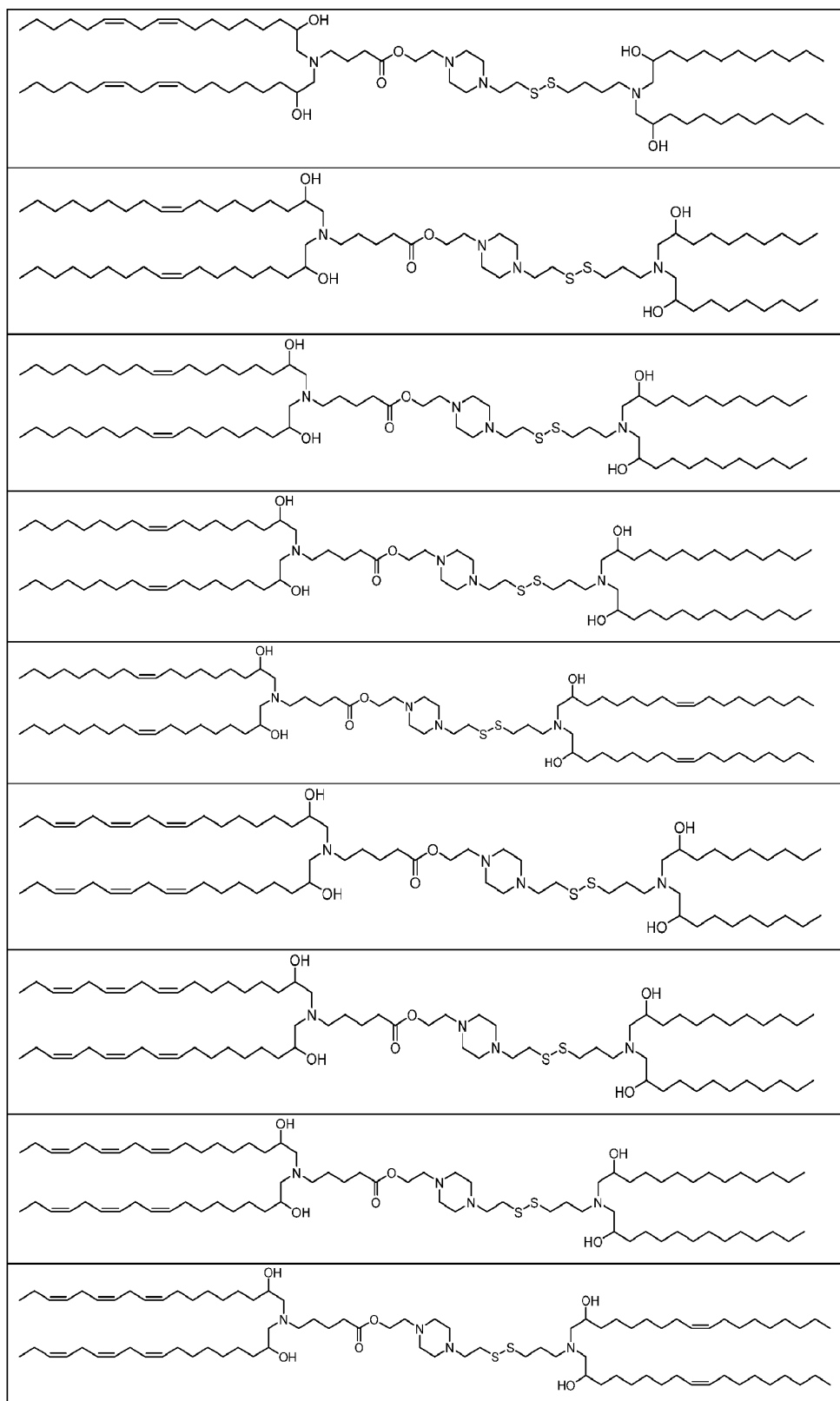


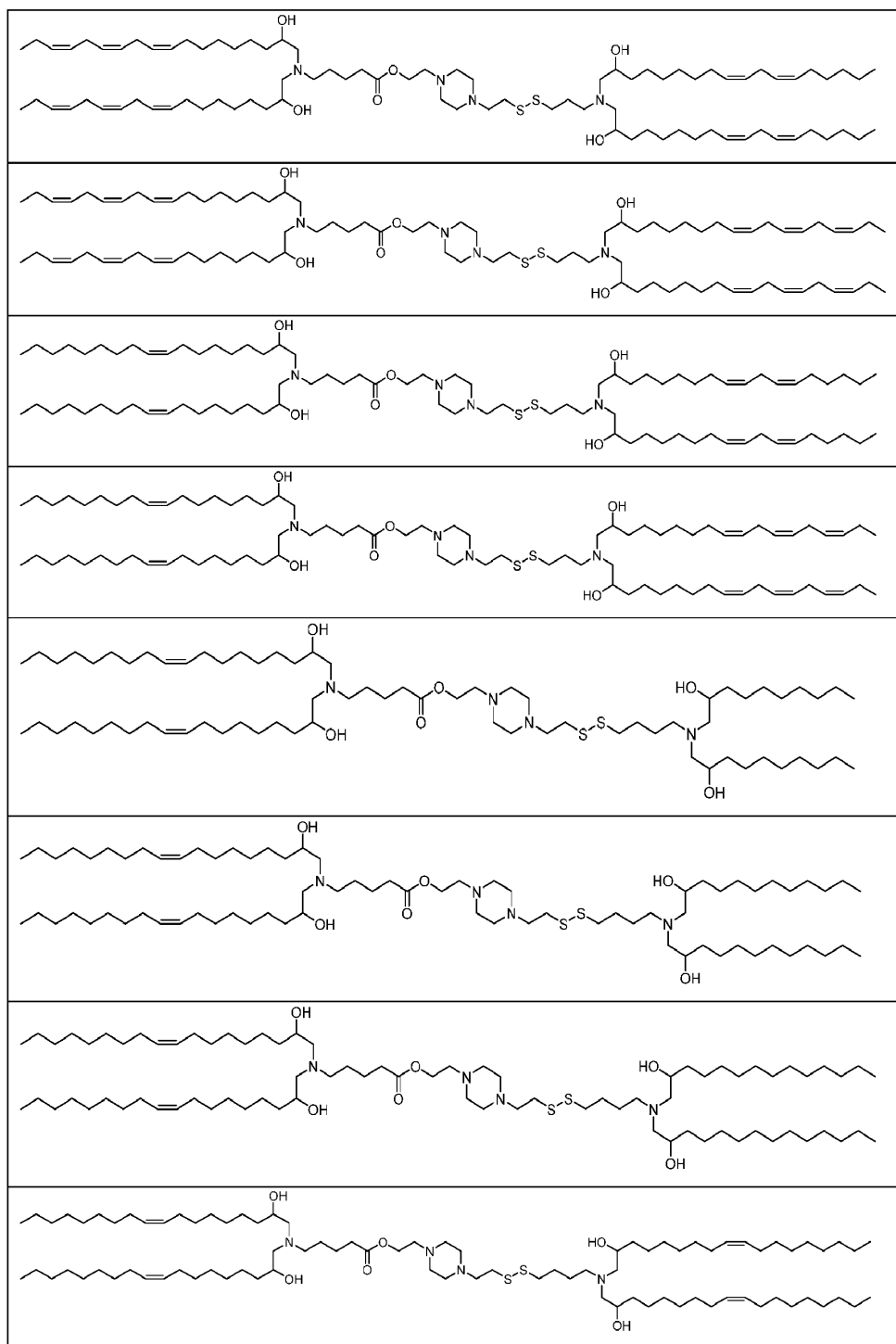


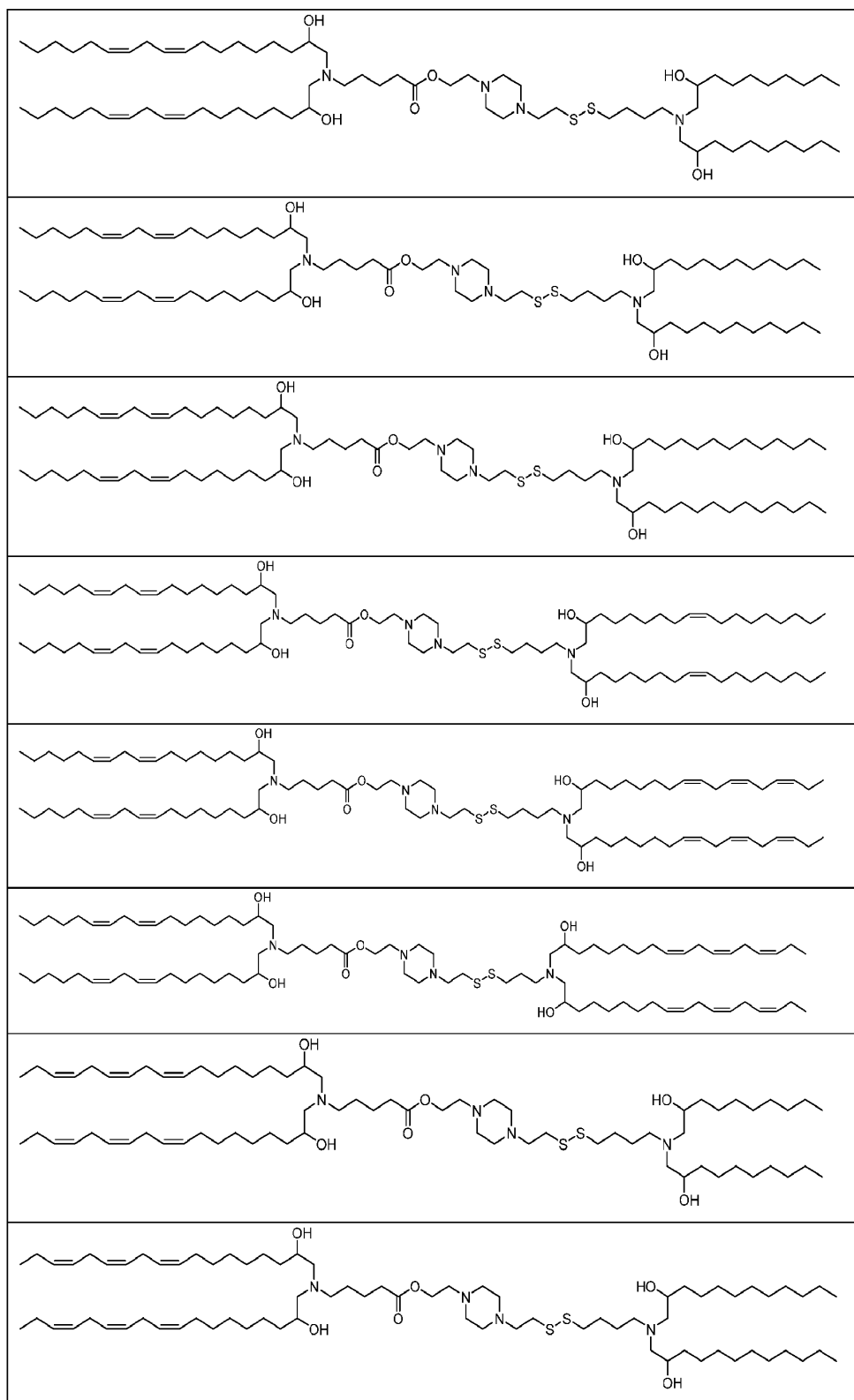


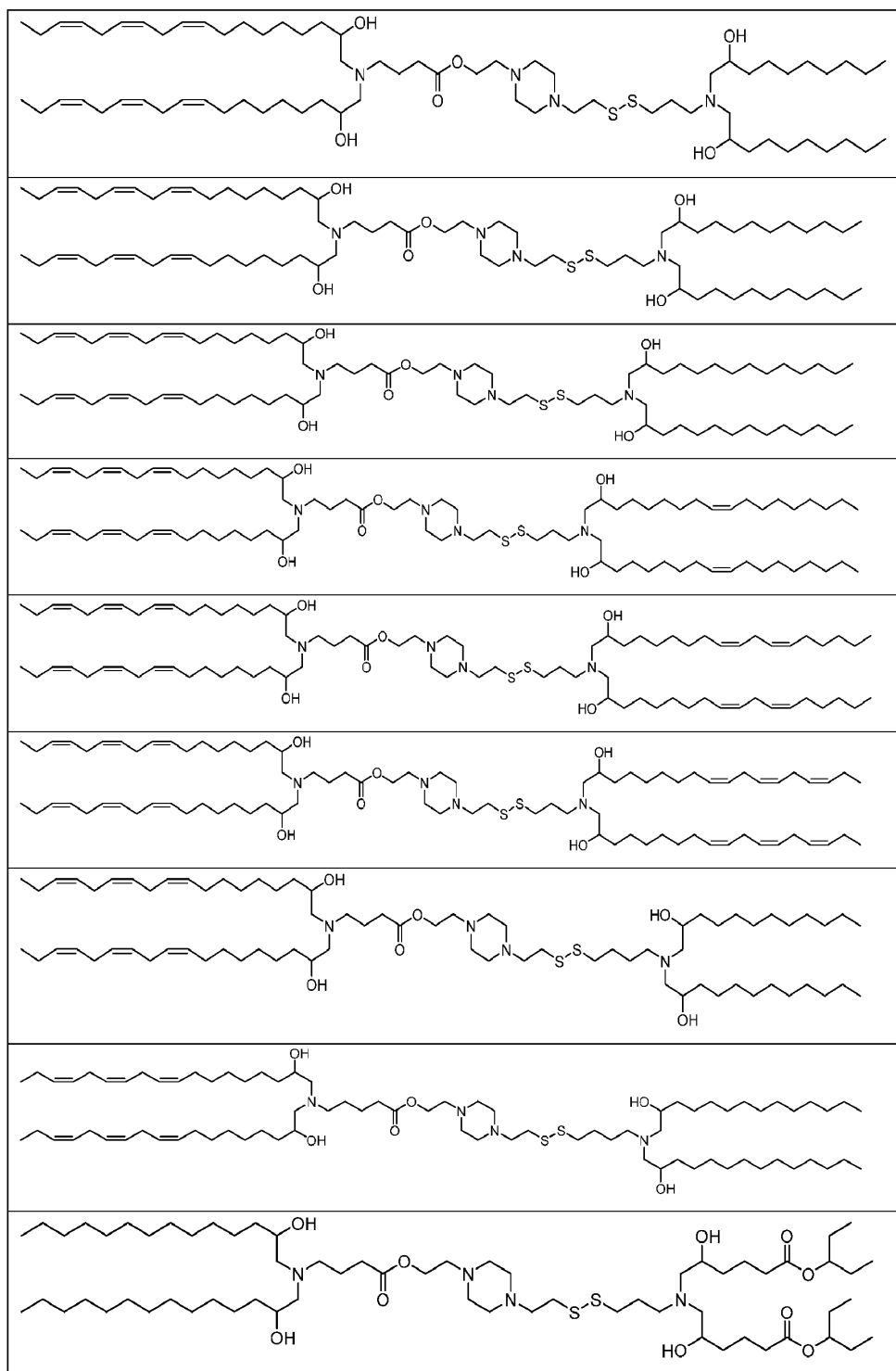


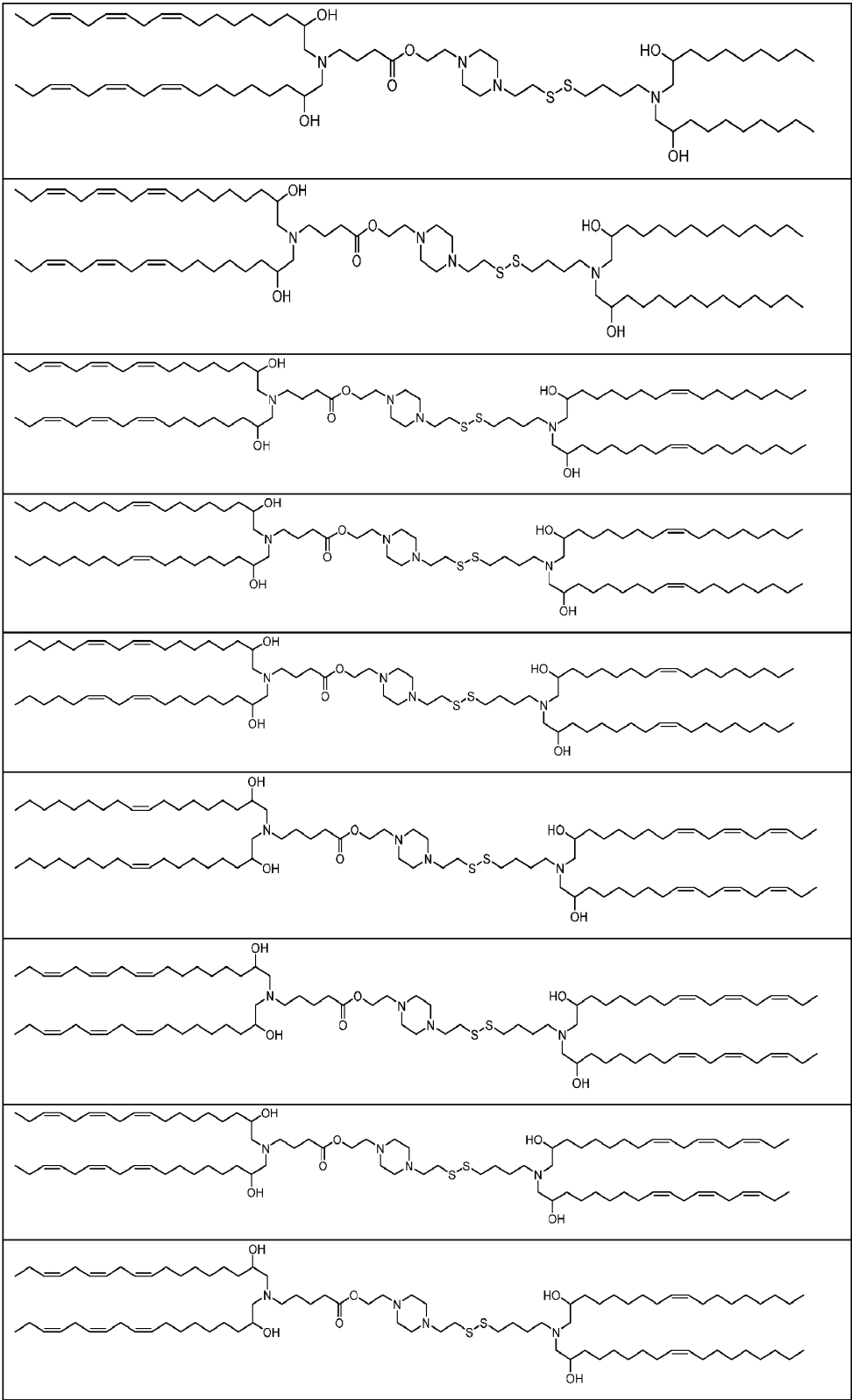


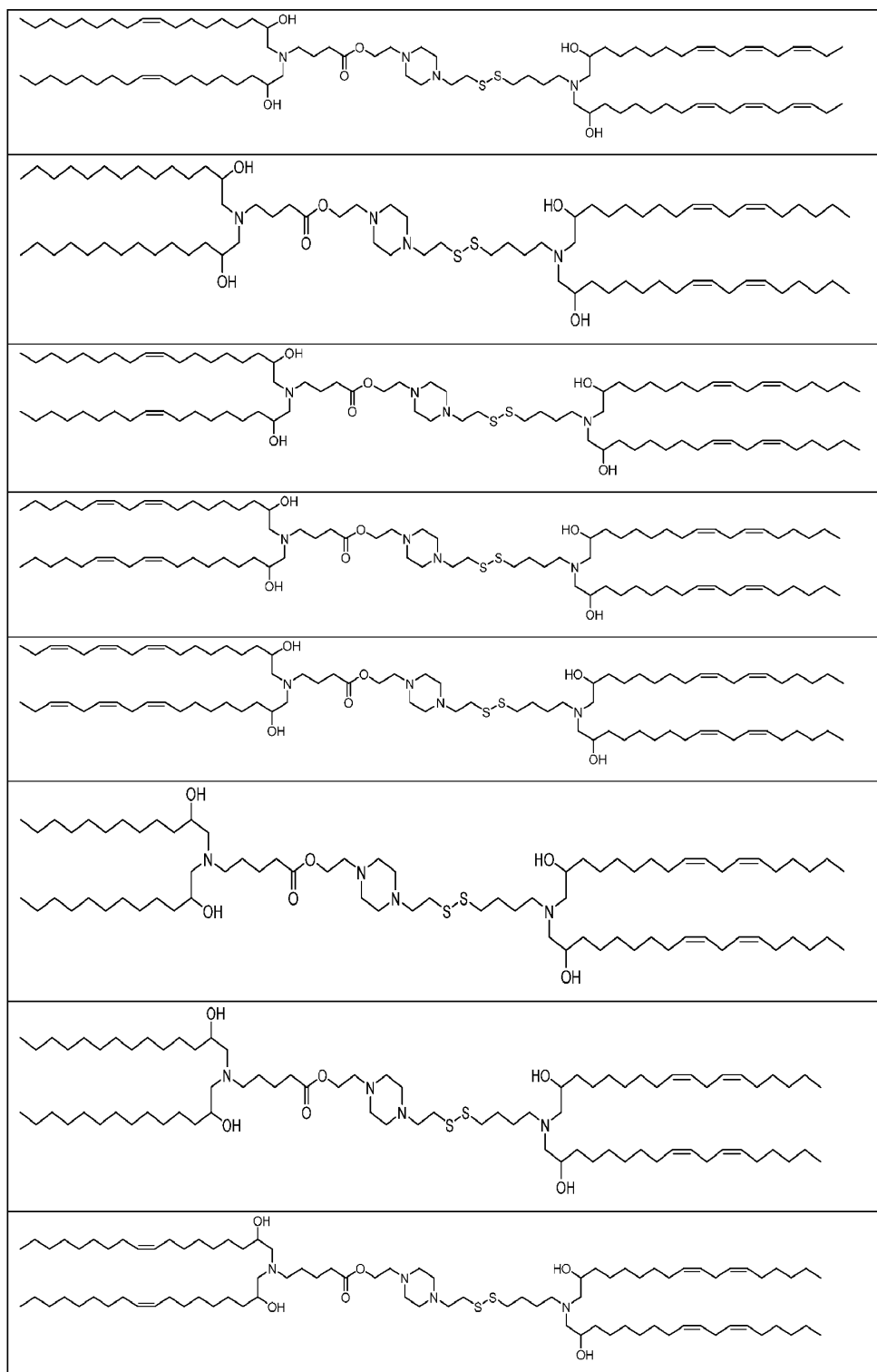


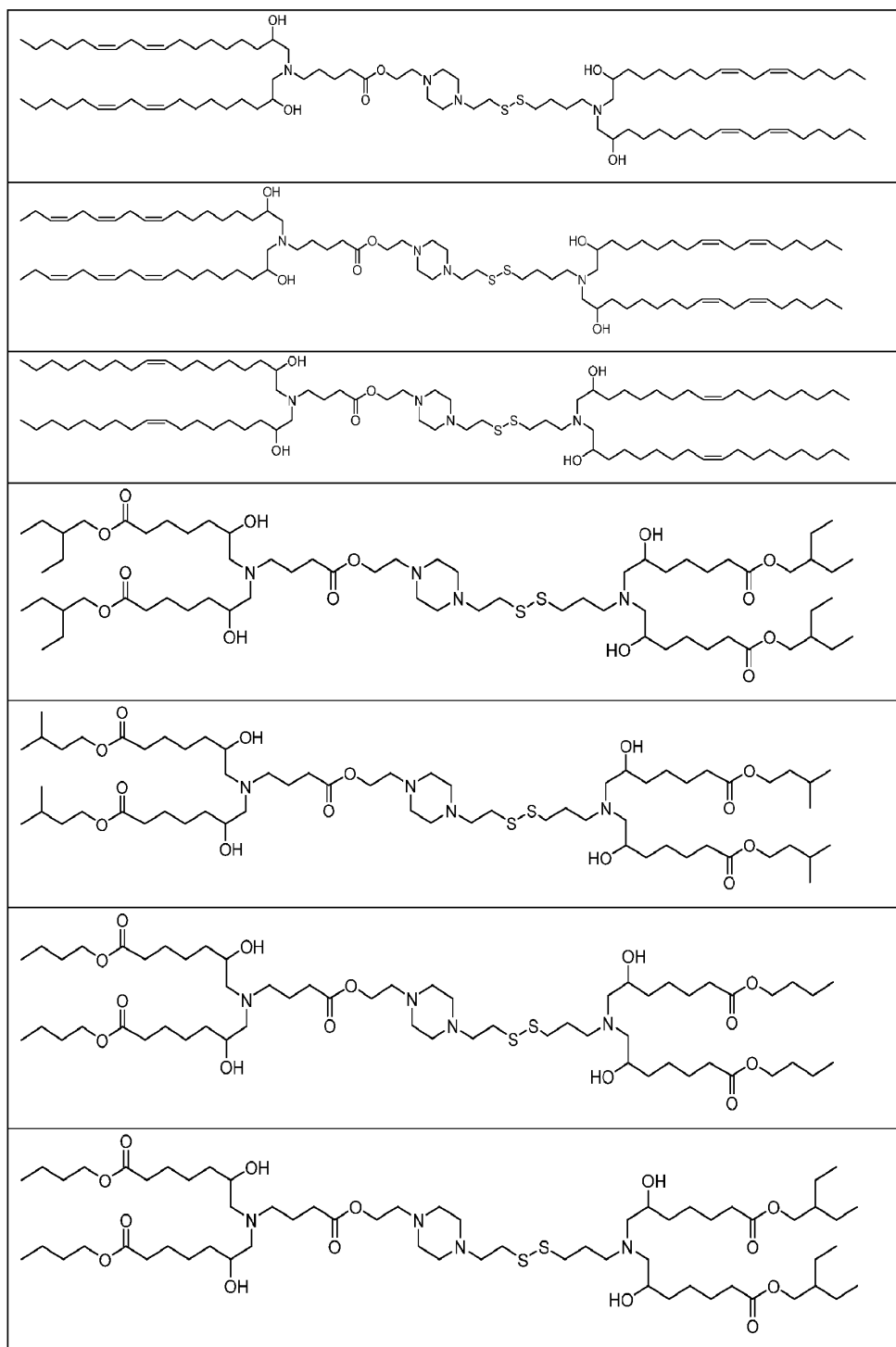


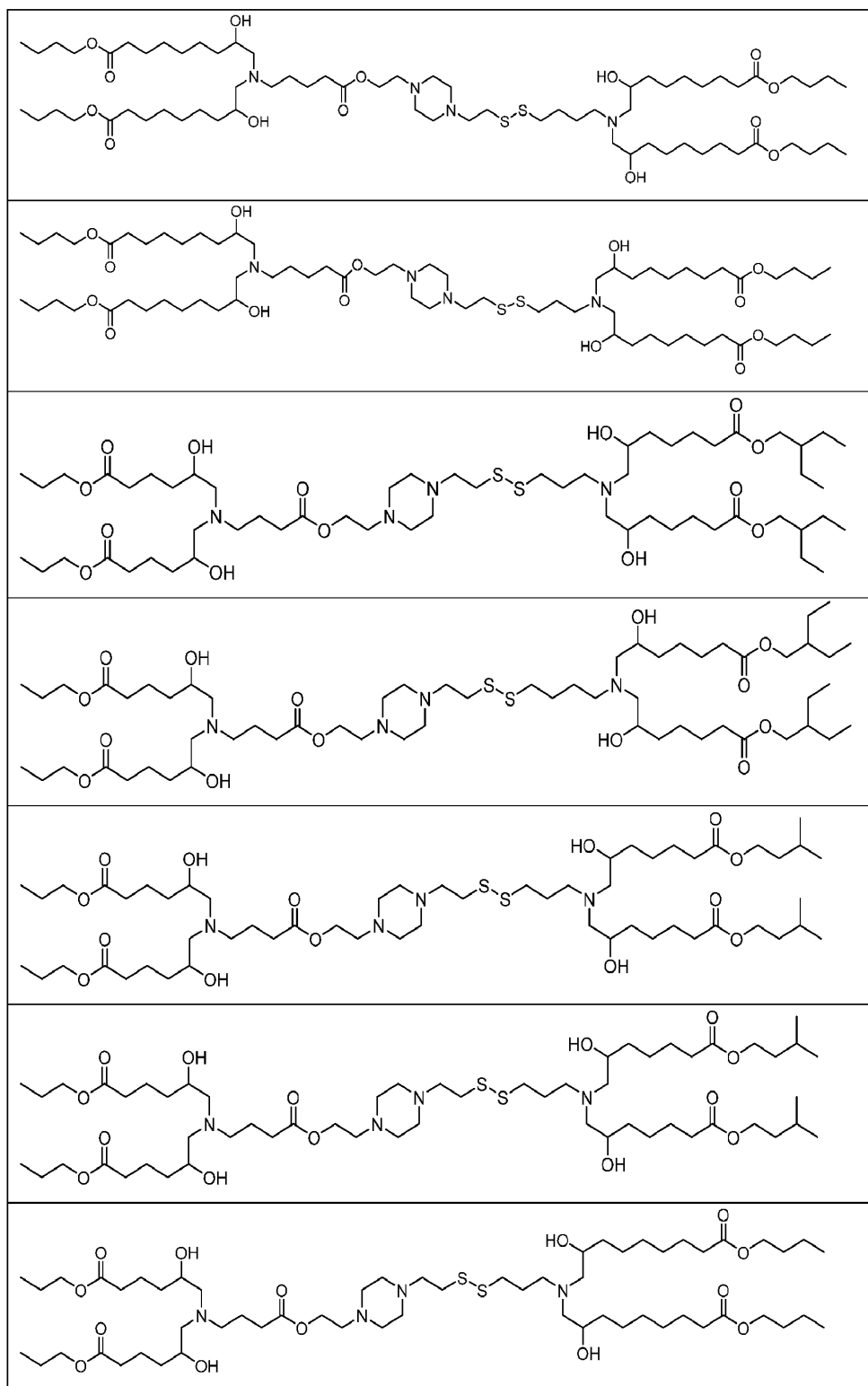


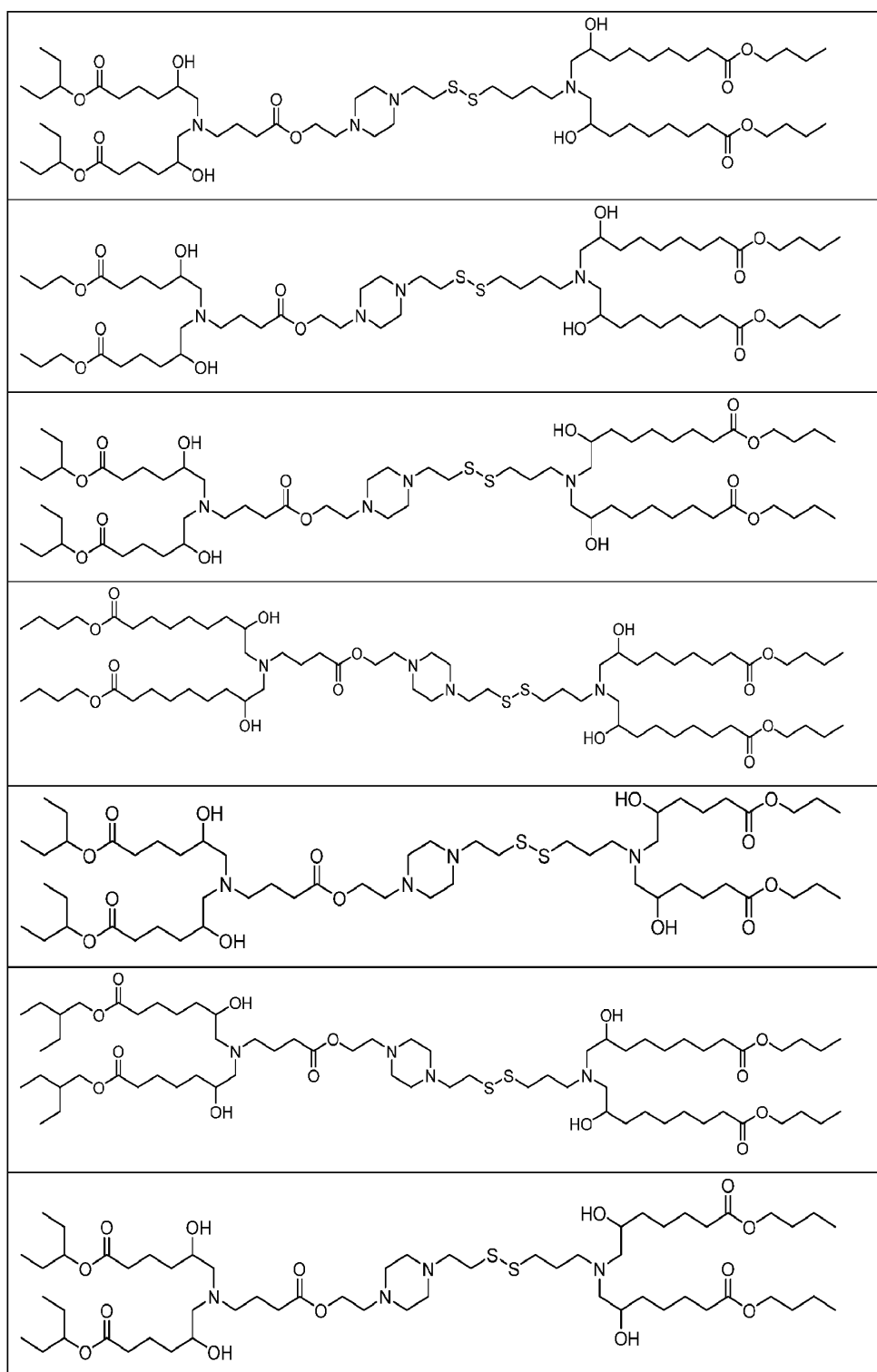


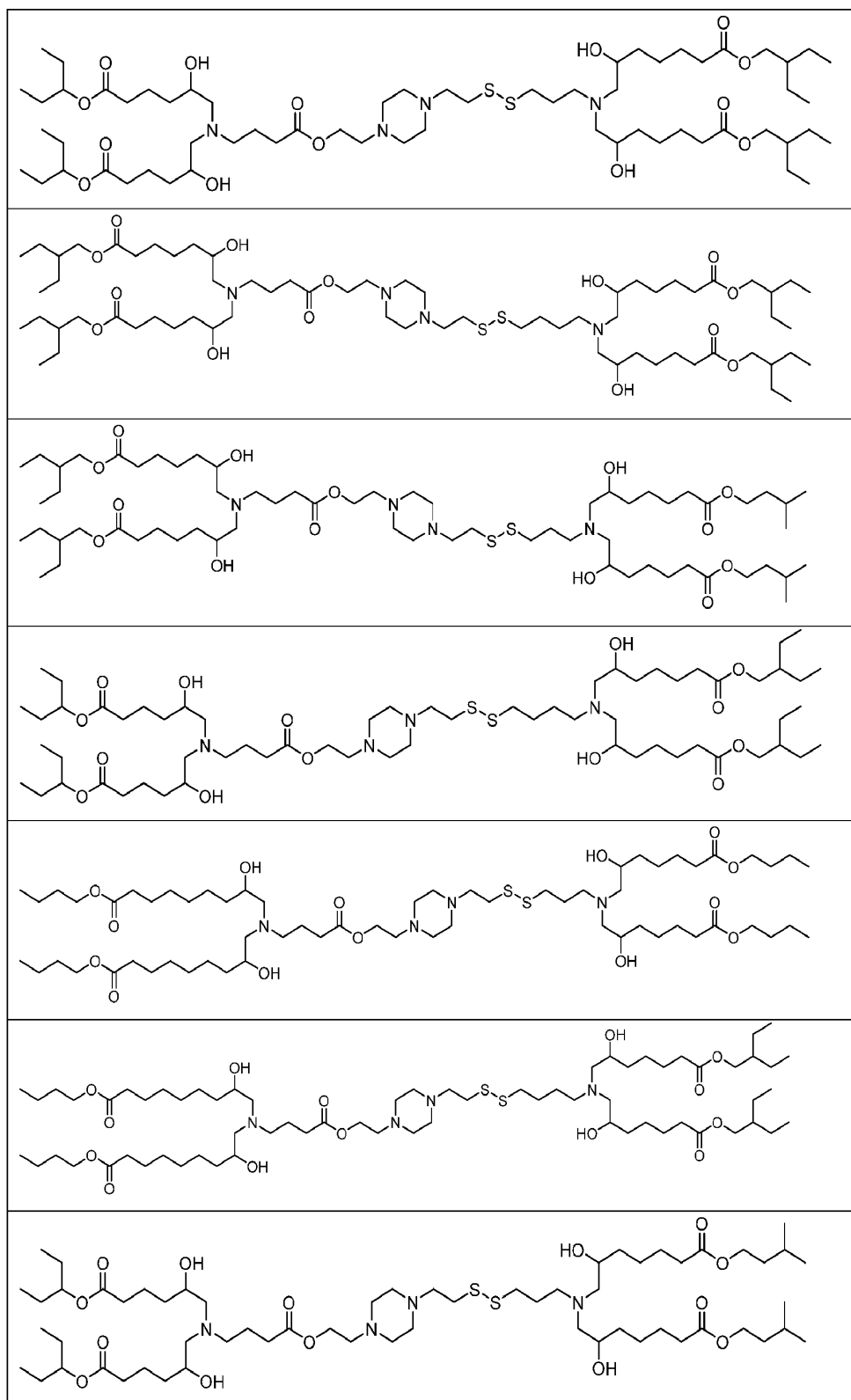












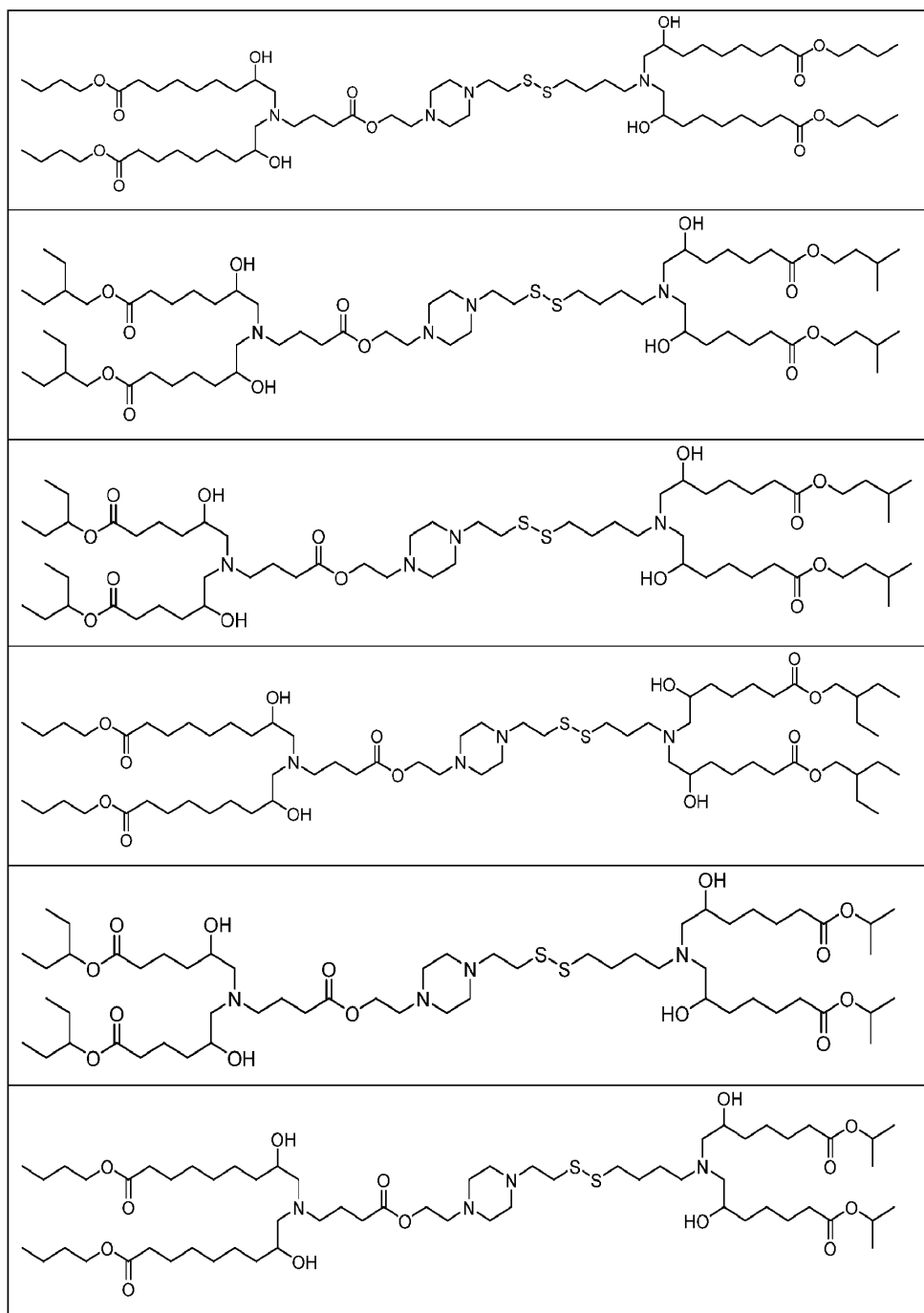
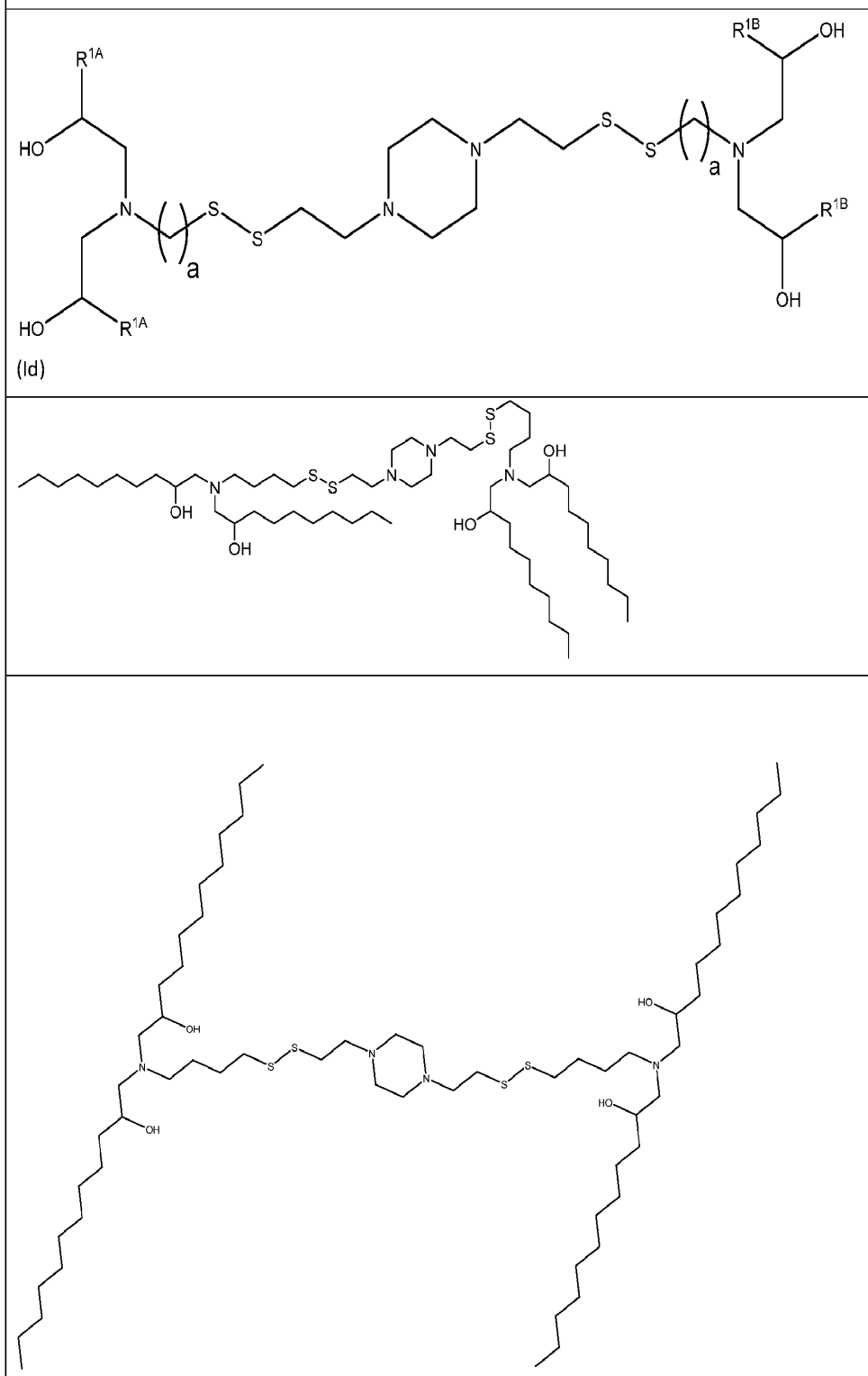


Table C – PIPES-based disulfide/disulfide cationic lipids with a piperazine core



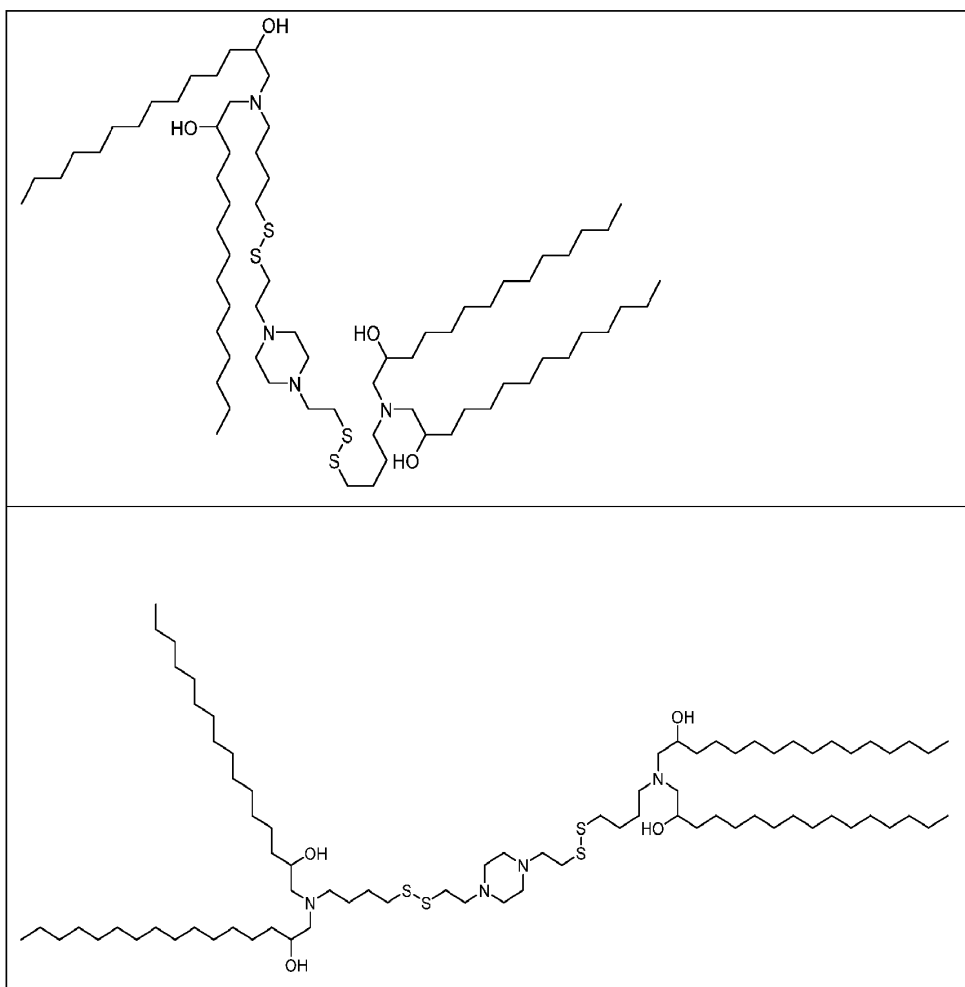
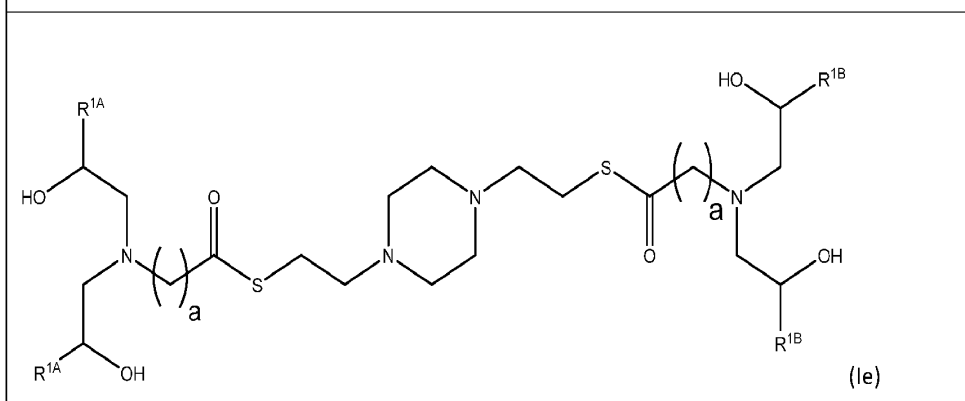
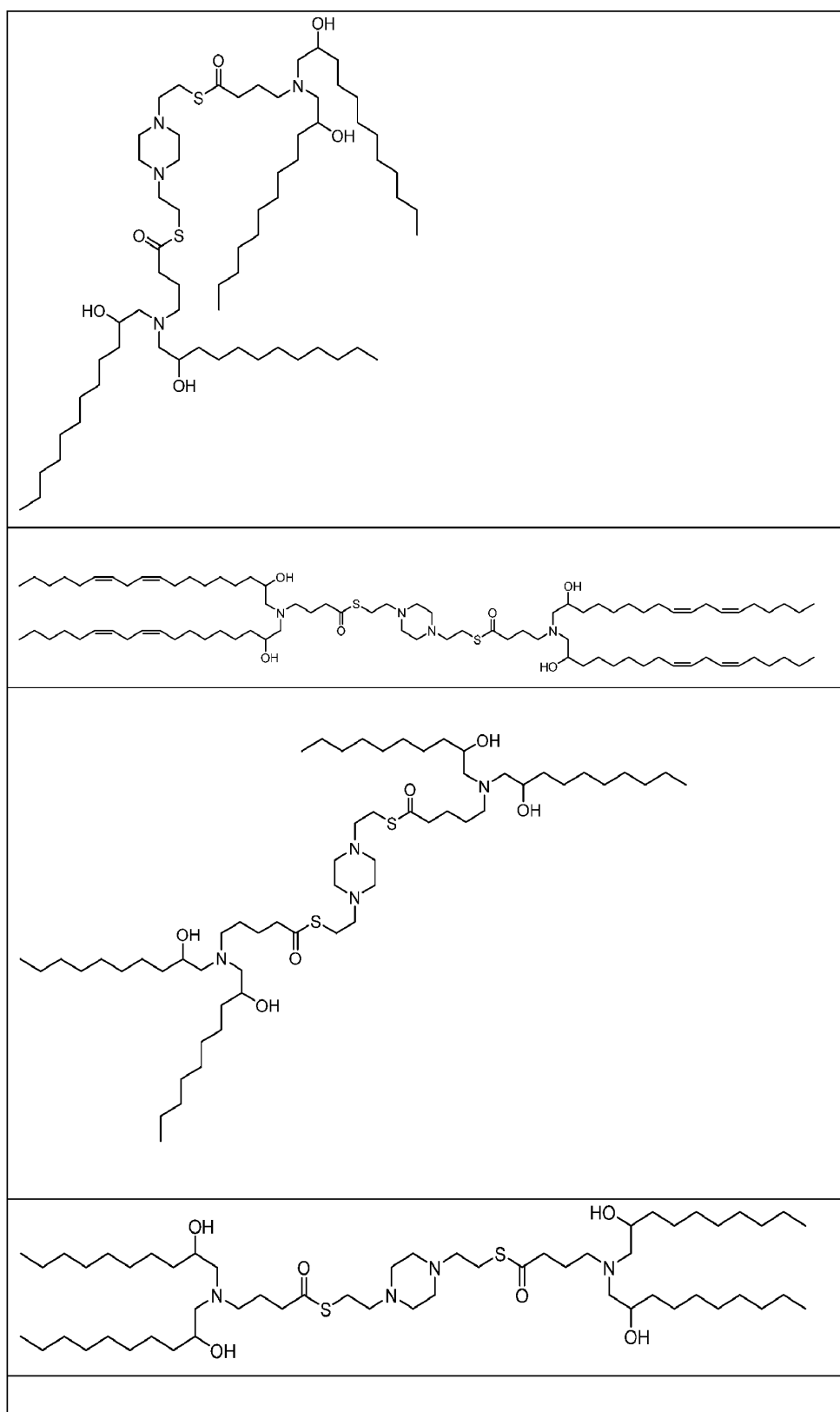


Table D – PIPES-based thioester/thioester cationic lipids with a piperazine core





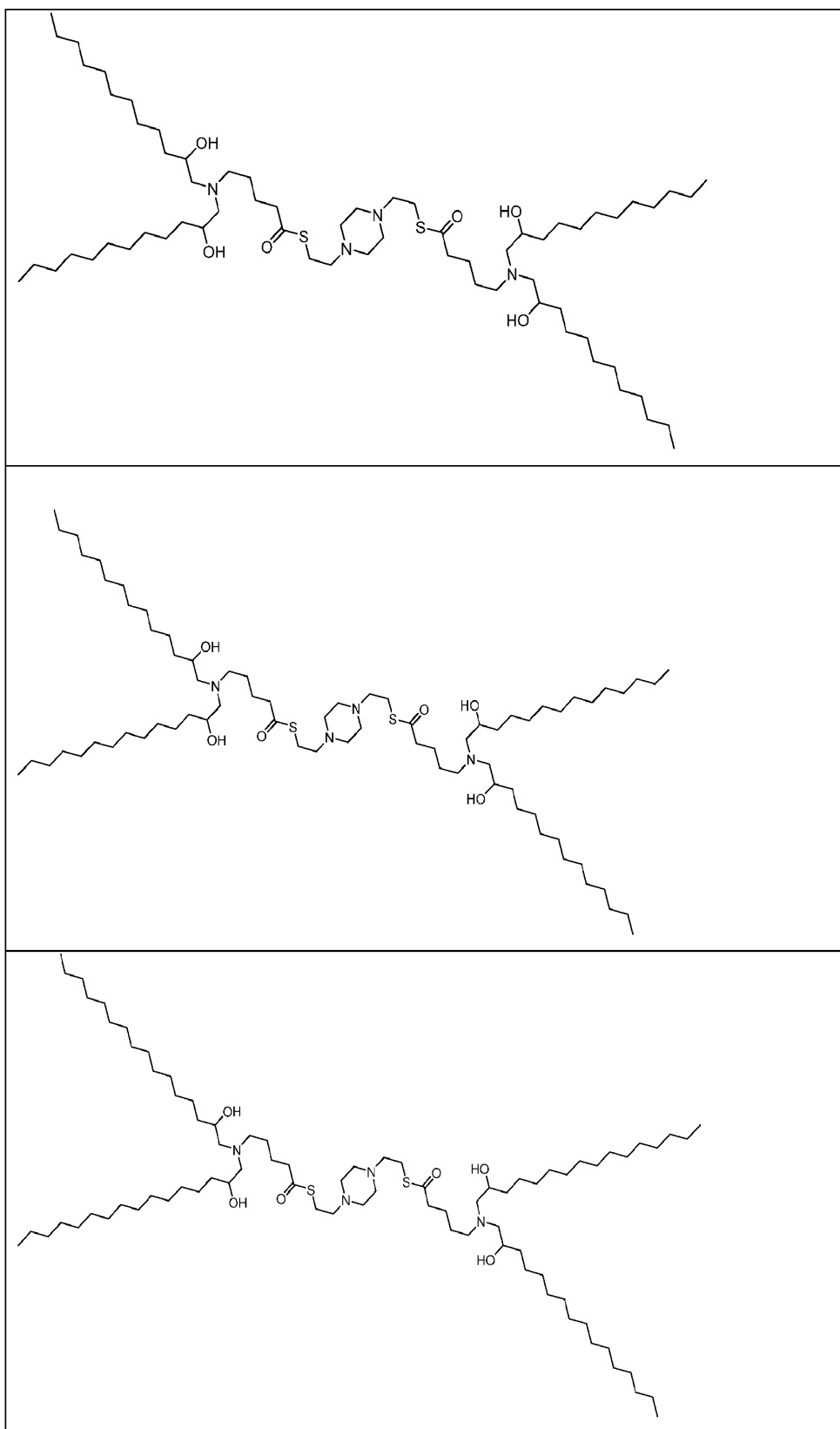
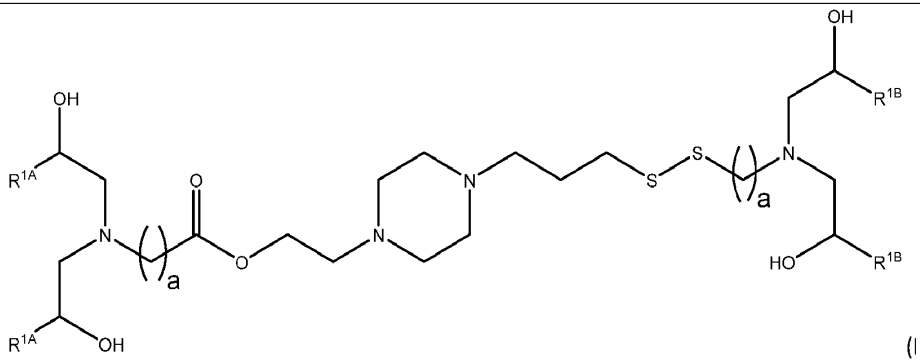
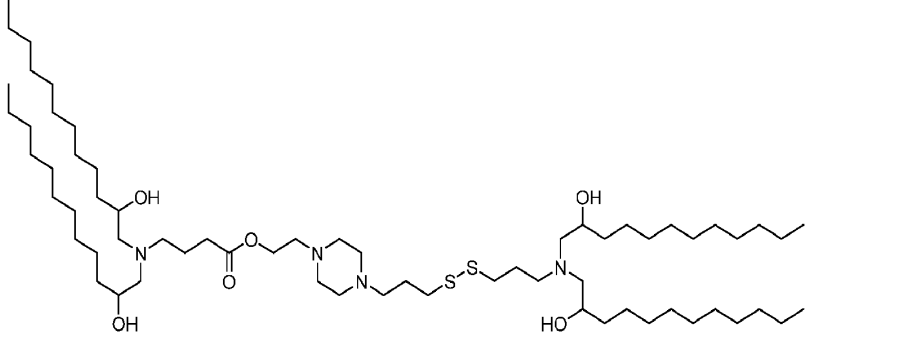
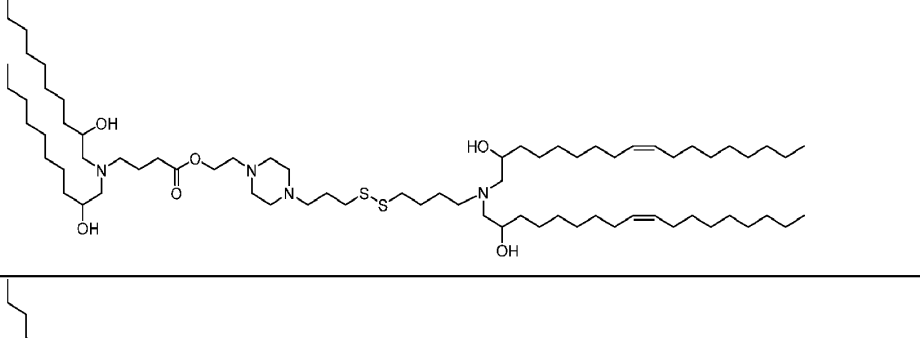
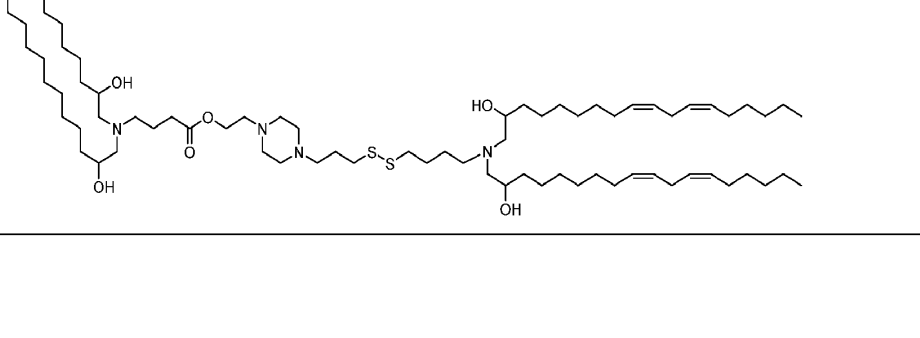
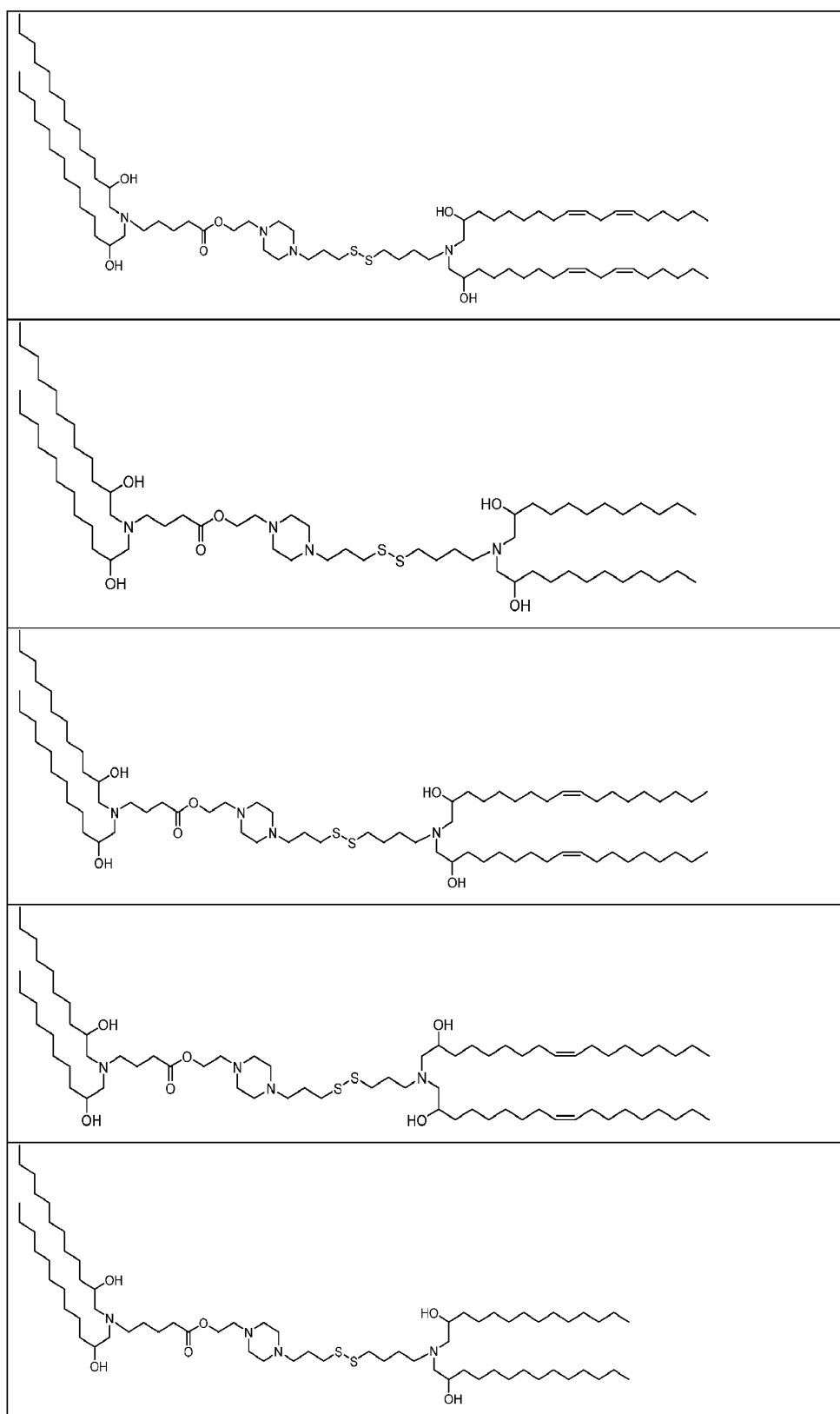


Table E – HEPPS-based ester/disulfide cationic lipids with a piperazine core	
 (IIC)	
	
	
	



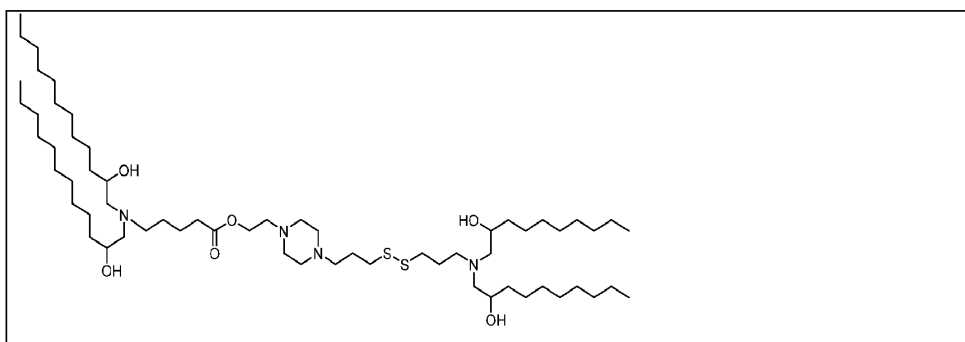
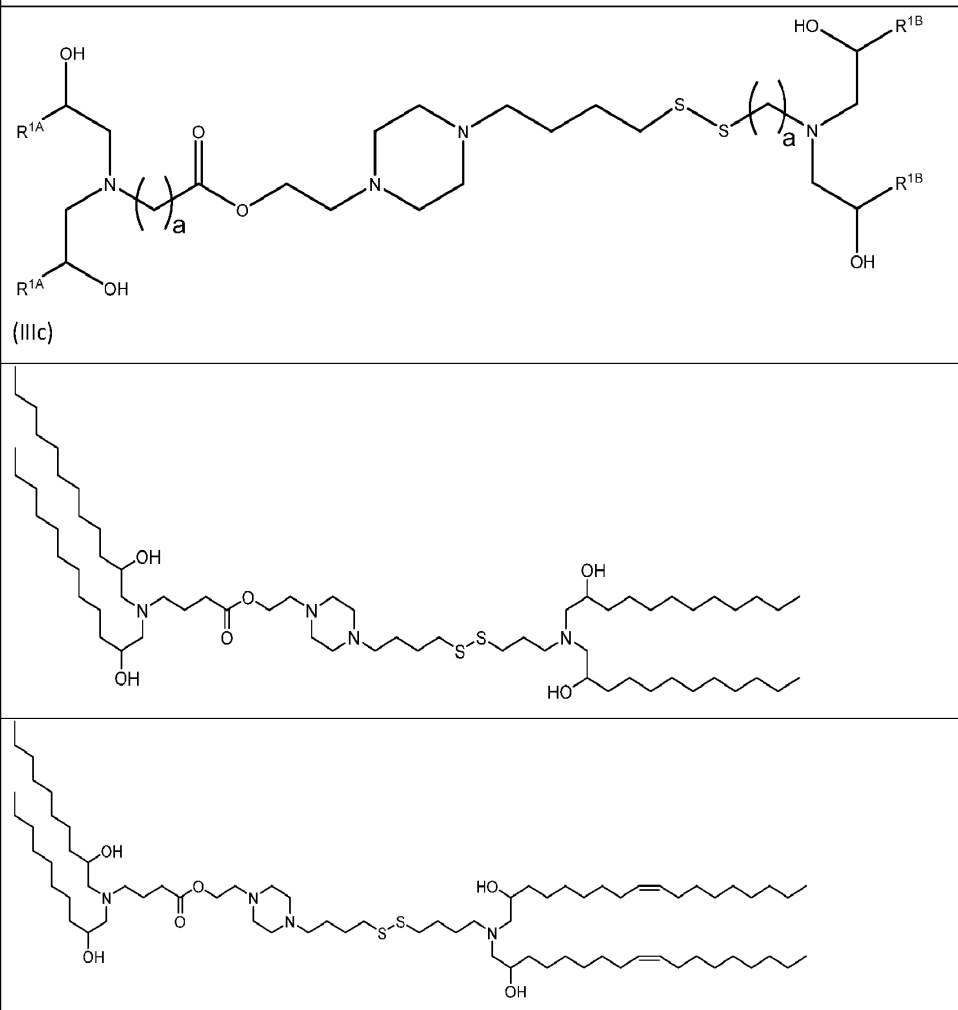
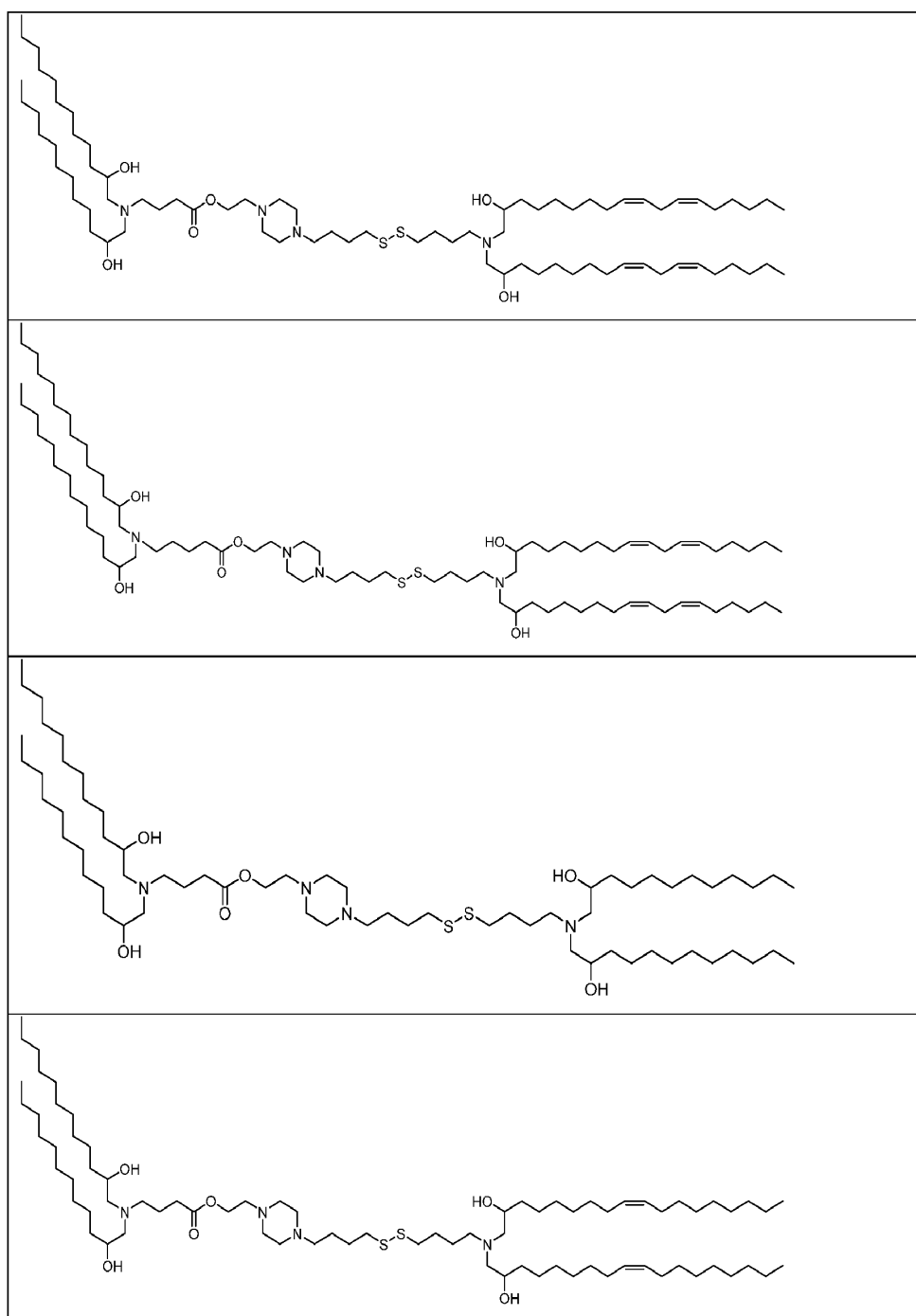
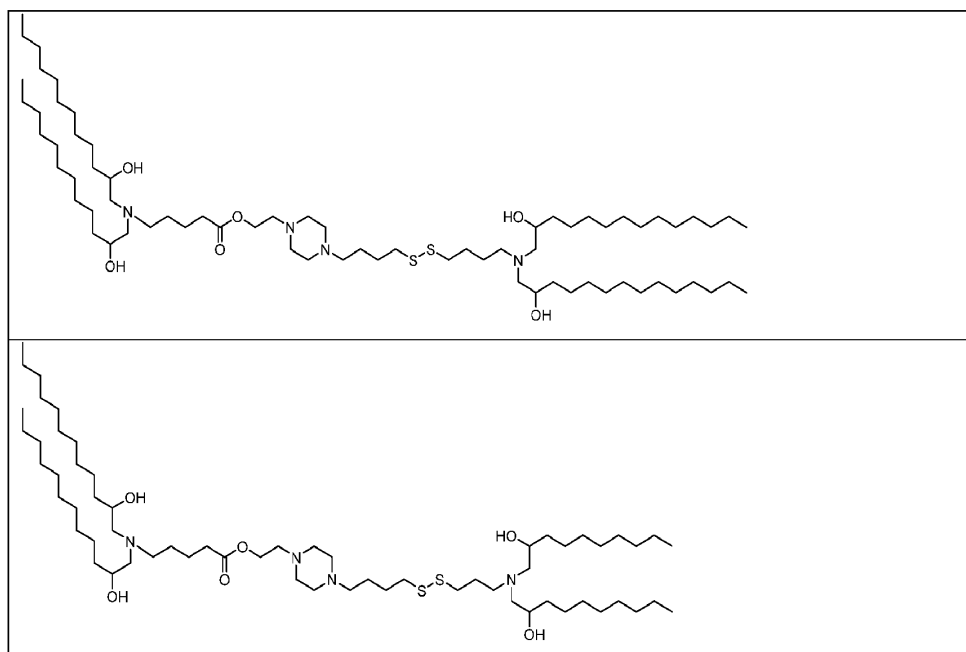


Table F – HEPBS-based ester/disulfide cationic lipids with a piperazine core







[0159] Any of the compounds identified in Tables A to F above may be provided in the form of a pharmaceutically acceptable salt and such salts are intended to be encompassed by the present invention.

[0160] Exemplary compounds include those described in Tables A-F above and compounds of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof of the invention described by examples 1-13.

[0161] Any of the compounds identified in Tables A to F above and compounds of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof of the invention described by examples 1-13 may be provided in the form of a pharmaceutically acceptable salt and such salts are intended to be encompassed by the present invention.

[0162] The compounds of the invention as described herein can be prepared according to methods known in the art, including the exemplary syntheses of the Examples provided herein.

Nucleic Acids

[0163] The compounds of the invention as described herein can be used to prepare compositions useful for the delivery of nucleic acids.

Synthesis of Nucleic Acids

[0164] Nucleic acids according to the present invention may be synthesized according to any known methods. For example, mRNAs according to the present invention may be synthesized via *in vitro* transcription (IVT). Briefly, IVT is typically performed with a linear or circular DNA template containing a promoter, a pool of ribonucleotide triphosphates, a buffer system that may include DTT and magnesium ions, and an appropriate RNA polymerase (*e.g.*, T3, T7, mutated T7 or SP6 RNA polymerase), DNase I, pyrophosphatase, and/or RNase inhibitor. The exact conditions will vary according to the specific application.

[0165] In some embodiments, for the preparation of mRNA according to the invention, a DNA template is transcribed *in vitro*. A suitable DNA template typically has a promoter, for example a T3, T7, mutated T7 or SP6 promoter, for *in vitro* transcription, followed by desired nucleotide sequence for desired mRNA and a termination signal.

[0166] Desired mRNA sequence(s) according to the invention may be determined and incorporated into a DNA template using standard methods. For example, starting from a desired amino acid sequence (*e.g.*, an enzyme sequence), a virtual reverse translation is carried out based on the degenerated genetic code. Optimization algorithms may then be used for selection of suitable codons. Typically, the G/C content can be optimized to achieve the highest possible G/C content on one hand, taking into the best possible account the frequency of the tRNAs according to codon usage on the other hand. The optimized RNA sequence can be established and displayed, for example, with the aid of an appropriate display device and compared with the original (wild-type) sequence. A secondary structure can also be analyzed to calculate stabilizing and destabilizing properties or, respectively, regions of the RNA.

Modified mRNA

[0167] In some embodiments, mRNA according to the present invention may be synthesized as unmodified or modified mRNA. Modified mRNA comprise nucleotide modifications in the RNA. A modified mRNA according to the invention can thus include nucleotide modification that are, for example, backbone modifications, sugar modifications or base modifications. In some embodiments, mRNAs may be synthesized from naturally occurring nucleotides and/or nucleotide analogues (modified nucleotides) including, but not limited to, purines (adenine (A), guanine (G)) or pyrimidines (thymine (T), cytosine (C), uracil (U)), and as modified nucleotides analogues or derivatives of purines and pyrimidines, such as *e.g.*, 1-methyl-adenine, 2-methyl-adenine, 2-methylthio-N-6-isopentenyl-adenine, N6-methyl-adenine, N6-isopentenyl-adenine, 2-thio-cytosine, 3-methyl-cytosine, 4-acetyl-

cytosine, 5-methyl-cytosine, 2,6-diaminopurine, 1-methyl-guanine, 2-methyl-guanine, 2,2-dimethyl-guanine, 7-methyl-guanine, inosine, 1-methyl-inosine, pseudouracil (5-uracil), dihydro-uracil, 2-thio-uracil, 4-thio-uracil, 5-carboxymethylaminomethyl-2-thio-uracil, 5-(carboxyhydroxymethyl)-uracil, 5-fluoro-uracil, 5-bromo-uracil, 5-carboxymethylaminomethyl-uracil, 5-methyl-2-thio-uracil, 5-methyl-uracil, N-uracil-5-oxyacetic acid methyl ester, 5-methylaminomethyl-uracil, 5-methoxyaminomethyl-2-thio-uracil, 5'-methoxycarbonylmethyl-uracil, 5-methoxy-uracil, uracil-5-oxyacetic acid methyl ester, uracil-5-oxyacetic acid (v), 1-methyl-pseudouracil, queuosine, beta-D-mannosyl-queuosine, wybutoxosine, and phosphoramidates, phosphorothioates, peptide nucleotides, methylphosphonates, 7-deazaguanosine, 5-methylcytosine and inosine. The preparation of such analogues is known to a person skilled in the art *e.g.*, from the U.S. Pat. No. 4,373,071, U.S. Pat. No. 4,401,796, U.S. Pat. No. 4,415,732, U.S. Pat. No. 4,458,066, U.S. Pat. No. 4,500,707, U.S. Pat. No. 4,668,777, U.S. Pat. No. 4,973,679, U.S. Pat. No. 5,047,524, U.S. Pat. No. 5,132,418, U.S. Pat. No. 5,153,319, U.S. Pat. Nos. 5,262,530 and 5,700,642, the disclosures of which are incorporated by reference in their entirety.

Pharmaceutical Formulations of Cationic Lipids and Nucleic Acids

[0168] In certain embodiments, the compounds of the invention as described herein, as well as pharmaceutical and liposomal compositions comprising such lipids, can be used in formulations to facilitate the delivery of encapsulated materials (*e.g.*, one or more polynucleotides such as mRNA) to, and subsequent transfection of one or more target cells. For example, in certain embodiments cationic lipids described herein (and compositions such as liposomal compositions comprising such lipids) are characterized as resulting in one or more of receptor-mediated endocytosis, clathrin-mediated and caveolae-mediated endocytosis, phagocytosis and macropinocytosis, fusogenicity, endosomal or lysosomal disruption and/or releasable properties that afford such compounds advantages relative other similarly classified lipids.

[0169] According to the present invention, a nucleic acid, *e.g.*, mRNA encoding a protein (*e.g.*, a full length, fragment or portion of a protein) as described herein may be delivered via a delivery vehicle comprising a compound of the invention as described herein.

[0170] As used herein, the terms “delivery vehicle,” “transfer vehicle,” “nanoparticle,” or grammatical equivalents thereof, are used interchangeably.

[0171] For example, the present invention provides a composition (*e.g.*, a pharmaceutical composition) comprising a compound described herein and one or more polynucleotides. A composition (*e.g.*, a pharmaceutical composition) may further comprise one or more cationic

lipids, one or more non-cationic lipids, one or more cholesterol-based lipids and/or one or more PEG-modified lipids.

[0172] In certain embodiments a composition exhibits an enhanced (*e.g.*, increased) ability to transfect one or more target cells. Accordingly, also provided herein are methods of transfecting one or more target cells. Such methods generally comprise the step of contacting the one or more target cells with the cationic lipids and/or pharmaceutical compositions disclosed herein (*e.g.*, a liposomal formulation comprising a compound described herein encapsulating one or more polynucleotides) such that the one or more target cells are transfected with the materials encapsulated therein (*e.g.*, one or more polynucleotides). As used herein, the terms “transfect” or “transfection” refer to the intracellular introduction of one or more encapsulated materials (*e.g.*, nucleic acids and/or polynucleotides) into a cell, or preferably into a target cell. The introduced polynucleotide may be stably or transiently maintained in the target cell. The term “transfection efficiency” refers to the relative amount of such encapsulated material (*e.g.*, polynucleotides) up-taken by, introduced into, and/or expressed by the target cell which is subject to transfection. In practice, transfection efficiency may be estimated by the amount of a reporter polynucleotide product produced by the target cells following transfection. In certain embodiments, the compounds and pharmaceutical compositions described herein demonstrate high transfection efficiencies thereby improving the likelihood that appropriate dosages of the encapsulated materials (*e.g.*, one or more polynucleotides) will be delivered to the site of pathology and subsequently expressed, while at the same time minimizing potential systemic adverse effects or toxicity associated with the compound or their encapsulated contents.

[0173] Following transfection of one or more target cells by, for example, the polynucleotides encapsulated in the one or more lipid nanoparticles comprising the pharmaceutical or liposomal compositions disclosed herein, the production of the product (*e.g.*, a polypeptide or protein) encoded by such polynucleotide may be preferably stimulated and the capability of such target cells to express the polynucleotide and produce, for example, a polypeptide or protein of interest is enhanced. For example, transfection of a target cell by one or more compounds or pharmaceutical compositions encapsulating mRNA will enhance (*i.e.*, increase) the production of the protein or enzyme encoded by such mRNA.

[0174] Further, delivery vehicles described herein (*e.g.*, liposomal delivery vehicles) may be prepared to preferentially distribute to other target tissues, cells or organs, such as the heart, lungs, kidneys, spleen. In embodiments, the lipid nanoparticles of the present invention may

be prepared to achieve enhanced delivery to the target cells and tissues. For example, polynucleotides (*e.g.*, mRNA) encapsulated in one or more of the compounds or pharmaceutical and liposomal compositions described herein can be delivered to and/or transfect targeted cells or tissues. In some embodiments, the encapsulated polynucleotides (*e.g.*, mRNA) are capable of being expressed and functional polypeptide products produced (and in some instances excreted) by the target cell, thereby conferring a beneficial property to, for example the target cells or tissues. Such encapsulated polynucleotides (*e.g.*, mRNA) may encode, for example, a hormone, enzyme, receptor, polypeptide, peptide or other protein of interest.

Liposomal Delivery Vehicles

[0175] In some embodiments, a composition is a suitable delivery vehicle. In embodiments, a composition is a liposomal delivery vehicle, *e.g.*, a lipid nanoparticle.

[0176] The terms “liposomal delivery vehicle” and “liposomal composition” are used interchangeably.

[0177] Enriching liposomal compositions with one or more of the cationic lipids disclosed herein may be used as a means of improving (*e.g.*, reducing) the toxicity or otherwise conferring one or more desired properties to such enriched liposomal composition (*e.g.*, improved delivery of the encapsulated polynucleotides to one or more target cells and/or reduced in vivo toxicity of a liposomal composition). Accordingly, also contemplated are pharmaceutical compositions, and in particular liposomal compositions, that comprise one or more of the cationic lipids disclosed herein.

[0178] Thus, in certain embodiments, the compounds of the invention as described herein may be used as a component of a liposomal composition to facilitate or enhance the delivery and release of encapsulated materials (*e.g.*, one or more therapeutic agents) to one or more target cells (*e.g.*, by permeating or fusing with the lipid membranes of such target cells).

[0179] As used herein, liposomal delivery vehicles, *e.g.*, lipid nanoparticles, are usually characterized as microscopic vesicles having an interior aqueous space sequestered from an outer medium by a membrane of one or more bilayers. Bilayer membranes of liposomes are typically formed by amphiphilic molecules, such as lipids of synthetic or natural origin that comprise spatially separated hydrophilic and hydrophobic domains (Lasic, Trends Biotechnol., 16: 307-321, 1998). Bilayer membranes of the liposomes can also be formed by amphiphilic polymers and surfactants (*e.g.*, polyerosomes, niosomes, *etc.*). In the context of the present invention, a liposomal delivery vehicle typically serves to transport a desired mRNA to a target cell or tissue.

[0180] In certain embodiments, such compositions (*e.g.*, liposomal compositions) are loaded with or otherwise encapsulate materials, such as for example, one or more biologically-active polynucleotides (*e.g.*, mRNA).

[0181] In embodiments, a composition (*e.g.*, a pharmaceutical composition) comprises an mRNA encoding a protein, encapsulated within a liposome. In embodiments, a liposome comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids and one or more PEG-modified lipids, and wherein at least one cationic lipid is a compound of the invention as described herein. In embodiments, a composition comprises an mRNA encoding for a protein (*e.g.*, any protein described herein). In embodiments, a composition comprises an mRNA encoding for cystic fibrosis transmembrane conductance regulator (CFTR) protein. In embodiments, a composition comprises an mRNA encoding for ornithine transcarbamylase (OTC) protein.

[0182] In embodiments, a composition (*e.g.*, a pharmaceutical composition) comprises a nucleic acid encapsulated within a liposome, wherein the liposome comprises a compound described herein.

[0183] In embodiments, a nucleic acid is an mRNA encoding a peptide or protein. In embodiments, an mRNA encodes a peptide or protein for use in the delivery to or treatment of the lung of a subject or a lung cell (*e.g.*, an mRNA encodes cystic fibrosis transmembrane conductance regulator (CFTR) protein). In embodiments, an mRNA encodes a peptide or protein for use in the delivery to or treatment of the liver of a subject or a liver cell (*e.g.*, an mRNA encodes ornithine transcarbamylase (OTC) protein). Still other exemplary mRNAs are described herein.

[0184] In embodiments, a liposomal delivery vehicle (*e.g.*, a lipid nanoparticle) can have a net positive charge.

[0185] In embodiments, a liposomal delivery vehicle (*e.g.*, a lipid nanoparticle) can have a net negative charge.

[0186] In embodiments, a liposomal delivery vehicle (*e.g.*, a lipid nanoparticle) can have a net neutral charge.

[0187] In embodiments, a lipid nanoparticle that encapsulates a nucleic acid (*e.g.*, mRNA encoding a peptide or protein) comprises one or more compounds of the invention as described herein.

[0188] For example, the amount of a compound of the invention as described herein in a composition can be described as a percentage ("wt%") of the combined dry weight of all

lipids of a composition (*e.g.*, the combined dry weight of all lipids present in a liposomal composition).

[0189] In embodiments of the pharmaceutical compositions described herein, a compound of the invention as described herein is present in an amount that is about 0.5 wt% to about 30 wt% (*e.g.*, about 0.5 wt% to about 20 wt%) of the combined dry weight of all lipids present in a composition (*e.g.*, a liposomal composition).

[0190] In embodiments, a compound of the invention as described herein is present in an amount that is about 1 wt% to about 30 wt%, about 1 wt% to about 20 wt%, about 1 wt% to about 15 wt%, about 1 wt% to about 10 wt%, or about 5 wt% to about 25 wt% of the combined dry weight of all lipids present in a composition (*e.g.*, a liposomal composition). In embodiments, a compound of the invention as described herein is present in an amount that is about 0.5 wt% to about 5 wt%, about 1 wt% to about 10 wt%, about 5 wt% to about 20 wt%, or about 10 wt% to about 20 wt% of the combined molar amounts of all lipids present in a composition such as a liposomal delivery vehicle.

[0191] In embodiments, the amount of a compound of the invention as described herein is present in an amount that is at least about 5 wt%, about 10 wt%, about 15 wt%, about 20 wt%, about 25 wt%, about 30 wt%, about 35 wt%, about 40 wt%, about 45 wt%, about 50 wt%, about 55 wt%, about 60 wt%, about 65 wt%, about 70 wt%, about 75 wt%, about 80 wt%, about 85 wt%, about 90 wt%, about 95 wt%, about 96 wt%, about 97 wt%, about 98 wt%, or about 99 wt% of the combined dry weight of total lipids in a composition (*e.g.*, a liposomal composition).

[0192] In embodiments, the amount of a compound of the invention as described herein is present in an amount that is no more than about 5 wt%, about 10 wt%, about 15 wt%, about 20 wt%, about 25 wt%, about 30 wt%, about 35 wt%, about 40 wt%, about 45 wt%, about 50 wt%, about 55 wt%, about 60 wt%, about 65 wt%, about 70 wt%, about 75 wt%, about 80 wt%, about 85 wt%, about 90 wt%, about 95 wt%, about 96 wt%, about 97 wt%, about 98 wt%, or about 99 wt% of the combined dry weight of total lipids in a composition (*e.g.*, a liposomal composition).

[0193] In embodiments, a composition (*e.g.*, a liposomal delivery vehicle such as a lipid nanoparticle) comprises about 0.1 wt% to about 20 wt% (*e.g.*, about 0.1 wt% to about 15 wt%) of a compound described herein. In embodiments, a delivery vehicle (*e.g.*, a liposomal delivery vehicle such as a lipid nanoparticle) comprises about 0.5 wt%, about 1 wt%, about 3 wt%, about 5 wt%, or about 10 wt% of a compound described herein. In embodiments, a delivery vehicle (*e.g.*, a liposomal delivery vehicle such as a lipid nanoparticle) comprises up

to about 0.5 wt%, about 1 wt%, about 3 wt%, about 5 wt%, about 10 wt%, about 15 wt%, or about 20 wt% of a compound described herein. In embodiments, the percentage results in an improved beneficial effect (*e.g.*, improved delivery to targeted tissues such as the liver or the lung).

[0194] The amount of a compound of the invention as described herein in a composition also can be described as a percentage ("mol%") of the combined molar amounts of total lipids of a composition (*e.g.*, the combined molar amounts of all lipids present in a liposomal delivery vehicle).

[0195] In embodiments of pharmaceutical compositions described herein, a compound of the invention as described herein is present in an amount that is about 0.5 mol% to about 50 mol% (*e.g.*, about 0.5 mol% to about 20 mol%) of the combined molar amounts of all lipids present in a composition such as a liposomal delivery vehicle.

[0196] In embodiments, a compound of the invention as described herein is present in an amount that is about 0.5 mol% to about 5 mol%, about 1 mol% to about 10 mol%, about 5 mol% to about 20 mol%, about 10 mol% to about 20 mol%, about 15 mol% to about 30 mol%, about 20 mol% to about 35 mol%, about 25 mol% to about 40 mol%, about 30 mol% to about 45 mol%, about 35 mol% to about 50 mol%, about 40 mol% to about 55 mol%, or about 45 mol% to about 60 mol% of the combined molar amounts of all lipids present in a composition such as a liposomal delivery vehicle. In embodiments, a compound of the invention as described herein is present in an amount that is about 1 mol% to about 60 mol%, 1 mol% to about 50 mol%, 1 mol% to about 40 mol%, 1 mol% to about 30 mol%, about 1 mol% to about 20 mol%, about 1 mol% to about 15 mol%, about 1 mol% to about 10 mol%, about 5 mol% to about 55 mol%, about 5 mol% to about 45 mol%, about 5 mol% to about 35 mol% or about 5 mol% to about 25 mol% of the combined dry weight of all lipids present in a composition such as a liposomal delivery vehicle

[0197] In certain embodiments, a compound of the invention as described herein can comprise from about 0.1 mol% to about 50 mol%, or from 0.5 mol% to about 50 mol%, or from about 1 mol% to about 25 mol%, or from about 1 mol% to about 10 mol% of the total amount of lipids in a composition (*e.g.*, a liposomal delivery vehicle).

[0198] In certain embodiments, a compound of the invention as described herein can comprise greater than about 0.1 mol%, or greater than about 0.5 mol%, or greater than about 1 mol%, greater than about 5 mol%, greater than about 10 mol%, greater than about 20 mol%, greater than about 30 mol%, or greater than about 40 mol% of the total amount of lipids in the lipid nanoparticle.

[0199] In certain embodiments, a compound as described can comprise less than about 60 mol%, or less than about 55 mol%, or less than about 50 mol%, or less than about 45 mol%, or less than about 40 mol%, or less than about 35 mol %, less than about 30 mol%, or less than about 25 mol%, or less than about 10 mol%, or less than about 5 mol%, or less than about 1 mol% of the total amount of lipids in a composition (*e.g.*, a liposomal delivery vehicle).

[0200] In embodiments, the amount of a compound of the invention as described herein is present in an amount that is at least about 5 mol%, about 10 mol%, about 15 mol%, about 20 mol%, about 25 mol%, about 30 mol%, about 35 mol%, about 40 mol%, about 45 mol%, about 50 mol%, about 55 mol%, about 60 mol%, about 65 mol%, about 70 mol%, about 75 mol%, about 80 mol%, about 85 mol%, about 90 mol%, about 95 mol%, about 96 mol%, about 97 mol%, about 98 mol%, or about 99 mol% of the combined dry weight of total lipids in a composition (*e.g.*, a liposomal composition).

[0201] In embodiments, the amount of a compound of the invention as described herein is present in an amount that is no more than about 5 mol%, about 10 mol%, about 15 mol%, about 20 mol%, about 25 mol%, about 30 mol%, about 35 mol%, about 40 mol%, about 45 mol%, about 50 mol%, about 55 mol%, about 60 mol%, about 65 mol%, about 70 mol%, about 75 mol%, about 80 mol%, about 85 mol%, about 90 mol%, about 95 mol%, about 96 mol%, about 97 mol%, about 98 mol%, or about 99 mol% of the combined dry weight of total lipids in a composition (*e.g.*, a liposomal composition).

[0202] In embodiments, the percentage results in an improved beneficial effect (*e.g.*, improved delivery to targeted tissues such as the liver or the lung).

[0203] In a typical embodiment, a composition of the invention (*e.g.*, a liposomal composition) comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids, and one or more PEG-modified lipids, wherein at least one cationic lipid is a compound of the invention as described herein. For example, a composition suitable for practicing the invention has four lipid components comprising a compound of the invention as described herein as the cationic lipid component, a non-cationic lipid, a cholesterol-based lipid and a PEG-modified lipid. The non-cationic lipid may be DOPE or DEPE. The cholesterol-based lipid may be cholesterol. The PEG-modified lipid may be DMG-PEG2K.

[0204] In further embodiments, pharmaceutical (*e.g.*, liposomal) compositions comprise one or more of a PEG-modified lipid, a non-cationic lipid and a cholesterol lipid. In other embodiments, such pharmaceutical (*e.g.*, liposomal) compositions comprise: one or more PEG-modified lipids; one or more non-cationic lipids; and one or more cholesterol lipids. In

yet further embodiments, such pharmaceutical (*e.g.*, liposomal) compositions comprise: one or more PEG-modified lipids and one or more cholesterol lipids.

[0205] In embodiments, a composition (*e.g.*, lipid nanoparticle) that encapsulates a nucleic acid (*e.g.*, mRNA encoding a peptide or protein) comprises one or more compounds of the invention as described herein and one or more lipids selected from the group consisting of a cationic lipid, a non-cationic lipid, and a PEGylated lipid.

[0206] In embodiments, a composition (*e.g.*, lipid nanoparticle) that encapsulates a nucleic acid (*e.g.*, mRNA encoding a peptide or protein) comprises one or more compound of the invention as described herein; one or more lipids selected from the group consisting of a cationic lipid, a non-cationic lipid, and a PEGylated lipid; and further comprises a cholesterol-based lipid. Typically, such a composition has four lipid components comprising a compound of the invention as described herein as the cationic lipid component, a non-cationic lipid (*e.g.*, DOPE), a cholesterol-based lipid (*e.g.*, cholesterol) and a PEG-modified lipid (*e.g.*, DMG-PEG2K).

[0207] In embodiments, a lipid nanoparticle that encapsulates a nucleic acid (*e.g.*, mRNA encoding a peptide or protein) comprises one or more compounds of the invention as described herein, as well as one or more lipids selected from the group consisting of a cationic lipid, a non-cationic lipid, a PEGylated lipid, and a cholesterol-based lipid.

[0208] According to various embodiments, the selection of cationic lipids, non-cationic lipids and/or PEG-modified lipids which comprise the lipid nanoparticle, as well as the relative molar ratio of such lipids to each other, is based upon the characteristics of the selected lipid(s), the nature of the intended target cells, the characteristics of the mRNA to be delivered. Additional considerations include, for example, the saturation of the alkyl chain, as well as the size, charge, pH, pKa, fusogenicity and toxicity of the selected lipid(s). Thus, the molar ratios may be adjusted accordingly.

[0209] In some embodiments, the ratio of cationic lipid(s) to non-cationic lipid(s) to cholesterol-based lipid(s) to PEG-modified lipid(s) may be between about 30:60:20:40:20-30:1-10, respectively. In some embodiments, the ratio of cationic lipid(s) to non-cationic lipid(s) to cholesterol-based lipid(s) to PEG-modified lipid(s) is approximately 40:30:20:10, respectively. In some embodiments, the ratio of cationic lipid(s) to non-cationic lipid(s) to cholesterol-based lipid(s) to PEG-modified lipid(s) is approximately 40:30:25:5, respectively. In some embodiments, the ratio of cationic lipid(s) to non-cationic lipid(s) to cholesterol-based lipid(s) to PEG-modified lipid(s) is approximately 40:32:25:3, respectively. In some

embodiments, the ratio of cationic lipid(s) to non-cationic lipid(s) to cholesterol-based lipid(s) to PEG-modified lipid(s) is approximately 50:25:20:5.

Cationic Lipids

[0210] In addition to any of the compounds of the invention as described herein, a composition may comprise one or more additional cationic lipids.

[0211] In some embodiments, liposomes may comprise one or more additional cationic lipids. As used herein, the phrase “cationic lipid” refers to any of a number of lipid species that have a net positive charge at a selected pH, such as physiological pH. Several cationic lipids have been described in the literature, many of which are commercially available.

[0212] Suitable additional cationic lipids for use in the compositions include the cationic lipids as described in the literature.

Helper Lipids

[0213] Compositions (*e.g.*, liposomal compositions) may also comprise one or more helper lipids. Such helper lipids include non-cationic lipids. As used herein, the phrase “non-cationic lipid” refers to any neutral, zwitterionic or anionic lipid. As used herein, the phrase “anionic lipid” refers to any of a number of lipid species that carry a net negative charge at a selected pH, such as physiological pH. Non-cationic lipids include, but are not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), 1,2-Dierucoyl-sn-glycero-3-phosphoethanolamine (DEPE), palmitoyl-oleoylphosphatidylcholine (POPC), palmitoyl-oleoyl-phosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), or a mixture thereof. A non-cationic or helper lipid suitable for practicing the invention is dioleoylphosphatidylethanolamine (DOPE). Alternatively, 1,2-Dierucoyl-sn-glycero-3-phosphoethanolamine (DEPE) can be used as a non-cationic or helper lipid.

[0214] In some embodiments, a non-cationic lipid is a neutral lipid, *i.e.*, a lipid that does not carry a net charge in the conditions under which the composition is formulated and/or administered.

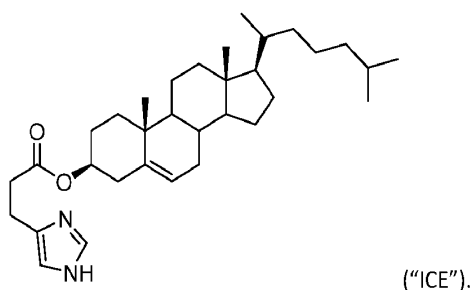
[0215] In some embodiments, a non-cationic lipid may be present in a molar ratio (mol%) of about 5% to about 90%, about 5% to about 70%, about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 10 % to about 70%, about 10% to about 50%, or about 10% to about 40% of the total lipids present in a composition. In some embodiments, total non-cationic lipids may be present in a molar ratio (mol%) of about 5% to about 90%, about 5% to about 70%, about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 10 % to about 70%, about 10% to about 50%, or about 10% to about 40% of the total lipids present in a composition. In some embodiments, the percentage of non-cationic lipid in a liposome may be greater than about 5 mol%, greater than about 10 mol%, greater than about 20 mol%, greater than about 30 mol%, or greater than about 40 mol%. In some embodiments, the percentage total non-cationic lipids in a liposome may be greater than about 5 mol%, greater than about 10 mol%, greater than about 20 mol%, greater than about 30 mol%, or greater than about 40 mol%. In some embodiments, the percentage of non-cationic lipid in a liposome is no more than about 5 mol%, no more than about 10 mol%, no more than about 20 mol%, no more than about 30 mol%, or no more than about 40 mol%. In some embodiments, the percentage total non-cationic lipids in a liposome may be no more than about 5 mol%, no more than about 10 mol%, no more than about 20 mol%, no more than about 30 mol%, or no more than about 40 mol%.

[0216] In some embodiments, a non-cationic lipid may be present in a weight ratio (wt%) of about 5% to about 90%, about 5% to about 70%, about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 10 % to about 70%, about 10% to about 50%, or about 10% to about 40% of the total lipids present in a composition. In some embodiments, total non-cationic lipids may be present in a weight ratio (wt%) of about 5% to about 90%, about 5% to about 70%, about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 10 % to about 70%, about 10% to about 50%, or about 10% to about 40% of the total lipids present in a composition. In some embodiments, the percentage of non-cationic lipid in a liposome may be greater than about 5 wt%, greater than about 10 wt%, greater than about 20 wt%, greater than about 30 wt%, or greater than about 40 wt%. In some embodiments, the percentage total non-cationic lipids in a liposome may be greater than about 5 wt%, greater than about 10 wt%, greater than about 20 wt%, greater than about 30 wt%, or greater than about 40 wt%. In some embodiments, the percentage of non-cationic lipid in a liposome is no more than about 5 wt%, no more than about 10 wt%, no more than about 20 wt%, no more than about 30 wt%, or no more than about 40 wt%. In some embodiments, the percentage total non-cationic lipids in a liposome may be no more than

about 5 wt%, no more than about 10 wt%, no more than about 20 wt%, no more than about 30 wt%, or no more than about 40 wt%.

Cholesterol-based Lipids

[0217] In some embodiments, a composition (*e.g.*, a liposomal composition) comprises one or more cholesterol-based lipids. For example, a suitable cholesterol-based lipid for practicing the invention is cholesterol. Other suitable cholesterol-based lipids include, for example, DC-Chol (N,N-dimethyl-N-ethylcarboxamidcholesterol), 1,4-bis(3-N-oleylamino-propyl)piperazine (Gao, *et al.* Biochem. Biophys. Res. Comm. 179, 280 (1991); Wolf *et al.* BioTechniques 23, 139 (1997); U.S. Pat. No. 5,744,335), or imidazole cholesterol ester (ICE), which has the following structure,



[0218] In some embodiments, a cholesterol-based lipid may be present in a molar ratio (mol%) of about 1% to about 30%, or about 5% to about 20% of the total lipids present in a liposome. In some embodiments, the percentage of cholesterol-based lipid in the lipid nanoparticle may be greater than about 5 mol%, greater than about 10 mol%, greater than about 20 mol%, greater than about 30 mol%, or greater than about 40 mol%. In some embodiments, the percentage of cholesterol-based lipid in the lipid nanoparticle may be no more than about 5 mol%, no more than about 10 mol%, no more than about 20 mol%, no more than about 30 mol%, or no more than about 40 mol%.

[0219] In some embodiments, a cholesterol-based lipid may be present in a weight ratio (wt%) of about 1% to about 30%, or about 5% to about 20% of the total lipids present in a liposome. In some embodiments, the percentage of cholesterol-based lipid in the lipid nanoparticle may be greater than about 5 wt%, greater than about 10 wt%, greater than about 20 wt%, greater than about 30 wt%, or greater than about 40 wt%. In some embodiments, the percentage of cholesterol-based lipid in the lipid nanoparticle may be no more than about 5 wt%, no more than about 10 wt%, no more than about 20 wt%, no more than about 30 wt%, or no more than about 40 wt%.

PEGylated Lipids

[0220] In some embodiments, a composition (*e.g.*, a liposomal composition) comprises one or more further PEGylated lipids. A suitable PEG-modified or PEGylated lipid for practicing the invention is 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2K).

[0221] For example, the use of polyethylene glycol (PEG)-modified phospholipids and derivatized lipids such as derivatized ceramides (PEG-CER), including N-octanoyl-sphingosine-1-[succinyl(methoxy polyethylene glycol)-2000] (C8 PEG-2000 ceramide) is also contemplated by the present invention in combination with one or more of compounds of the invention as described herein and, in some embodiments, other lipids together which comprise the liposome. In some embodiments, particularly useful exchangeable lipids are PEG-ceramides having shorter acyl chains (*e.g.*, C₁₄ or C₁₈).

[0222] Contemplated further PEG-modified lipids (also referred to herein as a PEGylated lipid, which term is interchangeable with PEG-modified lipid) include, but are not limited to, a polyethylene glycol chain of up to 5 kDa in length covalently attached to a lipid with alkyl chain(s) of C₆-C₂₀ length. In some embodiments, a PEG-modified or PEGylated lipid is PEGylated cholesterol or PEG-2K. The addition of such components may prevent complex aggregation and may also provide a means for increasing circulation lifetime and increasing the delivery of the lipid-nucleic acid composition to the target cell, (Klibanov *et al.* (1990) FEBS Letters, 268 (1): 235-237), or they may be selected to rapidly exchange out of the formulation in vivo (see U.S. Pat. No. 5,885,613).

[0223] PEG-modified phospholipid and derivatized lipids of the present invention may be present in a molar ratio (mol%) from about 0% to about 10%, about 0.5% to about 10%, about 1% to about 10%, about 2% to about 10%, or about 3% to about 5% of the total lipid present in the composition (*e.g.*, a liposomal composition).

Pharmaceutical Formulations and Therapeutic Uses

[0224] Compounds of the invention as described herein may be used in the preparation of compositions (*e.g.*, to construct liposomal compositions) that facilitate or enhance the delivery and release of encapsulated materials (*e.g.*, one or more therapeutic polynucleotides) to one or more target cells (*e.g.*, by permeating or fusing with the lipid membranes of such target cells).

[0225] For example, when a liposomal composition (*e.g.*, a lipid nanoparticle) comprises or is otherwise enriched with one or more of the compounds disclosed herein, the phase transition in the lipid bilayer of the one or more target cells may facilitate the delivery of the

encapsulated materials (*e.g.*, one or more therapeutic polynucleotides encapsulated in a lipid nanoparticle) into the one or more target cells.

[0226] Similarly, in certain embodiments compounds of the invention as described herein may be used to prepare liposomal vehicles that are characterized by their reduced toxicity *in vivo*. In certain embodiments, the reduced toxicity is a function of the high transfection efficiencies associated with the compositions disclosed herein, such that a reduced quantity of such composition may administered to the subject to achieve a desired therapeutic response or outcome.

[0227] Thus, pharmaceutical formulations comprising a compound described and nucleic acids provided by the present invention may be used for various therapeutic purposes. To facilitate delivery of nucleic acids *in vivo*, a compound described herein and nucleic acids can be formulated in combination with one or more additional pharmaceutical carriers, targeting ligands or stabilizing reagents. In some embodiments, a compound described herein can be formulated via pre-mixed lipid solution. In other embodiments, a composition comprising a compound described herein can be formulated using post-insertion techniques into the lipid membrane of the nanoparticles. Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition.

[0228] Suitable routes of administration include, for example, oral, rectal, vaginal, transmucosal, pulmonary including intratracheal or inhaled, or intestinal administration; parenteral delivery, including intradermal, transdermal (topical), intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, or intranasal. In particular embodiments, the intramuscular administration is to a muscle selected from the group consisting of skeletal muscle, smooth muscle and cardiac muscle. In some embodiments the administration results in delivery of the nucleic acids to a muscle cell. In some embodiments the administration results in delivery of the nucleic acids to a hepatocyte (*i.e.*, liver cell).

[0229] A common route for administering a liposomal composition of the invention may be intravenous delivery, in particular when treating metabolic disorders, especially those affecting the liver (*e.g.*, ornithine transcarbamylase (OTC) deficiency). Alternatively, depending on the disease or disorder to be treated, the liposomal composition may be administered via pulmonary delivery (*e.g.*, for the treatment of cystic fibrosis). For vaccination, a liposomal composition of the invention is typically administered

intramuscularly. Diseases or disorders affecting the eye may be treated by administering a liposomal composition of the invention intravitreally.

[0230] Alternatively or additionally, pharmaceutical formulations of the invention may be administered in a local rather than systemic manner, for example, via injection of the pharmaceutical formulation directly into a targeted tissue, preferably in a sustained release formulation. Local delivery can be affected in various ways, depending on the tissue to be targeted. Exemplary tissues in which delivered mRNA may be delivered and/or expressed include, but are not limited to the liver, kidney, heart, spleen, serum, brain, skeletal muscle, lymph nodes, skin, and/or cerebrospinal fluid. In embodiments, the tissue to be targeted is the liver. For example, aerosols containing compositions of the present invention can be inhaled (for nasal, tracheal, or bronchial delivery); compositions of the present invention can be injected into the site of injury, disease manifestation, or pain, for example; compositions can be provided in lozenges for oral, tracheal, or esophageal application; can be supplied in liquid, tablet or capsule form for administration to the stomach or intestines, can be supplied in suppository form for rectal or vaginal application; or can even be delivered to the eye by use of creams, drops, or even injection.

[0231] Compositions described herein can comprise mRNA encoding peptides including those described herein (*e.g.*, a polypeptide such as a protein).

[0232] In embodiments, a mRNA encodes a polypeptide.

[0233] In embodiments, a mRNA encodes a protein.

[0234] Exemplary peptides encoded by mRNA (*e.g.*, exemplary proteins encoded by mRNA) are described herein.

[0235] The present invention provides methods for delivering a composition having full-length mRNA molecules encoding a peptide or protein of interest for use in the treatment of a subject, *e.g.*, a human subject or a cell of a human subject or a cell that is treated and delivered to a human subject.

[0236] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes a peptide or protein for use in the delivery of or treatment with a vaccine for a subject or a cell of a subject. For example, in certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen from an infectious agent, such as a virus.

[0237] In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen associated with

a cancer of a subject or identified from a cancer cell of a subject. In certain embodiments the present invention provides a method for producing a therapeutic composition having full-length mRNA that encodes for an antigen determined from a subject's own cancer cell, *i.e.*, to provide a personalized cancer vaccine.

Delivery Methods

[0238] The route of delivery used in the methods of the invention allows for non-invasive, self-administration of the compounds of the invention. In some embodiments, the methods involve intratracheal or pulmonary administration by aerosolization, nebulization, or instillation of a compositions comprising mRNA encoding a therapeutic protein in a suitable transfection or lipid carrier vehicles as described above. In some embodiments, the protein is encapsulated with a liposome. In some embodiments, the liposome comprises a lipid, which is a compound of the invention. As used herein below, administration of a compound of the invention includes administration of a composition comprising a compound of the invention.

[0239] Although the local cells and tissues of the lung represent a potential target capable of functioning as a biological depot or reservoir for production and secretion of the protein encoded by the mRNA, applicants have discovered that administration of the compounds of the invention to the lung via aerosolization, nebulization, or instillation results in the distribution of even non-secreted proteins outside the lung cells. Without wishing to be bound by any particular theory, it is contemplated that nanoparticle compositions of the invention pass, through the lung airway-blood barrier, resulting in translation of the intact nanoparticle to non-lung cells and tissues, such as, *e.g.*, the heart, the liver, the spleen, where it results in the production of the encoded protein in these non-lung tissues. Thus, the utility of the compounds of the invention and methods of the invention extend beyond production of therapeutic protein in lung cells and tissues of the lung and can be used to delivery to non-lung target cells and/or tissues. They are useful in the management and treatment of a large number of diseases, and in particular peripheral diseases which result from both secreted and non-secreted protein and/or enzyme deficiencies (*e.g.*, one or more lysosomal storage disorders). In certain embodiments, the compounds of the invention, used in the methods of the invention result in the distribution of the mRNA encapsulated nanoparticles and production of the encoded protein in the liver, spleen, heart, and/or other non-lung cells. For example, administration of the compounds of the invention, by aerosolization, nebulization, or instillation to the lung will result in the composition itself and its protein product (*e.g.*, functional beta galactosidase protein) will be detectable in both the local cells and tissues of

the lung, as well as in peripheral target cells, tissues and organs as a result of translocation of the mRNA and delivery vehicle to non-lung cells.

[0240] In certain embodiments, the compounds of the invention may be employed in the methods of the invention to specifically target peripheral cells or tissues. Following the pulmonary delivery, it is contemplated the compounds of the invention cross the lung airway-blood barrier and distribute into cells other than the local lung cells. Accordingly, the compounds disclosed herein may be administered to a subject by way of the pulmonary route of administration, using a variety of approach known by those skilled in the art (*e.g.*, by inhalation), and distribute to both the local target cells and tissues of the lung, as well as in peripheral non-lung cells and tissues (*e.g.*, cells of the liver, spleen, kidneys, heart, skeletal muscle, lymph nodes, brain, cerebrospinal fluid, and plasma). As a result, both the local cells of the lung and the peripheral non-lung cells can serve as biological reservoirs or depots capable of producing and/or secreting a translation product encoded by one or more polynucleotides. Accordingly, the present invention is not limited to the treatment of lung diseases or conditions, but rather can be used as a non-invasive means of facilitating the delivery of polynucleotides, or the production of enzymes and proteins encoded thereby, in peripheral organs, tissues and cells (*e.g.*, hepatocytes) which would otherwise be achieved only by systemic administration. Exemplary peripheral non-lung cells include, but are not limited to, hepatocytes, epithelial cells, hematopoietic cells, epithelial cells, endothelial cells, bone cells, stem cells, mesenchymal cells, neural cells, cardiac cells, adipocytes, vascular smooth muscle cells, cardiomyocytes, skeletal muscle cells, beta cells, pituitary cells, synovial lining cells, ovarian cells, testicular cells, fibroblasts, B cells, T cells, reticulocytes, leukocytes, granulocytes and tumor cells.

[0241] Following administration of the composition to the subject, the protein product encoded by the mRNA (*e.g.*, a functional protein or enzyme) is detectable in the peripheral target tissues for at least about one to seven days or longer following administration of the compound to the subject. The amount of protein product necessary to achieve a therapeutic effect will vary depending on the condition being treated, the protein encoded, and the condition of the patient. For example, the protein product may be detectable in the peripheral target tissues at a concentration (*e.g.*, a therapeutic concentration) of at least 0.025-1.5 µg/ml (*e.g.*, at least 0.050 µg/ml, at least 0.075 µg/ml, at least 0.1 µg/ml, at least 0.2 µg/ml, at least 0.3 µg/ml, at least 0.4 µg/ml, at least 0.5 µg/ml, at least 0.6 µg/ml, at least 0.7 µg/ml, at least 0.8 µg/ml, at least 0.9 µg/ml, at least 1.0 µg/ml, at least 1.1 µg/ml, at least 1.2 µg/ml, at least 1.3 µg/ml, at least 1.4 µg/ml, or at least 1.5 µg/ml), for at least about 1, 2,

3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45 days or longer following administration of the compound to the subject.

[0242] It has been demonstrated that nucleic acids can be delivered to the lungs by intratracheal administration of a liquid suspension of the compound and inhalation of an aerosol mist produced by a liquid nebulizer or the use of a dry powder apparatus such as that described in U.S. patent 5,780,014, incorporated herein by reference.

[0243] In certain embodiments, the compounds of the invention may be formulated such that they may be aerosolized or otherwise delivered as a particulate liquid or solid prior to or upon administration to the subject. Such compounds may be administered with the assistance of one or more suitable devices for administering such solid or liquid particulate compositions (such as, *e.g.*, an aerosolized aqueous solution or suspension) to generate particles that are easily respirable or inhalable by the subject. In some embodiments, such devices (*e.g.*, a metered dose inhaler, jet-nebulizer, ultrasonic nebulizer, dry-powder-inhalers, propellant-based inhaler or an insufflator) facilitate the administration of a predetermined mass, volume or dose of the compositions (*e.g.*, about 0.5 mg/kg of mRNA per dose) to the subject. For example, in certain embodiments, the compounds of the invention are administered to a subject using a metered dose inhaler containing a suspension or solution comprising the compound and a suitable propellant. In certain embodiments, the compounds of the invention may be formulated as a particulate powder (*e.g.*, respirable dry particles) intended for inhalation. In certain embodiments, compositions of the invention formulated as respirable particles are appropriately sized such that they may be respirable by the subject or delivered using a suitable device (*e.g.*, a mean D50 or D90 particle size less than about 500 μ m, 400 μ m, 300 μ m, 250 μ m, 200 μ m, 150 μ m, 100 μ m, 75 μ m, 50 μ m, 25 μ m, 20 μ m, 15 μ m, 12.5 μ m, 10 μ m, 5 μ m, 2.5 μ m or smaller). In yet other embodiments, the compounds of the invention are formulated to include one or more pulmonary surfactants (*e.g.*, lamellar bodies). In some embodiments, the compounds of the invention are administered to a subject such that a concentration of at least 0.05 mg/kg, at least 0.1 mg/kg, at least 0.5 mg/kg, at least 1.0 mg/kg, at least 2.0 mg/kg, at least 3.0 mg/kg, at least 4.0 mg/kg, at least 5.0 mg/kg, at least 6.0 mg/kg, at least 7.0 mg/kg, at least 8.0 mg/kg, at least 9.0 mg/kg, at least 10 mg/kg, at least 15 mg/kg, at least 20 mg/kg, at least 25 mg/kg, at least 30 mg/kg, at least 35 mg/kg, at least 40 mg/kg, at least 45 mg/kg, at least 50 mg/kg, at least 55 mg/kg, at least 60 mg/kg, at least 65 mg/kg, at least 70 mg/kg, at least 75 mg/kg, at least 80 mg/kg, at least 85 mg/kg, at least 90 mg/kg, at least 95 mg/kg, or at least 100 mg/kg body weight is administered in a single dose. In some embodiments, the compounds of the

invention are administered to a subject such that a total amount of at least 0.1 mg, at least 0.5 mg, at least 1.0 mg, at least 2.0 mg, at least 3.0 mg, at least 4.0 mg, at least 5.0 mg, at least 6.0 mg, at least 7.0 mg, at least 8.0 mg, at least 9.0 mg, at least 10 mg, at least 15 mg, at least 20 mg, at least 25 mg, at least 30 mg, at least 35 mg, at least 40 mg, at least 45 mg, at least 50 mg, at least 55 mg, at least 60 mg, at least 65 mg, at least 70 mg, at least 75 mg, at least 80 mg, at least 85 mg, at least 90 mg, at least 95 mg or at least 100 mg mRNA is administered in one or more doses.

Synthesis of Compounds of the invention

[0244] The cationic lipid MC3 is the current gold standard for *in vivo* delivery of *e.g.* siRNA (see WO2010/144740). However, the synthesis of this lipid involves a six-step process and requires handling of a Grignard reagent. In contrast, the present invention provides cationic lipids that can be prepared from readily available starting reagents, such as “Good’s” buffers (see Table 1 below). These starting reagents can be coupled to cationic headgroups and lipid tails using coupling reactions, such as sulfonylation, acetylation and alkylation (see for example, Table 2 below).

Table 1: Examples of “Good” buffers

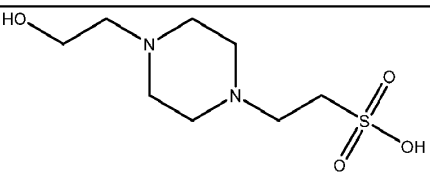
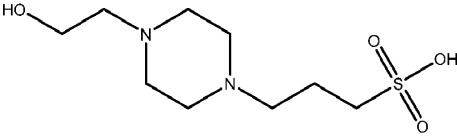
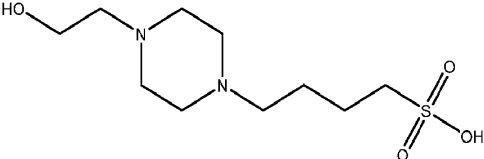
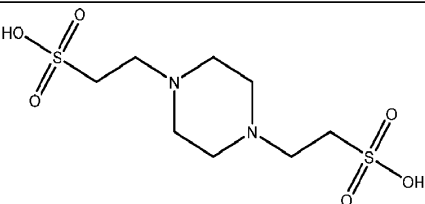
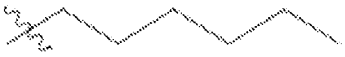
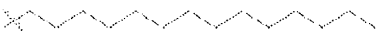
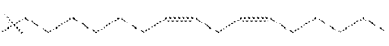
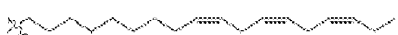
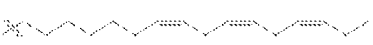
“Good” Buffer name	Structure
HEPES	
HEPPS	
HEPBS	
PIPES	

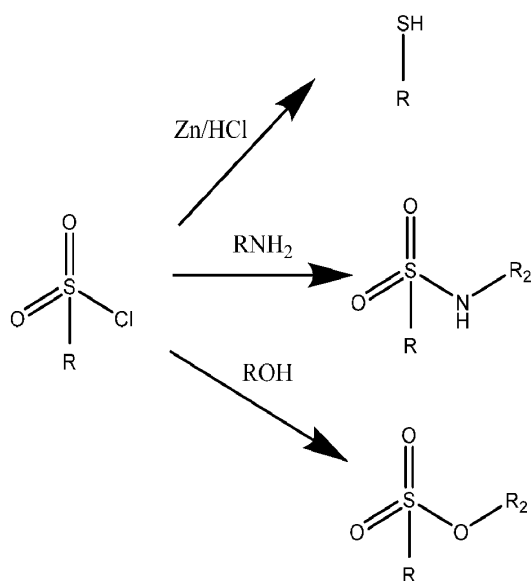
Table 2: Examples of lipid chains that are suitable for the present invention at the position R^{1A} and R^{1B}:

Carbon Chain	Full chain	R ^{1A} / R ^{1B} in compounds of the invention
Caprylic (C8)		
Capric (C10)		
Lauric (C12)		
Myristic (C14)		
Palmitic (C16)		
Stearic (C18)		
Oleic (C18:1) (9Z)		
Linoleic (C18:2) (9Z, 12Z)		
Linolenic (C18:3) (9Z, 12Z, 15Z)		

Pentan-3-yl hexanoate		

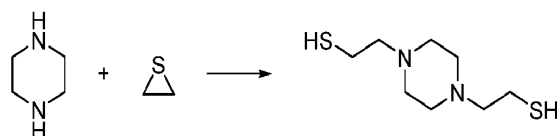
[0245] In embodiments, a cationic lipid described herein can be prepared by conjugating a “Good’s” Buffer with a lipid, for example the carboxylic acid of a lipid, under suitable conditions. Exemplary “Good’s” Buffers are described in Table 1, and exemplary lipid chains are described in Table 2. Accordingly, suitable cationic lipids include those resulting from any combination of the precursors described in Table 1 and Table 2.

[0246] In some embodiments, the sulfonic acid groups of compounds, such as “Good’s” buffers can be derivatized by forming a sulfonyl chloride using reagents, such as oxalyl chloride. The resulting sulfonyl chloride can undergo a number of reactions, including but not limited to reduction with Zn/HCl to form the corresponding thiol and coupling to nucleophiles, such as amines and alcohols to form the corresponding sulfonamides and sulfonates (see for example, **Scheme A** below):



[0247] Using the chemistry outlined in scheme 1 it is possible to derivatise the sulfonic acid starting reagents with a range of suitable cationic lipid head groups and lipid chains.

[0248] Furthermore, compounds such as “Good’s” buffers can be readily synthesized. For example, through nucleophilic ring opening of an episulfide with a piperazine (see for example, **Scheme B** below).



[0249] The compounds of the invention as described herein can be prepared according to methods known in the art, including the exemplary syntheses of the Examples provided herein.

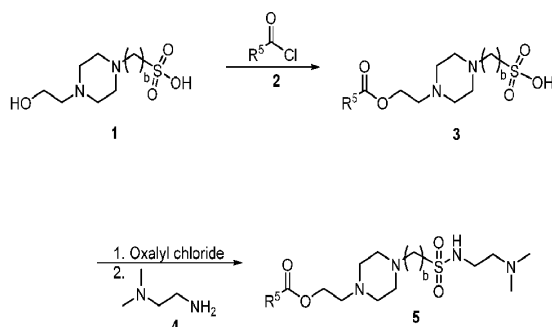
EXAMPLES

[0250] While certain compounds, compositions and methods of the present invention have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds of the invention and are not intended to limit the same.

Example 1: Generic synthesis scheme for HEPES/HEPPS/HEPBS-based cationic lipids

[0251] HEPES/HEPPS/HEPBS-based cationic lipids described herein may be prepared according to **Scheme 1**:

Scheme 1

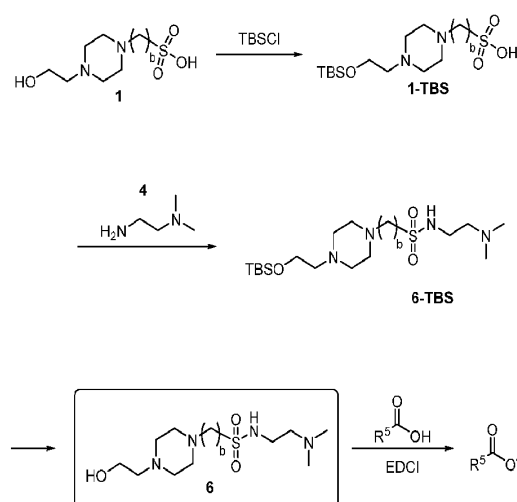


[0252] For example, a buffer compound such as compound 1 can first be reacted with an acyl chloride such as compound 2. Further treatment with a chlorinating agent such as oxalyl chloride can provide an electrophile, which can subsequently be reacted with a nucleophile such as compound 4 to afford the lipid 5.

Example 2: Alternative generic synthesis scheme for HEPES/HEPPS/HEPBS-based cationic lipids

[0253] Alternatively, HEPES/HEPPS/HEPBS-based cationic lipids described herein may be prepared according to Scheme 2:

Scheme 2

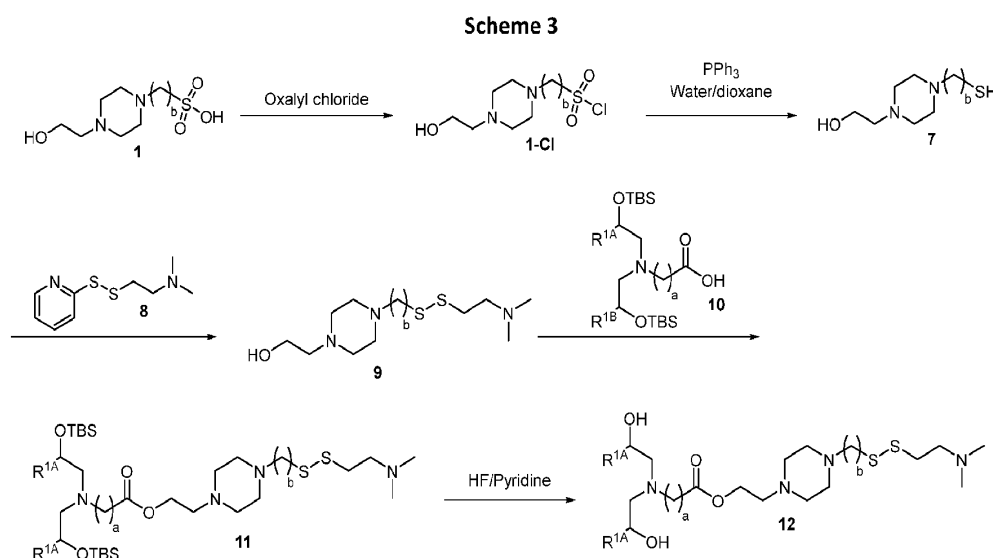


[0254] For example, protection of the hydroxyl group of a buffer compound such as compound 1 using protecting groups and conditions known in the art (such as TBSCl) can provide the corresponding hydroxyl protected buffer compound 1-TBS. The hydroxyl protected compound 1-TBS can be reacted with a nucleophile such as compound 4 to afford compound 6-TBS. Deprotection of the hydroxyl functionality can then provide compound 6.

Further reaction of compound 6 with a carboxylic acid, which can be activated with a reagent such as EDCI, affords lipid 5.

Example 3: Synthesis scheme for disulfide-containing HEPES/HEPPS/HEPBS-based cationic lipids

[0255] Disulfide-containing HEPES/HEPPS/HEPBS-based cationic lipids described herein may be prepared according to **Scheme 3**:

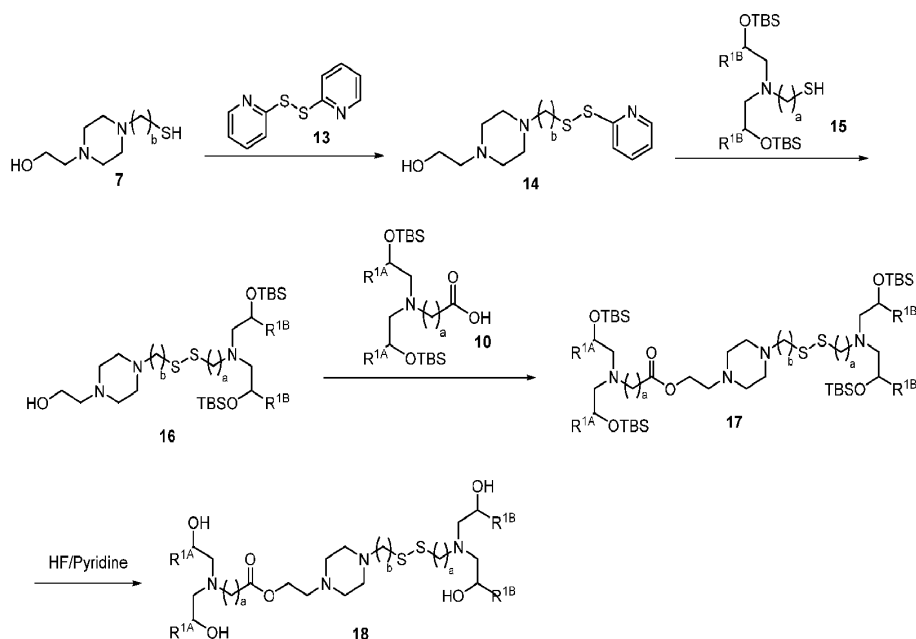


[0256] For example, treatment of buffer compound 1 with a chlorinating agent such as oxalyl chloride can provide the compound 1-Cl. Reduction of compound 1-Cl using, for example PPh_3 , water and dioxane can then afford the corresponding thiol 7. Further reaction of thiol 7 with compound 8 can then provide the nucleophilic compound 9. Reaction of 9 with a hydroxyl protected electrophile such as 10 can then provide compound 11, the subsequent deprotection of which (using, for example, HF and Pyridine) can provide the lipid 12.

Example 4: Further synthesis scheme for disulfide-containing HEPES/HEPPS/HEPBS-based cationic lipids

[0257] Disulfide-containing HEPES/HEPPS/HEPBS-based cationic lipids described herein may be prepared according to **Scheme 4**:

Scheme 4

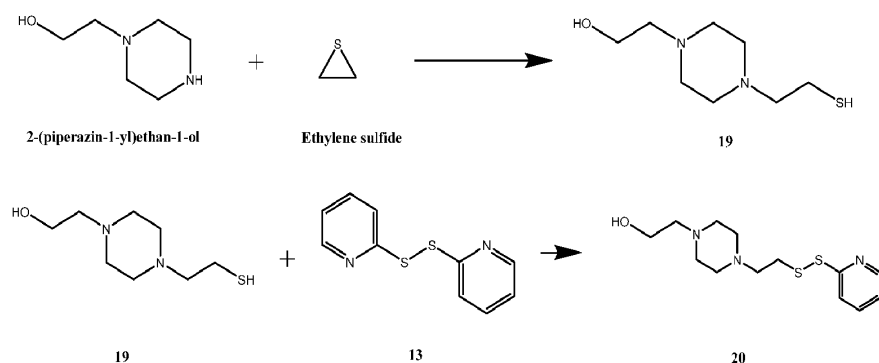


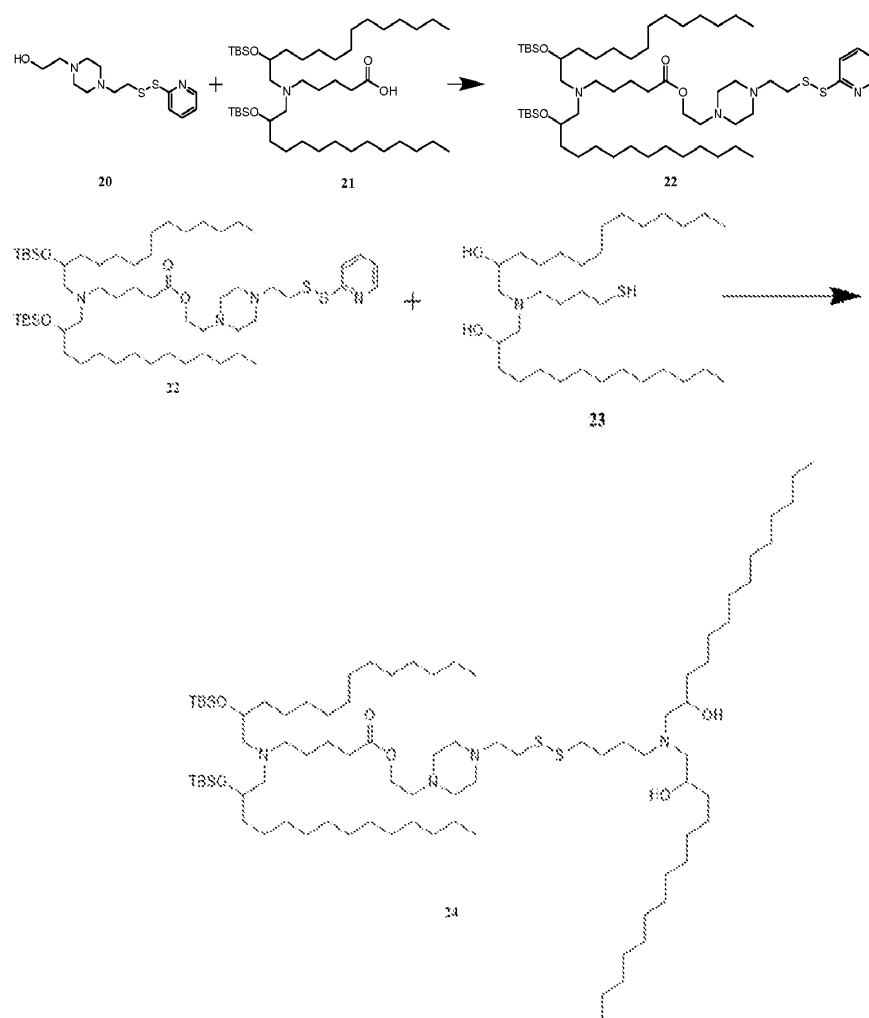
[0258] For example, reaction of thiol 7 with compound 13 can then provide the compound 14. Reaction of 14 with a hydroxyl protected compound such as 15 can then provide compound 16. Reacting compound 16 with a hydroxyl protected electrophile such as 10 can then provide compound 17, the subsequent deprotection of which (using, for example, HF and Pyridine) can provide the lipid 18.

Example 5: Further synthesis scheme for disulfide-containing HEPES-based cationic lipids

[0259] HEPES-based cationic lipids described herein may be prepared according to Scheme 5:

Scheme 5

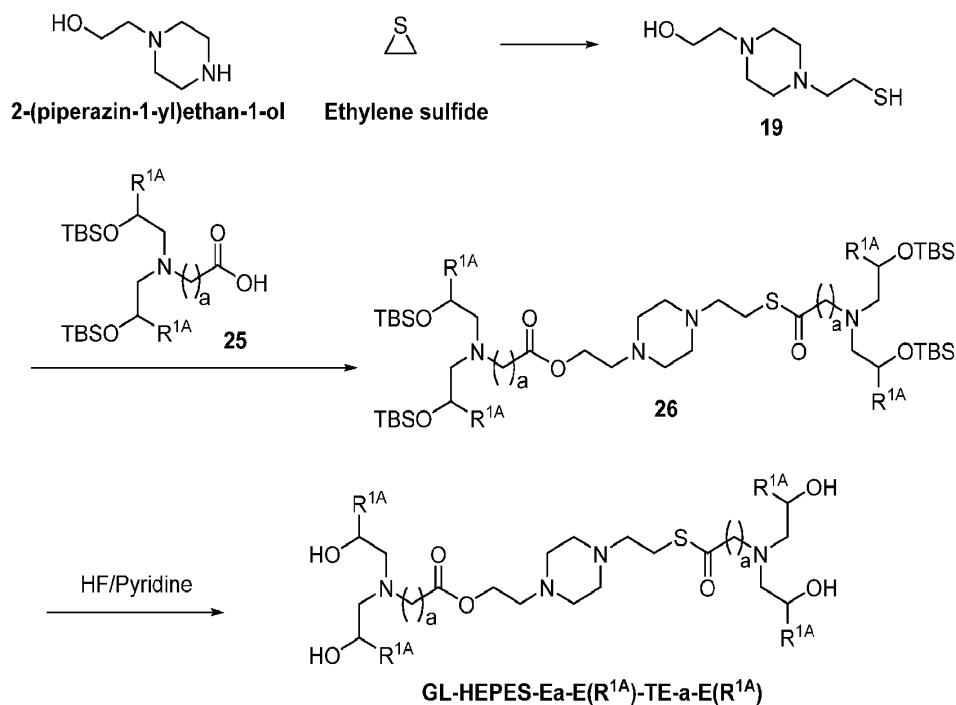




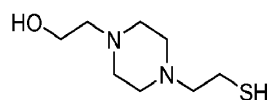
[0260] For example, reaction of 2-(piperazin-1-yl)ethan-1-ol with ethylene sulfide forms thiol 19. Reaction of thiol 19 with compound 13 can then provide compound 20. Reaction of compound 20 with a hydroxyl protected compound such as 21 can then provide compound 22. Reacting compound 22 with a thiol such as 23 can then provide compound 24. Compound 24 can subsequently be deprotected using, for example, HF and Pyridine.

Example 6: Synthesis schemes for HEPES-based ester/thioester cationic lipids

[0261] HEPES-based cationic lipids described herein may be prepared according to **Scheme 6**:

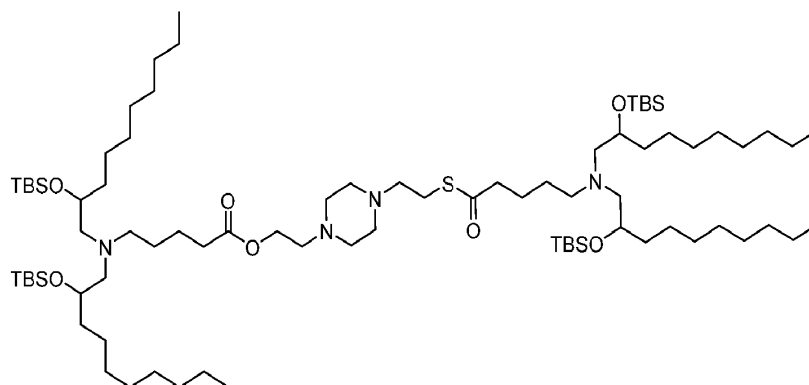
Scheme 6 - GL-HEPES-E/TE-a-E(R^{1A}) LipidsRepresentative Procedure for GL-HEPES-E/TE-a-E(R^{1A}) Lipids

Synthesis of 2-(4-(2-Mercaptoethyl)piperazin-1-yl)ethan-1-ol (19)



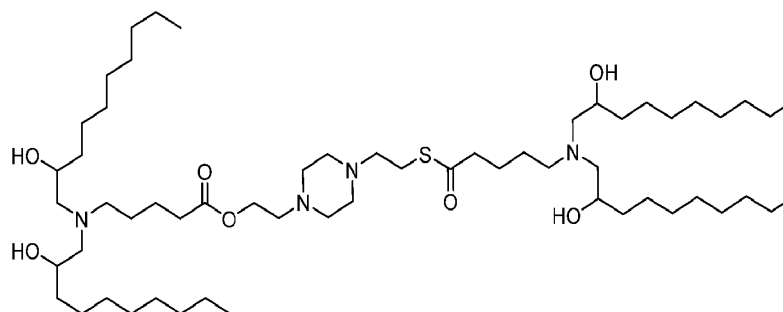
[0262] As set out in Scheme 6: To a solution of 2-(piperazin-1-yl)ethan-1-ol 1 (1.0 g, 7.68 mmol) in 100 mL anhydrous toluene at room temperature was added ethylene sulfide (508 mg, 8.45 mmol) in a pressure tube, and the mixture was heated at 50 °C for 48 h. The reaction mixture was cooled to room temperature, and solvent was evaporated under reduced pressure to 2-(4-(2-mercaptoethyl)piperazin-1-yl)ethan-1-ol as yellow oil which was used for the next step without further purification.

Synthesis of 2-(4-(7-(2-((tert-Butyldimethylsilyl)oxy)decyl)-2,2,3,3-tetramethyl-5-octyl-12-oxo-4-oxa-13-thia-7-aza-3-silapentadecan-15-yl)piperazin-1-yl)ethyl 5-(bis(2-((tert-butylidimethylsilyl)oxy)decyl)amino)pentanoate (26, when a=4)



[0263] As set out in Scheme 6: To a solution of 5-(bis(2-((tert-butylidimethylsilyl)oxy)decyl)amino)pentanoic acid 25 (830 mg, 1.26 mmol) in 10 mL anhydrous dichloromethane at 0 °C was added EDCI (0.43 g, 2.1 mmol) and DMAP (64 mg, 0.5 mmol). The reaction mixture was stirred for 15 min, and then 2-(4-(2-mercaptoethyl)piperazin-1-yl)ethan-1-ol 19 (100 mg, 0.52 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 16 h. MS and TLC analysis indicated completion of the reaction. After solvent was removed under reduced pressure, the crude was purified by flash column chromatography (40 g SiO₂: 0 to 80% ethyl acetate in hexanes gradient) to obtain 2-(4-(7-(2-((tert-butylidimethylsilyl)oxy)decyl)-2,2,3,3-tetramethyl-5-octyl-12-oxo-4-oxa-13-thia-7-aza-3-silapentadecan-15-yl)piperazin-1-yl)ethyl 5-(bis(2-((tert-butylidimethylsilyl)oxy)decyl)amino)pentanoate as white solid (330 mg, 42%).

Synthesis of 2-(4-(2-((5-(Bis(2-hydroxydodecyl)amino)pentanoyl)thio)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E/TE-4-E10)



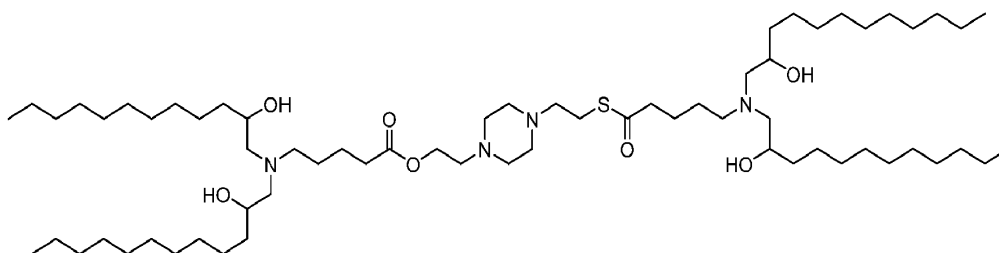
[0264] As set out in Scheme 6: To a solution of 2-(4-(7-(2-((tert-butylidimethylsilyl)oxy)decyl)-2,2,3,3-tetramethyl-5-octyl-12-oxo-4-oxa-13-thia-7-aza-3-silapentadecan-15-yl)piperazin-1-yl)ethyl 5-(bis(2-((tert-butylidimethylsilyl)oxy)decyl)amino)pentanoate 26 (321 mg, 0.22 mmol) in 10.0 mL anhydrous tetrahydrofuran at 0 °C was added hydrogen fluoride pyridine (70% HF, 3.0 mL). The reaction mixture was warmed to room temperature and stirred for 16 h. MS and TLC analysis indicated complete reaction. The reaction was quenched by slow addition of saturated sodium bicarbonate, and then the resulting mixture was extracted with dichloromethane. Combined organic layer was washed with brine and dried over anhydrous sodium sulfate. After concentration, the crude was purified by flash column chromatography (40 g SiO₂: 20 to 80% ethyl acetate in hexane gradient) to obtain 2-(4-(2-((5-(bis(2-hydroxydecyl)amino)pentanoyl)thio)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydecyl)amino)pentanoate as colorless oil (135 mg, 65%).

¹H NMR (300 MHz, Chloroform-d) δ 4.18 (t, 2H), 3.60 (m, 4H), 2.99 (t, 2H), 2.86 (bs, 2H), 2.63-2.27 (m, 26H), 1.74-1.17 (m, 64H), 0.86 (t, 12H).

APCI-MS analysis: Calculated C₅₈H₁₁₆N₄O₇S, [M+H] = 1013.6, Observed = 1013.8.

[0265] All the other HEPES-based ester/thioester lipids were prepared according the representative procedure in similar yields.

[0266] Analytical data for 2-(4-(2-((5-(Bis(2-hydroxydodecyl)amino)pentanoyl)thio)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E/TE-4-E12)



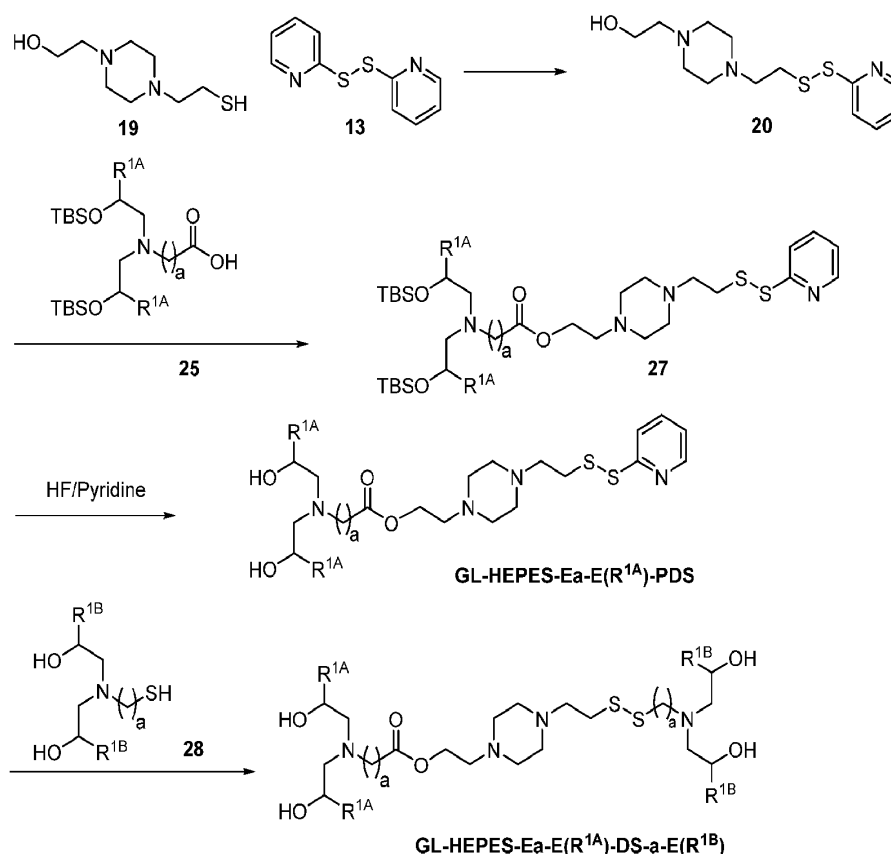
¹H NMR (300 MHz, Chloroform-d) δ 4.18 (t, 2H), 3.59 (m, 4H), 2.99 (t, 2H), 2.87 (bs, 2H), 2.63-2.27 (m, 26H), 1.70-1.15 (m, 80H), 0.86 (t, 12H).

APCI-MS analysis: Calculated C₆₆H₁₃₂N₄O₇S, [M+H] = 1125.8, Observed = 1125.9.

Example 7: Synthesis of HEPES-based ester/disulfide cationic lipids

[0267] HEPES-based cationic lipids described herein may be prepared according to

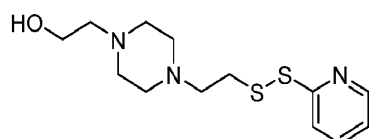
Scheme 7:

Scheme 7 - GL-HEPES-Ea-E(R^{1A})-PDS and GL-HEPES-Ea-E(R^{1A})-DS-a-E(R^{1B}) Lipids

Representative Procedure for GL-HEPES-Ea-E(R^{1A})-PDS and GL-HEPES-Ea-E(R^{1A})-DS-a-E(R^{1B})

Lipids:

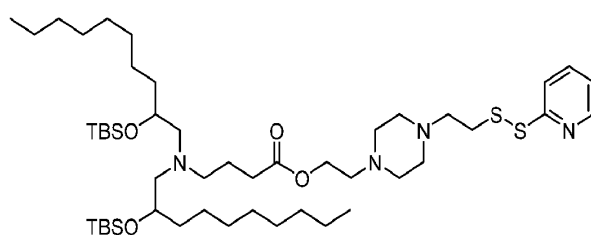
Synthesis of 2-(4-(2-(Pyridin-2-yl)ethoxy)ethyl)piperazine-1-yl)ethan-1-ol (20)



[0268] As set out in Schemes 7: To a solution of 2-(piperazin-1-yl)ethan-1-ol (5.0 g, 38.4 mmol) in 200 mL anhydrous toluene at room temperature was added ethylene sulfide (2.5 mL, 42.3 mmol) in a pressure tube, and the mixture was heated at 50 °C overnight. The reaction mixture was cooled to room temperature, pyridyl disulfide 6 (11.00 g, 49.9 mmol) was added, and the reaction mixture was stirred at room temperature for 3 days. MS and TLC

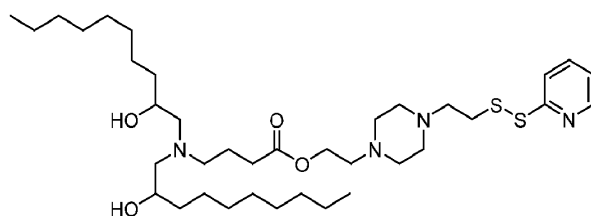
analysis indicated completion of the reaction. The reaction mixture was diluted with dichloromethane, washed with saturated sodium bicarbonate, water and brine. After dried over sodium sulfate, the solvent was removed under reduced pressure, and the crude was purified via flash column chromatography (SiO₂: 0 to 10% methanol in dichloromethane) to obtain 2-(4-(2-(pyridin-2-yl)disulfaneyl)ethyl)piperazin-1-yl)ethan-1-ol as colorless oil (2.86 g, 25%).

[0269] Synthesis of 2-(4-(2-(Pyridin-2-yl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-((tert-butyl)dimethylsilyl)oxy)decyl)amino)butanoate (27 when a=3)



[0270] As set out in Scheme 7: To a solution of 4-(bis(2-((tert-butyl)dimethylsilyl)oxy)decyl)amino)butanoic acid 25 (3.10 g, 4.81 mmol) in 40 mL dichloromethane, was added EDCI (1.54 g, 8.61 mmol) and dimethylaminopyridine (245 mg, 2.0 mmol), and the resulting solution was stirred at room temperature for 40 min. Then 2-(4-(2-(pyridin-2-yl)disulfaneyl)ethyl)piperazin-1-yl)ethan-1-ol (600 mg, 2.00 mmol) was added, and the reaction mixture was stirred overnight. MS and TLC analysis showed complete reaction. The reaction mixture was diluted with dichloromethane, and washed with saturated sodium bicarbonate, water and brine. After dried over sodium sulfate, the solvent was evaporated under vacuum, and the crude was purified via flash column chromatography (SiO₂: 0 to 10% methanol in dichloromethane gradient) to give 2-(4-(2-(pyridin-2-yl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-((tert-butyl)dimethylsilyl)oxy)decyl)amino)butanoate as tan oil (1.72 g, 93%).

[0271] Synthesis of 2-(4-(2-(Pyridin-2-yl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate (GL-HEPES-E3-E10-PDS)



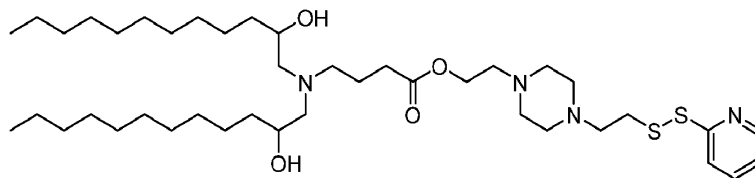
[0272] As set out in Scheme 7: To a solution of 2-(4-(2-(pyridin-2-yl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-(tert-butyl)dimethylsilyloxy)decyl)amino)butanoate 27 (1.72 g, 1.86 mmol) in 9 mL anhydrous tetrahydrofuran at 0 °C was added hydrogen fluoride pyridine (70% HF, 0.24 mL, 9.29 mmol). The reaction mixture was warmed to room temperature and stirred for 16 h. MS and TLC analysis indicated complete reaction. The reaction was quenched by slow addition of saturated sodium bicarbonate, and then the resulting mixture was extracted with dichloromethane. Combined organic layer was washed with brine and dried over anhydrous sodium sulfate. After concentration, 2-(4-(2-(pyridin-2-yl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate was obtained as tan oil (1.24 g, 95%), which was used for the next step without purification.

¹H NMR (300 MHz, Methanol-*d*₄) δ 8.39 (d, 1H), 7.89 (d, 1H), 7.81 (t, 1H), 7.12 (t, 1H), 4.23 (t, 2H), 3.61 (bs, 2H), 2.98 (t, 2H), 2.70-2.38 (m, 22H), 1.76 (m, 2H), 1.45-1.30 (m, 26H), 0.90 (t, 6H).

APCI-MS analysis: Calculated C₃₇H₆₈N₄O₄S₂, [M+H]⁺ = 697.5, Observed = 697.5.

All the other GL-HEPES-Ea-E(R^{1A})-PDS lipids were prepared according the representative procedure in similar yields.

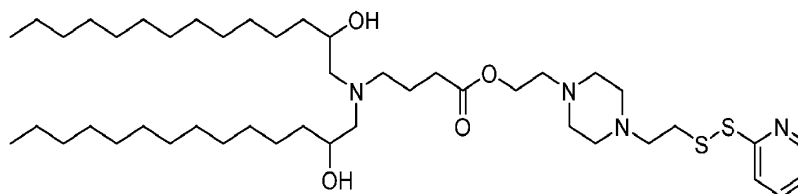
[0273] Analytical data for 2-(4-(2-(Pyridin-2-yl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-PDS)



¹H NMR (300 MHz, Chloroform-*d*) δ 8.45 (d, 1H), 7.74 (d, 1H), 7.62 (t, 1H), 7.07 (t, 1H), 4.19 (t, 2H), 3.67 (bs, 2H), 2.94 (t, 2H), 2.74-2.38 (m, 22H), 1.82 (m, 2H), 1.52-1.18 (m, 26H), 0.87 (t, 6H).

APCI-MS analysis: Calculated C₃₇H₆₈N₄O₄S₂, [M+H]⁺ = 697.5, Observed = 697.5.

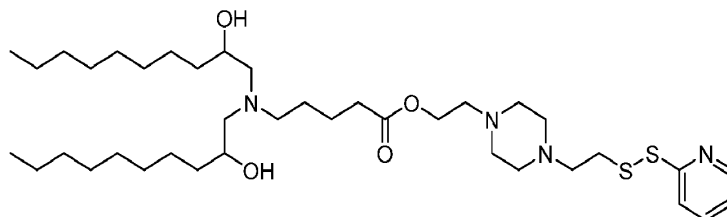
[0274] Analytical data for 2-(4-(2-(Pyridin-2-yl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-PDS)



¹H NMR (300 MHz, Methanol-d₄) δ 8.39 (t, 1H), 7.89 (d, 1H), 7.81 (t, 1H), 7.22 (t, 1H), 4.21 (t, 2H), 3.65 (bs, 2H), 2.98 (t, 2H), 2.70-2.38 (m, 22H), 1.79 (m, 2H), 1.45-1.28 (m, 42H), 0.90 (t, 6H).

APCI-MS analysis: Calculated C₄₅H₈₄N₄O₄S₂, [M+H] = 809.7, Observed = 809.7.

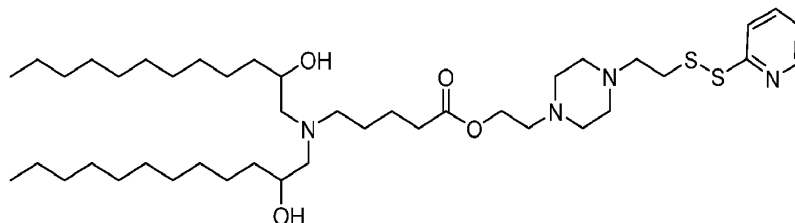
[0275] Analytical data for 2-(4-(2-(Pyridin-2-yldisulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-PDS)



¹H NMR (300 MHz, Methanol-d₄) δ 8.39 (t, 1H), 7.91 (d, 1H), 7.81 (t, 1H), 7.23 (t, 1H), 4.21 (t, 2H), 3.72 (bs, 2H), 2.98 (t, 2H), 2.80-2.38 (m, 20H), 1.62 (m, 2H), 1.45-1.28 (m, 30H), 0.90 (t, 6H).

APCI-MS analysis: Calculated C₃₈H₇₀N₄O₄S₂, [M+H] = 711.5, Observed = 711.5.

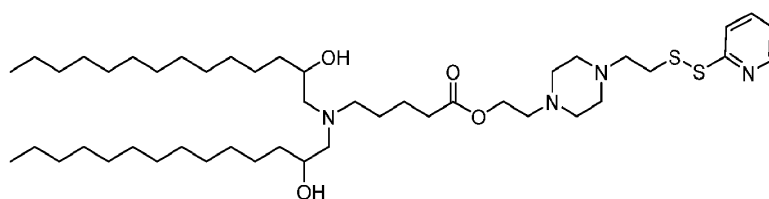
[0276] Analytical data for 2-(4-(2-(Pyridin-2-yldisulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-PDS)



¹H NMR (300 MHz, CD₃OD) δ 8.40 (d, 1H), 7.90 (d, 1H), 7.88 (t, 1H), 7.22 (t, 1H), 4.20 (t, 2H), 3.61 (m, 2H), 2.97 (t, 2H), 2.76-2.34 (m, 18H), 1.66-1.57 (m, 2H), 1.56-1.16 (m, 40H), 0.89 (t, 6H).

APCI-MS analysis: Calculated C₄₂H₇₈N₄O₄S₂, [M+H] = 767.5, Observed = 767.6.

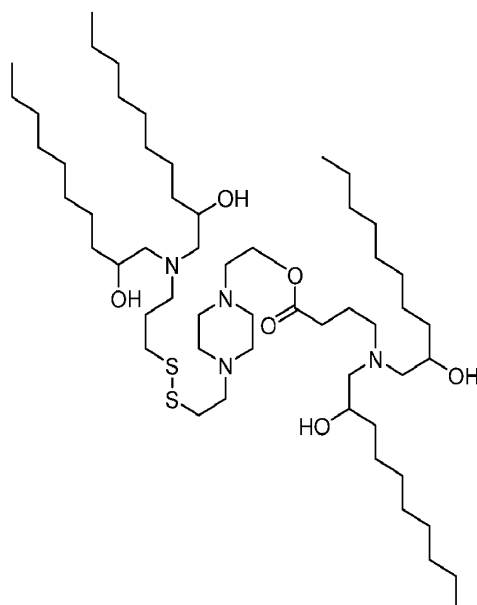
[0277] Analytical data for 2-(4-(2-(Pyridin-2-yldisulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-PDS)



^1H NMR (300 MHz, CD_3OD) δ 8.38 (t, 1H), 7.90 (d, 1H), 7.80 (t, 1H), 7.22 (t, 1H), 4.20 (t, 2H), 3.63 (m, 2H), 2.98 (t, 2H), 2.76-2.34 (m, 18H), 1.68-1.16 (m, 52H), 0.89 (t, 6H).

APCI-MS analysis: Calculated $\text{C}_{46}\text{H}_{86}\text{N}_4\text{O}_4\text{S}_2$, $[\text{M}+\text{H}] = 823.6$, Observed = 823.7.

[0278] Synthesis of 2-(4-(2-((3-(Bis(2-hydroxydecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate (GL-HEPES-E3-E10-DS-3-E10)



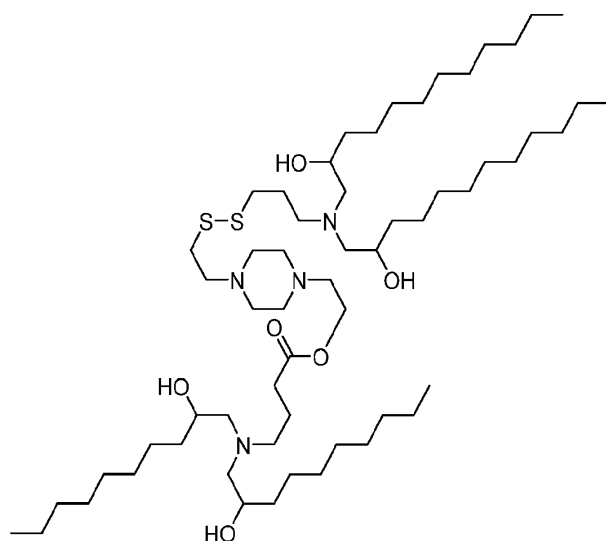
[0279] As set out in Scheme 7: To a solution of 2-(4-(2-(pyridin-2-yl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate GL-HEPES-E3-E10-PDS (150 mg, 0.215 mmol) in 1.5 mL chloroform, was added 1,1'-((4-mercaptopropyl)azanediyl)bis(decane-2-ol) 28 (148 mg, 0.323 mmol), and the reaction mixture was stirred at room temperature for 3 h. MS and TLC analysis indicated complete reaction. The reaction was concentrated to dryness, and the crude was purified with flash column chromatography (SiO_2 : 0 to 10% methanol in dichloromethane) to give 2-(4-(2-((3-(bis(2-hydroxydecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate as colorless oil (137 mg, 61%).

[0280] ^1H NMR (300 MHz, Methanol- d_4) δ 4.26 (t, 2H), 3.64 (bs, 4H), 2.85-2.46 (m, 30H), 1.90-1.79 (m, 4H), 1.46-1.31 (m, 56H), 0.91 (t, 12H).

[0281] APCI-MS analysis: Calculated $\text{C}_{55}\text{H}_{112}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 989.8$, Observed = 989.8.

[0282] All the other GL-HEPES-Ea-E(R)-DS-a-E(R') lipids were prepared according to the representative procedure in similar yields.

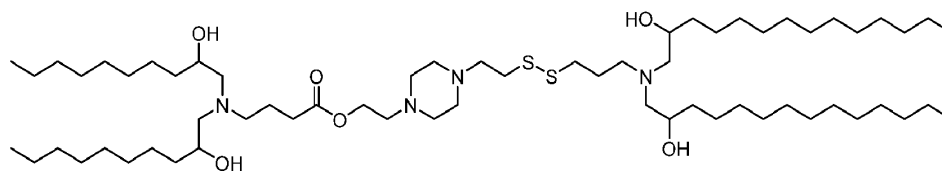
[0283] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate (GL-HEPES-E3-E10-DS-3-E12)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.63 (bs, 4H), 2.85-2.42 (m, 30H), 1.87-1.78 (m, 4H), 1.46-1.31 (m, 64H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{59}\text{H}_{120}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1045.9$, Observed = 1045.9.

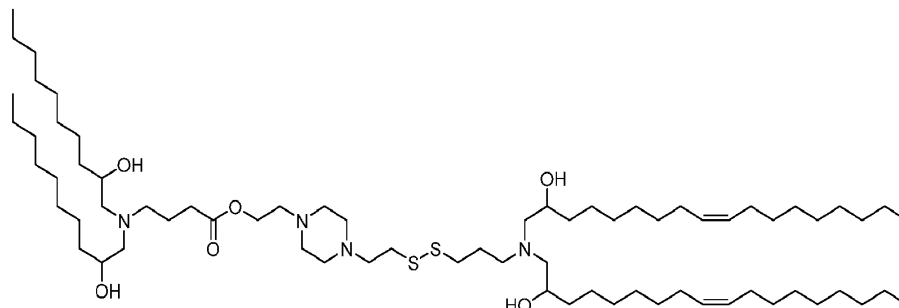
[0284] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate (GL-HEPES-E3-E10-DS-3-E14)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.63 (bs, 4H), 2.85-2.39 (m, 30H), 1.87-1.77 (m, 4H), 1.46-1.29 (m, 72H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{63}\text{H}_{128}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1101.9$, Observed = 1101.9.

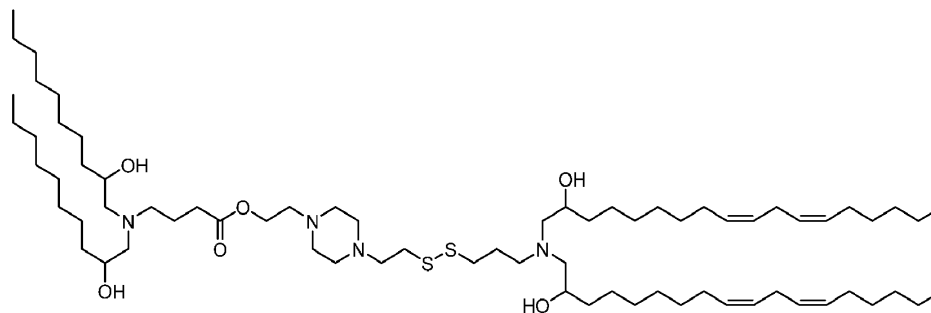
[0285] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate (GL-HEPES-E3-E10-DS-3-E18-1)



^1H NMR (300 MHz, Methanol- d_4) δ 5.38-5.33 (m, 4H) 4.22 (t, 2H), 3.65 (m, 4H), 2.89-2.40 (m, 30H), 2.04 (m, 8H), 1.87-1.78 (m, 4H), 1.46-1.31 (m, 72H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{140}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1210.0$, Observed = 1210.0.

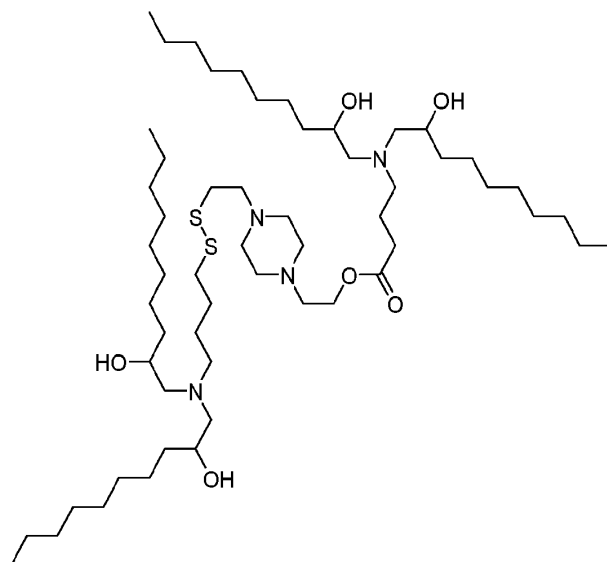
[0286] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate (GL-HEPES-E3-E10-DS-3-E18-2)



^1H NMR (300 MHz, Methanol- d_4) δ 5.39-5.33 (m, 8H) 4.22 (t, 2H), 3.65 (m, 4H), 2.85-2.39 (m, 30H), 2.07 (m, 8H) 1.87-1.78 (m, 4H), 1.46-1.31 (m, 64H), 0.90 (t, 12H)

APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{136}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1206.0$, Observed = 1206.0.

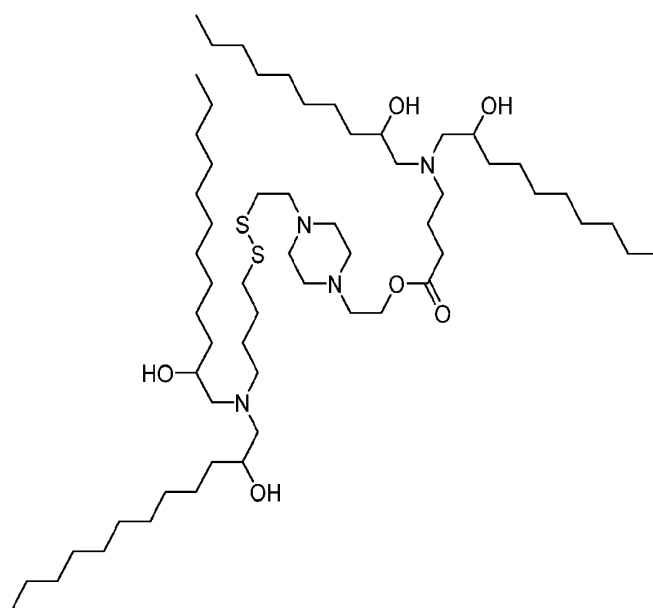
[0287] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate (GL-HEPES-E3-E10-DS-4-E10)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.63 (bs, 4H), 2.85-2.39 (m, 30H), 1.77 (m, 4H), 1.46-1.29 (m, 58H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{56}\text{H}_{114}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1003.8$, Observed = 1003.8.

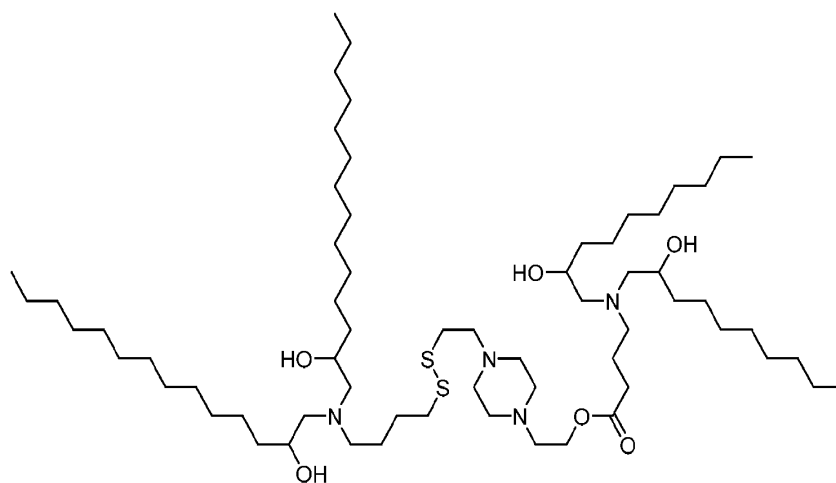
[0288] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E10-DS-4-E12)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.65 (bs, 4H), 2.85-2.39 (m, 30H), 1.77 (m, 4H), 1.46-1.29 (m, 66H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{60}\text{H}_{122}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1059.9$, Observed = 1059.9.

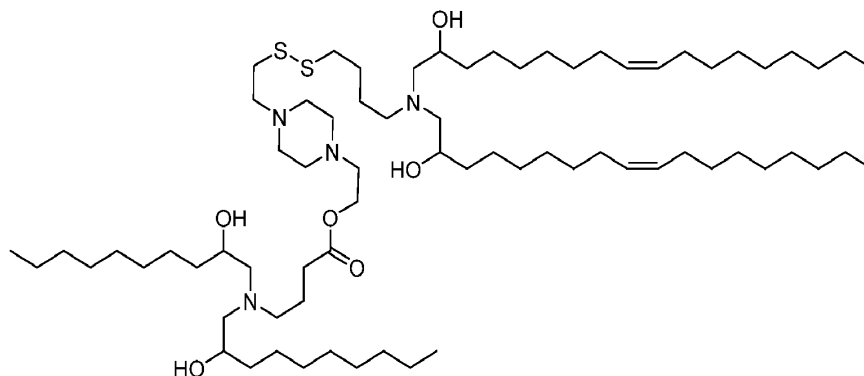
[0289] Analytical data for 2-{4-(2-((4-(bis(2-hydroxytetradecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate (GL-HEPES-E3-E10-DS-4-E14)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.69-3.58 (m, 4H), 2.85-2.39 (m, 30H), 1.83-1.72 (m, 4H), 1.46-1.29 (m, 74H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{130}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1115.9$, Observed = 1115.9.

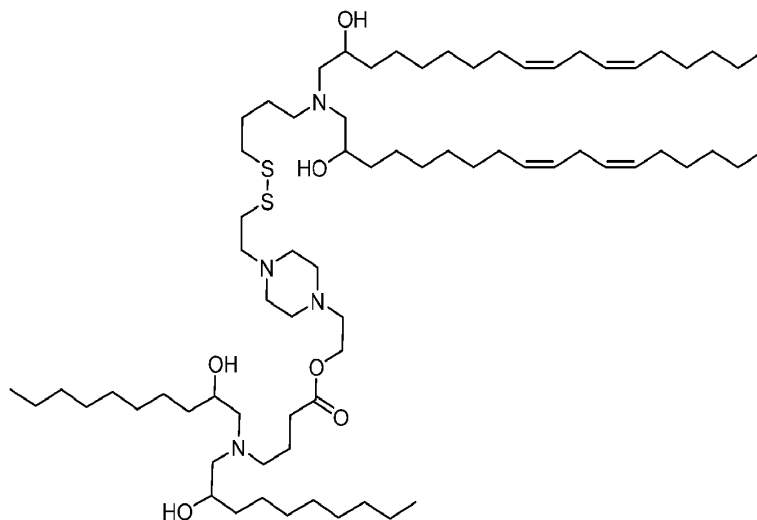
[0290] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate (GL-HEPES-E3-E10-DS-4-E18-1)



^1H NMR (300 MHz, Chloroform- d) δ 5.38-5.32 (m, 4H), 4.20 (t, 2H), 3.64 (bs, 4H), 2.85-2.33 (m, 30H), 2.05-1.93 (m, 8H), 1.83-1.72 (m, 2H), 1.48-1.21 (m, 76H), 0.87 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{142}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1224.0$, Observed = 1224.1.

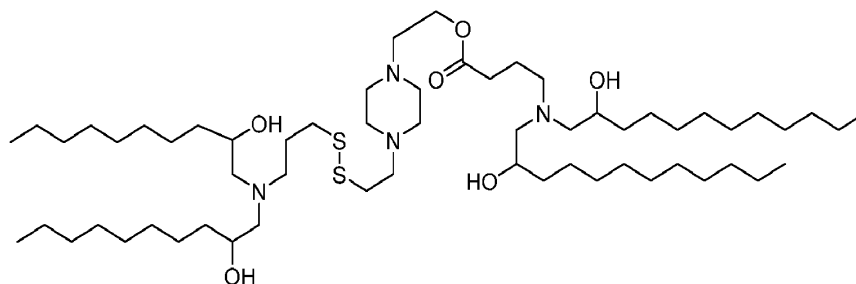
[0291] Analytical data for 2-(4-(2-((4-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate (GL-HEPES-E3-E10-DS-4-E18-2)



^1H NMR (300 MHz, Chloroform- d) δ 5.38-5.29 (m, 8H), 4.20 (t, 2H), 3.63 (bs, 4H), 2.85-2.33 (m, 30H), 2.09-1.95 (m, 8H), 1.83-1.72 (m, 2H), 1.48-1.21 (m, 66H), 0.87 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{138}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1220.0$, Observed = 1220.0.

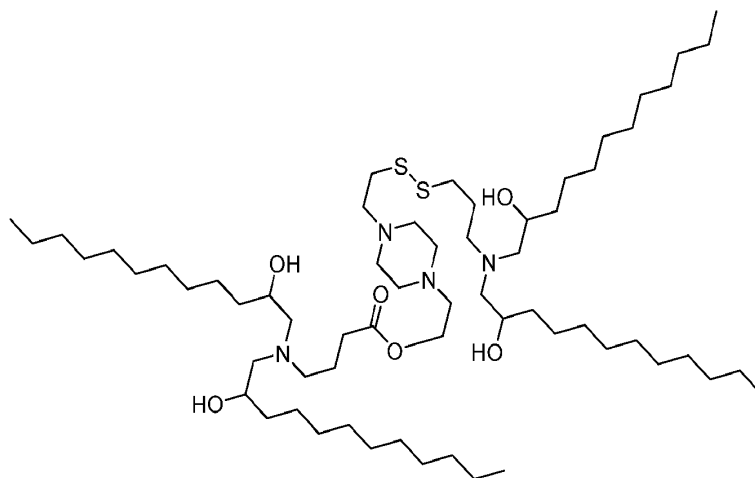
[0292] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-DS-3-E10)



$^1\text{H NMR}$ (300 MHz, Chloroform- d) δ 4.20 (t, 2H), 3.64 (bs, 4H), 2.85-2.33 (m, 30H), 1.92-1.76 (m, 4H), 1.53-1.18 (m, 64H), 0.87 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{59}\text{H}_{120}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1045.7$, Observed = 1045.9.

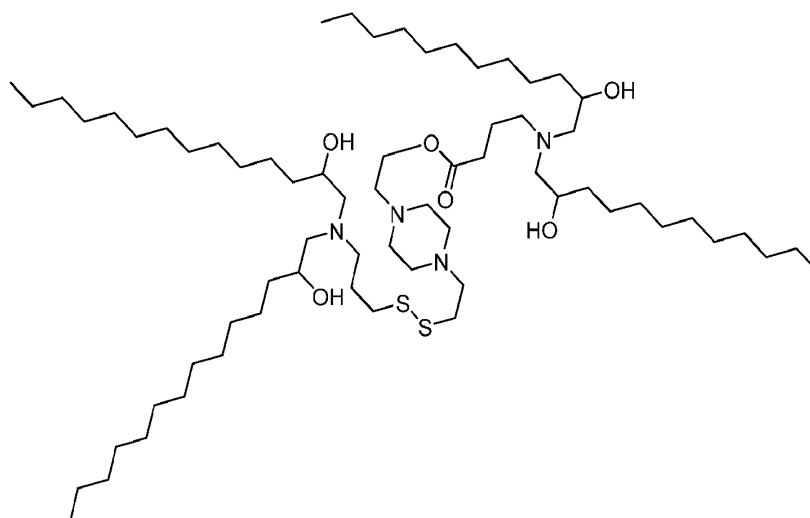
[0293] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-DS-3-E12)



$^1\text{H NMR}$ (300 MHz, Chloroform- d) δ 4.20 (t, 2H), 3.64 (bs, 4H), 2.85-2.33 (m, 30H), 1.92-1.76 (m, 4H), 1.53-1.18 (m, 72H), 0.87 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{63}\text{H}_{128}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1101.8$, Observed = 1102.0.

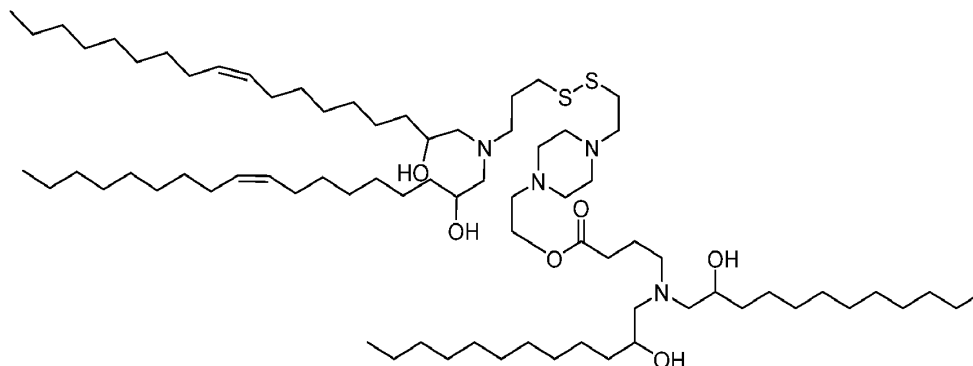
[0294] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-DS-3-E14)



^1H NMR (300 MHz, Chloroform- d) δ 4.19 (t, 2H), 3.64 (bs, 4H), 2.85-2.33 (m, 30H), 1.92-1.76 (m, 4H), 1.53-1.18 (m, 80H), 0.87 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{67}\text{H}_{136}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1157.9$, Observed = 1158.0.

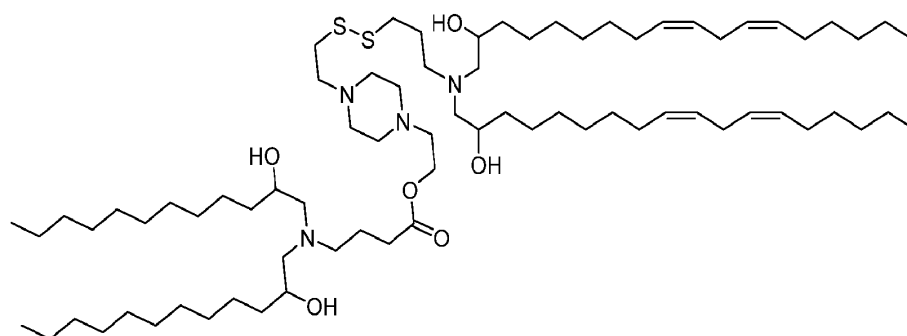
[0295] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-DS-3-E18-1)



^1H NMR (300 MHz, Chloroform- d) δ 5.38-5.33 (m, 4H) 4.20 (t, 2H), 3.64 (m, 4H), 2.89-2.35 (m, 30H), 2.04-1.65 (m, 8H), 1.87-1.78 (m, 4H), 1.52-1.18 (m, 72H), 0.87 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{140}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1266.1$, Observed = 1266.1.

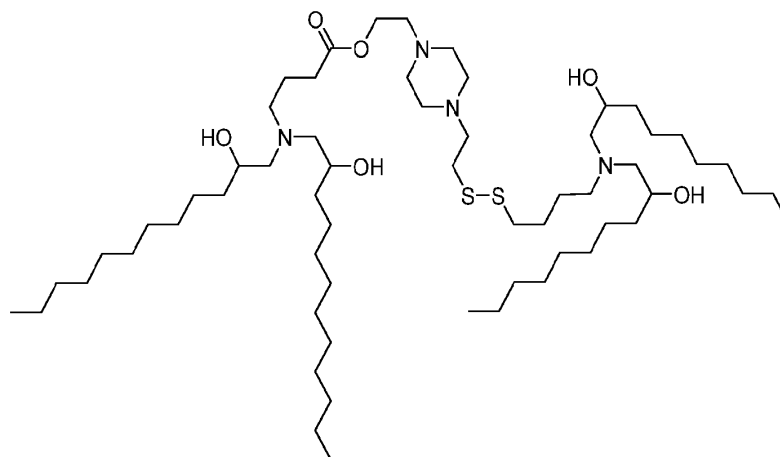
[0296] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-DS-3-E18-2)



^1H NMR (300 MHz, Chloroform- d) δ 5.40-5.31 (m, 8H) 4.20 (t, 2H), 3.63 (m, 4H), 2.85-2.33 (m, 30H), 2.10-1.96 (m, 8H) 1.87-1.78 (m, 4H), 1.52-1.21 (m, 72H), 0.88 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{75}\text{H}_{144}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1262.1$, Observed = 1262.1

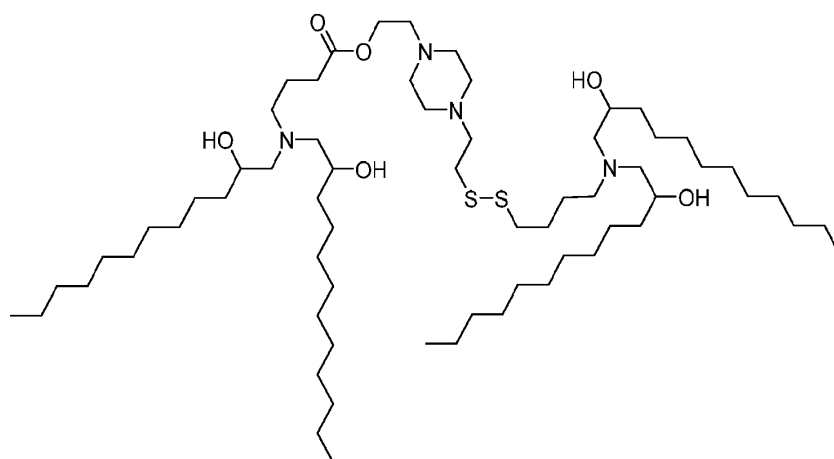
[0297] Analytical data for 2-(4-(2-((3-(bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-DS-4-E10)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.65 (bs, 4H), 2.85-2.39 (m, 30H), 1.77 (m, 4H), 1.46-1.29 (m, 66H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{60}\text{H}_{122}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1059.9$, Observed = 1059.9.

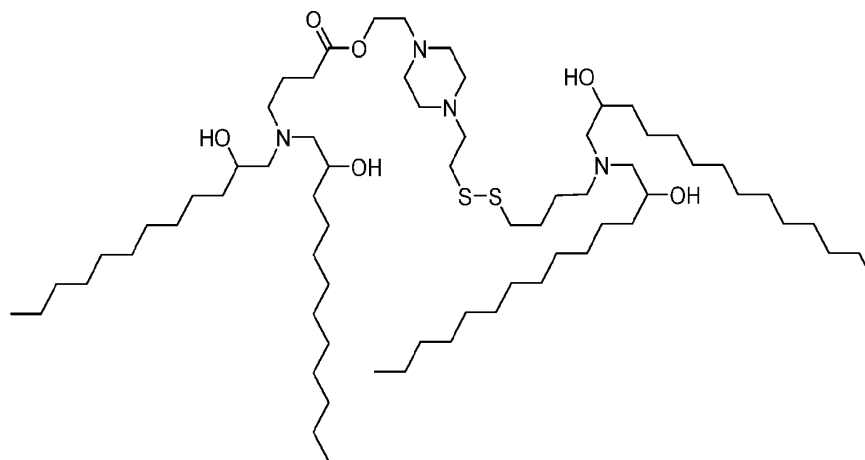
[0298] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-DS-4-E12)



¹H NMR (300 MHz, Methanol-d₄) δ 4.22 (t, 2H), 3.62 (bs, 4H), 2.87-2.35 (m, 30H), 1.77 (m, 4H), 1.65-1.29 (m, 74H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₆₄H₁₃₀N₄O₆S₂, [M+H] = 1116.0, Observed = 1116.0.

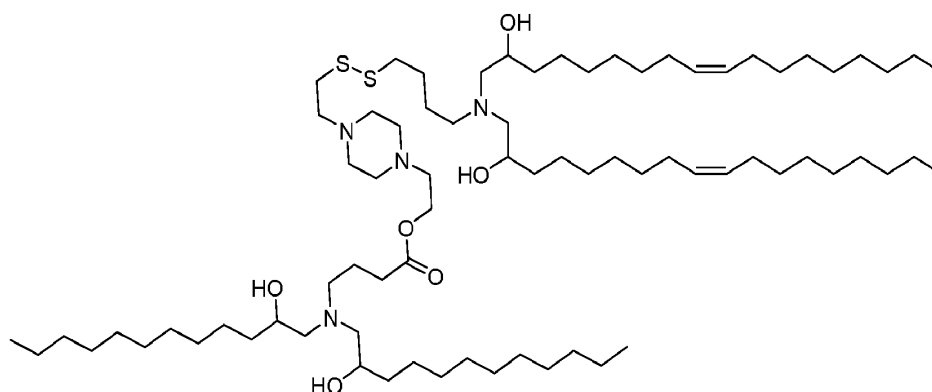
[0299] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxytetradecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-DS-4-E14)



¹H NMR (300 MHz, Methanol-d₄) δ 4.22 (t, 2H), 3.62 (bs, 4H), 2.87-2.35 (m, 30H), 1.77 (m, 4H), 1.65-1.29 (m, 82H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₆₈H₁₃₈N₄O₆S₂, [M+H] = 1172.0, Observed = 1172.0.

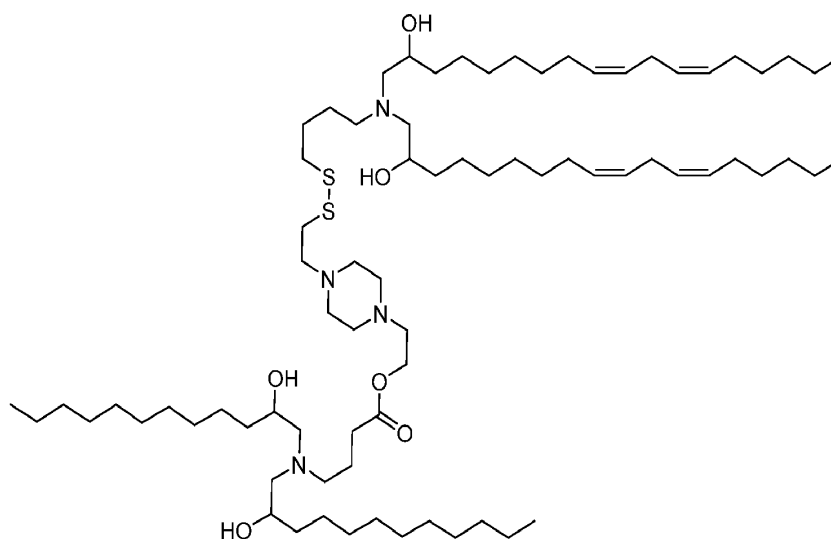
[0300] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-DS-4-E18-1)



^1H NMR (300 MHz, Chloroform- d) δ 5.38-5.32 (m, 4H), 4.20 (t, 2H), 3.65 (bs, 4H), 2.85-2.33 (m, 30H), 2.05-1.93 (m, 8H), 1.83-1.16 (m, 86H), 0.88 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{76}\text{H}_{150}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1280.1$, Observed = 1280.1.

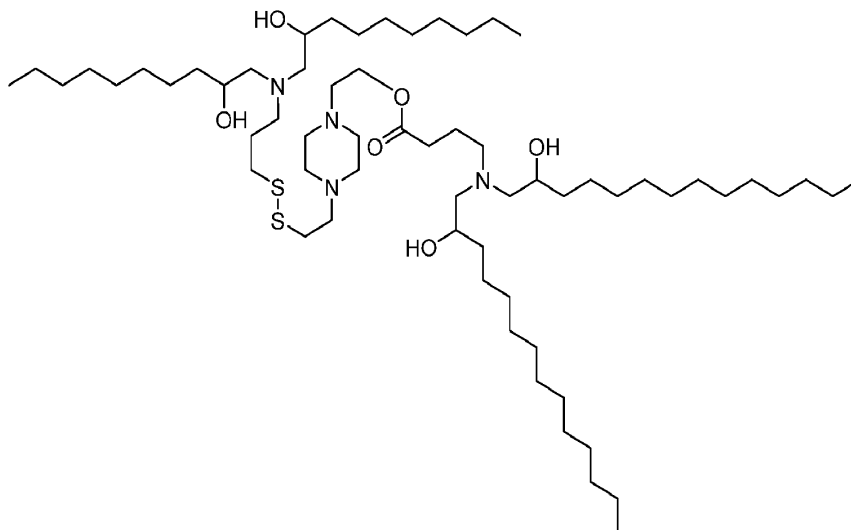
[0301] Analytical data for 2-(4-(2-((4-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-DS-4-E18-2)



^1H NMR (300 MHz, Chloroform- d) δ 5.38-5.29 (m, 8H), 4.20 (t, 2H), 3.65 (bs, 4H), 2.85-2.33 (m, 30H), 2.09-1.95 (m, 8H), 1.85-1.21 (m, 78H), 0.87 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{76}\text{H}_{146}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1276.1$, Observed = 1276.1.

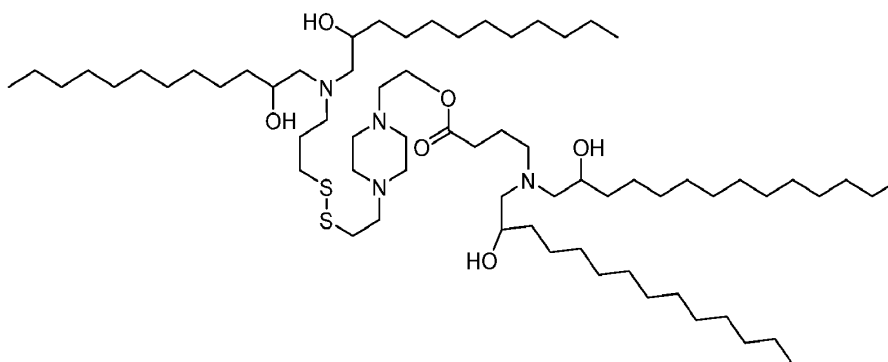
[0302] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-DS-3-E10)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.62 (bs, 4H), 2.87-2.35 (m, 30H), 1.92-1.75 (m, 4H), 1.46-1.30 (m, 72H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{63}\text{H}_{128}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1102.0$, Observed = 1102.0.

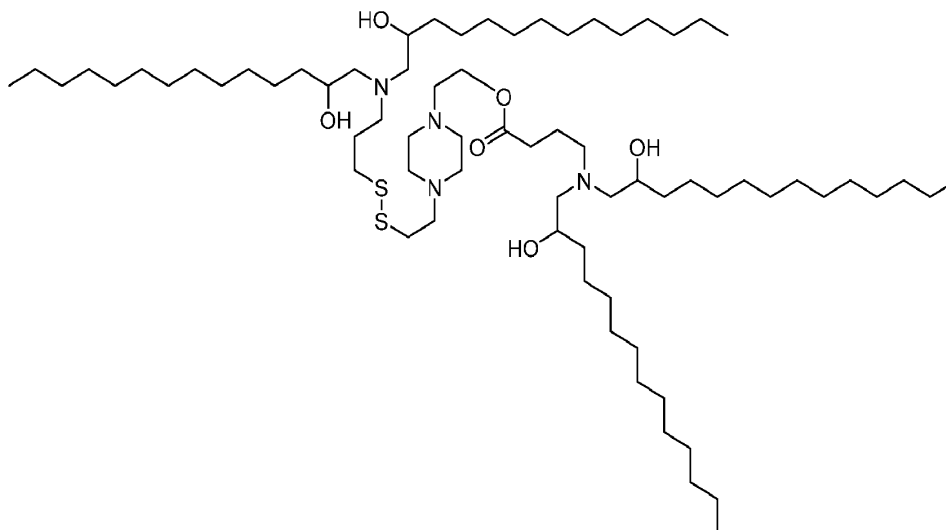
[0303] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-DS-3-E12)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.66 (bs, 4H), 2.87-2.35 (m, 30H), 1.92-1.75 (m, 4H), 1.46-1.30 (m, 80H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{67}\text{H}_{136}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1158.0$, Observed = 1158.0.

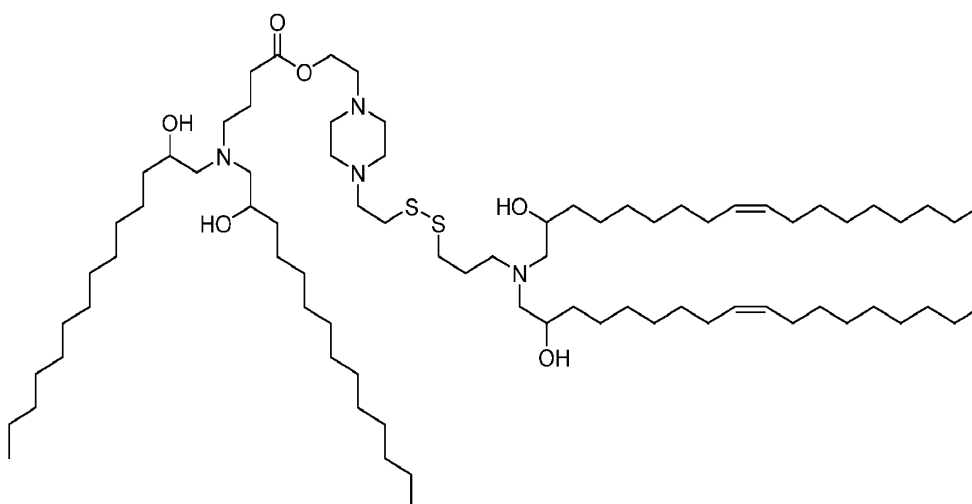
[0304] Analytical data for 2-(4-(2-((3-(bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-DS-3-E14)



¹H NMR (300 MHz, Methanol-d₄) δ 4.22 (t, 2H), 3.64 (bs, 4H), 2.87-2.35 (m, 30H), 1.92-1.75 (m, 4H), 1.46-1.30 (m, 88H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₇₁H₁₄₄N₄O₆S₂, [M+H]⁺ = 1214.1, Observed = 1214.1.

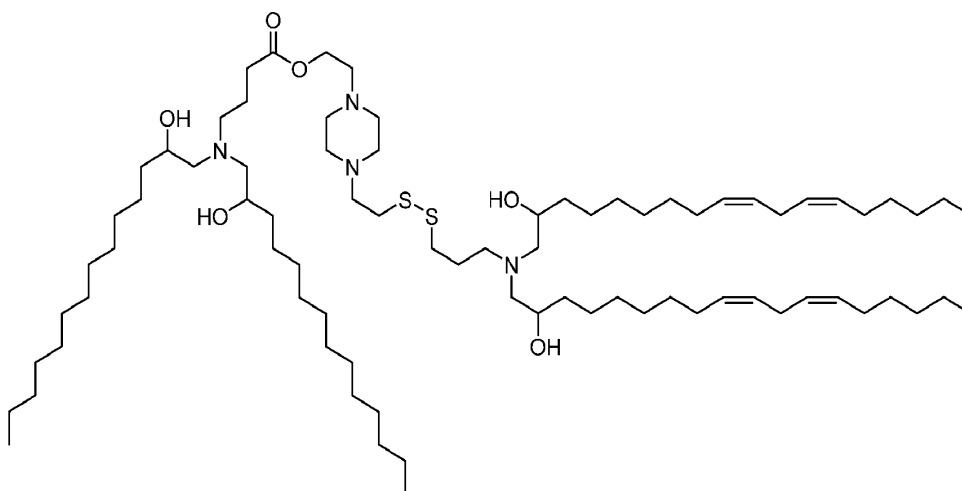
[0305] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-DS-3-E18-1)



^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 4H) 4.22 (t, 2H), 3.64 (bs, 4H), 2.87-2.35 (m, 30H), 2.05 (m, 8H), 1.92-1.75 (m, 4H), 1.46-1.30 (m, 88H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{79}\text{H}_{156}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1322.2$, Observed = 1322.2.

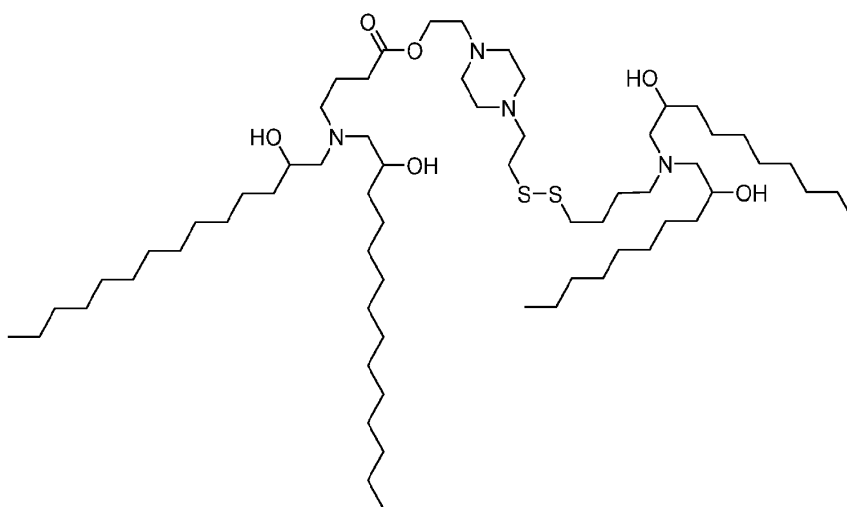
[0306] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-DS-3-E18-2)



^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 8H), 4.22 (t, 2H), 3.67 (bs, 4H), 2.87-2.40 (m, 30H), 2.06 (m, 8H), 1.92-1.75 (m, 4H), 1.46-1.30 (m, 80H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{79}\text{H}_{152}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1318.1$, Observed = 1318.1.

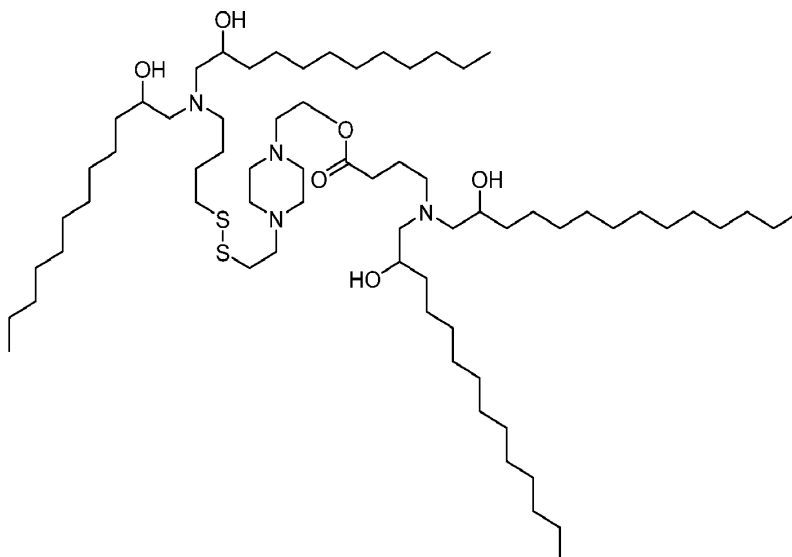
[0307] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-DS-4-E10)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.64 (bs, 4H), 2.87-2.35 (m, 30H), 1.77 (m, 4H), 1.63-1.29 (m, 74H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{130}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1116.0$, Observed = 1116.0.

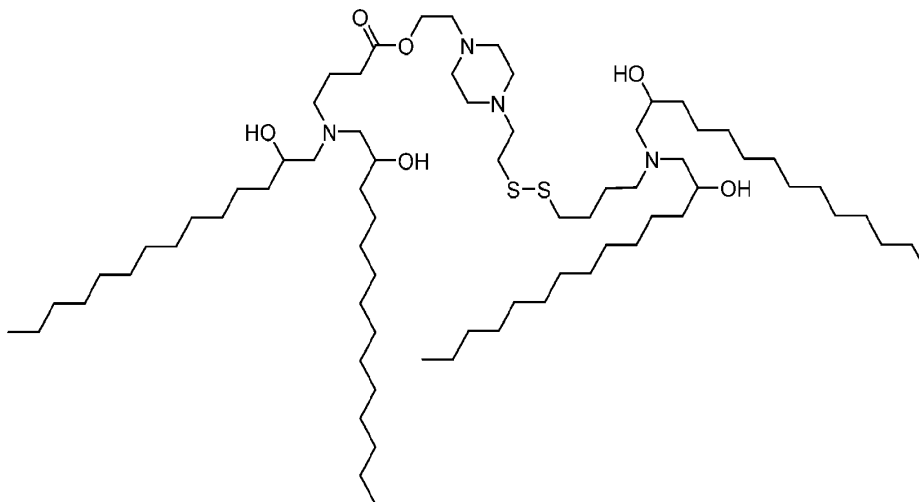
[0308] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-DS-4-E12)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.64 (bs, 4H), 2.87-2.35 (m, 30H), 1.77 (m, 4H), 1.63-1.29 (m, 82H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₆₈H₁₃₈N₄O₆S₂, [M+H] = 1172.0, Observed = 1172.0.

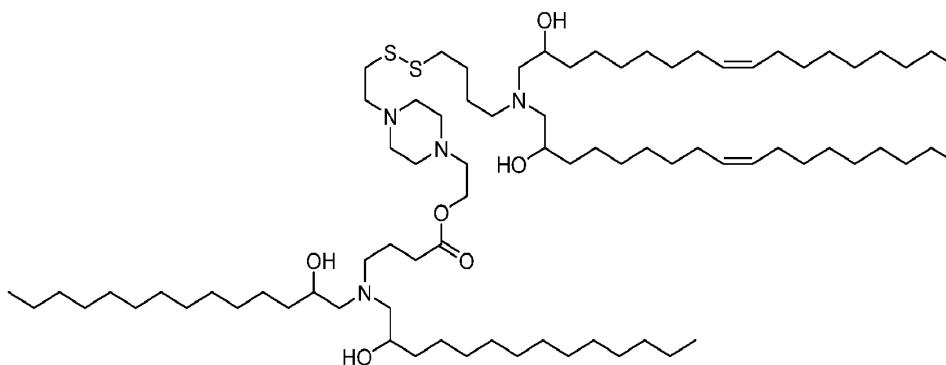
[0309] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxytetradecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-DS-4-E14)



¹H NMR (300 MHz, Methanol-d₃) δ 4.22 (t, 2H), 3.67 (bs, 4H), 2.87-2.40 (m, 30H), 1.80 (m, 4H), 1.63-1.29 (m, 90H), 0.91 (t, 12H).

APCI-MS analysis: Calculated C₇₂H₁₄₆N₄O₆S₂, [M+H] = 1226.1, Observed = 1226.1.

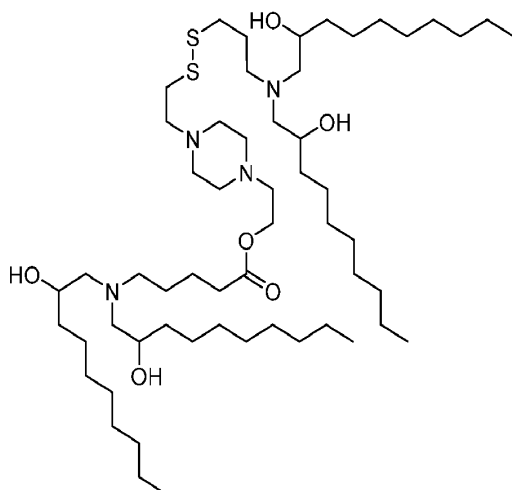
[0310] Analytical data for 2-(4-(2-((4-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-DS-4-E18-1)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 4H) 4.22 (t, 2H), 3.67 (bs, 4H), 2.87-2.40 (m, 30H), 2.05 (m, 8H), 1.92-1.75 (m, 4H), 1.46-1.30 (m, 90H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₈₀H₁₅₈N₄O₆S₂, [M+H] = 1336.2, Observed = 1336.2.

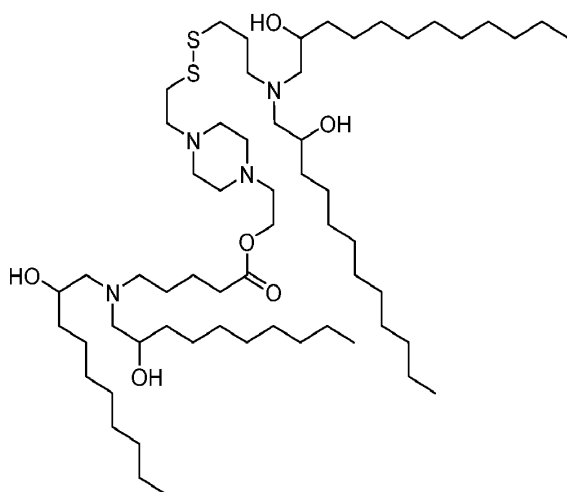
[0311] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-DS-3-E10)



¹H NMR (300 MHz, Methanol-d₄) δ 4.22 (t, 2H), 3.65 (bs, 4H), 2.87-2.40 (m, 30H), 1.89 (quint, 2H), 1.68-1.31 (m, 60H), 0.91 (t, 12H).

APCI-MS analysis: Calculated C₅₆H₁₁₄N₄O₆S₂, [M+H] = 1003.8, Observed = 1003.8.

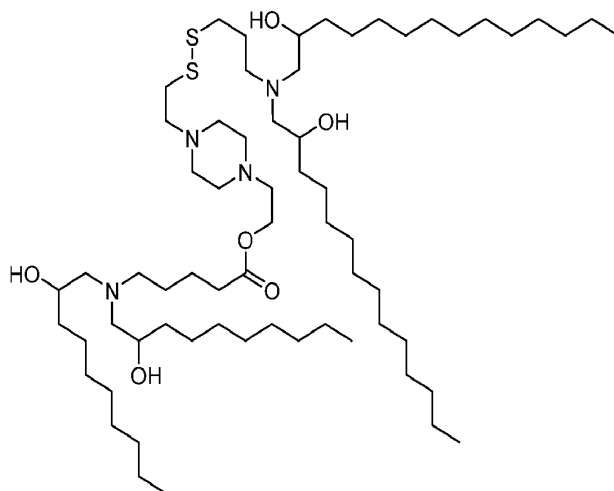
[0312] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-DS-3-E12)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.66 (bs, 4H), 2.87-2.36 (m, 30H), 1.87 (quint, 2H), 1.68-1.31 (m, 68H), 0.91 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{60}\text{H}_{122}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1059.9$, Observed = 1059.9.

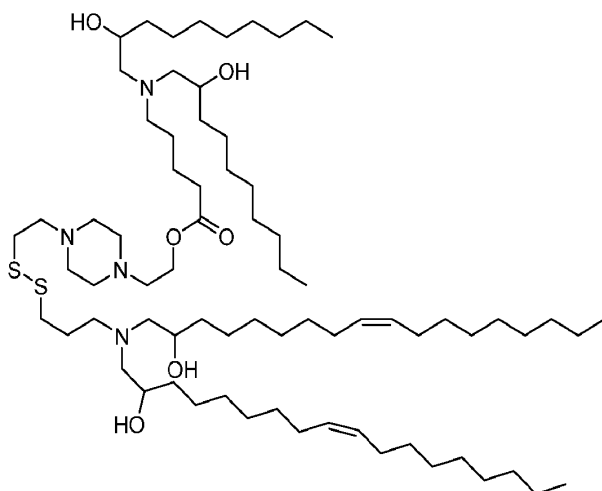
[0313] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-DS-3-E14)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.63 (bs, 4H), 2.87-2.36 (m, 30H), 1.87 (quint, 2H), 1.68-1.31 (m, 76H), 0.91 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{130}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1116.0$, Observed = 1116.0.

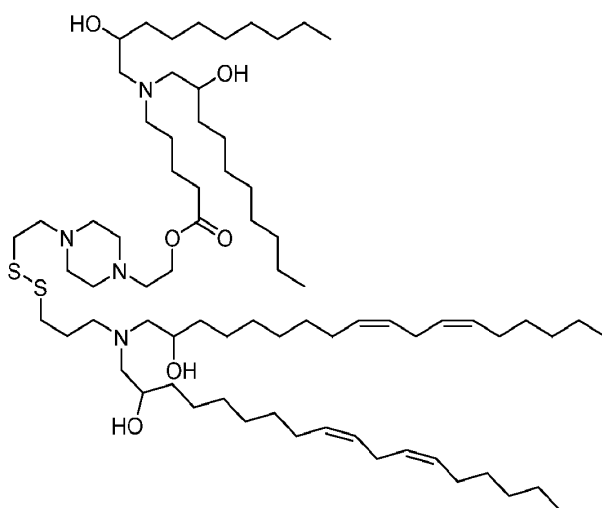
[0314] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-DS-3-E18-1)



^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 4H) 4.22 (t, 2H), 3.65 (bs, 4H), 2.87-2.35 (m, 30H), 2.05 (m, 8H), 1.87 (m, 2H), 1.67-1.30 (m, 76H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{142}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1224.1$ Observed = 1224.1.

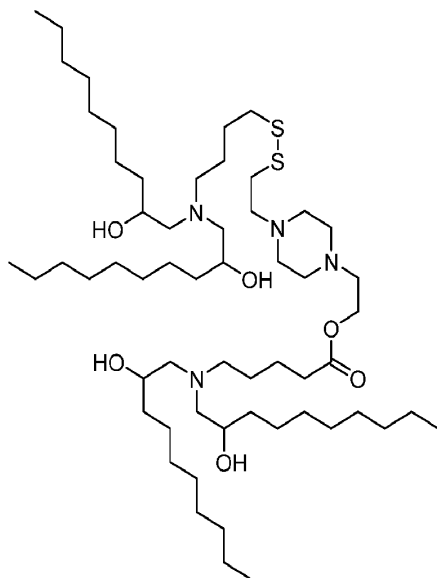
[0315] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-DS-3-E18-2)



^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 8H) 4.22 (t, 2H), 3.64 (bs, 4H), 2.87-2.35 (m, 30H), 2.06 (m, 8H), 1.87 (m, 2H), 1.67-1.30 (m, 68H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{138}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1220.0$ Observed = 1220.0.

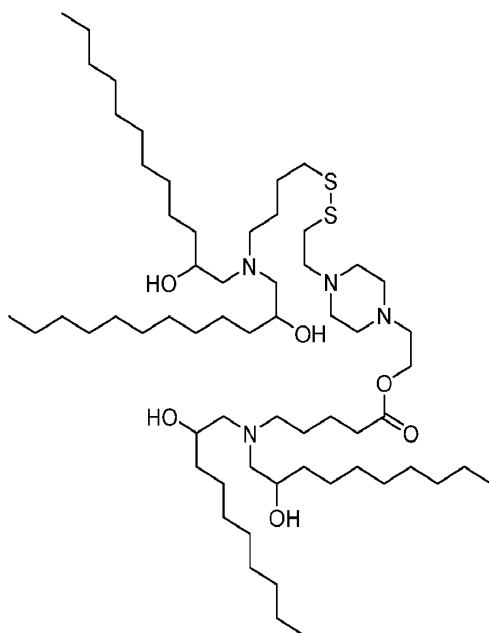
[0316] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-DS-4-E10)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.63 (bs, 4H), 2.87-2.36 (m, 30H), 1.75-1.31 (m, 64H), 0.91 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{57}\text{H}_{116}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1017.9$, Observed = 1017.9.

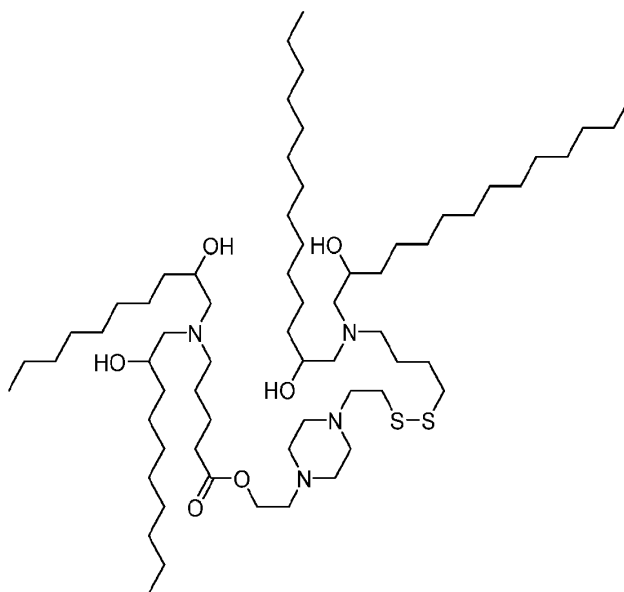
[0317] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-DS-4-E12)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.63 (bs, 4H), 2.87-2.36 (m, 30H), 1.75-1.31 (m, 72H), 0.91 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{61}\text{H}_{124}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1074.0$, Observed = 1074.0.

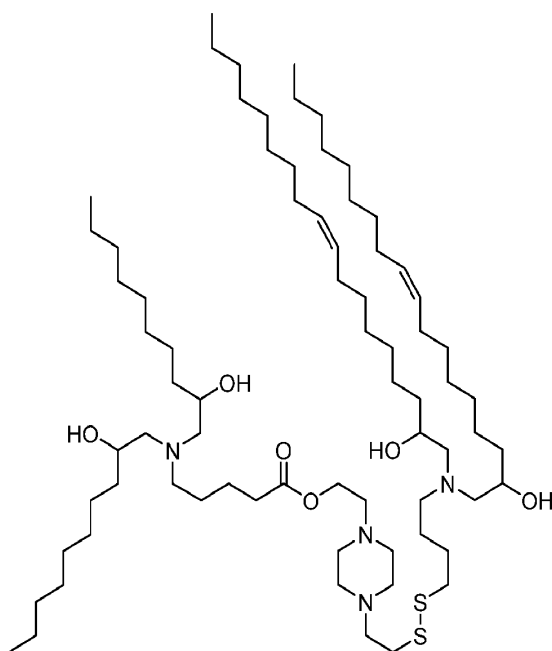
[0318] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxytetradecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-DS-4-E14)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.63 (bs, 4H), 2.87-2.36 (m, 30H), 1.75-1.31 (m, 80H), 0.91 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{132}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1130.0$, Observed = 1130.0.

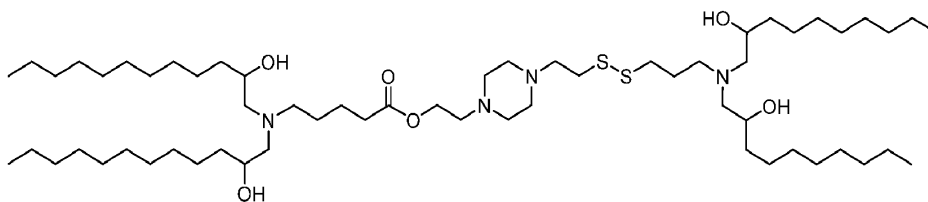
[0319] Analytical data for 2-(4-(2-((4-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-DS-4-E18-1)



^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 4H) 4.22 (t, 2H), 3.65 (bs, 4H), 2.87-2.35 (m, 30H), 2.04 (m, 8H), 1.75-1.30 (m, 80H), 0.90 (t, 12H)

APCI-MS analysis: Calculated $\text{C}_{73}\text{H}_{144}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1238.1$, Observed = 1238.1.

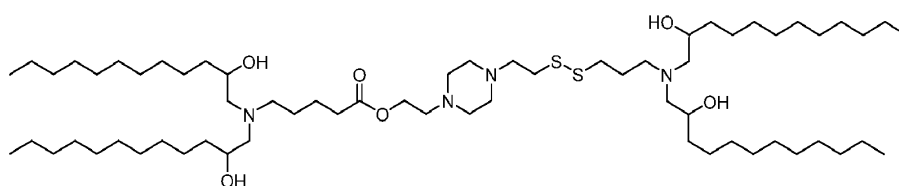
[0320] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-DS-3-E10)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.62 (bs, 4H), 2.86-2.36 (m, 30H), 1.92-1.84 (m, 2H), 1.68-1.20 (m, 68H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{60}\text{H}_{122}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1059.9$, Observed = 1059.9.

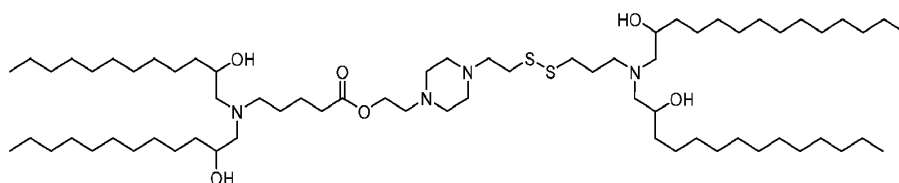
[0321] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-DS-3-E12)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.62 (bs, 4H), 2.86-2.36 (m, 30H), 1.92-1.84 (m, 2H), 1.68-1.20 (m, 76H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{130}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1115.9$, observed = 1116.0.

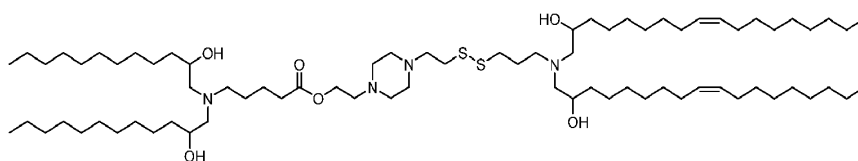
[0322] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-DS-3-E14)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.63 (bs, 4H), 2.86-2.36 (m, 30H), 1.92-1.84 (m, 2H), 1.68-1.20 (m, 84H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{138}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1172.0$, Observed = 1172.1.

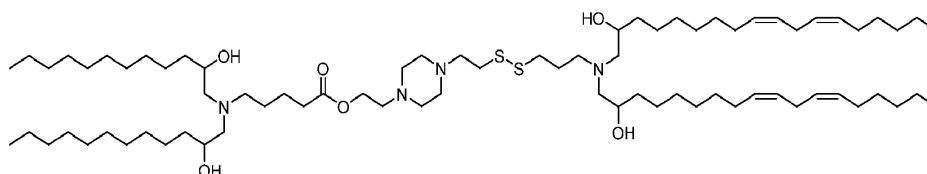
[0323] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-DS-3-E18-1)



¹H NMR (300 MHz, Methanol-d₄) δ 5.42-5.26 (m, 4H), 4.22 (t, 2H), 3.63 (bs, 4H), 2.86-2.36 (m, 30H), 2.08-1.94 (m, 8H), 1.89-1.80 (m, 2H), 1.68-1.23 (m, 84H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₇₅H₁₅₀N₄O₆S₂, [M+H] = 1280.1, Observed = 1281.1.

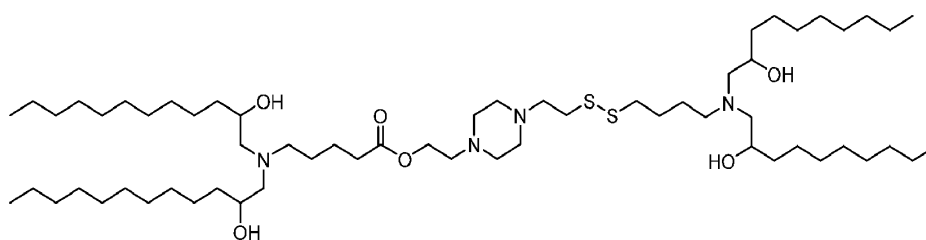
[0324] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-DS-3-E18-2)



¹H NMR (300 MHz, Methanol-d₄) δ 5.42-5.26 (m, 8H), 4.22 (t, 2H), 3.68 (bs, 4H), 2.86-2.36 (m, 30H), 2.12-2.02 (m, 8H), 1.91-1.85 (m, 2H), 1.68-1.23 (m, 76H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₇₆H₁₄₆N₄O₆S₂, [M+H] = 1276.0, Observed = 1276.1.

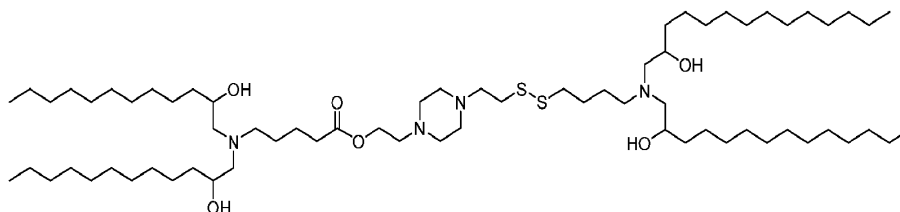
[0325] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-DS-4-E10)



¹H NMR (300 MHz, Methanol-d₄) δ 4.22 (t, 2H), 3.62 (bs, 4H), 2.86-2.33 (m, 30H), 1.78-1.20 (m, 72H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₆₁H₁₂₄N₄O₆S₂, [M+H] = 1073.9, Observed = 1073.9.

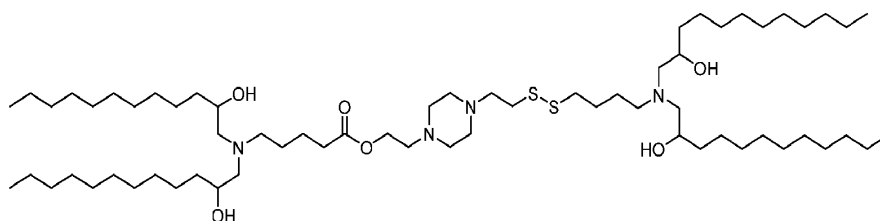
[0326] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxytetradecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-DS-4-E14)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.63 (bs, 4H), 2.86-2.33 (m, 30H), 1.78-1.20 (m, 88H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{69}\text{H}_{140}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1186.0$, Observed = 1186.0.

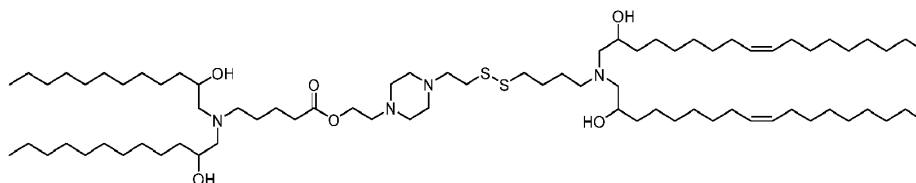
[0327] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-DS-4-E12)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.63 (bs, 4H), 2.86-2.33 (m, 30H), 1.78-1.20 (m, 80H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{132}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1129.9$, Observed = 1130.0.

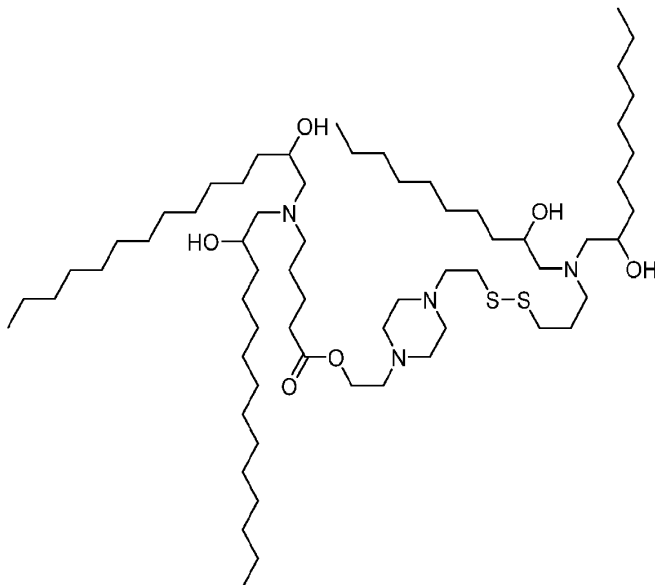
[0328] Analytical data for 2-(4-(2-((4-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-DS-4-E18-1)



^1H NMR (300 MHz, Methanol- d_4) δ 5.42-5.26 (m, 4H), 4.22 (t, 2H), 3.63 (bs, 4H), 2.86-2.36 (m, 30H), 2.08-1.93 (m, 8H), 1.78-1.23 (m, 88H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{77}\text{H}_{152}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1294.1$, Observed = 1294.1.

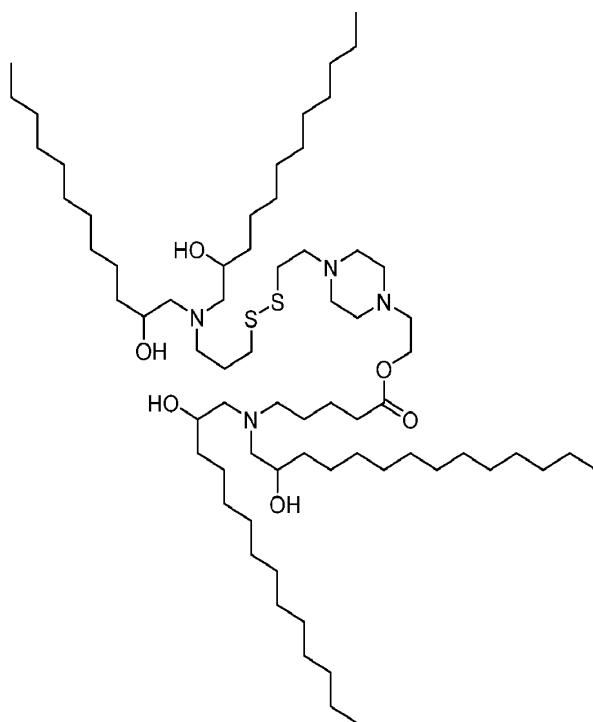
[0329] Analytical data for 2-(4-((3-(Bis(2-hydroxydecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-3-E10)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.63 (bs, 4H), 2.86-2.36 (m, 30H), 1.92-1.84 (m, 2H), 1.68-1.20 (m, 80H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{130}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1115.9$, Observed = 1116.0.

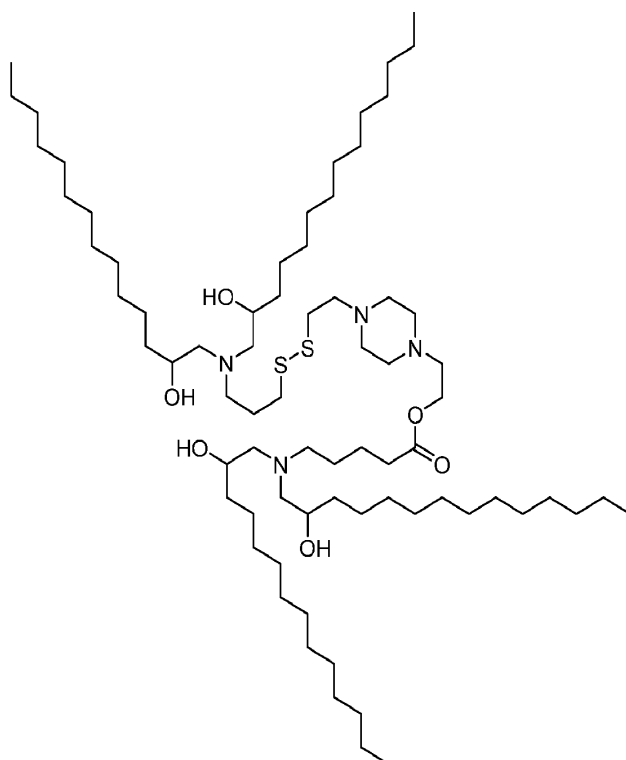
[0330] Analytical data for 2-(4-((3-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-3-E12)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.63 (bs, 4H), 2.86-2.36 (m, 30H), 1.92-1.84 (m, 2H), 1.68-1.20 (m, 84H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{138}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1172.0$, Observed = 1172.0.

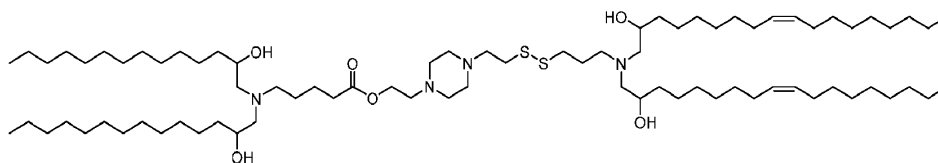
[0331] Analytical data for 2-(4-((3-(Bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-3-E14)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.65 (bs, 4H), 2.86-2.36 (m, 30H), 1.92-1.84 (m, 2H), 1.68-1.20 (m, 92H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{146}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1228.1$, Observed = 1228.1.

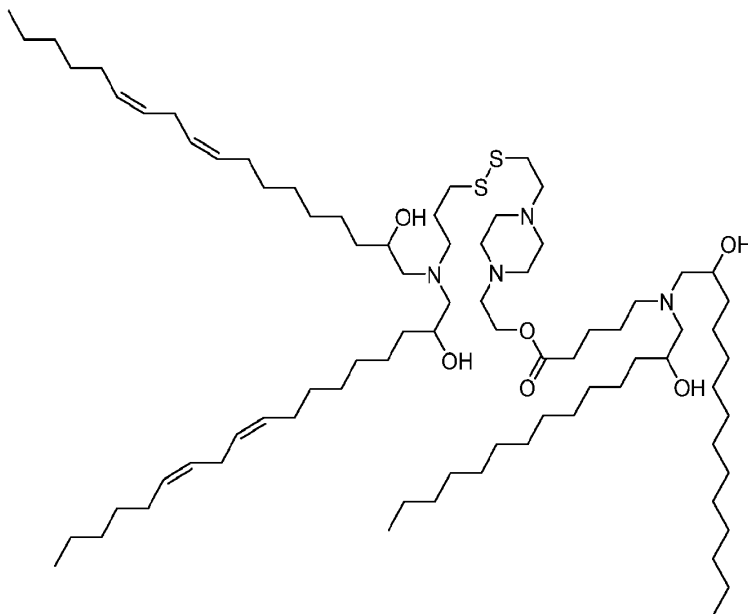
[0332] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-3-E18-1)



^1H NMR (300 MHz, Methanol- d_4) δ 5.42-5.26 (m, 4H), 4.22 (t, 2H), 3.68 (bs, 4H), 2.86-2.36 (m, 30H), 2.08-1.87 (m, 8H), 1.68-1.23 (m, 94H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{80}\text{H}_{158}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1336.1$, Observed = 1336.2.

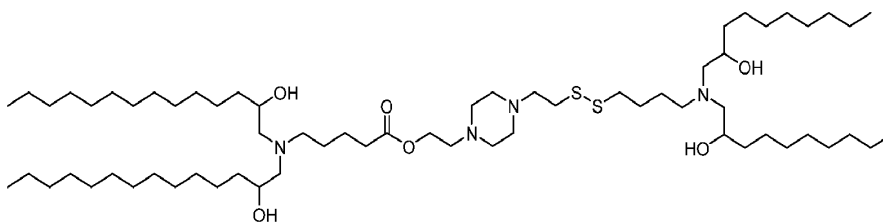
[0333] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-3-E18-2)



^1H NMR (300 MHz, Methanol- d_4) δ 5.42-5.26 (m, 8H), 4.22 (t, 2H), 3.68 (bs, 4H), 2.86-2.36 (m, 30H), 2.12-2.02 (m, 8H), 1.91-1.85 (m, 2H), 1.68-1.23 (m, 84H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{80}\text{H}_{154}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1332.1$, Observed = 1333.2.

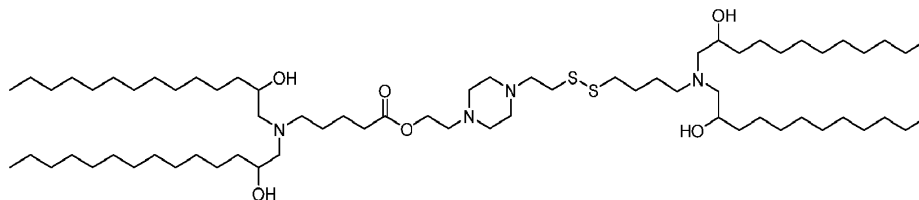
[0334] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-4-E10)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.65 (bs, 4H), 2.86-2.32 (m, 30H), 1.76-1.16 (m, 80H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{132}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1129.9$, Observed = 1129.9.

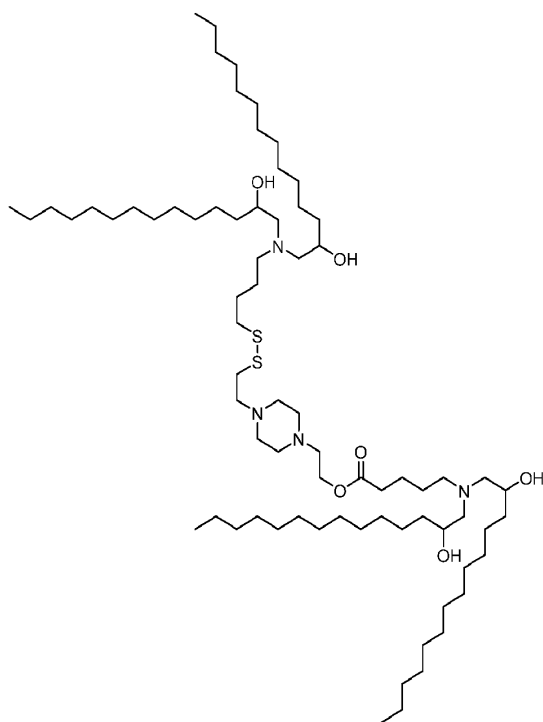
[0335] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-4-E12)



^1H NMR (300 MHz, Methanol- d_4) δ 4.22 (t, 2H), 3.66 (bs, 4H), 2.86-2.35 (m, 30H), 1.76-1.20 (m, 88H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{69}\text{H}_{140}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1186.0$, Observed = 1186.0.

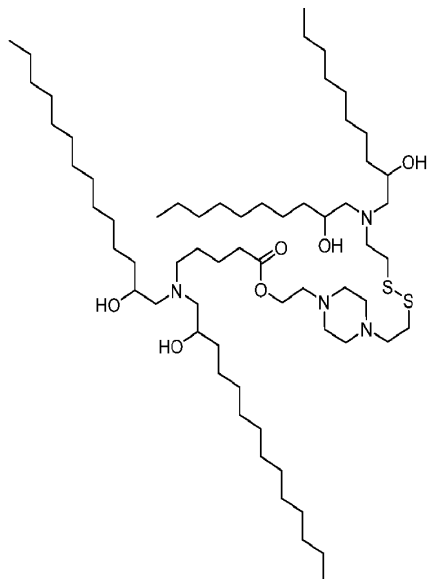
[0336] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxytetradecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-4-E14)



^1H NMR (300 MHz, Chloroform- d) δ 4.18 (t, 2H), 3.76-3.46 (m, 8H), 2.86-2.42 (m, 26H), 2.33 (t, 2H), 1.74-1.52 (m, 8H), 1.60-1.51 (m, 2H), 1.48-1.16 (m, 88H), 0.87 (t, 12H).

APCI-MS analysis: Calculated C₇₃H₁₄₈N₄O₆S₂, [M+H] = 1242.1, Observed = 1241.9.

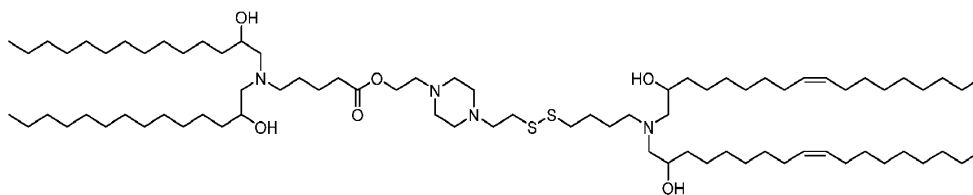
[0337] Analytical data for 2-(4-(2-((2-(Bis(2-hydroxydecyl)amino)ethyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-2-E10)



¹H NMR (300 MHz, Chloroform-d) δ 4.18 (t, 2H), 3.40-3.76 (m, 8H), 2.40-2.92 (m, 28H), 2.32 (t, 2H), 1.52-1.74 (m, 4H), 1.18-1.48 (m, 72H), 0.87 (t, 12H).

APCI-MS analysis: Calculated C₆₃H₁₂₈N₄O₆S₂, [M+H] = 1101.9, Observed = 1101.9.

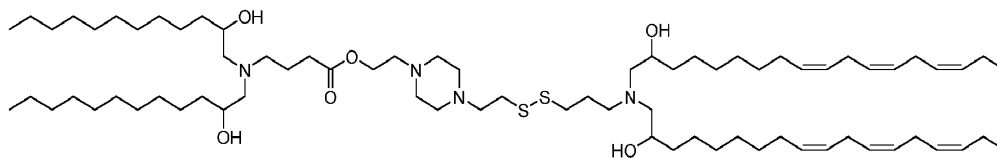
[0338] Analytical data for 2-(4-(2-((4-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-4-E18-1)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 4H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.87-2.35 (m, 30H), 2.03 (m, 8H), 1.61-1.30 (m, 96H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₈₁H₁₆₀N₄O₆S₂, [M+H] = 1350.2, Observed = 1350.2.

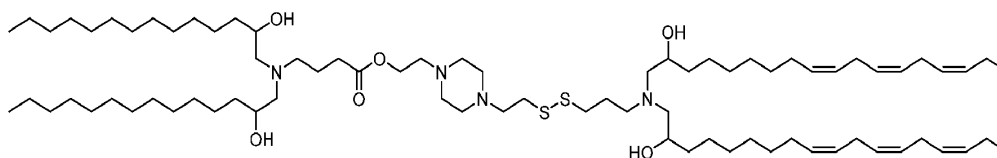
[0339] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-DS-3-E18-3)



^1H NMR (300 MHz, Methanol- d_3) δ 5.34 (m, 12H), 4.22 (t, 2H), 3.66 (s, br., 4H), 2.87-2.38 (m, 38H), 2.09 (m, 8H), 1.91-1.78 (m, 4H), 1.45-1.31 (m, 48H), 0.98 (t, 6H) 0.90 (t, 6H).

APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{132}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1202.0$, Observed = 1202.0.

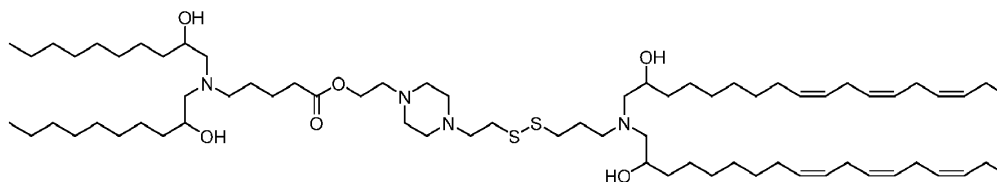
[0340] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-DS-3-E18-3)



^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 12H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.87-2.35 (m, 38H), 2.09 (m, 8H), 1.90-1.75 (m, 4H), 1.45-1.30 (m, 64H), 0.98 (t, 6H) 0.90 (t, 6H).

APCI-MS analysis: Calculated $\text{C}_{79}\text{H}_{148}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1314.1$, Observed = 1314.1.

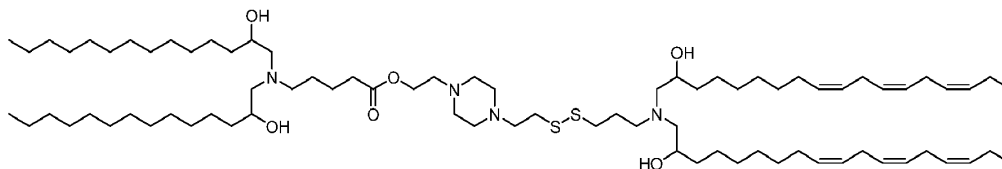
[0341] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-DS-3-E18-3)



^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 12H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.87-2.35 (m, 38H), 2.09 (m, 8H), 1.87 (quint, 2H), 1.65-1.31 (m, 52H), 0.98 (t, 6H) 0.90 (t, 6H).

APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{134}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1216.0$, Observed = 1216.0.

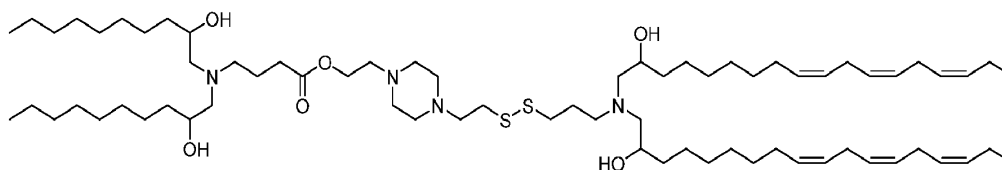
[0342] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-3-E18-3)



^1H NMR (300 MHz, Methanol- d_4) δ 5.36 (m, 12H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.87-2.35 (m, 38H), 2.09 (m, 8H), 1.87 (quint, 2H), 1.63-1.29 (m, 68H), 0.98 (t, 6H) 0.90 (t, 6H).

APCI-MS analysis: Calculated $\text{C}_{80}\text{H}_{150}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1328.1$, Observed = 1328.1.

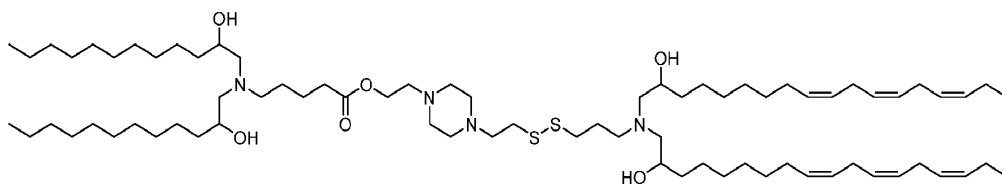
[0343] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)butanoate (GL-HEPES-E3-E10-DS-3-E18-3)



^1H NMR (300 MHz, Methanol- d_4) δ 5.34 (m, 12H), 4.22 (t, 2H), 3.66 (s, br., 4H), 2.87-2.38 (m, 38H), 2.09 (m, 8H), 1.91-1.78 (m, 4H), 1.45-1.31 (m, 48H), 0.98 (t, 6H) 0.90 (t, 6H).

APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{132}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1202.0$, Observed = 1202.0.

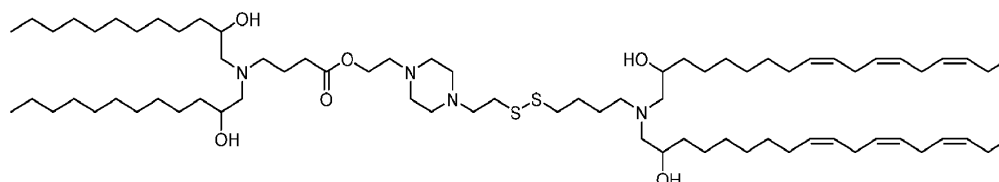
[0344] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-DS-3-E18-3)



^1H NMR (300 MHz, Methanol- d_4) δ 5.34 (m, 12H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.87-2.38 (m, 38H), 2.09 (m, 8H), 1.87 (m, 2H), 1.68-1.30 (m, 60H), 0.98 (t, 6H) 0.90 (t, 6H).

APCI-MS analysis: Calculated $\text{C}_{76}\text{H}_{142}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1272.0$, Observed = 1272.0.

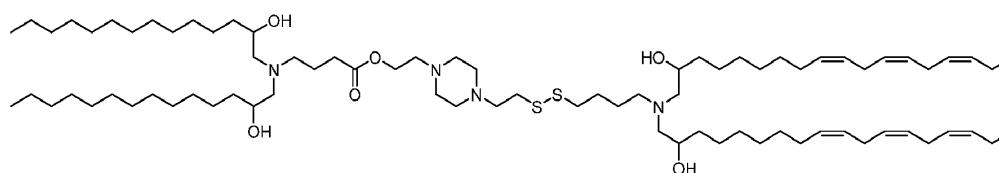
[0345] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E12-DS-4-E18-3)



^1H NMR (300 MHz, Methanol- d_4) δ 5.36 (m, 12H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.83-2.35 (m, 38H), 2.09 (m, 8H), 1.77-1.29 (m, 62H), 0.98 (t, 6H) 0.90 (t, 6H).

APCI-MS analysis: Calculated $\text{C}_{76}\text{H}_{142}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1272.0$, Observed=1272.1.

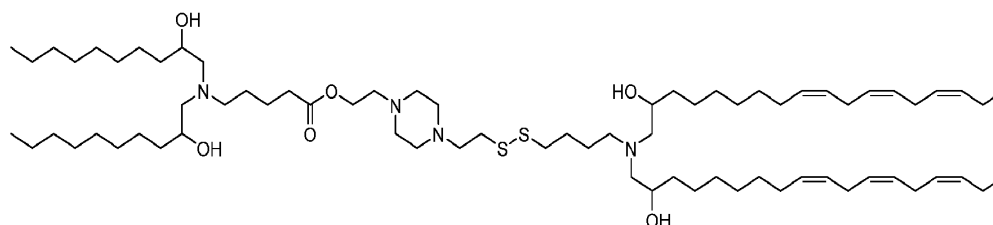
[0346] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-DS-4-E18-3)



^1H NMR (300 MHz, Methanol- d_4) δ 5.36 (m, 12H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.83-2.38 (m, 38H), 2.09 (m, 8H), 1.77-1.29 (m, 70H), 0.98 (t, 6H) 0.90 (t, 6H).

APCI-MS analysis: Calculated $\text{C}_{80}\text{H}_{150}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1328.1$, Observed = 1328.1.

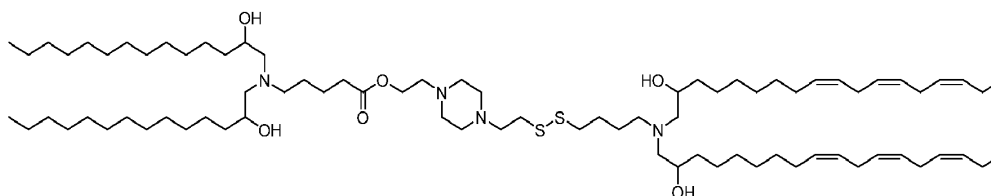
[0347] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-DS-4-E18-3)



^1H NMR (300 MHz, Methanol- d_4) δ 5.36 (m, 12H), 4.22 (t, 2H), 3.64 (s, br., 4H), 2.85-2.35 (m, 38H), 2.09 (m, 8H), 1.77-1.31 (m, 56H), 0.98 (t, 6H) 0.90 (t, 6H).

APCI-MS analysis: Calculated C₇₃H₁₃₆N₄O₆S₂ [M+H] = 1230.0, Observed = 1230.0.

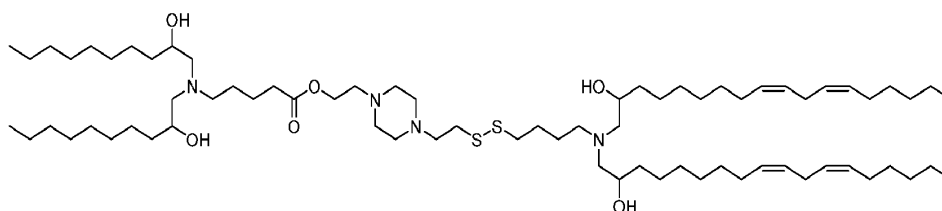
[0348] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-4-E18-3)



¹H NMR (300 MHz, Methanol-d₄) δ 5.36 (m, 12H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.83-2.35 (m, 38H), 2.09 (m, 8H), 1.77-1.31 (m, 72H), 0.98 (t, 6H) 0.90 (t, 6H).

APCI-MS analysis: Calculated C₈₁H₁₅₂N₄O₆S₂, [M+H] = 1342.1, Observed = 1342.1.

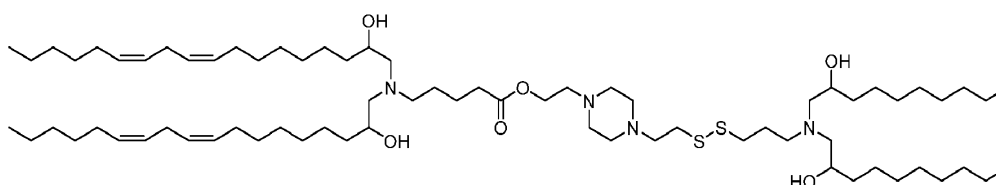
[0349] Analytical data for 2-(4-(2-((4-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydecyl)amino)pentanoate (GL-HEPES-E4-E10-DS-4-E18-2)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 8H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.87-2.35 (m, 34H), 2.03 (m, 8H), 1.61-1.30 (m, 68H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₇₃H₁₄₀N₄O₆S₂, [M+H] = 1234.0, Observed = 1234.0.

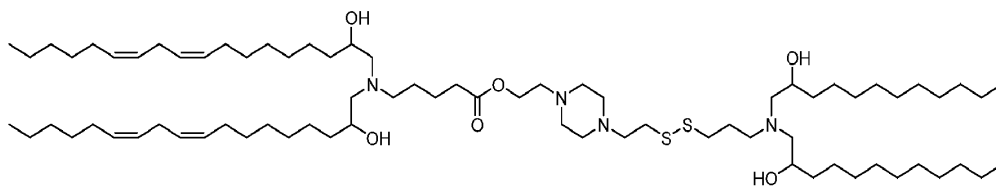
[0350] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-2-DS-3-E10)



^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 8H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.87-2.35 (m, 34H), 2.06 (m, 8H), 1.87 (quint, 2H) 1.68-1.32 (m, 64H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{138}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1220.0$, Observed = 1220.0.

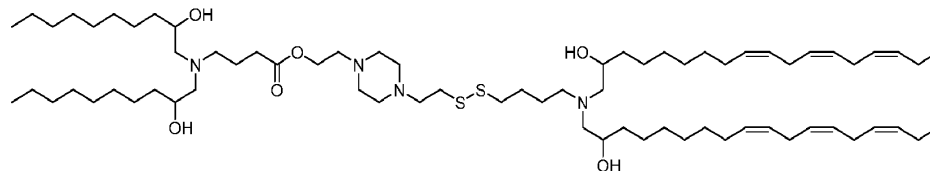
[0351] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-2-DS-3-E12)



^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 8H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.87-2.35 (m, 34H), 2.06 (m, 8H), 1.87 (quint, 2H) 1.68-1.32 (m, 72H), 0.90 (t, 12H).

APCI-MS analysis: Calculated $\text{C}_{76}\text{H}_{146}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1276.1$, Observed = 1276.1.

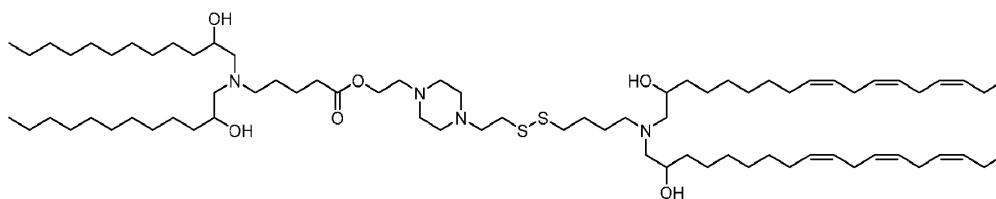
[0352] Analytical data for 2-(4-(2-((4-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate (GL-HEPES-E3-E10-DS-4-E18-3)



^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 12H), 4.22 (t, 2H), 3.66 (s, br., 4H), 2.83-2.37 (m, 38H), 2.09 (m, 8H), 1.78 (m, 4H), 1.63 (m, 2H), 1.47-1.30 (m, 48H), 1.00-0.88 (m, 12H).

APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{134}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1216.0$, Observed = 1216.0.

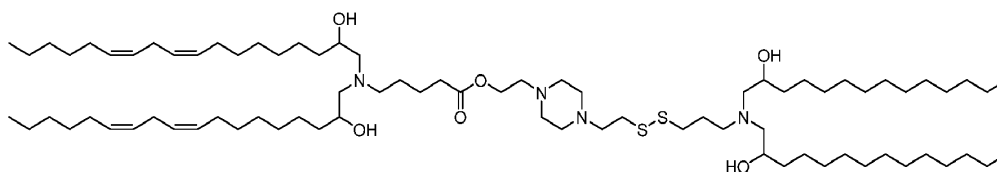
[0353] Analytical data for 2-(4-(2-((4-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-DS-4-E18-3)



^1H NMR (300 MHz, Methanol- d_4) δ 5.36 (m, 12H), 4.22 (t, 2H), 3.68 (s, br., 4H), 2.85-2.35 (m, 38H), 2.06 (m, 8H), 1.72-1.30 (m, 64H), 1.00-0.88 (m, 12H).

APCI-MS analysis: Calculated $\text{C}_{77}\text{H}_{144}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1286.0$, Observed = 1286.1.

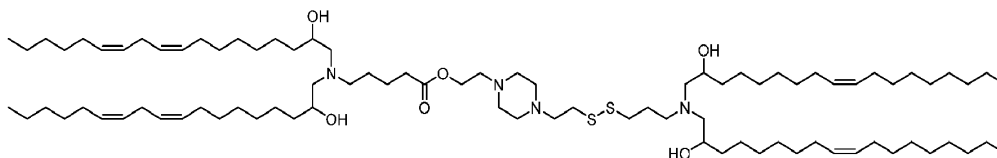
[0354] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-2-DS-3-E14)



^1H NMR (300 MHz, Methanol- d_4) δ 5.34 (m, 8H), 4.22 (t, 2H), 3.68 (s, br., 4H), 2.85-2.35 (m, 34H), 2.06 (m, 8H), 1.87 (t, 2H), 1.65-1.30 (m, 80H), 0.90 (m, 12H).

APCI-MS analysis: Calculated $\text{C}_{80}\text{H}_{154}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1332.1$, Observed = 1332.1.

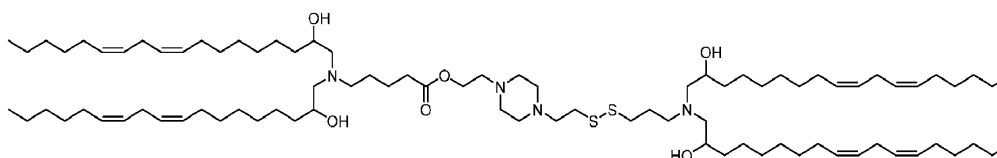
[0355] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-2-DS-3-E18-1)



^1H NMR (300 MHz, Methanol- d_4) δ 5.34 (m, 12H), 4.22 (t, 2H), 3.68 (s, br., 4H), 2.85-2.35 (m, 34H), 2.06 (m, 16H), 1.87 (t, 2H), 1.65-1.30 (m, 80H), 0.90 (m, 12H).

APCI-MS analysis: Calculated $\text{C}_{88}\text{H}_{166}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1440.2$, Observed = 1440.2.

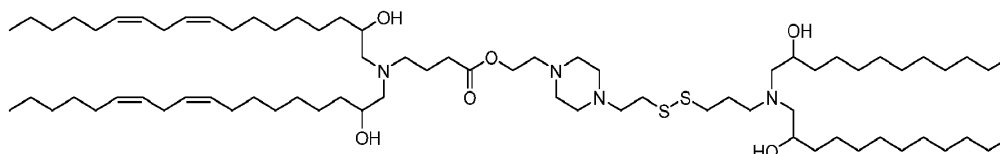
[0356] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-2-DS-3-E18-2)



¹H NMR (300 MHz, Methanol-d₄) δ 5.36 (m, 16H), 4.22 (t, 2H), 3.66 (s, br., 4H), 2.85-2.35 (m, 38H), 2.06 (m, 16H), 1.87 (t, 2H), 1.65-1.30 (m, 68H), 0.90 (m, 12H).

APCI-MS analysis: Calculated C₈₈H₁₆₂N₄O₆S₂, [M+H] = 1436.2, Observed = 1436.2.

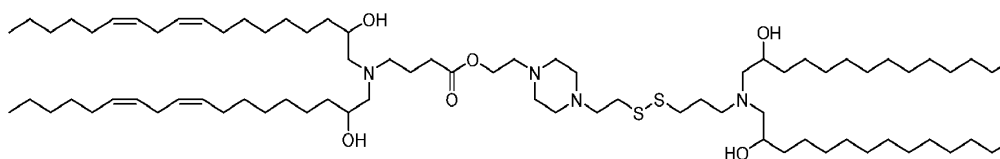
[0357] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanoate (GL-HEPES-E3-E18-2-DS-3-E12)



¹H NMR (300 MHz, Methanol-d₄) δ 5.36 (m, 8H), 4.22 (t, 2H), 3.66 (s, br., 4H), 2.85-2.35 (m, 34H), 2.06 (m, 8H), 1.87-1.65 (m, 4H), 1.48-1.30 (m, 68H), 0.91 (m, 12H).

APCI-MS analysis: Calculated C₇₅H₁₄₄N₄O₆S₂, [M+H] = 1262.1, Observed = 1262.1.

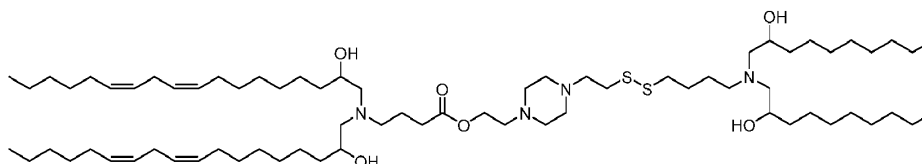
[0358] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanoate (GL-HEPES-E3-E18-2-DS-3-E14)



¹H NMR (300 MHz, Methanol-d₄) δ 5.36 (m, 8H), 4.22 (t, 2H), 3.66 (s, br., 4H), 2.85-2.35 (m, 34H), 2.06 (m, 8H), 1.87-1.65 (m, 4H), 1.48-1.30 (m, 76H), 0.91 (m, 12H).

APCI-MS analysis: Calculated C₇₉H₁₅₂N₄O₆S₂, [M+H] = 1318.1, Observed = 1318.1.

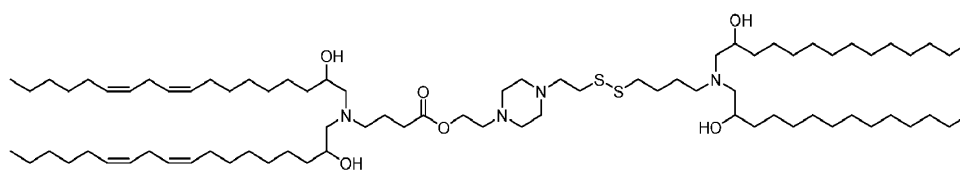
[0359] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanoate (GL-HEPES-E3-E18-2-DS-4-E10)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 8H), 4.22 (t, 2H), 3.66 (s, br., 4H), 2.83-2.37 (m, 34H), 2.09 (m, 8H), 1.78 (m, 4H), 1.63 (m, 2H), 1.62-1.30 (m, 60H), 0.91 (m, 12H).

APCI-MS analysis: Calculated C₇₂H₁₃₈N₄O₆S₂ [M+H] = 1220.0, Observed = 1219.9.

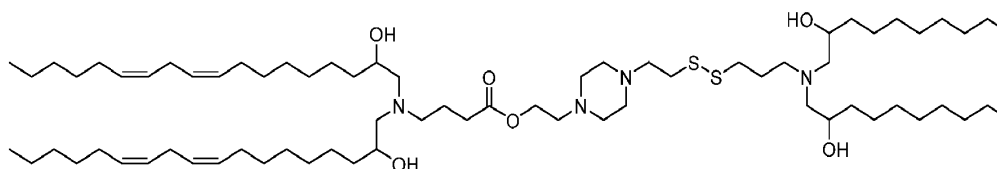
[0360] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxytetradecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanoate (GL-HEPES-E3-E18-2-DS-4-E14)



¹H NMR (300 MHz, Methanol-d₄) δ 5.36 (m, 8H), 4.23 (t, 2H), 3.64 (s, br., 4H), 2.85-2.37 (m, 34H), 2.09 (m, 8H), 1.78 (m, 4H), 1.63-1.30 (m, 78H), 0.91 (m, 12H).

APCI-MS analysis: Calculated C₈₀H₁₅₄N₄O₆S₂ [M+H] = 1332.1, Observed = 1332.1.

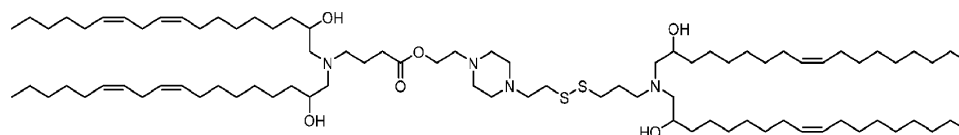
[0361] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanoate (GL-HEPES-E3-E18-2-DS-3-E10)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 8H), 4.22 (t, 2H), 3.66 (s, br., 4H), 2.85-2.37 (m, 34H), 2.09 (m, 8H), 1.92-1.77 (m, 4H), 1.47-1.30 (m, 60H), 0.91 (t, 12H).

[0362] APCI-MS analysis: Calculated C₇₁H₁₃₆N₄O₆S₂ [M+H] = 1206.0, Observed = 1206.0.

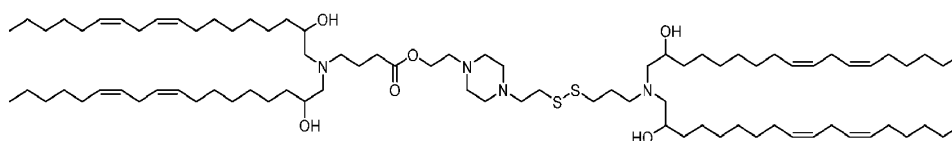
[0363] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanoate (GL-HEPES-E3-E18-2-DS-3-E18-1)



¹H NMR (300 MHz, Methanol-d₄) δ 5.36 (m, 12H), 4.23 (t, 2H), 3.66 (s, br., 4H), 2.83-2.37 (m, 34H), 2.08 (m, 16H), 1.95-1.78 (m, 4H), 1.47-1.30 (m, 76H), 0.91 (t, 12H).

APCI-MS analysis: Calculated C₈₇H₁₆₄N₄O₆S₂ [M+H] = 1426.2, Observed = 1426.2.

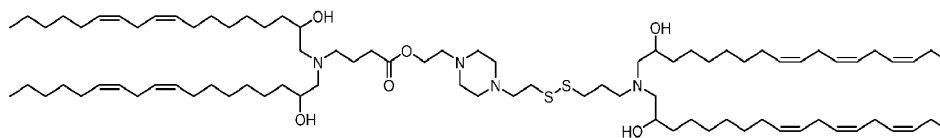
[0364] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanoate (GL-HEPES-E3-E18-2-DS-3-E18-2)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 16H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.85-2.37 (m, 38H), 2.08 (m, 16H), 1.90-1.75 (m, 4H), 1.47-1.30 (m, 64H), 0.91 (m, 12H).

APCI-MS analysis: Calculated C₈₇H₁₆₀N₄O₆S₂ [M+H] = 1422.2, Observed = 1422.1.

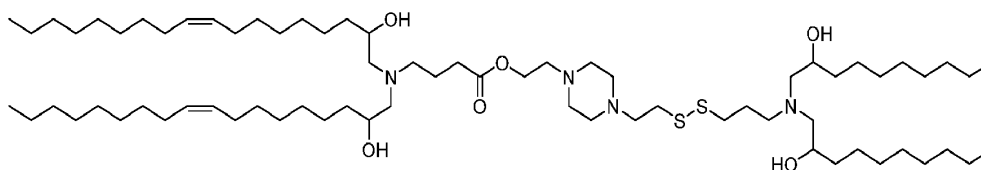
[0365] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanoate (GL-HEPES-E3-E18-2-DS-3-E18-3)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 20H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.84-2.37 (m, 42H), 2.09 (m, 16H), 1.87-1.77 (m, 4H), 1.47-1.30 (m, 52H), 1.00-0.88 (m, 12H).

APCI-MS analysis: Calculated C₈₇H₁₅₆N₄O₆S₂ [M+H] = 1418.1, Observed = 1418.2.

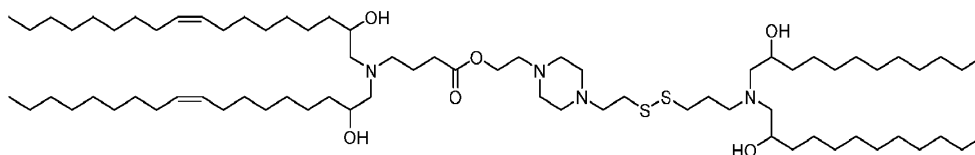
[0366] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butanoate (GL-HEPES-E3-E18-1-DS-3-E10)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 4H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.83-2.35 (m, 30H), 2.08 (m, 16H), 1.87-1.77 (m, 4H) 1.46-1.30 (m, 72H), 0.90 (m, 12H).

APCI-MS analysis: Calculated C₈₉H₁₆₀N₄O₆S₂ [M+H] = 1210.0, Observed = 1210.0.

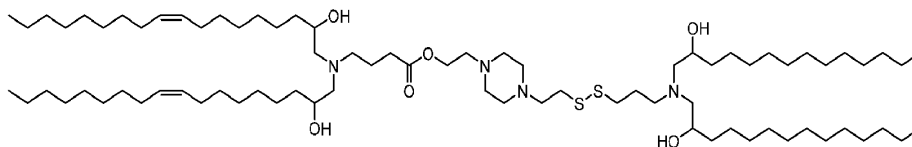
[0367] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butanoate (GL-HEPES-E3-E18-1-DS-3-E12)



¹H NMR (300 MHz, Methanol-d₄) δ 5.33 (m, 4H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.86-2.38 (m, 30H), 2.01 (m, 8H), 1.90-1.74 (m, 4H), 1.47-1.30 (m, 80H), 0.89 (t, 12H).

APCI-MS analysis: Calculated C₇₅H₁₄₈N₄O₆S₂ [M+H] = 1266.2, Observed = 1266.1.

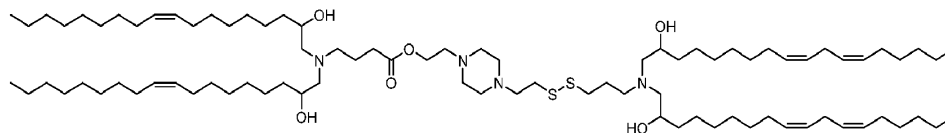
[0368] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butanoate (GL-HEPES-E3-E18-1-DS-3-E14)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 4H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.85-2.39 (m, 30H), 2.04 (m, 16H), 1.87-1.77 (m, 4H) 1.46-1.30 (m, 88H), 0.90 (m, 12H).

APCI-MS analysis: Calculated C₈₉H₁₆₀N₄O₆S₂ [M+H] = 1322.1, Observed = 1322.2.

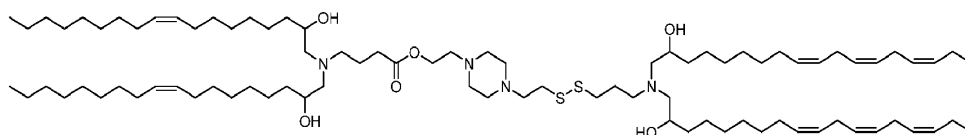
[0369] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butanoate (GL-HEPES-E3-E18-1-DS-3-E18-2)



¹H NMR (300 MHz, Methanol-d₄) δ 5.32 (m, 12H), 4.21 (t, 2H), 3.62 (s, br., 4H), 2.85-2.37 (m, 34H), 2.09 (m, 16H), 1.92-1.77 (m, 4H), 1.47-1.30 (m, 76H), 0.88 (t, 12H).

APCI-MS analysis: Calculated C₈₇H₁₆₄N₄O₆S₂ [M+H] = 1426.2, Observed = 1426.2.

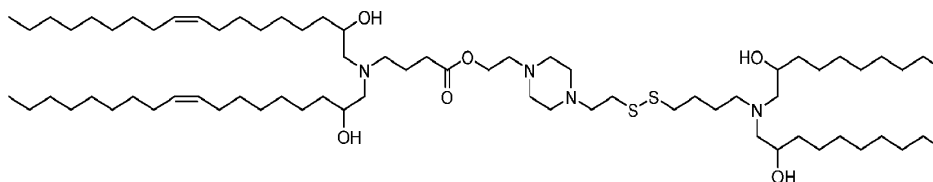
[0370] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butanoate (GL-HEPES-E3-E18-1-DS-3-E18-3)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 16H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.88-2.37 (m, 38H), 2.09 (m, 16H), 1.92-1.77 (m, 4H), 1.47-1.30 (m, 64H), 1.02-0.87 (m, 12H).

APCI-MS analysis: Calculated C₈₇H₁₆₀N₄O₆S₂ [M+H] = 1422.2, Observed = 1422.2.

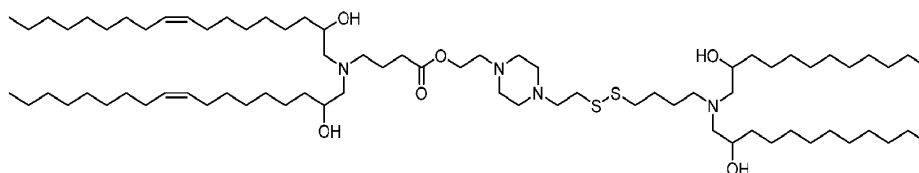
[0371] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butanoate (GL-HEPES-E3-E18-1-DS-4-E10)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 4H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.86-2.37 (m, 30H), 2.03 (m, 8H), 1.82-1.78 (m, 4H), 1.60-1.30 (m, 74H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₇₂H₁₄₂N₄O₆S₂ [M+H] = 1224.1, Observed = 1224.1.

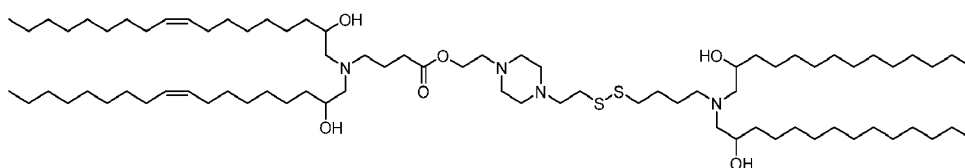
[0372] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butanoate (GL-HEPES-E3-E18-1-DS-4-E12)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 4H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.88-2.37 (m, 30H), 2.03 (m, 8H), 1.82-1.78 (m, 4H), 1.60-1.30 (m, 82H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₇₄H₁₅₀N₄O₆S₂ [M+H] = 1280.2, Observed = 1280.2.

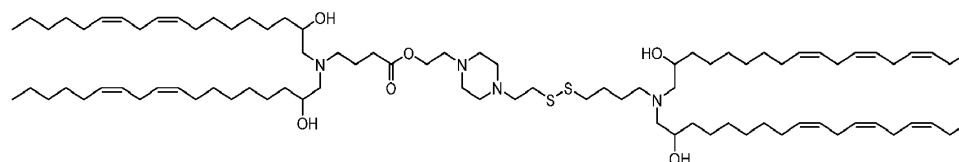
[0373] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxytetradecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butanoate (GL-HEPES-E3-E18-1-DS-4-E14)



¹H NMR (300 MHz, Methanol-d₄) δ 5.33 (m, 4H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.85-2.37 (m, 30H), 2.01 (m, 8H), 1.82-1.78 (m, 4H), 1.60-1.30 (m, 90H), 0.89 (t, 12H).

APCI-MS analysis: Calculated C₈₀H₁₅₈N₄O₆S₂ [M+H] = 1336.2, Observed = 1336.1.

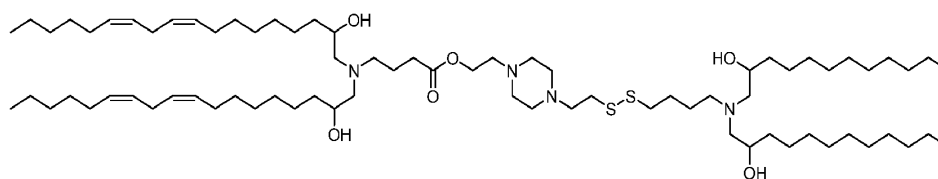
[0374] Analytical data for 2-(4-(2-((4-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanoate (GL-HEPES-E3-E18-2-DS-4-E18-3)



¹H NMR (300 MHz, Methanol-d₄) δ 5.36 (m, 20H), 4.23 (t, 2H), 3.64 (s, br., 4H), 2.85-2.37 (m, 42H), 2.09 (m, 16H), 1.78 (m, 4H), 1.60-1.35 (m, 54H), 1.00-0.88 (m, 12H).

APCI-MS analysis: Calculated C₈₈H₁₅₈N₄O₆S₂ [M+H] = 1432.2, Observed = 1432.1.

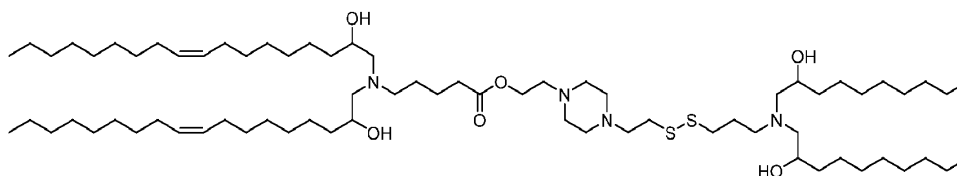
[0375] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanoate (GL-HEPES-E3-E18-2-DS-4-E12)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 8H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.83-2.37 (m, 34H), 2.08 (m, 8H), 1.74 (m, 4H) 1.59-1.30 (m, 70H), 0.90 (m, 12H).

APCI-MS analysis: Calculated C₇₆H₁₄₆N₄O₆S₂ [M+H] = 1276.1, Observed = 1276.0.

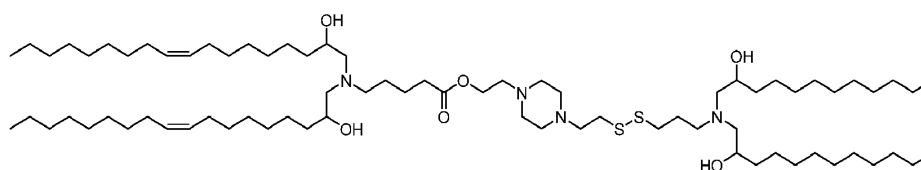
[0376] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydeacyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentanoate (GL-HEPES-E4-E18-1-DS-3-E10)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 4H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.86-2.37 (m, 30H), 2.03 (m, 8H), 1.86 (quint., 2H), 1.65-1.30 (m, 76H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₇₂H₁₄₂N₄O₆S₂ [M+H] = 1224.1, Observed = 1224.1.

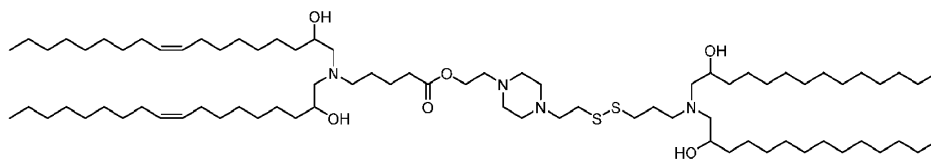
[0377] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentanoate (GL-HEPES-E4-E18-1-DS-3-E12)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 4H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.86-2.37 (m, 30H), 2.03 (m, 8H), 1.86 (quint., 2H), 1.65-1.30 (m, 84H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₇₆H₁₅₀N₄O₆S₂ [M+H] = 1280.2, Observed = 1280.1.

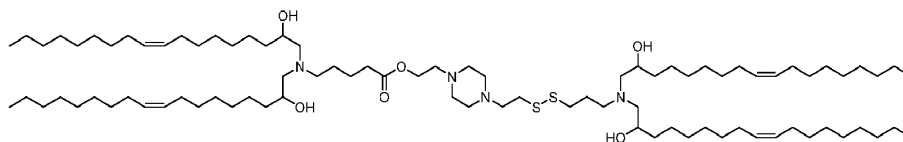
[0378] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentanoate (GL-HEPES-E4-E18-1-DS-3-E14)



¹H NMR (300 MHz, Methanol-d₄) δ 5.33 (m, 4H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.86-2.36 (m, 30H), 2.01 (m, 8H), 1.86 (quint., 2H), 1.64-1.30 (m, 92H), 0.89 (t, 12H).

APCI-MS analysis: Calculated C₈₀H₁₅₈N₄O₆S₂ [M+H] = 1336.2, Observed = 1336.2.

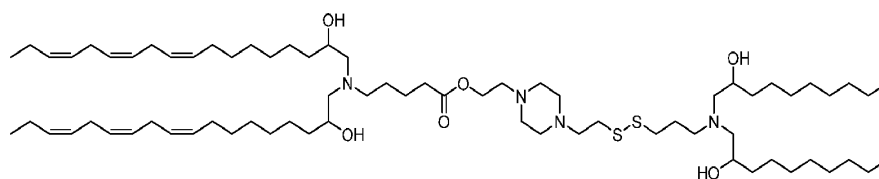
[0379] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentanoate (GL-HEPES-E4-E18-1-DS-3-E18-1)



¹H NMR (300 MHz, Methanol-d₄) δ 5.33 (m, 8H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.86-2.36 (m, 30H), 2.02 (m, 16H), 1.86 (quint., 2H), 1.64-1.30 (m, 92H), 0.89 (t, 12H).

APCI-MS analysis: Calculated C₈₈H₁₇₀N₄O₆S₂ [M+H] = 1444.3, Observed = 1444.3.

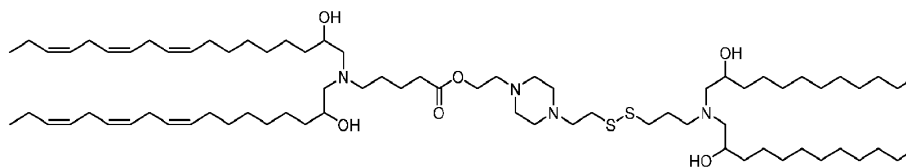
[0380] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-3-DS-3-E10)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 12H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.86-2.33 (m, 38H), 2.09 (m, 8H), 1.86 (quint., 2H), 1.65-1.31 (m, 52H), 1.01-0.87 (m, 12H).

APCI-MS analysis: Calculated C₇₂H₁₃₄N₄O₆S₂ [M+H] = 1216.0, Observed = 1216.0.

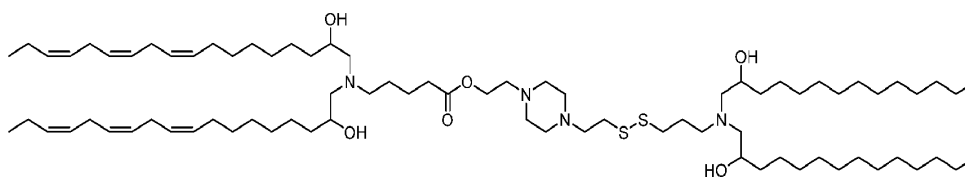
[0381] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-3-DS-3-E12)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 12H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.86-2.33 (m, 38H), 2.09 (m, 8H), 1.86 (quint., 2H), 1.65-1.31 (m, 60H), 1.01-0.87 (m, 12H).

APCI-MS analysis: Calculated C₇₆H₁₄₂N₄O₆S₂ [M+H] = 1272.0, Observed = 1272.1.

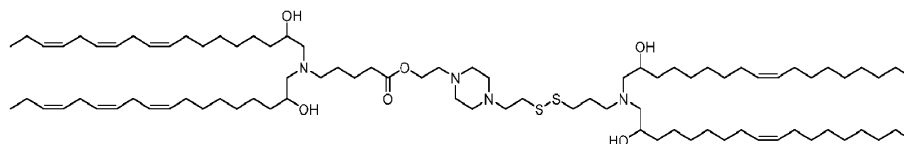
[0382] Analytical data for 2-(4-(2-((3-(Bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-3-DS-3-E14)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 12H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.86-2.33 (m, 38H), 2.09 (m, 8H), 1.86 (quint., 2H), 1.65-1.29 (m, 68H), 1.01-0.87 (m, 12H).

APCI-MS analysis: Calculated C₈₀H₁₅₀N₄O₆S₂ [M+H] = 1328.1, Observed = 1328.1.

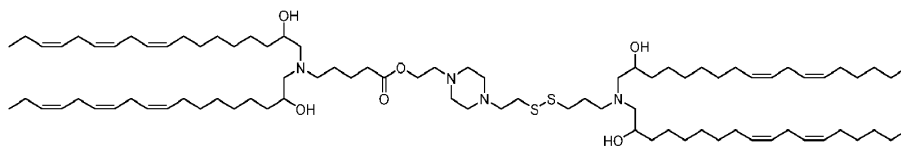
[0383] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-3-DS-3-E18-1)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 16H), 4.21 (t, 2H), 3.62 (s, br., 4H), 2.81-2.33 (m, 38H), 2.05 (m, 16H), 1.86 (quint., 2H), 1.64-1.27 (m, 68H), 1.01-0.87 (m, 12H).

APCI-MS analysis: Calculated C₈₈H₁₆₂N₄O₆S₂ [M+H] = 1436.2, Observed = 1436.2.

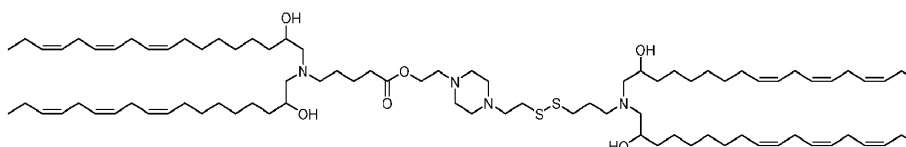
- [0384]** Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-3-DS-3-E18-2)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 20H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.86-2.33 (m, 42H), 2.09 (m, 16H), 1.86 (quint., 2H), 1.62-1.32 (m, 56H), 1.00-0.87 (m, 12H).

APCI-MS analysis: Calculated C₈₈H₁₅₈N₄O₆S₂ [M+H] = 1432.2, Observed = 1432.2.

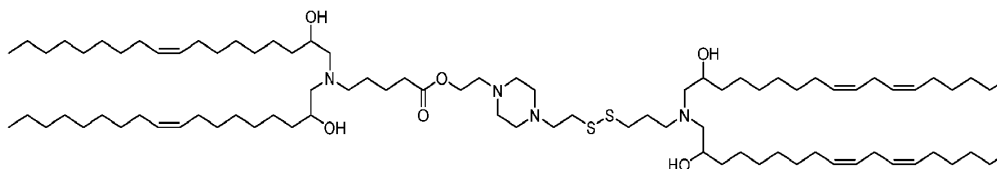
- [0385]** Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-3-DS-3-E18-3)



¹H NMR (300 MHz, Chloroform-d) δ 5.37 (m, 24H), 4.22 (t, 2H), 3.64 (s, br., 4H), 2.82-2.33 (m, 46H), 2.05 (m, 16H), 1.86 (quint., 2H), 1.62-1.32 (m, 44H), 0.97 (t, 12H).

APCI-MS analysis: Calculated C₈₈H₁₅₄N₄O₆S₂ [M+H] = 1428.2, Observed = 1428.2.

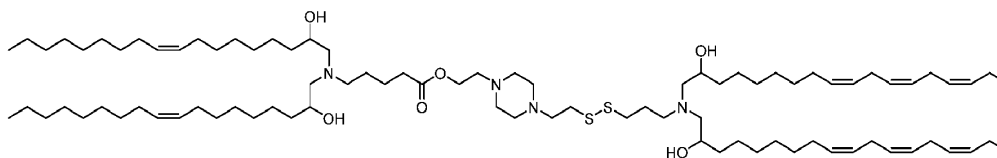
- [0386]** Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentanoate (GL-HEPES-E4-E18-1-DS-3-E18-2)



¹H NMR (300 MHz, Chloroform-d) δ 5.35 (m, 12H), 4.19 (t, 2H), 3.63 (s, br., 4H), 2.86-2.36 (m, 34H), 2.03 (m, 16H), 1.86 (quint., 2H), 1.61-1.30 (m, 80H), 0.88 (t, 12H).

APCI-MS analysis: Calculated C₈₈H₁₆₆N₄O₆S₂ [M+H] = 1440.2, Observed = 1440.3.

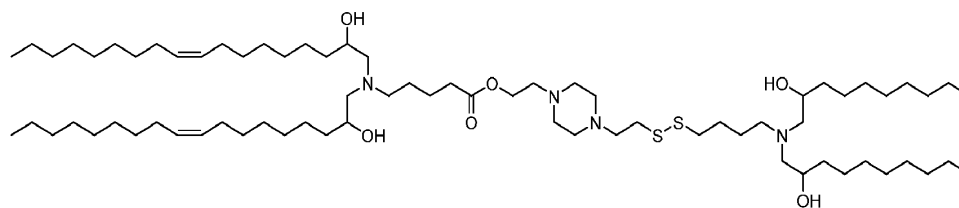
[0387] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentanoate (GL-HEPES-E4-E18-1-DS-3-E18-3)



¹H NMR (300 MHz, Chloroform-d) δ 5.36 (m, 16H), 4.19 (t, 2H), 3.61 (s, br., 4H), 2.86-2.36 (m, 38H), 2.01 (m, 16H), 1.86 (quint., 2H), 1.61-1.30 (m, 80H), 0.97 (t, 6H), 0.88 (t, 6H).

APCI-MS analysis: Calculated C₈₈H₁₆₂N₄O₆S₂ [M+H] = 1436.2, Observed = 1436.2.

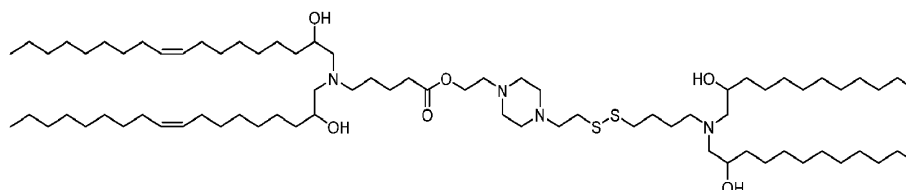
[0388] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentanoate (GL-HEPES-E4-E18-1-DS-4-E10)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 4H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.86-2.36 (m, 30H), 2.03 (m, 8H), 1.73-1.30 (m, 80H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₇₃H₁₄₄N₄O₆S₂ [M+H] = 1238.1, Observed = 1238.1.

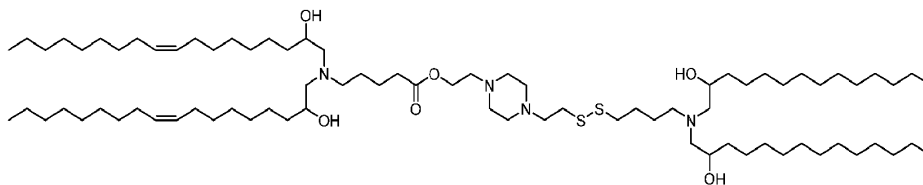
[0389] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentanoate (GL-HEPES-E4-E18-1-DS-4-E12)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 4H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.86-2.36 (m, 30H), 2.03 (m, 8H), 1.73-1.30 (m, 88H), 0.90 (t, 12H).

APCI-MS analysis: Calculated C₇₇H₁₅₂N₄O₆S₂ [M+H] = 1294.1, Observed = 1294.1.

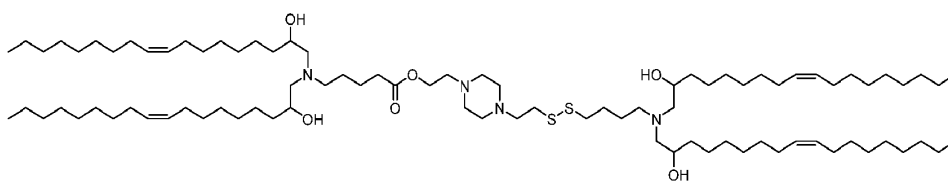
[0390] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxytetradecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentanoate (GL-HEPES-E4-E18-1-DS-4-E14)



¹H NMR (300 MHz, Chloroform-d) δ 5.34 (m, 4H), 4.19 (t, 2H), 3.63 (s, br., 4H), 2.86-2.33 (m, 30H), 2.00 (m, 8H), 1.70-1.25 (m, 96H), 0.88 (t, 12H).

APCI-MS analysis: Calculated C₈₁H₁₆₀N₄O₆S₂ [M+H] = 1350.2, Observed = 1350.2.

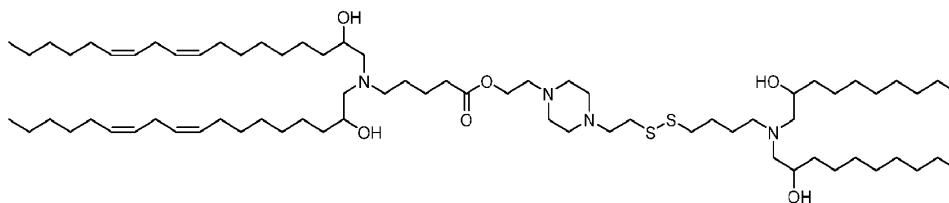
[0391] Analytical data for 2-(4-(2-((4-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentanoate (GL-HEPES-E4-E18-1-DS-4-E18-1)



¹H NMR (300 MHz, Chloroform-d) δ 5.34 (m, 8H), 4.19 (t, 2H), 3.62 (s, br., 4H), 2.83-2.31 (m, 30H), 2.00 (m, 16H), 1.70-1.26 (m, 96H), 0.88 (t, 12H).

APCI-MS analysis: Calculated C₈₉H₁₇₂N₄O₆S₂ [M+H] = 1458.3, Observed = 1458.3.

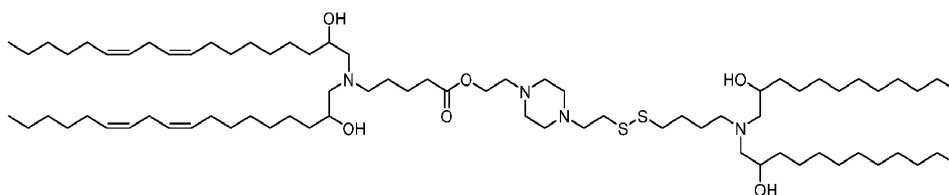
[0392] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-2-DS-4-E10)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 8H), 4.23 (t, 2H), 3.63 (s, br., 4H), 2.85-2.35 (m, 34H), 2.08 (m, 8H), 1.78 (m, 4H), 1.75-1.35 (m, 64H), 0.90 (m, 12H).

APCI-MS analysis: Calculated C₇₃H₁₄₀N₄O₆S₂ [M+H] = 1234.0, Observed = 1233.9.

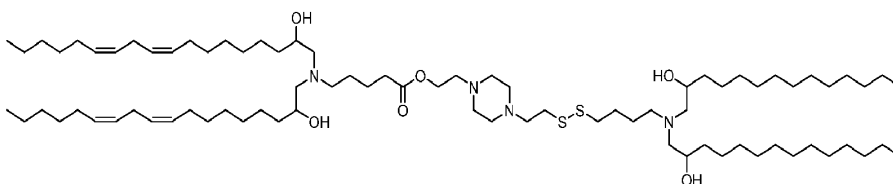
[0393] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-2-DS-4-E12)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 8H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.87-2.34 (m, 34H), 2.08 (m, 8H), 1.74-1.30 (m, 76H), 0.90 (m, 12H).

APCI-MS analysis: Calculated C₇₇H₁₄₈N₄O₆S₂ [M+H] = 1290.1, Observed = 1290.1.

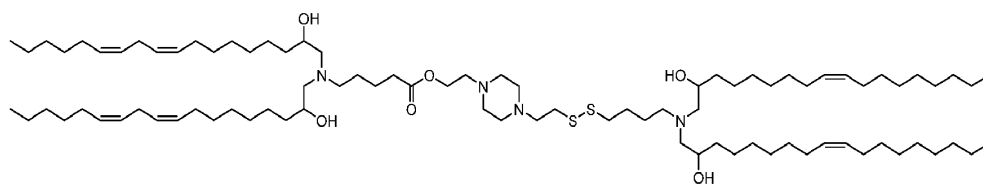
[0394] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxytetradecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-2-DS-4-E14)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 8H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.87-2.34 (m, 34H), 2.08 (m, 8H), 1.74-1.30 (m, 84H), 0.90 (m, 12H).

APCI-MS analysis: Calculated C₈₁H₁₅₆N₄O₆S₂ [M+H] = 1356.1, Observed = 1356.1.

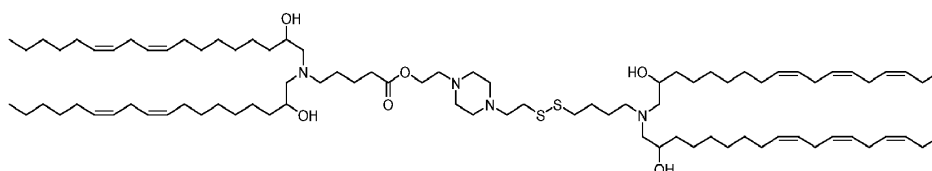
[0395] Analytical data for 2-(4-(2-((4-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-2-DS-4-E18-1)



¹H NMR (300 MHz, Methanol-d₄) δ 5.33 (m, 12H), 4.21 (t, 2H), 3.61 (s, br., 4H), 2.85-2.37 (m, 34H), 2.08 (m, 16H), 1.73-1.30 (m, 84H), 0.88 (m, 12H).

APCI-MS analysis: Calculated C₈₉H₁₆₈N₄O₆S₂ [M+H] = 1454.2, Observed = 1454.2.

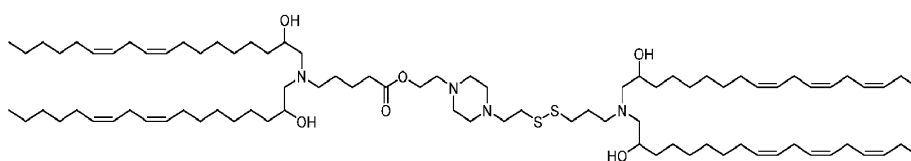
[0396] Analytical data for 2-(4-(2-((4-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-2-DS-4-E18-3)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 20H), 4.21 (t, 2H), 3.65 (s, br., 4H), 2.83-2.35 (m, 42H), 2.08 (m, 16H), 1.65-1.35 (m, 64H), 1.00-0.88 (m, 12H).

APCI-MS analysis: Calculated C₈₉H₁₆₀N₄O₆S₂ [M+H] = 1446.2, Observed = 1446.2.

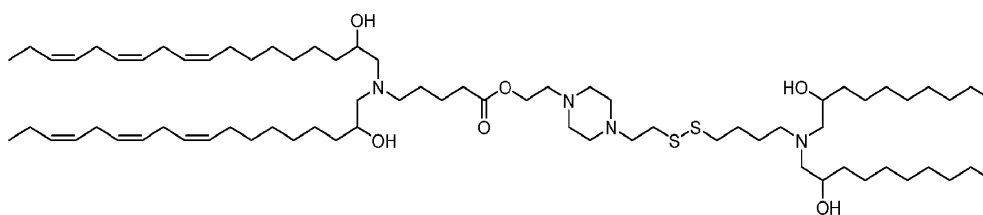
[0397] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-2-DS-3-E18-3)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 20H), 4.21 (t, 2H), 3.63 (s, br., 4H), 2.87-2.34 (m, 42H), 2.09 (m, 16H), 1.86 (m, 2H), 1.62-1.32 (m, 62H), 1.00-0.88 (m, 12H).

APCI-MS analysis: Calculated C₈₈H₁₅₈N₄O₆S₂ [M+H] = 1432.2, Observed = 1432.1.

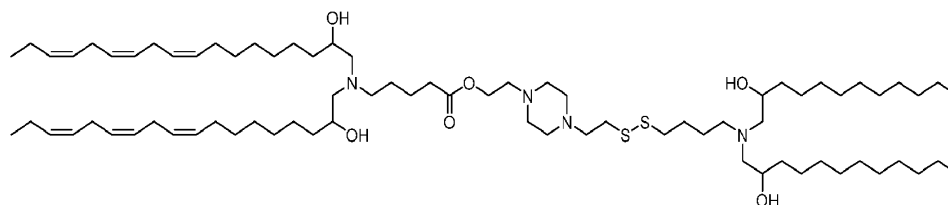
[0398] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-3-DS-4-E10)



¹H NMR (300 MHz, Chloroform-d) δ 5.35 (m, 12H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.83-2.33 (m, 38H), 2.09 (m, 8H), 1.75-1.31 (m, 56H), 1.00-0.87 (m, 12H).

APCI-MS analysis: Calculated C₇₃H₁₃₆N₄O₆S₂ [M+H] = 1231.0, Observed = 1231.1.

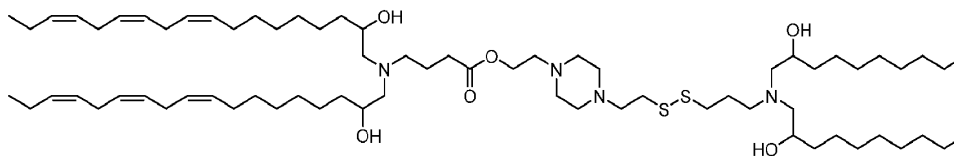
[0399] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-3-DS-4-E12)



¹H NMR (300 MHz, Chloroform-d) δ 5.34 (m, 12H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.83-2.35 (m, 38H), 2.09 (m, 8H), 1.75-1.29 (m, 64H), 1.00-0.87 (m, 12H).

APCI-MS analysis: Calculated C₇₇H₁₄₄N₄O₆S₂ [M+H] = 1268.1, Observed = 1268.1.

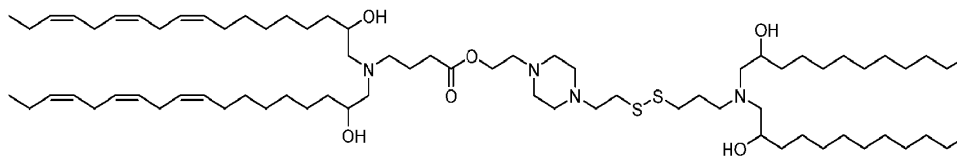
[0400] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butanoate (GL-HEPES-E3-E18-3-DS-3-E10)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 12H), 4.21 (t, 2H), 3.63 (s, br., 4H), 2.85-2.37 (m, 38H), 2.09 (m, 8H), 1.92-1.77 (m, 4H), 1.47-1.30 (m, 48H), 1.02-0.87 (m, 12H).

APCI-MS analysis: Calculated C₇₁H₁₃₂N₄O₆S₂ [M+H] = 1201.9, Observed = 1201.9.

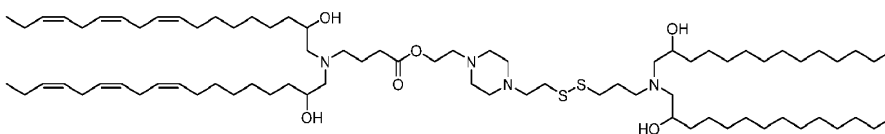
[0401] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydodecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butanoate (GL-HEPES-E3-E18-3-DS-3-E12)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 12H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.86-2.37 (m, 38H), 2.09 (m, 8H), 1.92-1.77 (m, 4H), 1.47-1.30 (m, 52H), 1.02-0.87 (m, 12H).

APCI-MS analysis: Calculated C₇₅H₁₄₀N₄O₆S₂ [M+H] = 1258.1, Observed = 1258.0.

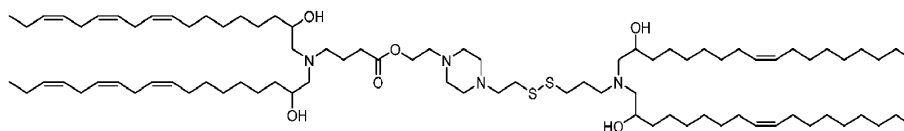
[0402] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxytetradecyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butanoate (GL-HEPES-E3-E18-3-DS-3-E14)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 12H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.86-2.37 (m, 38H), 2.09 (m, 8H), 1.88-1.76 (m, 4H), 1.60-1.30 (m, 64H), 1.02-0.87 (m, 12H).

APCI-MS analysis: Calculated C₇₉H₁₄₈N₄O₆S₂ [M+H] = 1314.2, Observed = 1314.1.

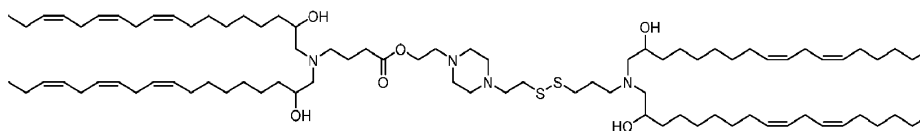
[0403] Analytical data for 2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butanoate (GL-HEPES-E3-E18-3-DS-3-E18-1)



¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 16H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.88-2.37 (m, 38H), 2.03 (m, 16H), 1.82-1.78 (m, 4H), 1.60-1.22 (m, 64H), 1.02-0.87 (m, 12H).

APCI-MS analysis: Calculated C₈₇H₁₆₀N₄O₆S₂ [M+H] = 1422.3, Observed = 1422.2.

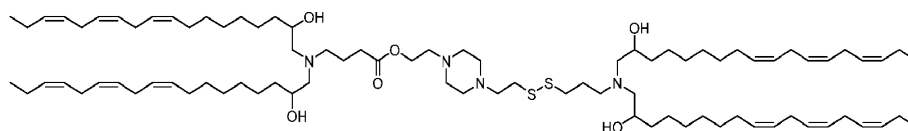
[0404] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butanoate (GL-HEPES-E3-E18-3-DS-3-E18-2)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 20H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.85-2.32 (m, 42H), 2.09 (m, 16H), 1.82-1.78 (m, 4H), 1.60-1.30 (m, 52H), 1.02-0.87 (m, 12H).

APCI-MS analysis: Calculated C₈₇H₁₅₆N₄O₆S₂ [M+H] = 1419.3, Observed = 1419.2.

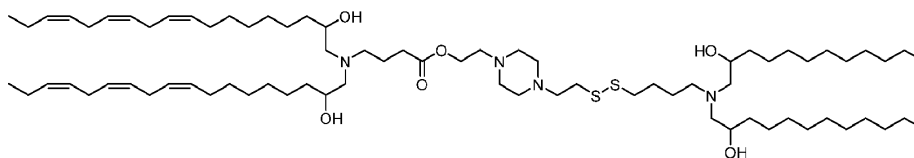
[0405] Analytical data for 2-(4-(2-((3-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butanoate (GL-HEPES-E3-E18-3-DS-3-E18-3)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 24H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.85-2.32 (m, 46H), 2.09 (m, 16H), 1.82-1.78 (m, 4H), 1.60-1.30 (m, 40H), 0.97 (t, 12H).

APCI-MS analysis: Calculated C₈₇H₁₅₂N₄O₆S₂ [M+H] = 1415.3, Observed = 1415.1.

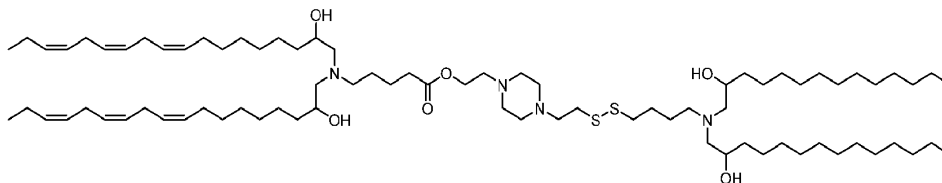
[0406] Analytical data for 2-(4-(2-((4-(Bis(2-hydroxydodecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butanoate (GL-HEPES-E3-E18-3-DS-4-E12)



¹H NMR (300 MHz, Methanol-d₄) δ 5.34 (m, 12H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.86-2.37 (m, 38H), 2.08 (m, 8H), 1.82 (m, 2H), 1.65-1.30 (m, 60H), 1.02-0.87 (m, 12H).

APCI-MS analysis: Calculated C₇₆H₁₄₂N₄O₆S₂ [M+H] = 1272.1, Observed = 1273.1.

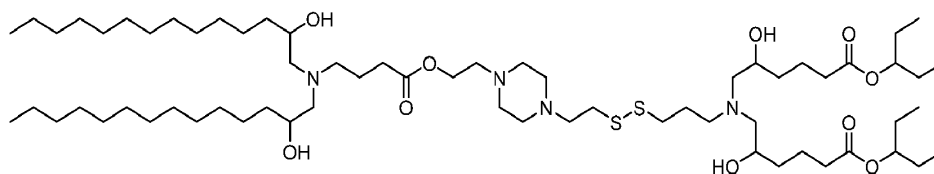
- [0407]** Analytical data for 2-(4-(2-((4-(Bis(2-hydroxytetradecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-3-DS-4-E14)



^1H NMR (300 MHz, Methanol- d_4) δ 5.34 (m, 12H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.86-2.38 (m, 38H), 2.08 (m, 8H), 1.77-1.25 (m, 72H), 1.02-0.87 (m, 12H).

APCI-MS analysis: Calculated $\text{C}_{81}\text{H}_{152}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1342.2$, Observed = 1343.2.

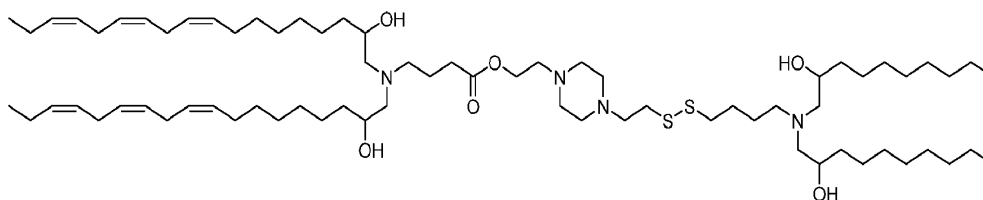
- [0408]** Analytical data for Di(pentan-3-yl) 6,6'-((3-((2-(4-(2-((4-(bis(2-hydroxytetradecyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(5-hydroxyhexanoate) (GL-HEPES-E3-E14-DS-3-E6-Ei5)



[0409] ^1H NMR (300 MHz, Methanol- d_4) δ 5.76 (quint., 2H), 4.22 (t, 2H), 3.66 (m, 4H), 2.86-2.36 (m, 32H), 1.93-1.25 (m, 66H), 0.89 (m, 18H).

[0410] APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{128}\text{N}_4\text{O}_{10}\text{S}_2$ $[\text{M}+\text{H}] = 1189.8$, Observed = 1189.8.

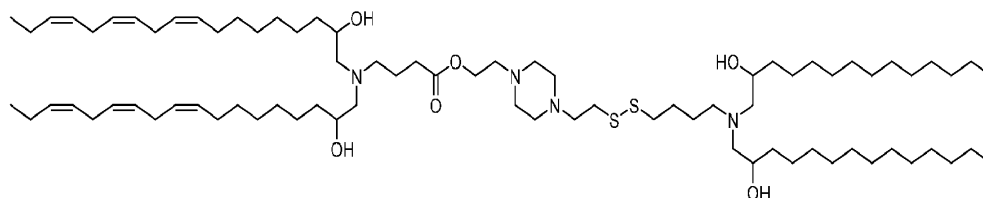
- 2-(4-(2-((4-(Bis(2-hydroxydecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butanoate (GL-HEPES-E3-E18-3-DS-4-E10)**



[0411] ^1H NMR (300 MHz, Methanol- d_4) δ 5.34 (m, 12H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.83-2.37 (m, 38H), 2.09 (m, 8H), 1.76 (m, 4H), 1.59-1.31 (m, 50H), 1.00-0.87 (m, 12H).

[0412] APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{134}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1216.0$, Observed = 1216.1.

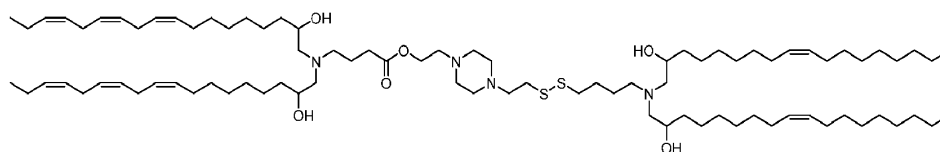
2-(4-(2-((4-(Bis(2-hydroxytetradecyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butanoate (GL-HEPES-E3-E18-3-DS-4-E14)



[0413] ^1H NMR (300 MHz, Methanol- d_4) δ 5.34 (m, 12H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.83-2.39 (m, 38H), 2.09 (m, 8H), 1.77 (m, 4H), 1.59-1.29 (m, 66H), 1.00-0.87 (m, 12H).

[0414] APCI-MS analysis: Calculated $\text{C}_{80}\text{H}_{150}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1328.1$, Observed = 1328.2.

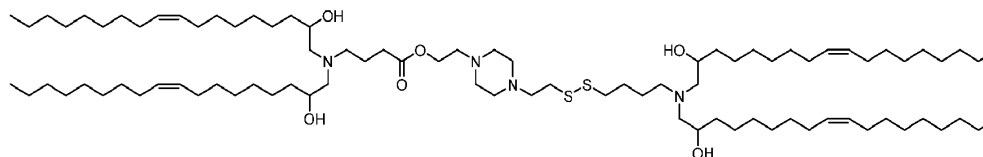
2-(4-(2-((4-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butanoate (GL-HEPES-E3-E18-3-DS-4-E18-1)



[0415] ^1H NMR (300 MHz, Methanol- d_4) δ 5.34 (m, 16H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.80-2.39 (m, 38H), 2.07 (m, 16H), 1.77 (m, 4H), 1.59-1.29 (m, 66H), 1.00-0.87 (m, 12H).

[0416] APCI-MS analysis: Calculated $\text{C}_{88}\text{H}_{162}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1435.2$, Observed = 1435.2.

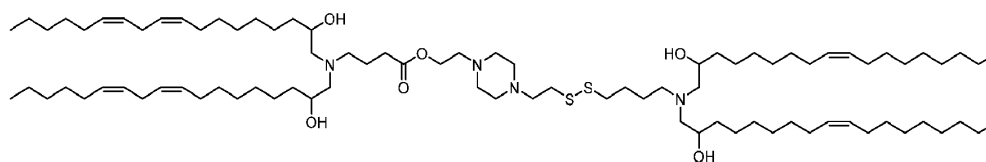
2-(4-(2-((4-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butanoate (GL-HEPES-E3-E18-1-DS-4-E18-1)



[0417] ^1H NMR (300 MHz, Methanol- d_4) δ 5.32 (m, 8H), 4.22 (t, 2H), 3.60 (s, br., 4H), 2.83-2.37 (m, 30H), 2.01 (m, 16H), 1.77 (m, 4H), 1.58-1.27 (m, 90H), 0.87 (t, 12H).

[0418] APCI-MS analysis: Calculated $\text{C}_{88}\text{H}_{170}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1444.3$, Observed = 1444.3.

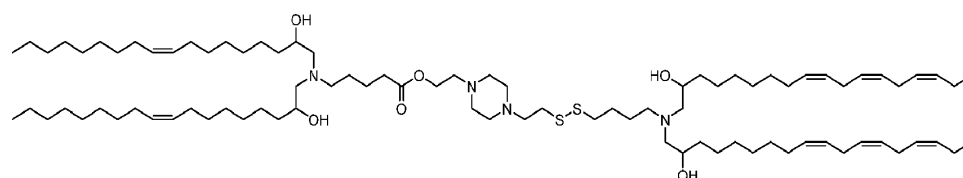
2-(4-(2-((4-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanoate (GL-HEPES-E3-E18-2-DS-4-E18-1)



[0419] ^1H NMR (300 MHz, Methanol- d_4) δ 5.33 (m, 12H), 4.22 (t, 2H), 3.61 (s, br., 4H), 2.83-2.35 (m, 34H), 2.05 (m, 16H), 1.77 (m, 4H), 1.58-1.28 (m, 78H), 0.87 (t, 12H).

[0420] APCI-MS analysis: Calculated $\text{C}_{88}\text{H}_{166}\text{N}_4\text{O}_6\text{S}_2$ [M+H] = 1440.2, Observed = 1440.2.

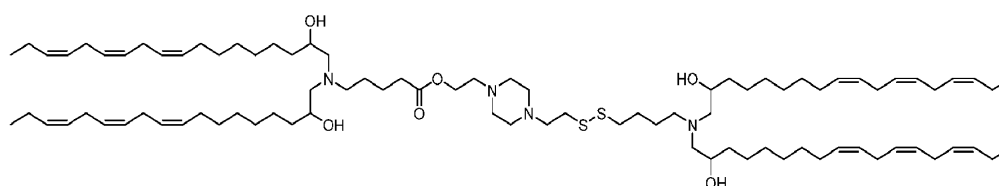
2-(4-(2-((4-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentanoate (GL-HEPES-E4-E18-1-DS-4-E18-3)



[0421] ^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 16H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.83-2.34 (m, 38H), 2.04 (m, 16H), 1.73-1.29 (m, 72H), 1.00-0.87 (m, 12H).

[0422] APCI-MS analysis: Calculated $\text{C}_{89}\text{H}_{164}\text{N}_4\text{O}_6\text{S}_2$ [M+H] = 1440.2, Observed = 1440.2.

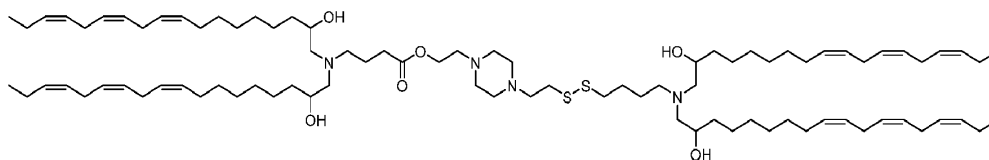
2-(4-(2-((4-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-3-DS-4-E18-3)



[0423] ^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 24H), 4.22 (t, 2H), 3.61 (s, br., 4H), 2.82-2.33 (m, 46H), 2.08 (m, 16H), 1.73-1.34 (m, 48H), 0.97 (t, 12H).

[0424] APCI-MS analysis: Calculated $\text{C}_{89}\text{H}_{156}\text{N}_4\text{O}_6\text{S}_2$ [M+H] = 1442.1, Observed = 1442.2.

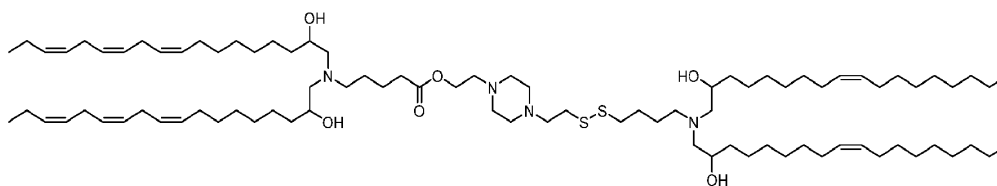
2-(4-(2-((4-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butanoate (GL-HEPES-E3-E18-3-DS-4-E18-3)



[0425] ^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 24H), 4.21 (t, 2H), 3.60 (s, br., 4H), 2.81-2.35 (m, 46H), 2.07 (m, 16H), 1.76 (m, 2H) 1.57-1.33 (m, 44H), 0.96 (t, 12H).

[0426] APCI-MS analysis: Calculated $\text{C}_{88}\text{H}_{154}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1428.1$, Observed = 1428.2.

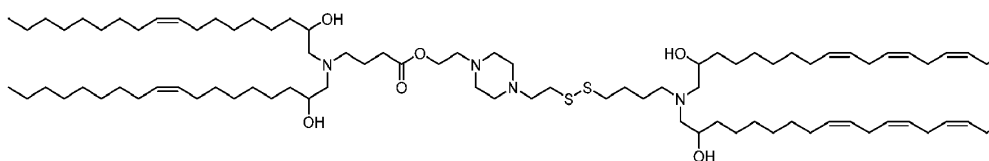
2-(4-(2-((4-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-3-DS-4-E18-1)



[0427] ^1H NMR (300 MHz, Methanol- d_4) δ 5.37 (m, 16H), 4.22 (t, 2H), 3.68 (s, br., 4H), 2.87-2.35 (m, 38H), 2.07 (m, 16H), 1.74-1.30 (m, 72H), 1.00-0.88 (m, 12H).

[0428] APCI-MS analysis: Calculated $\text{C}_{89}\text{H}_{164}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1450.2$, Observed = 1450.2.

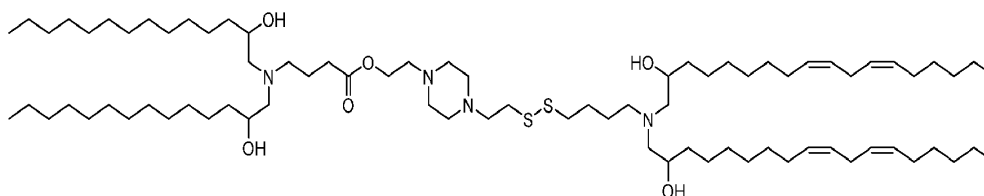
2-(4-(2-((4-(Bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butanoate (GL-HEPES-E3-E18-1-DS-4-E18-3)



[0429] ^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 16H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.87-2.38 (m, 38H), 2.07 (m, 16H), 1.74 (m, 4H) 1.60-1.30 (m, 66H), 1.00-0.88 (m, 12H).

[0430] APCI-MS analysis: Calculated $\text{C}_{88}\text{H}_{162}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1436.2$, Observed = 1436.2.

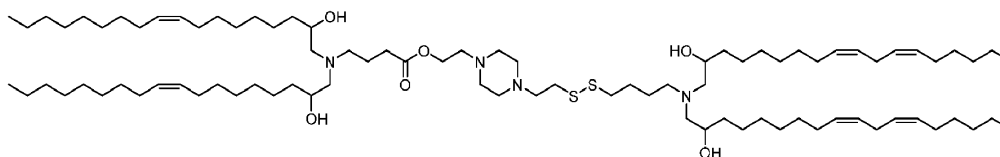
2-(4-(2-((4-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxytetradecyl)amino)butanoate (GL-HEPES-E3-E14-DS-4-E18-2)



[0431] ^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 8H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.83-2.35 (m, 34H), 2.08 (m, 8H), 1.79 (m, 3H) 1.63-1.29 (m, 79H), 0.91 (m, 12H).

[0432] APCI-MS analysis: Calculated $\text{C}_{80}\text{H}_{154}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1332.1$, Observed = 1332.1.

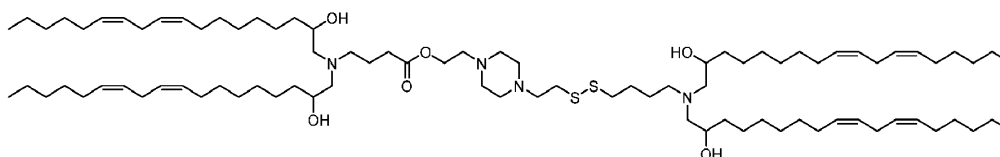
2-(4-(2-((4-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butanoate (GL-HEPES-E3-E18-1-DS-4-E18-2)



[0433] ^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 12H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.83-2.35 (m, 34H), 2.05 (m, 16H), 1.79 (m, 3H) 1.63-1.30 (m, 79H), 0.90 (t, 12H).

[0434] APCI-MS analysis: Calculated $\text{C}_{88}\text{H}_{166}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1440.2$, Observed = 1440.2.

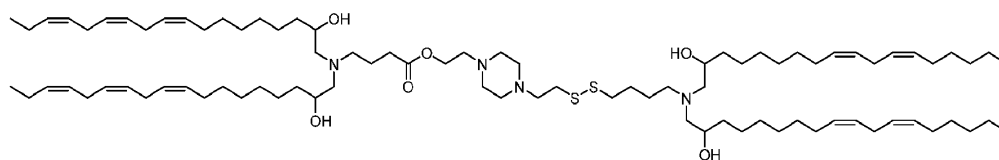
2-(4-(2-((4-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanoate (GL-HEPES-E3-E18-2-DS-4-E18-2)



[0435] ^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 16H), 4.22 (t, 2H), 3.64 (s, br., 4H), 2.85-2.35 (m, 38H), 2.08 (m, 16H), 1.80-1.32 (m, 70H), 0.91 (t, 12H).

[0436] APCI-MS analysis: Calculated $\text{C}_{88}\text{H}_{162}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1436.2$, Observed = 1436.2.

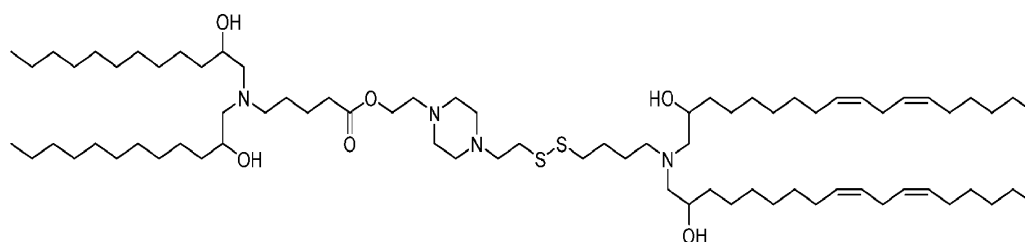
2-(4-(2-((4-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)butanoate (GL-HEPES-E3-E18-3-DS-4-E18-2)



[0437] ^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 20H), 4.22 (t, 2H), 3.62 (s, br., 4H), 2.83-2.35 (m, 42H), 2.08 (m, 16H), 1.75 (m, 3H), 1.58-1.35 (m, 55H), 1.01-0.89 (m, 12H).

[0438] APCI-MS analysis: Calculated $\text{C}_{88}\text{H}_{158}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1432.2$, Observed = 1432.2.

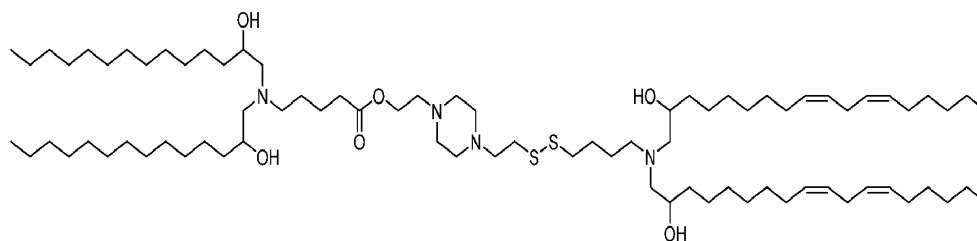
2-(4-(2-((4-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxydodecyl)amino)pentanoate (GL-HEPES-E4-E12-DS-4-E18-2)



[0439] ^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 8H), 4.22 (t, 2H), 3.63 (s, br., 4H), 2.85-2.33 (m, 34H), 2.08 (m, 8H), 1.71-1.30 (m, 76H), 0.90 (m, 12H).

[0440] APCI-MS analysis: Calculated $\text{C}_{77}\text{H}_{148}\text{N}_4\text{O}_6\text{S}_2$ $[\text{M}+\text{H}] = 1290.1$, Observed = 1290.1.

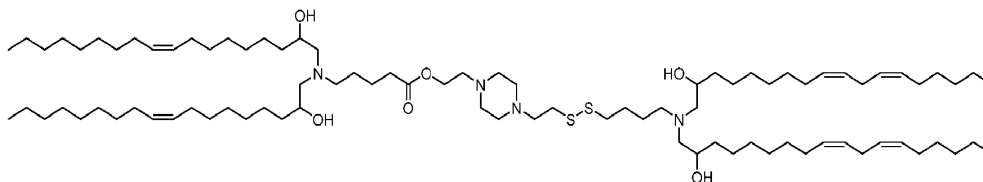
2-(4-(2-((4-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis(2-hydroxytetradecyl)amino)pentanoate (GL-HEPES-E4-E14-DS-4-E18-2)



[0441] ^1H NMR (300 MHz, Methanol- d_4) δ 5.35 (m, 8H), 4.22 (t, 2H), 3.64 (s, br., 4H), 2.85-2.33 (m, 34H), 2.08 (m, 8H), 1.71-1.30 (m, 84H), 0.90 (m, 12H).

[0442] APCI-MS analysis: Calculated C₈₁H₁₅₆N₄O₆S₂ [M+H] = 1346.1, Observed = 1346.2.

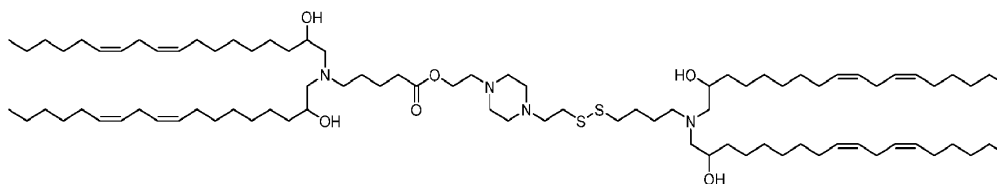
2-(4-(2-((4-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentanoate (GL-HEPES-E4-E18-1-DS-4-E18-2)



[0443] ¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 12H), 4.22 (t, 2H), 3.64 (s, br., 4H), 2.88-2.33 (m, 34H), 2.05 (m, 16H), 1.73-1.30 (m, 84H), 0.90 (m, 12H).

[0444] APCI-MS analysis: Calculated C₈₉H₁₆₈N₄O₆S₂ [M+H] = 1454.2, Observed = 1454.2.

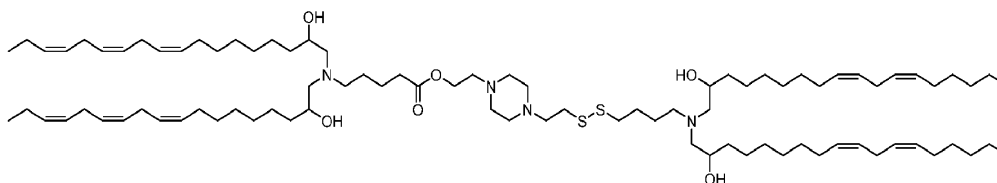
2-(4-(2-((4-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-2-DS-4-E18-2)



[0445] ¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 16H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.85-2.34 (m, 38H), 2.08 (m, 16H), 1.73-1.33 (m, 72H), 0.91 (m, 12H).

[0446] APCI-MS analysis: Calculated C₈₉H₁₆₄N₄O₆S₂ [M+H] = 1450.2, Observed = 1450.2.

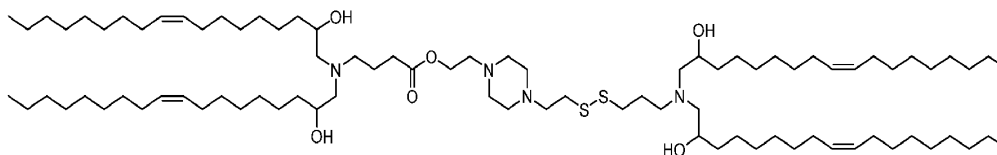
2-(4-(2-((4-(Bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 5-(bis((9Z,12Z,15Z)-2-hydroxyoctadeca-9,12,15-trien-1-yl)amino)pentanoate (GL-HEPES-E4-E18-3-DS-4-E18-2)



[0447] ¹H NMR (300 MHz, Methanol-d₄) δ 5.35 (m, 20H), 4.22 (t, 2H), 3.65 (s, br., 4H), 2.83-2.35 (m, 42H), 2.08 (m, 16H), 1.73-1.33 (m, 60H), 1.00-0.89 (m, 12H).

[0448] APCI-MS analysis: Calculated C₈₉H₁₆₀N₄O₆S₂ [M+H] = 1446.2, Observed = 1446.2.

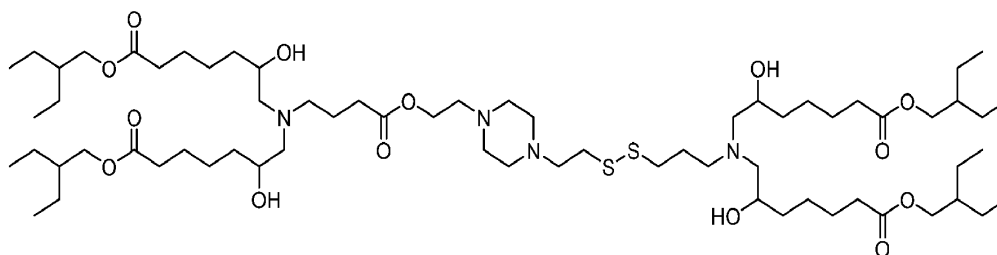
2-(4-(2-((3-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethyl 4-(bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butanoate (GL-HEPES-E3-E18-1-DS-3-E18-1)



[0449] ¹H NMR (300 MHz, Methanol-d₄) δ 5.32 (m, 8H), 4.21 (t, 2H), 3.64 (s, br., 4H), 2.85-2.38 (m, 30H), 2.08-1.78 (m, 20H), 1.31-1.27 (m, 88H), 0.88 (t, 12H).

[0450] APCI-MS analysis: Calculated C₇₁H₁₆₈N₄O₆S₂ [M+H] = 1430.2, Observed = 1430.3.

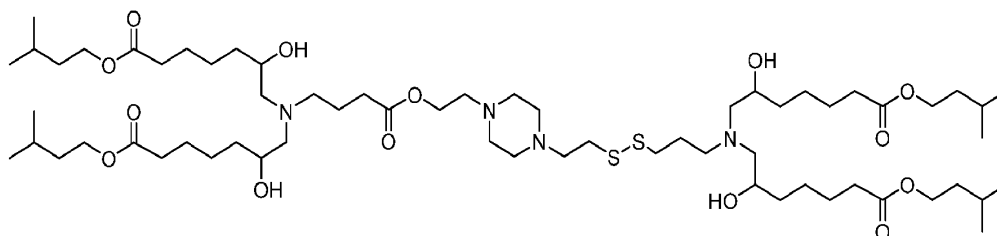
Bis(2-ethylbutyl) 7,7'-((3-((2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7-Es6-DS-3-E7-Es6)



[0451] ¹H NMR (300 MHz, Methanol-d₄) δ 4.21 (t, 2H), 4.01 (d, 8H), 3.62 (m, 4H), 2.88-2.50 (m, 22H), 2.45-2.28 (m, 16H), 1.89-1.73 (m, 4H), 1.64 (m, 8H), 1.56-1.45 (m, 12H), 1.37 (m, 24H), 0.91 (t, 24H).

[0452] APCI-MS analysis: Calculated C₆₇H₁₂₈N₄O₁₄S₂ [M+H] = 1277.9, Observed = 1277.8.

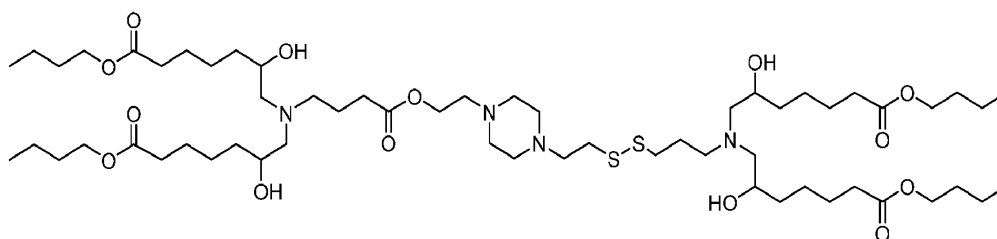
Diisopentyl 7,7'-((3-((2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7-Ei5-DS-3-E7-Ei5)



[0453] ^1H NMR (300 MHz, CDCl_3) δ 4.18 (t, 2H), 4.07 (t, 8H), 3.61 (m, 4H), 2.82-2.45 (m, 22H), 2.42-2.24 (m, 16H), 1.85-1.73 (m, 4H), 1.71-1.56 (m, 12H), 1.50 (m, 12H), 1.37 (m, 12H), 0.90 (d, 24H).

[0454] APCI-MS analysis: Calculated $\text{C}_{63}\text{H}_{120}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1221.8$, Observed = 1221.7.

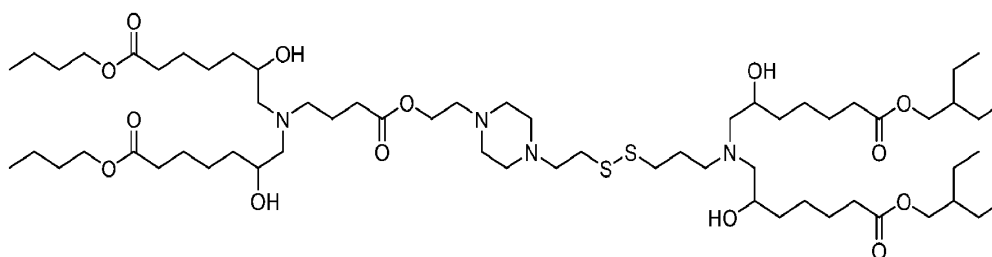
Dibutyl 7,7'-((3-((2-(4-(2-((4-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7-E4-DS-3-E7-E4)



[0455] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.05 (t, 8H), 3.61 (m, 4H), 2.84-2.46 (m, 22H), 2.43-2.26 (m, 16H), 1.89-1.73 (m, 4H), 1.69-1.55 (m, 18H), 1.44-1.30 (m, 22H), 0.92 (d, 12H).

[0456] APCI-MS analysis: Calculated $\text{C}_{59}\text{H}_{112}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1165.6$, Observed = 1165.7.

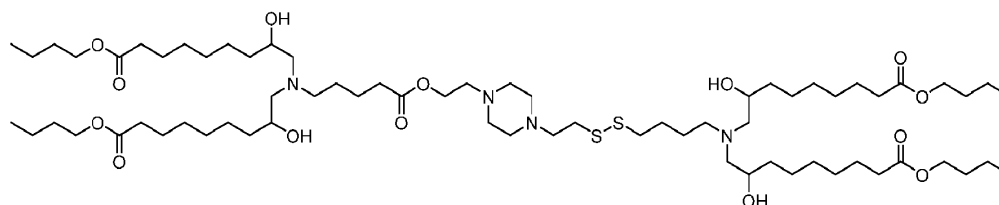
Dibutyl 7,7'-((4-(2-(4-(2-((3-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7-E4-DS-3-E7-Es6)



[0457] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.64 (m, 4H), 2.84-2.46 (m, 22H), 2.43-2.26 (m, 14H), 1.89-1.73 (m, 4H), 1.69-1.46 (m, 22H), 1.43-1.29 (m, 22H), 0.92 (t, 6H), 0.88 (t, 12H).

[0458] APCI-MS analysis: Calculated $\text{C}_{63}\text{H}_{120}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1221.7$, Observed = 1221.9.

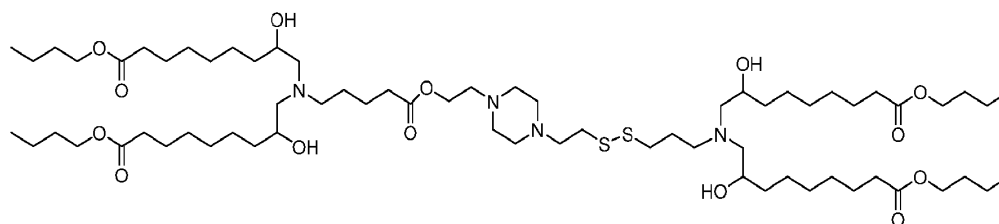
Dibutyl 9,9'-((5-(2-(4-(2-((4-(bis(9-butoxy-2-hydroxy-9-oxononyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9-E4-DS-4-E9-E4)



[0459] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.05 (t, 4H), 3.98 (d, 4H), 3.62 (m, 4H), 2.84-2.77 (m, 2H), 2.71-2.48 (m, 18H), 2.44-2.25 (m, 16H), 1.69-1.55 (m, 24H), 1.48-1.27 (m, 42H), 0.92 (t, 12H).

[0460] APCI-MS analysis: Calculated $\text{C}_{69}\text{H}_{132}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1305.9$, Observed = 1305.9.

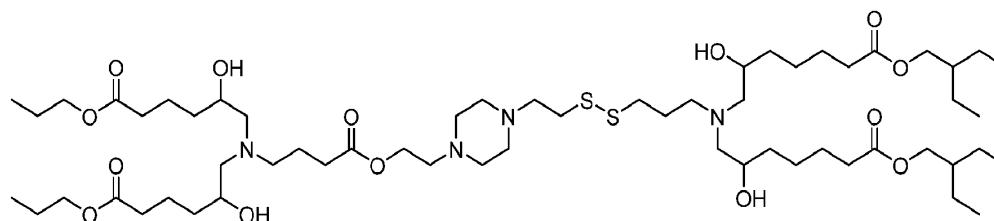
Dibutyl 9,9'-((3-((2-(4-(2-((5-(bis(9-butoxy-2-hydroxy-9-oxononyl)amino)pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9-E4-DS-3-E9-E4)



[0461] ^1H NMR (300 MHz, CDCl_3) δ 4.18 (t, 2H), 4.05 (t, 8H), 3.60 (m, 4H), 2.84-2.46 (m, 22H), 2.42-2.24 (m, 16H), 1.84 (m, 2H), 1.69-1.55 (m, 21H), 1.49-1.27 (m, 44H), 0.92 (t, 12H).

[0462] APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{130}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1291.9$, Observed = 1291.9.

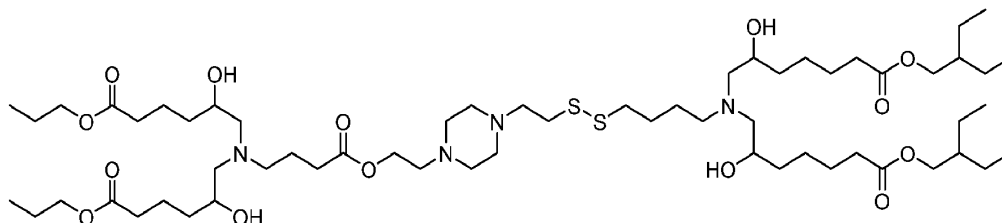
Bis(2-ethylbutyl) 7,7'-((3-((2-(4-(2-((4-(bis(2-hydroxy-6-oxo-6-propoxyhexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E6-E3-DS-3-E7-Es6)



[0463] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.04-3.97 (m, 8H), 3.64 (m, 4H), 2.84-2.46 (m, 20H), 2.42-2.26 (m, 16H), 1.88-1.29 (m, 44H), 0.95-0.85 (m, 18H).

[0464] APCI-MS analysis: Calculated $\text{C}_{59}\text{H}_{112}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1165.6$, Observed = 1165.8.

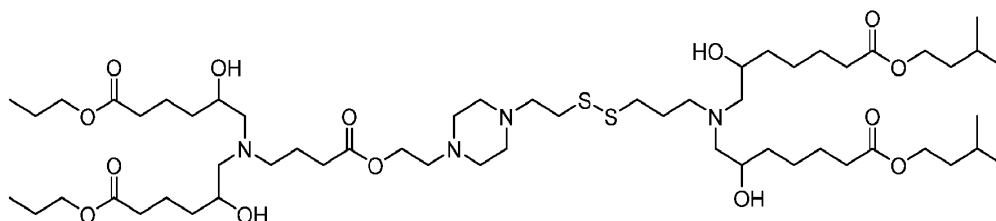
Bis(2-ethylbutyl) 7,7'-((4-((2-(4-(2-((4-bis(2-hydroxy-6-oxo-6-propoxyhexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E6-E3-DS-4-E7-Es6)



[0465] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.04-3.97 (m, 8H), 3.61 (m, 4H), 2.84-2.77 (m, 2H), 2.73-2.46 (m, 18H), 2.42-2.25 (m, 16H), 1.88-1.29 (m, 42H), 0.95-0.85 (m, 18H).

[0466] APCI-MS analysis: Calculated $\text{C}_{60}\text{H}_{114}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1179.7$, Observed = 1179.8.

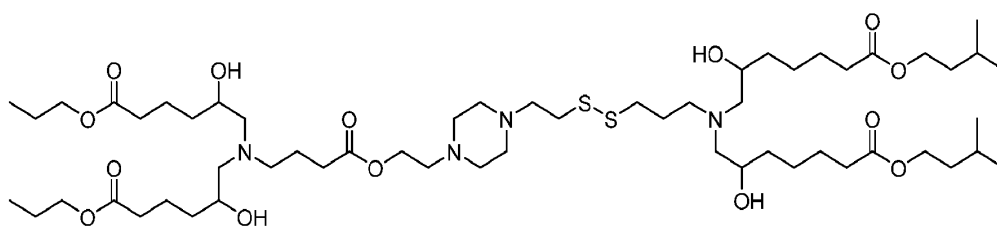
Diisopentyl 7,7'-((3-((2-(4-(2-((4-bis(2-hydroxy-6-oxo-6-propoxyhexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E6-E3-DS-3-E7-Ei5)



[0467] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.02 (t, 4H), 3.64 (m, 4H), 2.84-2.28 (m, 40H), 1.88-1.60 (m, 18H), 1.54-1.34 (m, 14H), 0.95-0.90 (m, 18H).

[0468] APCI-MS analysis: Calculated $\text{C}_{57}\text{H}_{108}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1137.6$, Observed = 1137.8.

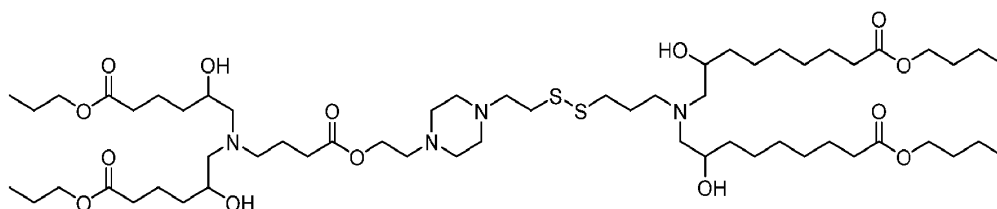
Diisopentyl 7,7'-((4-((2-(4-(2-((4-bis(2-hydroxy-6-oxo-6-propoxyhexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E6-E3-DS-4-E7-Ei5)



[0469] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.02 (t, 4H), 3.64 (m, 4H), 2.84-2.77 (m, 2H), 2.69-2.48 (m, 16H), 2.44-2.28 (m, 14H), 1.84-1.34 (m, 42H), 0.95-0.90 (m, 18H).

[0470] APCI-MS analysis: Calculated $\text{C}_{58}\text{H}_{110}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1151.6$, Observed = 1151.8.

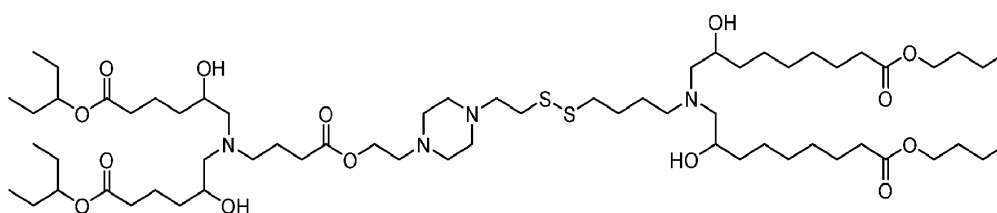
Dibutyl 9,9'-((3-((2-4-(2-((4-(bis(2-hydroxy-6-oxo-6-propoxyhexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E6-E3-DS-3-E9-E4)



[0471] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.05 (t, 4H), 4.02 (t, 4H), 3.64 (m, 4H), 2.84-2.28 (m, 36H), 1.88-1.54 (m, 24H), 1.48-1.28 (m, 22H), 0.95-0.90 (m, 12H).

[0472] APCI-MS analysis: Calculated $\text{C}_{59}\text{H}_{112}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1165.6$, Observed = 1165.8.

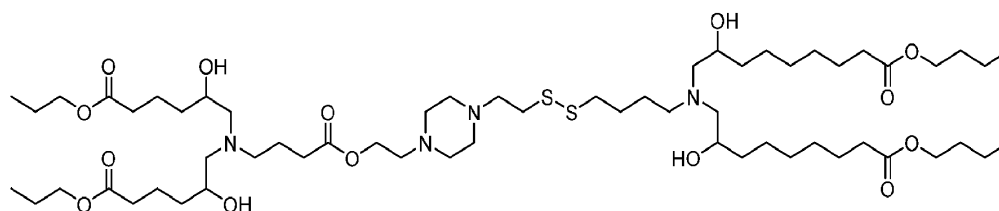
Dibutyl 9,9'-((4-((2-4-(2-((4-(bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E6-Es5-DS-4-E9-E4)



[0473] ^1H NMR (300 MHz, CDCl_3) δ 4.75 (quint, 2H), 4.19 (t, 2H), 4.05 (t, 4H), 3.62 (m, 4H), 2.82-2.24 (m, 38H), 1.86-1.50 (m, 28H), 1.48-1.27 (m, 22H), 0.92 (t, 6H), 0.86 (t, 12H).

[0474] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{122}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1235.8$, Observed = 1235.9.

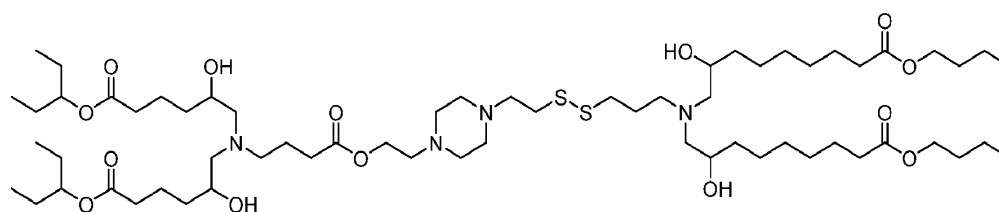
Dibutyl 9,9'-((4-((2-(4-(2-((4-(bis(2-hydroxy-6-oxo-6-propoxyhexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E6-E3-DS-4-E9-E4)



[0475] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.05 (t, 4H), 4.05 (t, 4H), 3.61 (m, 4H), 2.82-2.24 (m, 36H), 1.86-1.27 (m, 48H), 0.93 (t, 6H), 0.92 (t, 6H).

[0476] APCI-MS analysis: Calculated $\text{C}_{61}\text{H}_{114}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1179.7$, Observed = 1179.8.

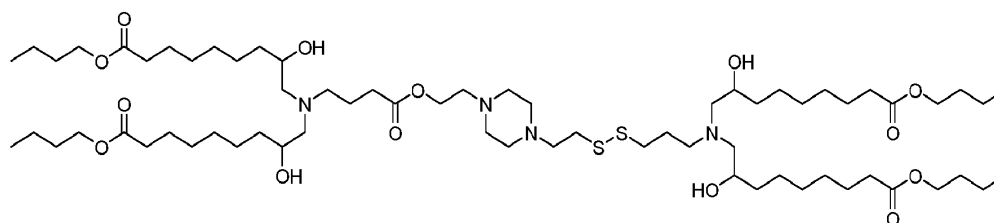
Dibutyl 9,9'-((4-((2-(4-(2-((4-(bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E6-Es5-DS-3-E9-E4)



[0477] ^1H NMR (300 MHz, CDCl_3) δ 4.75 (quint, 2H), 4.19 (t, 2H), 4.05 (t, 4H), 3.62 (m, 4H), 2.83-2.25 (m, 36H), 1.86-1.50 (m, 26H), 1.43-1.27 (m, 24H), 0.92 (t, 6H), 0.86 (t, 12H).

[0478] APCI-MS analysis: Calculated $\text{C}_{63}\text{H}_{120}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1221.7$, Observed = 1221.9.

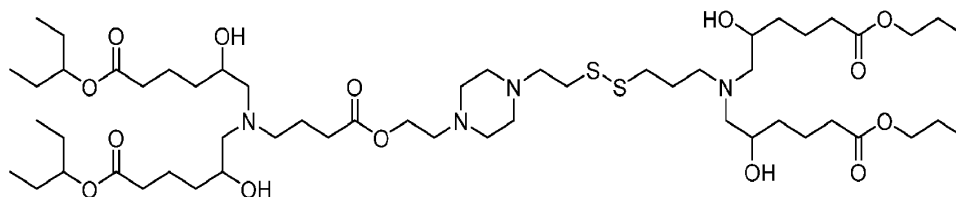
Dibutyl 9,9'-((3-((2-(4-(2-((4-(bis(9-butoxy-2-hydroxy-9-oxononyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-E4-DS-3-E9-E4)



[0479] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.05 (t, 8H), 3.61 (m, 4H), 2.83-2.24 (m, 36H), 1.89-1.74 (m, 4H), 1.68-1.53 (m, 16H), 1.46-1.27 (m, 42H), 0.93 (t, 12H).

[0480] APCI-MS analysis: Calculated $\text{C}_{67}\text{H}_{128}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1277.9$, Observed = 1277.9.

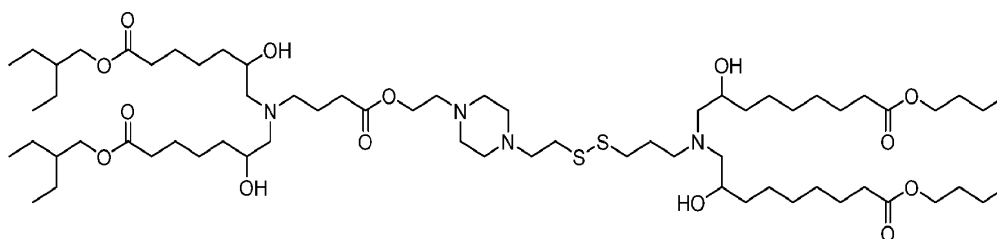
Di(pentan-3-yl) 6,6'-((4-(2-(4-(2-((3-(bis(2-hydroxy-6-oxo-6-propoxyhexyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(5-hydroxyhexanoate) (GL-HEPES-E3-E6-Es5-DS-3-E6-E3)



[0481] ^1H NMR (300 MHz, CDCl_3) δ 4.75 (quint, 2H), 4.19 (t, 2H), 4.02 (t, 4H), 3.64 (m, 4H), 2.83-2.28 (m, 36H), 1.89-1.30 (m, 34H), 0.93 (t, 6H), 0.86 (t, 12H).

[0482] APCI-MS analysis: Calculated $\text{C}_{55}\text{H}_{104}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1109.5$, Observed = 1109.6.

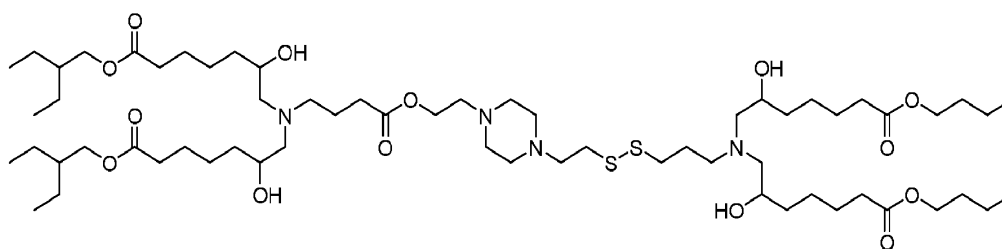
Dibutyl 9,9'-((3-((2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7-Es6-DS-3-E9-E4)



[0483] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.05 (t, 4H), 3.98 (d, 4H), 3.63 (m, 4H), 2.83-2.24 (m, 40H), 1.89-1.75 (m, 4H), 1.68-1.28 (m, 48H), 0.92 (t, 6H), 0.88 (t, 12H).

[0484] APCI-MS analysis: Calculated $\text{C}_{67}\text{H}_{128}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1277.9$, Observed = 1277.8.

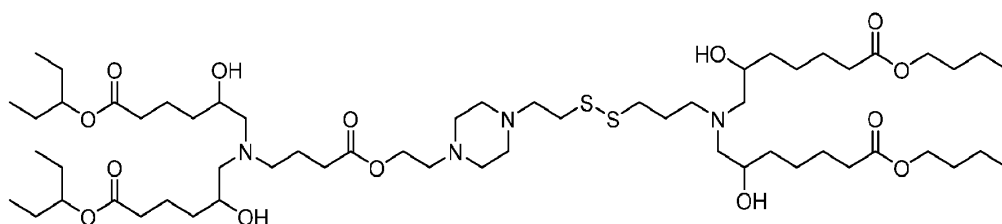
Dibutyl 7,7'-((3-((2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)-azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7-Es6-DS-3-E7-E4)



[0485] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.64 (m, 4H), 2.84-2.45 (m, 22H), 2.42-2.24 (m, 16H), 1.85-1.73 (m, 4H), 1.72-1.46 (m, 20H), 1.45-1.29 (m, 22H), 0.93 (t, 6H), 0.88 (t, 12H).

[0486] APCI-MS analysis: Calculated $\text{C}_{63}\text{H}_{120}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1221.8$, Observed = 1221.7.

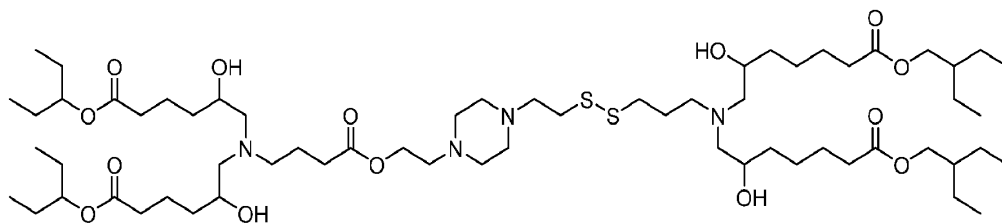
Dibutyl 7,7'-((3-((2-((4-(2-((4-(bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E6-Es5-DS-3-E7-E4)



[0487] ^1H NMR (300 MHz, CDCl_3) δ 4.75 (quint, 2H), 4.19 (t, 2H), 4.05 (t, 4H), 3.63 (m, 4H), 2.83-2.25 (m, 38H), 1.89-1.30 (m, 40H), 0.92 (t, 6H), 0.86 (t, 12H).

[0488] APCI-MS analysis: Calculated $\text{C}_{59}\text{H}_{112}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1165.6$, Observed = 1165.8.

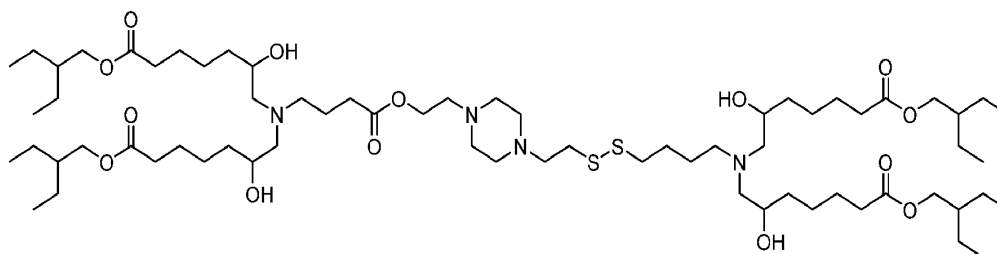
Bis(2-ethylbutyl) 7,7'-((3-((2-((4-(2-((4-(bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E6-Es5-DS-3-E7-Es6)



[0489] ^1H NMR (300 MHz, CDCl_3) δ 4.75 (quint, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.63 (m, 4H), 2.83-2.26 (m, 38H), 1.89-1.29 (m, 42H), 0.88 (t, 12H), 0.86 (t, 12H).

[0490] APCI-MS analysis: Calculated $\text{C}_{63}\text{H}_{120}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1221.7$, Observed = 1221.9.

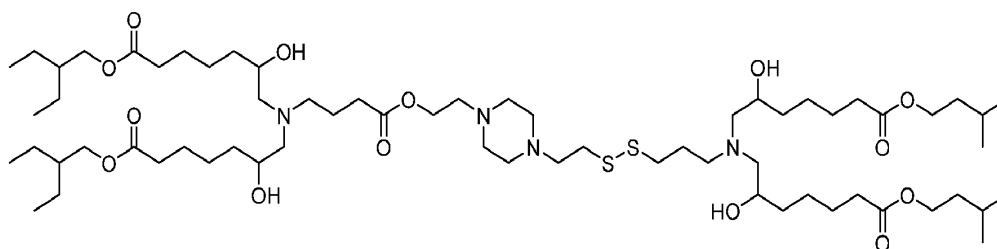
Bis(2-ethylbutyl) 7,7'-((4-((2-(4-(2-((4-bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7Es6-DS-4-E7Es6)



[0491] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 3.98 (d, 8H), 3.62 (m, 4H), 2.83-2.28 (m, 38H), 1.83-1.29 (m, 50H), 0.88 (t, 24H).

[0492] APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{130}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1291.9$, Observed = 1291.9.

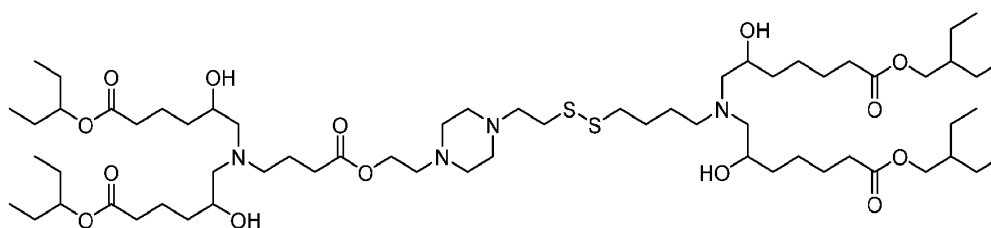
Bis(2-ethylbutyl) 7,7'-((4-((2-(4-(2-((3-bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7Es6-DS-3-E7Ei5)



[0493] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.99 (t, 4H), 3.63 (m, 4H), 2.83-2.26 (m, 38H), 1.88-1.29 (m, 44H), 0.91 (d, 12H), 0.88 (t, 12H).

[0494] APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{124}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1249.8$, Observed = 1249.9.

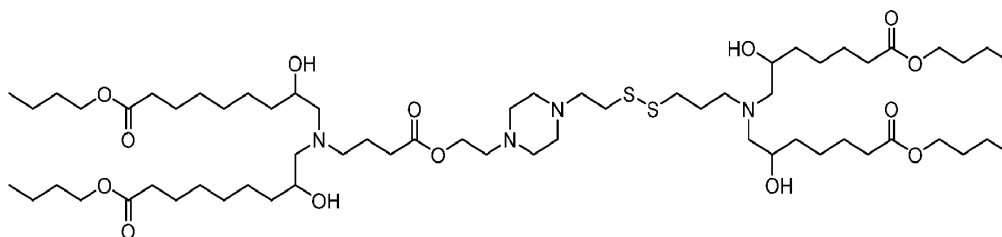
Dibutyl 7,7'-((3-((2-(4-(2-((4-bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E6-Es5-DS-4-E7-Es6)



[0495] ^1H NMR (300 MHz, CDCl_3) δ 4.75 (quint, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.62 (m, 4H), 2.83-2.28 (m, 38H), 1.86-1.29 (m, 44H), 0.88 (t, 12H), 0.86 (t, 12H).

[0496] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{122}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1235.8$, Observed = 1235.9.

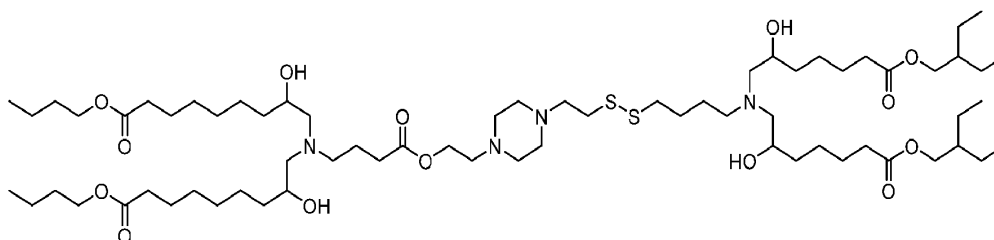
Dibutyl 9,9'-((4-(2-(4-(2-((3-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediy)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-E4-DS-3-E7-E4)



[0497] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.05 (t, 8H), 3.61 (m, 4H), 2.83-2.24 (m, 38H), 1.89-1.74 (m, 4H), 1.68-1.53 (m, 18H), 1.43-1.27 (m, 30H), 0.93 (t, 12H).

[0498] APCI-MS analysis: Calculated $\text{C}_{63}\text{H}_{120}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1221.7$, Observed = 1221.9.

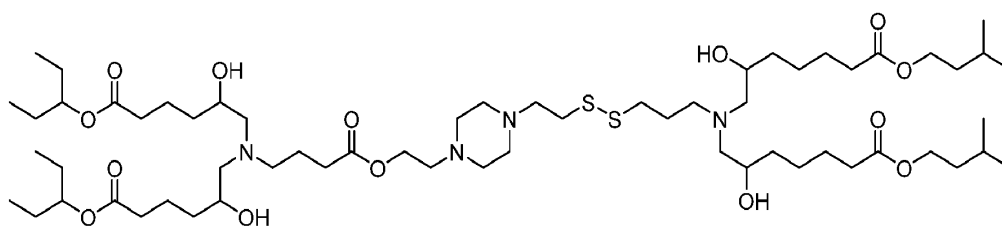
Dibutyl 9,9'-((4-(2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediy)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9E4-DS-4-E7Es6)



[0499] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.05 (t, 4H), 3.98 (d, 4H), 3.60 (m, 4H), 2.83-2.24 (m, 38H), 1.83-1.74 (m, 4H), 1.68-1.27 (m, 52H), 0.92 (t, 6H), 0.88 (t, 12H).

[0500] APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{130}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1291.9$, Observed = 1291.9.

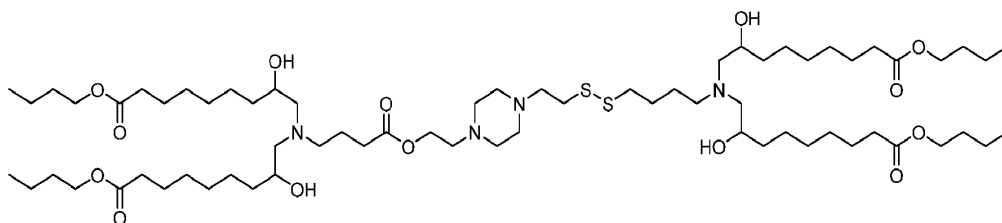
Diisopentyl 7,7'-((3-((2-(4-(2-((4-(bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediy)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E6Es5-DS-3-E7Ei5)



[0501] ^1H NMR (300 MHz, CDCl_3) δ 4.75 (quint, 2H), 4.19 (t, 2H), 4.08 (t, 4H), 3.62 (m, 4H), 2.83-2.27 (m, 38H), 1.88-1.34 (m, 38H), 0.91 (d, 12H), 0.86 (t, 12H).

[0502] APCI-MS analysis: Calculated $\text{C}_{61}\text{H}_{116}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1193.7$, Observed = 1193.8.

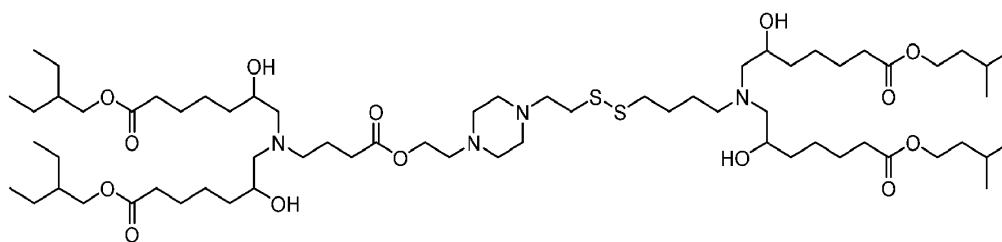
Dibutyl 9,9'-((4-((2-((4-((bis(9-butoxy-2-hydroxy-9-oxononyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9E4-DS-4-E9E4)



[0503] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.05 (t, 8H), 3.61 (m, 4H), 2.83-2.77 (m, 2H), 2.71-2.47 (m, 18H), 2.42-2.24 (m, 18H), 1.83-1.74 (m, 2H), 1.68-1.55 (m, 20H), 1.43-1.27 (m, 40H), 0.92 (t, 12H).

[0504] APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{130}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1291.9$, Observed = 1291.9.

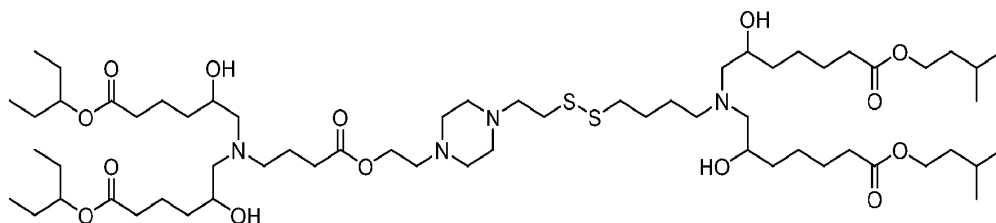
Bis(2-ethylbutyl) 7,7'-((4-((2-((4-((bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7Es6-DS-4-E7Ei5)



[0505] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.83-2.27 (m, 38H), 1.83-1.29 (m, 46H), 0.91 (d, 12H), 0.88 (t, 12H).

[0506] APCI-MS analysis: Calculated $\text{C}_{66}\text{H}_{126}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1263.8$, Observed = 1263.9.

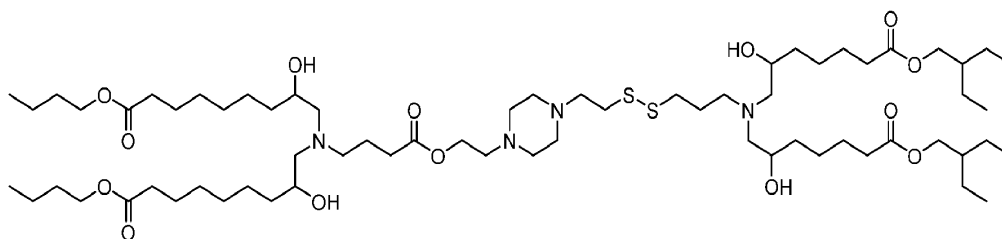
Diisopentyl 7,7'-((3-((2-(4-(2-((4-(bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E6-Es5-DS-4-E7-Ei5)



[0507] ^1H NMR (300 MHz, CDCl_3) δ 4.75 (quint, 2H), 4.19 (t, 2H), 4.08 (t, 4H), 3.62 (m, 4H), 2.83-2.27 (m, 38H), 1.86-1.34 (m, 40H), 0.91 (d, 12H), 0.86 (t, 12H).

[0508] APCI-MS analysis: Calculated $\text{C}_{62}\text{H}_{118}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1207.7$, Observed = 1207.8.

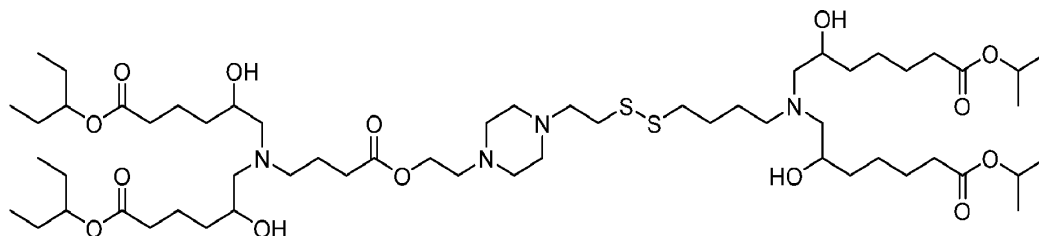
Dibutyl 9,9'-((4-(2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-E4-DS-3-E7-Es6)



[0509] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.05 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.83-2.24 (m, 38H), 1.89-1.74 (m, 4H), 1.68-1.27 (m, 50H), 0.92 (t, 6H), 0.88 (t, 12H).

[0510] APCI-MS analysis: Calculated $\text{C}_{67}\text{H}_{128}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1277.9$, Observed = 1277.9.

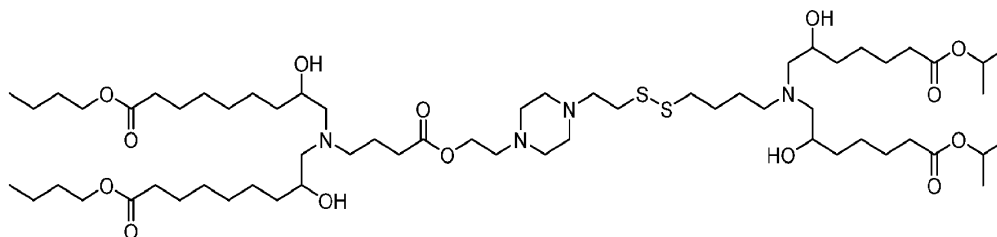
Diisopropyl 7,7'-((4-(2-(4-(2-((4-(bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E6-Es5-DS-4-E7-Ei3)



[0511] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.75 (quint, 2H), 4.19 (t, 2H), 3.62 (m, 4H), 2.83-2.24 (m, 38H), 1.85-1.34 (m, 34H), 1.22 (d, 12H), 0.86 (t, 12H).

[0512] APCI-MS analysis: Calculated $\text{C}_{58}\text{H}_{110}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1151.6$, Observed = 1151.8.

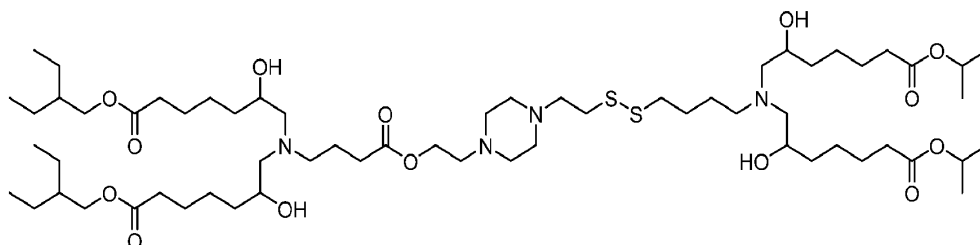
Dibutyl 9,9'-((4-(2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-E4-DS-4-E7-Ei3)



[0513] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (Hept, 2H), 4.19 (t, 2H), 4.05 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.83-2.24 (m, 38H), 1.84-1.74 (m, 2H), 1.68-1.27 (m, 38H), 1.22 (d, 12H), 0.92 (t, 12H).

[0514] APCI-MS analysis: Calculated $\text{C}_{62}\text{H}_{118}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1207.7$, Observed = 1207.9.

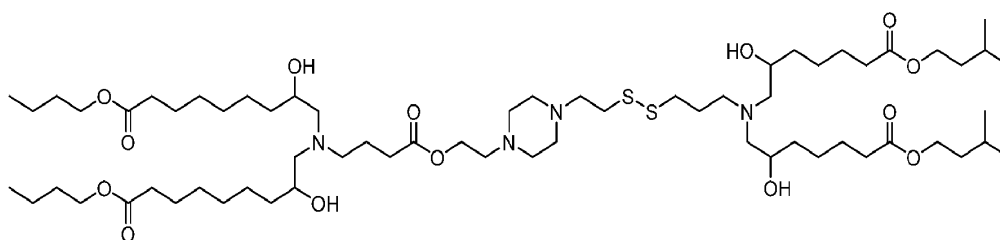
Bis(2-ethylbutyl) 7,7'-((4-(2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7-Es6-DS-4-E7-Ei3)



[0515] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.62 (m, 4H), 2.83-2.21 (m, 38H), 1.85-1.24 (m, 40H), 1.22 (d, 12H), 0.86 (t, 12H).

[0516] APCI-MS analysis: Calculated $\text{C}_{62}\text{H}_{118}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1207.8$, Observed = 1207.7.

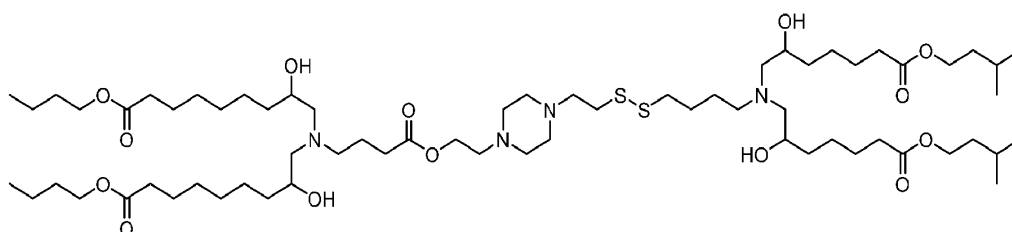
Dibutyl 9,9'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-E4-DS-3-E7-Ei5)



[0517] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 8H), 3.70 (m, 4H), 2.84-2.46 (m, 22H), 2.43-2.26 (m, 16H), 1.96-1.25 (m, 50H), 0.95-0.88 (m, 18H).

[0518] APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{124}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1249.8$, Observed = 1249.8.

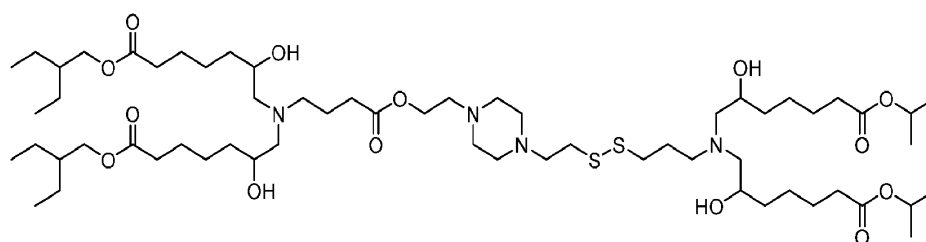
Dibutyl 9,9'-((4-(2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-E4-DS-4-E7-Ei5)



[0519] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (m, 8H), 3.75 (m, 4H), 2.84-2.46 (m, 22H), 2.43-2.25 (m, 16H), 1.92-1.25 (m, 52H), 0.95-0.88 (m, 18H).

[0520] APCI-MS analysis: Calculated $\text{C}_{66}\text{H}_{126}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1263.9$, Observed = 1263.7.

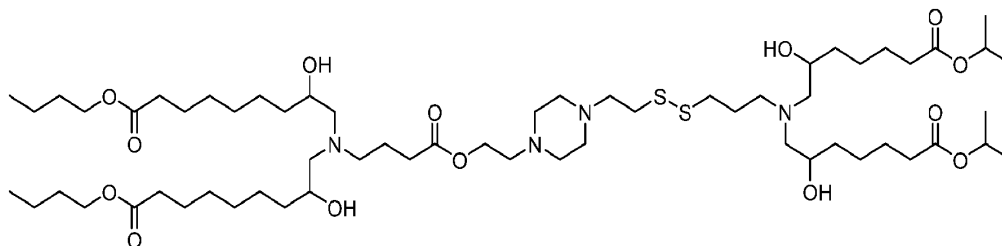
Bis(2-ethylbutyl) 7,7'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7-Es6-DS-3-E7-Ei3)



[0521] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.65 (m, 4H), 2.86-2.21 (m, 38H), 1.90-1.28 (m, 38H), 1.22 (d, 12H), 0.89 (t, 12H).

[0522] APCI-MS analysis: Calculated $\text{C}_{61}\text{H}_{116}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1193.7$, Observed = 1193.6.

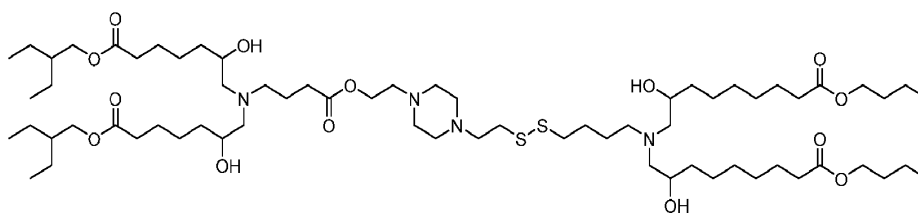
Dibutyl 9,9'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-E4-DS-3-E7-Ei3)



[0523] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.04 (d, 4H), 3.62 (m, 4H), 2.86-2.21 (m, 38H), 1.90-1.28 (m, 44H), 1.22 (d, 12H), 0.89 (t, 6H).

[0524] APCI-MS analysis: Calculated $\text{C}_{61}\text{H}_{116}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1193.7$, Observed = 1193.7.

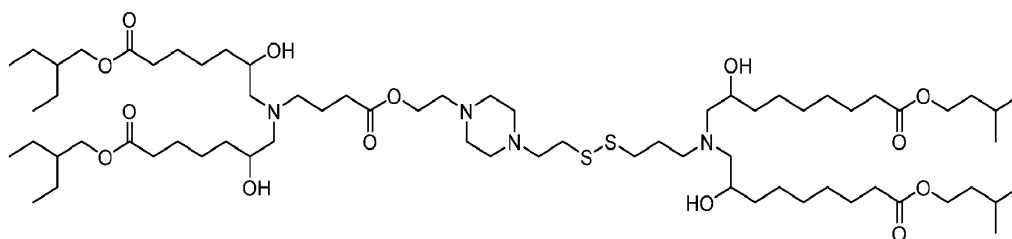
Dibutyl 9,9'-((4-(2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7-Es6-DS-4-E9-E4)



[0525] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.64 (m, 4H), 2.84-2.45 (m, 22H), 2.42-2.24 (m, 16H), 1.90-1.28 (m, 56H), 0.93 (t, 6H), 0.88 (t, 12H).

[0526] APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{130}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1291.9$, Observed = 1291.8.

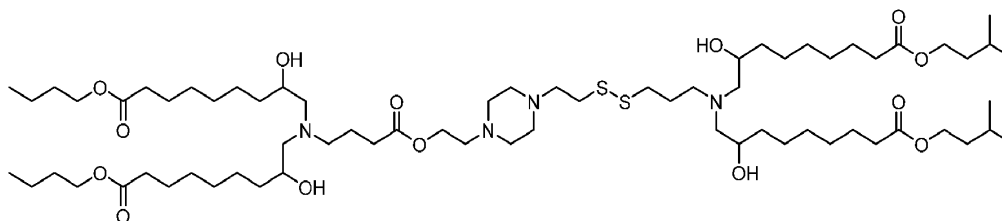
Diisopentyl 9,9'-((3-((2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7-Es6-DS-3-E9-Ei5)



[0527] ^1H NMR (300 MHz, CDCl_3) δ 4.20 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.68 (m, 4H), 2.88-2.45 (m, 22H), 2.42-2.24 (m, 16H), 1.95-1.26 (m, 52H), 0.95-0.86 (m, 24H).

[0528] APCI-MS analysis: Calculated $\text{C}_{69}\text{H}_{132}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1305.9$, Observed = 1305.8.

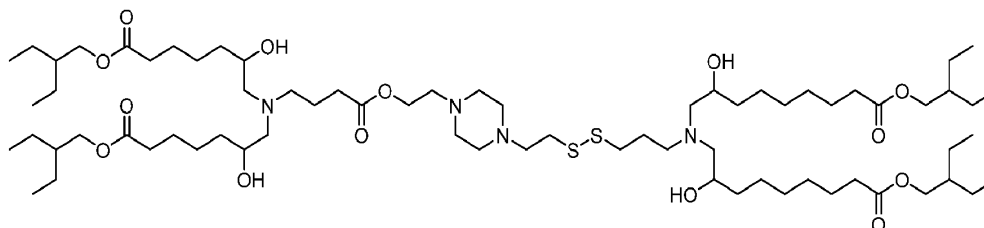
dibutyl 9,9'-((4-(2-(4-(2-((3-(bis(2-hydroxy-9-(isopentyloxy)-9-oxononyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediy)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-E4-DS-3-E9-Ei5)



[0529] ^1H NMR (300 MHz, CDCl_3) δ 4.20 (t, 2H), 4.06 (m, 8H), 3.64 (m, 4H), 2.88-2.45 (m, 22H), 2.42-2.24 (m, 16H), 1.95-1.26 (m, 58H), 0.95-0.88 (m, 18H).

[0530] APCI-MS analysis: Calculated $\text{C}_{69}\text{H}_{132}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1305.9$, Observed = 1305.8.

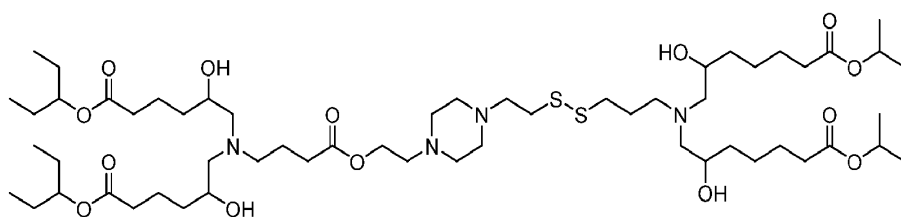
Bis(2-ethylbutyl) 9,9'-((3-((2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediy)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7-Es6-DS-3-E9-Es6)



[0531] ^1H NMR (300 MHz, CDCl_3) δ 4.21 (t, 2H), 3.98 (d, 8H), 3.74 (m, 4H), 2.82-2.46 (m, 22H), 2.43-2.25 (m, 16H), 1.99-1.25 (m, 56H), 0.93-0.85 (m, 24H).

[0532] APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{136}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1334.0$, Observed = 1133.8.

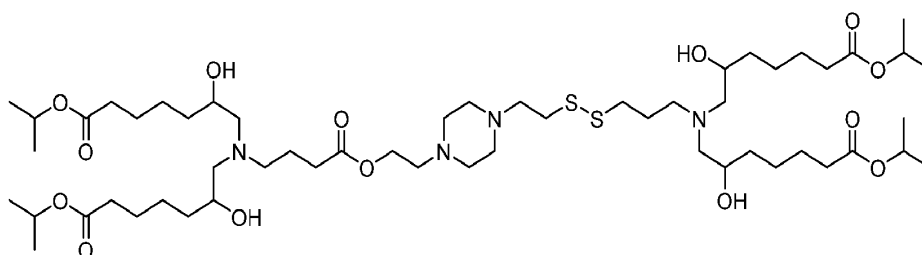
Diisopropyl 7,7'-((3-((2-(4-(2-((4-(bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediy)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E6-Es5-DS-3-E7-Ei3)



[0533] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.74 (pent, 2H), 4.19 (t, 2H), 3.65 (m, 4H), 3.32-3.00 (bs, 4H), 2.83-2.24 (m, 38H), 1.91-1.74 (m, 2H), 1.70-1.36 (m, 30H), 1.22 (d, 12H), 0.86 (t, 12H).

[0534] APCI-MS analysis: Calculated $\text{C}_{57}\text{H}_{108}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1137.6$, Observed = 1137.6.

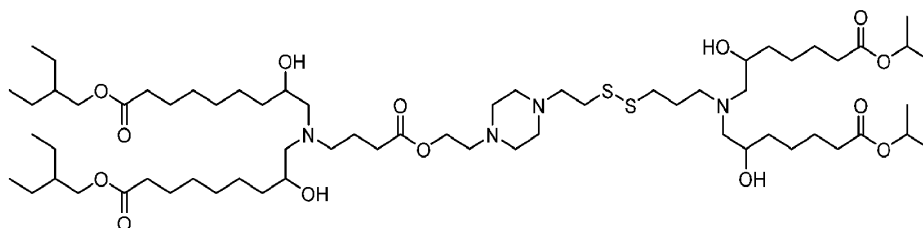
Diisopropyl 7,7'-((3-((2-(4-(2-((4-bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7-Ei3-DS-3-E7-Ei3)



[0535] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 4H), 4.19 (t, 2H), 3.68 (m, 4H), 3.32-3.00 (bs, 4H), 2.87-2.35 (m, 30H), 2.26 (t, 8H), 1.93-1.74 (m, 4H), 1.70-1.56 (m, 8H), 1.54-1.33 (m, 16H), 1.22 (d, 24H).

[0536] APCI-MS analysis: Calculated $\text{C}_{55}\text{H}_{104}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1109.5$, Observed = 1109.6.

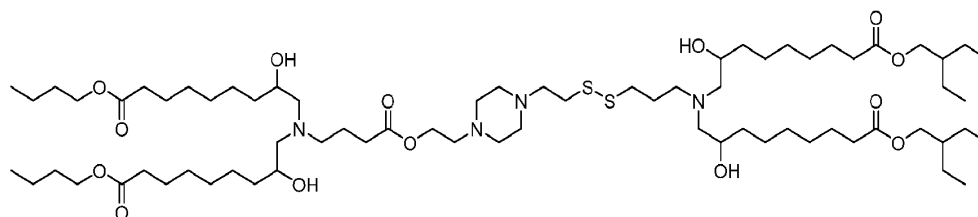
Bis(2-ethylbutyl) 9,9'-((4-(2-(4-(2-((3-bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-Es6-DS-3-E7-Ei3)



[0537] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.63 (m, 4H), 2.84-2.35 (m, 30H), 2.28 (q, 8H), 1.92-1.74 (m, 5H), 1.68-1.56 (m, 9H), 1.54-1.26 (m, 32H), 1.22 (d, 12H), 0.88 (t, 12H).

[0538] APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{124}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1249.8$, Observed = 1249.7.

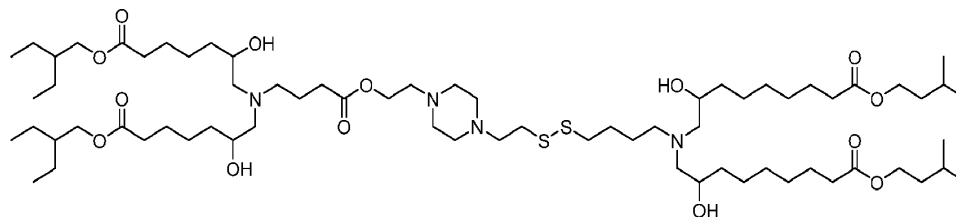
Dibutyl 9,9'-((4-(2-(4-(2-((3-(bis(9-(2-ethylbutoxy)-2-hydroxy-9-oxononyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-E4-DS-3-E9-Es6)



[0539] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.04 (t, 4H), 3.98 (d, 4H), 3.72 (m, 4H), 2.84-2.28 (m, 38H), 1.95-1.22 (m, 62H), 0.95-0.85 (m, 18H).

[0540] APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{136}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1334.0$, Observed = 1333.8.

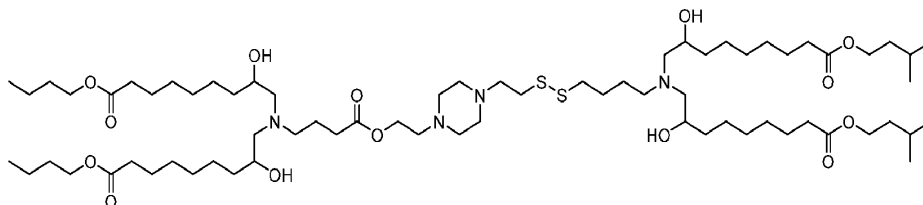
Diisopentyl 9,9'-((4-(2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7-Es6-DS-4-E9-Ei5)



[0541] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.62 (m, 4H), 2.84-2.28 (m, 38H), 1.95-1.22 (m, 54H), 0.95-0.86 (m, 24H).

[0542] APCI-MS analysis: Calculated $\text{C}_{70}\text{H}_{134}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1319.9$, Observed = 1319.8.

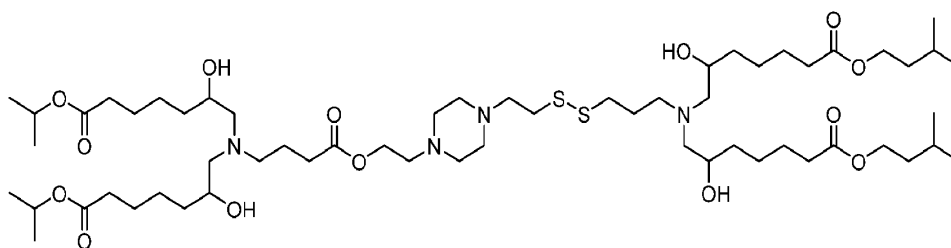
Dibutyl 9,9'-((4-(2-(4-(2-((4-(bis(2-hydroxy-9-(isopentyloxy)-9-oxononyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-E4-DS-4-E9-Ei5)



[0543] ^1H NMR (300 MHz, CDCl_3) δ 4.20 (t, 2H), 4.06 (m, 8H), 3.61 (m, 4H), 2.82-2.46 (m, 22H), 2.43-2.25 (m, 16H), 1.90-1.22 (m, 60H), 0.93-0.85 (m, 18H).

[0544] APCI-MS analysis: Calculated $\text{C}_{70}\text{H}_{134}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1319.9$, Observed = 1319.8.

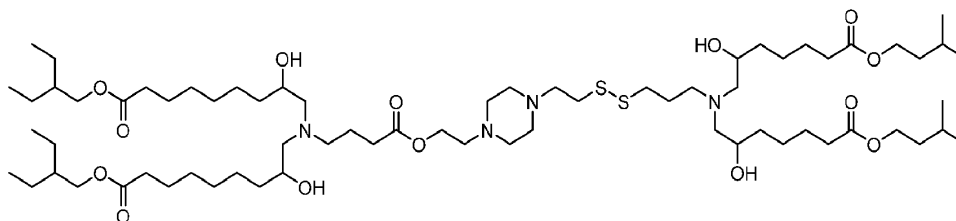
Diisopentyl 7,7'-((3-((2-(4-((4-bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)-azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7-Ei3-DS-3-E7-Ei5)



[0545] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.08 (t, 4H), 3.64 (m, 4H), 2.82-2.35 (m, 24H), 2.28 (t, 8H), 1.92-1.74 (m, 6H), 1.72-1.56 (m, 12H), 1.50 (q, 8H), 1.44-1.32 (m, 14H), 1.22 (d, 12H), 0.91 (d, 12H).

[0546] APCI-MS analysis: Calculated $\text{C}_{59}\text{H}_{112}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1165.6$, Observed = 1165.7.

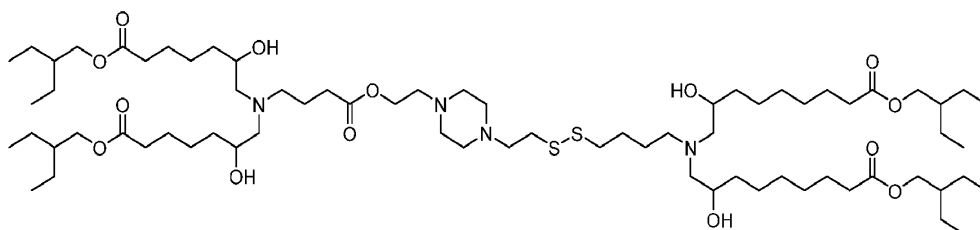
Bis(2-ethylbutyl) 9,9'-((4-(2-(4-((3-bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)-bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-Es6-DS-3-E7-Ei5)



[0547] ^1H NMR (300 MHz, CDCl_3) δ 4.20 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.65 (m, 4H), 2.84-2.32 (m, 32H), 2.29 (dt, 8H), 1.92-1.74 (m, 5H), 1.72-1.56 (m, 9H), 1.54-1.26 (m, 36H), 0.91 (d, 12H), 0.88 (t, 12H).

[0548] APCI-MS analysis: Calculated $\text{C}_{69}\text{H}_{132}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1305.9$, Observed = 1305.8.

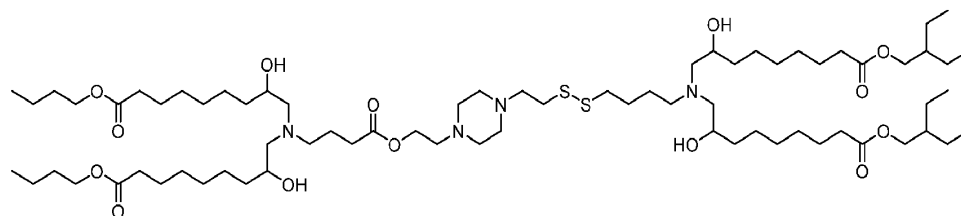
Bis(2-ethylbutyl) 9,9'-((4-((2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7-Es6-DS-4-E9-Es6)



[0549] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 3.98 (d, 8H), 3.74 (m, 4H), 2.83-2.25 (m, 38H), 1.90-1.22 (m, 58H), 0.88 (t, 24H).

[0550] APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{138}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1348.0$, Observed = 1347.9.

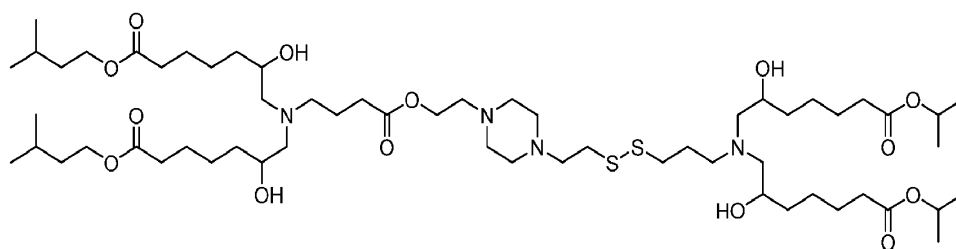
Dibutyl 9,9'-((4-((2-(4-(2-((4-(bis(9-butoxy-2-hydroxy-9-oxononyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-E4-DS-4-E9-Es6)



[0551] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.79 (m, 4H), 2.84-2.28 (m, 38H), 1.95-1.22 (m, 64H), 0.95-0.85 (m, 18H).

[0552] APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{138}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1348.0$, Observed = 1347.9.

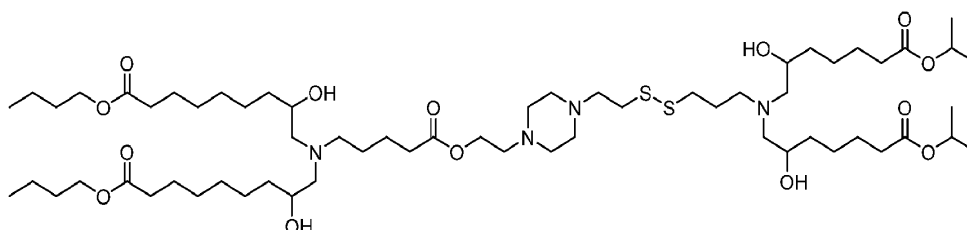
Diisopentyl 7,7'-((4-((2-(4-(2-((3-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)-bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7-Ei5-DS-3-E7-Ei3)



[0553] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.08 (t, 4H), 3.62 (m, 4H), 2.86-2.21 (m, 38H), 1.90-1.26 (m, 34H), 1.22 (d, 12H), 0.92 (d, 12H).

[0554] APCI-MS analysis: Calculated $\text{C}_{59}\text{H}_{112}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1165.7$, Observed = 1165.8.

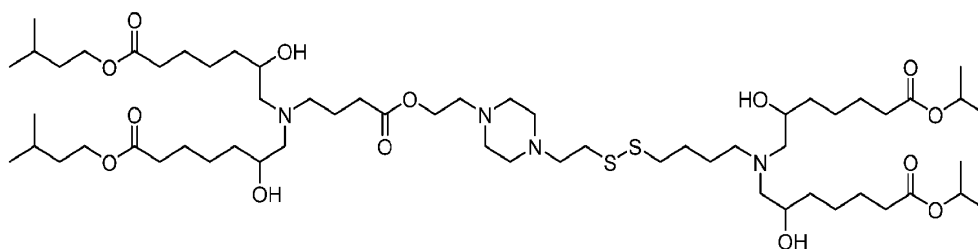
Dibutyl 9,9'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-pentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9-E4-DS-3-E7-Ei3)



[0555] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.18 (t, 2H), 4.05 (t, 4H), 3.64 (m, 4H), 2.86-2.21 (m, 38H), 1.90-1.28 (m, 46H), 1.22 (d, 12H), 0.90 (t, 6H).

[0556] APCI-MS analysis: Calculated $\text{C}_{62}\text{H}_{118}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1207.8$, Observed = 1207.8.

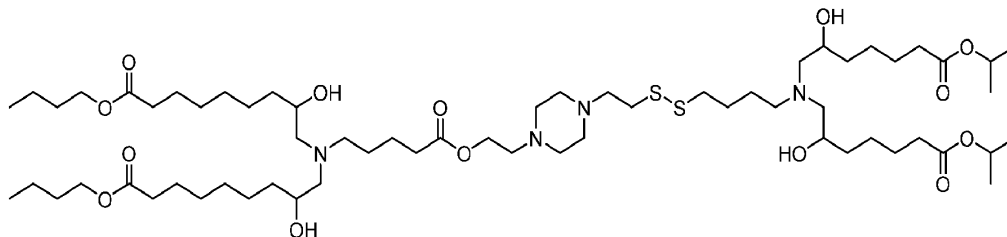
Diisopentyl 7,7'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7-Ei5-DS-4-E7-Ei3)



[0557] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.08 (t, 4H), 3.61 (m, 4H), 2.83-2.23 (m, 38H), 1.84-1.30 (m, 36H), 0.92 (d, 12H), 0.86 (d, 12H).

[0558] APCI-MS analysis: Calculated $\text{C}_{60}\text{H}_{114}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1179.7$, Observed = 1179.8.

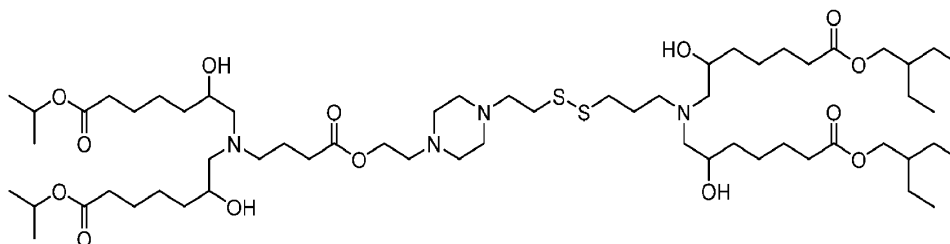
Dibutyl 9,9'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-pentyl)azanediy)bis(8-hydroxynonanoate) (GL (GL-HEPES-E4-E9-E4-DS-4-E7-Ei3)



[0559] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.05 (t, 4H), 3.60 (m, 4H), 2.86-2.21 (m, 38H), 1.86-1.25 (m, 48H), 1.21 (d, 12H), 0.92 (t, 6H).

[0560] APCI-MS analysis: Calculated $\text{C}_{63}\text{H}_{120}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1221.8$, Observed = 1221.8.

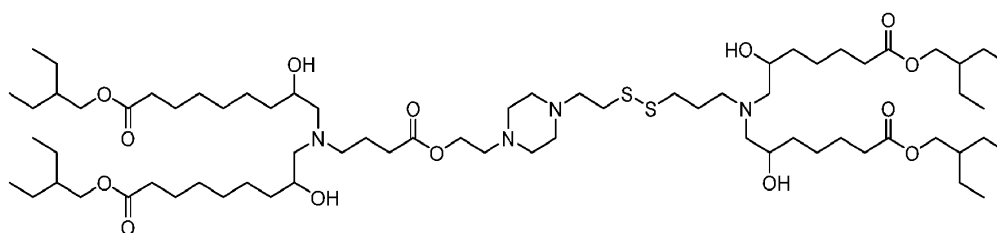
Bis(2-ethylbutyl) 7,7'-((3-((2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)-azanediy)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7-Ei3-DS-3-E7-Es6)



[0561] ^1H NMR (300 MHz, CDCl_3) δ 4.98 (hept, 2H), 4.21 (t, 2H), 3.97 (d, 4H), 3.74 (m, 6H), 2.92-2.38 (m, 28H), 2.29 (dt, 8H), 1.98-1.78 (m, 4H), 1.72-1.29 (m, 34H), 1.21 (d, 12H), 0.88 (t, 12H).

[0562] APCI-MS analysis: Calculated $\text{C}_{61}\text{H}_{116}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1193.7$, Observed = 1193.7.

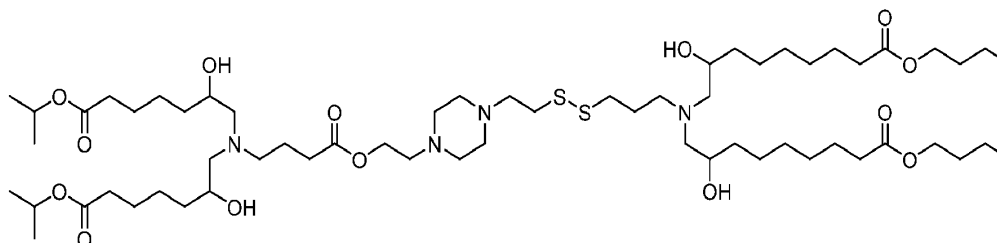
Bis(2-ethylbutyl) 9,9'-((4-(2-(4-(2-((3-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediy)-bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-Es6-DS-3-E7-Es6)



[0563] ^1H NMR (300 MHz, CDCl_3) δ 4.21 (t, 2H), 3.97 (d, 8H), 3.78 (m, 6H), 2.94-2.39 (m, 28H), 2.29 (dt, 8H), 1.92-1.74 (m, 4H), 1.72-1.26 (m, 52H), 0.88 (t, 24H).

[0564] APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{136}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1334.0$, Observed = 1333.8.

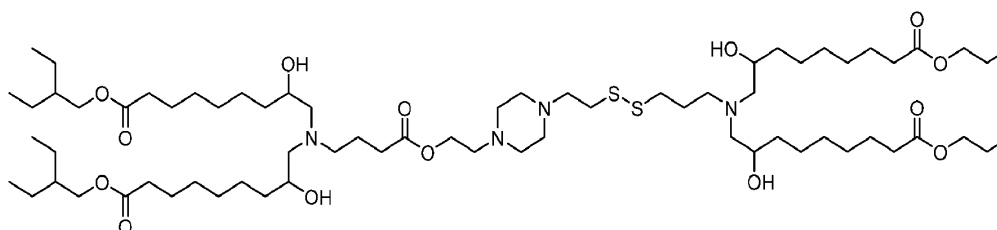
Dibutyl 9,9'-((3-((2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)-azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7-Ei3-DS-3-E9-E4)



[0565] ^1H NMR (300 MHz, CDCl_3) δ 4.98 (hept, 2H), 4.20 (t, 2H), 4.05 (t, 4H), 3.63 (bs, 4H), 2.82-2.24 (m, 36H), 1.92-1.74 (m, 6H), 1.68-1.55 (m, 12H), 1.50-1.27 (m, 28H), 1.22 (d, 12H), 0.92 (t, 6H).

[0566] APCI-MS analysis: Calculated $\text{C}_{61}\text{H}_{116}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1193.7$, Observed = 1193.8.

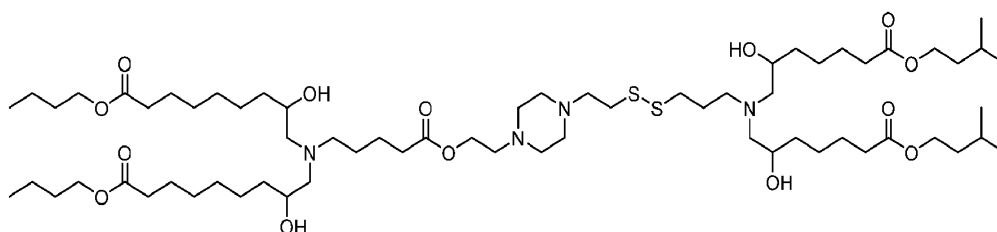
Bis(2-ethylbutyl) 9,9'-((4-(2-(4-(2-((3-(bis(2-hydroxy-9-oxo-9-propoxynonyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9-Es6-DS-3-E9-E4)



[0567] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.05 (t, 4H), 3.99 (d, 4H), 3.65 (bs, 4H), 2.84-2.39 (m, 28H), 2.29 (t, 4H), 2.28 (t, 4H), 1.92-1.74 (m, 6H), 1.68-1.55 (m, 14H), 1.52-1.24 (m, 44H), 0.92 (t, 6H), 0.88 (t, 12H).

[0568] APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{136}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1334.0$, Observed = 1334.0.

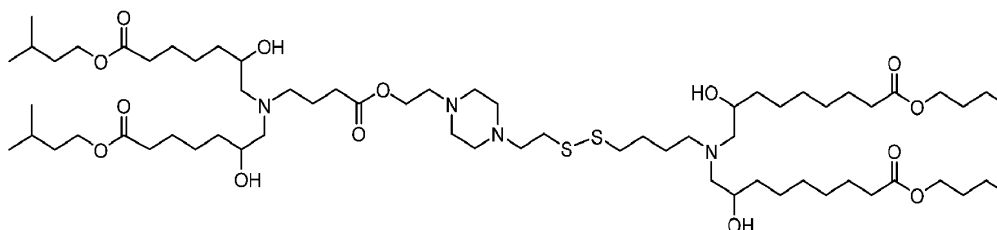
Dibutyl 9,9'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-isopentyloxy-7-oxoheptyl)amino)-propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-pentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9-E4-DS-3-E7-Ei5)



[0569] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 8H), 3.62 (m, 4H), 2.83-2.23 (m, 38H), 1.91-1.22 (m, 52H), 0.95-0.86 (m, 18H).

[0570] APCI-MS analysis: Calculated $\text{C}_{66}\text{H}_{126}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1263.9$, Observed = 1263.9.

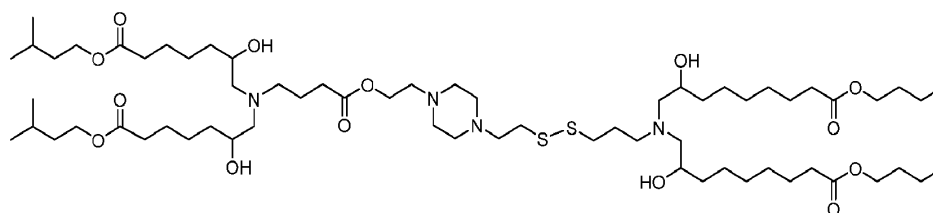
Dibutyl 9,9'-((4-(2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7-Ei5-DS-4-E9-E4)



[0571] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 8H), 3.61 (m, 4H), 2.85-2.23 (m, 38H), 1.89-1.25 (m, 52H), 0.95-0.86 (m, 18H).

[0572] APCI-MS analysis: Calculated $\text{C}_{66}\text{H}_{126}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1263.9$, Observed = 1263.9.

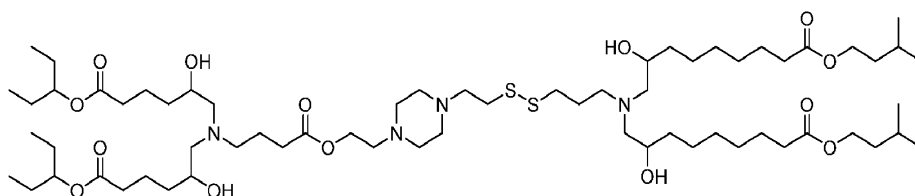
Dibutyl 9,9'-((4-(2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7-Ei5-DS-3-E9-E4)



[0573] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 8H), 3.62 (m, 4H), 2.85-2.23 (m, 38H), 1.90-1.25 (m, 50H), 0.95-0.86 (m, 18H).

[0574] APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{124}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1249.8$, Observed = 1249.9.

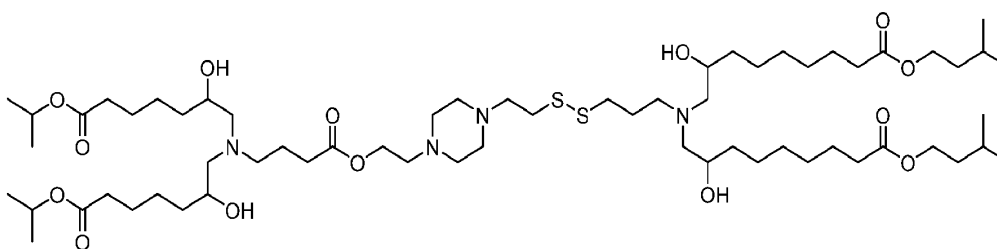
Diisopentyl 9,9'-((3-((2-(4-(2-((4-bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate)
(GL-HEPES-E3-E6Es5-DS-3-E9Ei5)



[0575] ^1H NMR (300 MHz, CDCl_3) δ 4.77 (pent, 2H), 4.19 (t, 2H), 4.08 (t, 4H), 3.65 (m, 4H), 2.85-2.25 (m, 38H), 1.90-1.24 (m, 46H), 0.91 (d, 12H), 0.86 (t, 12H).

[0576] APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{124}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1249.8$, Observed = 1249.8.

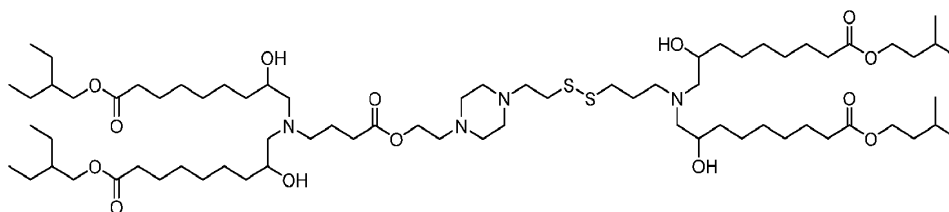
Diisopentyl 9,9'-((3-((2-(4-(2-((4-bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate)
(GL-HEPES-E3-E7Ei3-DS-3-E9Ei5)



[0577] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.08 (t, 4H), 3.65 (m, 4H), 2.85-2.24 (m, 40H), 1.92-1.78 (m, 4H), 1.72-1.26 (m, 36H), 1.23 (d, 12H), 0.91 (t, 12H).

[0578] APCI-MS analysis: Calculated $\text{C}_{63}\text{H}_{120}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1221.7$, Observed = 1221.8.

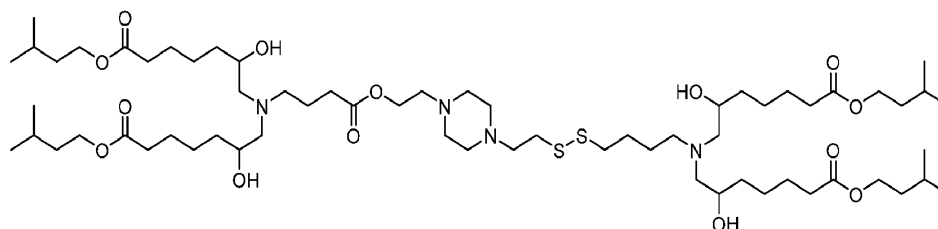
Bis(2-ethylbutyl) 9,9'-((4-(2-(4-(2-((3-(bis(2-hydroxy-9-(isopentyloxy)-9-oxononyl)-amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9Es6-DS-3-E9Ei5)



[0579] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.67 (m, 4H), 2.85-2.25 (m, 38H), 1.92-1.78 (m, 4H), 1.74-1.26 (m, 56H), 0.91 (d, 12H), 0.88 (t, 12H).

[0580] APCI-MS analysis: Calculated $\text{C}_{73}\text{H}_{140}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1362.0$, Observed = 1362.0.

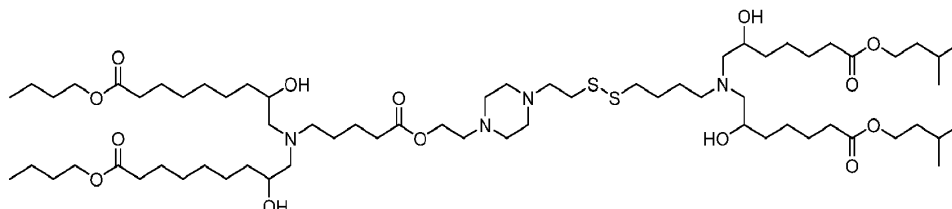
Diisopentyl 7,7'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-isopentyloxy-7-oxoheptyl)amino)butyl)-disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7Ei5-DS-4-E7Ei5)



[0581] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 8H), 3.63 (m, 4H), 2.84-2.46 (m, 22H), 2.43-2.26 (m, 16H), 1.84-1.33 (m, 42H), 0.91 (d, 24H).

[0582] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{122}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1235.8$, Observed = 1235.9.

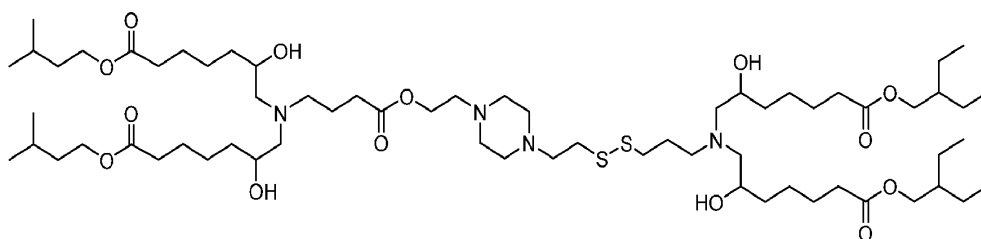
Dibutyl 9,9'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)butyl)-disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9E4-DS-4-E7Ei5)



[0583] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.05 (t, 4H), 3.62 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.25 (m, 16H), 1.79-1.25 (m, 54H), 0.92 (t, 6H), 0.90 (d, 12H).

[0584] APCI-MS analysis: Calculated $\text{C}_{67}\text{H}_{128}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1277.9$, Observed = 1277.9.

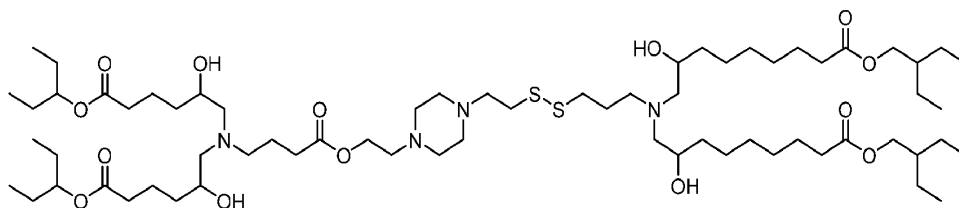
Bis(2-ethylbutyl) 7,7'-((3-((2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediy)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7Ei5-DS-3-E7Es6)



[0585] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.64 (m, 4H), 2.84-2.45 (m, 22H), 2.44-2.25 (m, 16H), 1.85-1.28 (m, 44H), 0.91 (d, 12H), 0.88 (t, 12H).

[0586] APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{124}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1249.8$, Observed = 1249.9.

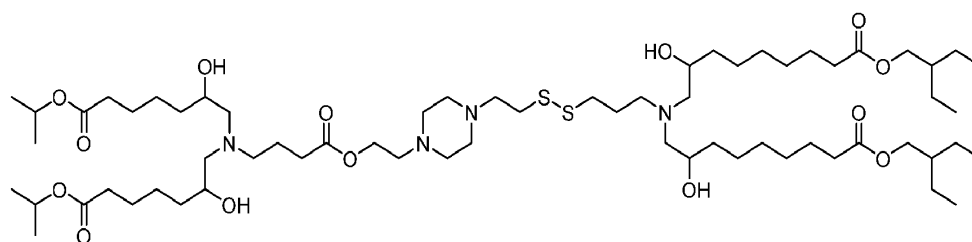
Bis(2-ethylbutyl) 9,9'-((3-((2-(4-(2-((4-(bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediy)bis(8-hydroxynonanoate) (GL-HEPES-E3-E6Es5-DS-3-E9Es6)



[0587] ^1H NMR (300 MHz, CDCl_3) δ 4.75 (pent, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.64 (m, 4H), 2.85-2.25 (m, 40H), 1.90-1.24 (m, 52H), 0.88 (d, 12H), 0.86 (t, 12H).

[0588] APCI-MS analysis: Calculated $\text{C}_{67}\text{H}_{128}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1277.9$, Observed = 1277.9.

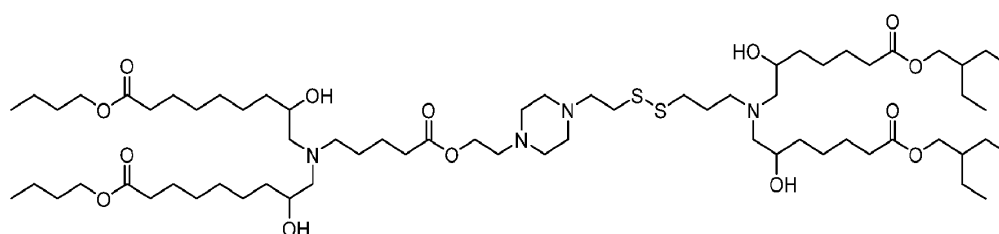
Bis(2-ethylbutyl) 9,9'-((3-((2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediy)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7Ei3-DS-3-E9Es6)



[0589] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 3.98 (t, 4H), 3.64 (m, 4H), 2.85-2.24 (m, 36H), 1.90-1.78 (m, 4H), 1.68-1.26 (m, 44H), 1.22 (d, 12H), 0.88 (t, 12H).

[0590] APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{124}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1249.8$, Observed = 1249.9.

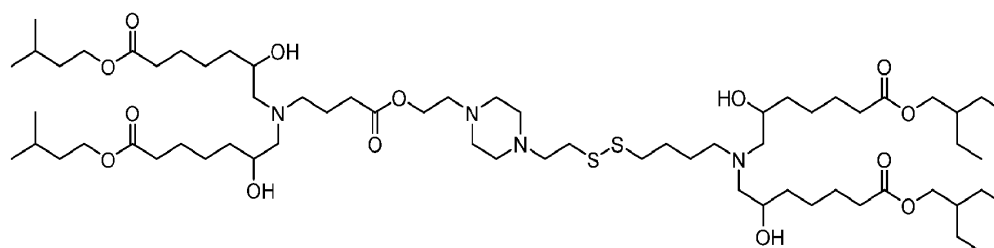
7-Oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)-azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9E4-DS-3-E7Es6)



[0591] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.62 (m, 4H), 2.85-2.45 (m, 22H), 2.44-2.24 (m, 16H), 1.92-1.25 (m, 58H), 0.92 (t, 6H), 0.88 (t, 12H).

[0592] APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{130}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1291.9$, Observed = 1291.9.

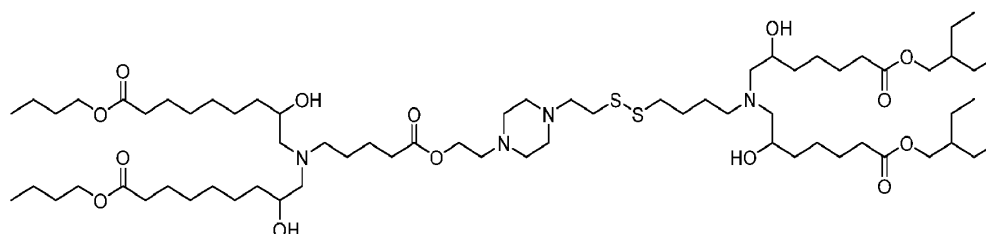
Bis(2-ethylbutyl) 7,7'-((4-((2-(4-(2-((4-bis(2-hydroxy-7-(isopentylloxy)-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7Ei5-DS-4-E7Es6)



[0593] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.84-2.45 (m, 22H), 2.44-2.25 (m, 16H), 1.86-1.28 (m, 46H), 0.91 (d, 12H), 0.88 (t, 12H).

[0594] APCI-MS analysis: Calculated $\text{C}_{66}\text{H}_{126}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1263.8$, Observed = 1263.9.

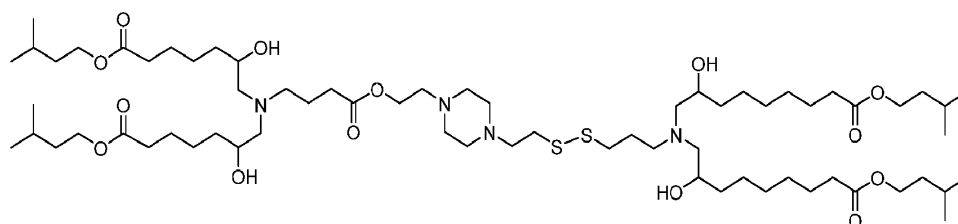
Dibutyl 9,9'-((5-(2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9E4-DS-4-E7Es6)



[0595] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.85-2.45 (m, 22H), 2.44-2.24 (m, 16H), 1.78-1.26 (m, 58H), 0.92 (t, 6H), 0.88 (m, 12H).

[0596] APCI-MS analysis: Calculated $\text{C}_{69}\text{H}_{132}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1305.9$, Observed = 1306.0.

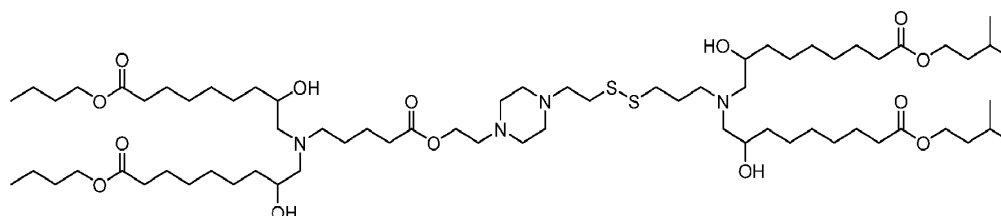
Diisopentyl 9,9'-((3-((2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7Ei5-DS-3-E9Ei5)



[0597] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 8H), 3.63 (m, 4H), 2.84-2.46 (m, 22H), 2.43-2.23 (m, 16H), 1.91-1.29 (m, 48H), 0.91 (d, 24H).

[0598] APCI-MS analysis: Calculated $\text{C}_{67}\text{H}_{128}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1277.9$, Observed = 1277.9.

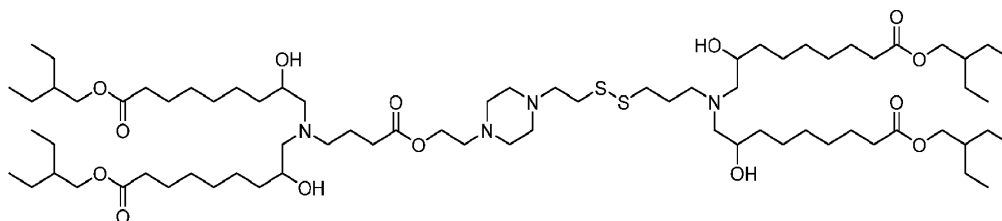
Dibutyl 9,9'-((5-(2-(4-(2-((3-(bis(2-hydroxy-9-(isopentyloxy)-9-oxononyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9E4-DS-3-E9Ei5)



[0599] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.06 (t, 4H), 3.61 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.25 (m, 16H), 1.91-1.25 (m, 60H), 0.92 (t, 6H), 0.91 (d, 12H).

[0600] APCI-MS analysis: Calculated $\text{C}_{70}\text{H}_{134}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1319.9$, Observed = 1319.9.

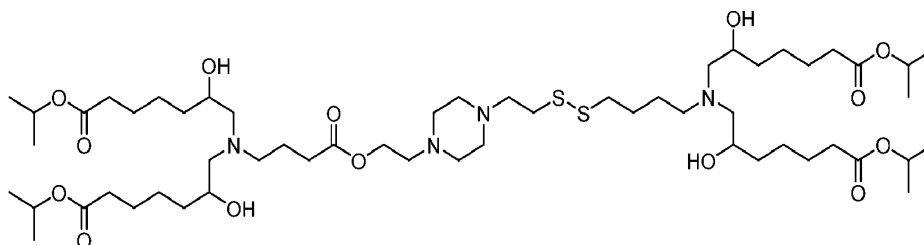
Bis(2-ethylbutyl) 9,9'-((3-((2-(4-(2-((4-(bis(9-(2-ethylbutoxy)-2-hydroxy-9-oxononyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9Es6-DS-3-E9Es6)



[0601] ^1H NMR (300 MHz, CDCl_3) δ 4.20 (t, 2H), 3.98 (d, 8H), 3.67 (m, 4H), 2.88-2.35 (m, 30H), 2.29 (t, 8H), 1.96-1.78 (m, 4H), 1.70-1.28 (m, 60H), 0.88 (t, 24H).

[0602] APCI-MS analysis: Calculated $\text{C}_{75}\text{H}_{144}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1390.1$, Observed = 1390.1.

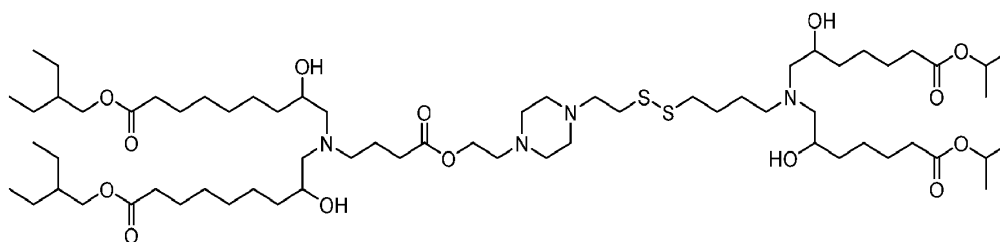
Diisopropyl 7,7'-((4-((2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7Ei3-DS-4-E7Ei3)



[0603] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 4H), 4.20 (t, 2H), 3.65 (m, 4H), 2.85-2.34 (m, 28H), 2.27 (t, 8H), 1.92-1.32 (m, 36H), 1.22 (d, 24H).

[0604] APCI-MS analysis: Calculated $\text{C}_{56}\text{H}_{106}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1123.6$, Observed = 1123.7.

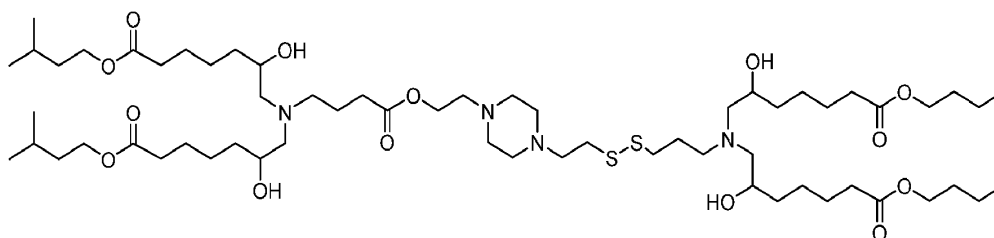
Bis(2-ethylbutyl) 9,9'-((4-((2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9Es6-DS-4-E7Ei3)



[0605] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.66 (m, 4H), 2.85-2.24 (m, 40H), 1.86-1.75 (m, 4H), 1.70-1.26 (m, 46H), 1.22 (d, 12H), 0.88 (t, 12H).

[0606] APCI-MS analysis: Calculated $\text{C}_{66}\text{H}_{126}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1263.8$, Observed = 1263.9.

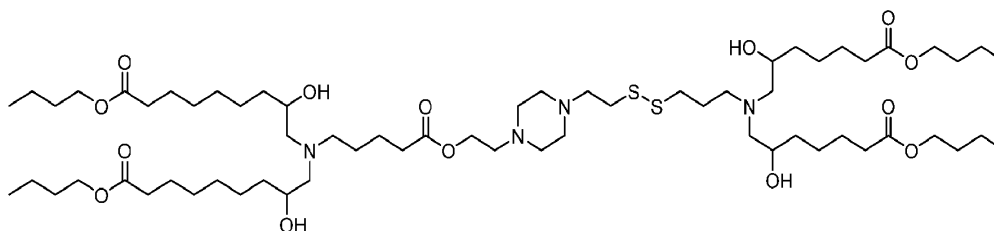
Dibutyl 7,7'-((3-((2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediy)bis(6-hydroxyheptanoate)
(GL-HEPES-E3-E7Ei5-DS-3-E7E4)



[0607] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.06 (t, 4H), 3.64 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.25 (m, 16H), 1.91-1.28 (m, 42H), 0.92 (t, 6H), 0.91 (d, 12H).

[0608] APCI-MS analysis: Calculated $\text{C}_{61}\text{H}_{116}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1193.7$, Observed = 1193.9.

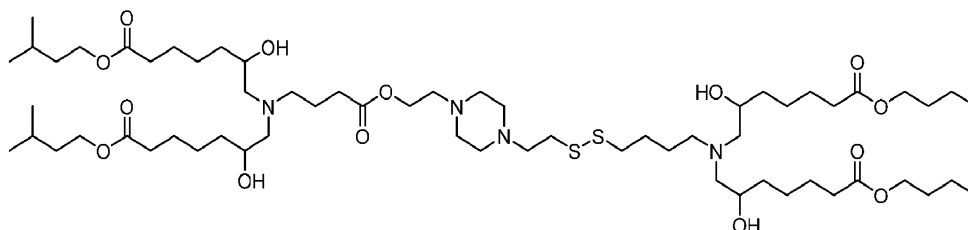
Dibutyl 9,9'-((5-(2-(4-(2-((3-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)-propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediy)bis(8-hydroxynonanoate)
(GL-HEPES-E4-E9E4-DS-3-E7E4)



[0609] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 8H), 3.66 (m, 4H), 2.85-2.46 (m, 22H), 2.43-2.23 (m, 16H), 1.90-1.70 (m, 4H), 1.69-1.25 (m, 54H), 0.92 (t, 12H).

[0610] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{122}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1235.8$, Observed = 1235.9.

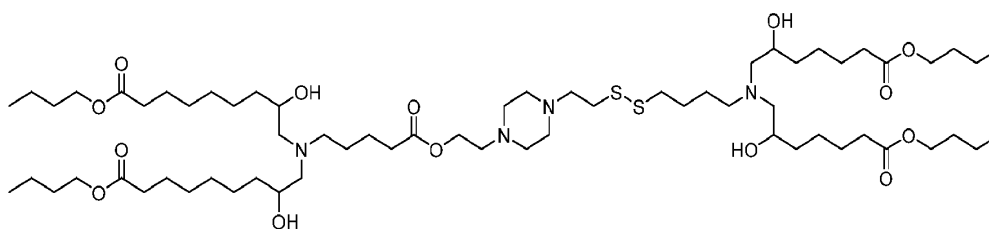
Dibutyl 7,7'-((4-((2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7Ei5-DS-4-E7E4)



[0611] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.06 (t, 4H), 3.62 (m, 4H), 2.82-2.46 (m, 22H), 2.43-2.25 (m, 16H), 1.86-1.22 (m, 44H), 0.92 (t, 6H), 0.91 (d, 12H).

[0612] APCI-MS analysis: Calculated $\text{C}_{62}\text{H}_{118}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1207.7$, Observed = 1207.8.

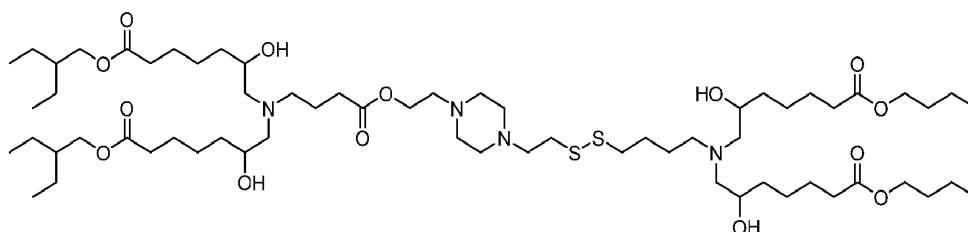
Dibutyl 9,9'-((5-(2-(4-(2-((4-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)-butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9E4-DS-4-E7E4)



[0613] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 8H), 3.61 (m, 4H), 2.83-2.25 (m, 38H), 1.85-1.25 (m, 56H), 0.92 (t, 12H).

[0614] APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{124}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1249.8$, Observed = 1249.9.

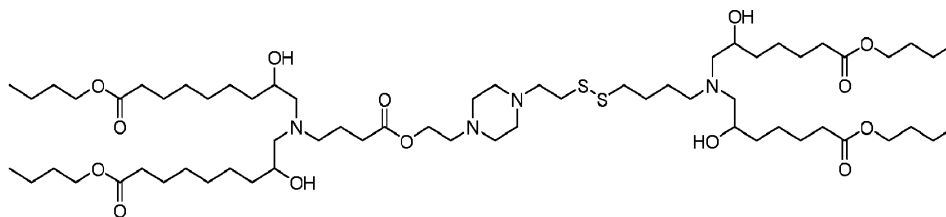
Dibutyl 7,7'-((4-((2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7Es6-DS-4-E7E4)



[0615] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.84-2.25 (m, 38H), 1.90-1.22 (m, 48H), 0.92 (t, 6H), 0.88 (t, 12H).

[0616] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{122}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1235.8$, Observed = 1235.9.

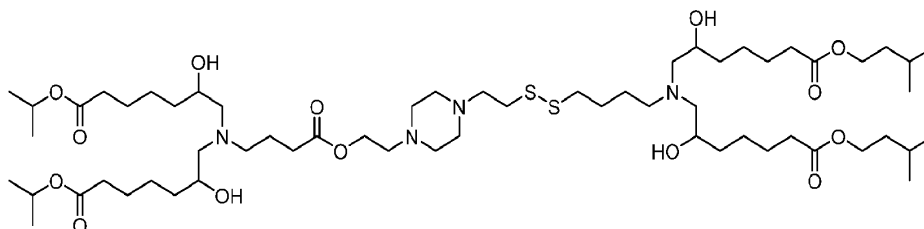
Dibutyl 9,9'-((4-(2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)-butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9E4-DS-4-E7E4)



[0617] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 8H), 3.61 (m, 4H), 2.83-2.25 (m, 38H), 1.85-1.25 (m, 54H), 0.92 (t, 12H).

[0618] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{122}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1235.8$, Observed = 1235.9.

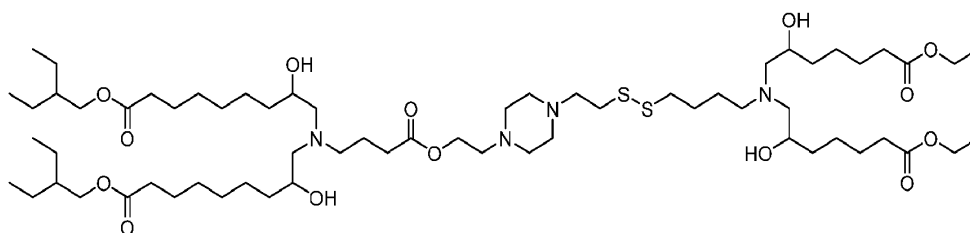
Diisopentyl 7,7'-((4-(2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7Ei3-DS-4-E7Ei5)



[0619] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 4H), 4.19 (t, 2H), 4.08 (t, 4H), 3.63 (m, 4H), 2.84-2.24 (m, 36H), 1.84-1.32 (m, 40H), 1.22 (d, 12H), 0.91 (d, 12H).

[0620] APCI-MS analysis: Calculated $\text{C}_{60}\text{H}_{114}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1179.7$, Observed = 1179.8.

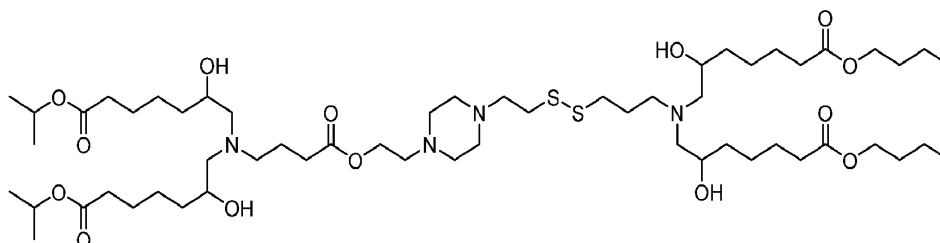
Bis(2-ethylbutyl) 9,9'-((4-(2-(4-(2-((4-(bis(7-ethoxy-2-hydroxy-7-oxoheptyl)amino)-butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9Es6-DS-4-E7Ei5)



[0621] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.85-2.24 (m, 40H), 1.84-1.26 (m, 56H), 0.91 (d, 12H), 0.88 (t, 12H).

[0622] APCI-MS analysis: Calculated $\text{C}_{70}\text{H}_{134}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1319.9$, Observed = 1320.0.

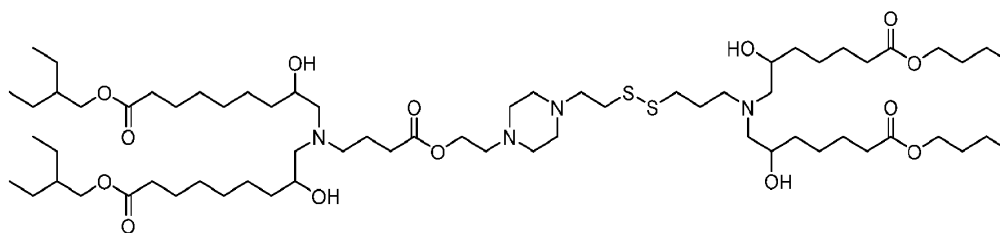
Dibutyl 7,7'-((3-((2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate)
(GL-HEPES-E3-E7Ei3-DS-3-E7E4)



[0623] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 4H), 4.20 (t, 2H), 4.06 (t, 4H), 3.64 (m, 4H), 2.87-2.24 (m, 36H), 1.90-1.32 (m, 40H), 1.22 (d, 12H), 0.92 (t, 6H).

[0624] APCI-MS analysis: Calculated $\text{C}_{57}\text{H}_{108}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1136.7$, Observed = 1137.8.

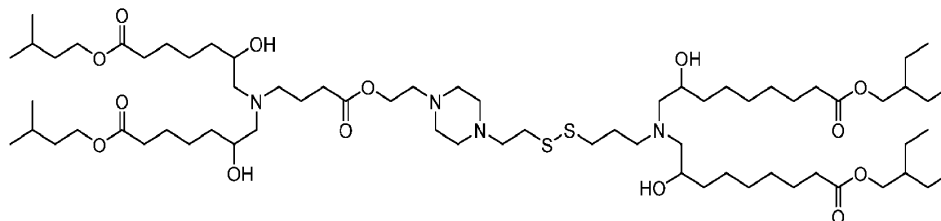
Bis(2-ethylbutyl) 9,9'-((4-(2-(4-(2-((3-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)-propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate)
(GL-HEPES-E3-E9Es6-DS-3-E7E4)



[0625] ^1H NMR (300 MHz, CDCl_3) δ 4.20 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.64 (m, 4H), 2.84-2.24 (m, 40H), 1.92-1.26 (m, 56H), 0.92 (d, 6H), 0.88 (t, 12H).

[0626] APCI-MS analysis: Calculated $\text{C}_{67}\text{H}_{128}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1277.8$, Observed = 1277.9.

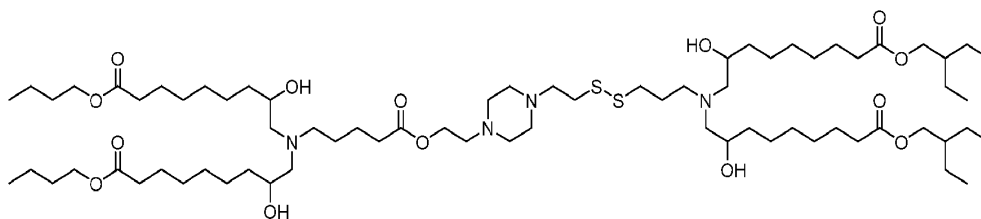
Bis(2-ethylbutyl) 9,9'-((3-((2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediy)bis(8-hydroxynonanoate)
(GL-HEPES-E3-E7Ei5-DS-3-E9Es6)



[0627] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.84-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.91-1.29 (m, 52H), 0.91 (d, 12H), 0.88 (t, 12H).

[0628] APCI-MS analysis: Calculated $\text{C}_{69}\text{H}_{132}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1305.9$, Observed = 1305.9.

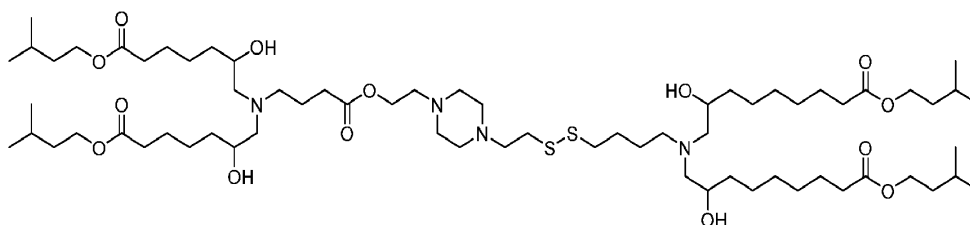
Dibutyl 9,9'-((5-(2-(4-(2-((3-(bis(9-(2-ethylbutoxy)-2-hydroxy-9-oxononyl)amino)-propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediy)bis(8-hydroxynonanoate)
(GL-HEPES-E4-E9E4-DS-3-E9Es6)



[0629] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.62 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.91-1.23 (m, 64H), 0.92 (t, 6H), 0.88 (t, 12H).

[0630] APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{138}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1348.0$, Observed = 1348.0.

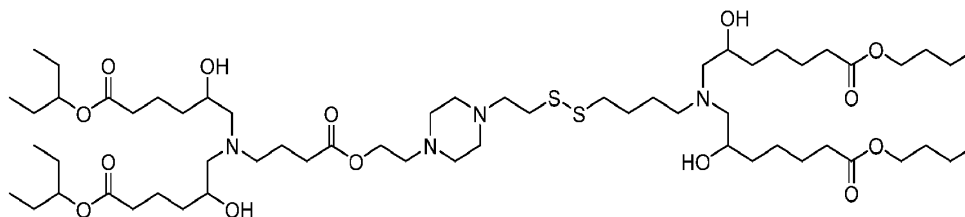
Diisopentyl 9,9'-((4-((2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediy)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7Ei5-DS-4-E9Ei5)



[0631] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 8H), 3.61 (m, 4H), 2.85-2.21 (m, 38H), 1.85-1.25 (m, 50H), 0.91 (d, 24H).

[0632] APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{130}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1291.9$, Observed = 1291.8.

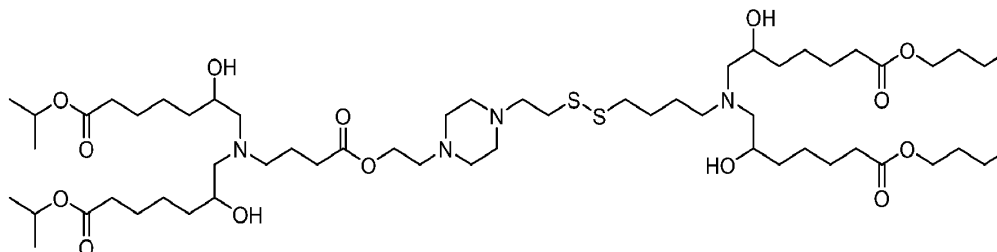
Dibutyl 7,7'-((4-((2-(4-(2-((4-(bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E6Es5-DS-4-E7E4)



[0633] ^1H NMR (300 MHz, CDCl_3) δ 4.75 (pent, 2H), 4.20 (t, 2H), 4.06 (t, 4H), 3.63 (m, 4H), 2.85-2.28 (m, 30H), 1.84-1.33 (m, 54H), 0.92 (d, 6H), 0.86 (t, 12H).

[0634] APCI-MS analysis: Calculated $\text{C}_{60}\text{H}_{114}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1179.7$, Observed = 1179.8.

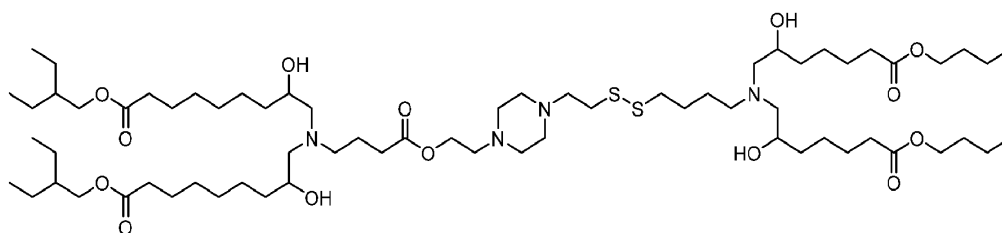
Dibutyl 7,7'-((4-((2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7Ei3-DS-4-E7E4)



[0635] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.06 (t, 4H), 3.62 (m, 4H), 2.84-2.24 (m, 34H), 1.85-1.30 (m, 40H), 1.22 (d, 12H), 0.92 (t, 12H).

[0636] APCI-MS analysis: Calculated $\text{C}_{58}\text{H}_{110}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1151.6$, Observed = 1151.7.

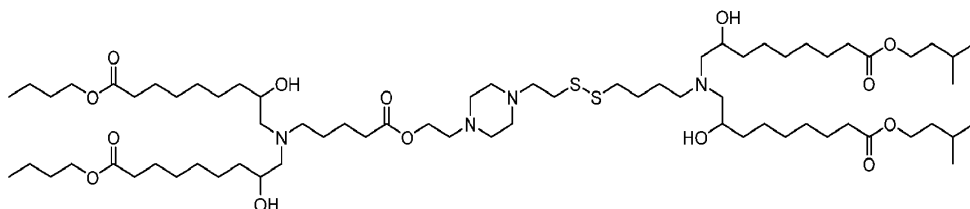
Bis(2-ethylbutyl) 9,9'-((4-((2-(4-(2-((4-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)-butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9Es6-DS-4-E7E4)



[0637] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.84-2.26 (m, 34H), 1.85-1.28 (m, 60H), 0.92 (t, 6H), 0.88 (t, 12H).

[0638] APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{130}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1291.9$, Observed = 1291.9.

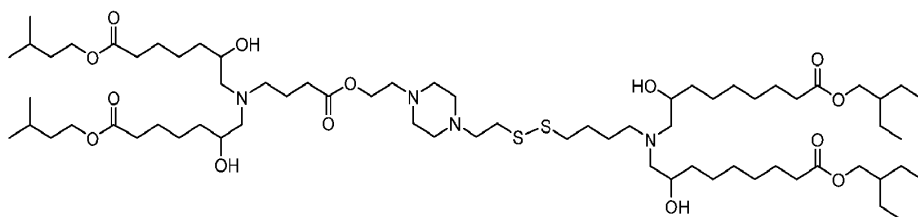
Dibutyl 9,9'-((5-(2-(4-(2-((4-bis(2-hydroxy-9-(isopentyloxy)-9-oxononyl)-amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)-bis(8-hydroxynonanoate) (GL-HEPES-E4-E9E4-DS-4-E9Ei5)



[0639] NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.06 (t, 4H), 3.61 (m, 4H), 2.85-2.25 (m, 38H), 1.80-1.25 (m, 62H), 0.92 (t, 6H), 0.91 (d, 12H).

[0640] APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{136}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1334.0$, Observed = 1334.0.

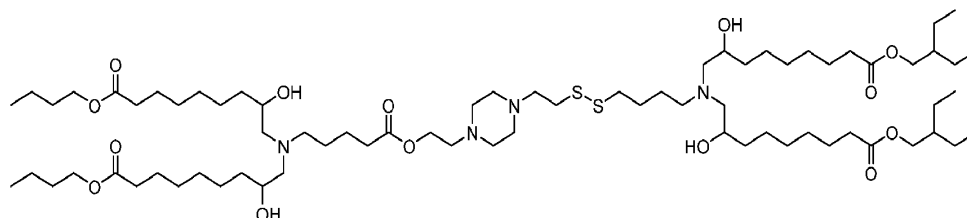
Bis(2-ethylbutyl) 9,9'-((4-((2-(4-(2-((4-bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7Ei5-DS-4-E9Es6)



[0641] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.84-2.45 (m, 22H), 2.44-2.25 (m, 16H), 1.83-1.28 (m, 54H), 0.91 (d, 12H), 0.88 (t, 12H).

[0642] APCI-MS analysis: Calculated $\text{C}_{70}\text{H}_{134}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1318.9$, Observed = 1319.0.

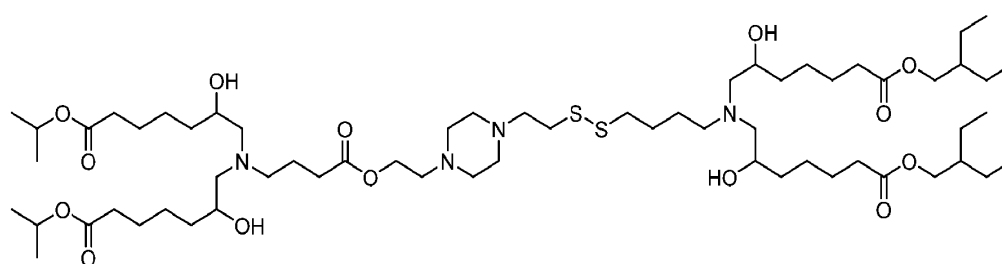
Dibutyl 9,9'-((5-(2-(4-(2-((4-(bis(9-(2-ethylbutoxy)-2-hydroxy-9-oxononyl)amino)-butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediy)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9E4-DS-4-E9Es6)



[0643] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.05 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.84-2.45 (m, 22H), 2.44-2.25 (m, 16H), 1.77-1.26 (m, 66H), 0.92 (t, 6H), 0.88 (t, 12H).

[0644] APCI-MS analysis: Calculated $\text{C}_{73}\text{H}_{140}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1362.0$, Observed = 1362.0.

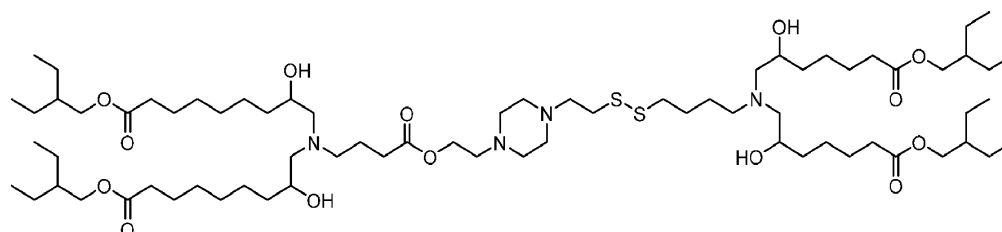
Bis(2-ethylbutyl) 7,7'-((4-(2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediy)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7Ei3-DS-4-E7Es6)



[0645] ^1H NMR (300 MHz, CDCl_3) δ 5.01 (hept, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.63 (m, 4H), 2.83-2.24 (m, 34H), 1.82-1.29 (m, 44H), 1.22 (d, 12H), 0.88 (t, 12H).

[0646] APCI-MS analysis: Calculated $\text{C}_{62}\text{H}_{118}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1207.7$, Observed = 1207.8.

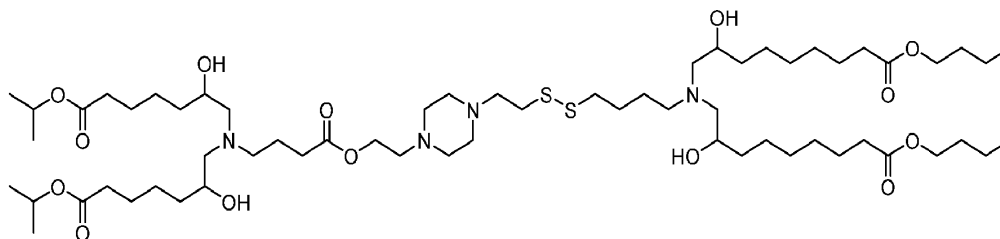
Bis(2-ethylbutyl) 9,9'-((4-(2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)-butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediy)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9Es6-DS-4-E7Es6)



[0647] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 3.98 (d, 8H), 3.61 (m, 4H), 2.84-2.26 (m, 36H), 1.83-1.28 (m, 64H), 0.88 (t, 24H).

[0648] APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{138}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1348.0$, Observed = 1348.0.

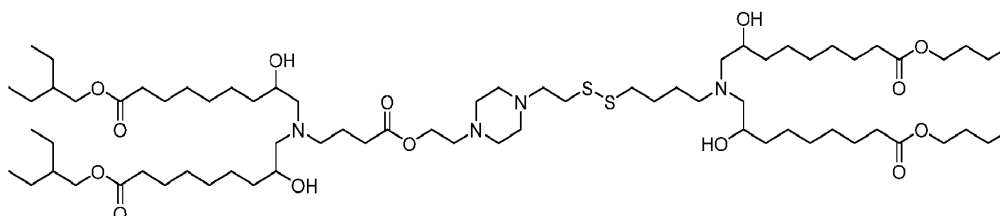
Dibutyl 9,9'-((4-((2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7Ei3-DS-4-E9E4)



[0649] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.06 (t, 4H), 3.61 (m, 4H), 2.83-2.24 (m, 36H), 1.82-1.27 (m, 52H), 1.22 (d, 12H), 0.93 (t, 6H).

[0650] APCI-MS analysis: Calculated $\text{C}_{62}\text{H}_{118}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1207.7$, Observed = 1207.8.

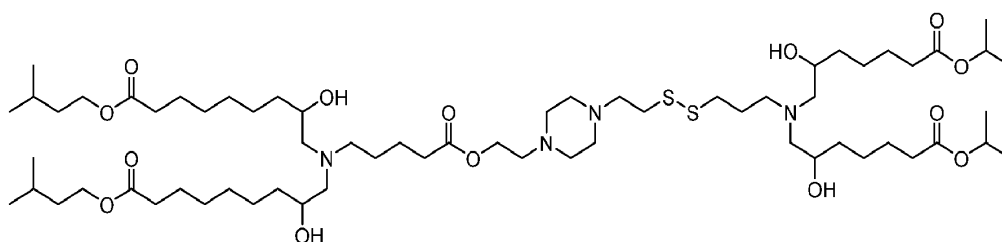
Dibutyl 9,9'-((4-((2-(4-(2-((4-(bis(9-(2-ethylbutoxy)-2-hydroxy-9-oxononyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9Es6-DS-4-E9E4)



[0651] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.62 (m, 4H), 2.84-2.25 (m, 36H), 1.83-1.28 (m, 70H), 0.92 (t, 6H), 0.88 (t, 12H).

[0652] APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{138}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1348.0$, Observed = 1348.0.

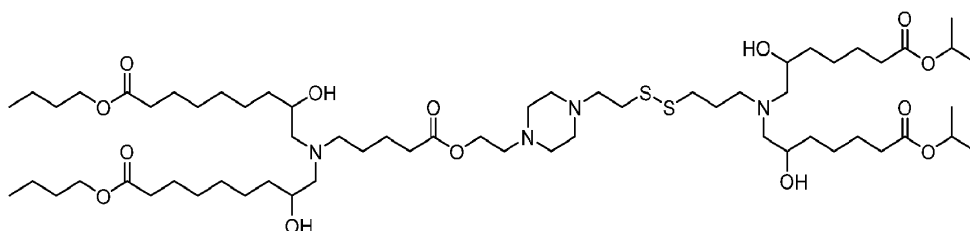
Diisopentyl 9,9'-((5-(2-(4-(2-((3-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Ei5-DS-3-E7Ei3)



[0653] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.08 (t, 4H), 3.62 (m, 4H), 2.85-2.22 (m, 38H), 1.85-1.24 (m, 44H), 1.22 (d, 12H), 0.91 (d, 12H).

[0654] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{122}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1235.8$, Observed = 1235.9.

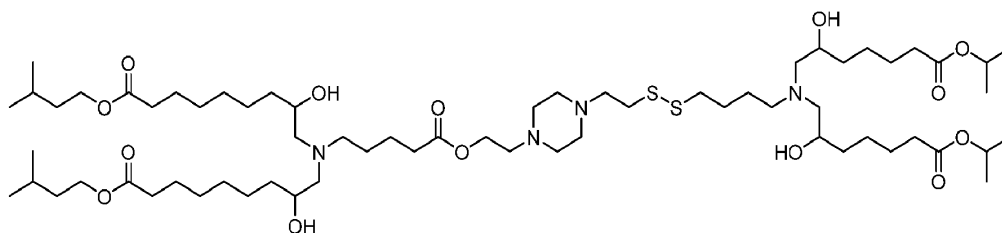
Dibutyl 9,9'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-pentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9E4-DS-3-E7Ei3)



[0655] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.18 (t, 2H), 4.05 (t, 4H), 3.64 (m, 4H), 2.86-2.21 (m, 38H), 1.90-1.28 (m, 46H), 1.22 (d, 12H), 0.90 (t, 6H).

[0656] APCI-MS analysis: Calculated $\text{C}_{62}\text{H}_{118}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1207.8$, Observed = 1207.8.

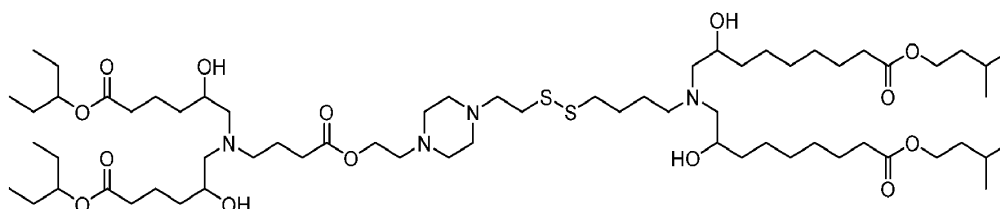
Diisopentyl 9,9'-((5-(2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Ei5-DS-4-E7Ei3)



[0657] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.08 (t, 4H), 3.61 (m, 4H), 2.85-2.22 (m, 38H), 1.78-1.24 (m, 46H), 1.22 (d, 12H), 0.91 (d, 12H).

[0658] APCI-MS analysis: Calculated $\text{C}_{65}\text{H}_{124}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1249.8$, Observed = 1249.9.

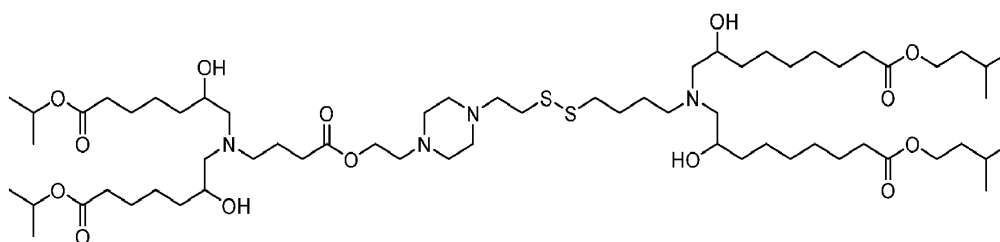
Diisopentyl 9,9'-((4-((2-(4-(2-((4-(bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E6Es5-DS-4-E9Ei5)



[0659] ^1H NMR (300 MHz, CDCl_3) δ 4.77 (pent, 2H), 4.19 (t, 2H), 4.08 (t, 4H), 3.62 (m, 4H), 2.85-2.25 (m, 36H), 1.86-1.24 (m, 54H), 0.91 (d, 12H), 0.86 (t, 12H).

[0660] APCI-MS analysis: Calculated $\text{C}_{66}\text{H}_{126}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1263.8$, Observed = 1263.9.

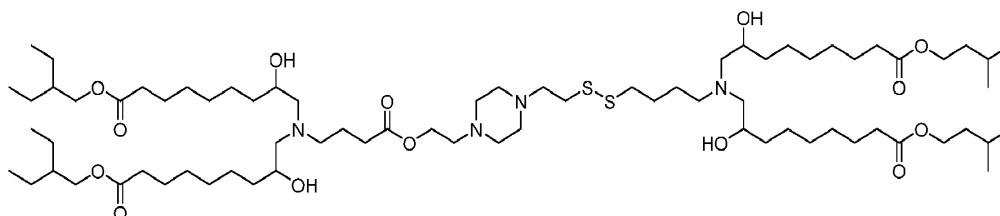
Diisopentyl 9,9'-((4-((2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7Ei3-DS-4-E9Ei5)



[0661] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.08 (t, 4H), 3.61 (m, 4H), 2.85-2.24 (m, 36H), 1.86-1.27 (m, 46H), 1.22 (d, 12H), 0.91 (t, 12H).

[0662] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{122}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1235.8$, Observed = 1235.9.

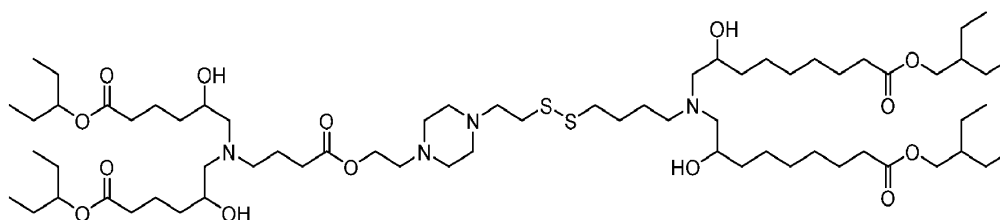
Bis(2-ethylbutyl) 9,9'-((4-((2-(4-(2-((4-(bis(2-hydroxy-9-(isopentyloxy)-9-oxononyl)amino)butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9Es6-DS-4-E9Ei5)



[0663] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.85-2.25 (m, 38H), 1.85-1.24 (m, 62H), 0.91 (d, 12H), 0.88 (t, 12H).

[0664] APCI-MS analysis: Calculated $\text{C}_{74}\text{H}_{142}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1376.0$, Observed = 1376.1.

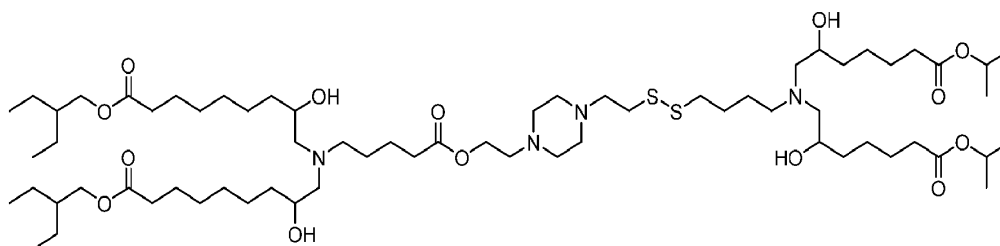
Bis(2-ethylbutyl) 9,9'-((4-((2-(4-(2-((4-(bis(2-hydroxy-6-oxo-6-(pentan-3-yloxy)hexyl)-amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E6Es5-DS-4-E9Es6)



[0665] ^1H NMR (300 MHz, CDCl_3) δ 4.75 (pent, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.61 (m, 4H), 2.85-2.25 (m, 38H), 1.86-1.24 (m, 52H), 0.88 (t, 12H), 0.86 (t, 12H).

[0666] APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{130}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1291.9$, Observed = 1291.9.

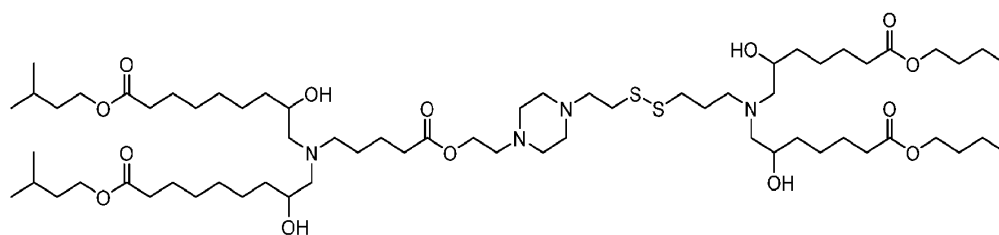
Bis(2-ethylbutyl) 9,9'-((5-(2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Es6-DS-4-E7Ei3)



[0667] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.62 (m, 4H), 2.85-2.23 (m, 38H), 1.83-1.25 (m, 50H), 1.22 (d, 12H), 0.88 (t, 12H).

[0668] APCI-MS analysis: Calculated $\text{C}_{67}\text{H}_{128}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1277.9$, Observed = 1277.9.

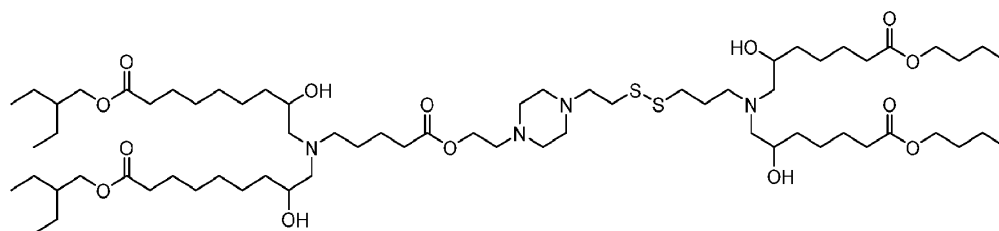
Diisopentyl 9,9'-((5-(2-(4-(2-((3-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)-propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Ei5-DS-3-E7E4)



[0669] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.06 (t, 4H), 3.65 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.91-1.23 (m, 52H), 0.92 (t, 6H), 0.91 (d, 12H).

[0670] APCI-MS analysis: Calculated $\text{C}_{66}\text{H}_{126}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1263.8$, Observed = 1263.9.

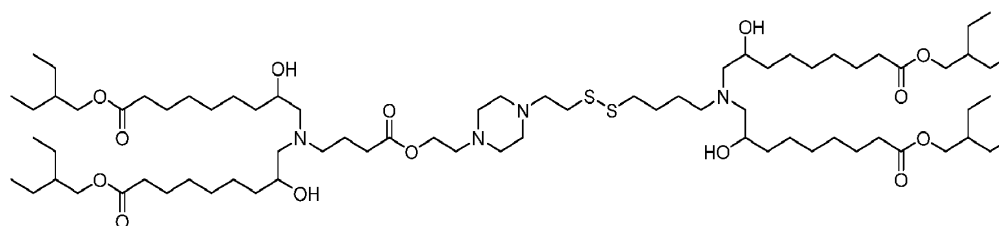
Bis(2-ethylbutyl) 9,9'-((5-(2-(4-(2-((3-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)-propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Es6-DS-3-E7E4)



[0671] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.63 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.91-1.23 (m, 62H), 0.93 (t, 6H), 0.88 (t, 12H).

[0672] APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{130}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1291.9$, Observed = 1291.9.

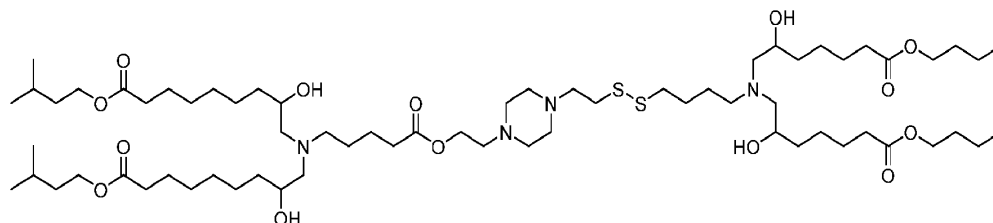
Bis(2-ethylbutyl) 9,9'-((4-((2-(4-(2-((4-(bis(9-(2-ethylbutoxy)-2-hydroxy-9-oxononyl)amino)butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E9Es6-DS-4-E9Es6)



[0673] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 3.98 (d, 8H), 3.62 (m, 4H), 2.85-2.25 (m, 36H), 1.85-1.24 (m, 72H), 0.88 (t, 24H).

[0674] APCI-MS analysis: Calculated $\text{C}_{76}\text{H}_{146}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1404.1$, Observed = 1404.0.

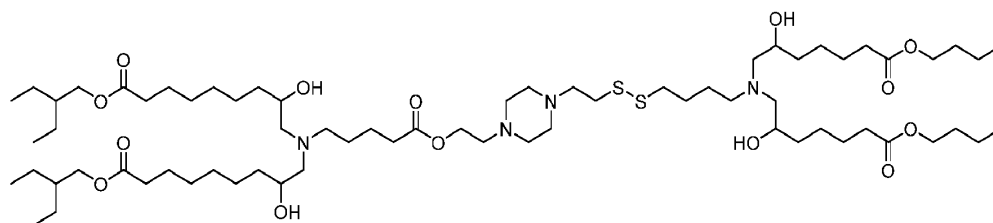
Diisopentyl 9,9'-((5-(2-(4-(2-((4-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)butyl)-disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Ei5-DS-4-E7E4)



[0675] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.06 (t, 4H), 3.61 (m, 4H), 2.84-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.80-1.25 (m, 54H), 0.92 (t, 6H), 0.91 (d, 12H).

[0676] APCI-MS analysis: Calculated $\text{C}_{67}\text{H}_{128}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1277.9$, Observed = 1278.0.

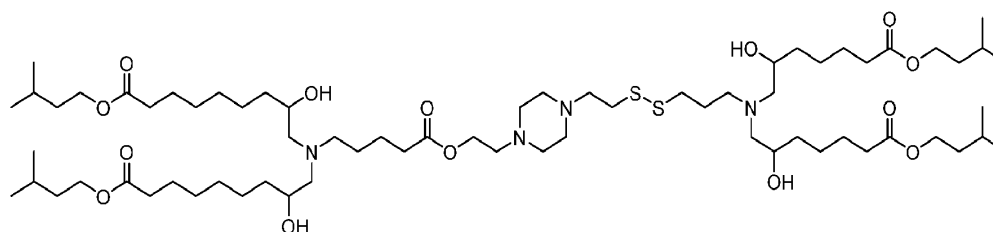
Bis(2-ethylbutyl) 9,9'-((5-(2-(4-(2-((4-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)butyl)-disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Es6-DS-4-E7E4)



[0677] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.80-1.23 (m, 58H), 0.92 (t, 6H), 0.88 (t, 12H).

[0678] APCI-MS analysis: Calculated $\text{C}_{69}\text{H}_{132}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1305.9$, Observed = 1306.0.

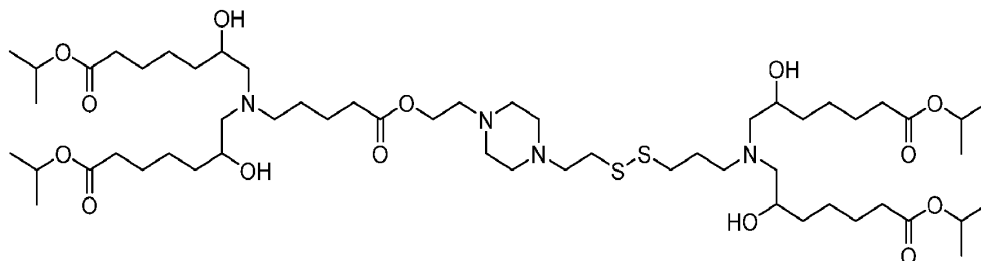
Diisopentyl 9,9'-((5-(2-(4-(2-((3-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Ei5-DS-3-E7Ei5)



[0679] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 8H), 3.62 (m, 4H), 2.85-2.23 (m, 38H), 1.91-1.25 (m, 54H), 0.91 (d, 24H).

[0680] APCI-MS analysis: Calculated C₆₈H₁₃₀N₄O₁₄S₂ [M+H] = 1291.9, Observed = 1292.0.

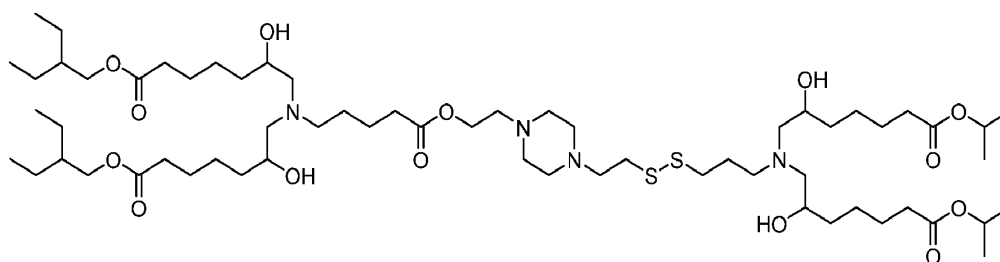
Diisopropyl 7,7'-((3-((2-(4-(2-((5-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate)
(GL-HEPES-E4-E7Ei3-DS-3-E7Ei3)



[0681] ¹H NMR (300 MHz, CDCl₃) δ 4.99 (hept, 4H), 4.19 (t, 2H), 3.62 (m, 4H), 2.85-2.24 (m, 34H), 1.95-1.32 (m, 38H), 1.22 (d, 24H).

[0682] APCI-MS analysis: Calculated C₅₆H₁₀₆N₄O₁₄S₂ [M+H] = 1123.6, Observed = 1123.7.

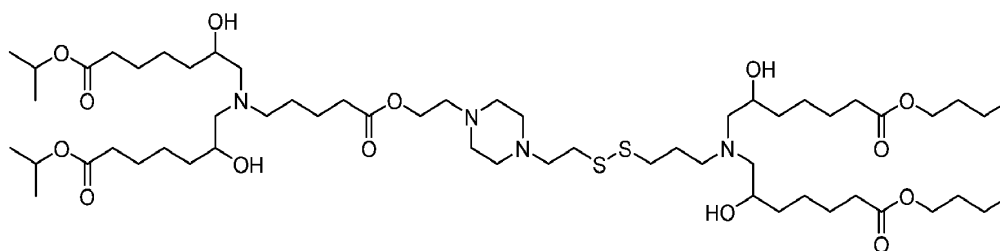
Bis(2-ethylbutyl) 7,7'-((5-(2-(4-(2-((3-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(6-hydroxyheptanoate)
(GL-HEPES-E4-E7Es6-DS-3-E7Ei3)



[0683] ¹H NMR (300 MHz, CDCl₃) δ 4.99 (hept, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.65 (m, 4H), 2.85-2.23 (m, 40H), 1.88-1.29 (m, 42H), 1.22 (d, 12H), 0.88 (t, 12H).

[0684] APCI-MS analysis: Calculated C₆₂H₁₁₈N₄O₁₄S₂ [M+H] = 1207.7, Observed = 1207.9.

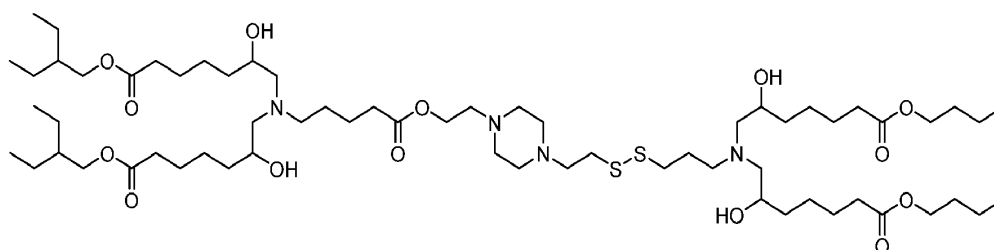
Dibutyl 7,7'-((3-((2-(4-(2-((5-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate)
(GL-HEPES-E4-E7Ei3-DS-3-E7E4)



[0685] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.06 (t, 4H), 3.62 (m, 4H), 2.85-2.24 (m, 40H), 1.95-1.30 (m, 40H), 1.22 (d, 12H), 0.92 (t, 6H).

[0686] APCI-MS analysis: Calculated $\text{C}_{58}\text{H}_{110}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1151.6$, Observed = 1151.8.

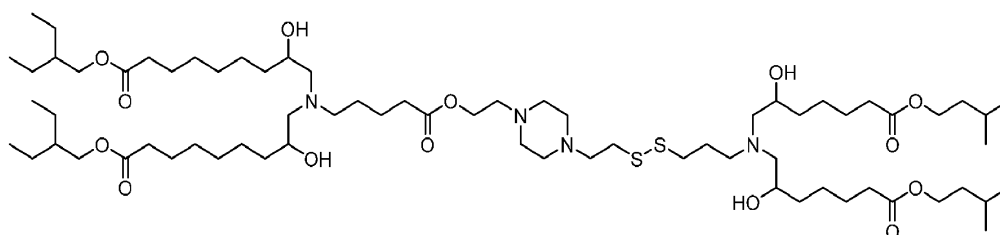
Dibutyl 7,7'-((3-((2-(4-(2-((5-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate)
(GL-HEPES-E4-E7Es6-DS-3-E7E4)



[0687] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.65 (m, 4H), 2.85-2.28 (m, 38H), 1.95-1.24 (m, 52H), 0.92 (t, 6H), 0.88 (t, 12H).

[0688] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{122}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1235.8$, Observed = 1235.8.

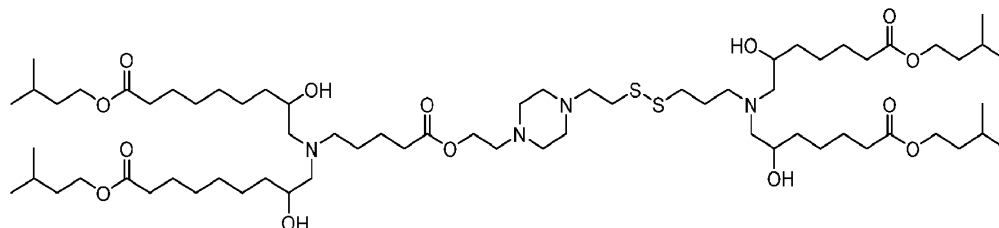
Bis(2-ethylbutyl) 9,9'-((5-(2-(4-(2-((3-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)-propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate)
(GL-HEPES-E4-E9Es6-DS-3-E7Ei5)



[0689] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.90-1.24 (m, 58H), 0.91 (d, 12H), 0.88 (t, 12H).

[0690] APCI-MS analysis: Calculated $\text{C}_{70}\text{H}_{134}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1319.9$, Observed = 1320.0.

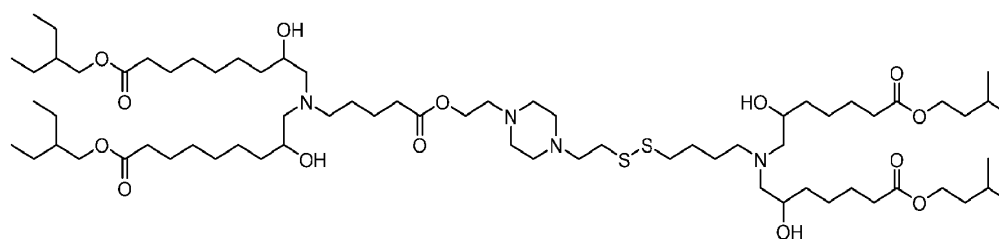
Diisopentyl 9,9'-((5-(2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)butyl)-disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediy)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Ei5-DS-4-E7Ei5)



[0691] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 8H), 3.62 (m, 4H), 2.85-2.23 (m, 38H), 1.79-1.25 (m, 56H), 0.91 (d, 24H).

[0692] APCI-MS analysis: Calculated $\text{C}_{69}\text{H}_{132}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1305.9$, Observed = 1305.9.

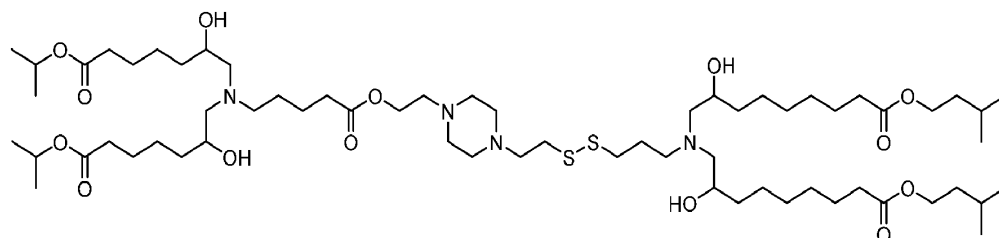
Bis(2-ethylbutyl) 9,9'-((5-(2-(4-(2-((4-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)-butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediy)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Es6-DS-4-E7Ei5)



[0693] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.80-1.24 (m, 60H), 0.91 (d, 12H), 0.88 (t, 12H).

[0694] APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{136}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1334.0$, Observed = 1333.9.

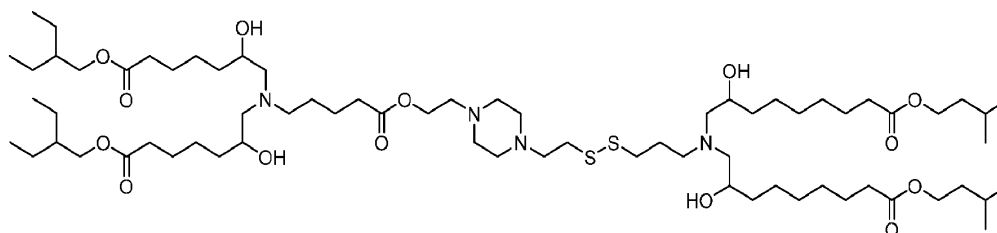
Diisopentyl 9,9'-((3-((2-(4-(2-((5-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediy)bis(8-hydroxynonanoate) (GL-HEPES-E4-E7Ei3-DS-3-E9Ei5)



[0695] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.08 (t, 8H), 3.63 (m, 4H), 2.85-2.24 (m, 40H), 1.95-1.27 (m, 40H), 1.22 (d, 12H), 0.91 (t, 12H).

[0696] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{122}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1235.8$, Observed = 1235.9.

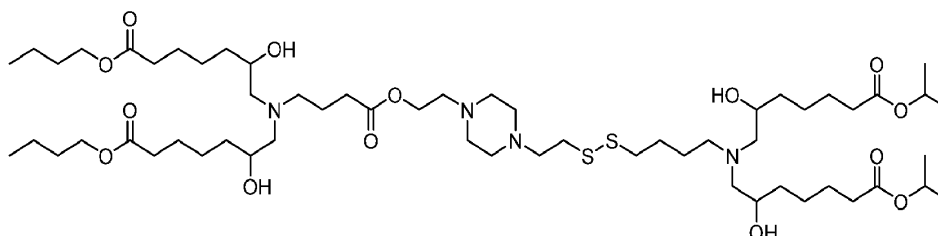
Diisopentyl 9,9'-((3-((2-(4-(2-((5-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate)) (GL-HEPES-E4-E7Es6-DS-3-E9Ei5)



[0697] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.63 (m, 4H), 2.85-2.25 (m, 40H), 1.95-1.24 (m, 52H), 0.91 (t, 12H), 0.88 (t, 12H).

[0698] APCI-MS analysis: Calculated $\text{C}_{70}\text{H}_{134}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1319.9$, Observed = 1320.0.

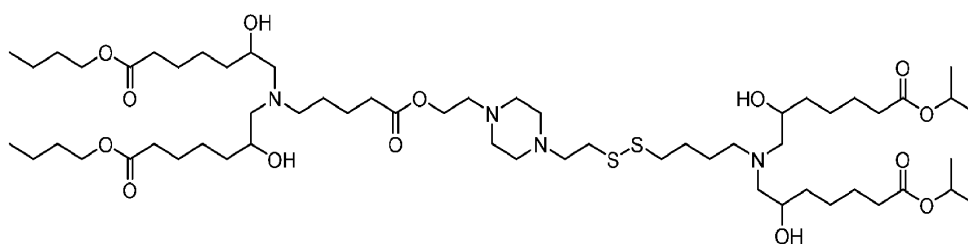
Dibutyl 7,7'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)propyl)-disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7E4-DS-4-E7Ei3)



[0699] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.06 (t, 4H), 3.63 (m, 4H), 2.85-2.22 (m, 38H), 1.85-1.28 (m, 38H), 1.22 (d, 12H), 0.92 (t, 6H).

[0700] APCI-MS analysis: Calculated $\text{C}_{58}\text{H}_{110}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1151.6$, Observed = 1151.1.

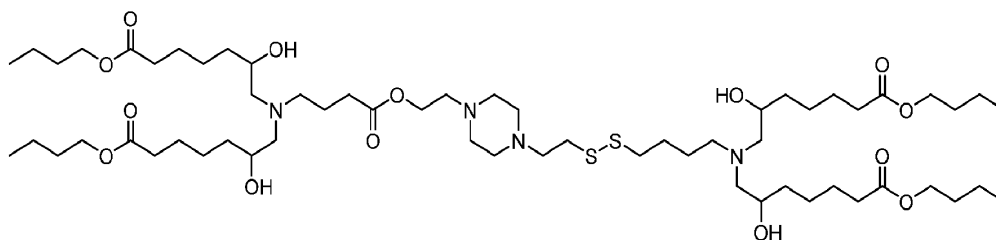
Dibutyl 7,7'-((5-(2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)butyl)-disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E4-E7E4-DS-4-E7Ei3)



[0701] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.06 (t, 4H), 3.62 (m, 4H), 2.85-2.22 (m, 38H), 1.85-1.24 (m, 40H), 1.22 (d, 12H), 0.92 (t, 6H).

[0702] APCI-MS analysis: Calculated $\text{C}_{59}\text{H}_{112}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1165.6$, Observed = 1165.2.

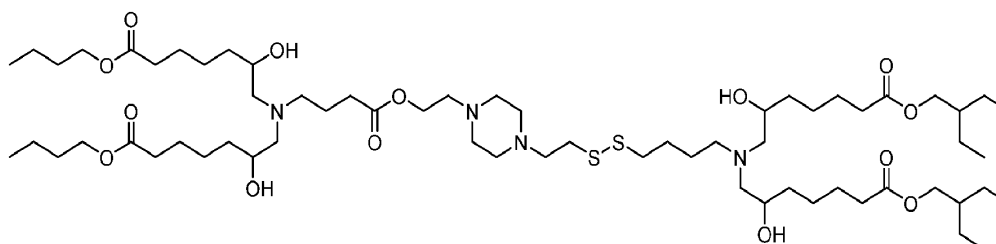
Dibutyl 7,7'-((4-((2-(4-(2-((4-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)butanoyl)-oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7E4-DS-4-E7E4)



[0703] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 8H), 3.62 (m, 4H), 2.85-2.22 (m, 38H), 1.85-1.24 (m, 50H), 0.92 (t, 12H).

[0704] APCI-MS analysis: Calculated $\text{C}_{60}\text{H}_{114}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1179.7$, Observed = 1179.0.

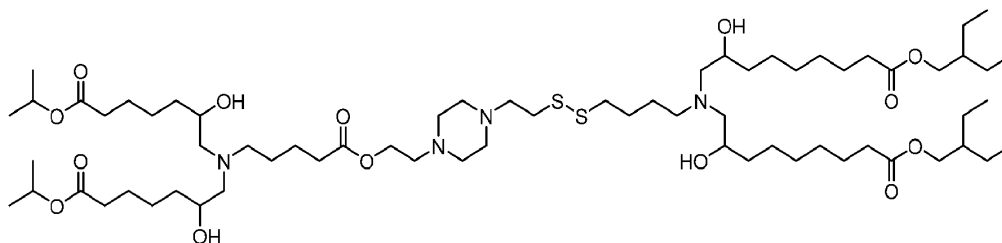
Dibutyl 7,7'-((4-(2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)butyl)-disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7E4-DS-4-E7Es6)



[0705] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.63 (m, 4H), 2.85-2.22 (m, 38H), 1.85-1.24 (m, 36H), 0.92 (t, 6H), 0.88 (t, 12H).

[0706] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{122}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1235.8$, Observed = 1235.0.

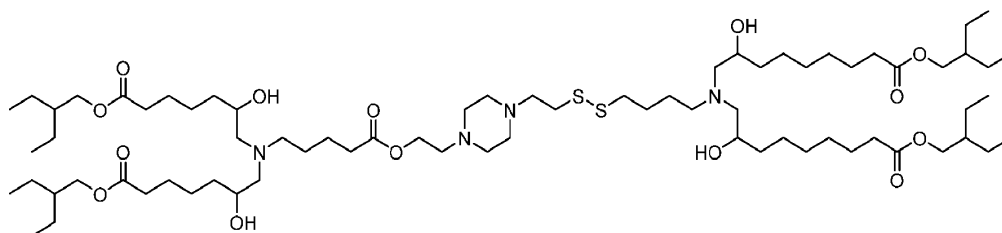
Bis(2-ethylbutyl) 9,9'-((4-((2-(4-(2-((5-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate)
(GL-HEPES-E4-E7Ei3-DS-4-E9Es6)



[0707] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.64 (m, 4H), 2.85-2.24 (m, 40H), 1.78-1.29 (m, 48H), 1.21 (d, 12H), 0.88 (t, 12H).

[0708] APCI-MS analysis: Calculated $\text{C}_{67}\text{H}_{128}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1277.9$, Observed = 1277.0.

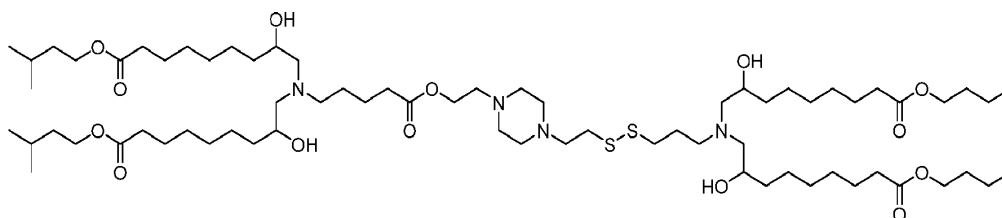
Bis(2-ethylbutyl) 9,9'-((4-((2-(4-(2-((5-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)-amino)pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E7Es6-DS-4-E9Es6)



[0709] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 3.98 (d, 8H), 3.63 (m, 4H), 2.85-2.25 (m, 40H), 1.90-1.24 (m, 62H), 0.88 (t, 24H).

[0710] APCI-MS analysis: Calculated $\text{C}_{73}\text{H}_{140}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1362.0$, Observed = 1361.2.

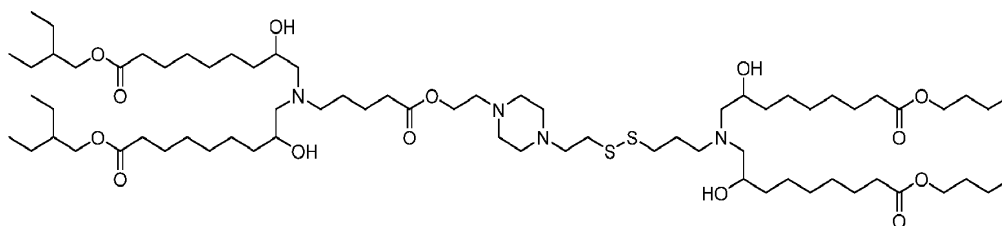
Dibutyl 9,9'-((3-((2-(4-(2-((5-(bis(2-hydroxy-9-(isopentyl)oxy)-9-oxononyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate)
(GL-HEPES-E4-E9Ei5-DS-3-E9E4)



[0711] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.06 (t, 4H), 3.64 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.90-1.23 (m, 64H), 0.92 (t, 6H), 0.91 (d, 12H).

[0712] APCI-MS analysis: Calculated $\text{C}_{70}\text{H}_{134}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1319.9$, Observed = 1319.0.

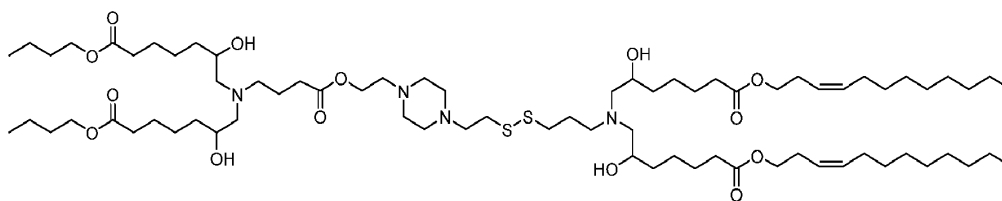
Dibutyl 9,9'-((3-((2-(4-(2-((5-(bis(9-(2-ethylbutoxy)-2-hydroxy-9-oxononyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Es6-DS-3-E9E4)



[0713] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.90-1.23 (m, 68H), 0.92 (t, 6H), 0.88 (d, 12H).

[0714] APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{138}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1348.0$, Observed = 1346.9.

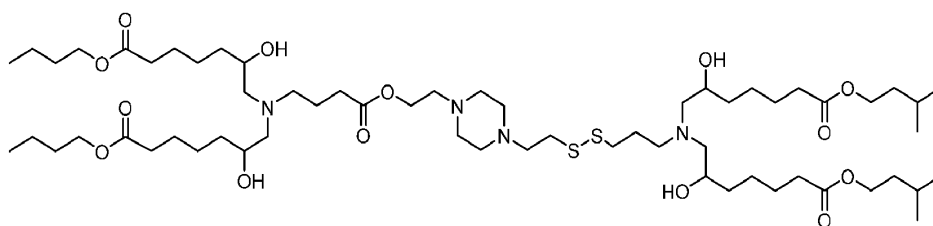
Dibutyl 7,7'-((4-(2-(4-(2-((3-(bis(7-(((Z)-dodec-3-en-1-yl)oxy)-2-hydroxy-7-oxoheptyl)-amino)propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7E4-DS-3-E7E12-1)



[0715] ^1H NMR (300 MHz, CDCl_3) δ 5.56-5.25 (m, 4H), 4.19 (t, 2H), 4.06 (t, 8H), 3.64 (m, 4H), 2.86-2.46 (m, 22H), 2.43-2.23 (m, 16H), 2.08-1.22 (m, 72H), 0.92 (t, 6H), 0.87 (t, 6H).

[0716] APCI-MS analysis: Calculated $\text{C}_{75}\text{H}_{140}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1386.1$, Observed = 1386.6.

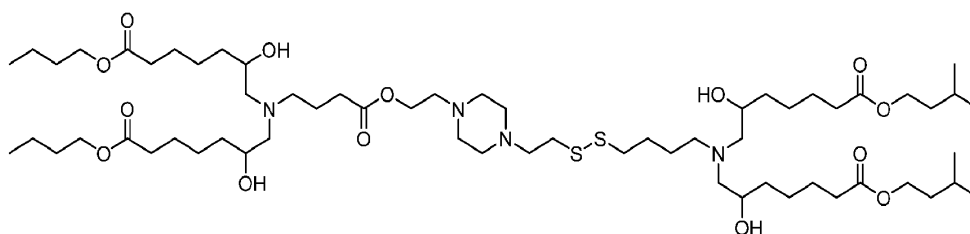
Dibutyl 7,7'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)propyl)-disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7E4-DS-3-E7E15)



[0717] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.06 (t, 4H), 3.64 (m, 4H), 2.87-2.46 (m, 22H), 2.45-2.26 (m, 16H), 1.91-1.31 (m, 46H), 0.92 (t, 6H), 0.91 (d, 12H).

[0718] APCI-MS analysis: Calculated $\text{C}_{61}\text{H}_{116}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1193.7$, Observed = 1193.4.

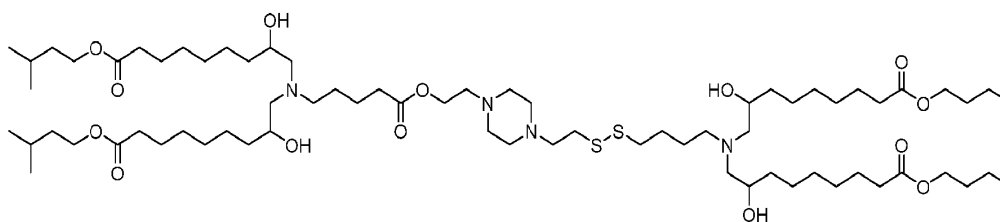
Dibutyl 7,7'-((4-(2-(4-(2-((4-bis(2-hydroxy-7-(isopentyloxy)-7-oxoheptyl)amino)-butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7E4-DS-4-E7Ei5)



[0719] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.06 (t, 4H), 3.63 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.25 (m, 16H), 1.91-1.28 (m, 48H), 0.92 (t, 6H), 0.91 (d, 12H).

[0720] APCI-MS analysis: Calculated $\text{C}_{62}\text{H}_{118}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1207.7$, Observed = 1207.4.

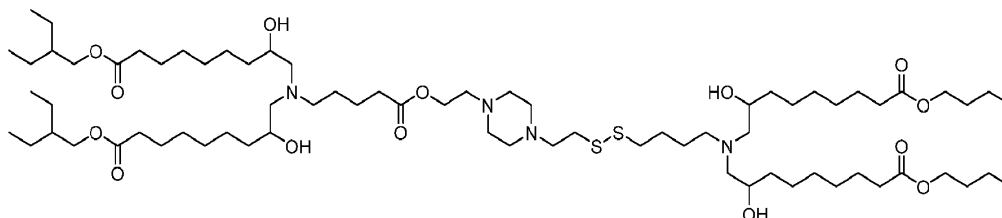
Dibutyl 9,9'-((4-(2-(4-(2-((5-bis(2-hydroxy-9-(isopentyloxy)-9-oxononyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Ei5-DS-4-E9E4)



[0721] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 4.05 (t, 4H), 3.64 (m, 4H), 2.86-2.23 (m, 40H), 1.75-1.23 (m, 64H), 0.92 (t, 6H), 0.91 (d, 12H).

[0722] APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{136}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1334.0$, Observed = 1333.7.

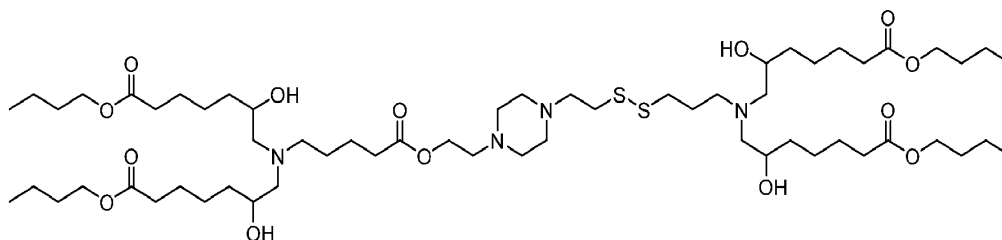
Dibutyl 9,9'-((3-((2-(4-(2-((5-(bis(9-(2-ethylbutoxy)-2-hydroxy-9-oxononyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(8-hydroxynonanoate)
(GL-HEPES-E4-E9Es6-DS-4-E9E4)



[0723] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 4H), 3.98 (d, 4H), 3.63 (m, 4H), 2.86-2.23 (m, 38H), 1.70-1.23 (m, 62H), 0.92 (t, 6H), 0.88 (t, 12H).

[0724] APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{136}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1362.0$, Observed = 1361.5.

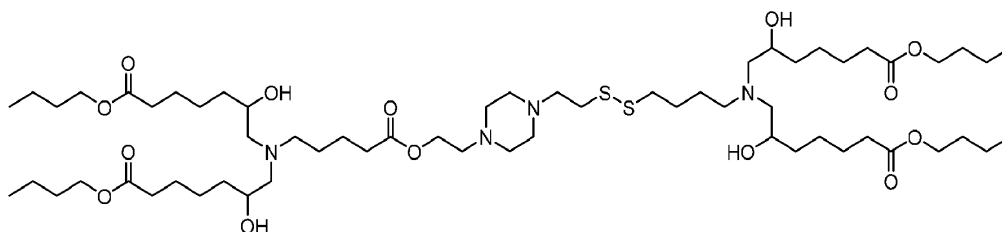
Dibutyl 7,7'-((3-((2-(4-(2-((5-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate)
(GL-HEPES-E4-E7E4-DS-3-E7E4)



[0725] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 8H), 3.64 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.26 (m, 16H), 1.91-1.30 (m, 50H), 0.92 (t, 12H).

[0726] APCI-MS analysis: Calculated $\text{C}_{60}\text{H}_{114}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1179.7$, Observed = 1179.4.

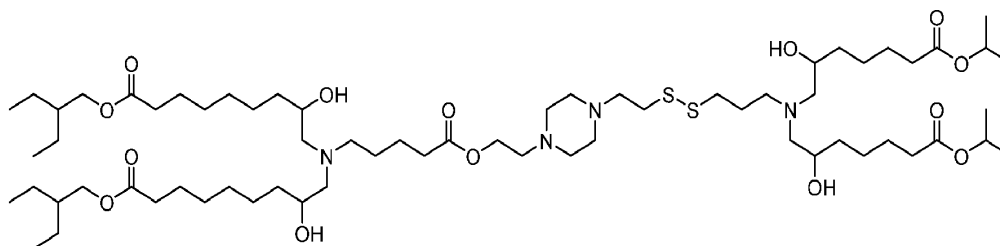
Dibutyl 7,7'-((5-(2-(4-(2-((4-(bis(7-butoxy-2-hydroxy-7-oxoheptyl)amino)butyl)-disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E4-E7E4-DS-4-E7E4)



[0727] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.06 (t, 8H), 3.63 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.26 (m, 16H), 1.81-1.30 (m, 52H), 0.92 (t, 12H).

[0728] APCI-MS analysis: Calculated $\text{C}_{61}\text{H}_{116}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1193.7$, Observed = 1193.5.

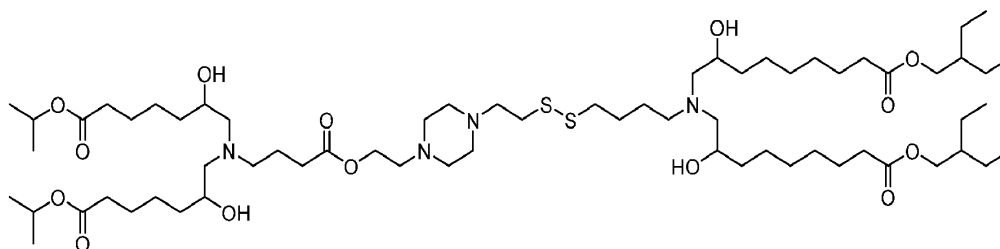
Bis(2-ethylbutyl) 9,9'-((5-(2-(4-(2-((3-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E4-E9Es6-DS-3-E7Ei3)



[0729] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.62 (m, 4H), 2.86-2.22 (m, 38H), 1.85-1.24 (m, 40H), 1.22 (d, 12H), 0.88 (t, 12H).

[0730] APCI-MS analysis: Calculated $\text{C}_{66}\text{H}_{126}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1263.8$, Observed = 1263.9.

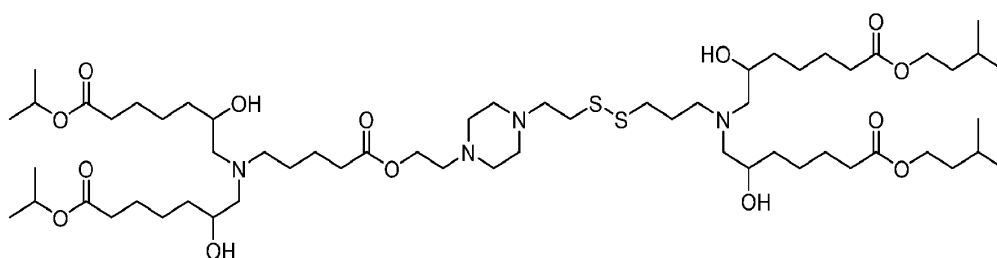
Bis(2-ethylbutyl) 9,9'-((4-((2-(4-(2-((4-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-butanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)butyl)azanediyl)bis(8-hydroxynonanoate) (GL-HEPES-E3-E7Ei3-DS-4-E9Es6)



[0731] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 3.98 (d, 4H), 3.61 (m, 4H), 2.85-2.24 (m, 38H), 1.86-1.27 (m, 48H), 1.22 (d, 12H), 0.88 (t, 12H).

[0732] APCI-MS analysis: Calculated $\text{C}_{66}\text{H}_{126}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1263.8$, Observed = 1263.9.

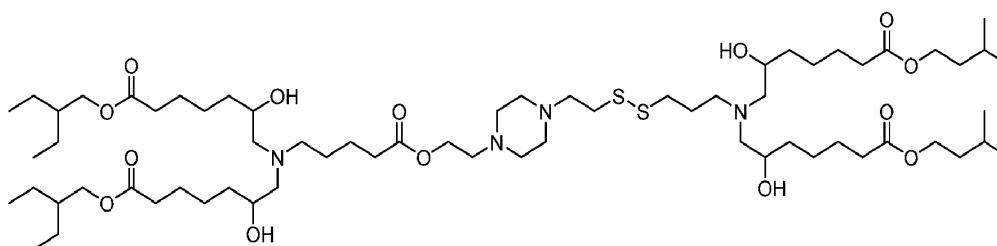
Diisopentyl 7,7'-((3-((2-(4-(2-((5-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E4-E7Ei3-DS-3-E7Ei5)



[0733] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.08 (t, 8H), 3.62 (m, 4H), 2.85-2.24 (m, 40H), 1.95-1.27 (m, 34H), 1.21 (d, 12H), 0.91 (d, 12H).

[0734] APCI-MS analysis: Calculated $\text{C}_{60}\text{H}_{114}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1179.7$, Observed = 1179.8.

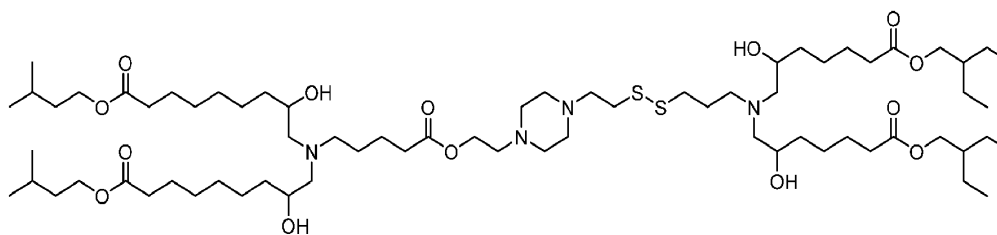
Diisopentyl 7,7'-((3-((2-((4-((5-bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate))
(GL-HEPES-E4-E7Es6-DS-3-E7Ei5)



[0735] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.63 (m, 4H), 2.85-2.25 (m, 40H), 1.95-1.24 (m, 48H), 0.91 (d, 12H), 0.88 (t, 12H).

[0736] APCI-MS analysis: Calculated $\text{C}_{66}\text{H}_{126}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1263.8$, Observed = 1263.9.

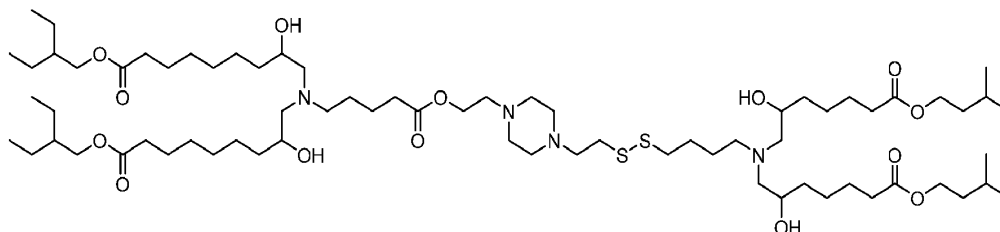
Diisopentyl 9,9'-((5-(2-(4-((3-bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)-propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate))
(GL-HEPES-E4-E9Ei5-DS-3-E7Es6)



[0737] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.62 (m, 4H), 2.85-2.23 (m, 38H), 1.79-1.25 (m, 58H), 0.91 (d, 12H), 0.88 (t, 12H).

[0738] APCI-MS analysis: Calculated $\text{C}_{70}\text{H}_{134}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1319.9$, Observed = 1320.0.

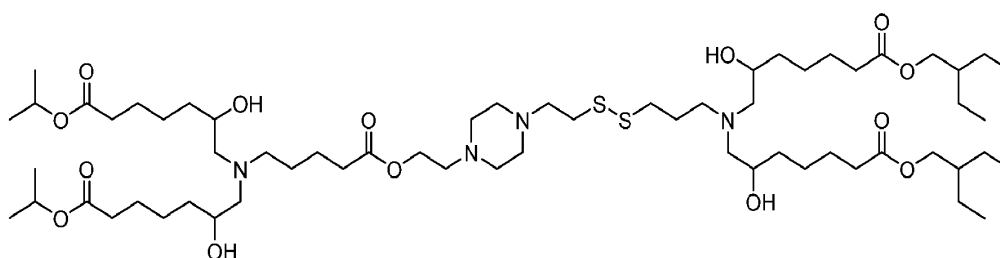
Bis(2-ethylbutyl) 9,9'-((5-(2-(4-(2-((3-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)-propyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate)
(GL-HEPES-E4-E9Es6-DS-3-E7Es6)



[0739] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 3.98 (d, 8H), 3.63 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.88-1.24 (m, 62H), 0.88 (t, 24H).

[0740] APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{138}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1348.0$, Observed = 1348.0.

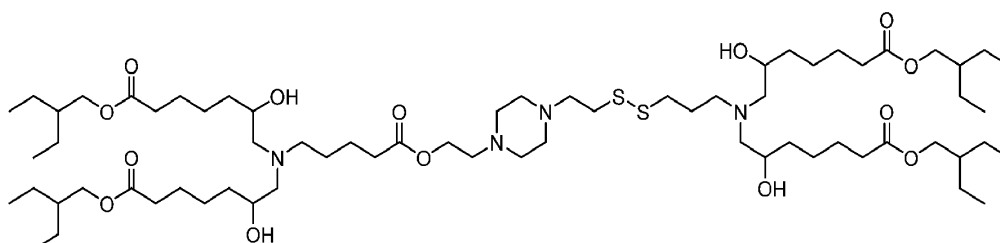
Bis(2-ethylbutyl) 7,7'-((3-((2-(4-(2-((5-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)-pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate)
(GL-HEPES-E4-E7Ei3-DS-3-E7Es6)



[0741] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 3.98 (d, 8H), 3.64 (m, 4H), 2.85-2.24 (m, 40H), 1.95-1.29 (m, 38H), 1.21 (d, 12H), 0.88 (t, 12H).

[0742] APCI-MS analysis: Calculated $\text{C}_{62}\text{H}_{118}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1207.7$, Observed = 1207.8.

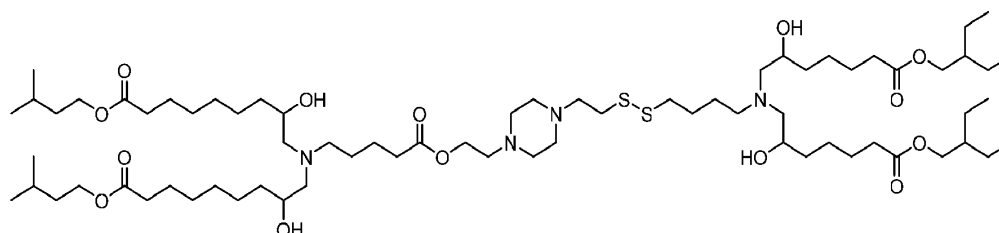
Bis(2-ethylbutyl) 7,7'-((3-((2-(4-(2-((5-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)-amino)pentanoyl)oxy)ethyl)piperazin-1-yl)ethyl)disulfaneyl)propyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E4-E7Es6-DS-3-E7Es6)



[0743] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 3.98 (d, 8H), 3.63 (m, 4H), 2.85-2.25 (m, 40H), 1.95-1.24 (m, 52H), 0.88 (t, 24H).

[0744] APCI-MS analysis: Calculated $\text{C}_{68}\text{H}_{130}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1291.9$, Observed = 1291.9.

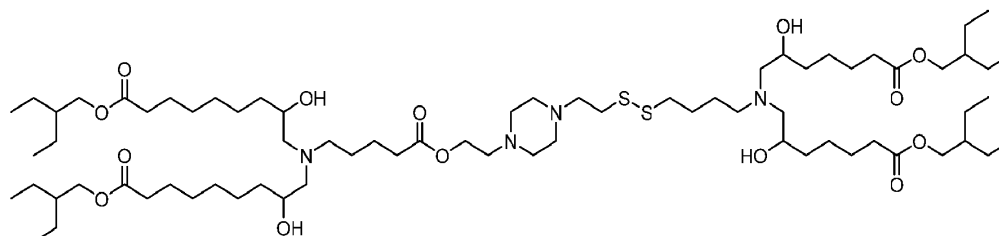
Diisopentyl 9,9'-((5-(2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)-butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate)
(GL-HEPES-E4-E9Ei5-DS-4-E7Es6)



[0745] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 4.08 (t, 4H), 3.98 (d, 4H), 3.61 (m, 4H), 2.85-2.23 (m, 40H), 1.79-1.25 (m, 52H), 0.91 (d, 12H), 0.88 (t, 12H).

[0746] APCI-MS analysis: Calculated $\text{C}_{71}\text{H}_{136}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1334.0$, Observed = 1334.0.

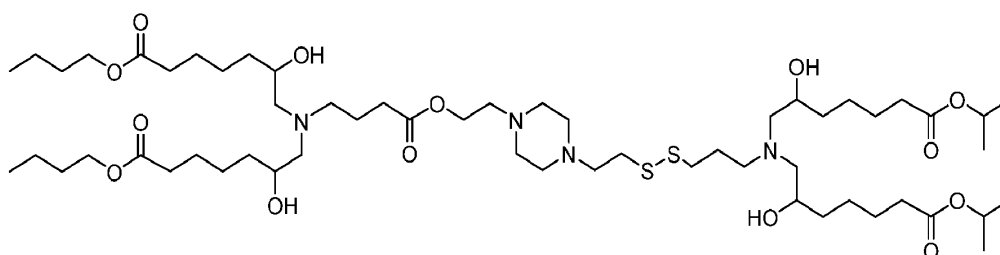
Bis(2-ethylbutyl) 9,9'-((5-(2-(4-(2-((4-(bis(7-(2-ethylbutoxy)-2-hydroxy-7-oxoheptyl)amino)-butyl)disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(8-hydroxynonanoate)
(GL-HEPES-E4-E9Es6-DS-4-E7Es6)



[0747] ^1H NMR (300 MHz, CDCl_3) δ 4.19 (t, 2H), 3.98 (d, 8H), 3.61 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.75-1.24 (m, 60H), 0.88 (t, 24H).

[0748] APCI-MS analysis: Calculated $\text{C}_{73}\text{H}_{140}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1362.0$, Observed = 1362.0.

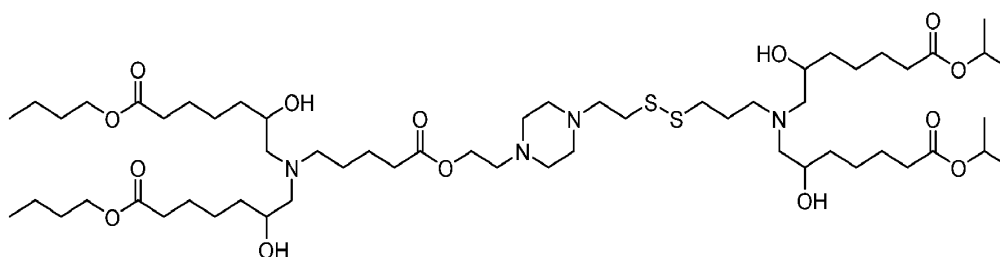
Dibutyl 7,7'-((4-(2-(4-(2-((3-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)propyl)-disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-4-oxobutyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E3-E7E4-DS-3-E7Ei3)



[0749] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.05 (t, 4H), 3.63 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.88-1.30 (m, 40H), 1.22 (d, 12H), 0.92 (t, 6H).

[0750] APCI-MS analysis: Calculated $\text{C}_{57}\text{H}_{108}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1137.6$, Observed = 1137.7.

Dibutyl 7,7'-((5-(2-(4-(2-((3-(bis(2-hydroxy-7-isopropoxy-7-oxoheptyl)amino)propyl)-disulfaneyl)ethyl)piperazin-1-yl)ethoxy)-5-oxopentyl)azanediyl)bis(6-hydroxyheptanoate) (GL-HEPES-E4-E7E4-DS-3-E7Ei3)



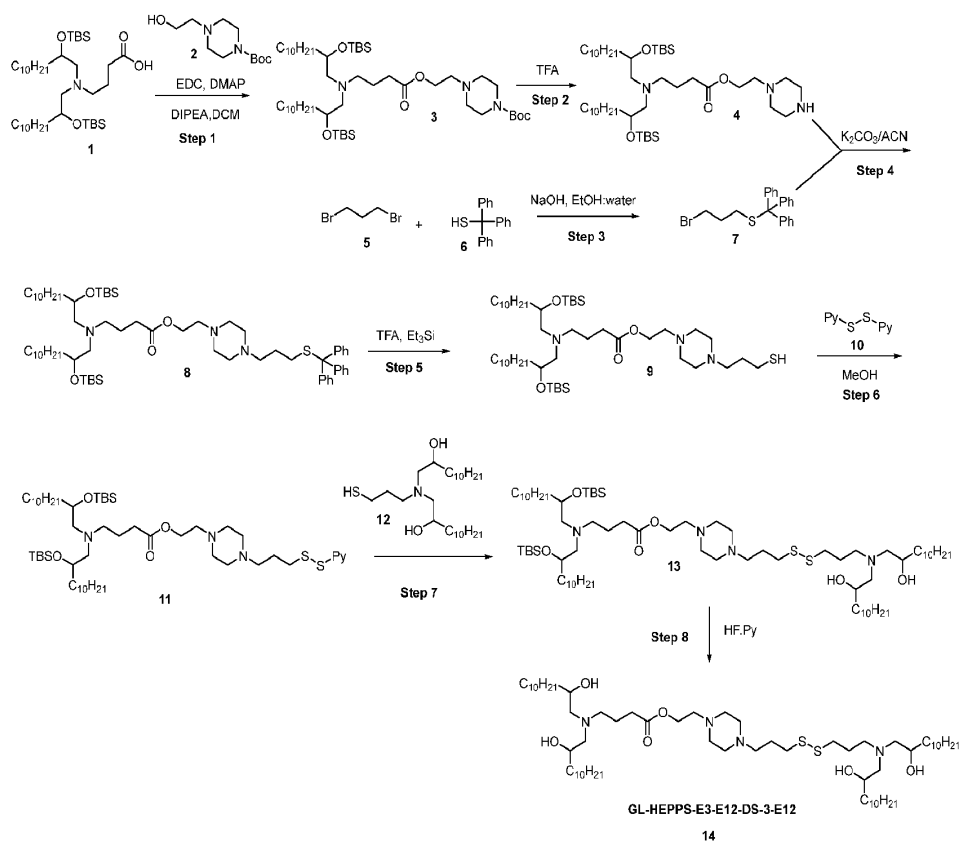
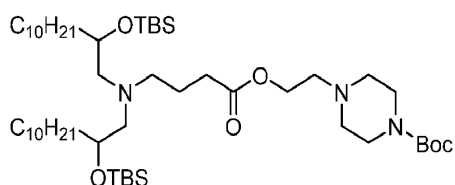
[0751] ^1H NMR (300 MHz, CDCl_3) δ 4.99 (hept, 2H), 4.19 (t, 2H), 4.05 (t, 4H), 3.63 (m, 4H), 2.86-2.46 (m, 22H), 2.45-2.23 (m, 16H), 1.88-1.30 (m, 42H), 1.22 (d, 12H), 0.92 (t, 6H).

[0752] APCI-MS analysis: Calculated $\text{C}_{58}\text{H}_{110}\text{N}_4\text{O}_{14}\text{S}_2$ $[\text{M}+\text{H}] = 1151.6$, Observed = 1151.7.

Example 8: Synthesis of HEPPS-based ester/disulfide cationic lipids

[0753] HEPPS-based cationic lipids described herein may be prepared according to **Scheme 8a**:

Scheme 8a

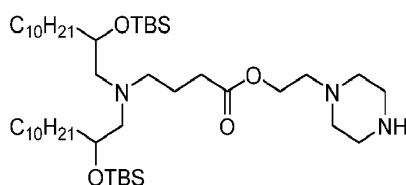
**Intermediate [3]:**

[0754] To a solution of [2] (1.25 g, 5.43 mmol) in DCM (25 mL) were added [1] (4.18 g, 5.97 mmol) in DCM (25 mL), EDC (1.56 g, 8.14 mmol), DMAP (0.133 g, 1.08 mmol), DIPEA (1.89 mL, 10.85 mmol) and stirred at room temperature for 14 hours. After completion of the reaction as monitored by MS. The reaction mixture was diluted with DCM (200 mL) washed with NaHCO_3 solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 , concentrated, and the crude compound was purified (eluent: 20%-60% EtOAc in hexanes) to obtain pure compound [3] as a color less oil (3.31 g, 67%). It was confirmed by MS analysis.

[0755] Results:

[0756] ESI-MS analysis: Calculated C₅₁H₁₀₆N₃O₆Si₂, [M+H] = 912.76, Observed = 912.7

Intermediate [4]:

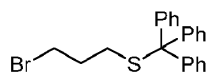


[0757] To a solution of [3] (3.3 g, 3.62 mmol) in DCM (75 mL) at 0 °C was added TFA (37.5 mL, 490.04 mmol). Then the reaction mixture was brought to room temperature and stirred for 14 hours. After completion of the reaction as monitored by MS. The reaction mixture was concentrated to obtain compound [3] with TFA salts (3.2 g, 80%). It was confirmed by MS analysis.

[0758] Results:

[0759] ESI-MS analysis: Calculated C₄₆H₉₈N₃O₄Si₂, [M+H] = 812.71, Observed = 812.7

Intermediate [7]:



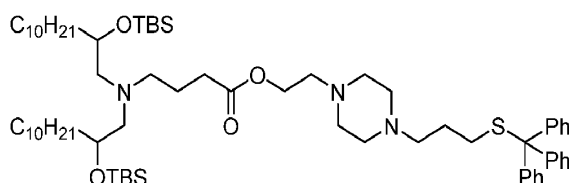
[0760] To a solution of [6] (5.0 g, 18.08 mmol) in EtOH (50 mL) and water (50 mL) was added a solution (in 25 mL water) of NaOH (1.44 g, 36.16 mmol). The reaction mixture was stirred for 10 min and added a solution (in 25 mL EtOH) of [5] (1.83 mL, 18.08 mmol) to reaction mixture and was stirred at room temperature for 4 h. After completion of the reaction as monitored by TLC (5% EtOAc/hexanes), the reaction mixture was diluted DCM and aqueous sodium bicarbonate solution, the organic layer was washed with brine. The organic layer was dried over anhydrous Na₂SO₄, concentrated under vacuum to give crude compound. To that was added MeOH (17 mL) and stirred for 15 min at 0-10 °C, the solid compound was filtered and dried under vacuum to give [7] (5.6 g, 79%) as a white solid.

[0761] Results:

[0762] ¹H NMR (400 MHz, CDCl₃): δ 7.42 (d, J = 7.6 Hz, 6H), 7.30-7.26 (m, 6H), 7.23-7.19 (m, 3H), 3.32 (t, J = 6.8 Hz, 2H), 2.32 (t, J = 6.8 Hz, 2H), 1.84-1.77 (m, 2H). LCMS: Purity 99.25 %,

[0763] Mass Expected = 397.37, Observed = 242.95 (m/z, CPh₃⁺).

Intermediate [8]:

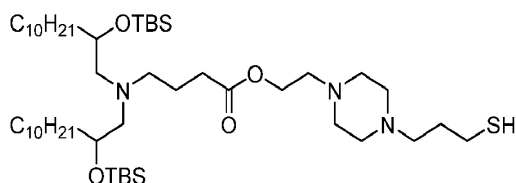


[0764] To a mixture of [4] (0.50 g, 0.615 mmol) and [7] (0.29 g, 0.74 mmol) in acetonitrile (6 mL) was added K₂CO₃ (0.68 g, 4.92 mmol). The reaction mixture was heated at 65 °C for 16 h. The reaction progress was monitored by MS. Then reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under vacuum to give crude product. The crude residue was purified (eluent: 20%-30% EtOAc in hexanes) to obtain pure compound [8] (0.411 g, 60%). It was confirmed by MS analysis.

[0765] Results:

[0766] ESI-MS analysis: Calculated C₆₈H₁₁₈N₃O₄Si₂, [M+H]⁺ = 1128.84, Observed = 1128.7

Intermediate [9]:

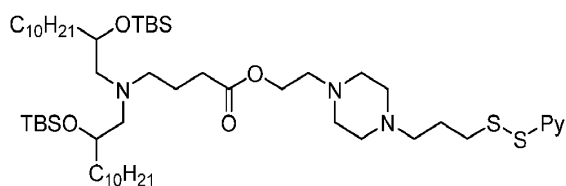


[0767] To a solution of [8] (0.2 g, .18 mmol) in DCM (1.5 mL) was slowly added TFA (1.5 mL, 19.60 mmol) at room temperature and stirred at room temperature for 0.5 hour. To that triethylsilane (35 mL, 0.22 mmol) was added slowly and stirred for 1 hour. After completion of the reaction as monitored by MS. The reaction mixture was concentrated to obtain crude product [9] (>0.157 g, quantitative). It was confirmed by MS analysis.

[0768] Results:

[0769] ESI-MS analysis: Calculated C₄₉H₁₀₄N₃O₄Si₂, [M+H]⁺ = 886.73, Observed = 886.7

Intermediate [11]:

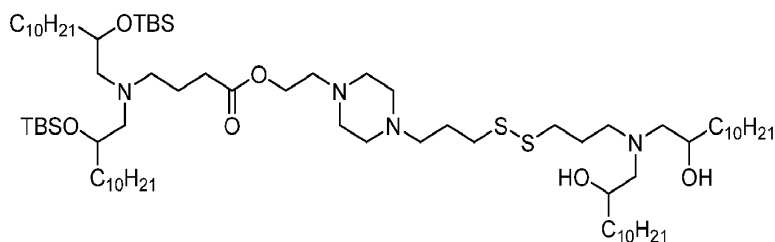


[0770] To a solution of [9] (0.157 g, 0.177 mmol) in MeOH (4 mL) was added [10] (0.058 g, 0.266 mmol) at room temperature and stirred for 2 hours. After completion of the reaction as monitored by MS. The reaction mixture was concentrated, and the crude compound was purified (eluent: 10% MeOH in DCM) to obtain pure product [11] as a colorless oil (0.138 g, 78%). It was confirmed by MS analysis.

[0771] Results:

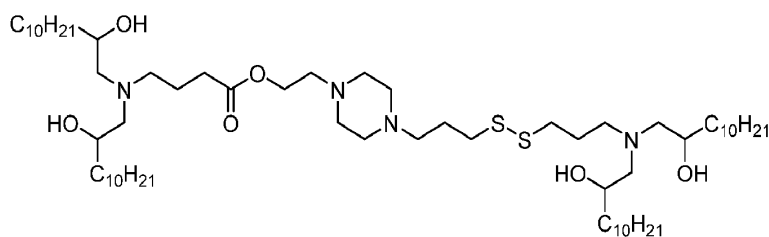
[0772] ESI-MS analysis: Calculated for C₅₄H₁₀₇N₄O₄S₂Si₂, [M+H] = 995.73; Observed = 995.7

Intermediate [13]:



[0773] To a solution of [11] (0.135 g, 0.135 mmol) in chloroform (4 mL) were added triethylamine (75 mL, 0.542 mmol) and [12] (0.125 g, 0.271 mmol) and stirred at room temperature for 2 hours. After completion of the reaction as monitored by MS. The reaction mixture was concentrated, and the crude compound was purified (eluent: 2-10% MeOH in DCM) to obtain pure compound [13] as a colorless oil (0.091 g, 50%). It was confirmed by MS analysis.

GL-HEPPS-E3-E12-DS-3-E12 [14]:



[0774] To a solution of [13] (90 mg, 0.067 mmol) in THF (4 mL) was slowly added HF.Py (70% HF) (1.5 mL) at 0 °C. Then reaction mixture was brought to room temperature and stirred for 16 hours. After completion of the reaction as monitored by MS. The reaction mixture was concentrated, and the obtained residue was dissolved in ethyl acetate, washed with NaHCO₃ solution, water and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified to obtain compound [14] (43 mg, 58%). It was confirmed by ¹H NMR and MS analysis.

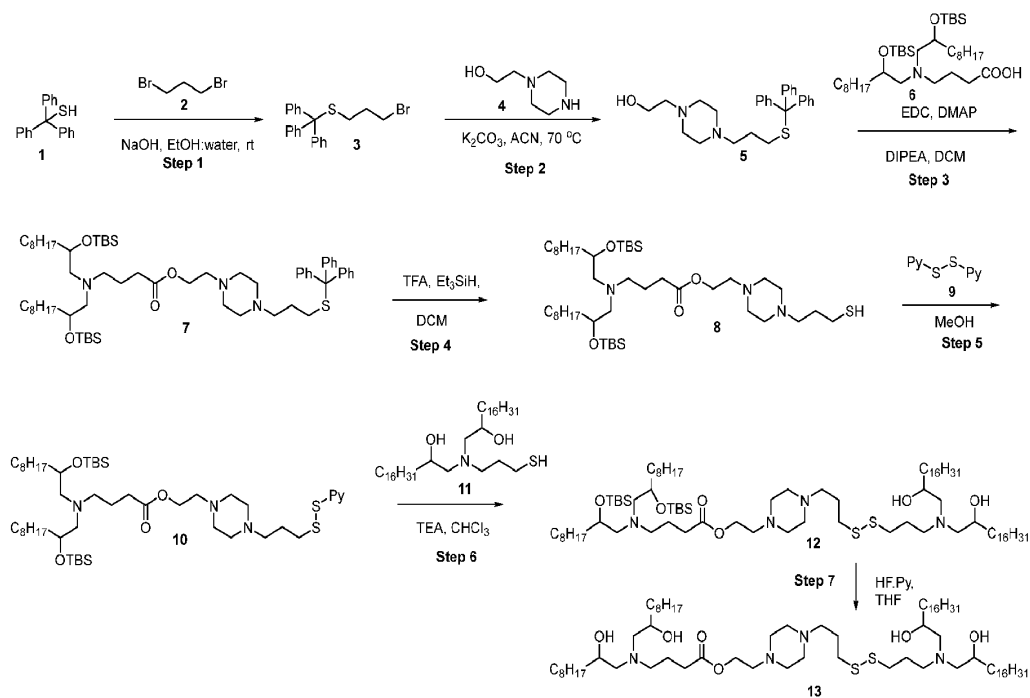
[0775] Results:

[0776] ¹H NMR (400 MHz, CDCl₃) δ 4.19 – 4.09 (m, 2H), 3.62 (brs, 4H), 2.82 – 2.26 (m, 28H), 2.01 – 1.71 (m, 6H), 1.47 – 1.10 (m, 72H), 0.81 (t, 12H).

[0777] ESI-MS analysis: Calculated for C₆₄H₁₃₁N₄O₆S₂, [M+H]⁺ = 1115.95; Observed = 1115.8.

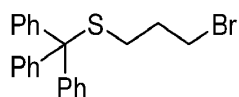
[0778] HEPPS-based cationic lipids described herein may also be prepared according to Scheme 8b:

Scheme 8b



GL-HEPPS-E3-E10-DS-3-E18:1

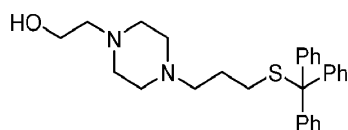
Intermediate [3]:



[0779] To a solution of triphenylmethanethiol (5.0 g, 18.08 mmol) in EtOH (50 mL) and water (50 mL) was added a solution (in 25 ml water) of NaOH (1.44 g, 36.16 mmol). The reaction mixture was stirred for 10 min and added a solution (in 25 ml EtOH) of 1,3-dibromopropane (3.65 g, 18.08 mmol) to reaction mixture. The reaction mixture was stirred for 4 h at RT. The progress of reaction was monitored by TLC (5% EtOAc/hexanes). The reaction mixture was diluted DCM and aqueous sodium bicarbonate solution, the organic layer was washed with brine. The organic layer was dried over sodium sulphate, concentrated under vacuum to give crude compound. To the crude was added MeOH (50 mL) and stirred for 15 min at 0-10 oC, the solid compound was filtered and dried under vacuum to give (3-bromopropyl)(triphenyl)sulfane (5.67 g, 79.0%) as a white solid.

[0780] Results: ^1H NMR (400 MHz, CDCl_3): δ 7.42 (d, J = 7.6 Hz, 6H), 7.30-7.26 (m, 6H), 7.23-7.19 (m, 3H), 3.32 (t, J = 6.8 Hz, 2H), 2.32 (t, J = 6.8 Hz, 2H), 1.84-1.77 (m, 2H). LCMS: Purity 99.25 %, Mass Expected = 397.37, Observed = 242.95 (m/z , CPh_3^+).

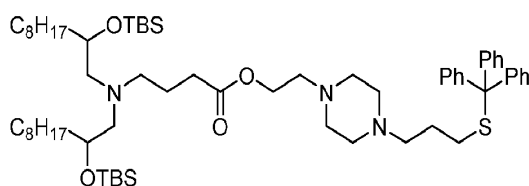
Intermediate [5]:



[0781] To a solution of (3-bromopropyl)(trityl)sulfane (13.0 g, 32.71 mmol) and 2-(piperazin-1-yl)ethan-1-ol (8.51 g, 65.43 mmol) in ACN (195 mL) was added K₂CO₃ (18.08 g, 130.84 mmol). The reaction mixture was heated at 70 °C for 16h. The reaction progress was monitored by TLC (2.5% MeOH in DCM). The reaction mixture was cooled to RT and filtered. The filtrate was concentrated under vacuum to give crude product. The crude was purified by flash chromatography (0 to 2.5 % MeOH in DCM) to give 2-(4-(3-(tritylthio)propyl)piperazin-1-yl)ethan-1-ol (12.0 g, 82.31%) as a white solid.

[0782] Results: ¹H NMR (400 MHz, DMSO-d₆): δ 7.34-7.29 (m, 12H), 7.25-7.22 (m, 3 H), 4.34 (t, J = 4.8 Hz, 1H), 3.47-3.42 (m, 2H), 2.32 (t, J = 6.4 Hz, 6H), 2.18-2.09 (m, 8H), 1.43-1.36 (m, 2H). LCMS: Purity 99.56 %, Mass Expected = 446.65, Observed = 447.25 (m/z, M+1).

Intermediate [7]:

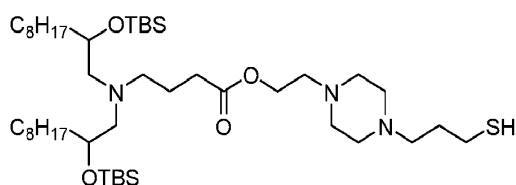


[0783] To a solution of [5] (1.0 g, 2.24 mmol) in DCM (7 mL) were added [6] (1.59 g, 2.46 mmol) in DCM (8 mL), EDC (0.64 g, 3.35 mmol), DMAP (55 mg, 0.44 mmol), DIPEA (0.78 mL, 4.47 mmol) and stirred at room temperature for 14 hours. After completion of the reaction as monitored by MS, the reaction mixture was rotovaped and resuspended in ethyl acetate. The organic layer was washed twice with water (The first water wash was extracted back with ethyl acetate). With the organic layers combined, the organic layer was washed twice with saturated NaHCO₃ solution and three times with brine. The organic layer was dried over anhydrous Na₂SO₄, concentrated, and the crude compound was purified (eluent: 0-25% EtOAc in hexanes) to obtain pure compound [7] as a colorless oil (1.89 g, 79%). It was confirmed by MS analysis.

[0784] Results:

[0785] ESI-MS analysis: Calculated $C_{64}H_{109}N_3O_4Si_2$, $[M+H] = 1073.82$, Observed = 1073.8

Intermediate [8]:

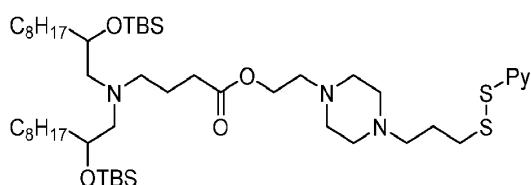


[0786] To a solution of [7] (0.50 g, 0.46 mmol) in DCM (4 mL), under a nitrogen atmosphere, was added TFA (4 mL) at room temperature and stirred at room temperature for 0.5 hour. To that, triethylsilane (0.13 mL, 0.82 mmol) was added and stirred for 1 hour. After completion of the reaction as monitored by MS, the reaction mixture was concentrated to obtain crude product [8] (>0.39 g, quantitative). It was confirmed by MS analysis.

[0787] Results:

[0788] ESI-MS analysis: Calculated $C_{45}H_{95}N_3O_4Si_2$, $[M+H] = 831.50$, Observed = 831.8

Intermediate [10]:

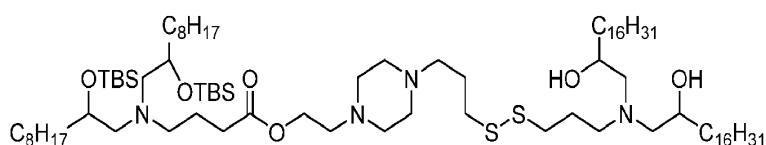


[0789] To a solution of [8] (0.39 g, 0.46 mmol) in MeOH (4 mL), under a nitrogen atmosphere, was added [9] (0.15 g, 0.70 mmol) at room temperature and stirred for 2 hours. After completion of the reaction as monitored by MS, the reaction mixture was concentrated and the crude compound was purified (eluent: 0-100% ethyl acetate in hexanes, then 0-10% MeOH in DCM) to obtain pure product [10] (0.41 g, 95%). It was confirmed by MS analysis.

[0790] Results:

[0791] ESI-MS analysis: Calculated for $C_{50}H_{98}N_4O_4S_2Si_2$, $[M+H] = 940.65$; Observed = 940.8

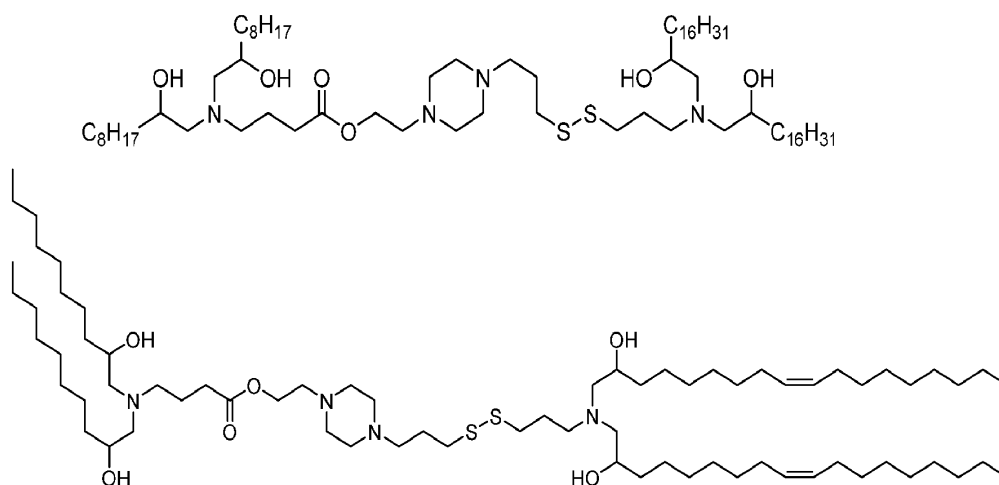
Intermediate [12]:



[0792] To a solution of [10] (0.41 g, 0.44 mmol) and [11] (0.68 g, 1.09 mmol) in chloroform (3 ml) was added triethylamine (0.250 ml, 1.75 mmol) and allowed to react at room temperature for 2 hours. After completion of the reaction, determined by MS, the reaction mixture was concentrated and purified to obtain [12] as colorless oil (0.170 g, 27% yield).

[0793] ESI-MS analysis: Calculated for $C_{84}H_{170}N_4O_6S_2Si_2$, $[M+H] = 1453.60$; Observed = 1453.8

GL-HEPPS-E3-E10-DS-3-E18:1 [13]:



[0794] To a 20 ml polypropylene scintillation vial was added [12] (0.170 g, 0.117 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (0.600 mL, 23.06 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate, diluted with ethyl acetate, washed with $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified to obtain compound [13] (0.115 g, 80%). It was confirmed by ^1H NMR and MS analysis.

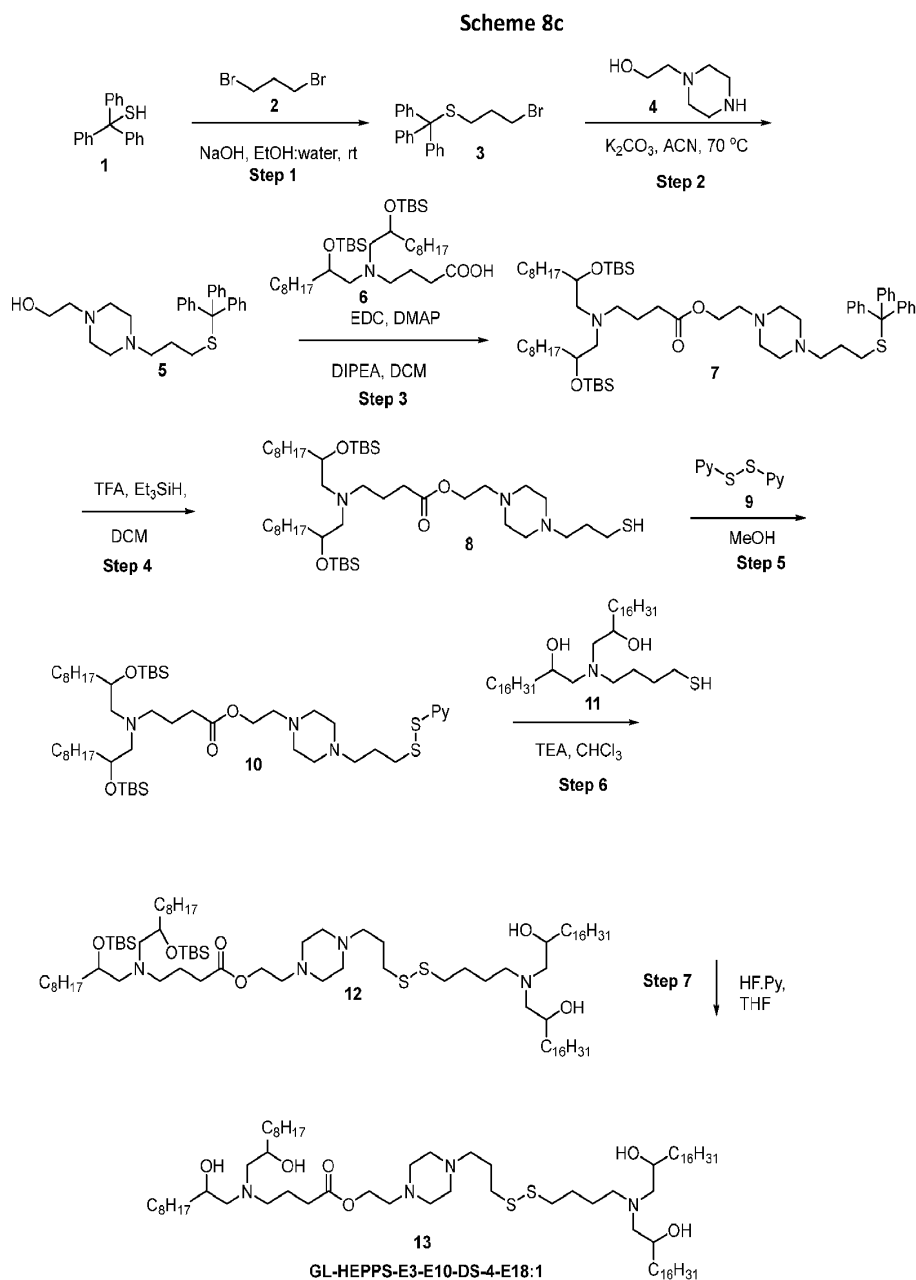
[0795] Results:

[0796] ^1H NMR (400 MHz, CDCl_3), 5.40-5.29 (m, 4H), 4.22 (t, 2H), 3.78 (brs, 4H), 3.07-2.32 (m, 26H), 2.15-1.72 (m, 14 H), 1.65-1.15 (m, 80H), 0.88 (t, 12H)

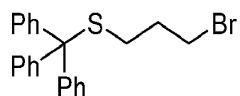
[0797] ESI-MS analysis: Calculated for $\text{C}_{72}\text{H}_{142}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1225.07$; Observed = 1225.8.

[0798] HEPPS-based cationic lipids described herein may also be prepared according to

Scheme 8c:



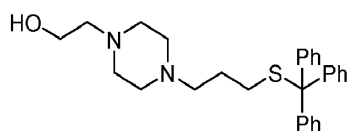
Intermediate [3]:



[0799] To a solution of triphenylmethanethiol (5.0 g, 18.08 mmol) in EtOH (50 mL) and water (50 mL) was added a solution (in 25 ml water) of NaOH (1.44 g, 36.16 mmol). The reaction mixture was stirred for 10 min and added a solution (in 25 ml EtOH) of 1,3-dibromopropane (3.65 g, 18.08 mmol) to reaction mixture. The reaction mixture was stirred for 4 h at RT. The progress of reaction was monitored by TLC (5% EtOAc/hexanes). The reaction mixture was diluted DCM and aqueous sodium bicarbonate solution, the organic layer was washed with brine. The organic layer was dried over sodium sulphate, concentrated under vacuum to give crude compound. To the crude was added MeOH (50 mL) and stirred for 15 min at 0-10 oC, the solid compound was filtered and dried under vacuum to give (3-bromopropyl)(trityl)sulfane (5.67 g, 79.0%) as a white solid.

[0800] Results: ¹H NMR (400 MHz, CDCl₃): δ 7.42 (d, J = 7.6 Hz, 6H), 7.30-7.26 (m, 6H), 7.23-7.19 (m, 3H), 3.32 (t, J = 6.8 Hz, 2H), 2.32 (t, J = 6.8 Hz, 2H), 1.84-1.77 (m, 2H). LCMS: Purity 99.25 %, Mass Expected = 397.37, Observed = 242.95 (m/z, CPh₃⁺).

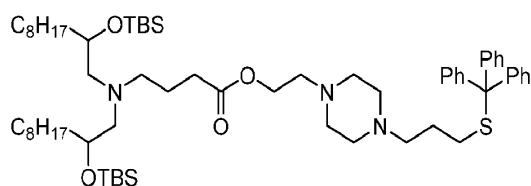
Intermediate [5]:



[0801] To a solution of (3-bromopropyl)(trityl)sulfane (13.0 g, 32.71 mmol) and 2-(piperazin-1-yl)ethan-1-ol (8.51 g, 65.43 mmol) in ACN (195 mL) was added K₂CO₃ (18.08 g, 130.84 mmol). The reaction mixture was heated at 70 oC for 16h. The reaction progress was monitored by TLC (2.5% MeOH in DCM)). The reaction mixture was cooled to RT and filtered. The filtrate was concentrated under vacuum to give crude product. The crude was purified by flash chromatography (0 to 2.5 % MeOH in DCM) to give 2-(4-(3-(tritylthio)propyl)piperazin-1-yl)ethan-1-ol (12.0 g, 82.31%) as a white solid.

[0802] Results: ¹H NMR (400 MHz, DMSO-d₆): δ 7.34-7.29 (m, 12H), 7.25-7.22 (m, 3 H), 4.34 (t, J = 4.8 Hz, 1H), 3.47-3.42 (m, 2H), 2.32 (t, J = 6.4 Hz, 6H), 2.18-2.09 (m, 8H), 1.43-1.36 (m, 2H). LCMS: Purity 99.56 %, Mass Expected = 446.65, Observed = 447.25 (m/z, M+1).

Intermediate [7]:

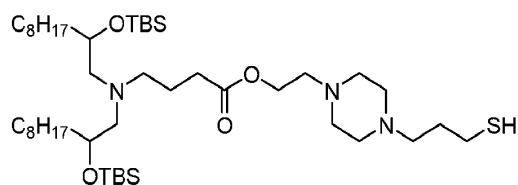


[0803] To a solution of [5] (1.0 g, 2.24 mmol) in DCM (7 mL) were added [6] (1.59 g, 2.46 mmol) in DCM (8 mL), EDC (0.64 g, 3.35 mmol), DMAP (55 mg, 0.44 mmol), DIPEA (0.78 mL, 4.47 mmol) and stirred at room temperature for 14 hours. After completion of the reaction as monitored by MS, the reaction mixture was rotovaped and resuspended in ethyl acetate. The organic layer was washed twice with water (The first water wash was extracted back with ethyl acetate). With the organic layers combined, the organic layer was washed twice with saturated NaHCO₃ solution and three times with brine. The organic layer was dried over anhydrous Na₂SO₄, concentrated, and the crude compound was purified (eluent: 0-25% EtOAc in hexanes) to obtain pure compound [7] as a color less oil (1.89 g, 79%). It was confirmed by MS analysis.

[0804] Results:

[0805] ESI-MS analysis: Calculated C₆₄H₁₀₉N₃O₄Si₂, [M+H] = 1073.82, Observed = 1073.8

Intermediate [8]:

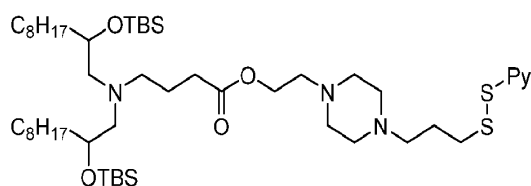


[0806] To a solution of [7] (0.50 g, 0.46 mmol) in DCM (4 mL), under a nitrogen atmosphere, was added TFA (4 mL) at room temperature and stirred at room temperature for 0.5 hour. To that, triethylsilane (0.13 mL, 0.82 mmol) was added and stirred for 1 hour. After completion of the reaction as monitored by MS, the reaction mixture was concentrated to obtain crude product [8] (>0.39 g, quantitative). It was confirmed by MS analysis.

[0807] Results:

[0808] ESI-MS analysis: Calculated C₄₅H₉₅N₃O₄Si₂, [M+H] = 831.50, Observed = 831.8

Intermediate [10]:

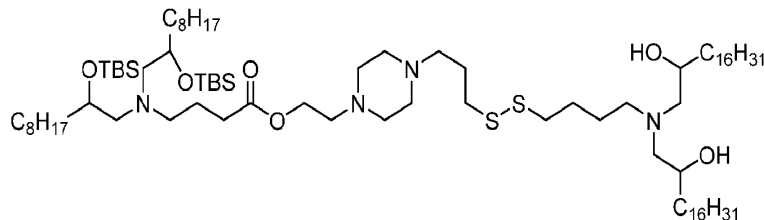


[0809] To a solution of [8] (0.39 g, 0.46 mmol) in MeOH (4 mL), under a nitrogen atmosphere, was added [9] (0.15 g, 0.70 mmol) at room temperature and stirred for 2 hours. After completion of the reaction as monitored by MS. The reaction mixture was concentrated, and the crude compound was purified (eluent: 0-100% ethyl acetate in hexanes, then 0-10% MeOH in DCM) to obtain pure product [10] (0.41 g, 95%). It was confirmed by MS analysis.

[0810] Results:

[0811] ESI-MS analysis: Calculated for $C_{50}H_{98}N_4O_4S_2Si_2$, $[M+H] = 940.65$; Observed = 940.8

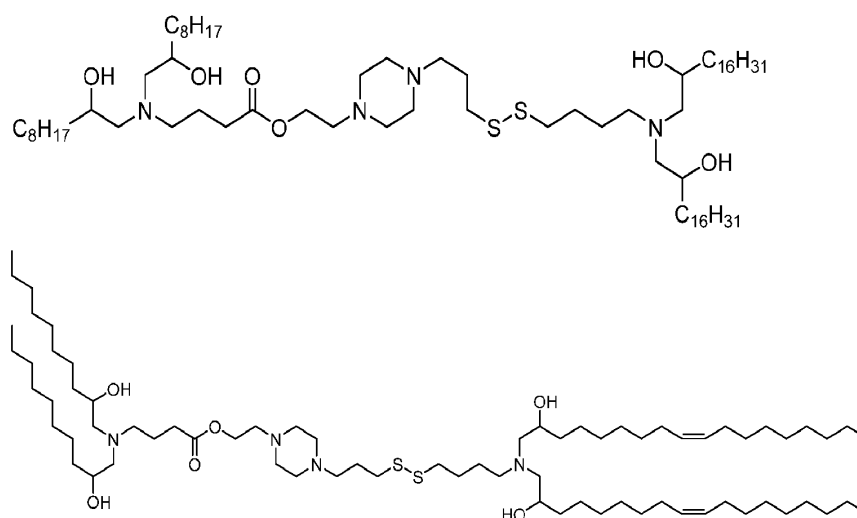
Intermediate [12]:



[0812] To a solution of [10] (0.40 g, 0.43 mmol) and [11] (0.73 g, 1.15 mmol) in chloroform (3 mL) was added triethylamine (0.300 mL, 2.13 mmol) and allowed to react at room temperature for 2 hours. After completion of the reaction, determined by MS, the reaction mixture was concentrated and purified to obtain [12] as colorless oil (0.330 g, 53% yield).

[0813] ESI-MS analysis: Calculated for $C_{85}H_{172}N_4O_6S_2Si_2$ $C_{84}H_{170}N_4O_6S_2Si_2$, $[M+H] = 1467.62$; Observed = 1467.8

GL-HEPPS-E3-E10-DS-4-E18:1 [13]:



[0814] To a 20 ml polypropylene scintillation vial was added [12] (0.330 g, 0.23 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 oC and HF/pyridine (1.2 mL, 44.3 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 oC and neutralized with solid sodium bicarbonate, diluted with ethyl acetate, washed with NaHCO₃ solution, water and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified to obtain compound [13] (0.130 g, 46%). It was confirmed by ¹H NMR and MS analysis.

[0815] Results:

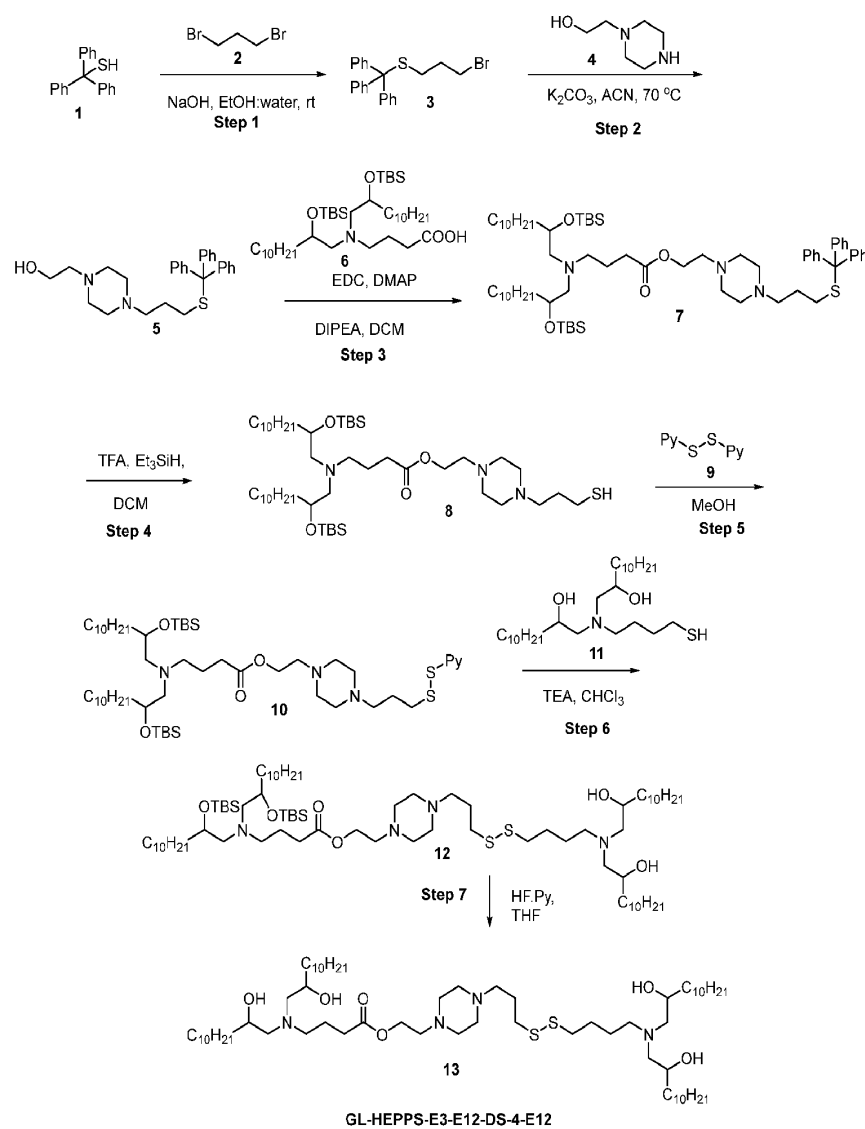
[0816] ¹H NMR (400 MHz, CDCl₃), 5.41-5.30 (m, 4H), 4.21 (t, 2H), 3.80 (brs, 4H), 3.05-2.24 (m, 26H), 2.15-1.65 (m, 16 H), 1.64-1.10 (m, 80H), 0.87 (t, 12H)

[0817] ESI-MS analysis: Calculated for C₇₃H₁₄₄N₄O₆S₂, [M+H] = 1239.10; Observed = 1239.8.

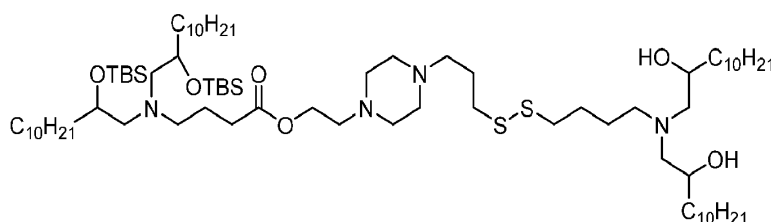
[0818] HEPPS-based cationic lipids described herein may also be prepared according to

Scheme 8d:

Scheme 8d

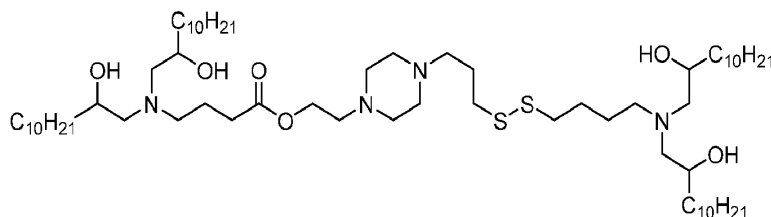


[0819] Intermediate **10** was synthesized using the same procedure as that used to synthesise intermediate **11** in Scheme 8a.

Intermediate [12]:

[0820] To a solution of **[10]** (0.545 g, 0.55 mmol) and **[11]** (0.590 g, 1.25 mmol) in chloroform was added triethylamine (0.35 ml, 2.48 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain **[12]** as colorless oil (0.270 g, % yield).

[0821] ESI-MS analysis: Calculated for $C_{77}H_{160}N_4O_6S_2Si_2$, $[M+H] = 1359.44$; Observed = 1539.8

GL-HEPPS-E3-E12-DS-4-E12 [13]:

[0822] To a 20 ml polypropylene scintillation vial was added **[12]** (0.300 g, 0.22 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (1.4 mL, 53.67 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified to obtain compound **[13]** (0.220 g, 88%). It was confirmed by ^1H NMR and MS analysis.

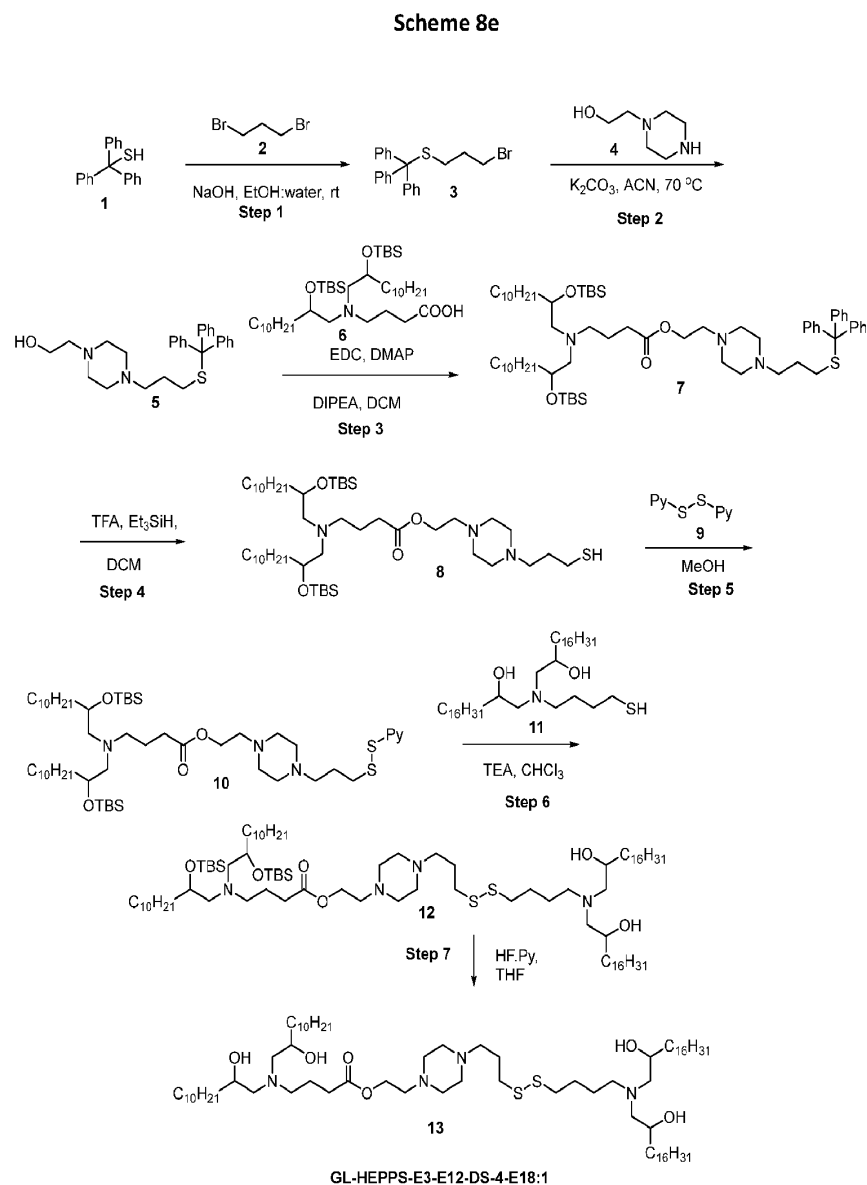
[0823] Results:

[0824] ^1H NMR (400 MHz, $CDCl_3$) 4.20 (t, 2H), 3.92 – 3.55 (br, 4H), 2.91 – 2.25 (m, 30H), 2.06 – 1.90 (br, 2H), 1.89–1.78 (m, 2H), 1.78 – 1.61 (br, 4H) 1.60 – 1.12 (m, 74H), 0.87 (t, 12H).

[0825] ESI-MS analysis: Calculated for $C_{65}H_{132}N_4O_6S_2$, $[M+H] = 1130.91$; Observed = 1130.8

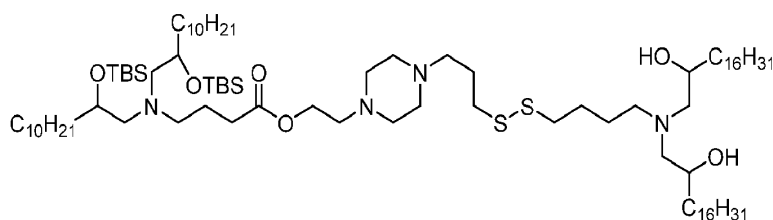
[0826] HEPPS-based cationic lipids described herein may also be prepared according to

Scheme 8e:



[0827] Intermediate **10** was synthesized using the same procedure as that used to synthesise intermediate **11** in **Scheme 8a**.

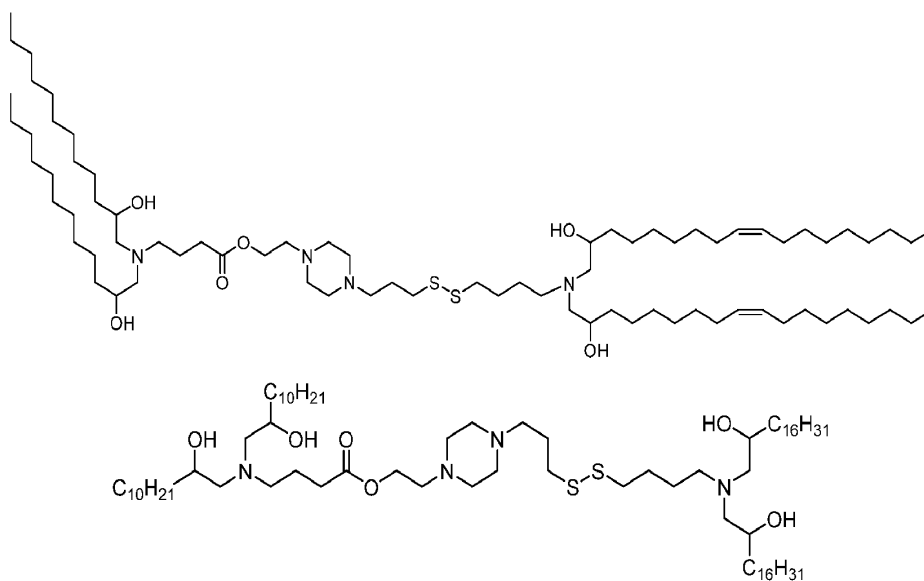
Intermediate [12]:



[0828] To a solution of **[10]** (0.421 g, 0.42 mmol) and **[11]** (0.540 g, 0.84 mmol) in chloroform was added triethylamine (0.29 ml, 2.12 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain **[12]** as an oil with yellow solid (Note: Yellow solid is pyridyl-disulfide and is removed in next step) (0.30 g, 46% yield).

[0829] ESI-MS analysis: Calculated for C₈₉H₁₈₀N₄O₆S₂Si₂, [M+H] = 1522.73; Observed = 1522.8

GL-HEPPS-E3-E12-DS-4-E18:1 [13]:



[0830] To a 20 ml polypropylene scintillation vial was added **[12]** (0.400 g, 0.26 mmol, 1.0 eq NOTE: ~ 0.100 g is Pyridyl Disulfide impurity, however calculations take everything into consideration) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and

HF/pyridine (1.4 mL, 51.74 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with NaHCO₃ solution, water and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified to obtain compound **[13]** (0.200 g, 59%). It was confirmed by ¹H NMR and MS analysis.

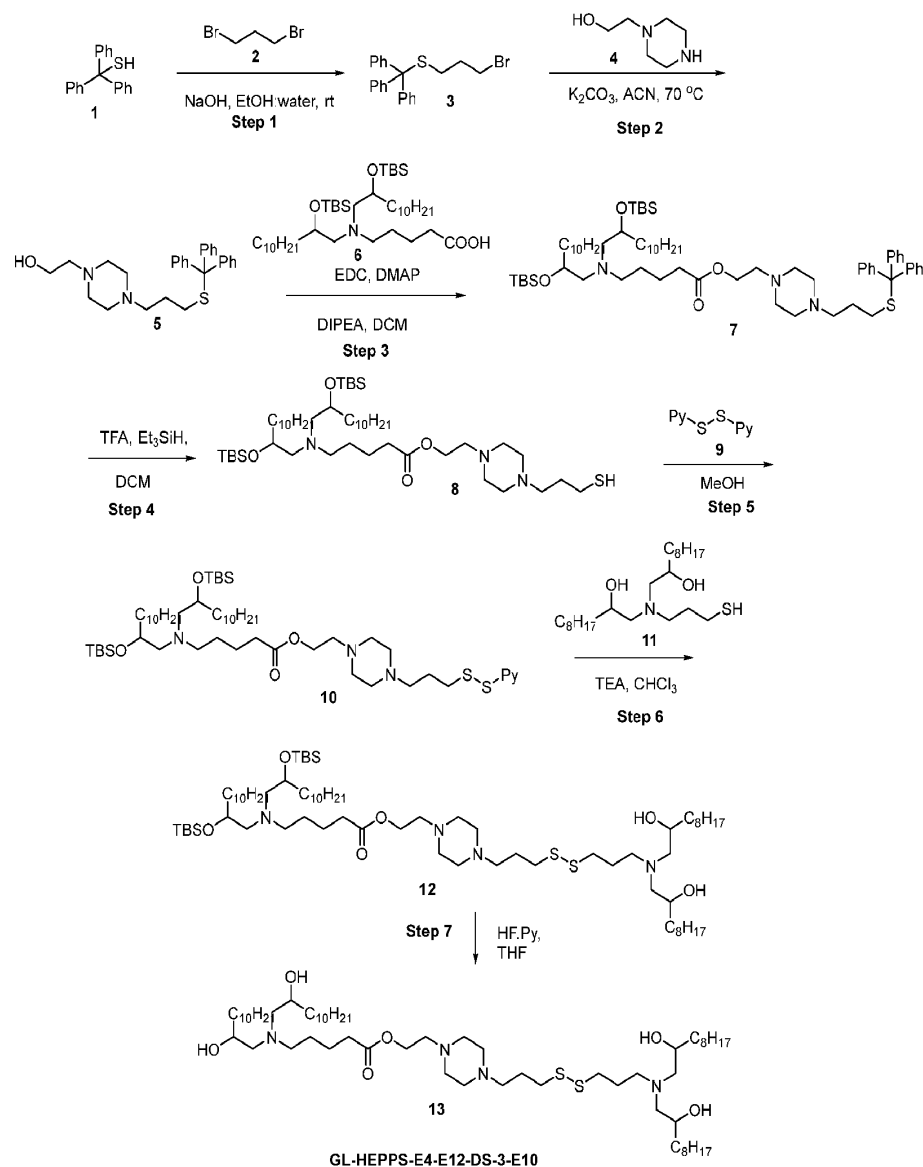
[0831] Results:

[0832] ¹H NMR (400 MHz, CDCl₃) 5.45 – 5.25 (m, 4H), 4.20 (t, 2H), 3.85 – 3.50 (br, 4H), 2.79 – 2.27 (m, 30H), 2.06 – 1.86 (br, 10H), 1.87–1.76 (m, 2H), 1.75 – 1.52 (br, 6H) 1.51 – 1.12 (m, 80H), 0.87 (t, 12H).

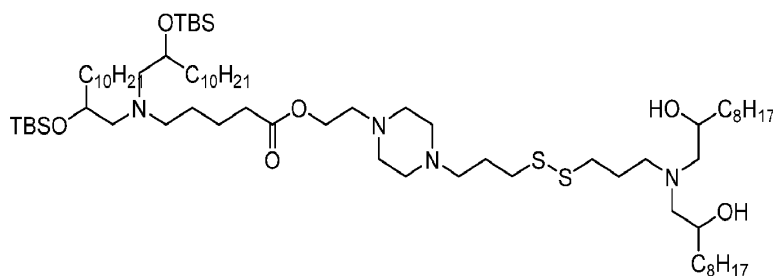
ESI-MS analysis: Calculated for C₇₇H₁₅₂N₄O₆S₂, [M+H] = 1294.21; Observed = 1294.8

[0833] HEPPS-based cationic lipids described herein may also be prepared according to **Scheme 8f**:

Scheme 8f

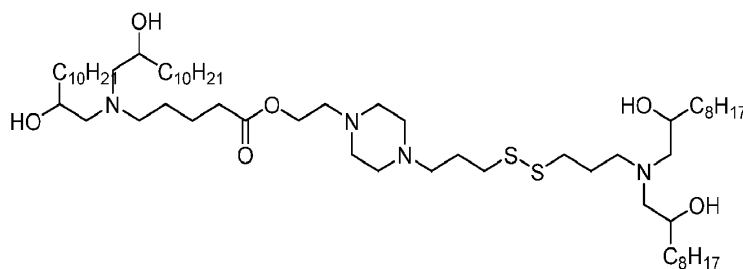


[0834] Intermediate 10 was synthesized using the same procedures as **Scheme 8a**, with the exception of using a 4-carbon spacer between the terminal carboxylate and tertiary amine for the coupling in Step 3.

Intermediate [12]:

[0835] To a solution of **[10]** (0.450 g, 0.44 mmol) and **[11]** (0.360 g, 0.89 mmol) in chloroform was added triethylamine (0.310 ml, 2.23 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain **[12]** as colorless oil (0.320 g, 55% yield).

[0836] ESI-MS analysis: Calculated for $C_{73}H_{152}N_4O_6S_2Si_2$, $[M+H] = 1302.33$; Observed = 1302.8

GL-HEPPS-E4-E12-DS-3-E10 [13]:

[0837] To a 20 ml polypropylene scintillation vial was added **[12]** (0.320 g, 0.24 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (1.25 mL, 48.42 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified to obtain compound **[13]** (0.180 g, 68%). It was confirmed by ^1H NMR and MS analysis.

[0838] Results:

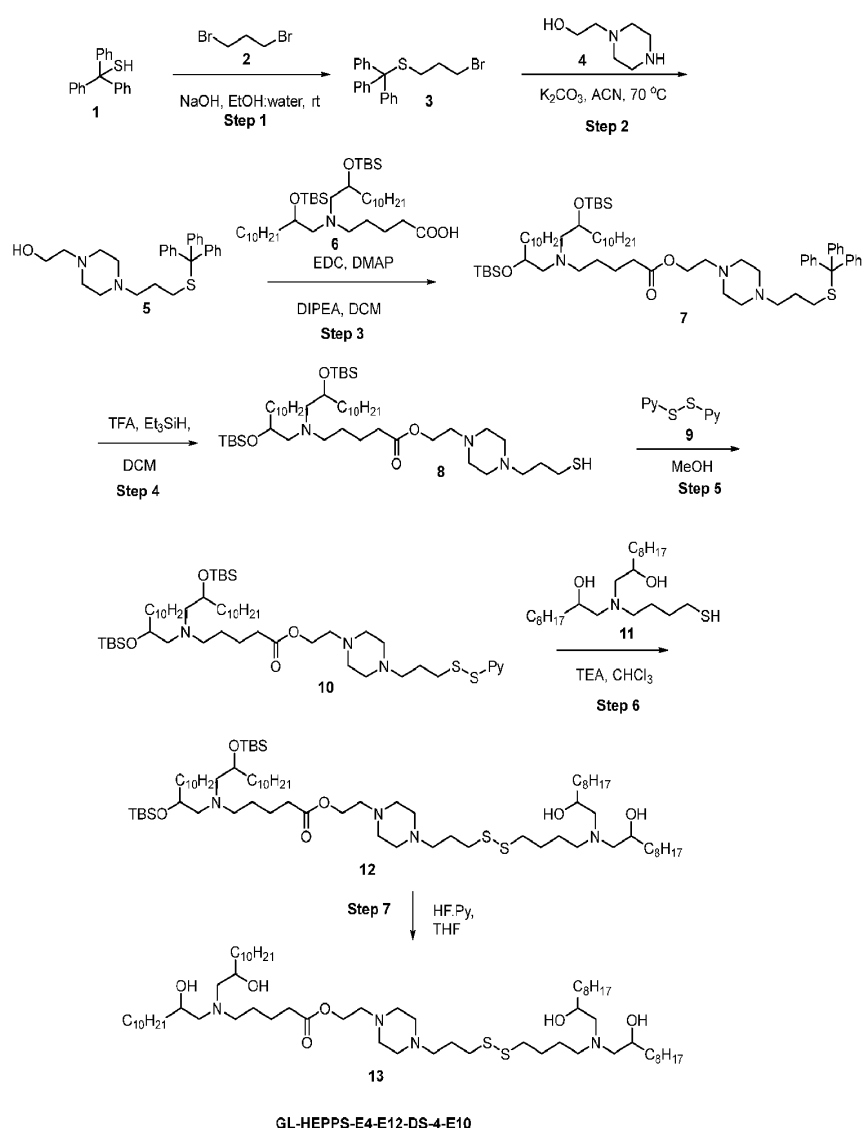
[0839] ^1H NMR (400 MHz, CDCl_3) 4.20 (t, 2H), 3.89 – 3.61 (br, 4H), 2.92 – 2.40 (m, 26H), 2.39 – 2.22 (m, 2H), 2.09 – 1.79 (m, 4H), 1.75–1.51 (br, 4H), 1.50 – 1.13 (m, 66H), 0.87 (t, 12H).

[0840] ESI-MS analysis: Calculated for $\text{C}_{61}\text{H}_{124}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1073.81$; Observed = 1073.7

[0841] HEPPS-based cationic lipids described herein may also be prepared according to

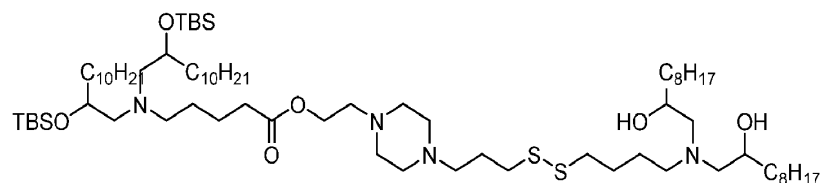
Scheme 8g:

Scheme 8g



[0842] Intermediate 10 was synthesized using the same procedures as Scheme 8f.

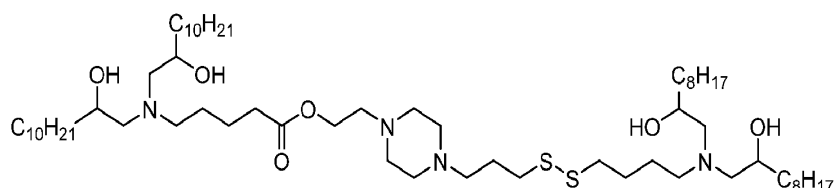
Intermediate [12]:



[0843] To a solution of [10] (0.450 g, 0.44 mmol) and [11] (0.372 g, 0.89 mmol) in chloroform was added triethylamine (0.310 ml, 2.23 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain [12] as colorless oil (0.373 g, 64% yield).

[0844] ESI-MS analysis: Calculated for $C_{74}H_{154}N_4O_6S_2Si_2$, $[M+H] = 1316.36$; Observed = 1316.8

GL-HEPPS-E4-E12-DS-4-E10 [13]:



[0845] To a 20 ml polypropylene scintillation vial was added [12] (0.373 g, 0.28 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (1.4 mL, 55.88 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified to obtain compound [13] (0.230 g, 75%). It was confirmed by ^1H NMR and MS analysis.

[0846] Results:

[0847] ^1H NMR (400 MHz, $CDCl_3$) 4.20 (t, 2H), 3.80 – 3.60 (br, 4H), 2.86 – 2.41 (m, 26H), 2.40 – 2.27 (t, 2H), 2.00 – 1.82 (m, 2H), 1.81–1.50 (m, 10H), 1.49 – 1.34 (m, 12H), 1.33 – 1.17 (m, 52H) 0.87 (t, 12H).

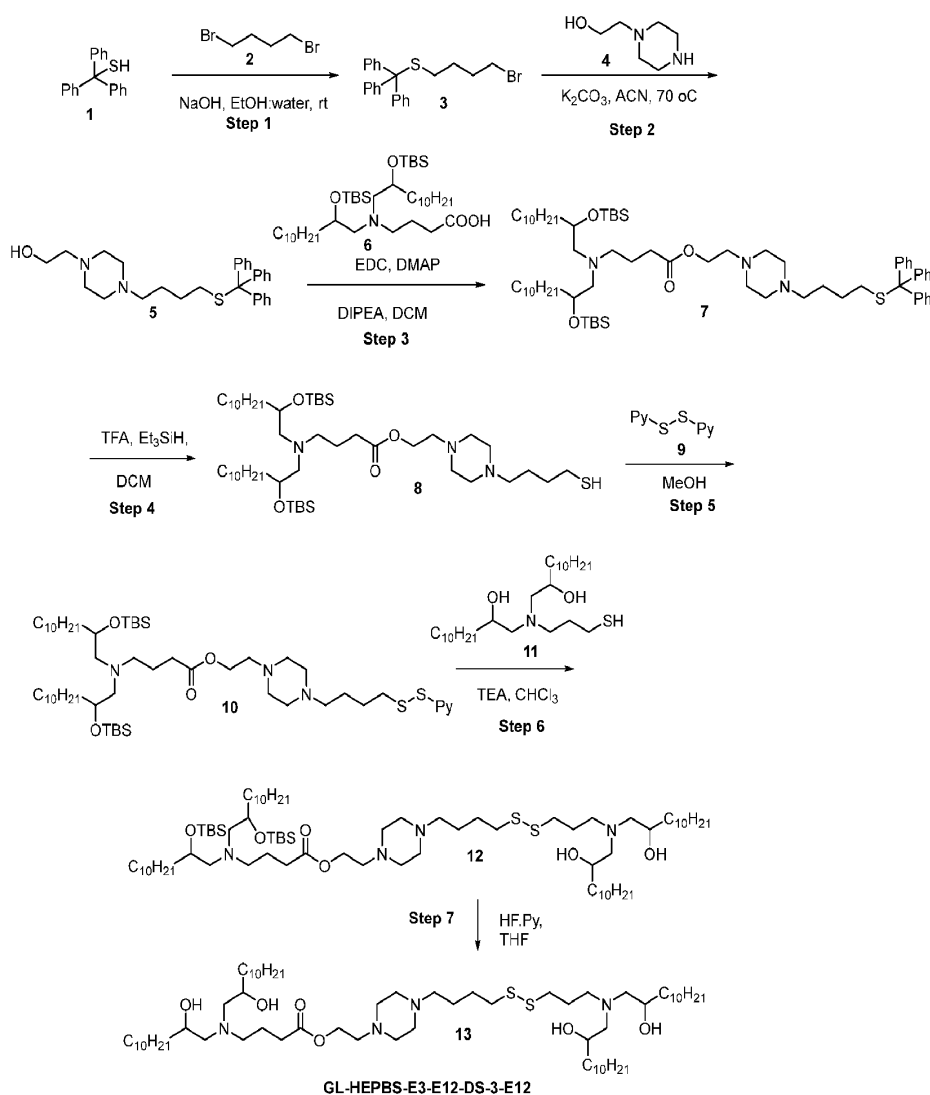
ESI-MS analysis: Calculated for $C_{62}H_{126}N_4O_6S_2$, $[M+H] = 1087.83$; Observed = 1087.8

Example 9: Synthesis of HEPBS-based ester/disulfide cationic lipids

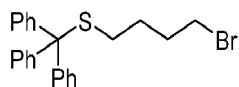
[0848] HEPBS-based cationic lipids described herein may be prepared according to **Scheme 9a**:

9a:

Scheme 9a



Intermediate [3]:

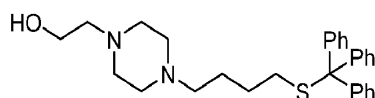


[0849] To a solution of triphenylmethanethiol (5.0 g, 18.08 mmol) in EtOH (40 mL) and water (40 mL) was added a solution (in 40 mL water) of NaOH (1.44 g, 36.16 mmol). The reaction mixture was stirred for 10 min and added a solution (in 40 mL EtOH) of 1,4-dibromobutane (3.65 g, 18.08 mmol) to reaction mixture. The reaction mixture was stirred for 4 hours at room temperature. The progress of reaction was monitored by TLC (5% EtOAc/hexanes). The reaction mixture was diluted DCM and aqueous sodium bicarbonate solution, the organic layer was washed with brine. The organic layer was dried over sodium sulphate, concentrated under vacuum to give crude compound. To the crude was added MeOH (15 mL) and stirred for 15 min at 0-10 °C, the solid compound was filtered and dried under vacuum to give [3] (5.1 g, 69%) as a white solid.

[0850] Results:

[0851] ¹H NMR (400 MHz, CDCl₃): δ 7.42-7.39 (m, 6H), 7.30-7.26 (m, 6H), 7.23-7.19 (m, 3H), 3.24 (t, 2H), 2.17 (t, 2H), 1.82-1.77 (m, 2H), 1.55-1.50 (m, 2H). LCMS: Purity 84.99 % (low ionization)

Intermediate [5]:



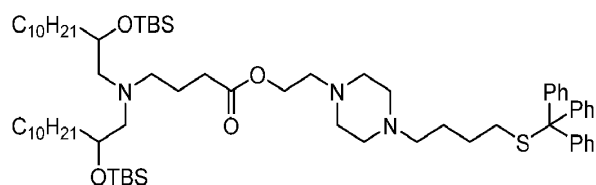
[0852] To a solution of [3] (5.0 g, 12.16 mmol) and [4] (3.16 g, 24.32 mmol) in ACN (75 mL) was added K₂CO₃ (6.72 g, 48.62 mmol). The reaction mixture was heated at 40 °C for 48 hours. The reaction progress was monitored by TLC (2.5% MeOH in DCM). The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under vacuum to give crude product. The crude was purified by flash chromatography (0 to 2.5 % MeOH in DCM) to give [5] (2.6 g, 46%) as a white solid.

[0853] Results:

[0854] ¹H NMR (400 MHz, DMSO-d₆): δ 7.41 (d, 6H), 7.28 (d, 6H), 7.20 (t, 3H), 3.59 (t, 2H), 2.73 (brs, 1H), 2.53-2.39 (m, 10H), 2.20-2.14 (m, 4H), 1.41 (brs, 4H). LCMS: Purity 98 %

[0855] ESI-MS analysis: Calculated C₂₉H₃₇N₂OS, [M+H] = 461.26, Observed = 461.29

Intermediate [7]:

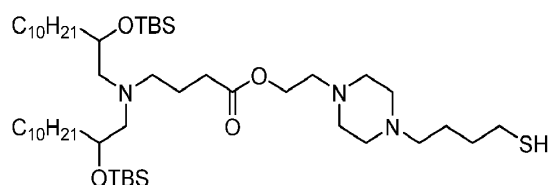


[0856] To a solution of [5] (0.5 g, 1.09 mmol) in DCM (8 mL) were added [6] (0.84 g, 1.19 mmol) in DCM (7 mL), EDC (0.31 g, 1.63 mmol), DMAP (27 mg, 0.22 mmol), DIPEA (0.38 mL, 2.17 mmol) and stirred at room temperature for 14 hours. After completion of the reaction as monitored by MS. The reaction mixture was diluted with DCM washed with NaHCO₃ solution, water and brine. The organic layer was dried over anhydrous Na₂SO₄, concentrated, and the crude compound was purified (eluent: 20% EtOAc in hexanes) to obtain pure compound [7] as a colorless oil (0.75 g, 60%). It was confirmed by MS analysis.

[0857] Results:

[0858] ESI-MS analysis: Calculated C₆₉H₁₂₀N₃O₄Si₂, [M+H] = 1142.85, Observed = 1142.8

Intermediate [8]:

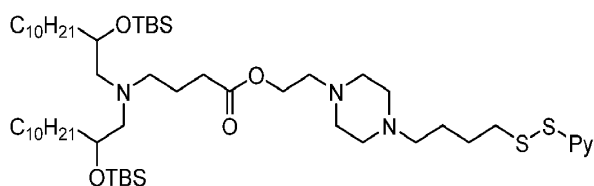


[0859] To a solution of [7] (0.75 g, 0.66 mmol) in DCM (6 mL) was slowly added TFA (6 mL) at room temperature and stirred at room temperature for 0.5 hour. To that triethylsilane (0.13 mL, 0.82 mmol) was added slowly and stirred for 1 hour. After completion of the reaction as monitored by MS. The reaction mixture was concentrated to obtain crude product [8] (>0.59 g, quantitative). It was confirmed by MS analysis.

[0860] Results:

[0861] ESI-MS analysis: Calculated C₅₀H₁₀₆N₃O₄Si₂, [M+H] = 900.74, Observed = 900.7

Intermediate [10]:

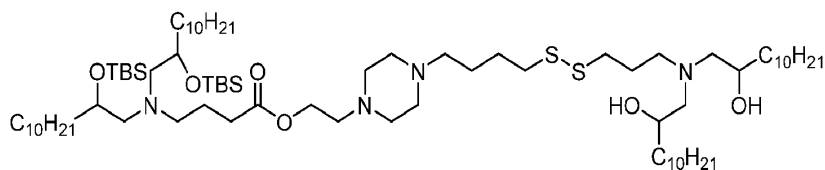


[0862] To a solution of [8] (0.59 g, 0.65 mmol) in MeOH (10 mL) was added [9] (0.22 g, 0.98 mmol) at room temperature and stirred for 2 hours. After completion of the reaction as monitored by MS. The reaction mixture was concentrated, and the crude compound was purified (eluent: 8% MeOH in DCM) to obtain pure product [10] (0.45 g, 68%). It was confirmed by MS analysis.

[0863] Results:

[0864] ESI-MS analysis: Calculated for $\text{C}_{55}\text{H}_{109}\text{N}_4\text{O}_4\text{S}_2\text{Si}_2$, $[\text{M}+\text{H}] = 1009.74$; Observed = 1009.7

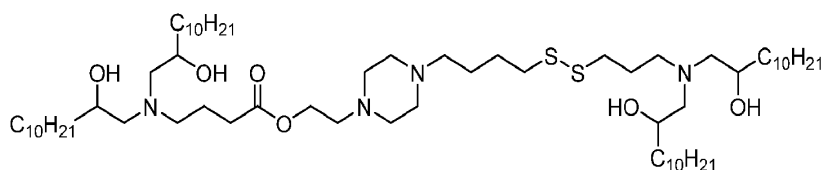
Intermediate [12]:



[0865] To a solution of [10] (0.320 g, 0.317 mmol) and [11] (0.364 g, 0.792 mmol) in chloroform was added triethylamine (0.180 mL, 1.27 mmol) and allowed to react at room temperature for 2 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain [12] as colorless oil (0.245 g, 57% yield).

[0866] ESI-MS analysis: Calculated for $\text{C}_{77}\text{H}_{161}\text{N}_4\text{O}_4\text{S}_2\text{Si}_2$, $[\text{M}+\text{H}] = 1326.15$; Observed = 1326.1

GL-HEPBS-E3-E12-DS-3-E12 [13]:



[0867] To a 20 ml polypropylene scintillation vial was added [12] (0.245 g, 0.180 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (0.930 mL, 35.541 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with NaHCO₃ solution, water and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified to obtain compound [13] (0.159 g, 78%). It was confirmed by ¹H NMR and MS analysis.

[0868] Results:

[0869] ¹H NMR (400 MHz, CDCl₃) 4.21 (t, 2H), 3.71 (brs, 4H), 2.89 – 2.29 (m, 28H), 1.98–1.65 (m, 8H), 1.53 – 1.17 (m, 72H), 0.87 (t, 12H).

[0870] ESI-MS analysis: Calculated for C₆₅H₁₃₃N₄O₆S₂, [M+H]⁺ = 1129.97; Observed = 1129.9

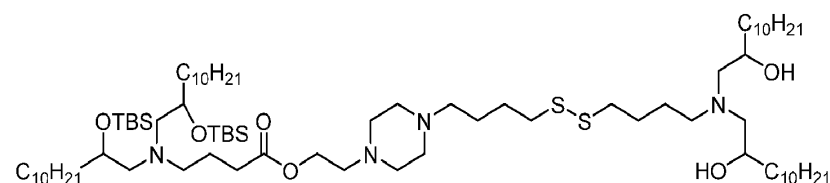
Scheme 9b:

The reaction scheme illustrates the synthesis of compound 13 from compound 1 through seven steps:

- Step 1:** Compound 1 (triphenylsulfhydryl) reacts with 1,4-dibromobutane (2) in the presence of NaOH, EtOH, water, at room temperature to form compound 3 (triphenylsulfide with a 4-bromobutyl chain).
- Step 2:** Compound 3 reacts with 4-(hydroxymethyl)piperazine (4) in the presence of K₂CO₃ in ACN at 70 °C to form compound 5 (a piperazine-linked sulfide).
- Step 3:** Compound 5 reacts with 6-(tert-butyldimethylsilyloxy)-2-hydroxy-2-(undecyloxy)hexanoic acid (6) in the presence of EDC and DMAP, followed by DIPEA in DCM, to form compound 7 (an ester-linked intermediate).
- Step 4:** Compound 7 is treated with TFA and Et₃SiH in DCM to form compound 8 (a thiol intermediate).
- Step 5:** Compound 8 reacts with pyridine disulfide (9) in MeOH to form compound 10 (a disulfide-linked intermediate).
- Step 6:** Compound 10 reacts with 1,4-bis(undecyloxy)-2,2-bis(hydroxymethyl)piperazine (11) in the presence of TEA and CHCl₃ to form compound 12 (a complex intermediate with multiple functional groups).
- Step 7:** Compound 12 is treated with HF·Py in THF to yield the final product, compound 13.

[0872] Intermediate 10 was synthesized using the same procedures as in Scheme 9a.

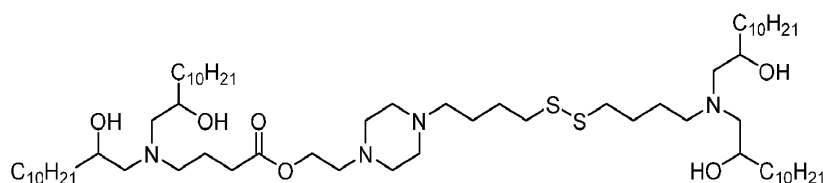
Intermediate [12]:



[0873] To a solution of [10] (0.45 g, 0.45 mmol) and [11] (0.528 g, 1.11 mmol) in chloroform was added triethylamine (0.31 ml, 2.23 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain [12] as a colorless oil (0.30 g, 50% yield).

[0874] ESI-MS analysis: Calculated for $C_{78}H_{162}N_4O_6S_2Si_2$, $[M+H] = 1373.46$; Observed = 1373.8

GL-HEPBS-E3-E12-DS-4-E12 [13]:



[0875] To a 20 ml polypropylene scintillation vial was added [12] (0.300 g, 0.22 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (1.2 mL, 43.10 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with NaHCO₃ solution, water and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified to obtain compound [13] (0.18 g, 72%). It was confirmed by ¹H NMR and MS analysis.

[0876] Results:

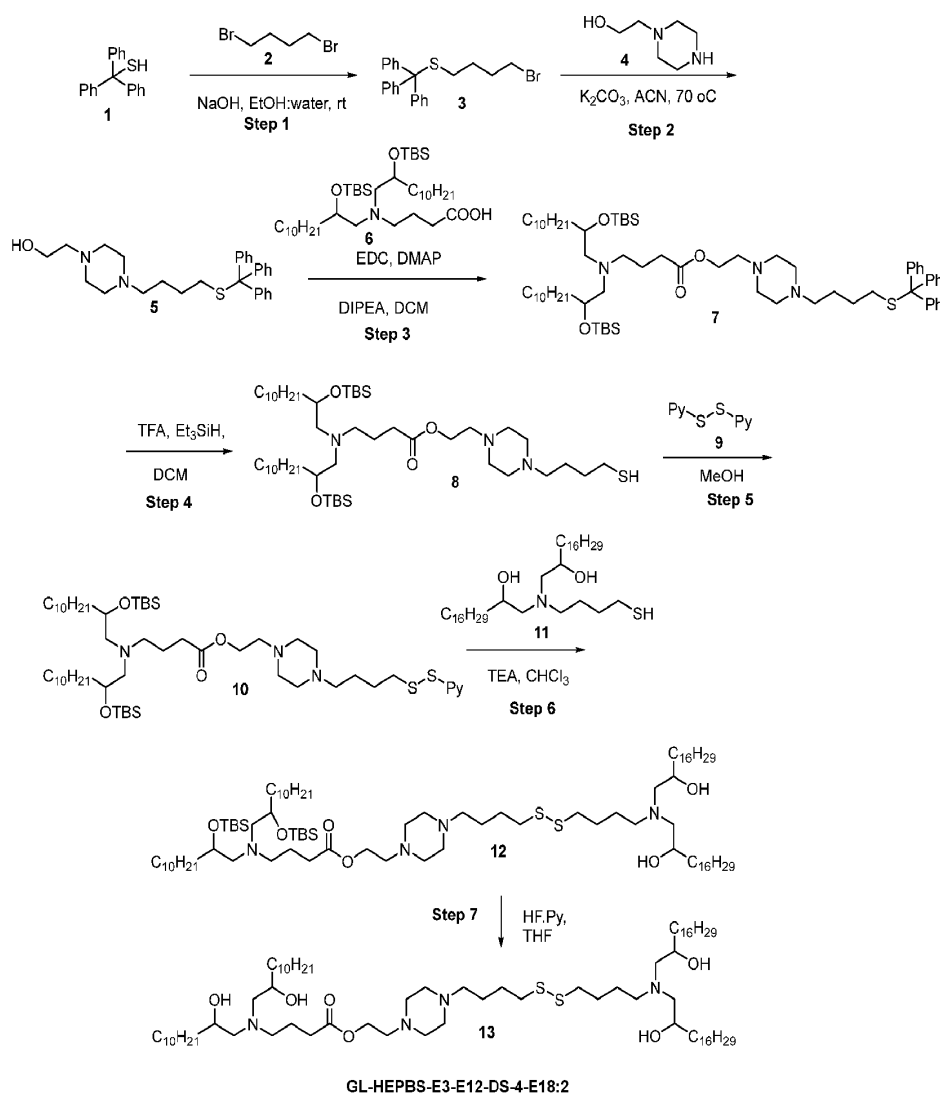
[0877] ¹H NMR (400 MHz, CDCl₃) 4.20 (t, 2H), 3.85 – 3.54 (br, 4H), 2.98 – 2.19 (m, 28H), 1.93–1.58 (m, 9H), 1.57 – 1.12 (m, 72H), 0.87 (t, 12H).

[0878] ESI-MS analysis: Calculated for $C_{66}H_{134}N_4O_6S_2$, $[M+H] = 1144.94$; Observed = 1144.8.

[0879] HEPBS-based cationic lipids described herein may also be prepared according to

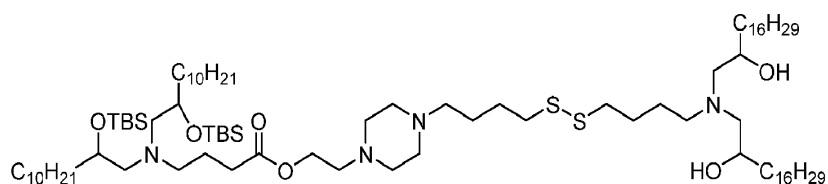
Scheme 9c:

Scheme 9c



[0880] Intermediate 10 was synthesized using the same procedures as in Schemes 9a and 9b.

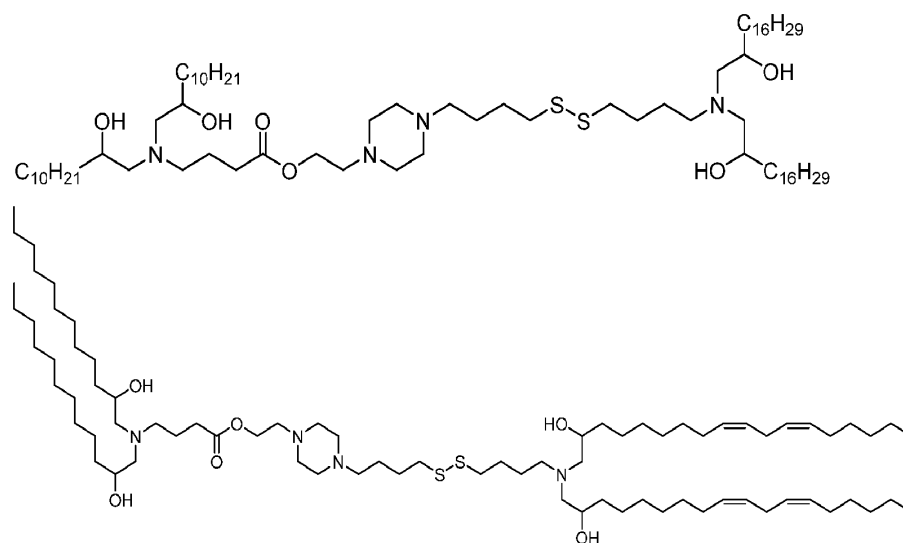
Intermediate [12]:



[0881] To a solution of [10] (0.348 g, 0.35 mmol) and [11] (0.438 g, 0.69 mmol) in chloroform was added triethylamine (0.24 ml, 1.72 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain [12] as colorless oil (0.31 g, 59% yield).

[0507] ESI-MS analysis: Calculated for C₉₀H₁₇₈N₄O₆S₂Si₂, [M+H] = 1533.73; Observed = 1533.8

GL-HEPBS-E3-E12-DS-4-E18:2 [13]:



[0882] To a 20 ml polypropylene scintillation vial was added [12] (0.310 g, 0.20 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (1.03 mL, 39.8 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with NaHCO₃ solution, water and brine. The organic layer was dried over

anhydrous Na₂SO₄ and concentrated. The crude product was purified to obtain compound [13] (0.23 g, 86%). It was confirmed by ¹H NMR and MS analysis.

[0883] Results:

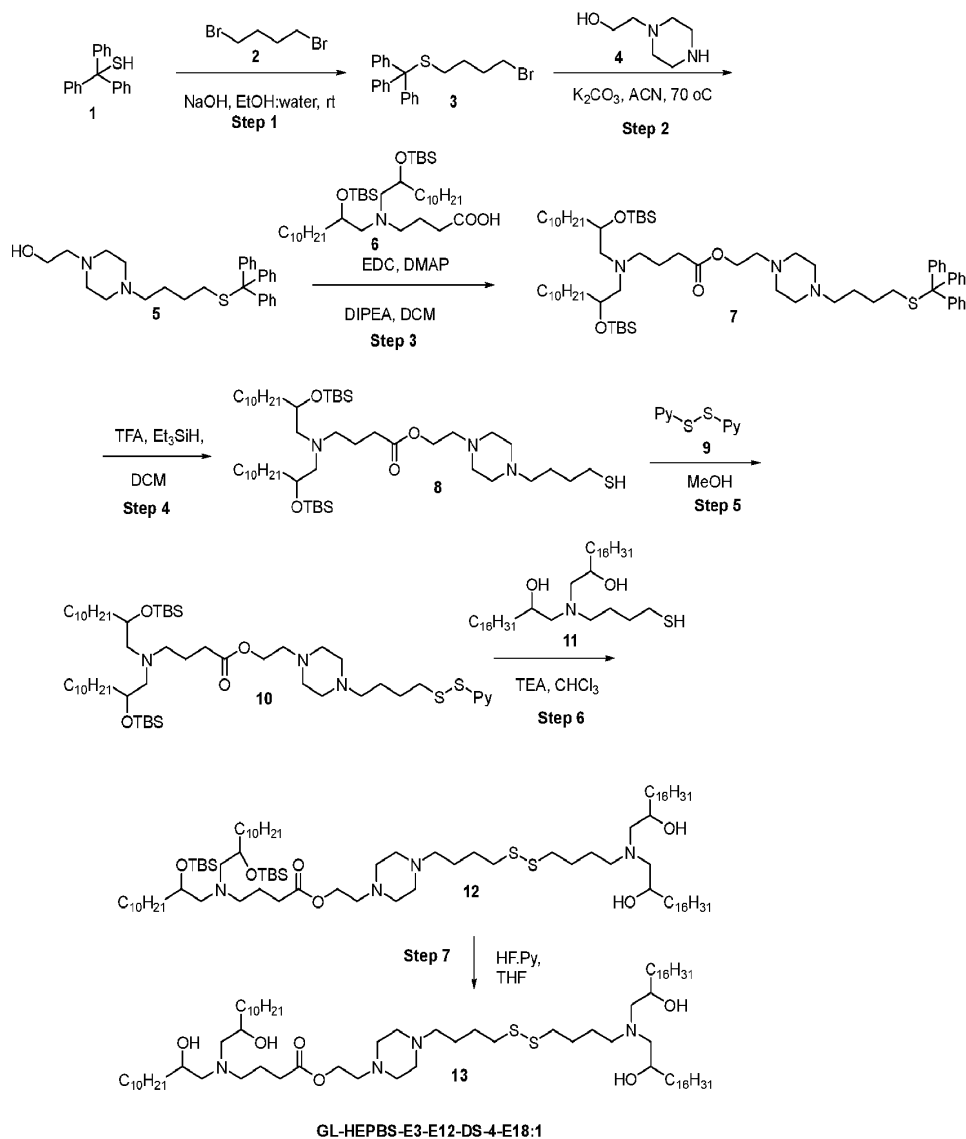
[0884] ¹H NMR (400 MHz, CDCl₃) 5.47 – 5.27 (m, 8H), 4.20 (t, 2H), 3.89 – 3.55 (br, 4H), 2.98 – 2.24 (m, 32H), 2.07 – 1.92 (m, 8H), 1.88–1.55 (m, 10H), 1.54 – 1.12 (m, 70H), 0.87 (t, 12H).

[0885] ESI-MS analysis: Calculated for C₇₈H₁₅₀N₄O₆S₂, [M+H] = 1305.20; Observed = 1305.8.

[0886] HEPBS-based cationic lipids described herein may also be prepared according to

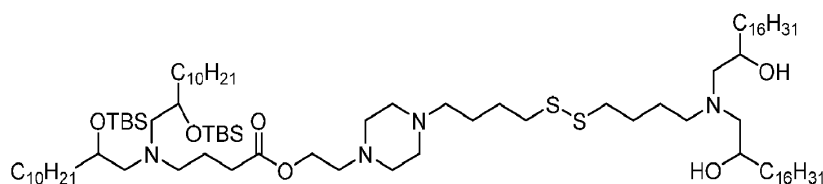
Scheme 9d:

Scheme 9d



[0887] Intermediate 10 was synthesized using the same procedures as in Schemes 9a, 9b and 9c.

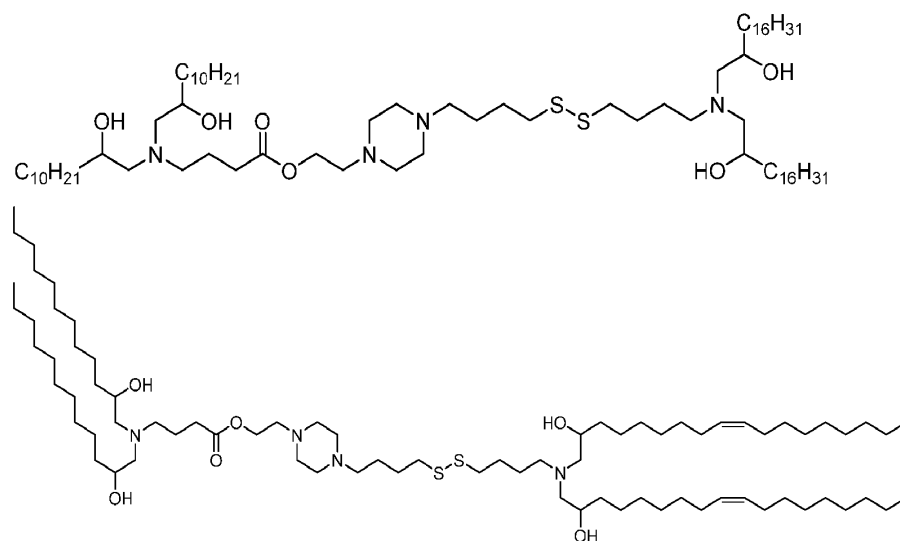
Intermediate [12]:



[0888] To a solution of [10] (0.418 g, 0.41 mmol) and [11] (0.529 g, 0.83 mmol) in chloroform was added triethylamine (0.34 ml, 2.44 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain [12] as colorless oil (0.400 g, 63% yield).

[0889] ESI-MS analysis: Calculated for C₉₀H₁₈₂N₄O₆S₂Si₂, [M+H] = 1537.76; Observed = 1537.8.

GL-HEPBS-E3-E12-DS-4-E18-1 [13]:



[0890] To a 20 ml polypropylene scintillation vial was added [12] (0.40 g, 0.26 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (1.3 mL, 51.27 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with NaHCO₃ solution, water and brine. The organic layer was dried over anhydrous

Na₂SO₄ and concentrated. The crude product was purified to obtain compound [13] (0.20 g, 59%). It was confirmed by ¹H NMR and MS analysis.

[0891] Results:

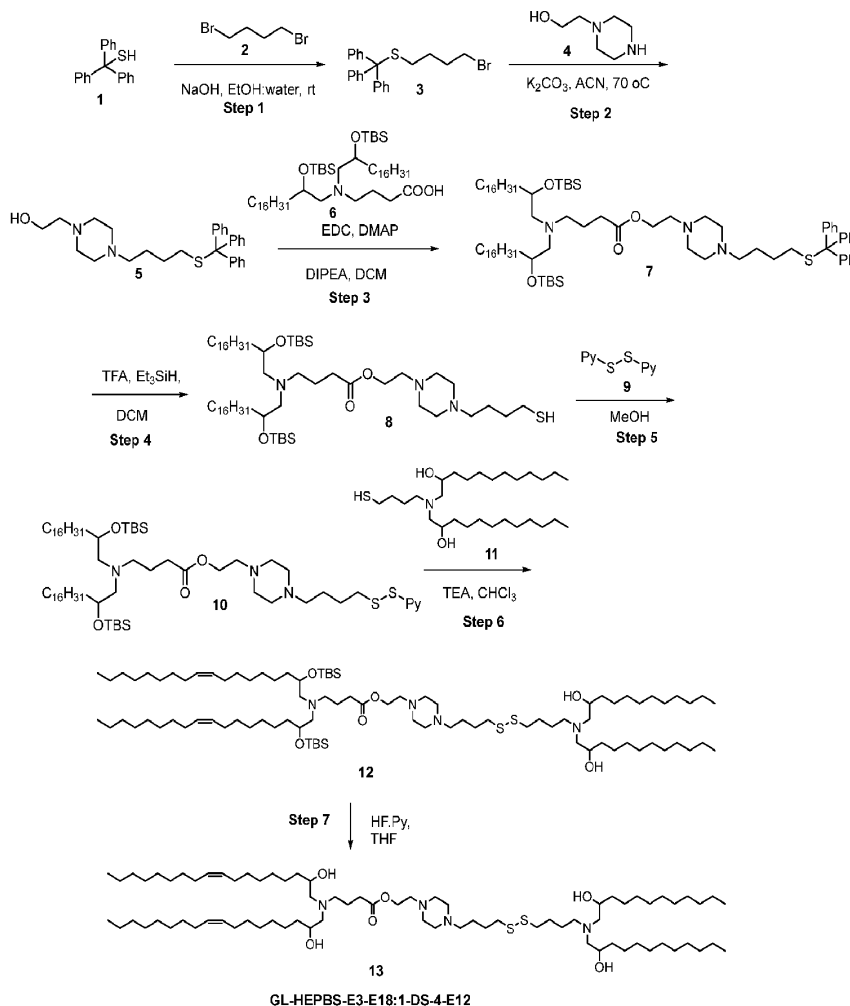
[0892] ¹H NMR (400 MHz, CDCl₃) 5.43 – 5.28 (m, 4H), 4.20 (t, 2H), 3.65 (br, 4H), 2.86 – 2.24 (m, 32H), 2.07 – 1.89 (m, 8H), 1.88–1.52 (m, 10H), 1.52 – 1.11 (m, 80H), 0.87 (t, 12H).

[0893] ESI-MS analysis: Calculated for C₇₈H₁₅₄N₄O₆S₂, [M+H]⁺ = 1309.23; Observed = 1309.8.

[0894] HEPBS-based cationic lipids described herein may also be prepared according to

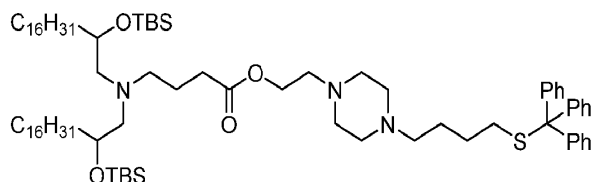
Scheme 9e:

Scheme 9e



[0895] Intermediate 5 was synthesized using the same procedures as in Schemes 9a, 9b, 9c, and 9d.

Intermediate [7]:

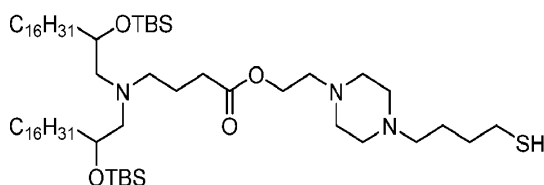


[0896] To a solution of [5] (1.06 g, 2.31 mmol) in DCM (12 mL) were added [6] (2.0 g, 2.31 mmol) in DCM (8 mL), EDC (0.665 g, 3.47 mmol), DMAP (56 mg, 0.463 mmol), DIPEA (0.810 mL, 4.63 mmol) and stirred at room temperature for 14 hours. After completion of the reaction as monitored by MS. The reaction mixture was diluted with DCM washed with NaHCO₃ solution, water and brine. The organic layer was dried over anhydrous Na₂SO₄, concentrated, and the crude compound was purified (eluent: 20% EtOAc in hexanes) to obtain pure compound [7] as a color less oil (2.13 g, 70%). It was confirmed by MS analysis.

Results:

[0897] ESI-MS analysis: Calculated C₈₁H₁₃₉N₃O₄Si₂, [M+H] = 1307.25, Observed = 1307.8

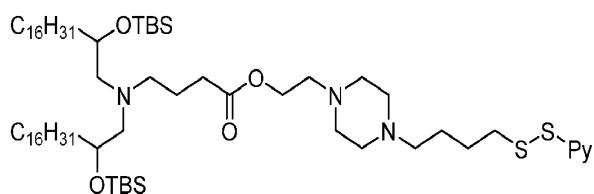
Intermediate [8]:



[0898] To a solution of [7] (2.13 g, 1.63 mmol) in DCM (6 mL) was slowly added TFA (6 mL) at room temperature and stirred at room temperature for 0.5 hour. To that triethylsilane (0.330 mL, 2.04 mmol) was added slowly and stirred for 1 hour. After completion of the reaction as monitored by MS. The reaction mixture was concentrated to obtain crude product [8] (quantitative). It was confirmed by MS analysis.

Results:

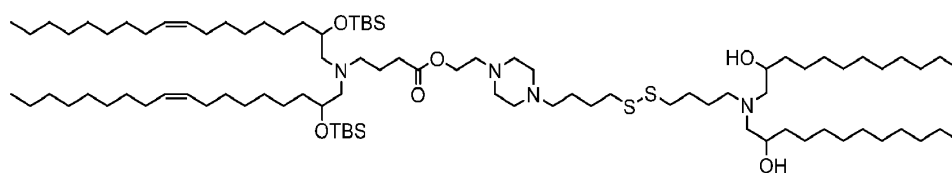
[0899] ESI-MS analysis: Calculated C₆₂H₁₂₅N₃O₄Si₂, [M+H] = 1064.93, Observed = 1064.8

Intermediate [10]:

[0900] To a solution of [8] (quantitative) in MeOH (4 mL) was added [9] (0.610 g, 2.77 mmol) at room temperature and stirred for 2 hours. After completion of the reaction as monitored by MS. The reaction mixture was concentrated, and the crude compound was purified (eluent:100% Ethyl Acetate, then 0-20 % Methanol in Ethyl Acetate) to obtain pure product [10] (1.85 g, 97%). It was confirmed by MS analysis.

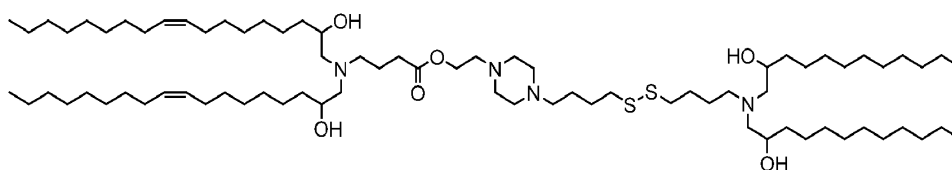
Results:

[0901] ESI-MS analysis: Calculated for C₆₇H₁₂₈N₄O₄S₂Si₂, [M+H] = 1174.08; Observed = 1174.8

Intermediate [12]:

[0902] To a solution of [10] (0.400 g, 0.341 mmol) and [11] (0.282 g, 0.596 mmol) in chloroform was added triethylamine (0.285 ml, 2.04 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and taken to the next step without purification (0.777 g Crude Material).

[0903] ESI-MS analysis: Calculated for C₉₀H₁₈₂N₄O₆S₂Si₂, [M+H] = 1536.76; Observed = 1536.8

GL-HEPBS-E3E18:1-DS-4-E12 [13]:

[0904] To a 20 ml polypropylene scintillation vial was added **[12]** (Crude Material, 0.777 g) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (2.0 mL, 76.92 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with NaHCO₃ solution, water and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified to obtain compound **[13]** (0.272 g, 61% Over Two Steps). It was confirmed by ¹H NMR and MS analysis.

Results:

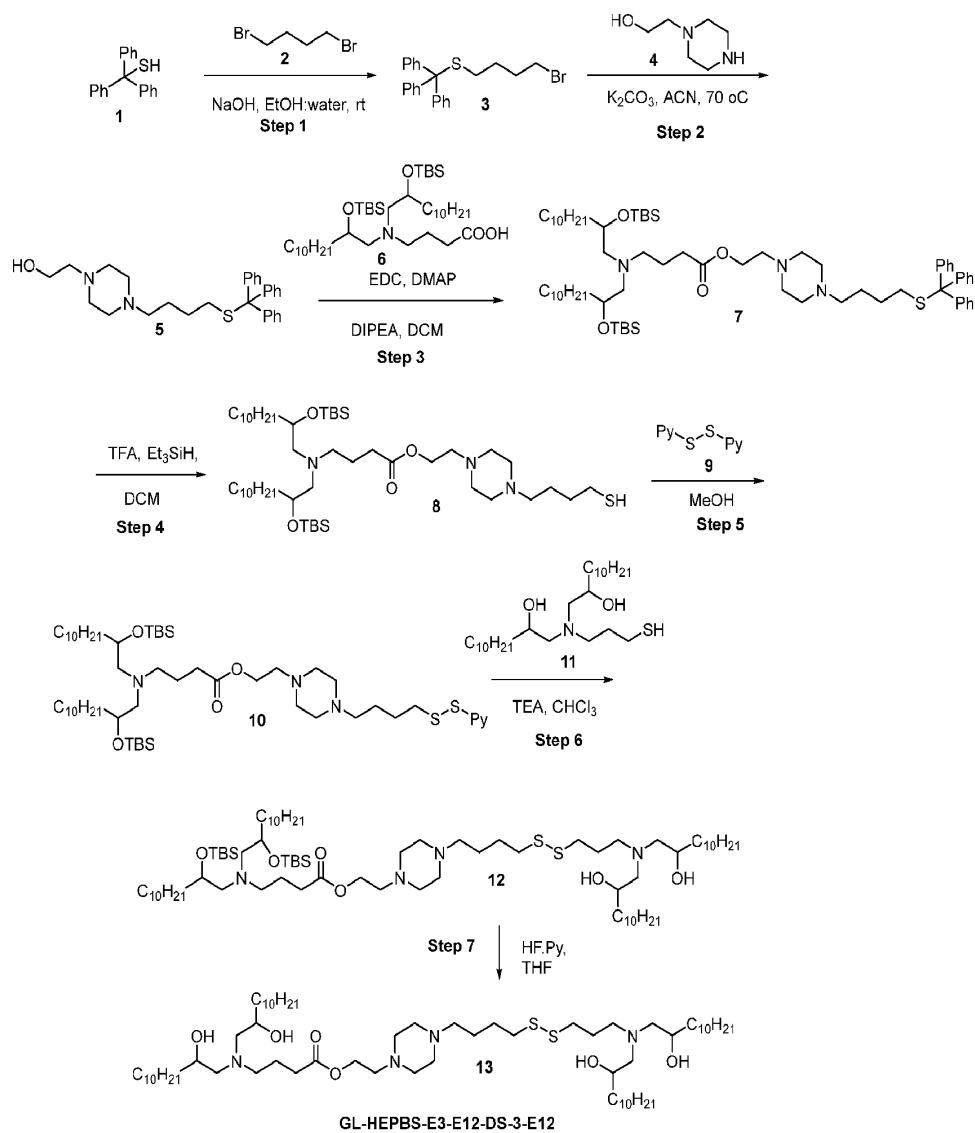
[0905] ¹H NMR (400 MHz, CDCl₃) 5.41 – 5.30 (m, 4H), 4.20 (t, 2H), 3.63 (m, 4H), 3.15 (br, 2H), 2.86 – 2.25 (m, 33H), 2.11 – 1.89 (m, 8H), 1.88 – 1.52 (m, 12H), 1.51 – 1.16 (m, 92H), 0.87 (t, 12H).

[0906] ESI-MS analysis: Calculated for C₇₈H₁₅₄N₄O₆S₂, [M+H] = 1308.23; Observed = 1308.8

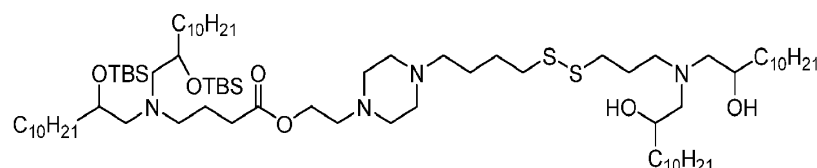
[0907] HEPBS-based cationic lipids described herein may also be prepared according to

Scheme 9f:

Scheme 9f

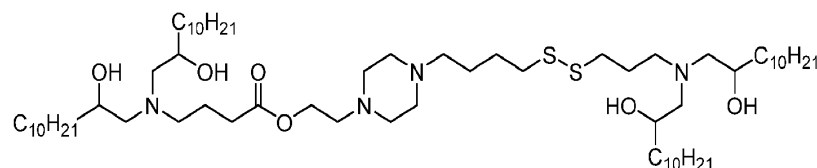


[0908] Intermediate 10 was synthesized using the same procedures as in Schemes 9a, 9b, 9c, and 9d.

Intermediate [12]:

[0909] To a solution of **[10]** (0.320 g, 0.317 mmol) and **[11]** (0.364 g, 0.792 mmol) in chloroform was added triethylamine (0.180 ml, 1.27 mmol) and allowed to react at room temperature for 2 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain **[12]** as colorless oil (0.245 g, 57% yield).

[0910] ESI-MS analysis: Calculated for $C_{77}H_{161}N_4O_4S_2Si_2$, $[M+H] = 1326.15$; Observed = 1326.1

GL-HEPBS-E3-E12-DS-3-E12 [13]:

[0911] To a 20 ml polypropylene scintillation vial was added **[12]** (0.245 g, 0.180 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (0.930 mL, 35.541 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified to obtain compound **[13]** (0.159 g, 78%). It was confirmed by ^1H NMR and MS analysis.

[0912] Results:

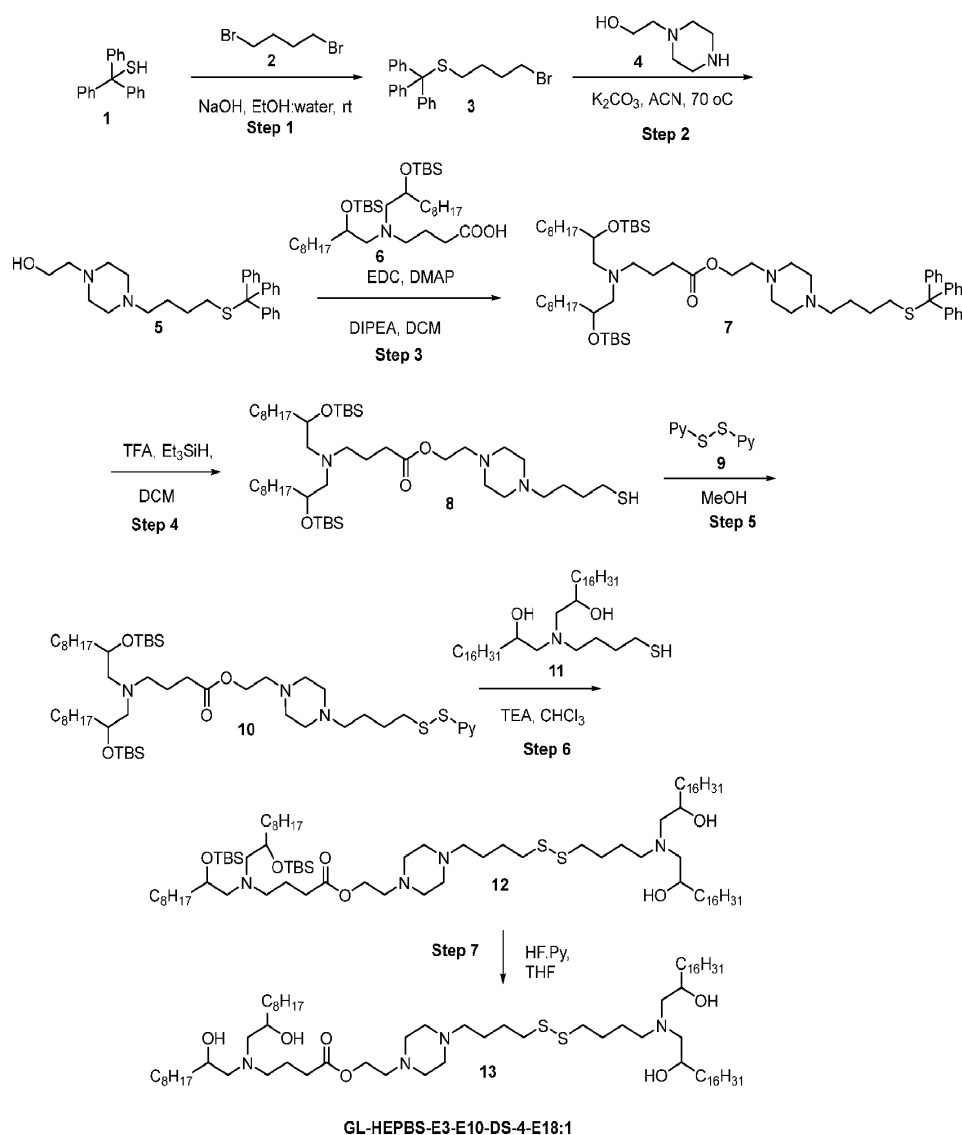
[0913] ^1H NMR (400 MHz, $CDCl_3$) 4.21 (t, 2H), 3.71 (brs, 4H), 2.89 – 2.29 (m, 28H), 1.98–1.65 (m, 8H), 1.53 – 1.17 (m, 72H), 0.87 (t, 12H).

[0914] ESI-MS analysis: Calculated for $C_{65}H_{133}N_4O_6S_2$, $[M+H] = 1129.97$; Observed = 1129.9

[0915] HEPBS-based cationic lipids described herein may also be prepared according to

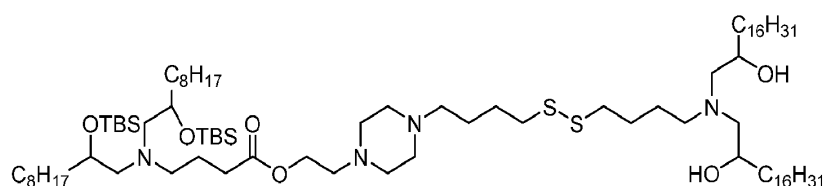
Scheme 9g:

Scheme 9g



[0916] Intermediate 10 was synthesized using the same procedures as **Scheme 9d**, with the exception of using an 8-carbon tail instead of the 10-carbon tail for the coupling in Step 3.

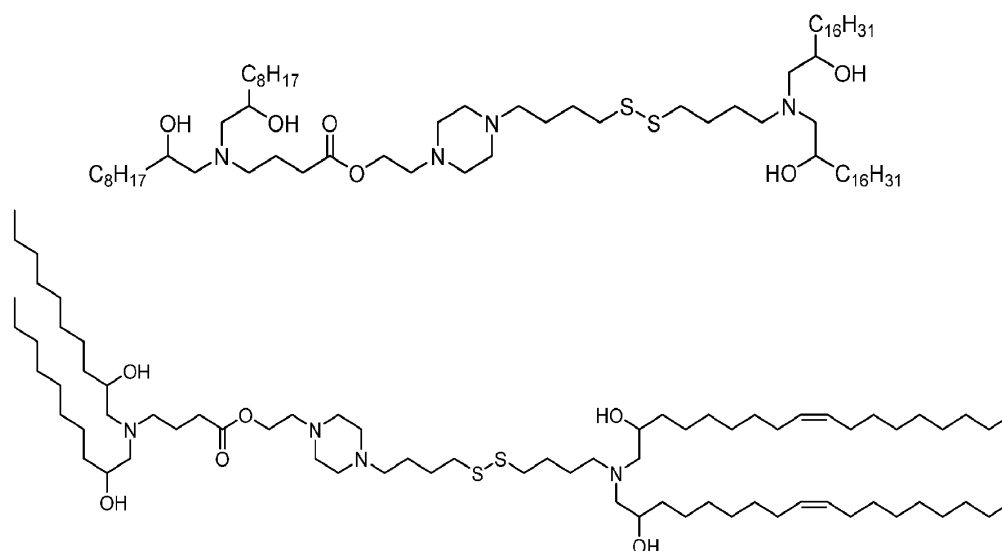
Intermediate [12]:



[0917] To a solution of **[10]** (0.348 g, 0.36 mmol) and **[11]** (0.430 g, 0.67 mmol) in chloroform was added triethylamine (0.260 ml, 1.86 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain **[12]** as colorless oil (0.200 g, 37% yield).

[0918] ESI-MS analysis: Calculated for $C_{86}H_{174}N_4O_6S_2Si_2$, $[M+H] = 1480.65$; Observed = 1480.8

GL-HEPBS-E3-E10-DS-4-E18:1 [13]:



[0919] To a 20 ml polypropylene scintillation vial was added **[12]** (0.200 g, 0.14 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (0.7 mL, 26.62 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous

Na_2SO_4 and concentrated. The crude product was purified to obtain compound [13] (0.160 g, 92%). It was confirmed by ^1H NMR and MS analysis.

[0920] Results:

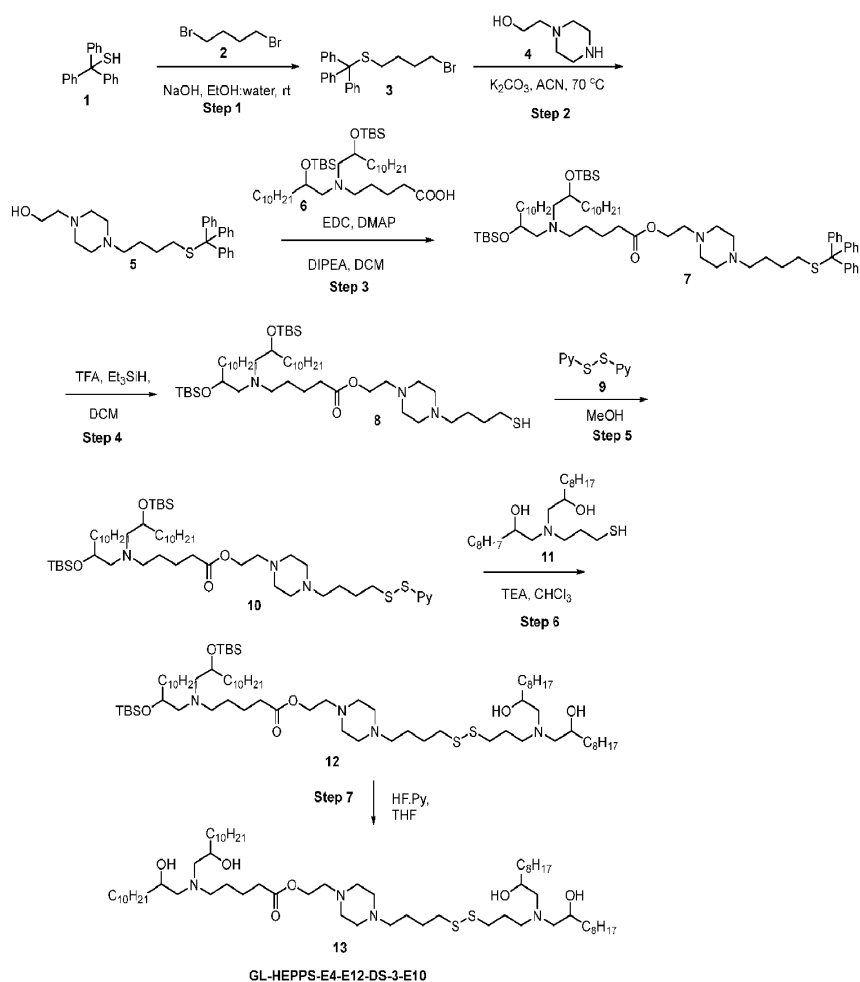
[0921] ^1H NMR (400 MHz, CDCl_3) 5.40 – 5.30 (m, 4H), 4.20 (t, 2H), 3.87 – 3.53 (br, 4H), 2.93 – 2.22 (m, 28H), 2.11 – 1.89 (m, 8H), 1.89–1.56 (m, 10H), 1.56 – 1.12 (m, 74H), 0.87 (t, 12H).

[0922] ESI-MS analysis: Calculated for $\text{C}_{74}\text{H}_{146}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1252.12$; Observed = 1252.8

[0923] HEPBS-based cationic lipids described herein may also be prepared according to

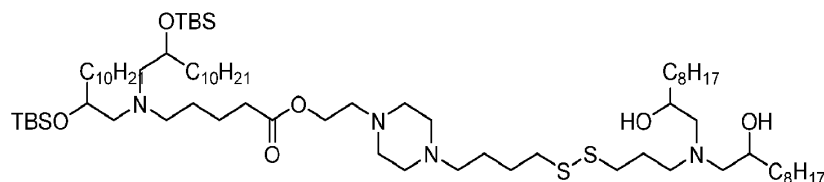
Scheme 9h:

Scheme 9h



[0924] Intermediate **10** was synthesized using the same procedures as **Scheme 9d**, with the exception of using a 4-carbon spacer between the carboxylic acid and tertiary amine in Step 3.

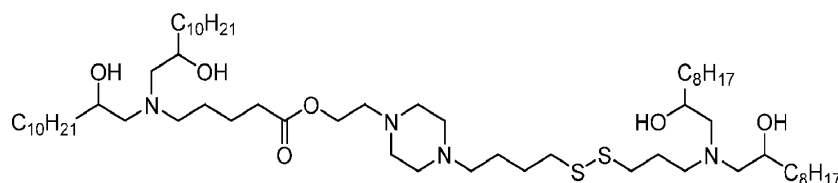
Intermediate **[12]**:



[0925] To a solution of **[10]** (0.500 g, 0.49 mmol) and **[11]** (0.355 g, 0.88 mmol) in chloroform was added triethylamine (0.340 ml, 2.44 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain **[12]** as colorless oil (0.245 g, 38% yield).

[0926] ESI-MS analysis: Calculated for $C_{74}H_{154}N_4O_6S_2Si_2$, $[M+H] = 1316.36$; Observed = 1316.8

GL-HEPBS-E4-E12-DS-3-E10 **[13]**:



[0927] To a 20 ml polypropylene scintillation vial was added **[12]** (0.245 g, 0.19 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (0.95 mL, 36.7 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified to obtain compound **[13]** (0.151 g, 75%). It was confirmed by ^1H NMR and MS analysis.

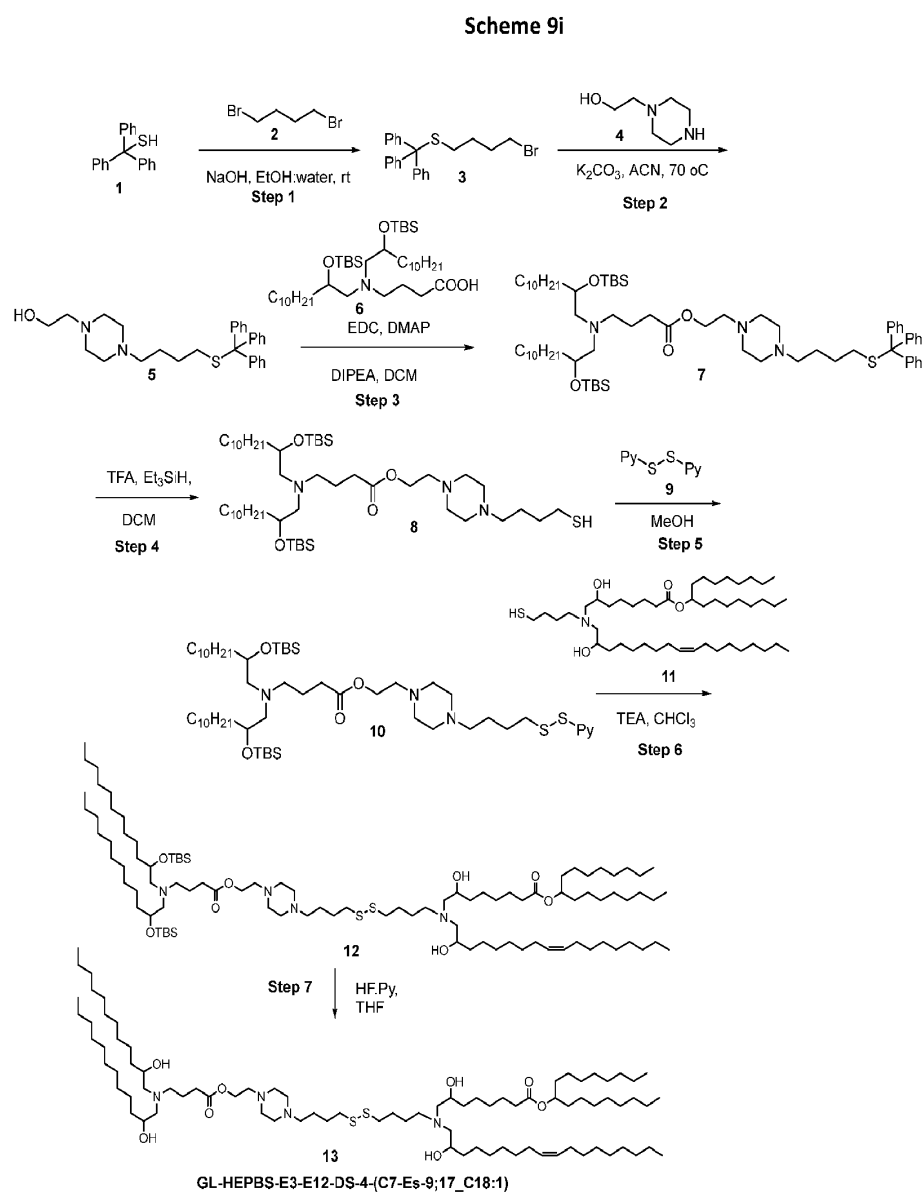
[0928] Results:

[0929] ^1H NMR (400 MHz, CDCl_3) 4.19 (t, 2H), 3.85 – 3.58 (br, 4H), 2.95 – 2.38 (m, 26H), 2.37 – 2.25 (m, 2H), 1.99 – 1.80 (m, 2H), 1.81 – 1.51 (br, 8H), 1.50 – 1.14 (m, 64H), 0.87 (t, 12H).

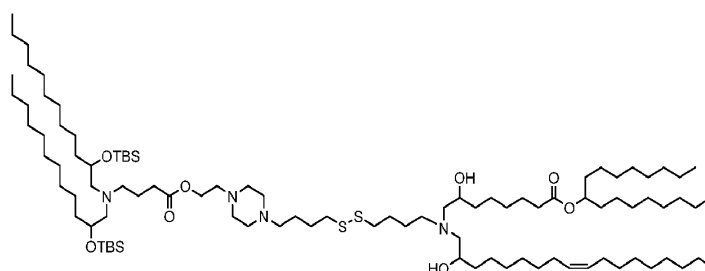
[0930] ESI-MS analysis: Calculated for $\text{C}_{62}\text{H}_{126}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1087.83$; Observed = 1087.8

[0931] HEPBS-based cationic lipids described herein may also be prepared according to

Scheme 9i:

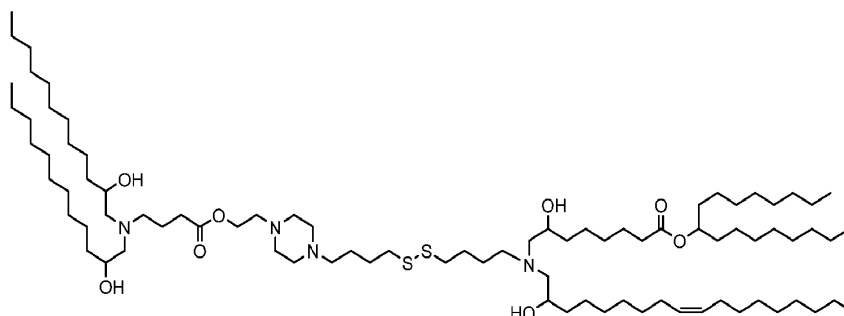


[0932] Intermediate 10 was synthesized using the same procedures as Scheme 9a.

Intermediate [12]:

[0933] To a solution of **[10]** (0.500 g, 0.495 mmol) and **[11]** (0.646 g, 0.841 mmol) in chloroform was added triethylamine (0.35 ml, 2.47 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain **[12]** as colorless oil (0.370 g, 45% yield).

[0934] ESI-MS analysis: Calculated for $C_{97}H_{196}N_4O_8S_2Si_2$, $[M+H] = 1666.95$; Observed = 1666.

GL-HEPBS-E3E12-DS-4-(C7-Es-9;17_C18:1) [13]:

[0935] To a 20 ml polypropylene scintillation vial was added **[12]** (0.370 g, 0.222 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (1.14 mL, 43.73 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified to obtain compound **[13]** (0.213 g, 66%). It was confirmed by ^1H NMR and MS analysis.

[0936] Results:

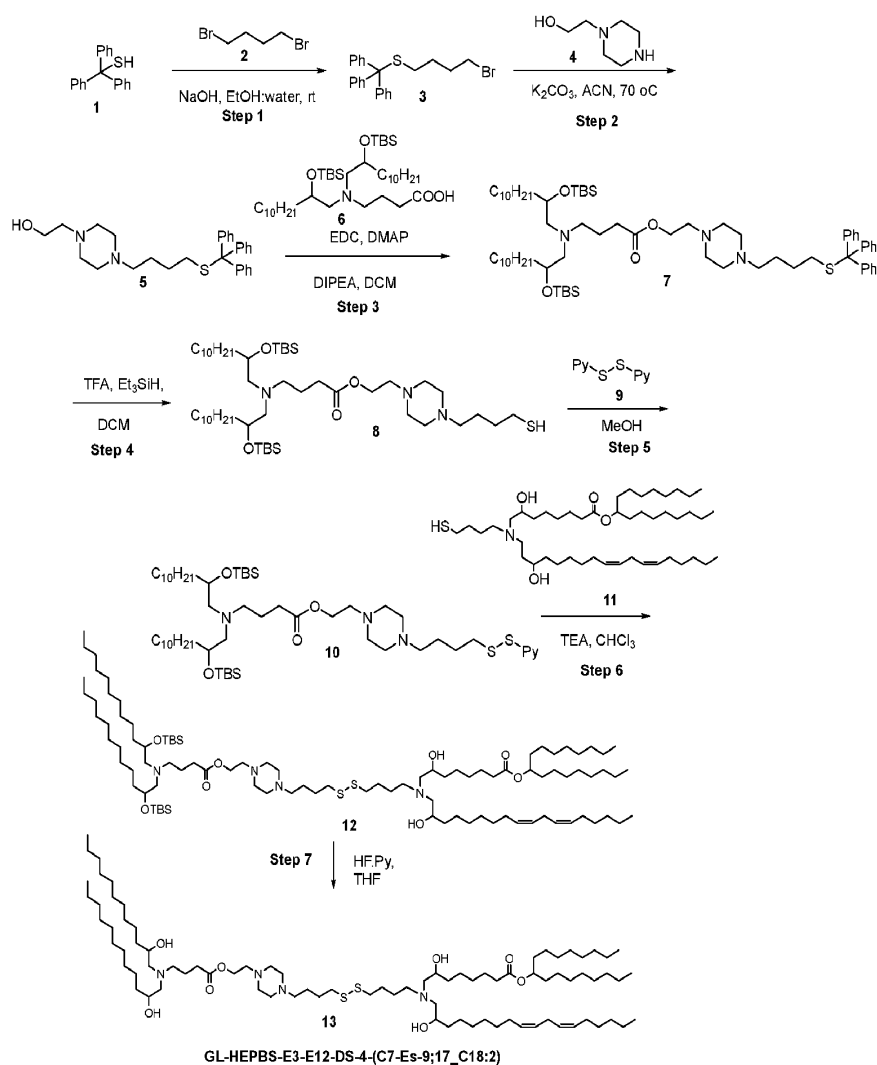
[0937] ^1H NMR (400 MHz, CDCl_3) 5.42 – 5.29 (m, 2H), 4.84 (quin, 1H), 4.20 (t, 2H), 3.68 (br, 3H), 2.92 – 2.31 (m, 22H), 2.30 – 2.23 (t, 2H), 2.03 – 1.91 (m, 4H), 1.88–1.76 (m, 2H), 1.75 – 1.55 (m, 12H), 1.54 – 1.11 (m, 102H), 0.86 (t, 15H).

[0938] ESI-MS analysis: Calculated for $\text{C}_{85}\text{H}_{168}\text{N}_4\text{O}_8\text{S}_2$, $[\text{M}+\text{H}] = 1438.42$; Observed = 1438.

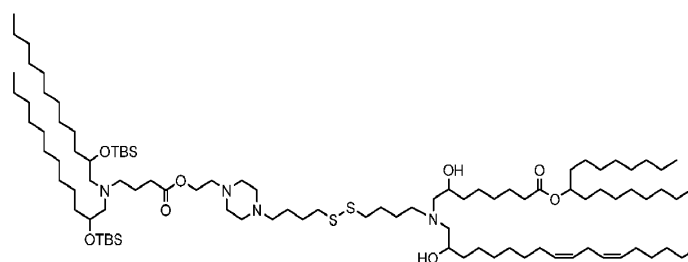
[0939] HEPBS-based cationic lipids described herein may also be prepared according to

Scheme 9j:

Scheme 9j

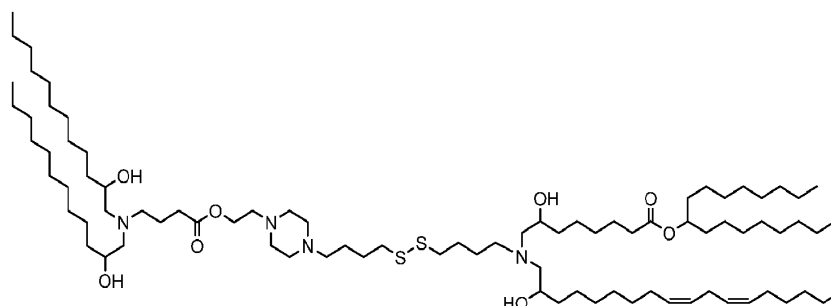


[0940] Intermediate 10 was synthesized using the same procedures as Scheme 9a.

Intermediate [12]:

[0941] To a solution of **[10]** (0.390 g, 0.386 mmol) and **[11]** (0.518 g, 0.675 mmol) in chloroform was added triethylamine (0.322 ml, 2.32 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and purified to obtain **[12]** as colorless oil (0.320 g, 50% yield).

[0942] ESI-MS analysis: Calculated for $C_{97}H_{134}N_4O_8S_2Si_2$, $[M+H] = 1664.93$; Observed = 1664.

GL-HEPBS-E3E12-DS-4-(C7-Es-9;17_C18:2) [13]:

[0943] To a 20 ml polypropylene scintillation vial was added **[12]** (0.320 g, 0.192 mmol, 1.0 eq) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (0.983 mL, 37.86 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified to obtain compound **[13]** (0.176 g, 64%). It was confirmed by ^1H NMR and MS analysis.

[0944] Results:

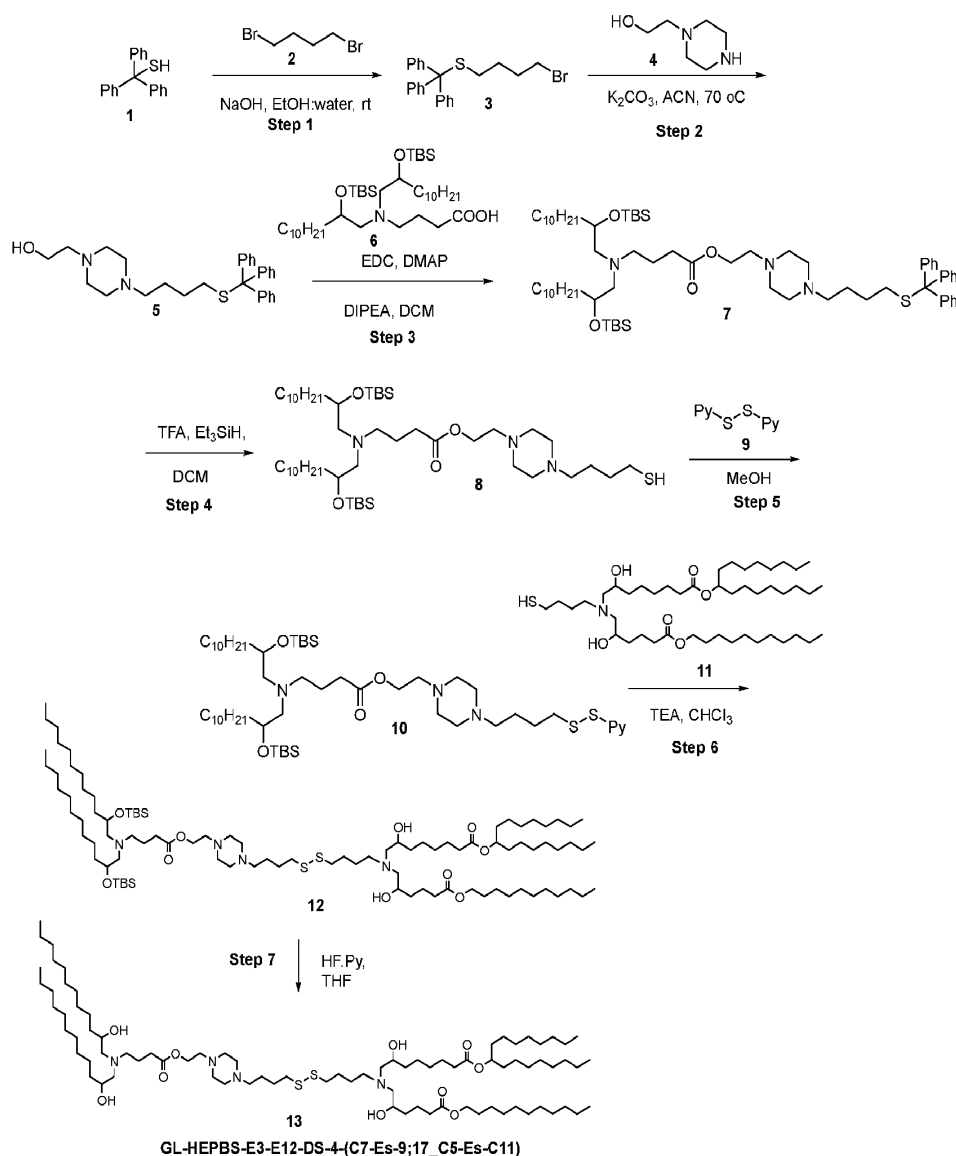
[0945] ^1H NMR (400 MHz, CDCl_3) 5.44 – 5.26 (m, 4H), 4.84 (quin, 1H), 4.20 (t, 2H), 3.70 (br, 4H), 2.99 – 2.33 (m, 32H), 2.32 – 2.24 (t, 2H), 2.09–1.92 (m, 4H), 1.91 – 1.56 (m, 12H), 1.55 – 1.14 (m, 90H), 0.87 (t, 15H).

[0946] ESI-MS analysis: Calculated for $\text{C}_{85}\text{H}_{166}\text{N}_4\text{O}_8\text{S}_2$, $[\text{M}+\text{H}] = 1436.40$; Observed = 1436.

[0947] HEPBS-based cationic lipids described herein may also be prepared according to

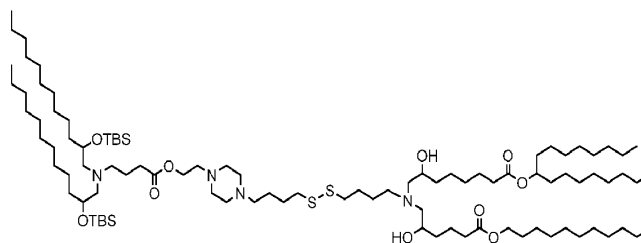
Scheme 9k:

Scheme 9k



[0948] Intermediate 10 was synthesized using the same procedures as Scheme 9a.

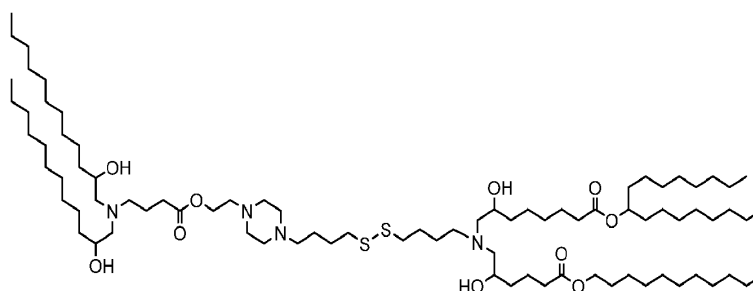
Intermediate [12]:



[0949] To a solution of [10] (0.190 g, 0.188 mmol) and [11] (0.259 g, 0.329 mmol) in chloroform was added triethylamine (0.157 ml, 1.13 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and taken to the next step without purification (0.317 g Crude Material).

[0950] ESI-MS analysis: Calculated for $C_{96}H_{194}N_4O_{10}S_2Si_2$, $[M+H] = 1684.92$; Observed = 1684.

GL-HEPBS-E3E12-DS-4-(C7-Es-9;17_C5-Es-C11) [13]:



[0951] To a 20 ml polypropylene scintillation vial was added [12] (Crude Material, 0.317 g) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (1.0 mL, 37.06 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified to obtain compound [13] (0.090 g, 33% Over Two Steps). It was confirmed by ^1H NMR and MS analysis.

[0952] Results:

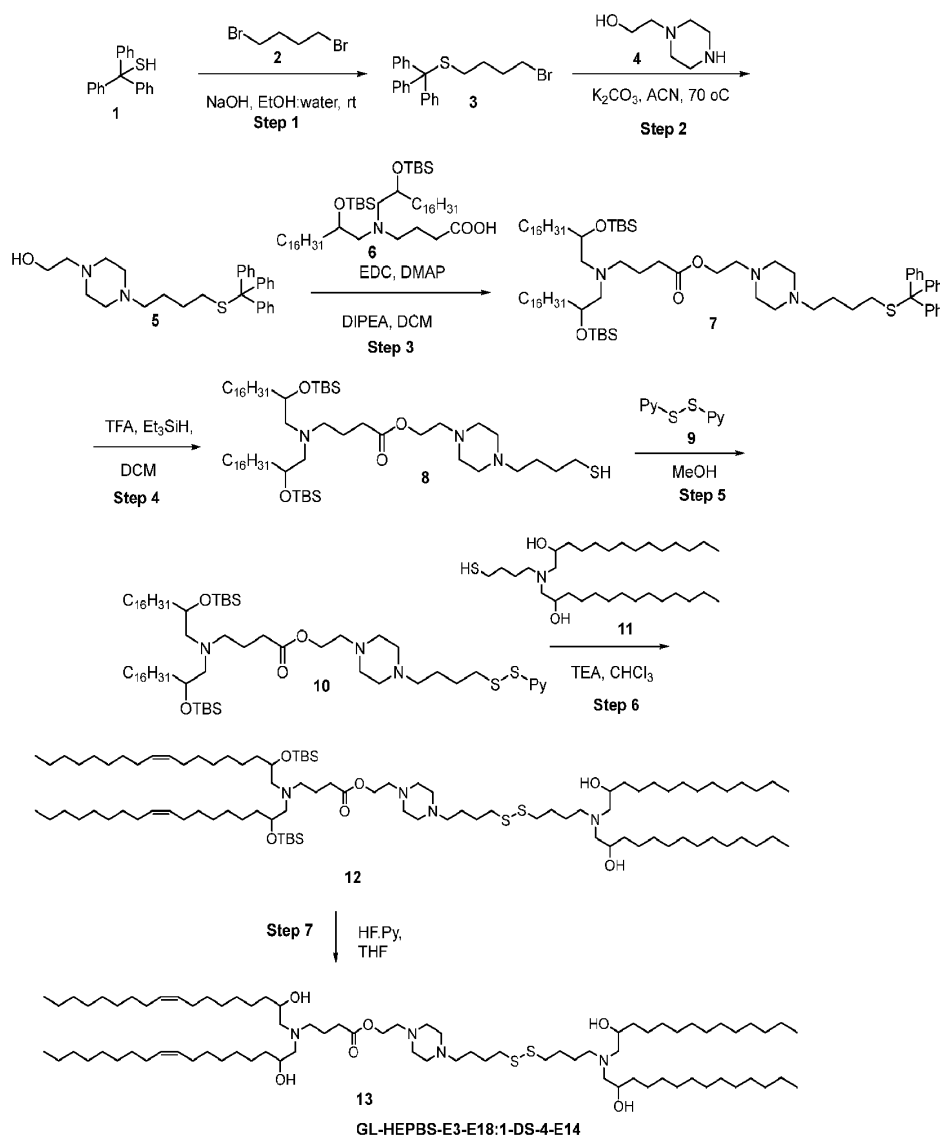
[0953] ^1H NMR (400 MHz, CDCl_3) 4.84 (quin, 1H), 4.20 (t, 2H), 4.04 (t, 2H), 3.98 – 3.65 (br, 3H), 3.20 – 2.47 (m, 22H), 2.46 – 2.19 (m, 6H), 1.99 – 1.66 (m, 10H), 1.65–1.56 (m, 5H), 1.55 – 1.13 (m, 80H), 0.87 (t, 15H).

[0954] ESI-MS analysis: Calculated for $\text{C}_{84}\text{H}_{166}\text{N}_4\text{O}_{10}\text{S}_2$, $[\text{M}+\text{H}] = 1456.39$; Observed = 1456.

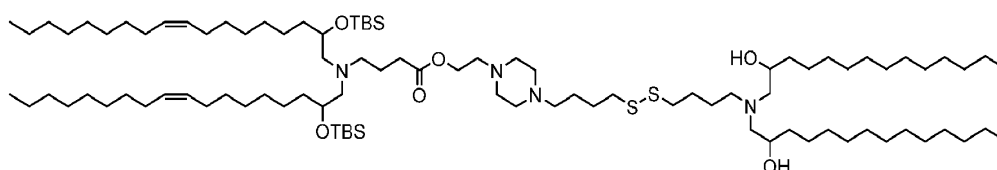
[0955] HEPBS-based cationic lipids described herein may also be prepared according to

Scheme 9l:

Scheme 9l

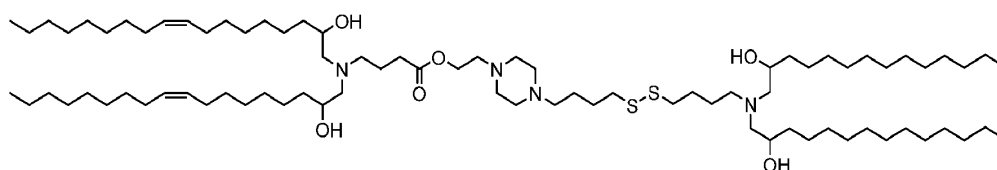


[0956] Intermediate 10 was synthesized using the same procedures as Scheme 9e.

Intermediate [12]:

[0957] To a solution of **[10]** (0.400 g, 0.341 mmol) and **[11]** (0.325 g, 0.613 mmol) in chloroform was added triethylamine (0.285 ml, 2.04 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and taken to the next step without purification (0.800 g, Crude Material).

[0958] ESI-MS analysis: Calculated for $C_{94}H_{190}N_4O_6S_2Si_2$, $[M+H] = 1592.87$; Observed = 1592.8

GL-HEPBS-E3E18:1-DS-4-E14 [13]:

[0959] To a 20 ml polypropylene scintillation vial was added **[12]** (Crude Material, 0.800 g) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (2.0 mL, 74.20 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified to obtain compound **[13]** (0.226 g, 48% Over Two Steps). It was confirmed by ^1H NMR and MS analysis.

[0960] Results:

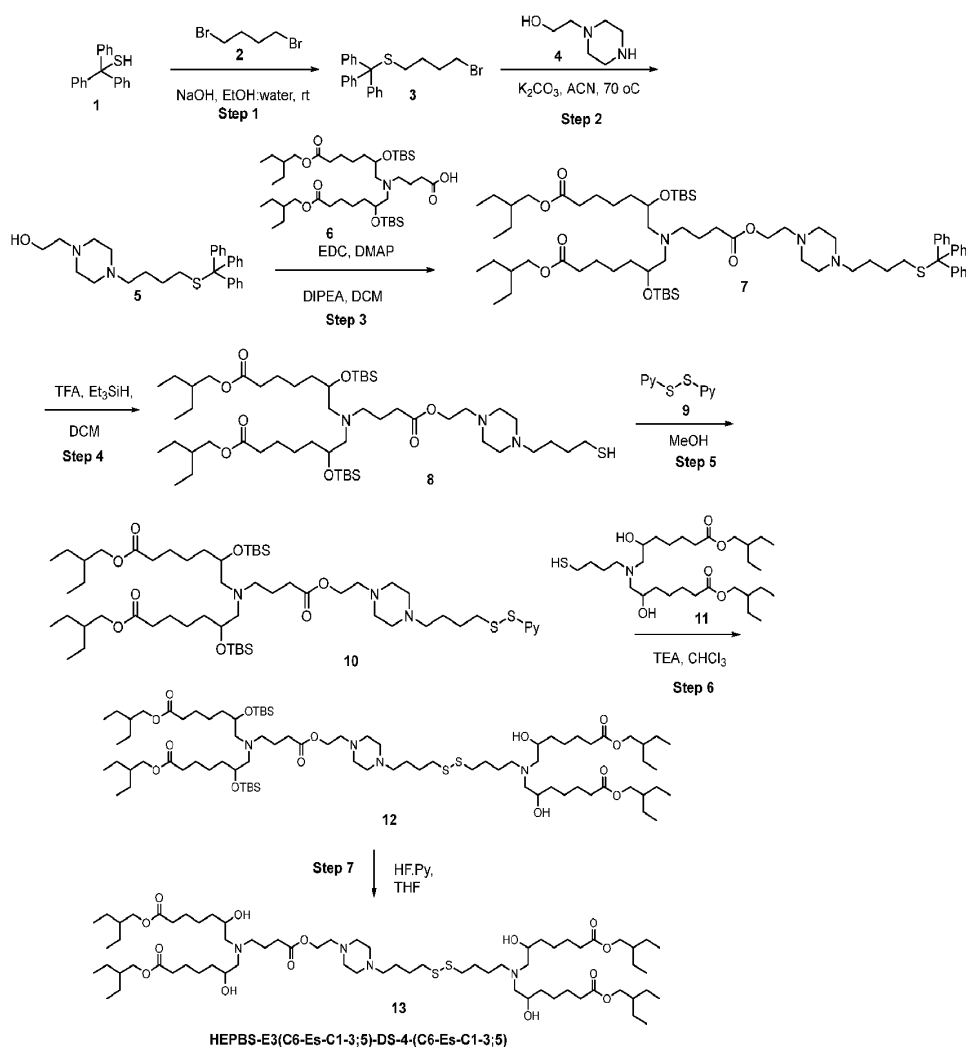
[0961] ^1H NMR (400 MHz, $CDCl_3$) 5.45 – 5.22 (m, 4H), 4.20 (t, 2H), 3.64 (br, 4H), 2.86 – 2.26 (m, 30H), 2.11 – 1.89 (m, 8H), 1.88 – 1.52 (m, 12H), 1.51 – 1.15 (m, 98H), 0.87 (t, 12H).

[0962] ESI-MS analysis: Calculated for $C_{82}H_{162}N_4O_6S_2$, $[M+H] = 1364.34$; Observed = 1364.8

[0963] HEPBS-based cationic lipids described herein may also be prepared according to

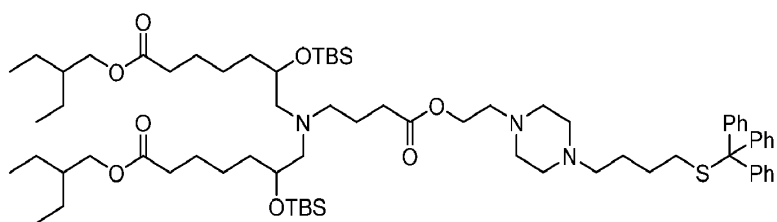
Scheme 9m:

Scheme 9m



[0964] Intermediate 5 was synthesized using the same procedures as Scheme 9l.

Intermediate [7]:

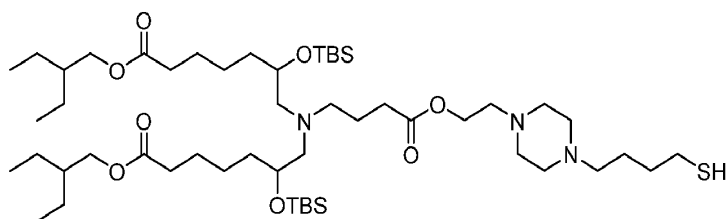


[0965] To a solution of **[5]** (0.613 g, 1.33 mmol) in DCM (7 mL) were added **[6]** (1.0 g, 1.26 mmol) in DCM (8 mL), EDC (0.364 g, 1.90 mmol), DMAP (31 mg, 0.253 mmol), DIPEA (0.442 mL, 2.54 mmol) and stirred at room temperature for 14 hours. After completion of the reaction as monitored by MS. The reaction mixture was diluted with DCM washed with NaHCO₃ solution, water and brine. The organic layer was dried over anhydrous Na₂SO₄, concentrated, and the crude compound was purified (eluent: 20% EtOAc in hexanes) to obtain pure compound **[7]** as a color less oil (0.77 g, 49%). It was confirmed by MS analysis.

[0966] Results:

[0967] ESI-MS analysis: Calculated $C_{71}H_{119}N_3O_8SSi_2$, $[M+H] = 1230.98$, Observed = 1230.8

Intermediate [8]:

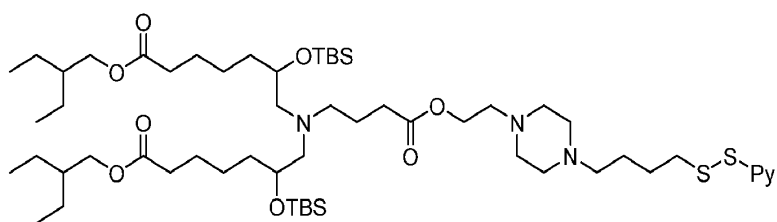


[0968] To a solution of **[7]** (0.77 g, 0.625 mmol) in DCM (3 mL) was slowly added TFA (3 mL) at room temperature and stirred at room temperature for 0.5 hour. To that triethylsilane (0.124 mL, 0.782 mmol) was added slowly and stirred for 1 hour. After completion of the reaction as monitored by MS. The reaction mixture was concentrated to obtain crude product **[8]** (quantitative). It was confirmed by MS analysis.

[0969] Results:

[0970] ESI-MS analysis: Calculated $C_{52}H_{105}N_3O_8SSi_2$, $[M+H] = 988.66$, Observed = 988.66

Intermediate [10]:

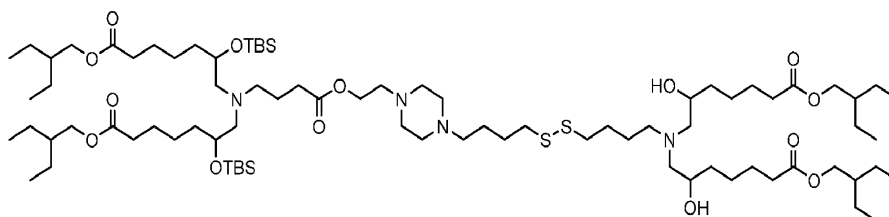


[0971] To a solution of **[8]** (quantitative) in MeOH (4 mL) was added **[9]** (0.234 g, 1.06 mmol) at room temperature and stirred for 2 hours. After completion of the reaction as monitored by MS. The reaction mixture was concentrated, and the crude compound was purified (eluent: 100% Ethyl Acetate, then 0-20 % Methanol in Ethyl Acetate) to obtain pure product **[10]** (0.691 g, Quantitative Yield). It was confirmed by MS analysis.

[0972] Results:

[0973] ESI-MS analysis: Calculated for $C_{57}H_{108}N_4O_8S_2Si_2$, $[M+H] = 1097.80$; Observed = 1097.8

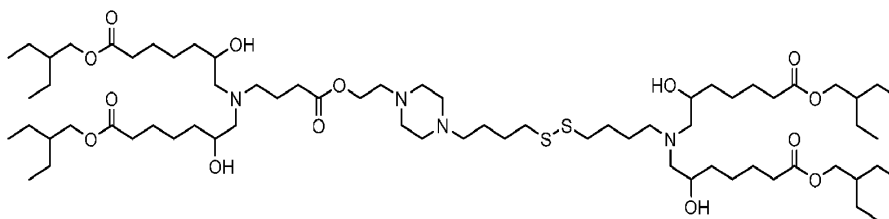
Intermediate **[12]**:



[0974] To a solution of **[10]** (0.350 g, 0.319 mmol) and **[11]** (0.322 g, 0.574 mmol) in chloroform was added triethylamine (0.266 mL, 1.91 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and taken to the next step without purification (0.800 g Crude Material).

[0975] ESI-MS analysis: Calculated for $C_{82}H_{162}N_4O_{14}S_2Si_2$, $[M+H] = 1548.50$; Observed = 1548.8

GL-HEPBS-E3(C6-Es-C1-3;5)-DS-4-(C6-Es-C1-3;5) **[13]**:



[0976] To a 20 ml polypropylene scintillation vial was added **[12]** (Crude Material, 0.800 g) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (2.0 mL, 76.33 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with NaHCO₃ solution, water and brine. The organic layer was dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified to obtain compound **[13]** (0.196 g, 46% Over Two Steps). It was confirmed by ¹H NMR and MS analysis.

[0977] Results:

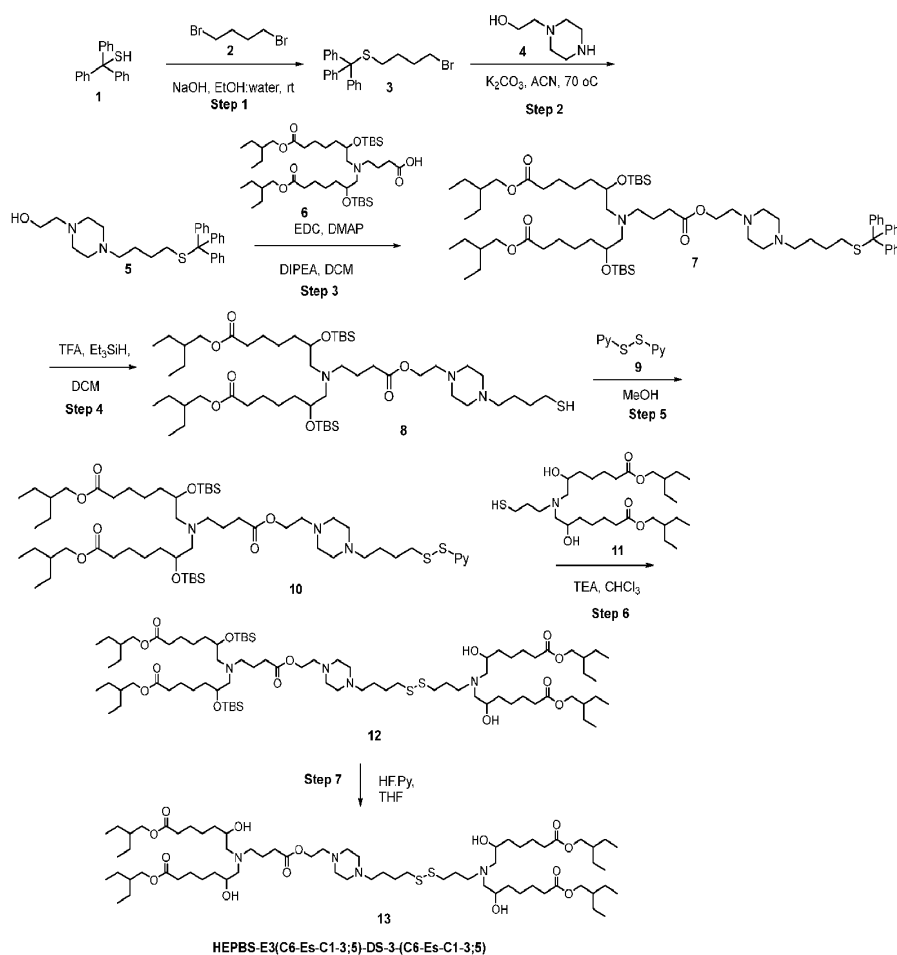
[0978] ¹H NMR (400 MHz, CDCl₃) 4.19 (t, 2H), 3.97 (d, 8H), 3.64 (br, 4H), 2.76 – 2.22 (m, 36H), 1.86 – 1.74 (m, 2H), 1.73 – 1.56 (m, 15H), 1.55 – 1.44 (m, 9H), 1.43 – 1.26 (m, 28H), 0.87 (t, 24H).

[0979] ESI-MS analysis: Calculated for C₇₀H₁₃₄N₄O₁₄S₂, [M+H] = 1319.98; Observed = 1319.8

[0980] HEPBS-based cationic lipids described herein may also be prepared according to

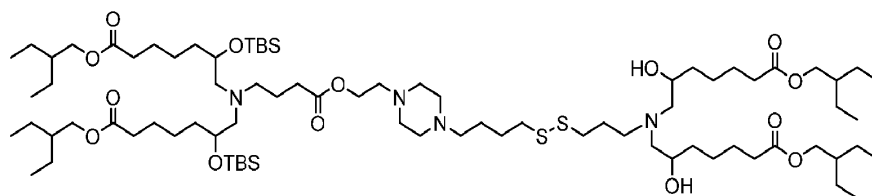
Scheme 9n:

Scheme 9n



[0981] Intermediate 10 was synthesized using the same procedures as Scheme 9m.

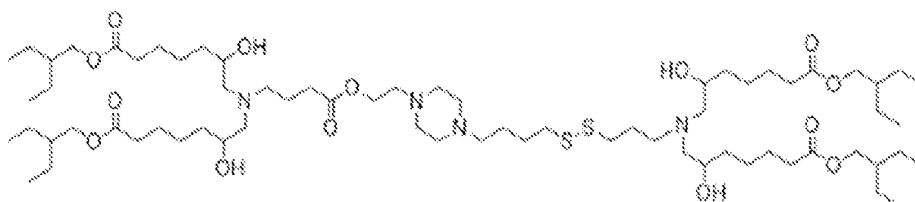
Intermediate [12]:



[0982] To a solution of [10] (0.320 g, 0.291 mmol) and [11] (0.287 g, 0.525 mmol) in chloroform was added triethylamine (0.243 ml, 1.75 mmol) and allowed to react at room temperature for 2.5 hours. After completion of the reaction, the reaction mixture was concentrated and taken to the next step without purification (0.800 g Crude Material).

[0983] ESI-MS analysis: Calculated for $C_{81}H_{160}N_4O_{14}S_2Si_2$, $[M+H] = 1534.48$; Observed = 1534.8

GL-HEPBS-E3(C6-Es-C1-3;5)-DS-3-(C6-Es-C1-3;5) [13]:



[0984] To a 20 ml polypropylene scintillation vial was added [12] (Crude Material, 0.800 g) along with 4 mL of dry tetrahydrofuran. The vial was cooled to 0-5 °C and HF/pyridine (2.0 mL, 77.03 mmol) was added dropwise. After addition, the reaction vial was allowed to warm to room temperature and stirred for 18 hours. Afterwards, the reaction mixture was cooled back to 0 °C and neutralized with solid sodium bicarbonate solid, diluted with ethyl acetate, washed with $NaHCO_3$ solution, water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified to obtain compound [13] (0.211 g, 55% Over Two Steps). It was confirmed by ^1H NMR and MS analysis.

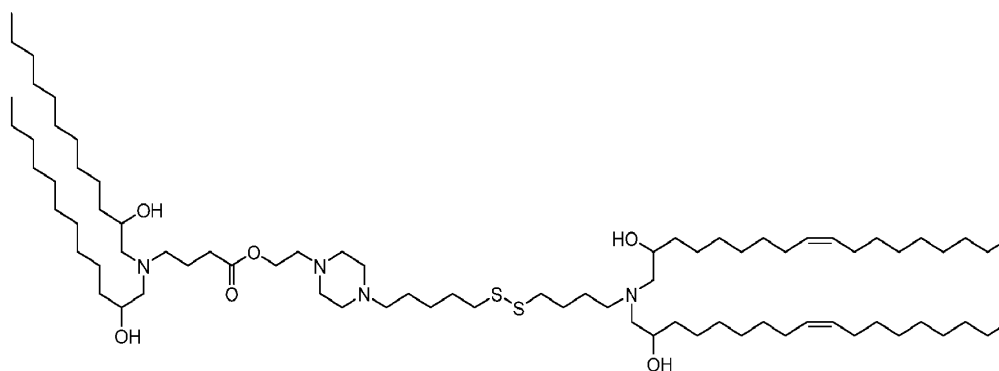
[0985] Results:

[0986] ^1H NMR (400 MHz, $CDCl_3$) 4.19 (t, 2H), 3.97 (d, 8H), 3.64 (br, 4H), 2.85 – 2.23 (m, 36H), 1.89 – 1.74 (m, 4H), 1.73 – 1.55 (m, 12H), 1.55 – 1.44 (m, 8H), 1.43 – 1.28 (m, 30H), 0.87 (t, 24H).

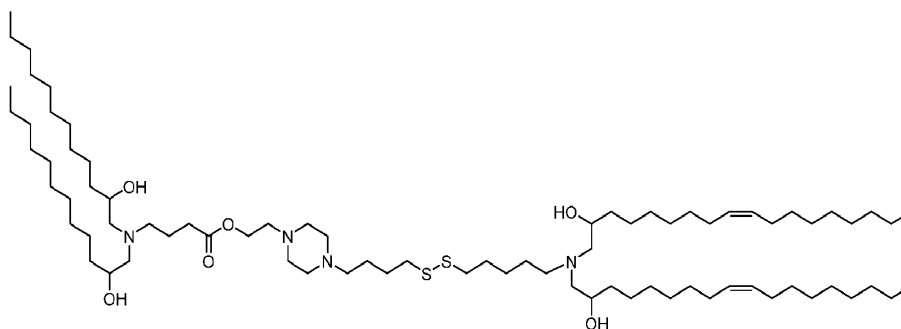
[0987] ESI-MS analysis: Calculated for $C_{69}H_{132}N_4O_{14}S_2$, $[M+H] = 1305.95$; Observed = 1305.8

[0988] Further representative examples:

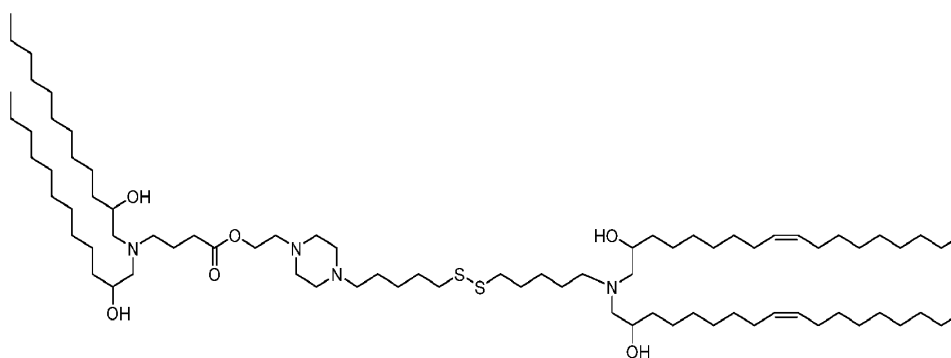
2-(4-(5-((4-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)butyl)disulfaneyl)pentyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate:



[0989] 2-(4-{4-((5-{Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentyl)disulfaneyl)butyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate:



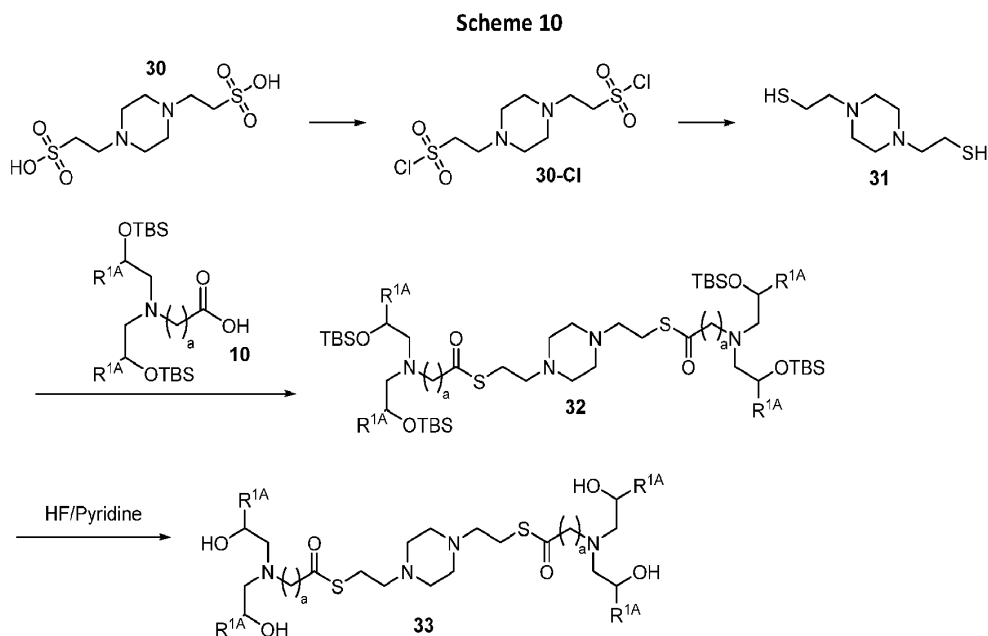
[0990] 2-(4-(5-((5-(Bis((Z)-2-hydroxyoctadec-9-en-1-yl)amino)pentyl)disulfaneyl)pentyl)piperazin-1-yl)ethyl 4-(bis(2-hydroxydodecyl)amino)butanoate:



Example 10: Generic synthesis scheme for PIPES-based cationic lipids

[0991] PIPES-based cationic lipids described herein may be prepared according to **Scheme 10**

10:

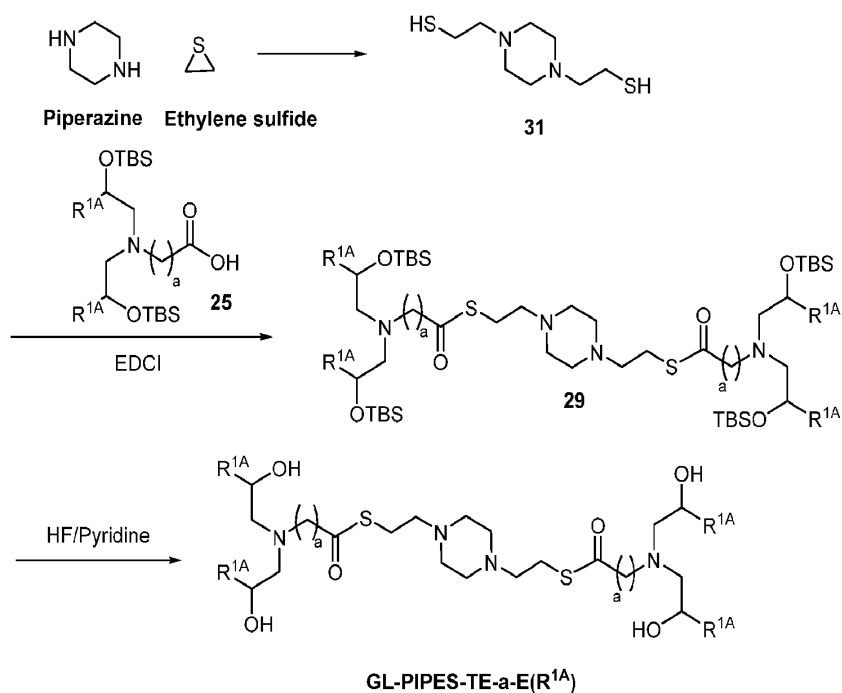


[0992] For example, compound **30** can be chlorinated and then reduced to provide the dithiol **31**. Reaction of compound **31** with a hydroxyl protected electrophile such as **10** provides compound **32**, the subsequent deprotection of which (using, for example, HF and Pyridine) can provide the lipid thioester **33**.

Example 11: Synthesis schemes for further PIPES-based thioester cationic lipids

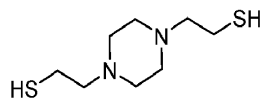
[0993] PIPES-based cationic lipids described herein may be prepared according to

Scheme 11:

Scheme 11 - GL-PIPES-TE-a-E(R^{1A}) Lipids

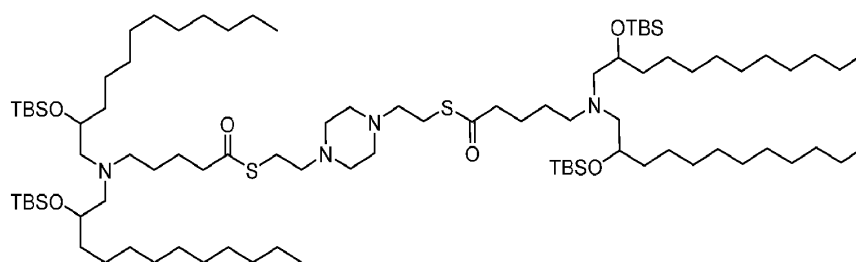
[0994] Representative Procedure for GL-PIPES-TE-a-E(R^{1A}) Lipids

[0995] Synthesis of 2,2'-(Piperazine-1,4-diyl)bis(ethane-1-thiol) (31)



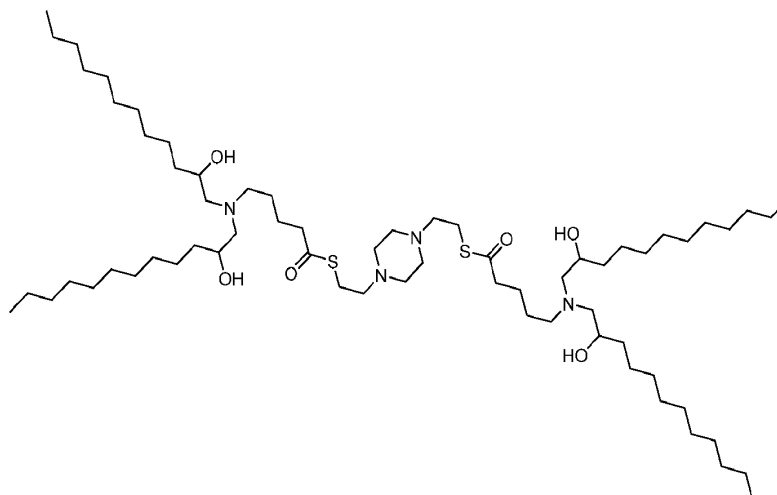
[0996] As set out in Scheme 9: To a solution of piperazine (1.0 g, 11.6 mmol) in 10 mL anhydrous toluene at room temperature was added ethylene sulfide (1.4 g, 23.2 mmol) in a pressure tube, and the mixture was heated at 50 °C for 48 h. The reaction mixture was cooled to room temperature, and solvent was evaporated under reduced pressure to get 2,2'-(piperazine-1,4-diyl)bis(ethane-1-thiol) as yellow oil which was used for the next step without further purification.

[0997] Synthesis of S,S'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl) bis(5-(bis(2-((tert-butyl)dimethylsilyl)oxy)dodecyl)amino)pentanethioate) (29, when a=4)



[0998] As set out in Scheme 9: To a solution of 5-(bis(2-((tert-butyldimethylsilyl)oxy)dodecyl)amino)pentanoic acid 25 (0.7 g, 0.85 mmol) in 10 mL anhydrous dichloromethane at 0 °C was added EDCI (0.72 g, 5.8 mmol) and DMAP (25 mg, 0.1 mmol). The reaction mixture was stirred for 15 min, and then 2,2'-(piperazine-1,4-diyl)bis(ethane-1-thiol) 31 (82 mg, 0.4 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 16 h. MS and TLC analysis indicated completion of the reaction. After solvent was removed under reduced pressure, the crude was purified by flash column chromatography (40 g SiO₂: 0 to 80% ethyl acetate in hexanes gradient) to obtain S,S'-(piperazine-1,4-diylbis(ethane-2,1-diyl)) bis(5-(bis(2-((tert-butyldimethylsilyl)oxy)dodecyl)amino)pentanethioate) as white solid (360 mg, 56%).

[0999] Synthesis of S,S'-(Piperazine-1,4-diylbis(ethane-2,1-diyl)) bis(5-(bis(2-hydroxydodecyl)amino)pentanethioate) (GL-PIPES-TE-4-E12)



[01000] As set out in Scheme 9: To a solution of S,S'-(piperazine-1,4-diylbis(ethane-2,1-diyl)) bis(5-(bis(2-((tert-butyldimethylsilyl)oxy)dodecyl)amino)pentanethioate) 29 (350 mg, 0.22 mmol) in 10.0 mL anhydrous tetrahydrofuran at 0 °C was added hydrogen fluoride pyridine (70% HF, 3.0 mL). The reaction mixture was warmed to room temperature and stirred for 16

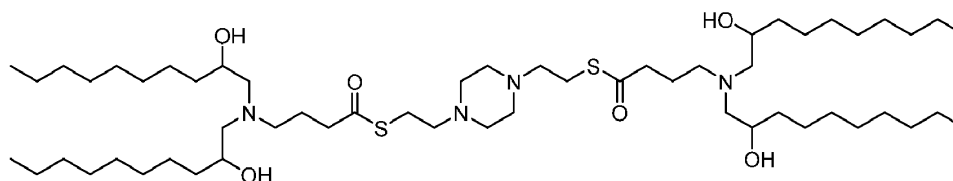
h. MS and TLC analysis indicated complete reaction. The reaction was quenched by slow addition of saturated sodium bicarbonate, and then the resulting mixture was extracted with dichloromethane (2 x 100 mL). Combined organic layer was washed with brine (100 mL) and dried over anhydrous sodium sulfate. After concentration, the crude was purified by flash column chromatography (40 g SiO₂: 20 to 80% ethyl acetate in hexane gradient) to obtain *S,S'*-(piperazine-1,4-diylbis(ethane-2,1-diyl)) bis(5-(bis(2-hydroxydodecyl)amino)pentanethioate) as colorless oil (134 mg, 54%).

[01001] ¹H NMR (300 MHz, Chloroform-*d*) δ 3.54-3.67 (m, 4H), 2.99 (t, 4H), 2.83 (bs, 4H), 2.46-2.62 (m, 20H), 2.34-2.42 (m, 6H), 1.58-1.74 (m, 4H), 1.16-1.52 (m, 78H), 0.86 (t, 12H).

[01002] APCI-MS analysis: Calculated C₆₆H₁₃₂N₄O₆S₂, [M+H] = 1141.9, observed = 1141.8.

[01003] All the other PIPES-based thioester lipids were prepared according the representative procedure in similar yields.

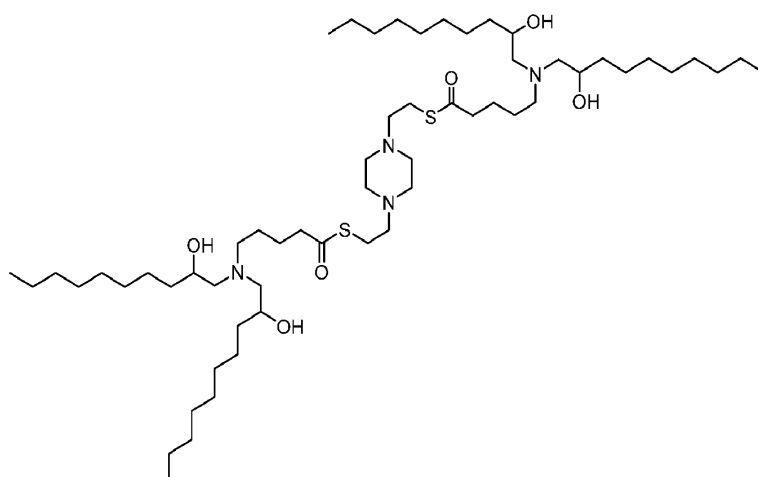
[01004] Analytical data for *S,S'*-(Piperazine-1,4-diylbis(ethane-2,1-diyl)) bis(4-(bis(2-hydroxydecyl)amino)butanethioate) (GL-PIPES-TE-3-E10)



[01005] ¹H NMR (300 MHz, Chloroform-*d*) δ 3.54-3.67 (m, 4H), 3.00 (t, 4H), 2.77-2.84 (m, 2H), 2.32-2.69 (m, 34H), 1.74-1.87 (m, 2H), 1.14-1.50 (m, 50H), 0.87 (t, 12H).

APCI-MS analysis: Calculated C₅₆H₁₁₂N₄O₆S₂, [M+H] = 1001.8, observed = 1001.7.

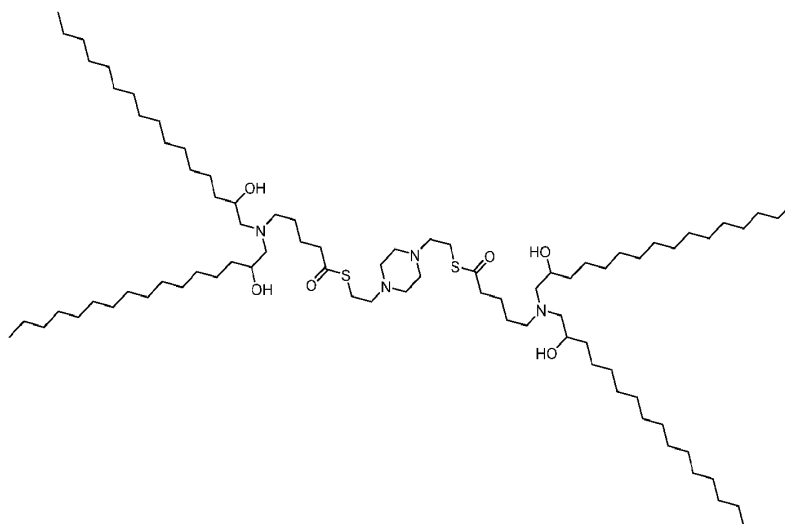
[01006] Analytical data for *S,S'*-(Piperazine-1,4-diylbis(ethane-2,1-diyl)) bis(5-(bis(2-hydroxydecyl)amino)pentanethioate) (GL-PIPES-TE-4-E10)



[01007] ^1H NMR (300 MHz, Chloroform- d) δ 3.54-3.67 (m, 4H), 2.98 (t, 4H), 2.70-2.88 (bs, 4H), 2.32-2.62 (m, 28H), 1.56-1.73 (m, 3H), 1.18-1.52 (m, 59H), 0.85 (t, 12H).

[01008] APCI-MS analysis: Calculated $\text{C}_{58}\text{H}_{116}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1029.8$, observed = 1029.7.

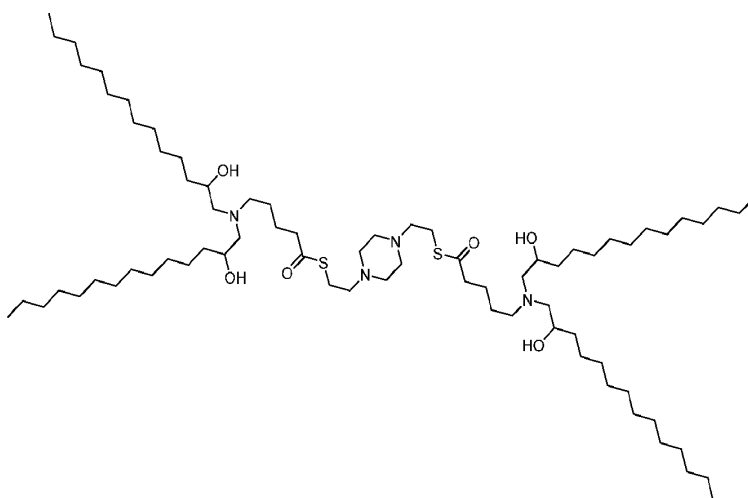
[01009] Analytical data for *S,S'*-(Piperazine-1,4-diylbis(ethane-2,1-diyl)) bis(5-(bis(2-hydroxyhexadecyl)amino)pentanethioate) (GL-PIPES-TE-4-E16)



[01010] ^1H NMR (300 MHz, Chloroform- d) δ 3.54-3.67 (m, 4H), 3.00 (t, 4H), 2.32-2.82 (m, 30H), 1.57-1.73 (m, 4H), 1.16-1.54 (m, 110H), 0.86 (t, 12H).

[01011] APCI-MS analysis: Calculated $\text{C}_{82}\text{H}_{164}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1366.2$, observed = 1367.0.

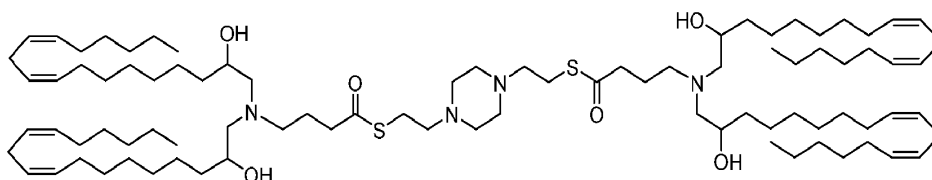
[01012] Analytical data for *S,S'*-(Piperazine-1,4-diylbis(ethane-2,1-diyl)) bis(5-(bis(2-hydroxytetradecyl)amino)pentanethioate) (GL-PIPES-TE-4-E14)



[01013] ¹H NMR (300 MHz, Chloroform-*d*) δ 3.54-3.67 (m, 4H), 2.99 (t, 4H), 2.81 (bs, 4H), 2.44-2.60 (m, 20H), 2.32-2.41 (m, 6H), 1.57-1.73 (m, 4H), 1.18-1.54 (m, 92H), 0.86 (t, 12H).

[01014] APCI-MS analysis: Calculated C₇₄H₁₄₈N₄O₆S₂, [M+H] = 1254.0, observed = 1254.0.

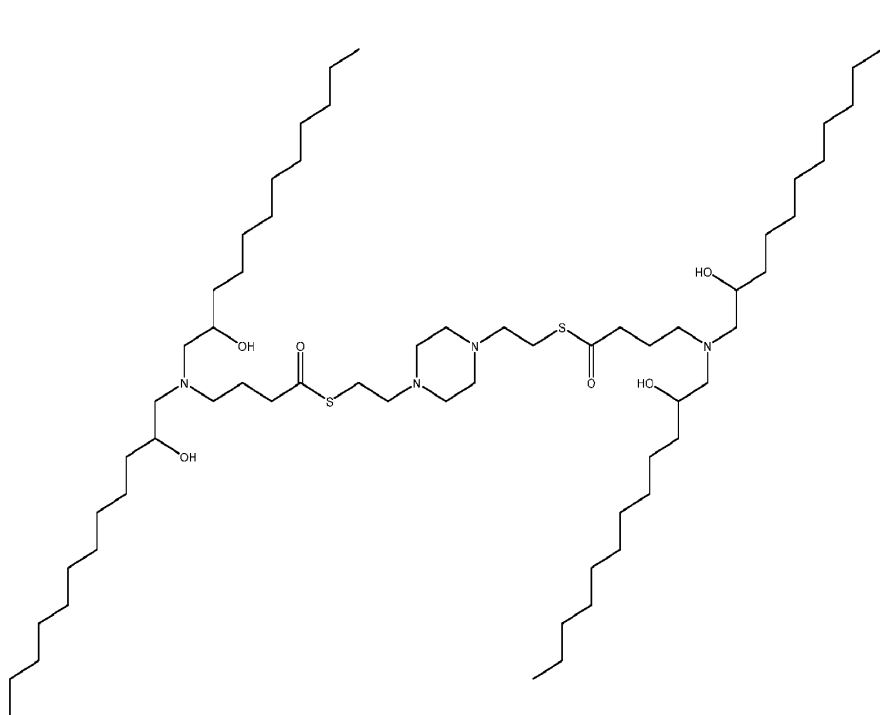
[01015] Analytical data for *S,S'*-(Piperazine-1,4-diylbis(ethane-2,1-diyl)) bis(4-(bis((9Z,12Z)-2-hydroxyoctadeca-9,12-dien-1-yl)amino)butanethioate) (GL-PIPES-TE-3-E18-2)



[01016] ¹H NMR (300 MHz, Chloroform-*d*) δ 5.26-5.44 (m, 16H), 3.54-3.67 (m, 4H), 3.15 (bs, 4H), 3.00 (t, 4H), 2.75 (t, 8H), 2.34-2.64 (m, 26H), 2.00-2.06 (m, 16H), 1.74-1.88 (m, 4H), 1.18-1.50 (m, 66H), 0.87 (t, 12H).

[01017] APCI-MS analysis: Calculated C₈₈H₁₆₀N₄O₆S₂, [M+H] = 1433.1, observed = 1434.0.

[01018] Analytical data for *S,S'*-(Piperazine-1,4-diylbis(ethane-2,1-diyl)) bis(4-(bis(2-hydroxydodecyl)amino)butanethioate) (GL-PIPES-TE-3-E12)

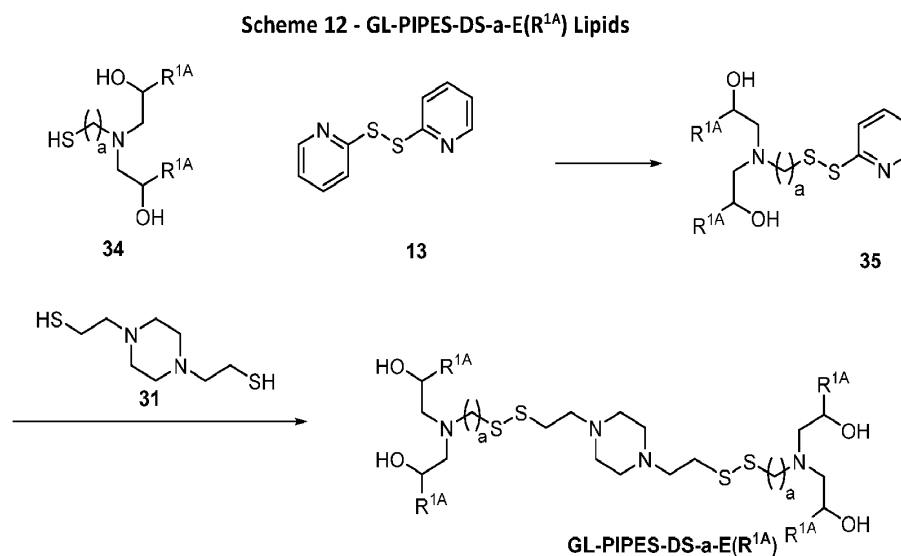


[01019] ^1H NMR (300 MHz, Chloroform- d) δ 3.54-3.66 (m, 4H), 2.99 (t, 4H), 2.45-2.62 (m, 18H), 2.30-2.41 (m, 6H), 1.68-1.86 (m, 4H), 1.16-1.50 (m, 76H), 0.86 (t, 12H).

[01020] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{128}\text{N}_4\text{O}_6\text{S}_2$, $[\text{M}+\text{H}] = 1113.9$, observed = 1114.0.

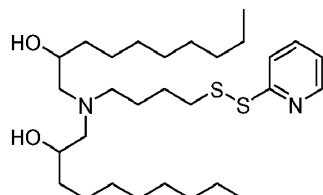
Example 12: Synthesis scheme for PIPES-based disulfide cationic lipids

[01021] PIPES-based cationic lipids described herein may be prepared according to

Scheme 12:

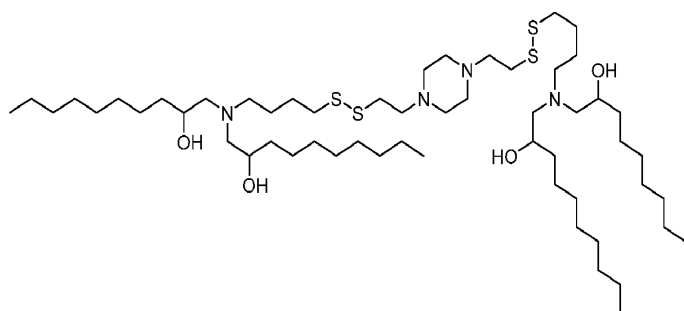
[01022] Representative Procedure for GL-PIPES-DS-a-E(R^{1A}) Lipids

[01023] Synthesis of 1,1'-((4-(Pyridin-2-yl)disulfaneyl)butyl)azanediyl)bis(decane-2-ol) (35, when a=4)



[01024] As set out in Scheme 12: To a solution of 1,1'-((4-mercaptobutyl)azanediyl)bis(decane-2-ol) 34 (0.80 g, 1.92 mmol) in 10 mL chloroform at room temperature was added pyridyl disulfide 13 (1.27 g, 5.75 mmol). The reaction mixture was stirred for 24 h. MS and TLC analysis indicated completion of the reaction. After solvent was removed under reduced pressure, the crude was purified via flash column chromatography (80 g SiO₂: 20 to 100% ethyl acetate in hexanes gradient) to obtain 1,1'-((4-(pyridin-2-yl)disulfaneyl)butyl)azanediyl)bis(decane-2-ol) as yellow liquid (653 mg, 65%).

[01025] Synthesis of 1,1',1'',1'''-((((Piperazine-1,4-diylbis(ethane-2,1-diyl))bis(disulfaneyl))bis(butane-4,1-diyl))bis(azanetriyl))tetrakis(decane-2-ol) (GL-PIPES-DS-4-E10)



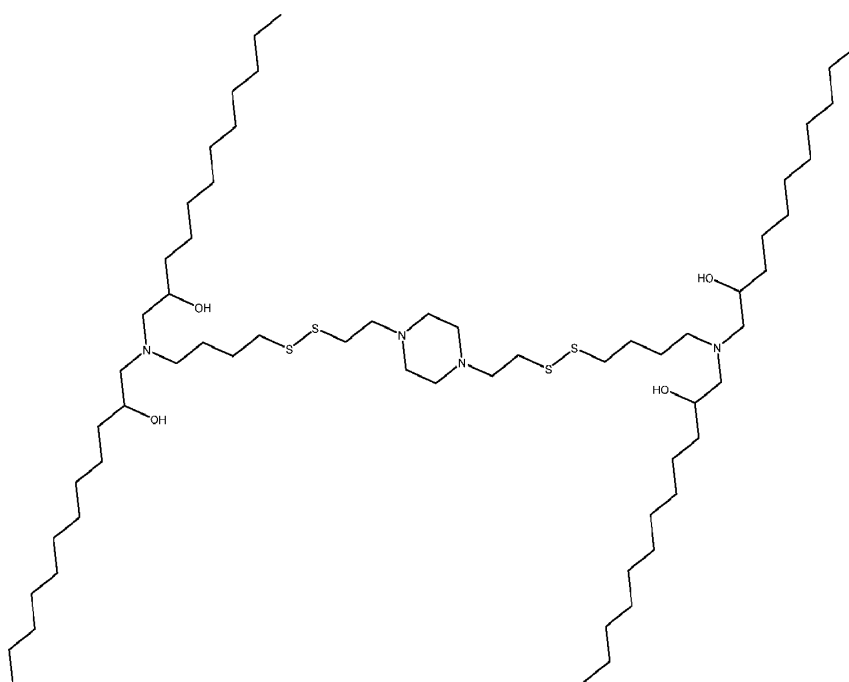
[01026] As set out in Scheme 12: To a solution of 1,1'-((4-(pyridin-2-yl)disulfaneyl)butyl)azanediyl)bis(decane-2-ol) 35 (500 mg, 0.95 mmol) in chloroform (5.0 mL) at room temperature was added 2,2'-(piperazine-1,4-diyl)bis(ethane-1-thiol) 31 (82 mg, 0.4 mmol). The reaction mixture was stirred for 3 h. MS and TLC analysis indicated completion of the reaction. After concentration, the crude was purified via flash column chromatography (40 g SiO₂: 0 to 10% methanol in dichloromethane gradient) to give 1,1',1'',1'''-(((piperazine-1,4-diylbis(ethane-2,1-diyl))bis(disulfaneyl))bis(butane-4,1-diyl))bis(azanetriyl))tetrakis(decane-2-ol) as colorless oil (56 mg, 15%).

[01027] ¹H NMR (300 MHz, Chloroform-d) δ 3.64-3.76 (m, 4H), 3.45 (bs, 4H), 2.47-2.81 (m, 29H), 1.54-1.77 (m, 7H), 1.14-1.48 (m, 56H), 0.87 (t, 12H).

[01028] APCI-MS analysis: Calculated C₅₆H₁₁₆N₄O₄S₄, [M+H] = 1037.8, observed = 1037.7.

[01029] All the other PIPES-based disulfide lipids were prepared according the representative procedure in similar yields.

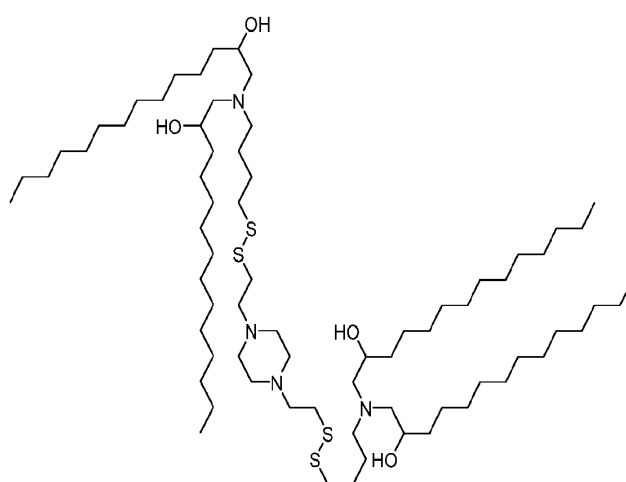
[01030] Analytical data for 1,1',1'',1'''-(((Piperazine-1,4-diylbis(ethane-2,1-diyl))bis(disulfaneyl))bis(butane-4,1-diyl))bis(azanetriyl))tetrakis(dodecane-2-ol) (GL-PIPES-DS-4-E12)



[01031] ^1H NMR (300 MHz, Chloroform- d) δ 3.55-3.64 (m, 4H), 2.77-2.82 (m, 4H), 2.65-2.71 (m, 10H), 2.45-2.60 (m, 14H), 2.35-2.42 (m, 8H), 1.62-1.74 (m, 4H), 1.51-1.60 (m, 2H), 1.17-1.48 (m, 70H), 0.87 (t, 12H).

[01032] APCI-MS analysis: Calculated $\text{C}_{64}\text{H}_{132}\text{N}_4\text{O}_4\text{S}_4$, $[\text{M}+\text{H}] = 1149.9$, observed = 1149.1.

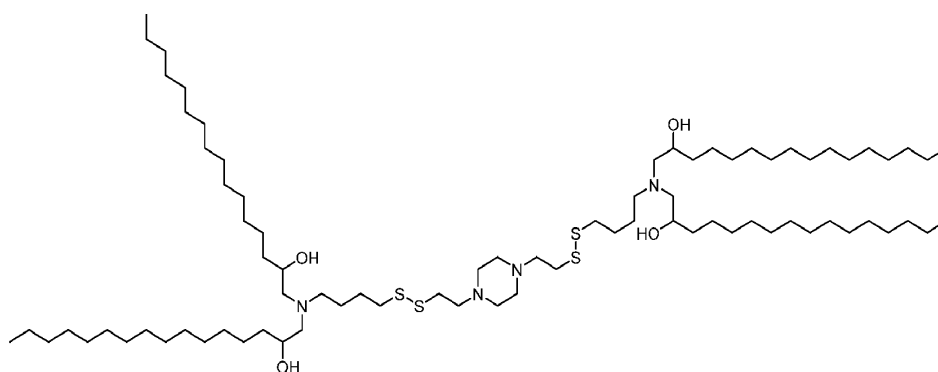
[01033] Analytical data for 1,1',1'',1'''-((((Piperazine-1,4-diylbis(ethane-2,1-diyl))bis(disulfanediy))bis(butane-4,1-diyl))bis(azanetriyl))tetrakis(tetradecan-2-ol) (GL-PIPES-DS-4-E14)



[01034] ^1H NMR (300 MHz, Chloroform- d) δ 3.58-3.68 (m, 4H), 3.28 (bs, 4H), 2.77-2.82 (m, 4H), 2.38-2.72 (m, 26H), 1.52-1.77 (m, 8H), 1.18-1.48 (m, 90H), 0.86 (t, 12H).

[01035] APCI-MS analysis: Calculated $\text{C}_{72}\text{H}_{148}\text{N}_4\text{O}_4\text{S}_4$, $[\text{M}+\text{H}] = 1262.0$, observed = 1261.8.

[01036] Analytical data for 1,1',1'',1'''-((((Piperazine-1,4-diylbis(ethane-2,1-diyl))bis(disulfanediy))bis(butane-4,1-diyl))bis(azanetriyl))tetrakis(hexadecan-2-ol) (GL-PIPES-DS-4-E16)

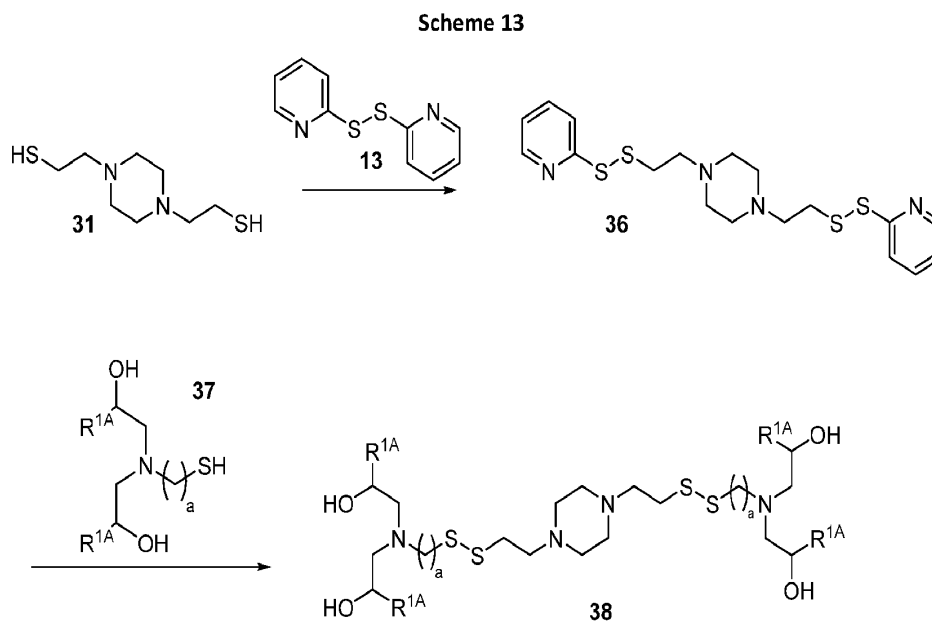


[01037] ^1H NMR (300 MHz, Chloroform- d) δ 3.58-3.68 (m, 4H), 3.21 (bs, 4H), 2.38-2.82 (m, 32H), 1.52-1.77 (m, 8H), 1.18-1.48 (m, 104H), 0.86 (t, 12H).

[01038] APCI-MS analysis: Calculated $\text{C}_{80}\text{H}_{164}\text{N}_4\text{O}_4\text{S}_4$, $[\text{M}+\text{H}] = 1374.1$, observed = 1375.0.

Example 13: Alternative synthesis scheme for PIPES-based cationic lipids

[01039] Alternatively, PIPES-based cationic lipids described herein may be prepared according to **Scheme 13**:



[01040] For example, reaction of dithiol **31** with compound **13** can provide the disulphide containing compound **36**. Subsequent reaction of **36** with nucleophile **37** can provide the lipid disulphide **38**.

Example 14: Lipid Nanoparticle Formulation

[01041] Cationic lipids described herein can be used in the preparation of lipid nanoparticles according to methods known in the art. For example, suitable methods include methods described in International Publication No. WO 2018/089801, which is hereby incorporated by reference in its entirety.

[01042] One exemplary process for lipid nanoparticle formulation is *Process A* of WO 2018/089801 (see, e.g., **Example 1** and Figure 1 of WO 2018/089801). *Process A* ("A") relates to a conventional method of encapsulating mRNA by mixing mRNA with a mixture of lipids, without first pre-forming the lipids into lipid nanoparticles. In an exemplary process, an ethanol lipid solution and an aqueous buffered solution of mRNA were prepared separately. A solution of mixture of lipids (cationic lipid, helper lipids, zwitterionic lipids, PEG lipids etc.) was prepared by dissolving lipids in ethanol. The mRNA solution was prepared by dissolving the mRNA in citrate buffer. The mixtures were then both heated to 65 °C prior to mixing. Then, these two solutions were mixed using a pump system. In some instances, the two

solutions were mixed using a gear pump system. In certain instances the two solutions were mixing using a 'T' junction (or "Y" junction). The mixture was then purified by diafiltration with a TFF process. The resultant formulation concentrated and stored at 2-8 °C until further use.

[01043] A second exemplary process for lipid nanoparticle formulation is *Process B* of WO 2018/089801 (see, e.g., Example 2 and Figure 2 of WO 2018/089801). Process B ("B") refers to a process of encapsulating messenger RNA (mRNA) by mixing pre-formed lipid nanoparticles with mRNA. A range of different conditions, such as varying temperatures (i.e., heating or not heating the mixture), buffers, and concentrations, may be employed in Process B. In an exemplary process, lipids dissolved in ethanol and citrate buffer were mixed using a pump system. The instantaneous mixing of the two streams resulted in the formation of empty lipid nanoparticles, which was a self-assembly process. The resultant formulation mixture was empty lipid nanoparticles in citrate buffer containing alcohol. The formulation was then subjected to a TFF purification process wherein buffer exchange occurred. The resulting suspension of pre-formed empty lipid nanoparticles was then mixed with mRNA using a pump system. For certain cationic lipids, heating the solution post-mixing resulted in a higher percentage of lipid nanoparticles containing mRNA and a higher total yield of mRNA.

[01044] The Polydispersity Index (PDI) of lipid nanoparticles can be determined by diluting the formulation in 10% trehalose at about 0.1 mg/ml mRNA concentration and then measuring the size on Malvern zetasizer.

[01045] The lipid nanoparticle size can be obtained with Malvern Zetasizer Nano-ZS. The encapsulation efficiency of mRNA in lipid nanoparticles can be determined using Invitrogen RiboGreen assay kit. The unencapsulated mRNA was detected directly. The total mRNA was measured after lysis of lipid nanoparticles in the presence 0.45% w/v of Triton X-100. The encapsulation efficiency was calculated as (Total mRNA – unencapsulated mRNA) / Total mRNA x 100%.

Example 15: Delivery of human erythropoietin (hEPO) mRNA by intramuscular (IM) administration

[01046] Lipid nanoparticle (LNP) formulations encapsulating hEPO mRNA were prepared by Process A as described above for IM administration. The LNP compositions administered comprised 1.5% PEG, 40% Cationic lipid, 28.5% Cholesterol, and 30% DOPE an N/P ratio of 4. After LNP formulation, the nanoparticles were initially buffer exchanged with 20% EtOH, and then with a final buffer exchange in 10% Trehalose. The LNPs were characterized for size, PDI, encapsulation, and mRNA concentration. For the hEPO animal dosing studies, the LNPs

were diluted to 3.33ug/mL in 10% trehalose. Mice were dosed intramuscularly with 0.1ug in 30uL volume into the right gastrocnemius muscle. Blood samples were collected 6 hours and 24 hours post injection to measure the amount of hEPO protein produced in the serum. The EPO protein amounts were detected using an ELISA assay from commercially available kits.

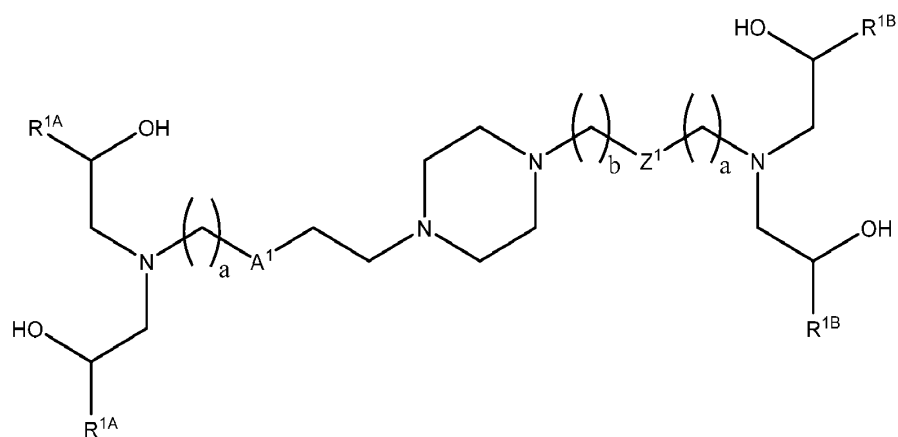
Figure 1 shows that lipid nanoparticles comprising lipids described herein are highly effective in delivering hEPO mRNA and show high levels of hEPO protein expression at 6 hours post-IM injection dose.

[01047] From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

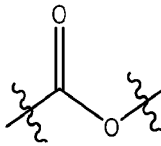
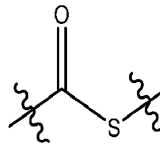
[01048] All references, patents or applications, U.S. or foreign, cited in the application are hereby incorporated by reference as if written herein in their entireties. Where any inconsistencies arise, material literally disclosed herein controls.

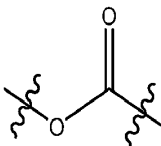
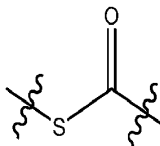
CLAIMS

1. A compound having a structure according to Formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

A^1 is selected from , , and -S-S-, wherein the left hand side of each depicted structure is bound to the $-(CH_2)_a-$;

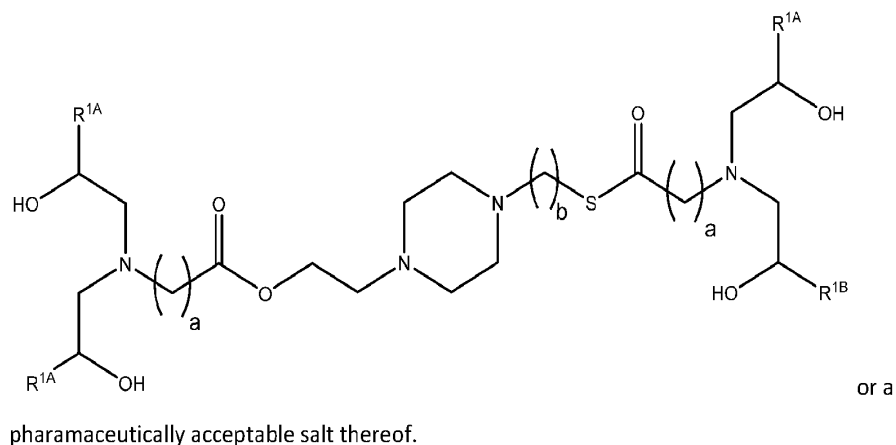
Z^1 is selected from , , and -S-S-, wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$;

R^{1A} and R^{1B} are each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted acyl and $-W^1-X^1-Y^1$; each W^1 is independently selected from optionally substituted alkyl and optionally substituted alkenyl, each X^1 is independently selected from $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to W^1 , each Y^1 is independently selected from hydrogen, $-*O-(C=O)$ -optionally substituted alkyl, $-(C=O)-O$ -optionally substituted alkyl, $-*O-(C=O)$ -optionally substituted alkenyl, and $-(C=O)-O$ -optionally substituted alkenyl, wherein the atom marked with a * is connected to X^1 ;

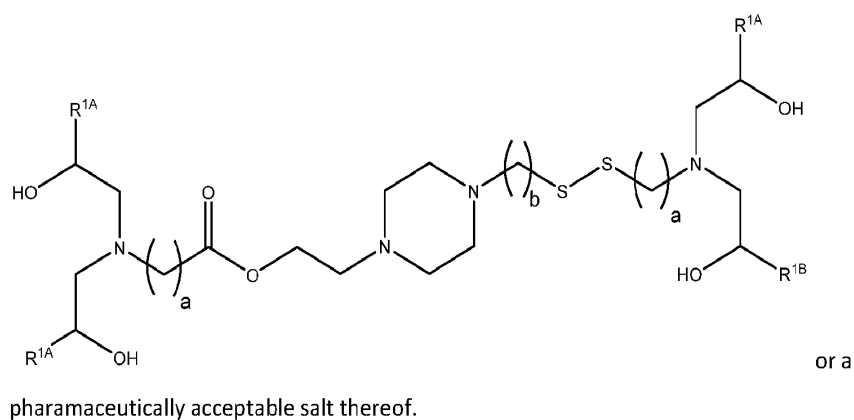
b is 1, 2, 3, 4 or 5; and

each a is independently selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

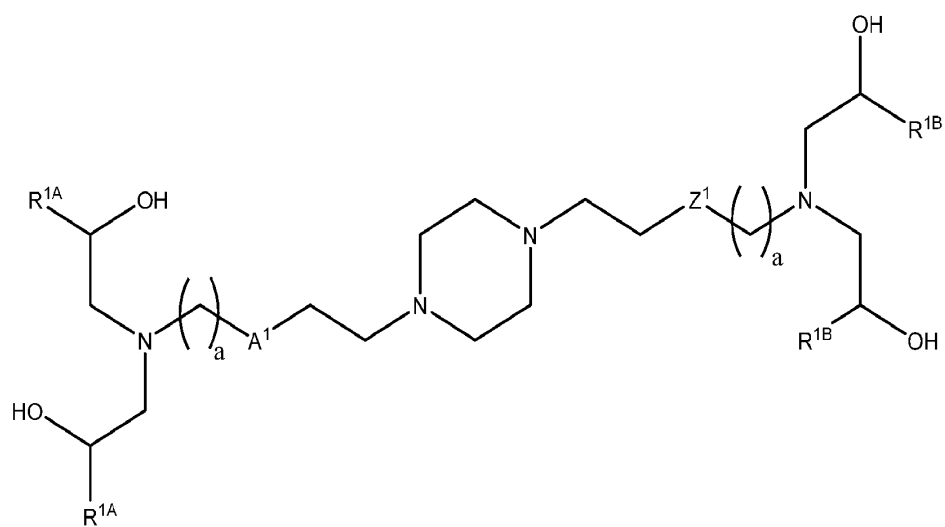
2. The compound of claim 1, wherein the compound has a structure according to Formula (li):



3. The compound of claim 1, wherein the compound has a structure according to Formula (lii):

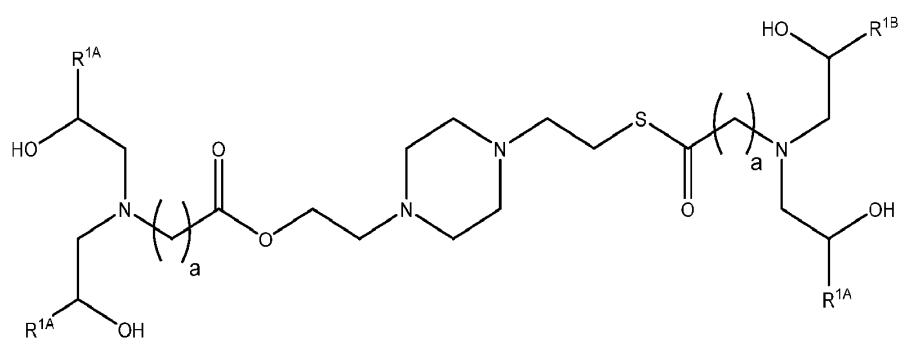


4. The compound of claim 1, wherein the compound has a structure according to Formula (la):



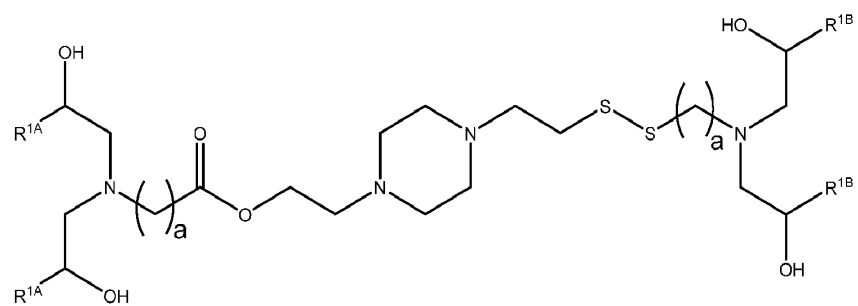
or a pharmaceutically acceptable salt thereof.

5. The compound of claim 1, 2 or 4, wherein the compound has a structure according to Formula (1b):



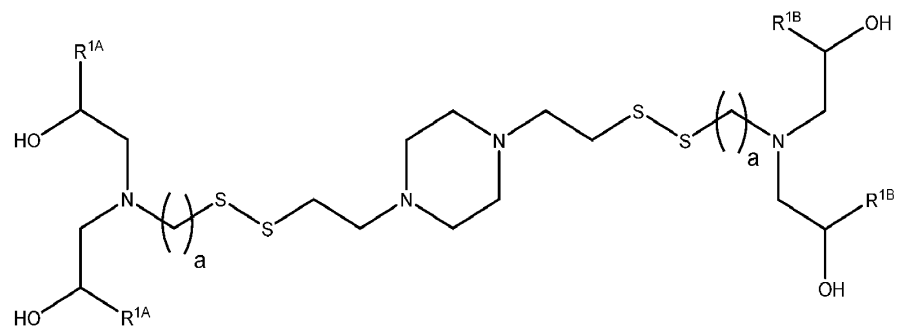
or a pharmaceutically acceptable salt thereof.

6. The compound of claim 1, 3 or 4, wherein the compound has a structure according to Formula (1c):



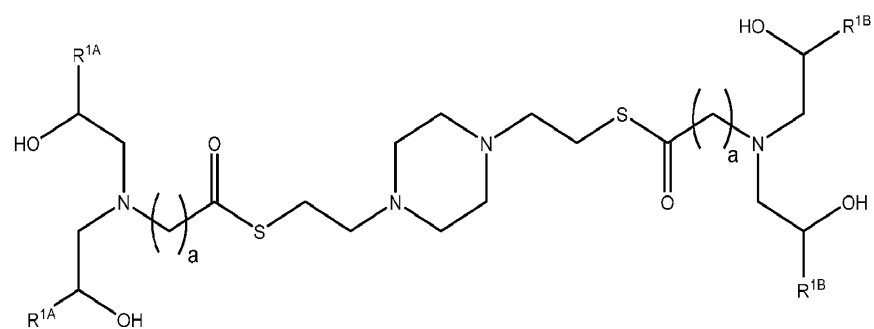
or a pharmaceutically acceptable salt thereof.

7. The compound of claim 1 or claim 4, wherein the compound has a structure according to Formula (Id):



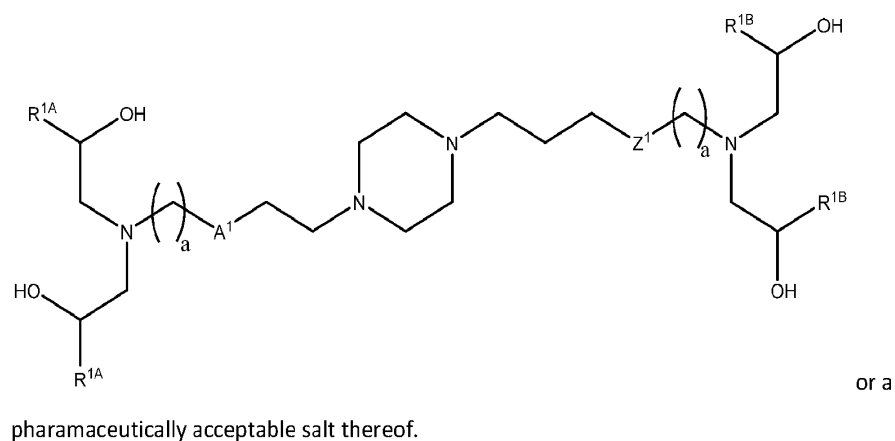
or a pharmaceutically acceptable salt thereof.

8. The compound of claim 1 or claim 4, wherein the compound has a structure according to Formula (Ie):

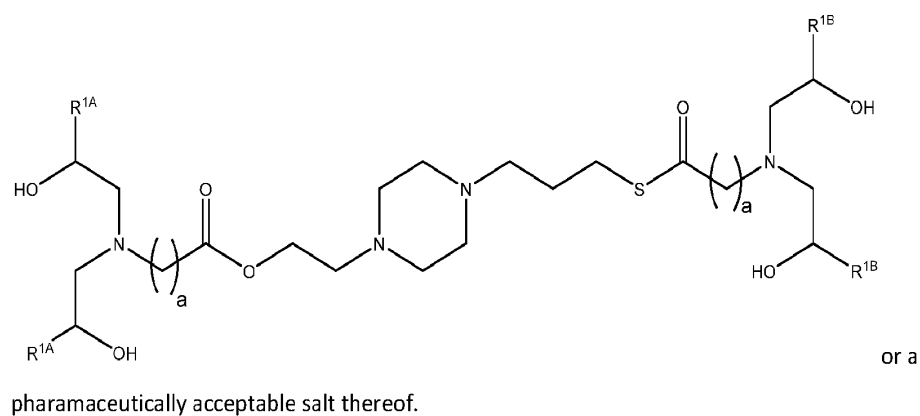


or a pharmaceutically acceptable salt thereof.

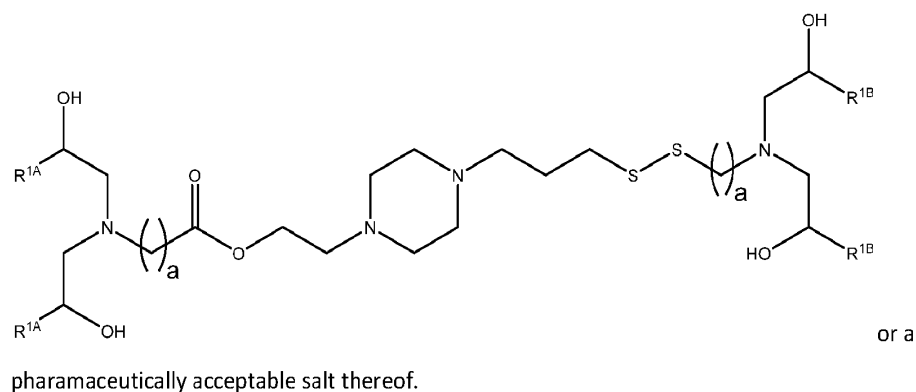
9. The compound of claim 1, wherein the compound has a structure according to Formula (IIa):



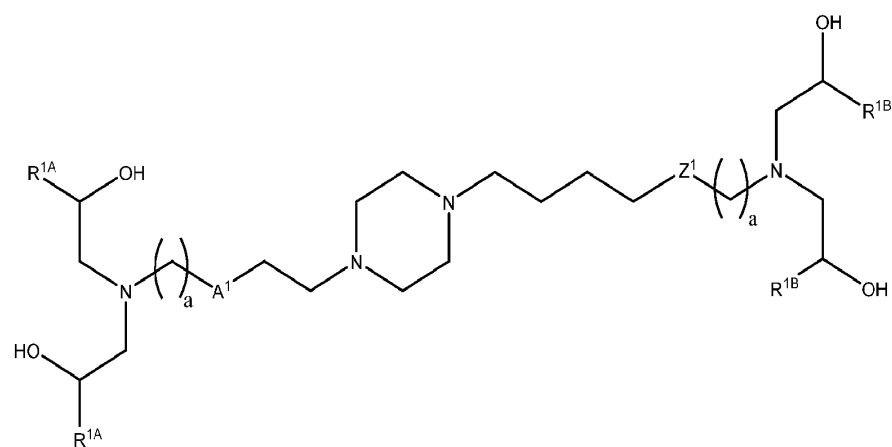
10. The compound of claim 1 or claim 2, wherein the compound has a structure according to Formula (IIb):



11. The compound of claim 1 or claim 3, wherein the compound has a structure according to Formula (IIc):

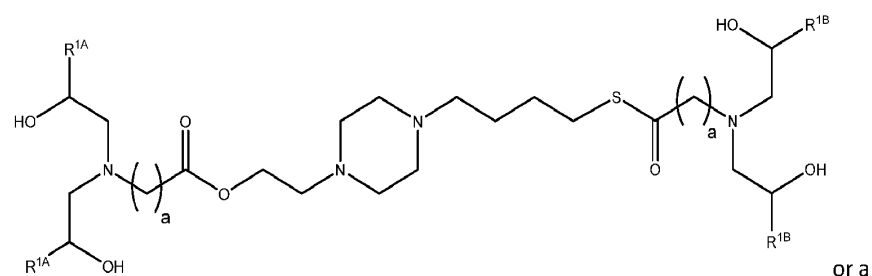


12. The compound of claim 1, wherein the compound has a structure according to Formula (IIIa):



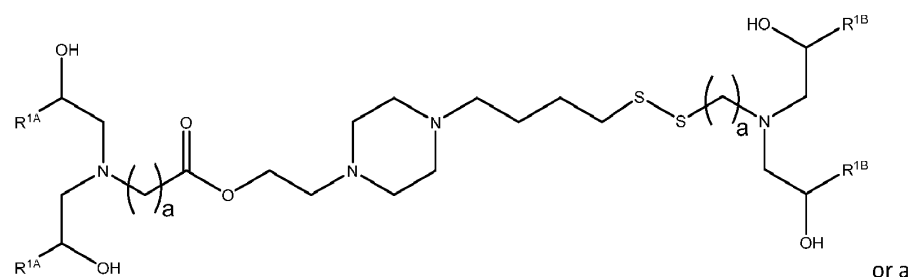
or a pharmaceutically acceptable salt thereof.

13. The compound of claim 1 or claim 2, wherein the compound has a structure according to Formula (IIIb):



pharmaceutically acceptable salt thereof.

14. The compound of claim 1 or claim 3, wherein the compound has a structure according to Formula (IIIc):

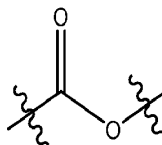


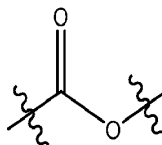
pharmaceutically acceptable salt thereof.

15. The compound of claim 1, 4, 9 or 12 or a pharmaceutically acceptable salt thereof, wherein A^1 and Z^1 are the same.

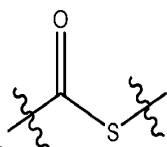
16. The compound of claim 1, 4, 9 or 12 or a pharmaceutically acceptable salt thereof, wherein A^1 and Z^1 are different.

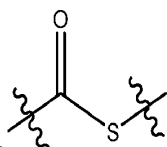
17. The compound of any one of claims 1, 4, 9, 12 and 15-16 or a pharmaceutically



acceptable salt thereof, wherein A^1 is , wherein the left hand side of each depicted structure is bound to the $-(CH_2)_a-$.

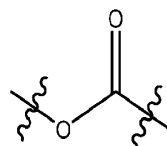
18. The compound of any one of claims 1, 4, 9, 12 and 15-16 or a pharmaceutically

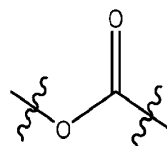


acceptable salt thereof, wherein A^1 is , wherein the left hand side of each depicted structure is bound to the $-(CH_2)_a-$.

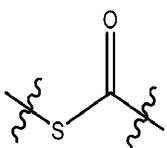
19. The compound of any one of claims 1, 4, 9, 12 and 15-16 or a pharmaceutically acceptable salt thereof, wherein A^1 is $-S-S-$, wherein the left hand side of each depicted structure is bound to the $-(CH_2)_a-$.

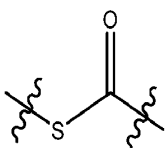
20. The compound of any one of claims 1, 4, 9, 12 and 15-19 or a pharmaceutically



acceptable salt thereof, wherein Z^1 is , wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

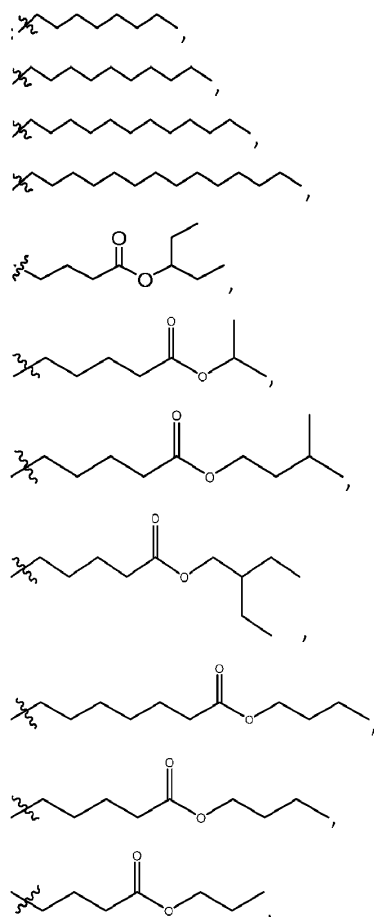
21. The compound of any one of claims 1, 4, 9, 12 and 15-19 or a pharmaceutically

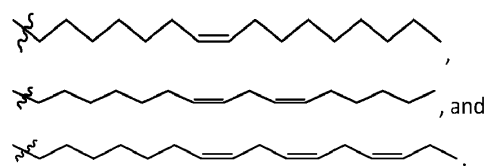


acceptable salt thereof, wherein Z^1 is , wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

22. The compound of any one of claims 1, 4, 9, 12 and 15-19 or a pharmaceutically acceptable salt thereof, wherein Z^1 is $-S-S-$, wherein the right hand side of each depicted structure is bound to the $-(CH_2)_a-$.

23. The compound of any one of the preceding claims or a pharmaceutically acceptable salt thereof, wherein each a is independently selected from 2, 3 and 4.
24. The compound of any one of the preceding claims or a pharmaceutically acceptable salt thereof, wherein each a is the same.
25. The compound of any one of claims 1-23 or a pharmaceutically acceptable salt thereof, wherein each a is different.
26. The compound of any one of the preceding claims or a pharmaceutically acceptable salt thereof, wherein R^{1A} and R^{1B} are
- (i) each independently selected from optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, and optionally substituted acyl; or
 - (ii) each independently selected from optionally substituted alkyl and optionally substituted alkenyl.
27. The compound of any one of claims 1 to 25 or a pharmaceutically acceptable salt thereof, wherein R^{1A} and R^{1B} are each independently selected from:

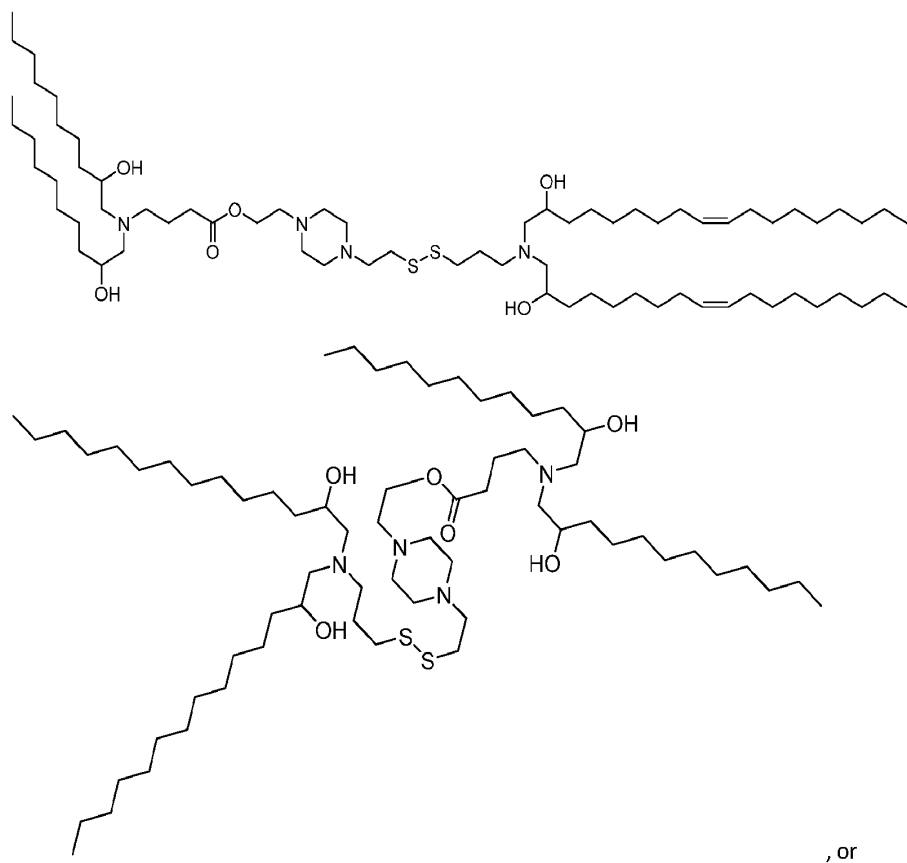


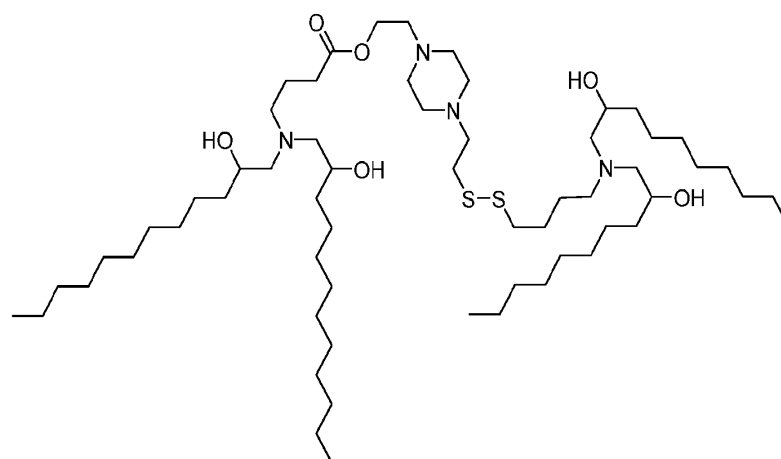


28. A compound selected from those listed in:

- (i) Tables A-F or a pharmaceutically acceptable salt thereof; or
- (ii) Tables A-F or a pharmaceutically acceptable salt thereof, and compounds of Formula (I), (II), (III), (Ia), (Ib), (Ic), (Id), (Ie), (IIa), (IIb), (IIc), (IIIa), (IIIb), (IIIc) or a pharmaceutically acceptable salt thereof of the invention described by examples 1-13.

29. A compound having the structure:





or a

pharmaceutically acceptable salt thereof.

30. A composition comprising the cationic lipid of any one of the preceding claims, one or more non-cationic lipids, one or more cholesterol-based lipids and one or more PEG-modified lipids.

31. The composition of claim 30, wherein the composition is a lipid nanoparticle, optionally a liposome.

32. The composition of claim 30 or claim 31, wherein the one or more cationic lipid(s) constitute(s) about 30 mol %-60 mol % of the lipid nanoparticle.

33. The composition of any one of claims 30-32, wherein the one or more non-cationic lipid(s) constitute(s) 10 mol %-50 mol % of the lipid nanoparticle.

34. The composition of any one of claims 30-33, wherein the one or more PEG-modified lipid(s) constitute(s) 1 mol %-10 mol % of the lipid nanoparticle.

35. The composition of any one of claims 30-34, wherein the cholesterol-based lipid constitutes 10 mol %-50 mol % of the lipid nanoparticle.

36. The composition of any one of claims 30-35, wherein the lipid nanoparticle encapsulates a nucleic acid, optionally an mRNA encoding a peptide or protein.

37. The composition of any one of claims 30-36, wherein the lipid nanoparticle encapsulates an mRNA encoding a peptide or protein, optionally for use in a vaccine.

38. The composition of claim 37, wherein the lipid nanoparticles have an encapsulation percentage for mRNA of

- (i) at least 70%;
- (ii) at least 75%;
- (iii) at least 80%;
- (iv) at least 85%;

(v) at least 90%; or

(vi) at least 95%.

39. The composition of any one of claims 36-38 for use in therapy.

40. The composition of any one of claims 36-38 for use in a method of treating or preventing a disease amenable to treatment or prevention by the peptide or protein encoded by the mRNA, optionally wherein the mRNA encodes an antigen and/or the disease is (a) a protein deficiency, optionally wherein the protein deficiency affects the liver, lung, brain or muscle, (b) an autoimmune disease, (c) an infectious disease, or (d) cancer.

41. The composition for use according to claim 39 or 40, wherein the composition is administered intravenously, intrathecally or intramuscular, or by pulmonary delivery, optionally through nebulization.

42. A method for treating or preventing a disease wherein said method comprises administering to a subject in need thereof the composition of any one of claims 36-38 and wherein the disease is amenable to treatment or prevention by the peptide or protein encoded by the mRNA, optionally wherein the mRNA encodes an antigen and/or the disease is (a) a protein deficiency, optionally wherein the protein deficiency affects the liver, lung, brain or muscle, (b) an autoimmune disease, (c) an infectious disease, or (d) cancer.

43. The method of claim 42, wherein the composition is administered intravenously, intrathecally or intramuscular, or by pulmonary delivery, optionally through nebulization.