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(54) Title: LIPID COMPOUNDS AND METHODS OF MAKING AND USE THEREOF

(57) Abstract: Disclosed herein are lipid compounds and compositions comprising lipid compounds and methods of making and use thereof.



WO 2024/124148 A1

LIPID COMPOUNDS AND METHODS OF MAKING AND USE THEREOF

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with Government Support under Grant No. GM144117 awarded
5 by the National Institutes of Health. The Government has certain rights in the invention.

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Serial No.
63/386,755 filed December 9, 2022, the disclosure of which is expressly incorporated herein by
10 reference.

TECHNICAL FIELD

This application generally relates to lipid formulations that can be used in drug delivery
and screening.
15

BACKGROUND

Efficient delivery of vaccines, gene therapeutics, mRNA, and drug delivery is a key step
and challenge for the application of mRNA therapeutics. Despite promising data from ongoing
clinical trials, there are currently problems restricting the widespread use of oligonucleotides in
20 therapeutic and diagnostic contexts. First, free RNAs are susceptible to nuclease digestion in
plasma, facilitating degradation of the therapeutic agent. Second, these oligonucleotides are often
unable to access the intracellular compartment where the relevant translation machinery resides.

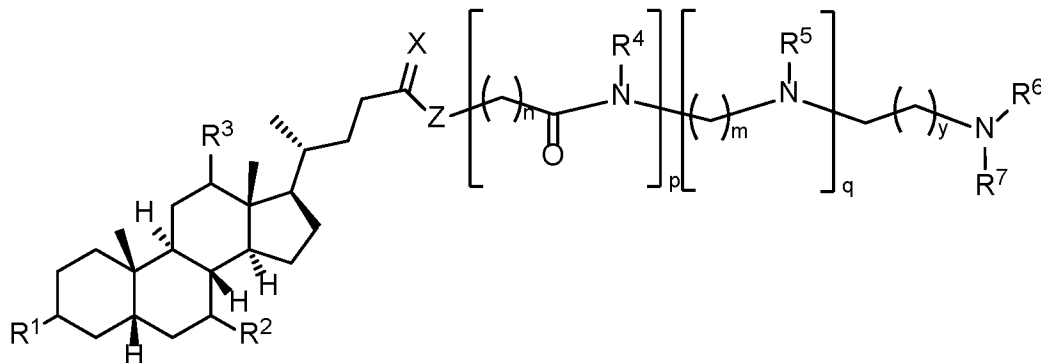
As a result, lipid nanoparticles formed from cationic lipids with other lipid components
have been used to as a possible way to traverse these barriers in delivery and increase the cellular
25 uptake of oligonucleotides. However, the efficacy of these delivery systems typically stems from
the compositional structure of the base lipid molecule, and new compositions and methods are
needed for delivering mRNA to cells for treating various disease states. Thus, there remains a need
for improved cationic lipids and lipid nanoparticles for the delivery of oligonucleotides.

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SUMMARY

In accordance with the purposes of the disclosed compounds and methods as embodied and broadly described herein, the disclosed subject matter relates to compounds and methods of making and use thereof.

Disclosed herein are compounds defined by Formula I, or a pharmaceutically acceptable salt thereof:

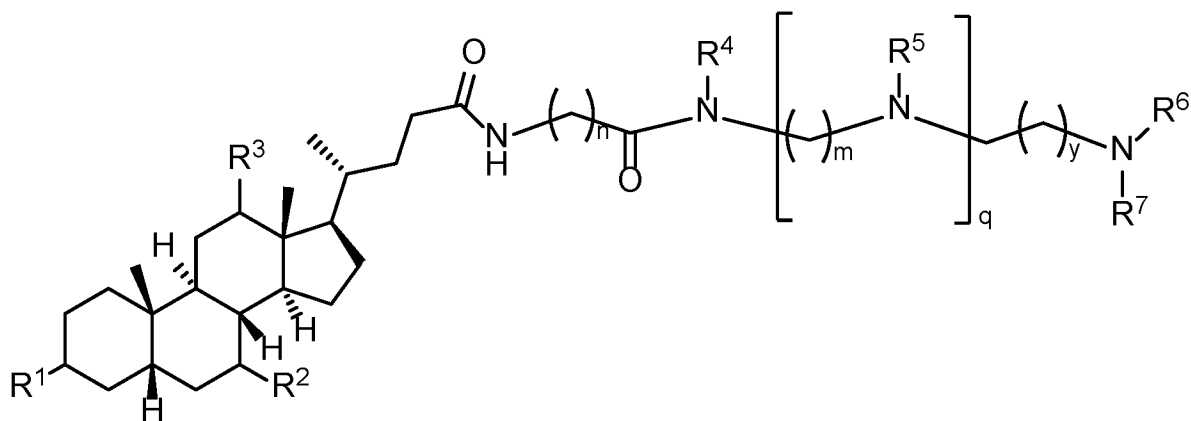


I

wherein

- X and Z are each independently O, NR^a , or S;
 R^a , when present, is hydrogen or substituted or unsubstituted $\text{C}_1\text{-C}_{10}$ alkyl;
 R^1 , R^2 , and R^3 are each independently H, OH, halogen, substituted or unsubstituted $\text{C}_1\text{-C}_3$ alkyl, or substituted or unsubstituted $\text{C}_1\text{-C}_3$ alkoxy;
 p is 0 or 1;
 q is an integer from 0 to 2;
 n is an integer from 1 to 10;
 m in each case is independently an integer from 1 to 10;
 y is an integer from 1 to 10;
 R^4 , when present, is hydrogen or a substituted or unsubstituted $\text{C}_1\text{-C}_{18}$ alkyl;
 each R^5 , when present, is independently a substituted or unsubstituted $\text{C}_8\text{-C}_{18}$ alkyl; and
 R^6 and R^7 are each independently a substituted or unsubstituted $\text{C}_8\text{-C}_{18}$ alkyl.

In some examples, the compound is defined by Formula II, or a pharmaceutically acceptable salt thereof:



II

wherein R^1 , R^2 , and R^3 are each independently H, OH, halogen, substituted or unsubstituted C_1 - C_3 alkyl, or substituted or unsubstituted C_1 - C_3 alkoxy;

q is an integer from 0 to 2;

n is an integer from 1 to 5;

m in each case is independently an integer from 1 to 10;

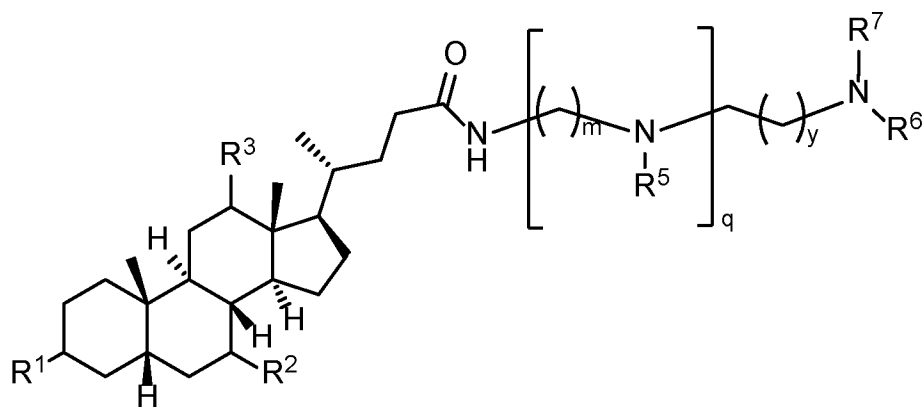
y is an integer from 1 to 10;

R^4 is hydrogen or a substituted or unsubstituted C_1 - C_{18} alkyl;

each R^5 , when present, is independently a substituted or unsubstituted C_8 - C_{18} alkyl; and

R^6 and R^7 are each independently a substituted or unsubstituted C_8 - C_{18} alkyl.

In some examples, the compound is defined by Formula III, or a pharmaceutically acceptable salt thereof:



III

wherein R^1 , R^2 , and R^3 are each independently H, OH, halogen, substituted or unsubstituted C₁-C₃ alkyl, or substituted or unsubstituted C₁-C₃ alkoxy;

q is an integer from 0 to 2;

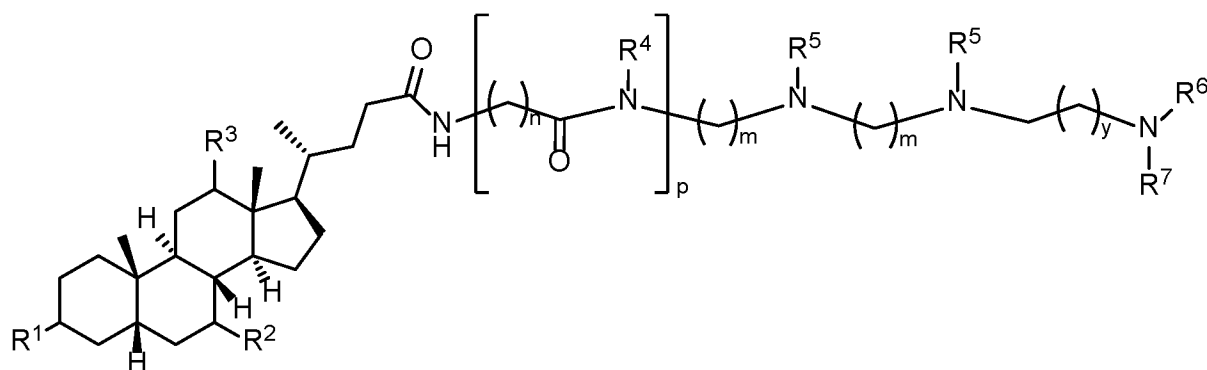
m in each case is independently an integer from 1 to 7;

5 y is an integer from 1 to 10;

each R^5 , when present, is independently a substituted or unsubstituted C₈-C₁₈ alkyl; and

R^6 and R^7 are each independently a substituted or unsubstituted C₈-C₁₈ alkyl.

In some examples, the compound is defined by Formula IV, or a pharmaceutically acceptable salt thereof:



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IV

wherein R^1 , R^2 , and R^3 are each independently H, OH, halogen, substituted or unsubstituted C₁-C₃ alkyl, or substituted or unsubstituted C₁-C₃ alkoxy;

each m is independently an integer from 1 to 7;

15 y is an integer from 1 to 10;

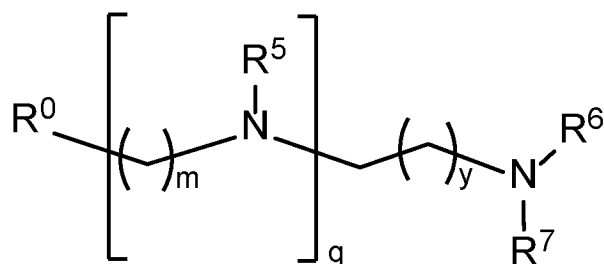
n is an integer from 1 to 10;

each R^5 is independently a substituted or unsubstituted C₈-C₁₈ alkyl; and

R^6 and R^7 are each independently a substituted or unsubstituted C₈-C₁₈ alkyl.

In some examples, the compound is defined by Formula V, or a pharmaceutically acceptable salt thereof:

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V

wherein q is an integer from 0 to 2;

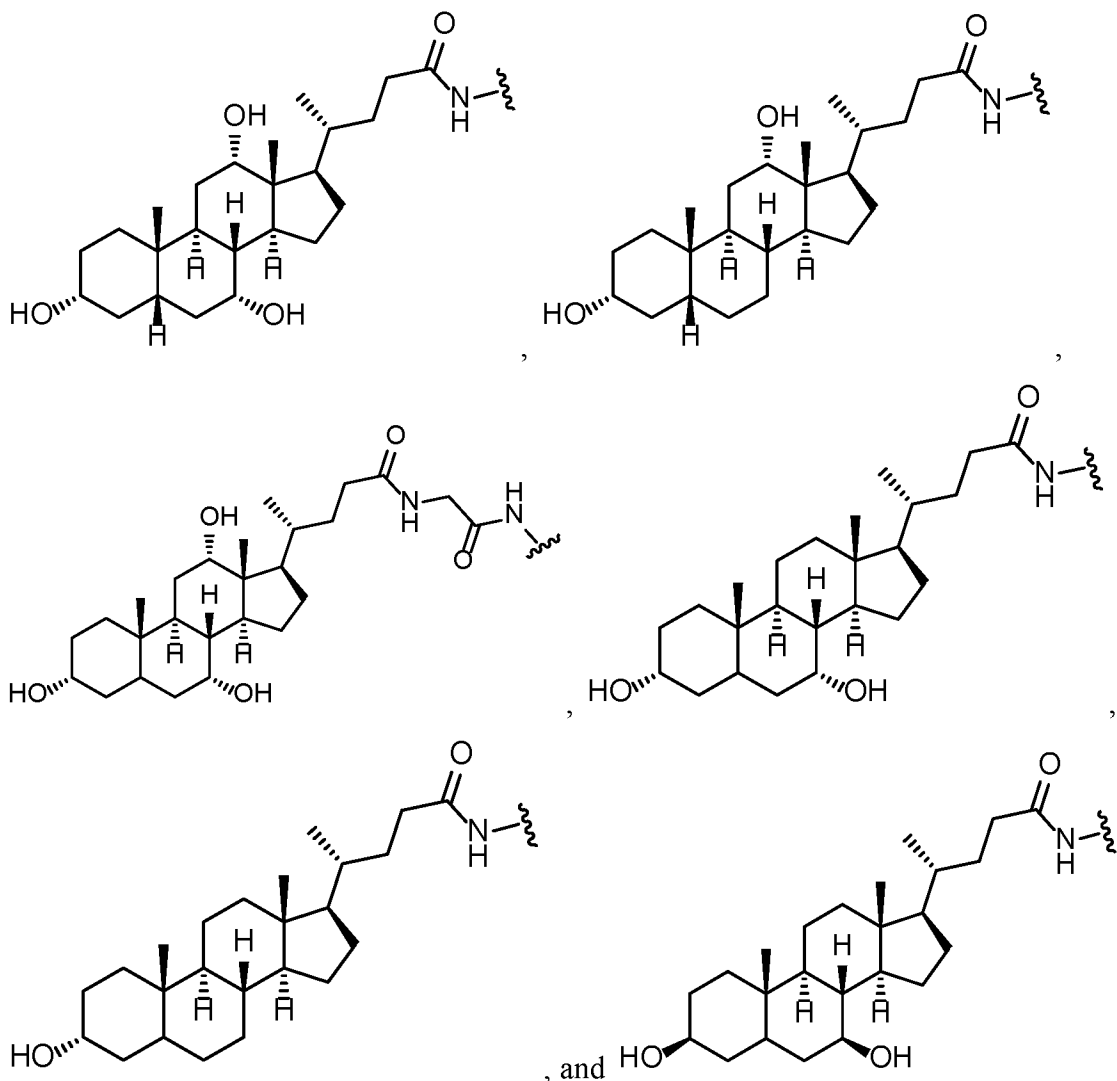
y is an integer from 1 to 7;

each m is independently an integer from 1 to 7;

each R⁵, when present, is independently a substituted or unsubstituted C₈-C₁₈ alkyl;

5 R⁶ and R⁷ are each independently substituted or unsubstituted C₈-C₁₈ alkyl;

and R⁰ is selected from the group consisting of:



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Also disclosed herein are compositions comprising any of the compounds disclosed herein. Also disclosed herein are compositions comprising any of the compounds disclosed herein further comprising an agent (e.g. mRNA).

Also disclosed herein are methods of making any of the compounds and compositions disclosed herein.

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Also disclosed herein are lipid nanoparticles comprising any of the compounds disclosed herein, a non-cationic lipid; a polyethylene glycol-lipid; and a sterol. In some examples, the non-cationic lipid comprises 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (SOPE), DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine), 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), 1,2-dioleoyl-5/7-glycero-3-phospho-(1'-rac-glycerol) (DOPG), or combinations thereof.

Also disclosed herein are pharmaceutical compositions comprising a pharmaceutically acceptable carrier and an effective amount of any of the compounds and compositions disclosed herein.

Also disclosed herein are methods for delivering an agent into a cell, comprising: introducing into the cell any of the compositions, nanoparticles or pharmaceutically acceptable compositions described herein.

Additional advantages of the disclosed compounds, compositions and methods will be set forth in part in the description which follows, and in part will be obvious from the description. The advantages of the disclosed compounds, compositions and methods will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the disclosed compounds, compositions and methods, as claimed.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several aspects of the disclosure, and together with the description, serve to explain the principles of the disclosure.

FIG. 1 shows exemplary synthetic routes for several bile acids derived lipids described in Example 1.

FIG. 2 shows luciferase mRNA delivery in the Hep3B cell line.

FIG. 3 shows luciferase mRNA delivery in the JAWS2 cell line.

FIG. 4 shows luciferase mRNA delivery in the C2C12 cell line.

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DETAILED DESCRIPTION

The compounds, compositions and methods described herein may be understood more readily by reference to the following detailed description of specific aspects of the disclosed subject matter and the Examples included therein.

Before the present compounds, compositions and methods are disclosed and described, it is to be understood that the aspects described below are not limited to specific synthetic methods or specific reagents, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

Also, throughout this specification, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which the disclosed matter pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

General Definitions

In this specification and in the claims that follow, reference will be made to a number of terms, which shall be defined to have the following meanings.

As used herein, the article “a,” “an,” and “the” means “at least one,” unless the context in which the article is used clearly indicates otherwise.

The term “nucleic acid” as used herein means a polymer composed of nucleotides, e.g. deoxyribonucleotides or ribonucleotides.

The terms “ribonucleic acid” and “RNA” as used herein mean a polymer composed of ribonucleotides.

The terms “deoxyribonucleic acid” and “DNA” as used herein mean a polymer composed of deoxyribonucleotides.

The term “oligonucleotide” denotes single- or double-stranded nucleotide multimers of from about 2 to up to about 100 nucleotides in length. Suitable oligonucleotides may be prepared by the phosphoramidite method described by Beaucage and Carruthers, *Tetrahedron Lett.*,

22:1859-1862 (1981), or by the triester method according to Matteucci, et al., *J. Am. Chem. Soc.*,
103:3185 (1981), both incorporated herein by reference, or by other chemical methods using either
a commercial automated oligonucleotide synthesizer or VLSIPS™ technology. When
oligonucleotides are referred to as “double-stranded,” it is understood by those of skill in the art
5 that a pair of oligonucleotides exist in a hydrogen-bonded, helical array typically associated with,
for example, DNA. In addition to the 100% complementary form of double-stranded
oligonucleotides, the term “double-stranded,” as used herein is also meant to refer to those forms
which include such structural features as bulges and loops, described more fully in such
biochemistry texts as Stryer, *Biochemistry*, Third Ed., (1988), incorporated herein by reference for
10 all purposes.

The term “polynucleotide” refers to a single or double stranded polymer composed of
nucleotide monomers. In some embodiments, the polynucleotide is composed of nucleotide
monomers of generally greater than 100 nucleotides in length and up to about 8,000 or more
nucleotides in length.

15 The term “polypeptide” refers to a compound made up of a single chain of D- or L-amino
acids or a mixture of D- and L-amino acids joined by peptide bonds.

The term “complementary” refers to the topological compatibility or matching together of
interacting surfaces of a probe molecule and its target. Thus, the target and its probe can be
described as complementary, and furthermore, the contact surface characteristics are
20 complementary to each other.

The term “hybridization” refers to a process of establishing a non-covalent, sequence-
specific interaction between two or more complementary strands of nucleic acids into a single
hybrid, which in the case of two strands is referred to as a duplex.

25 The term “anneal” refers to the process by which a single-stranded nucleic acid sequence
pairs by hydrogen bonds to a complementary sequence, forming a double-stranded nucleic acid
sequence, including the reformation (renaturation) of complementary strands that were separated
by heat (thermally denatured).

The term “melting” refers to the denaturation of a double-stranded nucleic acid sequence
due to high temperatures, resulting in the separation of the double strand into two single strands
30 by breaking the hydrogen bonds between the strands.

The term “target” refers to a molecule that has an affinity for a given probe. Targets may be naturally-occurring or man-made molecules. Also, they can be employed in their unaltered state or as aggregates with other species.

The term “promoter” or “regulatory element” refers to a region or sequence determinants
5 located upstream or downstream from the start of transcription and which are involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. Promoters need not be of bacterial origin, for example, promoters derived from viruses or from other organisms can be used in the compositions, systems, or methods described herein. The term “regulatory element” is intended to include promoters, enhancers, internal ribosomal entry sites
10 (IRES), and other expression control elements (e.g. transcription termination signals, such as polyadenylation signals and poly-U sequences). Such regulatory elements are described, for example, in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, Calif. (1990). Regulatory elements include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of
15 the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). A tissue-specific promoter may direct expression primarily in a desired tissue of interest, such as muscle, neuron, bone, skin, blood, specific organs (e.g. liver, pancreas), or particular cell types (e.g. lymphocytes). Regulatory elements may also direct expression in a temporal-dependent manner, such as in a cell-cycle dependent or developmental stage-dependent manner, which may
20 or may not also be tissue or cell-type specific. In some embodiments, a vector comprises one or more pol III promoter (e.g. 1, 2, 3, 4, 5, or more pol I promoters), one or more pol II promoters (e.g. 1, 2, 3, 4, 5, or more pol II promoters), one or more pol I promoters (e.g. 1, 2, 3, 4, 5, or more pol I promoters), or combinations thereof. Examples of pol III promoters include, but are not limited to, U6 and H1 promoters. Examples of pol II promoters include, but are not limited to, the
25 retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer) [see, e.g., Boshart et al, *Cell*, 41:521-530 (1985)], the SV40 promoter, the dihydrofolate reductase promoter, the β -actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF1 α promoter. Also encompassed by the term “regulatory element” are enhancer elements, such as WPRE; CMV
30 enhancers; the R-U5' segment in LTR of HTLV-I (*Mol. Cell. Biol.*, Vol. 8(1), p. 466-472, 1988); SV40 enhancer; and the intron sequence between exons 2 and 3 of rabbit β -globin (*Proc. Natl. Acad. Sci. USA.*, Vol. 78(3), p. 1527-31, 1981). It is appreciated by those skilled in the art that the

design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression desired, etc.

The term “recombinant” refers to a human manipulated nucleic acid (e.g. polynucleotide) or a copy or complement of a human manipulated nucleic acid (e.g. polynucleotide), or if in reference to a protein (i.e. a “recombinant protein”), a protein encoded by a recombinant nucleic acid (e.g. polynucleotide). In embodiments, a recombinant expression cassette comprising a promoter operably linked to a second nucleic acid (e.g. polynucleotide) may include a promoter that is heterologous to the second nucleic acid (e.g. polynucleotide) as the result of human manipulation (e.g., by methods described in Sambrook et al., *Molecular Cloning—A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., (1989) or Current Protocols in Molecular Biology Volumes 1-3, John Wiley & Sons, Inc. (1994-1998)). In another example, a recombinant expression cassette may comprise nucleic acids (e.g. polynucleotides) combined in such a way that the nucleic acids (e.g. polynucleotides) are extremely unlikely to be found in nature. For instance, human manipulated restriction sites or plasmid vector sequences may flank or separate the promoter from the second nucleic acid (e.g. polynucleotide). One of skill will recognize that nucleic acids (e.g. polynucleotides) can be manipulated in many ways and are not limited to the examples above.

The term “expression cassette” refers to a nucleic acid construct, which when introduced into a host cell, results in transcription and/or translation of a RNA or polypeptide, respectively. In embodiments, an expression cassette comprising a promoter operably linked to a second nucleic acid (e.g. polynucleotide) may include a promoter that is heterologous to the second nucleic acid (e.g. polynucleotide) as the result of human manipulation (e.g., by methods described in Sambrook et al., *Molecular Cloning—A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., (1989) or Current Protocols in Molecular Biology Volumes 1-3, John Wiley & Sons, Inc. (1994-1998)). In some embodiments, an expression cassette comprising a terminator (or termination sequence) operably linked to a second nucleic acid (e.g. polynucleotide) may include a terminator that is heterologous to the second nucleic acid (e.g. polynucleotide) as the result of human manipulation. In some embodiments, the expression cassette comprises a promoter operably linked to a second nucleic acid (e.g. polynucleotide) and a terminator operably linked to the second nucleic acid (e.g. polynucleotide) as the result of human manipulation. In some embodiments, the expression cassette comprises an endogenous promoter. In some embodiments, the expression cassette comprises an endogenous terminator. In some embodiments, the

expression cassette comprises a synthetic (or non-natural) promoter. In some embodiments, the expression cassette comprises a synthetic (or non-natural) terminator.

The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, preferably 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher identity over a specified region when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (see, e.g., NCBI web site or the like). Such sequences are then said to be “substantially identical.” This definition also refers to, or may be applied to, the complement of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 10 amino acids or 20 nucleotides in length, or more preferably over a region that is 10-50 amino acids or 20-50 nucleotides in length. As used herein, percent (%) amino acid sequence identity is defined as the percentage of amino acids in a candidate sequence that are identical to the amino acids in a reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared can be determined by known methods.

For sequence comparisons, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Preferably, default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1977) *Nuc. Acids Res.* 25:3389-3402, and Altschul et al. (1990) *J. Mol. Biol.* 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al. (1990) *J. Mol. Biol.* 215:403-410). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01.

The phrase “codon optimized” as it refers to genes or coding regions of nucleic acid molecules for the transformation of various hosts, refers to the alteration of codons in the gene or coding regions of polynucleic acid molecules to reflect the typical codon usage of a selected organism without altering the polypeptide encoded by the DNA. Such optimization includes replacing at least one, or more than one, or a significant number, of codons with one or more codons that are more frequently used in the genes of that selected organism.

Nucleic acid is “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, “operably linked” means that the DNA sequences being linked are near each other, and, in the case of a secretory leader, contiguous and in reading phase. However, operably linked nucleic acids (e.g. enhancers and coding sequences) do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. In embodiments, a promoter is operably linked with a coding sequence when it is capable of affecting (e.g. modulating relative to the absence of the promoter) the expression of a protein from that coding sequence (i.e., the coding sequence is under the transcriptional control of the promoter).

The term "nucleobase" refers to the part of a nucleotide that bears the Watson/Crick base-pairing functionality. The most common naturally-occurring nucleobases, adenine (A), guanine (G), uracil (U), cytosine (C), and thymine (T) bear the hydrogen-bonding functionality that binds one nucleic acid strand to another in a sequence specific manner.

As used throughout, by a "subject" (or a “host”) is meant an individual. Thus, the "subject" can include, for example, domesticated animals, such as cats, dogs, etc., livestock (e.g., cattle, horses, pigs, sheep, goats, etc.), laboratory animals (e.g., mouse, rabbit, rat, guinea pig, etc.) mammals, non-human mammals, primates, non-human primates, rodents, birds, reptiles, amphibians, fish, and any other animal. The subject can be a mammal such as a primate or a human.

The term “about” as used herein when referring to a measurable value such as an amount, a percentage, and the like, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, or $\pm 1\%$ from the measurable value.

A nucleic acid sequence is “heterologous” to a second nucleic acid sequence if it originates from a foreign species, or, if from the same species, is modified by human action from its original form. For example, a heterologous promoter (or heterologous 5’ untranslated region (5’UTR)) operably linked to a coding sequence refers to a coding sequence from a species different from that from which the promoter was derived, or, if from the same species, a coding sequence which is different from naturally occurring allelic variants.

As used herein, the terms “treating” or “treatment” of a subject includes the administration of a drug to a subject with the purpose of curing, healing, alleviating, relieving, altering, remedying, ameliorating, improving, stabilizing or affecting a disease or disorder, or a symptom of a disease or disorder. The terms “treating” and “treatment” can also refer to reduction in severity and/or frequency of symptoms, elimination of symptoms and/or underlying cause, and improvement or remediation of damage.

As used herein, the term “preventing” a disease, a disorder, or unwanted physiological event in a subject refers to the prevention of a disease, a disorder, or unwanted physiological event or prevention of a symptom of a disease, a disorder, or unwanted physiological event.

“Effective amount” of an agent refers to a sufficient amount of an agent to provide a desired effect. The amount of agent that is “effective” will vary from subject to subject, depending on many factors such as the age and general condition of the subject, the particular agent or agents, and the like. Thus, it is not always possible to specify a quantified “effective amount.” However, an appropriate “effective amount” in any subject case may be determined by one of ordinary skill in the art using routine experimentation. Also, as used herein, and unless specifically stated otherwise, an “effective amount” of an agent can also refer to an amount covering both therapeutically effective amounts and prophylactically effective amounts. An “effective amount” of an agent necessary to achieve a therapeutic effect may vary according to factors such as the age, sex, and weight of the subject. Dosage regimens can be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. In certain embodiments, the effective amount per dose varies from about 0.001 mg/kg to about 1000 mg/kg,

from about 0.01 mg/kg to about 750 mg/kg, from about 0.1 mg/kg to about 500 mg/kg, from about 1.0 mg/kg to about 250 mg/kg, and from about 10.0 mg/kg to about 150 mg/kg.

"Pharmaceutically acceptable" component can refer to a component that is not biologically or otherwise undesirable, i.e., the component may be incorporated into a pharmaceutical formulation of the invention and administered to a subject as described herein without causing significant undesirable biological effects or interacting in a deleterious manner with any of the other components of the formulation in which it is contained. When used in reference to administration to a human, the term generally implies the component has met the required standards of toxicological and manufacturing testing or that it is included on the Inactive Ingredient Guide prepared by the U.S. Food and Drug Administration.

"Pharmaceutically acceptable carrier" (sometimes referred to as a "carrier") means a carrier or excipient that is useful in preparing a pharmaceutical or therapeutic composition that is generally safe and non-toxic, and includes a carrier that is acceptable for veterinary and/or human pharmaceutical or therapeutic use. The terms "carrier" or "pharmaceutically acceptable carrier" can include, but are not limited to, phosphate buffered saline solution, water, emulsions (such as an oil/water or water/oil emulsion) and/or various types of wetting agents. As used herein, the term "carrier" encompasses, but is not limited to, any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, lipid, stabilizer, or other material well known in the art for use in pharmaceutical formulations and as described further herein.

"Therapeutic agent" refers to any composition that has a beneficial biological effect. Beneficial biological effects include both therapeutic effects, e.g., treatment of a disorder or other undesirable physiological condition, and prophylactic effects, e.g., prevention of a disorder or other undesirable physiological condition. The terms also encompass pharmaceutically acceptable, pharmacologically active derivatives of beneficial agents specifically mentioned herein, including, but not limited to, salts, esters, amides, proagents, active metabolites, isomers, fragments, analogs, and the like. When the term "therapeutic agent" is used, or when a particular agent is specifically identified, it is to be understood that the term includes the agent per se as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, proagents, conjugates, active metabolites, isomers, fragments, analogs, etc.

As used herein, the term "controlled-release" or "controlled-release drug delivery" or "extended release" refers to release or administration of a drug from a given dosage form in a controlled fashion in order to achieve the desired pharmacokinetic profile in vivo. An aspect of

“controlled” drug delivery is the ability to manipulate the formulation and/or dosage form in order to establish the desired kinetics of drug release.

The phrases "concurrent administration", "administration in combination", "simultaneous administration" or "administered simultaneously" as used herein, means that the compounds are administered at the same point in time or immediately following one another.

The term “antibodies” is used herein in a broad sense and includes both polyclonal and monoclonal antibodies. In addition to intact immunoglobulin molecules, also included in the term “antibodies” are fragments or polymers of those immunoglobulin molecules, and human or humanized versions of immunoglobulin molecules or fragments thereof. The antibodies can be tested for their desired activity using the *in vitro* assays described herein, or by analogous methods, after which their *in vivo* therapeutic and/or prophylactic activities are tested according to known clinical testing methods. There are five major classes of human immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG-1, IgG-2, IgG-3, and IgG-4; IgA-1 and IgA-2. One skilled in the art would recognize the comparable classes for mouse. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively.

The term “monoclonal antibody” as used herein refers to an antibody obtained from a substantially homogeneous population of antibodies, i.e., the individual antibodies within the population are identical except for possible naturally occurring mutations that may be present in a small subset of the antibody molecules. The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, as long as they exhibit the desired antagonistic activity.

The disclosed monoclonal antibodies can be made using any procedure which produces monoclonal antibodies. For example, disclosed monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized

in vitro.

The monoclonal antibodies may also be made by recombinant DNA methods. DNA encoding the disclosed monoclonal antibodies can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Libraries of antibodies or active antibody fragments can also be generated and screened using phage display techniques, e.g., as described in U.S. Patent No. 5,804,440 to Burton et al. and U.S. Patent No. 6,096,441 to Barbas et al.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art. For instance, digestion can be performed using papain. Examples of papain digestion are described in WO 94/29348 published Dec. 22, 1994 and U.S. Pat. No. 4,342,566. Papain digestion of antibodies typically produces two identical antigen binding fragments, called Fab fragments, each with a single antigen binding site, and a residual Fc fragment. Pepsin treatment yields a fragment that has two antigen combining sites and is still capable of cross-linking antigen.

As used herein, the term “antibody or antigen binding fragment thereof” or “antibody or fragments thereof” encompasses chimeric antibodies and hybrid antibodies, with dual or multiple antigen or epitope specificities, and fragments, such as F(ab')₂, Fab', Fab, Fv, sFv, scFv and the like, including hybrid fragments. Thus, fragments of the antibodies that retain the ability to bind their specific antigens are provided. For example, fragments of antibodies which maintain binding activity are included within the meaning of the term “antibody or antigen binding fragment thereof.” Such antibodies and fragments can be made by techniques known in the art and can be screened for specificity and activity according to the methods set forth in the Examples and in general methods for producing antibodies and screening antibodies for specificity and activity (See Harlow and Lane. *Antibodies, A Laboratory Manual*. Cold Spring Harbor Publications, New York, (1988)).

Also included within the meaning of “antibody or antigen binding fragment thereof” are conjugates of antibody fragments and antigen binding proteins (single chain antibodies). Also included within the meaning of “antibody or antigen binding fragment thereof” are immunoglobulin single variable domains, such as for example a nanobody.

The fragments, whether attached to other sequences or not, can also include insertions,

deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues, provided the activity of the antibody or antibody fragment is not significantly altered or impaired compared to the non-modified antibody or antibody fragment. These modifications can provide for some additional property, such as to remove/add amino acids capable of disulfide bonding, to increase its bio-longevity, to alter its secretory characteristics, etc. In any case, the antibody or antibody fragment must possess a bioactive property, such as specific binding to its cognate antigen. Functional or active regions of the antibody or antibody fragment may be identified by mutagenesis of a specific region of the protein, followed by expression and testing of the expressed polypeptide. Such methods are readily apparent to a skilled practitioner in the art and can include site-specific mutagenesis of the nucleic acid encoding the antibody or antibody fragment. (Zoller, M.J. *Curr. Opin. Biotechnol.* 3:348-354, 1992).

As used herein, the term “antibody” or “antibodies” can also refer to a human antibody and/or a humanized antibody. Many non-human antibodies (e.g., those derived from mice, rats, or rabbits) are naturally antigenic in humans, and thus can give rise to undesirable immune responses when administered to humans. Therefore, the use of human or humanized antibodies in the methods serves to lessen the chance that an antibody administered to a human will evoke an undesirable immune response.

Chemical Definitions

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

The organic moieties mentioned when defining variable positions within the general formulae described herein (e.g., the term “halogen”) are collective terms for the individual substituents encompassed by the organic moiety. The prefix C_n-C_m preceding a group or moiety indicates, in each case, the possible number of carbon atoms in the group or moiety that follows.

The term “ion,” as used herein, refers to any molecule, portion of a molecule, cluster of molecules, molecular complex, moiety, or atom that contains a charge (positive, negative, or both at the same time within one molecule, cluster of molecules, molecular complex, or moiety (e.g., zwitterions)) or that can be made to contain a charge. Methods for producing a charge in a molecule, portion of a molecule, cluster of molecules, molecular complex, moiety, or atom are disclosed herein and can be accomplished by methods known in the art, e.g., protonation,

deprotonation, oxidation, reduction, alkylation, acetylation, esterification, de-esterification, hydrolysis, etc.

The term “anion” is a type of ion and is included within the meaning of the term “ion.” An “anion” is any molecule, portion of a molecule (e.g., zwitterion), cluster of molecules, molecular complex, moiety, or atom that contains a net negative charge or that can be made to contain a net negative charge. The term “anion precursor” is used herein to specifically refer to a molecule that can be converted to an anion via a chemical reaction (e.g., deprotonation).

The term “cation” is a type of ion and is included within the meaning of the term “ion.” A “cation” is any molecule, portion of a molecule (e.g., zwitterion), cluster of molecules, molecular complex, moiety, or atom, that contains a net positive charge or that can be made to contain a net positive charge. The term “cation precursor” is used herein to specifically refer to a molecule that can be converted to a cation via a chemical reaction (e.g., protonation or alkylation).

As used herein, the term “substituted” is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, and aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described below. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this disclosure, the heteroatoms, such as nitrogen, can have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms. This disclosure is not intended to be limited in any manner by the permissible substituents of organic compounds. Also, the terms “substitution” or “substituted with” include the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., a compound that does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

“Z¹,” “Z²,” “Z³,” and “Z⁴” are used herein as generic symbols to represent various specific substituents. These symbols can be any substituent, not limited to those disclosed herein, and when they are defined to be certain substituents in one instance, they can, in another instance, be defined as some other substituents.

The term “aliphatic” as used herein refers to a non-aromatic hydrocarbon group and includes branched and unbranched, alkyl, alkenyl, or alkynyl groups.

As used herein, the term “alkyl” refers to saturated, straight-chained or branched saturated hydrocarbon moieties. Unless otherwise specified, C₁-C₂₄ (e.g., C₂-C₂₂, C₄-C₂₂, C₆-C₂₂, C₈-C₂₂, C₁₀-C₂₂, C₁₂-C₂₂, C₁₄-C₂₂, C₁₆-C₂₂, C₂-C₂₀, C₄-C₂₀, C₆-C₂₀, C₈-C₂₀, C₁₀-C₂₀, C₁₂-C₂₀, C₁₄-C₂₀, C₁₆-C₂₀, C₁-C₂₀, C₁-C₁₈, C₁-C₁₆, C₁-C₁₄, C₁-C₁₂, C₁-C₁₀, C₁-C₈, C₁-C₆, or C₁-C₄) alkyl groups are intended. Examples of alkyl groups include methyl, ethyl, propyl, 1-methyl-ethyl, butyl, 1-methyl-propyl, 2-methyl-propyl, 1,1-dimethyl-ethyl, pentyl, 1-methyl-butyl, 2-methyl-butyl, 3-methyl-butyl, 2,2-dimethyl-propyl, 1-ethyl-propyl, hexyl, 1,1-dimethyl-propyl, 1,2-dimethyl-propyl, 1-methyl-pentyl, 2-methyl-pentyl, 3-methyl-pentyl, 4-methyl-pentyl, 1,1-dimethyl-butyl, 1,2-dimethyl-butyl, 1,3-dimethyl-butyl, 2,2-dimethyl-butyl, 2,3-dimethyl-butyl, 3,3-dimethyl-butyl, 1-ethyl-butyl, 2-ethyl-butyl, 1,1,2-trimethyl-propyl, 1,2,2-trimethyl-propyl, 1-ethyl-1-methyl-propyl, 1-ethyl-2-methyl-propyl, heptyl, octyl, nonyl, decyl, dodecyl, tetradecyl, hexadecyl, eicosyl, tetracosyl, and the like. Alkyl substituents may be unsubstituted or substituted with one or more chemical moieties. The alkyl group can be substituted with one or more groups including, but not limited to, hydroxyl, halogen, acetal, acyl, alkyl, alkoxy, alkenyl, alkynyl, aryl, heteroaryl, aldehyde, amino, cyano, carboxylic acid, ester, ether, carbonate ester, carbamate ester, ketone, nitro, phosphonyl, silyl, sulfo-oxo, sulfonyl, sulfone, sulfoxide, or thiol, as described below, provided that the substituents are sterically compatible and the rules of chemical bonding and strain energy are satisfied.

Throughout the specification “alkyl” is generally used to refer to both unsubstituted alkyl groups and substituted alkyl groups; however, substituted alkyl groups are also specifically referred to herein by identifying the specific substituent(s) on the alkyl group. For example, the term “halogenated alkyl” or “haloalkyl” specifically refers to an alkyl group that is substituted with one or more halides (halogens; e.g., fluorine, chlorine, bromine, or iodine). The term “alkoxyalkyl” specifically refers to an alkyl group that is substituted with one or more alkoxy groups, as described below. The term “alkylamino” specifically refers to an alkyl group that is substituted with one or more amino groups, as described below, and the like. When “alkyl” is used in one instance and a specific term such as “alkylalcohol” is used in another, it is not meant to imply that the term “alkyl” does not also refer to specific terms such as “alkylalcohol” and the like.

This practice is also used for other groups described herein. That is, while a term such as “cycloalkyl” refers to both unsubstituted and substituted cycloalkyl moieties, the substituted moieties can, in addition, be specifically identified herein; for example, a particular substituted

cycloalkyl can be referred to as, *e.g.*, an “alkylcycloalkyl.” Similarly, a substituted alkoxy can be specifically referred to as, *e.g.*, a “halogenated alkoxy,” a particular substituted alkenyl can be, *e.g.*, an “alkenylalcohol,” and the like. Again, the practice of using a general term, such as “cycloalkyl,” and a specific term, such as “alkylcycloalkyl,” is not meant to imply that the general term does not also include the specific term.

As used herein, the term “alkenyl” refers to unsaturated, straight-chained, or branched hydrocarbon moieties containing a double bond. Unless otherwise specified, C₂-C₂₄ (*e.g.*, C₂-C₂₂, C₂-C₂₀, C₂-C₁₈, C₂-C₁₆, C₂-C₁₄, C₂-C₁₂, C₂-C₁₀, C₂-C₈, C₂-C₆, or C₂-C₄) alkenyl groups are intended. Alkenyl groups may contain more than one unsaturated bond. Examples include ethenyl, 1-propenyl, 2-propenyl, 1-methylethenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1-methyl-1-propenyl, 2-methyl-1-propenyl, 1-methyl-2-propenyl, 2-methyl-2-propenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-methyl-1-butenyl, 2-methyl-1-butenyl, 3-methyl-1-butenyl, 1-methyl-2-butenyl, 2-methyl-2-butenyl, 3-methyl-2-butenyl, 1-methyl-3-butenyl, 2-methyl-3-butenyl, 3-methyl-3-butenyl, 1,1-dimethyl-2-propenyl, 1,2-dimethyl-1-propenyl, 1,2-dimethyl-2-propenyl, 1-ethyl-1-propenyl, 1-ethyl-2-propenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl, 5-hexenyl, 1-methyl-1-pentenyl, 2-methyl-1-pentenyl, 3-methyl-1-pentenyl, 4-methyl-1-pentenyl, 1-methyl-2-pentenyl, 2-methyl-2-pentenyl, 3-methyl-2-pentenyl, 4-methyl-2-pentenyl, 1-methyl-3-pentenyl, 2-methyl-3-pentenyl, 3-methyl-3-pentenyl, 4-methyl-3-pentenyl, 1-methyl-4-pentenyl, 2-methyl-4-pentenyl, 3-methyl-4-pentenyl, 4-methyl-4-pentenyl, 1,1-dimethyl-2-butenyl, 1,1-dimethyl-3-butenyl, 1,2-dimethyl-1-butenyl, 1,2-dimethyl-2-butenyl, 1,2-dimethyl-3-butenyl, 1,3-dimethyl-1-butenyl, 1,3-dimethyl-2-butenyl, 1,3-dimethyl-3-butenyl, 2,2-dimethyl-3-butenyl, 2,3-dimethyl-1-butenyl, 2,3-dimethyl-2-butenyl, 2,3-dimethyl-3-butenyl, 3,3-dimethyl-1-butenyl, 3,3-dimethyl-2-butenyl, 1-ethyl-1-butenyl, 1-ethyl-2-butenyl, 1-ethyl-3-butenyl, 2-ethyl-1-butenyl, 2-ethyl-2-butenyl, 2-ethyl-3-butenyl, 1,1,2-trimethyl-2-propenyl, 1-ethyl-1-methyl-2-propenyl, 1-ethyl-2-methyl-1-propenyl, and 1-ethyl-2-methyl-2-propenyl. The term “vinyl” refers to a group having the structure –CH=CH₂; 1-propenyl refers to a group with the structure –CH=CH-CH₃; and 2-propenyl refers to a group with the structure –CH₂-CH=CH₂. Asymmetric structures such as (Z¹Z²)C=C(Z³Z⁴) are intended to include both the *E* and *Z* isomers. This can be presumed in structural formulae herein wherein an asymmetric alkene is present, or it can be explicitly indicated by the bond symbol C=C. Alkenyl substituents may be unsubstituted or substituted with one or more chemical moieties. Examples of suitable substituents include, for example, alkyl, alkoxy, alkenyl, alkynyl, aryl, heteroaryl, acetal, acyl, aldehyde, amino, cyano,

carboxylic acid, ester, ether, carbonate ester, carbamate ester, halide, hydroxyl, ketone, nitro, phosphonyl, silyl, sulfo-oxo, sulfonyl, sulfone, sulfoxide, or thiol, as described below, provided that the substituents are sterically compatible and the rules of chemical bonding and strain energy are satisfied.

5 As used herein, the term “alkynyl” represents straight-chained or branched hydrocarbon moieties containing a triple bond. Unless otherwise specified, C₂-C₂₄ (e.g., C₂-C₂₄, C₂-C₂₀, C₂-C₁₈, C₂-C₁₆, C₂-C₁₄, C₂-C₁₂, C₂-C₁₀, C₂-C₈, C₂-C₆, or C₂-C₄) alkynyl groups are intended. Alkynyl groups may contain more than one unsaturated bond. Examples include C₂-C₆-alkynyl, such as ethynyl, 1-propynyl, 2-propynyl (or propargyl), 1-butylnyl, 2-butylnyl, 3-butylnyl, 1-methyl-2-
10 propynyl, 1-pentylnyl, 2-pentylnyl, 3-pentylnyl, 4-pentylnyl, 3-methyl-1-butylnyl, 1-methyl-2-butylnyl, 1-methyl-3-butylnyl, 2-methyl-3-butylnyl, 1,1-dimethyl-2-propynyl, 1-ethyl-2-propynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, 5-hexynyl, 3-methyl-1-pentylnyl, 4-methyl-1-pentylnyl, 1-methyl-2-pentylnyl, 4-methyl-2-pentylnyl, 1-methyl-3-pentylnyl, 2-methyl-3-pentylnyl, 1-methyl-4-pentylnyl, 2-methyl-4-pentylnyl, 3-methyl-4-pentylnyl, 1,1-dimethyl-2-butylnyl, 1,1-
15 dimethyl-3-butylnyl, 1,2-dimethyl-3-butylnyl, 2,2-dimethyl-3-butylnyl, 3,3-dimethyl-1-butylnyl, 1-ethyl-2-butylnyl, 1-ethyl-3-butylnyl, 2-ethyl-3-butylnyl, and 1-ethyl-1-methyl-2-propynyl. Alkynyl substituents may be unsubstituted or substituted with one or more chemical moieties. Examples of suitable substituents include, for example, alkyl, alkoxy, alkenyl, alkynyl, aryl, heteroaryl, acetal, acyl, aldehyde, amino, cyano, carboxylic acid, ester, ether, carbonate ester, carbamate ester, halide,
20 hydroxyl, ketone, nitro, phosphonyl, silyl, sulfo-oxo, sulfonyl, sulfone, sulfoxide, or thiol, as described below.

As used herein, the term “aryl,” as well as derivative terms such as aryloxy, refers to groups that include a monovalent aromatic carbocyclic group of from 3 to 50 carbon atoms. Aryl groups can include a single ring or multiple condensed rings. In some embodiments, aryl groups include
25 C₆-C₁₀ aryl groups. Examples of aryl groups include, but are not limited to, benzene, phenyl, biphenyl, naphthyl, tetrahydronaphthyl, phenylcyclopropyl, phenoxybenzene, and indanyl. The term “aryl” also includes “heteroaryl,” which is defined as a group that contains an aromatic group that has at least one heteroatom incorporated within the ring of the aromatic group. Examples of heteroatoms include, but are not limited to, nitrogen, oxygen, sulfur, and phosphorus. The term
30 “non-heteroaryl,” which is also included in the term “aryl,” defines a group that contains an aromatic group that does not contain a heteroatom. The aryl substituents may be unsubstituted or substituted with one or more chemical moieties. Examples of suitable substituents include, for

example, alkyl, alkoxy, alkenyl, alkynyl, aryl, heteroaryl, acetal, acyl, aldehyde, amino, cyano, carboxylic acid, ester, ether, carbonate ester, carbamate ester, halide, hydroxyl, ketone, nitro, phosphonyl, silyl, sulfo-oxo, sulfonyl, sulfone, sulfoxide, or thiol as described herein. The term “biaryl” is a specific type of aryl group and is included in the definition of aryl. Biaryl refers to two aryl groups that are bound together *via* a fused ring structure, as in naphthalene, or are attached *via* one or more carbon-carbon bonds, as in biphenyl.

The term “cycloalkyl” as used herein is a non-aromatic carbon-based ring composed of at least three carbon atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, etc. The term “heterocycloalkyl” is a cycloalkyl group as defined above where at least one of the carbon atoms of the ring is substituted with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. The cycloalkyl group and heterocycloalkyl group can be substituted or unsubstituted. The cycloalkyl group and heterocycloalkyl group can be substituted with one or more groups including, but not limited to, alkyl, alkoxy, alkenyl, alkynyl, aryl, heteroaryl, acetal, acyl, aldehyde, amino, cyano, carboxylic acid, ester, ether, carbonate ester, carbamate ester, halide, hydroxyl, ketone, nitro, phosphonyl, silyl, sulfo-oxo, sulfonyl, sulfone, sulfoxide, or thiol as described herein.

The term “cycloalkenyl” as used herein is a non-aromatic carbon-based ring composed of at least three carbon atoms and containing at least one double bond, *i.e.*, C=C. Examples of cycloalkenyl groups include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, cyclohexadienyl, and the like. The term “heterocycloalkenyl” is a type of cycloalkenyl group as defined above and is included within the meaning of the term “cycloalkenyl,” where at least one of the carbon atoms of the ring is substituted with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. The cycloalkenyl group and heterocycloalkenyl group can be substituted or unsubstituted. The cycloalkenyl group and heterocycloalkenyl group can be substituted with one or more groups including, but not limited to, alkyl, alkoxy, alkenyl, alkynyl, aryl, heteroaryl, acetal, acyl, aldehyde, amino, cyano, carboxylic acid, ester, ether, carbonate ester, carbamate ester, halide, hydroxyl, ketone, nitro, phosphonyl, silyl, sulfo-oxo, sulfonyl, sulfone, sulfoxide, or thiol as described herein.

The term “cyclic group” is used herein to refer to either aryl groups, non-aryl groups (*i.e.*, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl groups), or both. Cyclic groups have one or more ring systems (e.g., monocyclic, bicyclic, tricyclic, polycyclic, etc.) that can be

substituted or unsubstituted. A cyclic group can contain one or more aryl groups, one or more non-aryl groups, or one or more aryl groups and one or more non-aryl groups.

The term “acyl” as used herein is represented by the formula $-C(O)Z^1$ where Z^1 can be a hydrogen, hydroxyl, alkoxy, alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above. As used herein, the term “acyl” can be used interchangeably with “carbonyl.” Throughout this specification “C(O)” or “CO” is a shorthand notation for $C=O$.

The term “acetal” as used herein is represented by the formula $(Z^1Z^2)C(=OZ^3)(=OZ^4)$, where Z^1 , Z^2 , Z^3 , and Z^4 can be, independently, a hydrogen, halogen, hydroxyl, alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The term “alkanol” as used herein is represented by the formula Z^1OH , where Z^1 can be an alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

As used herein, the term “alkoxy” as used herein is an alkyl group bound through a single, terminal ether linkage; that is, an “alkoxy” group can be defined as to a group of the formula Z^1-O- , where Z^1 is unsubstituted or substituted alkyl as defined above. Unless otherwise specified, alkoxy groups wherein Z^1 is a C_1 - C_{24} (e.g., C_2 - C_{22} , C_4 - C_{22} , C_6 - C_{22} , C_8 - C_{22} , C_{10} - C_{22} , C_{12} - C_{22} , C_{14} - C_{22} , C_{16} - C_{22} , C_2 - C_{20} , C_4 - C_{20} , C_6 - C_{20} , C_8 - C_{20} , C_{10} - C_{20} , C_{12} - C_{20} , C_{14} - C_{20} , C_{16} - C_{20} , C_1 - C_{20} , C_1 - C_{18} , C_1 - C_{16} , C_1 - C_{14} , C_1 - C_{12} , C_1 - C_{10} , C_1 - C_8 , C_1 - C_6 , or C_1 - C_4) alkyl group are intended. Examples include methoxy, ethoxy, propoxy, 1-methyl-ethoxy, butoxy, 1-methyl-propoxy, 2-methyl-propoxy, 1,1-dimethyl-ethoxy, pentoxy, 1-methyl-butyloxy, 2-methyl-butoxy, 3-methyl-butoxy, 2,2-di-methyl-propoxy, 1-ethyl-propoxy, hexoxy, 1,1-dimethyl-propoxy, 1,2-dimethyl-propoxy, 1-methyl-pentoxy, 2-methyl-pentoxy, 3-methyl-pentoxy, 4-methyl-penoxy, 1,1-dimethyl-butoxy, 1,2-dimethyl-butoxy, 1,3-dimethyl-butoxy, 2,2-dimethyl-butoxy, 2,3-dimethyl-butoxy, 3,3-dimethyl-butoxy, 1-ethyl-butoxy, 2-ethylbutoxy, 1,1,2-trimethyl-propoxy, 1,2,2-trimethyl-propoxy, 1-ethyl-1-methyl-propoxy, and 1-ethyl-2-methyl-propoxy.

The term “aldehyde” as used herein is represented by the formula $-C(O)H$. Throughout this specification “C(O)” is a shorthand notation for $C=O$.

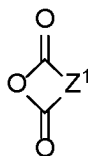
The terms “amine” or “amino” as used herein are represented by the formula $-NZ^1Z^2Z^3$, where Z^1 , Z^2 , and Z^3 can each be substitution group as described herein, such as hydrogen, an

alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The terms “amide” or “amido” as used herein are represented by the formula —C(O)NZ¹Z², where Z¹ and Z² can each be substitution group as described herein, such as hydrogen,
 5 an alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The term “anhydride” as used herein is represented by the formula Z¹C(O)OC(O)Z² where Z¹ and Z², independently, can be an alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

10 The term “cyclic anhydride” as used herein is represented by the formula:



where Z¹ can be an alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The term “azide” as used herein is represented by the formula —N=N=N.

15 The term “carboxylic acid” as used herein is represented by the formula —C(O)OH.

A “carboxylate” or “carboxyl” group as used herein is represented by the formula —C(O)O[•].

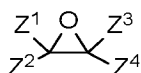
A “carbonate ester” group as used herein is represented by the formula Z¹OC(O)OZ².

The term “cyano” as used herein is represented by the formula —CN.

20 The term “ester” as used herein is represented by the formula —OC(O)Z¹ or —C(O)OZ¹, where Z¹ can be an alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The term “ether” as used herein is represented by the formula Z¹OZ², where Z¹ and Z² can be, independently, an alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl,
 25 heterocycloalkyl, or heterocycloalkenyl group described above.

The term “epoxy” or “epoxide” as used herein refers to a cyclic ether with a three atom ring and can be represented by the formula:



where Z^1 , Z^2 , Z^3 , and Z^4 can be, independently, an alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above

The term “ketone” as used herein is represented by the formula $Z^1C(O)Z^2$, where Z^1 and Z^2 can be, independently, an alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The term “halide” or “halogen” or “halo” as used herein refers to fluorine, chlorine, bromine, and iodine.

The term “hydroxyl” as used herein is represented by the formula $—OH$.

The term “nitro” as used herein is represented by the formula $—NO_2$.

The term “phosphonyl” is used herein to refer to the phospho-oxo group represented by the formula $—P(O)(OZ^1)_2$, where Z^1 can be hydrogen, an alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The term “silyl” as used herein is represented by the formula $—SiZ^1Z^2Z^3$, where Z^1 , Z^2 , and Z^3 can be, independently, hydrogen, alkyl, alkoxy, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The term “sulfonyl” or “sulfone” is used herein to refer to the sulfo-oxo group represented by the formula $—S(O)_2Z^1$, where Z^1 can be hydrogen, an alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The term “sulfide” as used herein is comprises the formula $—S—$.

The term “thiol” as used herein is represented by the formula $—SH$.

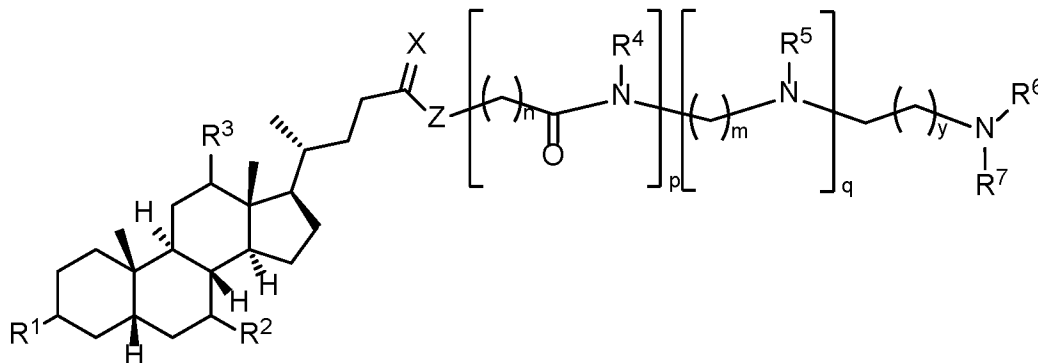
“ R^1 ,” “ R^2 ,” “ R^3 ,” “ R^n ,” etc., where n is some integer, as used herein can, independently, possess one or more of the groups listed above. For example, if R^1 is a straight chain alkyl group, one of the hydrogen atoms of the alkyl group can optionally be substituted with a hydroxyl group, an alkoxy group, an amine group, an alkyl group, a halide, and the like. Depending upon the groups that are selected, a first group can be incorporated within second group or, alternatively, the first group can be pendant (i.e., attached) to the second group. For example, with the phrase “an alkyl group comprising an amino group,” the amino group can be incorporated within the backbone of the alkyl group. Alternatively, the amino group can be attached to the backbone of the alkyl group. The nature of the group(s) that is (are) selected will determine if the first group is embedded or attached to the second group.

Unless stated to the contrary, a formula with chemical bonds shown only as solid lines and not as wedges or dashed lines contemplates each possible stereoisomer or mixture of stereoisomer

(e.g., each enantiomer, each diastereomer, each meso compound, a racemic mixture, or scalemic mixture).

Compounds

Disclosed herein are compounds and methods of making and uses thereof. For example,
5 disclosed herein are compounds defined by Formula I, or a pharmaceutically acceptable salt thereof:



I

wherein

- 10 X and Z are each independently O, NR^a, or S;
R^a, when present, is hydrogen or substituted or unsubstituted C₁-C₁₀ alkyl;
R¹, R², and R³ are each independently H, OH, halogen, substituted or unsubstituted C₁-C₃ alkyl, or substituted or unsubstituted C₁-C₃ alkoxy;
p is an integer from 0 to 5;
15 q is an integer from 0 to 5;
n in each case is independently an integer from 1 to 10;
m in each case is independently an integer from 1 to 10;
y is an integer from 1 to 10;
each R⁴, when present, is independently hydrogen or a substituted or unsubstituted C₁-C₁₈
20 alkyl;
each R⁵, when present, is independently a substituted or unsubstituted C₈-C₁₈ alkyl; and
R⁶ and R⁷ are each independently a substituted or unsubstituted C₈-C₁₈ alkyl.

In some examples of Formula I, X and Z are each independently is O, NR^a, or S. In some examples of Formula I, X is O. In some examples of Formula I, Z is NR^a. In some examples of
25 Formula I, X is O and Z is NR^a. In some examples of Formula I, R^a is hydrogen or substituted or

unsubstituted C₁-C₁₀ alkyl. In some examples of Formula I, R^a is hydrogen. Thus, in some examples of Formula I, X is O and Z is NH. In some examples of Formula I, X and Z are both O.

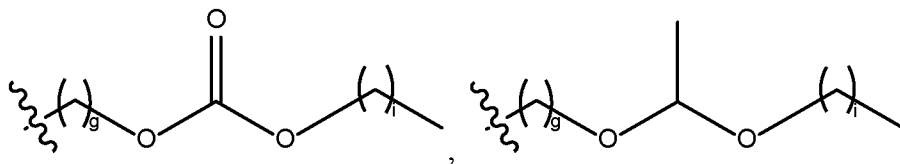
In some examples of Formula I, R¹ is OH. In some examples of Formula I, R¹ is H. In some examples of Formula I, R¹ is a halogen. In some examples of Formula I, R¹ is a substituted or unsubstituted C₁-C₃ alkyl. In some examples of Formula I, R¹ is a substituted or unsubstituted C₁-C₃ alkoxy.

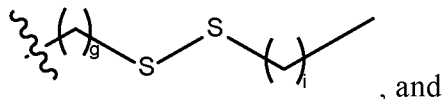
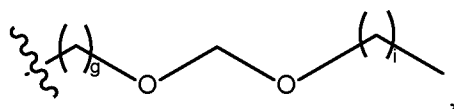
In some examples of Formula I, R² is OH. In some examples of Formula I, R² is H. In some examples of Formula I, R² is a halogen. In some examples of Formula I, R² is a substituted or unsubstituted C₁-C₃ alkyl. In some examples of Formula I, R² is a substituted or unsubstituted C₁-C₃ alkoxy.

In some examples of Formula I, R³ is OH. In some examples of Formula I, R³ is H. In some examples of Formula I, R³ is a halogen. In some examples of Formula I, R³ is a substituted or unsubstituted C₁-C₃ alkyl. In some examples of Formula I, R³ is a substituted or unsubstituted C₁-C₃ alkoxy.

In some examples of Formula I, R¹, R², and R³ are all the same. In some examples of Formula I, R¹, R², and R³ are different. In some examples of Formula I, only two of R¹, R², and R³ are the same. In some examples of Formula I, each of R¹, R², and R³ are different.

In some examples of Formula I, R⁶ and R⁷ are each independently an unsubstituted C₈-C₁₈ alkyl or a linear or branched C₈-C₁₈ alkyl substituted with one or more substituents selected from the group consisting of amine, amide, ester, ether, and carbonate ester. In some examples of Formula I, R⁶ and R⁷ are each independently an unsubstituted C₈-C₁₈ alkyl. In some examples of Formula I, R⁶ and R⁷ are each independently a linear or branched C₈-C₁₈ alkyl substituted with one or more substituents selected from the group consisting of amine, amide, ester, ether, and carbonate ester. In some examples of Formula I, R⁶ and R⁷ are each independently an unsubstituted C₈-C₁₈ alkyl. In some examples of Formula I, R⁶ and R⁷ are each independently an unsubstituted linear C₈-C₁₈ alkyl (e.g., a C₁₀-C₁₄ alkyl, or a C₁₂ alkyl). In some examples of Formula I, R⁶ and R⁷ are independently selected from the group consisting of:





5 wherein G and I each independently represent integers from 1 to 9 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9). In some examples of Formula I, R^6 and R^7 are the same. In some examples of Formula I, R^6 and R^7 are different.

In some examples of Formula I, y is from 1 to 7. In some examples of Formula I, y is from 1 to 6. In some examples of Formula I, y is from 1 to 5. In some examples of Formula I, y is from 1 to 4. In some examples of Formula I, y is from 1 to 3. In some examples of Formula I, y is 1.

In some examples of Formula I, n is from 1 to 7. In some examples of Formula I, n is from 1 to 6. In some examples of Formula I, n is from 1 to 5. In some examples of Formula I, n is from 1 to 4. In some examples of Formula I, n is from 1 to 3. In some examples of Formula I, n is 1.

In some examples of Formula I, m is from 1 to 7. In some examples of Formula I, m is from 1 to 6. In some examples of Formula I, m is from 1 to 5. In some examples of Formula I, m is from 1 to 4. In some examples of Formula I, m is from 1 to 3. In some examples of Formula I, m is 1. In some examples of Formula I, R^5 is a substituted or unsubstituted C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl). In some examples of Formula I, R^5 is an unsubstituted C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl).

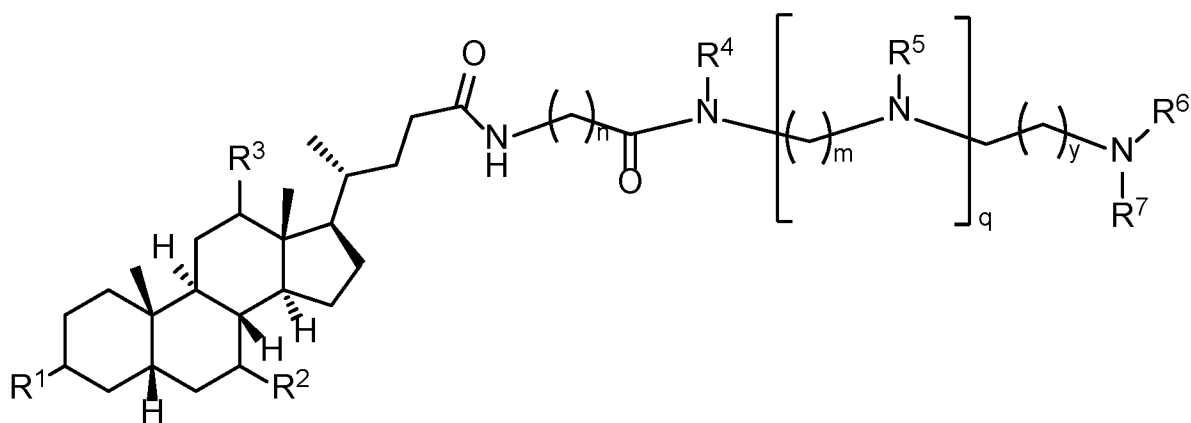
20 In some examples of Formula I, each R^4 , when present, is independently a substituted or unsubstituted C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl). In some examples of Formula I, each R^4 , when present, is independently an unsubstituted C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl). In some examples of Formula I, each R^4 , when present, is hydrogen.

In some examples of Formula I, p is from 0 to 4. In some examples of Formula I, p is from 0 to 3. In some examples of Formula I, p is from 0 to 2. In some examples of Formula I, p is from 0 to 1. In some examples of Formula I, p is 0. In some examples of Formula I, p is 1. In some examples of Formula I, p is 2 or more (e.g., 2, 3, 4, 5). In some examples of Formula I, where p is 2 or more, each R^4 can be the same. In some examples of Formula I, where p is 2 or more, at least

one R^4 is different. In some examples of Formula I, where p is 2 or more, each n is the same. In some examples of Formula I, where p is 2 or more, at least one n is different.

In some examples of Formula I, q is from 0 to 4. In some examples of Formula I, q is from 0 to 3. In some examples of Formula I, q is from 0 to 2. In some examples of Formula I, q is from 0 to 1. In some examples of Formula I, q is 0. In some examples of Formula I, q is 1. In some examples of Formula I, q is 2 or more. In some examples of Formula I when q is 2 or more, each m is the same. In some examples of Formula I when q is 2, at least one m is different. For example, when q is 2, the first m can be an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, 7) and the second m can represent the same or different integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, 7). In some examples of Formula I when q is 2 or more, each R^5 is the same. In some examples of Formula I when q is 2 or more, at least one R^5 is different.

In some examples, the compound is defined by Formula II, or a pharmaceutically acceptable salt thereof:



II

wherein R^1 , R^2 , and R^3 are each independently H, OH, halogen, substituted or unsubstituted C_1 - C_3 alkyl, or substituted or unsubstituted C_1 - C_3 alkoxy;

q is an integer from 0 to 5;

n is an integer from 1 to 5;

m in each case is independently an integer from 1 to 10;

y is an integer from 1 to 10;

R^4 is hydrogen or a substituted or unsubstituted C_1 - C_{18} alkyl;

each R^5 , when present, is independently a substituted or unsubstituted C_8 - C_{18} alkyl; and

R^6 and R^7 are each independently a substituted or unsubstituted C_8 - C_{18} alkyl.

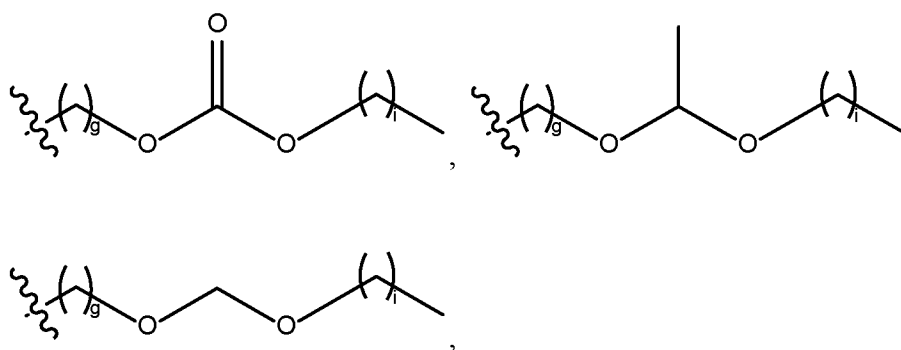
In some examples of Formula II, R^1 is OH. In some examples of Formula II, R^1 is H. In some examples of Formula II, R^1 is a halogen. In some examples of Formula II, R^1 is a substituted or unsubstituted C_1 - C_3 alkyl. In some examples of Formula II, R^1 is a substituted or unsubstituted C_1 - C_3 alkoxy.

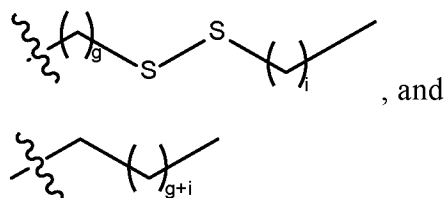
5 In some examples of Formula II, R^2 is OH. In some examples of Formula II, R^2 is H. In some examples of Formula II, R^2 is a halogen. In some examples of Formula II, R^2 is a substituted or unsubstituted C_1 - C_3 alkyl. In some examples of Formula II, R^2 is a substituted or unsubstituted C_1 - C_3 alkoxy.

10 In some examples of Formula II, R^3 is OH. In some examples of Formula II, R^3 is H. In some examples of Formula II, R^3 is a halogen. In some examples of Formula II, R^3 is a substituted or unsubstituted C_1 - C_3 alkyl. In some examples of Formula II, R^3 is a substituted or unsubstituted C_1 - C_3 alkoxy.

In some examples of Formula II, R^1 , R^2 , and R^3 are all the same. In some examples of Formula II, R^1 , R^2 , and R^3 are different. In some examples of Formula II, only two of R^1 , R^2 , and R^3 are the same. In some examples of Formula II, each of R^1 , R^2 , and R^3 are different.

In some examples of Formula II, R^6 and R^7 are each independently an unsubstituted C_8 - C_{18} alkyl or a linear or branched C_8 - C_{18} alkyl substituted with one or more substituents selected from the group consisting of amine, amide, ester, ether, and carbonate ester. In some examples of Formula II, R^6 and R^7 are each independently an unsubstituted C_8 - C_{18} alkyl. In some examples of Formula II, R^6 and R^7 are each independently a linear or branched C_8 - C_{18} alkyl substituted with one or more substituents selected from the group consisting of amine, amide, ester, ether, and carbonate ester. In some examples of Formula II, R^6 and R^7 are each independently an unsubstituted C_8 - C_{18} alkyl. In some examples of Formula II, R^6 and R^7 are each independently an unsubstituted linear C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl). In some examples of Formula II, R^6 and R^7 are independently selected from the group consisting of:





wherein G and I each independently represent integers from 1 to 9 (e.g., 1, 2, 3, 4, 5, 6, 7,

5 8, 9).

In some examples of Formula II, R^6 and R^7 are the same. In some examples of Formula II, R^6 and R^7 are different.

In some examples of Formula II, y is from 1 to 7. In some examples of Formula II, y is from 1 to 6. In some examples of Formula II, y is from 1 to 5. In some examples of Formula II, y is from 1 to 4. In some examples of Formula II, y is from 1 to 3. In some examples of Formula II, y is 1.

In some examples of Formula II, R^4 is a substituted or unsubstituted C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl). In some examples of Formula II, R^4 is an unsubstituted C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl). In some examples of Formula II, R^4 is hydrogen.

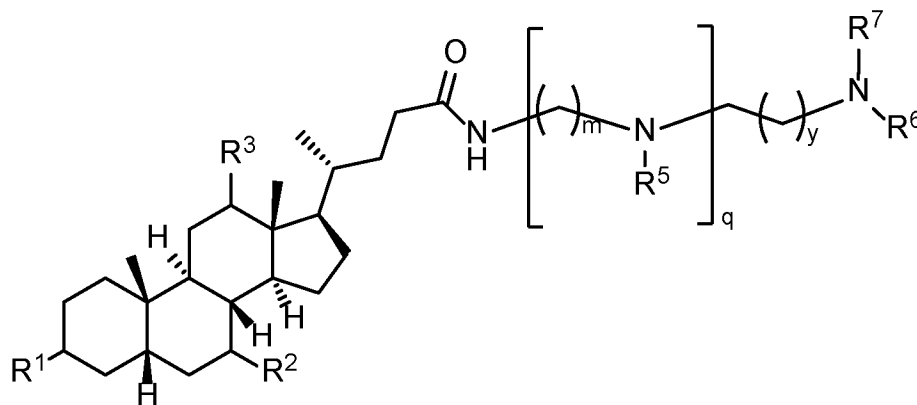
15 In some examples of Formula II, n is from 1 to 4. In some examples of Formula II, n is from 1 to 3. In some examples of Formula II, n is 1.

In some examples of Formula II, m is from 1 to 7. In some examples of Formula II, m is from 1 to 6. In some examples of Formula II, m is from 1 to 5. In some examples of Formula II, m is from 1 to 4. In some examples of Formula II, m is from 1 to 3. In some examples of Formula II, m is 1. In some examples of Formula II, R^5 is a substituted or unsubstituted C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl). In some examples of Formula II, R^5 is an unsubstituted C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl).

20 In some examples of Formula II, q is from 0 to 4. In some examples of Formula II, q is from 0 to 3. In some examples of Formula II, q is from 0 to 2. In some examples of Formula II, q is from 0 to 1. In some examples of Formula II, q is 0. In some examples of Formula II, q is 1. In some examples of Formula II, q is 2 or more (e.g., 2, 3, 4, 5). In some examples of Formula II when q is 2 or more, each m is the same. In some examples of Formula II when q is 2 or more, at least one m is different. For example, when q is 2, the first m can be an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, 7) and the second m can represent the same or different integer from 1 to 7 (e.g., 1,

2, 3, 4, 5, 6, 7). In some examples of Formula II when q is 2 or more, each R^5 is the same. In some examples of Formula II when q is 2 or more, at least one R^5 is different.

In some examples, the compound is defined by Formula III, or a pharmaceutically acceptable salt thereof:



III

wherein R^1 , R^2 , and R^3 are each independently H, OH, halogen, substituted or unsubstituted C_1 - C_3 alkyl, or substituted or unsubstituted C_1 - C_3 alkoxy;

q is an integer from 0 to 5;

m in each case is independently an integer from 1 to 7;

y is an integer from 1 to 10;

each R^5 , when present, is independently a substituted or unsubstituted C_8 - C_{18} alkyl; and

R^6 and R^7 are each independently a substituted or unsubstituted C_8 - C_{18} alkyl.

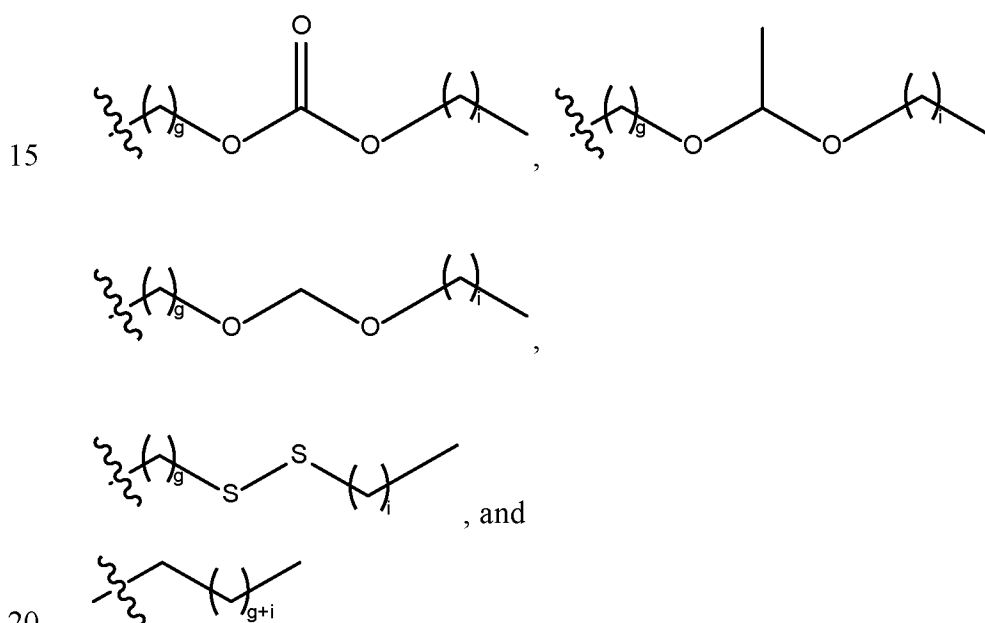
In some examples of Formula III, R^1 is OH. In some examples of Formula III, R^1 is H. In some examples of Formula III, R^1 is a halogen. In some examples of Formula III, R^1 is a substituted or unsubstituted C_1 - C_3 alkyl. In some examples of Formula III, R^1 is a substituted or unsubstituted C_1 - C_3 alkoxy.

In some examples of Formula III, R^2 is OH. In some examples of Formula III, R^2 is H. In some examples of Formula III, R^2 is a halogen. In some examples of Formula III, R^2 is a substituted or unsubstituted C_1 - C_3 alkyl. In some examples of Formula III, R^2 is a substituted or unsubstituted C_1 - C_3 alkoxy.

In some examples of Formula III, R^3 is OH. In some examples of Formula III, R^3 is H. In some examples of Formula III, R^3 is a halogen. In some examples of Formula III, R^3 is a substituted or unsubstituted C_1 - C_3 alkyl. In some examples of Formula III, R^3 is a substituted or unsubstituted C_1 - C_3 alkoxy.

In some examples of Formula III, R^1 , R^2 , and R^3 are all the same. In some examples of Formula III, R^1 , R^2 , and R^3 are different. In some examples of Formula III, only two of R^1 , R^2 , and R^3 are the same. In some examples of Formula III, each of R^1 , R^2 , and R^3 are different.

In some examples of Formula III, R^6 and R^7 are each independently an unsubstituted C_8 - C_{18} alkyl or a linear or branched C_8 - C_{18} alkyl substituted with one or more substituents selected from the group consisting of amine, amide, ester, ether, and carbonate ester. In some examples of Formula III, R^6 and R^7 are each independently an unsubstituted C_8 - C_{18} alkyl. In some examples of Formula III, R^6 and R^7 are each independently a linear or branched C_8 - C_{18} alkyl substituted with one or more substituents selected from the group consisting of amine, amide, ester, ether, and carbonate ester. In some examples of Formula III, R^6 and R^7 are each independently an unsubstituted C_8 - C_{18} alkyl. In some examples of Formula III, R^6 and R^7 are each independently an unsubstituted linear C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl). In some examples of Formula III, R^6 and R^7 are independently selected from the group consisting of:



wherein G and I each independently represent integers from 1 to 9 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9).

In some examples of Formula III, R^6 and R^7 are the same. In some examples of Formula III, R^6 and R^7 are different.

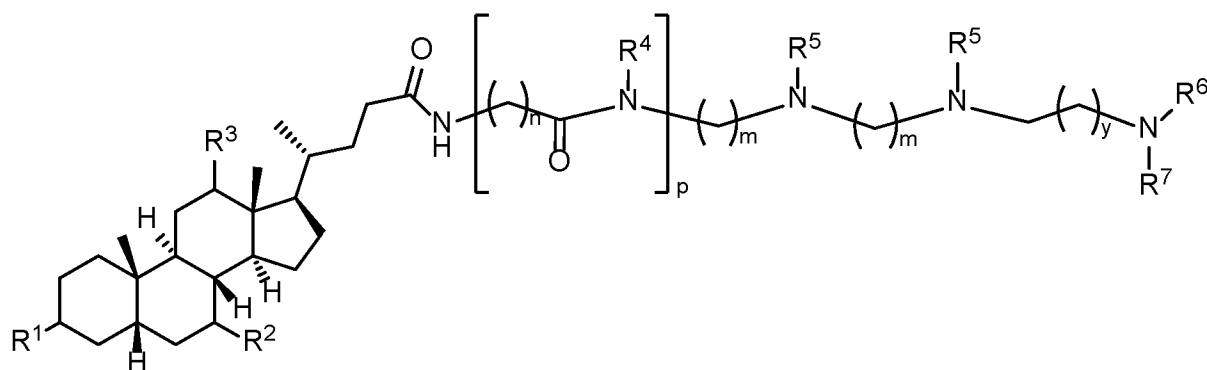
25 In some examples of Formula III, y is from 1 to 7. In some examples of Formula III, y is from 1 to 6. In some examples of Formula III, y is from 1 to 5. In some examples of Formula III, y is from 1 to 4. In some examples of Formula III, y is from 1 to 3. In some examples of Formula III, y is from 1 to 2. In some examples of Formula III, y is 1.

y is from 1 to 4. In some examples of Formula III, y is from 1 to 3. In some examples of Formula III, y is 1.

In some examples of Formula III, m is from 1 to 7. In some examples of Formula III, m is from 1 to 6. In some examples of Formula III, m is from 1 to 5. In some examples of Formula III, m is from 1 to 4. In some examples of Formula III, m is from 1 to 3. In some examples of Formula III, m is 1. In some examples of Formula III, R⁵ is a substituted or unsubstituted C₈-C₁₈ alkyl (e.g., a C₁₀-C₁₄ alkyl, or a C₁₂ alkyl). In some examples of Formula III, R⁵ is an unsubstituted C₈-C₁₈ alkyl (e.g., a C₁₀-C₁₄ alkyl, or a C₁₂ alkyl).

In some examples of Formula III, q is from 0 to 4. In some examples of Formula III, q is from 0 to 3. In some examples of Formula III, q is from 0 to 2. In some examples of Formula III, q is from 0 to 1. In some examples of Formula III, q is 0. In some examples of Formula III, q is 1. In some examples of Formula III, q is 2 or more (e.g., 2, 3, 4, 5). In some examples of Formula III when q is 2 or more, each m is the same. In some examples of Formula III when q is 2 or more, at least one m is different. For example, when q is 2, the first m can be an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, 7) and the second m can represent the same or different integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, 7). In some examples of Formula III when q is 2 or more, each R⁵ is the same. In some examples of Formula III when q is 2 or more, at least one R⁵ is different.

In some examples, the compound is defined by Formula IV, or a pharmaceutically acceptable salt thereof:



IV

wherein R¹, R², and R³ are each independently H, OH, halogen, substituted or unsubstituted C₁-C₃ alkyl, or substituted or unsubstituted C₁-C₃ alkoxy;

p is an integer from 0 to 5.

each m is independently an integer from 1 to 7;

y is an integer from 1 to 10;

n in each case is independently an integer from 1 to 10;

each R^4 , when present, is independently hydrogen or a substituted or unsubstituted C_1 - C_{18} alkyl;

each R^5 is independently a substituted or unsubstituted C_8 - C_{18} alkyl; and

5 R^6 and R^7 are each independently a substituted or unsubstituted C_8 - C_{18} alkyl.

In some examples of Formula IV, R^1 is OH. In some examples of Formula IV, R^1 is H. In some examples of Formula IV, R^1 is a halogen. In some examples of Formula IV, R^1 is a substituted or unsubstituted C_1 - C_3 alkyl. In some examples of Formula IV, R^1 is a substituted or unsubstituted C_1 - C_3 alkoxy.

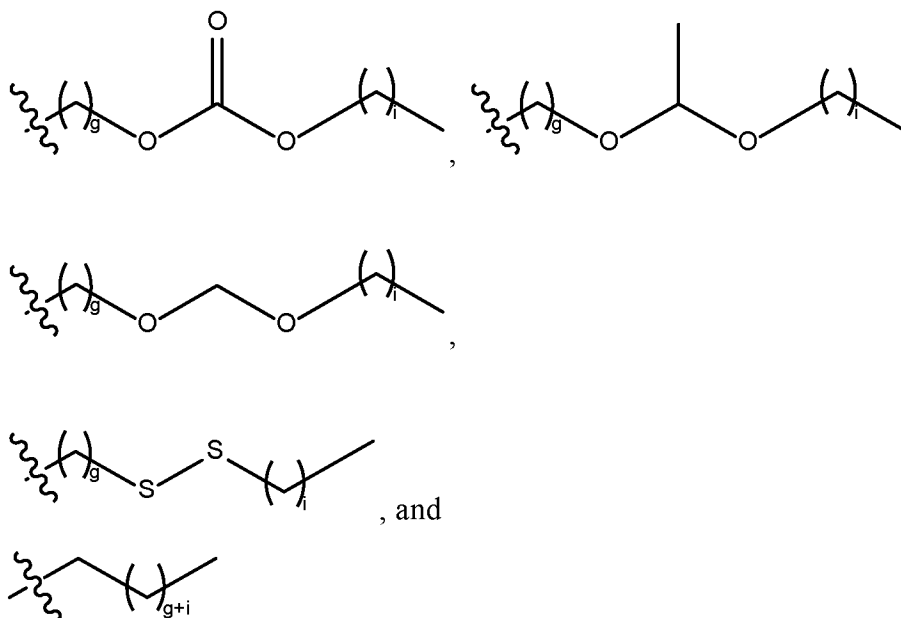
10 In some examples of Formula IV, R^2 is OH. In some examples of Formula IV, R^2 is H. In some examples of Formula IV, R^2 is a halogen. In some examples of Formula IV, R^2 is a substituted or unsubstituted C_1 - C_3 alkyl. In some examples of Formula IV, R^2 is a substituted or unsubstituted C_1 - C_3 alkoxy.

15 In some examples of Formula IV, R^3 is OH. In some examples of Formula IV, R^3 is H. In some examples of Formula IV, R^3 is a halogen. In some examples of Formula IV, R^3 is a substituted or unsubstituted C_1 - C_3 alkyl. In some examples of Formula IV, R^3 is a substituted or unsubstituted C_1 - C_3 alkoxy.

20 In some examples of Formula IV, R^1 , R^2 , and R^3 are all the same. In some examples of Formula IV, R^1 , R^2 , and R^3 are different. In some examples of Formula IV, only two of R^1 , R^2 , and R^3 are the same. In some examples of Formula IV, each of R^1 , R^2 , and R^3 are different.

25 In some examples of Formula IV, R^6 and R^7 are each independently an unsubstituted C_8 - C_{18} alkyl or a linear or branched C_8 - C_{18} alkyl substituted with one or more substituents selected from the group consisting of amine, amide, ester, ether, and carbonate ester. In some examples of Formula IV, R^6 and R^7 are each independently an unsubstituted C_8 - C_{18} alkyl. In some examples of Formula IV, R^6 and R^7 are each independently a linear or branched C_8 - C_{18} alkyl substituted with one or more substituents selected from the group consisting of amine, amide, ester, ether, and carbonate ester. In some examples of Formula IV, R^6 and R^7 are each independently an unsubstituted C_8 - C_{18} alkyl. In some examples of Formula IV, R^6 and R^7 are each independently an unsubstituted linear C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl). In some examples of Formula IV, R^6 and R^7 are independently selected from the group consisting of:

30



wherein G and I each independently represent integers from 1 to 9 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9).

In some examples of Formula IV, R^6 and R^7 are the same. In some examples of Formula IV, R^6 and R^7 are different.

In some examples of Formula IV, each R^4 , when present, is independently a substituted or unsubstituted C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl). In some examples of Formula IV, each R^4 , when present, is independently an unsubstituted C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl). In some examples of Formula IV, each R^4 , when present, is hydrogen.

In some examples of Formula IV, y is from 1 to 7. In some examples of Formula IV, y is from 1 to 6. In some examples of Formula IV, y is from 1 to 5. In some examples of Formula IV, y is from 1 to 4. In some examples of Formula IV, y is from 1 to 3. In some examples of Formula IV, y is 1.

In some examples of Formula IV, n is from 1 to 7. In some examples of Formula IV, n is from 1 to 6. In some examples of Formula IV, n is from 1 to 5. In some examples of Formula IV, n is from 1 to 4. In some examples of Formula IV, n is from 1 to 3. In some examples of Formula IV, n is 1.

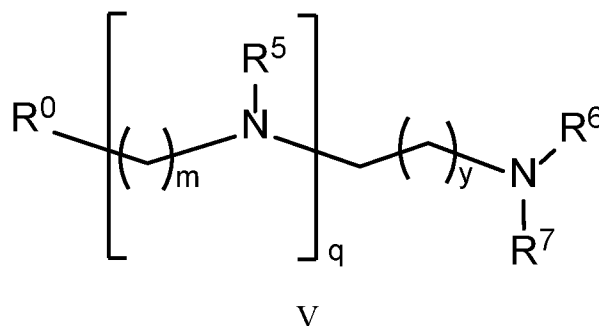
In some examples of Formula IV, m is from 1 to 7. In some examples of Formula IV, m is from 1 to 6. In some examples of Formula IV, m is from 1 to 5. In some examples of Formula IV, m is from 1 to 4. In some examples of Formula IV, m is from 1 to 3. In some examples of Formula IV, m is 1. In some examples of Formula IV, R^5 is a substituted or unsubstituted C_8 - C_{18} alkyl (e.g.,

a C₁₀-C₁₄ alkyl, or a C₁₂ alkyl). In some examples of Formula IV, R⁵ is an unsubstituted C₈-C₁₈ alkyl (e.g., a C₁₀-C₁₄ alkyl, or a C₁₂ alkyl).

In some examples of Formula IV, p is from 0 to 4. In some examples of Formula IV, p is from 0 to 3. In some examples of Formula IV, p is from 0 to 2. In some examples of Formula IV, p is from 0 to 1. In some examples of Formula IV, p is 0. In some examples of Formula IV, p is 1. In some examples of Formula IV, p is 2 or more (e.g., 2, 3, 4, 5). In some examples of Formula IV, where p is 2 or more, each R⁴ can be the same. In some examples of Formula IV, where p is 2 or more, at least one R⁴ is different. In some examples of Formula IV, where p is 2 or more, each n is the same. In some examples of Formula IV, where p is 2 or more, at least one n is different.

In some examples of Formula IV, each m is the same. In some examples of Formula IV, each m is different. For example, the first m can be an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, 7) and the second m can represent the same or different integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, 7). In some examples of Formula IV, each R⁵ is the same. In some examples of Formula IV, each R⁵ is different.

In some examples, the compound is defined by Formula V, or a pharmaceutically acceptable salt thereof:



wherein q is an integer from 0 to 5;

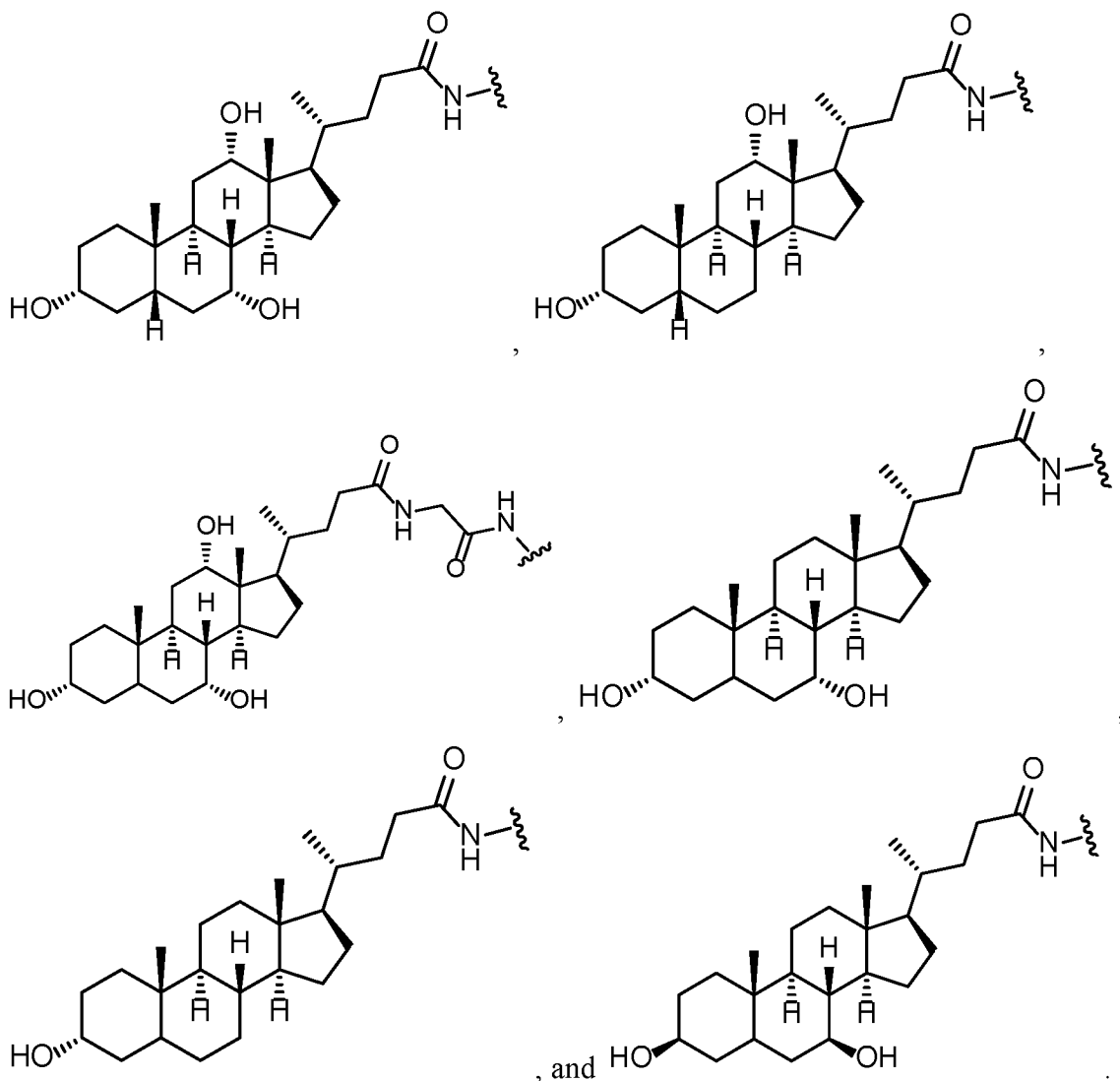
y is an integer from 1 to 7;

each m is independently an integer from 1 to 7;

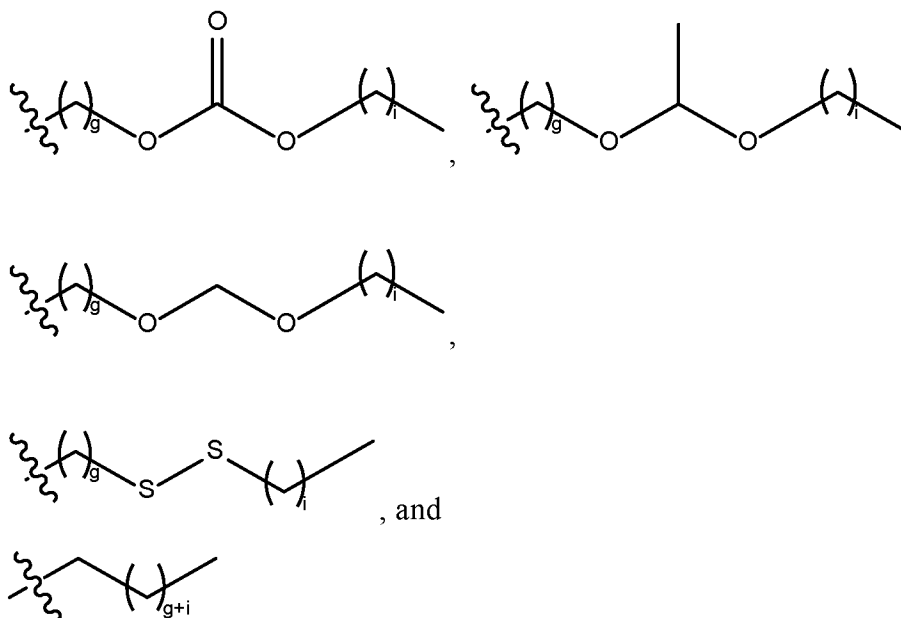
each R⁵, when present, is independently a substituted or unsubstituted C₈-C₁₈ alkyl;

R⁶ and R⁷ are each independently substituted or unsubstituted C₈-C₁₈ alkyl;

and R⁰ is selected from the group consisting of:



In some examples of Formula V, R^6 and R^7 are each independently an unsubstituted C₈-C₁₈ alkyl or a linear or branched C₈-C₁₈ alkyl substituted with one or more substituents selected from the group consisting of amine, amide, ester, ether, and carbonate ester. In some examples of Formula V, R^6 and R^7 are each independently an unsubstituted C₈-C₁₈ alkyl. In some examples of Formula V, R^6 and R^7 are each independently a linear or branched C₈-C₁₈ alkyl substituted with one or more substituents selected from the group consisting of amine, amide, ester, ether, and carbonate ester. In some examples of Formula V, R^6 and R^7 are each independently an unsubstituted C₈-C₁₈ alkyl. In some examples of Formula V, R^6 and R^7 are each independently an unsubstituted linear C₈-C₁₈ alkyl (e.g., a C₁₀-C₁₄ alkyl, or a C₁₂ alkyl). In some examples of Formula V, R^6 and R^7 are independently selected from the group consisting of:



wherein G and I each independently represent integers from 1 to 9 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9).

In some examples of Formula V, R^6 and R^7 are the same. In some examples of Formula V, R^6 and R^7 are different.

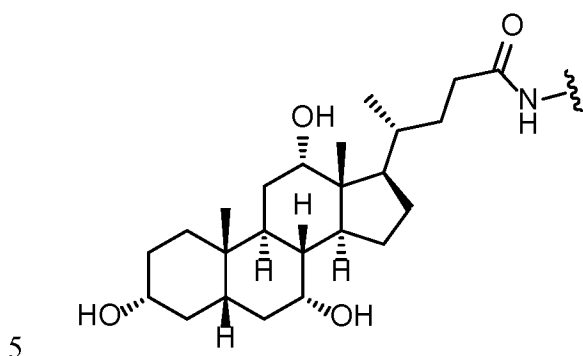
In some examples of Formula V, y is from 1 to 7. In some examples of Formula V, y is from 1 to 6. In some examples of Formula V, y is from 1 to 5. In some examples of Formula V, y is from 1 to 4. In some examples of Formula V, y is from 1 to 3. In some examples of Formula V, y is 1.

In some examples of Formula V, m is from 1 to 7. In some examples of Formula V, m is from 1 to 6. In some examples of Formula V, m is from 1 to 5. In some examples of Formula V, m is from 1 to 4. In some examples of Formula V, m is from 1 to 3. In some examples of Formula V, m is 1. In some examples of Formula V, R^5 is a substituted or unsubstituted C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl). In some examples of Formula V, R^5 is an unsubstituted C_8 - C_{18} alkyl (e.g., a C_{10} - C_{14} alkyl, or a C_{12} alkyl).

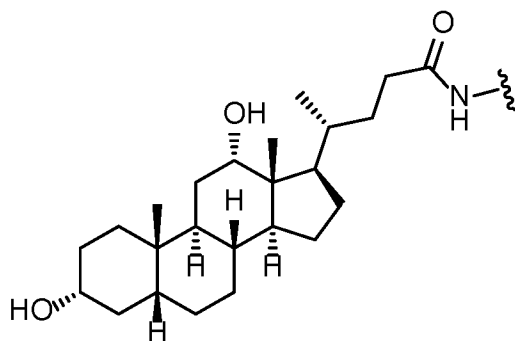
In some examples of Formula V, q is from 0 to 4. In some examples of Formula V, q is from 0 to 3. In some examples of Formula V, q is from 0 to 2. In some examples of Formula V, q is from 0 to 1. In some examples of Formula V, q is 0. In some examples of Formula V, q is 1. In some examples of Formula V, q is 2 or more. In some examples of Formula V when q is 2 or more, each m is the same. In some examples of Formula V when q is 2, at least one m is different. For example, when q is 2, the first m can be an integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, 7) and the second m can represent the same or different integer from 1 to 7 (e.g., 1, 2, 3, 4, 5, 6, 7). In some

examples of Formula V when q is 2 or more, each R^5 is the same. In some examples of Formula V when q is 2 or more, at least one R^5 is different.

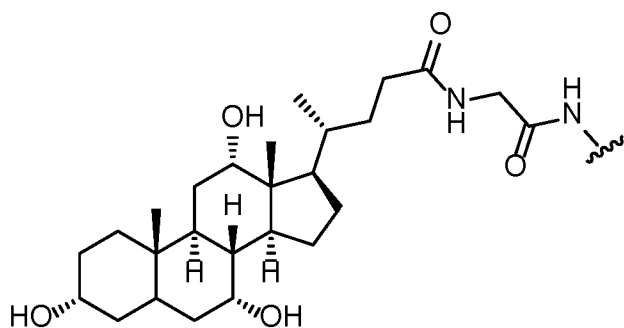
In some examples of Formula V, R^0 is:



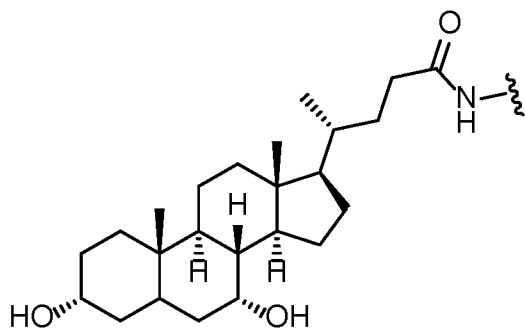
In some examples of Formula V, R^0 is:



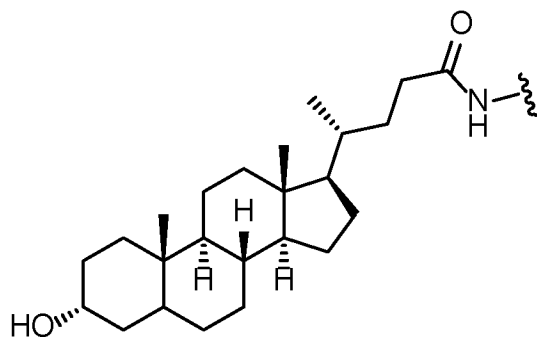
In some examples of Formula V, R^0 is:



In some examples of Formula V, R⁰ is:

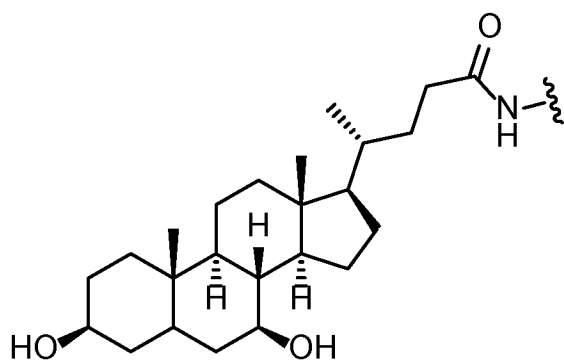


In some examples of Formula V, R⁰ is:

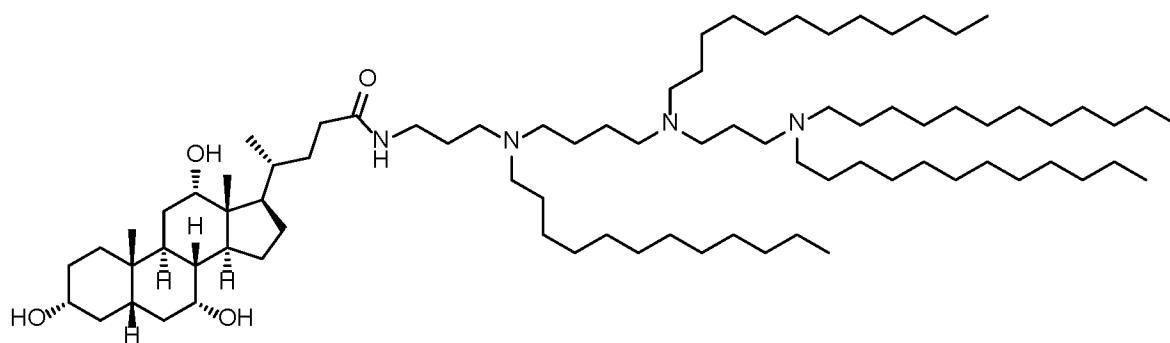
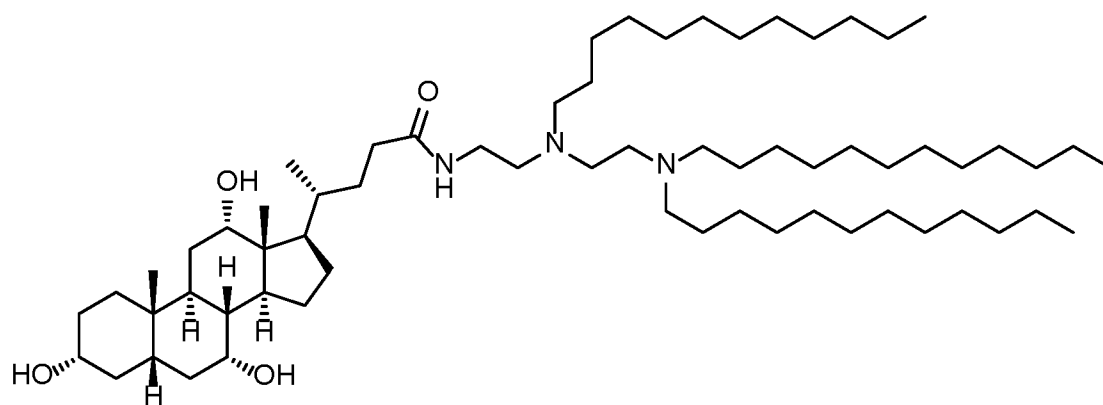
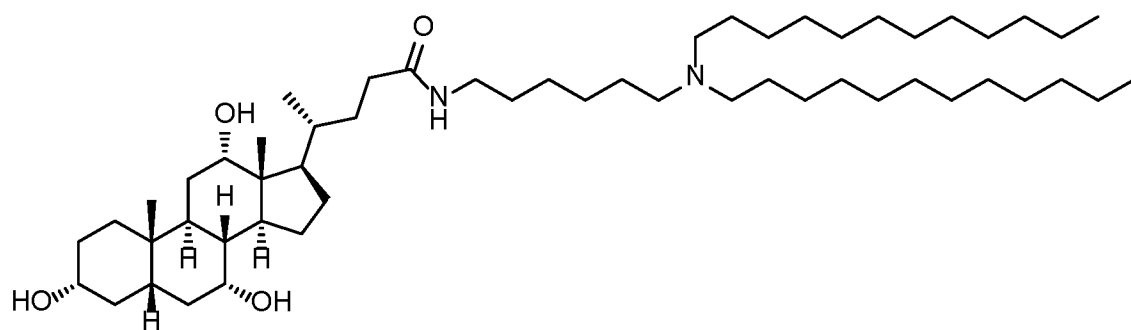
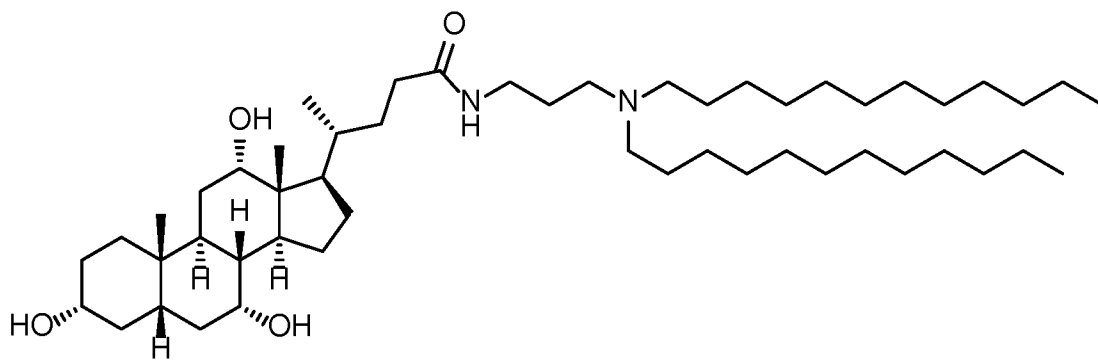


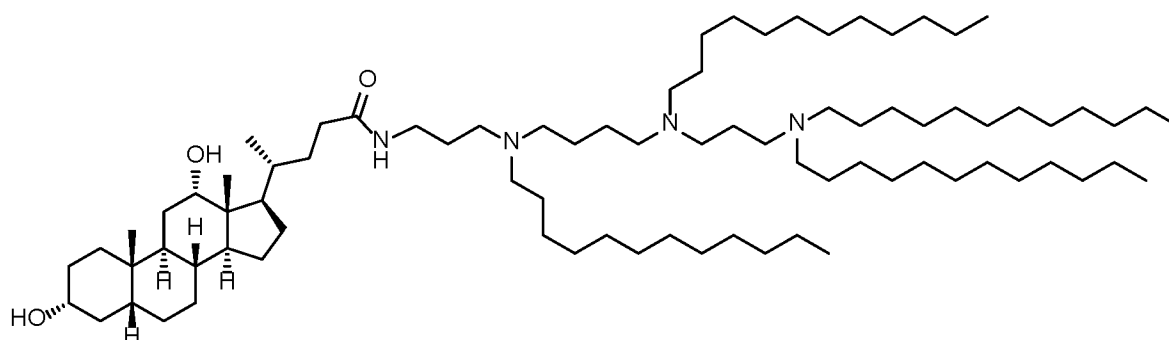
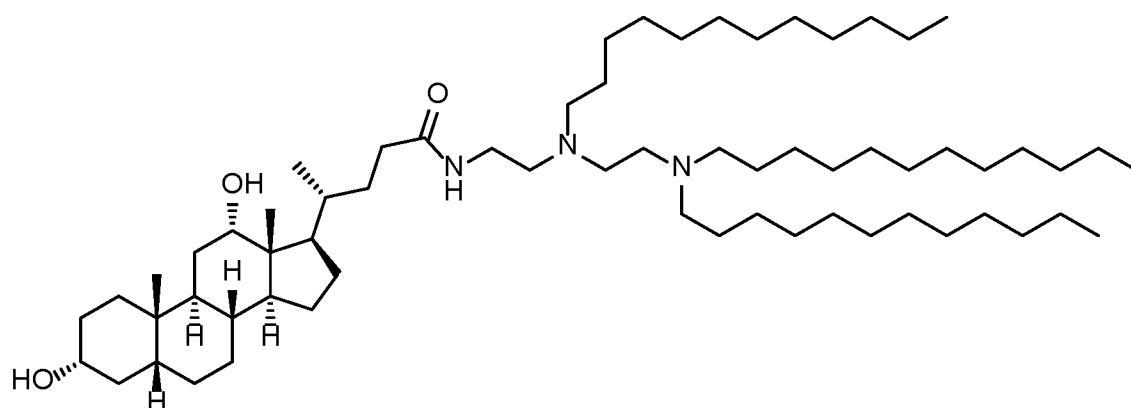
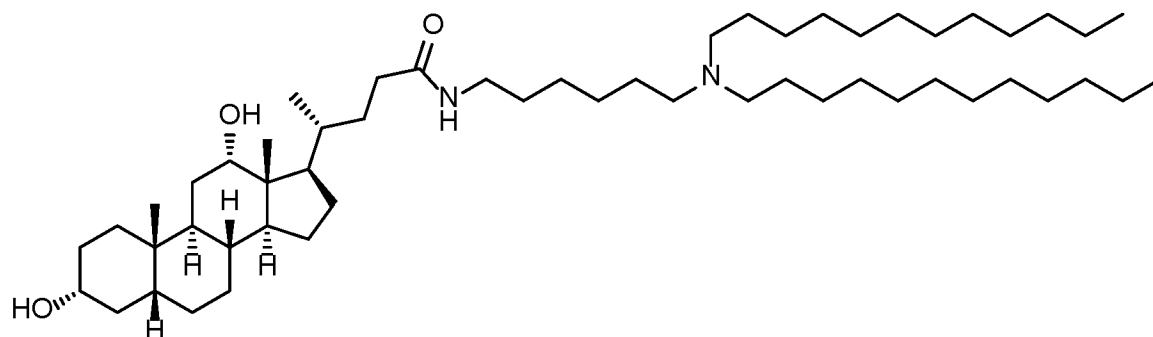
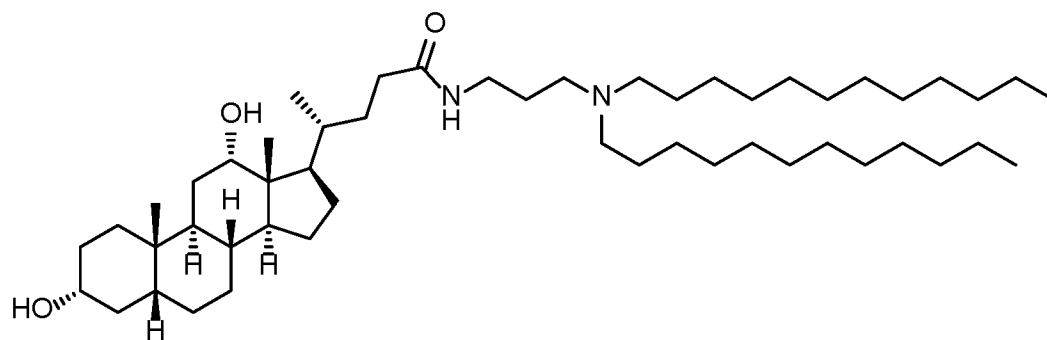
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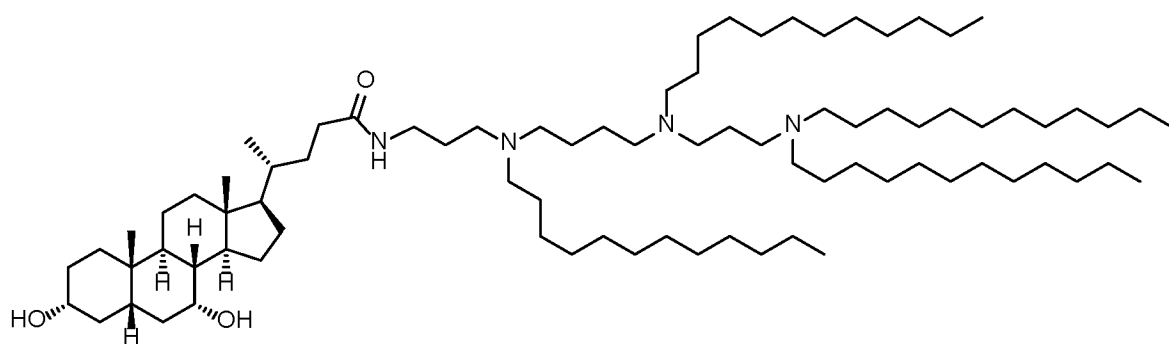
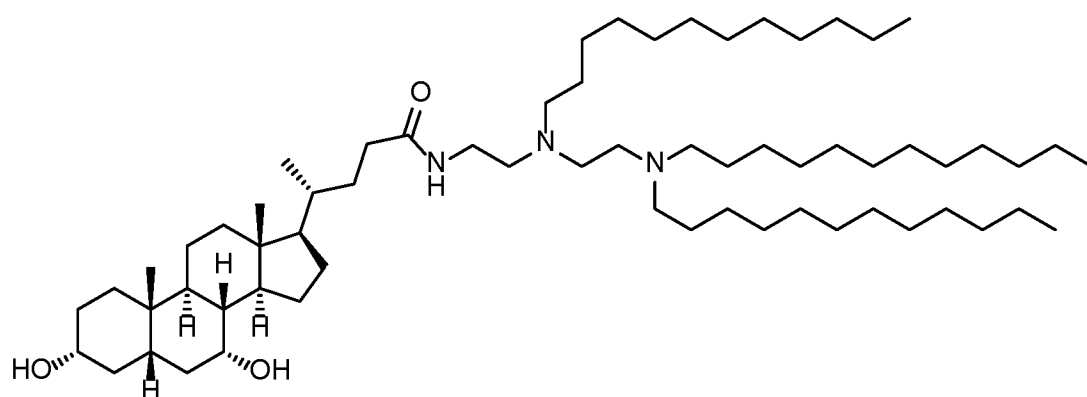
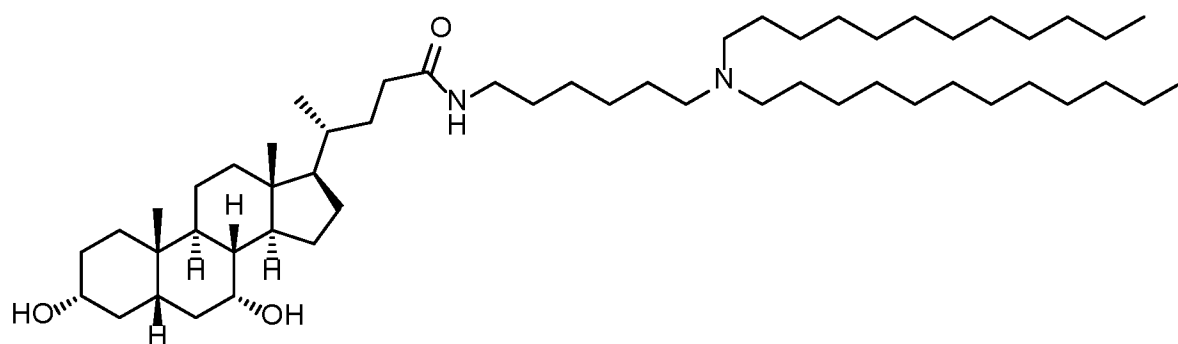
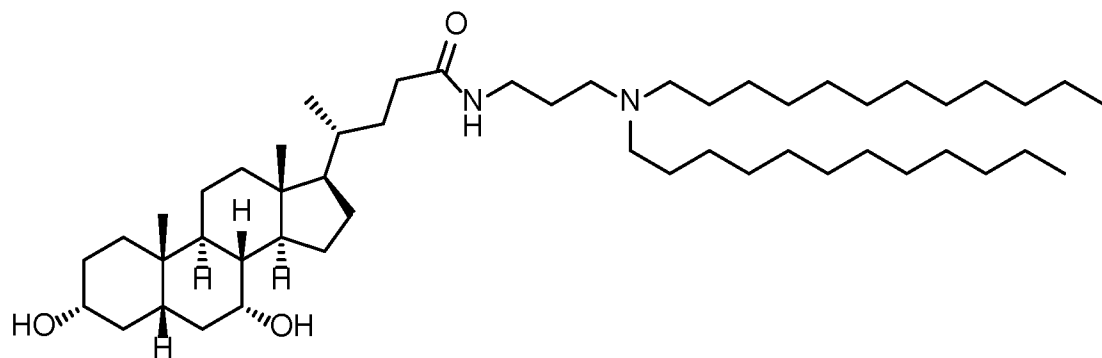
In some examples of Formula V, R⁰ is:

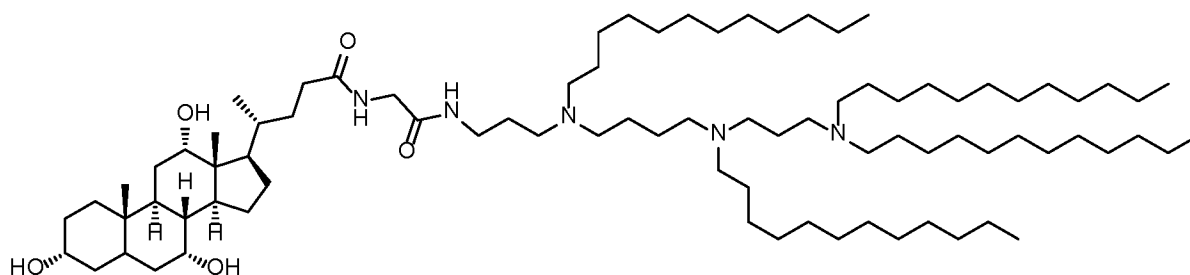
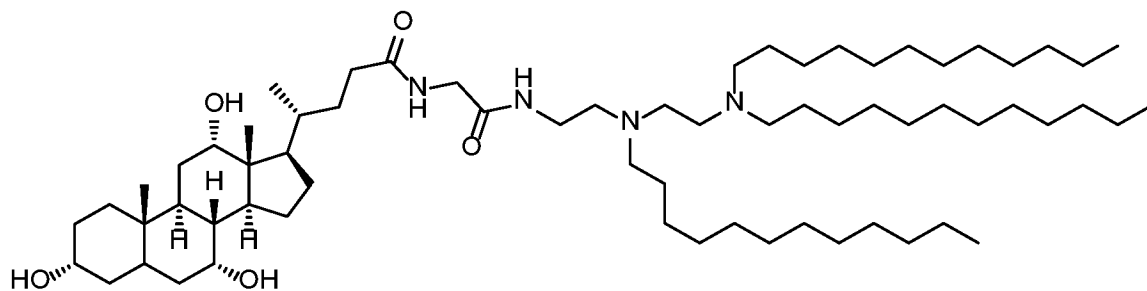
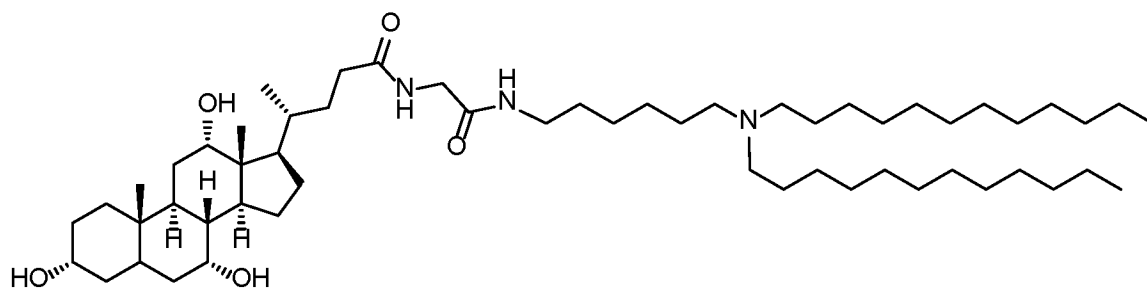
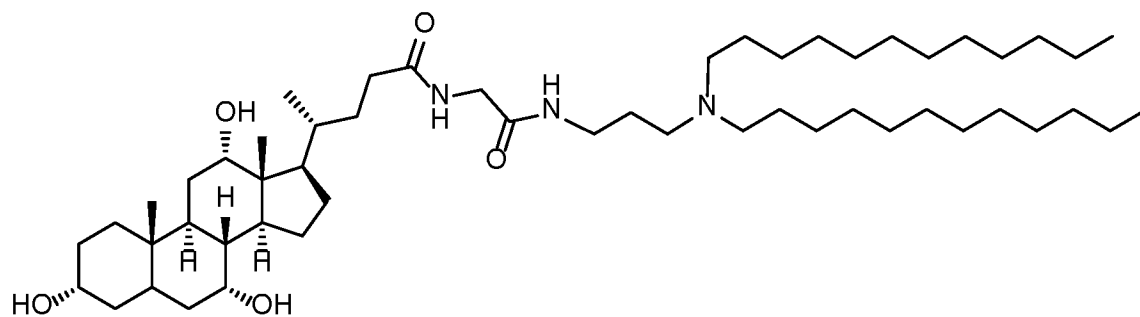


In some examples, the compound is selected from the group consisting of:



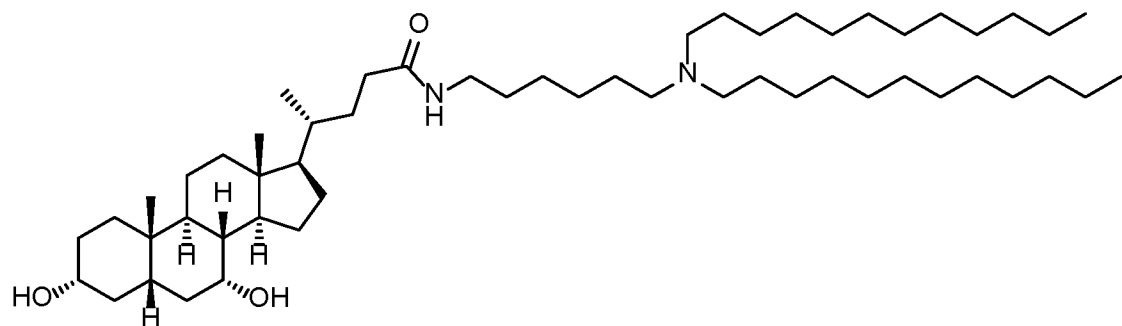
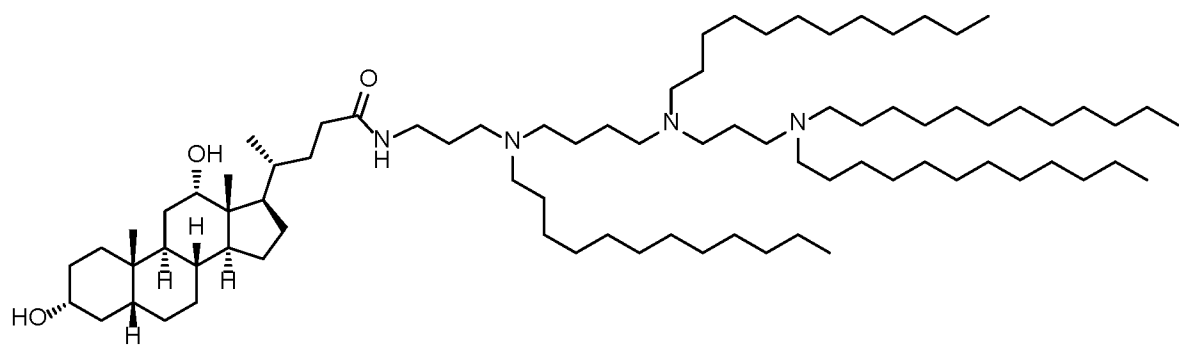
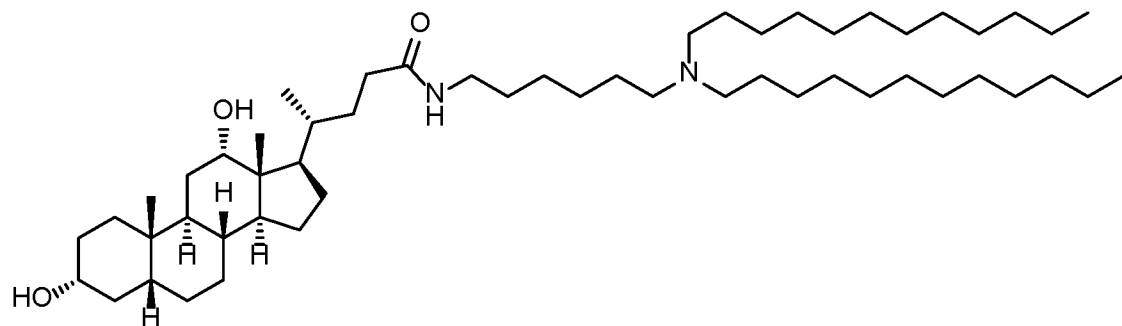
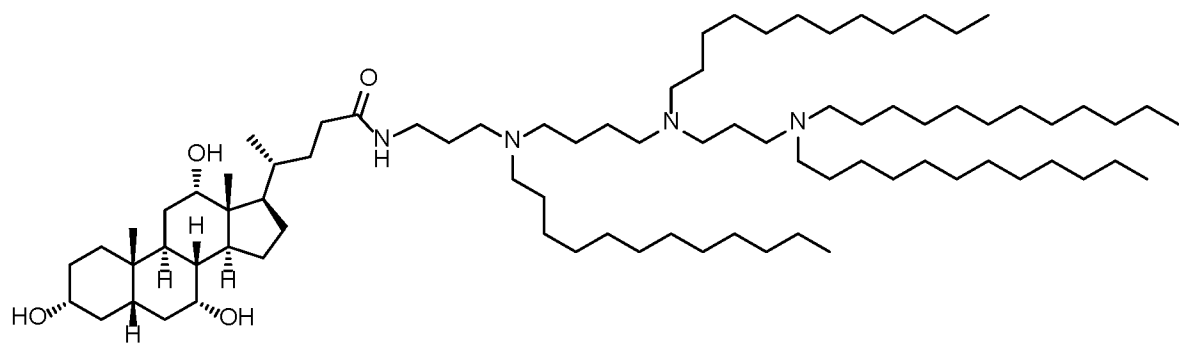


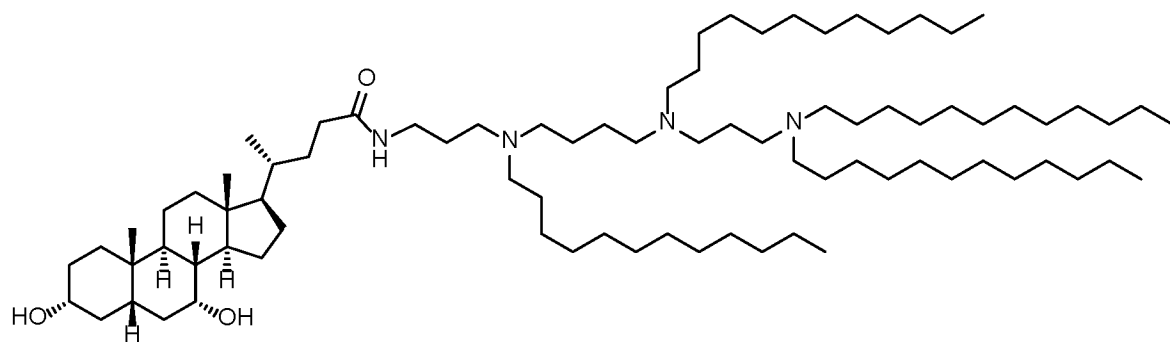




5 and pharmaceutically acceptable salts thereof, and combinations thereof.

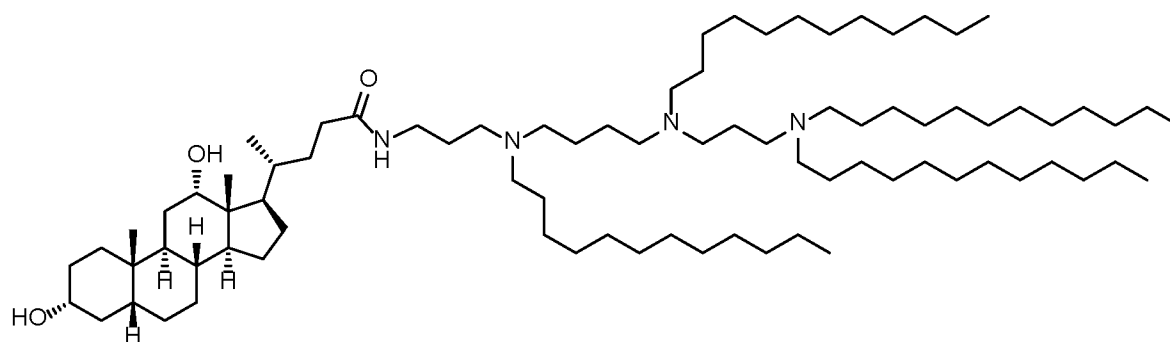
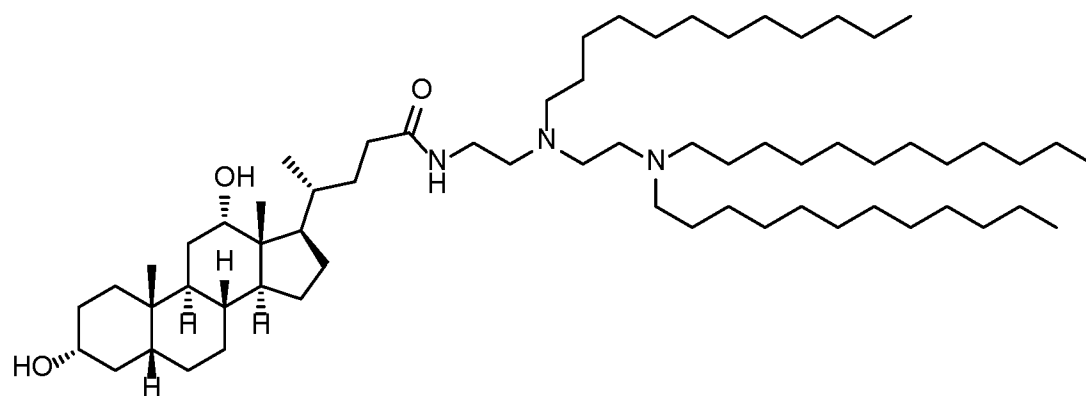
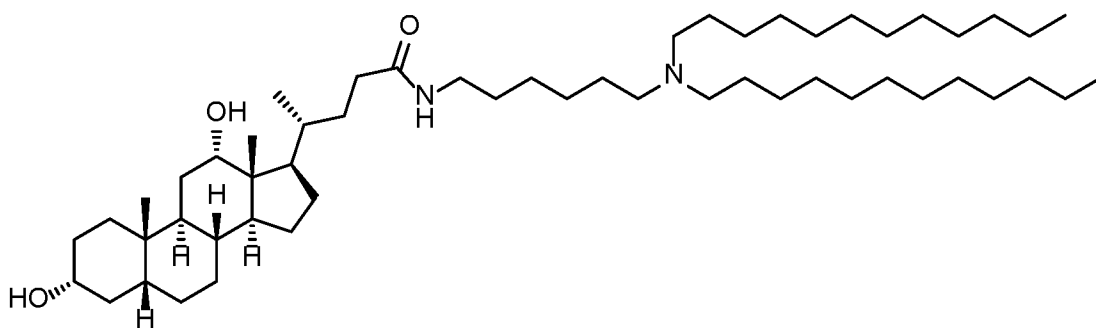
In some examples, the compound is selected from the group consisting of:

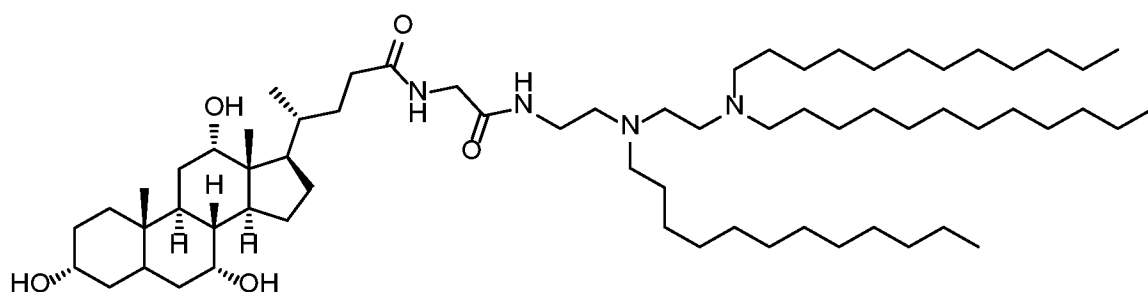
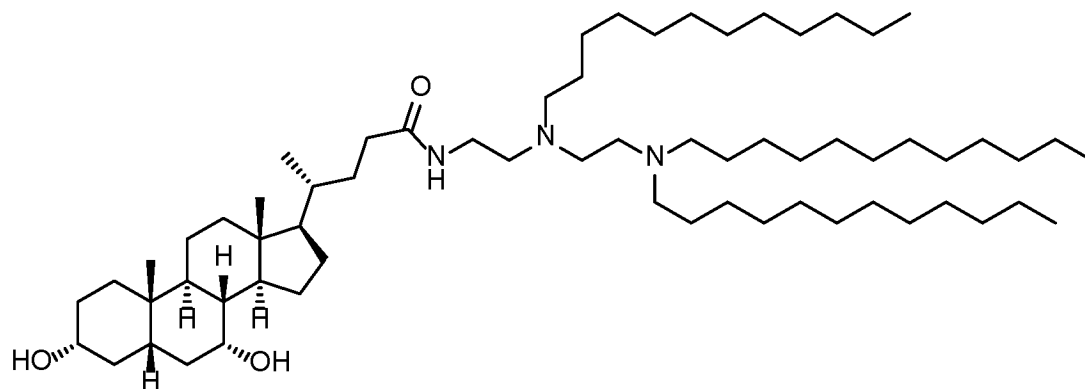




and pharmaceutically acceptable salts thereof, and combinations thereof.

In some examples, the compound is selected from the group consisting of:

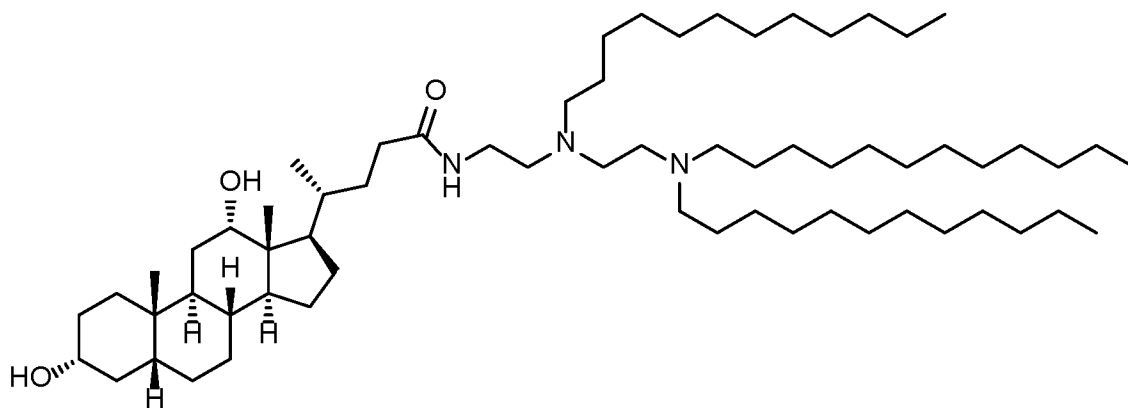




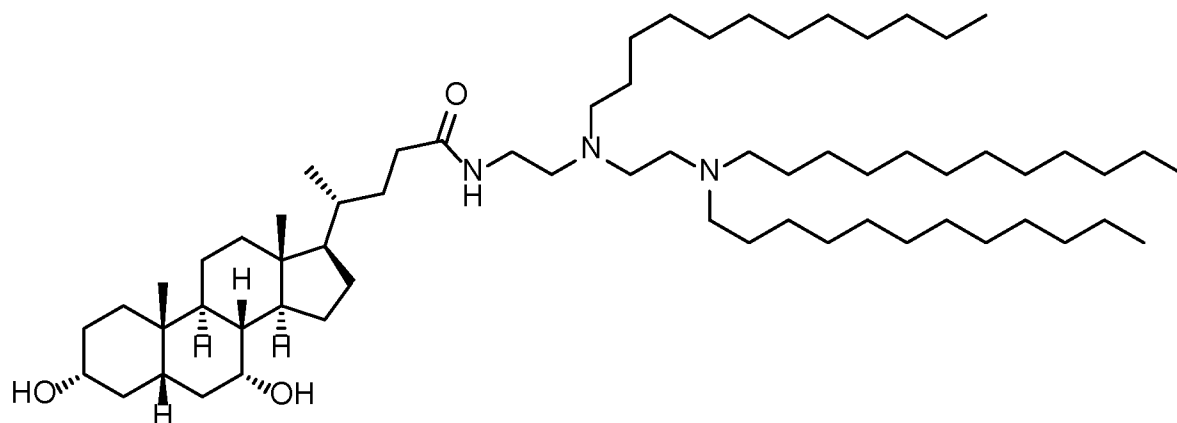
, and

pharmaceutically acceptable salts thereof, and combinations thereof.

In some examples, the compound is selected from the group consisting of:

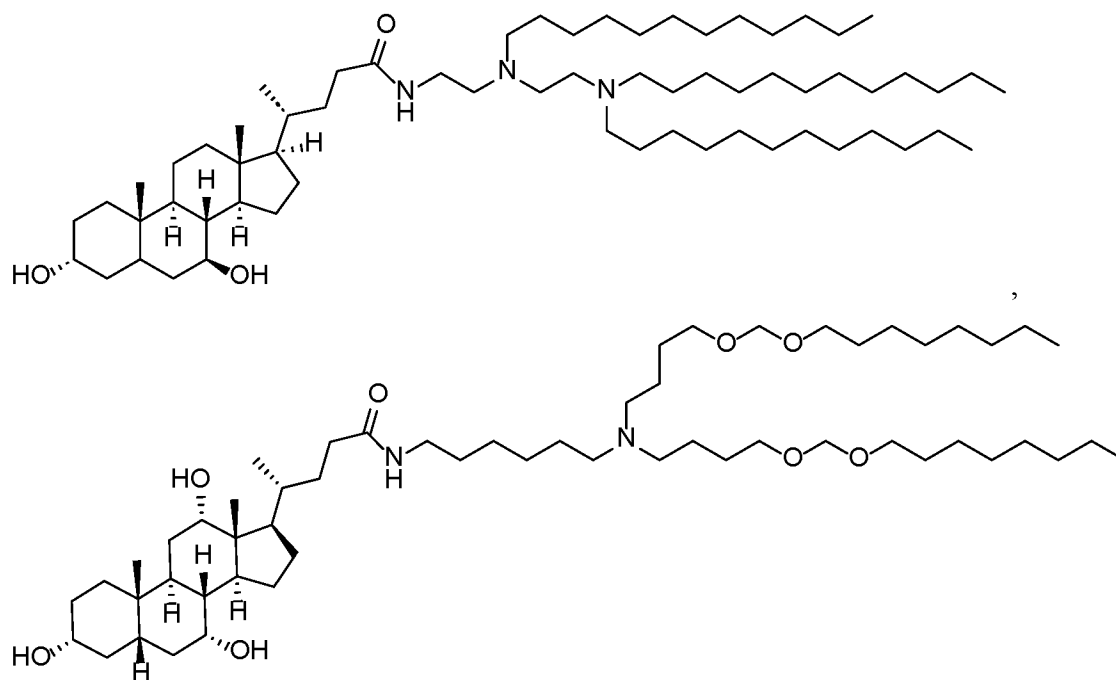


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and pharmaceutically acceptable salts thereof, and combinations thereof.

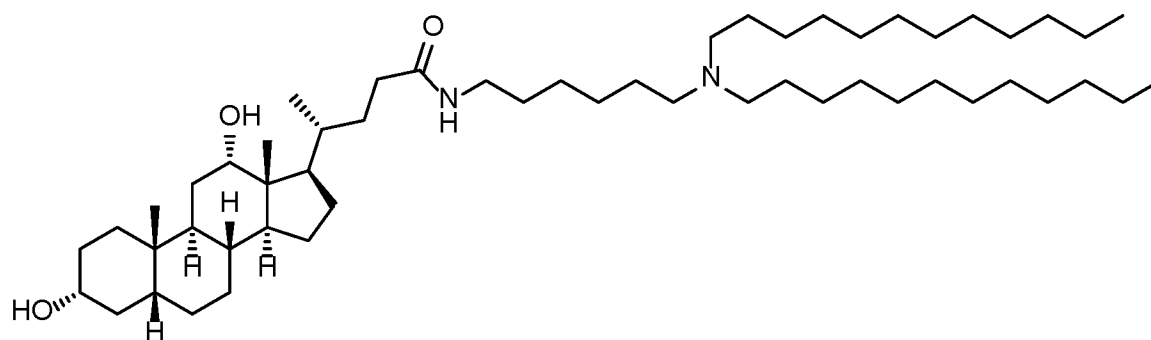
In some examples, the compound is selected from the group consisting of:



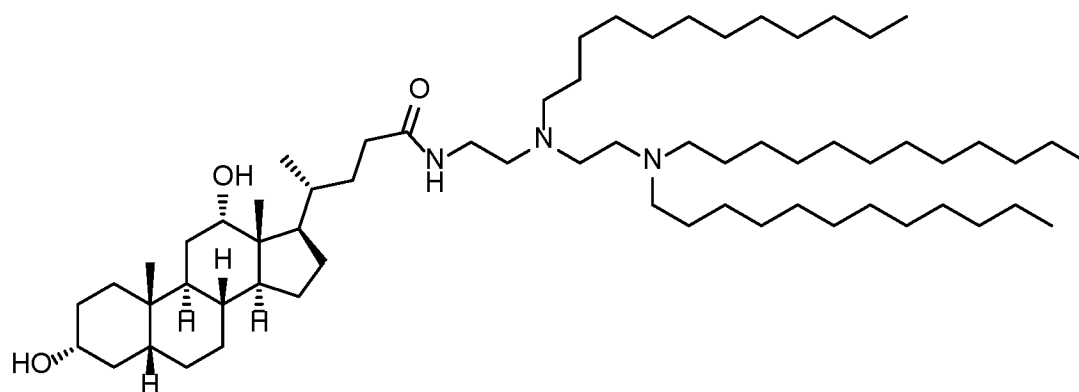
, and

pharmaceutically acceptable salts thereof, and combinations thereof.

5 In some examples, the compound comprises:



In some examples, the compound comprises:



Lipid Nanoparticles

Also disclosed herein is a lipid nanoparticle (e.g., one or more nanoparticles) comprising any of the compounds disclosed herein.

In one aspect, the disclosure provides a nanoparticle comprising:

- 5 a compound of any one of Formulas I-V;
a non-cationic lipid;
a polyethylene glycol-lipid; and
a sterol.

10 In one aspect, the disclosure provides a nanoparticle comprising: a compound of Formula I; a non-cationic lipid; a polyethylene glycol-lipid; and a sterol.

In one aspect, the disclosure provides a nanoparticle comprising: a compound of Formula II; a non-cationic lipid; a polyethylene glycol-lipid; and a sterol.

In one aspect, the disclosure provides a nanoparticle comprising: a compound of Formula III; a non-cationic lipid; a polyethylene glycol-lipid; and a sterol.

15 In one aspect, the disclosure provides a nanoparticle comprising: a compound of Formula IV; a non-cationic lipid; a polyethylene glycol-lipid; and a sterol.

In one aspect, the disclosure provides a nanoparticle comprising: a compound of Formula V; a non-cationic lipid; a polyethylene glycol-lipid; and a sterol.

20 The various compounds of Formulas I-V, including the specific example compounds, are described in the Compounds section above. In some embodiments, the nanoparticle comprises a compound of Formulas I-V in a molar ratio of about 10% to about 40%. In some embodiments, the nanoparticle comprises a compound of Formulas I-V in a molar ratio of about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, or about 40%. In one embodiment, the nanoparticle comprises a compound of Formulas I-V in a molar ratio of about 20%.

25 In some embodiments, the nanoparticle comprises a non-cationic lipid. In some embodiments, the non-cationic lipid interacts with the lipids as a helper lipid. In some embodiments, the non-cationic lipid can include, but is not limited to, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (SOPE), DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine), 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), 1,2-

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dioleoyl-5/7-glycero-3-phospho-(l'-rac-glycerol) (DOPG), or combinations thereof. In one embodiment, the non-cationic lipid is 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE). In one embodiment, the non-cationic lipid is 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE). In one embodiment, the non-cationic lipid is 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC). In one embodiment, the non-cationic lipid is 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (SOPE). While several non-cationic lipids are described here, additional non-cationic lipids can be used in combination with the compounds disclosed herein.

In some embodiments, the nanoparticle comprises a non-cationic lipid in a molar ratio of about 10% to about 40%. In some embodiments, the nanoparticle comprises a non-cationic lipid in a molar ratio of about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, or about 40%. In one embodiment, the nanoparticle comprises a non-cationic lipid in a molar ratio of about 30%.

In some embodiments, the nanoparticle includes a polyethylene glycol-lipid (PEG-lipid). PEG-lipid is incorporated to form a hydrophilic outer layer and stabilize the particles. Nonlimiting examples of polyethylene glycol-lipids include PEG-modified lipids such as PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, and PEG-modified dialkylglycerols. Representative polyethylene glycol-lipids include DMG-PEG, DLPE-PEGs, DMPE-PEGs, DPPC-PEGs, and DSPE-PEGs. In one embodiment, the polyethylene glycol-lipid is 1,2-dimyristoyl-*sn*-glycerol, methoxypolyethylene glycol (DMG-PEG). In one embodiment, the polyethylene glycol-lipid is 1,2-dimyristoyl-*sn*-glycerol, methoxypolyethylene glycol-2000 (DMG-PEG2000). DMG-PEGXXXX means 1,2-dimyristoyl-*sn*-glycerol, methoxypolyethylene glycol-XXXX, wherein XXXX signifies the molecular weight of the polyethylene glycol moiety, e.g. DMG-PEG2000 or DMG-PEG5000.

In some embodiments, the nanoparticle comprises a polyethylene glycol-lipid in a molar ratio of about 0% to about 5%. In some embodiments, the nanoparticle comprises a polyethylene glycol-lipid in a molar ratio of about 0%, about 0.25%, about 0.5%, about 0.75%, about 1%, about 1.5%, about 2%, about 3%, about 4%, or about 5%. In one embodiment, the nanoparticle comprises a polyethylene glycol-lipid in a molar ratio of about 0.75%.

In some embodiments, the nanoparticle includes a sterol. Sterols are well known to those skilled in the art and generally refers to those compounds having a

perhydrocyclopentanophenanthrene ring system and having one or more OH substituents. Examples of sterols include, but are not limited to, cholesterol, campesterol, ergosterol, sitosterol, and the like.

5 In some embodiments, the sterol is selected from a cholesterol-based lipid. In some embodiments, the one or more cholesterol-based lipids are selected from cholesterol, PEGylated cholesterol, DC-Choi (N,N-dimethyl-N-ethylcarboxamidocholesterol), 1,4-bis(3-N-oleylamino-propyl)piperazine, or combinations thereof.

The sterol can be used to tune the particle permeability and fluidity base on its function in cell membranes. In one embodiment, the sterol is cholesterol.

10 In some embodiments, the nanoparticle comprises a sterol in a molar ratio of about 25% to about 50%. In some embodiments, the nanoparticle comprises a sterol in a molar ratio of about 25%, about 30%, about 35%, about 40%, about 45%, or about 50%. In one embodiment, the nanoparticle comprises a sterol in a molar ratio of about 40%.

In one embodiment, the disclosure provides a nanoparticle comprising:
15 a compound of Formulas I-V;
1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE);
1,2-dimyristoyl-*sn*-glycerol, methoxypolyethylene glycol (DMG-PEG₂₀₀₀); and
cholesterol.

In one embodiment, the disclosure provides a nanoparticle comprising:
20 a compound of Formulas I-V;
1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE);
1,2-dimyristoyl-*sn*-glycerol, methoxypolyethylene glycol (DMG-PEG₂₀₀₀); and
cholesterol.

In one embodiment, the disclosure provides a nanoparticle comprising:
25 a compound of Formulas I-V;
1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC);
1,2-dimyristoyl-*sn*-glycerol, methoxypolyethylene glycol (DMG-PEG₂₀₀₀); and
cholesterol.

In one embodiment, the nanoparticle further comprises an agent. In one embodiment, the
30 nanoparticle further comprises a therapeutic agent. In one embodiment, the nanoparticle further comprises a diagnostic agent.

The agents delivered into cells can be a polynucleotide. Polynucleotides or oligonucleotides that can be introduced according to the methods herein include DNA, cDNA, and RNA sequences of all types. For example, the polynucleotide can be double stranded DNA, single-stranded DNA, complexed DNA, encapsulated DNA, naked RNA, encapsulated RNA, messenger RNA (mRNA), tRNA, short interfering RNA (siRNA), double stranded RNA (dsRNA), micro-RNA (miRNA), antisense RNA (asRNA) and combinations thereof. The polynucleotides can also be DNA constructs, such as expression vectors, expression vectors encoding a desired gene product (e.g., a gene product homologous or heterologous to the subject into which it is to be introduced), and the like. In one embodiment, the agent is an mRNA.

The nanoparticle can be of any shape. (e.g., a sphere, a rod, a quadrilateral, an ellipse, a triangle, a polygon, etc.). In some examples, the nanoparticle can have a regular shape, an irregular shape, an isotropic shape, an anisotropic shape, or a combination thereof. In some examples, the lipid particle are substantially spherical in shape.

The lipid particles can have an average particle size. “Average particle size” and “mean particle size” are used interchangeably herein, and generally refer to the statistical mean particle size of the particles in a population of particles. For example, the average particle size for a plurality of particles with a substantially spherical shape can comprise the average diameter of the plurality of particles. For a particle with a substantially spherical shape, the diameter of a particle can refer, for example, to the hydrodynamic diameter. As used herein, the hydrodynamic diameter of a particle can refer to the largest linear distance between two points on the surface of the particle. Mean particle size can be measured using methods known in the art, such as evaluation by scanning electron microscopy, transmission electron microscopy, and/or dynamic light scattering.

The lipid particles can, for example, have an average particle size of 30 nanometers (nm) or more (e.g., 40 nm or more, 50 nm or more, 60 nm or more, 70 nm or more, 80 nm or more, 90 nm or more, 100 nm or more, 110 nm or more, 120 nm or more, 130 nm or more, 140 nm or more, 150 nm or more, 160 nm or more, 170 nm or more, 180 nm or more, 190 nm or more, 200 nm or more, 225 nm or more, 250 nm or more, 275 nm or more, 300 nm or more, 325 nm or more, 350 nm or more, 375 nm or more, 400 nm or more, 425 nm or more, 450 nm or more, 475 nm or more, 500 nm or more, 550 nm or more, 600 nm or more, 650 nm or more, 700 nm or more, or 750 nm or more). In some examples, the lipid particles can have an average particle size of 800 nm or less (e.g., 750 nm or less, 700 nm or less, 650 nm or less, 600 nm or less, 550

nm or less, 500 nm or less, 475 nm or less, 450 nm or less, 425 nm or less, 400 nm or less, 375 nm or less, 350 nm or less, 325 nm or less, 300 nm or less, 275 nm or less, 250 nm or less, 225 nm or less, 200 nm or less, 190 nm or less, 180 nm or less, 170 nm or less, 160 nm or less, 150 nm or less, 140 nm or less, 130 nm or less, 120 nm or less, 110 nm or less, 100 nm or less, 90 nm or less, 80 nm or less, 70 nm or less, 60 nm or less, 50 nm or less, or 40 nm or less). The average particle size of the lipid particles can range from any of the minimum values described above to any of the maximum values described above. For example, the lipid particles can have an average particle size of from 30 nm to 800 nm (e.g., from 30 nm to 425 nm, from 425 nm to 800 nm, from 30 nm to 200 nm, from 200 nm to 400 nm, from 400 nm to 600 nm, from 600 nm to 800 nm, from 50 nm to 800 nm, from 30 nm to 750 nm, or from 50 nm to 750 nm).

With respect to particle size distribution characterization, a parameter used to define the size range of the lipid particles is called the “polydispersity index” (PDI). The term “polydispersity” (or “dispersity” as recommended by IUPAC) is used to describe the degree of non-uniformity of a size distribution of particles. PDI is basically a representation of the distribution of size populations within a given sample. The numerical value of PDI ranges from 0.0 (for a perfectly uniform sample with respect to the particle size) to 1.0 (for a highly polydisperse sample with multiple particle size populations).

In some examples, the lipid particles can have a polydispersity index of 0.5 or less (e.g., 0.49 or less, 0.48 or less, 0.47 or less, 0.46 or less, 0.45 or less, 0.44 or less, 0.43 or less, 0.42 or less, 0.41 or less, 0.40 or less, 0.39 or less, 0.38 or less, 0.37 or less, 0.36 or less, 0.35 or less, 0.34 or less, 0.33 or less, 0.32 or less, 0.31 or less, 0.30 or less, 0.29 or less, 0.28 or less, 0.27 or less, 0.26 or less, 0.25 or less, 0.24 or less, 0.23 or less, 0.22 or less, 0.21 or less, 0.20 or less, 0.19 or less, 0.18 or less, 0.17 or less, 0.16 or less, 0.15 or less, 0.14 or less, 0.13 or less, 0.12 or less, 0.11 or less, 0.10 or less, 0.09 or less, 0.08 or less, 0.07 or less, 0.06 or less, 0.05 or less, 0.04 or less, 0.03 or less, 0.02 or less, or 0.01 or less).

In some examples, the lipid particles can be substantially monodisperse. “Monodisperse” and “homogeneous size distribution,” as used herein, and generally describe a population of particles where all of the particles are the same or nearly the same size. As used herein, a monodisperse distribution refers to particle distributions in which 80% of the distribution (e.g., 85% of the distribution, 90% of the distribution, or 95% of the distribution) lies within 25% of the median particle size (e.g., within 20% of the median particle size, within 15% of the median particle size, within 10% of the median particle size, or within 5% of the median particle size).

Compositions

Compositions, as described herein, comprising an active compound and an excipient of some sort may be useful in a variety of medical and non-medical applications. For example, pharmaceutical compositions comprising an active compound and an excipient may be useful in the delivery of an effective amount of an agent to a subject in need thereof. Nutraceu-
5 tical compositions comprising an active compound and an excipient may be useful in the delivery of an effective amount of a nutraceutical, e.g., a dietary supplement, to a subject in need thereof. Cosmetic compositions comprising an active compound and an excipient may be formulated as a cream, ointment, balm, paste, film, or liquid, etc., and may be useful in the application of make-
10 up, hair products, and materials useful for personal hygiene, etc. Compositions comprising an active compound and an excipient may be useful for non-medical applications, e.g., such as an emulsion or emulsifier, useful, for example, as a food component, for extinguishing fires, for disinfecting surfaces, for oil cleanup, etc.

In certain embodiments, the composition further comprises an agent, as described herein. For example, in certain embodiments, the agent is a small molecule, organometallic compound, nucleic acid, protein, peptide, polynucleotide, metal, targeting agent, an isotopically labeled chemical compound, drug, vaccine, immunological agent, or an agent useful in bioprocessing. In certain embodiments, the agent is a polynucleotide. In certain embodiments, the polynucleotide is DNA or RNA. In certain embodiments, the RNA is RNAi, dsRNA, siRNA, shRNA, miRNA, or
20 antisense RNA. In certain embodiments, the polynucleotide and the one or more active compounds are not covalently attached.

In one aspect, the disclosure provides a composition comprising:
a compound of Formulas I-V; and
an agent.

25 In one aspect, the disclosure provides a composition comprising:
a nanoparticle, comprising a compound of Formulas I-V; and
an agent.

In another aspect, disclosed herein is a composition comprising:
a nanoparticle, comprising a compound of Formulas I-V; and an agent,

30 wherein the agent comprises an mRNA encoding at least one antigenic polypeptide or an immunogenic fragment thereof capable of inducing an immune response to the antigenic polypeptide.

In some embodiments, the mRNA encoding at least one antigenic polypeptide or an immunogenic fragment thereof capable of inducing an immune response to the antigenic polypeptide is encapsulated by the nanoparticle.

In some aspects, disclosed herein is a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a nanoparticle comprising an mRNA at least one antigenic polypeptide or an immunogenic fragment thereof capable of inducing an immune response to the antigenic polypeptide.

Agents

Agents to be delivered by the compounds, compositions, and systems described herein may be therapeutic, diagnostic, or prophylactic agents. Any chemical compound to be administered to a subject may be delivered using the particles or nanoparticles described herein. The agent may be an organic molecule (e.g., a therapeutic agent, a drug), inorganic molecule, nucleic acid, protein, amino acid, peptide, polypeptide, polynucleotide, targeting agent, isotopically labeled organic or inorganic molecule, vaccine, immunological agent, etc.

In certain embodiments, the agents are organic molecules with pharmaceutical activity, e.g., a drug. In certain embodiments, the drug is an antibiotic, anti-viral agent, anesthetic, steroidal agent, anti-inflammatory agent, anti-neoplastic agent, anti-cancer agent, antigen, vaccine, antibody, decongestant, antihypertensive, sedative, birth control agent, progestational agent, anticholinergic, analgesic, anti-depressant, anti-psychotic, β -adrenergic blocking agent, diuretic, cardiovascular active agent, vasoactive agent, non-steroidal anti-inflammatory agent, nutritional agent, etc.

In certain embodiments of the present disclosure, the agent to be delivered may be a mixture of agents.

Diagnostic agents include gases; metals; commercially available imaging agents used in positron emissions tomography (PET), computer assisted tomography (CAT), single photon emission computerized tomography, x-ray, fluoroscopy, and magnetic resonance imaging (MRI); and contrast agents. Examples of suitable materials for use as contrast agents in MRI include gadolinium chelates, as well as iron, magnesium, manganese, copper, and chromium. Examples of materials useful for CAT and x-ray imaging include iodine-based materials.

Therapeutic and prophylactic agents include, but are not limited to, antibiotics, nutritional supplements, and vaccines. Vaccines may comprise isolated proteins or peptides, inactivated

organisms and viruses, dead organisms and viruses, genetically altered organisms or viruses, cell extracts, and RNA encoding at least one antigenic polypeptide or an immunogenic fragment thereof (e.g., an immunogenic fragment capable of inducing an immune response to the antigenic polypeptide). Therapeutic and prophylactic agents may be combined with interleukins, interferon, cytokines, and adjuvants such as cholera toxin, alum, Freund's adjuvant, etc. Prophylactic agents include antigens of such bacterial organisms as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium diphtheriae*, *Listeria monocytogenes*, *Bacillus anthracis*, *Clostridium tetani*, *Clostridium botulinum*, *Clostridium perfringens*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Haemophilus parainfluenzae*, *Bordetella pertussis*, *Francisella tularensis*, *Yersinia pestis*, *Vibrio cholerae*, *Legionella pneumophila*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Treponema pallidum*, *Leptospira interrogans*, *Borrelia burgdorferi*, *Campylobacter jejuni*, and the like; antigens of such viruses as smallpox, influenza A and B, respiratory syncytial virus, parainfluenza, measles, HIV, varicella-zoster, herpes simplex 1 and 2, cytomegalovirus, Epstein-Barr virus, rotavirus, rhinovirus, adenovirus, papillomavirus, poliovirus, mumps, rabies, rubella, coxsackieviruses, equine encephalitis, Japanese encephalitis, yellow fever, Rift Valley fever, hepatitis A, B, C, D, and E virus, and the like; antigens of fungal, protozoan, and parasitic organisms such as *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Candida albicans*, *Candida tropicalis*, *Nocardia asteroides*, *Rickettsia rickettsii*, *Rickettsia typhi*, *Mycoplasma pneumoniae*, *Chlamydia psittaci*, *Chlamydia trachomatis*, *Plasmodium falciparum*, *Trypanosoma brucei*, *Entamoeba histolytica*, *Toxoplasma gondii*, *Trichomonas vaginalis*, *Schistosoma mansoni*, and the like. These antigens may be in the form of whole killed organisms, peptides, proteins, glycoproteins, carbohydrates, or combinations thereof.

In some aspects, the agent is a ribonucleic acid (RNA) (e.g., mRNA) polynucleotide having an open reading frame encoding at least one (e.g., at least 2, 3, 4 or 5) antigenic polypeptide or an immunogenic fragment thereof (e.g., an immunogenic fragment capable of inducing an immune response to the antigenic polypeptide).

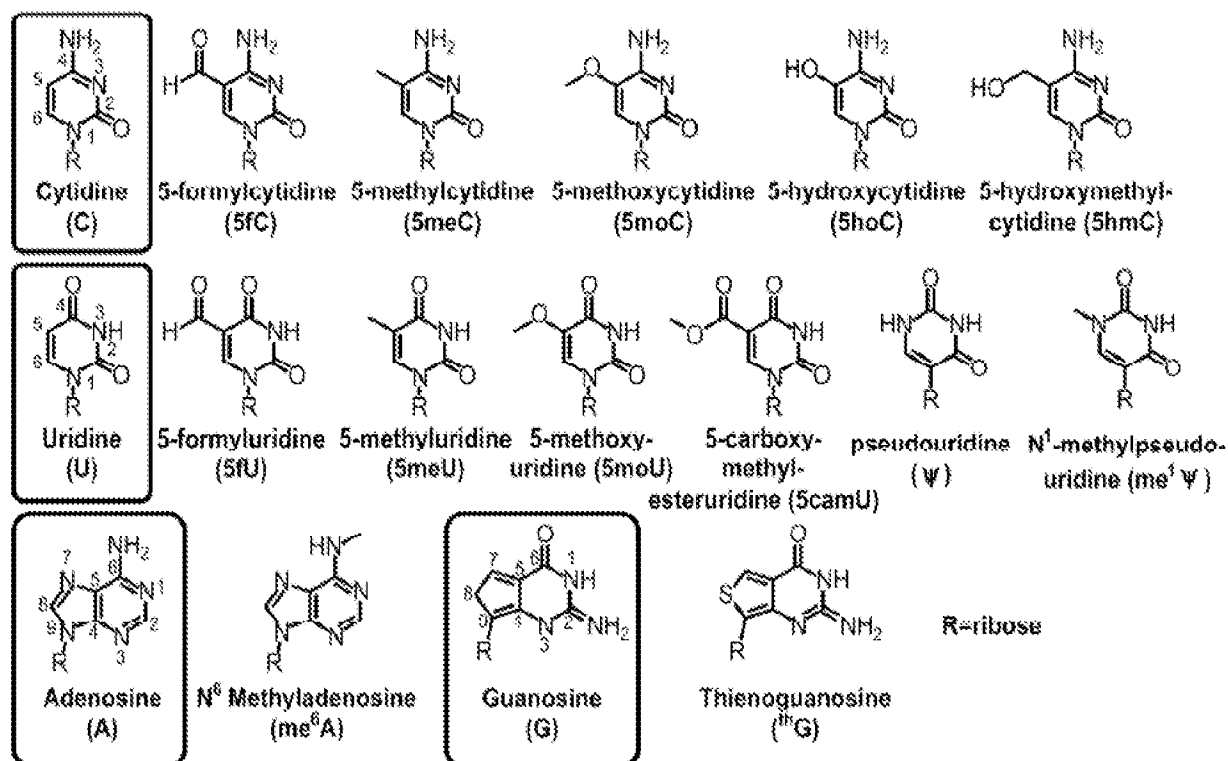
In some embodiments, the nucleic acids disclosed herein comprise at least one chemically modified nucleotide. In some embodiments, the at least one chemically modified nucleotide comprises a chemically modified nucleobase, a chemically modified ribose, a chemically modified phosphodiester linkage, or a combination thereof.

In one embodiment, the at least one chemically modified nucleotide is a chemically modified nucleobase.

In one embodiment, the chemically modified nucleobase is selected from 5-formylcytidine (5fC), 5-methylcytidine (5meC), 5-methoxycytidine (5moC), 5-hydroxycytidine (5hoC), 5-hydroxymethylcytidine (5hmC), 5-formyluridine (5fU), 5-methyluridine (5meU), 5-methoxyuridine (5moU), 5-carboxymethylesteruridine (5camU), pseudouridine (Ψ), N¹-methylpseudouridine (me¹ Ψ), N⁶-methyladenosine (me⁶A), or thienoguanosine (thG).

In some embodiments, the chemically modified nucleobase is 5-methoxyuridine (5moU). In some embodiments, the chemically modified nucleobase is pseudouridine (Ψ). In some
10 embodiments, the chemically modified nucleobase is N¹-methylpseudouridine (me¹ Ψ).

The structures of these modified nucleobases are shown below:

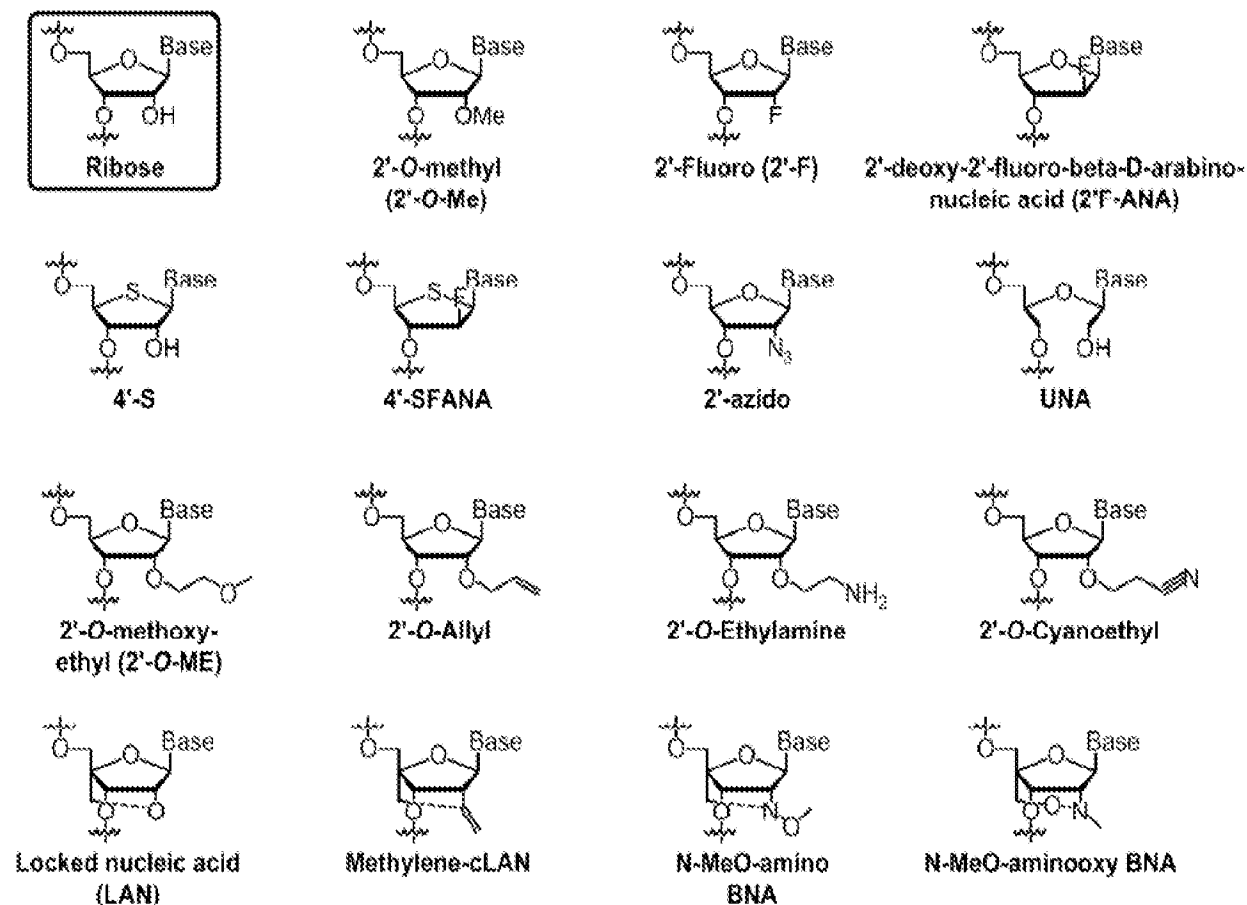


In one embodiment, the at least one chemically modified nucleotide is a chemically
15 modified ribose.

In one embodiment, the chemically modified ribose is selected from 2'-O-methyl (2'-O-Me), 2'-Fluoro (2'-F), 2'-deoxy-2'-fluoro-beta-D-arabino-nucleic acid (2'F-ANA), 4'-S, 4'-SFANA, 2'-azido, UNA, 2'-O-methoxy-ethyl (2'-O-ME), 2'-O-Allyl, 2'-O-Ethylamine, 2'-O-Cyanoethyl, Locked nucleic acid (LAN), Methylene-cLAN, N-MeO-amino BNA, or N-MeO-

aminoxy BNA. In one embodiment, the chemically modified ribose is 2'-O-methyl (2'-O-Me). In one embodiment, the chemically modified ribose is 2'-Fluoro (2'-F).

The structures of these modified riboses are shown below:

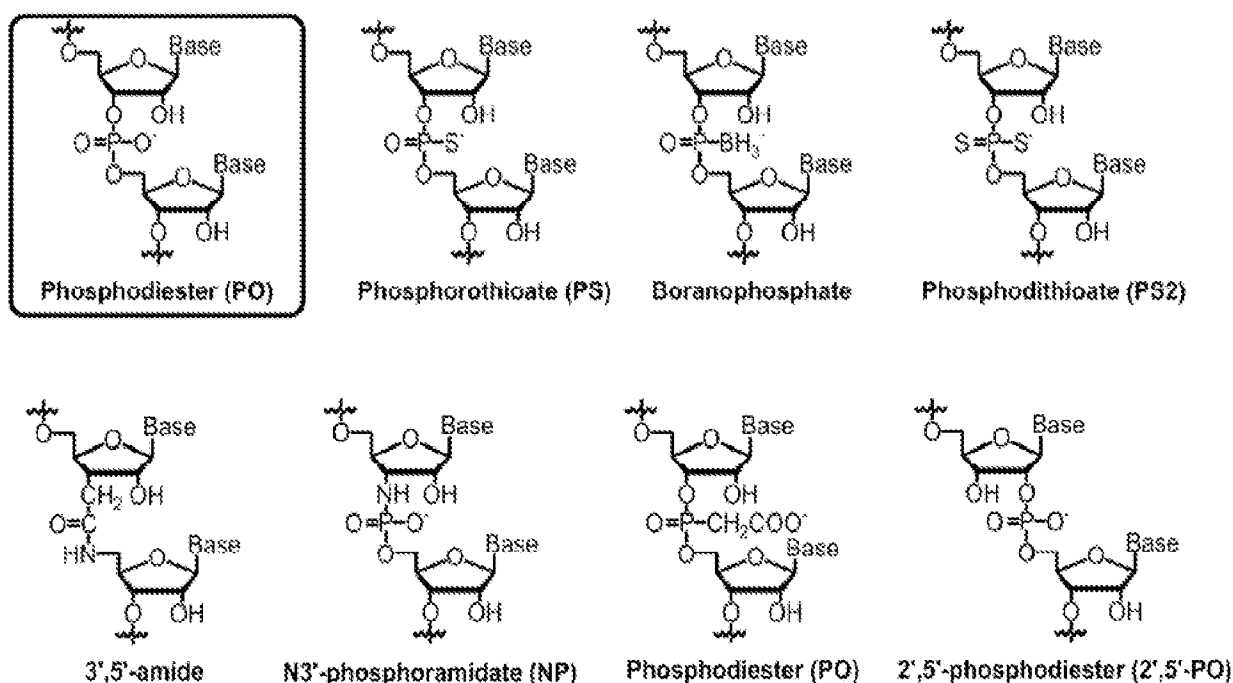


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In one embodiment, the at least one chemically modified nucleotide is a chemically modified phosphodiester linkage.

In one embodiment, the chemically modified phosphodiester linkage is selected from phosphorothioate (PS), boranophosphate, phosphodithioate (PS2), 3',5'-amide, N3'-phosphoramidate (NP), Phosphodiester (PO), or 2',5'-phosphodiester (2',5'-PO). In one embodiment, the chemically modified phosphodiester linkage is phosphorothioate.

The structures of these modified phosphodiester linkages are shown below:



Methods of Making

Also disclosed herein are methods of making any of the compounds or compositions disclosed herein. Also disclosed herein are methods of making any of the lipid particles disclosed herein. Also disclosed herein are methods of making any of the pharmaceutical compositions disclosed herein.

The compounds described herein can be prepared in a variety of ways known to one skilled in the art of organic synthesis or variations thereon as appreciated by those skilled in the art. The compounds described herein can be prepared from readily available starting materials. Optimum reaction conditions can vary with the particular reactants or solvents used, but such conditions can be determined by one skilled in the art.

Variations on the compounds described herein include the addition, subtraction, or movement of the various constituents as described for each compound. Similarly, when one or more chiral centers are present in a molecule, the chirality of the molecule can be changed. Additionally, compound synthesis can involve the protection and deprotection of various chemical groups. The use of protection and deprotection, and the selection of appropriate protecting groups can be determined by one skilled in the art. The chemistry of protecting groups can be found, for example, in Wuts and Greene, *Protective Groups in Organic Synthesis*, 4th Ed., Wiley & Sons, 2006, which is incorporated herein by reference in its entirety.

The starting materials and reagents used in preparing the disclosed compounds and compositions are either available from commercial suppliers such as Katchem (Prague, Czech Republic), Aldrich Chemical Co., (Milwaukee, WI), Acros Organics (Morris Plains, NJ), Fisher Scientific (Pittsburgh, PA), Sigma (St. Louis, MO), Pfizer (New York, NY), GlaxoSmithKline (Raleigh, NC), Merck (Whitehouse Station, NJ), Johnson & Johnson (New Brunswick, NJ), Aventis (Bridgewater, NJ), AstraZeneca (Wilmington, DE), Novartis (Basel, Switzerland), Wyeth (Madison, NJ), Bristol-Myers-Squibb (New York, NY), Roche (Basel, Switzerland), Lilly (Indianapolis, IN), Abbott (Abbott Park, IL), Schering Plough (Kenilworth, NJ), or Boehringer Ingelheim (Ingelheim, Germany), or are prepared by methods known to those skilled in the art following procedures set forth in references such as Fieser and Fieser's Reagents for Organic Synthesis, Volumes 1-17 (John Wiley and Sons, 1991); Rodd's Chemistry of Carbon Compounds, Volumes 1-5 and Supplementals (Elsevier Science Publishers, 1989); Organic Reactions, Volumes 1-40 (John Wiley and Sons, 1991); March's Advanced Organic Chemistry, (John Wiley and Sons, 4th Edition); and Larock's Comprehensive Organic Transformations (VCH Publishers Inc., 1989). Other materials, such as the pharmaceutical excipients disclosed herein can be obtained from commercial sources.

Reactions to produce the compounds described herein can be carried out in solvents, which can be selected by one of skill in the art of organic synthesis. Solvents can be substantially nonreactive with the starting materials (reactants), the intermediates, or products under the conditions at which the reactions are carried out, *i.e.*, temperature and pressure. Reactions can be carried out in one solvent or a mixture of more than one solvent. Product or intermediate formation can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (*e.g.*, ^1H or ^{13}C) infrared spectroscopy, spectrophotometry (*e.g.*, UV-visible), or mass spectrometry, or by chromatography such as high performance liquid chromatography (HPLC) or thin layer chromatography.

Methods of Use

Also disclosed herein are methods of use of any of the compounds or compositions disclosed herein.

In one aspect, provided herein is a method for the delivery of an agent (for example, a polynucleotide) into a cell comprising;

introducing into the cell a composition comprising;

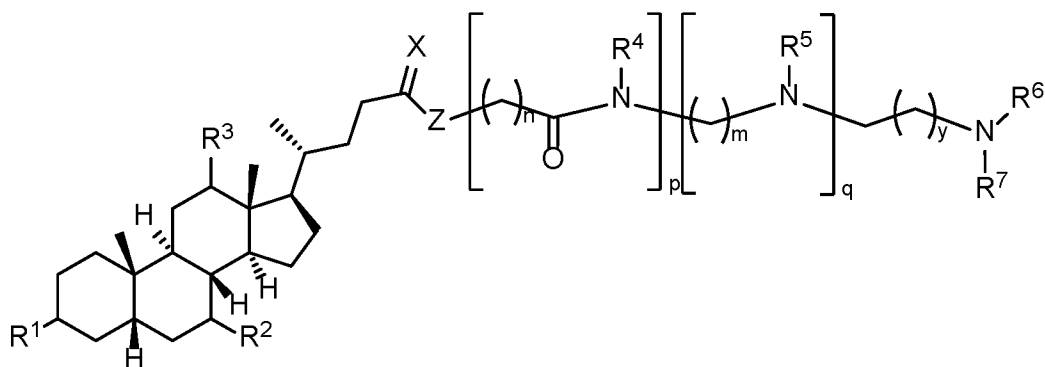
a nanoparticle, comprising;
 a compound of Formulas I-V;
 a non-cationic lipid;
 a polyethylene glycol-lipid;
 a sterol; and
 an agent.

In one aspect, disclosed herein is a method for the delivery of an agent into a cell comprising;

introducing into the cell a composition comprising;

a nanoparticle comprising;

a compound of Formula I, or a pharmaceutically acceptable salt thereof:



I

wherein

X and Z are each independently O, NR^a, or S;
 R^a, when present, is hydrogen or substituted or unsubstituted C₁-C₁₀ alkyl;
 R¹, R², and R³ are each independently H, OH, halogen, substituted or unsubstituted C₁-C₃ alkyl, or substituted or unsubstituted C₁-C₃ alkoxy;
 p is an integer from 0 to 5;
 q is an integer from 0 to 5;
 n is an integer from 1 to 10;
 m in each case is independently an integer from 1 to 10;
 y is an integer from 1 to 10;
 R⁴, when present, is hydrogen or a substituted or unsubstituted C₁-C₁₈ alkyl;
 each R⁵, when present, is independently a substituted or unsubstituted C₈-C₁₈ alkyl; and

R⁶ and R⁷ are each independently a substituted or unsubstituted C₈-C₁₈ alkyl.
a non-cationic lipid;
a polyethylene glycol-lipid;
a sterol; and
5 an agent.

In some embodiments, a nanoparticle comprising any compound as described in the Compounds section above, is used in the methods herein, for delivery of an agent into a cell.

In some embodiments, the agent is a polynucleotide. In some embodiments, the agent is an RNA. In some embodiments, the agent is an mRNA. In some embodiments, the agent is a
10 therapeutic agent, diagnostic agent, or prophylactic agent.

In some embodiments, provided herein are methods for the delivery of polynucleotides. In some embodiments, provided herein are methods for the delivery of polynucleotides (for example, mRNA) to correct a mutation in a genome. For example, mRNAs can be delivered to correct mutations that cause hemophilia (due to mutations in the genes encoding Factor VIII (F8; hemophilia A) or Factor IX (F9; hemoglobin B). In some embodiments, provided herein are
15 methods for the delivery of polynucleotides. In some embodiments, provided herein are methods for the delivery of polynucleotides (for example, mRNA) to provide expression of the mRNA (and translation to produce a protein) in a cell. In some embodiments, provided herein are methods for the delivery of polynucleotides (for example, mRNA) to induce an immune response in a subject.

20 In some embodiments, the RNA (e.g., mRNA) polynucleotide having an open reading frame encoding at least one (e.g., at least 2, 3, 4 or 5) hMPV, PIV, RSV, MeV, and/or a BetaCoV (e.g., MERS-CoV, SARS-CoV, SARS-CoV2, HCoV-OC43, HCoV-229E, HCoV-NL63, HCoV-NL, HCoV-NH, HCoV-HKU1) antigenic polypeptide, or any combination of two or more of the antigenic polypeptides.

25 In one aspect, the methods described herein are used to treat cancer, for example, melanoma, lung cancer (including lung adenocarcinoma, basal cell carcinoma, squamous cell carcinoma, large cell carcinoma, bronchioloalveolar carcinoma, bronchogenic carcinoma, non-small-cell carcinoma, small cell carcinoma, mesothelioma); breast cancer (including ductal carcinoma, lobular carcinoma, inflammatory breast cancer, clear cell carcinoma, mucinous
30 carcinoma, serosal cavities breast carcinoma); colorectal cancer (colon cancer, rectal cancer, colorectal adenocarcinoma); anal cancer; pancreatic cancer (including pancreatic adenocarcinoma, islet cell carcinoma, neuroendocrine tumors); prostate cancer; prostate adenocarcinoma; ovarian

carcinoma (ovarian epithelial carcinoma or surface epithelial-stromal tumor including serous tumor, endometrioid tumor and mucinous cystadenocarcinoma, sex-cord-stromal tumor); liver and bile duct carcinoma (including hepatocellular carcinoma, cholangiocarcinoma, hemangioma); esophageal carcinoma (including esophageal adenocarcinoma and squamous cell carcinoma); oral and oropharyngeal squamous cell carcinoma; salivary gland adenoid cystic carcinoma; bladder cancer; bladder carcinoma; carcinoma of the uterus (including endometrial adenocarcinoma, 5 ocular, uterine papillary serous carcinoma, uterine clear-cell carcinoma, uterine sarcomas, leiomyosarcomas, mixed mullerian tumors); glioma, glioblastoma, medulloblastoma, and other tumors of the brain; kidney cancers (including renal cell carcinoma, clear cell carcinoma, Wilm's tumor); cancer of the head and neck (including squamous cell carcinomas); cancer of the stomach (gastric cancers, stomach adenocarcinoma, gastrointestinal stromal tumor); testicular cancer; germ cell tumor; neuroendocrine tumor; cervical cancer; carcinoids of the gastrointestinal tract, breast, and other organs; signet ring cell carcinoma; mesenchymal tumors including sarcomas, fibrosarcomas, haemangioma, angiomas, haemangiopericytoma, pseudoangiomatous stromal 10 hyperplasia, myofibroblastoma, fibromatosis, inflammatory myofibroblastic tumor, lipoma, angiolipoma, granular cell tumor, neurofibroma, schwannoma, angiosarcoma, liposarcoma, rhabdomyosarcoma, osteosarcoma, leiomyoma, leiomyosarcoma, skin, including melanoma, cervical, retinoblastoma, head and neck cancer, pancreatic, brain, thyroid, testicular, renal, bladder, soft tissue, adrenal gland, urethra, cancers of the penis, myxosarcoma, chondrosarcoma, 15 osteosarcoma, chordoma, malignant fibrous histiocytoma, lymphangiosarcoma, mesothelioma, squamous cell carcinoma; epidermoid carcinoma, malignant skin adnexal tumors, adenocarcinoma, hepatoma, hepatocellular carcinoma, renal cell carcinoma, hypernephroma, cholangiocarcinoma, transitional cell carcinoma, choriocarcinoma, seminoma, embryonal cell carcinoma, glioma anaplastic; glioblastoma multiforme, neuroblastoma, medulloblastoma, 20 malignant meningioma, malignant schwannoma, neurofibrosarcoma, parathyroid carcinoma, medullary carcinoma of thyroid, bronchial carcinoid, pheochromocytoma, Islet cell carcinoma, malignant carcinoid, malignant paraganglioma, melanoma, Merkel cell neoplasm, cystosarcoma phylloide, salivary cancers, thymic carcinomas, and cancers of the vagina among others.

In some embodiments, the compositions and methods described herein are useful in 30 treating or preventing a cancer. In some cases, the cancer is a circulating cancer cell (circulating tumor cell). In some cases, the cancer is a metastatic cancer cell.

In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human.

In some embodiments, the antibody or antigen binding fragment thereof and the nanoparticle are administered by intramuscularly injection or systematically.

5 In some embodiments, the method further comprises administering an additional therapeutic agent. In some embodiments, the additional therapeutic agent comprises an additional immunotherapeutic agent. In some embodiments, the immunotherapeutic agent is selected from an anti-CD40 antibody, an anti-PDL1 antibody, an anti-PD1 antibody, an anti-CTLA4 antibody, or a combination thereof.

10 In one embodiment, the immunotherapeutic agent is an anti-PDL1 antibody. In one embodiment, the anti-PDL1 antibody is selected from atezolizumab, durvalumab, or avelumab. In one embodiment, the anti-PDL1 antibody is atezolizumab (MPDL3280A)(Roche). In one embodiment, the anti-PDL1 antibody is durvalumab (MEDI4736). In one embodiment, the anti-PDL1 antibody is avelumab (MS0010718C).

15 In one embodiment, the immunotherapeutic agent is a programmed death protein 1 (PD-1) inhibitor or programmed death protein ligand 1 or 2 inhibitor. PD-1 inhibitors are known in the art, and include, for example, nivolumab (BMS), pembrolizumab (Merck), pidilizumab (CureTech/Teva), AMP-244 (Amplimmune/GSK), BMS-936559 (BMS), and MEDI4736 (Roche/Genentech).

20 In one embodiment, the immunotherapeutic agent is an anti-PD1 antibody. In one embodiment, the anti-PD1 antibody is nivolumab. In one embodiment, the anti-PD1 antibody is pembrolizumab.

In one embodiment, the immunotherapeutic agent is an anti-CTLA4 antibody. In one embodiment, the anti-CTLA4 antibody is ipilimumab.

25 In some embodiments, the additional therapeutic agent is an anti-neoplastic agent. For example, the anti-neoplastic agent can be selected from the group consisting of Abiraterone Acetate, Abitrexate (Methotrexate), Abraxane (Paclitaxel Albumin-stabilized Nanoparticle Formulation), ABVD, ABVE, ABVE-PC, AC, AC-T, Adcetris (Brentuximab Vedotin), ADE, Ado-Trastuzumab Emtansine, Adriamycin (Doxorubicin Hydrochloride), Adrucil (Fluorouracil),
30 Afatinib Dimaleate, Afinitor (Everolimus), Akynzeo (Netupitant and Palonosetron Hydrochloride), Aldara (Imiquimod), Aldesleukin, Alemtuzumab, Alimta (Pemetrexed Disodium), Aloxi (Palonosetron Hydrochloride), Ambochlorin (Chlorambucil), Amboclorin

(Chlorambucil), Aminolevulinic Acid, Anastrozole, Aprepitant, Aredia (Pamidronate Disodium), Arimidex (Anastrozole), Aromasin (Exemestane), Arranon (Nelarabine), Arsenic Trioxide, Arzerra (Ofatumumab), Asparaginase Erwinia chrysanthemi, Avastin (Bevacizumab), Axitinib, Azacitidine, BEACOPP, Becenum (Carmustine), Beleodaq (Belinostat), Belinostat, 5 Bendamustine Hydrochloride, BEP, Bevacizumab, Bexarotene, Bexxar (Tositumomab and Iodine I 131 Tositumomab), Bicalutamide, BiCNU (Carmustine), Bleomycin, Blinatumomab, Blincyto (Blinatumomab), Bortezomib, Bosulif (Bosutinib), Bosutinib, Brentuximab Vedotin, Busulfan, Busulfex (Busulfan), Cabazitaxel, Cabozantinib-S-Malate, CAF, Campath (Alemtuzumab), Camptosar (Irinotecan Hydrochloride), Capecitabine, CAPOX, Carboplatin, CARBOPLATIN- 10 TAXOL, Carfilzomib, Carmubris (Carmustine), Carmustine, Carmustine Implant, Casodex (Bicalutamide), CeeNU (Lomustine), Ceritinib, Cerubidine (Daunorubicin Hydrochloride), Cervarix (Recombinant HPV Bivalent Vaccine), Cetuximab, Chlorambucil, CHLORAMBUCIL-PREDNISONE, CHOP, Cisplatin, Clafen (Cyclophosphamide), Clofarabine, Clofarex (Clofarabine), Clolar (Clofarabine), CMF, Cometriq (Cabozantinib-S-Malate), COPP, COPP- 15 ABV, Cosmegen (Dactinomycin), Crizotinib, CVP, Cyclophosphamide, Cyfos (Ifosfamide), Cytamza (Ramucirumab), Cytarabine, Cytarabine, Liposomal, Cytosar-U (Cytarabine), Cytosan (Cyclophosphamide), Dabrafenib, Dacarbazine, Dacogen (Decitabine), Dactinomycin, Dasatinib, Daunorubicin Hydrochloride, Decitabine, Degarelix, Denileukin Diftitox, Denosumab, DepoCyt (Liposomal Cytarabine), DepoFoam (Liposomal Cytarabine), Dexrazoxane Hydrochloride, 20 Dinutuximab, Docetaxel, Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride, Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), DTIC-Dome (Dacarbazine), Efudex (Fluorouracil), Elitek (Rasburicase), Ellence (Epirubicin Hydrochloride), Eloxatin (Oxaliplatin), Eltrombopag Olamine, Emend (Aprepitant), Enzalutamide, Epirubicin Hydrochloride, EPOCH, Erbitux (Cetuximab), Eribulin Mesylate, 25 Erivedge (Vismodegib), Erlotinib Hydrochloride, Erwinaze (Asparaginase Erwinia chrysanthemi), Etopophos (Etoposide Phosphate), Etoposide, Etoposide Phosphate, Evacet (Doxorubicin Hydrochloride Liposome), Everolimus, Evista (Raloxifene Hydrochloride), Exemestane, Fareston (Toremifene), Farydak (Panobinostat), Faslodex (Fulvestrant), FEC, Femara (Letrozole), Filgrastim, Fludara (Fludarabine Phosphate), Fludarabine Phosphate, 30 Fluoroplex (Fluorouracil), Fluorouracil, Folex (Methotrexate), Folex PFS (Methotrexate), FOLFIRI, FOLFIRI-BEVACIZUMAB, FOLFIRI-CETUXIMAB, FOLFIRINOX, FOLFOX, Folutyn (Pralatrexate), FU-LV, Fulvestrant, Gardasil (Recombinant HPV Quadrivalent Vaccine),

Gardasil 9 (Recombinant HPV Nonavalent Vaccine), Gazyva (Obinutuzumab), Gefitinib, Gemcitabine Hydrochloride, GEMCITABINE-CISPLATIN, GEMCITABINE-OXALIPLATIN, Gemtuzumab Ozogamicin, Gemzar (Gemcitabine Hydrochloride), Gilotrif (Afatinib Dimaleate), Gleevec (Imatinib Mesylate), Gliadel (Carmustine Implant), Gliadel wafer (Carmustine Implant),

5 Glucarpidase, Goserelin Acetate, Halaven (Eribulin Mesylate), Herceptin (Trastuzumab), HPV Bivalent Vaccine, Recombinant, HPV Nonavalent Vaccine, Recombinant, HPV Quadrivalent Vaccine, Recombinant, Hycamtin (Topotecan Hydrochloride), Hyper-CVAD, Ibrance (Palbociclib), Ibritumomab Tiuxetan, Ibrutinib, ICE, Iclusig (Ponatinib Hydrochloride), Idamycin (Idarubicin Hydrochloride), Idarubicin Hydrochloride, Idelalisib, Ifex (Ifosfamide), Ifosfamide,

10 Ifosfamidum (Ifosfamide), Imatinib Mesylate, Imbruvica (Ibrutinib), Imiquimod, Inlyta (Axitinib), Interferon Alfa-2b, Recombinant, Intron A (Recombinant Interferon Alfa-2b), Iodine I 131 Tositumomab and Tositumomab, Ipilimumab, Iressa (Gefitinib), Irinotecan Hydrochloride, Istodax (Romidepsin), Ixabepilone, Ixempria (Ixabepilone), Jakafi (Ruxolitinib Phosphate), Jevtana (Cabazitaxel), Kadcyra (Ado-Trastuzumab Emtansine), Keoxifene (Raloxifene

15 Hydrochloride), Kepivance (Palifermin), Keytruda (Pembrolizumab), Kyprolis (Carfilzomib), Lanreotide Acetate, Lapatinib Ditosylate, Lenalidomide, Lenvatinib Mesylate, Lenvima (Lenvatinib Mesylate), Letrozole, Leucovorin Calcium, Leukeran (Chlorambucil), Leuprolide Acetate, Levulan (Aminolevulinic Acid), Linfolizin (Chlorambucil), LipoDox (Doxorubicin Hydrochloride Liposome), Liposomal Cytarabine, Lomustine, Lupron (Leuprolide Acetate),

20 Lupron Depot (Leuprolide Acetate), Lupron Depot-Ped (Leuprolide Acetate), Lupron Depot-3 Month (Leuprolide Acetate), Lupron Depot-4 Month (Leuprolide Acetate), Lynparza (Olaparib), Marqibo (Vincristine Sulfate Liposome), Matulane (Procarbazine Hydrochloride), Mechlorethamine Hydrochloride, Megace (Megestrol Acetate), Megestrol Acetate, Mekinist (Trametinib), Mercaptopurine, Mesna, Mesnex (Mesna), Methazolastone (Temozolomide),

25 Methotrexate, Methotrexate LPF (Methotrexate), Mexate (Methotrexate), Mexate-AQ (Methotrexate), Mitomycin C, Mitoxantrone Hydrochloride, Mitozytrex (Mitomycin C), MOPP, Mozobil (Plerixafor), Mustargen (Mechlorethamine Hydrochloride), Mutamycin (Mitomycin C), Myleran (Busulfan), Mylosar (Azacitidine), Mylotarg (Gemtuzumab Ozogamicin), Nanoparticle Paclitaxel (Paclitaxel Albumin-stabilized Nanoparticle Formulation), Navelbine (Vinorelbine

30 Tartrate), Nelarabine, Neosar (Cyclophosphamide), Netupitant and Palonosetron Hydrochloride, Neupogen (Filgrastim), Nexavar (Sorafenib Tosylate), Nilotinib, Nivolumab, Nolvadex (Tamoxifen Citrate), Nplate (Romiplostim), Obinutuzumab, Odomzo (Sonidegib), OEPA,

Ofatumumab, OFF, Olaparib, Omacetaxine Mepesuccinate, Oncaspar (Pegaspargase),
 Ondansetron Hydrochloride, Ontak (Denileukin Diftitox), Opdivo (Nivolumab), OPPA,
 Oxaliplatin, Paclitaxel, Paclitaxel Albumin-stabilized Nanoparticle Formulation, PAD,
 Palbociclib, Palifermin, Palonosetron Hydrochloride, Palonosetron Hydrochloride and Netupitant,
 5 Pamidronate Disodium, Panitumumab, Panobinostat, Paraplat (Carboplatin), Paraplatin
 (Carboplatin), Pazopanib Hydrochloride, Pegaspargase, Peginterferon Alfa-2b, PEG-Intron
 (Peginterferon Alfa-2b), Pembrolizumab, Pemetrexed Disodium, Perjeta (Pertuzumab),
 Pertuzumab, Platinol (Cisplatin), Platinol-AQ (Cisplatin), Plerixafor, Pomalidomide, Pomalyst
 (Pomalidomide), Ponatinib Hydrochloride, Pralatrexate, Prednisone, Procarbazine Hydrochloride,
 10 Proleukin (Aldesleukin), Prolia (Denosumab), Promacta (Eltrombopag Olamine), Provenge
 (Sipuleucel-T), Purinethol (Mercaptopurine), Purixan (Mercaptopurine), Radium 223 Dichloride,
 Raloxifene Hydrochloride, Ramucirumab, Rasburicase, R-CHOP, R-CVP, Recombinant Human
 Papillomavirus (HPV) Bivalent Vaccine, Recombinant Human Papillomavirus (HPV) Nonavalent
 Vaccine, Recombinant Human Papillomavirus (HPV) Quadrivalent Vaccine, Recombinant
 15 Interferon Alfa-2b, Regorafenib, R-EPOCH, Revlimid (Lenalidomide), Rheumatrex
 (Methotrexate), Rituxan (Rituximab), Rituximab, Romidepsin, Romiplostim, Rubidomycin
 (Daunorubicin Hydrochloride), Ruxolitinib Phosphate, Sclerosol Intrapleural Aerosol (Talc),
 Siltuximab, Sipuleucel-T, Somatuline Depot (Lanreotide Acetate), Sonidegib, Sorafenib Tosylate,
 Sprycel (Dasatinib), STANFORD V, Sterile Talc Powder (Talc), Steritalc (Talc), Stivarga
 20 (Regorafenib), Sunitinib Malate, Sutent (Sunitinib Malate), Sylatron (Peginterferon Alfa-2b),
 Sylvant (Siltuximab), Synovir (Thalidomide), Synribo (Omacetaxine Mepesuccinate), TAC,
 Tafenlar (Dabrafenib), Talc, Tamoxifen Citrate, Tarabine PFS (Cytarabine), Tarceva (Erlotinib
 Hydrochloride), Targretin (Bexarotene), Tassigna (Nilotinib), Taxol (Paclitaxel), Taxotere
 (Docetaxel), Temodar (Temozolomide), Temozolomide, Temsirolimus, Thalidomide, Thalomid
 25 (Thalidomide), Thiotepa, Toposar (Etoposide), Topotecan Hydrochloride, Toremifene, Torisel
 (Temsirrolimus), Tositumomab and Iodine I 131 Tositumomab, Totect (Dexrazoxane
 Hydrochloride), TPF, Trametinib, Trastuzumab, Treanda (Bendamustine Hydrochloride),
 Trisenox (Arsenic Trioxide), Tykerb (Lapatinib Ditosylate), Unituxin (Dinutuximab), Vandetanib,
 VAMP, Vectibix (Panitumumab), VeIP, Velban (Vinblastine Sulfate), Velcade (Bortezomib),
 30 Velsar (Vinblastine Sulfate), Vemurafenib, VePesid (Etoposide), Viadur (Leuprolide Acetate),
 Vidaza (Azacitidine), Vinblastine Sulfate, Vincasar PFS (Vincristine Sulfate), Vincristine Sulfate,
 Vincristine Sulfate Liposome, Vinorelbine Tartrate, VIP, Vismodegib, Voraxaze (Glucarpidase),

Vorinostat, Votrient (Pazopanib Hydrochloride), Wellcovorin (Leucovorin Calcium), Xalkori (Crizotinib), Xeloda (Capecitabine), XELIRI, XELOX, Xgeva (Denosumab), Xofigo (Radium 223 Dichloride), Xtandi (Enzalutamide), Yervoy (Ipilimumab), Zaltrap (Ziv-Aflibercept), Zelboraf (Vemurafenib), Zevalin (Ibritumomab Tiuxetan), Zinecard (Dexrazoxane Hydrochloride), Ziv-Aflibercept, Zofran (Ondansetron Hydrochloride), Zoladex (Goserelin Acetate), Zoledronic Acid, Zolinza (Vorinostat), Zometa (Zoledronic Acid), Zydelig (Idelalisib), Zykadia (Ceritinib), and Zytiga (Abiraterone Acetate).

In one embodiment, provided herein is a method of treating an inflammation disorder, including autoimmune diseases in a subject. The method comprises administering to said subject a therapeutically effective amount of a compound, a combination of compounds, or a composition provided herein, or a pharmaceutically acceptable form thereof, or a pharmaceutical composition as provided herein. Examples of autoimmune diseases include but are not limited to acute disseminated encephalomyelitis (ADEM), Addison's disease, antiphospholipid antibody syndrome (APS), aplastic anemia, autoimmune hepatitis, autoimmune skin disease, coeliac disease, Crohn's disease, Diabetes mellitus (type 1), Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome (GBS), Hashimoto's disease, lupus erythematosus, multiple sclerosis, myasthenia gravis, opsoclonus myoclonus syndrome (OMS), optic neuritis, Ord's thyroiditis, oemphigus, polyarthritis, primary biliary cirrhosis, psoriasis, rheumatoid arthritis, Reiter's syndrome, Takayasu's arteritis, temporal arteritis (also known as "giant cell arteritis"), warm autoimmune hemolytic anemia, Wegener's granulomatosis, alopecia universalis (e.g., inflammatory alopecia), Chagas disease, chronic fatigue syndrome, dysautonomia, endometriosis, hidradenitis suppurativa, interstitial cystitis, neuromyotonia, sarcoidosis, scleroderma, ulcerative colitis, vitiligo, and vulvodynia. Other disorders include bone-resorption disorders and thrombosis.

Inflammation takes on many forms and includes, but is not limited to, acute, adhesive, atrophic, catarrhal, chronic, cirrhotic, diffuse, disseminated, exudative, fibrinous, fibrosing, focal, granulomatous, hyperplastic, hypertrophic, interstitial, metastatic, necrotic, obliterative, parenchymatous, plastic, productive, proliferous, pseudomembranous, purulent, sclerosing, seroplastic, serous, simple, specific, subacute, suppurative, toxic, traumatic, and/or ulcerative inflammation.

Exemplary inflammatory conditions include, but are not limited to, inflammation associated with acne, anemia (e.g., aplastic anemia, haemolytic autoimmune anaemia), asthma, arteritis (e.g., polyarteritis, temporal arteritis, periarteritis nodosa, Takayasu's arteritis), arthritis

(e.g., crystalline arthritis, osteoarthritis, psoriatic arthritis, gout flare, gouty arthritis, reactive arthritis, rheumatoid arthritis and Reiter's arthritis), ankylosing spondylitis, amylosis, amyotrophic lateral sclerosis, autoimmune diseases, allergies or allergic reactions, atherosclerosis, bronchitis, bursitis, chronic prostatitis, conjunctivitis, Chagas disease, chronic obstructive pulmonary disease, 5 cermatomyositis, diverticulitis, diabetes (e.g., type I diabetes mellitus, type 2 diabetes mellitus), a skin condition (e.g., psoriasis, eczema, burns, dermatitis, pruritus (itch)), endometriosis, Guillain-Barre syndrome, infection, ischaemic heart disease, Kawasaki disease, glomerulonephritis, gingivitis, hypersensitivity, headaches (e.g., migraine headaches, tension headaches), ileus (e.g., postoperative ileus and ileus during sepsis), idiopathic thrombocytopenic purpura, interstitial 10 cystitis (painful bladder syndrome), gastrointestinal disorder (e.g., selected from peptic ulcers, regional enteritis, diverticulitis, gastrointestinal bleeding, eosinophilic gastrointestinal disorders (e.g., eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic colitis), gastritis, diarrhea, gastroesophageal reflux disease (GORD, or its synonym GERD), inflammatory bowel disease (IBD) (e.g., Crohn's disease, ulcerative colitis, collagenous colitis, 15 lymphocytic colitis, ischaemic colitis, diversion colitis, Behcet's syndrome, indeterminate colitis) and inflammatory bowel syndrome (IBS)), lupus, multiple sclerosis, morphea, myasthenia gravis, myocardial ischemia, nephrotic syndrome, pemphigus vulgaris, pernicious anaemia, peptic ulcers, polymyositis, primary biliary cirrhosis, neuroinflammation associated with brain disorders (e.g., Parkinson's disease, Huntington's disease, and Alzheimer's disease), prostatitis, chronic 20 inflammation associated with cranial radiation injury, pelvic inflammatory disease, polymyalgia rheumatic, reperfusion injury, regional enteritis, rheumatic fever, systemic lupus erythematosus, scleroderma, scleroderma, sarcoidosis, spondyloarthropathies, Sjogren's syndrome, thyroiditis, transplantation rejection, tendonitis, trauma or injury (e.g., frostbite, chemical irritants, toxins, scarring, burns, physical injury), vasculitis, vitiligo and Wegener's granulomatosis. In certain 25 embodiments, the inflammatory disorder is selected from arthritis (e.g., rheumatoid arthritis), inflammatory bowel disease, inflammatory bowel syndrome, asthma, psoriasis, endometriosis, interstitial cystitis and prostatitis. In certain embodiments, the inflammatory condition is an acute inflammatory condition (e.g., for example, inflammation resulting from infection). In certain embodiments, the inflammatory condition is a chronic inflammatory condition (e.g., conditions 30 resulting from asthma, arthritis and inflammatory bowel disease). The compounds can also be useful in treating inflammation associated with trauma and non-inflammatory myalgia.

Immune disorders, such as auto-immune disorders include, but are not limited to, arthritis (including rheumatoid arthritis, spondyloarthropathies, gouty arthritis, degenerative joint diseases such as osteoarthritis, systemic lupus erythematosus, Sjogren's syndrome, ankylosing spondylitis, undifferentiated spondylitis, Behcet's disease, haemolytic autoimmune anaemias, multiple sclerosis, amyotrophic lateral sclerosis, amylosis, acute painful shoulder, psoriatic, and juvenile arthritis), asthma, atherosclerosis, osteoporosis, bronchitis, tendonitis, bursitis, skin condition (e.g., psoriasis, eczema, burns, dermatitis, pruritus (itch)), enuresis, eosinophilic disease, gastrointestinal disorder (e.g., selected from peptic ulcers, regional enteritis, diverticulitis, gastrointestinal bleeding, eosinophilic gastrointestinal disorders (e.g., eosinophilic esophagitis, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic colitis), gastritis, diarrhea, gastroesophageal reflux disease (GORD, or its synonym GERD), inflammatory bowel disease (IBD) (e.g., Crohn's disease, ulcerative colitis, collagenous colitis, lymphocytic colitis, ischaemic colitis, diversion colitis, Behcet's syndrome, indeterminate colitis) and inflammatory bowel syndrome (IBS)), relapsing polychondritis (e.g., atrophic polychondritis and systemic polychondromalacia), and disorders ameliorated by a gastroprokinetic agent (e.g., ileus, postoperative ileus and ileus during sepsis; gastroesophageal reflux disease (GORD, or its synonym GERD); eosinophilic esophagitis, gastroparesis such as diabetic gastroparesis; food intolerances and food allergies and other functional bowel disorders, such as non-ulcerative dyspepsia (NUD) and non-cardiac chest pain (NCCP, including costo-chondritis)).

In some examples, the compound or composition can be administered to the subject in an amount of 1 microgram (μg) per kilogram (kg) of body weight of the subject per day ($\mu\text{g}/\text{kg}/\text{day}$) or more (e.g., 2 $\mu\text{g}/\text{kg}/\text{day}$ or more, 3 $\mu\text{g}/\text{kg}/\text{day}$ or more, 4 $\mu\text{g}/\text{kg}/\text{day}$ or more, 5 $\mu\text{g}/\text{kg}/\text{day}$ or more, 10 $\mu\text{g}/\text{kg}/\text{day}$ or more, 15 $\mu\text{g}/\text{kg}/\text{day}$ or more, 20 $\mu\text{g}/\text{kg}/\text{day}$ or more, 25 $\mu\text{g}/\text{kg}/\text{day}$ or more, 30 $\mu\text{g}/\text{kg}/\text{day}$ or more, 35 $\mu\text{g}/\text{kg}/\text{day}$ or more, 40 $\mu\text{g}/\text{kg}/\text{day}$ or more, 45 $\mu\text{g}/\text{kg}/\text{day}$ or more, 50 $\mu\text{g}/\text{kg}/\text{day}$ or more, 60 $\mu\text{g}/\text{kg}/\text{day}$ or more, 70 $\mu\text{g}/\text{kg}/\text{day}$ or more, 80 $\mu\text{g}/\text{kg}/\text{day}$ or more, 90 $\mu\text{g}/\text{kg}/\text{day}$ or more, 100 $\mu\text{g}/\text{kg}/\text{day}$ or more, 125 $\mu\text{g}/\text{kg}/\text{day}$ or more, 150 $\mu\text{g}/\text{kg}/\text{day}$ or more, 175 $\mu\text{g}/\text{kg}/\text{day}$ or more, 200 $\mu\text{g}/\text{kg}/\text{day}$ or more, 225 $\mu\text{g}/\text{kg}/\text{day}$ or more, 250 $\mu\text{g}/\text{kg}/\text{day}$ or more, 300 $\mu\text{g}/\text{kg}/\text{day}$ or more, 350 $\mu\text{g}/\text{kg}/\text{day}$ or more, 400 $\mu\text{g}/\text{kg}/\text{day}$ or more, 450 $\mu\text{g}/\text{kg}/\text{day}$ or more, 500 $\mu\text{g}/\text{kg}/\text{day}$ or more, 600 $\mu\text{g}/\text{kg}/\text{day}$ or more, 700 $\mu\text{g}/\text{kg}/\text{day}$ or more, 800 $\mu\text{g}/\text{kg}/\text{day}$ or more, 900 $\mu\text{g}/\text{kg}/\text{day}$ or more, 1 milligram (mg)/kg/day or more, 2 mg/kg/day or more, 3 mg/kg/day or more, 4 mg/kg/day or more, 5 mg/kg/day or more, 6 mg/kg/day or more, 7 mg/kg/day or more, 8 mg/kg/day or more, or 9 mg/kg/day or more). In some examples, the compound or composition

can be administered to the subject in an amount of 10 milligrams (mg) per kilogram (kg) of body weight of the subject per day (mg/kg/day) or less (e.g., 9 mg/kg/day or less, 8 mg/kg/day or less, 7 mg/kg/day or less, 6 mg/kg/day or less, 5 mg/kg/day or less, 4 mg/kg/day or less, 3 mg/kg/day or less, 2 mg/kg/day or less, 1 mg/kg/day or less, 900 µg/kg/day or less, 800 µg/kg/day or less, 700 µg/kg/day or less, 600 µg/kg/day or less, 500 µg/kg/day or less, 450 µg/kg/day or less, 400 µg/kg/day or less, 350 µg/kg/day or less, 300 µg/kg/day or less, 250 µg/kg/day or less, 225 µg/kg/day or less, 200 µg/kg/day or less, 175 µg/kg/day or less, 150 µg/kg/day or less, 125 µg/kg/day or less, 100 µg/kg/day or less, 90 µg/kg/day or less, 80 µg/kg/day or less, 70 µg/kg/day or less, 60 µg/kg/day or less, 50 µg/kg/day or less, 45 µg/kg/day or less, 40 µg/kg/day or less, 35 µg/kg/day or less, 30 µg/kg/day or less, 25 µg/kg/day or less, 20 µg/kg/day or less, 15 µg/kg/day or less, 10 µg/kg/day or less, 5 µg/kg/day or less, 4 µg/kg/day or less, 3 µg/kg/day or less, or 2 µg/kg/day or less).

The amount of the compound or composition administered to the subject can range from any of the minimum values described above to any of the maximum values described above. For example, the compound or composition can be administered to the subject in an amount of from 1 microgram (µg) per kilogram (kg) of body weight of the subject per day to 10 milligrams (mg)/kg/day (e.g., from 1 µg/kg/day to 100 µg/kg/day, from 100 µg/kg/day to 10 mg/kg/day, from 1 µg/kg/day to 10 µg/kg/day, from 10 µg/kg/day to 100 µg/kg/day, from 100 µg/kg/day to 1 mg/kg/day, from 1 mg/kg/day to 10 mg/kg/day, from 5 µg/kg/day to 10 mg/kg/day, from 1 µg/kg/day to 5 mg/kg/day, or from 5 to 5 mg/kg/day).

It is understood, however, that the specific dose level for any particular subject will depend upon a variety of factors. Such factors include the age, body weight, general health, sex, and diet of the subject. Other factors include the time and route of administration, rate of excretion, drug combination, and the type and severity of the particular disease or disorder.

Compositions, Formulations, Methods of Administration, and Kits

In vivo application of the disclosed compounds, and compositions containing them, can be accomplished by any suitable method and technique presently or prospectively known to those skilled in the art. For example, the disclosed compounds can be formulated in a physiologically- or pharmaceutically-acceptable form and administered by any suitable route known in the art including, for example, oral, nasal, rectal, topical, and parenteral routes of administration. As used herein, the term parenteral includes subcutaneous, intradermal, intravenous, intramuscular, intraperitoneal, and intrasternal administration, such as by injection. Administration of the

disclosed compounds or compositions can be a single administration, or at continuous or distinct intervals as can be readily determined by a person skilled in the art.

The compounds disclosed herein, and compositions comprising them, can also be administered utilizing liposome technology, slow release capsules, implantable pumps, and biodegradable containers. These delivery methods can, advantageously, provide a uniform dosage over an extended period of time. The compounds can also be administered in their salt derivative forms or crystalline forms.

The compounds disclosed herein can be formulated according to known methods for preparing pharmaceutically acceptable compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E.W. Martin (1995) describes formulations that can be used in connection with the disclosed methods. In general, the compounds disclosed herein can be formulated such that an effective amount of the compound is combined with a suitable excipient in order to facilitate effective administration of the compound. The compositions used can also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspension, suppositories, injectable and infusible solutions, and sprays. The preferred form depends on the intended mode of administration and application. The compositions can also include conventional pharmaceutically-acceptable carriers and diluents which are known to those skilled in the art.

Examples of carriers or diluents for use with the compounds include ethanol, dimethyl sulfoxide, glycerol, alumina, starch, saline, and equivalent carriers and diluents. To provide for the administration of such dosages for the desired application, compositions disclosed herein can comprise between about 0.1% and 100% by weight of the total of one or more of the subject compounds based on the weight of the total composition including carrier or diluent.

The pharmaceutical carrier employed can be, for example, a solid, liquid, or gas. Examples of solid carriers include lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, and stearic acid. Examples of liquid carriers are sugar syrup, peanut oil, olive oil, and water. Examples of gaseous carriers include carbon dioxide and nitrogen.

Formulations suitable for administration include, for example, aqueous sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions, which can include suspending agents and thickening agents. The formulations can be

presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and can be stored in a freeze dried (lyophilized) condition requiring only the condition of the sterile liquid carrier, for example, water for injections, prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powder, granules, tablets, *etc.* It should be understood that in addition to the excipients particularly mentioned above, the compositions disclosed herein can include other agents conventional in the art having regard to the type of formulation in question.

Compounds disclosed herein, and compositions comprising them, can be delivered to a cell either through direct contact with the cell or via a carrier means. Carrier means for delivering compounds and compositions to cells are known in the art.

For the treatment of oncological disorders, the compounds or compositions disclosed herein can be administered to a patient in need of treatment in combination with other antitumor or anticancer substances and/or with radiation and/or photodynamic therapy and/or with surgical treatment to remove a tumor. These other substances or treatments can be given at the same as or at different times from the compounds or compositions disclosed herein. For example, the compounds or compositions disclosed herein can be used in combination with mitotic inhibitors such as taxol or vinblastine, alkylating agents such as cyclophosphamide or ifosfamide, antimetabolites such as 5-fluorouracil or hydroxyurea, DNA intercalators such as adriamycin or bleomycin, topoisomerase inhibitors such as etoposide or camptothecin, antiangiogenic agents such as angiostatin, antiestrogens such as tamoxifen, and/or other anti-cancer drugs or antibodies, such as, for example, GLEEVEC (Novartis Pharmaceuticals Corporation) and HERCEPTIN (Genentech, Inc.), respectively, or an immunotherapeutic such as ipilimumab and bortezomib.

In certain examples, compounds and compositions disclosed herein can be locally administered at one or more anatomical sites, such as sites of unwanted cell growth (such as a tumor site or benign skin growth, *e.g.*, injected or topically applied to the tumor or skin growth), optionally in combination with a pharmaceutically acceptable carrier such as an inert diluent. Compounds and compositions disclosed herein can be systemically administered, such as intravenously or orally, optionally in combination with a pharmaceutically acceptable carrier such as an inert diluent, or an assimilable edible carrier for oral delivery. They can be enclosed in hard or soft shell gelatin capsules, can be compressed into tablets, or can be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound can be

combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, aerosol sprays, and the like.

The tablets, troches, pills, capsules, and the like can also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; diluents such as dicalcium phosphate; a
5 disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring can be added. When the unit dosage form is a capsule, it can contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials can be present as
10 coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules can be coated with gelatin, wax, shellac, or sugar and the like. A syrup or elixir can contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and
15 substantially non-toxic in the amounts employed. In addition, the active compound can be incorporated into sustained-release preparations and devices.

Compounds and compositions disclosed herein, including pharmaceutically acceptable salts thereof, can be administered intravenously, intramuscularly, or intraperitoneally by infusion or injection. Solutions of the active agent or its salts can be prepared in water, optionally mixed
20 with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient, which are
25 adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. The ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils,
30 nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. Optionally, the prevention of the action of

microorganisms can be brought about by various other antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the inclusion of agents that delay absorption, for example, aluminum monostearate and gelatin.

Pharmaceutical compositions disclosed herein suitable for injectable use include sterile aqueous solutions or dispersions. Furthermore, the compositions can be in the form of sterile powders for the extemporaneous preparation of such sterile injectable solutions or dispersions. In some examples, the final injectable form can be sterile and can be effectively fluid for easy syringability. In some examples, the pharmaceutical compositions can be stable under the conditions of manufacture and storage; thus, they can be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol and liquid polyethylene glycol), vegetable oils, and suitable mixtures thereof.

Sterile injectable solutions are prepared by incorporating a compound and/or agent disclosed herein in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

Pharmaceutical compositions disclosed herein can be in a form suitable for topical use such as, for example, an aerosol, cream, ointment, lotion, dusting powder, mouth washes, gargles, solution, tincture, and the like. In some examples, the compositions can be in a form suitable for use in transdermal devices. In some examples, it will be desirable to administer them topically to the skin as compositions, in combination with a dermatologically acceptable carrier, which can be a solid or a liquid. Compounds and agents and compositions disclosed herein can be applied topically to a subject's skin. These formulations can be prepared, utilizing any of the compounds disclosed herein or pharmaceutically acceptable salts thereof, via conventional processing methods.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the compounds can be dissolved or dispersed at effective

levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers, for example.

5 Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

10 Pharmaceutical compositions disclosed herein can be in a form suitable for rectal administration wherein the carrier is a solid. In some examples, the mixture forms unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art. The suppositories can be conveniently formed by first admixing the composition with the softened or melted carriers) followed by chilling and shaping in molds.

15 In addition to the aforementioned carrier ingredients, the pharmaceutical formulations described above can include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders, surface-active agents, thickeners, lubricants, preservatives (including anti-oxidants) and the like. Furthermore, other adjuvants can be included to render the formulation isotonic with the blood of the intended recipient. Compositions containing any of the compounds disclosed herein, and/or pharmaceutically acceptable salts
20 thereof, can also be prepared in powder or liquid concentrate form.

 Useful dosages of the compounds and agents and pharmaceutical compositions disclosed herein can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art.

25 The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms or disorder are affected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be
30 adjusted by the individual physician in the event of any counterindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days.

Also disclosed are kits that comprise a compound disclosed herein in one or more containers. The disclosed kits can optionally include pharmaceutically acceptable carriers and/or diluents. In one embodiment, a kit includes one or more other components, adjuncts, or adjuvants as described herein. In one embodiment, a kit includes instructions or packaging materials that describe how to administer a compound or composition of the kit. Containers of the kit can be of any suitable material, *e.g.*, glass, plastic, metal, *etc.*, and of any suitable size, shape, or configuration. In one embodiment, a compound and/or agent disclosed herein is provided in the kit as a solid, such as a tablet, pill, or powder form. In another embodiment, a compound and/or agent disclosed herein is provided in the kit as a liquid or solution. In one embodiment, the kit comprises an ampoule or syringe containing a compound and/or agent disclosed herein in liquid or solution form.

In some examples, the kit further comprises at least one agent, wherein the compound and the agent are co-formulated.

In some examples, the compound and the agent are co-packaged.

The kits can also comprise compounds and/or products co-packaged, co-formulated, and/or co-delivered with other components. For example, a drug manufacturer, a drug reseller, a physician, a compounding shop, or a pharmacist can provide a kit comprising a disclosed compound and/or product and another component for delivery to a patient.

It is contemplated that the disclosed kits can be used in connection with the disclosed methods of making, the disclosed methods of using, and/or the disclosed compositions.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

The examples below are intended to further illustrate certain aspects of the systems and methods described herein, and are not intended to limit the scope of the claims.

EXAMPLES

The following examples are set forth below to illustrate the methods and results according to the disclosed subject matter. These examples are not intended to be inclusive of all aspects of the subject matter disclosed herein, but rather to illustrate representative methods and results. These examples are not intended to exclude equivalents and variations of the present invention which are apparent to one skilled in the art.

Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric. There are numerous variations and combinations of measurement conditions, e.g., component concentrations, temperatures, pressures and other measurement ranges and conditions that can be used to optimize the described process.

Example 1 – Bile acid derived lipid nanomaterials and uses thereof

Efficient delivery of mRNA is a key step and challenge for the applicant of mRNA therapeutics. Despite promising data from ongoing clinical trials, the clinical use of mRNA requires the discovery and development of more efficient delivery systems.

Described herein are various bile acids derived lipid nanomaterials for gene therapy and drug delivery applications. Exemplary synthetic routes and characterizations are explained below and in Figure 1.

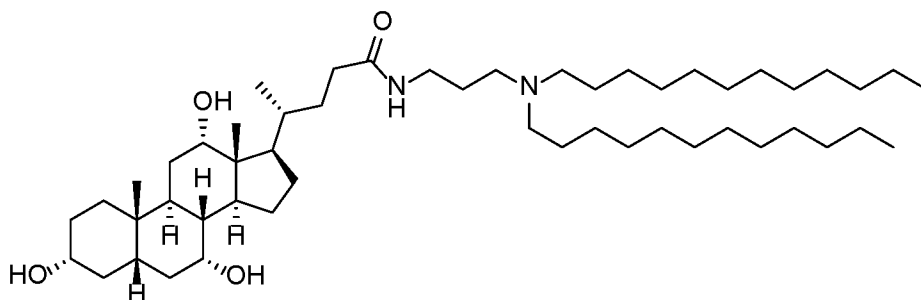
General procedures:

To a solution of Boc protected amines in anhydrous CH_2Cl_2 was added trimethylamine, Dodecyl aldehyde, and sodium triacetoxyborohydride under Ar. The reacting mixture was stirred at room temperature overnight and was evaporated under reduced pressure. The resulting product was washed with NaHCO_3 and brine, then purified by Combiflash column chromatography with a RediSep Gold Resolution silica column (Teledyne Isco) using gradient elution from 100% CH_2Cl_2 to and $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ (90/10/0.3, v/v/v) to afford Boc protected intermediate lipids.

The boc protected intermediate lipids were then dissolved in CH_2Cl_2 and trifluoroacetic acid was added, the mixture was kept stirring for 2.5 h. After the reaction was completed (checked by TLC), the solvent was removed under reduced pressure. The product intermediate lipids were used directly without further purification.

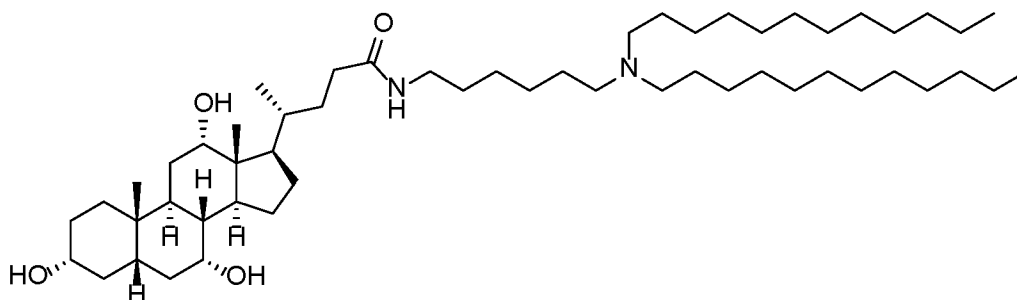
To a solution of bile acid, dimethylformamide, N,N-Diisopropylethylamine. N,N,N',N'-Tetramethyl-O-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)uronium tetrafluoroborate (TDBTU) were added. The resulting mixture was stirred for 20 min at room temperature. Then intermediate lipid was added, and the solution was stirred at room temperature overnight. The resulting mixture was added, dropwise, into 5% NaHCO_3 and the precipitate collected by filtration, and washed 3 times with deionized water. The resulting solid was further purified by Combiflash column

chromatography using a silica column (Buchi) with gradient elution from 100% CH₂Cl₂ to CH₂Cl₂/MeOH/NH₄OH (90/10/0.3, v/v/v) to give desired products.

BAL1

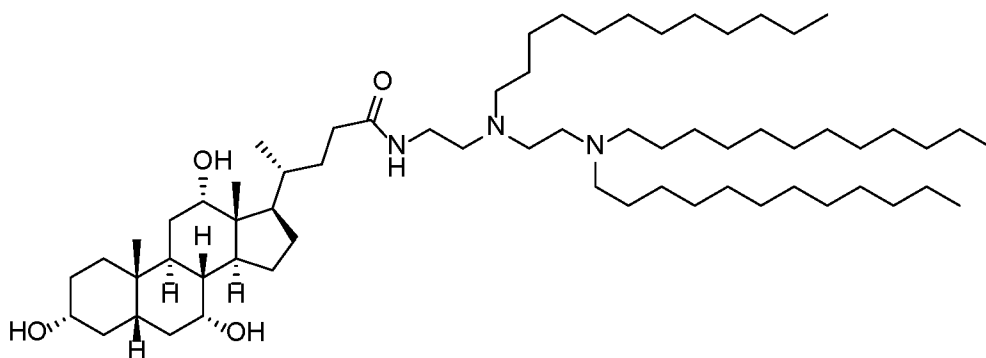
5

¹H NMR (400 MHz, CDCl₃) δ 4.00 (s, 1H), 3.86 (s, 1H), 3.46 (t, 1H), 3.41 – 3.24 (m, 2H), 2.58 (s, 2H), 2.53 – 2.39 (m, 4H), 2.30 – 0.94 (m, 76H), 0.94 – 0.86 (m, 12H), 0.71 (s, 3H). ESI-MS for C₅₁H₉₆N₂O₄ ([M+H⁺]) Calculated 801.8 Found: 801.9.

BAL2

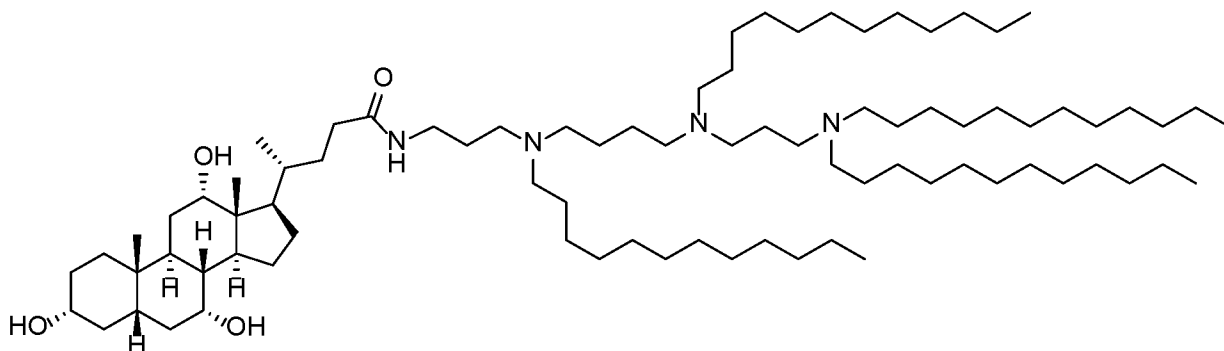
10

¹H NMR (300 MHz, CDCl₃) δ 3.97 (s, 1H), 3.85 (s, 1H), 3.44 (s, 1H), 3.28 – 3.16 (m, 2H), 2.49 – 2.33 (m, 6H), 2.28 – 0.96 (m, 87H), 0.97 – 0.80 (m, 12H), 0.69 (s, 3H). MALDI-MS for C₅₄H₁₀₂N₂O₄ [M + H⁺] Calculated 843.79; Found: 843.84.

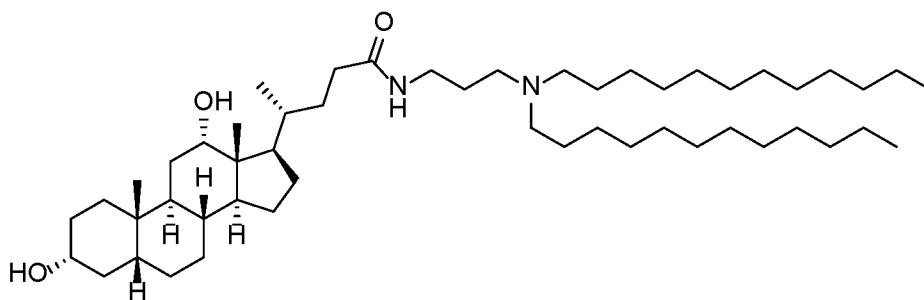
BAL3

15

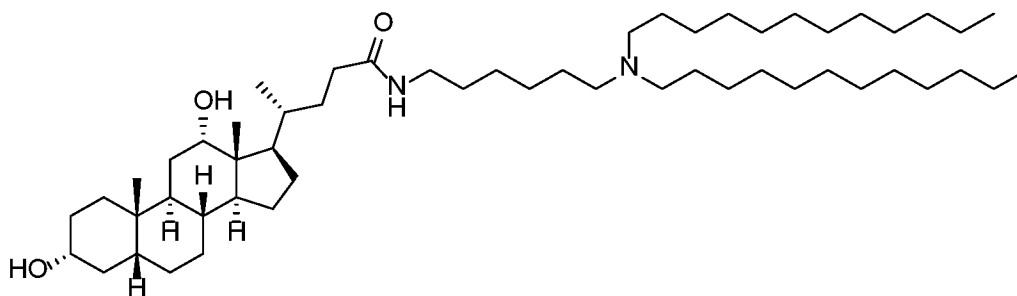
^1H NMR (300 MHz, CDCl_3) δ 4.00 (s, 1H), 3.87 (s, 1H), 3.49 (s, 1H), 3.27 (s, 2H), 2.53 (m, 6H), 2.46 (m, 6H), 2.33 – 0.96 (m, 125H), 0.96 – 0.82 (m, 15H), 0.71 (s, 3H). MALDI-MS for $\text{C}_{64}\text{H}_{123}\text{N}_3\text{O}_4$ $[\text{M} + \text{H}^+]$ Calculated: 999.96; Found: 999.95.

BAL4

^1H NMR (300 MHz, CDCl_3) δ 4.03 – 3.90 (m, 2H), 3.85 (s, 1H), 3.43 (s, 1H), 3.30 (t, $J = 11.6$ Hz, 2H), 2.54 (m, 18H), 2.33 – 0.97 (m, 122H), 0.89 (m, 18H), 0.69 (s, 3H). ESI-MS for $\text{C}_{82}\text{H}_{160}\text{N}_4\text{O}_4$ $[\text{M} + \text{H}^+]$ Calculated 1265.3 Found 1265.2.

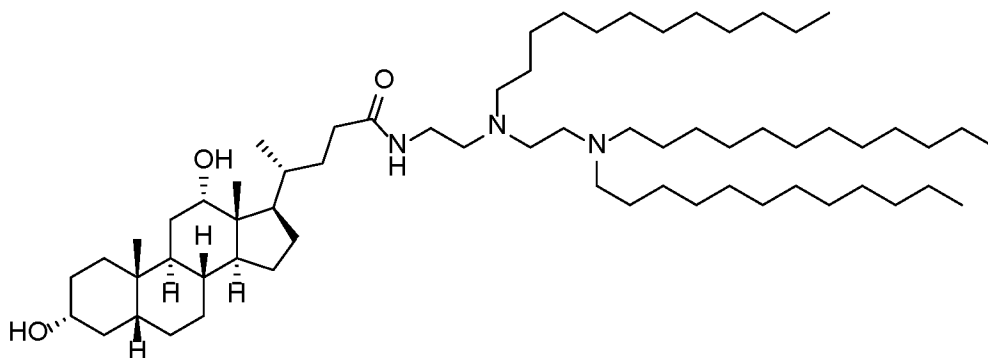
BAL5

^1H NMR (300 MHz, CDCl_3) δ 4.00 (s, 1H), 3.63 (s, 1H), 3.35 (m, 2H), 2.52 (t, $J = 5.7$ Hz, 2H), 2.45 – 2.32 (m, 4H), 2.32 – 1.01 (m, 82H), 0.98 – 0.81 (m, 12H), 0.70 (s, 3H). MALDI-MS for $\text{C}_{51}\text{H}_{96}\text{N}_2\text{O}_3$ $[\text{M} + \text{H}^+]$ Calculated: 785.75; Found: 785.74

BAL6

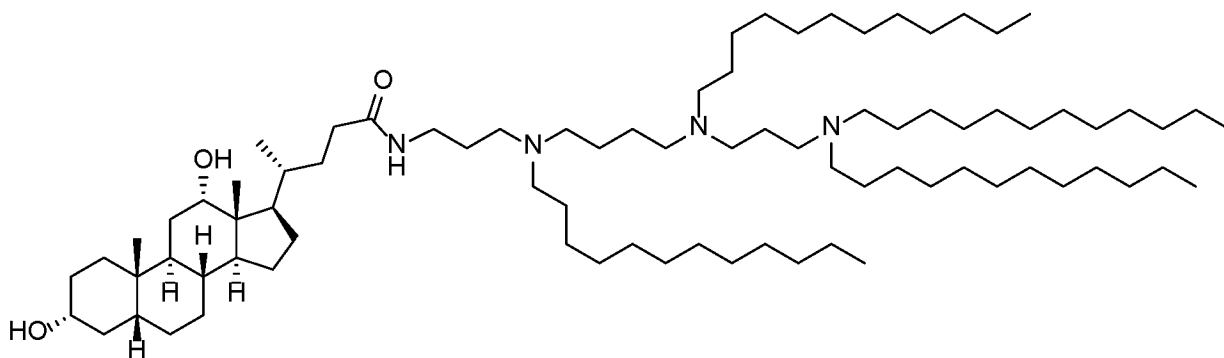
Chemical Formula: $\text{C}_{54}\text{H}_{102}\text{N}_2\text{O}_3$

^1H NMR (300 MHz, CDCl_3) δ 3.99 (s, 1H), 3.69 – 3.57 (m, 1H), 3.25 (m, 2H), 2.38 (m, 6H), 2.30 – 0.96 (m, 89H), 0.94 – 0.85 (m, 9H), 0.70 (s, 3H). MALDI-MS for $\text{C}_{54}\text{H}_{102}\text{N}_2\text{O}_3$ [$\text{M} + \text{H}^+$] Calculated 827.80; Found: 827.79.

BAL7

Chemical Formula: $\text{C}_{64}\text{H}_{123}\text{N}_3\text{O}_3$

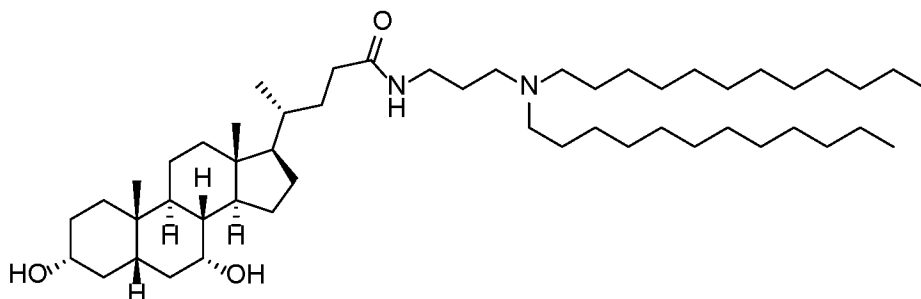
^1H NMR (300 MHz, CDCl_3) δ 3.99 (s, 1H), 3.69 – 3.56 (m, 1H), 3.26 (s, 2H), 2.50 (t, $J = 7.9$ Hz, 6H), 2.47 – 2.36 (m, 6H), 2.33 – 0.79 (m, 115H), 0.69 (s, 3H). ESI-MS for $\text{C}_{64}\text{H}_{123}\text{N}_3\text{O}_3$ [$\text{M} + \text{H}^+$] Calculated: 982.96; Found: 983.00

BAL8

Chemical Formula: $\text{C}_{82}\text{H}_{160}\text{N}_4\text{O}_3$

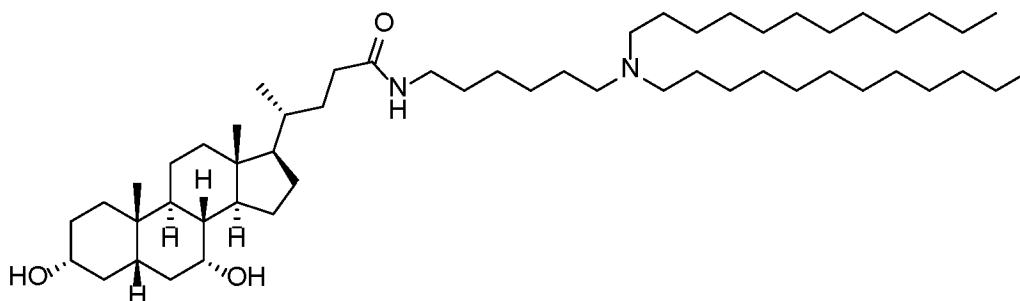
^1H NMR (300 MHz, CDCl_3) δ 3.98 (s, 1H), 3.66 – 3.54 (m, 1H), 3.30 (t, $J = 11.6$ Hz, 2H), 2.54 (m, 18H), 2.33 – 0.97 (m, 122H), 0.89 (m, 15H), 0.69 (s, 3H). ESI-MS for $\text{C}_{82}\text{H}_{160}\text{N}_4\text{O}_3$ [$\text{M} + \text{H}^+$] Calculated: 1250.25; Found: 1250.25

BAL9



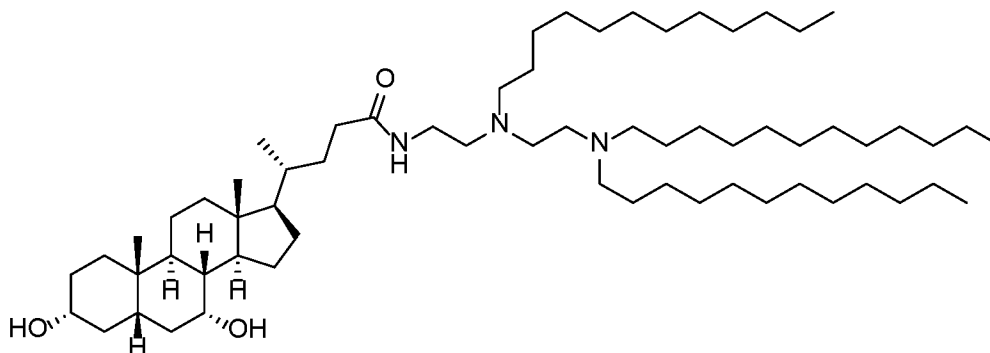
^1H NMR (300 MHz, CDCl_3) δ 3.87 (s, 1H), 3.48 (s, 1H), 3.35 (m, 2H), 2.52 (t, $J = 5.7$ Hz, 2H), 2.45 – 2.29 (m, 3H), 2.29 – 1.03 (m, 67H), 0.99 – 0.79 (m, 12H), 0.68 (s, 3H). MALDI-MS $\text{C}_{51}\text{H}_{96}\text{N}_2\text{O}_3$ $[\text{M} + \text{H}^+]$ Calculated: 785.75; Found: 785.74.

5 **BAL10**



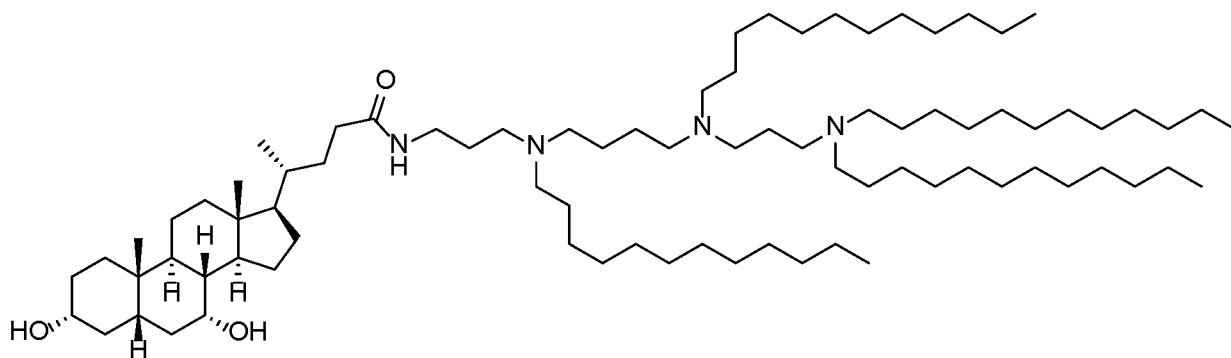
^1H NMR (300 MHz, CDCl_3) δ 3.86 (s, 1H), 3.47 (s, 1H), 3.24 (m, 2H), 2.45 – 2.31 (m, 6H), 2.22 – 1.03 (m, 86H), 0.96 – 0.82 (m, 12H), 0.67 (s, 3H). MALDI-MS for $\text{C}_{54}\text{H}_{102}\text{N}_2\text{O}_3$ $[\text{M} + \text{H}^+]$ Calculated 827.8; Found: 827.8

10 **BAL11**



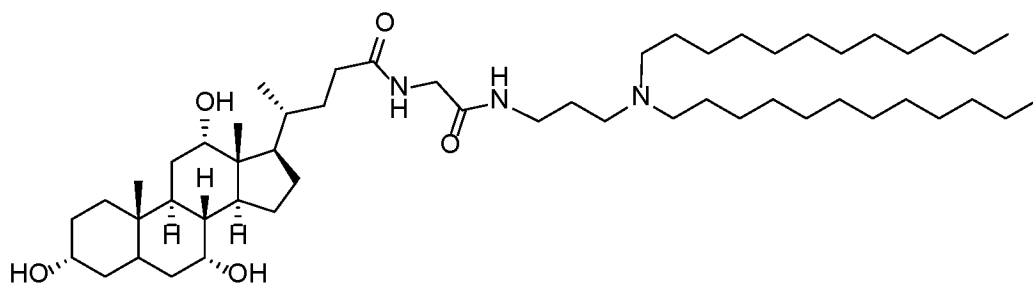
^1H NMR (300 MHz, CDCl_3) δ 3.87 (s, 1H), 3.47 (s, 1H), 3.26 (m, 2H), 2.51 (t, 6H), 2.48 – 2.33 (m, 6H), 2.30 – 0.78 (m, 109H), 0.68 (s, 3H). ESI-MS for $\text{C}_{64}\text{H}_{123}\text{N}_3\text{O}_3$ $[\text{M} + \text{H}^+]$ Calculated: 983.0; Found: 983.0

15 **BAL12**



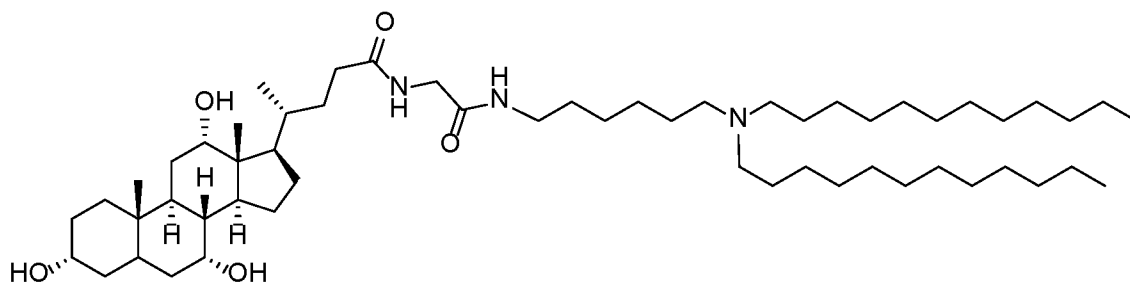
^1H NMR (300 MHz, CDCl_3) δ 3.99 (s, 1H), 3.43 (s, 1H), 3.30 (t, $J = 11.6$ Hz, 2H), 2.54 (m, 18H), 2.33 – 0.97 (m, 122H), 0.89 (m, 15H), 0.69 (s, 3H). MALDI-MS for $\text{C}_{82}\text{H}_{160}\text{N}_4\text{O}_3$ [$\text{M} + \text{H}^+$] Calculated: 1250.25; Found: 1250.29.

5 **BAL13**



^1H NMR (300 MHz, CDCl_3) δ 3.97 (s, 1H), 3.93 – 3.81 (m, 2H), 3.45 (s, 1H), 3.36 (d, $J = 5.7$ Hz, 2H), 2.54 (d, $J = 6.1$ Hz, 2H), 2.49 – 2.39 (m, 4H), 2.36 – 0.78 (m, 89H), 0.70 (s, 3H). MALDI-MS for $\text{C}_{53}\text{H}_{99}\text{N}_3\text{O}_5$ [$\text{M} + \text{H}^+$] Calculated: 858.77; Found: 858.78.

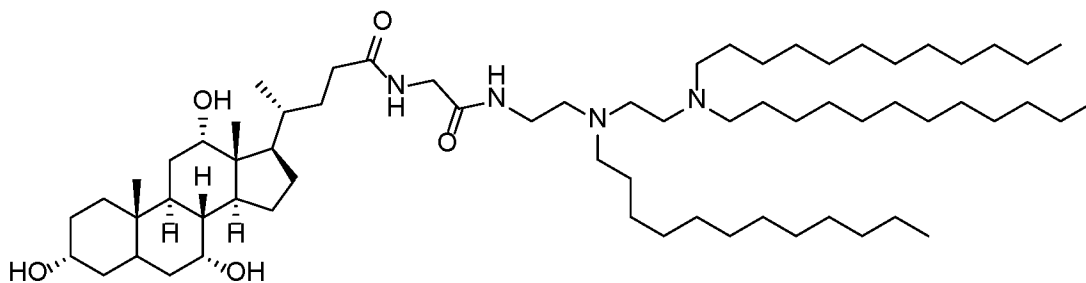
10 **BAL14**



Chemical Formula: $\text{C}_{56}\text{H}_{105}\text{N}_3\text{O}_5$

^1H NMR (300 MHz, CDCl_3) δ 4.03 – 3.90 (m, 2H), 3.86 (s, 2H), 3.42 (s, 1H), 3.23 (m, 2H), 2.38 (m, 6H), 2.28 – 0.97 (m, 82H), 0.96–0.89 (m, 12H), 0.68 (s, 3H). MALDI-MS for $\text{C}_{56}\text{H}_{105}\text{N}_3\text{O}_5$ [$\text{M} + \text{H}^+$] Calculated 900.8; Found: 900.9.

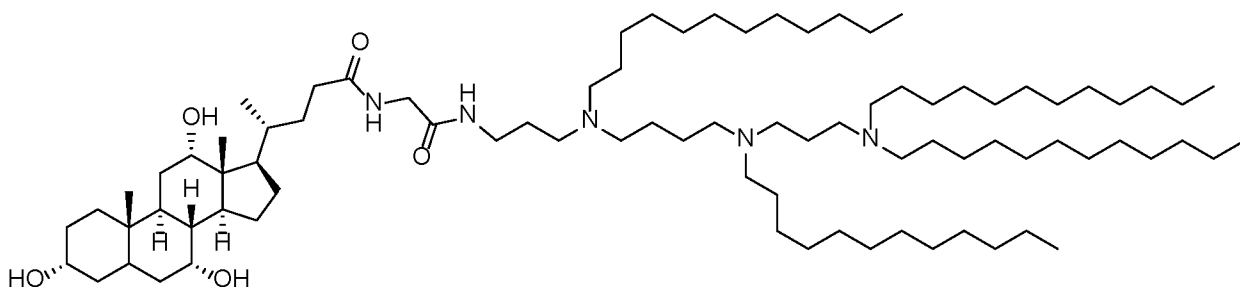
15 **BAL15**



Chemical Formula: $C_{66}H_{126}N_4O_5$

1H NMR (300 MHz, $CDCl_3$) δ 4.03 – 3.90 (m, 2H), 3.85 (s, 1H), 3.43 (s, 1H), 3.30 (s, 2H), 2.52 (t, 6H), 2.41 (m, 6H), 2.35 – 0.79 (m, 100H), 0.70 (s, 3H). ESI-MS $C_{66}H_{126}N_4O_5$ for $[M + H^+]$ Calculated: 1055.98; Found: 1056.00.

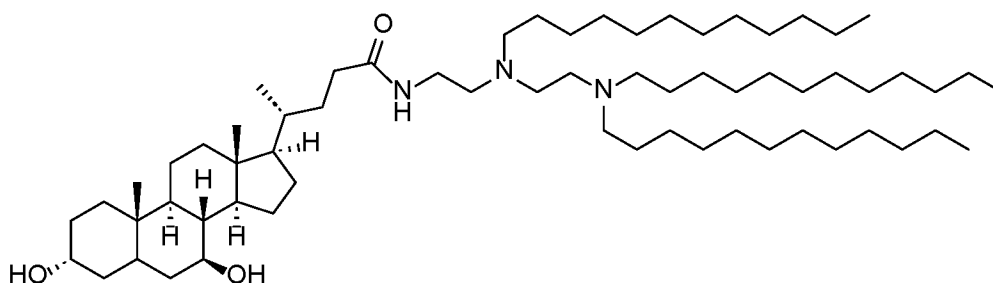
5 **BAL16**



Chemical Formula: $C_{84}H_{163}N_5O_5$

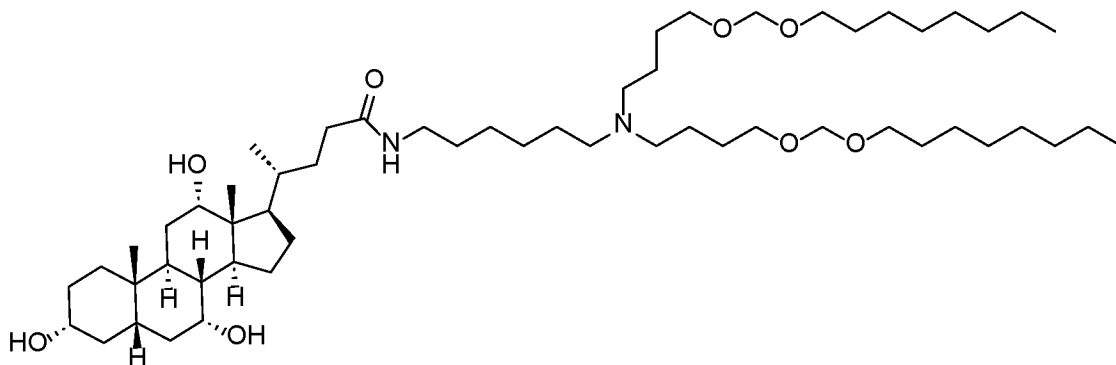
1H NMR (300 MHz, $CDCl_3$) δ 4.03 – 3.90 (m, 2H), 3.85 (s, 1H), 3.43 (s, 1H), 3.30 (t, $J = 11.6$ Hz, 2H), 2.54 (m, 18H), 2.33 – 0.97 (m, 122H), 0.89 (m, 15H), 0.69 (s, 3H). ESI-MS for $C_{84}H_{163}N_5O_5$ $[M+H^+]$ calculated 1265.3 Found 1265.2.

10 **BAL17**



1H NMR (300 MHz, $CDCl_3$) δ 3.60 (s, 2H), 3.28 (m, 2H), 2.51 (t, 6H), 2.48 – 2.33 (m, 6H), 2.30 – 0.78 (m, 95H), 0.70 (s, 3H).

15 **BAL18**



^1H NMR (400 MHz, CDCl_3) δ 4.67 (s, 4H), 3.99 (s, 1H), 3.82 (s, 1H), 3.59 (m, 4H), 3.49 (m, 6H), 3.33 (s, 1H), 3.05 (m, 8H), 2.39–0.91 (m, 118H), 0.68 (s, 3H).

5

In vitro screenings

Lipid nanoparticles (LNPs) with synthesized bile acid derived lipids, DOPE, cholesterol and DMG-PEG were prepared and screened in various cell lines using mRNA-encoding firefly luciferase (Figures 2-4). BAL6 shows more effective mRNA delivery efficiency than other LNPs in Hep3B cell line (Figure 2). In both JAWS2 (Figure 3) and C2C12 cell lines (Figure 4), BAL7 and BAL11 show high mRNA delivery efficiency.

Other advantages which are obvious and which are inherent to the invention will be evident to one skilled in the art. It will be understood that certain features and sub-combinations are of utility and may be employed without reference to other features and sub-combinations. This is contemplated by and is within the scope of the claims. Since many possible embodiments may be made of the invention without departing from the scope thereof, it is to be understood that all matter herein set forth or shown in the accompanying drawings is to be interpreted as illustrative and not in a limiting sense.

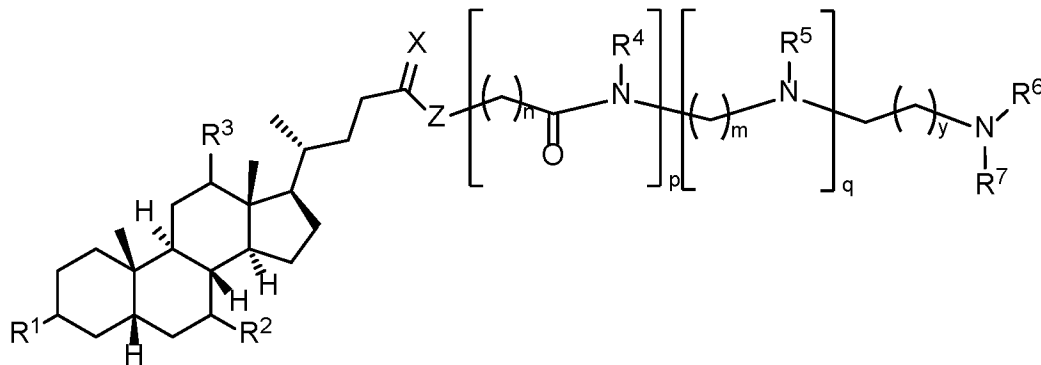
The methods of the appended claims are not limited in scope by the specific methods described herein, which are intended as illustrations of a few aspects of the claims and any methods that are functionally equivalent are intended to fall within the scope of the claims. Various modifications of the methods in addition to those shown and described herein are intended to fall within the scope of the appended claims. Further, while only certain representative method steps disclosed herein are specifically described, other combinations of the method steps also are intended to fall within the scope of the appended claims, even if not specifically recited. Thus, a combination of steps, elements, components, or constituents may be explicitly mentioned herein

or less, however, other combinations of steps, elements, components, and constituents are included, even though not explicitly stated.

CLAIMS

What is claimed is:

1. A compound defined by Formula I, or a pharmaceutically acceptable salt thereof:



I

wherein

X and Z are each independently O, NR^a, or S;

R^a, when present, is hydrogen or substituted or unsubstituted C₁-C₁₀ alkyl;

R¹, R², and R³ are each independently H, OH, halogen, substituted or unsubstituted C₁-C₃ alkyl, or substituted or unsubstituted C₁-C₃ alkoxy;

p is an integer from 0 to 5;

q is an integer from 0 to 5;

n is an integer from 1 to 10;

m in each case is independently an integer from 1 to 10;

y is an integer from 1 to 10;

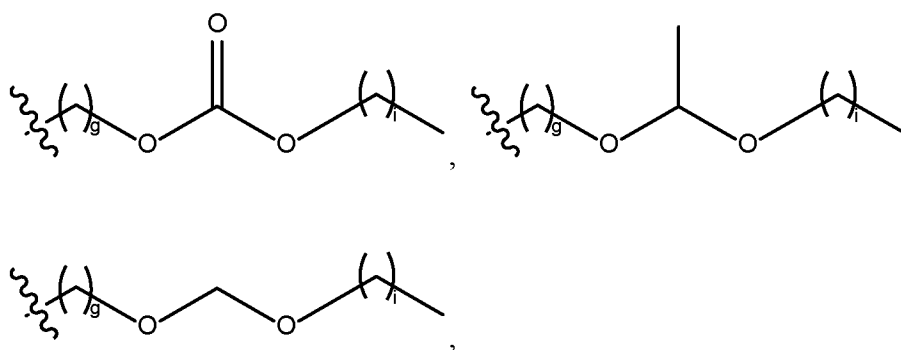
each R⁴, when present, is independently hydrogen or a substituted or unsubstituted C₁-C₁₈ alkyl;

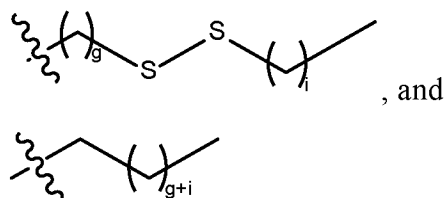
each R⁵, when present, is independently a substituted or unsubstituted C₈-C₁₈ alkyl; and

R⁶ and R⁷ are each independently a substituted or unsubstituted C₈-C₁₈ alkyl.

2. The compound of claim 1, wherein X is O.
3. The compound of claim 1 or claim 2, wherein Z is NR^a.
4. The compound of any one of claims 1-3, wherein R¹ is OH.

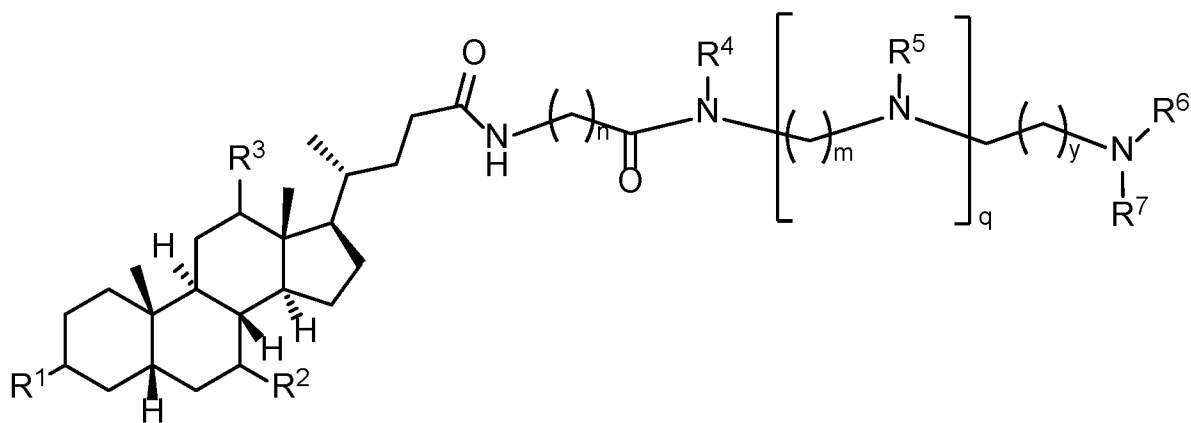
5. The compound of any one of claims 1-4, wherein R^2 is OH.
6. The compound of any one of claims 1-5, wherein R^3 is OH.
7. The compound of any one of claims 1-6, wherein R^1 , R^2 , and R^3 are all the same.
8. The compound of any one of claims 1-6, wherein R^1 , R^2 , and R^3 are different.
9. The compound of any one of claims 1-8, wherein R^6 and R^7 are each independently an unsubstituted C₈-C₁₈ alkyl or a linear or branched C₈-C₁₈ alkyl substituted with one or more substituents selected from the group consisting of amine, amide, ester, ether, and carbonate ester.
10. The compound of any one of claims 1-9, wherein R^6 and R^7 are each independently an unsubstituted C₈-C₁₈ alkyl.
11. The compound of any one of claims 1-10, wherein R^6 and R^7 are each independently an unsubstituted linear C₈-C₁₈ alkyl.
12. The compound of any one of claims 1-11, wherein R^6 and R^7 are each independently an unsubstituted linear C₁₀-C₁₄ alkyl.
13. The compound of any one of claims 1-12, wherein R^6 and R^7 are each an unsubstituted C₁₂ alkyl.
14. The compound of any one of claims 1-12, wherein R^6 and R^7 are independently selected from the group consisting of:





wherein G and I each independently represent integers from 1 to 9.

15. The compound of any one of claims 1-14, wherein R^6 and R^7 are the same.
16. The compound of any one of claims 1-15, wherein y is from 1 to 6.
17. The compound of any one of claims 1-16, wherein p is 0 or 1, such as 0.
18. The compound of any one of claims 1-17, wherein the compound is further defined by Formula II, or a pharmaceutically acceptable salt thereof:

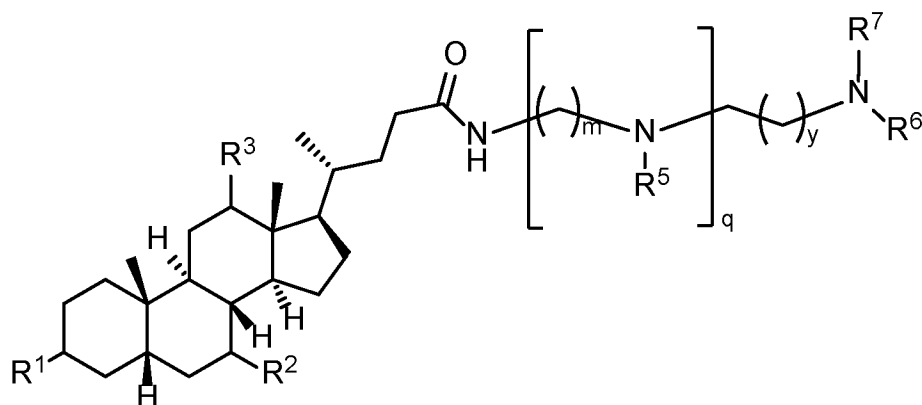


II

wherein n is an integer from 1 to 5.

19. The compound of claim 18, wherein n is from 1 to 3.
20. The compound of any one of claims 18-19, wherein R^4 is hydrogen.
21. The compound of any one of claims 18-19, wherein R^4 is an unsubstituted C_1 - C_5 alkyl.

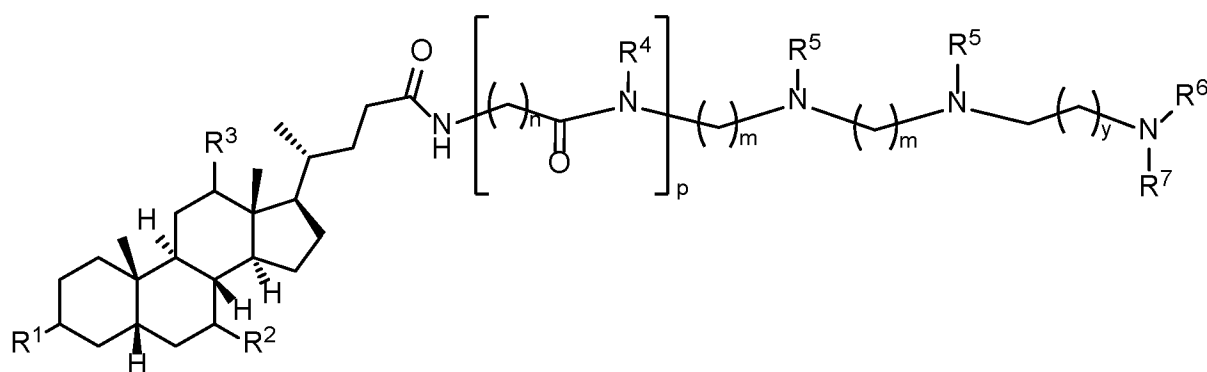
22. The compound of claim 21, wherein the compound is further defined by Formula III, or a pharmaceutically acceptable salt thereof:



III

wherein m in each case is independently an integer from 1 to 7.

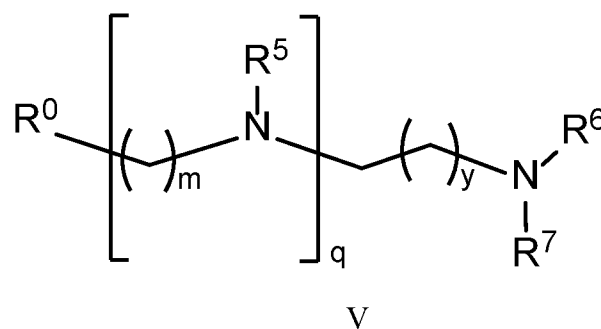
23. The compound of any one of claims 1-22, wherein q is an integer from 0 to 2.
24. The compound of any one of claims 1-23, wherein q is 0.
25. The compound of any one of claims 1-23, wherein q is 1.
26. The compound of any one of claims 1-23, wherein q is 2.
27. The compound of claim 26, wherein the compound is further defined by Formula IV, or a pharmaceutically acceptable salt thereof:



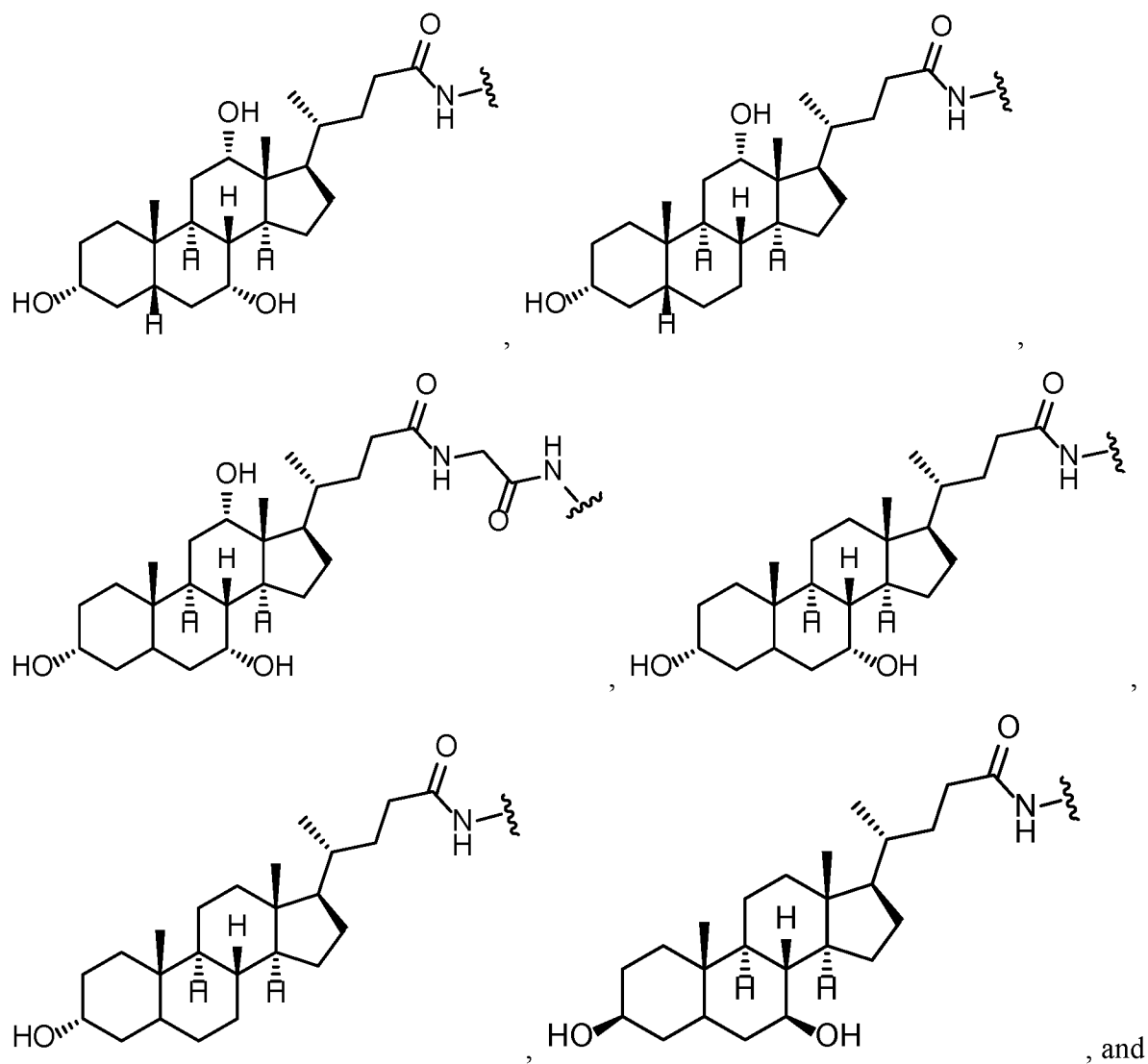
IV

wherein each m is independently an integer from 1 to 7; and
each R^5 is independently a substituted or unsubstituted C_8 - C_{18} alkyl.

28. The compound of any one of claims 26-27, wherein each m is the same.
29. The compound of any one of claims 26-27, wherein each m is different.
30. The compound of any one of claims 26-29, wherein each R^5 is the same.
31. The compound of any one of claims 26-29, wherein each R^5 is different.
32. The compound of any one of claims 1-31, wherein the compound is further defined by Formula V, or a pharmaceutically acceptable salt thereof:

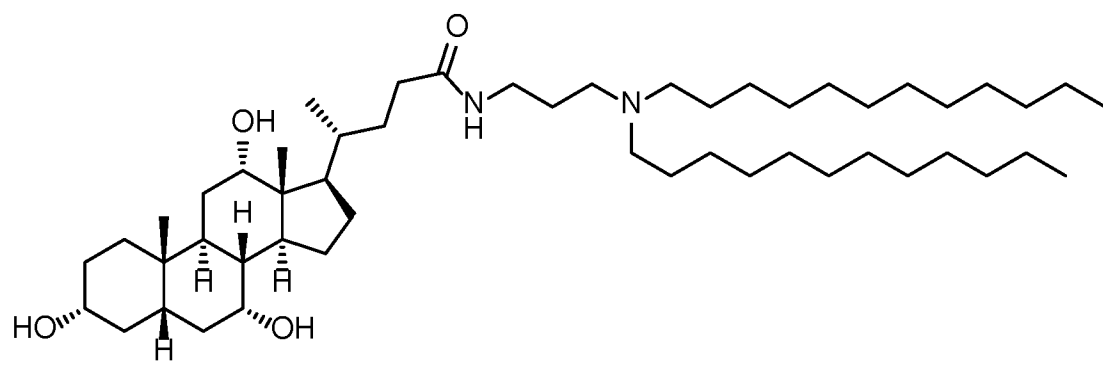


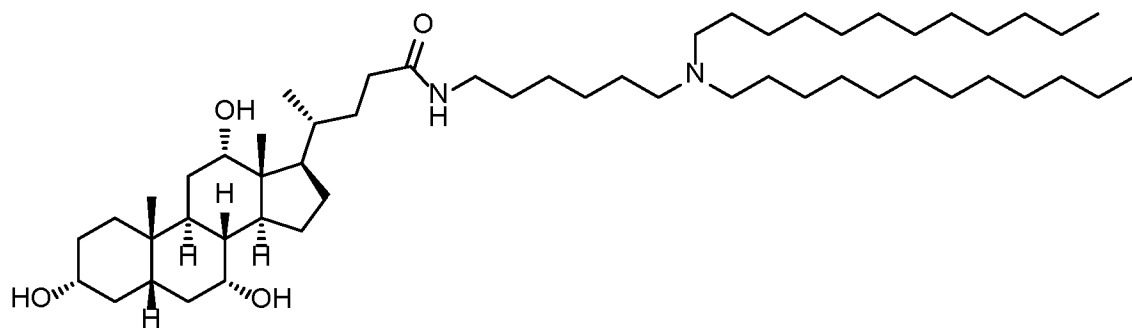
wherein q is an integer from 0 to 5;
 y is an integer from 1 to 7;
each m is independently an integer from 1 to 7;
each R^5 , when present, is independently a substituted or unsubstituted C_8 - C_{18} alkyl;
 R^6 and R^7 are each independently substituted or unsubstituted C_8 - C_{18} alkyl
and R^0 is selected from the group consisting of:



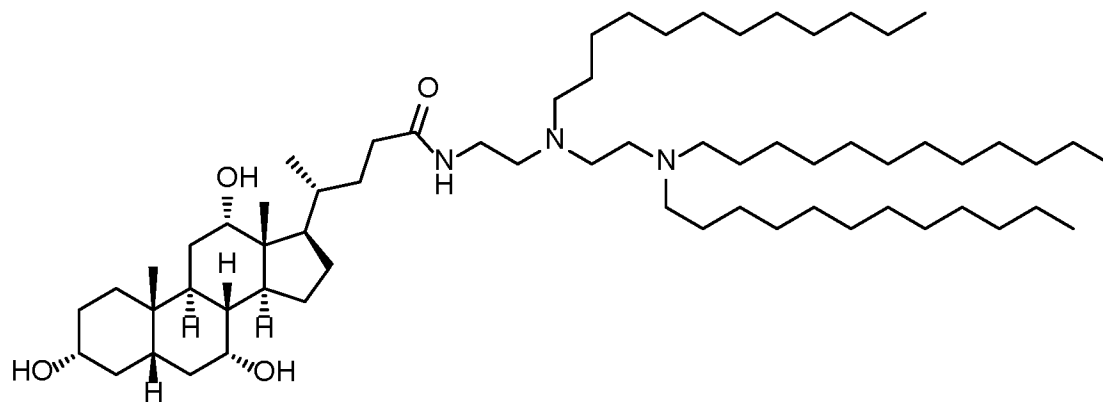
pharmaceutically acceptable salts thereof.

33. The compound of any one of claims 1-32, wherein the compound is selected from the group consisting of:

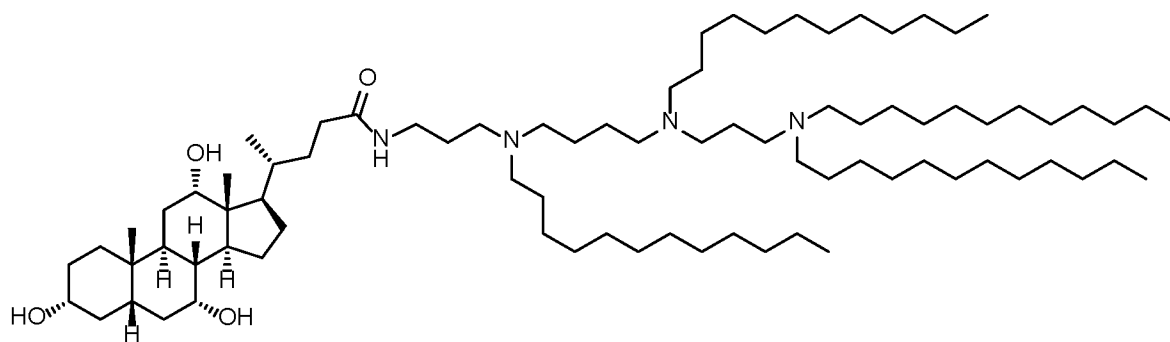




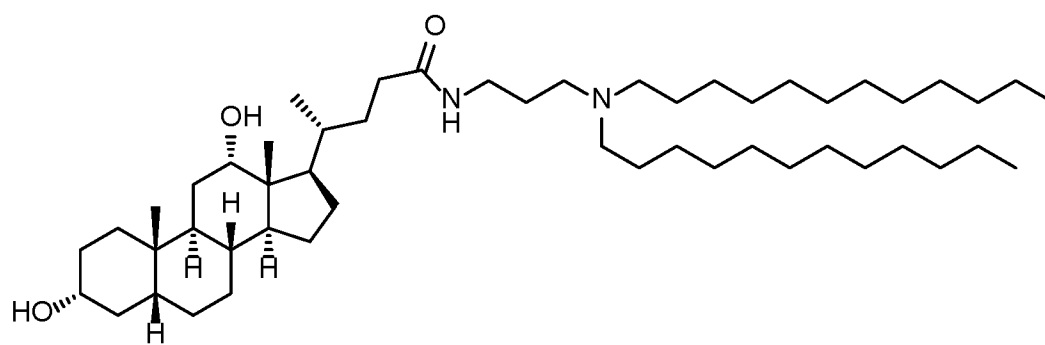
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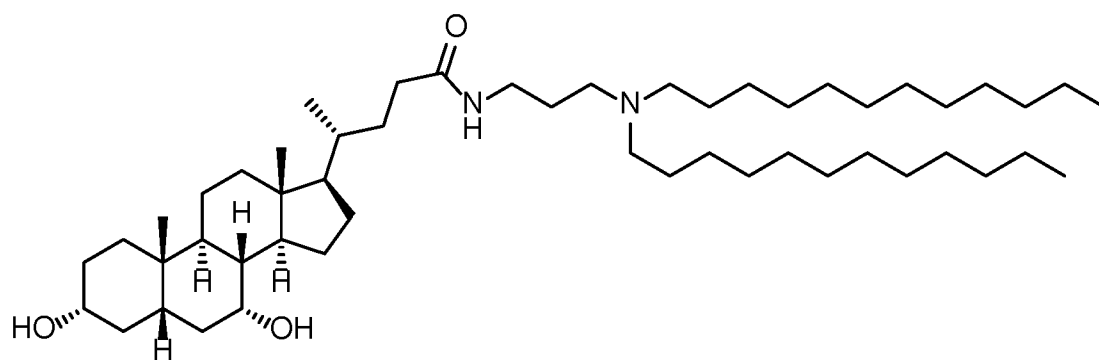
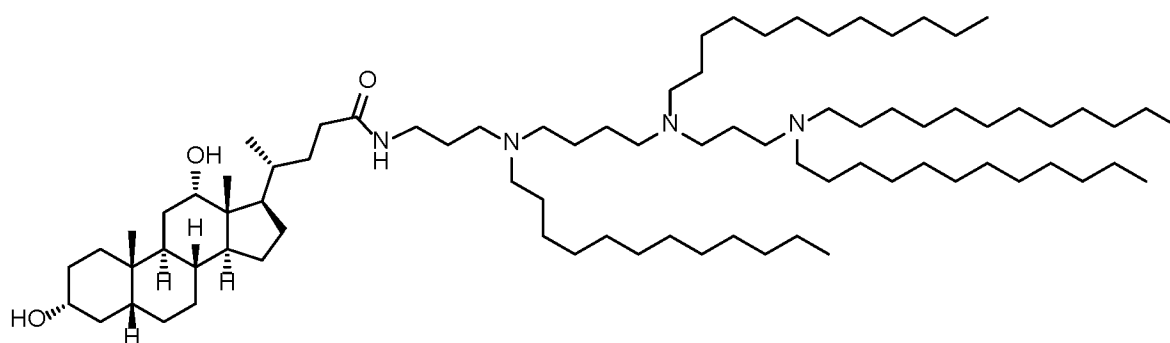
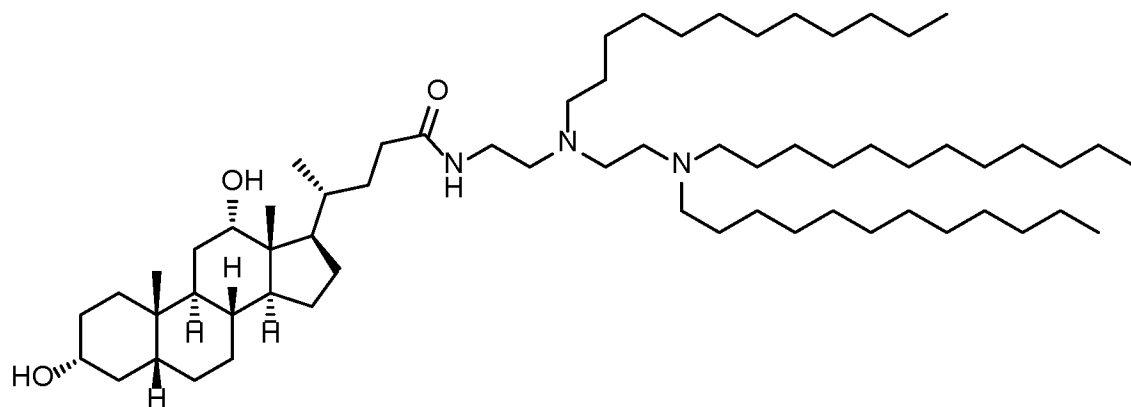
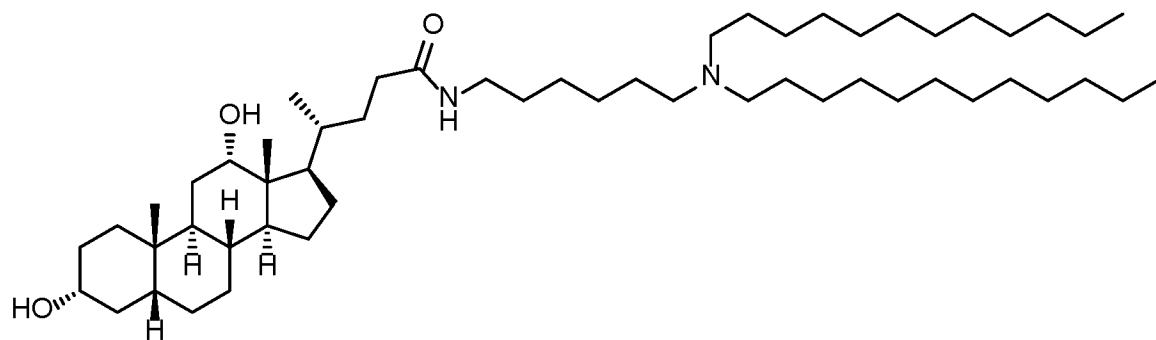
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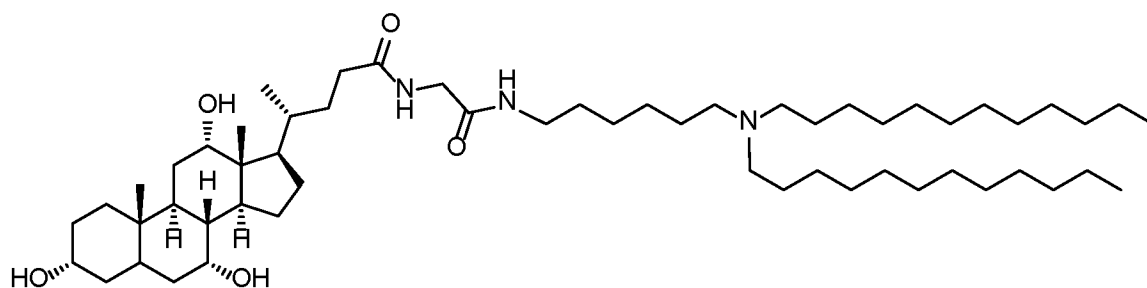
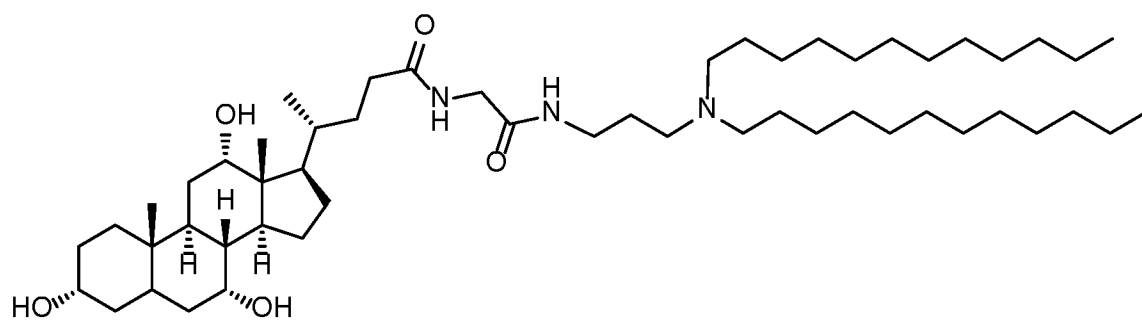
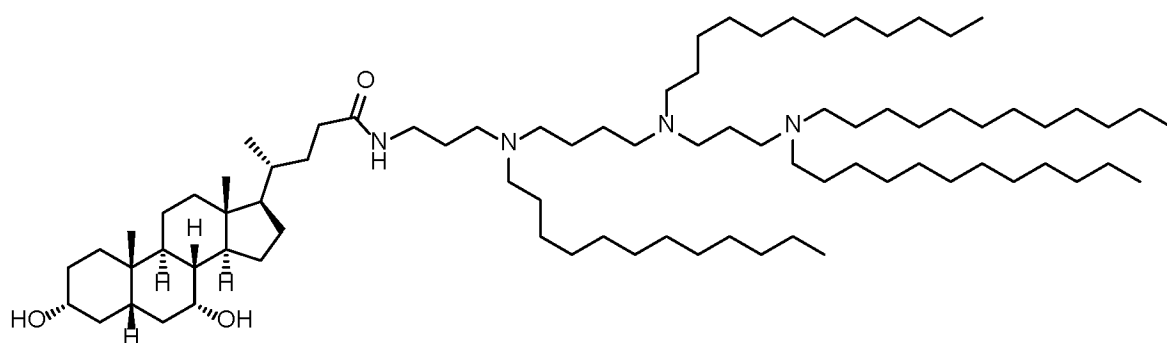
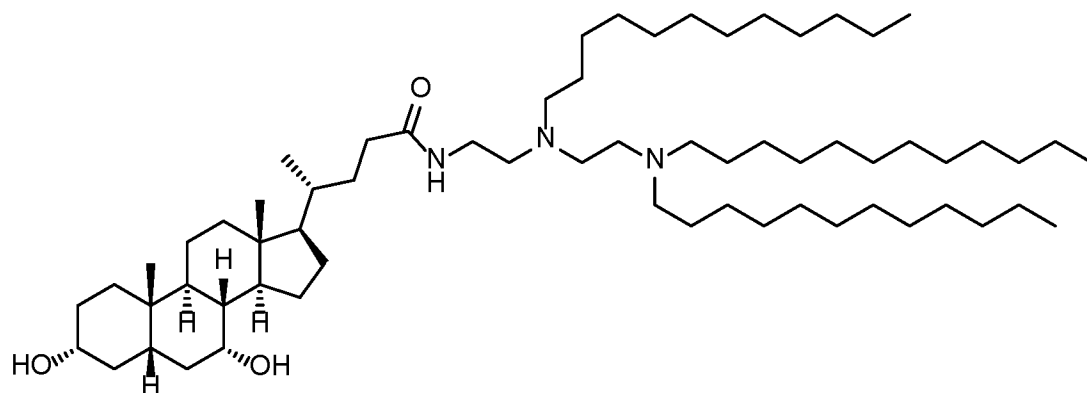
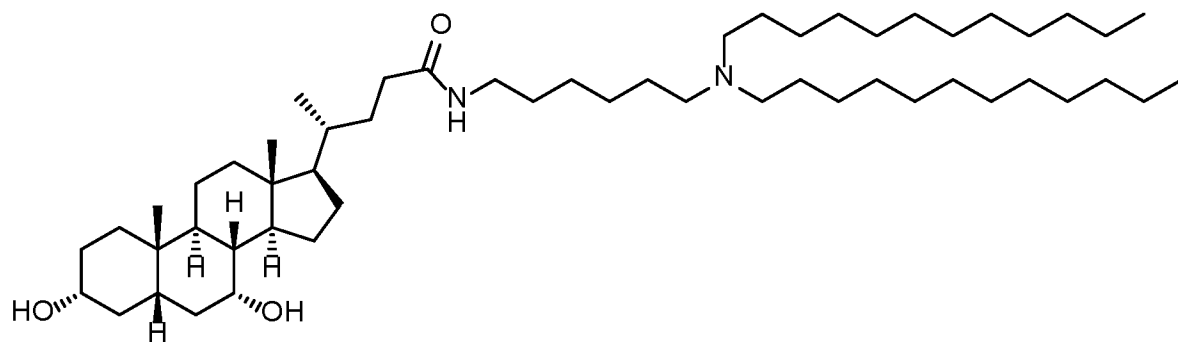


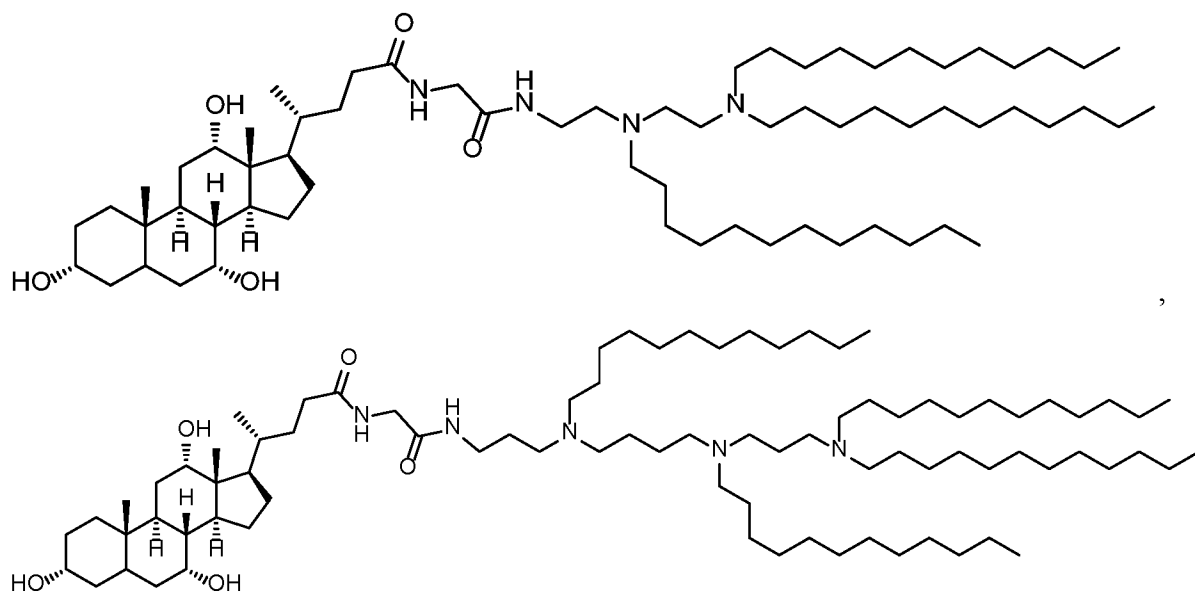
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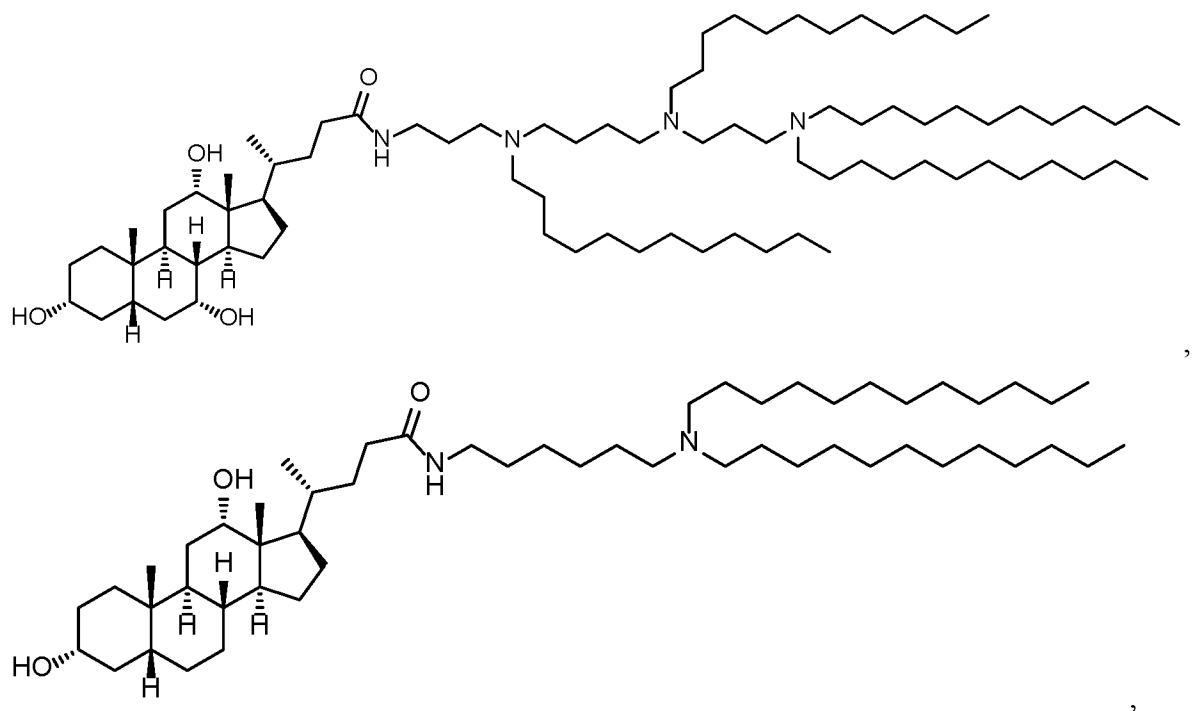


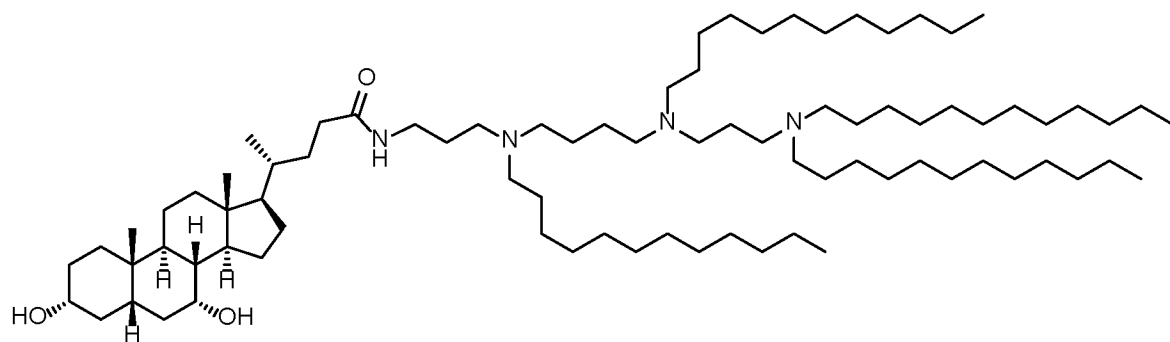
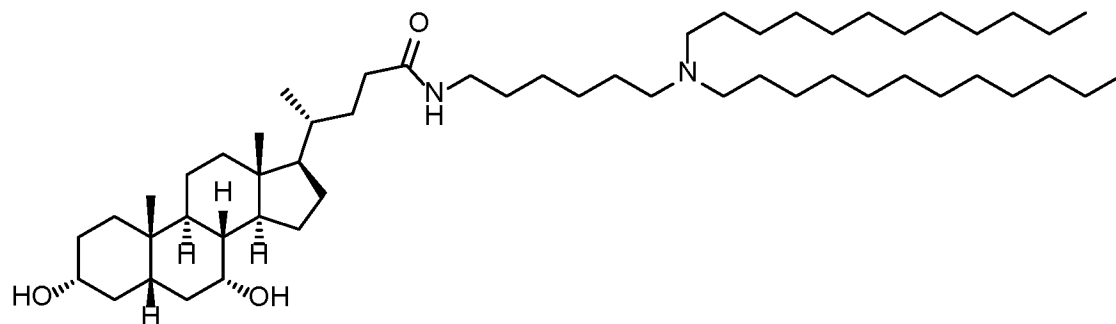
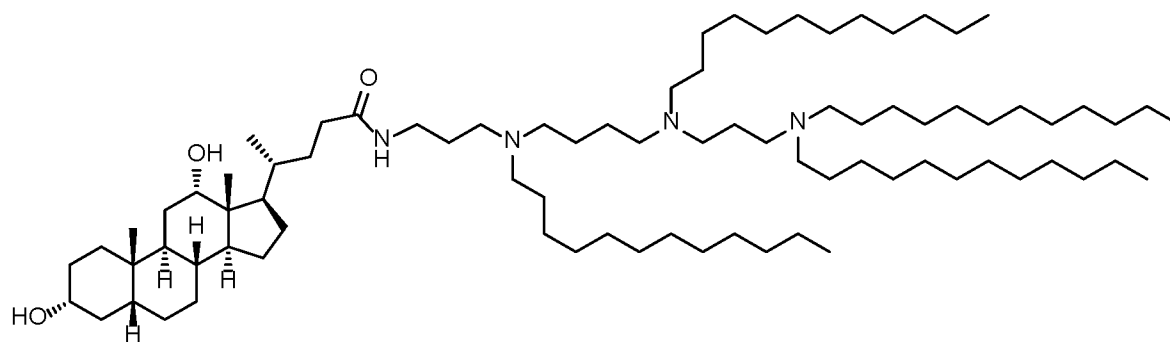




and pharmaceutically acceptable salts thereof, and combinations thereof.

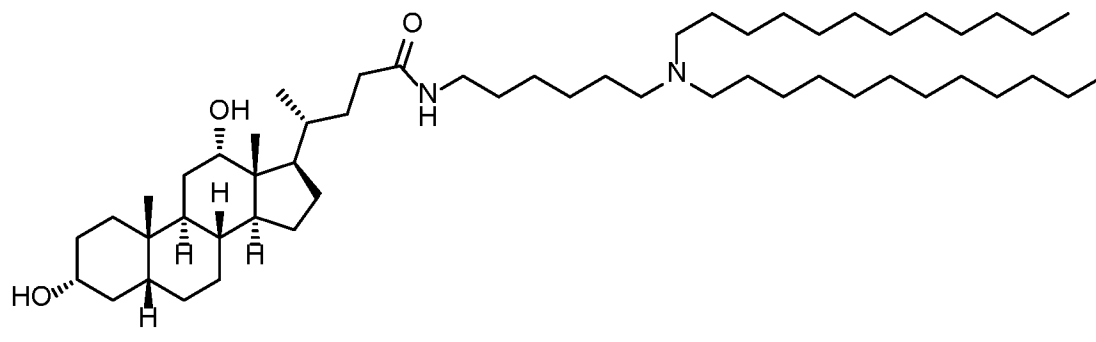
34. The compound of any one of claims 1-33, wherein the compound is selected from the group consisting of:

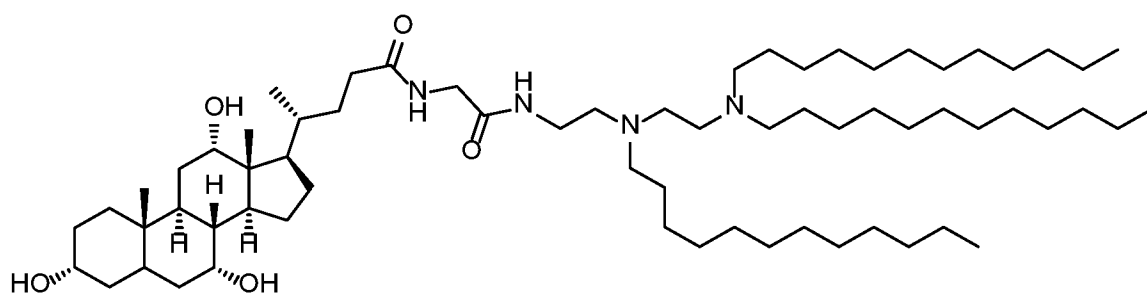
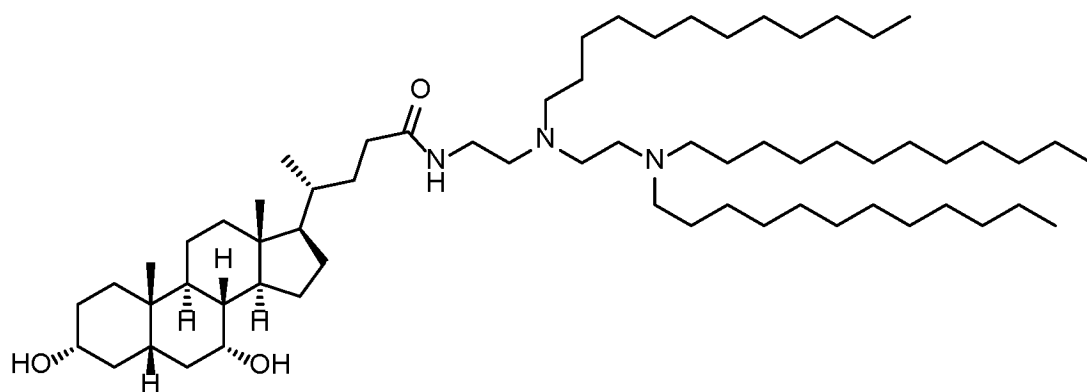
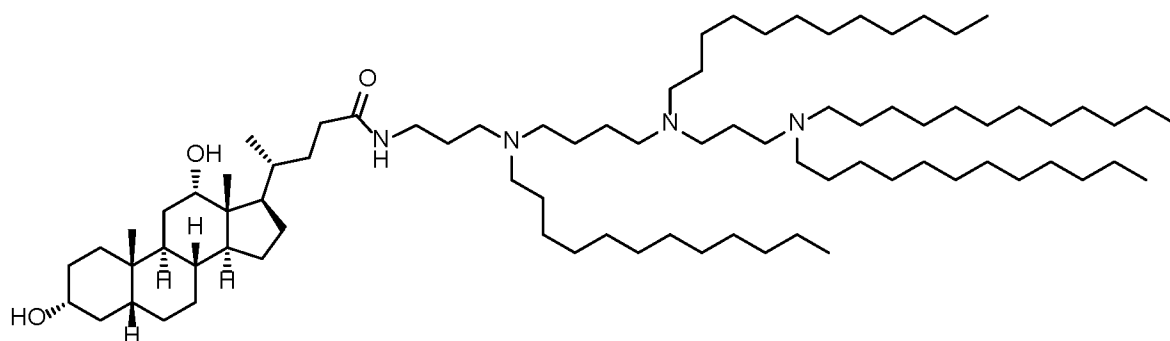
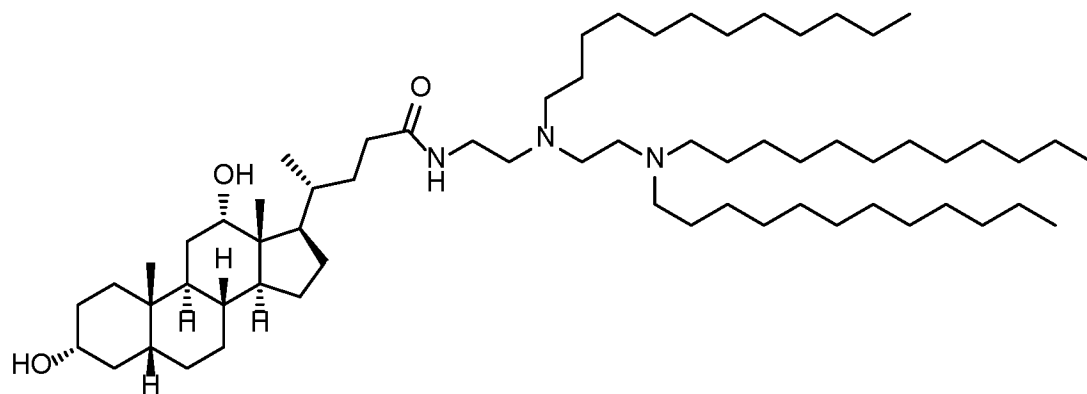




and pharmaceutically acceptable salts thereof, and combinations thereof.

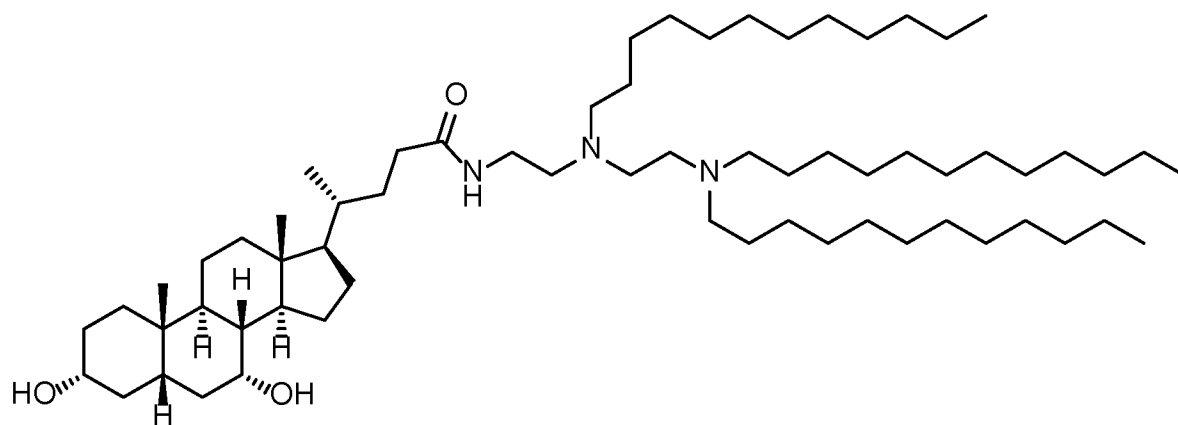
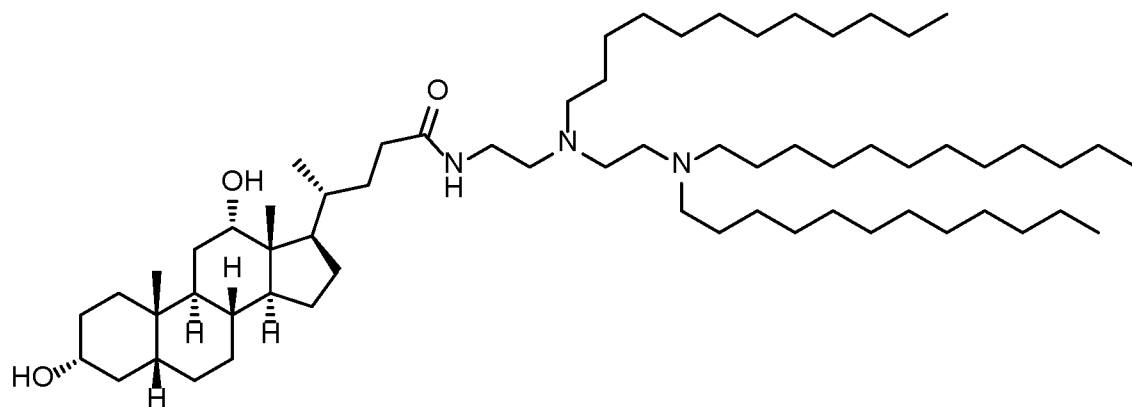
35. The compound of any one of claims 1-33, wherein the compound is selected from the group consisting of:





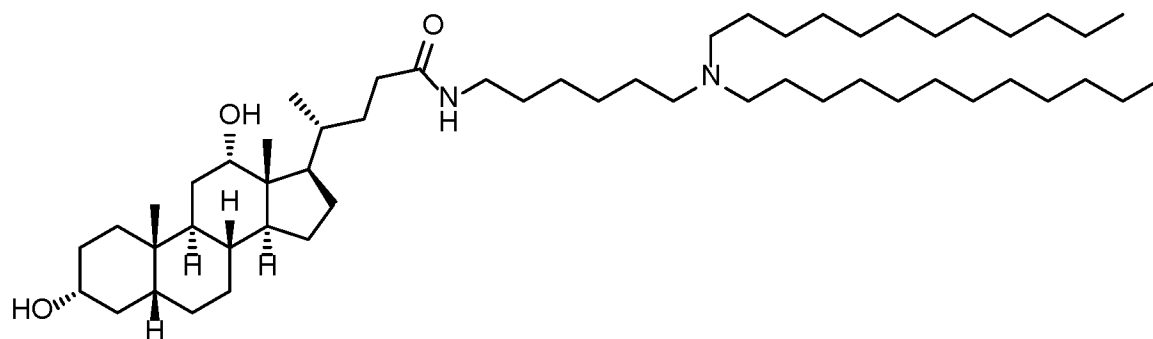
, and
pharmaceutically acceptable salts thereof, and combinations thereof.

36. The compound of any one of claims 1-33, wherein the compound is selected from the group consisting of:



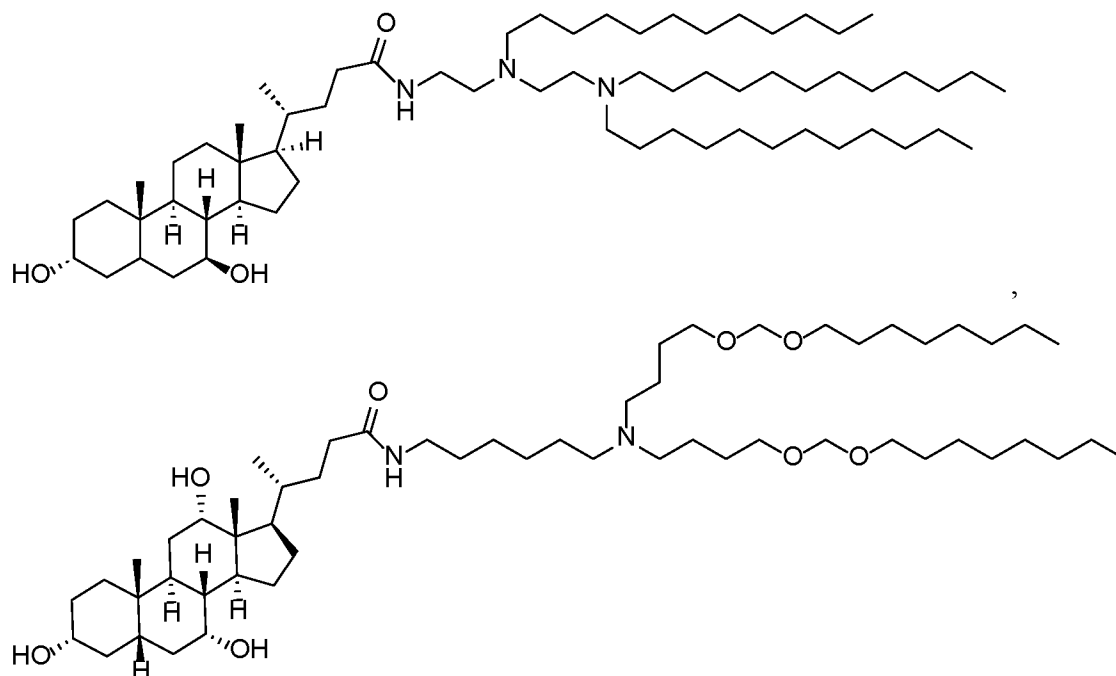
and pharmaceutically acceptable salts thereof, and combinations thereof.

37. The compound of any one of claims 1-33, wherein the compound comprises:



pharmaceutically acceptable salts thereof.

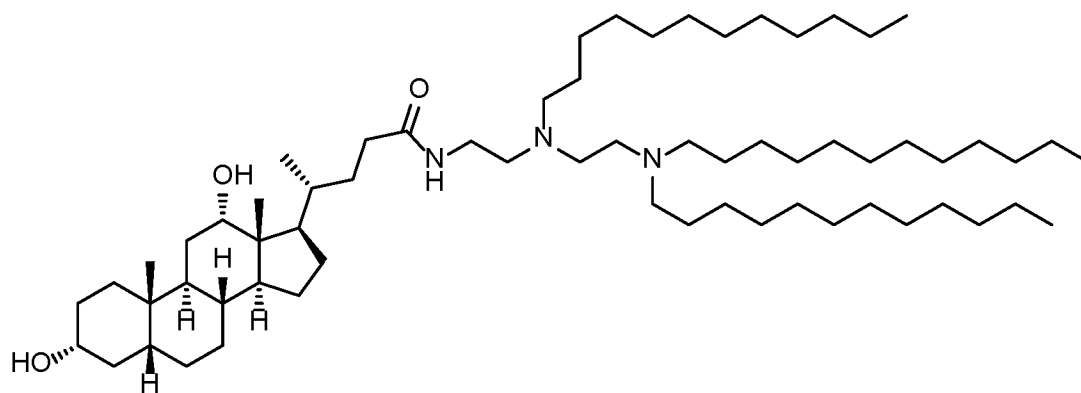
38. The compound of any one of claims 1-33, wherein the compound is selected from the group consisting of:



, and

pharmaceutically acceptable salts thereof, and combinations thereof.

39. The compound of any one of claims 1-33, wherein the compound comprises:



, or

pharmaceutically acceptable salts thereof.

40. A composition comprising:
the compound of any of claims 1-39; and
an agent.

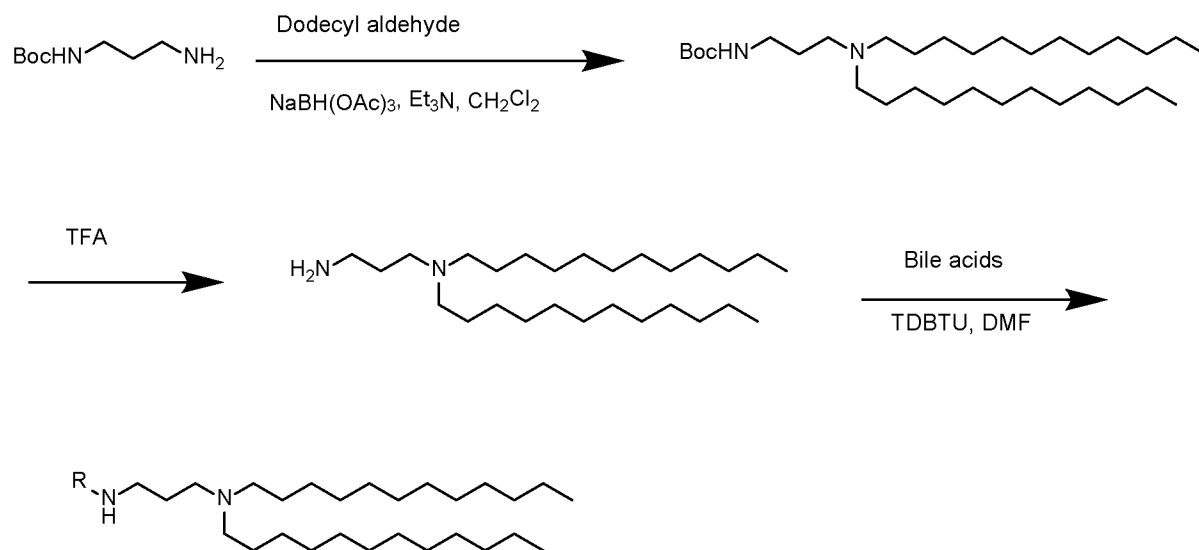
41. The composition of claim 40, wherein the agent is a polynucleotide.

42. The composition of any of claims 40-41, wherein the agent is an RNA.
43. The composition of any of claims 40-42, wherein the agent is an mRNA.
44. A method of making the compound of any one of claims 1-39.
45. A lipid nanoparticle comprising:
the compound of any of claims 1-39;
a non-cationic lipid;
a polyethylene glycol-lipid; and
a sterol.
46. The lipid nanoparticle of claim 45, further comprising an agent.
47. The lipid nanoparticle of claim 46, wherein the agent is a polynucleotide.
48. The lipid nanoparticle of any of claims 46-47, wherein the agent is an RNA.
49. The lipid nanoparticle of any of claims 46-48, wherein the agent is an mRNA.
50. The lipid nanoparticle of claim 49, wherein the mRNA is encapsulated by the nanoparticle.
51. The lipid nanoparticle of any of claims 45-50, wherein the non-cationic lipid comprises 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (SOPE), DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine), 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), 1,2-dioleoyl-5/7-glycero-3-phospho-(1'-rac-glycerol) (DOPG), or combinations thereof.
52. The lipid nanoparticle of any of claims 45-51, wherein the sterol comprises a cholesterol-based lipid.

53. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of the compounds of any one of claims 1-39, the composition of any one of claims 40-43, or the lipid nanoparticles of any one of claims 45-52.

54. A method for delivering an agent into a cell, comprising:
introducing into the cell the composition of any one of claims 40-43, or the lipid nanoparticle of any one of claims 45-52 or the pharmaceutically acceptable composition of claim 53.

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R=

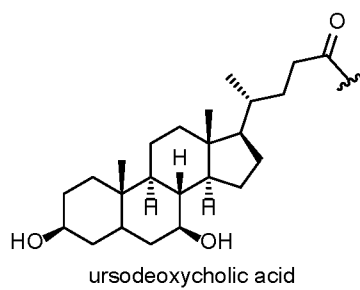
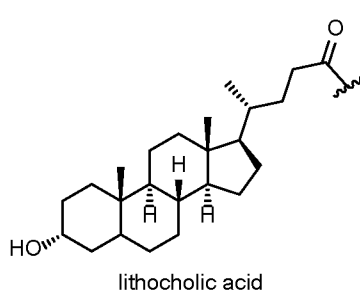
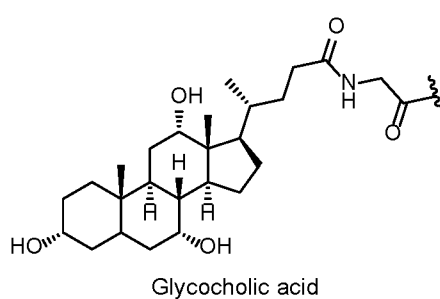
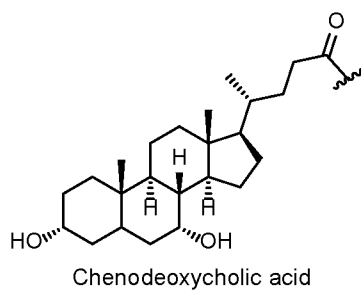
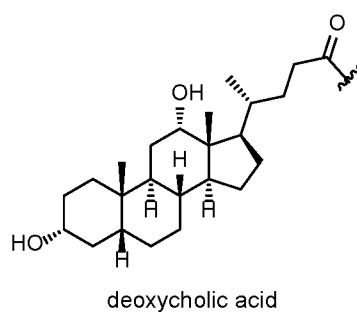
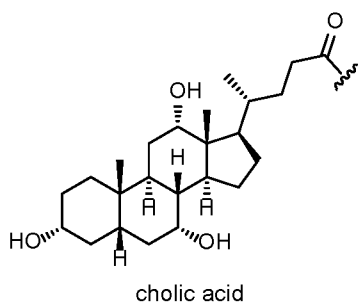


FIG. 1

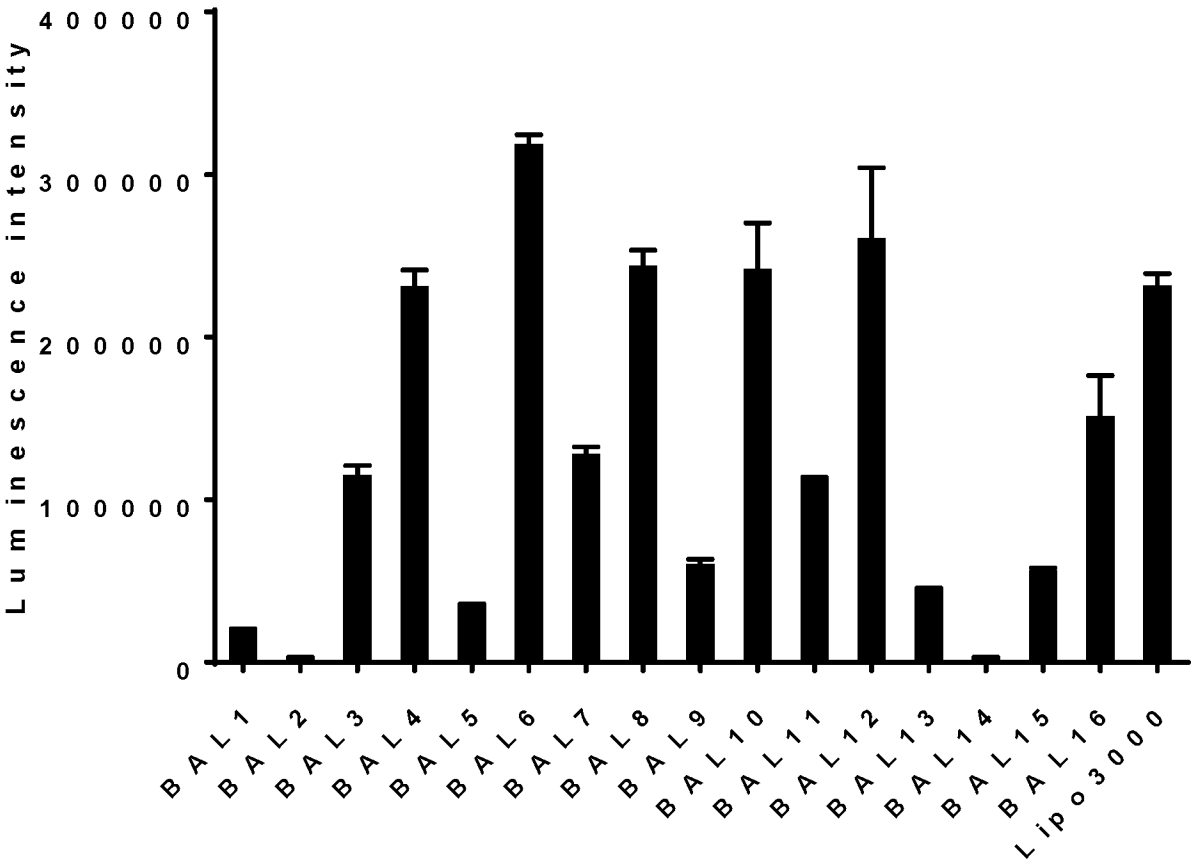


FIG. 2

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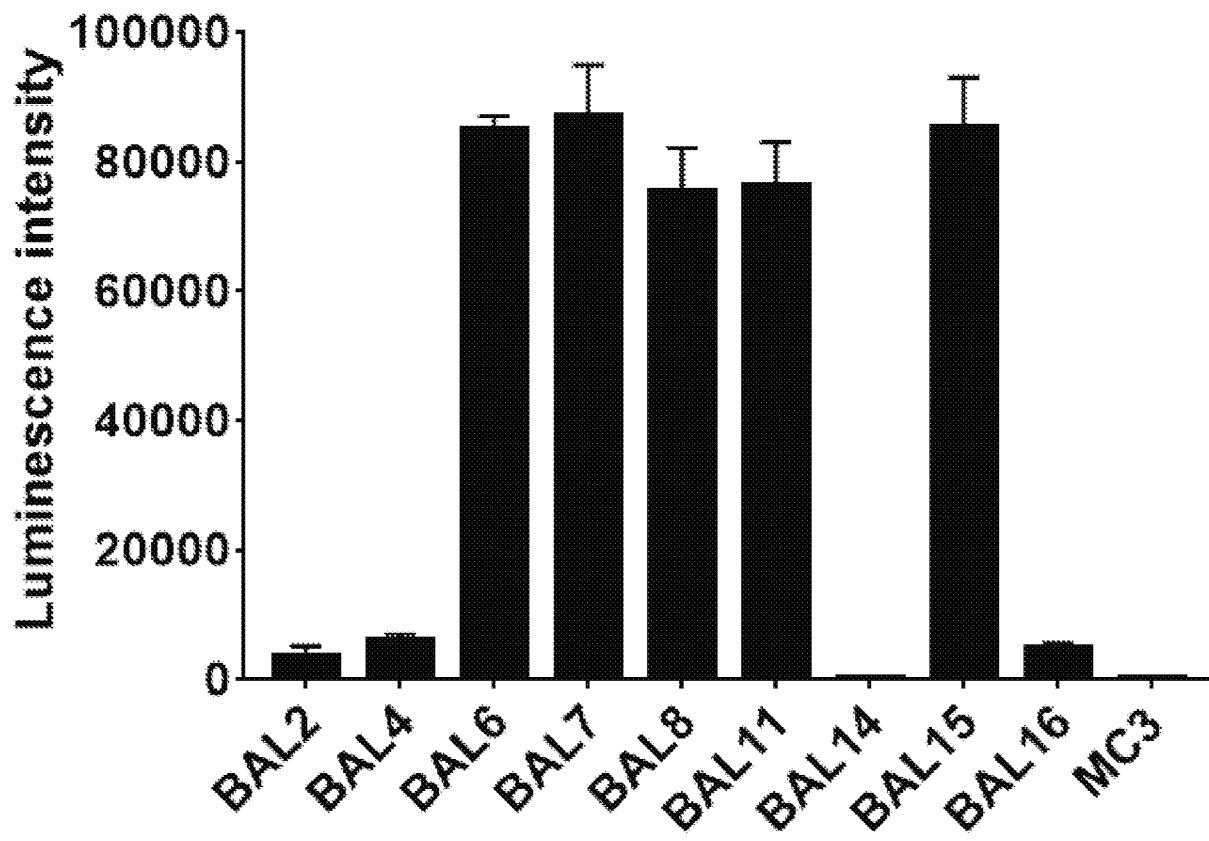


FIG. 3

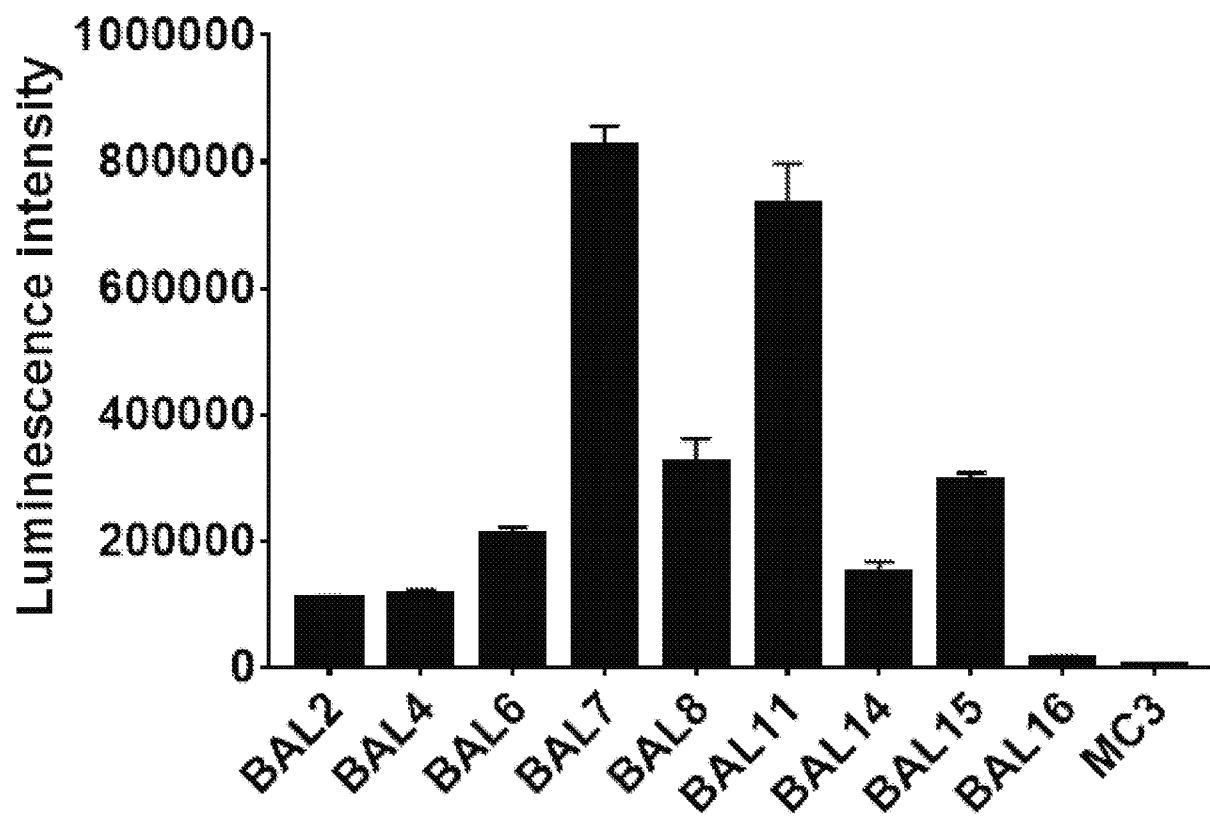


FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/83144

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 9/127, C07J 41/00 (2024.01)

ADD. C12N 15/09 (2024.01)

CPC - INV. A61K 9/127, A61K 9/1271, C07J 41/00

ADD. C12N 15/09

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2020/0131223 A1 (Insight Health Limited), 30 April 2020 (30.04.2020), entire document, especially para[0016]-[0025], [0041]-[0042]	1-3
A	WO 2001/49324 A2 (Woodle et al.), 12 July 2001 (12.07.2001), entire document, especially page 45, ln 25-33	1-3
A	US 2022/0378939 A1 (Translate Bio, Inc.), 01 December 2022 (01.12.2022), entire document, especially para[0015], [0054]	1-3
A	US 3,144,471 (Nelson, N. A.), 01 August 1964 (01.08.1964), entire document, especially col 1, ln 10-53	1-3
A	"Pubchem CID 153303806", Create date: 13 August 2020 (13.08.2020), entire document, especially page 2, compound listed	1-3
A	WO 2019/129913 A1 (Universidad Del Pais Vasco), 04 July 2019 (04.07.2019), entire document, especially page 13, para 1; see Formula (I)	1-3

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

"D" document cited by the applicant in the international application

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

14 February 2024

Date of mailing of the international search report

APR 10 2024

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Kari Rodriguez

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 23/83144

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-54
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.