

LIPIDS FOR USE IN LIPID NANOPARTICLE FORMULATIONS

BACKGROUND

Technical Field

The present disclosure generally relates to novel cationic lipids that can be used in combination with other lipid components, such as neutral lipids, cholesterol, and polymer conjugated lipids, to form lipid nanoparticles with oligonucleotides, to facilitate the intracellular delivery of therapeutic nucleic acids (*e.g.*, oligonucleotides, messenger RNA) both *in vitro* and *in vivo*.

Description of the Related Art

There are many challenges associated with the delivery of nucleic acids to affect a desired response in a biological system. Nucleic acid-based therapeutics have enormous potential but there remains a need for more effective delivery of nucleic acids to appropriate sites within a cell or organism in order to realize this potential. Therapeutic nucleic acids include, *e.g.*, messenger RNA (mRNA), antisense oligonucleotides, ribozymes, DNazymes, plasmids, immune stimulating nucleic acids, antagomir, antimir, mimic, supermir, and aptamers. Some nucleic acids, such as mRNA or plasmids, can be used to effect expression of specific cellular products as would be useful in the treatment of, for example, diseases related to a deficiency of a protein or enzyme. The therapeutic applications of translatable nucleotide delivery are extremely broad as constructs can be synthesized to produce any chosen protein sequence, whether or not indigenous to the system. The expression products of the nucleic acid can augment existing levels of protein, replace missing or non-functional versions of a protein, or introduce new protein and associated functionality in a cell or organism.

Some nucleic acids, such as miRNA inhibitors, can be used to effect expression of specific cellular products that are regulated by miRNA as would be useful in the treatment of, for example, diseases related to deficiency of protein or enzyme. The therapeutic applications of miRNA inhibition are extremely broad as constructs can be synthesized to inhibit one or more miRNA that would in turn regulate the expression of mRNA products. The inhibition of endogenous miRNA can augment its downstream target endogenous protein expression and restore proper function in a cell or organism as a means to treat disease associated to a specific miRNA or a group of miRNA.

Other nucleic acids can down-regulate intracellular levels of specific mRNA and, as a result, down-regulate the synthesis of the corresponding proteins through processes such as RNA interference (RNAi) or complementary binding of antisense RNA. The therapeutic applications of antisense oligonucleotide and RNAi are also extremely broad, since oligonucleotide constructs
5 can be synthesized with any nucleotide sequence directed against a target mRNA. Targets may include mRNAs from normal cells, mRNAs associated with disease-states, such as cancer, and mRNAs of infectious agents, such as viruses. To date, antisense oligonucleotide constructs have shown the ability to specifically down-regulate target proteins through degradation of the cognate mRNA in both *in vitro* and *in vivo* models. In addition, antisense oligonucleotide constructs are
10 currently being evaluated in clinical studies.

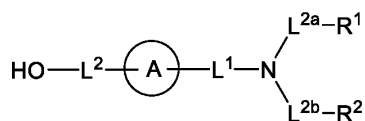
However, two problems currently face using oligonucleotides in therapeutic contexts. First, free RNAs are susceptible to nuclease digestion in plasma. Second, free RNAs have limited ability to gain access to the intracellular compartment where the relevant translation machinery resides. Lipid nanoparticles formed from cationic lipids with other lipid components, such as
15 neutral lipids, cholesterol, PEG, PEGylated lipids, and oligonucleotides have been used to block degradation of the RNAs in plasma and facilitate the cellular uptake of the oligonucleotides.

There remains a need for improved cationic lipids and lipid nanoparticles for the delivery of oligonucleotides. Preferably, these lipid nanoparticles would provide optimal drug to lipid ratios, protect the nucleic acid from degradation and clearance in serum, be suitable for systemic
20 delivery, and provide intracellular delivery of the nucleic acid. In addition, these lipid-nucleic acid particles should be well-tolerated and provide an adequate therapeutic index, such that patient treatment at an effective dose of the nucleic acid is not associated with unacceptable toxicity and/or risk to the patient. The present disclosure provides these and related advantages.

BRIEF SUMMARY

25 In brief, the present disclosure provides lipid compounds, including stereoisomers, pharmaceutically acceptable salts, or tautomers thereof, which can be used alone or in combination with other lipid components such as neutral lipids, charged lipids, steroids (including for example, all sterols) and/or their analogs, and/or polymer conjugated lipids to form lipid nanoparticles for the delivery of therapeutic agents. In some instances, the lipid
30 nanoparticles are used to deliver nucleic acids such as antisense and/or messenger RNA. Methods for use of such lipid nanoparticles for treatment of various diseases or conditions, such as those caused by infectious entities and/or insufficiency of a protein, are also provided.

In one embodiment, compounds having the following Structure (I) are provided:



(I)

or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof, wherein R^1 , R^2 , L^1 , L^2 , L^{2a} , L^{2b} , and A are as defined herein.

Pharmaceutical compositions comprising one or more of the foregoing compounds of Structure (I) and a therapeutic agent are also provided. In some embodiments, the pharmaceutical compositions further comprise one or more components selected from neutral lipids, charged lipids, steroids, and polymer conjugated lipids. Such compositions are useful for formation of lipid nanoparticles for the delivery of the therapeutic agent.

In other embodiments, the present disclosure provides a method for administering a therapeutic agent to a patient in need thereof, the method comprising preparing a composition of lipid nanoparticles comprising the compound of Structure (I) and a therapeutic agent and delivering the composition to the patient. Such methods are useful for inducing expression of a protein in a subject, for example for expressing an antigen for purposes of vaccination or a gene editing protein.

These and other aspects of the disclosure will be apparent upon reference to the following detailed description.

DETAILED DESCRIPTION

In the following description, certain specific details are set forth to provide a thorough understanding of various embodiments of the disclosure. However, one skilled in the art will understand that the embodiments of the disclosure may be practiced without these details.

The present disclosure is based, in part, upon the discovery of novel cationic (amino) lipids that provide advantages when used in lipid nanoparticles for the *in vivo* delivery of an active or therapeutic agent such as a nucleic acid into a cell of a mammal. In particular, embodiments of the present disclosure provide nucleic acid-lipid nanoparticle compositions comprising one or more of the novel cationic lipids described herein that provide increased activity of the nucleic acid and improved tolerability of the compositions *in vivo*, resulting in a significant increase in the therapeutic index as compared to nucleic acid-lipid nanoparticle compositions previously described.

In particular embodiments, the present disclosure provides novel cationic lipids that enable the formulation of improved compositions for the *in vitro* and *in vivo* delivery of mRNA and/or other oligonucleotides. In some embodiments, these improved lipid nanoparticle compositions are useful for expression of protein encoded by mRNA. In other embodiments, these improved lipid nanoparticles compositions are useful for upregulation of endogenous protein expression by delivering miRNA inhibitors targeting one specific miRNA or a group of miRNA regulating one target mRNA or several mRNA. In other embodiments, these improved lipid nanoparticle compositions are useful for down-regulating (*e.g.*, silencing) the protein levels and/or mRNA levels of target genes. In some other embodiments, the lipid nanoparticles are also useful for delivery of mRNA and plasmids for expression of transgenes. In yet other embodiments, the lipid nanoparticle compositions are useful for inducing a pharmacological effect resulting from expression of a protein, *e.g.*, increased production of red blood cells through the delivery of a suitable erythropoietin mRNA, or protection against infection through delivery of mRNA encoding for a suitable antibody.

The lipid nanoparticles and compositions of the present disclosure may be used for a variety of purposes, including the delivery of encapsulated or associated (*e.g.*, complexed) therapeutic agents such as nucleic acids to cells, both *in vitro* and *in vivo*. Accordingly, embodiments of the present disclosure provide methods of treating or preventing diseases or disorders in a subject in need thereof by contacting the subject with a lipid nanoparticle that encapsulates or is associated with a suitable therapeutic agent, wherein the lipid nanoparticle comprises one or more of the novel cationic lipids described herein.

As described herein, embodiments of the lipid nanoparticles of the present disclosure are particularly useful for the delivery of nucleic acids, including, *e.g.*, mRNA, antisense oligonucleotide, plasmid DNA, microRNA (miRNA), miRNA inhibitors (antagomirs/antimirs), messenger-RNA-interfering complementary RNA (micRNA), DNA, multivalent RNA, dicer substrate RNA, complementary DNA (cDNA), etc. Therefore, the lipid nanoparticles and compositions of the present disclosure may be used to induce expression of a desired protein both *in vitro* and *in vivo* by contacting cells with a lipid nanoparticle comprising one or more novel cationic lipids described herein, wherein the lipid nanoparticle encapsulates or is associated with a nucleic acid that is expressed to produce the desired protein (*e.g.*, a messenger RNA or plasmid encoding the desired protein). Alternatively, the lipid nanoparticles and compositions of the present disclosure may be used to decrease the expression of target genes and proteins both *in vitro* and *in vivo* by contacting cells with a lipid nanoparticle comprising one

or more novel cationic lipids described herein, wherein the lipid nanoparticle encapsulates or is associated with a nucleic acid that reduces target gene expression (*e.g.*, an antisense oligonucleotide or small interfering RNA (siRNA)). The lipid nanoparticles and compositions of the present disclosure may also be used for co-delivery of different nucleic acids (*e.g.* mRNA and plasmid DNA) separately or in combination, such as may be useful to provide an effect requiring colocalization of different nucleic acids (*e.g.* mRNA encoding for a suitable gene modifying enzyme and DNA segment(s) for incorporation into the host genome).

Nucleic acids for use with this disclosure may be prepared according to any available technique. For mRNA, the primary methodology of preparation is, but not limited to, enzymatic synthesis (also termed *in vitro* transcription) which currently represents the most efficient method to produce long sequence-specific mRNA. *In vitro* transcription describes a process of template-directed synthesis of RNA molecules from an engineered DNA template comprised of an upstream bacteriophage promoter sequence (*e.g.* including but not limited to that from the T7, T3, and SP6 coliphage) linked to a downstream sequence encoding the gene of interest. Template DNA can be prepared for *in vitro* transcription from a number of sources with appropriate techniques which are well known in the art including, but not limited to, plasmid DNA and polymerase chain reaction amplification (see Linpinsel, J.L and Conn, G.L., General protocols for preparation of plasmid DNA template and Bowman, J.C., Azizi, B., Lenz, T.K., Ray, P., and Williams, L.D. in RNA *in vitro* transcription and RNA purification by denaturing PAGE in Recombinant and *in vitro* RNA syntheses Methods v. 941 Conn G.L. (ed), New York, N.Y. Humana Press, 2012)

Transcription of the RNA occurs *in vitro* using the linearized DNA template in the presence of the corresponding RNA polymerase and adenosine, guanosine, uridine, and cytidine ribonucleoside triphosphates (rNTPs) under conditions that support polymerase activity while minimizing potential degradation of the resultant mRNA transcripts. *In vitro* transcription can be performed using a variety of commercially available kits including, but not limited to RiboMax Large Scale RNA Production System (Promega), MegaScript Transcription kits (Life Technologies) as well as with commercially available reagents including RNA polymerases and rNTPs. The methodology for *in vitro* transcription of mRNA is well known in the art. (see, *e.g.* Losick, R., 1972, *In vitro* transcription, Ann Rev Biochem v.41 409-46; Kamakaka, R. T. and Kraus, W. L. 2001. *In Vitro* Transcription. Current Protocols in Cell Biology. 2:11.6:11.6.1–11.6.17; Beckert, B. And Masquida, B., (2010) Synthesis of RNA by *In Vitro* Transcription in RNA in Methods in Molecular Biology v. 703 (Neilson, H. Ed), New York, N.Y. Humana Press,

2010; Brunelle, J.L. and Green, R., 2013, Chapter Five – *In vitro* transcription from plasmid or PCR-amplified DNA, Methods in Enzymology v. 530, 101-114; all of which are incorporated herein by reference).

The desired *in vitro* transcribed mRNA is then purified from the undesired components of
5 the transcription or associated reactions (including unincorporated rNTPs, protein enzyme, salts, short RNA oligos *etc.*). Techniques for the isolation of the mRNA transcripts are well known in the art. Well known procedures include phenol/chloroform extraction or precipitation with either alcohol (ethanol, isopropanol) in the presence of monovalent cations or lithium chloride. Additional, non-limiting examples of purification procedures which can be used include size
10 exclusion chromatography (Lukavsky, P.J. and Puglisi, J.D., 2004, Large-scale preparation and purification of polyacrylamide-free RNA oligonucleotides, RNA v.10, 889-893), silica-based affinity chromatography and polyacrylamide gel electrophoresis (Bowman, J.C., Azizi, B., Lenz, T.K., Ray, P., and Williams, L.D. in RNA *in vitro* transcription and RNA purification by denaturing PAGE in Recombinant and *in vitro* RNA syntheses Methods v. 941 Conn G.L. (ed),
15 New York, N.Y. Humana Press, 2012). Purification can be performed using a variety of commercially available kits including, but not limited to SV Total Isolation System (Promega) and *In Vitro* Transcription Cleanup and Concentration Kit (Norgen Biotek).

Furthermore, while reverse transcription can yield large quantities of mRNA, the products can contain a number of aberrant RNA impurities associated with undesired polymerase
20 activity which may need to be removed from the full-length mRNA preparation. These include short RNAs that result from abortive transcription initiation as well as double-stranded RNA (dsRNA) generated by RNA-dependent RNA polymerase activity, RNA-primed transcription from RNA templates and self-complementary 3' extension. It has been demonstrated that these contaminants with dsRNA structures can lead to undesired immunostimulatory activity through
25 interaction with various innate immune sensors in eukaryotic cells that function to recognize specific nucleic acid structures and induce potent immune responses. This in turn, can dramatically reduce mRNA translation since protein synthesis is reduced during the innate cellular immune response. Therefore, additional techniques to remove these dsRNA contaminants have been developed and are known in the art including but not limited to scaleable
30 HPLC purification (see, *e.g.*, Kariko, K., Muramatsu, H., Ludwig, J. And Weissman, D., 2011, Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA, Nucl Acid Res, v. 39 e142; Weissman, D., Pardi, N., Muramatsu, H., and Kariko, K., HPLC Purification of *in vitro*

transcribed long RNA in Synthetic Messenger RNA and Cell Metabolism Modulation in Methods in Molecular Biology v.969 (Rabinovich, P.H. Ed), 2013). HPLC purified mRNA has been reported to be translated at much greater levels, particularly in primary cells and *in vivo*.

A significant variety of modifications have been described in the art which are used to alter specific properties of *in vitro* transcribed mRNA and improve its utility. These include but are not limited to modifications to the 5' and 3' termini of the mRNA. Endogenous eukaryotic mRNA typically contains a cap structure on the 5'-end of a mature molecule which plays an important role in mediating binding of the mRNA Cap Binding Protein (CBP), which is in turn responsible for enhancing mRNA stability in the cell and efficiency of mRNA translation.

Therefore, highest levels of protein expression are achieved with capped mRNA transcripts. The 5'-cap contains a 5'-5'-triphosphate linkage between the 5'-most nucleotide and guanine nucleotide. The conjugated guanine nucleotide is methylated at the N7 position. Additional modifications include methylation of the ultimate and penultimate most 5'-nucleotides on the 2'-hydroxyl group.

Multiple distinct cap structures can be used to generate the 5'-cap of *in vitro* transcribed synthetic mRNA. 5'-capping of synthetic mRNA can be performed co-transcriptionally with chemical cap analogs (*i.e.*, capping during *in vitro* transcription). For example, the Anti-Reverse Cap Analog (ARCA) cap contains a 5'-5'-triphosphate guanine-guanine linkage where one guanine contains an N7 methyl group as well as a 3'-O-methyl group. However, up to 20% of transcripts remain uncapped during this co-transcriptional process and the synthetic cap analog is not identical to the 5'-cap structure of an authentic cellular mRNA, potentially reducing translatability and cellular stability. Alternatively, synthetic mRNA molecules may also be enzymatically capped post-transcriptionally. These may generate a more authentic 5'-cap structure that more closely mimics, either structurally or functionally, the endogenous 5'-cap which have enhanced binding of cap binding proteins, increased half-life, reduced susceptibility to 5' endonucleases and/or reduced 5' decapping. Numerous synthetic 5'-cap analogs have been developed and are known in the art to enhance mRNA stability and translatability (see, *e.g.*, Grudzien-Nogalska, E., Kowalska, J., Su, W., Kuhn, A.N., Slepnev, S.V., Darynkiewicz, E., Sahin, U., Jemielity, J., and Rhoads, R.E., Synthetic mRNAs with superior translation and stability properties in Synthetic Messenger RNA and Cell Metabolism Modulation in Methods in Molecular Biology v.969 (Rabinovich, P.H. Ed), 2013).

On the 3'-terminus, a long chain of adenine nucleotides (poly-A tail) is normally added to mRNA molecules during RNA processing. Immediately after transcription, the 3' end of the

transcript is cleaved to free a 3' hydroxyl to which poly-A polymerase adds a chain of adenine nucleotides to the RNA in a process called polyadenylation. The poly-A tail has been extensively shown to enhance both translational efficiency and stability of mRNA (see Bernstein, P. and Ross, J., 1989, Poly (A), poly (A) binding protein and the regulation of mRNA stability, Trends Bio Sci v. 14 373-377; Guhaniyogi, J. And Brewer, G., 2001, Regulation of mRNA stability in mammalian cells, Gene, v. 265, 11-23; Dreyfus, M. And Regnier, P., 2002, The poly (A) tail of mRNAs: Bodyguard in eukaryotes, scavenger in bacteria, Cell, v.111, 611-613).

Poly (A) tailing of *in vitro* transcribed mRNA can be achieved using various approaches including, but not limited to, cloning of a poly (T) tract into the DNA template or by post-transcriptional addition using Poly (A) polymerase. The first case allows *in vitro* transcription of mRNA with poly (A) tails of defined length, depending on the size of the poly (T) tract, but requires additional manipulation of the template. The latter case involves the enzymatic addition of a poly (A) tail to *in vitro* transcribed mRNA using poly (A) polymerase which catalyzes the incorporation of adenine residues onto the 3' termini of RNA, requiring no additional manipulation of the DNA template, but results in mRNA with poly(A) tails of heterogeneous length. 5'-capping and 3'-poly (A) tailing can be performed using a variety of commercially available kits including, but not limited to Poly (A) Polymerase Tailing kit (EpiCenter), mMESSAGE mMACHINE T7 Ultra kit and Poly (A) Tailing kit (Life Technologies) as well as with commercially available reagents, various ARCA caps, Poly (A) polymerase, etc.

In addition to 5' cap and 3' poly adenylation, other modifications of the *in vitro* transcripts have been reported to provide benefits as related to efficiency of translation and stability. It is well known in the art that pathogenic DNA and RNA can be recognized by a variety of sensors within eukaryotes and trigger potent innate immune responses. The ability to discriminate between pathogenic and self-DNA and RNA has been shown to be based, at least in part, on structure and nucleoside modifications since most nucleic acids from natural sources contain modified nucleosides. In contrast, *in vitro* synthesized RNA lacks these modifications, thus rendering it immunostimulatory which in turn can inhibit effective mRNA translation as outlined above. The introduction of modified nucleosides into *in vitro* transcribed mRNA can be used to prevent recognition and activation of RNA sensors, thus mitigating this undesired immunostimulatory activity and enhancing translation capacity (see, e.g., Kariko, K. And Weissman, D. 2007, Naturally occurring nucleoside modifications suppress the immunostimulatory activity of RNA: implication for therapeutic RNA development, Curr Opin Drug Discov Devel, v.10 523-532; Pardi, N., Muramatsu, H., Weissman, D., Kariko, K., *In vitro*

transcription of long RNA containing modified nucleosides in Synthetic Messenger RNA and Cell Metabolism Modulation in Methods in Molecular Biology v.969 (Rabinovich, P.H. Ed), 2013); Kariko, K., Muramatsu, H., Welsh, F.A., Ludwig, J., Kato, H., Akira, S., Weissman, D., 2008, Incorporation of Pseudouridine Into mRNA Yields Superior Nonimmunogenic Vector
5 With Increased Translational Capacity and Biological Stability, Mol Ther v.16, 1833-1840. The modified nucleosides and nucleotides used in the synthesis of modified RNAs can be prepared monitored and utilized using general methods and procedures known in the art. A large variety of nucleoside modifications are available that may be incorporated alone or in combination with other modified nucleosides to some extent into the *in vitro* transcribed mRNA (see, *e.g.*, US
10 Publication No. 2012/0251618). *In vitro* synthesis of nucleoside-modified mRNA has been reported to have reduced ability to activate immune sensors with a concomitant enhanced translational capacity.

Other components of mRNA which can be modified to provide benefit in terms of translatability and stability include the 5' and 3' untranslated regions (UTR). Optimization of the
15 UTRs (favorable 5' and 3' UTRs can be obtained from cellular or viral RNAs), either both or independently, have been shown to increase mRNA stability and translational efficiency of *in vitro* transcribed mRNA (see, *e.g.*, Pardi, N., Muramatsu, H., Weissman, D., Kariko, K., *In vitro* transcription of long RNA containing modified nucleosides in Synthetic Messenger RNA and Cell Metabolism Modulation in Methods in Molecular Biology v.969 (Rabinovich, P.H. Ed),
20 2013).

In addition to mRNA, other nucleic acid payloads may be used for this disclosure. For oligonucleotides, methods of preparation include but are not limited to chemical synthesis and enzymatic, chemical cleavage of a longer precursor, *in vitro* transcription as described above, etc. Methods of synthesizing DNA and RNA nucleotides are widely used and well known in the art
25 (see, *e.g.*, Gait, M. J. (ed.) Oligonucleotide synthesis: a practical approach, Oxford [Oxfordshire], Washington, D.C.: IRL Press, 1984; and Herdewijn, P. (ed.) Oligonucleotide synthesis: methods and applications, Methods in Molecular Biology, v. 288 (Clifton, N.J.) Totowa, N.J.: Humana Press, 2005; both of which are incorporated herein by reference).

For plasmid DNA, preparation for use with this disclosure commonly utilizes but is not
30 limited to expansion and isolation of the plasmid DNA *in vitro* in a liquid culture of bacteria containing the plasmid of interest. The presence of a gene in the plasmid of interest that encodes resistance to a particular antibiotic (penicillin, kanamycin, etc) allows those bacteria containing the plasmid of interest to selective grow in antibiotic-containing cultures. Methods of isolating

plasmid DNA are widely used and well known in the art (see, *e.g.* Heilig, J., Elbing, K. L. and Brent, R. (2001) Large-Scale Preparation of Plasmid DNA. *Current Protocols in Molecular Biology*. 41:II:1.7.1.7.1–1.7.16; Rozkov, A., Larsson, B., Gillström, S., Björnstedt, R. and Schmidt, S. R. (2008), Large-scale production of endotoxin-free plasmids for transient
5 expression in mammalian cell culture. *Biotechnol. Bioeng.*, 99: 557–566; and US Patent No. 6,197,553). Plasmid isolation can be performed using a variety of commercially available kits including, but not limited to Plasmid Plus (Qiagen), GenJET plasmid MaxiPrep (Thermo) and PureYield MaxiPrep (Promega) kits as well as with commercially available reagents.

Various exemplary embodiments of the cationic lipids of the present disclosure, lipid
10 nanoparticles and compositions comprising the same, and their use to deliver active or therapeutic agents such as nucleic acids to modulate gene and protein expression, are described in further detail below.

As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

15 Unless the context requires otherwise, throughout the present specification and claims, the word “comprise” and variations thereof, such as, “comprises” and “comprising” are to be construed in an open and inclusive sense, that is, as “including, but not limited to”.

Reference throughout this specification to “one embodiment” or “an embodiment” means that a particular feature, structure, or characteristic described in connection with the embodiment
20 is included in at least one embodiment of the present disclosure. Thus, the appearances of the phrases “in one embodiment” or “in an embodiment” in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

25 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this disclosure belongs. As used in the specification and claims, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise.

The phrase “induce expression of a desired protein” refers to the ability of a nucleic acid
30 to increase expression of the desired protein. To examine the extent of protein expression, a test sample (*e.g.*, a sample of cells in culture expressing the desired protein) or a test mammal (*e.g.*, a mammal such as a human or an animal model such as a rodent (*e.g.*, mouse) or a non-human primate (*e.g.*, monkey) model) is contacted with a nucleic acid (*e.g.*, nucleic acid in combination

with a lipid of the present disclosure). Expression of the desired protein in the test sample or test animal is compared to expression of the desired protein in a control sample (*e.g.*, a sample of cells in culture expressing the desired protein) or a control mammal (*e.g.*, a mammal such as a human or an animal model such as a rodent (*e.g.*, mouse) or non-human primate (*e.g.*, monkey) model) that is not contacted with or administered the nucleic acid. When the desired protein is present in a control sample or a control mammal, the expression of a desired protein in a control sample or a control mammal may be assigned a value of 1.0. In particular embodiments, inducing expression of a desired protein is achieved when the ratio of desired protein expression in the test sample or the test mammal to the level of desired protein expression in the control sample or the control mammal is greater than 1, for example, about 1.1, 1.5, 2.0, 5.0 or 10.0. When a desired protein is not present in a control sample or a control mammal, inducing expression of a desired protein is achieved when any measurable level of the desired protein in the test sample or the test mammal is detected. One of ordinary skill in the art will understand appropriate assays to determine the level of protein expression in a sample, for example dot blots, northern blots, in situ hybridization, ELISA, immunoprecipitation, enzyme function, and phenotypic assays, or assays based on reporter proteins that can produce fluorescence or luminescence under appropriate conditions.

The phrase “inhibiting expression of a target gene” refers to the ability of a nucleic acid to silence, reduce, or inhibit the expression of a target gene. To examine the extent of gene silencing, a test sample (*e.g.*, a sample of cells in culture expressing the target gene) or a test mammal (*e.g.*, a mammal such as a human or an animal model such as a rodent (*e.g.*, mouse) or a non-human primate (*e.g.*, monkey) model) is contacted with a nucleic acid that silences, reduces, or inhibits expression of the target gene. Expression of the target gene in the test sample or test animal is compared to expression of the target gene in a control sample (*e.g.*, a sample of cells in culture expressing the target gene) or a control mammal (*e.g.*, a mammal such as a human or an animal model such as a rodent (*e.g.*, mouse) or non-human primate (*e.g.*, monkey) model) that is not contacted with or administered the nucleic acid. The expression of the target gene in a control sample or a control mammal may be assigned a value of 100%. In particular embodiments, silencing, inhibition, or reduction of expression of a target gene is achieved when the level of target gene expression in the test sample or the test mammal relative to the level of target gene expression in the control sample or the control mammal is about 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, or 0%. In other words, the nucleic acids are capable of silencing, reducing, or inhibiting the expression of a

target gene by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% in a test sample or a test mammal relative to the level of target gene expression in a control sample or a control mammal not contacted with or administered the nucleic acid. Suitable assays for determining the level of target gene expression include, without limitation, examination of protein or mRNA levels using techniques known to those of skill in the art, such as, *e.g.*, dot blots, northern blots, in situ hybridization, ELISA, immunoprecipitation, enzyme function, as well as phenotypic assays known to those of skill in the art.

An “effective amount” or “therapeutically effective amount” of an active agent or therapeutic agent such as a therapeutic nucleic acid is an amount sufficient to produce the desired effect, *e.g.*, an increase or inhibition of expression of a target sequence in comparison to the normal expression level detected in the absence of the nucleic acid. An increase in expression of a target sequence is achieved when any measurable level is detected in the case of an expression product that is not present in the absence of the nucleic acid. In the case where the expression product is present at some level prior to contact with the nucleic acid, an increase in expression is achieved when the fold increase in value obtained with a nucleic acid such as mRNA relative to control is about 1.05, 1.1, 1.2, 1.3, 1.4, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 75, 100, 250, 500, 750, 1000, 5000, 10000 or greater. Inhibition of expression of a target gene or target sequence is achieved when the value obtained with a nucleic acid such as antisense oligonucleotide relative to the control is about 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, or 0%. Suitable assays for measuring expression of a target gene or target sequence include, *e.g.*, examination of protein or RNA levels using techniques known to those of skill in the art such as dot blots, northern blots, in situ hybridization, ELISA, immunoprecipitation, enzyme function, fluorescence or luminescence of suitable reporter proteins, as well as phenotypic assays known to those of skill in the art.

The term “nucleic acid” as used herein refers to a polymer containing at least two deoxyribonucleotides or ribonucleotides in either single- or double-stranded form and includes DNA, RNA, and hybrids thereof. DNA may be in the form of antisense molecules, plasmid DNA, cDNA, PCR products, or vectors. RNA may be in the form of small hairpin RNA (shRNA), messenger RNA (mRNA), antisense RNA, miRNA, micRNA, multivalent RNA, dicer substrate RNA or viral RNA (vRNA), and combinations thereof. Nucleic acids include nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are

synthetic, naturally occurring, and non-naturally occurring, and which have similar binding properties as the reference nucleic acid. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2'-O-methyl ribonucleotides, and peptide-nucleic acids (PNAs). Unless specifically limited, the term

5 encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions), alleles, orthologs, single nucleotide polymorphisms, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate

10 codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res., 19:5081 (1991); Ohtsuka et al., J. Biol. Chem., 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes, 8:91-98 (1994)). "Nucleotides" contain a sugar deoxyribose (DNA) or ribose (RNA), a base, and a phosphate group. Nucleotides are linked

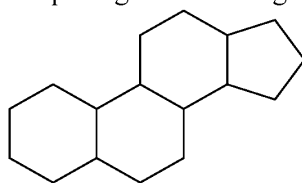
15 together through the phosphate groups. "Bases" include purines and pyrimidines, which further include natural compounds adenine, thymine, guanine, cytosine, uracil, inosine, and natural analogs, and synthetic derivatives of purines and pyrimidines, which include, but are not limited to, modifications which place new reactive groups such as, but not limited to, amines, alcohols, thiols, carboxylates, and alkylhalides.

20 The term "gene" refers to a nucleic acid (*e.g.*, DNA or RNA) sequence that comprises partial length or entire length coding sequences necessary to produce a polypeptide or precursor polypeptide.

"Gene product," as used herein, refers to a product of a gene such as an RNA transcript or a polypeptide.

25 The term "lipid" refers to a group of organic compounds that include, but are not limited to, esters of fatty acids and are generally characterized by being poorly soluble in water, but soluble in many organic solvents. They are usually divided into at least three classes: (1) "simple lipids," which include fats and oils as well as waxes; (2) "compound lipids," which include phospholipids and glycolipids; and (3) "derived lipids" such as steroids.

30 A "steroid" is a compound comprising the following carbon skeleton:



Non-limiting examples of steroids include cholesterol, and the like.

A “cationic lipid” refers to a lipid capable of being positively charged. Exemplary cationic lipids include one or more amine group(s) which bear the positive charge. Preferred cationic lipids are ionizable such that they can exist in a positively charged or neutral form depending on pH. The ionization of the cationic lipid affects the surface charge of the lipid nanoparticle under different pH conditions. This charge state can influence plasma protein absorption, blood clearance and tissue distribution (Semple, S.C., et al., *Adv. Drug Deliv Rev* 32:3-17 (1998)) as well as the ability to form endosomolytic non-bilayer structures (Hafez, I.M., et al., *Gene Ther* 8:1188-1196 (2001)) critical to the intracellular delivery of nucleic acids.

The term “lipid nanoparticle” refers to particles having at least one dimension on the order of nanometers (*e.g.*, 1-1,000 nm) which include one or more of the compounds of formula (I) or other specified cationic lipids. In some embodiments, lipid nanoparticles are included in a formulation that can be used to deliver an active agent or therapeutic agent, such as a nucleic acid (*e.g.*, mRNA) to a target site of interest (*e.g.*, cell, tissue, organ, tumor, and the like). In some embodiments, the lipid nanoparticles of the disclosure comprise a nucleic acid. Such lipid nanoparticles typically comprise a compound of Structure (I) and one or more excipient selected from neutral lipids, charged lipids, steroids and polymer conjugated lipids. In some embodiments, the active agent or therapeutic agent, such as a nucleic acid, may be encapsulated in the lipid portion of the lipid nanoparticle or an aqueous space enveloped by some or all of the lipid portion of the lipid nanoparticle, thereby protecting it from enzymatic degradation or other undesirable effects induced by the mechanisms of the host organism or cells *e.g.* an adverse immune response.

In various embodiments, the lipid nanoparticles have a mean diameter of from about 30 nm to about 150 nm, from about 40 nm to about 150 nm, from about 50 nm to about 150 nm, from about 60 nm to about 130 nm, from about 70 nm to about 110 nm, from about 70 nm to about 100 nm, from about 80 nm to about 100 nm, from about 90 nm to about 100 nm, from about 70 to about 90 nm, from about 80 nm to about 90 nm, from about 70 nm to about 80 nm, or about 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, or 150 nm, and are substantially non-toxic. In certain embodiments, nucleic acids, when present in the lipid nanoparticles, are resistant in aqueous solution to degradation with a nuclease. Lipid nanoparticles comprising nucleic acids and their method of preparation are disclosed in, *e.g.*, U.S. Patent Publication Nos. 2004/0142025, 2007/0042031 and PCT Pub. Nos. WO

2013/016058 and WO 2013/086373, the full disclosures of which are herein incorporated by reference in their entirety for all purposes.

As used herein, “lipid encapsulated” refers to a lipid nanoparticle that provides an active agent or therapeutic agent, such as a nucleic acid (*e.g.*, mRNA), with full encapsulation, partial
5 encapsulation, or both. In an embodiment, the nucleic acid (*e.g.*, mRNA) is fully encapsulated in the lipid nanoparticle.

The term “polymer conjugated lipid” refers to a molecule comprising both a lipid portion and a polymer portion. An example of a polymer conjugated lipid is a pegylated lipid. The term “pegylated lipid” refers to a molecule comprising both a lipid portion and a polyethylene glycol
10 portion. Pegylated lipids are known in the art and include 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG) and the like.

The term “neutral lipid” refers to any of a number of lipid species that exist either in an uncharged or neutral zwitterionic form at a selected pH. At physiological pH, such lipids include, but are not limited to, phosphatidylcholines such as 1,2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), phosphatidylethanolamines such as 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), sphingomyelins (SM), ceramides, steroids such as sterols and their derivatives. Neutral lipids may be synthetic or naturally derived.

20 The term “charged lipid” refers to any of a number of lipid species that exist in either a positively charged or negatively charged form independent of the pH within a useful physiological range *e.g.* pH ~3 to pH ~9. Charged lipids may be synthetic or naturally derived. Examples of charged lipids include phosphatidylserines, phosphatidic acids, phosphatidylglycerols, phosphatidylinositols, sterol hemisuccinates, dialkyl trimethylammonium-propanes, (*e.g.* DOTAP, DOTMA), dialkyl dimethylaminopropanes, ethyl phosphocholines,
25 dimethylaminoethane carbamoyl sterols (*e.g.* DC-Chol).

As used herein, the term “aqueous solution” refers to a composition comprising water.

“Serum-stable” in relation to nucleic acid-lipid nanoparticles means that the nucleotide is not significantly degraded after exposure to a serum or nuclease assay that would significantly
30 degrade free DNA or RNA. Suitable assays include, for example, a standard serum assay, a DNase assay, or an RNase assay.

“Systemic delivery,” as used herein, refers to delivery of a therapeutic product that can result in a broad exposure of an active agent within an organism. Some techniques of

administration can lead to the systemic delivery of certain agents, but not others. Systemic delivery means that a useful, preferably therapeutic, amount of an agent is exposed to most parts of the body. Systemic delivery of lipid nanoparticles can be by any means known in the art including, for example, intravenous, intraarterial, subcutaneous, and intraperitoneal delivery. In
5 some embodiments, systemic delivery of lipid nanoparticles is by intravenous delivery.

“Local delivery,” as used herein, refers to delivery of an active agent directly to a target site within an organism. For example, an agent can be locally delivered by direct injection into a disease site such as a tumor, other target site such as a site of inflammation, or a target organ such as the liver, heart, pancreas, kidney, and the like. Local delivery can also include topical
10 applications or localized injection techniques such as intramuscular, subcutaneous, or intradermal injection. Local delivery does not preclude a systemic pharmacological effect.

“Alkyl” refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms that is saturated (*i.e.*, contains no double and/or triple bonds), having from one to twenty-four carbon atoms (C₁-C₂₄ alkyl), one to sixteen carbon atoms (C₁-C₁₆
15 alkyl), one to twelve carbon atoms (C₁-C₁₂ alkyl), six to twenty-four carbon atoms (C₆-C₂₄ alkyl), one to eight carbon atoms (C₁-C₈ alkyl) or one to six carbon atoms (C₁-C₆ alkyl) and which is attached to the rest of the molecule by a single bond, *e.g.*, methyl, ethyl, n-propyl, 1-methylethyl (iso propyl), n-butyl, n-pentyl, 1,1-dimethylethyl (t-butyl), 3-methylhexyl, 2-methylhexyl, and the like. Unless stated otherwise specifically in the specification, an alkyl group is optionally
20 substituted.

“Alkylhydroxyl” refers to an alkyl group, as defined herein, comprising at least one hydroxyl (OH) substituent.

“Alkenyl” refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms that contains at least one carbon-carbon double, having from one to
25 twenty-four carbon atoms (C₂-C₂₄ alkenyl), one to twelve carbon atoms (C₂-C₁₂ alkenyl), six to twenty-four carbon atoms (C₆-C₂₄ alkenyl), two to sixteen carbon atoms (C₂-C₁₆ alkenyl), four to twelve carbon atoms (C₄-C₁₂ alkenyl), one to eight carbon atoms (C₂-C₈ alkenyl) or one to six carbon atoms (C₂-C₆ alkenyl) and which is attached to the rest of the molecule by a single bond, *e.g.*, ethenyl, n-propenyl, 1-methylethenyl, n-butenyl, n-pentenyl, 1,1-dimethylethenyl, 3-
30 methylhexenyl, 2-methylhexenyl, and the like. Unless stated otherwise specifically in the specification, an alkenyl group is optionally substituted.

“Alkynyl” refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms that contains at least one carbon-carbon triple bond, having from one

to twenty-four carbon atoms (C₂-C₂₄ alkynyl), one to twelve carbon atoms (C₂-C₁₂ alkynyl), one to eight carbon atoms (C₂-C₈ alkynyl) or one to six carbon atoms (C₂-C₆ alkynyl) and which is attached to the rest of the molecule by a single bond, *e.g.*, ethynyl, *n*-propynyl, 1-methylethynyl, *n*-butynyl, *n*-pentynyl, 1,1-dimethylethynyl, 3-methylhexynyl, 2-methylhexynyl, and the like.

- 5 Unless stated otherwise specifically in the specification, an alkynyl group is optionally substituted.

“Alkylene” or “alkylene chain” refers to a straight or branched divalent saturated hydrocarbon chain linking the rest of the molecule to a radical group, consisting solely of carbon and hydrogen. In some embodiments, an alkylene chain has from one to twenty-four carbon
10 atoms (C₁-C₂₄ alkylene), one to fifteen carbon atoms (C₁-C₁₅ alkylene), one to twelve carbon atoms (C₁-C₁₂ alkylene), one to eight carbon atoms (C₁-C₈ alkylene), one to six carbon atoms (C₁-C₆ alkylene), four to six carbon atoms (C₄-C₆ alkylene), two to four carbon atoms (C₂-C₄ alkylene), one to two carbon atoms (C₁-C₂ alkylene), *e.g.*, methylene, ethylene, propylene, *n*-butylene, and the like. The alkylene chain is attached to the rest of the molecule through a
15 single bond and to the radical group through a single bond. The points of attachment of the alkylene chain to the rest of the molecule and to the radical group can be through one carbon or any two carbons within the chain. Unless stated otherwise specifically in the specification, an alkylene chain is optionally substituted. “Cycloalkyl” or “carbocyclic ring” refers to a stable non aromatic monocyclic or polycyclic hydrocarbon radical consisting solely of carbon and hydrogen
20 atoms, which may include fused or bridged ring systems, having from three to fifteen ring carbon atoms (C₃-C₁₅), from three to ten ring carbon atoms (C₃-C₁₀) or from three to eight ring carbon atoms (C₃-C₈), and which is saturated or unsaturated and attached to the rest of the molecule by a single bond. Monocyclic radicals include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Polycyclic radicals include, for example, adamantyl,
25 norbornyl, decalyl, 7,7-dimethyl bicyclo[2.2.1]heptanyl, and the like. Unless otherwise stated specifically in the specification, a cycloalkyl group is optionally substituted.

“Aryl” refers to a carbocyclic ring system radical comprising hydrogen, 6 to 18 carbon atoms and at least one aromatic ring. For purposes of this disclosure, the aryl radical is a monocyclic, bicyclic, tricyclic, or tetracyclic ring system, which may include fused or bridged
30 ring systems. Aryl radicals include, but are not limited to, aryl radicals derived from aceanthrylene, acenaphthylene, acephenanthrylene, anthracene, azulene, benzene, chrysene, fluoranthene, fluorene, *as*-indacene, *s*-indacene, indane, indene, naphthalene, phenalene, phenanthrene, pleiadene, pyrene, and triphenylene.

“Arylalkyl” refers to a radical of the formula $-R_b-R_c$ where R_b is an alkylene or alkenylene as defined above and R_c is one or more aryl radicals as defined above, for example, benzyl, diphenylmethyl and the like. Unless stated otherwise specifically in the specification, an arylalkyl group is optionally substituted.

5 “Heterocyclyl” or “heterocyclic ring” refers to a stable 3- to 18-membered non-aromatic ring radical having one to twelve ring carbon atoms (*e.g.*, two to twelve) and from one to six ring heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur. Unless stated otherwise specifically in the specification, the heterocyclyl radical is a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused, spirocyclic (“spiro-heterocyclyl”) and/or bridged ring systems; and the nitrogen, carbon or sulfur atoms in the heterocyclyl radical is optionally oxidized; the nitrogen atom is optionally quaternized; and the heterocyclyl radical is partially or fully saturated. Examples of such heterocyclyl radicals include, but are not limited to, dioxolanyl, thienyl[1,3]dithianyl, decahydroisoquinolyl, imidazoliny, imidazolidiny, isothiazolidiny, isoxazolidiny, morpholiny, octahydroindolyl, octahydroisindolyl, 15 2-oxopiperaziny, 2-oxopiperidiny, 2-oxopyrrolidiny, oxazolidiny, piperidiny, piperaziny, 4-piperidonyl, pyrrolidiny, pyrazolidiny, quinuclidiny, thiazolidiny, tetrahydrofuryl, trithianyl, tetrahydropyrany, thiomorpholiny, thiamorpholiny, 1-oxo-thiomorpholiny, and 1,1-dioxo-thiomorpholiny. Unless stated otherwise specifically in the specification, a heterocyclyl group is optionally substituted.

20 The term “substituted” used herein means any of the above groups (*e.g.*, alkyl, alkylhydroxyl, alkenyl, alkynyl, alkylene, cycloalkyl, aryl, aralkyl or heterocyclyl) wherein at least one hydrogen atom is replaced by a bond to a non-hydrogen atoms such as, but not limited to: a halogen atom such as F, Cl, Br, and I; oxo groups ($=O$); hydroxyl groups ($-OH$); alkoxy groups ($-OR^a$, where R^a is C_1 - C_{12} alkyl or cycloalkyl); carboxyl groups ($-OC(=O)R^a$ or $-C(=O)OR^a$, where R^a is H, C_1 - C_{12} alkyl or cycloalkyl); amine groups ($-NR^aR^b$, where R^a and R^b are each independently H, C_1 - C_{12} alkyl or cycloalkyl); C_1 - C_{12} alkyl groups; and cycloalkyl groups. In some embodiments the substituent is a C_1 - C_{12} alkyl group. In other embodiments, the substituent is a cycloalkyl group. In other embodiments, the substituent is a halo group, such as fluoro. In other embodiments, the substituent is an oxo group. In other embodiments, the substituent is a hydroxyl group. In other embodiments, the substituent is an alkoxy group. In other embodiments, the substituent is a carboxyl group. In other embodiments, the substituent is an amine group.

30

“Optional” or “optionally” (*e.g.*, optionally substituted) means that the subsequently described event or circumstances may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, “optionally substituted alkyl” means that the alkyl radical may or may not be substituted and that the description includes both substituted alkyl radicals and alkyl radicals having no substitution. In some embodiments, “optionally substituted” means a particular radical is substituted with one or more substituents selected from the group consisting of halo (*e.g.*, F, Cl, Br, and I), oxo (=O), hydroxyl (-OH), alkoxy (-OR^a, where R^a is C₁-C₁₂ alkyl), cycloalkoxy (-OR^a, where R^a is C₃-C₈ cycloalkyl), carboxyl (-OC(=O)R^a or -C(=O)OR^a, where R^a is H, C₁-C₁₂ alkyl, or C₃-C₈ cycloalkyl), amine (-NR^aR^b, where R^a and R^b are each independently H, C₁-C₁₂ alkyl, or C₃-C₈ cycloalkyl), C₁-C₁₂ alkyl, and C₃-C₈ cycloalkyl.

In some embodiments, “optionally substituted” means substituted with one or more halo substituents. In some embodiments, “optionally substituted” means substituted with one or more oxo substituents. In some embodiments, “optionally substituted” means substituted with one or more hydroxyl substituents. In certain embodiments, “optionally substituted” means substituted with one or more alkoxy substituents. In some embodiments, “optionally substituted” means substituted with one or more cycloalkoxy substituents. In certain embodiments, “optionally substituted” means substituted with one or more carboxy substituents. In some embodiments, “optionally substituted” means substituted with one or more amine substituents. In certain embodiments, “optionally substituted” means substituted with one or more C₁-C₁₂ alkyl substituents. In some embodiments, “optionally substituted” means substituted with one or more C₃-C₈ cycloalkyl substituents.

When a functional group is described as “optionally substituted,” and in turn, substituents on the functional group are also “optionally substituted” and so on, for the purposes of this disclosure, such iterations are limited to five, preferably such iterations are limited to two. In some embodiments, such iterations are limited to one. In some embodiments, such iterations are limited to zero.

This disclosure is also meant to encompass all pharmaceutically acceptable compounds of the compound of Structure (I) being isotopically labelled by having one or more atoms replaced by an atom having a different atomic mass or mass number. Examples of isotopes that can be incorporated into the disclosed compounds include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine, chlorine, and iodine, such as ²H, ³H, ¹¹C, ¹³C, ¹⁴C, ¹³N, ¹⁵N, ¹⁵O, ¹⁷O, ¹⁸O, ³¹P, ³²P, ³⁵S, ¹⁸F, ³⁶Cl, ¹²³I, and ¹²⁵I, respectively. These radiolabeled compounds could

be useful to help determine or measure the effectiveness of the compounds, by characterizing, for example, the site or mode of action, or binding affinity to pharmacologically important site of action. Certain isotopically labelled compounds of Structure (I), for example, those incorporating a radioactive isotope, are useful in drug and/or substrate tissue distribution studies. The
5 radioactive isotopes tritium, *i.e.*, ^3H , and carbon-14, *i.e.*, ^{14}C , are particularly useful for this purpose in view of their ease of incorporation and ready means of detection.

Substitution with heavier isotopes such as deuterium, *i.e.*, ^2H , may afford certain therapeutic advantages resulting from greater metabolic stability, for example, increased *in vivo* half-life or reduced dosage requirements, and hence may be preferred in some circumstances.

10 Substitution with positron emitting isotopes, such as ^{11}C , ^{18}F , ^{15}O and ^{13}N , can be useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy. Isotopically labeled compounds of Structure (I) can generally be prepared by conventional techniques known to those skilled in the art or by processes analogous to those described in the Preparations and Examples as set out below using an appropriate isotopically labeled reagent in
15 place of the non-labeled reagent previously employed.

This disclosure is also meant to encompass the *in vivo* metabolic products of the disclosed compounds. Such products may result from, for example, the oxidation, reduction, hydrolysis, amidation, esterification, and the like of the administered compound, primarily due to enzymatic processes. Accordingly, the disclosure includes compounds produced by a process comprising
20 administering a compound of this disclosure to a mammal for a period sufficient to yield a metabolic product thereof. Such products are typically identified by administering a radiolabeled compound of the disclosure in a detectable dose to an animal, such as rat, mouse, guinea pig, monkey, or to human, allowing sufficient time for metabolism to occur, and isolating its conversion products from the urine, blood, or other biological samples.

25 “Stable compound” and “stable structure” are meant to indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

“Mammal” includes humans and both domestic animals such as laboratory animals and household pets (*e.g.*, cats, dogs, swine, cattle, sheep, goats, horses, rabbits), and non-domestic
30 animals such as wildlife and the like.

“Pharmaceutically acceptable carrier, diluent or excipient” includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavor enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic

agent, solvent, or emulsifier which has been approved by the United States Food and Drug Administration as being acceptable for use in humans or domestic animals.

“Pharmaceutically acceptable salt” includes both acid and base addition salts.

“Pharmaceutically acceptable acid addition salt” refers to those salts which retain the biological effectiveness and properties of the free bases, which are not biologically or otherwise undesirable, and which are formed with inorganic acids such as, but are not limited to, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as, but not limited to, acetic acid, 2,2-dichloroacetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, 4-acetamidobenzoic acid, camphoric acid, camphor-10-sulfonic acid, capric acid, caproic acid, caprylic acid, carbonic acid, cinnamic acid, citric acid, cyclamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, gluconic acid, glucuronic acid, glutamic acid, glutaric acid, 2-oxo-glutaric acid, glycerophosphoric acid, glycolic acid, hippuric acid, isobutyric acid, lactic acid, lactobionic acid, lauric acid, maleic acid, malic acid, malonic acid, mandelic acid, methanesulfonic acid, mucic acid, naphthalene-1,5-disulfonic acid, naphthalene-2-sulfonic acid, 1-hydroxy-2-naphthoic acid, nicotinic acid, oleic acid, orotic acid, oxalic acid, palmitic acid, pamoic acid, propionic acid, pyroglutamic acid, pyruvic acid, salicylic acid, 4-aminosalicylic acid, sebacic acid, stearic acid, succinic acid, tartaric acid, thiocyanic acid, *p*-toluenesulfonic acid, trifluoroacetic acid, undecylenic acid, and the like.

“Pharmaceutically acceptable base addition salt” refers to those salts which retain the biological effectiveness and properties of the free acids, which are not biologically or otherwise undesirable. These salts are prepared from addition of an inorganic base or an organic base to the free acid. Salts derived from inorganic bases include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Preferred inorganic salts are the ammonium, sodium, potassium, calcium, and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as ammonia, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, diethanolamine, ethanolamine, deanol, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, benethamine, benzathine, ethylenediamine, glucosamine, methylglucamine, theobromine, triethanolamine,

tromethamine, purines, piperazine, piperidine, *N*-ethylpiperidine, polyamine resins and the like. Particularly preferred organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline and caffeine.

Often crystallizations produce a solvate of the compound of the disclosure. As used
5 herein, the term “solvate” refers to an aggregate that comprises one or more molecules of a compound of the disclosure with one or more molecules of solvent. The solvent may be water, in which case the solvate may be a hydrate. Alternatively, the solvent may be an organic solvent. Thus, the compounds of the present disclosure may exist as a hydrate, including a monohydrate, dihydrate, hemihydrate, sesquihydrate, trihydrate, tetrahydrate and the like, as well as the
10 corresponding solvated forms. The compound of the disclosure may be true solvates, while in other cases, the compound of the disclosure may merely retain adventitious water or be a mixture of water plus some adventitious solvent.

A “pharmaceutical composition” refers to a formulation of a compound of the disclosure and a medium generally accepted in the art for the delivery of the biologically active compound
15 to mammals, *e.g.*, humans. Such a medium includes all pharmaceutically acceptable carriers, diluents or excipients therefor.

“Effective amount” or “therapeutically effective amount” refers to that amount of a compound of the disclosure which, when administered to a mammal, preferably a human, is sufficient to effect treatment in the mammal, preferably a human. The amount of a lipid
20 nanoparticle of the disclosure which constitutes a “therapeutically effective amount” will vary depending on the compound, the condition and its severity, the manner of administration, and the age of the mammal to be treated, but can be determined routinely by one of ordinary skill in the art having regard to his own knowledge and to this disclosure.

“Treating” or “treatment” as used herein covers the treatment of the disease or condition
25 of interest in a mammal, preferably a human, having the disease or condition of interest, and includes:

- (i) preventing the disease or condition from occurring in a mammal, in particular, when such mammal is predisposed to the condition but has not yet been diagnosed as having it;
- (ii) inhibiting the disease or condition, *i.e.*, arresting its development;
- 30 (iii) relieving the disease or condition, *i.e.*, causing regression of the disease or condition; or
- (iv) relieving the symptoms resulting from the disease or condition, *i.e.*, relieving pain without addressing the underlying disease or condition. As used herein, the terms “disease” and

“condition” may be used interchangeably or may be different in that the particular malady or condition may not have a known causative agent (so that etiology has not yet been worked out) and it is therefore not yet recognized as a disease but only as an undesirable condition or syndrome, wherein a more or less specific set of symptoms have been identified by clinicians.

5 The compounds of the disclosure, or their pharmaceutically acceptable salts may contain one or more asymmetric centers and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (*R*)- or (*S*)- or, as (*D*)- or (*L*)- for amino acids. The present disclosure is meant to include all such possible isomers, as well as their racemic and optically pure forms. Optically active (+) and (-), (*R*)- and
10 (*S*)-, or (*D*)- and (*L*)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques, for example, chromatography and fractional crystallization. Conventional techniques for the preparation/isolation of individual enantiomers include chiral synthesis from a suitable optically pure precursor or resolution of the racemate (or the racemate of a salt or derivative) using, for example, chiral high-pressure liquid
15 chromatography (HPLC). When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both *E* and *Z* geometric isomers. Likewise, all tautomeric forms are also intended to be included.

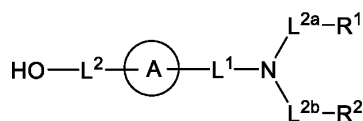
 A “stereoisomer” refers to a compound made up of the same atoms bonded by the same
20 bonds but having different three-dimensional structures, which are not interchangeable. The present disclosure contemplates various stereoisomers and mixtures thereof and includes “enantiomers”, which refers to two stereoisomers whose molecules are non-superimposable mirror images of one another.

 A “tautomer” refers to a proton shift from one atom of a molecule to another atom of the
25 same molecule. The present disclosure includes tautomers of any said compounds.

Compounds

 In an aspect, the disclosure provides novel lipid compounds which are capable of combining with other lipid components such as neutral lipids, charged lipids, steroids, and/or polymer conjugated lipids to form lipid nanoparticles with oligonucleotides. Without wishing to
30 be bound by theory, it is thought that these lipid nanoparticles shield oligonucleotides from degradation in the serum and provide for effective delivery of oligonucleotides to cells *in vitro* and *in vivo*.

One embodiment provides a compound having the following Structure (I):



(I)

or a pharmaceutically acceptable salt or stereoisomer thereof, wherein:

- 5 A is a 3-10 membered carbocyclic or oxygen-containing heterocyclic ring optionally substituted with one or more fluoro, hydroxyl, C₁-C₆ alkyl and/or C₁-C₆ alkylhydroxyl substituents;

R¹ is -NR^aC(=O)R³ or -C(=O)NR^bR^c;

R² is -NR^dC(=O)R⁴ or -C(=O)NR^eR^f;

- 10 R³ and R⁴ are each independently C₆-C₂₄ alkyl or C₆-C₂₄ alkenyl;

R^a, R^b, R^d and R^e are each independently H, C₁-C₁₆ alkyl or C₂-C₁₆ alkenyl;

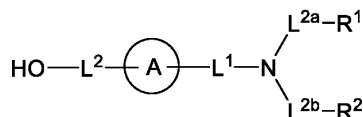
R^c and R^f are each independently C₁-C₁₆ alkyl or C₂-C₁₆ alkenyl;

L¹ and L² are each independently a direct bond or C₁-C₆ alkylene; and

L^{2a} and L^{2b} are each independently C₄-C₁₂ alkylene;

- 15 wherein each alkyl, alkylene and alkenyl is optionally substituted with fluoro.

One embodiment provides a compound having the following structure (I):



(I)

or a pharmaceutically acceptable salt or stereoisomer thereof, wherein:

- 20 A is a 3-10 membered carbocyclic or oxygen-containing heterocyclic ring optionally substituted with one or more fluoro, hydroxyl, C₁-C₆ alkyl, or C₁-C₆ alkylhydroxyl substituents (including combinations thereof – i.e., one or more fluoro substituents and one or more hydroxyl substituents, *etc.*);

R¹ is -NR^aC(=O)R³ or -C(=O)NR^bR^c;

- 25 R² is -NR^dC(=O)R⁴ or -C(=O)NR^eR^f;

R³ and R⁴ are each independently C₆-C₂₄ alkyl or C₆-C₂₄ alkenyl;

R^a, R^b, R^d and R^e are each independently H, C₁-C₂₀ alkyl or C₂-C₂₀ alkenyl;

R^c and R^f are each independently C₁-C₂₀ alkyl or C₂-C₂₀ alkenyl;

L¹ and L² are each independently a direct bond or C₁-C₆ alkylene; and

L^{2a} and L^{2b} are each independently C_4 - C_{12} alkylene;

wherein each alkyl, alkylene, and alkenyl is optionally substituted with one or more fluoro.

In some embodiment, R^1 is $-C(=O)NR^bR^c$. In certain embodiments, R^1 is $-NR^aC(=O)R^3$. In some embodiments, R^2 is $-C(=O)NR^eR^f$. In certain embodiments, $-NR^dC(=O)R^4$. In certain
 5 embodiments, $-C(=O)NR^bR^c$ and R^2 is $-C(=O)NR^eR^f$. In certain embodiments, R^1 is $-NR^aC(=O)R^3$ and R^2 is $-NR^dC(=O)R^4$. In certain embodiments, R^1 is $-NR^aC(=O)R^3$ and R^2 is $-C(=O)NR^eR^f$.

In some embodiments, R^a is C_8 - C_{12} alkyl. In certain embodiments, R^a is C_8 , C_9 or C_{10} alkyl. In some embodiments, R^b is C_8 - C_{12} alkyl. In certain embodiments, R^b is C_8 , C_9 or C_{10}
 10 alkyl. In some embodiments, R^c is C_8 - C_{12} alkyl. In certain embodiments, R^c is C_8 , C_9 or C_{10} alkyl. In some embodiments, R^d is C_8 - C_{12} alkyl. In certain embodiments, R^d is C_8 , C_9 or C_{10} alkyl. In some embodiments, R^e is C_8 - C_{12} alkyl. In certain embodiments, R^e is C_8 , C_9 or C_{10} alkyl. In some embodiments, R^f is C_8 - C_{12} alkyl. In certain embodiments, R^f is C_8 , C_9 or C_{10} alkyl.

In some embodiments, R^a , R^b , R^c , R^d , R^e and R^f are each independently C_1 - C_{20} alkyl. In
 15 certain embodiments, R^a , R^b , R^c , R^d , R^e and R^f are each independently C_8 - C_{19} alkyl. In some embodiments, R^a , R^b , R^c , R^d , R^e and R^f are each independently C_8 , C_9 , C_{10} , or C_{19} alkyl. In certain embodiments, R^3 and R^4 are each independently C_6 - C_{19} alkyl.

In some embodiments, R^3 is C_6 - C_{12} alkyl. In certain embodiments, R^3 is C_8 - C_{10} alkyl. In
 20 certain embodiments, R^3 is C_8 , C_9 or C_{10} alkyl. In some embodiments, R^4 is C_6 - C_{12} alkyl. In certain embodiments, R^4 is C_8 - C_{10} alkyl. In certain embodiments, R^4 is C_8 , C_9 or C_{10} alkyl.

In some embodiments, L^1 is a direct bond. In certain embodiments, L^1 is C_1 - C_6 alkylene. In
 certain embodiments, L^1 is C_1 or C_2 alkylene. In some embodiments, L^2 is a direct bond. In
 certain embodiments, L^2 is C_1 - C_6 alkylene. In certain embodiments, L^2 is C_1 or C_2 alkylene.

In some embodiments, L^{2a} is C_6 - C_{10} alkylene. In certain embodiments, L^{2a} is C_6 , C_7 , C_8 ,
 25 C_9 or C_{10} alkylene. In some embodiments, L^{2b} is C_6 - C_{10} alkylene. In certain embodiments, L^{2b} is C_6 , C_7 , C_8 , C_9 , or C_{10} alkylene.

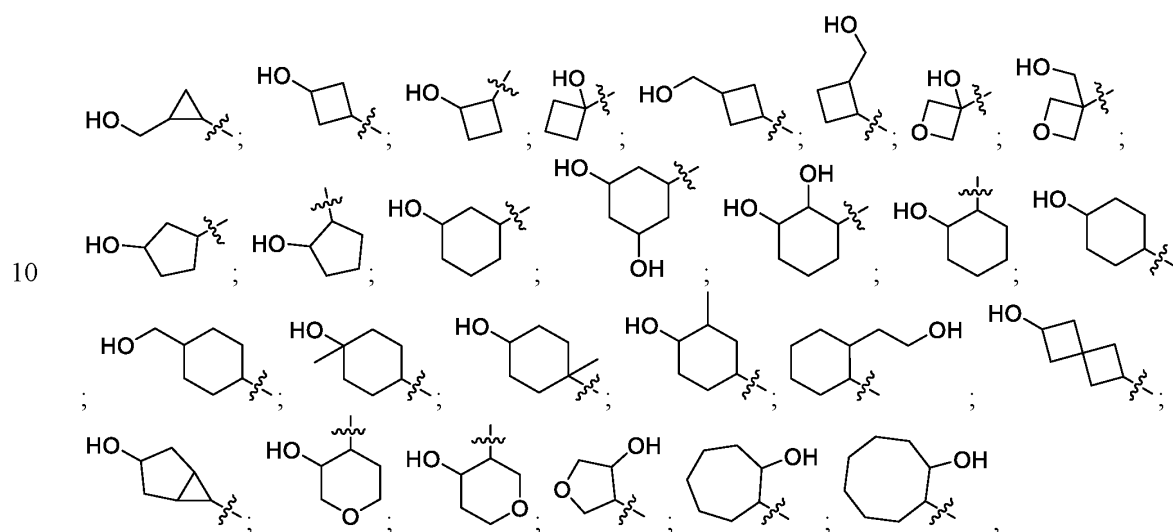
In some embodiments, A is a C_3 - C_{10} carbocyclic ring optionally substituted with one or
 more hydroxyl and/or C_1 - C_6 alkylhydroxyl substituents. In certain embodiments, A is
 monocyclic. In certain embodiments, A is bicyclic. In certain embodiments, A is spirocyclic.

30 In some embodiments, A is a C_3 - C_8 carbocyclic ring optionally substituted with one or
 more hydroxyl and/or C_1 - C_6 alkylhydroxyl substituents. In certain embodiments, A is
 cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, or cyclooctyl.

In some embodiments, A is a 3-10 membered oxygen-containing heterocyclic ring optionally substituted with one or more hydroxyl and/or C₁-C₆ alkylhydroxyl substituents. A is a 3-6 membered oxygen-containing heterocyclic ring optionally substituted with one or more hydroxyl and/or C₁-C₆ alkylhydroxyl substituents. In certain embodiments, A is oxiranyl, oxetananyl, tetrahydrofuranyl or tetrahydropyranyl.

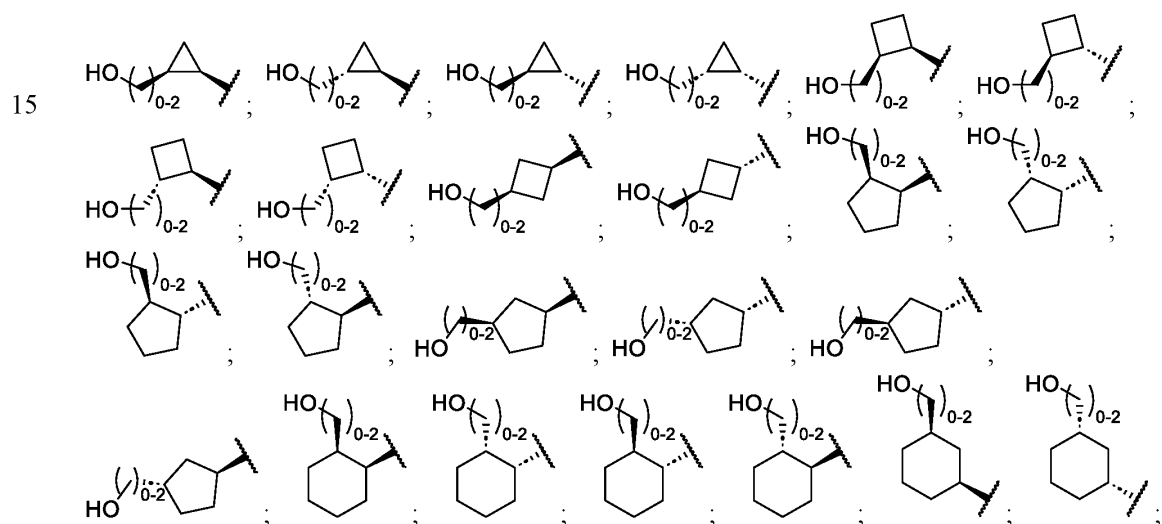
In some embodiments, A is unsubstituted. In certain embodiments, A is substituted with hydroxyl.

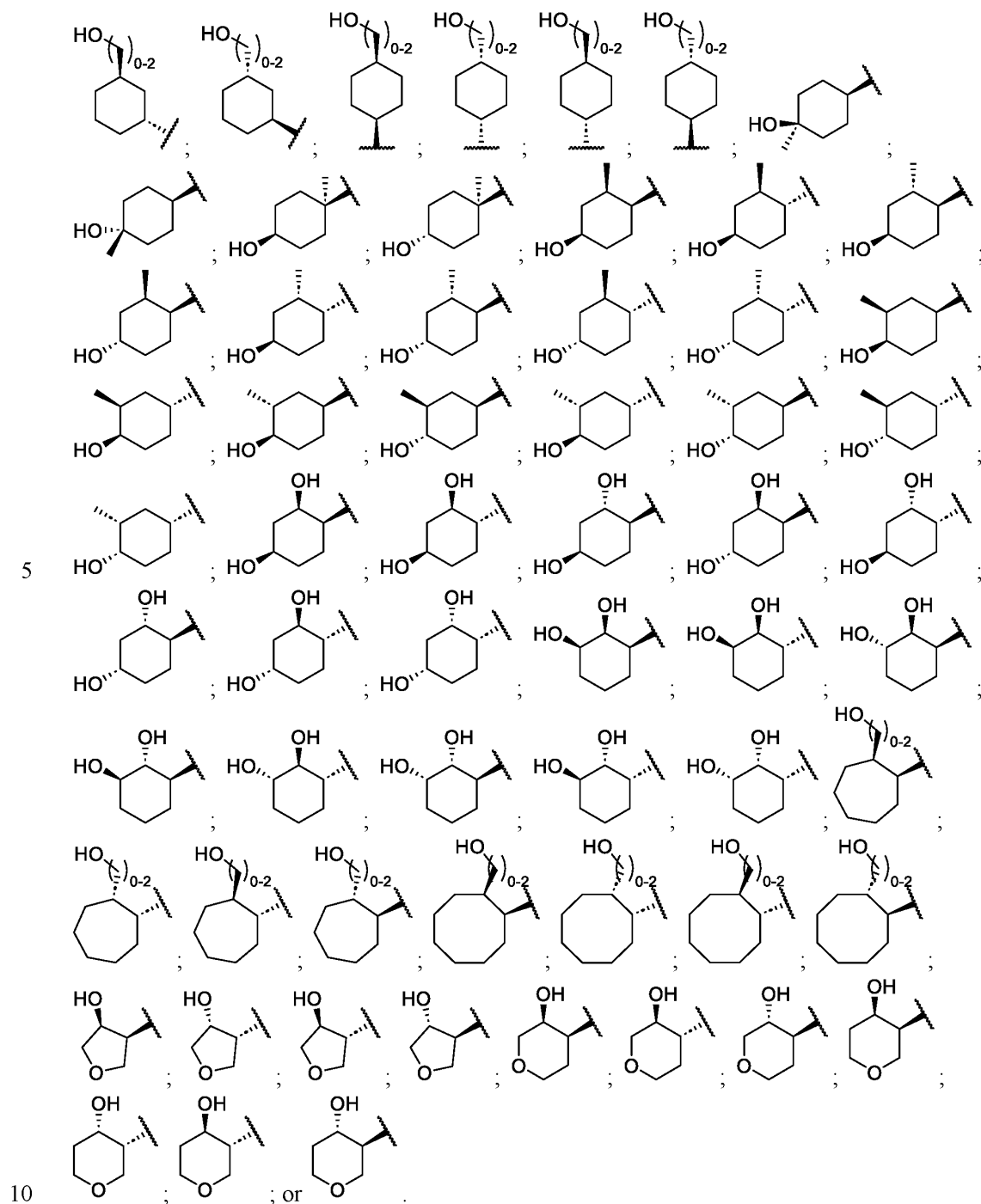
In some embodiments, $\text{HO}-\text{L}^2-\text{A}$ has one of the following structures:



or a stereoisomer thereof.

In certain embodiments, $\text{HO}-\text{L}^2-\text{A}$ has one of the following structures:





In some embodiments, R^a is substituted with one or more fluorine atoms. In some
 embodiments, R^b is substituted with one or more fluorine atoms. In some embodiments, R^c is
 substituted with one or more fluorine atoms. In some embodiments, R^d is substituted with one or
 more fluorine atoms. In some embodiments, R^e is substituted with one or more fluorine atoms. In
 15 some embodiments, R^f is substituted with one or more fluorine atoms. In some embodiments, L^1
 is substituted with one or more fluorine atoms. In some embodiments, L^2 is substituted with one

or more fluorine atoms. In some embodiments, L^{2a} is substituted with one or more fluorine atoms. In some embodiments, L^{2b} is substituted with one or more fluorine atoms.

In various different embodiments, the compound has one of the structures set forth in Table 1 below.

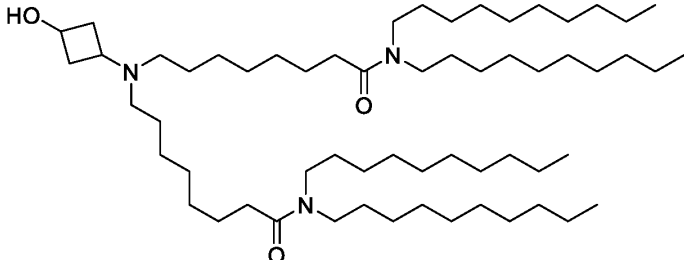
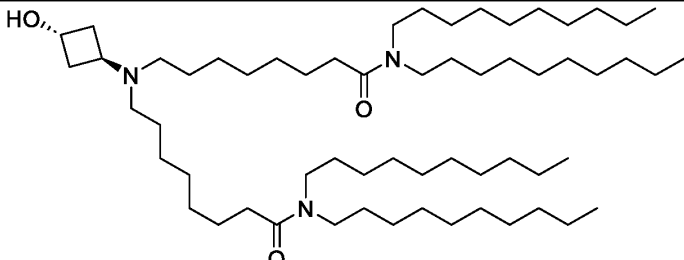
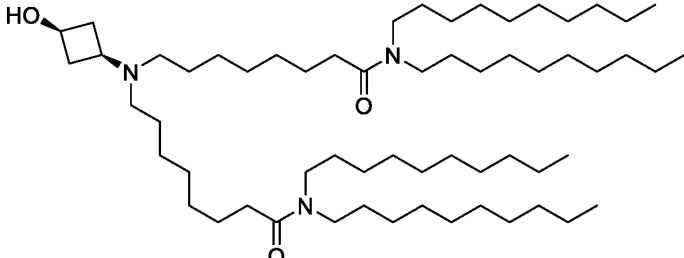
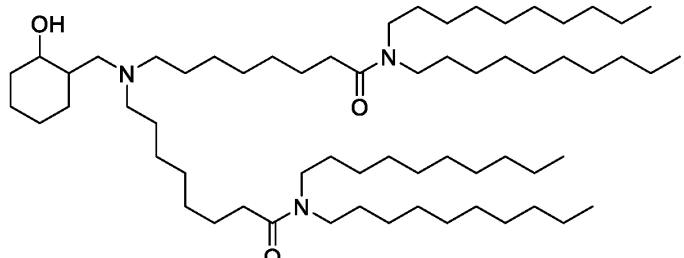
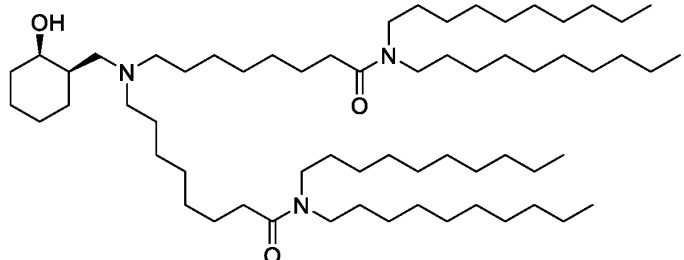
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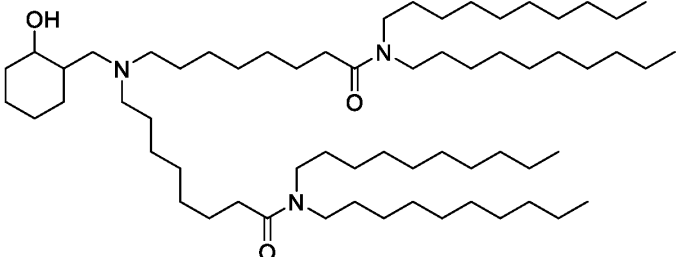
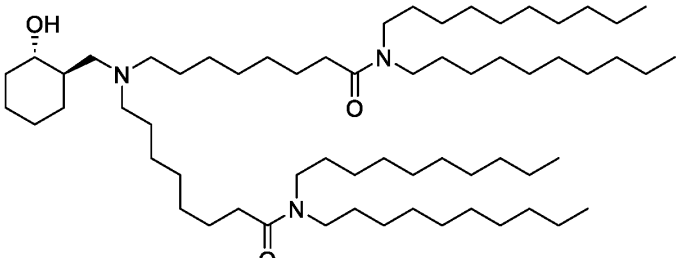
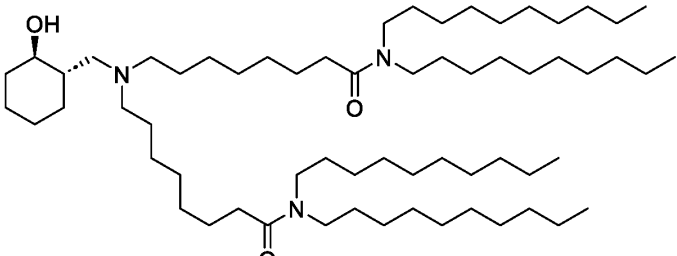
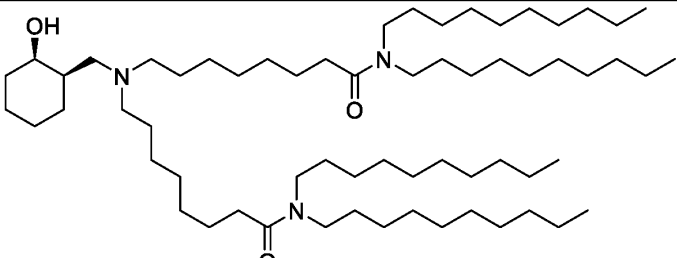
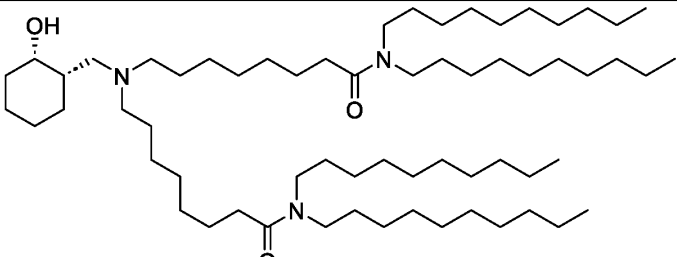
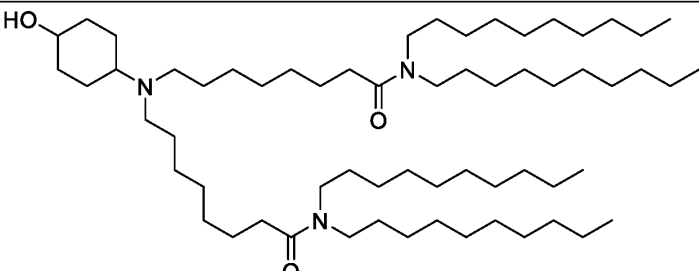
Table 1

No.	Structure	Name
I-1		8,8'-((2-(3-hydroxycyclobutyl)ethyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,3 <i>R</i>) I-1		8,8'-((2-((1 <i>S</i> ,3 <i>R</i>)-3-hydroxycyclobutyl)ethyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,3 <i>S</i>) I-1		8,8'-((2-((1 <i>R</i> ,3 <i>S</i>)-3-hydroxycyclobutyl)ethyl)azanediyl)bis(N,N-didecyloctanamide)
I-2		8,8'-(((3-hydroxycyclobutyl)methyl)azanediyl)bis(N,N-didecyloctanamide)

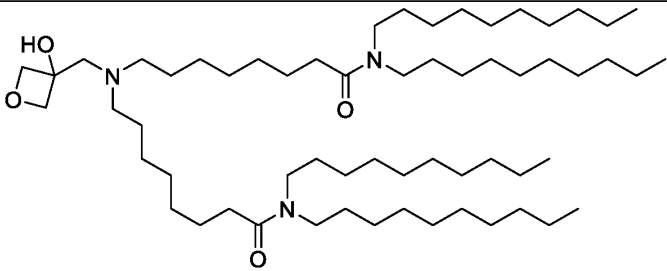
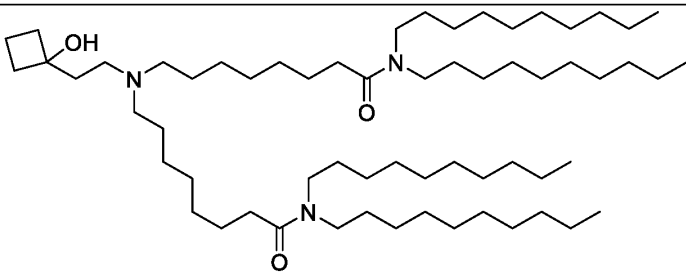
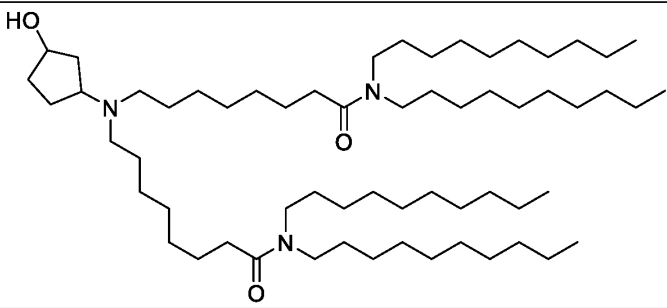
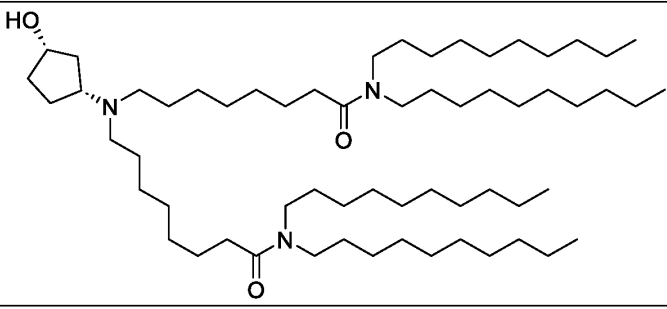
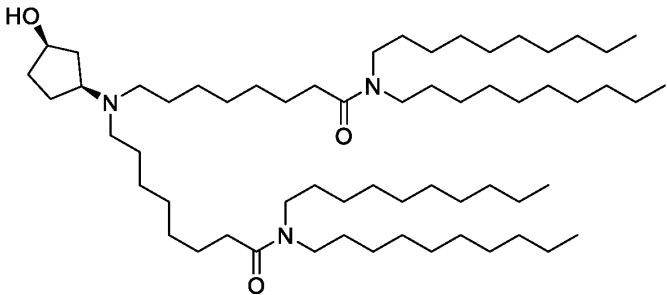
No.	Structure	Name
(1 <i>S</i> ,3 <i>S</i>) I-2		8,8'-((((1 <i>S</i> ,3 <i>S</i>)-3-hydroxycyclobutyl)methyl)azanediyl)bis(N,N-didecyl octanamide)
(1 <i>R</i> ,3 <i>R</i>) I-2		8,8'-((((1 <i>R</i> ,3 <i>R</i>)-3-hydroxycyclobutyl)methyl)azanediyl)bis(N,N-didecyl octanamide)
I-3		8,8'-(((3-hydroxycyclohexyl)methyl)azanediyl)bis(N,N-didecyl octanamide)
(1 <i>S</i> ,3 <i>R</i>) I-3		8,8'-((((1 <i>S</i> ,3 <i>R</i>)-3-hydroxycyclohexyl)methyl)azanediyl)bis(N,N-didecyl octanamide)
(1 <i>R</i> ,3 <i>S</i>) I-3		8,8'-((((1 <i>R</i> ,3 <i>S</i>)-3-hydroxycyclohexyl)methyl)azanediyl)bis(N,N-didecyl octanamide)
(1 <i>R</i> ,3 <i>R</i>) I-3		8,8'-((((1 <i>R</i> ,3 <i>R</i>)-3-hydroxycyclohexyl)methyl)azanediyl)bis(N,N-didecyl octanamide)

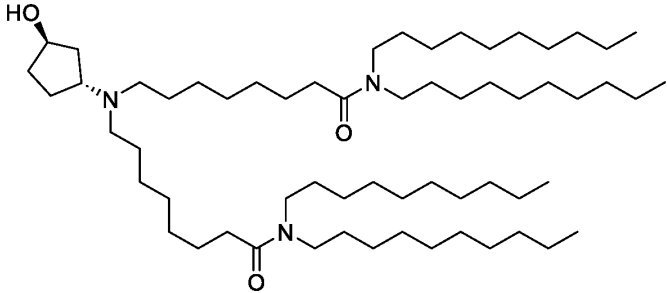
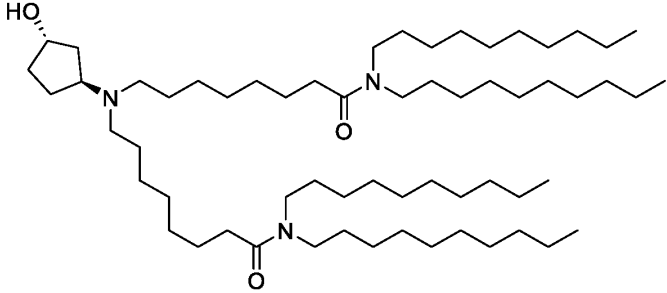
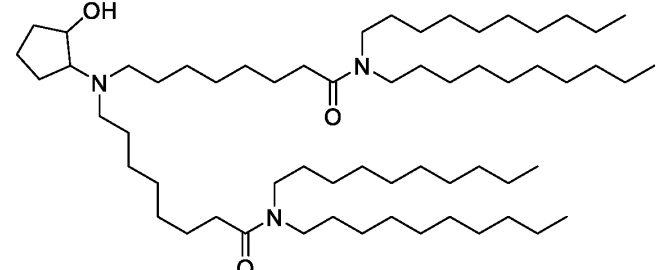
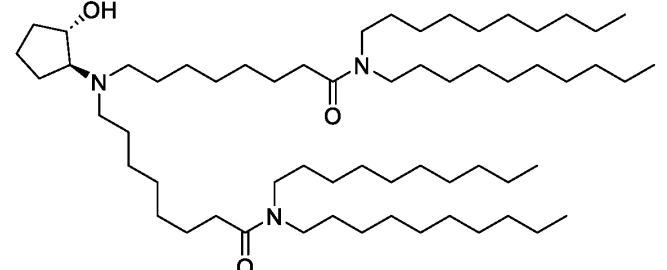
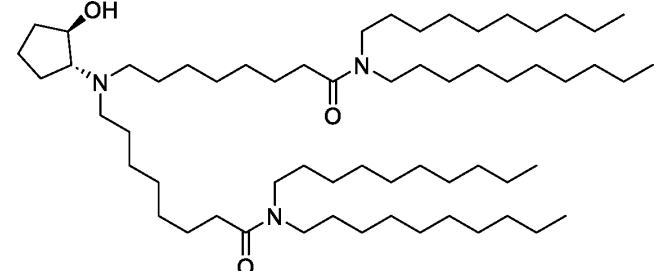
No.	Structure	Name
(1 <i>S</i> ,3 <i>S</i>) I-3		8,8'-((((1 <i>S</i> ,3 <i>S</i>)-3-hydroxycyclohexyl)methyl)azanediyloxy)bis(N,N-didecyloctanamide)
I-4		8,8'-(((3-hydroxycyclopentyl)methyl)azanediyloxy)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,3 <i>R</i>) I-4		8,8'-((((1 <i>S</i> ,3 <i>R</i>)-3-hydroxycyclopentyl)methyl)azanediyloxy)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,3 <i>S</i>) I-4		8,8'-((((1 <i>S</i> ,3 <i>S</i>)-3-hydroxycyclopentyl)methyl)azanediyloxy)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,3 <i>R</i>) I-4		8,8'-((((1 <i>R</i> ,3 <i>R</i>)-3-hydroxycyclopentyl)methyl)azanediyloxy)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,3 <i>S</i>) I-4		8,8'-((((1 <i>R</i> ,3 <i>S</i>)-3-hydroxycyclopentyl)methyl)azanediyloxy)bis(N,N-didecyloctanamide)

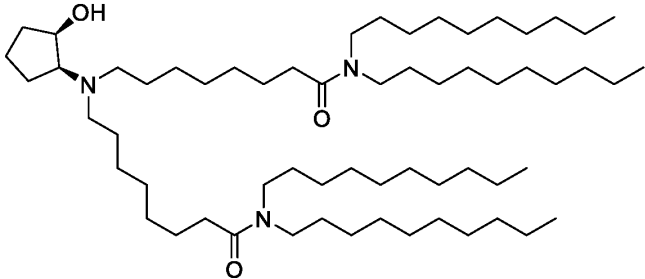
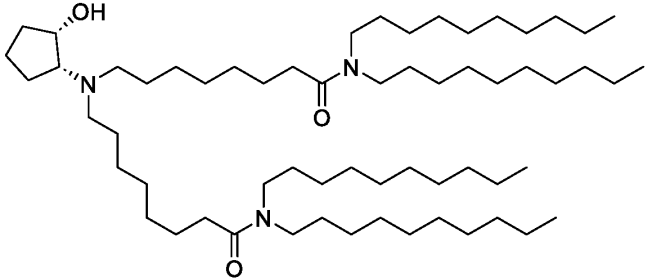
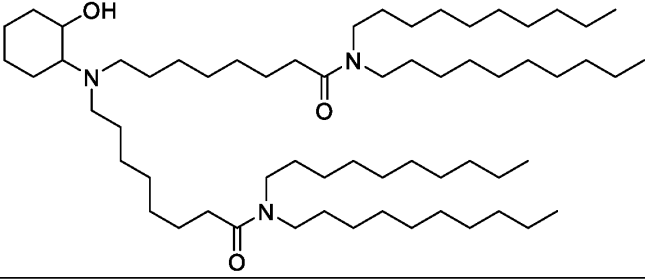
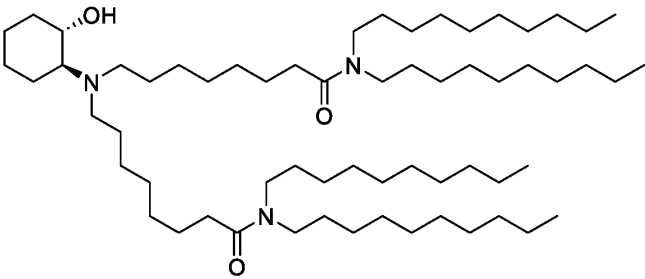
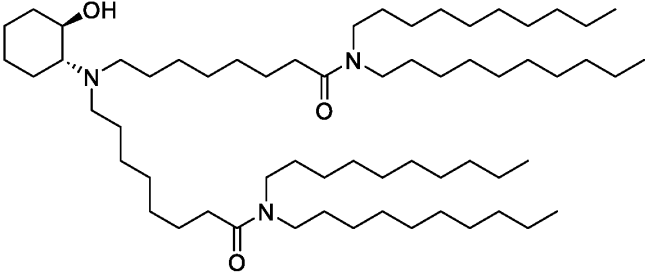
No.	Structure	Name
I-5		8,8'-((3-hydroxycyclobutyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,3 <i>R</i>) I-5		8,8'-(((1 <i>R</i> ,3 <i>R</i>)-3-hydroxycyclobutyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,3 <i>S</i>) I-5		8,8'-(((1 <i>S</i> ,3 <i>S</i>)-3-hydroxycyclobutyl)azanediyl)bis(N,N-didecyloctanamide)
I-6		8,8'-(((2-hydroxycyclohexyl)methyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>R</i>) I-6		8,8'-(((1 <i>R</i> ,2 <i>R</i>)-2-hydroxycyclohexyl)methyl)azanediyl)bis(N,N-didecyloctanamide)

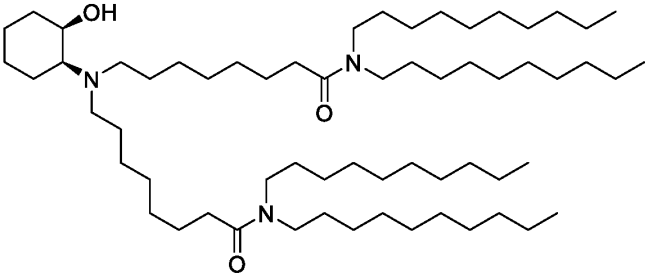
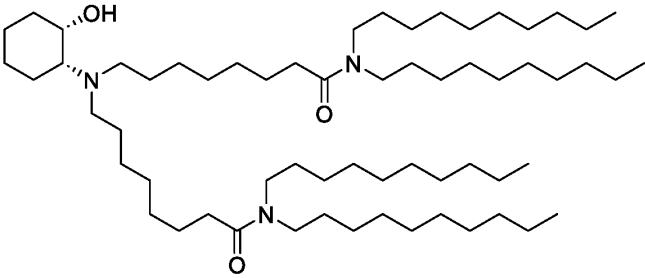
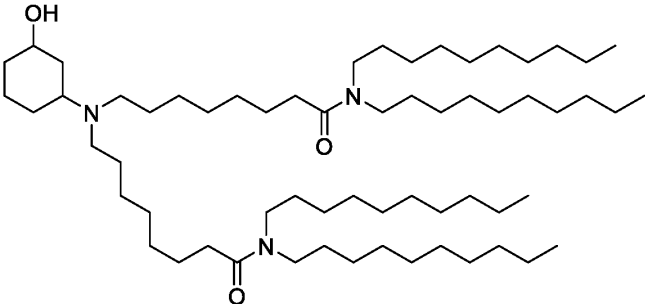
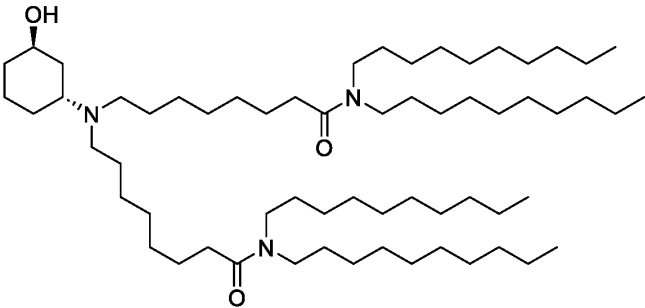
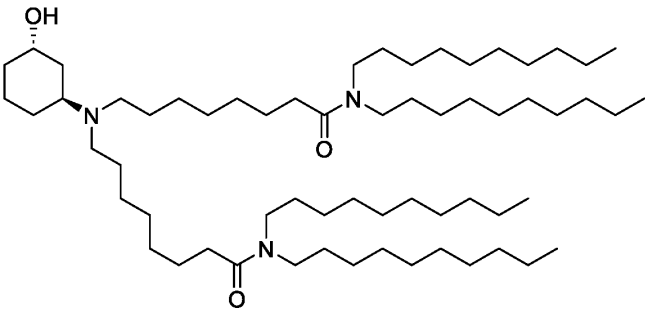
No.	Structure	Name
I-8		8,8'-(((2-hydroxycyclohexyl)methyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>S</i>) I-8		8,8'-(((1 <i>R</i> ,2 <i>S</i>)-2-hydroxycyclohexyl)methyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>R</i>) I-8		8,8'-(((1 <i>S</i> ,2 <i>R</i>)-2-hydroxycyclohexyl)methyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>R</i>) I-8		8,8'-(((1 <i>R</i> ,2 <i>R</i>)-2-hydroxycyclohexyl)methyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>S</i>) I-8		8,8'-(((1 <i>S</i> ,2 <i>S</i>)-2-hydroxycyclohexyl)methyl)azanediyl)bis(N,N-didecyloctanamide)
I-9		8,8'-((4-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)

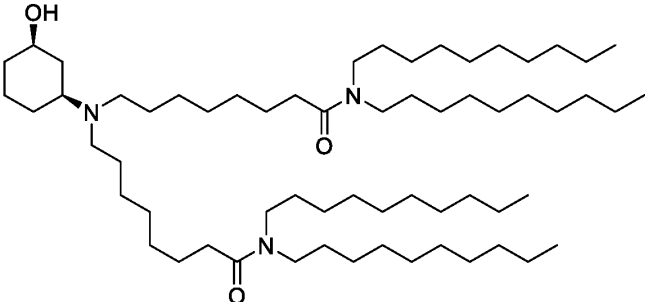
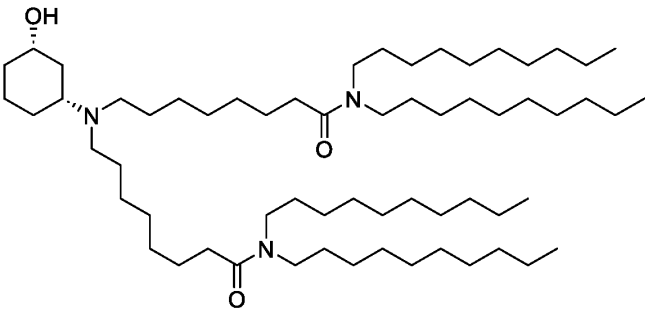
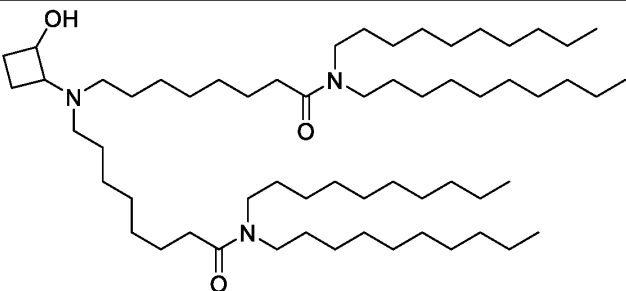
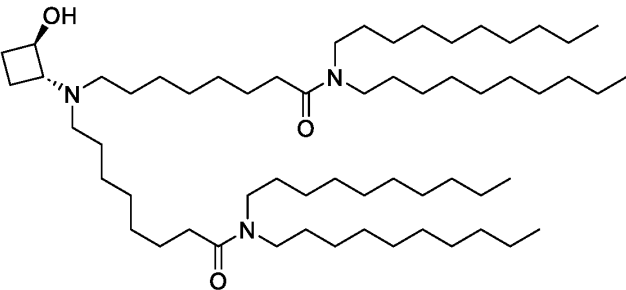
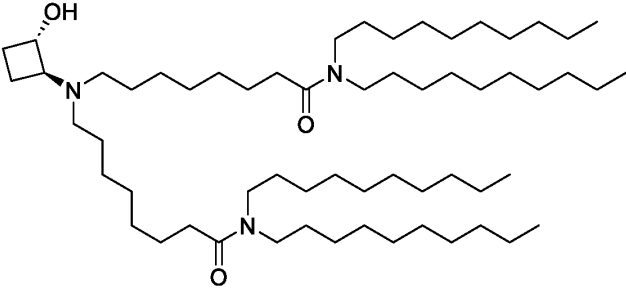
No.	Structure	Name
(1 <i>R</i> ,4 <i>R</i>) I-9		8,8'-(((1 <i>R</i> ,4 <i>R</i>)-4-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,4 <i>S</i>) I-9		8,8'-(((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
I-10		8,8'-((3-hydroxycyclobutyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,3 <i>S</i>) I-10		8,8'-(((1 <i>S</i> ,3 <i>S</i>)-3-hydroxycyclobutyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,3 <i>R</i>) I-10		8,8'-(((1 <i>R</i> ,3 <i>R</i>)-3-hydroxycyclobutyl)azanediyl)bis(N,N-didecyloctanamide)
I-11		8,8'-(((1-hydroxycyclobutyl)methyl)azanediyl)bis(N,N-didecyloctanamide)

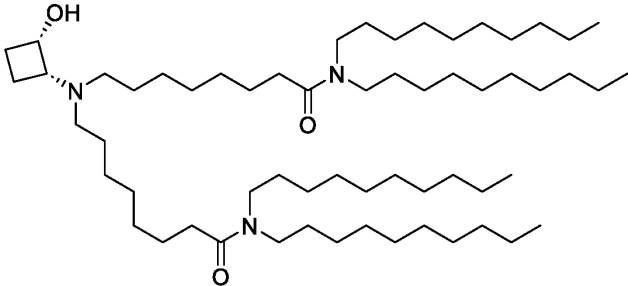
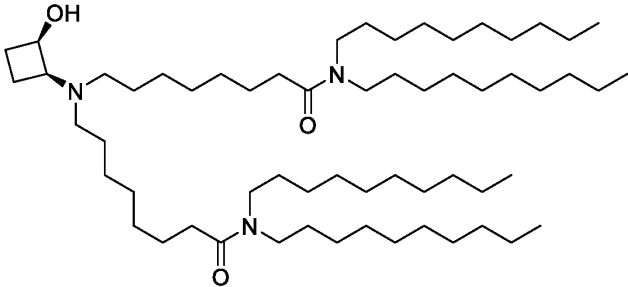
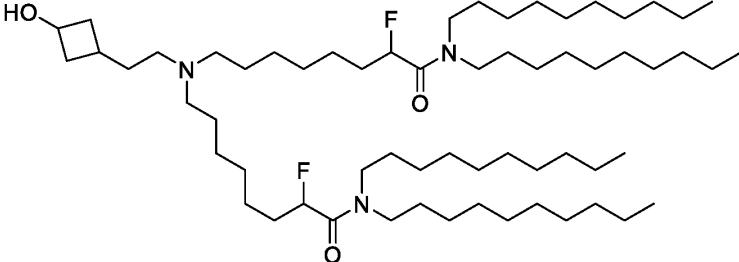
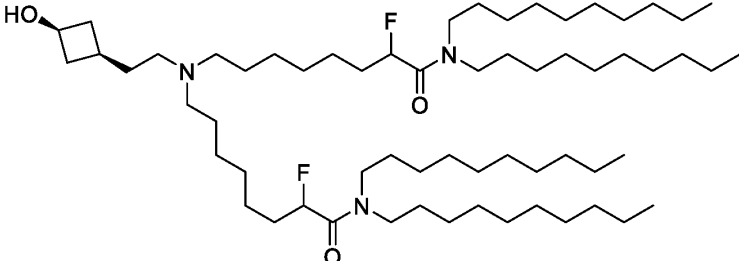
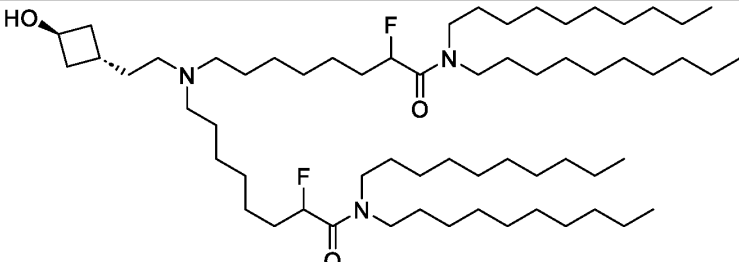
No.	Structure	Name
I-12		8,8'-(((3-hydroxyoxetan-3-yl)methyl)azanediyl)bis(N,N-didecyloctanamide)
I-13		8,8'-((2-(1-hydroxycyclobutyl)ethyl)azanediyl)bis(N,N-didecyloctanamide)
I-14		8,8'-((3-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,3 <i>S</i>) I-14		8,8'-(((1 <i>R</i> ,3 <i>S</i>)-3-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,3 <i>R</i>) I-14		8,8'-(((1 <i>S</i> ,3 <i>R</i>)-3-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)

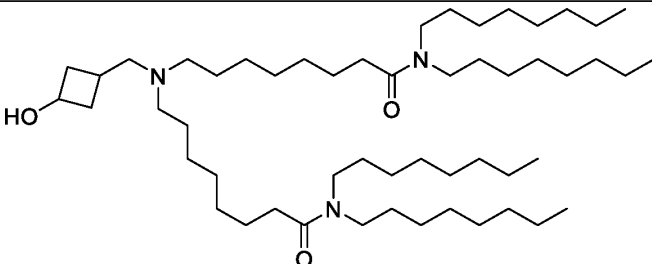
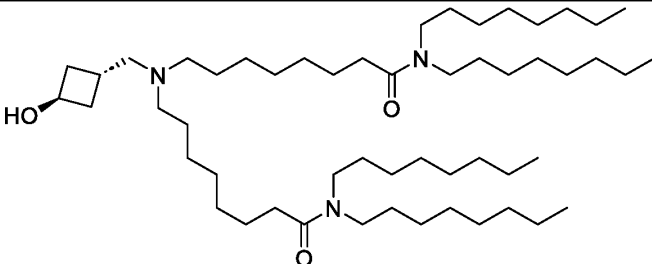
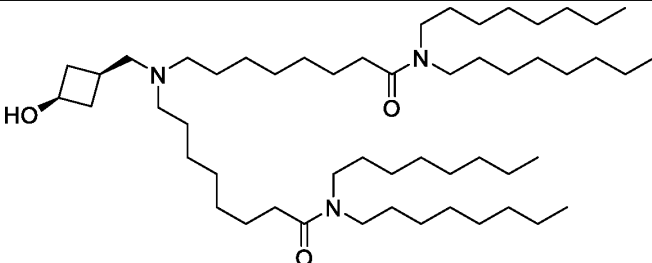
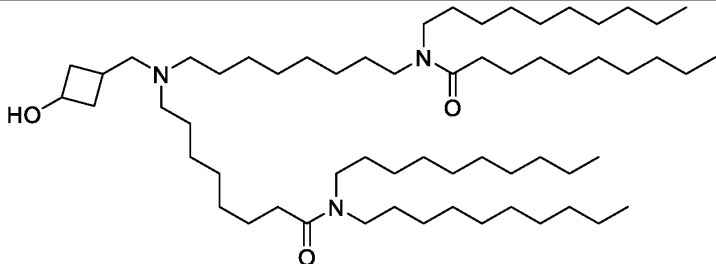
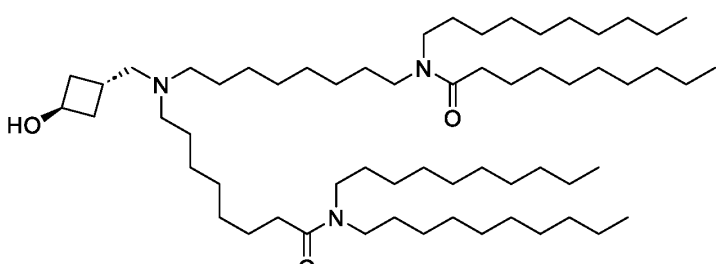
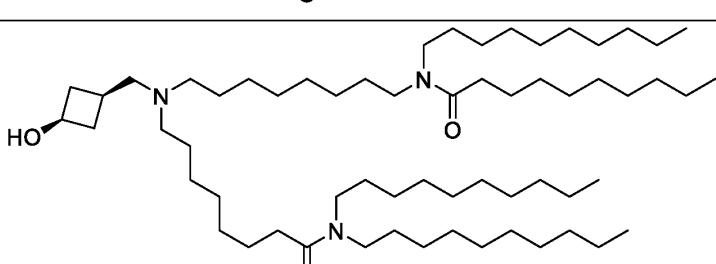
No.	Structure	Name
(1 <i>R</i> ,3 <i>R</i>) I-14		8,8'-(((1 <i>R</i> ,3 <i>R</i>)-3-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,3 <i>S</i>) I-14		8,8'-(((1 <i>S</i> ,3 <i>S</i>)-3-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)
I-15		8,8'-((2-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>S</i>) I-15		8,8'-(((1 <i>S</i> ,2 <i>S</i>)-2-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>R</i>) I-15		8,8'-(((1 <i>R</i> ,2 <i>R</i>)-2-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)

No.	Structure	Name
(1 <i>S</i> ,2 <i>R</i>) I-15		8,8'-(((1 <i>S</i> ,2 <i>R</i>)-2-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>S</i>) I-15		8,8'-(((1 <i>R</i> ,2 <i>S</i>)-2-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)
I-16		8,8'-((2-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>S</i>) I-16		8,8'-(((1 <i>S</i> ,2 <i>S</i>)-2-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>R</i>) I-16		8,8'-(((1 <i>R</i> ,2 <i>R</i>)-2-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)

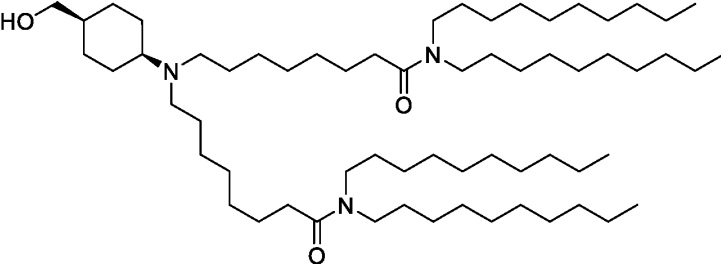
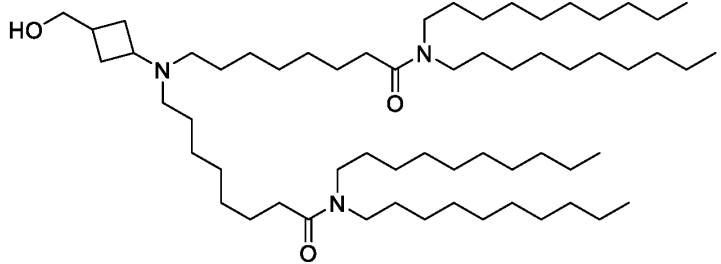
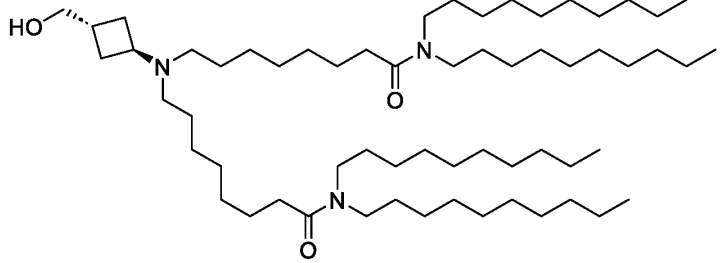
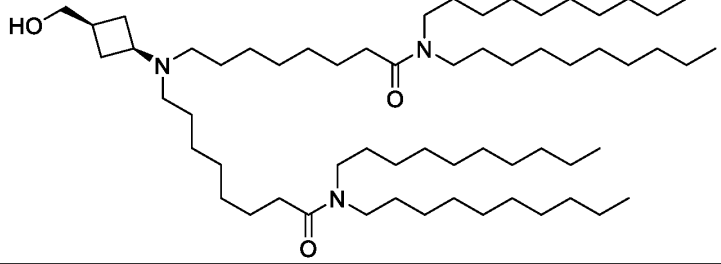
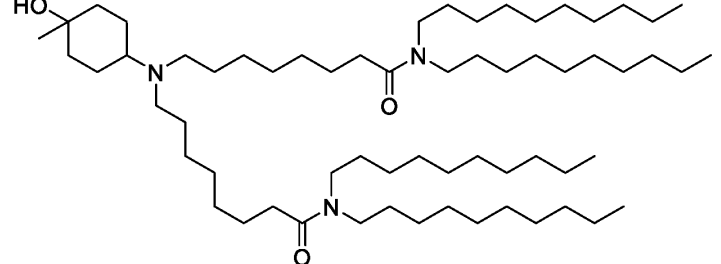
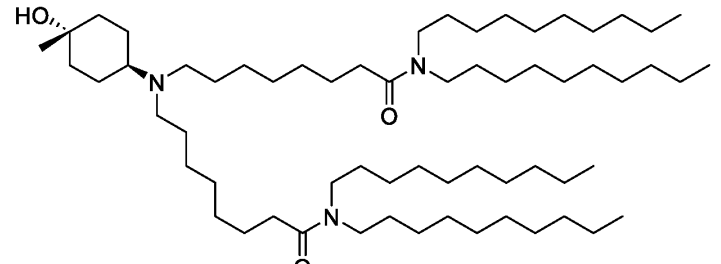
No.	Structure	Name
(1 <i>S</i> ,2 <i>R</i>) I-16		8,8'-(((1 <i>S</i> ,2 <i>R</i>)-2-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>S</i>) I-16		8,8'-(((1 <i>R</i> ,2 <i>S</i>)-2-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
I-17		8,8'-((3-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,3 <i>R</i>) I-17		8,8'-(((1 <i>R</i> ,3 <i>R</i>)-3-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,3 <i>S</i>) I-17		8,8'-(((1 <i>S</i> ,3 <i>S</i>)-3-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)

No.	Structure	Name
(1 <i>S</i> ,3 <i>R</i>) I-17		8,8'-(((1 <i>S</i> ,3 <i>R</i>)-3-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,3 <i>S</i>) I-17		8,8'-(((1 <i>R</i> ,3 <i>S</i>)-3-hydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
I-18		8,8'-((2-hydroxycyclobutyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>R</i>) I-18		8,8'-(((1 <i>R</i> ,2 <i>R</i>)-2-hydroxycyclobutyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>S</i>) I-18		8,8'-(((1 <i>S</i> ,2 <i>S</i>)-2-hydroxycyclobutyl)azanediyl)bis(N,N-didecyloctanamide)

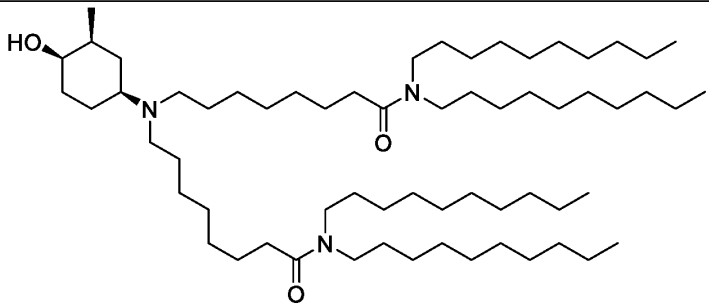
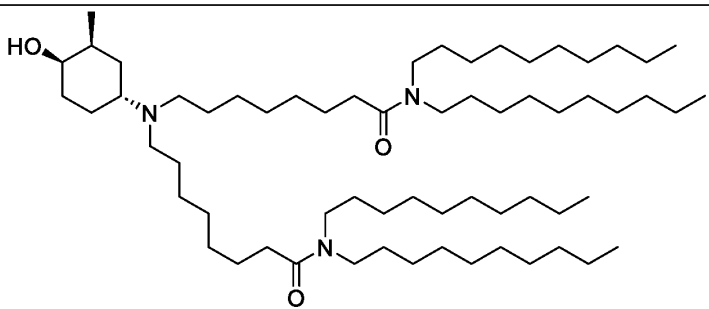
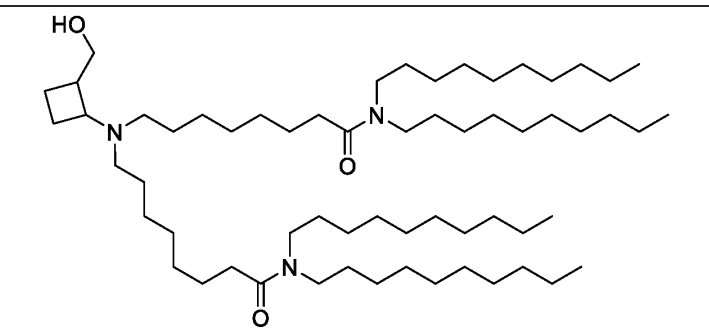
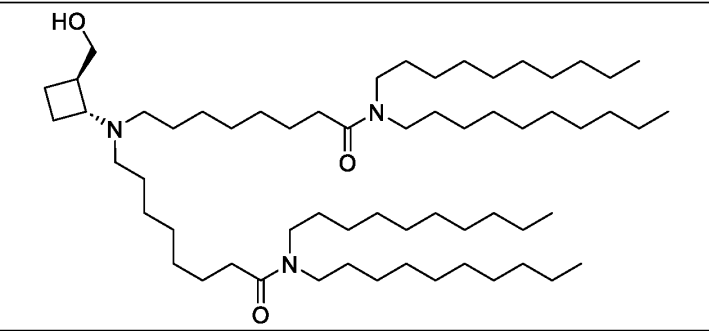
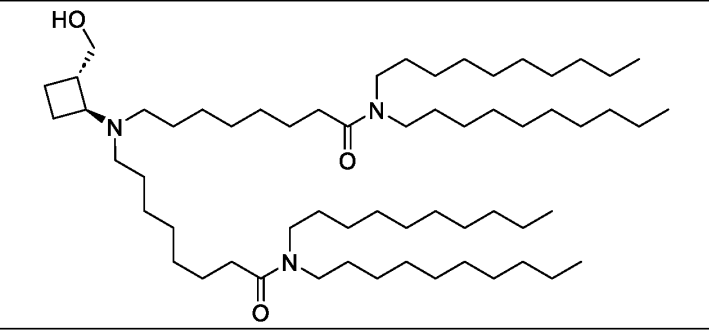
No.	Structure	Name
(1 <i>R</i> ,2 <i>S</i>) I-18		8,8'-(((1 <i>R</i> ,2 <i>S</i>)-2-hydroxycyclobutyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>R</i>) I-18		8,8'-(((1 <i>S</i> ,2 <i>R</i>)-2-hydroxycyclobutyl)azanediyl)bis(N,N-didecyloctanamide)
I-19		8,8'-((2-(3-hydroxycyclobutyl)ethyl)azanediyl)bis(N,N-didecyl-2-fluorooctanamide)
(1 <i>S</i> ,3 <i>R</i>) I-19		8,8'-((2-((1 <i>S</i> ,3 <i>R</i>)-3-hydroxycyclobutyl)ethyl)azanediyl)bis(N,N-didecyl-2-fluorooctanamide)
(1 <i>R</i> ,3 <i>S</i>) I-19		8,8'-((2-((1 <i>R</i> ,3 <i>S</i>)-3-hydroxycyclobutyl)ethyl)azanediyl)bis(N,N-didecyl-2-fluorooctanamide)

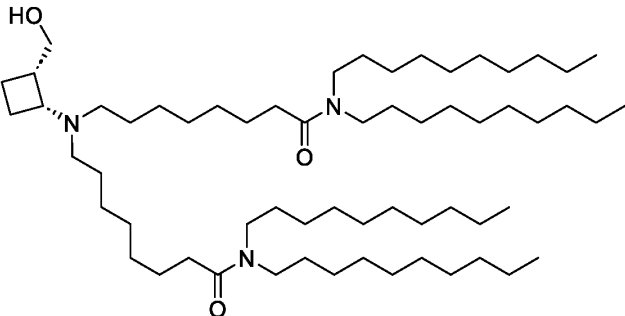
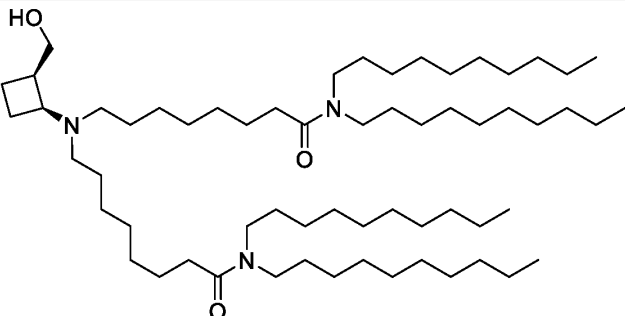
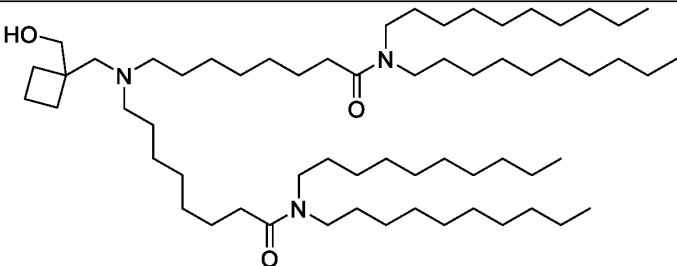
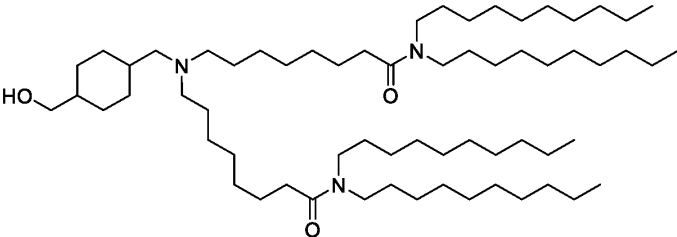
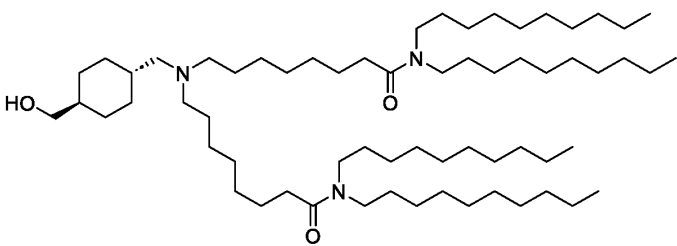
No.	Structure	Name
I-20		8,8'-(((3-hydroxycyclobutyl)methyl)azanediyl)bis(N,N-dioctyloctanamide)
(1 <i>R</i> ,3 <i>R</i>) I-20		8,8'-((((1 <i>R</i> ,3 <i>R</i>)-3-hydroxycyclobutyl)methyl)azanediyl)bis(N,N-dioctyloctanamide)
(1 <i>S</i> ,3 <i>S</i>) I-20		8,8'-((((1 <i>S</i> ,3 <i>S</i>)-3-hydroxycyclobutyl)methyl)azanediyl)bis(N,N-dioctyloctanamide)
I-21		N-decyl-N-(8-((8-(didecylamino)-8-oxooctyl)((3-hydroxycyclobutyl)methyl)amino)octyl)decanamide
(1 <i>R</i> ,3 <i>R</i>) I-21		N-decyl-N-(8-((8-(didecylamino)-8-oxooctyl)((1 <i>R</i> ,3 <i>R</i>)-3-hydroxycyclobutyl)methyl)amino)octyl)decanamide
(1 <i>S</i> ,3 <i>S</i>) I-21		N-decyl-N-(8-((8-(didecylamino)-8-oxooctyl)((1 <i>S</i> ,3 <i>S</i>)-3-hydroxycyclobutyl)methyl)amino)octyl)decanamide

No.	Structure	Name
		octyl)decanamide
I-22		N,N'-((((3-hydroxycyclobutyl)methyl)azanediyl)bis(octane-8,1-diyl))bis(N-decyldecanamide)
(1 <i>R</i> ,3 <i>R</i>) I-22		N,N'-((((1 <i>R</i> ,3 <i>R</i>)-3-hydroxycyclobutyl)methyl)azanediyl)bis(octane-8,1-diyl))bis(N-decyldecanamide)
(1 <i>S</i> ,3 <i>S</i>) I-22		N,N'-((((1 <i>S</i> ,3 <i>S</i>)-3-hydroxycyclobutyl)methyl)azanediyl)bis(octane-8,1-diyl))bis(N-decyldecanamide)
I-23		8,8'-((4-(hydroxymethyl)cyclohexyl)azanediyloxy)bis(N,N-didecyldecanamide)
(1 <i>R</i> ,4 <i>R</i>) I-23		8,8'-(((1 <i>R</i> ,4 <i>R</i>)-4-(hydroxymethyl)cyclohexyl)azanediyloxy)bis(N,N-didecyldecanamide)

No.	Structure	Name
(1 <i>S</i> ,4 <i>S</i>) I-23		8,8'-(((1 <i>S</i> ,4 <i>S</i>)-4-(hydroxymethyl)cyclohexyl)azane diyl)bis(N,N-didecyloctanamide)
I-24		8,8'-((3-(hydroxymethyl)cyclobutyl)azane diyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,3 <i>R</i>) I-24		8,8'-(((1 <i>R</i> ,3 <i>R</i>)-3-(hydroxymethyl)cyclobutyl)azane diyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,3 <i>S</i>) I-24		8,8'-(((1 <i>S</i> ,3 <i>S</i>)-3-(hydroxymethyl)cyclobutyl)azane diyl)bis(N,N-didecyloctanamide)
I-25		8,8'-((4-hydroxy-4-methylcyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,4 <i>R</i>) I-25		8,8'-(((1 <i>R</i> ,4 <i>R</i>)-4-hydroxy-4-methylcyclohexyl)azanediyl)bis(N,N-didecyloctanamide)

No.	Structure	Name
(1 <i>S</i> ,4 <i>S</i>) I-25		8,8'-(((1 <i>S</i> ,4 <i>S</i>)-4-hydroxy-4-methylcyclohexyl)azanediyl)bis(<i>N,N</i> -didecyl octanamide)
I-26		8,8'-((4-hydroxy-1-methylcyclohexyl)azanediyl)bis(<i>N,N</i> -didecyl octanamide)
(1 <i>R</i> ,4 <i>R</i>) I-26		8,8'-(((1 <i>R</i> ,4 <i>R</i>)-4-hydroxy-1-methylcyclohexyl)azanediyl)bis(<i>N,N</i> -didecyl octanamide)
(1 <i>S</i> ,4 <i>S</i>) I-26		8,8'-(((1 <i>S</i> ,4 <i>S</i>)-4-hydroxy-1-methylcyclohexyl)azanediyl)bis(<i>N,N</i> -didecyl octanamide)
I-27		8,8'-((4-hydroxy-3-methylcyclohexyl)azanediyl)bis(<i>N,N</i> -didecyl octanamide)

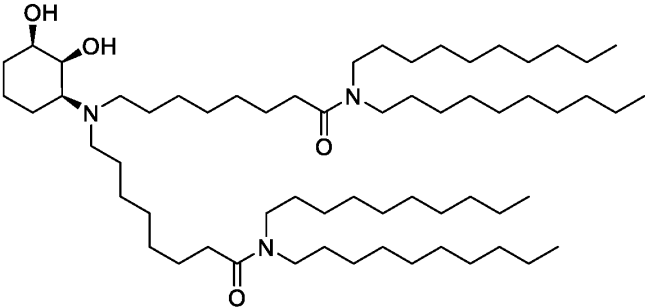
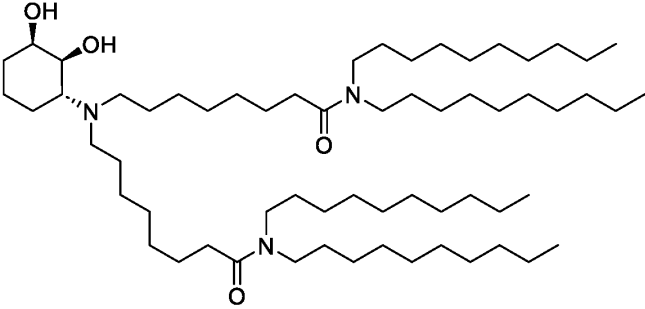
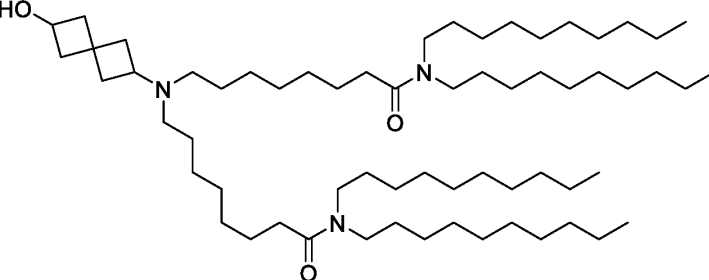
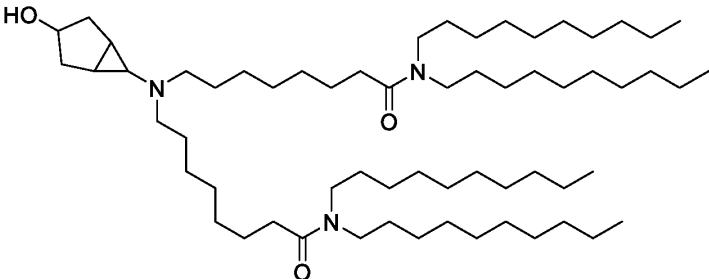
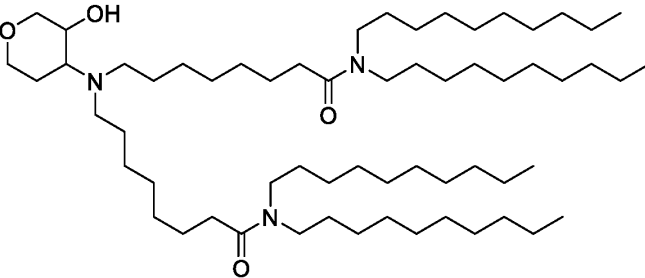
No.	Structure	Name
(1 <i>S</i> ,3 <i>S</i> ,4 <i>R</i>) I-27		8,8'- (((1 <i>S</i> ,3 <i>S</i> ,4 <i>R</i>)-4- hydroxy-3- methylcyclohexy l)azanediyl)bis(N ,N- didecyloctanami de)
(1 <i>R</i> ,3 <i>S</i> ,4 <i>R</i>) I-27		8,8'- (((1 <i>R</i> ,3 <i>S</i> ,4 <i>R</i>)-4- hydroxy-3- methylcyclohexy l)azanediyl)bis(N ,N- didecyloctanami de)
I-28		8,8'-((2- (hydroxymethyl) cyclobutyl)azane diyl)bis(N,N- didecyloctanami de)
(1 <i>R</i> ,2 <i>R</i>) I-28		8,8'-(((1 <i>R</i> ,2 <i>R</i>)-2- (hydroxymethyl) cyclobutyl)azane diyl)bis(N,N- didecyloctanami de)
(1 <i>S</i> ,2 <i>S</i>) I-28		8,8'-(((1 <i>S</i> ,2 <i>S</i>)-2- (hydroxymethyl) cyclobutyl)azane diyl)bis(N,N- didecyloctanami de)

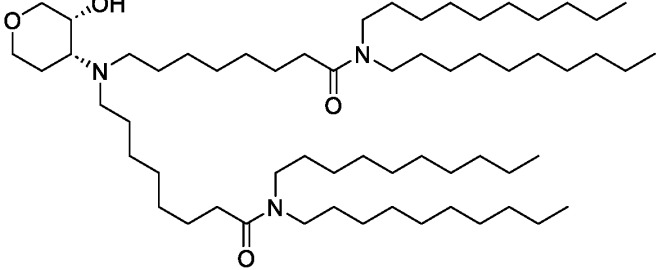
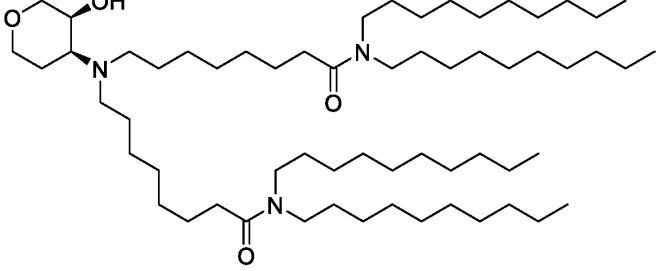
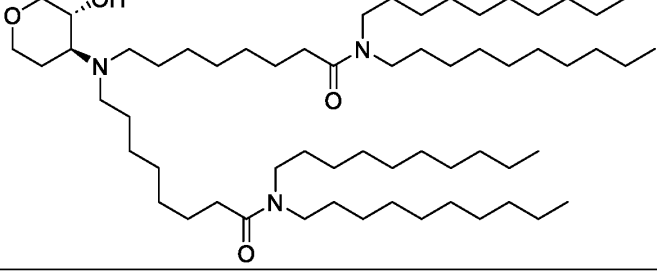
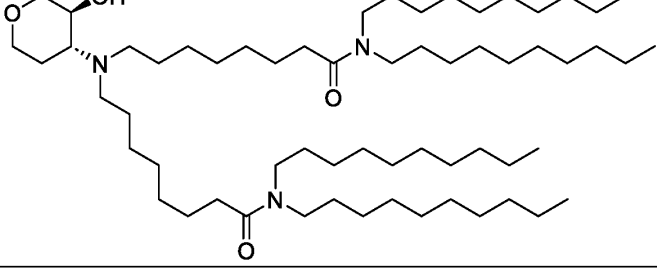
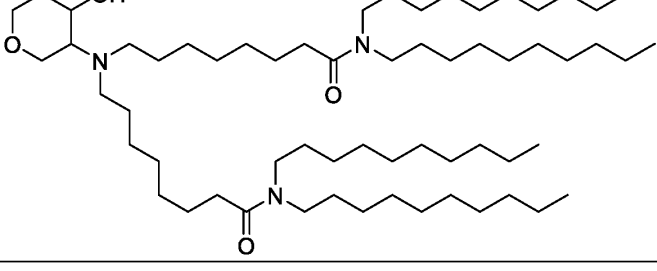
No.	Structure	Name
(1 <i>R</i> ,2 <i>S</i>) I-28		8,8'-(((1 <i>R</i> ,2 <i>S</i>)-2-(hydroxymethyl)cyclobutyl)azane diyl)bis(<i>N,N</i> -didecyloctanamide)
(1 <i>S</i> ,2 <i>R</i>) I-28		8,8'-(((1 <i>S</i> ,2 <i>R</i>)-2-(hydroxymethyl)cyclobutyl)azane diyl)bis(<i>N,N</i> -didecyloctanamide)
I-29		8,8'-(((1-(hydroxymethyl)cyclobutyl)methyl)azanediyl)bis(<i>N,N</i> -didecyloctanamide)
I-30		8,8'-(((4-(hydroxymethyl)cyclohexyl)methyl)azanediyl)bis(<i>N,N</i> -didecyloctanamide)
(1 <i>R</i> ,4 <i>R</i>) I-30		8,8'-(((1 <i>R</i> ,4 <i>R</i>)-4-(hydroxymethyl)cyclohexyl)methyl)azanediyl)bis(<i>N,N</i> -didecyloctanamide)

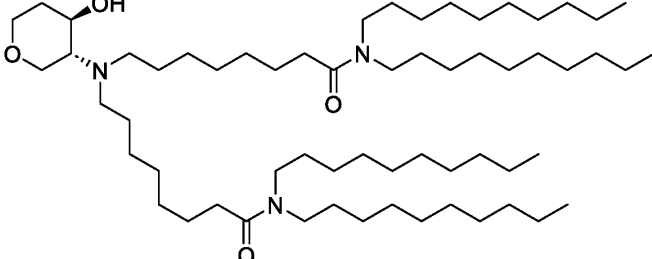
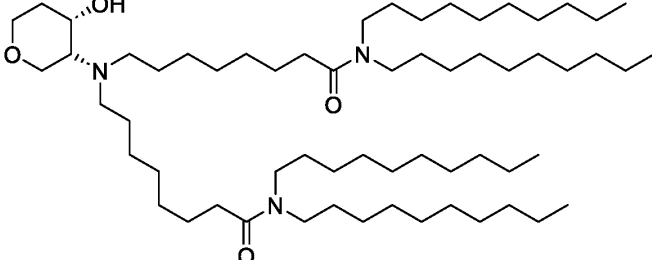
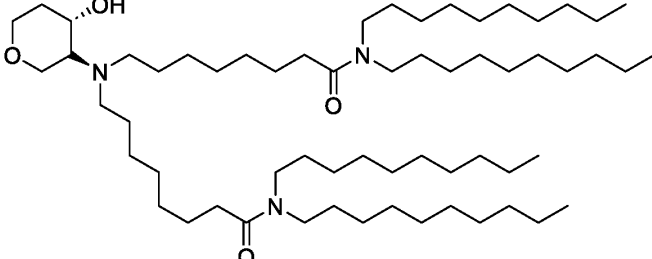
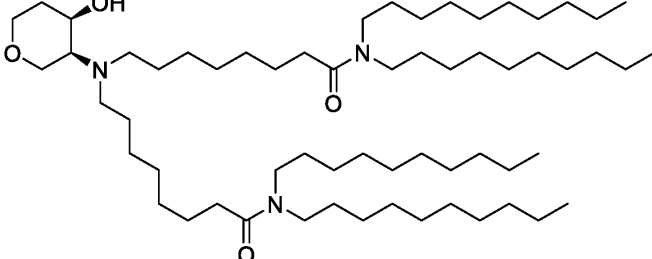
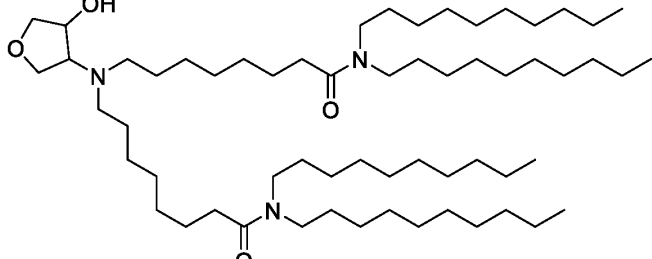
No.	Structure	Name
(1 <i>S</i> ,4 <i>S</i>) I-30		8,8'-(((1 <i>S</i> ,4 <i>S</i>)-4-(hydroxymethyl)cyclohexyl)methyl)azanediyl)bis(N,N-didecyloctanamide)
I-31		8,8'-((2-(2-hydroxycyclohexyl)ethyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>R</i>) I-31		8,8'-((2-((1 <i>R</i> ,2 <i>R</i>)-2-hydroxycyclohexyl)ethyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>S</i>) I-31		8,8'-((2-((1 <i>S</i> ,2 <i>S</i>)-2-hydroxycyclohexyl)ethyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>S</i>) I-31		8,8'-((2-((1 <i>R</i> ,2 <i>S</i>)-2-hydroxycyclohexyl)ethyl)azanediyl)bis(N,N-didecyloctanamide)

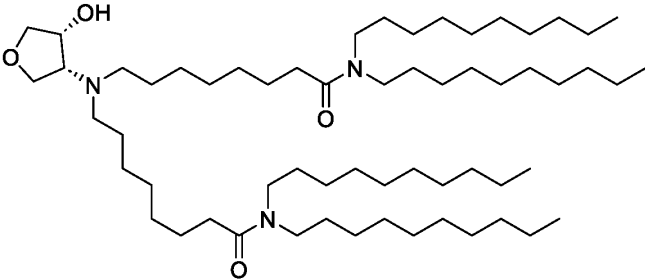
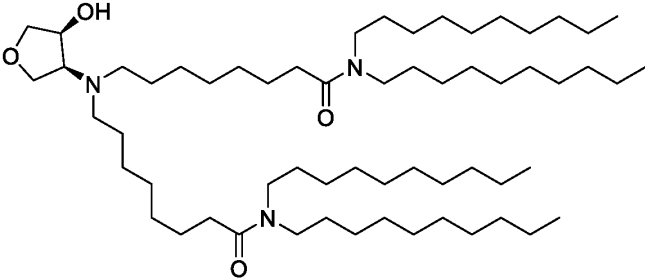
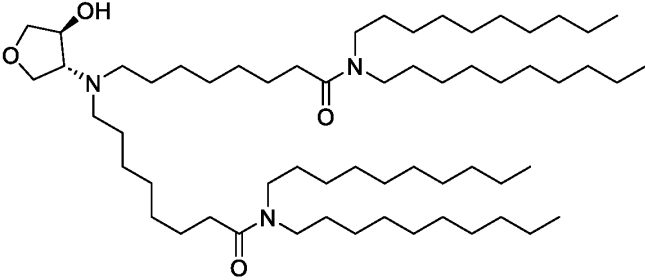
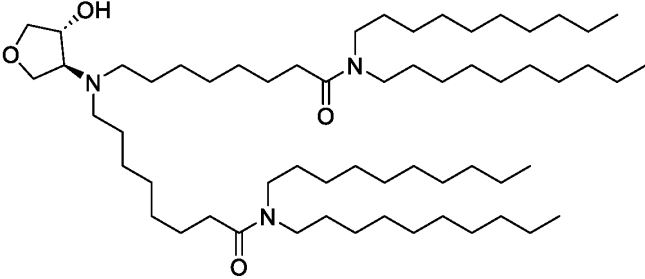
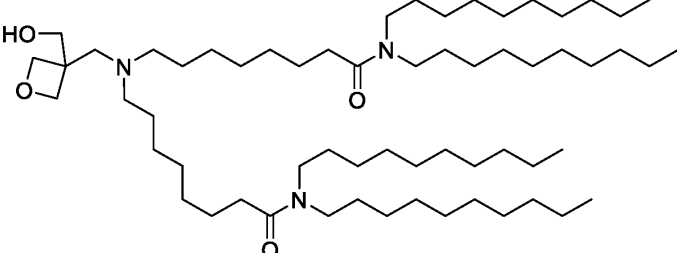
No.	Structure	Name
(1 <i>S</i> ,2 <i>R</i>) I-31		8,8'-((2-((1 <i>S</i> ,2 <i>R</i>)-2-hydroxycyclohexyl)ethyl)azanediyl)bis(N,N-didecyloctanamide)
I-32		8,8'-((3-(2-hydroxycyclohexyl)propyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>R</i>) I-32		8,8'-((3-((1 <i>R</i> ,2 <i>R</i>)-2-hydroxycyclohexyl)propyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>S</i>) I-32		8,8'-((3-((1 <i>S</i> ,2 <i>S</i>)-2-hydroxycyclohexyl)propyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>S</i>) I-32		8,8'-((3-((1 <i>R</i> ,2 <i>S</i>)-2-hydroxycyclohexyl)propyl)azanediyl)bis(N,N-didecyloctanamide)

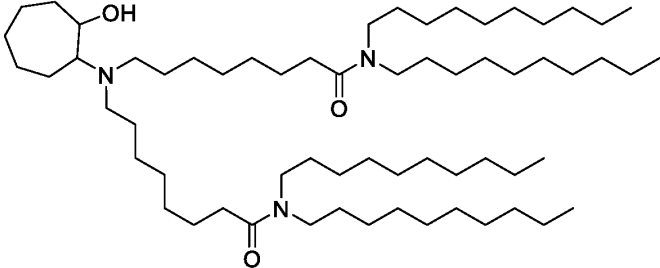
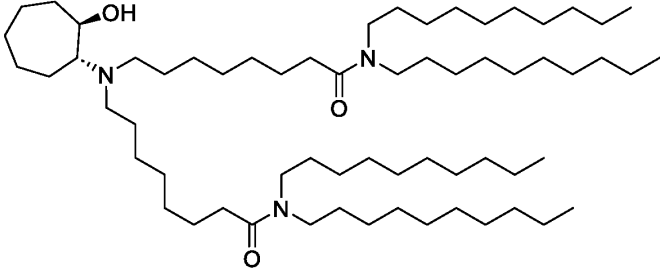
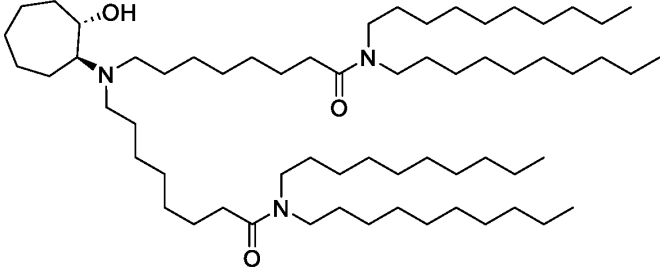
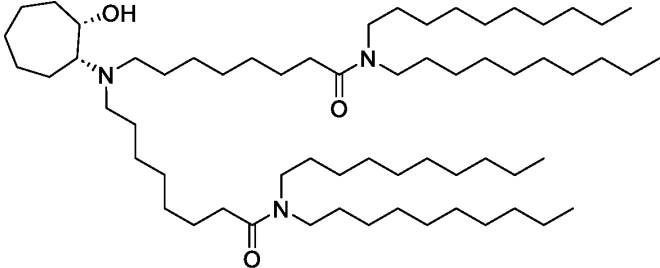
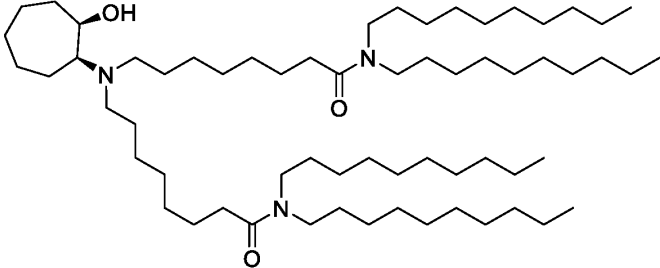
No.	Structure	Name
(1 <i>S</i> ,2 <i>R</i>) I-33		8,8'-(((1 <i>S</i> ,2 <i>R</i>)-2-(2-hydroxyethyl)cyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
I-34		8,8'-((3,5-dihydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>r</i> ,3 <i>R</i> ,5 <i>S</i>) I-34		8,8'-(((1 <i>r</i> ,3 <i>R</i> ,5 <i>S</i>)-3,5-dihydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>s</i> ,3 <i>R</i> ,5 <i>S</i>) I-34		8,8'-(((1 <i>s</i> ,3 <i>R</i> ,5 <i>S</i>)-3,5-dihydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)
I-35		8,8'-((2,3-dihydroxycyclohexyl)azanediyl)bis(N,N-didecyloctanamide)

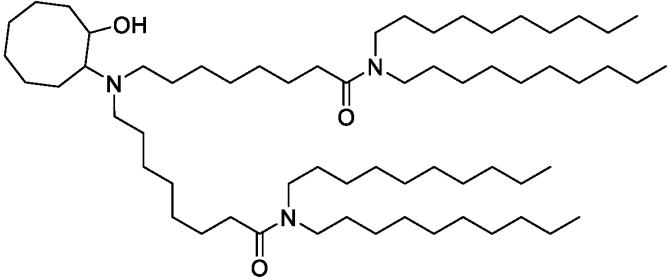
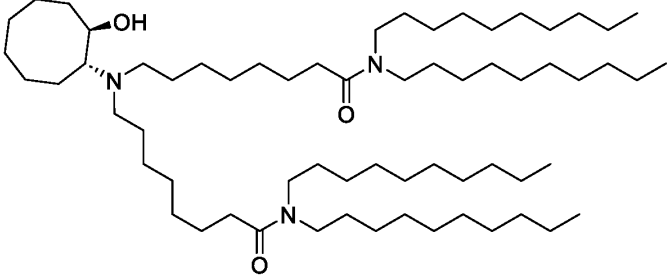
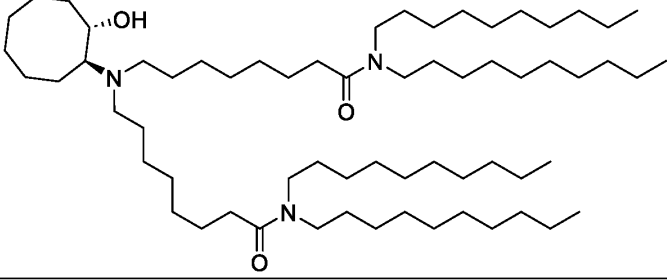
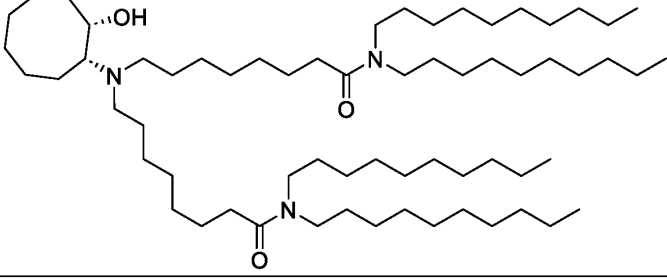
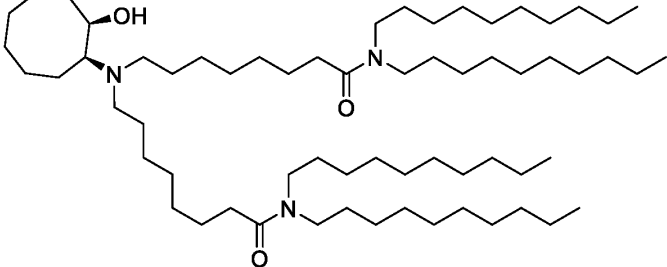
No.	Structure	Name
(1 <i>S</i> ,2 <i>S</i> ,3 <i>R</i>) I-35		8,8'- (((1 <i>S</i> ,2 <i>S</i> ,3 <i>R</i>)-2,3- dihydroxycyclohexyl)azanediyl)bi s(N,N- didecyloctanami de)
(1 <i>R</i> ,2 <i>S</i> ,3 <i>R</i>) I-35		8,8'- (((1 <i>R</i> ,2 <i>S</i> ,3 <i>R</i>)-2,3- dihydroxycyclohexyl)azanediyl)bi s(N,N- didecyloctanami de)
I-36		8,8'-(((6- hydroxyspiro[3.3]]heptan-2- yl)azanediyl)bis(N,N- didecyloctanami de)
I-37		8,8'-(((3- hydroxybicyclo[3.1.0]hexan-6- yl)azanediyl)bis(N,N- didecyloctanami de)
I-38		8,8'-(((3- hydroxytetrahydr o-2H-pyran-4- yl)azanediyl)bis(N,N- didecyloctanami de)

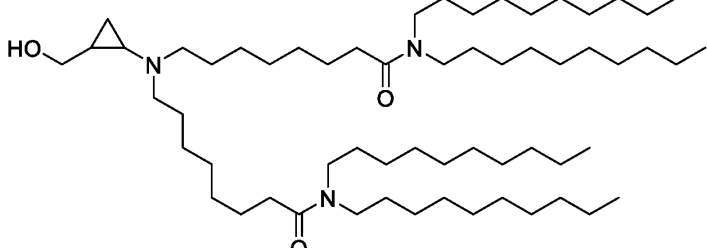
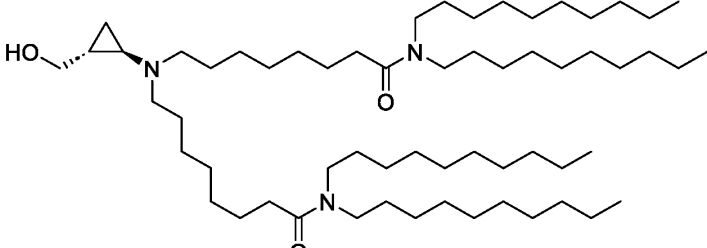
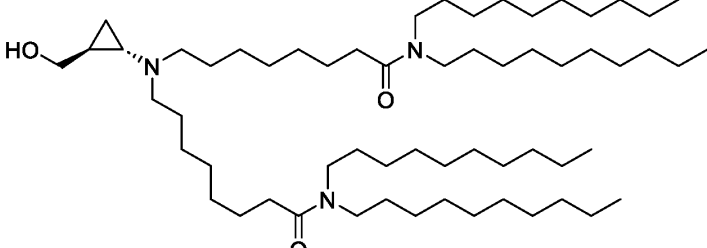
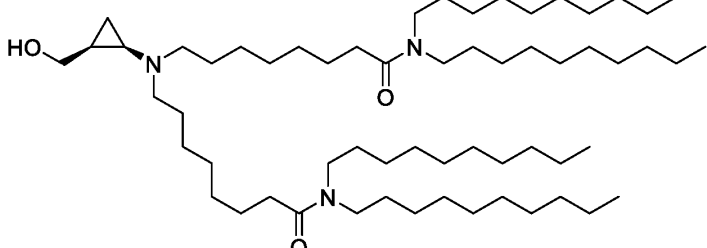
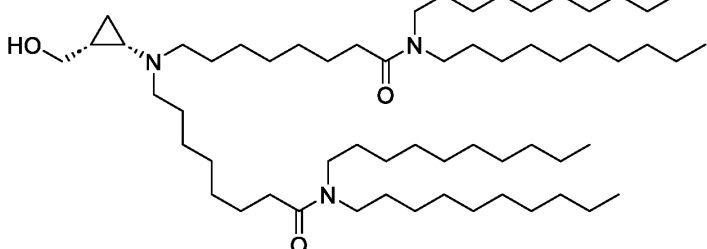
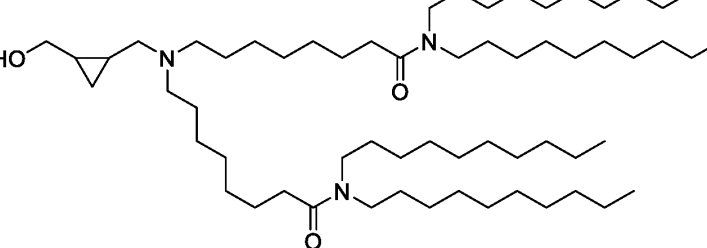
No.	Structure	Name
(3 <i>R</i> ,4 <i>R</i>) I-38		8,8'-(((3 <i>R</i> ,4 <i>R</i>)-3-hydroxytetrahydro-2H-pyran-4-yl)azanediyl)bis(N,N-didecyloctanamide)
(3 <i>S</i> ,4 <i>S</i>) I-38		8,8'-(((3 <i>S</i> ,4 <i>S</i>)-3-hydroxytetrahydro-2H-pyran-4-yl)azanediyl)bis(N,N-didecyloctanamide)
(3 <i>R</i> ,4 <i>S</i>) I-38		8,8'-(((3 <i>R</i> ,4 <i>S</i>)-3-hydroxytetrahydro-2H-pyran-4-yl)azanediyl)bis(N,N-didecyloctanamide)
(3 <i>S</i> ,4 <i>R</i>) I-38		8,8'-(((3 <i>S</i> ,4 <i>R</i>)-3-hydroxytetrahydro-2H-pyran-4-yl)azanediyl)bis(N,N-didecyloctanamide)
I-39		8,8'-((4-hydroxytetrahydro-2H-pyran-3-yl)azanediyl)bis(N,N-didecyloctanamide)

No.	Structure	Name
(3 <i>R</i> ,4 <i>R</i>) I-39		8,8'-(((3 <i>R</i> ,4 <i>R</i>)-4-hydroxytetrahydro-2H-pyran-3-yl)azanediyl)bis(N,N-didecyloctanamide)
(3 <i>S</i> ,4 <i>S</i>) I-39		8,8'-(((3 <i>S</i> ,4 <i>S</i>)-4-hydroxytetrahydro-2H-pyran-3-yl)azanediyl)bis(N,N-didecyloctanamide)
(3 <i>R</i> ,4 <i>S</i>) I-39		8,8'-(((3 <i>R</i> ,4 <i>S</i>)-4-hydroxytetrahydro-2H-pyran-3-yl)azanediyl)bis(N,N-didecyloctanamide)
(3 <i>S</i> ,4 <i>R</i>) I-39		8,8'-(((3 <i>S</i> ,4 <i>R</i>)-4-hydroxytetrahydro-2H-pyran-3-yl)azanediyl)bis(N,N-didecyloctanamide)
I-40		8,8'-((4-hydroxytetrahydrofuran-3-yl)azanediyl)bis(N,N-didecyloctanamide)

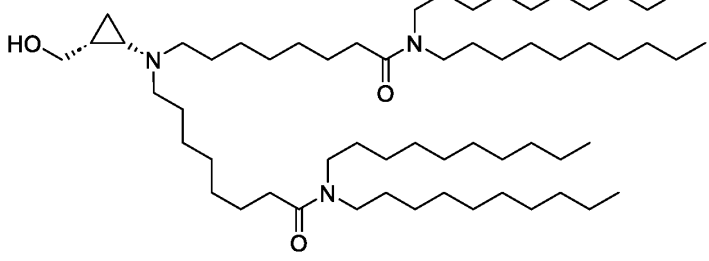
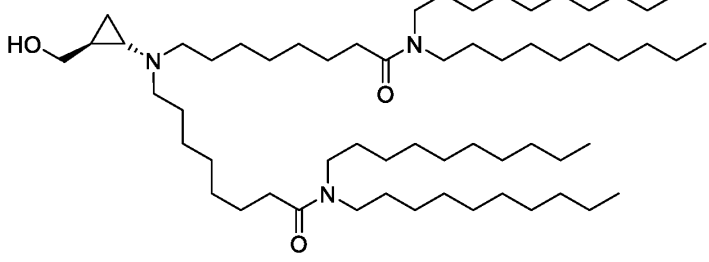
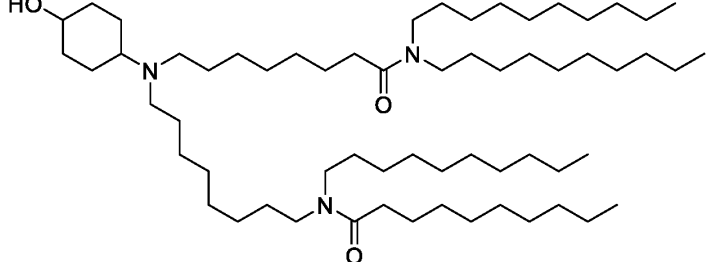
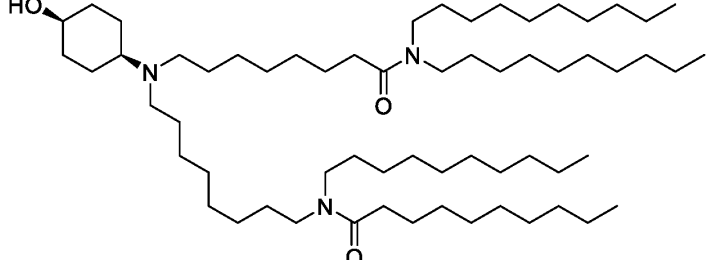
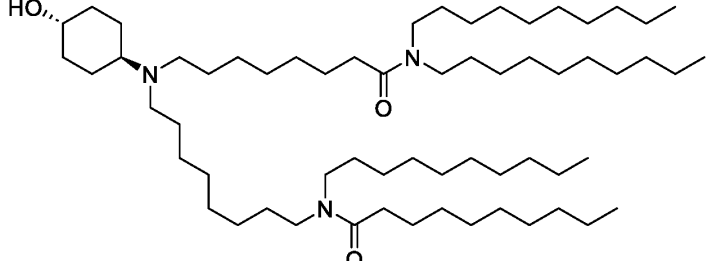
No.	Structure	Name
(3 <i>R</i> ,4 <i>R</i>) I-40		8,8'-(((3 <i>R</i> ,4 <i>R</i>)-4-hydroxytetrahydrofuran-3-yl)azanediyl)bis(N,N-didecyloctanamide)
(3 <i>S</i> ,4 <i>S</i>) I-40		8,8'-(((3 <i>S</i> ,4 <i>S</i>)-4-hydroxytetrahydrofuran-3-yl)azanediyl)bis(N,N-didecyloctanamide)
(3 <i>R</i> ,4 <i>S</i>) I-40		8,8'-(((3 <i>R</i> ,4 <i>S</i>)-4-hydroxytetrahydrofuran-3-yl)azanediyl)bis(N,N-didecyloctanamide)
(3 <i>S</i> ,4 <i>R</i>) I-40		8,8'-(((3 <i>S</i> ,4 <i>R</i>)-4-hydroxytetrahydrofuran-3-yl)azanediyl)bis(N,N-didecyloctanamide)
I-41		8,8'-(((3-(hydroxymethyl)oxetan-3-yl)methyl)azanediyl)bis(N,N-didecyloctanamide)

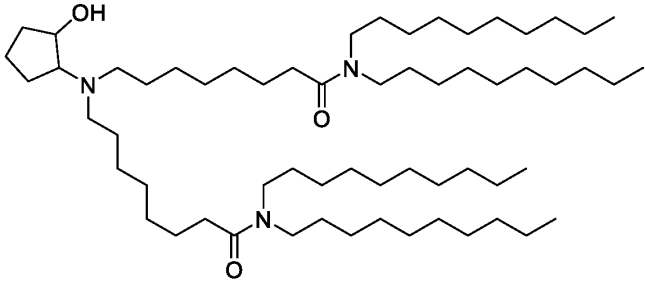
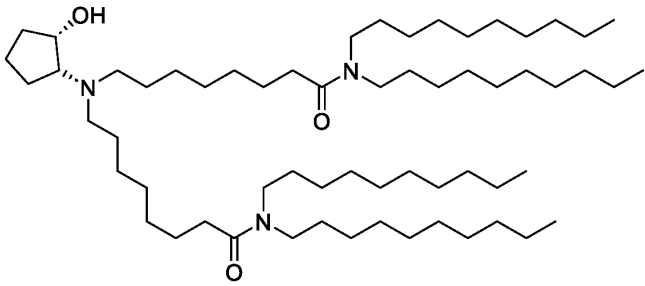
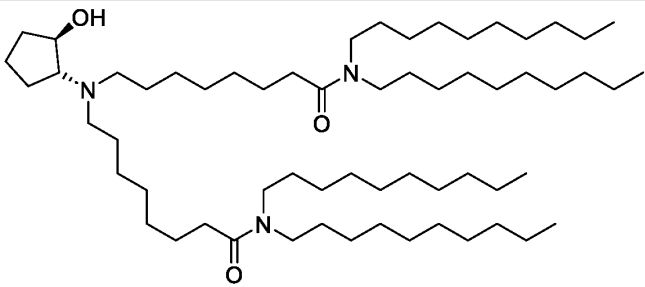
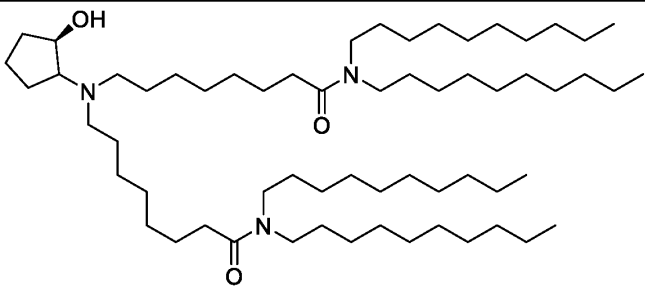
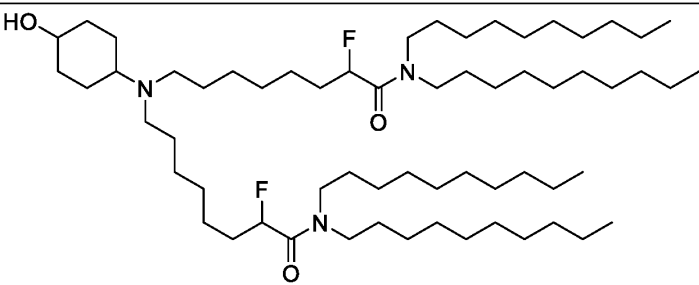
No.	Structure	Name
I-42		8,8'-((2-hydroxycycloheptyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>R</i>) I-42		8,8'-(((1 <i>R</i> ,2 <i>R</i>)-2-hydroxycycloheptyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>S</i>) I-42		8,8'-(((1 <i>S</i> ,2 <i>S</i>)-2-hydroxycycloheptyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>S</i>) I-42		8,8'-(((1 <i>R</i> ,2 <i>S</i>)-2-hydroxycycloheptyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>R</i>) I-42		8,8'-(((1 <i>S</i> ,2 <i>R</i>)-2-hydroxycycloheptyl)azanediyl)bis(N,N-didecyloctanamide)

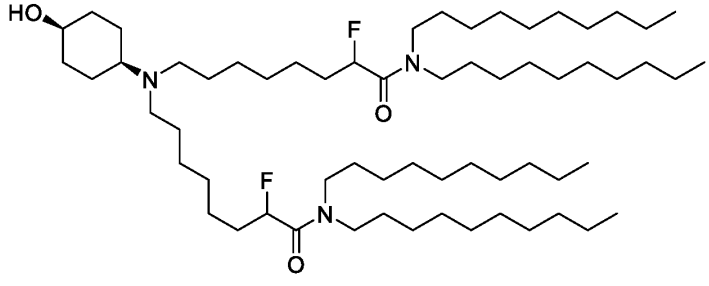
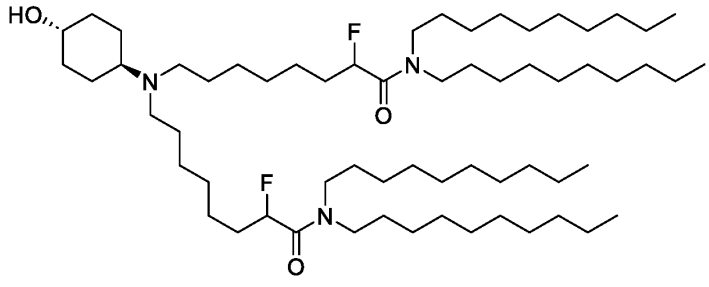
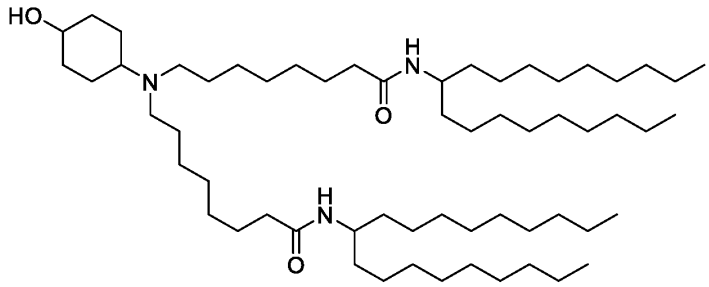
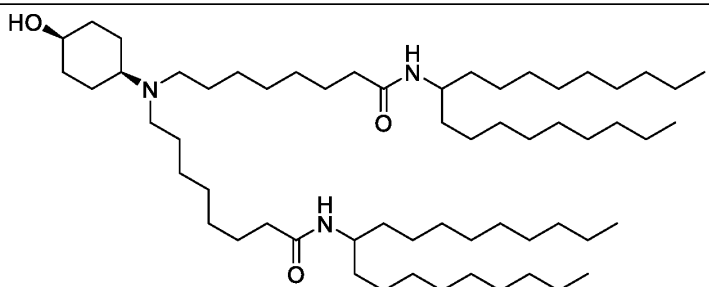
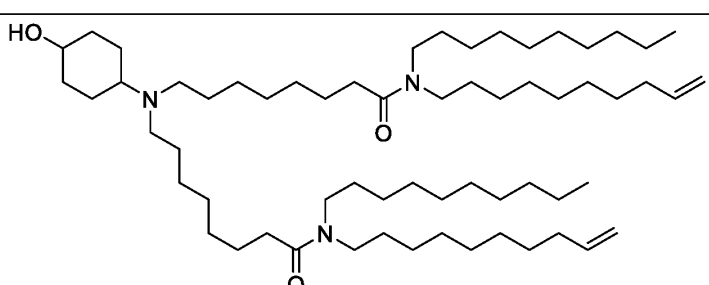
No.	Structure	Name
I-43		8,8'-((2-hydroxycyclooctyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>R</i>) I-43		8,8'-(((1 <i>R</i> ,2 <i>R</i>)-2-hydroxycyclooctyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>S</i>) I-43		8,8'-(((1 <i>S</i> ,2 <i>S</i>)-2-hydroxycyclooctyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>S</i>) I-43		8,8'-(((1 <i>R</i> ,2 <i>S</i>)-2-hydroxycyclooctyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>R</i>) I-43		8,8'-(((1 <i>S</i> ,2 <i>R</i>)-2-hydroxycyclooctyl)azanediyl)bis(N,N-didecyloctanamide)

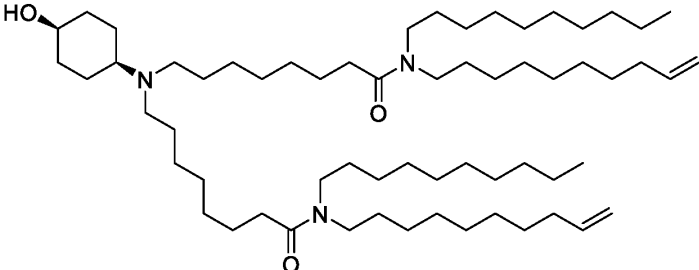
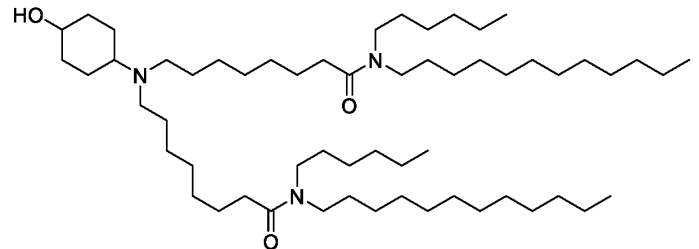
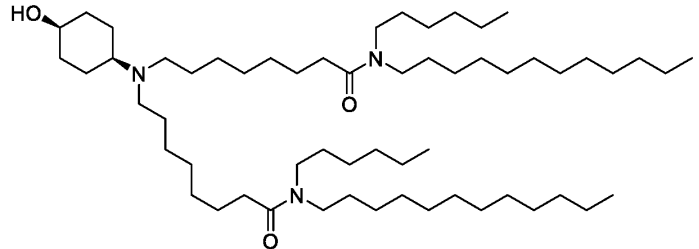
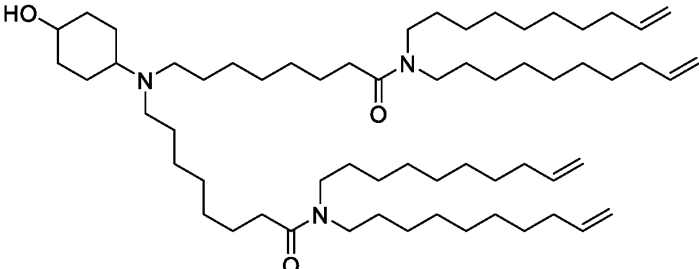
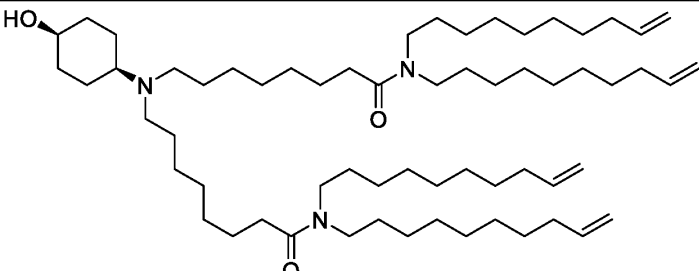
No.	Structure	Name
I-44		8,8'-((2-(hydroxymethyl)cyclopropyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>R</i>) I-44		8,8'-(((1 <i>R</i> ,2 <i>R</i>)-2-(hydroxymethyl)cyclopropyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>S</i>) I-44		8,8'-(((1 <i>S</i> ,2 <i>S</i>)-2-(hydroxymethyl)cyclopropyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>S</i>) I-44		8,8'-(((1 <i>R</i> ,2 <i>S</i>)-2-(hydroxymethyl)cyclopropyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>R</i>) I-44		8,8'-(((1 <i>S</i> ,2 <i>R</i>)-2-(hydroxymethyl)cyclopropyl)azanediyl)bis(N,N-didecyloctanamide)
I-45		8,8'-(((2-(hydroxymethyl)cyclopropyl)methyl)azanediyl)bis(N,N-didecyloctanamide)

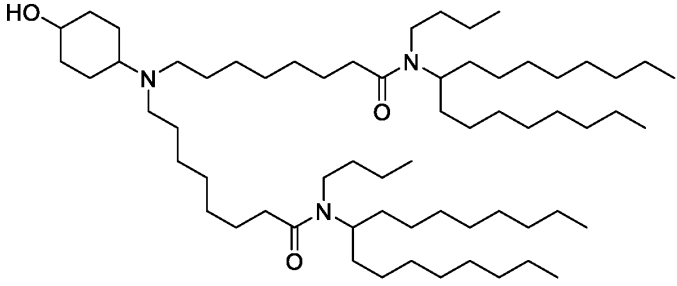
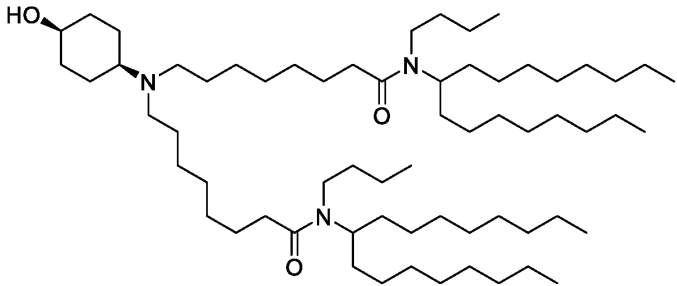
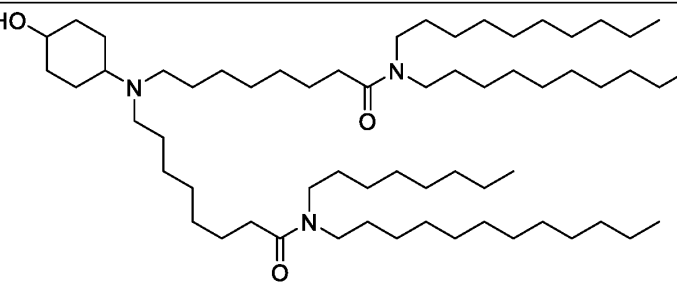
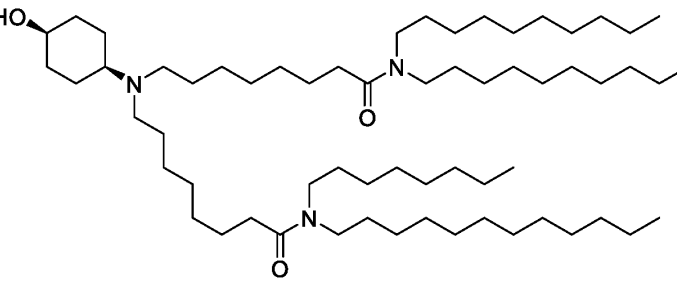
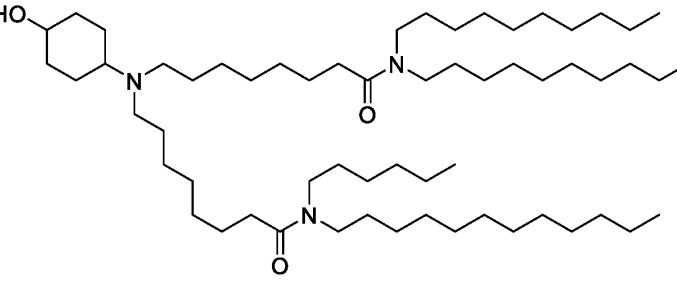
No.	Structure	Name
(1 <i>R</i> ,2 <i>R</i>) I-45		8,8'-(((1 <i>R</i> ,2 <i>R</i>)-2-(hydroxymethyl)cyclopropyl)methyl)azanediyl)bis(N,N-didecyl octanamide)
(1 <i>S</i> ,2 <i>S</i>) I-45		8,8'-(((1 <i>S</i> ,2 <i>S</i>)-2-(hydroxymethyl)cyclopropyl)methyl)azanediyl)bis(N,N-didecyl octanamide)
(1 <i>R</i> ,2 <i>S</i>) I-45		8,8'-(((1 <i>R</i> ,2 <i>S</i>)-2-(hydroxymethyl)cyclopropyl)methyl)azanediyl)bis(N,N-didecyl octanamide)
(1 <i>S</i> ,2 <i>R</i>) I-45		8,8'-(((1 <i>S</i> ,2 <i>R</i>)-2-(hydroxymethyl)cyclopropyl)methyl)azanediyl)bis(N,N-didecyl octanamide)
I-46		8,8'-((2-(hydroxymethyl)cyclopropyl)azanediyl)bis(N,N-didecyl octanamide)

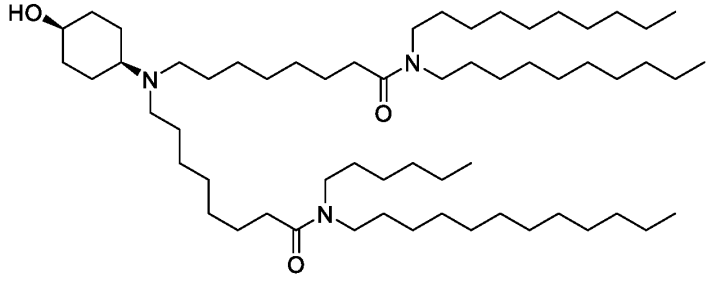
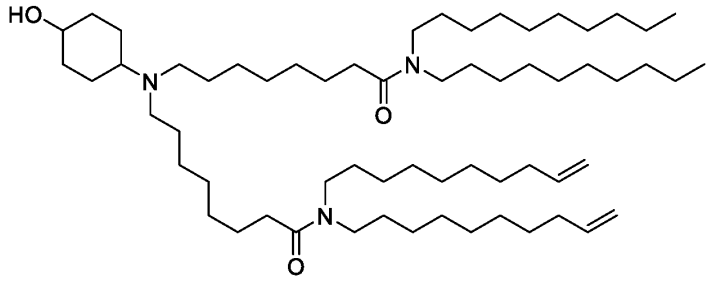
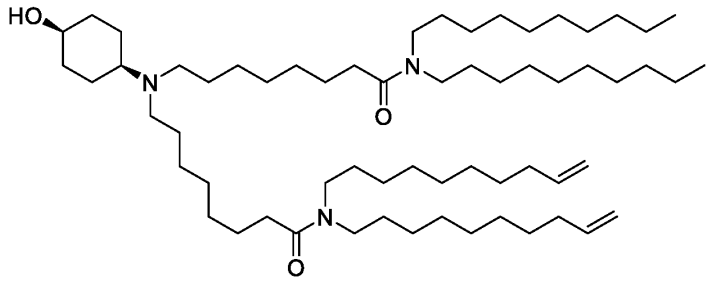
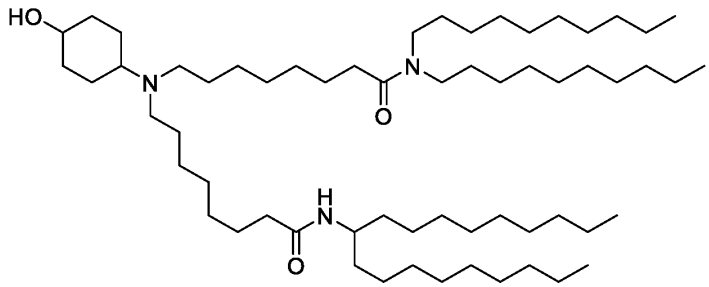
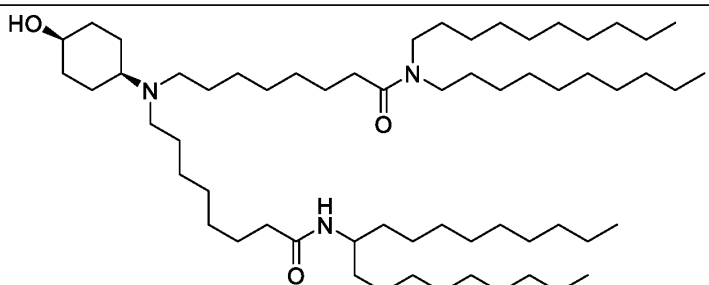
No.	Structure	Name
(1 <i>S</i> ,2 <i>R</i>) I-46		8,8'-(((1 <i>S</i> ,2 <i>R</i>)-2-(hydroxymethyl)cyclopropyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>S</i> ,2 <i>S</i>) I-46		8,8'-(((1 <i>S</i> ,2 <i>S</i>)-2-(hydroxymethyl)cyclopropyl)azanediyl)bis(N,N-didecyloctanamide)
I-47		N-decyl-N-(8-((8-(didecylamino)-8-oxooctyl)(4-hydroxycyclohexyl)amino)octyl)dodecanamide
(1 <i>S</i> ,4 <i>S</i>) I-47		N-decyl-N-(8-((8-(didecylamino)-8-oxooctyl)((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)amino)octyl)dodecanamide
(1 <i>R</i> ,4 <i>R</i>) I-47		N-decyl-N-(8-((8-(didecylamino)-8-oxooctyl)((1 <i>R</i> ,4 <i>R</i>)-4-hydroxycyclohexyl)amino)octyl)dodecanamide

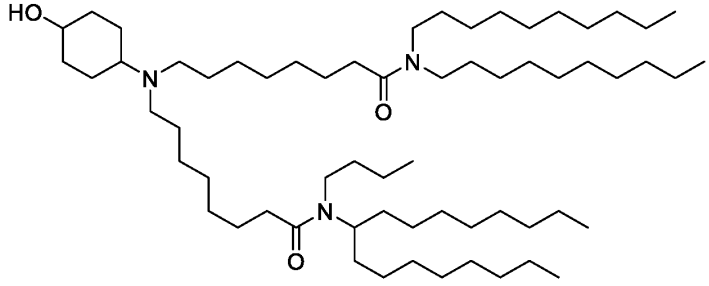
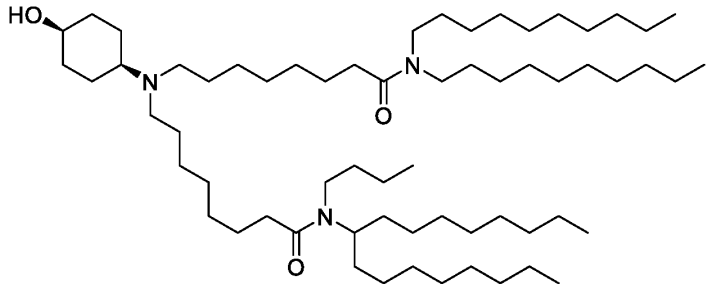
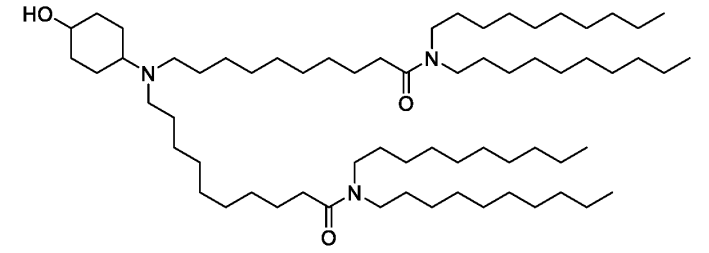
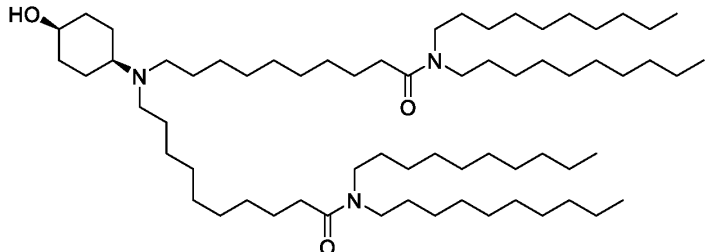
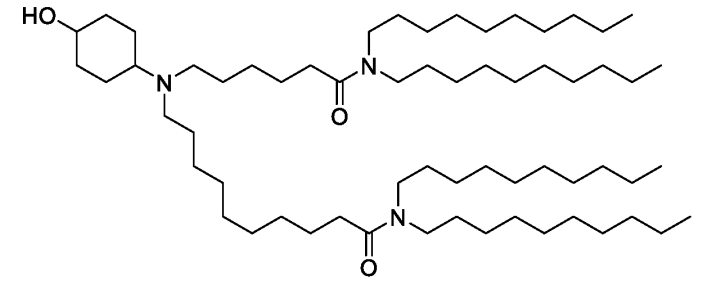
No.	Structure	Name
(rac)- (1 <i>RS</i> ,2 <i>SR</i>) I-48		8,8'-((2-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>S</i>) I-48		8,8'-(((1 <i>R</i> ,2 <i>S</i>)-2-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)
(1 <i>R</i> ,2 <i>R</i>) I-48		8,8'-(((1 <i>R</i> ,2 <i>R</i>)-2-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)
(rac)- (1 <i>RS</i> ,2 <i>R</i>) I-48		8,8'-(((2 <i>R</i>)-2-hydroxycyclopentyl)azanediyl)bis(N,N-didecyloctanamide)
I-49		8,8'-((4-hydroxycyclohexyl)azanediyl)bis(N,N-didecyl-2-fluorooctanamide)

No.	Structure	Name
(1 <i>S</i> ,4 <i>S</i>) I-49		8,8'-(((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)azanediyl)bis(N,N-didecyl-2-fluorooctanamide)
(1 <i>R</i> ,4 <i>R</i>) I-49		8,8'-(((1 <i>R</i> ,4 <i>R</i>)-4-hydroxycyclohexyl)azanediyl)bis(N,N-didecyl-2-fluorooctanamide)
I-50		8,8'-((4-hydroxycyclohexyl)azanediyl)bis(N-(nonadecan-10-yl)octanamide)
(1 <i>S</i> ,4 <i>S</i>) I-50		8,8'-(((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)azanediyl)bis(N-(nonadecan-10-yl)octanamide)
I-51		8,8'-((-4-hydroxycyclohexyl)azanediyl)bis(N-(dec-9-en-1-yl)-N-decyloctanamide)

No.	Structure	Name
(1 <i>S</i> ,4 <i>S</i>) I-51		8,8'-(((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)azanediyl)bis(N-(dec-9-en-1-yl)-N-decyloctanamide)
I-52		8,8'-((-4-hydroxycyclohexyl)azanediyl)bis(N-dodecyl-N-hexyloctanamide)
(1 <i>S</i> ,4 <i>S</i>) I-52		8,8'-(((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)azanediyl)bis(N-dodecyl-N-hexyloctanamide)
I-53		8,8'-((-4-hydroxycyclohexyl)azanediyl)bis(N,N-di(dec-9-en-1-yl)octanamide)
(1 <i>S</i> ,4 <i>S</i>) I-53		8,8'-(((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)azanediyl)bis(N,N-di(dec-9-en-1-yl)octanamide)

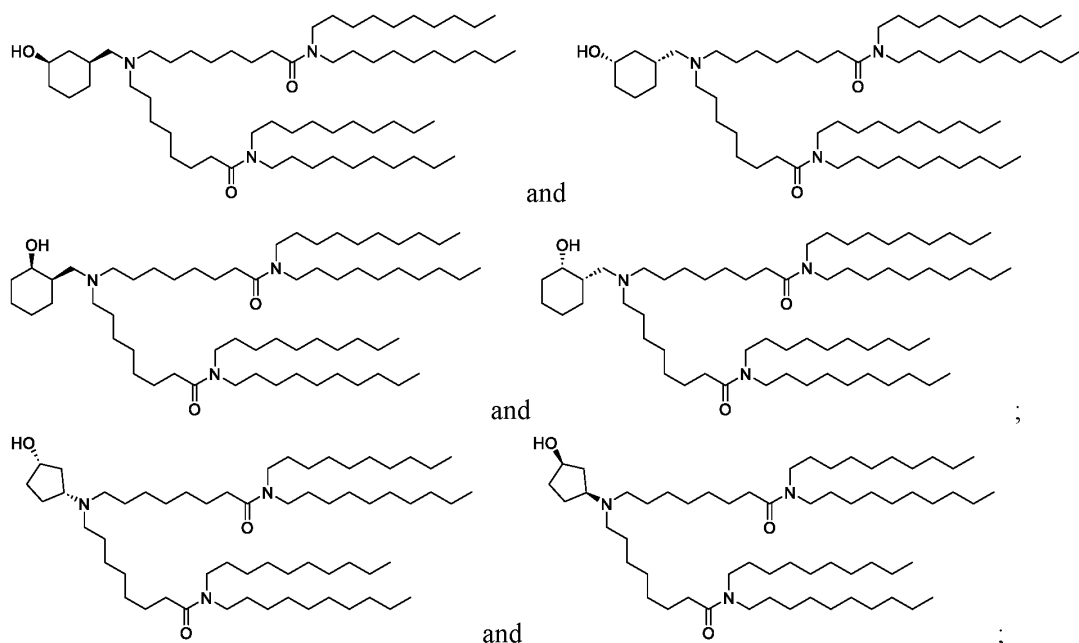
No.	Structure	Name
I-54		8,8'-((4-hydroxycyclohexyl)azanediyl)bis(N-butyl-N-(heptadecan-9-yl)octanamide)
(1 <i>S</i> ,4 <i>S</i>) I-54		8,8'-(((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)azanediyl)bis(N-butyl-N-(heptadecan-9-yl)octanamide)
I-55		N,N-didecyl-8-((8-(dodecyl(octyl)amino)-8-oxooctyl)-(4-hydroxycyclohexyl)amino)octanamide
(1 <i>S</i> ,4 <i>S</i>) I-55		N,N-didecyl-8-((8-(dodecyl(octyl)amino)-8-oxooctyl)((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)amino)octanamide
I-56		N,N-didecyl-8-((8-(dodecyl(hexyl)amino)-8-oxooctyl)-(4-hydroxycyclohexyl)amino)octanamide

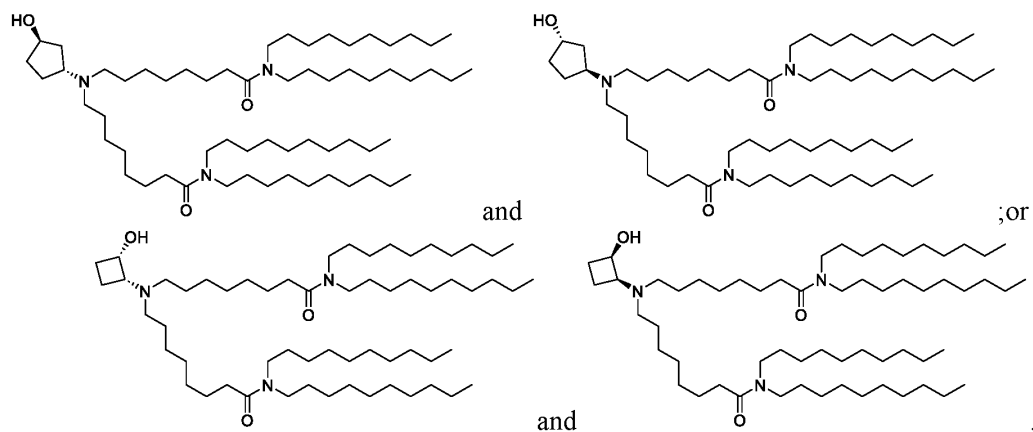
No.	Structure	Name
(1 <i>S</i> ,4 <i>S</i>) I-56		N,N-didecyl-8-((8-(dodecyl(hexyl)amino)-8-oxooctyl)((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)amino)octanamide
I-57		N,N-di(dec-9-en-1-yl)-8-((8-(didecylamino)-8-oxooctyl)(4-hydroxycyclohexyl)amino)octanamide
(1 <i>S</i> ,4 <i>S</i>) I-57		N,N-di(dec-9-en-1-yl)-8-((8-(didecylamino)-8-oxooctyl)((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)amino)octanamide
I-58		N,N-didecyl-8-((4-hydroxycyclohexyl)(8-(nonadecan-10-ylamino)-8-oxooctyl)amino)octanamide
(1 <i>S</i> ,4 <i>S</i>) I-58		N,N-didecyl-8-(((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)(8-(nonadecan-10-ylamino)-8-oxooctyl)amino)octanamide

No.	Structure	Name
I-59		N-butyl-8-((8-(didecylamino)-8-oxooctyl)(4-hydroxycyclohexyl)amino)-N-(heptadecan-9-yl)octanamide
(1 <i>S</i> ,4 <i>S</i>) I-59		N-butyl-8-((8-(didecylamino)-8-oxooctyl)((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)amino)-N-(heptadecan-9-yl)octanamide
I-60		10,10'-((4-hydroxycyclohexyl)azanediyl)bis(N,N-didecyldecanamide)
(1 <i>S</i> ,4 <i>S</i>) I-60		10,10'-(((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)azanediyl)bis(N,N-didecyldecanamide)
I-61		N,N-didecyl-10-((6-(didecylamino)-6-oxohexyl)(4-hydroxycyclohexyl)amino)decanamide

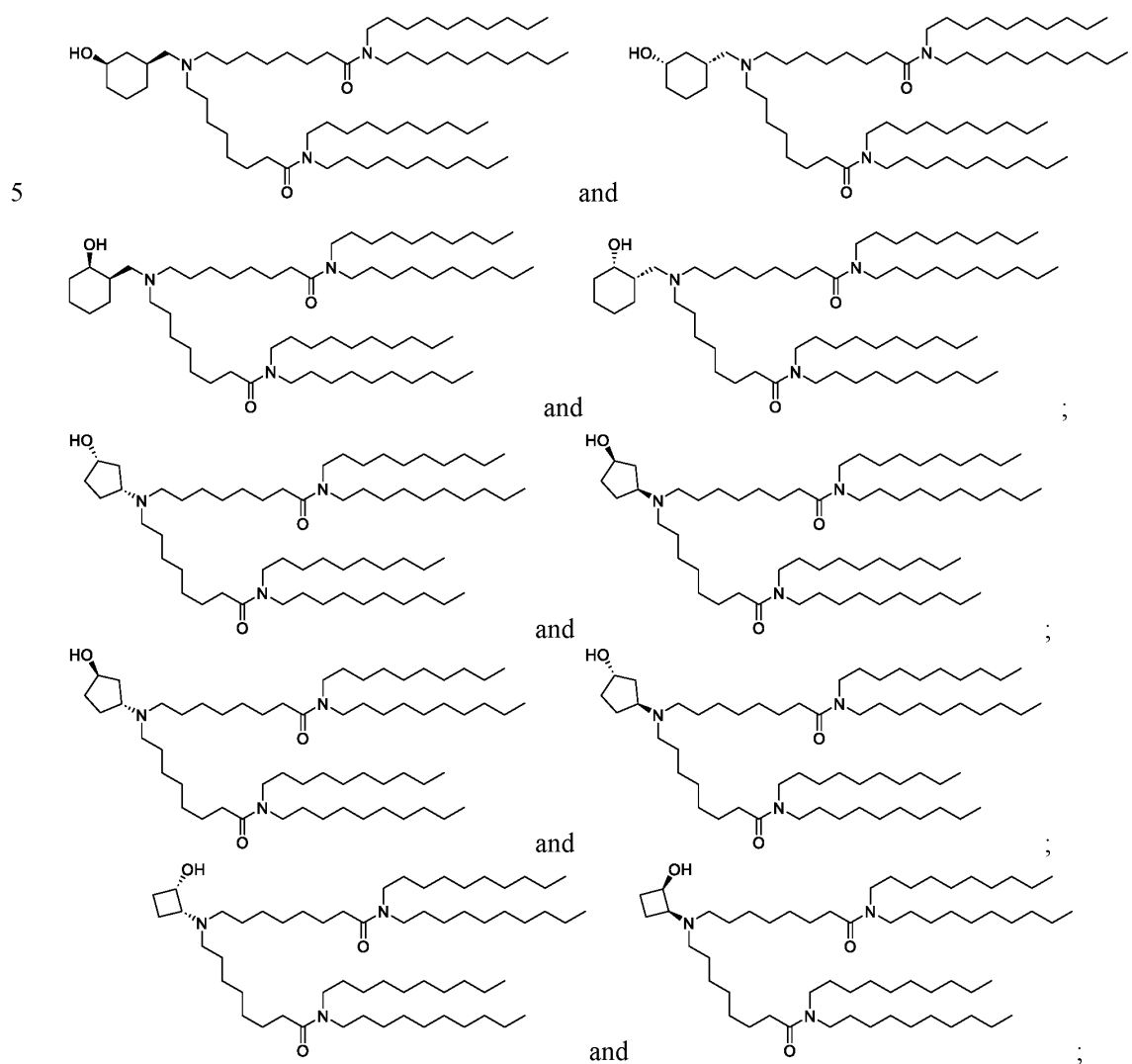
No.	Structure	Name
(1 <i>S</i> ,4 <i>S</i>) I-61		N,N-didecyl-10-((6-(didecylamino)-6-oxohexyl)((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)amino)decanamide
(1 <i>S</i> ,4 <i>S</i>) I-62		8,8'-(((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)azanediyl)bis(N-dodecyl-N-octyloctanamide)
I-63		N,N'-((((1 <i>S</i> ,4 <i>S</i>)-4-hydroxycyclohexyl)azanediyl)bis(octane-8,1-diyl))bis(N-decyldecanamide)

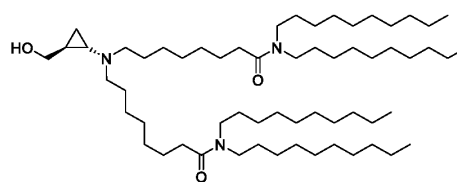
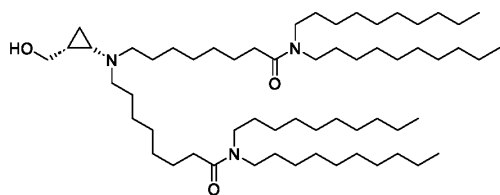
One embodiment provides a racemic mixture of one of the following enantiomeric pairs of Structure (I):





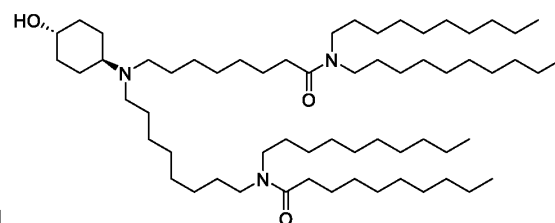
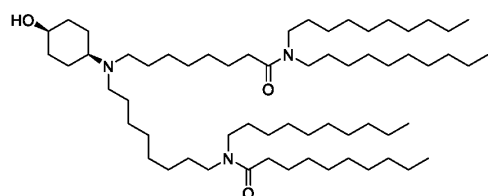
One embodiment provides a racemic mixture of one of the following enantiomeric pairs or combinations of Structure (I):





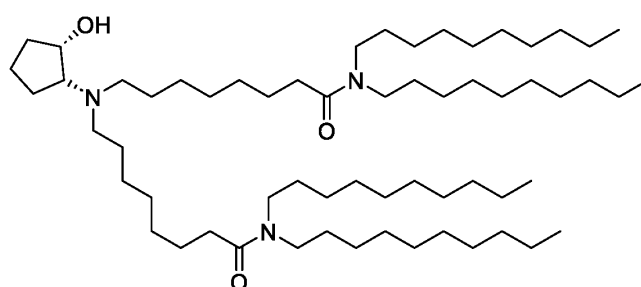
and

;

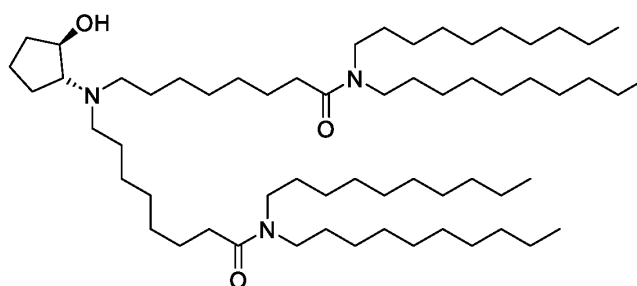


and

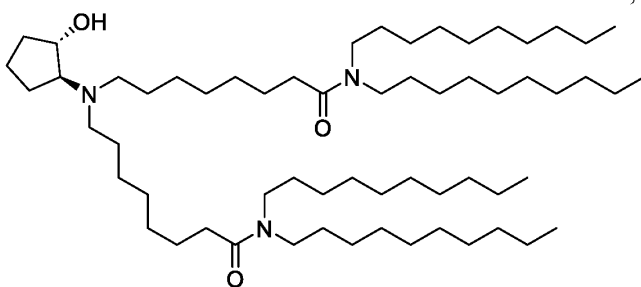
;



,

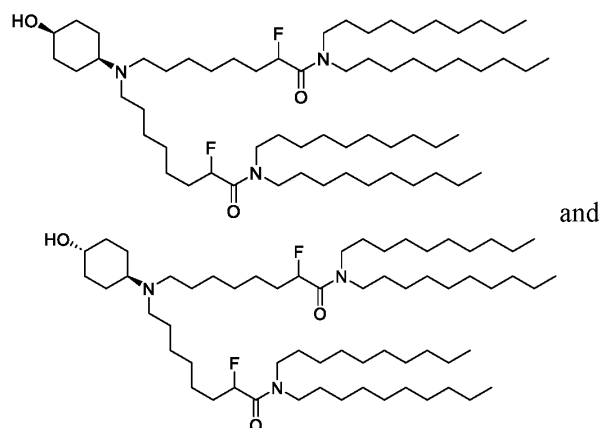


, and



;

5



It is understood that any embodiment of the compounds of Structure (I), as set forth above, and any specific substituent and/or variable in the compound Structure (I), as set forth above, may be independently combined with other embodiments and/or substituents and/or variables of compounds of Structure (I) to form embodiments of the disclosure not specifically set forth above. In addition, in the event that a list of substituents and/or variables is listed for any particular A group, R group or L group, in a particular embodiment and/or claim, it is understood that each individual substituent and/or variable may be deleted from the particular embodiment and/or claim and that the remaining list of substituents and/or variables will be considered to be within the scope of embodiments of the disclosure.

It is understood that in the present description, combinations of substituents and/or variables of the depicted formulae are permissible only if such contributions result in stable compounds.

For the purposes of administration, the compounds of the present disclosure (typically in the form of lipid nanoparticles in combination with a therapeutic agent) may be administered as a raw chemical or may be formulated as pharmaceutical compositions. Pharmaceutical compositions of the present disclosure comprise a compound of Structure (I) and one or more pharmaceutically acceptable carrier, diluent, or excipient. The compound of Structure (I) is present in the composition in an amount which is effective to form a lipid nanoparticle and deliver the therapeutic agent, *e.g.*, for treating a particular disease or condition of interest. Appropriate concentrations and dosages can be readily determined by one skilled in the art.

An embodiment provides a composition comprising a compound of Structure (I) and a therapeutic agent. In some embodiments, the composition further comprises one or more excipient selected from neutral lipids, steroids, and polymer conjugated lipids.

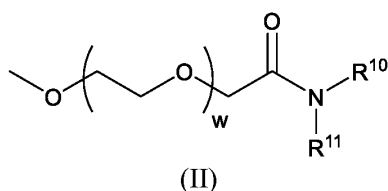
In some embodiments, the therapeutic agent comprises a nucleic acid. In certain embodiments, the nucleic acid is selected from antisense and messenger RNA.

In certain embodiments, the composition comprises one or more neutral lipids selected from DSPC, DPPC, DMPC, DOPC, POPC, DOPE and SM. In some embodiments, the neutral lipid is DSPC. In some embodiments, the molar ratio of the compound to the neutral lipid ranges from about 2:1 to about 8:1. In certain embodiments, the steroid is cholesterol. In some

5 embodiments, the molar ratio of the compound to cholesterol ranges from about 2:1 to 1:1. In certain embodiments, the polymer conjugated lipid is a pegylated lipid. In various embodiments, the polymer conjugated lipid is a pegylated lipid. For example, some embodiments include a pegylated diacylglycerol (PEG-DAG) such as

10 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG), a pegylated phosphatidylethanolamine (PEG-PE), a PEG succinate diacylglycerol (PEG-S-DAG) such as 4-O-(2',3'-di(tetradecanoyloxy)propyl-1-O-(ω -methoxy(polyethoxy)ethyl)butanedioate (PEG-S-DMG), a pegylated ceramide (PEG-cer), or a PEG dialkoxypentylcarbamate such as ω -methoxy(polyethoxy)ethyl-N-(2,3-di(tetradecanoyloxy)propyl)carbamate or 2,3-di(tetradecanoyloxy)propyl-N-(ω -methoxy(polyethoxy)ethyl)carbamate. In some embodiments, the

15 molar ratio of the compound to the pegylated lipid ranges from about 100:1 to about 10:1 or from about 100:1 to about 25:1. In some embodiments, the pegylated lipid is PEG-DMG. In certain embodiments, the pegylated lipid has the following Structure (II):



20 or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof, wherein:

R^{10} and R^{11} are each independently an unbranched or branched, alkyl, alkenyl, or alkynyl containing from 10 to 30 carbon atoms, wherein the alkyl, alkenyl, or alkynyl is optionally interrupted by one or more ester bonds; and

w has a value ranging from 30 to 60.

25 In some embodiments, R^{10} and R^{11} are each independently unbranched alkyl chains containing from 12 to 16 carbon atoms.

In certain embodiments, the lipid nanoparticle or composition comprises a plurality of compounds of Structure (II) and the average value of w for the plurality is about 49. In some embodiments, the plurality of compounds of Structure (II) has an average value of w ranging

30 from 40 to 55. In some embodiments, the plurality of compounds of Structure (II) has an average value of w ranging from 40 to 50, or 42 to 48. In some embodiments, the plurality of compounds

of Structure (II) has an average value of w ranging from 30 to 55, 30 to 50, 30 to 45, 30 to 40, or 30 to 35. In some embodiments, the plurality of compounds of Structure (II) has an average value of w ranging from 35 to 55, 40 to 55, 42 to 55, 45 to 55, or 48 to 55. In some embodiments, the average w is about 45 (*e.g.*, 43, 44, 45, 46, or 47). In some embodiments, the average w is about 43-47. In some embodiments, the average w is about 40-50. In some embodiments, the average w is about 48-55.

Synthesis of pegylated lipids can be found in US Patent No. 9,738,593, the disclosure of which is hereby incorporated by reference.

Methods of Administration

Administration of the compositions of the disclosure can be carried out via any of the accepted modes of administration of agents for serving similar utilities. The pharmaceutical compositions of the disclosure may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suspensions, suppositories, injections, inhalants, gels, microspheres, and aerosols. Typical routes of administering such pharmaceutical compositions include, without limitation, oral, topical, transdermal, inhalation, parenteral, sublingual, buccal, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intradermal, intrasternal injection or infusion techniques. Pharmaceutical compositions of the disclosure are formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will be administered to a subject or patient take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of a compound of the disclosure in aerosol form may hold a plurality of dosage units. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington: The Science and Practice of Pharmacy*, 20th Edition (Philadelphia College of Pharmacy and Science, 2000). The composition to be administered will, in any event, contain a therapeutically effective amount of a compound of the disclosure, or a pharmaceutically acceptable salt thereof, for treatment of a disease or condition of interest in accordance with the teachings of this disclosure.

One embodiment provides a method for administering a therapeutic agent to a patient in need thereof, the method comprising preparing or providing the lipid nanoparticle or composition of the disclosure and administering the composition to the patient.

One embodiment provides a pharmaceutical composition comprising the lipid nanoparticle of the disclosure and a pharmaceutically acceptable diluent or excipient.

Another embodiment provides a method for inducing expression of a protein in a patient in need thereof, comprising administering the pharmaceutical composition of the disclosure to
5 the patient, wherein the lipid nanoparticle comprises an mRNA encoding the protein. In some embodiments, the protein is an antigen and the method is for inducing an immune response in the patient. In certain embodiments, the protein is an antigen and the method is for vaccinating the patient against a pathogen. In some embodiments, the protein is for gene editing.

A pharmaceutical composition of the disclosure may be in the form of a solid or liquid. In
10 one aspect, the carrier(s) are particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) may be liquid, with the compositions being, for example, an oral syrup, injectable liquid, or an aerosol, which is useful in, for example, inhalatory administration.

When intended for oral administration, the pharmaceutical composition is preferably in either solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included
15 within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the pharmaceutical composition may be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer, or the like form. Such a solid composition will typically contain one or more inert diluents or edible carriers. In addition, one or more of the following may be present: binders such as
20 carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, gum tragacanth or gelatin; excipients such as starch, lactose or dextrans, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent.

25 When the pharmaceutical composition is in the form of a capsule, for example, a gelatin capsule, it may contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol or oil.

The pharmaceutical composition may be in the form of a liquid, for example, an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery
30 by injection, as two examples. When intended for oral administration, preferred composition contain, in addition to the present compounds, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one

or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

The liquid pharmaceutical compositions of the disclosure, whether they be solutions, suspensions or other like form, may include one or more of the following adjuvants: sterile
5 diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid;
10 buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose; agents to act as cryoprotectants such as sucrose or trehalose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. Physiological saline is a preferred adjuvant. An injectable pharmaceutical composition is preferably sterile.

15 A liquid pharmaceutical composition of the disclosure intended for either parenteral or oral administration should contain an amount of a compound of the disclosure such that a suitable dosage will be obtained.

The pharmaceutical composition of the disclosure may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment or
20 gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device.

25 The pharmaceutical composition of the disclosure may be intended for rectal administration, in the form, for example, of a suppository, which will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

30 The pharmaceutical composition of the disclosure may include various materials, which modify the physical form of a solid or liquid dosage unit. For example, the composition may include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and may be selected from, for example, sugar, shellac, and

other enteric coating agents. Alternatively, the active ingredients may be encased in a gelatin capsule.

The pharmaceutical composition of the disclosure in solid or liquid form may include an agent that binds to the compound of the disclosure and thereby assists in the delivery of the compound. Suitable agents that may act in this capacity include a monoclonal or polyclonal antibody, or a protein.

The pharmaceutical composition of the disclosure may consist of dosage units that can be administered as an aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery may be by a liquefied or compressed gas or by a suitable pump system that dispenses the active ingredients. Aerosols of compounds of the disclosure may be delivered in single phase, bi-phasic, or tri-phasic systems to deliver the active ingredient(s). Delivery of the aerosol includes the necessary container, activators, valves, sub-containers, and the like, which together may form a kit. One skilled in the art, without undue experimentation may determine preferred aerosols.

The pharmaceutical compositions of the disclosure may be prepared by methodology well known in the pharmaceutical art. For example, a pharmaceutical composition intended to be administered by injection can be prepared by combining the lipid nanoparticles of the disclosure with sterile, distilled water or other carrier to form a solution. A surfactant may be added to facilitate the formation of a homogeneous solution or suspension. Surfactants are compounds that non-covalently interact with the compound of the disclosure to facilitate dissolution or homogeneous suspension of the compound in the aqueous delivery system.

The compositions of the disclosure, or their pharmaceutically acceptable salts, are administered in a therapeutically effective amount, which will vary depending upon a variety of factors including the activity of the specific therapeutic agent employed; the metabolic stability and length of action of the therapeutic agent; the age, body weight, general health, sex, and diet of the patient; the mode and time of administration; the rate of excretion; the drug combination; the severity of the particular disorder or condition; and the subject undergoing therapy.

Compositions of the disclosure may also be administered simultaneously with, prior to, or after administration of one or more other therapeutic agents. Such combination therapy includes administration of a single pharmaceutical dosage formulation of a composition of the disclosure and one or more additional active agents, as well as administration of the composition of the disclosure and each active agent in its own separate pharmaceutical dosage formulation. For example, a composition of the disclosure and the other active agent can be administered to the

patient together in a single oral dosage composition such as a tablet or capsule, or each agent administered in separate oral dosage formulations. Where separate dosage formulations are used, the compounds of the disclosure and one or more additional active agents can be administered at essentially the same time, *i.e.*, concurrently, or at separately staggered times, *i.e.*, sequentially;

5 combination therapy is understood to include all these regimens.

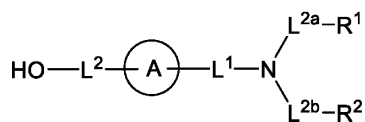
Preparation methods for the above compounds and compositions are described herein below and/or known in the art.

It will be appreciated by those skilled in the art that in the process described herein the functional groups of intermediate compounds may need to be protected by suitable protecting groups. Such functional groups include hydroxy, amino, mercapto and carboxylic acid. Suitable protecting groups for hydroxy include trialkylsilyl or diarylalkylsilyl (for example, *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl or trimethylsilyl), tetrahydropyranyl, benzyl, and the like. Suitable protecting groups for amino, amidino and guanidino include *t*-butoxycarbonyl, benzyloxycarbonyl, and the like. Suitable protecting groups for mercapto include -C(O)-R" (where R" is alkyl, aryl or arylalkyl), *p*-methoxybenzyl, trityl and the like. Suitable protecting groups for carboxylic acid include alkyl, aryl or arylalkyl esters. Protecting groups may be added or removed in accordance with standard techniques, which are known to one skilled in the art and as described herein. The use of protecting groups is described in detail in Green, T.W. and P.G.M. Wutz, *Protective Groups in Organic Synthesis* (1999), 3rd Ed., Wiley. As one of skill in the art would appreciate, the protecting group may also be a polymer resin such as a Wang resin, Rink resin or a 2-chlorotrityl-chloride resin.

It will also be appreciated by those skilled in the art, although such protected derivatives of compounds of this disclosure may not possess pharmacological activity as such, they may be administered to a mammal and thereafter metabolized in the body to form compounds of the disclosure which are pharmacologically active. Such derivatives may therefore be described as "prodrugs". All prodrugs of compounds of this disclosure are included within the scope of the disclosure.

Furthermore, all compounds of the disclosure which exist in free base or acid form can be converted to their pharmaceutically acceptable salts by treatment with the appropriate inorganic or organic base or acid by methods known to one skilled in the art. Salts of the compounds of the disclosure can be converted to their free base or acid form by standard techniques.

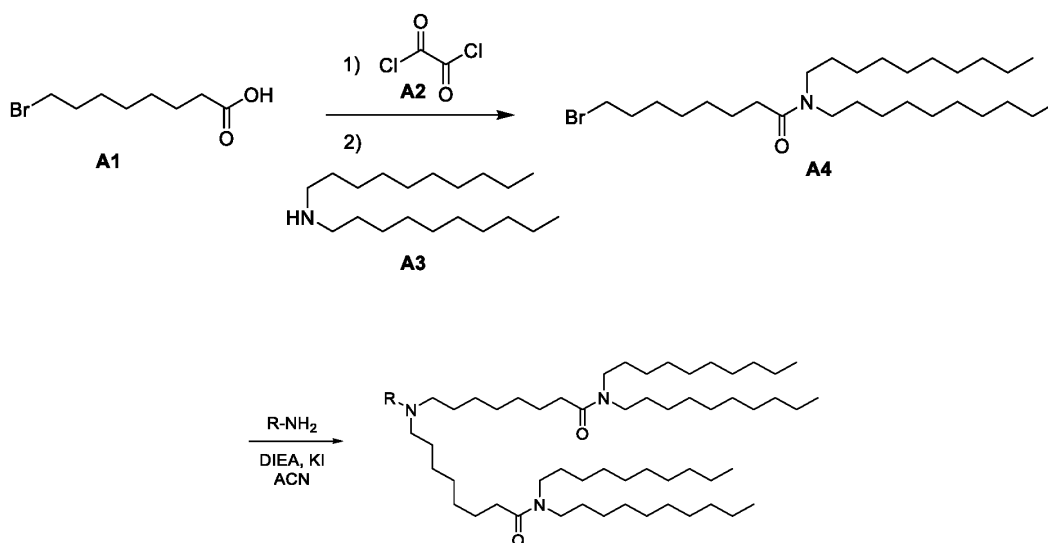
The following Reaction Scheme illustrates methods to make compounds of this disclosure, *i.e.*, compounds of Structure (I):



(I)

wherein R^1 , R^2 , L^1 , L^2 , L^{2a} , L^{2b} , and A are as defined herein. It is understood that one skilled in the art may be able to make these compounds by similar methods or by combining other methods known to one skilled in the art. It is also understood that one skilled in the art would be able to make, in a similar manner as described below, other compounds of Structure (I) not specifically illustrated below by using the appropriate starting components and modifying the parameters of the synthesis as needed. In general, starting components may be obtained from sources such as Sigma Aldrich, Lancaster Synthesis, Inc., Maybridge, Matrix Scientific, TCI, and Fluorochem USA, etc. or synthesized according to sources known to those skilled in the art (see, *e.g.*, Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th edition (Wiley, December 2000)) or prepared as described in this disclosure.

GENERAL REACTION SCHEME 1



Embodiments of the compounds of Structure (I) can be prepared according to General Reaction Scheme 1 ("Procedure A").

Procedure A

Referring to General Reaction Scheme 1, compound starting materials A1 can be purchased from commercial sources or prepared according to methods familiar to one of ordinary

skill in the art. To a mixture of starting material **A1** was added **A2** the reaction mixture is stirred at room temperature for 1 h. The reaction mixture was concentrated and used in the next step without further purification. To the mixture was added **A3** and the reaction mixture was stirred at room temperature for 17 h. The reaction mixture was concentrated, and the crude material was resuspended in hexanes. The resultant solids were filtered, and the filtrate was purified via automated flash chromatography to give **A4**.

A mixture of **A4** and the appropriate amine was heated at 75 °C for 24 h to 72 h. The reaction mixture was concentrated, and the resultant solids were filtered and the filtrate was purified by automated flash chromatography to give the desired compounds of Structure (I).

It should be noted that various alternative strategies for preparation of compounds of structure (I) are available to those of ordinary skill in the art. For example, other compounds of structure (I) can be prepared according to analogous methods using the appropriate starting material and as shown in the Examples that follow. The use of protecting groups as needed and other modification to the above General Reaction Scheme will be readily apparent to one of ordinary skill in the art. The following examples are provided for purpose of illustration and not limitation.

EXAMPLE 1

LUCIFERASE MRNA IN VIVO EVALUATION USING LIPID NANOPARTICLE COMPOSITIONS

A lipid of structure (I), DSPC, cholesterol and PEG-lipid are solubilized in ethanol at a molar ratio of 50:10:38.5:1.5 or 47.5:10:40.7:1.8. Lipid nanoparticles (LNP) are prepared at a total lipid to mRNA weight ratio of approximately 10:1 to 40:1. Briefly, the mRNA is diluted to 0.2 mg/mL in 10 to 50 mM citrate buffer, pH 4 to 6 or 10 to 25 mM acetate buffer, pH 4. Syringe pumps are used to mix the ethanolic lipid solution with the mRNA aqueous solution at a ratio of about 1:5 to 1:3 (vol/vol) with total flow rates above 15 mL/min. The ethanol is then removed, and the external buffer replaced with PBS by dialysis. Finally, the lipid nanoparticles are filtered through a 0.2 µm pore sterile filter.

Studies are performed in 6–8-week-old female C57BL/6 mice (Charles River) 8–10-week-old CD-1 mice (Charles River or Inotiv) according to guidelines established by an institutional animal care committee (ACC) and the Canadian Council on Animal Care (CCAC). Varying doses of mRNA-lipid nanoparticle are systemically administered by tail vein injection and animals euthanized at a specific time point (e.g., 4 hours) post-administration. Liver and

spleen are collected in pre-weighed tubes, weights determined, immediately snap frozen in liquid nitrogen, and stored at -80 °C until processing for analysis.

For liver, approximately 50 mg is dissected for analyses in a 2 mL FastPrep tubes (MP Biomedicals, Solon OH). ¼” ceramic sphere (MP Biomedicals) is added to each tube and 500-
 5 750 µL of Glo Lysis Buffer – GLB (Promega, Madison WI) equilibrated to room temperature is added to liver tissue. Liver tissues are homogenized with the FastPrep24 instrument (MP Biomedicals) at 2×6.0 m/s for 15 seconds. Homogenate is incubated at room temperature for 5 minutes prior to a 1:4 to 1:6 dilution in GLB and assessed using SteadyGlo Luciferase assay system (Promega). Specifically, 50 µL of diluted tissue homogenate is reacted with 50 µL of
 10 SteadyGlo substrate, shaken for 10 seconds followed by 5-minute incubation and then luminescence was quantitated using a FilterMax F5 Microplate Reader (Molecular Devices, USA). The amount of protein assayed is determined by using the BCA protein assay kit (Pierce, Rockford, IL). Relative luminescence units (RLU) were then normalized to total µg protein or weight (g) of tissue assayed. To convert RLU to ng luciferase a standard curve is generated with
 15 QuantiLum Recombinant Luciferase (Promega).

The FLuc mRNA (L-7202) from Trilink Biotechnologies will express a luciferase protein, originally isolated from the firefly, *photinus pyralis*. Fluc is commonly used in mammalian cell culture to measure both gene expression and cell viability. It emits bioluminescence in the presence of the substrate, luciferin. This capped and polyadenylated
 20 mRNA is modified with 5-methoxyuridine and optimized for mammalian systems.

EXAMPLE 2

IMMUNOGLOBULIN G (IgG) mRNA IN VIVO EVALUATION USING LIPID NANOPARTICLE COMPOSITIONS

A lipid of structure (I), DSPC, cholesterol and PEG-lipid are solubilized in ethanol at a
 25 molar ratio of 50:10:38.5:1.5 or 47.5:10:40.7:1.8. Lipid nanoparticles (LNP) are prepared at a total lipid to mRNA weight ratio of approximately 10:1 to 40:1. Briefly, the mRNA is diluted to 0.2 mg/mL in 10 to 50 mM citrate buffer, pH 4 to 6 or 10 to 25 mM acetate buffer, pH 4 to 6. Syringe pumps are used to mix the ethanolic lipid solution with the mRNA aqueous solution at a ratio of about 1:5 to 1:3 (vol/vol) with total flow rates above 15 mL/min. The ethanol is then
 30 removed, and the external buffer replaced with PBS by dialysis. Finally, the lipid nanoparticles are filtered through a 0.2 µm pore sterile filter.

Studies are performed in 6–8-week-old CD-1/ICR mice (Envigo, Charles River, or Inotiv) according to guidelines established by an institutional animal care committee (ACC) and the Canadian Council on Animal Care (CCAC). Varying doses of mRNA-lipid nanoparticle are systemically administered by tail vein injection and animals euthanized at a specific time point (e.g., 24 hours) post-administration. The whole blood is collected, and the serum subsequently separated by centrifuging the tubes of the whole blood at 2000 x g for 10 minutes at 4 °C and stored at -80 °C until use for analysis.

For immunoglobulin G (IgG) ELISA (Life Diagnostics Human IgG ELISA kit), the serum samples are diluted at 100 to 20,000 folds with 1x diluent solution. 100 µL of diluted serum is dispensed into anti-human IgG coated 96-well plate in duplicate alongside human IgG standards and incubated in a plate shaker at 150 rpm at 25 °C for 45 minutes. The wells are washed 5 times with 1x wash solution using a plate washer (400 µL/well). 100 µL of HRP conjugate is added into each well and incubated in a plate shaker at the same condition above. The wells are washed 5 times again with 1x wash solution using a plate washer (400 µL/well). 100 µL of TMB reagent is added into each well and incubated in a plate shaker at the same condition above. The reaction is stopped by adding 100 µL of Stop solution to each well. The absorbance is read at 450 nm (A450) with a microplate reader. The amount of human IgG in mouse serum is determined by plotting A450 values for the assay standard against human IgG concentration.

EXAMPLE 3

DETERMINATION OF pKa OF FORMULATED LIPIDS

As described elsewhere, the pKa of formulated lipids is correlated with the effectiveness of LNPs for delivery of nucleic acids (*see* Jayaraman et al, *Angewandte Chemie, International Edition* (2012), 51(34), 8529-8533; Semple et al, *Nature Biotechnology* 28, 172–176 (2010)). In some embodiments, the preferred range of pKa is ~5 to ~7. The pKa of each lipid may be determined in lipid nanoparticles using an assay based on fluorescence of 2-(p-toluidino)-6-naphthalene sulfonic acid (TNS). Lipid nanoparticles comprising compound of structure (I)/DSPC/cholesterol/PEG-lipid (50/10/38.5/1.5 or 47.5:10:40.7:1.8 mol%) in PBS at a concentration of 0.4 mM total lipid are prepared using the in-line process as described in Example 1. TNS is prepared as a 100 µM stock solution in distilled water. Vesicles are diluted to 24 µM lipid in 2 mL of buffered solutions containing 10 mM HEPES, 10 mM MES, 10 mM ammonium acetate, and 130 mM NaCl, where the pH ranged from 2.5 to 11. An aliquot of the

TNS solution is added to give a final concentration of 1 μ M and following vortex mixing fluorescence intensity is measured at room temperature in a SLM Aminco Series 2 Luminescence Spectrophotometer using excitation and emission wavelengths of 321 nm and 445 nm. A sigmoidal best fit analysis is applied to the fluorescence data and the pK_a is measured as the pH giving rise to half-maximal fluorescence intensity.

EXAMPLE 4

DETERMINATION OF EFFICACY OF LIPID NANOPARTICLE FORMULATIONS

CONTAINING VARIOUS CATIONIC LIPIDS USING AN IN VIVO

LUCIFERASE/IGG mRNA EXPRESSION RODENT MODEL

Representative compounds of the disclosure shown in Table 2 were formulated using the following molar ratio: 50% cationic lipid / 10% distearoylphosphatidylcholine (DSPC) / 38.5% Cholesterol / 1.5% PEG lipid 2-[2-(ω -methoxy(polyethyleneglycol₂₀₀₀)ethoxy]-N,N-ditetradecylacetamide) or 47.5% cationic lipid / 10% DSPC / 40.7% Cholesterol / 1.8% PEG lipid. Relative activity was determined by measuring luciferase expression in the liver 4 hours following administration via tail vein injection as described in Example 1 or by measuring the amount of human IgG in mouse serum as described in example 2. The activity was compared at a dose of 1.0 or 0.5 or 0.3 mg mRNA/kg and expressed as ng luciferase/g liver measured 4 hours after administration, as described in Example 1 or as μ g IgG/mL serum measured 24 hours after administration, as described in Example 2. Compound numbers in Table 2 refer to the compound numbers of Table 1.

Table 2. Novel Cationic Lipids and Associated Activity

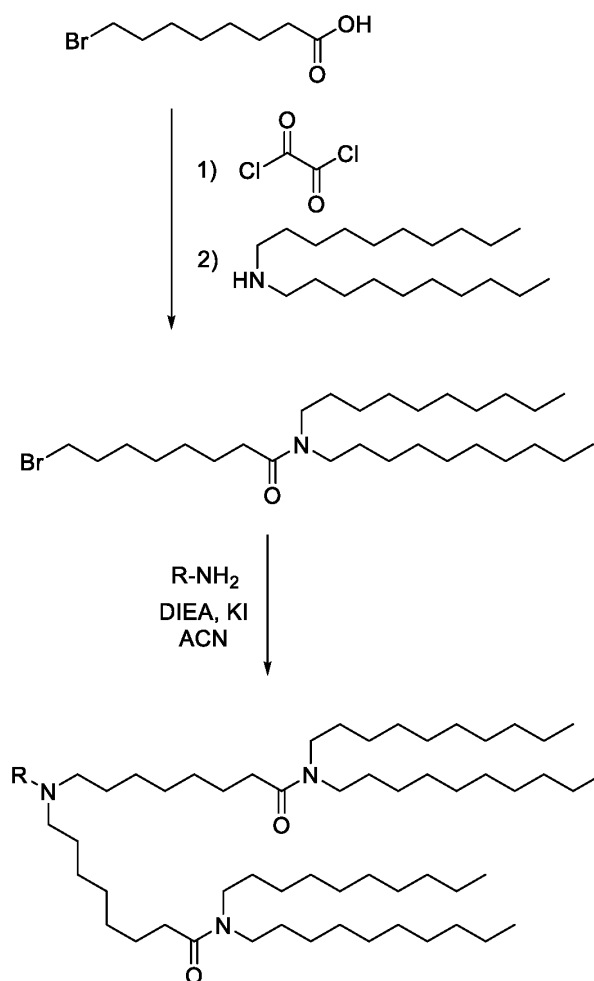
Compound No.	Liver Luc @ 0.3 mg/kg (ng/g)	Liver Luc @ 1.0 mg/kg (ng/g)	Serum IgG @ 0.3 mg/kg (ug/mL)	Serum IgG @ 1.0 mg/kg (ug/mL)
(1 <i>S</i> ,3 <i>R</i>) I-1	18927 $\pm$ 2102	79233 $\pm$ 12026	-	-
(1 <i>S</i> ,3 <i>S</i>) I-2	10872 $\pm$ 2411	91854 $\pm$ 15303	-	-
(1 <i>R</i> ,3 <i>R</i>) I-2	12651 $\pm$ 3017	82113 $\pm$ 14450	-	-
I-4	-	-	32.39 $\pm$ 3.12	155.20 $\pm$ 15.34
(1 <i>R</i> ,3 <i>R</i>) I-5	-	-	44.52 $\pm$ 9.89	215.61 $\pm$ 28.80
I-9	-	-	35.77 $\pm$ 4.50	-
(1 <i>R</i> ,4 <i>R</i>) I-9	-	-	45.52 $\pm$ 6.25	204.57 $\pm$ 48.15

Compound No.	Liver Luc @ 0.3 mg/kg (ng/g)	Liver Luc @ 1.0 mg/kg (ng/g)	Serum IgG @ 0.3 mg/kg (ug/mL)	Serum IgG @ 1.0 mg/kg (ug/mL)
(1 <i>S</i> ,4 <i>S</i>) I-9	-	-	97.57 ± 19.26	257.42 ± 91.86
(1 <i>S</i> ,3 <i>S</i>) I-10	-	-	27.62 ± 9.67	187.67 ± 66.41
(<i>rac</i>)-(1 <i>RS</i> ,3 <i>SR</i>) I-14	-	-	15.74 ± 5.71	66.16 ± 16.66
(<i>rac</i>)-(1 <i>RS</i> ,3 <i>RS</i>) I-14	-	-	11.48 ± 1.87	45.96 ± 11.03
(1 <i>S</i> ,2 <i>R</i>) I-15	-	-	33.97 ± 18.40	225.79 ± 54.78
(1 <i>S</i> ,2 <i>R</i>) I-16	-	-	19.42 ± 7.21	88.40 ± 26.36
(1 <i>R</i> ,3 <i>R</i>) I-17	-	-	27.97 ± 3.37	178.14 ± 33.27
(1 <i>S</i> ,3 <i>R</i>) I-19	-	-	30.68 ± 8.05	267.70 ± 45.90
(1 <i>S</i> ,3 <i>S</i>) I-21	-	-	71.49 ± 12.31	-
(1 <i>R</i> ,3 <i>R</i>) I-22	-	-	19.88 ± 2.53	-
(1 <i>S</i> ,3 <i>S</i>) I-22	-	-	50.48 ± 11.93	-
(1 <i>S</i> ,4 <i>S</i>) I-23	-	-	34.48 ± 9.25	273.72 ± 52.70
(1 <i>R</i> ,4 <i>R</i>) I-23	-	-	5.15 ± 1.83	37.79 ± 6.41
(1 <i>R</i> ,3 <i>R</i>) I-24	-	-	6.93 ± 2.48	19.77 ± 5.43
(1 <i>S</i> ,3 <i>S</i>) I-24	-	-	20.09 ± 5.90	57.71 ± 10.58
(1 <i>S</i> ,4 <i>S</i>) I-25	-	-	39.96 ± 10.72	194.38 ± 27.83
(1 <i>R</i> ,4 <i>R</i>) I-25	-	-	14.03 ± 2.71	105.77 ± 19.44
(1 <i>S</i> ,3 <i>R</i> ,5 <i>S</i>) I-34	-	-	7.06 ± 4.68	57.07 ± 24.86
I-36	-	-	25.76 ± 8.65	171.89 ± 45.93
(<i>rac</i>)-(1 <i>RS</i> ,2 <i>SR</i>) I-45	-	-	15.84 ± 3.20	-
(<i>rac</i>)-(1 <i>RS</i> ,2 <i>RS</i>) I-45	-	-	10.95 ± 4.31	-
(1 <i>S</i> ,4 <i>S</i>) I-47	-	-	55.53 ± 11.50	158.59 ± 40.38
(1 <i>R</i> ,4 <i>R</i>) I-47	-	-	16.24 ± 2.93	-
(<i>rac</i>)-(1 <i>RS</i> ,2 <i>SR</i>) I-48	-	-	43.32 ± 11.04	161.75 ± 26.50

Compound No.	Liver Luc @ 0.3 mg/kg (ng/g)	Liver Luc @ 1.0 mg/kg (ng/g)	Serum IgG @ 0.3 mg/kg (ug/mL)	Serum IgG @ 1.0 mg/kg (ug/mL)
(1 <i>R</i> ,2 <i>S</i>) I-48	-	-	28.32 ± 8.81	-
(1 <i>R</i> ,2 <i>R</i>) I-48	-	-	25.27 ± 7.50	92.5 ± 20.18
(<i>rac</i>)-(1 <i>R</i> ,2 <i>R</i>) I-48	-	-	32.85 ± 6.90	216.8 ± 46.48
(1 <i>S</i> ,4 <i>S</i>) I-49	-	-	60.49 ± 26.40	332.8 ± 57.13
(1 <i>R</i> ,4 <i>R</i>) I-49	-	-	28.94 ± 13.21	161.09 ± 11.20
(1 <i>S</i> ,4 <i>S</i>) I-50	-	-	29.82 ± 7.79	-
(1 <i>S</i> ,4 <i>S</i>) I-51	-	-	80.37 ± 26.77	-
(1 <i>S</i> ,4 <i>S</i>) I-52	-	-	40.24 ± 18.53	-
(1 <i>S</i> ,4 <i>S</i>) I-53	-	-	81.56 ± 44.40	-
(1 <i>S</i> ,4 <i>S</i>) I-54	-	-	43.22 ± 12.97	-
(1 <i>S</i> ,4 <i>S</i>) I-55	-	-	72.2 ± 14.87	-
(1 <i>S</i> ,4 <i>S</i>) I-56	-	-	52.9 ± 17.00	-
(1 <i>S</i> ,4 <i>S</i>) I-57	-	-	84.4 ± 35.10	-
(1 <i>S</i> ,4 <i>S</i>) I-58	-	-	61.75 ± 11.64	-
(1 <i>S</i> ,4 <i>S</i>) I-59	-	-	84.23 ± 17.22	-
(1 <i>S</i> ,4 <i>S</i>) I-60	-	-	15.65 ± 5.31	-
(1 <i>S</i> ,4 <i>S</i>) I-61	-	-	40.66 ± 9.70	-
(1 <i>S</i> ,4 <i>S</i>) I-62	-	-	48.49 ± 8.83	-
(1 <i>S</i> ,4 <i>S</i>) I-63	-	-	39.32 ± 8.99	-

EXAMPLE 5

SYNTHETIC PATHWAY FOR COMPOUNDS I-1 TO I-18



5 Synthesis of 8-bromo-N,N-didecyloctanamide (Intermediate 1)

To a mixture of 8-bromooctanoic acid (47.3 mmol, 10.5 g) and DMF (cat.) in DCM (100 mL) was added oxalyl chloride (142 mmol, 18.0 g) and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated to give 8-bromooctanoyl chloride which was used in the next step without further purification.

To a mixture of didecylamine (69.1 mmol, 20.6 g), triethylamine (377 mmol, 52.5 mL), and N,N-dimethylpyridin-4-amine (cat. 8.0 mg) in DCM (100 mL) was added 8-bromooctanoyl chloride (62.8 mmol, 15.2 g) in DCM (60 mL) and the reaction mixture was stirred at room temperature for 17 h. The reaction mixture was concentrated, and the crude material was resuspended in hexanes. The resultant solids were filtered and the filtrate was purified via

automated flash chromatography (5% to 25% EtOAc in hexanes) to give 8-bromo-N,N-didecyloctanamide (26g, 82%).

General Procedure A

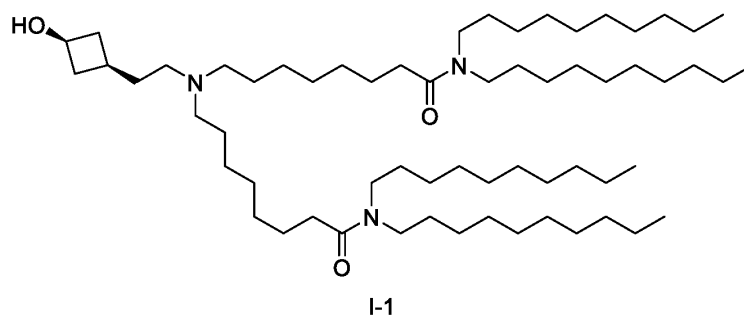
A mixture of 8-bromo-N,N-didecyloctanamide (1.0 to 2.0 eq), the appropriate amine (1.0 eq), diisopropylethylamine (“DIEA” 3.0 to 5.0 eq), and potassium iodide (0.0 to 3.0 eq) in ACN (0.1 M) was heated at 75 °C for 24 h to 72 h. The reaction mixture was concentrated and the crude material was suspended in a mixture of hexanes to EtOAc to Et₃N (95:5:1). The resultant solids were filtered, and the filtrate was purified by automated flash chromatography to give the desired compounds.

10 General Procedure B

A mixture of 8-bromo-N,N-didecyloctanamide (1.0 to 2.0 eq), the appropriate amine (1.0 eq), DIEA (3.0 to 5.0 eq) and potassium iodide (3.0 to 4.0 eq) in ACN (0.5 M) was heated via microwave irradiation at 140 °C to 160 °C for 30 min to 3 hours. The reaction mixture was concentrated and the crude material was suspended in a mixture of hexanes:EtOAc:Et₃N (95:5:1). The resultant solids were filtered, and the filtrate was purified by automated flash chromatography to give the desired compound.

EXAMPLE 6

8,8'-((2-((1*S*,3*R*)-3-HYDROXYCYCLOBUTYL)ETHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE) (COMPOUND (1*S*,3*R*)-I-1)



20

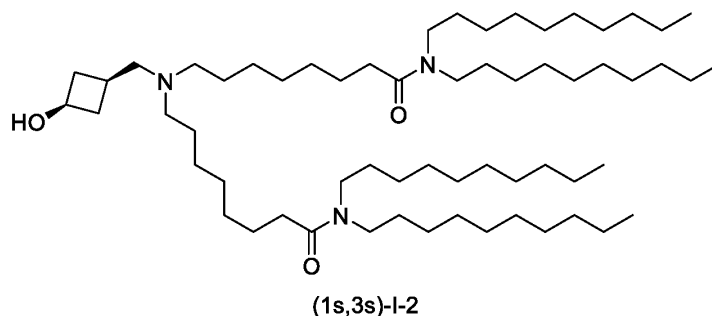
Synthesis of (1*S*,3*R*)-I-1

Compound (1*S*,3*R*)-I-1 was synthesized according to **General Procedure A** using (1*r*,3*s*)-3-(2-aminoethyl)cyclobutan-1-ol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (yield 12%). ¹H NMR (400

MHz, CDCl₃) δ 4.−3 − 4.03 (m, 1H), 3.32 − 3.23 (m, 4H), 3.23 − 3.15 (m, 4H), 2.52 − 2.41 (m, 2H), 2.39 − 2.22 (m, 10H), 1.72 − 1.45 (m, 22H), 1.44 − 1.16 (m, 72H), 0.95 − 0.83 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₂H₁₂₃N₃O₃ = 958.0. Found [M+H]⁺ = 959.1

EXAMPLE 7

5 8,8'-((((1S,3S)-3-HYDROXYCYCLOBUTYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE) (COMPOUND (1S,3S)-I-2)

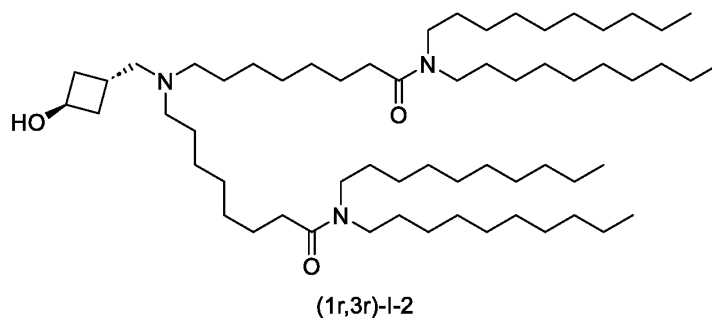


Synthesis of (1S,3S)-I-2

Compound (1S,3S)-I-2 was synthesized according to **General Procedure A** using
 10 (1S,3S)-3-(aminomethyl)cyclobutan-1-ol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (yield 44%). ¹H NMR (600 MHz, CDCl₃) δ 4.18 − 4.10 (m, 1H), 3.33 − 3.27 (m, 4H), 3.24 − 3.19 (m, 4H), 2.52 − 2.44 (m, 4H), 2.40 − 2.34 (m, 4H), 2.32 − 2.26 (m, 4H), 1.96 − 1.88 (m, 1H), 1.69 − 1.60 (m, 5H), 1.60 − 1.48 (m, 11H), 1.46 − 1.38 (m, 4H), 1.38 − 1.19 (m, 69H), 0.93 − 0.87 (m, 12H). *m/z* calcd. for
 15 Chemical Formula: C₆₁H₁₂₁N₃O₃ = 943.9. Found [M+H]⁺ = 945.0.

EXAMPLE 8

8,8'-((((1R,3R)-3-HYDROXYCYCLOBUTYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE) (COMPOUND (1R,3R)-I-2)

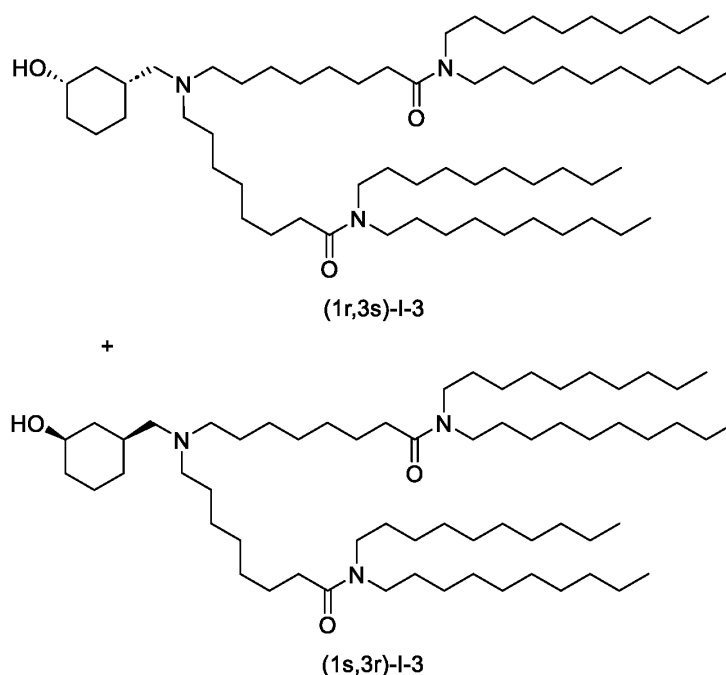


Synthesis of (1*R*,3*R*)-I-2

Compound **(1*R*,3*R*)-I-2** was synthesized according to **General Procedure A** using (1*S*,3*S*)-3-(aminomethyl)cyclobutan-1-ol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (yield 40%). ¹H NMR (600 MHz, CDCl₃) δ 4.44 - 4.36 (m, 1H), 3.33 - 3.27 (m, 4H), 3.24 - 3.19 (m, 4H), 2.41 (s, 3H), 2.39 - 2.33 (m, 4H), 2.32 - 2.26 (m, 4H), 2.13 - 1.99 (m, 4H), 1.69 - 1.61 (m, 7H), 1.60 - 1.48 (m, 8H), 1.45 - 1.20 (m, 74H), 0.94 - 0.87 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₁H₁₂₁N₃O₃ = 943.9. Found [M+H]⁺ = 944.9.

EXAMPLE 9

10 **8,8'-((((1*R*,3*S*)-3-HYDROXYCYCLOHEXYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)**
8,8'-((((1*S*,3*R*)-3-HYDROXYCYCLOHEXYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (RAC)-(1*RS*, 2*SR*)-I-3)



15

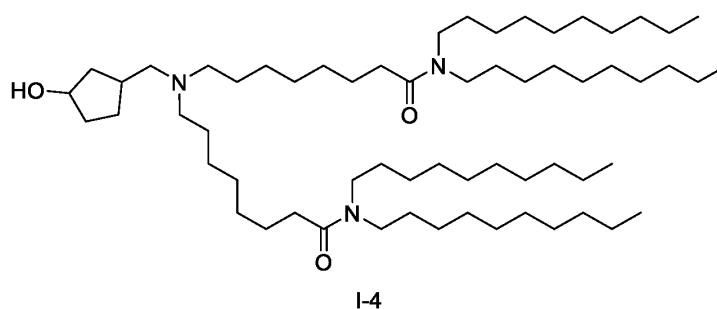
Synthesis of (rac)-(1*RS*,2*SR*)-I-3

A mixture of Compound **I-3** enantiomers was synthesized according to **General Procedure A** using a mixture of (1*R*,3*S*)-3-(aminomethyl)cyclohexan-1-ol and (1*S*,3*R*)-3-(aminomethyl)cyclohexan-1-ol. Purification via automated flash chromatography (5% to 65%

EtOAc in hexanes with 1% Et₃N) followed by a second purification via automated flash chromatography (10% to 100% EtOAc in hexanes) gave the titled compound (yield 27%). ¹H NMR (400 MHz, CDCl₃) δ 3.63 – 3.51 (m, 1H), 3.33 – 3.24 (m, 4H), 3.25 – 3.14 (m, 4H), 2.40 – 2.21 (m, 8H), 2.20 – 2.08 (m, 3H), 2.01 – 1.93 (m, 1H), 1.77 (dt, *J* = 13.0, 3.4 Hz, 1H), 1.71 – 1.59 (m, 10H), 1.57 – 1.44 (m, 10H), 1.42 – 1.06 (m, 80H), 0.97 – 0.64 (m, 15H). *m/z* calcd. for Chemical Formula: C₆₃H₁₂₅N₃O₃ = 972.0. Found [M+H]⁺ = 973.1.

EXAMPLE 10

8,8'-(((3-HYDROXYCYCLOPENTYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE) (COMPOUND I-4)



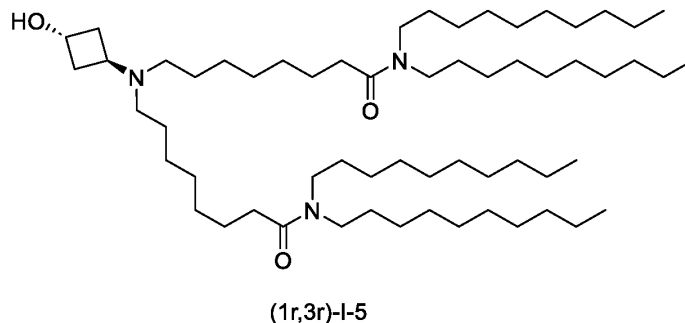
10

Synthesis of I-4

Compound **I-4** was synthesized according to **General Procedure A** using 3-(aminomethyl)cyclopentan-1-ol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) followed by a second purification via automated flash chromatography (5% to 100% EtOAc in hexanes) gave the titled compound (yield 39%). ¹H NMR (400 MHz, CDCl₃) δ 4.39 – 4.31 (m, 1H), 4.29 – 4.20 (m, 1H), 3.31 – 3.24 (m, 4H), 3.23 – 3.14 (m, 4H), 2.45 – 2.30 (m, 6H), 2.29 – 2.21 (m, 5H), 2.18 – 1.97 (m, 1H), 1.97 – 1.84 (m, 1H), 1.86 – 1.08 (m, 96H), 0.94 – 0.83 (m, 13H). *m/z* calcd. for Chemical Formula: C₆₂H₁₂₃N₃O₃ = 958.0. Found [M+H]⁺ = 959.1.

EXAMPLE 11

8,8'-(((1*R*,3*R*)-3-HYDROXYCYCLOBUTYL)AZANEDIYL)BIS(*N*,*N*-DIDECYLOCTANAMIDE)
(COMPOUND (1*R*,3*R*)-I-5)

5 Synthesis of (1*R*,3*R*)-I-5

Compound (1*R*,3*R*)-I-5 was synthesized according to **General Procedure A** using (1*r*,3*r*)-3-aminocyclobutan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) followed by a second purification via automated flash chromatography (5% to 100% EtOAc in hexanes) gave the titled compound (yield 10%).

- 10 ¹H NMR (400 MHz, CDCl₃) δ 4.43 – 4.34 (m, 1H), 3.49 - 3.37 (m, 1H), 3.32 – 3.23 (m, 4H), 3.23 – 3.15 (m, 4H), 2.30 – 2.22 (m, 4H), 2.22 - 2.12 (m, 2H), 2.08 - 1.97 (m, 2H), 1.79 - 1.69 (m, 1H), 1.68 – 1.56 (m, 7H), 1.57 - 1.43 (m, 8H), 1.44 – 1.14 (m, 75H), 0.92 - 0.83 (m, 12H).
m/z calcd. for Chemical Formula: C₆₀H₁₁₉N₃O₃ = 929.9. Found [M+H]⁺ = 931.1.

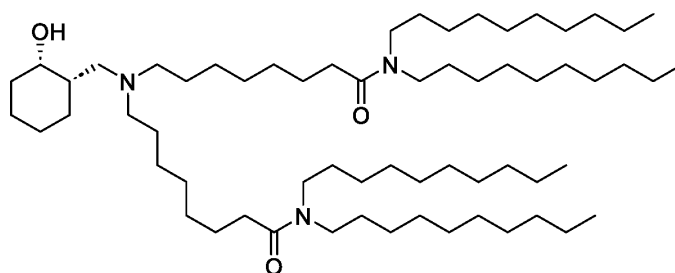
EXAMPLE 12

8,8'-((((1S,2S)-2-HYDROXYCYCLOHEXYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

8,8'-((((1R,2R)-2-HYDROXYCYCLOHEXYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

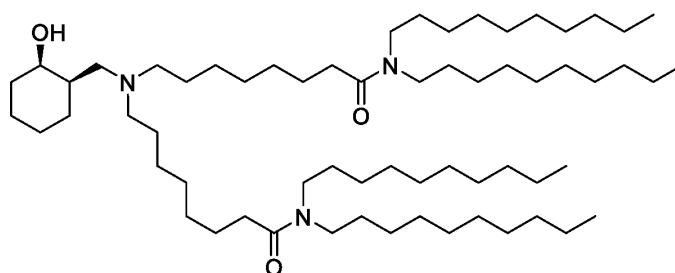
5

(COMPOUND (RAC)-(1RS,2RS)-I-6)



(1S,2S)-I-6

+



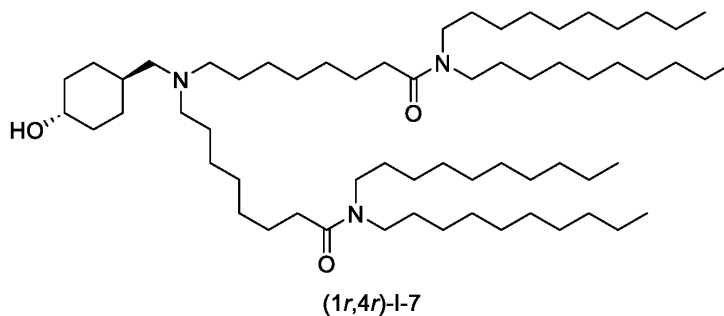
(1R,2R)-I-6

Synthesis of (rac)-(1RS,2RS)-I-6

A mixture of Compound **I-6** enantiomers was synthesized according to **General**
 10 **Procedure A** using a mixture of (1R,2R)-2-(aminomethyl)cyclohexan-1-ol hydrochloride and
 (1S,2S)-2-(aminomethyl)cyclohexan-1-ol hydrochloride. Purification via automated flash
 chromatography (10% to 100% EtOAc in hexanes). A second purification via automated flash
 chromatography (5% to 100% EtOAc in hexanes) gave the titled compound (yield 11%). ¹H
 NMR (400 MHz, CDCl₃) δ 3.84 - 3.75 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.96
 15 (dd, *J* = 13.1, 10.5 Hz, 1H), 2.60 - 2.48 (m, 2H), 2.31 - 2.20 (m, 7H), 2.17 - 2.07 (m, 1H), 1.78 -
 1.39 (m, 24H), 1.36 - 1.18 (m, 74H), 0.95 - 0.83 (m, 12H). *m/z* calcd. for Chemical Formula:
 C₆₃H₁₂₅N₃O₃ = 972.0. Found [M+H]⁺ = 973.1.

EXAMPLE 13

8,8'-((((1*R*,4*R*)-4-HYDROXYCYCLOHEXYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1*R*,4*R*)-I-7)



5

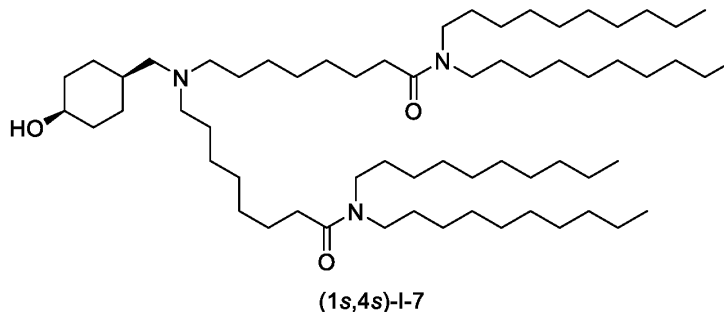
Synthesis of (1*R*,4*R*)-I-7

Compound (1*R*,4*R*)-I-7 was synthesized according to **General Procedure A** using (1*r*,4*r*)-4-(aminomethyl)cyclohexan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 100% EtOAc in hexanes). A second purification via automated flash chromatography (2% to 10% MeOH in DCM) gave the titled compound (yield 28%). ¹H NMR (400 MHz, CDCl₃) δ 3.59 – 3.49 (m, 1H), 3.32 – 3.23 (m, 4H), 3.23 – 3.15 (m, 4H), 2.35 – 2.23 (m, 8H), 2.11 (d, *J* = 6.9 Hz, 2H), 2.03 – 1.92 (m, 2H), 1.89 – 1.79 (m, 2H), 1.69 – 1.59 (m, 7H), 1.57 – 1.46 (m, 8H), 1.45 – 1.14 (m, 79H), 0.94 – 0.81 (m, 15H). *m/z* calcd. for Chemical Formula: C₆₃H₁₂₅N₃O₃ = 972.0. Found [M+H]⁺ = 973.0.

15

EXAMPLE 14

8,8'-((((1*S*,4*S*)-4-HYDROXYCYCLOHEXYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1*S*,4*S*)-I-7)

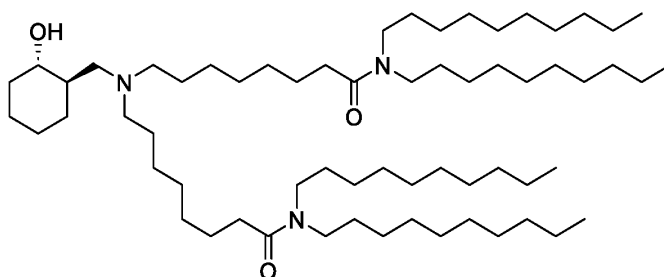


Synthesis of (1*S*,4*S*)-I-7

Compound **(1*S*,4*S*)-I-7** was synthesized according to **General Procedure A** using (1*S*,4*S*)-4-(aminomethyl)cyclohexan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 100% EtOAc in hexanes) gave the titled compound (yield 26%). ¹H NMR (400 MHz, CDCl₃) δ 3.95 (s, 1H), 3.32 – 3.23 (m, 4H), 3.23 – 3.15 (m, 4H), 2.36 – 2.22 (m, 8H), 2.17 (d, *J* = 6.8 Hz, 2H), 1.73 – 1.43 (m, 24H), 1.43 – 1.15 (m, 81H), 0.94 – 0.82 (m, 14H). *m/z* calcd. for Chemical Formula: C₆₃H₁₂₅N₃O₃ = 972.0. Found [M+H]⁺ = 973.1.

EXAMPLE 15

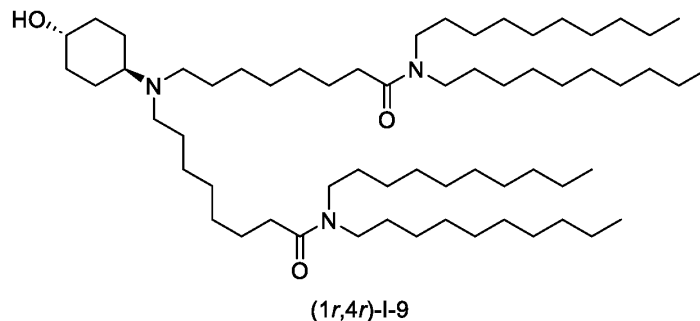
8,8'-((((1*R*,2*S*)-2-HYDROXYCYCLOHEXYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1*R*,2*R*)-I-8)

**(1*R*,2*S*)-I-8****Synthesis of (1*R*,2*S*)-I-8**

Compound **(1*R*,2*S*)-I-8** was synthesized according to **General Procedure A** using (1*S*,2*R*)-2-(aminomethyl)cyclohexan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N). A second purification via automated flash chromatography (5% to 100% EtOAc in hexanes) gave the titled compound (yield 22%). ¹H NMR (400 MHz, CDCl₃) δ 3.41 – 3.32 (m, 1H), 3.32 – 3.24 (m, 4H), 3.23 – 3.15 (m, 4H), 2.64 – 2.54 (m, 2H), 2.49 – 2.40 (m, 1H), 2.33 – 2.23 (m, 5H), 2.22 – 2.11 (m, 2H), 1.96 – 1.87 (m, 1H), 1.74 – 1.57 (m, 8H), 1.57 – 1.38 (m, 15H), 1.37 – 1.16 (m, 75H), 0.90 – 0.9 (m, 15H). *m/z* calcd. for Chemical Formula: C₆₃H₁₂₅N₃O₃ = 972.0. Found [M+H]⁺ = 973.1.

EXAMPLE 16

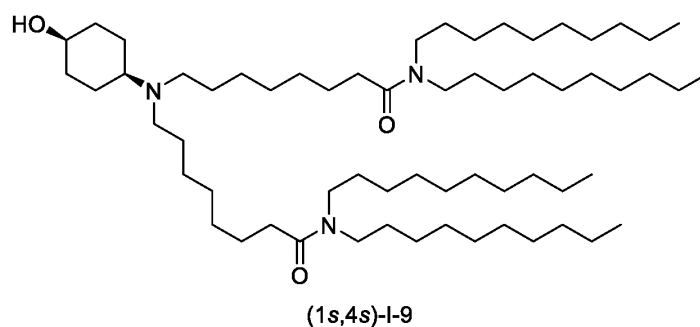
8,8'-(((1*R*,4*R*)-4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1*R*,4*R*)-I-9)

5 **Synthesis of (1*R*,4*R*)-I-9**

Compound **(1*R*,4*R*)-I-9** was synthesized according to **General Procedure A** using (1*r*,4*r*)-4-aminocyclohexan-1-ol. Purification via automated flash chromatography (5% to 100% EtOAc in hexanes) gave the titled compound (yield 42%). ¹H NMR (400 MHz, CDCl₃) δ 3.60 - 3.49 (m, 1H), 3.32 - 3.24 (m, 4H), 3.22 - 3.14 (m, 4H), 2.49 - 2.40 (m, 1H), 2.40 - 2.33 (m, 4H), 2.31 - 2.22 (m, 4H), 2.05 - 1.94 (m, 2H), 1.81 - 1.71 (m, 2H), 1.68 - 1.58 (m, 6H), 1.57 - 1.45 (m, 9H), 1.44 - 1.19 (m, 82H), 0.94 - 0.81 (m, 13H). *m/z* calcd. for Chemical Formula: C₆₂H₁₂₃N₃O₃ = 958.0. Found [M+H]⁺ = 959.0.

EXAMPLE 17

8,8'-(((1*S*,4*S*)-4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1*S*,4*S*)-I-9)

**Synthesis of (1*S*,4*S*)-I-9**

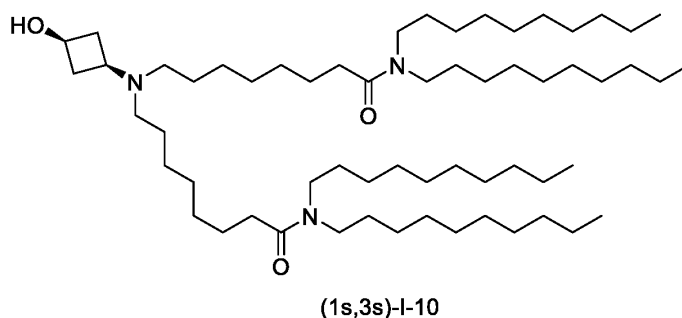
Compound **(1*S*,4*S*)-I-9** was synthesized according to **General Procedure A** using (1*s*,4*s*)-4-aminocyclohexan-1-ol. Purification via automated flash chromatography (5% to 100%

EtOAc in hexanes) gave the titled compound (yield 42%). ^1H NMR (400 MHz, CDCl_3) δ 4.01 - 3.95 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.49 - 2.38 (m, 5H), 2.26 (t, $J = 7.6$ Hz, 4H), 1.89 - 1.77 (m, 2H), 1.71 - 1.58 (m, 8H), 1.57 - 1.44 (m, 13H), 1.44 - 1.17 (m, 77H), 0.92 - 0.82 (m, 13H). m/z calcd. for Chemical Formula: $\text{C}_{62}\text{H}_{123}\text{N}_3\text{O}_3 = 958.0$. Found $[\text{M}+\text{H}]^+ = 959.0$.

5

EXAMPLE 18

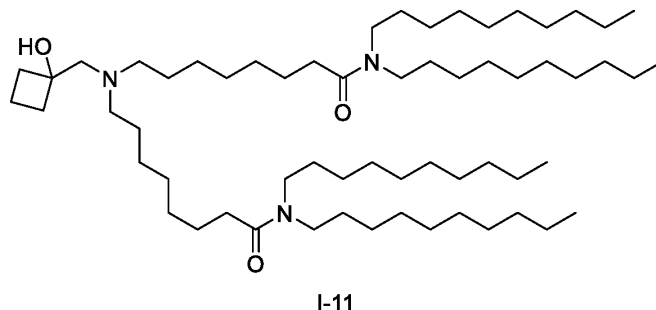
8,8'-(((1*S*,3*S*)-3-HYDROXYCYCLOBUTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1*S*,3*S*)-I-10)

**Synthesis of (1*S*,3*S*)-I-10**

10 Compound **(1*S*,3*S*)-I-10** was synthesized according to **General Procedure A** using (1*S*,3*S*)-3-aminocyclobutan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 100% EtOAc in hexanes) gave the titled compound (yield 47%). ^1H NMR (400 MHz, CDCl_3) δ 4.00 - 3.88 (m, 1H), 3.32 - 3.24 (m, 4H), 3.22 - 3.15 (m, 4H), 2.64 - 2.45 (m, 3H), 2.40 - 2.32 (m, 4H), 2.31 - 2.22 (s, 4H), 1.95 - 1.84 (m, 1H), 1.82 - 1.58 (m, 8H), 1.58 - 1.43 (m, 15 8H), 1.43 - 1.18 (m, 74H), 0.94 - 0.81 (m, 12H). m/z calcd. for Chemical Formula: $\text{C}_{60}\text{H}_{119}\text{N}_3\text{O}_3 = 929.9$. Found $[\text{M}+\text{H}]^+ = 931.0$.

EXAMPLE 19

8,8'-(((1-HYDROXYCYCLOBUTYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND I-11)

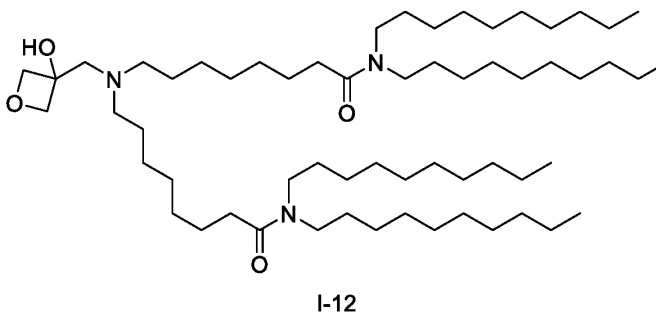
5 **Synthesis of I-11**

Compound **I-11** was synthesized according to **General Procedure A** using 1-(aminomethyl)cyclobutan-1-ol. The crude material was purified via automated reverse phase flash chromatography (10% to 100% MeOH in water with 0.1% TFA). The collected fractions were concentrated and partitioned between EtOAc and sat. NaHCO₃. Purification via automated
 10 flash chromatography (0% to 10% MeOH in DCM) gave the titled compound (yield 28%).- ¹H NMR (400 MHz, CDCl₃) δ 3.32 – 3.24 (m, 4H), 3.23 – 3.15 (m, 4H), 2.57 (s, 2H), 2.52 – 2.42 (m, 4H), 2.31 – 2.21 (m, 4H), 2.20 - 2.09 (m, 2H), 2.04 – 1.92 (m, 2H), 1.86 – 1.72 (m, 1H), 1.69 – 1.36 (m, 19H), 1.36 – 1.17 (m, 67H), 0.92 - 0.84 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₁H₁₂₁N₃O₃ = 943.9. Found [M+H]⁺ = 945.0.

15

EXAMPLE 20

8,8'-(((3-HYDROXYOXETAN-3-YL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND I-12)

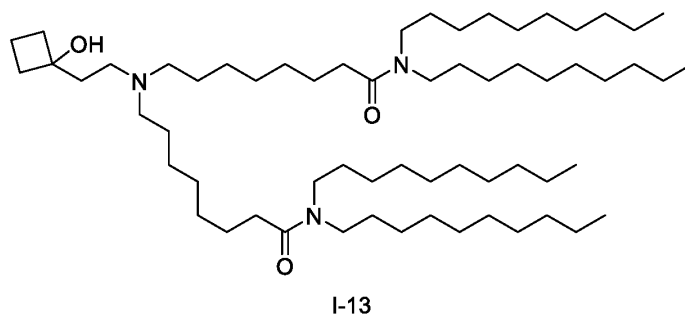


Synthesis of I-12

Compound **I-12** was synthesized according to **General Procedure A** using 1-(aminomethyl)cyclobutan-1-ol. Purification via automated flash chromatography (0% to 10% MeOH in DCM) gave the titled compound (yield 54%). ¹H NMR (400 MHz, CDCl₃) δ 4.72 (d, *J* = 6.6 Hz, 2H), 4.47 (d, *J* = 6.7 Hz, 2H), 3.32 – 3.25 (m, 4H), 3.22 – 3.15 (m, 4H), 2.82 (s, 2H), 2.39 (t, *J* = 7.5 Hz, 4H), 2.26 (t, *J* = 7.6 Hz, 4H), 1.68 - 1.59 (m, 4H), 1.57 - 1.46 (m, 8H), 1.44 – 1.36 (m, 4H), 1.36 - 1.18 (m, 67H), 0.92 - 0.83 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₀H₁₁₉N₃O₄ = 945.9. Found [M+H]⁺ = 946.9.

EXAMPLE 21

10 **8,8'-((2-(1-HYDROXYCYCLOBUTYL)ETHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)**
(**COMPOUND I-13**)

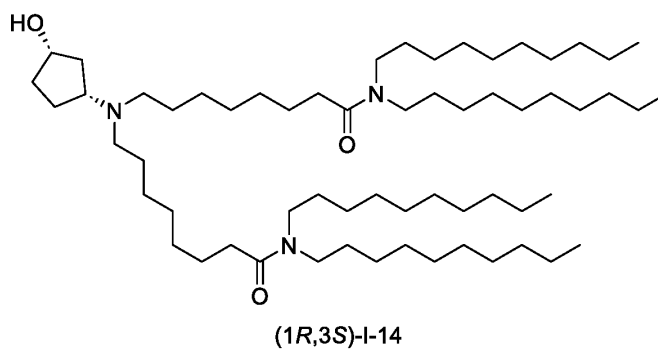
**Synthesis of I-13**

15 Compound **I-13** was synthesized according to **General Procedure A** using 1-(2-aminoethyl)cyclobutan-1-ol. Purification via automated flash chromatography (0% to 10% MeOH in DCM) gave the titled compound (yield 98%). ¹H NMR (400 MHz, CDCl₃) δ 3.31 – 3.23 (m, 4H), 3.22 – 3.14 (m, 4H), 2.27 (t, *J* = 7.4 Hz, 4H), 2.15 – 2.01 (m, 6H), 1.81 – 1.44 (m, 20H), 1.40 – 1.17 (m, 71H), 0.94 - 0.81 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₂H₁₂₃N₃O₃ = 958.0. Found [M+H]⁺ = 958.9.

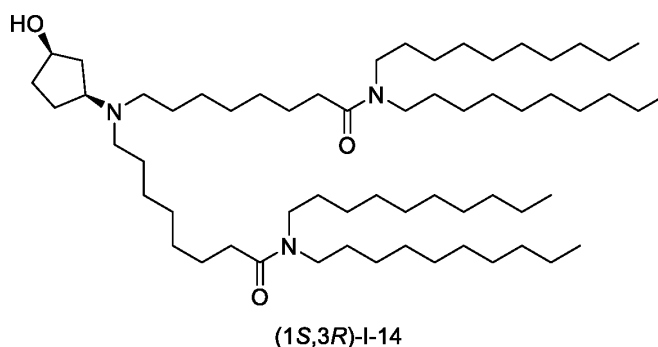
EXAMPLE 22

8,8'-(((1R,3S)-3-HYDROXYCYCLOPENTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

8,8'-(((1S,3R)-3-HYDROXYCYCLOPENTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (*RAC*)-(1*RS*,3*SR*)-I-14)

+



5

Synthesis of (*rac*)-(1*RS*,3*SR*)-I-14

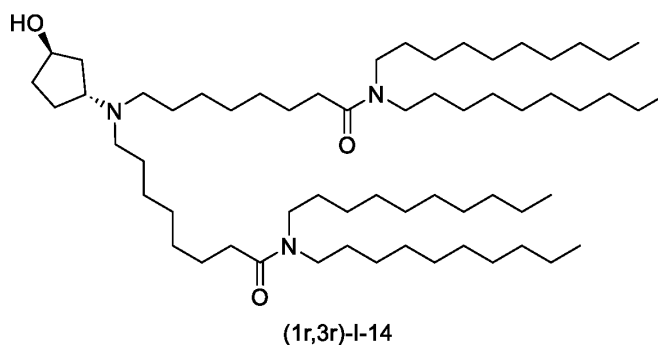
A mixture Compound **I-14** enantiomers was synthesized according to **General**

Procedure A using a mixture of (1*S*,3*R*)-3-aminocyclopentan-1-ol hydrochloride and (1*R*,3*S*)-3-aminocyclopentan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (yield 28%). ¹H NMR (400 MHz, CDCl₃) δ 4.24 - 4.17 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 3.07 - 3.00 (m, 1H), 2.55 - 2.41 (m, 4H), 2.26 (t, *J* = 7.6 Hz, 4H), 1.92 - 1.05 (m, 92H), 0.93 - 0.80 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₁H₁₂₁N₃O₃ = 943.9. Found [M+H]⁺ = 945.2.

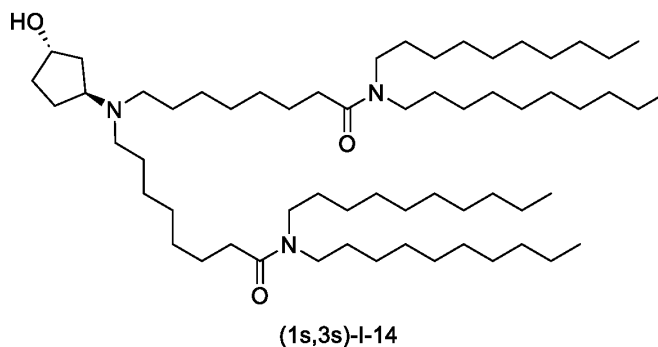
EXAMPLE 23

8,8'-(((1R,3R)-3-HYDROXYCYCLOPENTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

8,8'-(((1S,3S)-3-HYDROXYCYCLOPENTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (*RAC*)-(1*RS*,3*RS*)-I-14)

+



5

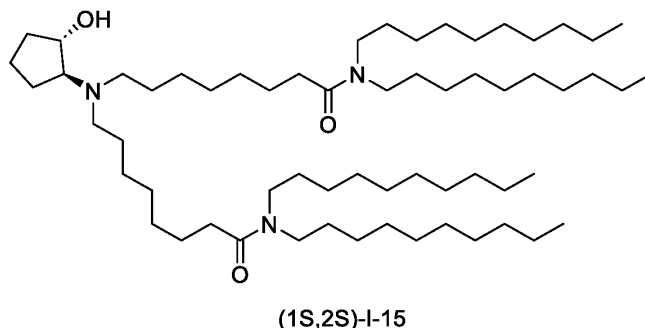
Synthesis of (*rac*)-(1*RS*,3*RS*)-I-14

A mixture of Compound **I-14** enantiomers was synthesized according to **General**

Procedure A using a mixture of (1R,3R)-3-aminocyclopentan-1-ol hydrochloride and (1S,3S)-3-aminocyclopentan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (yield 33%). ¹H NMR (400 MHz, CDCl₃) δ 4.39 - 4.32 (m, 1H), 3.43 - 3.24 (m, 5H), 3.23 - 3.15 (m, 4H), 2.48 - 2.40 (m, 4H), 2.31 - 2.22 (m, 4H), 2.07 - 1.89 (m, 2H), 1.88 - 1.77 (m, 1H), 1.70 - 1.37 (m, 23H), 1.37 - 1.16 (m, 68H), 0.93 - 0.83 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₁H₁₂₁N₃O₃ = 943.9. Found [M+H]⁺ = 945.3.

EXAMPLE 24

8,8'-(((1*S*,2*S*)-2-HYDROXYCYCLOPENTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1*S*,2*S*)-I-15)

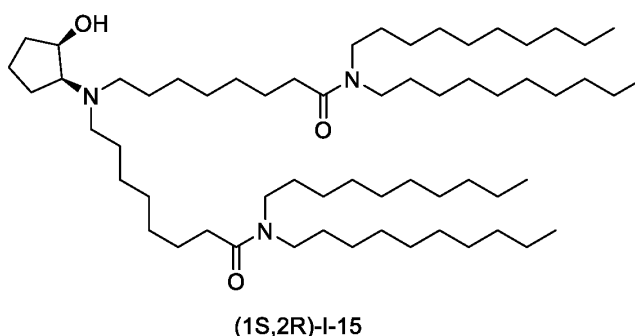
5 **Synthesis of (1*S*,2*S*)-I-15**

Compound (1*S*,2*S*)-I-15 was synthesized according to **General Procedure A** using (1*S*,2*S*)-2-aminocyclopentan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) followed by a second automated flash chromatography (10% to 100% EtOAc in hexanes) gave the titled compound (yield 12%).

- 10 ¹H NMR (400 MHz, CDCl₃) δ 3.94 - 3.83 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.87 - 2.76 (m, 1H), 2.52 - 2.33 (m, 4H), 2.27 (t, *J* = 7.6 Hz, 4H), 1.95 - 1.03 (m, 94H), 0.96 - 0.81 (m, 12H). *m/z* calculated for Chemical Formula: C₆₁H₁₂₁N₃O₃ = 943.9. Found [M+H]⁺ = 945.3.

EXAMPLE 25

15 **8,8'-(((1*S*,2*R*)-2-HYDROXYCYCLOPENTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)**
(COMPOUND (1*S*,2*R*)-I-15)

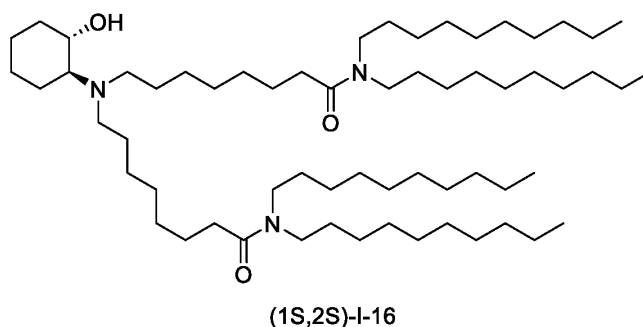


Synthesis of (1*S*,2*R*)-I-15

Compound **(1*S*,2*R*)-I-15** was synthesized according to **General Procedure A** using (1*R*,2*S*)-2-aminocyclopentan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) followed by a second automated flash chromatography (100% EtOAc) gave the titled compound (yield 16%). ¹H NMR (400 MHz, CDCl₃) δ 4.01 - 3.97 (m, 1H), 3.32 - 3.24 (m, 4H), 3.23 - 3.15 (m, 4H), 2.77 - 2.63 (m, 1H), 2.62 - 2.49 (m, 4H), 2.31 - 2.23 (m, 4H), 1.92 - 1.05 (m, 94H), 0.92 - 0.81 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₁H₁₂₁N₃O₃ = 943.9. Found [M+H]⁺ = 945.3.

EXAMPLE 26

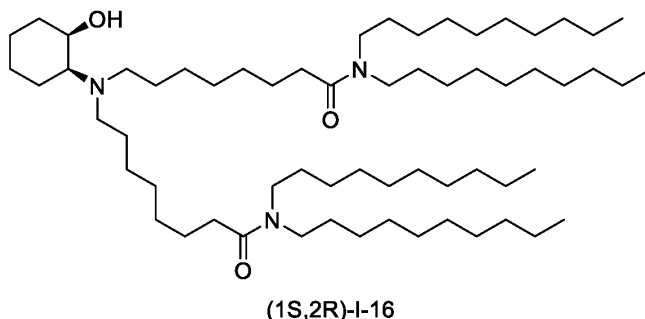
**8,8'-(((1*S*,2*S*)-2-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1*S*,2*S*)-I-16)**

**Synthesis of (1*S*,2*S*)-I-16**

Compound **(1*S*,2*S*)-I-16** was synthesized according to **General Procedure A** using (1*S*,2*S*)-2-aminocyclohexan-1-ol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) followed by a second automated flash chromatography (0% to 100% EtOAc) followed by a third automated flash chromatography (0% to 10% MeOH in DCM) gave the titled compound (yield 13%). ¹H NMR (400 MHz, CDCl₃) δ 3.80 - 3.02 (m, 10H), 2.61 - 2.18 (m, 8H), 2.17 - 2.05 (m, 2H), 2.02 - 1.00 (m, 96H), 0.92 - 0.83 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₂H₁₂₃N₃O₃ = 958.0. Found [M+H]⁺ = 959.3.

EXAMPLE 27

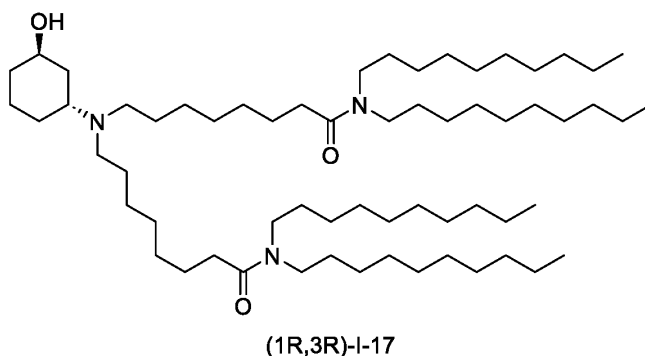
8,8'-(((1*S*,2*R*)-2-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1*S*,2*R*)-I-16)

5 **Synthesis of (1*S*,2*R*)-I-16**

Compound **(1*S*,2*R*)-I-16** was synthesized according to **General Procedure A** using (1*R*,2*S*)-2-aminocyclohexan-1-ol hydrochloride. Purification via automated flash chromatography (10% to 100% EtOAc in hexanes) followed by a second automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (yield 17%). ¹H NMR (400 MHz, CDCl₃) δ 3.94 - 3.86 (m, 1H), 3.32 - 3.24 (m, 4H), 3.23 - 3.15 (m, 4H), 2.61 - 2.47 (m, 4H), 2.37 - 2.31 (m, 1H), 2.30 - 2.23 (m, 4H), 2.05 - 1.97 (m, 1H), 1.80 - 1.02 (m, 97H), 0.94 - 0.84 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₂H₁₂₃N₃O₃ = 958.0. Found [M+H]⁺ = 959.3.

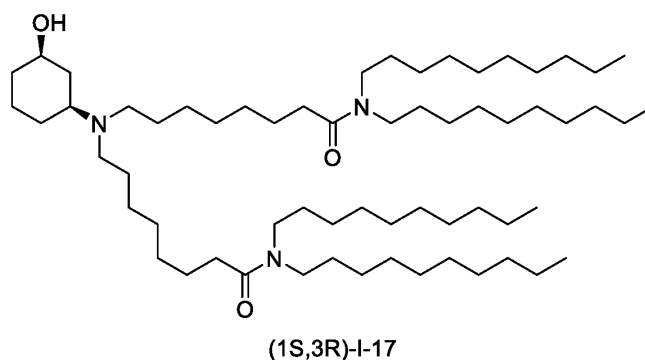
EXAMPLE 28

8,8'-(((1*R*,3*R*)-3-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1*R*,3*R*)-I-17)



Compound **(1*R*,3*R*)-I-17** was synthesized according to **General Procedure A** using (1*R*,3*R*)-3-aminocyclohexan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (yield 29%). ¹H NMR (400 MHz, CDCl₃) δ 4.27 - 4.19 (m, 1H), 3.32 – 3.23 (m, 4H), 3.23 – 3.15 (m, 4H), 3.01 - 2.86 (m, 1H), 2.38 (t, *J* = 7.4 Hz, 4H), 2.31 - 2.22 (m, 4H), 1.88 – 1.05 (m, 97H), 0.92 - 0.83 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₂H₁₂₃N₃O₃ = 958.0. Found [M+H]⁺ = 959.4.

8,8'-(((1*S*,3*R*)-3-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1*S*,3*R*)-I-17)



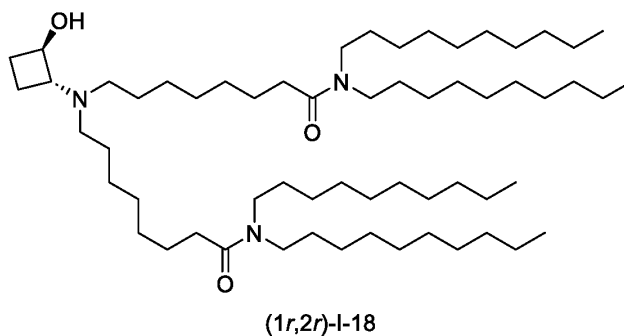
Compound **(1S,3R)-I-17** was synthesized according to **General Procedure A** using (1R,3S)-3-aminocyclohexan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (yield 36%). ¹H NMR (400 MHz, CDCl₃) δ 3.70 - 3.60 (m, 1H), 3.32 – 3.24 (m, 4H), 3.22 – 3.14 (m, 4H), 2.61 - 2.51 (m, 1H), 2.48 - 2.38 (m, 4H), 2.31 – 2.22 (m, 4H), 2.00 – 1.73 (m, 3H), 1.70 – 1.01 (m, 89H), 0.93 - 0.80 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₂H₁₂₃N₃O₃ = 958.0. Found [M+H]⁺ = 959.2.

EXAMPLE 30

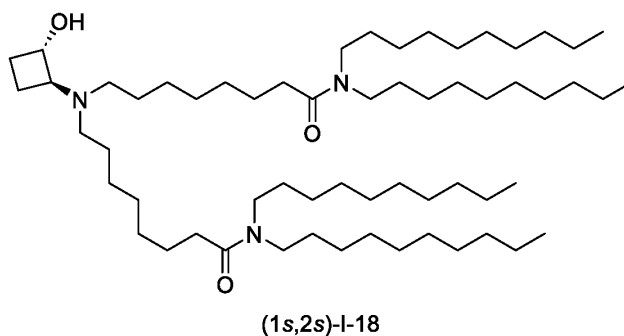
8,8'-(((1R,2R)-2-HYDROXYCYCLOBUTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

8,8'-(((1S,2S)-2-HYDROXYCYCLOBUTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (RAC)-(1RS,2RS)-I-18)



+



5

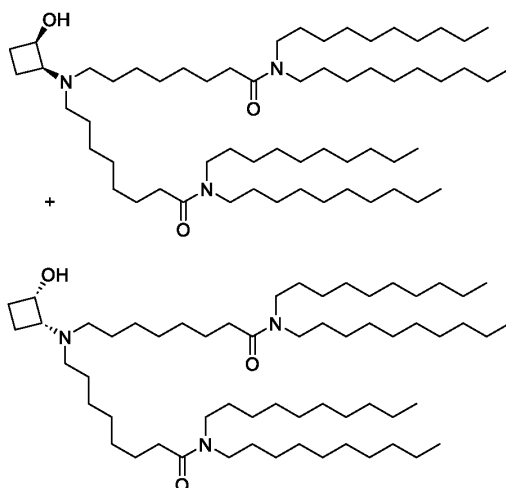
Synthesis of (rac)-(1RS,2RS)-I-18

A mixture of Compound **I-18** enantiomers was synthesized according to **General Procedure A** using a mixture of (1R,2R)-2-aminocyclobutan-1-ol hydrochloride and (1S,2S)-2-aminocyclobutan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (yield 30%). ¹H NMR (400 MHz, CDCl₃) δ 4.02 - 3.93 (m, 1H), 3.32 - 3.22 (m, 4H), 3.23 - 3.13 (m, 4H), 2.90 (q, *J* = 8.0 Hz, 1H), 2.60 - 2.35 (m, 4H), 2.26 (t, *J* = 7.5 Hz, 4H), 2.16 - 2.06 (m, 1H), 1.80 (q, *J* = 9.2 Hz, 1H), 1.74 - 1.05 (m, 90H), 0.94 - 0.80 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₀H₁₁₉N₃O₃ = 929.9. Found [M+H]⁺ = 931.2.

EXAMPLE 31

8,8'-(((1S,2R)-2-HYDROXYCYCLOBUTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

8,8'-(((1R,2S)-2-HYDROXYCYCLOBUTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (*RAC*)-(1*RS*,2*SR*)-I-18)

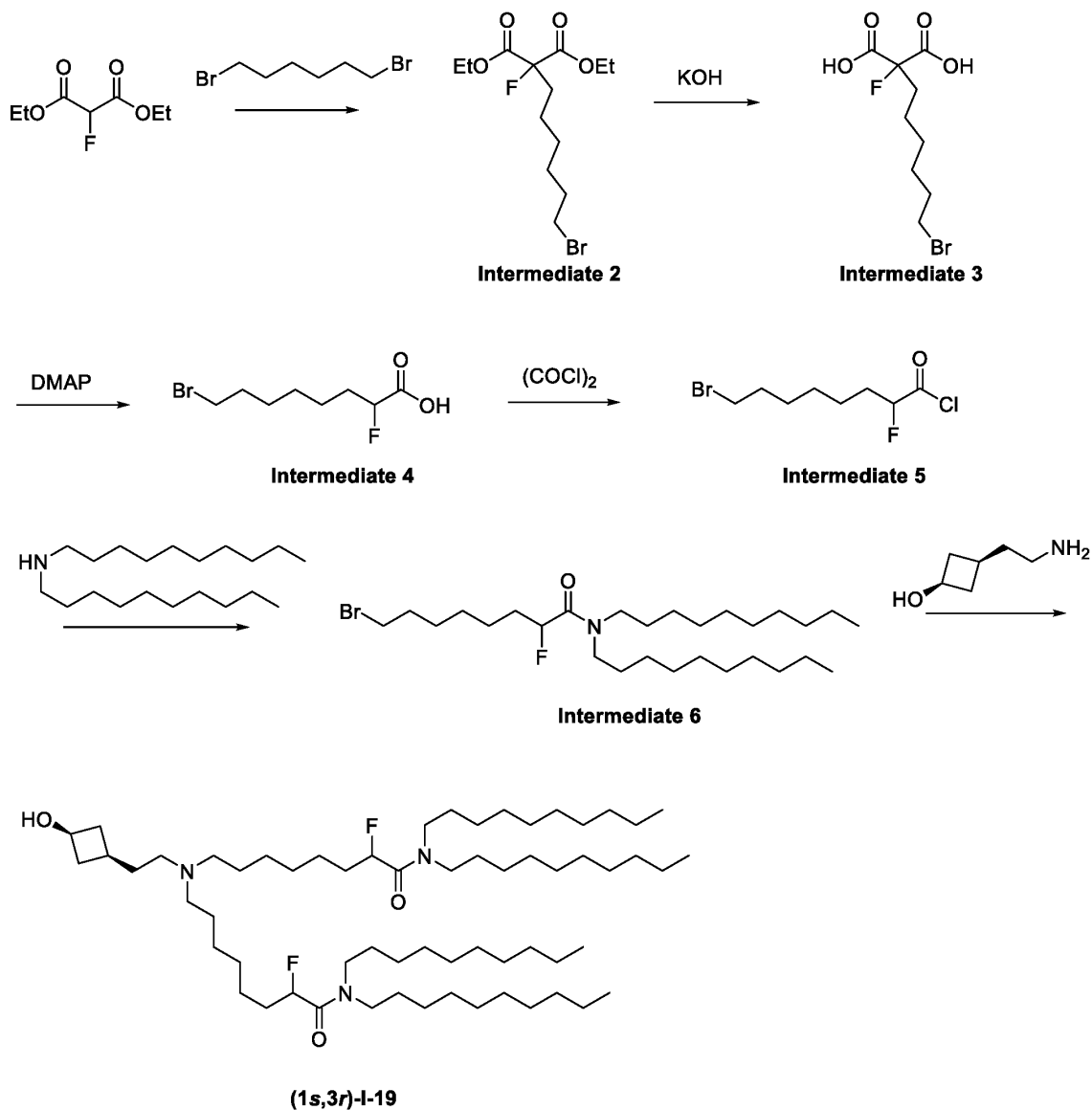
5

Synthesis of (*rac*)-(1*RS*,2*SR*)-I-18

A mixture of Compound **I-18** enantiomers was synthesized according to **General Procedure A** using a mixture of (1*R*,2*S*)-2-aminocyclobutan-1-ol hydrochloride and (1*S*,2*R*)-2-aminocyclobutan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) followed by a second purification via reverse phase flash chromatography 50% to 100% MeOH in water with 0.1% TFA) followed by a third purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (yield 15%). ¹H NMR (400 MHz, CDCl₃) δ 4.08 - 4.00 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 3.12 - 3.02 (m, 1H), 2.51 - 2.30 (m, 4H), 2.30 - 2.22 (m, 4H), 2.12 (p, *J* = 10.0 Hz, 1H), 2.01 - 1.89 (m, 1H), 1.87 - 1.76 (m, 1H), 1.72 - 1.05 (m, 90H), 0.92 - 0.83 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₀H₁₁₉N₃O₃ = 929.9. Found [M+H]⁺ = 931.2.

15

EXAMPLE 32

8,8'-((2-((1*S*,3*R*)-3-HYDROXYCYCLOBUTYL)ETHYL)AZANEDIYL)BIS(*N*,*N*-DIDECYL-2-FLUOROOCETANAMIDE) (COMPOUND (1*S*,3*R*)-I-19)

5 Synthesis of Intermediate 2

A mixture of diethyl 2-fluoromalonate (56.1 mmol, 10.0 g), 1,6-dibromohexane (168 mmol, 26 mL), and sodium methoxide (61.7 mmol, 3.3 g) in EtOH (110 mL) was stirred at room temperature for 24 h. The reaction mixture was concentrated, and the crude material was partitioned between DCM and water. The organic layer was separated, dried over Na₂SO₄,

filtered, and concentrated. Purification via automated flash chromatography (0% to 25% EtOAc in hexanes) gave Intermediate 2 (11.7 g, 61%).

Synthesis of Intermediate 3

A mixture of Intermediate 2 (5.9 mmol, 2.0 g) and KOH (11.8 mmol, 660 mg) in MeOH (20 mL), THF (2 mL), and water (4 mL) was stirred at room temperature for 3 h. The reaction mixture was concentrated, and the crude material was diluted with 0.1M NaOH (20 mL). The aqueous layer was washed with DCM (3 x 10 mL), acidified with 1M HCl, then extracted with EtOAc (2 x 20 mL). The combined EtOAc layers were dried over Na₂SO₄, filtered, and concentrated to give Intermediate 3 (1.57 g, 94%) which was used in the next step without further purification.

Synthesis of Intermediate 4

A mixture of Intermediate 3 (4.1 mmol, 1.2 g) and DMAP (cat.) in DMF (3 mL), was heated at 180 °C for 12 min. The reaction mixture was partitioned between EtOAc and 1M HCl. The organic layer was separated, dried over Na₂SO₄, filtered, and concentrated to give Intermediate 4 (960 mg, 98%) which was used in the next step without further purification.

Synthesis of Intermediate 5

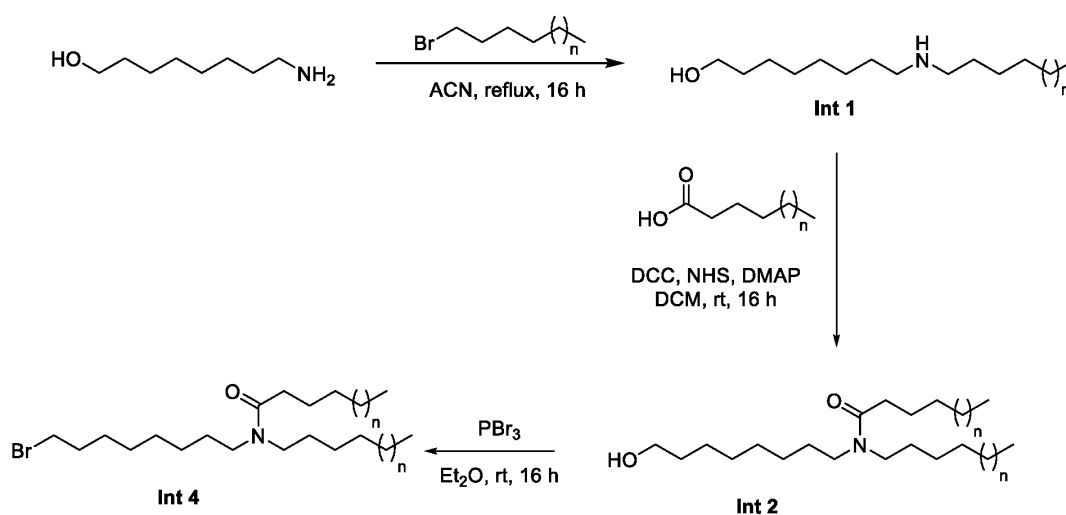
A mixture of Intermediate 4 (4.0 mmol, 960 mg), oxalyl chloride (12 mmol, 1.0 mL), and DMF (cat.) in DCM (10 mL) was stirred at room temperature for 20 min. The reaction mixture was concentrated to give Intermediate 5 which was used in the next step without further purification.

Synthesis of Intermediate 6

To mixture of didecylamine (4.0 mmol, 1.2 g), triethylamine (24 mmol, 3.4 mL), and DMAP (cat.) in DCM (10 mL) was added a solution of crude **Intermediate 5** (4.0 mmol) in DCM (5 mL). The reaction mixture was stirred at room temperature for 1 h. The reaction was concentrated and purified via automated flash chromatography (5% to 25% EtOAc in hexanes) gave **Intermediate 6** (1.2 g, 58% over 2 steps).

Synthesis of (1*S*,3*R*)-I-19

Compound (1*S*,3*R*)-I-19 was synthesized according to **General Procedure A** using **Intermediate 6** and (1*R*,3*S*)-3-(2-aminoethyl)cyclobutan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (yield 63%). ¹H NMR (400 MHz, CDCl₃) δ 5.03 (ddd, *J* = 49.4, 8.5, 4.3 Hz, 2H), 4.14 – 4.04 (m, 1H), 3.38 - 3.12 (m, 8H), 2.51 - 2.42 (m, 2H), 2.39 - 2.25 (m, 5H), 2.01 – 1.15 (m, 87H), 0.92 - 0.83 (m, 12H). *m/z* calcd. for Chemical Formula: C₆₂H₁₂₁F₂N₃O₃ = 993.9. Found [M+H]⁺ = 995.0.

EXAMPLE 33**10 SYNTHETIC PATHWAY FOR COMPOUNDS (1*R*,3*R*)-I-22 & (1*S*,3*S*)-I-22****General procedure for the preparation of Int 1**

To a solution of 8-aminooctanol (1.0 eq) in ACN (7 mL/mmol) was added appropriate bromide (1.0 eq) and the reaction mixture was refluxed overnight under N₂. Then, the reaction mixture was concentrated to afford the crude product. The solid crude product was purified by column chromatography (DCM/3% NH₃ in MeOH, 100:0 to 80:20).

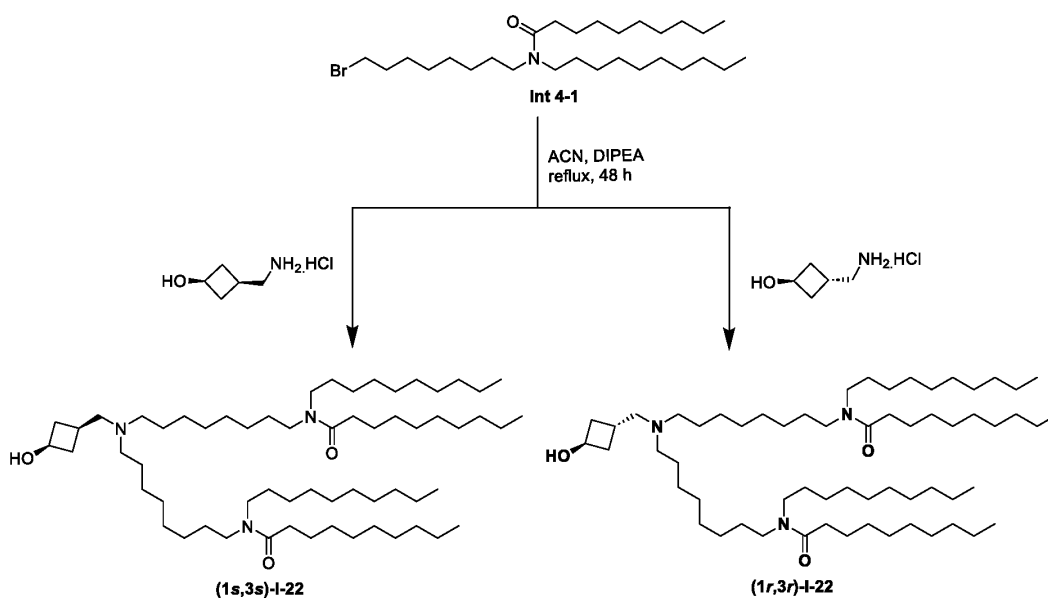
General procedure for the preparation of Int 2

To a solution of acid (0.9 eq) in DCM (2.5 mL/mmol) were added N-hydroxysuccinimide (0.9 eq), 4-dimethylaminopyridine (0.9 eq) and dicyclohexylcarbodiimide (0.9 eq) at RT under N₂. The resulting mixture was stirred at RT until complete conversion. The precipitate was filtered off, and the filtrate was added dropwise to a solution of the Int 1 (1.0 eq) in DCM

(2.3 mL/mmol) at RT. The reaction mixture was stirred overnight at RT under N₂. Then, the organic phase was washed with HCl (aq., 1 mol/L), Na₂CO₃ (aq.), and dried over Na₂SO₄. The organic phase was concentrated. The pale-yellow solid was purified by column chromatography (Hex/EtOAc, 100:0 to 0:100).

5 General procedure for the preparation of Int 4

To a solution of **Int 2** (1.0 eq) in diethyl ether (8.0 mL/mmol) was added PBr₃ (2-3 eq) dropwise at 0 °C under N₂. The reaction mixture was slowly warmed up to RT and stirred at RT under N₂ until the completion of the reaction. Then, ice-cooled water was added gradually until a clear solution was obtained. The aqueous phase was extracted with ethyl acetate three times,
 10 dried over Na₂SO₄, and concentrated under reduced pressure. The crude product was purified by column chromatography (Hex/EtOAc, 100 to 0 to 90 to 10).



Synthesis of N-(8-bromooctyl)-N-decyldecanamide (Int 4-1)

Int 4-1 was prepared following the general procedure for the preparation **Int 4**.

15 General procedure for the preparation of the compounds (1R,3R)-I-22 and (1S,3S)-I-22

To a solution of **Int 4-1** (1.5 eq) in ACN (5.5 mL/mmol) were added DIPEA (3.8 eq.) and the desired alkylation reagent (1.0 eq). The reaction was carried out in a sealed tube at 80 °C for 24 h. Then, more **Int 4-1** (0.5 eq) in ACN (2.0 mL/mmol) was added to the reaction mixture and stirring continued for another 24 h at 80 °C. The reaction mixture was then cooled to RT and

concentrated under reduced pressure. The crude product was purified by column chromatography (Hex/1% NEt₃ in EtOAc, 95:5 to 0:100).

Synthesis of *N,N'*-((((1*S*,3*S*)-3-hydroxycyclobutyl)methyl)azanediyl)bis(octane-8,1-diyl))bis(*N*-decyldecanamide) ((1*S*,3*S*)-I-22)

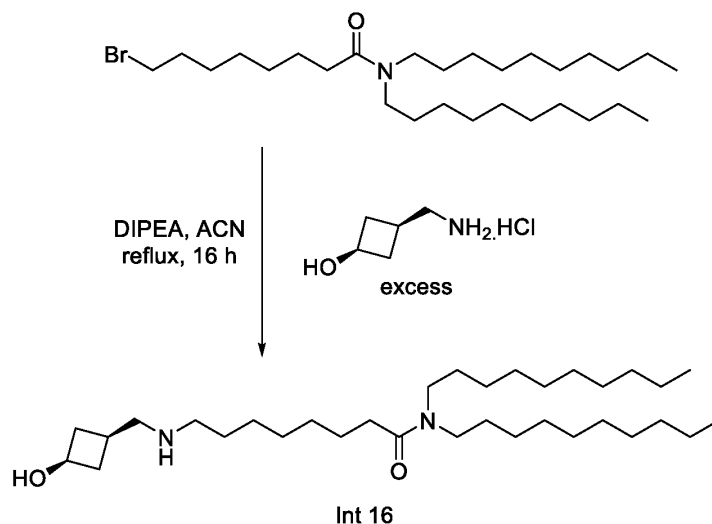
5 Compound **(1*S*,3*S*)-I-22** was prepared according to the general procedure from **Int 4-1** (0.5 g, 0.994 mmol), (1*S*, 3*S*)-3-(aminomethyl) cyclobutan-1-ol hydrochloride (0.047 g, 0.338 mmol) and DIPEA (0.83 mL, 5.97 mmol). The product was obtained as a colorless oil (0.2 g, 0.211 mmol, 21.3 %). ¹H NMR (600 MHz, Chloroform-*d*) δ 4.12 (p, *J* = 7.4 Hz, 1H), 3.31 – 3.24 (t, *J* = 8.5 Hz, 4H), 3.19 (t, *J* = 6.3 Hz, 4H), 2.45 (m, 4H), 2.35 (m, 4H), 2.29 – 2.23 (t, *J* = 7.1 Hz, 4H), 1.89 (m, 1H), 1.63 (m, 4H), 1.51 (m, 10H), 1.39 (m, 4H), 1.35 – 1.19 (m, 70H), 0.87 (t, *J* = 6.0 Hz, 12H). ESI-MS: MW for C₆₁H₁₂₁N₃O₃ [M+H]⁺ calc. 944.95, found 945.09.

Synthesis of *N,N'*-((((1*R*,3*R*)-3-hydroxycyclobutyl)methyl)azanediyl)bis(octane-8,1-diyl))bis(*N*-decyldecanamide) ((1*R*,3*R*)-I-22)

15 Compound **(1*R*,3*R*)-I-22** was prepared according to the general procedure from **Int 4-1** (0.5 g, 0.994 mmol), (1*R*, 3*R*)-3-(aminomethyl)cyclobutan-1-ol hydrochloride (0.047 g, 0.338 mmol) and DIPEA (0.83 mL, 5.97 mmol). The product was obtained as a colorless oil (0.22 g, 0.233 mmol, 23.4 %). ¹H NMR (600 MHz, CDCl₃) δ 4.41 – 4.33 (p, *J* = 7.4 Hz, 1H), 3.28 (t, *J* = 8.5 Hz, 4H), 3.19 (t, *J* = 6.3 Hz, 4H), 2.39 (m, 3H), 2.37 – 2.30 (m, 4H), 2.26 (t, *J* = 7.1 Hz, 4H), 2.07 (m, 2H), 2.03 (m, 2H), 1.70 – 1.57 (m, 7H), 1.57 – 1.45 (m, 8H), 1.37 (m, 4H), 1.34 – 1.21 (m, 70H), 0.90 – 0.85 (t, *J* = 6.0 Hz, 12H). ESI-MS: MW for C₆₁H₁₂₁N₃O₃ [M+H]⁺ calc. 944.95, found 945.02.

EXAMPLE 34

N-DECYL-N-(8-((8-(DIDECYLAMINO)-8-OXOOCTYL) (((1S,3S)-3-HYDROXYCYCLOBUTYL) METHYL) AMINO) OCTYL) DECANAMIDE
(COMPOUND (1*S*,3*S*)-I-21)

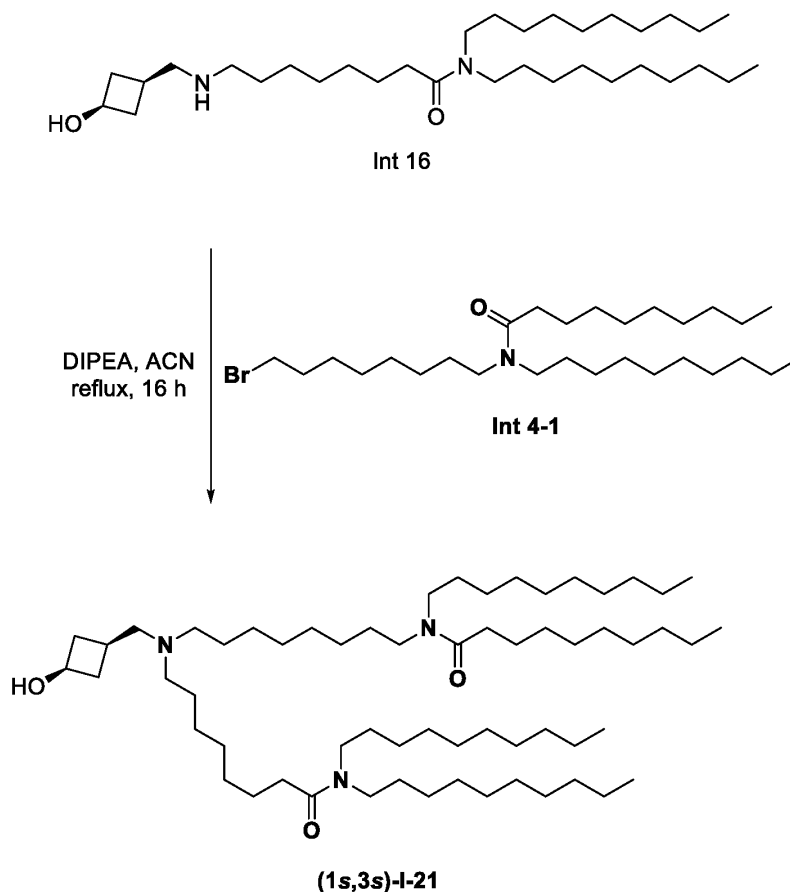


5

Synthesis of N,N-didecyl-8-((((1*S*,3*S*)-3-hydroxycyclobutyl)methyl)amino)octanamide (Int-16)

N,N-didecyl-8-((((1*S*,3*S*)-3-hydroxycyclobutyl)methyl)amino)octanamide was prepared from 8-bromo-N,N-didecyloctanamide (example 5, 0.4 g, 0.796 mmol), (1*S*, 3*S*)-3-aminomethyl cyclobutan-1-ol hydrochloride (0.384 g, 2.785 mmol) and DIPEA (0.44 mL, 3.183 mmol). The product was obtained as a colorless oil (0.3 g, 0.574 mmol, 72%). ESI-MS: MW for C₃₃H₆₆N₂O₂ [M+H]⁺ calc. 523.52, found 523.61.

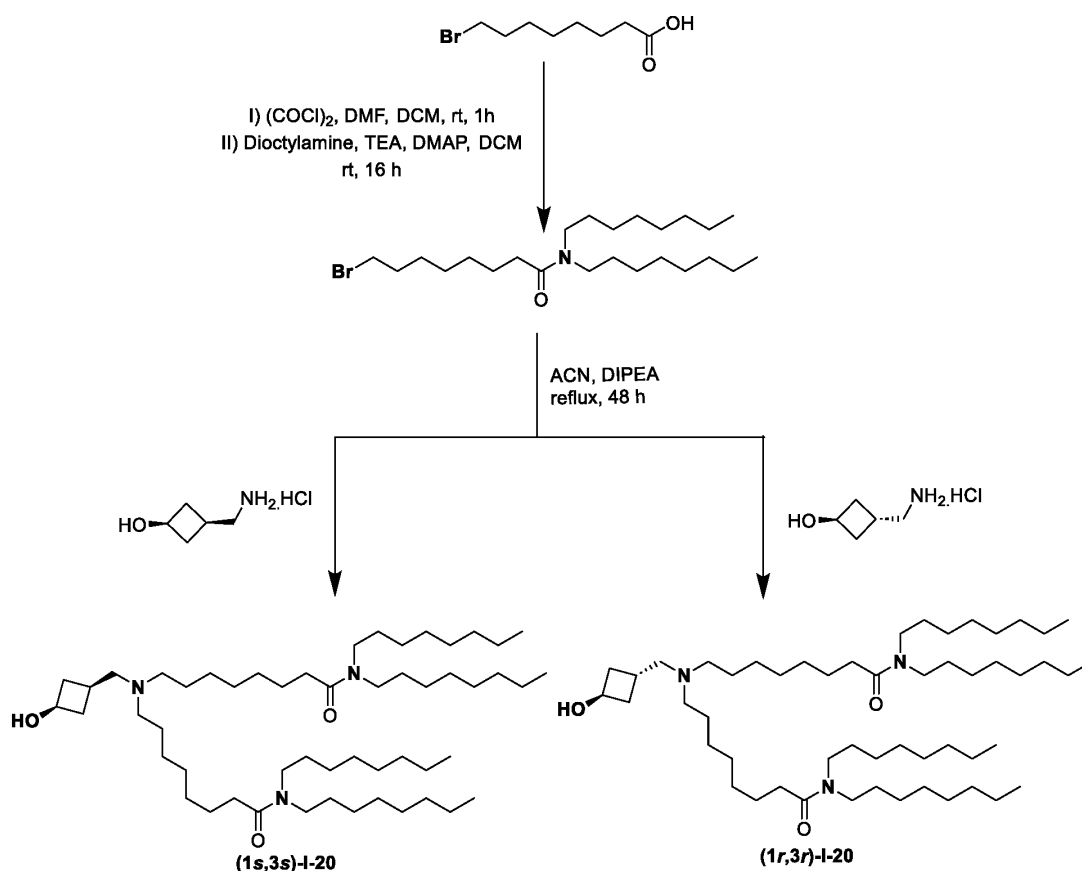
10



Synthesis of N-decyl-N-(8-((8-(didecylamino)-8-oxooctyl)((1S,3S)-3-hydroxycyclobutyl)methyl)amino)octyl)decanamide ((1S,3S)-I-21)

Compound (1S,3S)-I-21 was prepared from **Int 4-1** (0.32 g, 0.631 mmol), **Int 16** (0.3 g, 0.574 mmol) and DIPEA (0.4 mL, 2.296 mmol). The product was obtained as colorless oil (0.19 g, 0.201 mmol, 35%). ¹H NMR (600 MHz, CDCl₃) δ 4.25 – 4.15 (p, *J* = 7.4 Hz, 1H), 3.31 – 3.23 (t, *J* = 8.5 Hz, 4H), 3.19 (t, *J* = 6.3 Hz, 4H), 3.05 (m, 1H), 2.88 (m, 3H), 2.60 (m, 2H), 2.30 – 2.23 (m, 4H), 1.75 (s, 5H), 1.68 – 1.56 (m, 8H), 1.50 (m, 9H), 1.40 – 1.18 (m, 70H), 0.88 (t, *J* = 6.0 Hz, 12H). ESI-MS: MW for C₆₁H₁₂₁N₃O₃ [M+H]⁺ calc. 944.95, found 945.09.

EXAMPLE 35

SYNTHETIC PATHWAY FOR COMPOUNDS (1*R*,3*R*)-I-20 & (1*S*,3*S*)-I-20

Synthesis of 8-bromo-N,N-diethyloctanamide

8-Bromo-N,N-diethyloctanamide was prepared according to the procedure described in the example 5, from 8-bromooctanoic acid (2.00 g, 9.0 mmol), DMF (1 drop), oxalyl chloride (2.30 mL, 27 mmol), diethylamine (2.39 g, 9.9 mmol), triethylamine (7.50 mL, 54 mmol), DMAP (0.02 g, 0.16 mmol) and DCM (50 mL). The product 8-bromo-N,N-diethyloctanamide was obtained as colorless oil (3.12 g, 7.0 mmol, 78%). ESI-MS: MW for C₂₄H₄₉BrNO [M+H]⁺ calc. 446.30 & 448.29 found 446.36 & 448.38 (bromine pattern).

Synthesis of 8,8'-((((1*S*,3*S*)-3-hydroxycyclobutyl)methyl)azanediyl)bis(N,N-diethyloctanamide) ((1*S*,3*S*)-I-20)

Compound (1*S*,3*S*)-I-20 was prepared from 8-bromo-N,N-diethyloctanamide (0.447 g, 1.0 mmol), (1*S*, 3*S*)-3-(aminomethyl) cyclobutan-1-ol hydrochloride (0.050 g, 0.36 mmol) and DIPEA (1 mL, 6 mmol). The product (117 mg, 0.14 mmol, 39 %) was obtained as a yellowish

oil. ^1H NMR (600 MHz, CDCl_3) δ 4.11 (p, $J = 7.4$ Hz, 1H), 3.31 – 3.25 (m, 4H), 3.22 – 3.16 (m, 4H), 2.50 – 2.42 (m, 4H), 2.39 – 2.31 (m, 4H), 2.29 – 2.24 (m, 4H), 1.95 – 1.85 (m, 1H), 1.73 – 1.58 (m, 7H), 1.57 – 1.45 (m, 11H), 1.43 – 1.36 (m, 4H), 1.35 – 1.20 (m, 55H), 0.88 (dt, $J = 9.4$, 7.0 Hz, 12H). ESI-MS: MW for $\text{C}_{53}\text{H}_{105}\text{N}_3\text{O}_3$ $[\text{M}+\text{H}]^+$ calc. 832.83, found 832.85.

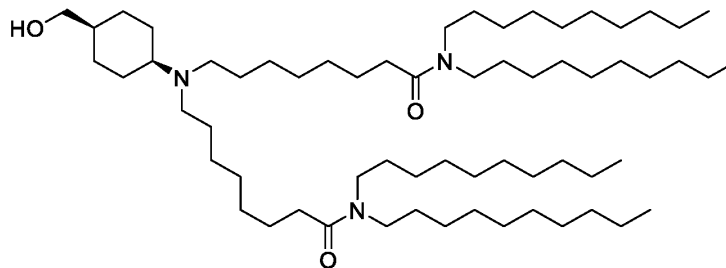
5 Synthesis of 8,8'-((((1R,3R)-3-hydroxycyclobutyl)methyl)azanediyl)bis(N,N-dioctyloctanamide) ((1R,3R)-I-20)

Compound (1R,3R)-I-20 was prepared from 8-bromo-N,N-dioctyloctanamide (0.447 g, 1.0 mmol), (1R, 3R)-3-(aminomethyl) cyclobutan-1-ol hydrochloride (0.050 g, 0.36 mmol) and DIPEA (1 mL, 6 mmol). The product (182 mg, 0.22 mmol, 60 %) was obtained as a yellowish oil. ^1H NMR (600 MHz, CDCl_3) δ 4.37 (p, $J = 7.4$ Hz, 1H), 3.32 – 3.24 (m, 4H), 3.21 – 3.15 (m, 4H), 2.38 (s, 3H), 2.36 – 2.31 (m, 4H), 2.30 – 2.23 (m, 4H), 2.10 – 2.04 (m, 2H), 2.04 – 1.96 (m, 3H), 1.69 – 1.57 (m, 8H), 1.56 – 1.46 (m, 8H), 1.38 (dt, $J = 14.7$, 7.5 Hz, 4H), 1.35 – 1.19 (m, 54H), 0.88 (dt, $J = 9.2$, 7.0 Hz, 12H). ESI-MS: MW for $\text{C}_{53}\text{H}_{105}\text{N}_3\text{O}_3$ $[\text{M}+\text{H}]^+$ calc. 832.83, found 832.85

15

EXAMPLE 36

8,8'-((((1S,4S)-4-(HYDROXYMETHYL)CYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE) (COMPOUND (1S,4S)-I-23)



20 Synthesis of (1S,4S)-I-23

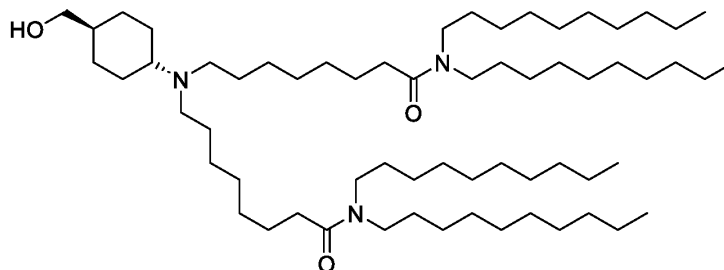
Compound **(1S,4S)-I-23** was synthesized according to **General Procedure A** using ((1s,4s)-4-aminocyclohexyl)methanol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et_3N) gave the titled compound (55 mg, 21%). ^1H NMR (400 MHz, CDCl_3) δ 3.60 (d, $J = 7.0$ Hz, 2H), 3.32 – 3.23 (m, 4H), 3.23 – 3.15 (m, 4H), 2.50 – 2.37 (m, 5H), 2.27 – 2.26 (m, 4H), 1.83 – 1.05 (m, 99H), 0.92 – 0.83 (m, 12H). ESI-MS: m/z calcd for $\text{C}_{63}\text{H}_{125}\text{N}_3\text{O}_3 = 972.0$, found $[\text{M}+\text{H}]^+ = 973.2$.

25

EXAMPLE 37

8,8'-(((1*R*,4*R*)-4-(HYDROXYMETHYL)CYCLOHEXYL)AZANEDIYL)BIS(*N*,*N*-
DIDECYLOCTANAMIDE)

(COMPOUND (1*R*,4*R*)-I-23)



5

Synthesis of (1*R*,4*R*)-I-23

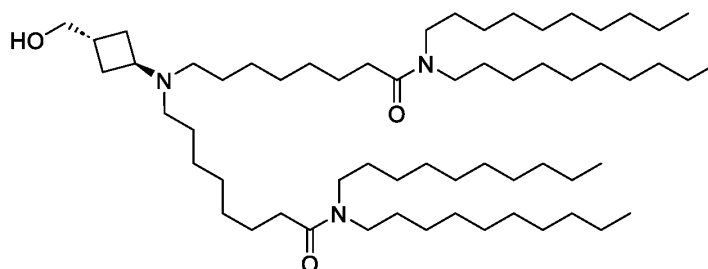
Compound (1*R*,4*R*)-I-23 was synthesized according to **General Procedure A** using ((1*r*,4*r*)-4-aminocyclohexyl)methanol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (165 mg, 63%). ¹H NMR (400 MHz, CDCl₃) δ 3.48 - 3.41 (m, 2H), 3.33 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.44 - 2.36 (m, 5H), 2.27 - 2.26 (m, 4H) 1.89 - 1.78 (m, 4H), 1.70 - 1.13 (m, 92H), 1.03 - 0.77 (m, 14H). ESI-MS: *m/z* calcd for C₆₃H₁₂₅N₃O₃ = 972.0, found [M+H]⁺ = 973.3.

15

EXAMPLE 38

8,8'-(((1*R*,3*R*)-4-(HYDROXYMETHYL)CYCLOBUTYL)AZANEDIYL)BIS(*N*,*N*-
DIDECYLOCTANAMIDE)

(COMPOUND (1*R*,3*R*)-I-24)

Synthesis of (1*R*,3*R*)-I-24

Compound (1*R*,3*R*)-I-24 was synthesized according to **General Procedure A** using ((1*r*,3*r*)-3-aminocyclobutyl)methanol. Purification via automated flash chromatography (5% to

20

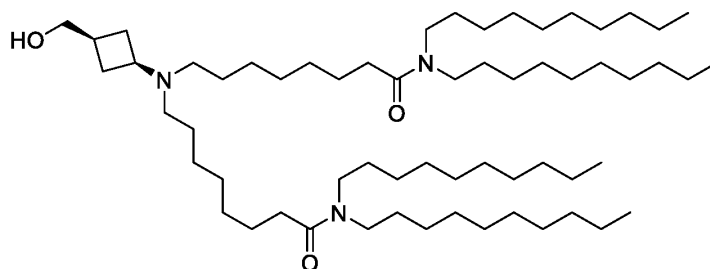
65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (198 mg, 77%). ¹H NMR (400 MHz, CDCl₃) δ 3.72 - 3.62 (m, 2H), 3.32 - 3.23 (m, 4H), 3.23 - 3.13 (m, 4H), 2.40 - 2.32 (m, 4H), 2.26 (t, *J* = 7.6 Hz, 5H), 2.09 - 1.95 (m, 2H), 1.93 - 1.84 (m, 2H), 1.69 - 1.04 (m, 88H), 0.92 - 0.81 (m, 12H). ESI-MS: *m/z* calcd for C₆₁H₁₂₁N₃O₃ = 943.9, found [M+H]⁺ = 945.2.

5

EXAMPLE 39

8,8'-(((1*S*,3*S*)-3-(HYDROXYMETHYL)CYCLOBUTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (1*S*,3*S*)-I-24)

**Synthesis of (1*S*,3*S*)-I-24**

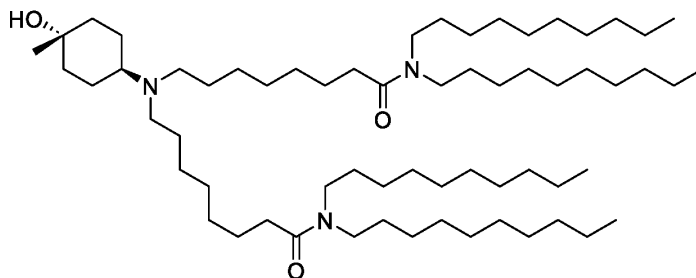
10 Compound **(1*S*,3*S*)-I-24** was synthesized according to **General Procedure A** using ((1*S*,3*S*)-3-aminocyclobutyl)methanol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (195 mg, 76%). ¹H NMR (400 MHz, CDCl₃) δ 3.58 (d, *J* = 4.1 Hz, 2H), 3.34 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.99 - 2.89 (m, 1H), 2.42 - 2.34 (m, 4H), 2.31 - 2.16 (m, 7H), 1.75 - 1.03 (m, 88H), 0.92 - 0.82 (m, 12H).

15 ESI-MS: *m/z* calcd for C₆₁H₁₂₁N₃O₃ = 943.9, found [M+H]⁺ = 945.2.

EXAMPLE 40

8,8'-(((1*R*,4*R*)-4-HYDROXY-4-METHYLCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (1*R*,4*R*)-I-25)



20

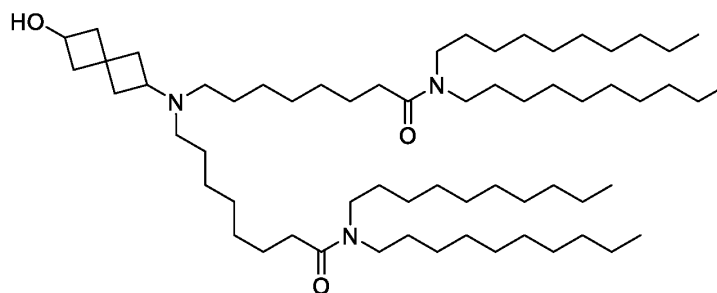
Synthesis of (1R,4R)-I-25

Compound **(1R,4R)-I-25** was synthesized according to **General Procedure A** using (1r,4r)-4-amino-1-methylcyclohexan-1-ol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (150 mg, 34%). ¹H NMR (400 MHz, CDCl₃) δ 3.32 – 3.23 (m, 4H), 3.23 – 3.15 (m, 4H), 2.56 – 2.35 (m, 5H), 2.31 – 2.22 (m, 4H), 1.80 – 1.01 (m, 89H), 0.93 - 0.79 (m, 12H). ESI-MS: *m/z* calcd for C₆₃H₁₂₅N₃O₃ = 972.0, found [M+H]⁺ = 973.2.

EXAMPLE 41

8,8'-((6-HYDROXYSPIRO[3.3]HEPTAN-2-YL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

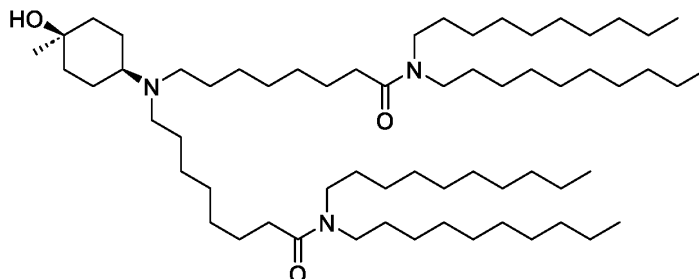
10

(COMPOUND I-36)**Synthesis of I-36**

Compound **I-36** was synthesized according to **General Procedure A** using 6-aminospiro[3.3]heptan-2-ol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (185 mg, 42%). ¹H NMR (400 MHz, CDCl₃) δ 4.22 - 4.12 (m, 1H), 3.32 – 3.23 (m, 4H), 3.23 – 3.14 (m, 4H), 2.98 – 2.87 (m, 1H), 2.48 - 2.37 (m, 1H), 2.35 - 2.20 (m, 9H), 2.13 - 1.97 (m, 2H), 1.94 – 1.79 (m, 4H), 1.69 – 1.02 (m, 87H), 0.92 - 0.84 (m, 12H). ESI-MS: *m/z* calcd for C₆₃H₁₂₃N₃O₃ = 970.0, found [M+H]⁺ = 971.2.

EXAMPLE 42

8,8'-(((1S,4S)-4-HYDROXY-4-METHYLCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1S,4S)-I-25)



5

Synthesis of (1S,4S)-I-25

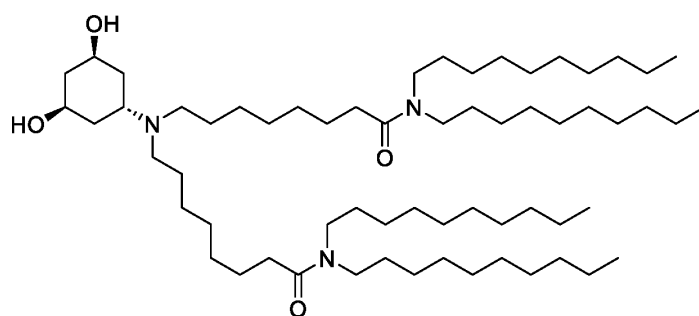
Compound (1S,4S)-I-25 was synthesized according to **General Procedure A** using (1S,4S)-4-amino-1-methylcyclohexan-1-ol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (145 mg, 33%). ¹H NMR (400 MHz, CDCl₃) δ 3.32 – 3.23 (m, 4H), 3.23 – 3.15 (m, 4H), 2.45 – 2.37 (m, 5H), 2.30 – 2.22 (m, 4H), 1.74 – 1.04 (m, 99H), 0.92 – 0.84 (m, 12H). ESI-MS: *m/z* calcd for C₆₃H₁₂₅N₃O₃ = 972.0, found [M+H]⁺ = 973.2.

10

EXAMPLE 43

8,8'-(((1R,3R,5S)-3,5-DIHYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)
(COMPOUND (1R,3R,5S)-I-34)

15



Synthesis of (1R,3R,5S)-I-34

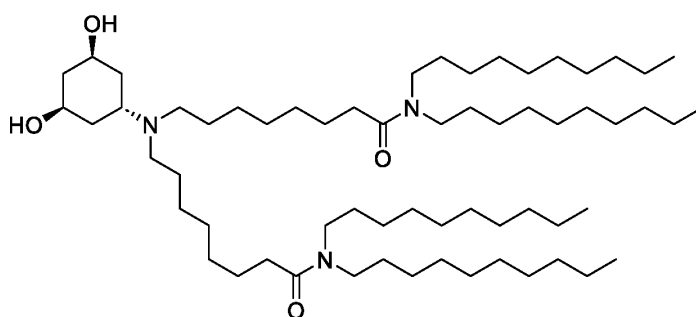
Compound (1R,3R,5S)-I-34 was synthesized according to **General Procedure A** using (1R,3S,5r)-5-aminocyclohexane-1,3-diol hydrochloride. Purification via automated flash

chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) followed by a second purification via automated flash chromatography (0% to 10% MeOH in chloroform) gave the titled compound (46 mg, 12%). ¹H NMR (400 MHz, CDCl₃) δ 3.80 - 3.60 (m, 2H), 3.31 - 3.15 (m, 8H), 3.05 - 2.36 (m, 5H), 2.34 - 2.04 (m, 8H), 2.01 - 1.06 (m, 93H), 0.92 - 0.84 (m, 12H). ESI-
 5 MS: *m/z* calcd for C₆₂H₁₂₃N₃O₄ = 974.0, found [M+H]⁺ = 975.2.

EXAMPLE 44

8,8'-(((1S,3R,5S)-3,5-DIHYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (1S,3R,5S)-I-34)



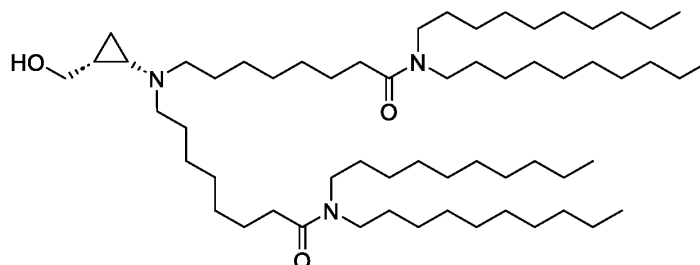
10 Synthesis of (1S,3R,5S)-I-34

Compound **(1S,3R,5S)-I-34** was synthesized according to **General Procedure A** using (1R,3S,5s)-5-aminocyclohexane-1,3-diol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (85 mg, 22%). ¹H NMR (400 MHz, CDCl₃) δ 4.28 (s, 2H), 3.74 (s, 2H), 3.51 - 3.34 (m, 1H), 3.31 - 3.15
 15 (m, 8H), 2.46 - 2.38 (m, 4H), 2.27 (t, *J* = 7.4 Hz, 4H), 2.09 - 1.97 (m, 3H), 1.72 - 1.02 (m, 92H), 0.92 - 0.83 (m, *J* = 6.9, 2.9 Hz, 12H). ESI-MS: *m/z* calcd for C₆₂H₁₂₃N₃O₄ = 974.0, found [M+H]⁺ = 975.2.

EXAMPLE 45

8,8'-(((1S,2R)-2-(HYDROXYMETHYL)CYCLOPROPYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (1S,2R)-I-46)



5

Synthesis of (1S,2R)-I-46

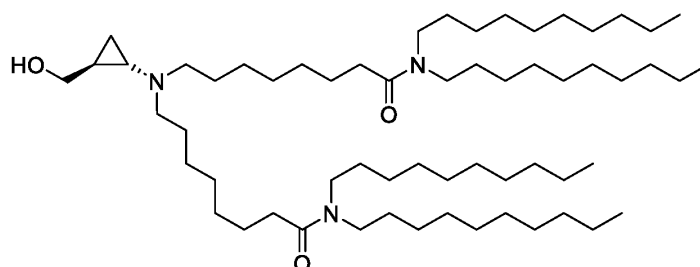
Compound (**1S,2R**)-**1-46** was synthesized according to **General Procedure A** using ((1R,2S)-2-aminocyclopropyl)methanol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) followed by a second purification via automated flash chromatography (0% to 10% MeOH in chloroform) gave the titled compound (107 mg, 29%). ¹H NMR (400 MHz, CDCl₃) δ 4.14 – 4.07 (m, 1H), 3.98 - 3.88 (m, 1H), 3.32 – 3.22 (m, 4H), 3.23 – 3.14 (m, 4H), 2.60 – 2.47 (m, 4H), 2.31 – 2.23 (m, 4H), 2.05 – 1.98 (m, 1H), 1.72 – 1.00 (m, 90H), 0.96 - 0.84 (m, 14H), 0.70 - 0.59 (m, 1H). ESI-MS: *m/z* calcd for C₆₀H₁₁₉N₃O₃ = 929.9, found [M+H]⁺ = 931.1.

15

EXAMPLE 46

8,8'-(((1S,2S)-2-(HYDROXYMETHYL)CYCLOPROPYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (1S,2S)-I-46)

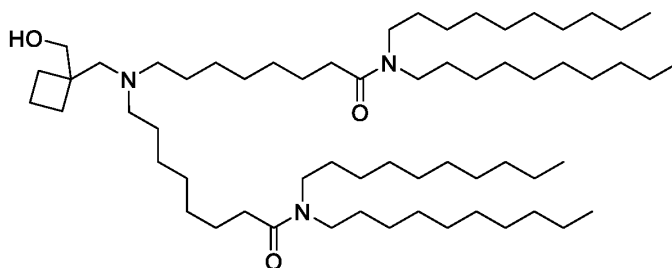


Synthesis of (1s,2s)-I-46

Compound **(1S,2S)-I-46** was synthesized according to **General Procedure A** using ((1S,2S)-2-aminocyclopropyl)methanol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) followed by a second purification via automated flash chromatography (0% to 10% MeOH in chloroform) gave the titled compound (65 mg, 18%). ¹H NMR (400 MHz, CDCl₃) δ 3.57 – 3.37 (m, 2H), 3.32 – 3.23 (m, 4H), 3.23 – 3.15 (m, 4H), 2.56 – 2.51 (m, 4H), 2.27 (t, *J* = 7.5 Hz, 4H), 1.89 – 1.01 (m, 91H), 0.92 – 0.83 (m, 12H), 0.73 – 0.60 (m, 1H), 0.51 – 0.38 (m, 1H). ESI-MS: *m/z* calcd for C₆₀H₁₁₉N₃O₃ = 929.9, found [M+H]⁺ = 931.1.

EXAMPLE 47

8,8'-(((1-(HYDROXYMETHYL)CYCLOBUTYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

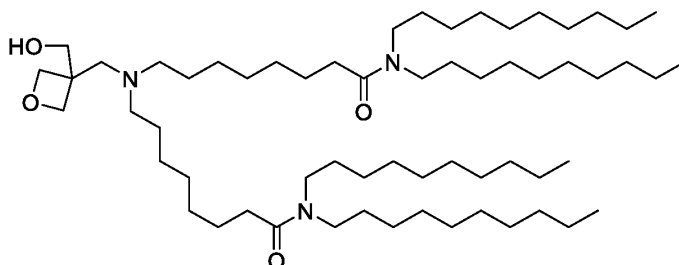
(COMPOUND I-29)**Synthesis of I-29**

Compound **I-29** was synthesized according to **General Procedure A** using (1-(aminomethyl)cyclobutyl)methanol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (168 mg, 44%). ¹H NMR (400 MHz, CDCl₃) δ 6.17 (broad s, 1H), 3.75 (s, 2H), 3.32 – 3.23 (m, 4H), 3.23 – 3.14 (m, 4H), 2.55 (s, 2H), 2.41 – 2.31 (m, 4H), 2.31 – 2.20 (m, 4H), 2.03 – 1.89 (m, 1H), 1.87 – 1.74 (m, 5H), 1.69 – 1.04 (m, 90H), 0.92 – 0.83 (m, 12H). ESI-MS: *m/z* calcd for C₆₂H₁₂₃N₃O₃ = 958.0, found [M+H]⁺ = 959.2.

EXAMPLE 48

8,8'-(((3-(HYDROXYMETHYL)OXETAN-3-YL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND I-41)



5

Synthesis of I-41

Compound **I-41** was synthesized according to **General Procedure A** using (3-(aminomethyl)oxetan-3-yl)methanol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (150 mg, 39%). ¹H NMR (400 MHz, CDCl₃) δ 5.69 (broad s, 1H), 4.47 (d, *J* = 6.0 Hz, 2H), 4.37 (d, *J* = 6.0 Hz, 2H), 4.04 (s, 2H), 3.32 – 3.24 (m, 4H), 3.23 – 3.15 (m, 4H), 2.84 (s, 2H), 2.36 – 2.22 (m, 8H), 1.72 – 0.99 (m, 90H), 0.92 – 0.83 (m, 12H). ESI-MS: *m/z* calcd for C₆₁H₁₂₁N₃O₄ = 959.9, found [M+H]⁺ = 961.2.

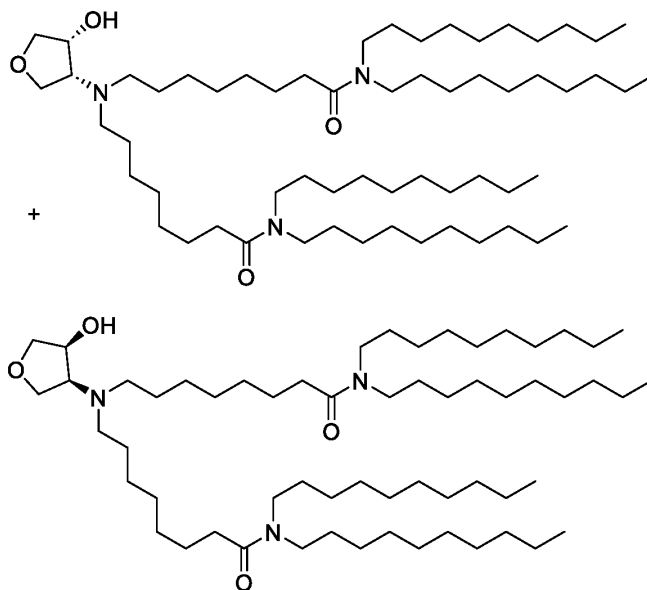
EXAMPLE 49

8,8'-(((3R,4R)-4-HYDROXYTETRAHYDROFURAN-3-YL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

8,8'-(((3S,4S)-4-HYDROXYTETRAHYDROFURAN-3-YL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

5

(COMPOUND (RAC)-(3RS,4RS)-I-40)



Synthesis of (rac)-(3RS,4RS)-I-40

- A mixture of Compound **I-40** enantiomers was synthesized according to **General**
- 10 **Procedure A** using a mixture of (3R,4R)-4-aminotetrahydrofuran-3-ol and (3S,4S)-4-aminotetrahydrofuran-3-ol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (94 mg, 25%). ¹H NMR (400 MHz, CDCl₃) δ 4.14 - 4.07 (m, 1H), 4.00 - 3.91 (m, 2H), 3.89 - 3.81 (m, 1H), 3.71 (dd, *J* = 9.7, 7.9 Hz, 1H), 3.32 - 3.23 (m, 4H), 3.21 - 3.17 (m, 4H), 3.16 - 3.07 (m, 1H), 2.58 - 2.42 (m, 4H), 2.31 - 2.23
- 15 (m, 4H), 1.73 - 1.05 (m, 86H), 0.92 - 0.81 (m, 12H). ESI-MS: *m/z* calcd for C₆₀H₁₁₉N₃O₄ = 945.9, found [M+H]⁺ = 947.2.

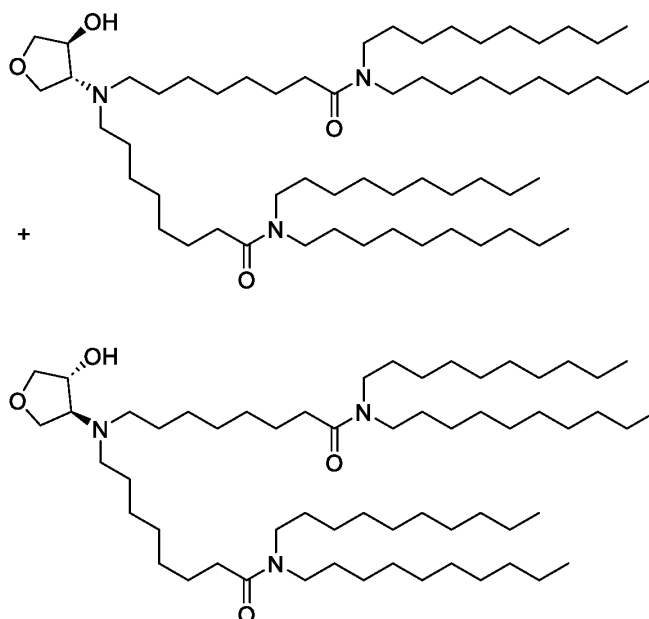
EXAMPLE 50

8,8'-(((3R,4S)-4-HYDROXYTETRAHYDROFURAN-3-YL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

8,8'-(((3S,4R)-4-HYDROXYTETRAHYDROFURAN-3-YL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

5

(COMPOUND (RAC)-(3RS,4SR)-I-40)



Synthesis of (rac)-(3RS,4SR)-I-40

A mixture of Compound **I-40** enantiomers was synthesized according to **General Procedure A** using a mixture of (3S,4R)-4-aminotetrahydrofuran-3-ol hydrochloride and (3R,4S)-4-aminotetrahydrofuran-3-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) followed by a second purification via automated flash chromatography (50% to 100% EtOAc in hexanes) gave the titled compound (90 mg, 24%). ¹H NMR (400 MHz, CDCl₃) δ 4.34 - 4.29 (m, 1H), 4.05 - 3.19 (m, 2H), 3.70 - 3.59 (m, 2H), 3.32 - 3.24 (m, 5H), 3.23 - 3.15 (m, 4H), 2.55 - 2.42 (m, 4H), 2.27 (t, *J* = 7.4 Hz, 4H), 1.97 - 1.00 (m, 87H), 0.92 - 0.81 (m, 12H). ESI-MS: *m/z* calcd for C₆₀H₁₁₉N₃O₄ = 945.9, found [M+H]⁺ = 947.2.

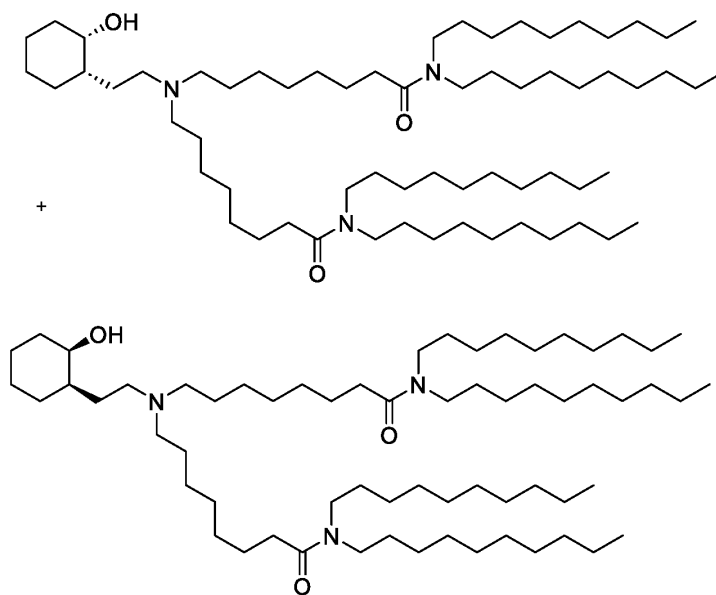
EXAMPLE 51

8,8'-((2-((1R,2R)-2-HYDROXYCYCLOHEXYL)ETHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

8,8'-((2-((1S,2S)-2-HYDROXYCYCLOHEXYL)ETHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

5

(COMPOUND (RAC)-(1RS,2RS)-I-31)



Synthesis of (rac)-(1RS,2RS)-I-31

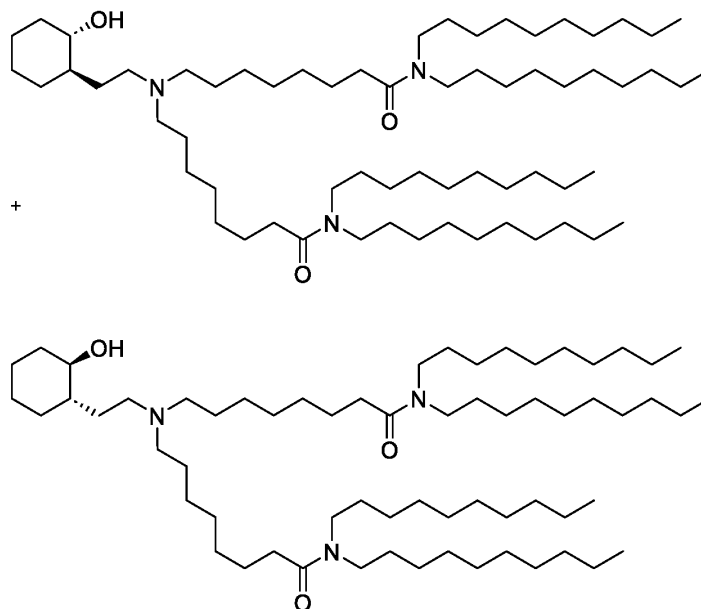
- A mixture of Compound **I-31** enantiomers was synthesized according to **General**
- 10 **Procedure B** using rac-(1R,2S)-2-(2-aminoethyl)cyclohexan-1-ol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (86 mg, 29%). ¹H NMR (400 MHz, CDCl₃) δ 7.95 (broad s, 1H), 3.32 – 3.23 (m, 4H), 3.23 – 3.14 (m, 4H), 3.10 – 3.00 (m, 1H), 2.60 – 2.40 (m, 4H), 2.36 – 2.22 (m, 6H), 2.02 - 1.93 (m, 1H), 1.77 – 0.98 (m, 96H), 0.92 - 0.83 (m, 12H). ESI-MS: *m/z* calcd for C₆₄H₁₂₇N₃O₃ = 986.0, found
- 15 [M+H]⁺ = 987.2.

EXAMPLE 52

8,8'-((2-((1R,2S)-2-HYDROXYCYCLOHEXYL)ETHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

8,8'-((2-((1S,2R)-2-HYDROXYCYCLOHEXYL)ETHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE) (COMPOUND (RAC)-(1RS,2SR)-I-31)

5



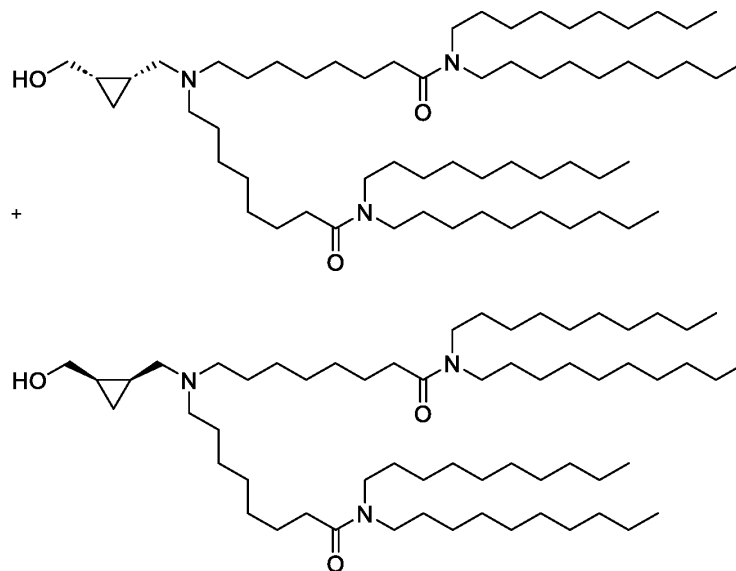
Synthesis of (rac)-(1RS,2SR)-I-31

A mixture of Compound **I-31** enantiomers was synthesized according to **General Procedure B** using rac-(1R,2R)-2-(2-aminoethyl)cyclohexan-1-ol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (92 mg, 25%). ¹H NMR (400 MHz, CDCl₃) δ 7.95 (broad s, 1H), 3.32 – 3.23 (m, 4H), 3.23 – 3.14 (m, 4H), 3.10 – 2.99 (m, 1H), 2.60 – 2.38 (m, 4H), 2.36 – 2.22 (m, 6H), 2.01 – 1.93 (m, 1H), 1.77 – 0.97 (m, 97H), 0.92 – 0.83 (m, 12H). ESI-MS: *m/z* calcd for C₆₄H₁₂₇N₃O₃ = 986.0, found [M+H]⁺ = 987.2.

EXAMPLE 53

8,8'-((((1R,2S)-2-(HYDROXYMETHYL)CYCLOPROPYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

5 8,8'-((((1S,2R)-2-(HYDROXYMETHYL)CYCLOPROPYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE) (COMPOUND (RAC)-(1RS,2SR)-I-45)



Synthesis of (rac)-(1RS,2SR)-I-45

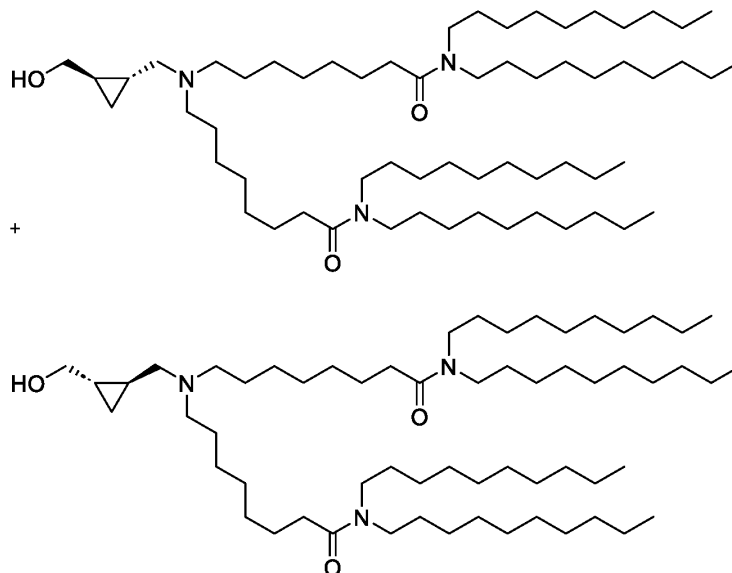
A mixture of Compound **I-45** enantiomers was synthesized according to **General Procedure B** using rac-((1R,2S)-2-(aminomethyl)cyclopropyl)methanol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (112 mg, 31%). ¹H NMR (400 MHz, CDCl₃) δ 6.19 (broad s, 1H), 4.03 - 3.91 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.12 (m, 4H), 3.12 - 3.00 (m, 1H), 2.75 - 2.58 (m, 3H), 2.34 - 2.13 (m, 7H), 1.73 - 0.96 (m, 88H), 0.92 - 0.75 (m, 13H), 0.26 - 0.13 (m, 1H). ESI-MS: *m/z* calcd for C₆₁H₁₂₁N₃O₃ = 943.9, found [M+H]⁺ = 945.2.

EXAMPLE 54

8,8'-((((1R,2R)-2-(HYDROXYMETHYL)CYCLOPROPYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

5

8,8'-((((1S,2S)-2-(HYDROXYMETHYL)CYCLOPROPYL)METHYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE) (COMPOUND (*RAC*)-(1*RS*,2*RS*)-I-45)



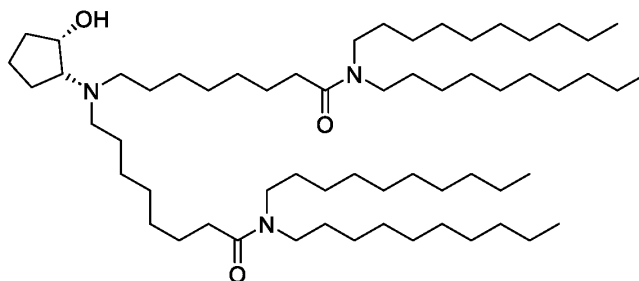
Synthesis of (*rac*)-(1*RS*,2*RS*)-I-45

A mixture of Compound **I-45** enantiomers was synthesized according to **General Procedure B** using *rac*-((1*R*,2*R*)-2-(aminomethyl)cyclopropyl)methanol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (110 mg, 31%). ¹H NMR (400 MHz, CDCl₃) δ 3.54 – 3.42 (m, 2H), 3.32 – 3.23 (m, 4H), 3.23 – 3.15 (m, 4H), 2.51 – 2.39 (m, 5H), 2.34 – 2.22 (m, 5H), 2.00 – 1.02 (m, 86H), 0.98 – 0.71 (m, 14H), 0.50 – 0.41 (m, 1H), 0.040 – 0.31 (m, 1H). ESI-MS: *m/z* calcd for C₆₁H₁₂₁N₃O₃ = 943.9, found [M+H]⁺ = 945.2.

EXAMPLE 55

8,8'-(((1R,2S)-2-HYDROXYCYCLOPENTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (1R,2S)-I-48)



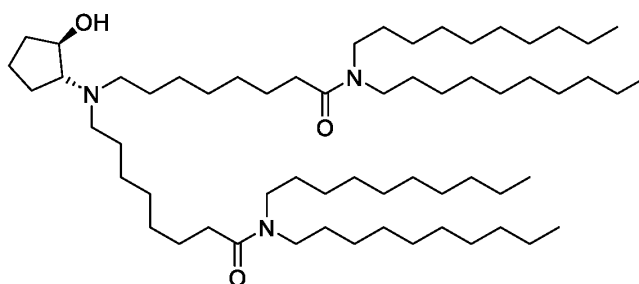
5 Synthesis of (1R,2S)-I-48

Compound (1R,2S)-I-48 was synthesized according to **General Procedure B** using (1S,2R)-2-aminocyclopentan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) followed by a second flash chromatography (100% EtOAc) gave the titled compound (61 mg, 18%). ¹H NMR (400 MHz, CDCl₃) δ 4.05 - 3.94 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.75 - 2.66 (m, 1H), 2.62 - 2.48 (m, 4H), 2.31 - 2.22 (m, 4H), 1.92 - 1.01 (m, 100H), 0.92 - 0.83 (m, 12H). ESI-MS: *m/z* calcd for C₆₁H₁₂₁N₃O₃ = 943.9, found [M+H]⁺ = 945.3.

EXAMPLE 56

8,8'-(((1R,2R)-2-HYDROXYCYCLOPENTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (1R,2R)-I-48)



Synthesis of (1R,2R)-I-48

Compound (1R,2R)-I-48 was synthesized according to **General Procedure B** using (1R,2R)-2-aminocyclopentan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) gave the titled compound (111

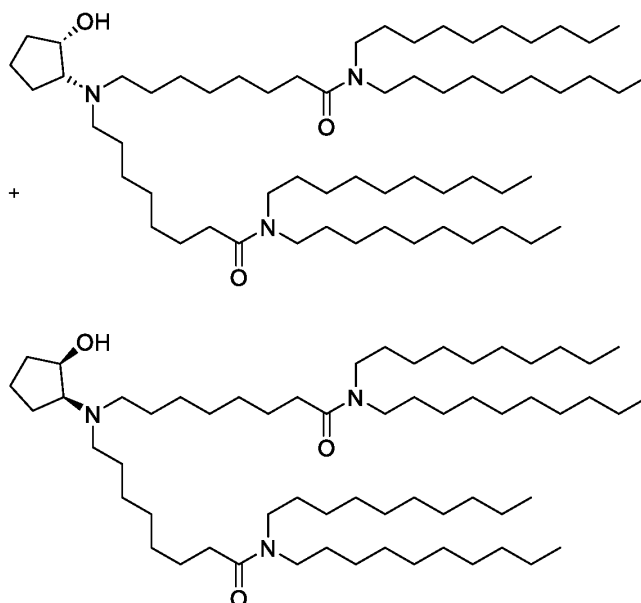
mg, 33%). ^1H NMR (400 MHz, CDCl_3) δ 3.95 - 3.83 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.87 - 2.74 (m, 1H), 2.55 - 2.20 (m, 9H), 1.95 - 1.85 (m, 1H), 1.81 - 1.02 (m, 97H), 0.92 - 0.83 (m, 12H). ESI-MS: m/z calcd for $\text{C}_{61}\text{H}_{121}\text{N}_3\text{O}_3$ = 943.9, found $[\text{M}+\text{H}]^+$ = 945.3.

EXAMPLE 57

5 **8,8'-(((1R,2S)-2-HYDROXYCYCLOPENTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)**

8,8'-(((1S,2R)-2-HYDROXYCYCLOPENTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (RAC)-(1RS,2SR)-I-48)



Synthesis of (rac)-(1RS,2SR)-I-48

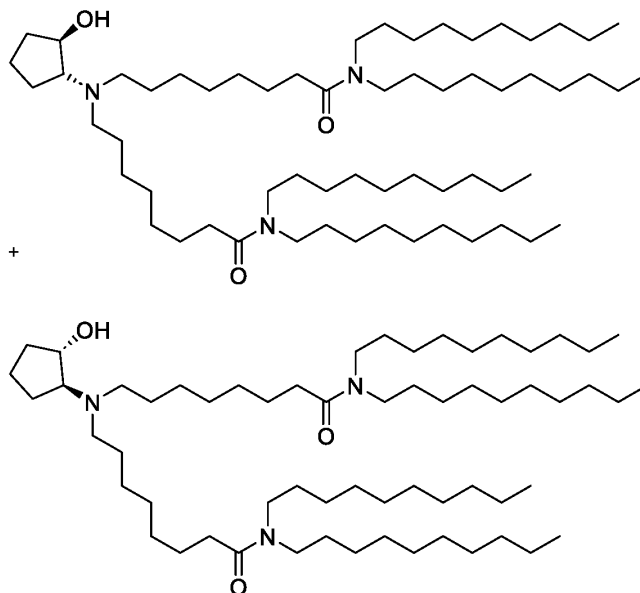
10 A mixture of Compound **I-48** enantiomers was synthesized according to **General Procedure A** using rac-(1R,2S)-2-aminocyclopentan-1-ol hydrochloride. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N) followed by a second flash chromatography (50% to 100% EtOAc in hexanes) gave the titled compound (68 mg, 20%). ^1H NMR (400 MHz, CDCl_3) δ 4.04 - 3.95 (m, 1H), 3.31 - 3.24 (m, 4H), 3.23 - 3.15 (m, 4H), 2.73 - 2.67 (m, 1H), 2.62 - 2.49 (m, 4H), 2.31 - 2.22 (m, 4H), 1.91 - 1.00 (m, 96H), 0.92 - 0.83 (m, 12H). ESI-MS: m/z calcd for $\text{C}_{61}\text{H}_{121}\text{N}_3\text{O}_3$ = 943.9, found $[\text{M}+\text{H}]^+$ = 945.3.

15

EXAMPLE 58

8,8'-(((1R,2R)-2-HYDROXYCYCLOPENTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

8,8'-(((1S,2S)-2-HYDROXYCYCLOPENTYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND (*RAC*)-(1*RS*,2*RS*)-I-48)

5

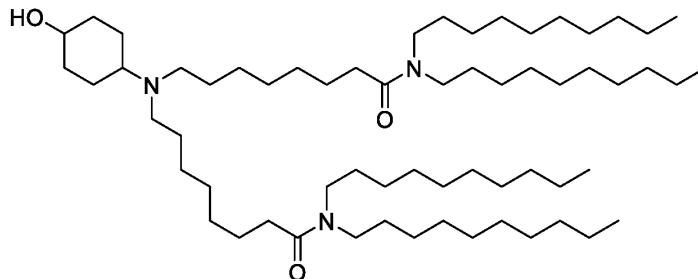
Synthesis of (*rac*)-(1*RS*,2*RS*)-I-48

A mixture of Compound **I-48** enantiomers was synthesized according to **General Procedure A** using *rac*-(1*R*,2*R*)-2-aminocyclopentan-1-ol. Purification via automated flash chromatography (5% to 65% EtOAc in hexanes with 1% Et₃N gave the titled compound (130 mg, 39%). ¹H NMR (400 MHz, CDCl₃) δ 3.94 - 3.81 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.88 - 2.75 (m, 1H), 2.55 - 2.34 (m, 4H), 2.31 - 2.26 (t, *J* = 7.6 Hz, 5H), 1.96 - 1.85 (m, 1H), 1.79 - 1.02 (m, 96H), 0.92 - 0.83 (m, 12H). ESI-MS: *m/z* calcd for C₆₁H₁₂₁N₃O₃ = 943.9, found [M+H]⁺ = 945.2.

EXAMPLE 59

8,8'-((4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLOCTANAMIDE)

(COMPOUND I-9)



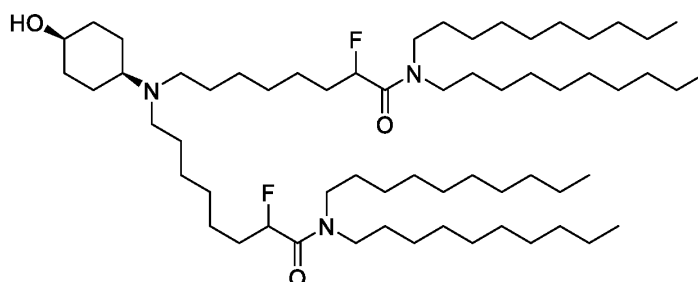
5 Synthesis of I-9

Compound **I-9** was prepared by mixing Compound **(1S,4S)-I-9** (25mg) and Compound **(1R,4R)-I-9** (25 mg) together to give the titled compound (47mg, 94%). ¹H NMR (400 MHz, CDCl₃) δ 3.98 (broad s, 0.5H), 3.54 (broad s, 0.5H), 3.32 – 3.23 (m, 4H), 3.23 – 3.15 (m, 4H), 2.46 - 2.32 (m, 5H), 2.30 – 2.22 (m, 4H), 2.06 - 1.92 (m, 1H), 1.89 – 1.73 (m, 2H), 1.71 – 1.07 (m, 105H), 0.94 - 0.83 (m, 12H). ESI-MS: *m/z* calcd for C₆₂H₁₂₃N₃O₃ = 958.0, found [M+H]⁺ = 959.2.

EXAMPLE 60

8,8'-(((1S,4S)-4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYL-2-FLUORO-OCTANAMIDE)

(COMPOUND (1S,4S)-I-49)



Synthesis of (1S,4S)-I-49

Compound **(1S,4S)-I-49** synthesized according to **General Procedure A** using **Intermediate 6** (Example 32) and (1S,4S)-4-aminocyclohexan-1-ol. Purification via automated flash chromatography (5% to 100% EtOAc in hexanes with 1% Et₃N) gave the titled compound

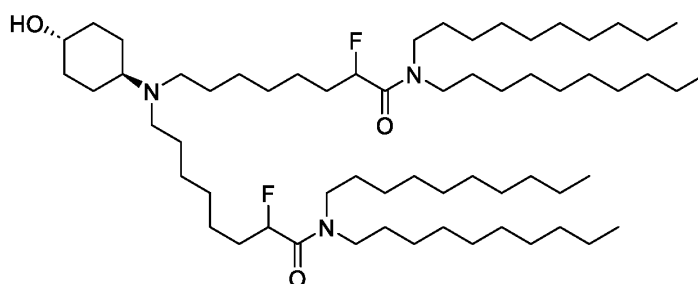
(155mg, 54%). ^1H NMR (400 MHz, CDCl_3) δ 5.12 (dd, $J = 8.4, 4.4$ Hz, 1H), 4.99 (dd, $J = 8.5, 4.3$ Hz, 1H), 4.01 (s, 1H), 3.41 - 3.14 (m, 8H), 2.52 - 2.40 (m, 5H), 2.02 - 1.73 (m, 6H), 1.72 - 1.09 (m, 92H), 0.95 - 0.86 (m, 12H). ESI-MS: m/z calcd for $\text{C}_{62}\text{H}_{121}\text{F}_2\text{N}_3\text{O}_3 = 993.9$. Found $[\text{M}+\text{H}]^+ = 995.3$.

5

EXAMPLE 61

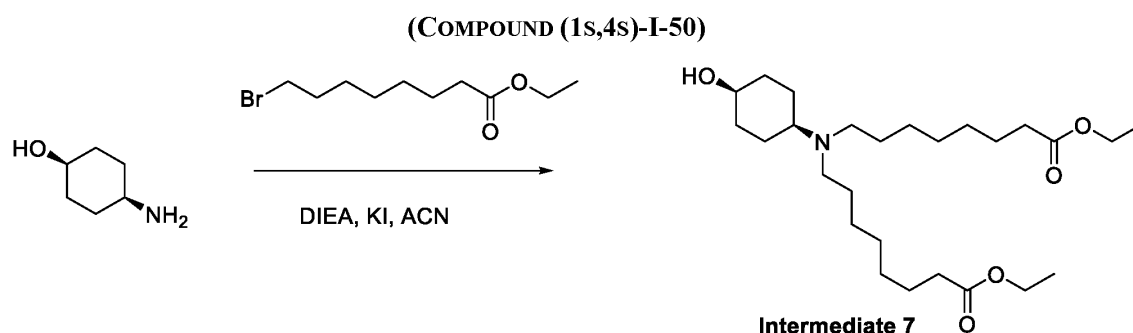
8,8'-(((1R,4R)-4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYL-2-FLUOROOCETANAMIDE)

(COMPOUND (1R,4R)-I-49)

10 **Synthesis of (1R,4R)-I-49**

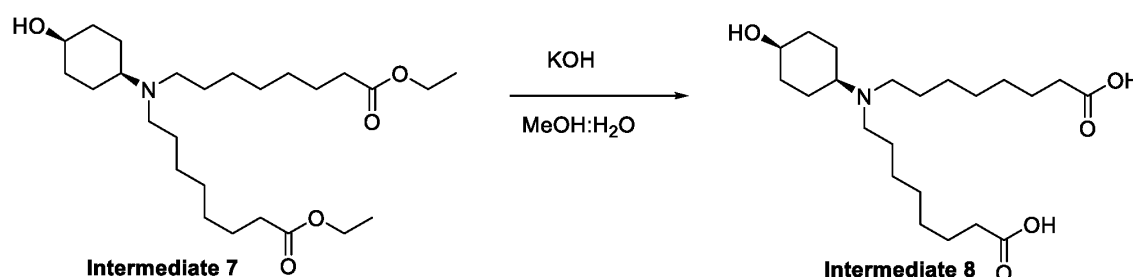
Compound **(1R,4R)-I-49** synthesized according to **General Procedure A** using **Intermediate 6** (Example 32) and (1r,4r)-4-aminocyclohexan-1-ol. Purification via automated flash chromatography (5% to 100% EtOAc in hexanes with 1% Et_3N) gave the titled compound (170 mg, 60%). ^1H NMR (400 MHz, CDCl_3) δ 5.12 (dd, $J = 8.4, 4.4$ Hz, 1H), 4.99 (dd, $J = 8.5, 4.3$ Hz, 1H), 3.57 (broad s, 1H), 3.41 - 3.14 (m, 8H), 2.54 - 2.31 (m, 5H), 2.09 - 1.72 (m, 8H), 1.69 - 1.08 (m, 90H), 0.95 - 0.86 (m, 12H). ESI-MS: m/z calcd for $\text{C}_{62}\text{H}_{121}\text{F}_2\text{N}_3\text{O}_3 = 993.9$. Found $[\text{M}+\text{H}]^+ = 995.2$.

EXAMPLE 62

8,8'-(((1*S*,4*S*)-4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N-(NONADECAN-10-YL)OCTANAMIDE)

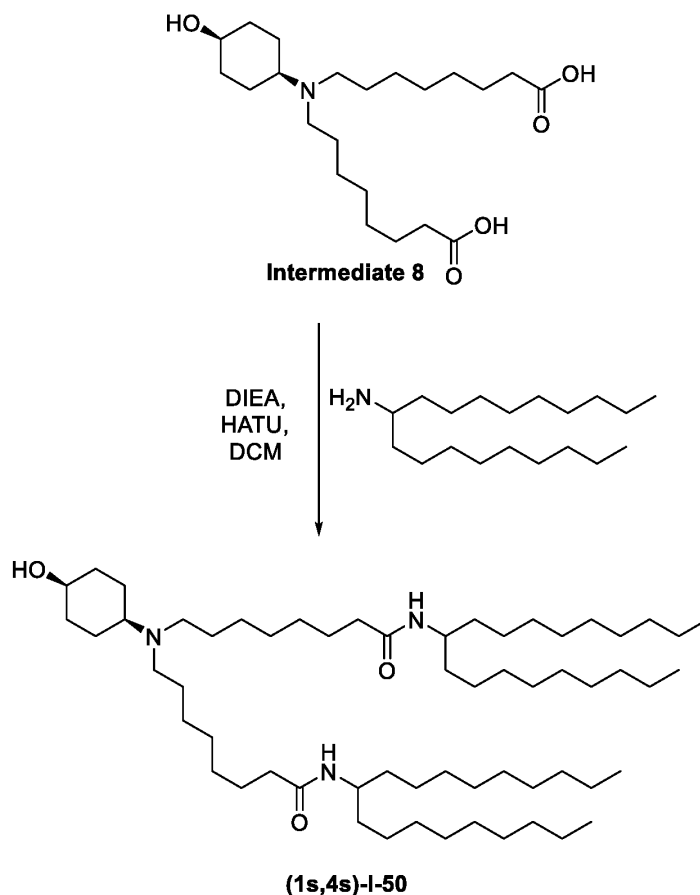
5 Synthesis of Intermediate 7

A mixture of (1*S*,4*S*)-4-aminocyclohexan-1-ol (4.34 mmol, 500 mg), ethyl 8-bromooctanoate (8.68 mmol, 2.18 g), DIEA (13.5 mmol, 2.34 mL), and potassium iodide (13.0 mmol, 2.16 g) in acetonitrile (8.7 mL) was stirred at 75 °C for 19 h. The reaction mixture was concentrated, and the crude material was suspended in dichloromethane and filtered. The filtrate was purified via automated flash chromatography (10% to 100% EtOAc in hexanes with 1% Et₃N) to give **Intermediate 7** (1.28 g, 65%).



Synthesis of Intermediate 8

A mixture of **Intermediate 7** (0.44 mmol, 200 mg) and potassium hydroxide (1.34 mmol, 75 mg) in methanol (0.88 mL) and water (0.88 mL) was stirred at room temperature for 20 h. The reaction mixture was concentrated to give **Intermediate 8** which was used in the subsequent step without further purification.



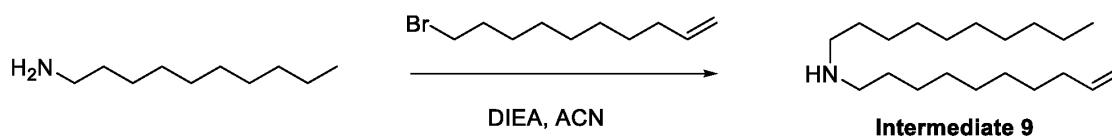
Synthesis of (1s,4s)-I-50

A mixture of **Intermediate 8** (0.25 mmol, 100 mg), nonadecan-10-amine (0.5 mmol, 142 mg), DIEA (0.75 mmol, 0.13 mL) and HATU (0.5 mmol, 190 mg) in dichloromethane (1.25 mL) was stirred at room temperature for 20 h. The reaction mixture was concentrated, and the crude material was partitioned between EtOAc and sat. NaHCO₃. The organic layer was separated, dried over Na₂SO₄ and concentrated. Purification via flash chromatography (5% to 100% EtOAc in hexanes with 1% Et₃N) gave compound **(1s,4s)-I-50** (75 mg, 32%). ¹H NMR (400 MHz, CDCl₃) δ 5.25 (d, *J* = 9.2 Hz, 2H), 4.05 – 3.88 (m, 3H), 2.58 - 2.39 (m, 5H), 2.18 (t, *J* = 7.6 Hz, 4H), 1.92 - 1.83 (m, 2H), 1.77 – 1.05 (m, 105H), 0.95 - 0.84 (m, 15H). ESI-MS: *m/z* calcd for C₆₀H₁₁₉N₃O₃ = 929.9. Found [M+H]⁺ = 931.2.

EXAMPLE 63

8,8'-(((1*s*,4*s*)-4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N-(DEC-9-EN-1-YL)-N-DECYLOCTANAMIDE)

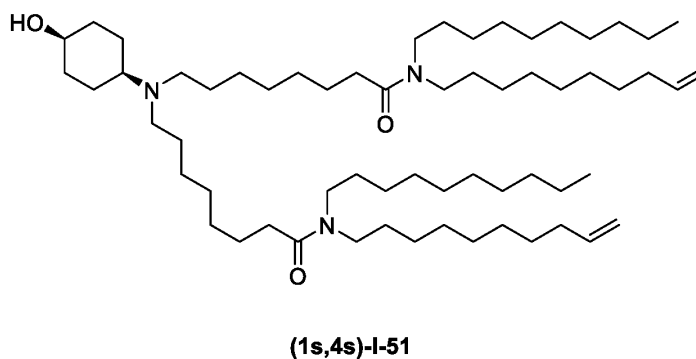
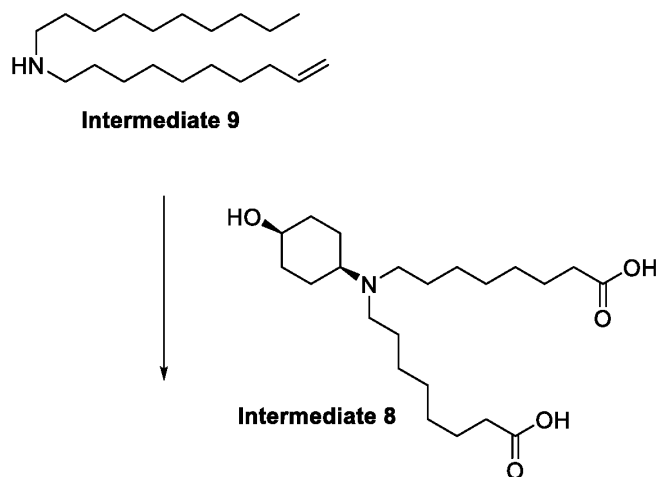
(COMPOUND (1*s*,4*s*)-I-51)



Synthesis of Intermediate 9

A mixture of decylamine (13.7 mmol, 2.15 g), 10-bromo-1-decene (4.6 mmol, 1.0 g) and DIEA (13.7 mmol, 2.38 mL) in acetonitrile (9.1 mL) was stirred at room temperature for 72 h. The reaction mixture was concentrated, and the crude material was purified via flash chromatography (0% to 20% EtOAc in hexanes with 1% Et₃N). A second purification via flash chromatography (1% to 15% MeOH in DCM) gave **Intermediate 9** (538 mg, 40%).

10



Synthesis of (1s,4s)-I-51

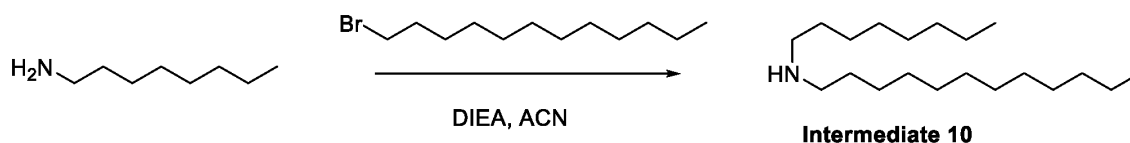
A mixture of **Intermediate 8** (0.25 mmol, 100 mg), **Intermediate 9** (0.5 mmol, 148 mg), DIEA (1.50 mmol, 0.26 mL) and HATU (0.65 mmol, 247 mg) in dichloromethane (2.5 mL) was stirred at room temperature for 20 h. The reaction mixture was concentrated, and the crude material was partitioned between EtOAc and sat. NaHCO₃. The organic layer was separated, dried over Na₂SO₄ and concentrated. Purification via flash chromatography (5% to 100% EtOAc in hexanes with 1% Et₃N) gave compound **(1s,4s)-I-51** (95 mg, 40%). ¹H NMR (400 MHz, CDCl₃) δ 5.88 - 5.73 (m, 2H), 5.04 - 4.88 (m, 4H), 4.01 - 3.92 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.49 - 2.38 (m, 5H), 2.30 - 2.22 (m, 4H), 2.09 - 1.97 (m, 4H), 1.92 - 1.75 (m, 2H), 1.76 - 1.07 (m, 89H), 0.93 - 0.81 (m, 6H). ESI-MS: *m/z* calcd for C₆₂H₁₁₉N₃O₃ = 953.9. Found [M+H]⁺ = 955.2.

EXAMPLE 64

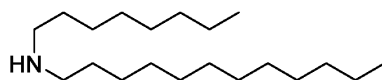
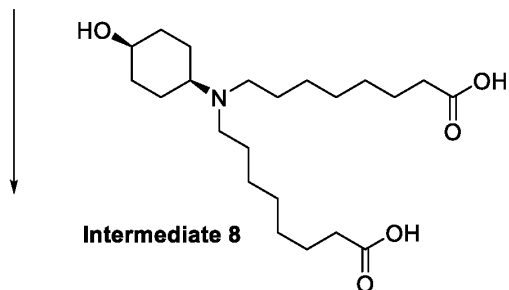
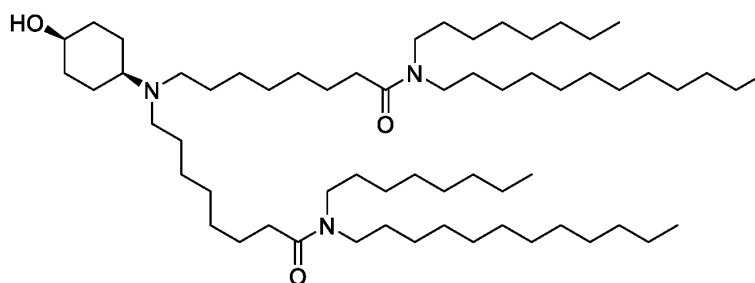
8,8'-(((1s,4s)-4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N-DODECYL-N-OCTYLOCTANAMIDE)

(COMPOUND (1s,4s)-I-62)

15

**Synthesis of Intermediate 10**

A mixture of octylamine (24.1 mmol, 3.11 g), 1-bromododecane (8.0 mmol, 2.0 g) and DIEA (24.1 mmol, 4.2 mL) in acetonitrile (16 mL) was stirred at 50 °C for 20 h. The reaction mixture was concentrated, and the crude material was purified via flash chromatography (1% to 15% MeOH in DCM) to give **Intermediate 10** (1.75 g, 73%).

**Intermediate 10****Intermediate 8****(1S,4S)-I-62****Synthesis of (1S,4S)-I-62**

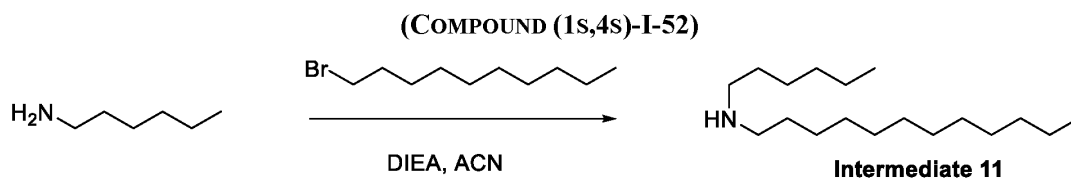
Compound **(1S,4S)-I-62** was prepared from **Intermediate 8** and **Intermediate 10** following the procedure outlined for compound **(1S,4S)-I-51** (Example 63). Yield (67 mg, 28%).

- ¹H NMR (400 MHz, CDCl₃) δ 4.02 - 3.92 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.50 - 2.38 (m, 5H), 2.31 - 2.22 (m, 4H), 1.86 - 1.79 (m, 2H), 1.72 - 1.10 (m, 96H), 0.92 - 0.83 (m, 12H). ESI-MS: *m/z* calcd for C₆₂H₁₂₃N₃O₃ = 958.0. Found [M+H]⁺ = 959.3.

EXAMPLE 65

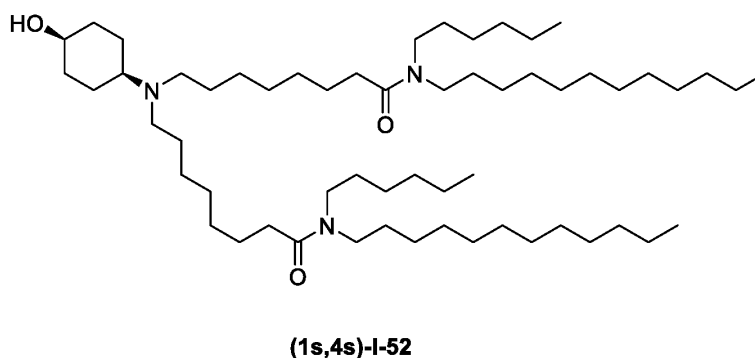
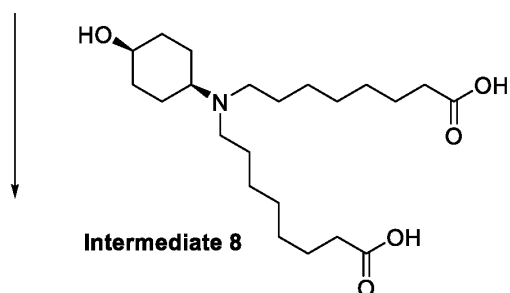
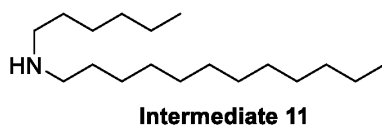
8,8'-(((1S,4S)-4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N-DECYL-N-HEXYLOCTANAMIDE)

10



Synthesis of Intermediate 11

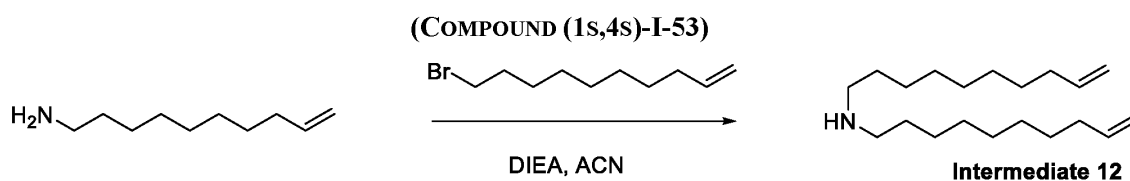
A mixture of hexylamine (24.1 mmol, 2.44 g), 1-bromododecane (8.0 mmol, 2.0 g) and DIEA (24.1 mmol, 4.2 mL) in acetonitrile (16 mL) was stirred at 50 °C for 20 h. The reaction mixture was concentrated, and the crude material was purified via flash chromatography (1% to 5 15% MeOH in DCM) to give **Intermediate 11** (1.7 g, 79%).

**Synthesis of (1S,4S)-I-52**

Compound **(1S,4S)-I-52** was prepared from **Intermediate 8** and **Intermediate 11** following the procedure outlined for compound **(1s,4s)-I-51** (Example 63). Yield (33 mg, 15%).

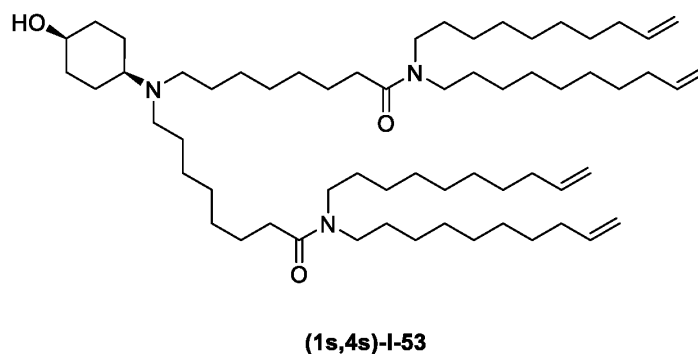
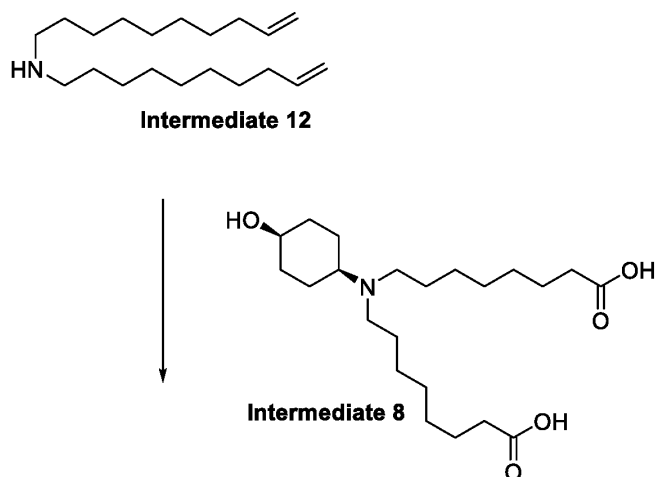
10 ¹H NMR (400 MHz, CDCl₃) δ 4.02 - 3.91 (m, 1H), 3.32 - 3.24 (m, 4H), 3.23 - 3.15 (m, 4H), 2.51 - 2.38 (m, 5H), 2.31 - 2.22 (m, 4H), 1.89 - 1.79 (m, 2H), 1.76 - 1.05 (m, 88H), 0.94 - 0.83 (m, 12H). ESI-MS: *m/z* calcd for C₅₈H₁₁₅N₃O₃ = 901.9. Found [M+H]⁺ = 903.2.

8,8'-(((1S,4S)-4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DI(DEC-9-EN-1-YL)OCTANAMIDE)



5 Synthesis of Intermediate 12

A mixture of dec-9-en-1-amine (6.84 mmol, 1.06 g), 10-bromodec-1-ene (2.28 mmol, 500 mg) and DIEA (6.84 mmol, 1.19 mL) in acetonitrile (4.6 mL) was stirred at room temperature for 20 h. The reaction mixture was concentrated, and the crude material was purified via flash chromatography (1% to 15% MeOH in DCM). A second purification via flash chromatography (1% to 10% MeOH in CHCl₃) gave **Intermediate 12** (0.36 g, 54%).

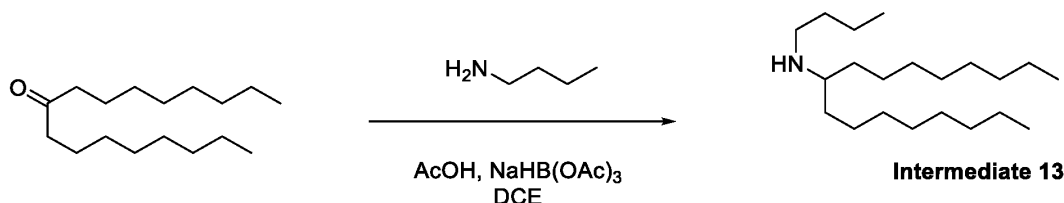


Synthesis of (1S,4S)-I-53

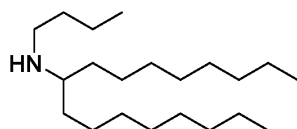
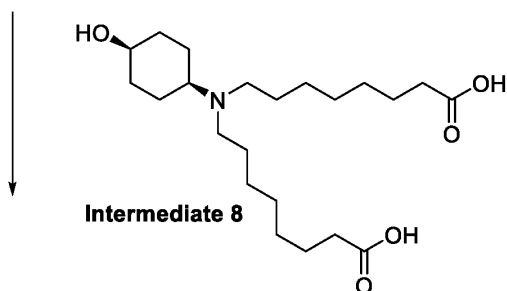
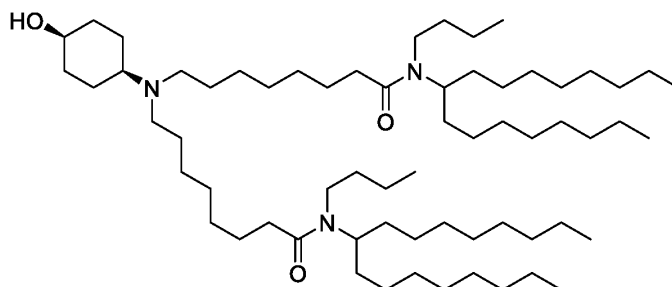
Compound **(1S,4S)-I-53** was prepared from **Intermediate 8** and **Intermediate 12** following the procedure outlined for compound **(1S,4S)-I-51** (Example 63). Yield (150 mg, 48%). ¹H NMR (400 MHz, CDCl₃) δ 5.83 (m, 4H), 5.07 – 4.90 (m, 8H), 4.05 – 3.96 (m, 1H), 3.34 – 3.26 (m, 4H), 3.25 – 3.17 (m, 4H), 2.51 – 2.40 (m, 5H), 2.33 – 2.25 (m, 4H), 2.12 – 2.00 (m, 8H), 1.90 – 1.81 (m, 2H), 1.74 – 1.06 (m, 84H). ESI-MS: *m/z* calcd for C₆₂H₁₁₅N₃O₃ = 949.9. Found [M+H]⁺ = 951.2.

EXAMPLE 67

**8,8'-(((1S,4S)-4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N-BUTYL-N-(HEPTADECAN-9-
YL)OCTANAMIDE) (COMPOUND (1S,4S)-I-54)**

**Synthesis of Intermediate 13**

A mixture of heptadecan-9-one (5.89 mmol, 1.5 g), butylamine (8.84 mmol, 0.87 mL), acetic acid (8.84 mmol, 0.51 mL) and sodium triacetoxyborohydride (23.6 mmol, 5.0 g) in dichloroethane (29.5 mL) was stirred at room temperature for 20 h. An additional portion of sodium triacetoxyborohydride (9.4 mmol, 2.0 g) was added and the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was concentrated, and the crude material was partitioned between EtOAc and sat. NaHCO₃. The organic layer was separated, dried over Na₂SO₄ and concentrated. Purification via flash chromatography (1% to 10% MeOH in CHCl₃) gave **Intermediate 13** (1.29 g, 70%).

**Intermediate 13****Intermediate 8****(1S,4S)-I-54**

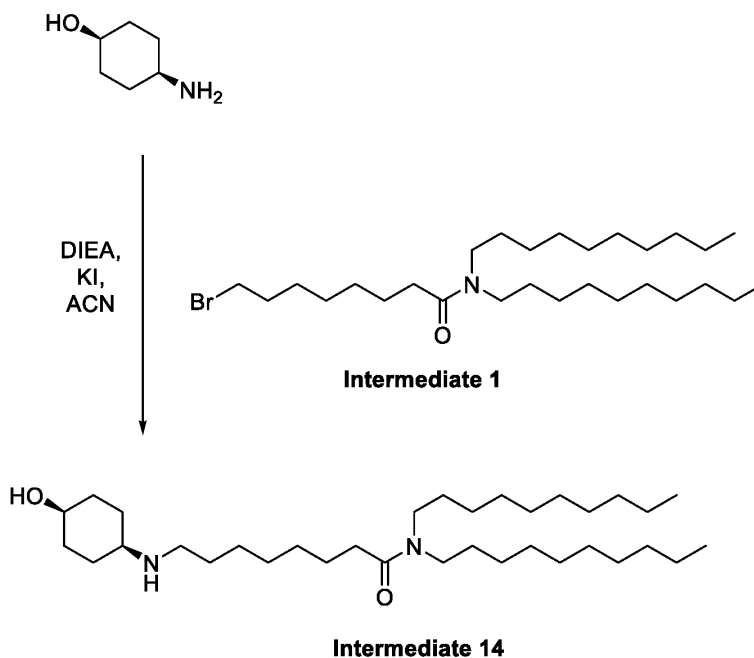
Synthesis of (1S,4S)-I-54

Compound **(1S,4S)-I-54** was prepared from **Intermediate 8** and **Intermediate 13** following the procedure outlined for compound **(1S,4S)-I-51** (Example 63). Yield (130 mg, 35%). ¹H NMR (400 MHz, CDCl₃) δ 4.45 (broad s, 1H), 4.05 - 3.96 (m, 1H), 3.70 - 3.58 (m, 1H), 3.12 - 3.00 (m, 4H), 2.58 - 2.38 (m, 5H), 2.35 - 2.26 (m, 4H), 1.89 - 1.81 (m, 2H), 1.77 - 1.06 (m, 97H), 1.02 - 0.85 (m, 18H). ESI-MS: *m/z* calcd for C₆₄H₁₂₇N₃O₃ = 986.0. Found [M+H]⁺ = 987.2.

EXAMPLE 68

N,N-DIDECYL-8-((8-(DODECYL(OCTYL)AMINO)-8-OXOOCTYL)((1S,4S)-4-HYDROXYCYCLOHEXYL)AMINO)OCTANAMIDE

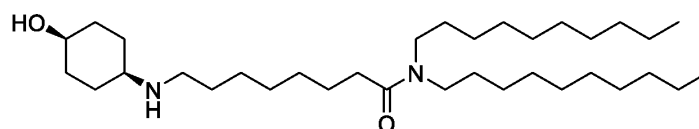
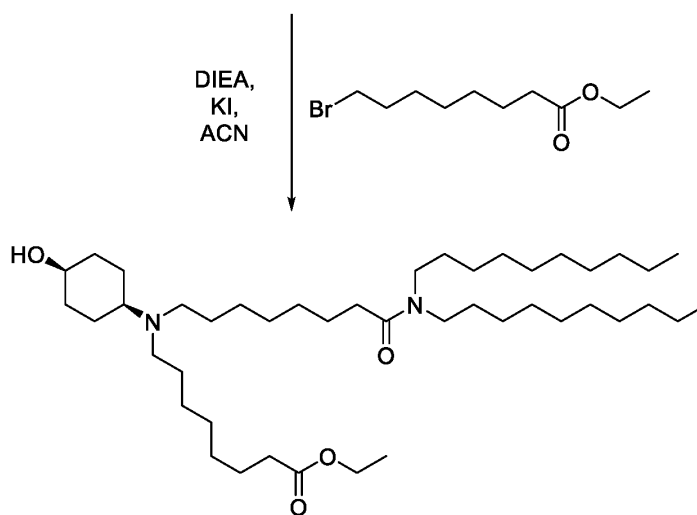
(COMPOUND (1S,4S)-I-55)



5

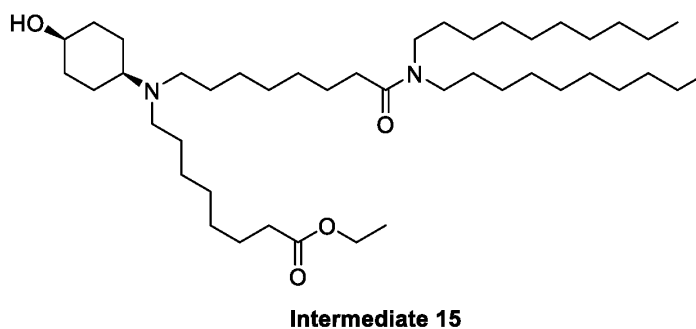
Synthesis of Intermediate 14

A mixture of (1S,4S)-4-aminocyclohexan-1-ol (18.3 mmol, 2.11 g), **Intermediate 1** (4.58 mmol, 2.3 g), DIEA (14.2 mmol, 2.47 mL), and potassium iodide (13.7 mmol, 2.28 g) in acetonitrile (9.2 mL) was heated at 75 °C for 20 h. The reaction mixture was concentrated and the resultant crude was suspended in 95:5:1 (hexanes:EtOAc:Et₃N) and filtered. The filtrate was purified via flash chromatography (100% EtOAc) followed by a second flash chromatography (1% to 10% MeOH in CHCl₃) to give **Intermediate 14** (1.0 g, 41%).

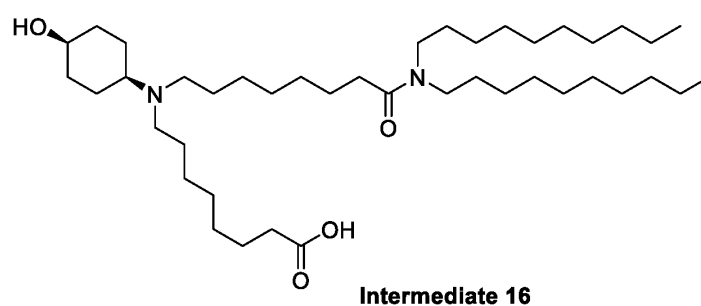
**Intermediate 14****Intermediate 15**

Synthesis of Intermediate 15

A mixture of **Intermediate 14** (1.6 mmol, 850 mg), ethyl 8-bromooctanoate (1.6 mmol, 400 mg), DIEA (4.9 mmol, 0.86 mL), and potassium iodide (4.8 mmol, 790 mg) was heated at 75 °C for 20 h. The reaction mixture was concentrated and the resultant crude was suspended in 95:5:1 (hexanes:EtOAc:Et₃N) and filtered. The filtrate was purified via flash chromatography (5% to 100% EtOAc in hexanes with 1% Et₃N) to give **Intermediate 15** (700 mg, 63%).

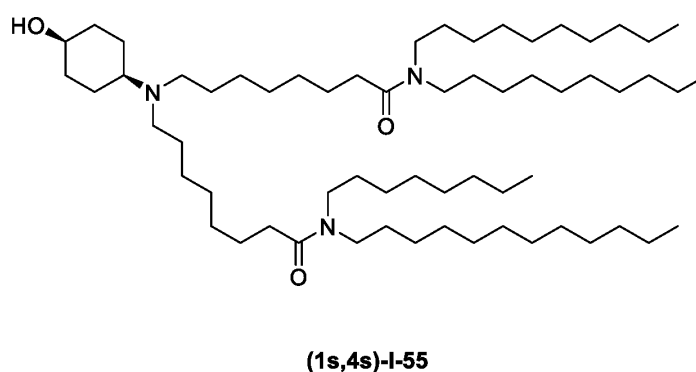
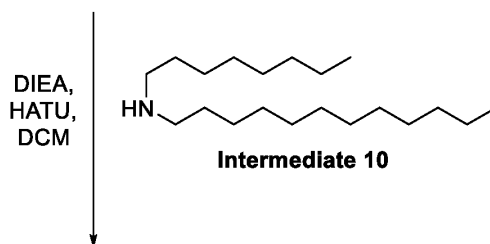
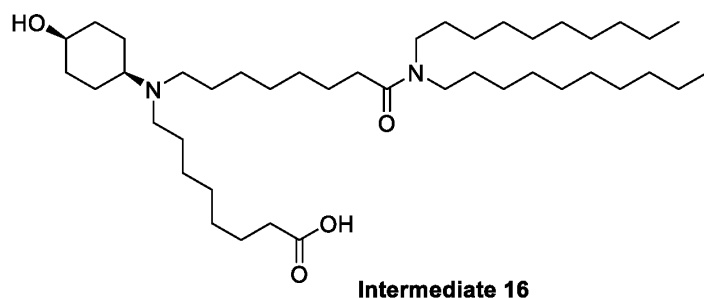


KOH
MeOH:H₂O:THF



Synthesis of Intermediate 16

A mixture of **Intermediate 15** (0.99 mmol, 0.70 g) and sodium hydroxide (25 mmol, 1 g) in methanol:water (4 mL:0.4 mL) was heated at 60 °C for 2 h. The reaction mixture was diluted
5 with water and extracted with EtOAc. The organic layer was separated, dried over Na₂SO₄ and concentrated to give **Intermediate 16** (600 mg, 89%).



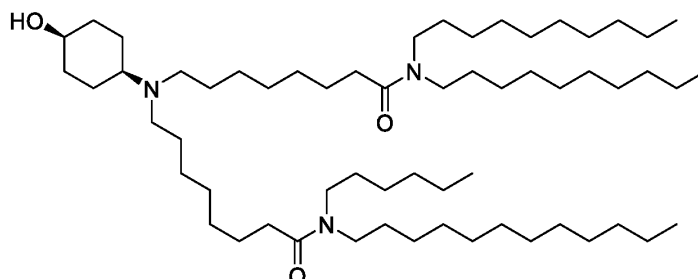
Synthesis of (1S,4S)-I-55

A mixture of **Intermediate 16** (0.12 mmol, 80 mg), **Intermediate 10** (0.12 mmol, 35 mg), DIEA (0.47 mmol, 0.082 mL) and HATU (0.15 mmol, 58 mg) in dichloromethane (1.2 mL) was stirred at room temperature for 20 h. The reaction mixture was concentrated, and the crude material was partitioned between EtOAc and sat. NaHCO₃. The organic layer was separated, dried over Na₂SO₄ and concentrated. Purification via flash chromatography (5% to 100% EtOAc in hexanes with 1% Et₃N) gave the titled compound (33 mg, 29%). ¹H NMR (400 MHz, CDCl₃) δ 4.05 - 3.98 (m, 1H), 3.35 - 3.27 (m, 4H), 3.26 - 3.18 (m, 4H), 2.57 - 2.40 (m, 5H), 2.34 - 2.26 (m, 4H), 1.92 - 1.82 (m, 2H), 1.78 - 1.09 (m, 95H), 0.96 - 0.87 (m, 12H). ESI-MS: *m/z* calcd for C₆₂H₁₂₃N₃O₃ = 958.0. Found [M+H]⁺ = 959.2.

EXAMPLE 69

N,N-DIDECYL-8-((8-(DODECYL(HEXYL)AMINO)-8-OXOOCTYL)((1S,4S)-4-HYDROXYCYCLOHEXYL)AMINO)OCTANAMIDE

(COMPOUND (1S,4S)-I-56)



5

Synthesis of (1S,4S)-I-56

Compound **(1S,4S)-I-56** was prepared from **Intermediate 16** and **Intermediate 11** following the procedure outlined for compound **(1S,4S)-I-55** (Example 68). Yield (26 mg, 24%).

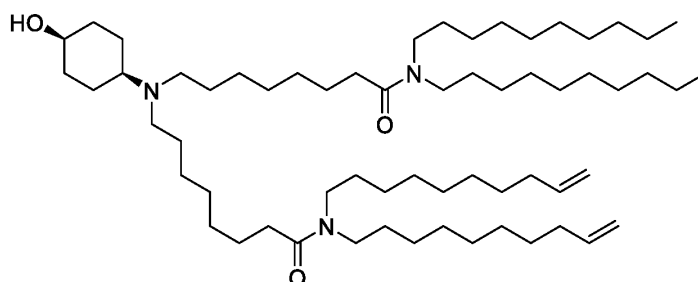
¹H NMR (400 MHz, CDCl₃) δ 4.05 - 3.98 (m, 1H), 3.35 - 3.27 (m, 4H), 3.27 - 3.18 (m, 4H), 2.57 - 2.40 (m, 5H), 2.34 - 2.26 (m, 4H), 1.92 - 1.82 (m, 2H), 1.77 - 1.09 (m, 91H), 0.91 (td, *J* = 6.9, 2.8 Hz, 12H). ESI-MS: *m/z* calcd for C₆₀H₁₁₉N₃O₃ = 929.9. Found [M+H]⁺ = 931.2.

10

EXAMPLE 70

N,N-DI(DEC-9-EN-1-YL)-8-((8-(DIDECYLAMINO)-8-OXOOCTYL)((1S,4S)-4-HYDROXYCYCLOHEXYL)AMINO)OCTANAMIDE

(COMPOUND (1S,4S)-I-57)



15

Synthesis of (1S,4S)-I-57

Compound **(1S,4S)-I-57** was prepared from **Intermediate 16** and **Intermediate 12** following the procedure outlined for compound **(1S,4S)-I-55** (Example 68). Yield (30 mg, 21%).

¹H NMR (400 MHz, CDCl₃) δ 5.92 - 5.77 (m, 2H), 5.08 - 4.92 (m, 4H), 4.01 (s, 1H), 3.35 - 3.27

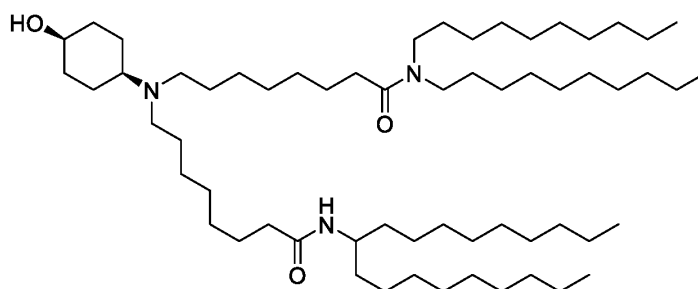
20

(m, 4H), 3.27 – 3.18 (m, 4H), 2.59 – 2.39 (m, 4H), 2.34 – 2.26 (m, 4H), 2.13 – 2.01 (m, 4H), 1.93 – 1.80 (m, 2H), 1.79 – 1.07 (m, 85H), 0.91 (td, $J = 6.8, 3.1$ Hz, 6H). ESI-MS: m/z calcd for $C_{62}H_{119}N_3O_3 = 953.9$. Found $[M+H]^+ = 955.2$.

EXAMPLE 71

5 **N,N-DIDECYL-8-(((1S,4S)-4-HYDROXYCYCLOHEXYL)(8-(NONADECAN-10-YLAMINO)-8-
OXOOCTYL)AMINO)OCTANAMIDE**

(COMPOUND (1S,4S)-I-58)



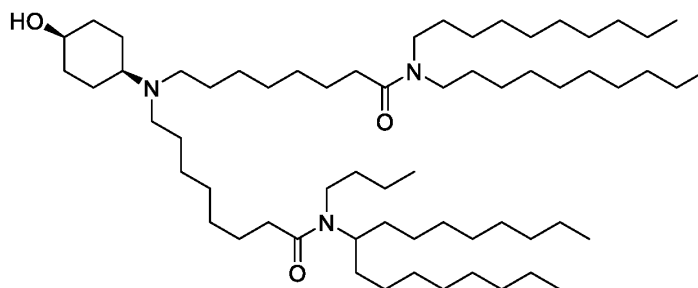
Synthesis of (1S,4S)-I-58

10 Compound **(1S,4S)-I-58** was prepared from **Intermediate 16** and nonadecan-10-amine following the procedure outlined for compound **(1S,4S)-I-55** (Example 68). Yield (36 mg, 26%).
 1H NMR (400 MHz, $CDCl_3$) δ 5.22 (d, $J = 9.1$ Hz, 1H), 4.03 – 3.84 (m, 2H), 3.28 (t, $J = 7.7$ Hz, 2H), 3.20 (t, $J = 7.8$ Hz, 2H), 2.55 – 2.37 (m, 5H), 2.27 (t, $J = 7.6$ Hz, 2H), 2.16 (t, $J = 7.6$ Hz, 2H), 1.88 – 1.80 (m, 2H), 1.75 – 1.03 (m, 93H), 0.93 – 0.84 (m, 12H). ESI-MS: m/z calcd for
 15 $C_{61}H_{121}N_3O_3 = 943.9$. Found $[M+H]^+ = 945.3$.

EXAMPLE 72

**N-BUTYL-8-((8-(DIDECYLAMINO)-8-OXOOCTYL)((1S,4S)-4-HYDROXYCYCLOHEXYL)AMINO)-N-
(HEPTADECAN-9-YL)OCTANAMIDE**

(COMPOUND (1S,4S)-I-59)

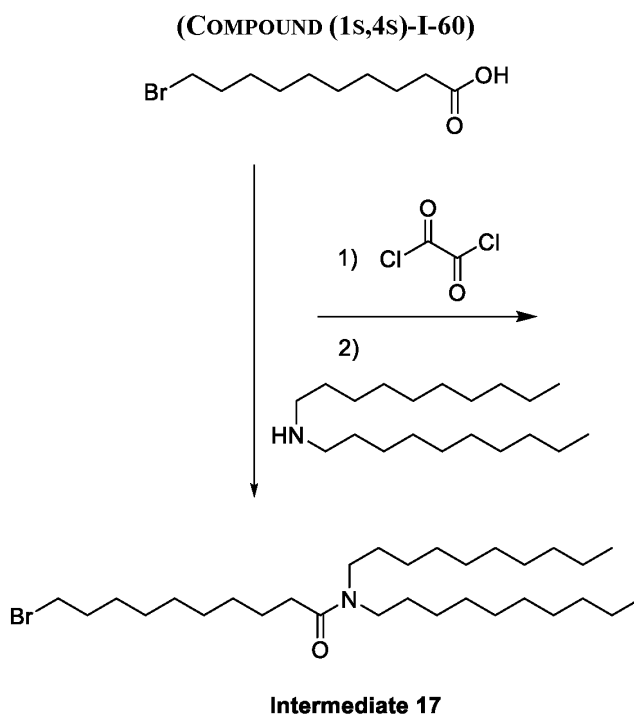


Synthesis of (1S,4S)-I-59

Compound **(1S,4S)-I-59** was prepared from **Intermediate 16** and **Intermediate 13** following the procedure outlined for compound **(1S,4S)-I-55** (Example 68). Yield (39 mg, 27%). ¹H NMR (400 MHz, CDCl₃) δ 4.02 - 3.96 (m, 1H), 3.67 - 3.57 (m, 1H), 3.28 (t, *J* = 7.7 Hz, 2H), 3.20 (t, *J* = 7.8 Hz, 2H), 3.12 - 2.97 (m, 2H), 2.55 - 2.36 (m, 5H), 2.33 - 2.23 (m, 4H), 1.90 - 1.78 (m, 2H), 1.76 - 1.05 (m, 97H), 1.00 - 0.84 (m, 15H). ESI-MS: *m/z* calcd for C₆₃H₁₂₅N₃O₃ = 972.0. Found [M+H]⁺ = 973.3.

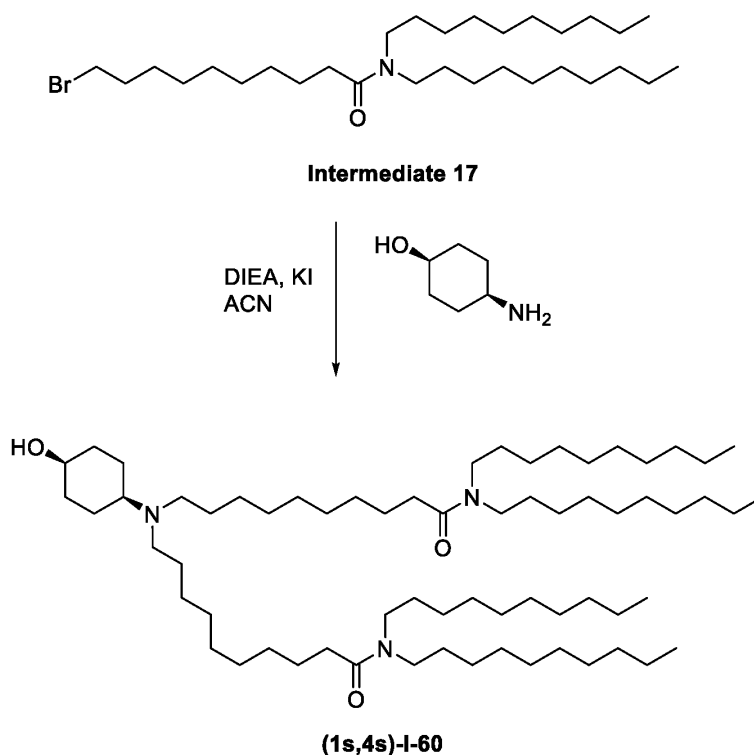
EXAMPLE 73

10,10'-(((1*S*,4*S*)-4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(N,N-DIDECYLDECANAMIDE)



Synthesis of Intermediate 17

Intermediate 17 was prepared from 10-bromodecanoic acid following the procedure outlined for **Intermediate 1** (Example 5). Yield (5.07 g, 40%).

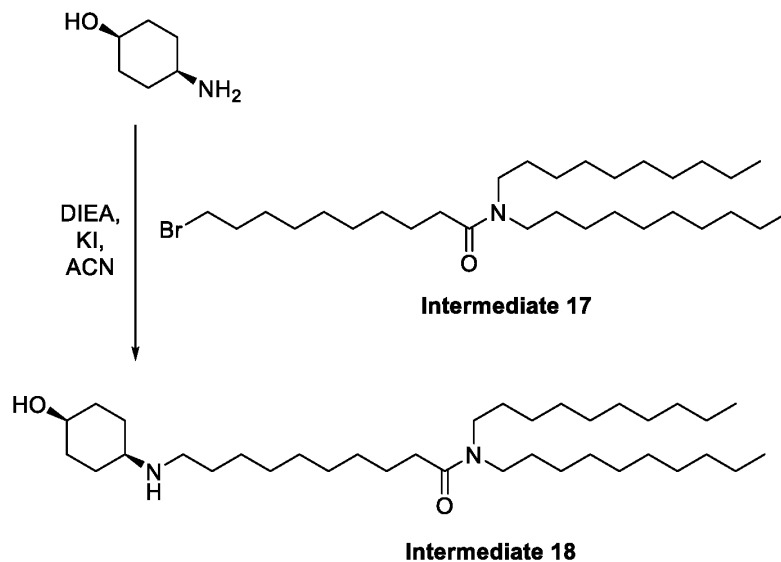


Synthesis of (1S,4S)-I-60

Compound **(1S,4S)-I-60** was synthesized according to **General Procedure A** using a mixture of **Intermediate 17** and (1S,4S)-4-aminocyclohexan-1-ol. Purification via automated
5 flash chromatography (5% to 100% EtOAc in hexanes with 1% Et₃N) gave the titled compound (yield 97 mg, 33%). ¹H NMR (400 MHz, CDCl₃) δ 4.04 - 3.98 (m, 1H), 3.34 - 3.26 (m, 4H), 3.25 - 3.17 (m, 4H), 2.55 - 2.38 (m, 5H), 2.33 - 2.25 (m, 4H), 1.92 - 1.80 (m, 2H), 1.77 - 1.05 (m, 106H), 0.95 - 0.86 (m, 12H). ESI-MS: *m/z* calcd for C₆₆H₁₃₁N₃O₃ = 1014.0. Found [M+H]⁺ = 1015.3.

EXAMPLE 74

N,N-DIDECYL-10-((6-(DIDECYLAMINO)-6-OXOHXYL)((1S,4S)-4-HYDROXYCYCLOHEXYL)AMINO)DECANAMIDE (COMPOUND (1S,4S)-I-61)

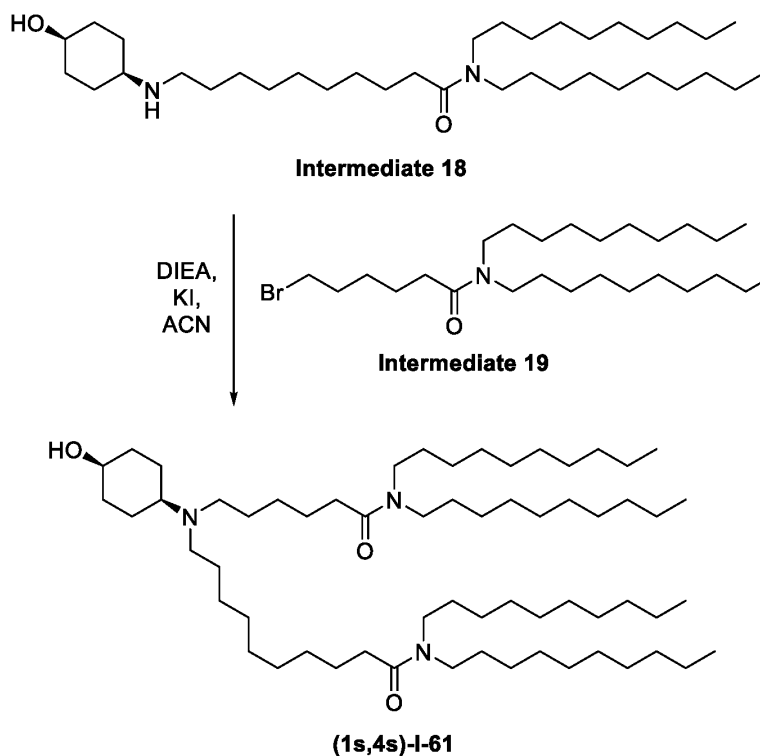


5 Synthesis of Intermediate 18

A mixture of (1S,4S)-4-aminocyclohexan-1-ol (3.77 mmol, 434 mg), **Intermediate 17** (0.94 mmol, 0.50 g), DIEA (2.92 mmol, 0.510 mL), and potassium iodide (2.83 mmol, 469 mg) in acetonitrile (1.9 mL) was heated via microwave irradiation at 140 °C for 1 h. The reaction mixture was concentrated and the resultant crude was suspended in 95:5:1 (hexanes:EtOAc:Et₃N) and filtered. The filtrate was purified via flash chromatography (25% to 100% EtOAc in hexanes) followed by a second flash chromatography (1% to 10% MeOH in CHCl₃) to give **Intermediate 18** (440 mg, 83%).

Synthesis of Intermediate 19

Intermediate 19 was prepared from 6-bromohexanoic acid following the procedure outlined for **Intermediate 1** (Example 5). Yield (4.6 g, 76%).

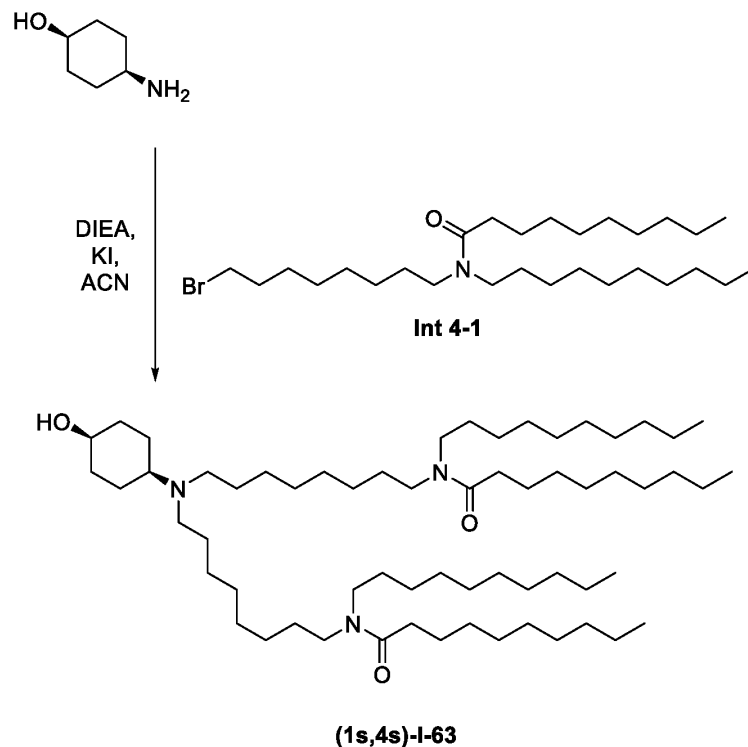


Synthesis of (1s,4s)-I-61

Compound **(1S,4S)-I-61** was synthesized from **Intermediate 18** and **Intermediate 19** following the procedure outlined for **Intermediate 15** (Example 68). Yield (90 mg, 53%). ¹H NMR (400 MHz, CDCl₃) δ 4.02 - 3.94 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.52 - 2.37 (m, 5H), 2.31 - 2.22 (m, 4H), 1.90 - 1.77 (m, 2H), 1.76 - 1.04 (m, 97H), 0.92 - 0.83 (m, 12H). ESI-MS: *m/z* calcd for C₆₂H₁₂₃N₃O₃ = 958.0. Found [M+H]⁺ = 959.3.

EXAMPLE 75

N,N'-((((1S,4S)-4-HYDROXYCYCLOHEXYL)AZANEDIYL)BIS(OCTANE-8,1-DIYL))BIS(N-DECYLDECANAMIDE) (COMPOUND (1S,4S)-I-63)

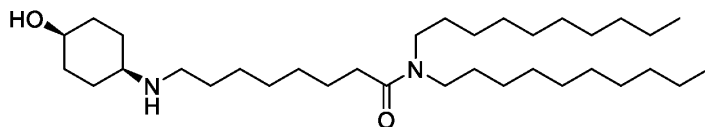


5 Synthesis of (1S,4S)-I-63

Compound (1S,4S)-I-63 was prepared from Int 4-1 (Example 33) and (1S,4S)-4-aminocyclohexan-1-ol according to **General Procedure B**. Purification via automated flash chromatography (5% to 100% EtOAc in hexanes with 1% Et₃N) gave the titled compound (122 mg, 59%). ¹H NMR (400 MHz, CDCl₃) δ 4.02 - 3.95 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.54 - 2.34 (m, 5H), 2.31 - 2.22 (m, 4H), 1.89 - 1.77 (m, 2H), 1.74 - 1.04 (m, 94H), 0.92 - 0.84 (m, 12H). ESI-MS: *m/z* calcd for C₆₂H₁₂₃N₃O₃ = 958.0, found [M+H]⁺ = 959.3.

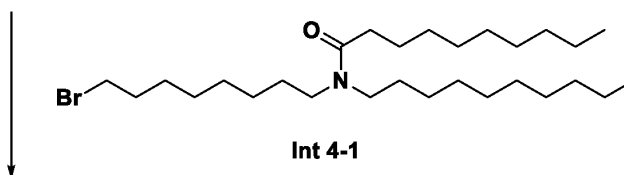
EXAMPLE 76

N-DECYL-N-(8-((8-(DIDECYLAMINO)-8-OXOOCTYL)((1*s*,4*s*)-4-HYDROXYCYCLOHEXYL)AMINO)OCTYL)DECANAMIDE (COMPOUND (1*s*,4*s*)-I-47)

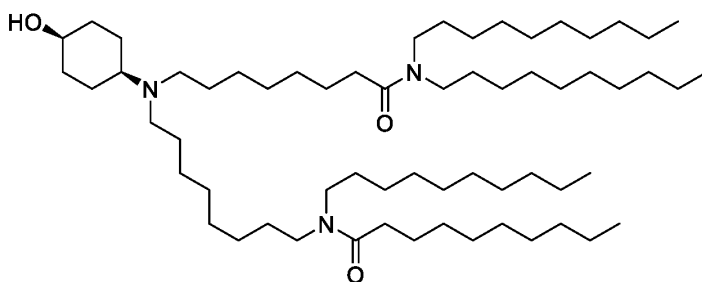


Intermediate 14

DIEA, KI, ACN



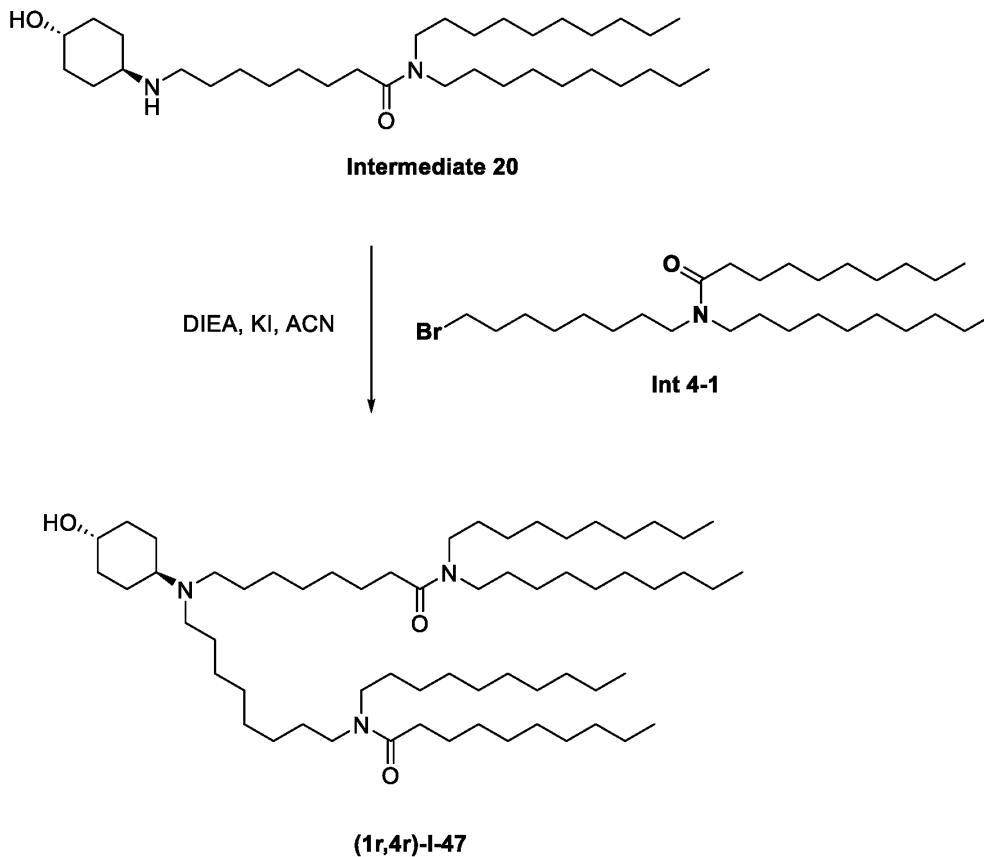
Int 4-1

(1*s*,4*s*)-I-475 Synthesis of (1*s*,4*s*)-I-47

A mixture of **Intermediate 14** (0.21 mmol, 112 mg), **Int 4-1** (0.14 mmol, 70 mg), DIEA (0.43 mmol, 0.075 mL), and potassium iodide (0.42 mmol, 69 mg) was heated via microwave irradiation at 160 °C for 3 h. The reaction mixture was concentrated and the resultant crude was suspended in 95:5:1 (hexanes:EtOAc:Et₃N) and filtered. The filtrate was purified via flash chromatography (5% to 100% EtOAc in hexanes with 1% Et₃N) to give the titled compound (45 mg, 34%). ¹H NMR (400 MHz, CDCl₃) δ 4.03 - 3.94 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.52 - 2.35 (m, 5H), 2.31 - 2.22 (m, 4H), 1.89 - 1.78 (m, 2H), 1.73 - 1.02 (m, 97H), 0.92 - 0.84 (m, 12H). ESI-MS: *m/z* calcd for C₆₂H₁₂₃N₃O₃ = 958.0, found [M+H]⁺ = 959.2.

EXAMPLE 77

N-DECYL-N-(8-((8-(DIDECYLAMINO)-8-OXOOCTYL)((1R,4R)-4-HYDROXYCYCLOHEXYL)AMINO)OCTYL)DECANAMIDE (COMPOUND (1R,4R)-I-47)

5 **Synthesis of Intermediate 20**

A mixture of (1R,4R)-4-aminocyclohexan-1-ol (3.99 mmol, 458 mg), **Intermediate 1** (1.0 mmol, 500 mg), DIEA (3.1 mmol, 0.54 mL), and potassium iodide (3.0 mmol, 495 mg) in acetonitrile (2.0 mL) was heated via microwave irradiation at 140 °C for 40 min. The reaction mixture was concentrated and the resultant crude was suspended in 95:5:1
 10 (hexanes:EtOAc:Et₃N) and filtered. The filtrate was purified via flash chromatography (10% to 100% EtOAc in hexanes) followed by a second flash chromatography (1% to 12% MeOH in dichloromethane) to give **Intermediate 20** (363 mg, 68%).

Synthesis of (1R,4R)-I-47

A mixture of **Intermediate 20** (0.24 mmol, 130 mg), **Int 4-1** (0.29 mmol, 146 mg), DIEA
 15 (0.75 mmol, 0.13 mL), and potassium iodide (0.73 mmol, 121 mg) was heated via microwave

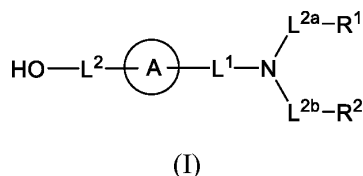
irradiation at 140 °C for 40 min. The reaction mixture was concentrated and the resultant crude was suspended in 95:5:1 (hexanes:EtOAc:Et₃N) and filtered. The filtrate was purified via flash chromatography (5% to 100% EtOAc in hexanes with 1% Et₃N) to give the titled compound (160 mg, 69%). ¹H NMR (400 MHz, CDCl₃) δ 3.60 - 3.49 (m, 1H), 3.32 - 3.23 (m, 4H), 3.23 - 3.15 (m, 4H), 2.51 - 2.31 (m, 5H), 2.31 - 2.21 (m, 4H), 2.06 - 1.93 (m, 2H), 1.84 - 1.02 (m, 97H), 0.92 - 0.81 (m, 12H). ESI-MS: *m/z* calcd for C₆₂H₁₂₃N₃O₃ = 958.0, found [M+H]⁺ = 959.2.

The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification, including US Provisional Patent Application No. 63/500,511 filed May 5, 2023, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications, and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

1. A compound having the following structure (I):



or a pharmaceutically acceptable salt or stereoisomer thereof, wherein:

A is a 3-10 membered carbocyclic or oxygen-containing heterocyclic ring optionally substituted with one or more fluoro, hydroxyl, C₁-C₆ alkyl, or C₁-C₆ alkylhydroxyl substituents;

R¹ is -NR^aC(=O)R³ or -C(=O)NR^bR^c;

R² is -NR^dC(=O)R⁴ or -C(=O)NR^eR^f;

R³ and R⁴ are each independently C₆-C₂₄ alkyl or C₆-C₂₄ alkenyl;

R^a, R^b, R^d and R^e are each independently H, C₁-C₂₀ alkyl or C₂-C₂₀ alkenyl;

R^c and R^f are each independently C₁-C₂₀ alkyl or C₂-C₂₀ alkenyl;

L¹ and L² are each independently a direct bond or C₁-C₆ alkylene; and

L^{2a} and L^{2b} are each independently C₄-C₁₂ alkylene;

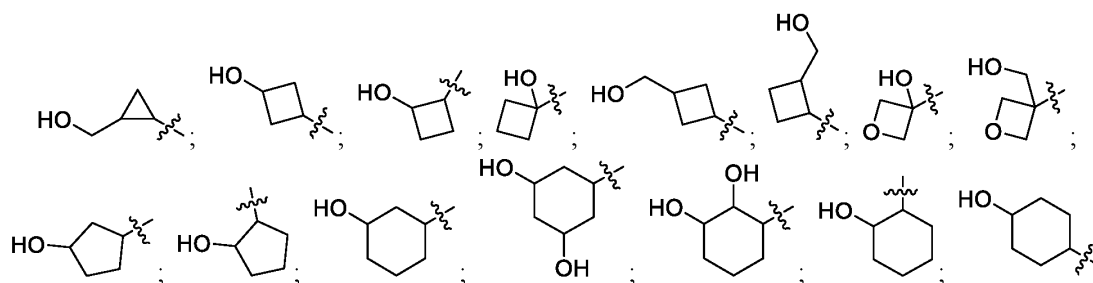
wherein each alkyl, alkylene, and alkenyl is optionally substituted with one or more fluoro.

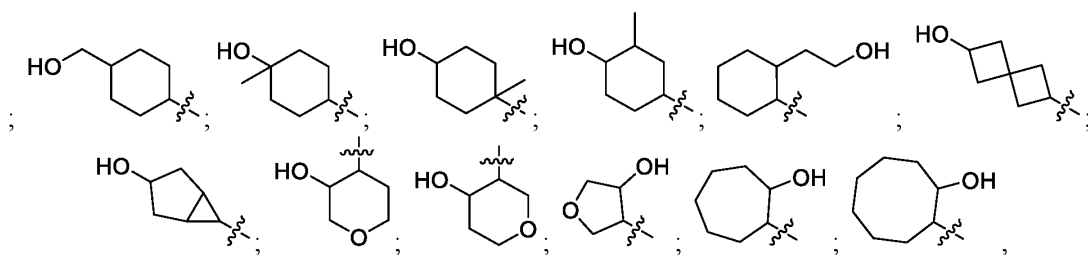
2. The compound of claim 1, wherein R¹ is -C(=O)NR^bR^c and R² is -C(=O)NR^eR^f.
3. The compound of claim 1, wherein R¹ is -NR^aC(=O)R³ and R² is -NR^dC(=O)R⁴.
4. The compound of claim 1, wherein R¹ is -NR^aC(=O)R³ and R² is -C(=O)NR^eR^f.
5. The compound of any one of claims 1-4, wherein R^a, R^b, R^c, R^d, R^e and R^f are each independently C₁-C₂₀ alkyl.
6. The compound of claim 5, wherein R^a, R^b, R^c, R^d, R^e and R^f are each independently C₈-C₁₉ alkyl.
7. The compound of claim 6, wherein R^a, R^b, R^c, R^d, R^e and R^f are each independently C₈, C₉, C₁₀, or C₁₉ alkyl.

8. The compound of any one of claims 1 or 3-7, wherein R^3 and R^4 are each independently C_6 - C_{19} alkyl.
9. The compound of claim 8, wherein R^3 and R^4 are each independently C_8 - C_{10} alkyl.
10. The compound of claim 9, wherein R^3 and R^4 are each independently C_8 , C_9 or C_{10} alkyl.
11. The compound of any one of claims 1-10, wherein L^1 is a direct bond.
12. The compound of any one of claims 1-10, wherein L^1 is C_1 - C_6 alkylene.
13. The compound of any one of claims 1-10, wherein L^1 is C_1 or C_2 alkylene.
14. The compound of any one of claims 1-13, wherein L^2 is a direct bond.
15. The compound of any one of claims 1-13, wherein L^2 is C_1 - C_6 alkylene.
16. The compound of any one of claims 1-13, wherein L^2 is C_1 or C_2 alkylene.
17. The compound of any one of claims 1-16, wherein L^{2a} and L^{2b} are each independently C_6 - C_{10} alkylene.
18. The compound of any one of claims 1-16, wherein L^{2a} and L^{2b} are each independently C_6 , C_7 , C_8 , C_9 , or C_{10} alkylene.
19. The compound of any one of claims 1-18, wherein A is a C_3 - C_{10} carbocyclic ring optionally substituted with one or more hydroxyl or C_1 - C_6 alkylhydroxyl substituents.
20. The compound of claim 19, wherein A is monocyclic.
21. The compound of claim 19, wherein A is bicyclic.

22. The compound of claim 19, wherein A is spirocyclic.
23. The compound of claim 19, wherein A is a C₃-C₈ carbocyclic ring optionally substituted with one or more hydroxyl, or C₁-C₆ alkylhydroxyl substituents.
24. The compound of claim 19, wherein A is cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, or cyclooctyl.
25. The compound of any one of claims 1-18, wherein A is a 3-10 membered oxygen-containing heterocyclic ring optionally substituted with one or more hydroxyl, or C₁-C₆ alkylhydroxyl substituents.
26. The compound of claim 24, wherein A is a 3-6 membered oxygen-containing heterocyclic ring optionally substituted with one or more hydroxyl, or C₁-C₆ alkylhydroxyl substituents.
27. The compound of claim 24, wherein A is oxiranyl, oxetaneyl, tetrahydrofuranyl, or tetrahydropyranyl.
28. The compound of any one of claims 1-27, wherein A is unsubstituted.
29. The compound of any one of claims 1-27, wherein A is substituted with hydroxyl.

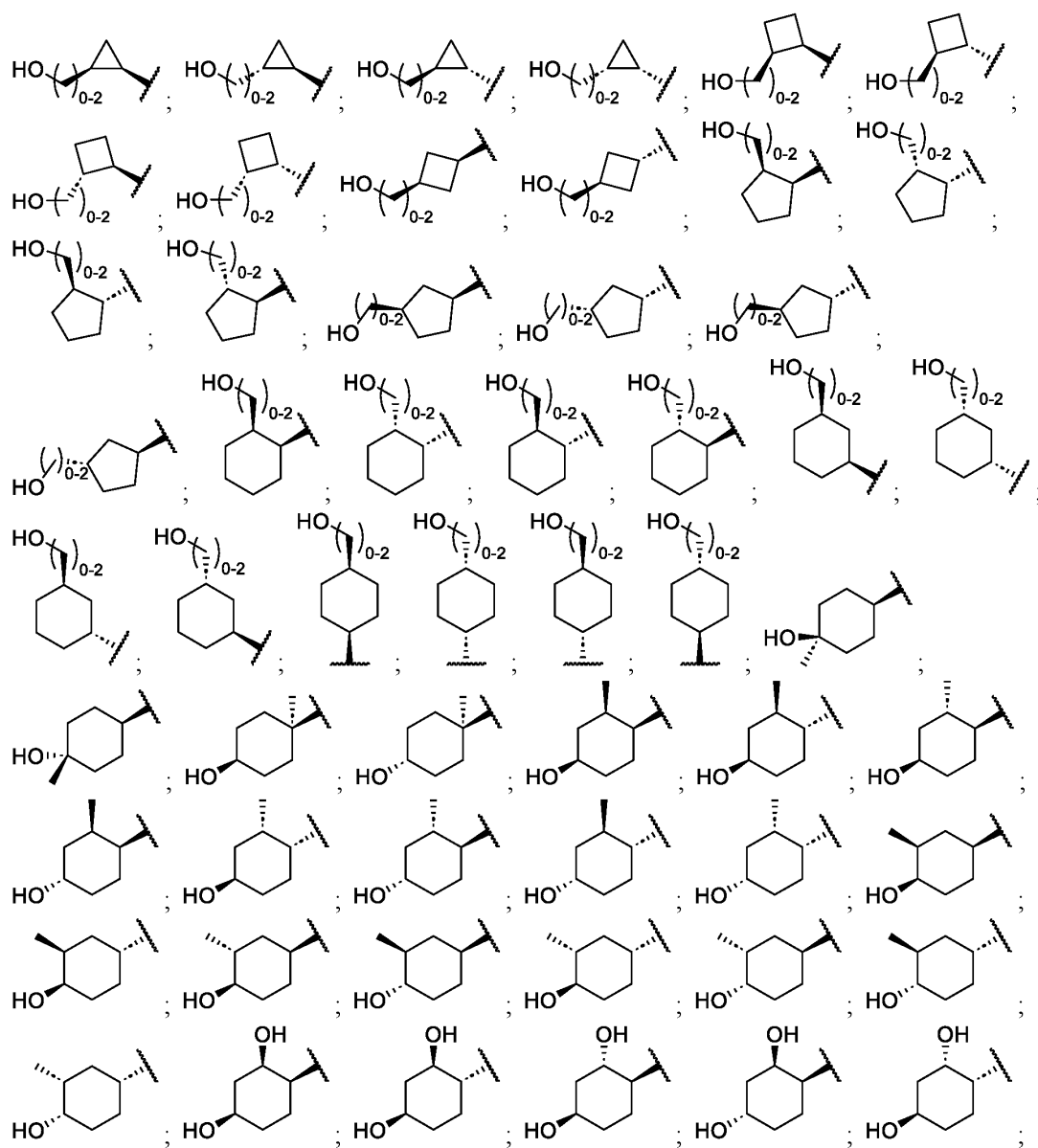
30. The compound of any one of claims 1-29, wherein $\text{HO}-\text{L}^2-\text{A}$ has one of the following structures:

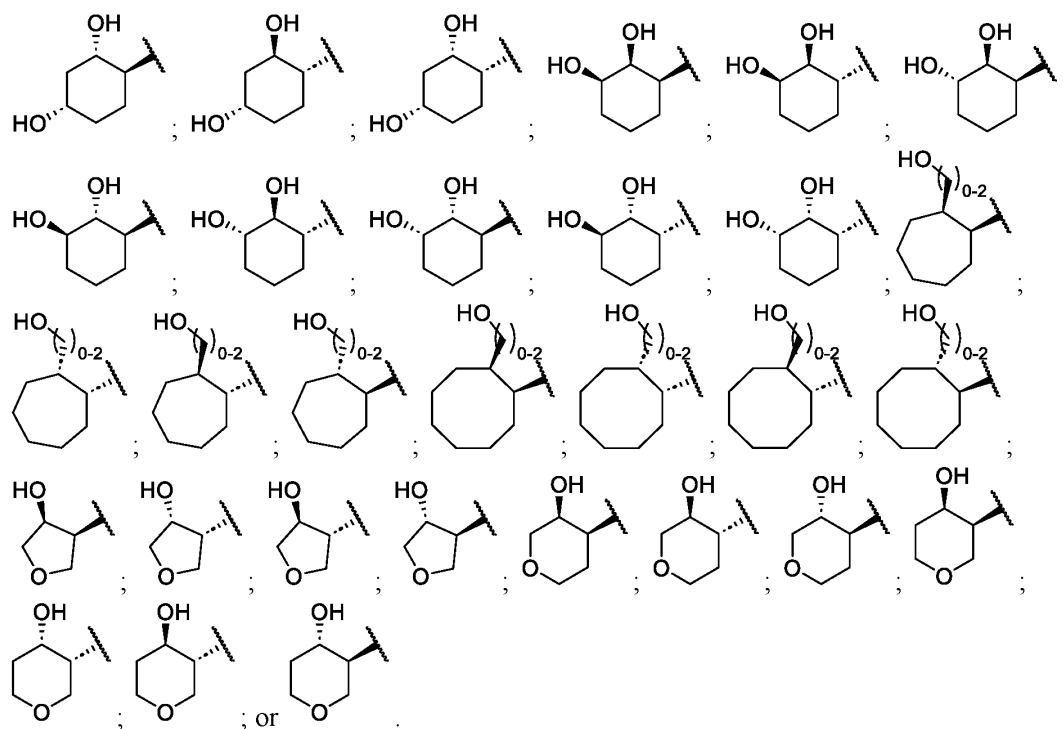




or a stereoisomer thereof.

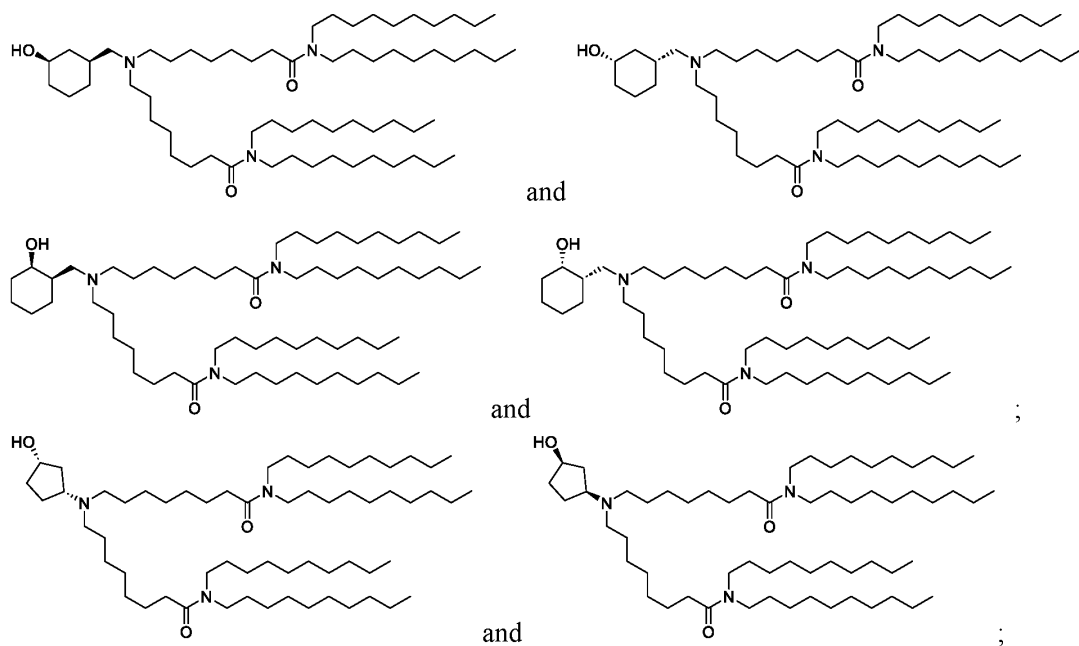
31. The compound of any one of claims 1-30, wherein $\text{HO}-\text{L}^2-\text{A}$ has one of the following structures:



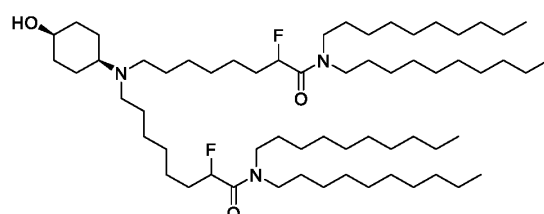
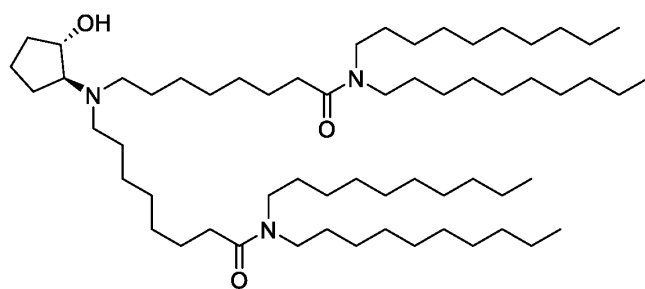


32. The compound of any one of claims 1-31, wherein one or more of R^a , R^b , R^c , R^d , R^e , R^f , L^1 , L^2 , L^{2a} and L^{2b} are substituted with one or more fluorine atoms.

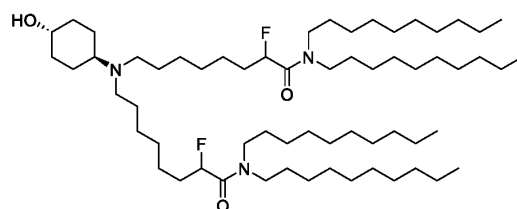
33. The compound of claim 1, wherein the compound is a racemic mixture of one of the following enantiomeric pairs or combinations of Structure (I):



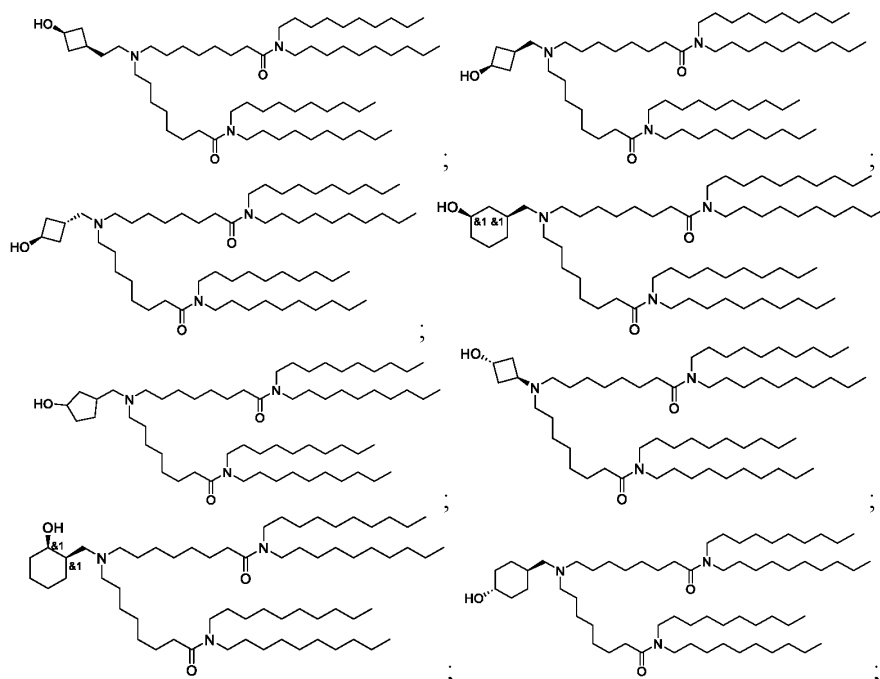


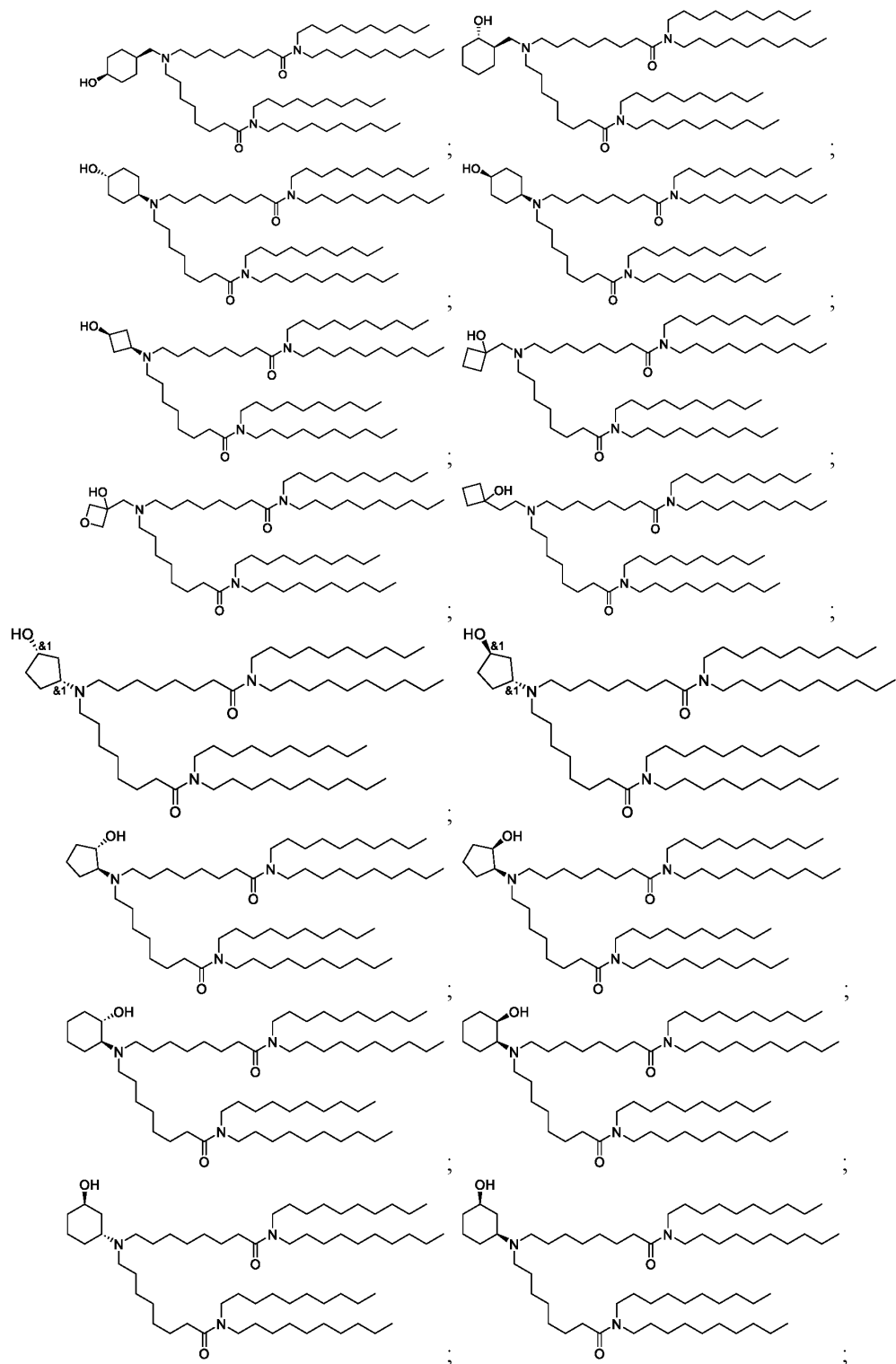


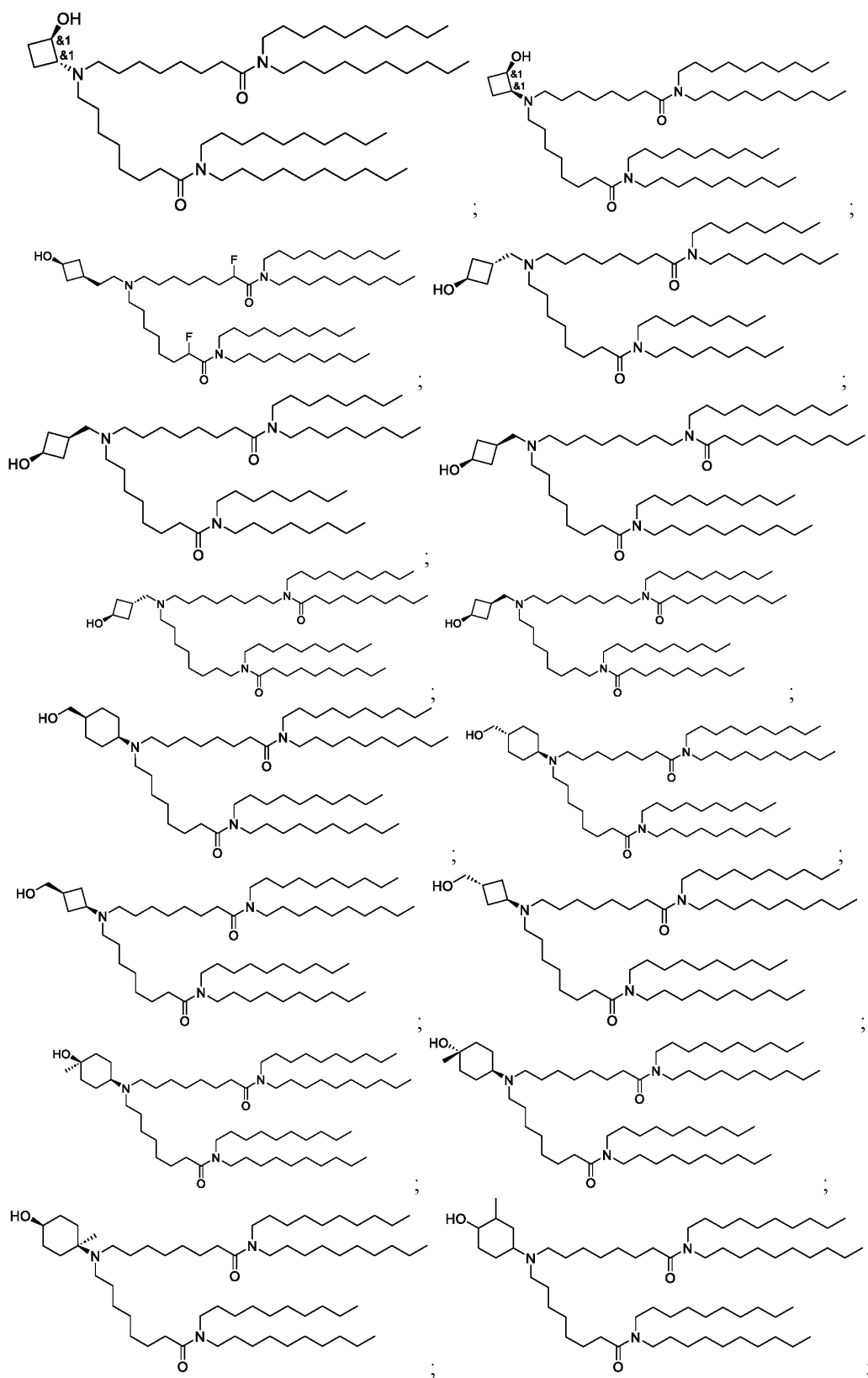
and

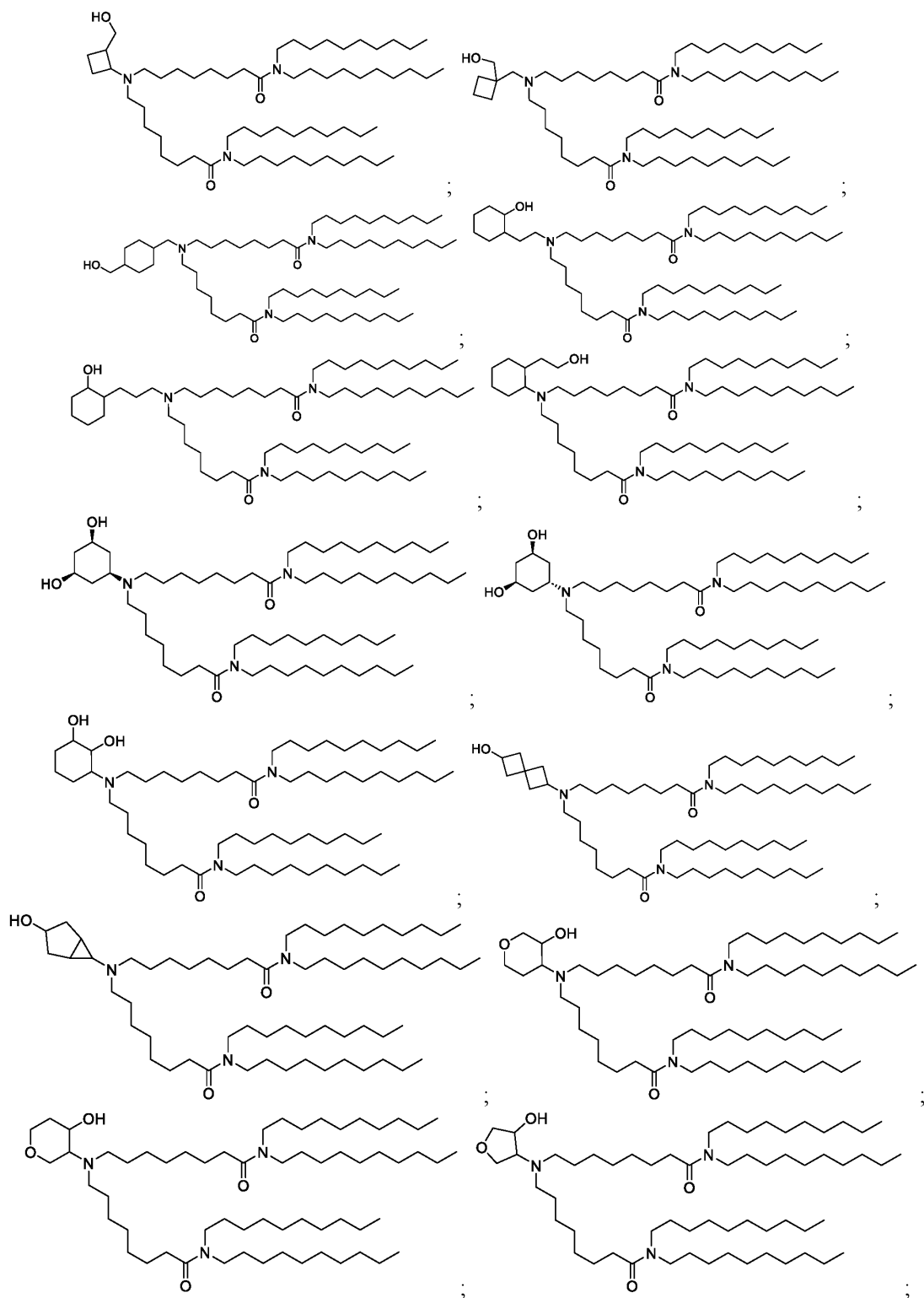


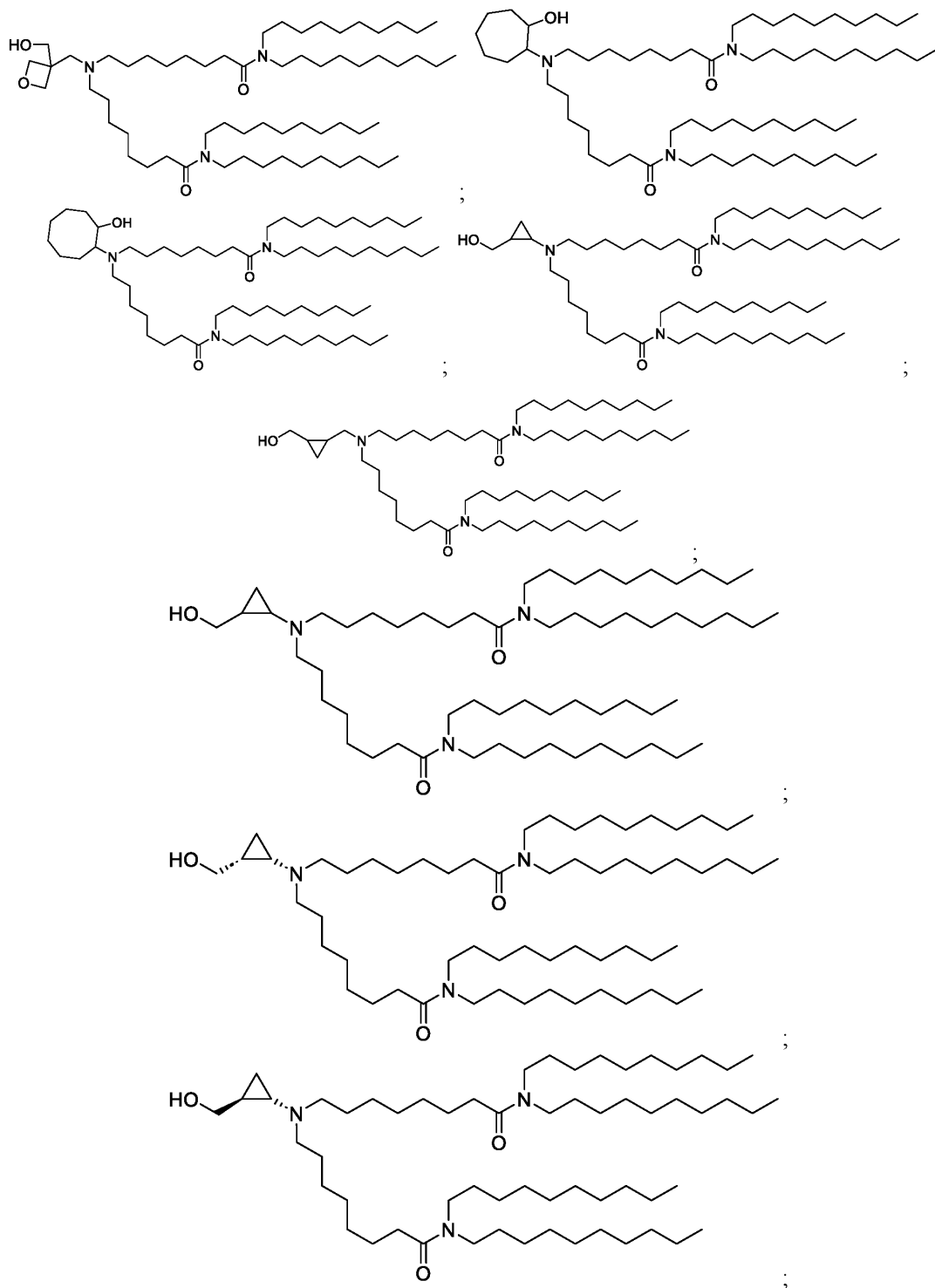
34. The compound of claim 1, having one of the following structures:

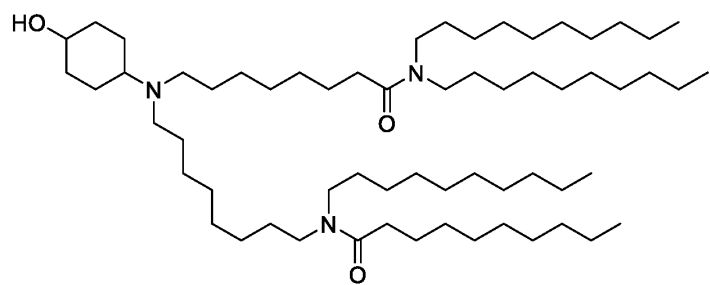




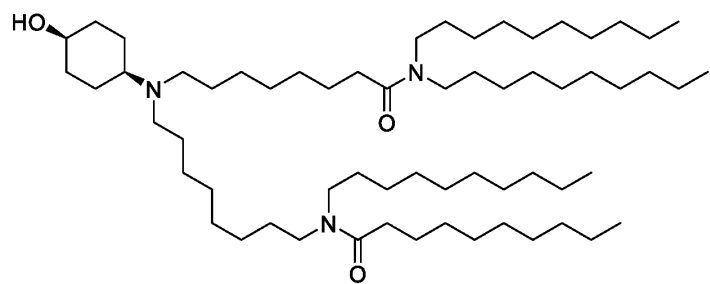




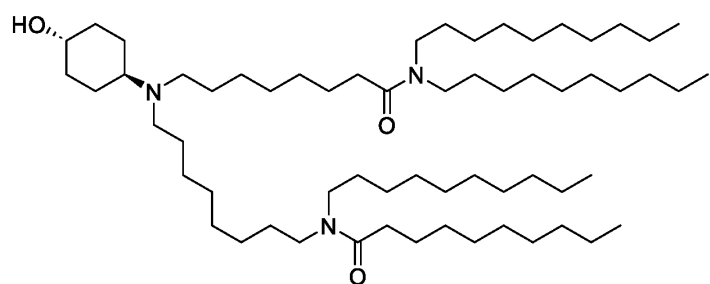




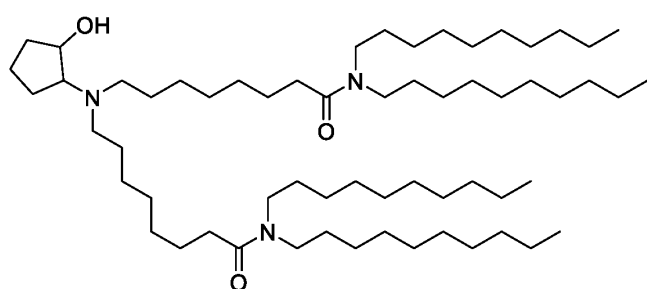
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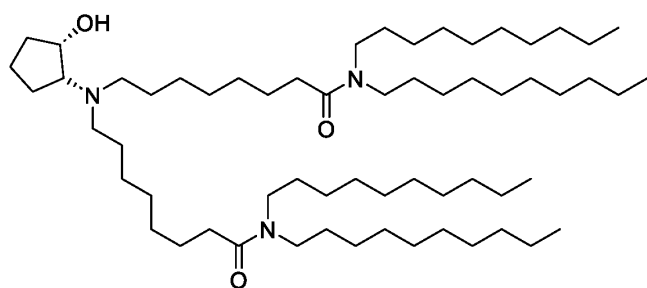
;



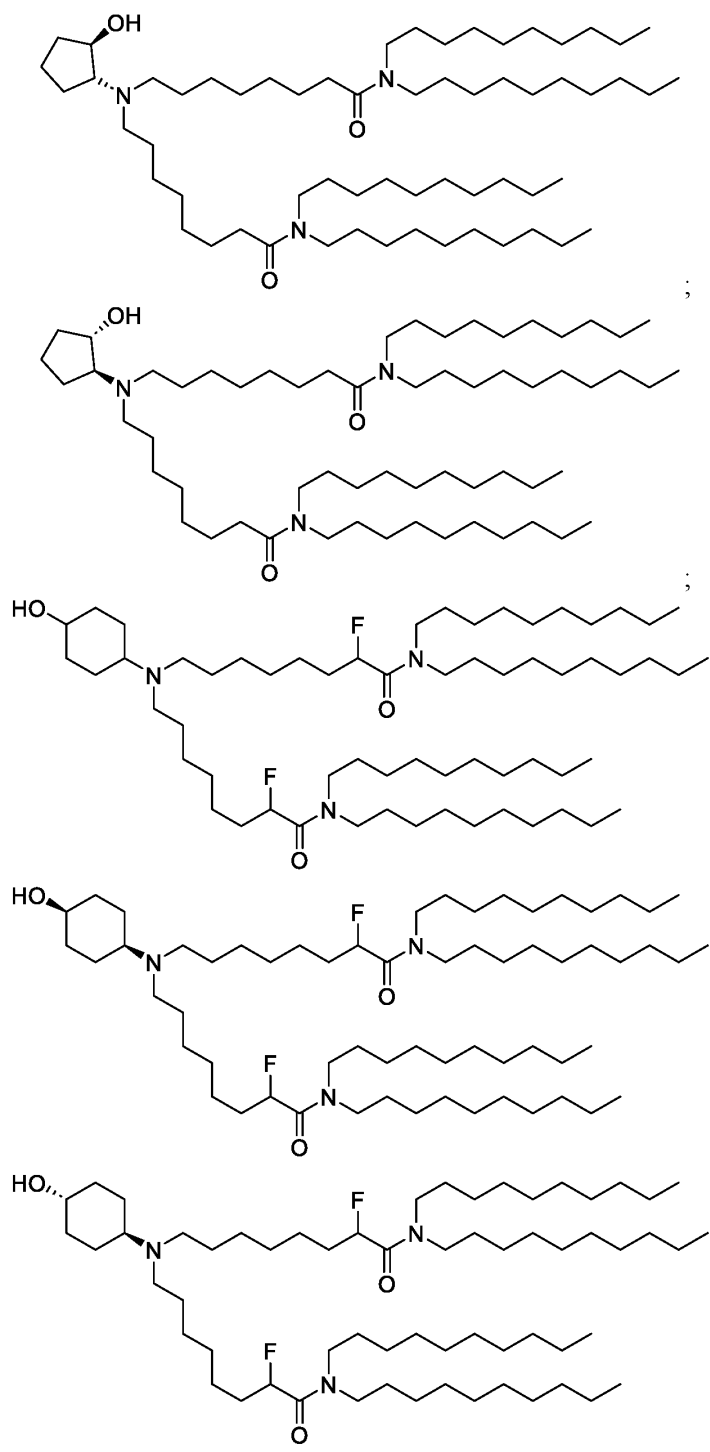
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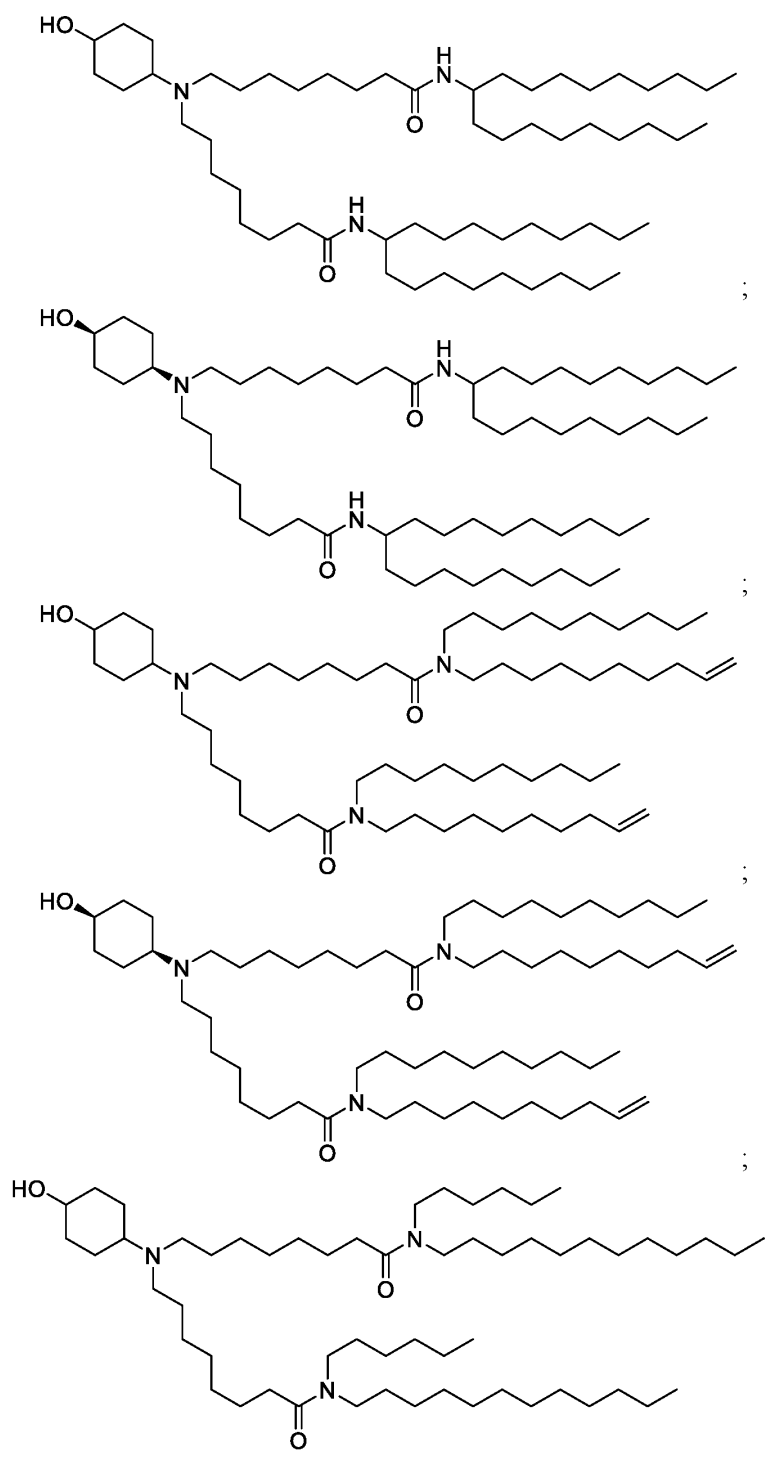


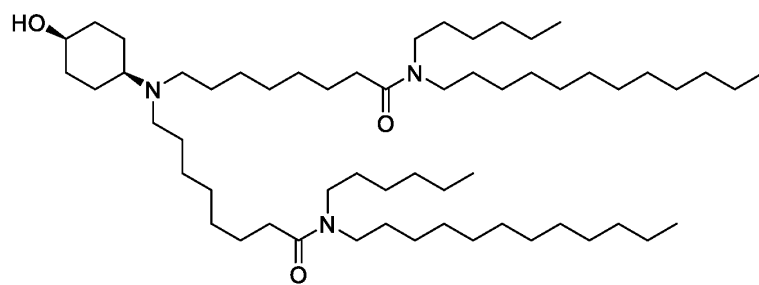
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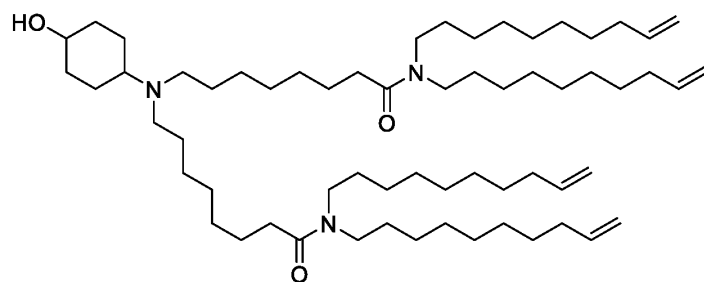
;



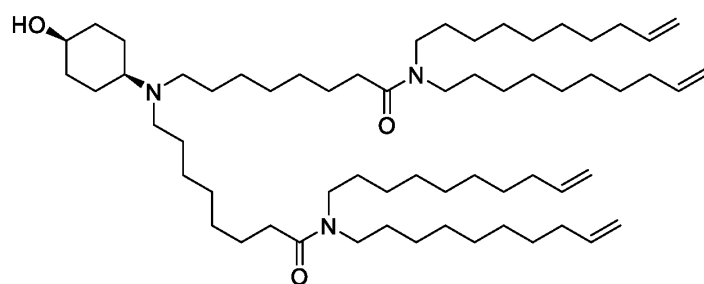




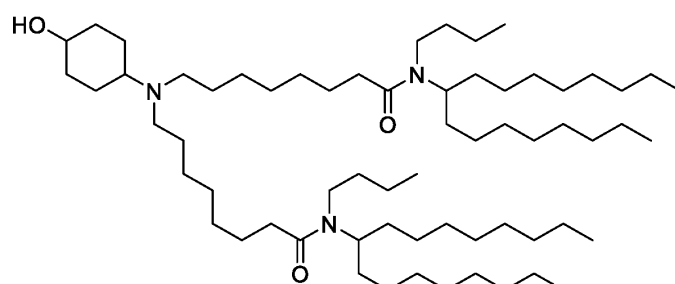
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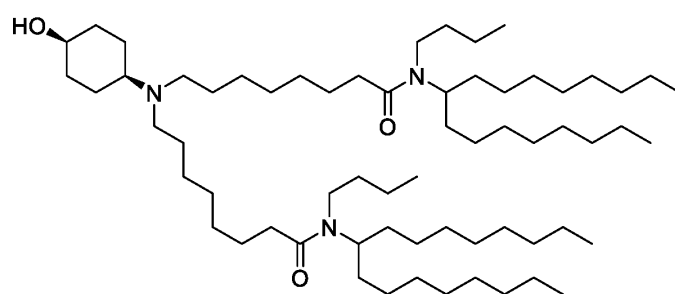
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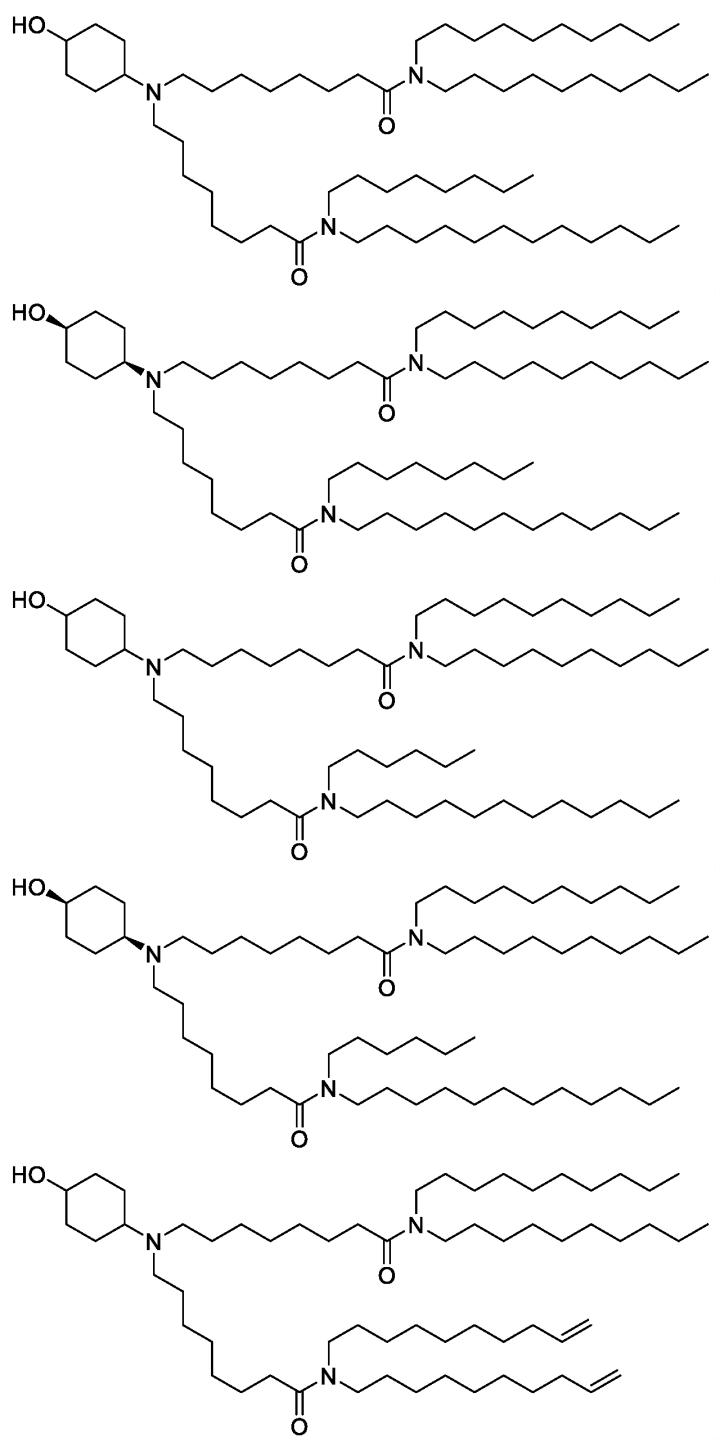
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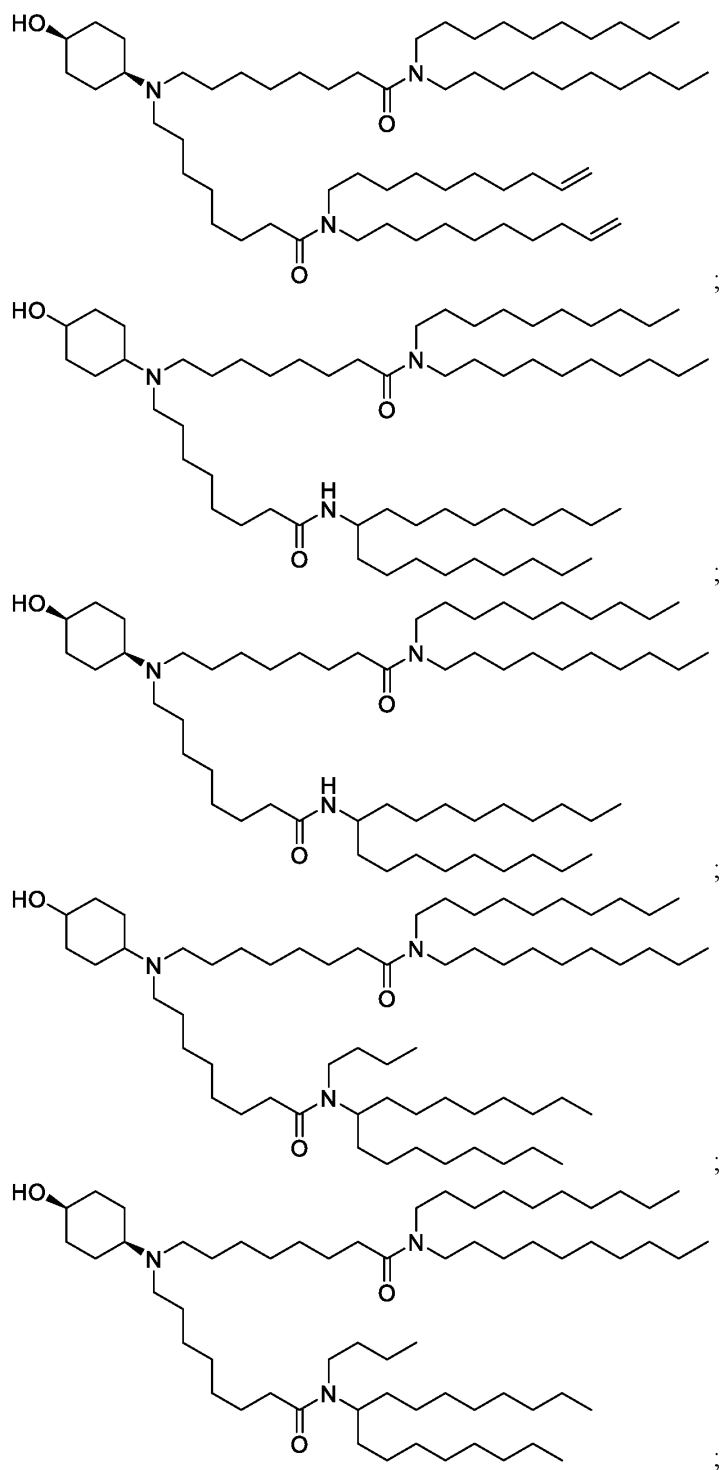


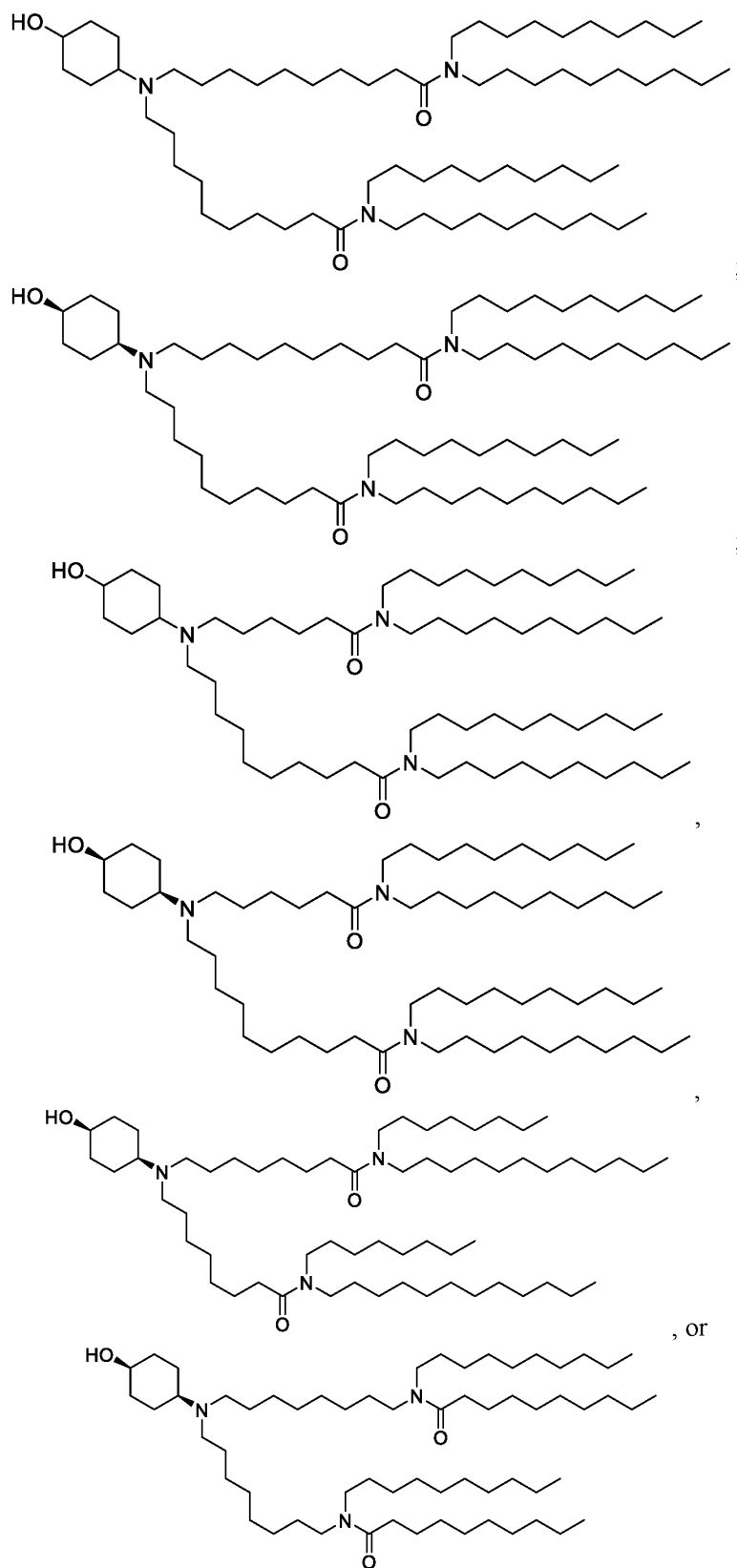
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;







35. A lipid nanoparticle comprising the compound of any one of claims 1-34 and a therapeutic agent.

36. A composition comprising the compound of any one of claims 1-34, or the nanoparticle of claim 35, and a therapeutic agent.

37. The lipid nanoparticle or composition of any one of claims 35 or 36, further comprising one or more excipient selected from neutral lipids, steroids and polymer conjugated lipids.

38. The lipid nanoparticle or composition of claim 37, comprising one or more neutral lipids selected from DSPC, DPPC, DMPC, DOPC, POPC, DOPE and SM.

39. The lipid nanoparticle or composition of claim 38, wherein the neutral lipid is DSPC.

40. The lipid nanoparticle or composition of any one of claims 37-39, wherein the molar ratio of the compound to the neutral lipid ranges from about 2:1 to about 8:1.

41. The lipid nanoparticle or composition of any one of claims 37-40, wherein the steroid is cholesterol.

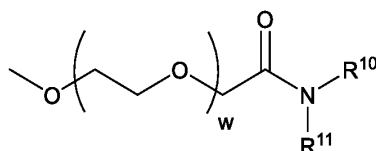
42. The lipid nanoparticle or composition of claim 41, wherein the molar ratio of the compound to cholesterol ranges from 5:1 to 1:1 or from 2:1 to 1:1.

43. The lipid nanoparticle or composition of any one of claims 37-42, wherein the polymer conjugated lipid is a pegylated lipid.

44. The lipid nanoparticle or composition of claim 43, wherein the molar ratio of the compound to pegylated lipid ranges from about 100:1 to about 20:1 or from about 100:1 to about 10:1.

45. The lipid nanoparticle or composition of claim 43 or 44, wherein the pegylated lipid is PEG-DAG, PEG-PE, PEG-S-DAG, PEG-cer or a PEG dialkoxypyrrolcarbamate.

46. The lipid nanoparticle or composition of claim 43 or 44, wherein the pegylated lipid has the following Structure (II):



(II)

or a pharmaceutically acceptable salt, tautomer, or stereoisomer thereof, wherein:

R¹⁰ and R¹¹ are each independently a straight or branched, alkyl, alkenyl or alkynyl from 10 to 30 carbon atoms, wherein the alkyl, alkenyl or alkynyl is optionally interrupted by one or more ester bonds; and

w has a value ranging from 30 to 60.

47. The lipid nanoparticle or composition of claim 46, wherein R¹⁰ and R¹¹ are each independently straight alkyl chain containing from 12 to 16 carbon atoms.

48. The lipid nanoparticle or composition of claim 46 or 47, wherein the lipid nanoparticle or composition comprises a plurality of compounds of Structure (II) and the average value of w for the plurality is about 49.

49. The lipid nanoparticle or composition of any one of claims 35-48, wherein the therapeutic agent comprises a nucleic acid.

50. The lipid nanoparticle or composition of claim 49, wherein the nucleic acid is selected from antisense and messenger RNA.

51. A method for administering a therapeutic agent to a patient in need thereof, the method comprising preparing or providing the lipid nanoparticle or composition of any one of claims 35-50 and administering the composition to the patient.

52. A pharmaceutical composition comprising the lipid nanoparticle of claim 35 and a pharmaceutically acceptable diluent or excipient.

53. A method for inducing expression of a protein in a patient in need thereof, comprising administering the pharmaceutical composition of claim 52 to the patient, wherein the lipid nanoparticle comprises an mRNA encoding the protein.

54. The method of claim 53, wherein the protein is an antigen and the method is for inducing an immune response in the patient.

55. The method of claim 53, wherein the protein is an antigen and the method is for vaccinating the patient against a pathogen.

56. The method of claim 53, wherein the protein is for gene editing.