

Constrained Ionizable Cationic Lipids and Lipid Nanoparticles**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to U.S. provisional application number 63/588,282, filed October 5, 2023; U.S. provisional application number 63/632,931, filed April 11, 2024; and U.S. provisional application number 63/654,744, filed May 31, 2024; the disclosures of each of which are expressly incorporated by reference herein.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing that has been submitted electronically and is hereby incorporated by reference in its entirety. The Sequence Listing was created on September 27, 2024, is named "24-0221-WO.xml", and is 11,729 bytes in size.

BACKGROUND

[0003] Lipid formulations have been used in the laboratory for delivering nucleic acids into cells. Early formulations based on the cationic lipid 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) and the ionizable, fusogenic lipid dioleoylphosphatidyl ethanolamine (DOPE) had a large particle size and were problematic when used *in vivo*, exhibiting too rapid clearance, tropism for the lung, and toxicity. Lipid nanoparticles (LNPs) comprising ionizable cationic lipids have been developed to address these issues to the extent that RNA-based products, such as the siRNA ONPATRO® and two mRNA-based SARS-CoV-2 vaccines have received regulatory approval and entered the marketplace.

[0004] However, there is limited ability to control which tissues or cells take up the LNP once administered. LNP administered intravenously are taken up primarily in the liver, lung, or spleen depending to a significant degree on net charge and particle size. It is possible to direct >90% of LNP to the liver by a combination of formulation and intravenous administration, for example. Intramuscular administration can provide a clinically useful level of local delivery and expression. LNP can be redirected to other tissues or cell types by conjugating to the LNP a binding moiety with specificity for the target tissue or cell type, for example, conjugating a polypeptide containing an antigen binding domain from an antibody. Nonetheless, avoiding uptake by the liver remains a challenge. Moreover, with current systems only a minor portion of the encapsulated nucleic acid is successfully delivered to the cells of interest and into the cytoplasm. Current formulations may release only 2-5% of the administered RNA into the cytoplasm (see for example Gilleron *et al.*, 2013, *Nat. Biotechnol.* 31:638-646 and Munson *et al.*, 2021, *Commun. Biol.* 4:211-224). There are remaining issues of off-target delivery, poor efficiency of release of nucleic acid into the cytoplasm, and toxicity associated with

accumulation of the component lipids.

[0005] Thus, there exists a need to address issues of off-target delivery, poor efficiency of release of nucleic acid into the cytoplasm, and toxicity associated with accumulation of the component lipids.

SUMMARY

[0006] This disclosure is directed towards fulfilling the needs for addressing issues of off-target delivery, poor efficiency of release of therapeutic agents and provides further related advantages.

[0007] In certain aspects, this disclosure provides constrained ionizable cationic lipids having a structure of formula M2 set forth herein.

[0008] In some embodiments, this disclosure provides ionizable cationic lipids CICL-207, CICL-215, CICL-216, CICL-217, CICL-218, CICL-219, CICL-220, CICL-221, CICL-222, CICL-223, CICL-224, CICL-225, CICL-238, CICL-239, CICL-242, CICL-243, CICL-244, CICL-245, CICL-246, CICL-247, CICL-248, and CICL-249 set forth herein.

[0009] In certain aspects, this disclosure provides methods of synthesizing ionizable cationic lipids as described herein, e.g., CICL-207, CICL-215, CICL-216, CICL-217, CICL-218, CICL-219, CICL-220, CICL-221, CICL-222, CICL-223, CICL-224, CICL-225, CICL-238, CICL-239, CICL-242, CICL-243, CICL-244, CICL-245, CICL-246, CICL-247, CICL-248, and CICL-249 set forth herein.

[0010] In certain aspects, this disclosure provides lipid nanoparticles (LNPs) and targeted lipid nanoparticles (tLNPs) incorporating the ionizable cationic lipids disclosed in this disclosure.

[0011] In certain aspects, this disclosure provides methods for preparing LNPs and tLNPs as described herein.

[0012] In certain embodiments, this disclosure provides methods of delivering a biologically active payload (e.g., nucleic acid molecules encoding a therapeutic agent) into a cell comprising contacting the cell with an LNP or tLNP of this disclosure.

[0013] These and other features, objects, and advantages of this disclosure will become better understood from the description that follows. In the description, reference is made to the accompanying drawings, which form a part hereof and in which there is shown by way of illustration, not limitation, embodiments of this disclosure. The description of preferred embodiments is not intended to limit this disclosure from covering all modifications,

equivalents, and alternatives. Reference should therefore be made to the claims recited herein for interpreting the scope of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The disclosure is better understood and features, aspects, and advantages other than those set forth above will become apparent when consideration is given to the following description of the drawings.

[0015] Figures 1A-1C depict the transfection rate (percentage of cells expressing the mRNA) versus expression level (as molecules of equivalent soluble fluorochrome (MESF)) for four tLNP compositions varying in the ionizable cationic lipid and/or PEG-lipid component, as indicated. The full lipid compositions are described in Table 7. Results in splenic T cells (Figure 1A), CD45⁺ liver cells (Figure 1B) and liver Kupffer cells (CD45⁺/CD11⁺ liver cells; Figure 1C) from C57BL/6 mice administered the tLNPs are shown. The binding moiety of the tLNPs was an anti-CD5 antibody and the payload was an mRNA encoding mCherry.

[0016] Figures 2A-2C depict the transfection rate (percentage of cells expressing the mRNA) versus expression level (as molecules of equivalent soluble fluorochrome (MESF)) for two tLNP compositions varying only in the ionizable cationic lipid component, as indicated. The full lipid compositions are described in Table 7. Results in splenic T cells (Figure 2A), CD45⁺ liver cells (Figure 2B) and liver Kupffer cells (CD45⁺/CD11⁺ liver cells; Figure 2C) from C57BL/6 mice administered the tLNPs are shown. The binding moiety of the tLNPs was an anti-CD5 antibody and the payload was an mRNA encoding mCherry.

[0017] Figures 3A-3B depict compiled transfection rate data (percentage of cells expressing the mRNA; Figure 3A) and expression level (MESF; Figure 3B) for tLNP incorporating various ionizable cationic lipids, or different amounts of ionizable cationic lipid, normalized to the result obtained for tLNP comprising 58% C1CL-207 as the ionizable cationic lipid in each experiment. The binding moiety of the tLNPs was an anti-CD5 antibody and the payload was an mRNA encoding mCherry.

[0018] Figures 4A-4C depict transfection rate for tLNP incorporating various ionizable cationic lipids versus measured pKa of the tLNP for splenic T cells (Figure 4A), CD45⁺ liver cells (Figure 4B) and liver Kupffer cells (CD45⁺/CD11⁺ liver cells; Figure 4C) from C57BL/6 mice administered the tLNPs. The full lipid compositions are described in Table 7. The binding moiety of the tLNPs was an anti-CD5 antibody and the payload was an mRNA encoding mCherry.

[0019] Figures 5A-5C depict the transfection rate (percentage of cells expressing the

mRNA) versus expression level (as MESF or mean fluorescence intensity (MFI)) for tLNP compositions varying only in the ionizable cationic lipid component, as indicated. The full lipid composition was C1CL:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL {58:10:30.5:1.4:0.1}. Results in splenic T cells (Figure 5A), CD45⁺ liver cells (Figure 5B) and liver Kupffer cells (CD45⁺/CD11⁺ liver cells; Figure 5C) from C57BL/6 mice administered the tLNPs are shown. The binding moiety of the tLNPs was an anti-CD5 antibody and the payload was an mRNA encoding mCherry.

[0020] Figures 6A-6C depict the transfection rate (percentage of cells expressing the mRNA) for tLNP comprising different amounts of ionizable cationic lipid for two ionizable cationic lipids and compensatory changes in cholesterol content. The full lipid compositions are described in Table 7. Results in splenic T cells (Figure 6A), CD45⁺ liver cells (Figure 6B) and liver Kupffer cells (CD45⁺/CD11⁺ liver cells; Figure 6C) from C57BL/6 mice administered the tLNPs are shown.

[0021] Figures 7A-7H portray the results of studies in NCG mice engrafted with human peripheral blood mononuclear cells (PBMCs) and dosed with tLNP composition 58% C1CL-1 (F9) and 58% C1CL-207 (F50) targeted to CD8 and encapsulating an mRNA encoding either mCherry or an anti-CD19 CAR. Figures 7A-7B depict mCherry or anti-CD19 CAR engineering rate in bar graphs for CD8⁺ T cells in spleen and blood from NCG mice engrafted with human PBMCs following administration of tLNP targeted to CD8⁺ cells. Results for engineering rate in splenic T and blood cells are displayed in Figures 7A and 7B, respectively. Figures 7C-7F depict anti-CD19 CAR expression as MFI and CAR molecules per cell (based on MESH) in bar graphs for CD8⁺ T cells in spleen and blood from NCG mice engrafted with human PBMCs following administration of tLNP targeted to CD8⁺ cells. Results for CAR expression MFI in splenic T and blood cells are displayed in Figures 7C and 7D, respectively. Results for CAR molecules per cell in splenic T cells and blood cells are displayed in Figures 7E and 7F, respectively. Figures 7G-7H depict B cells depletion in bar graphs in spleen for 6 hours after the mice received the tLNP dose. The number of B cells per μ L spleen cell suspension and percentage of B cells are displayed in Figures 7G and 7H respectively.

[0022] Figures 8A-8E present whole-animal images (Figures 8A-8B, prone and supine, respectively) and individual organ bioluminescence images collected at 6 and 24 hours post injection (Figures 8C-8D, respectively) and individual organ bioluminescence quantitation (Figure 8E) for C57BL/6 mice administered BF1 LNPs, CD5-targeted BF1 tLNPs, F50 LNPs, or CD5-targeted F50 tLNPs at 6 hours and 24 hours after administration.

[0023] Figure 9 depicts in vivo transfection efficiency as % mCherry positive cells in bone

marrow CD34⁺ cells (left panel) and CD34⁺CD117⁺ cells (right panel) in humanized CD34⁺ NCG mice after administration/transfection with mCherry mRNA using a tLNP decorated with an anti-CD117 monoclonal antibody. The tLNPs comprised either C1CL-1 (F9) or C1CL-207 (F50) lipid compositions and targeted CD117⁺ hematopoietic stem and progenitor cells (HSPCs). The bars represent the mean from the results of three mice (F9 tLNP mCherry and F50 tLNP mCherry groups), or one mouse (PBS group).

[0024] Figure 10 depicts transfection efficiency as % mCherry positive cells post CD117-targeted tLNP transfection in CD34⁺ NCG mice. The tLNPs comprised the C1CL-1 (F9) or C1CL-207 (F50) lipid compositions and encapsulated mCherry mRNA and targeted CD117⁺ hematopoietic stem and progenitor cells. Data is plotted for bone marrow CD34⁺CD38^{low} cells (upper left panel), CD117⁺CD34⁺CD38^{low} cells (upper right panel) and CD117-CD34⁺CD38^{low} cells (lower panel).

[0025] Figure 11 depicts percent B2M (beta-2-microglobulin) knock-out in bone marrow CD34⁺ (left panel) or CD34⁺CD38^{low} in humanized CD34⁺ NCG mice after administration/transfection of CD117-targeted tLNPs encapsulating gene editing payloads (SpCas9 mRNA + sgRNA targeting B2M locus), given in 30µg or 60µg of the tLNPs. The tLNPs comprised either C1CL-1 (F9) or C1CL-207 (F50) lipid compositions. The bars represent the mean of one to three mice with the symbols indicating results from mice engrafted with human CD34⁺ HSPC from three different donors (triangles, squares and circles).

[0026] Figures 12A-12L depict the levels of various liver enzymes, acute phase proteins, and cytokines in rats administered the F50 LNP. Figures 12A-12B plot mean liver enzyme levels in rat serum at 24 hours after dosage of F50 LNP composition for aspartate aminotransferase (AST) (Figure 12A) and alanine amino transferase (ALT) (Figure 12B). Figures 12C-12E depict the levels of three acute phase proteins in rat plasma after administration of the indicated doses of the F50 LNP at 6 and 24 hours after administration, specifically α1-acid glycoprotein (Figure 12C), α2-macroglobulin (Figure 12D), and haptoglobin (Figure 12E). Figures 12F-12H show the levels of three pro-inflammatory cytokines in rat plasma after administration of the indicated doses of the F50 LNP at 6 and 24 hours after administration, specifically interleukin-6 (Figure 12F), interleukin-1β (Figure 12G), tumor necrosis factor-α (Figure 12H).

DETAILED DESCRIPTION

[0027] This disclosure provides constrained ionizable cationic lipids, referred to as ionizable cationic lipids throughout, methods for synthesizing them, as well as intermediates

useful in synthesis of these lipids and methods of synthesizing the intermediates. This disclosure provides ionizable cationic lipids as a component of lipid nanoparticles (LNPs) that can be used for delivering a biologically active payload (e.g., nucleic acid molecules encoding a therapeutic agent) into cells *in vivo* or *ex vivo*. LNP compositions are also disclosed herein, including LNPs comprising a functionalized PEG-lipid to enable conjugation of a binding moiety to generate targeted LNPs (tLNPs), that is LNPs containing a binding moiety that directs the tLNP to a desired tissue or cell type (e.g., immune cells such as T cells or stem cells such as hematopoietic stem cells (HSCs)). Also disclosed herein are methods of delivering a nucleic acid into a cell comprising contacting the cell with an LNP or tLNP of this disclosure. The LNP and tLNP of this disclosure can be used for *in vivo*, *ex vivo*, or extracorporeal transfection. Also disclosed herein are methods for preparing LNPs and tLNPs comprising the ionizable cationic lipids as described herein.

[0028] Prior to setting forth this disclosure in more detail, it can be helpful to provide abbreviations and definitions of certain terms to be used herein. Additional abbreviations are set forth throughout this disclosure.

Abbreviations

[0029] Abbreviations used herein include:

[0030] BOC₂O – Di-tert-butyl dicarbonate

[0031] CDCl₃ – Deuteriochloroform

[0032] CDI – carbonyl diimidazole

[0033] CH₃CN – Acetonitrile

[0034] CHOL – Cholesterol

[0035] DMAP – 4-Dimethylaminopyridine

[0036] DMG – Dimyristoyl glycerol

[0037] DSG – Distearoyl glycerol

[0038] DSPC – Distearoylphosphatidylcholine

[0039] DSPE – Distearoylphosphatidylethanolamine

[0040] EDC-HCl – 1-Ethyl-3-(3'-dimethylaminopropyl)carbodiimide · HCl

[0041] EtOAc – Ethyl acetate

- [0042]** EtOH – Ethanol
- [0043]** Et₂O – Diethyl ether
- [0044]** Et₃N – Triethylamine
- [0045]** MAL – Maleimide
- [0046]** Me₃N – Trimethylamine
- [0047]** MeOH – Methanol
- [0048]** MeOTf – methyl trifluoromethanesulfonate
- [0049]** Pd/C – Palladium on carbon
- [0050]** PEG – Polyethylene glycol
- [0051]** PhCH₃ – Toluene
- [0052]** TFA – Trifluoroacetic acid
- [0053]** THF – Tetrahydrofuran

[0054] While this disclosure is capable of being embodied in various forms, the description below of several embodiments is made with the understanding that the disclosure is to be considered as an exemplification of the innovations disclosed herein and is not intended to limit the disclosure to the specific embodiments illustrated.

[0055] Headings are provided for convenience only and are not to be construed to limit the embodiments in any manner. Embodiments illustrated under any heading can be combined with embodiments illustrated under any other heading.

[0056] To the extent any materials incorporated herein by reference conflict with this disclosure, this disclosure controls.

Definitions

[0057] Prior to setting forth this disclosure in more detail, provided are definitions of certain terms to be used herein. Additional definitions are set forth throughout this disclosure.

[0058] As used in the specification and claims, the singular form “a,” “an,” and “the” includes plural references unless the context clearly dictates otherwise. It should be understood that the terms “a” and “an” as used herein refer to “one or more” of the enumerated components.

[0059] The use of the alternative (e.g., “or”) should be understood to mean either one, both, or any combination thereof of the alternatives.

[0060] The term “about” as used herein in the context of a number refers to a range centered on that number and spanning 10% less than that number and 10% more than that number. The term “about” used in the context of a range refers to an extended range spanning 10% less than that of the lowest number listed in the range and 10% more than the greatest number listed in the range.

[0061] Throughout this disclosure, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Also, any number range of this disclosure relating to any physical feature, such as polymer subunits, size, or thickness, are to be understood to include any integer within the recited range, unless otherwise indicated. Throughout this disclosure, numerical ranges are inclusive of their recited endpoints, unless specifically stated otherwise.

[0062] Unless the context requires otherwise, throughout this specification and claims, the word “comprise” and variations thereof, such as, “comprises” and “comprising” are to be construed in an open, inclusive sense, that is, as “including, but not limited to.” As used herein, the terms “include” and “comprise” are used synonymously.

[0063] The phrase “at least one of” when followed by a list of items or elements refers to an open-ended set of one or more of the elements in the list, which can, but does not necessarily, include more than one of the elements.

[0064] “Derivative,” as used herein, refers to a chemically or biologically modified version of a compound that is structurally similar to a parent compound and (actually or theoretically) derivable from that parent compound. Generally, a “derivative” differs from an “analogue” in that a parent compound may be the starting material to generate a “derivative,” whereas the parent compound is not necessarily used as the starting material to generate an “analogue.” A derivative may have different chemical or physical properties than the parent compound. For example, a derivative may be more hydrophilic or hydrophobic, or it may have altered reactivity as compared to the parent compound. Although a derivative can be obtained by physical (for example, biological or chemical) modification of the parent compound, a derivative can also be conceptually derived, for example, as when a protein sequence is designed based on one or more known sequences, an encoding nucleic acid is constructed, and the derived protein obtained by expression of the encoding nucleic acid.

[0065] As used herein “expansion” refers to proliferation of cells increasing their number. Activating agents can be used to stimulate proliferation (among other metabolic changes) but can also result in activation induced death upon initial exposure so that there is no immediate expansion. For T cells treated *in vitro* with activating agents such as IL-2 or CD3/CD28 activators, doubling time can be about 24 hours (which is fairly typical of mammalian cells *in vitro* generally); *in vivo* doubling time can be substantially shorter, depending on the presence and type of stimulation. Accordingly, during a limited time of extracorporeal manipulation, even when activating agents are used, such protocols will be effectively expansion-less.

[0066] As used herein an “exogenous protein” refers to a synthetic, recombinant, or other peptide or protein that is not produced by a wild-type cell of that type or is expressed at a lower level in a wild-type cell than in a cell containing the exogenous polypeptide. In some embodiments, an exogenous peptide is a peptide or protein encoded by a nucleic acid that was introduced into the cell, which nucleic acid is optionally not retained by the cell. As used herein “peptide” refers to a chain of amino acids less than 50 amino acids in length, while “protein” and “polypeptide” refer to a chain of amino acids at least 50 amino acids in length.

[0067] As used herein “extracorporeal” is used in reference to cells, such as peripheral blood or bone marrow cells, harvested or extracted from the body and the manipulation or modification of those cells prior to their intended return (reinfusion). Manipulation and modification of cells generally relates to cell separation and washing procedures and exposure to activation agents (e.g., biological response modifiers (BRMs)) and transfection agents (e.g., LNPs, tLNPs), over a time interval of several hours, for example, less than 6 hours, less than 5 hours, less than 4 hours, less than 3 hours, less than 2 hours, or less than 1 hour; and in space to a single institution. Extracorporeal is used in contradistinction to *ex vivo* which, as used herein, includes more extensive manipulation including extended periods of cell culture and expansion, and/or refrigerated or cryogenic storage or shipment, over several days or longer.

[0068] As used herein “transfection” or “transfecting” refers to the introduction of nucleic acids into cells by non-viral methods. Transfection can be mediated by calcium phosphate, cationic polymers, magnetic beads, electroporation, and lipid-based reagents. In preferred embodiments disclosed herein transfection is mediated by solid lipid nanoparticles (LNP) including targeted LNP (tLNP) (which can also be used to deliver non-nucleic acid payloads into cells). The term transfection is used in distinction to transduction – transfer of genetic material from cell to cell or virus to cell – and transformation – the uptake of extracellular genetic material by the natural processes of a cell. As used herein, phrases such as “delivering

a nucleic acid into a cell” are synonymous with transfection.

[0069] “Reprogramming,” as used herein with respect to immune cells, refers to changing the functionality of an immune cell with respect to antigenic specificity by causing expression of an exogenous T cell receptor (TCR), a chimeric antigen receptor (CAR), or an immune cell engager (“reprogramming agents”). Generally, T lymphocytes and natural killer (NK) cells could be reprogrammed with a TCR, a CAR, or an immune cell engager while only a CAR or an immune cell engager would be used in reprogramming monocytes. As used herein with respect to stem cells, for example hematopoietic stem cells (HSC) or mesenchymal stem cells (MSC), “reprogramming” refers to correction or amelioration of a genetic defect (for example, a hemoglobinopathy) so that the modified or corrected gene and gene product are the reprogramming agents. Reprogramming can be transient or durable depending on the nature of the engineering agent.

[0070] “Engineering agent,” as used herein, refers to agents that confer the expression of a reprogramming agent by an immune cell, particularly a non-B lymphocyte or monocyte. Engineering agents can include nucleic acids, including mRNA, that encode the reprogramming agent. Engineering agents can also include nucleic acids that are or encode components of gene editing systems such as RNA-guided nucleases, guide RNA, and nucleic acid templates for knocking-in a reprogramming agent or knocking-out an endogenous antigen receptor. Gene editing systems comprise base-editors, prime-editors or gene-writers. RNA-guided nucleases include CRISPR nucleases such as Cas9, Cas12, Cas13, Cas3, CasMINI, Cas7-11, and CasX. For transient expression of a reprogramming agent, such as a CAR, an mRNA encoding the reprogramming agent can be used as the engineering agent. For durable expression of the reprogramming agent, such as an exogenous, modified, or corrected gene (and its gene product), the engineering agent can comprise mRNA-encoded RNA-directed nucleases, guide RNAs, nucleic acid templates and other components of gene/genome editing systems.

[0071] Examples of gene editing components that are encoded by a nucleic acid molecule include an mRNA encoding an RNA-guided nuclease, a gene or base editing protein, a prime editing protein, a Gene Writer protein (e.g., a modified or modularized non-long terminal repeat (LTR) retrotransposon), a retrotransposase, an RNA writer, a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a meganuclease, a transposase, a retrotransposon, a reverse transcriptase (e.g., M-MLV reverse transcriptase), a nickase or inactive nuclease (e.g., Cas9, nCas9, dCas9), a DNA recombinase, a CRISPR nuclease (e.g., Cas9, Cas12, Cas13, Cas3, CasMINI, Cas7-11, CasX), a DNA nickase, a Cas9 nickase (e.g.,

D10A or H840A), or any fusion or combination thereof. Other components include a guide RNA (gRNA), a single guide RNA (sgRNA), a prime editing guide RNA (pegRNA), a clustered regularly interspaced short palindromic repeat (CRISPR) RNA (crRNA), a trans-activating clustered regularly interspaced short palindromic repeat (CRISPR) RNA (tracrRNA), or a DNA molecule to be inserted or serve as a template for double-strand break (DSB) repair at a specific genomic locus. Genome-, gene-, and base-editing technology are reviewed in Anzalone *et al.*, *Nature Biotechnology* 38:824-844, 2020, Sakuma, *Gene and Genome Editing* 3-4:100017, 2022, and Zhou *et al.*, *MedComm* 3(3):e155, 2022, each of which is incorporated by reference for all that they teach about the components and uses of this technology to the extent that it does not conflict with the present disclosure.

[0072] “Conditioning agent,” as used herein, refers to a biological response modifier (BRM) that enhances the efficiency of engineering an immune cell, expands the number of immune cells available to be engineered or the number of engineered cells in a target tissue (for example, a tumor, fibrotic tissue, or tissue undergoing autoimmune attack), promotes activity of the engineered cell in a target tissue, or broadens the range of operative mechanisms contributing to a therapeutic immune reaction. A conditioning agent may be provided by delivering an encoding nucleic acid in a tLNP. Exemplary BRMs include cytokines, such as IL-7, IL-15, or IL-18.

[0073] “Immune cell,” as used herein, can refer to any cell of the immune system. However, particular aspects can exclude polymorphonuclear leukocytes and/or B cells, or be limited to non-B lymphocytes such as T cells and/or NK cells, or to monocytes such as dendritic cells and/or macrophages in their various forms.

[0074] As used herein, “lipid nanoparticle” (LNP) means a solid particle, as distinct from a liposome having an aqueous lumen. The core of an LNP, like the lumen of a liposome, is surrounded by a layer of lipid that can be, but is not necessarily, a continuous lipid monolayer, a bilayer, or multi-layer having three or more lipid layers.

[0075] As used herein, a “binding moiety” or “targeting moiety” refers to a protein, polypeptide, oligopeptide, peptide, carbohydrate, nucleic acid, or combination thereof that is capable of specifically binding to a target or multiple targets. A binding domain includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a biological molecule or another target of interest. Exemplary binding moieties of this disclosure include an antibody, a Fab', F(ab')₂, Fab, Fv, rIgG, scFv, hcAbs (heavy chain antibodies), a single domain antibody, VHH, VNAR, sdAbs, nanobody, receptor ectodomains or ligand-binding portions thereof, or ligands (e.g., cytokines, chemokines). A “Fab” (fragment

antigen binding) is the part of an antibody that binds to antigens and includes the variable region and CH1 of the heavy chain linked to the light chain via an inter-chain disulfide bond. In other embodiments, a binding moiety comprises a ligand-binding domain of a receptor or a receptor ligand. In some embodiments, a binding moiety can have more than one specificity including, for example, bispecific or multispecific binders. A variety of assays are known for identifying binding moieties of this disclosure that specifically bind a particular target, including Western blot, ELISA, and Biacore® analysis. A binding moiety, such as a binding moiety comprising immunoglobulin light and heavy chain variable domains (e.g., scFv), can be incorporated into a variety of protein scaffolds or structures as described herein, such as an antibody or an antigen binding fragment thereof, a scFv-Fc fusion protein, or a fusion protein comprising two or more of such immunoglobulin binding domains.

[0076] As used herein, “antibody” refers to a protein comprising an immunoglobulin domain having hypervariable regions determining the specificity with which the antibody binds antigen; so-called complementarity determining regions (CDRs). The term antibody can thus refer to intact or whole antibodies as well as antibody fragments and constructs comprising an antigen binding portion of a whole antibody. While the canonical natural antibody has a pair of heavy and light chains, camelids (camels, alpacas, llamas, etc.) produce antibodies with both the canonical structure and antibodies comprising only heavy chains. The variable region of the camelid heavy chain only antibody has a distinct structure with a lengthened CDR3 referred to as VHH or, when produced as a fragment, a nanobody. Antigen binding fragments and constructs of antibodies include F(ab)₂, F(ab), minibodies, Fv, single-chain Fv (scFv), diabodies, and VH. Such elements can be combined to produce bi- and multi-specific reagents, such as Bispecific T-Cell Engagers (BiTEs). The term “monoclonal antibody” arose out of hybridoma technology but is now used to refer to any singular molecular species of antibody regardless of how it was originated or produced. Antibodies can be obtained through immunization, selection from a naïve or immunized library (for example, by phage display), alteration of an isolated antibody-encoding sequence, or any combination thereof. Numerous antibodies that could be used as binding moieties are known in the art. An excellent source of information about antibodies for an International Non-proprietary Name (INN) has been proposed or recommended, including sequence information, is Wilkinson & Hale, 2022, *MAbs* 14(1):2123299, including its Supplementary Tables, which is incorporated by reference herein for all that it teaches about individual antibodies and the various antibody formats that can be constructed. U.S. Patent No. 11,326,182 and especially its Table 9 entitled “Cancer, Inflammation and Immune System Antibodies,” is a source of sequence and other information for a wide range of antibodies including many that do not have

an INN and is incorporated herein by reference for all that it teaches about individual antibodies.

[0077] An antibody or other binding moiety (or a fusion protein thereof) “specifically binds” a target if it binds the target with an affinity or K_a (i.e., an equilibrium association constant of a particular binding interaction with units of $1/M$) equal to or greater than $10^5 M^{-1}$, while not significantly binding other components present in a test sample. Binding domains (or fusion proteins thereof) can be classified as “high affinity” binding domains (or fusion proteins thereof) and “low affinity” binding domains (or fusion proteins thereof). “High affinity” binding domains refer to those binding domains with a K_a of at least $10^8 M^{-1}$, at least $10^9 M^{-1}$, at least $10^{10} M^{-1}$, at least $10^{11} M^{-1}$, at least $10^{12} M^{-1}$, or at least $10^{13} M^{-1}$, preferably at least $10^8 M^{-1}$ or at least $10^9 M^{-1}$. “Low affinity” binding domains refer to those binding domains with a K_a of up to $10^8 M^{-1}$, up to $10^7 M^{-1}$, up to $10^6 M^{-1}$, up to $10^5 M^{-1}$. Alternatively, affinity can be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (e.g., $10^{-5} M$ to $10^{-13} M$). Affinities of binding domain polypeptides and fusion proteins according to this disclosure can be readily determined using conventional techniques (see, e.g., Scatchard *et al.*, 1949, Ann. N.Y. Acad. Sci. 51:660; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

[0078] As used herein, “payload” refers to a negatively charged biologically active agent that can interact with cationic lipids, such as the ionizable cationic lipids of this disclosure, to become encapsulated within lipid nanoparticles comprising the cationic lipid. The negatively charged, biologically active agent can be a small organic molecule, or a macromolecule such as a nucleic acid, a carbohydrate, or a peptide or polypeptide. In many embodiments, the payload can be one or more nucleic acid molecules, RNA or DNA, including mRNA and guide RNA (gRNA) molecules.

[0079] As used herein, “biologically active agent” refers to any substance, or a component of a combination of substances, that affects a metabolic or physiologic response in a living organism or cultured cells thereof.

[0080] As used herein, “therapeutic agent” is a substance the biological activity of which can potentially cure, ameliorate, stabilize, prevent, or otherwise beneficially impact a disease, pathological condition, or other disorder.

[0081] For simplicity, chemical moieties are defined and referred to throughout primarily as univalent chemical moieties (e.g., alkyl, aryl, and the like). Nevertheless, such terms can also be used to convey corresponding multivalent moieties under the appropriate structural

circumstances clear to those skilled in the art. For example, while an “alkyl” moiety generally refers to a monovalent radical (e.g. $\text{CH}_3\text{-CH}_2\text{-}$), in certain circumstances a bivalent linking moiety can be “alkyl,” in which case those skilled in the art will understand the alkyl to be a divalent radical (e.g., $\text{-CH}_2\text{-CH}_2\text{-}$), which is equivalent to the term “alkylene.” (Similarly, in circumstances in which a divalent moiety is required and is stated as being “aryl,” those skilled in the art will understand that the term “aryl” refers to the corresponding divalent moiety, arylene.) All atoms are understood to have their normal number of valences for bond formation (i.e., 4 for carbon, 3 for N, 2 for O, and 2, 4, or 6 for S, depending on the oxidation state of the S).

[0082] The term “alkyl” as employed herein refers to saturated straight and branched chain aliphatic groups having from 1 to 12 carbon atoms. As such, “alkyl” encompasses C_1 , C_2 , C_3 , C_4 , C_5 , C_6 , C_7 , C_8 , C_9 , C_{10} , C_{11} and C_{12} groups.

[0083] The term “alkenyl” as used herein means an unsaturated straight or branched chain aliphatic group with one or more carbon-carbon double bonds, having from 2 to 12 carbon atoms. As such, “alkenyl” encompasses C_2 , C_3 , C_4 , C_5 , C_6 , C_7 , C_8 , C_9 , C_{10} , C_{11} and C_{12} groups.

[0084] In some embodiments, the hydrocarbon chain is unsubstituted. In other embodiments, one or more hydrogens of the alkyl or alkenyl group can be substituted with the same or different substituents.

[0085] Alkynoic refers to a carboxylic acid moiety comprising one or more carbon-carbon triple bonds. In some embodiments, hydrogens are unsubstituted. In other embodiments, one or more hydrogens of the alkynoic group can be substituted with the same or different substituents.

[0086] Amide refers to a carboxylic acid derivative comprising a carbonyl group of a carboxylic acid bonded to an amine moiety.

[0087] Ester refers to a carboxylic acid derivative comprising a carbonyl group bond to an alkyloxy group to form an ester bond -C(=O)-O- .

[0088] Head group refers to the hydrophilic or polar portion of a lipid.

[0089] Sterol refers to a subgroup of steroids that contain at least one hydroxyl (OH) group. Examples of sterols include, without limitation, cholesterol, ergosterol, β -sitosterol, stigmasterol, stigmastanol, 20-hydroxycholesterol, 22-hydroxycholesterol, and the like.

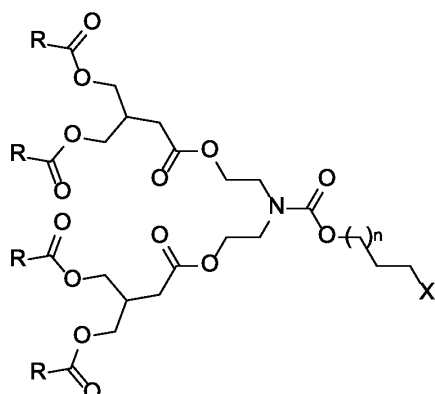
[0090] As standard, the bond represented as a solid wedge extends above the plane and

the bond represented as a dashed wedge extends below the plane when depicting absolute stereochemistry, throughout.

[0091] As compared to similar molecules in which two branching tails are unconnected to each other, in the constrained ionizable lipids described herein, a ring comprising a central branchpoint nitrogen atom connects the two branching tail groups to each other, reducing their freedom of movement. As used herein, “constrained” refers to this limited freedom of movement of the two tail groups extending from a ring comprising a nitrogen atom.

Ionizable Cationic Lipids

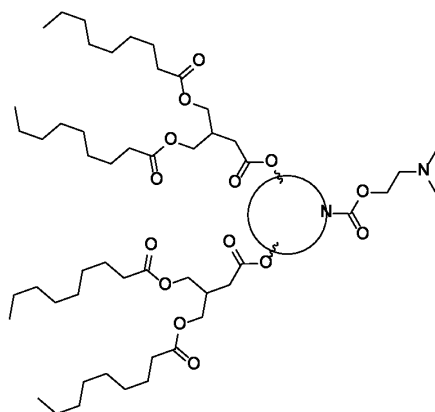
[0092] In designing families of chemical compounds, it is common to have a constant or near-constant core and vary the pendant groups attached to it. A different approach has been taken with the ionizable cationic lipids disclosed herein. A family of ionizable cationic lipids within the general structure of Formula 1b are provided where R represents hydrophobic tails of the lipid, n represents an integer from 0 to 4, and X constitutes the polar headgroup comprising an ionizable amine.



Formula 1b

[0093] Such lipids are useful as a component of lipid nanoparticles (LNPs) for delivery of nucleic acids into cells. It is convenient to draw the structure of the lipids as shown here both for compactness and because it illustrates the conical shape that is understood promotes endosomal escape of the contents of LNPs comprising such lipids. However, this is only one possible conformation the lipids can adopt. Entropy will favor adoption of multiple conformations.

[0094] The orientation of the three groups extending from the central branchpoint nitrogen atom in Formula 1b can be constrained by forming a ring comprising that nitrogen atom and to which the two branching tail groups are attached, for example, Formula 1c:

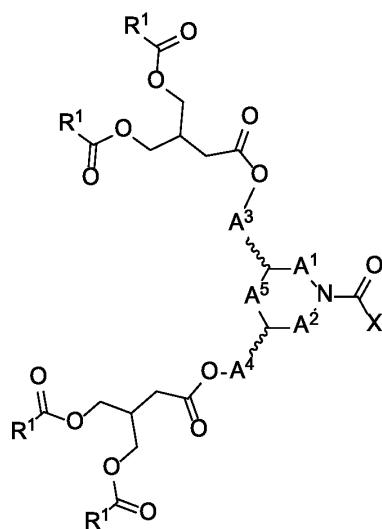


Formula 1c

[0095] By introducing this constraint, the number of conformations the lipids can adopt is reduced. This can increase the physical stability of LNPs or tLNPs incorporating such constrained lipids as compared to LNPs or tLNPs incorporating similar lipids lacking a constraining ring.

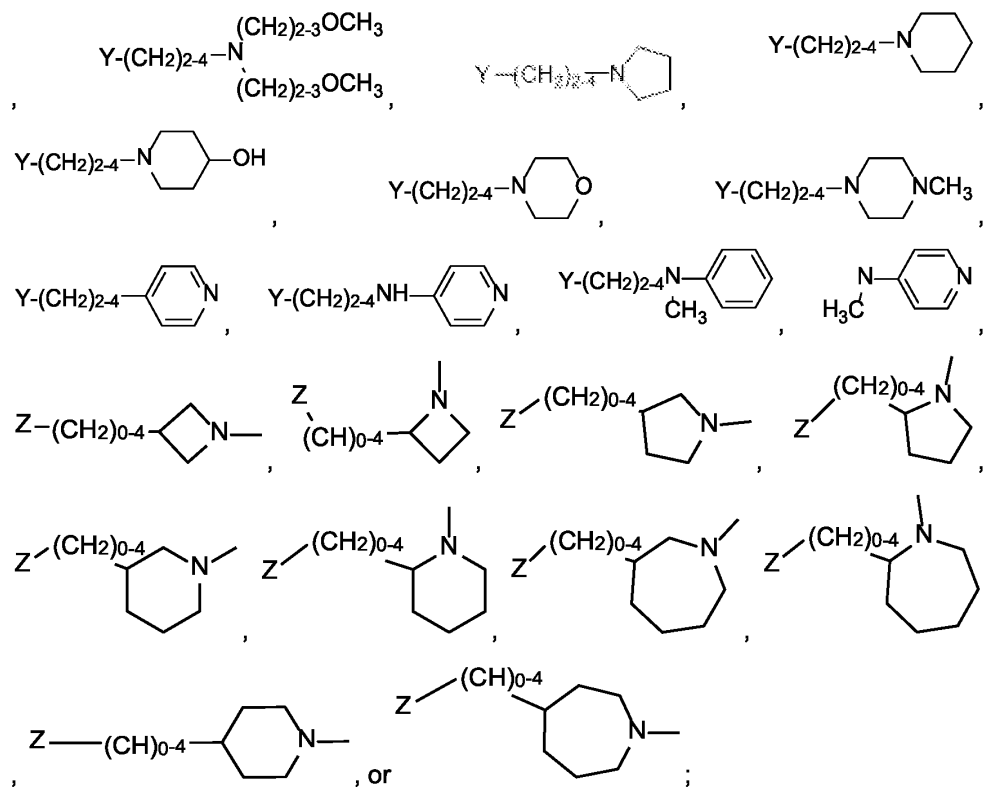
[0096] The size of the ring can be varied as can the positioning of the tail groups' attachment positions, whether the tail groups are directly attached to the ring or if additional carbons are interposed, whether the tail groups are cis or trans so that the substituents are axial or equatorial, or whether the tail groups are attached symmetrically or asymmetrically on the ring. However, these variations should preserve the high degree of biodegradability believed to be conferred by these branched tail groups, and the head group can be altered to attain a favorable c-pKa and cLogD.

[0097] In certain aspects, the constrained ionizable cationic lipids of this disclosure have a structure of formula M2:



M2,

wherein X is

$$\text{Y}-(\text{CH}_2)_{2-4}-\text{N} \begin{array}{l} (\text{CH}_2)_{0-3}\text{CH}_3 \\ (\text{CH}_2)_{0-3}\text{CH}_3 \end{array}, \quad \text{N} \begin{array}{c} \diagup \quad \diagdown \\ \text{---} \quad \text{---} \\ \diagdown \quad \diagup \end{array} \text{N}-(\text{CH}_2)_{0-3}\text{CH}_3 \quad \text{Y}-(\text{CH}_2)_{2-4}-\text{N} \begin{array}{l} (\text{CH}_2)_{2-3}\text{OCH}_3 \\ (\text{CH}_2)_{0-3}\text{CH}_3 \end{array}$$


Y is O, S, NH, or NCH₃;

Z is O, NH, or NCH₃;

each R¹ is independently selected from C₇-C₁₁ alkyl or C₇-C₁₁ alkenyl;

each A¹, A², A³, and A⁴ is independently selected from (CH₂)₀ and (CH₂)₁,

A⁵ is selected from (CH₂)₀₋₄, CH=CH, and CH₂-CH=CH-CH₂; and

a wavy bond indicates that any relative or absolute stereo-configuration of the corresponding ring atom, or a mixture of stereo-configurations, can be assumed.

[0098] In various embodiments, A¹ through A⁴ are selected so that there are only two main chain atoms between the ring nitrogen and each nearest ester oxygen in the nearest tail group.

[0099] In certain embodiments, A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is (CH₂)₁₋₄ or CH₂-CH=CH-CH₂.

[00100] In certain embodiments, A¹ is (CH₂)₀, A² is (CH₂)₁, A³ is (CH₂)₁, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁.

[00101] In certain embodiments, A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₀.

[00102] In certain embodiments, A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁.

[00103] In certain embodiments, A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₂ or CH=CH.

[00104] Ionizable cationic lipids of this disclosure have a branched structure to give the lipid a conical rather than cylindrical three-dimensional shape and such structure helps promote endosomolytic activity. The greater the endosomolytic activity, the more efficient release of the nucleotide cargo.

[00105] In certain embodiments, the ionizable cationic lipid is substantially enantiomerically pure (e.g., at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% pure). In certain embodiments, the ionizable cationic lipid is a racemic mixture. In certain embodiments, the ionizable cationic lipid is a mixture of two or more stereoisomers. In certain embodiments, at least two of the two or more stereoisomers are diastereomers. In certain embodiments, at least two of the two or more stereoisomers are enantiomers.

[00106] Ionizable cationic lipids as described herein, can be useful as a component of lipid nanoparticles for delivering nucleic acids, including DNA, mRNA, or siRNA into cells. The

ionizable cationic lipids can have a c-pKa (calculated pKa) in the range of from about 6, 7, or 8 to about 9, 10, or 11. For example, in various embodiments as described herein, the ionizable cationic lipids have a c-pKa ranging from about 6 to about 10, about 7 to about 10, about 8 to about 10, about 8 to about 9, 6 to 10, 7 to 10, 8 to 10, or 8 to 9. In certain embodiments, the ionizable cationic lipids have a c-pKa ranging from about 8.2 to about 9.0 or from 8.2 to 9.0. In certain embodiments, the ionizable cationic lipids have a c-pKa ranging from about 8.4 to about 8.7 or from 8.4 to 8.7. The ionizable cationic lipids as described herein can have cLogD ranging from about 9 to about 18, for example, ranging from about 10 to about 18, or about 10 to about 16, to about 10 to about 14, or about 11 to about 18, or about 11 to about 15, or about 11 to about 14. The ionizable cationic lipids as described herein can have cLogD ranging from 9 to 18, for example, ranging from 10 to 18, or 10 to 16, to 10 to 14, or 11 to 18, or 11 to 15, or 11 to 14. In certain embodiments, the ionizable cationic lipids have a cLogD ranging from about 13.6 to about 14.4 or from 13.6 to 14.4. In certain embodiments, the ionizable cationic lipids as described herein can have a c-pKa ranging from about 8 to about 11 or from 8 to 11 and a cLogD ranging from about 9 to about 18 or from 9 to 18. For example, in certain embodiments, the ionizable cationic lipids have a c-pKa ranging from about 8.4 to about 8.7 or from 8.4 to 8.7 and cLogD ranging from about 13.6 to about 14.4 or from 13.6 to 14.4. These ranges can lead to a measured pKa in the LNP ranging from about 6 to about 7 or from 6 to 7, which facilitates ionization in an endosome after delivery into a cell.

[00107] In some embodiments, somewhat greater basicity can be desirable and can be obtained from ionizable cationic lipids with c-pKa and cLogD in the stated ranges. In some embodiments cLogD is about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, or in a range bound by any pair of these values. Lipid design also accounts for potential biodegradability pathways of target lipids, utilizing esterases in plasma, liver and other tissues. Another consideration in lipid design is the fate of fragments of ionizable lipids resulting after esterase cleavage(s). Preferably, the resulting fragments are rapidly cleared from the body without the intervention of hepatic oxidative metabolism.

[00108] As used herein, cLogD is a calculated measure of lipophilicity that accounts for the state of ionization of the molecule at a particular pH, which is a predictor of partitioning of the lipid between water and octanol as a function of pH. More specifically, cLogD is calculated at a specified pH based on cLogP and c-pKa. (LogP is the partition coefficient of a molecule between aqueous (e.g., water) and lipophilic phases (e.g., octanol)). Numerous software packages are available to provide values of cLogD. When higher basicity of the ionizable lipid is desired, it should be balanced by greater lipophilicity as represented by a higher cLogD

value. Balance of basicity and lipophilicity is needed for optimal functioning of the LNP for both stability of the particle and release of the biologically active payload (e.g., one or more species of nucleic acid molecule that can encode a therapeutic agent) upon uptake by a cell. Accordingly, as R¹ increases from C6-C10, the overall lipophilicity of the ionizable cationic lipid will increase, as represented by cLogD. This can be balanced by alterations in X, which result in higher c-pKa based on the basicity of the head group. Each of the ionizable cationic lipid species described herein have a cLogD and c-pKa value within the desired range(s), as described herein. Specific cLogD and c-pKa values have been calculated using ACD Labs Structure Designer v 12.0 for ionic cationic lipids of the disclosure. cLogP was calculated using ACD Labs Version B; cLogD was calculated at pH 7.4. Table 1 shows cLogD and c-pKa for C1CL-207, C1CL-215, C1CL-216, C1CL-217, C1CL-218, C1CL-219, C1CL-220, C1CL-221, C1CL-222, C1CL-223, C1CL-224, and C1CL-225.

Table 1. cLogD and c-pKa for C1CL-207, C1CL-215, C1CL-216, C1CL-217, C1CL-218, C1CL-219, C1CL-220, C1CL-221, C1CL-222, C1CL-223, C1CL-224, C1CL-225, C1CL-238, C1CL-239, C1CL-242, C1CL-243, C1CL-244, C1CL-245, C1CL-246, C1CL-247, C1CL-248, and C1CL-249.

Lipid	c-LogD (pH 7.4)	c-pKa
C1CL-207	13.75	8.42
C1CL-215	13.67	8.42
C1CL-216	13.67	8.42
C1CL-217	14.34	8.62
C1CL-218	13.71	8.49
C1CL-219	13.71	8.49
C1CL-220	13.80	8.50
C1CL-221	13.67	8.42
C1CL-222	13.67	8.42
C1CL-223	13.75	8.42
C1CL-224	13.75	8.42
C1CL-225	13.75	8.42
C1CL-238	13.80	8.50
C1CL-239	14.77	7.16
C1CL-242	13.95	8.42
C1CL-243	13.95	8.42
C1CL-244	14.50	8.50
C1CL-245	14.50	8.50
C1CL-246	14.95	8.49
C1CL-247	14.95	8.49
C1CL-248	14.94	8.50
C1CL-249	14.94	8.50

[00109] Different constituents for X, Y, and R¹ allow for tuning of cLogD and c-pKa to

achieve a desired value of measured pKa within a LNP or a tLNP. For example, to make a

lipid with a head group of $\text{Y}^-(\text{CH}_2)_2-\text{N}(\text{CH}_3)_2$ less basic, the following head groups could be used

instead:

$\text{N} \begin{array}{c} \diagdown \\ | \\ \diagup \end{array} \text{N}-(\text{CH}_2)_{0-3}\text{CH}_3$, $\text{Y}^-(\text{CH}_2)_{2-4}-\text{N} \begin{array}{c} \diagdown \\ | \\ \diagup \end{array} \text{OH}$, $\text{Y}^-(\text{CH}_2)_{2-4}-\text{N} \begin{array}{c} \diagdown \\ | \\ \diagup \end{array} \text{O}$,

$\text{Y}-(\text{CH}_2)_{2-4}-\text{N} \begin{array}{c} \diagdown \\ | \\ \diagup \end{array} \text{NCH}_3$, $\text{Y}-(\text{CH}_2)_{2-4}-\text{NH}-\text{C}_5\text{H}_4\text{N}$, or $\text{Y}-(\text{CH}_2)_2\text{N} \begin{array}{l} (\text{CH}_2)_2\text{OCH}_3 \\ (\text{CH}_2)_2\text{OCH}_3 \end{array}$

[00110] Conversely, to make a lipid with a head group of $Y^-(CH_2)_2-N(CH_3)_2$ more basic, the following head groups could be used instead: $Y^-(CH_2)_3-N$ (pyrrolidine ring) or $Y^-(CH_2)_4-N$ (piperidine ring).

[00111] The addition of CH₂ groups in X will tend to increase basicity of the lipid which in turn will tend to increase measured pKa. The addition of CH₂ groups in R¹ will tend to increase the lipophilicity (cLogD) of the lipid which in turn will tend to decrease measured pKa of the LNP or tLNP.

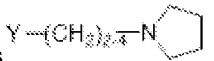
[00112] The use of further head groups to modify calculated pKa and target a desired measured pKa range is set out in Example 65.

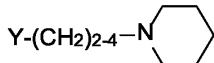
[00113] In some embodiments as described herein, X is
$$\text{Y}-(\text{CH}_2)_{2-4}-\text{N} \begin{array}{l} \text{(CH}_2\text{)}_{0-3}\text{CH}_3 \\ \text{(CH}_2\text{)}_{0-3}\text{CH}_3 \end{array} \text{ . For example, in some embodiments X is } \text{Y}(\text{CH}_2)_2-\text{N} \begin{array}{l} \text{CH}_3 \\ \text{CH}_3 \end{array} \text{ .}$$

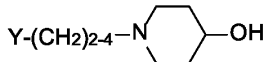
[00114] In some embodiments as described herein, X is . In some embodiments X is .

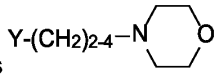
[00115] In some embodiments as described herein, X is
$$Y-(CH_2)_{2-4}-N \begin{matrix} (CH_2)_{2-3}OCH_3 \\ (CH_2)_{0-3}CH_3 \end{matrix}$$

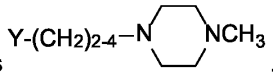
[00116] In some embodiments as described herein, X is
$$Y-(CH_2)_{2-4}-N \begin{matrix} (CH_2)_{2-3}OCH_3 \\ (CH_2)_{2-3}OCH_3 \end{matrix}$$

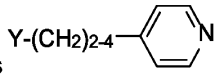
[00117] In some embodiments as described herein, X is .

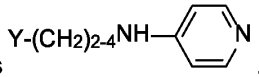
[00118] In some embodiments as described herein, X is .

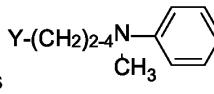
[00119] In some embodiments as described herein, X is .

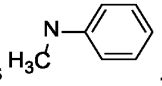
[00120] In some embodiments as described herein, X is .

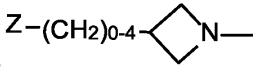
[00121] In some embodiments as described herein, X is .

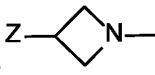
[00122] In some embodiments as described herein, X is .

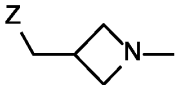
[00123] In some embodiments as described herein, X is .

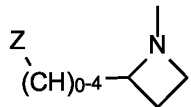
[00124] In some embodiments as described herein, X is .

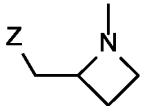
[00125] In some embodiments as described herein, X is .

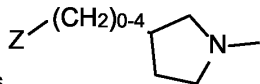
[00126] In some embodiments as described herein, X is .

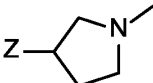
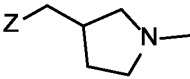
In some embodiments as described herein, X is . In some embodiments as

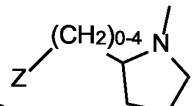
described herein, X is .

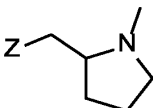
[00127] In some embodiments as described herein, X is . In

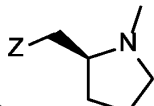
some embodiments as described herein, X is .

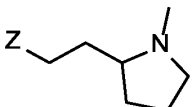
[00128] In some embodiments as described herein, X is .

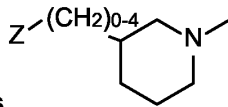
. In some embodiments as described herein, X is . In some embodiments as described herein, X is .

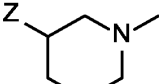
[00129] In some embodiments as described herein, X is . In

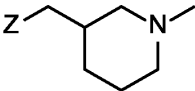
some embodiments as described herein, X is . In some embodiments as

described herein, X is . In some embodiments as described herein, X is

.

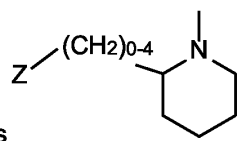
[00130] In some embodiments as described herein, X is . In

some embodiments as described herein, X is . In some embodiments as

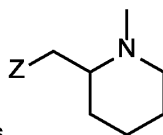
described herein, X is .

[00131]

In some embodiments as described herein, X is

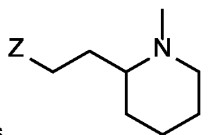


In some embodiments as described herein, X is

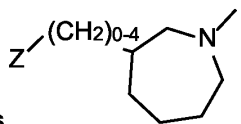


. In some embodiments as

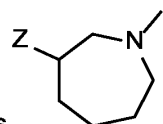
described herein, X is

**[00132]**

In some embodiments as described herein, X is

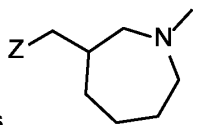


In some embodiments as described herein, X is

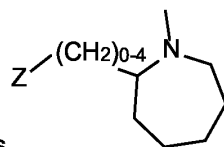


. In some embodiments as

described herein, X is

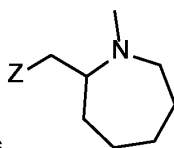
**[00133]**

In some embodiments as described herein, X is



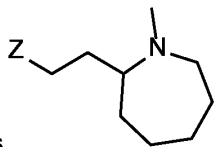
. In

some embodiments as described herein, X is

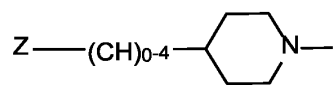


. In some embodiments as

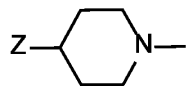
described herein, X is



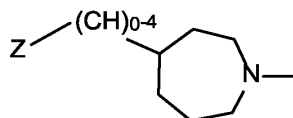
[00134] In some embodiments as described herein, X is



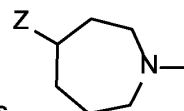
. In some embodiments as described herein, X is



[00135] In some embodiments as described herein, X is



. In some embodiments as described herein, X is



[00136] As described above, Y can be selected from O, S, NH, or NCH₃. In some embodiments, Y is O. In some other embodiments, Y is S.

[00137] In some embodiments, X is $Y^-(CH_2)_2-N^+(CH_3)_2$ and Y is O. In some embodiments X

is $Y^-(CH_2)_2-N^+(CH_3)_2$ and Y is S.

[00138] As described above, Z can be selected from O, NH, or NCH₃. In some embodiments, Z is O.

[00139] In some embodiments as described herein, X is $Z-(CH_2)_{0-4}-\text{C}_4\text{H}_8\text{N}$

and Z is O. In some embodiments as described herein, X is $Z-\text{C}_4\text{H}_8\text{N}$ and Z is O. In

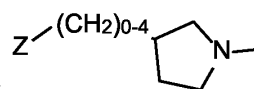
some embodiments as described herein, X is $Z-\text{C}_4\text{H}_8\text{N}$ and Z is O.

[00140] In some embodiments, X is $Z-(CH)_{0-2}-\text{C}_4\text{H}_7\text{N}$ and Z is O. In some

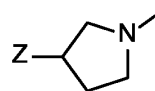
embodiments as described herein, X is $Z-\text{C}_4\text{H}_7\text{N}$ and Z is O.

[00141]

In some embodiments as described herein, X is

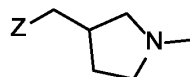


and Z is O. In some embodiments as described herein, X is



and Z is O. In

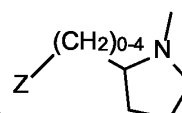
some embodiments as described herein, X is



and Z is O.

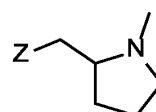
[00142]

In some embodiments as described herein, X is



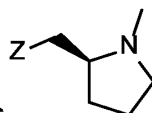
and Z

is O. In some embodiments as described herein, X is



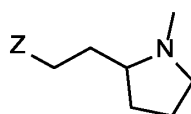
and Z is O. In some

embodiments as described herein, X is



and Z is O. In some embodiments as

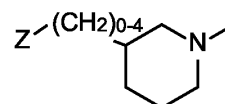
described herein, X is



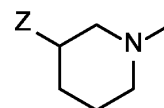
and Z is O.

[00143]

In some embodiments as described herein, X is

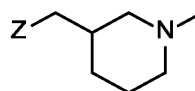


and Z is O. In some embodiments as described herein, X is



and Z is O. In

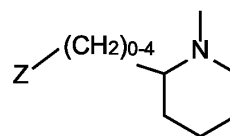
some embodiments as described herein, X is



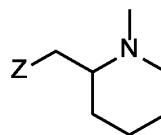
and Z is O.

[00144]

In some embodiments as described herein, X is

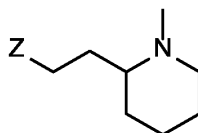


and Z is O. In some embodiments as described herein, X is



and Z is O. In

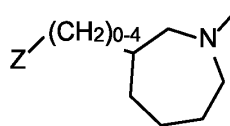
some embodiments as described herein, X is



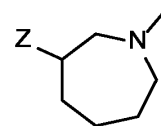
and Z is O.

[00145]

In some embodiments as described herein, X is

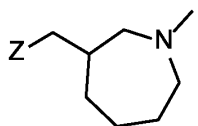


and Z is O. In some embodiments as described herein, X is



and Z is O. In

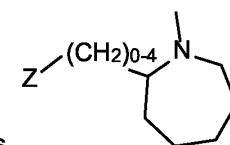
some embodiments as described herein, X is



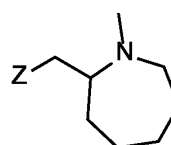
and Z is O.

[00146]

In some embodiments as described herein, X is

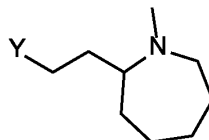


and Z is O. In some embodiments as described herein, X is



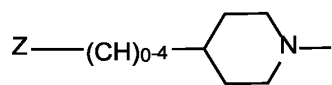
and Y is O. In

some embodiments as described herein, X is

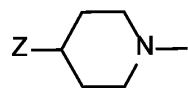


and Z is O.

[00147] In some embodiments a described herein, X is

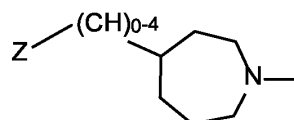


and Z is O. In some embodiments as described herein, X is

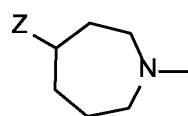


and Z is O.

[00148] In some embodiments as described herein, X is



and Z is O. In some embodiments as described herein, X is



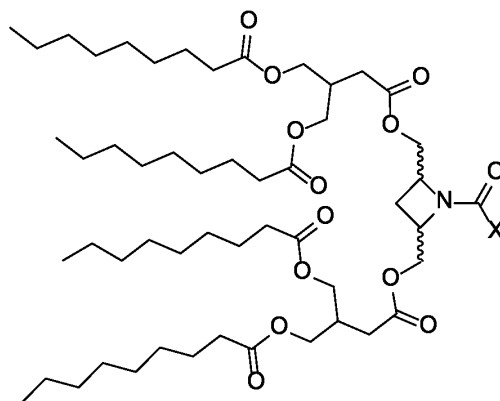
and Z is O.

[00149] As described above, each R^1 is independently selected from $\text{C}_7\text{-C}_{11}$ alkyl or $\text{C}_7\text{-C}_{11}$ alkene. In some embodiments, each R^1 is independently selected from $\text{C}_7\text{-C}_{11}$ alkyl, e.g., $\text{C}_7\text{-C}_{10}$ alkyl, or $\text{C}_7\text{-C}_9$ alkyl. In certain embodiments, each R^1 is independently selected from a linear $\text{C}_7\text{-C}_{11}$ alkyl, e.g., a linear $\text{C}_7\text{-C}_{10}$ alkyl, or a linear $\text{C}_7\text{-C}_9$ alkyl. In some embodiments as described herein, each R^1 is independently selected from $(\text{CH}_2)_{6-8}\text{CH}_3$. In some of these and other embodiments, R^1 is $(\text{CH}_2)_7\text{CH}_3$. In some embodiments, each R^1 is independently selected from a linear $\text{C}_7\text{-C}_{11}$ alkenyl, e.g., a linear $\text{C}_7\text{-C}_{10}$ alkenyl, or a linear $\text{C}_7\text{-C}_9$ alkenyl. For example, in some embodiments, each R^1 is a linear C_8 alkenyl. In certain other embodiments, each R^1 is independently selected from a branched $\text{C}_7\text{-C}_{11}$ alkyl, e.g., $\text{C}_7\text{-C}_{10}$ alkyl, or $\text{C}_7\text{-C}_9$ alkyl. For example, in some embodiments, each R^1 is a branched C_8 alkyl. In certain embodiments, each R^1 is independently selected from a branched $\text{C}_7\text{-C}_{11}$ alkenyl, e.g., $\text{C}_7\text{-C}_{10}$ alkenyl, or $\text{C}_7\text{-C}_9$ alkenyl. For example, in some embodiments, each R^1 is a branched C_8 alkenyl. In some embodiments, wherein R^1 is a branched alkyl or alkenyl, the branch point is positioned so that ester carbonyls are not in an α position relative to the branch point, for example they are in a β position relative to the branch point.

[00150] In certain embodiments as described herein, each R^1 is the same. In certain embodiments, each R^1 nearest a common branch point is the same, but those nearest a first common branch point differ from those nearest a second common branch point. In certain embodiments, each R^1 nearest a common branch point is different but the pair of R^1 's nearest a first common branch point is the same the pair nearest a second common branch point.

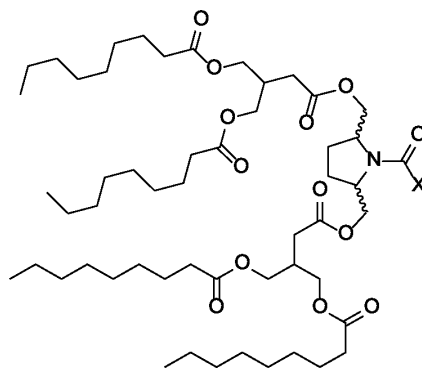
[00151] As described above, in some embodiments as described herein, the ionizable

cationic lipid has a structure of formula M2, wherein A^1 is $(CH_2)_0$, A^2 is $(CH_2)_0$, A^3 is $(CH_2)_1$, A^4 is $(CH_2)_1$, and A^5 is $(CH_2)_{1-4}$ or $CH_2-CH=CH-CH_2$. For example, in some embodiments as described herein, A^1 is $(CH_2)_0$, A^2 is $(CH_2)_0$, A^3 is $(CH_2)_1$, A^4 is $(CH_2)_1$, and A^5 is $(CH_2)_1$. In some embodiments as described herein, the ionizable cationic lipid has the structure:



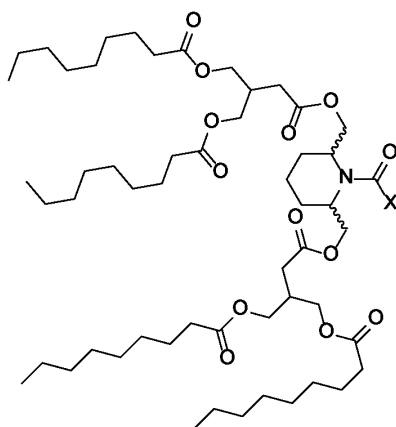
wherein X is as described herein and wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

[00152] In certain embodiments of formula M2 as described herein, A^1 is $(CH_2)_0$, A^2 is $(CH_2)_0$, A^3 is $(CH_2)_1$, A^4 is $(CH_2)_1$, and A^5 is $(CH_2)_2$. For example, in some embodiments, the ionizable cationic lipid has the structure:



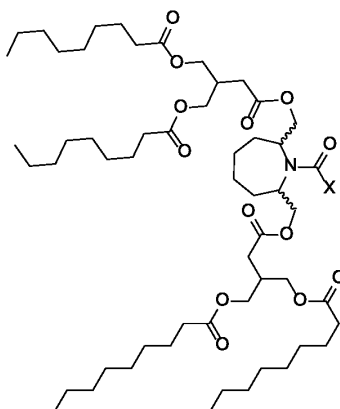
wherein X is as described herein and wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

[00153] In certain embodiments of formula M2 as described herein, A^1 is $(CH_2)_0$, A^2 is $(CH_2)_0$, A^3 is $(CH_2)_1$, A^4 is $(CH_2)_1$, and A^5 is $(CH_2)_3$. For example, in some embodiments, the ionizable cationic lipid has the structure:



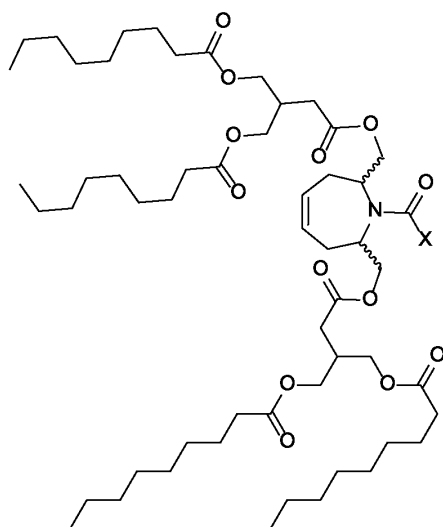
wherein X is as described herein and wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

[00154] In certain embodiments of formula M2 as described herein, A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is (CH₂)₄. For example, in some embodiments, the ionizable cationic lipid has the structure:



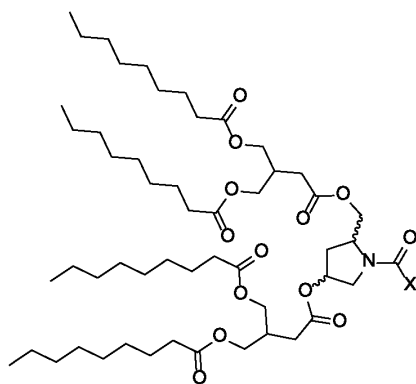
wherein X is as described herein and wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

[00155] In certain embodiments of formula M2 as described herein, A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is CH₂-CH=CH-CH₂. For example, in some embodiments, the ionizable cationic lipid has the structure:



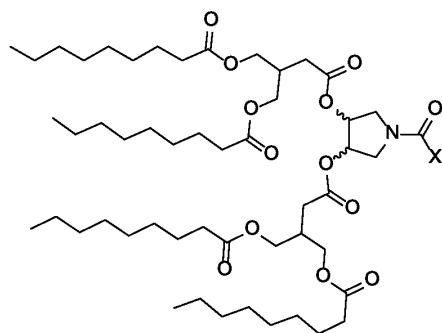
wherein X is as described herein and wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

[00156] As described above, in some embodiments as described herein, the ionizable cationic lipid has a structure of formula M2, wherein A¹ is (CH₂)₀, A² is (CH₂)₁, A³ is (CH₂)₁, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁. For example, in some embodiments, the ionizable cationic lipid has the structure:



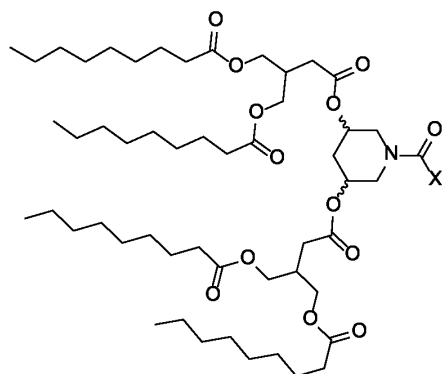
wherein X is as described herein and wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

[00157] As described above, in some embodiments as described herein, the ionizable cationic lipid has a structure of formula M2, wherein A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₀. For example, in some embodiments, the ionizable cationic lipid has the structure:



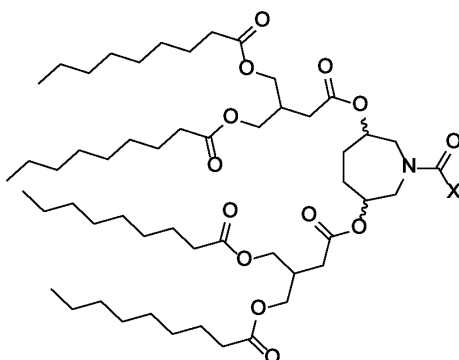
wherein X is as described herein and wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

[00158] As described above, in some embodiments as described herein, the ionizable cationic lipid has a structure of formula M2, wherein A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁. For example, in some embodiments, the ionizable cationic lipid has the structure:



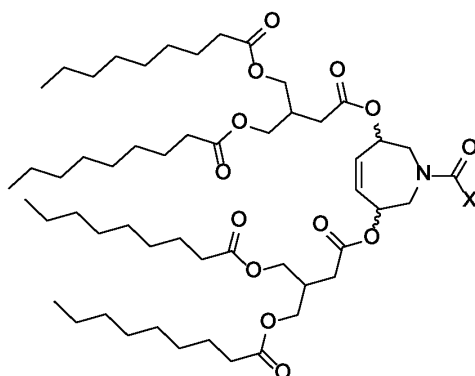
wherein X is as described herein and wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

[00159] As described above, in some embodiments as described herein, the ionizable cationic lipid has a structure of formula M2 wherein A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₂ or CH=CH. For example, in some embodiments as described herein, A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₂. In some embodiments as described herein, the ionizable cationic lipid has the structure:



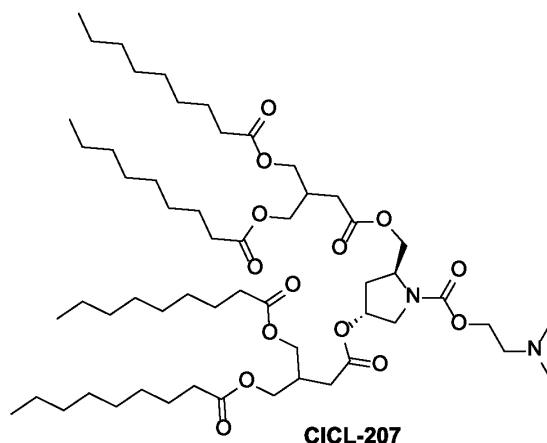
wherein X is as described herein and wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

[00160] In certain embodiments of formula M2 as described herein, A^1 is $(CH_2)_1$, A^2 is $(CH_2)_1$, A^3 is $(CH_2)_0$, A^4 is $(CH_2)_0$, and A^5 is $CH=CH$. For example, in some embodiments, the ionizable cationic lipid has the structure:



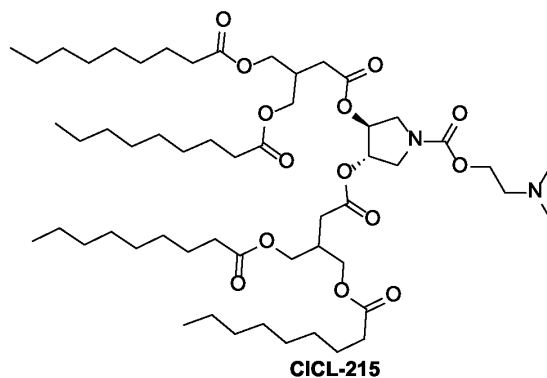
wherein X is as described herein and wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

[00161] In some embodiments, the ionizable cationic lipid has the structure C1CL-207:



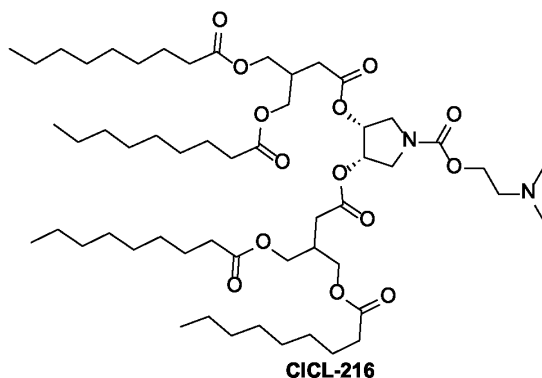
C1CL-207 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Other embodiments include diastereomers of these compounds (e.g., C1CL-223 and C1CL-224) as well as mixtures including two or more of the various stereoisomers.

[00162] In some embodiments, the ionizable cationic lipid has the structure C1CL-215:



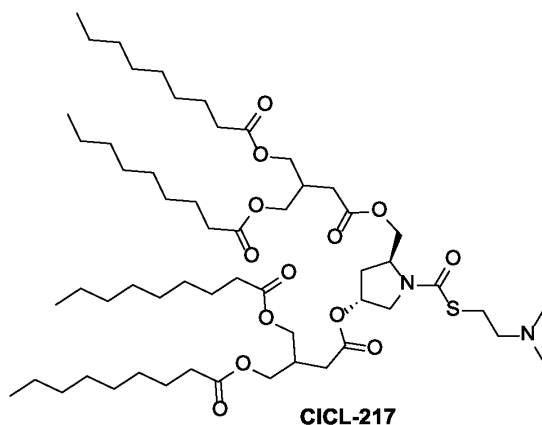
C1CL-215 is an example of a lipid as disclosed herein in which A³, A⁴, and A⁵ are absent, A¹ and A² are CH₂, and the tail groups are symmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include its enantiomer and other mixtures thereof. Still other embodiments include diastereomers of these compounds (e.g., C1CL-216) as well as mixtures including two or more of the various stereoisomers.

[00163] In some embodiments, the ionizable cationic lipid has the structure C1CL-216:



C1CL-216 is an example of a lipid as disclosed herein in which A³, A⁴, and A⁵ are absent, A¹ and A² are CH₂, and the tail groups are symmetrically placed relative to the ring nitrogen in a cis configuration. C1CL-216 is symmetric with the two nominal cis configurations being superimposable, due to the tail groups being identical. Other embodiments include diastereomers of this compound (e.g., C1CL-215) as well as mixtures including two or more of the various stereoisomers.

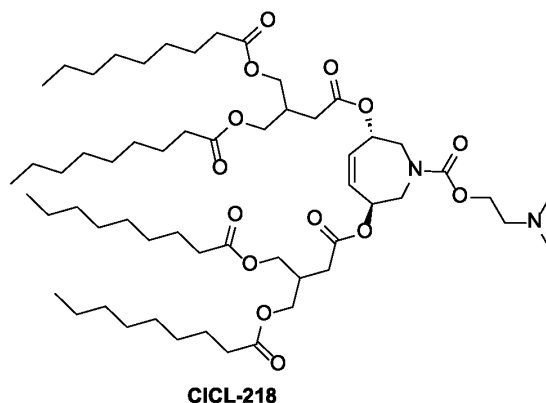
[00164] In some embodiments, the ionizable cationic lipid has the structure C1CL-217:



C1CL-217 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. This lipid differs from C1CL-207 in that Y is S instead of O. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include enantiomer of C1CL-217 and diastereomers thereof, and mixtures of stereoisomers (e.g.,

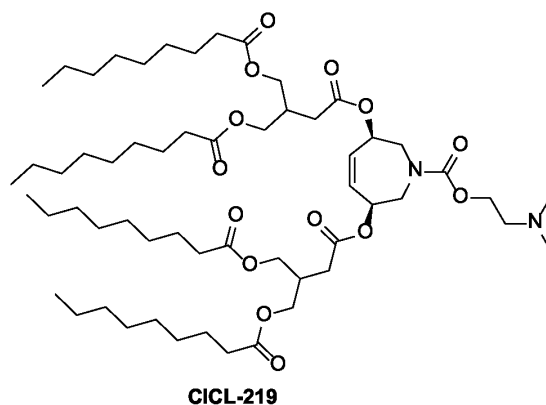
racemate, or other mixtures of various stereoisomers with various ratios). Other embodiments include diastereomers of these compounds as well as mixtures including two or more of the various stereoisomers.

[00165] In some embodiments, the ionizable cationic lipid has the structure C1CL-218:



C1CL-218 is an example of a lipid as disclosed herein in which A^3 and A^4 are absent, A^1 and A^2 are CH_2 , A^5 is $\text{CH}=\text{CH}$, and the tail groups are symmetrically placed relative to the ring nitrogen in a trans configuration. C1CL-218 was synthesized as a racemate so that the structure drawn above indicates the relative stereochemistry for this lipid. Other embodiments include the absolute stereochemistry drawn above, its enantiomer and mixtures thereof. Still other embodiments are diastereomers of these compounds (e.g., C1CL-219) as well as mixtures including two or more of the various stereoisomers.

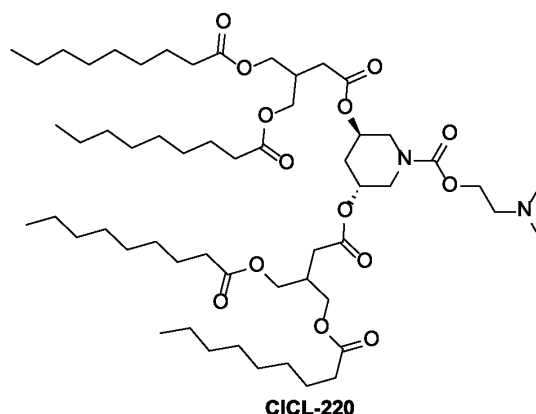
[00166] In some embodiments, the ionizable cationic lipid has the structure C1CL-219:



C1CL-219 is an example of a lipid as disclosed herein in which A^3 and A^4 are absent, A^1 and A^2 are CH_2 , A^5 is $\text{CH}=\text{CH}$, and the tail groups are symmetrically placed relative to the ring

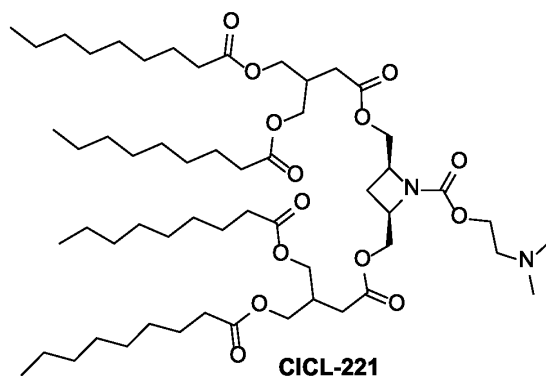
nitrogen in a cis configuration. C1CL-219 is symmetric with the two nominal cis configurations being superimposable, due to the tail groups being identical. Other embodiments include diastereomers of this compound (e.g., C1CL-218) as well as mixtures including two or more of the various stereoisomers.

[00167] In some embodiments, the ionizable cationic lipid has the structure C1CL-220:



C1CL-220 is an example of a lipid as disclosed herein in which A³ and A⁴ are absent, A¹, A², and A⁵ are CH₂, and the tail groups are symmetrically placed relative to the ring nitrogen in a trans configuration. C1CL-220 and the structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include its enantiomer and other mixtures thereof. Still other embodiments include diastereomers of these compounds as well as mixtures including two or more of the various stereoisomers.

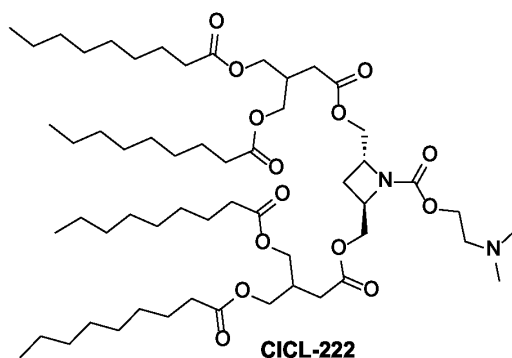
[00168] In some embodiments, the ionizable cationic lipid has the structure C1CL-221:



C1CL-221 is an example of a lipid as disclosed herein in which A¹ and A² are absent, A³, A⁴, and A⁵ are CH₂, and the tail groups are symmetrically placed relative to the ring nitrogen in a

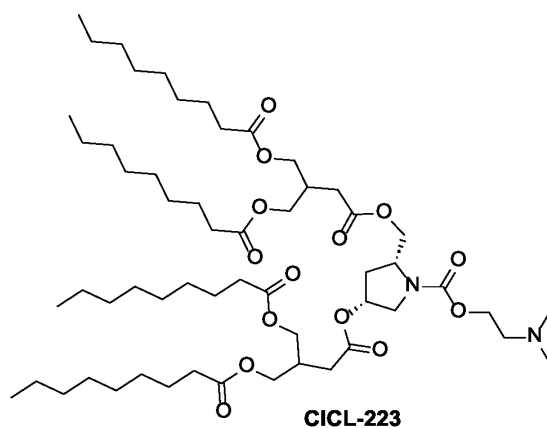
cis configuration. C1CL-221 is symmetric with the two nominal cis configurations being superimposable, due to the tail groups being identical. Other embodiments include diastereomers of this compound (e.g., C1CL-222) as well as mixtures including two or more of the various stereoisomers.

[00169] In some embodiments, the ionizable cationic lipid has the structure C1CL-222:



C1CL-221 is an example of a lipid as disclosed herein in which A¹ and A² are absent, A³, A⁴, and A⁵ are CH₂, and the tail groups are symmetrically placed relative to the ring nitrogen in a trans configuration. C1CL-222 was synthesized as a racemate so that the structure drawn above indicates the relative stereochemistry for this lipid. Other embodiments include the absolute stereochemistry drawn above, its enantiomer, and mixtures thereof. Still other embodiments include diastereomers of these compounds (e.g., C1CL-221) as well as mixtures including two or more of the various stereoisomers.

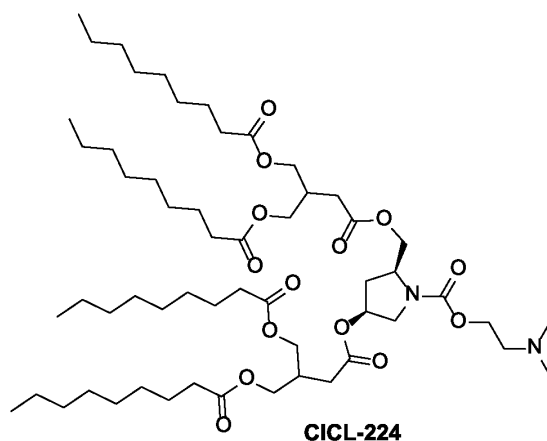
[00170] In some embodiments, the ionizable cationic lipid has the structure C1CL-223:



C1CL-223 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, A², A³,

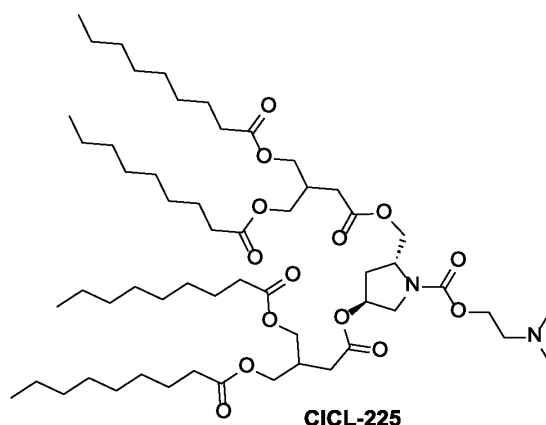
and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a cis configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of CICL-223 (CICL-224) and mixtures of the enantiomers (e.g., racemate, or other mixtures with various ratios). Other embodiments include diastereomers of these compounds (e.g., CICL-207 and CICL-225) as well as mixtures including two or more of the various stereoisomers.

[00171] In some embodiments, the ionizable cationic lipid has the structure CICL-224:



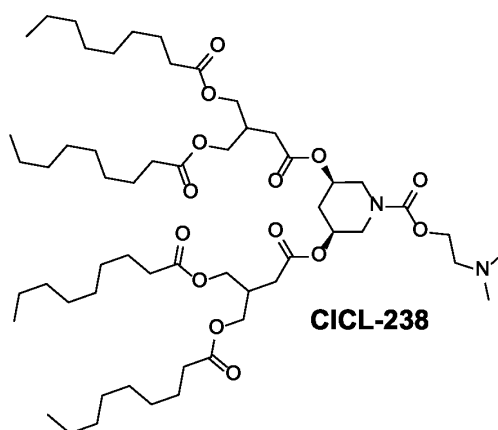
CICL-224 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a cis configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of CICL-224 (CICL-223) and mixtures of the enantiomers (e.g., racemate, or other mixtures with various ratios). Other embodiments include diastereomers of these compounds (e.g., CICL-207 and CICL-225) as well as mixtures including two or more of the various stereoisomers.

[00172] In some embodiments, the ionizable cationic lipid has the structure CICL-225:



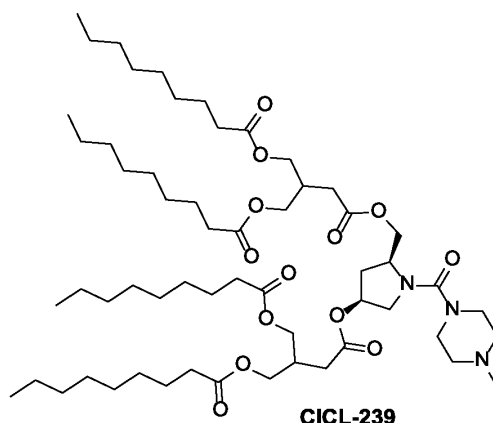
C1CL-225 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-225 (C1CL-207) and mixtures of the enantiomers (e.g., racemate, or other mixtures with various ratios). Other embodiments include diastereomers of these compounds (e.g., C1CL-223 and C1CL-224) as well as mixtures including two or more of the various stereoisomers.

[00173] In some embodiments, the ionizable cationic lipid has the structure C1CL-238:



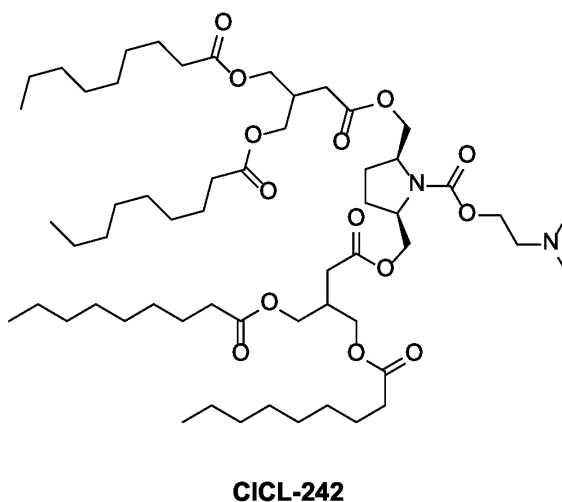
C1CL-238 is an example of a lipid as disclosed herein in which A¹, A², and A⁵ are CH₂, A³ and A⁴ are absent, and the tail groups are symmetrically placed relative to the ring nitrogen in a cis configuration. C1CL-238 is symmetric with the two nominal cis configurations being superimposable, due to the tail groups being identical. Other embodiments include diastereomers of this compound (e.g., C1CL-220) as well as mixtures including two or more of the various stereoisomers.

[00174] In some embodiments, the ionizable cationic lipid has the structure C1CL-239:



C1CL-239 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a cis configuration. Differing from many of the exemplified lipids the group Y of C1CL-239 is N-methyl-piperazine. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-239 and mixtures of the enantiomers (e.g., racemate, or other mixtures with various ratios). Other embodiments include diastereomers of these compounds as well as mixtures including two or more of the various stereoisomers.

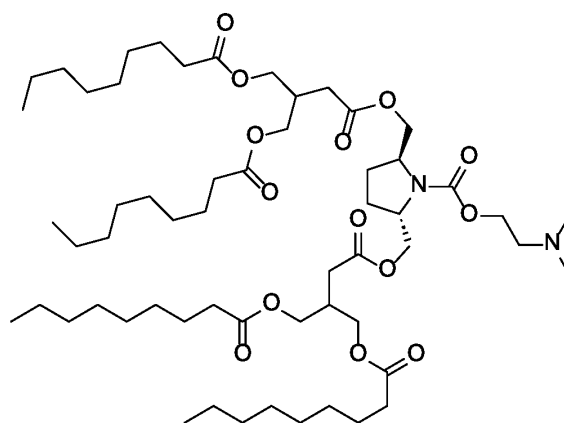
[00175] In some embodiments, the ionizable cationic lipid has the structure C1CL-242:



C1CL-242 is an example of a lipid as disclosed herein in which A¹ and A² are absent, A³ and A⁴ are CH₂, and A⁵ is CH₂CH₂, and the tail groups are symmetrically placed relative to the ring

nitrogen in a cis configuration. CICLE-242 is symmetric with the two nominal cis configurations being superimposable, due to the tail groups being identical. Other embodiments include diastereomers of this compound (e.g., CICLE-243) as well as mixtures including two or more of the various stereoisomers.

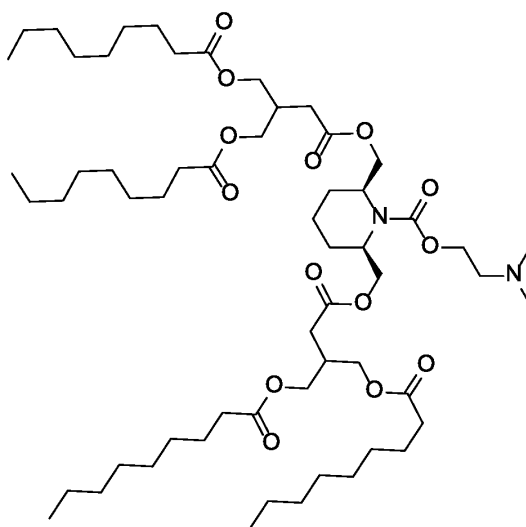
[00176] In some embodiments, the ionizable cationic lipid has the structure CICLE-243:



CICLE-243

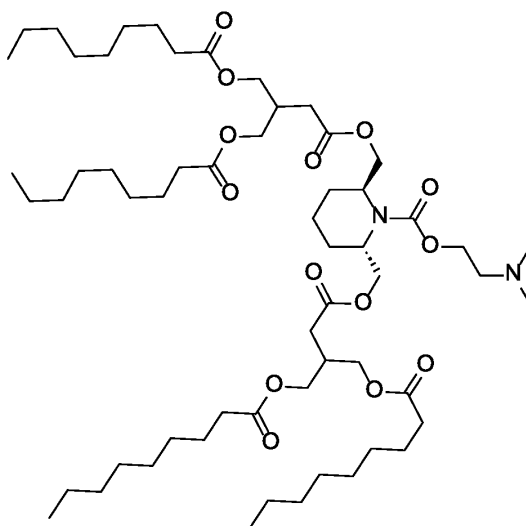
CICLE-243 is an example of a lipid as disclosed herein in which A^1 and A^2 are absent, A^3 and A^4 are CH_2 , and A^5 is CH_2CH_2 , and the tail groups are symmetrically placed relative to the ring nitrogen in a trans configuration. CICLE-243 is an optical antipode, so that the structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include: its enantiomer, and mixtures of the enantiomers (e.g., racemate, or other mixtures with various ratios). Still other embodiments include diastereomers of these compounds (e.g., CICLE-242) as well as mixtures including two or more of the various stereoisomers.

[00177] In some embodiments, the ionizable cationic lipid has the structure CICLE-244:

**C1CL-244**

C1CL-244 is an example of a lipid as disclosed herein in which A¹ and A² are absent, A³ and A⁴ are CH₂, and A⁵ is CH₂CH₂CH₂, and the tail groups are symmetrically placed relative to the ring nitrogen in a cis configuration. C1CL-244 is symmetric with the two nominal cis configurations being superimposable, due to the tail groups being identical. Other embodiments include diastereomers of this compound (e.g., C1CL-245) as well as mixtures including two or more of the various stereoisomers.

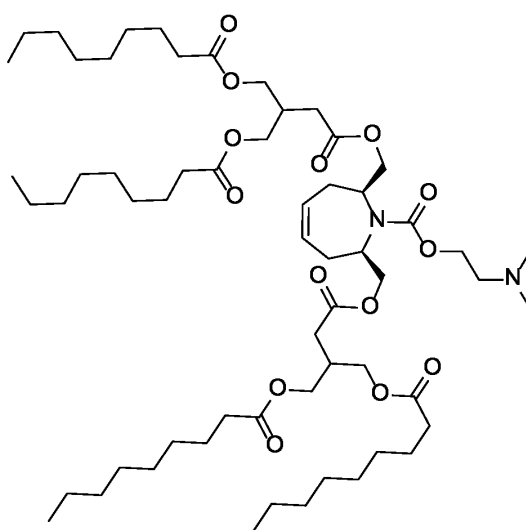
[00178] In some embodiments, the ionizable cationic lipid has the structure C1CL-245:



CICL-245

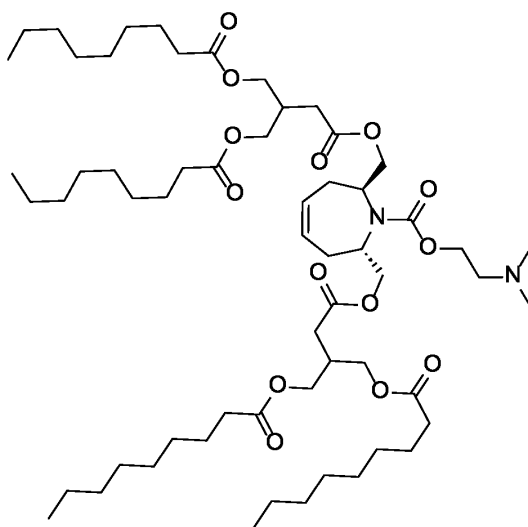
CICL-245 is an example of a lipid as disclosed herein in which A^1 and A^2 are absent, A^3 and A^4 are CH_2 , and A^5 is $\text{CH}_2\text{CH}_2\text{CH}_2$, and the tail groups are symmetrically placed relative to the ring nitrogen in a trans configuration. CICL-245 is a racemate, so that the structure drawn above indicates the relative stereochemistry for this lipid. Other embodiments include: its enantiomers, and mixtures thereof. Still other embodiments include diastereomers of these compounds (e.g., CICL-244) as well as mixtures including two or more of the various stereoisomers.

[00179] In some embodiments, the ionizable cationic lipid has the structure CICL-246:

**CICL-246**

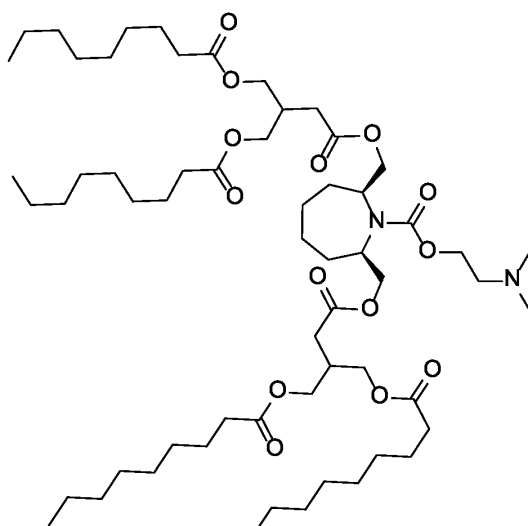
CICL-246 is an example of a lipid as disclosed herein in which A^1 and A^2 are absent, A^3 and A^4 are CH_2 , and A^5 is $\text{CH}_2\text{CH}=\text{CHCH}_2$, and the tail groups are symmetrically placed relative to the ring nitrogen in a cis configuration. CICL-246 is symmetric with the two nominal cis configurations being superimposable, due to the tail groups being identical. Other embodiments include diastereomers of this compound (e.g., CICL-247) as well as mixtures including two or more of the various stereoisomers.

[00180] In some embodiments, the ionizable cationic lipid has the structure CICL-247:

**CICL-247**

CICL-247 is an example of a lipid as disclosed herein in which A¹ and A² are absent, A³ and A⁴ are CH₂, and A⁵ is CH₂CH=CHCH₂, and the tail groups are symmetrically placed relative to the ring nitrogen in a trans configuration. CICL-247 is a racemate, so that the structure drawn above indicates the relative stereochemistry for this lipid. Other embodiments include: its enantiomers, and mixtures thereof. Still other embodiments include diastereomers of these compounds (e.g., CICL-246) as well as mixtures including two or more of the various stereoisomers.

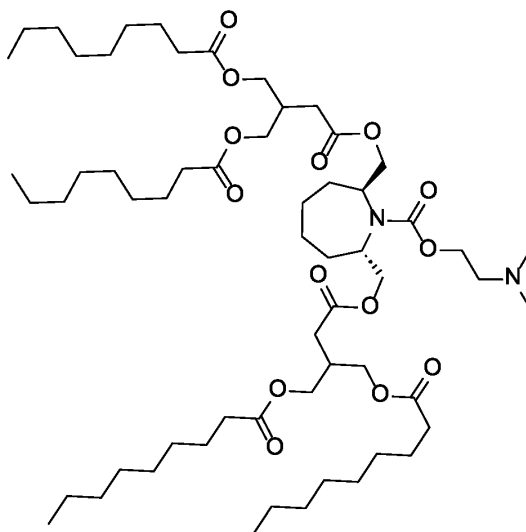
[00181] In some embodiments, the ionizable cationic lipid has the structure C1CL-248:



CICL-248

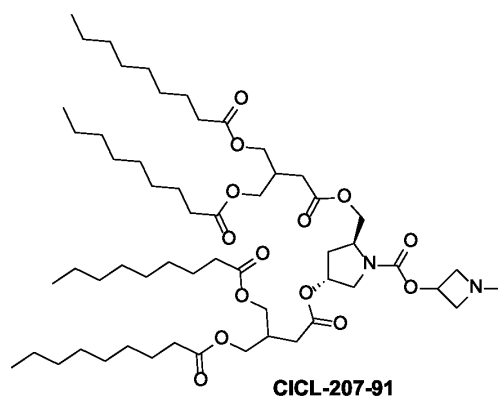
CICL-248 is an example of a lipid as disclosed herein in which A¹ and A² are absent, A³ and A⁴ are CH₂, and A⁵ is CH₂CH₂CH₂CH₂, and the tail groups are symmetrically placed relative to the ring nitrogen in a cis configuration. CICL-248 is symmetric with the two nominal cis configurations being superimposable, due to the tail groups being identical. Other embodiments include diastereomers of this compound (e.g., CICL-249) as well as mixtures including two or more of the various stereoisomers.

[00182] In some embodiments, the ionizable cationic lipid has the structure CICL-249:

**CICL-249**

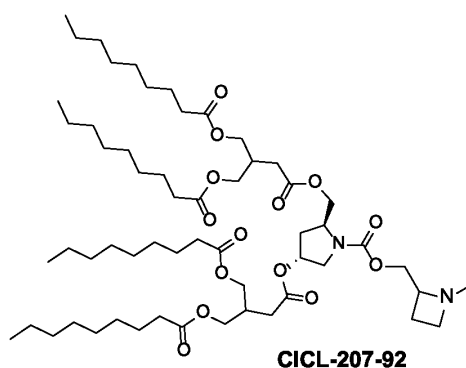
CICL-249 is an example of a lipid as disclosed herein in which A¹ and A² are absent, A³ and A⁴ are CH₂, and A⁵ is CH₂CH₂CH₂CH₂, and the tail groups are symmetrically placed relative to the ring nitrogen in a trans configuration. CICL-249 is a racemate, so that the structure drawn above indicates the relative stereochemistry for this lipid. Other embodiments include: its enantiomers, and mixtures thereof. Still other embodiments include diastereomers of these compounds (e.g., CICL-248) as well as mixtures including two or more of the various stereoisomers.

[00183] In some embodiments, the ionizable cationic lipid has the structure CICL-207-91:



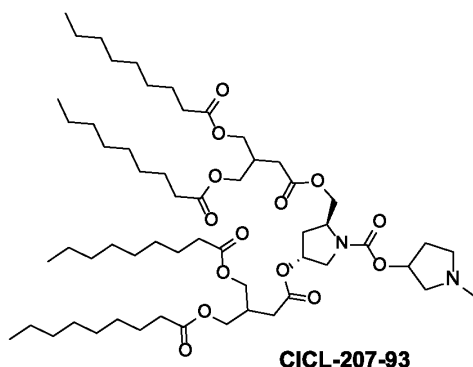
[00184] C1CL-207-91 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-91 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-91, and mixtures thereof.

[00185] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-92:



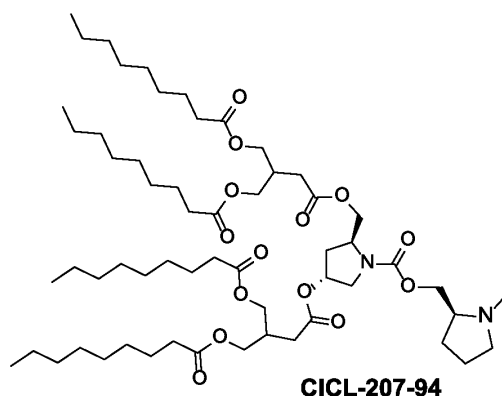
[00186] C1CL-207-92 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-92 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-92, and mixtures thereof.

[00187] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-93:



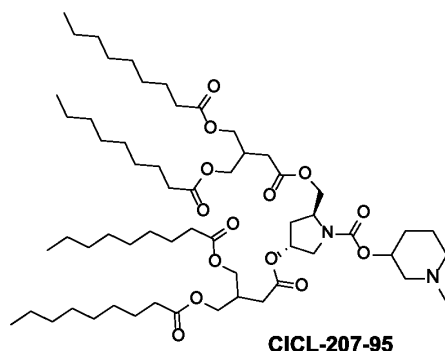
[00188] C1CL-207-93 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-93 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-93, and mixtures thereof.

[00189] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-94:



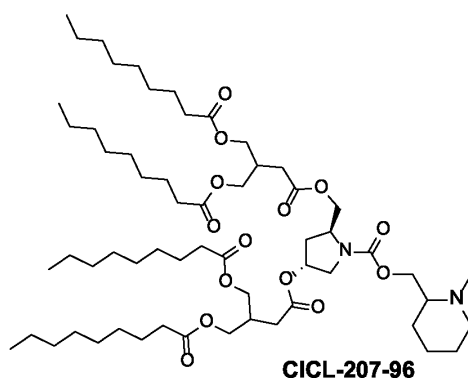
[00190] C1CL-207-94 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-94 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-94, and mixtures thereof.

[00191] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-95:



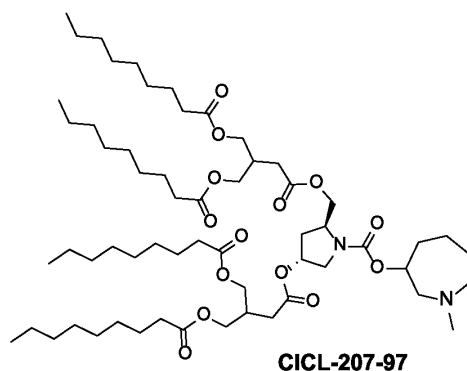
[00192] CICAL-207-95 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of CICAL-207-95 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of CICAL-207-95, and mixtures thereof.

[00193] In some embodiments, the ionizable cationic lipid has the structure CICAL-207-96:



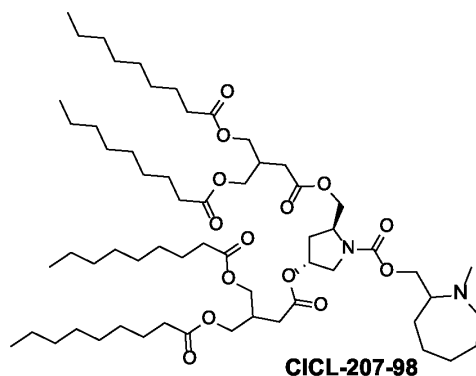
[00194] CICAL-207-96 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of CICAL-207-96 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of CICAL-207-96, and mixtures thereof.

[00195] In some embodiments, the ionizable cationic lipid has the structure CICAL-207-97:



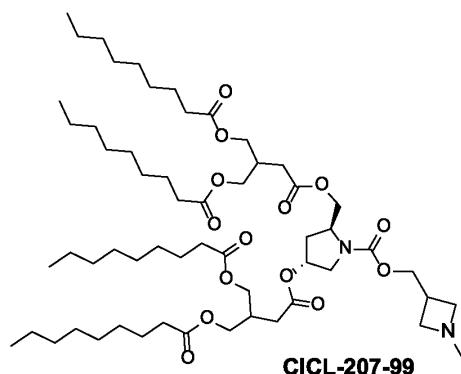
[00196] C1CL-207-97 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-97 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-97, and mixtures thereof.

[00197] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-98:



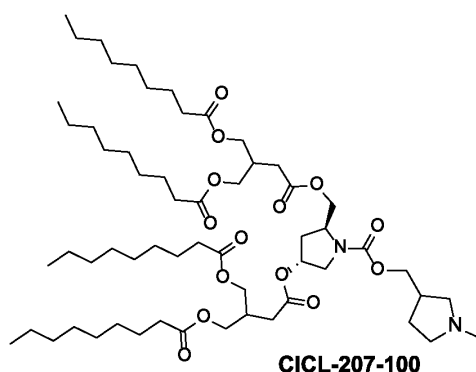
[00198] C1CL-207-98 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-98 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-98, and mixtures thereof.

[00199] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-99:



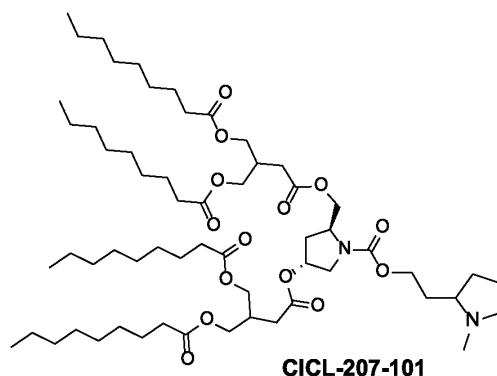
[00200] C1CL-207-99 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-99 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-99, and mixtures thereof.

[00201] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-100:



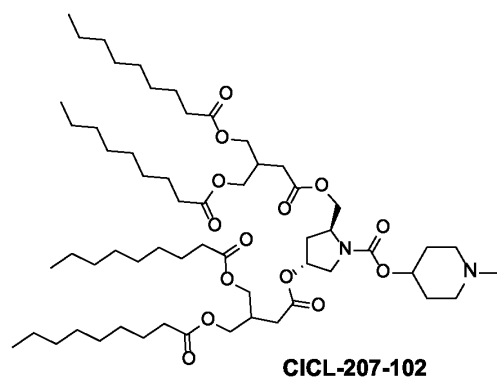
[00202] C1CL-207-100 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-100 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-100, and mixtures thereof.

[00203] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-101:



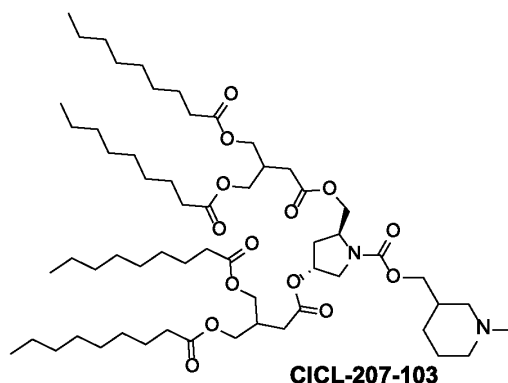
[00204] C1CL-207-101 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-101 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-101, and mixtures thereof.

[00205] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-102:



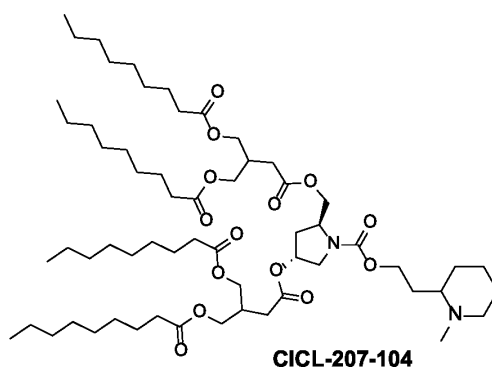
[00206] C1CL-207-102 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-102 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-102, and mixtures thereof.

[00207] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-103:



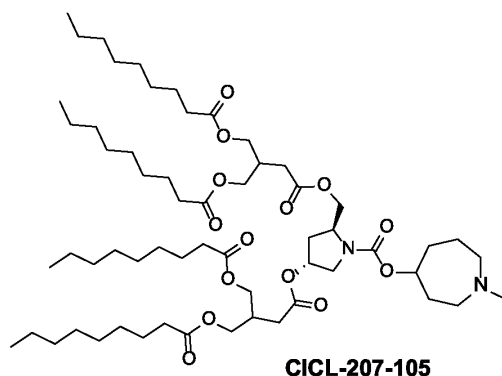
[00208] C1CL-207-103 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-103 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-103, and mixtures thereof.

[00209] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-104:



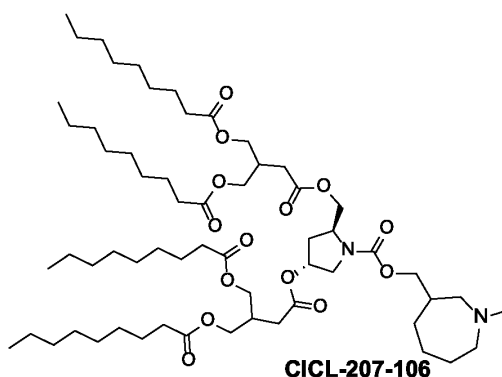
[00210] C1CL-207-104 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-104 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-104, and mixtures thereof.

[00211] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-105:



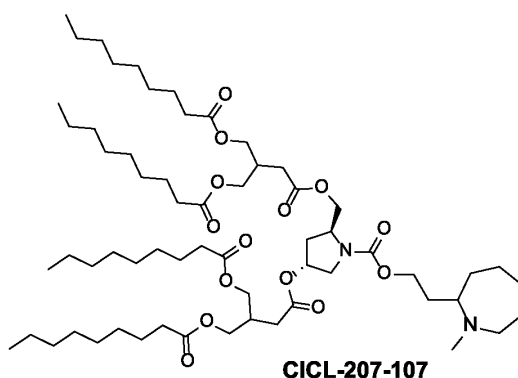
[00212] C1CL-207-105 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-105 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-105, and mixtures thereof.

[00213] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-106:



[00214] C1CL-207-106 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-106 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-106, and mixtures thereof.

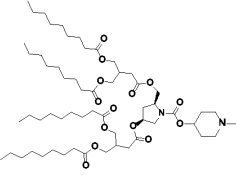
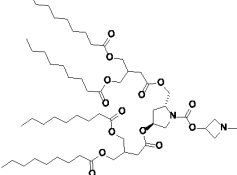
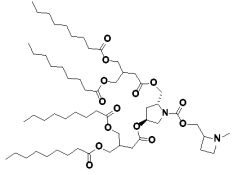
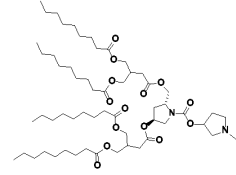
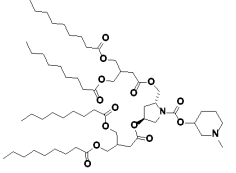
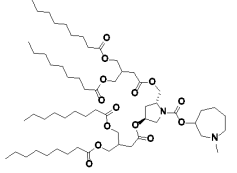
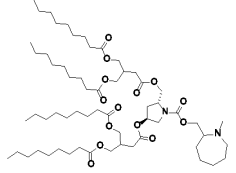
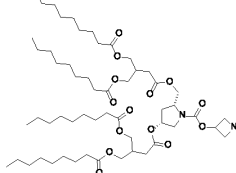
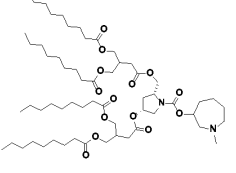
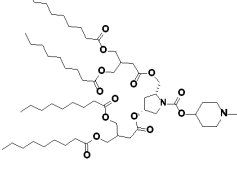
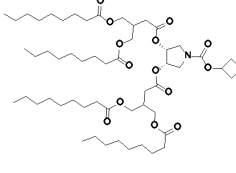
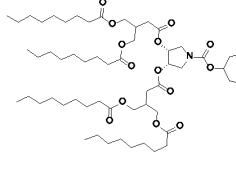
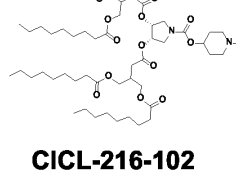
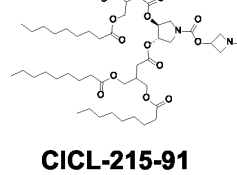
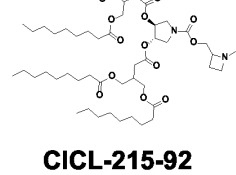
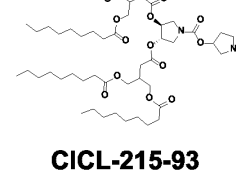
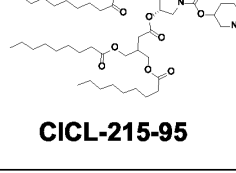
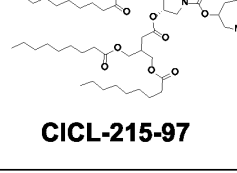
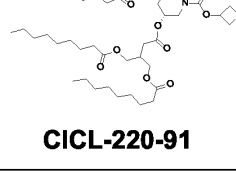
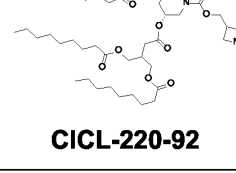
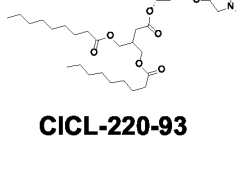
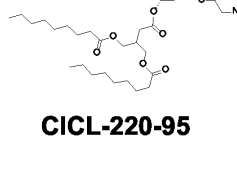
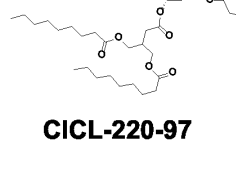
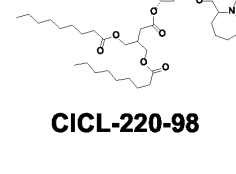
[00215] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-107:

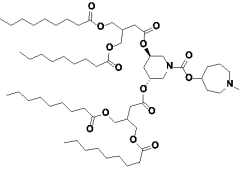
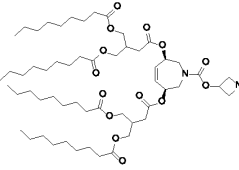
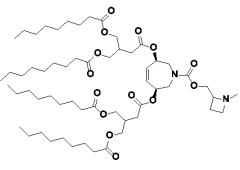
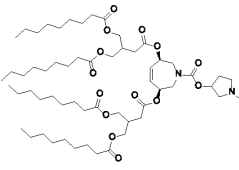
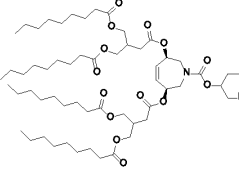
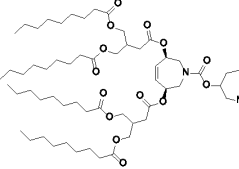
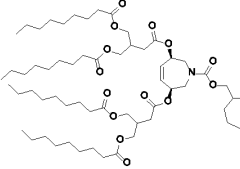
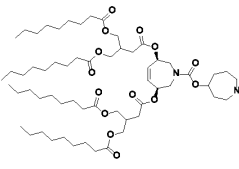
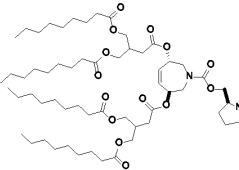
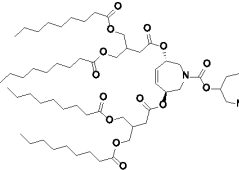
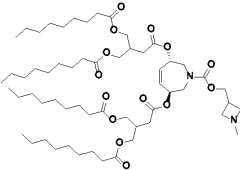
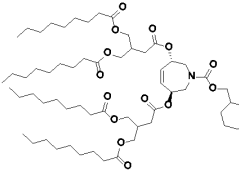
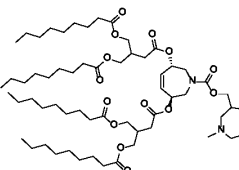
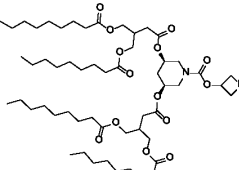
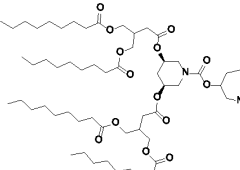
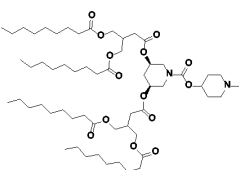
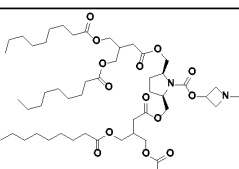
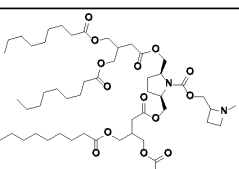
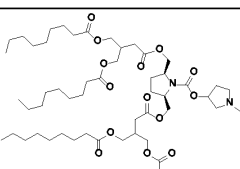
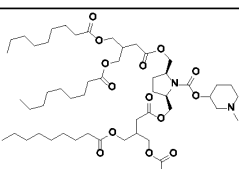
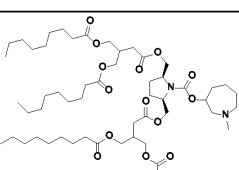
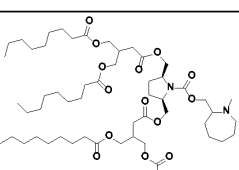
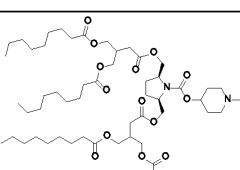
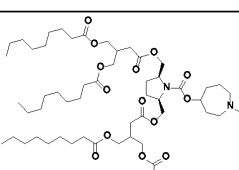


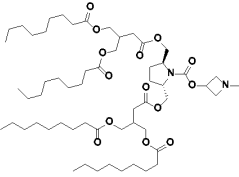
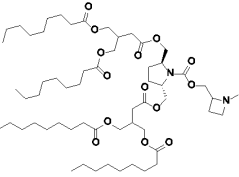
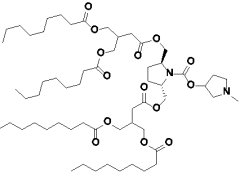
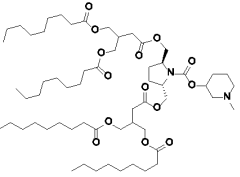
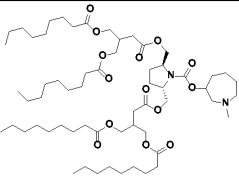
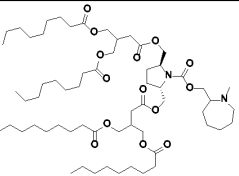
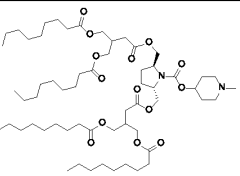
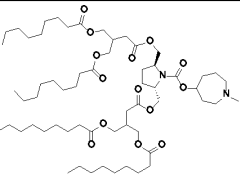
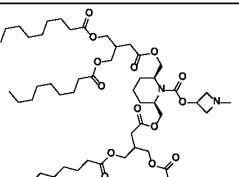
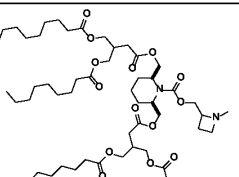
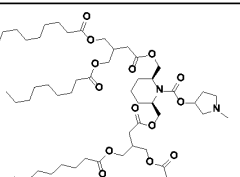
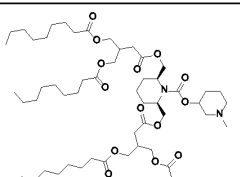
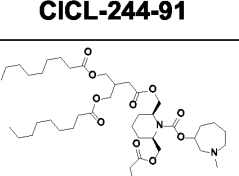
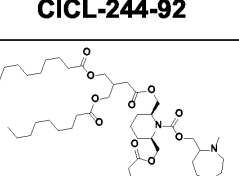
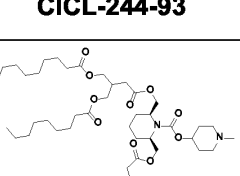
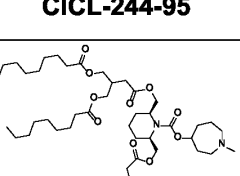
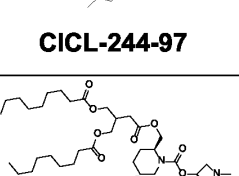
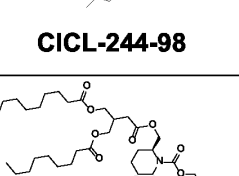
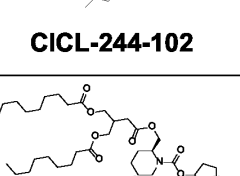
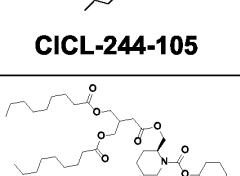
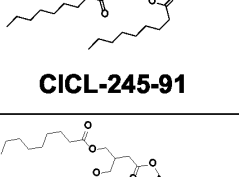
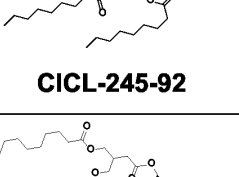
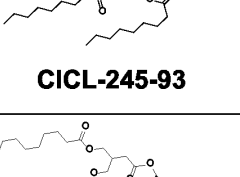
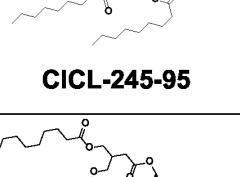
[00216] CICAL-207-107 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of CICAL-207-107 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of CICAL-207-107, and mixtures thereof.

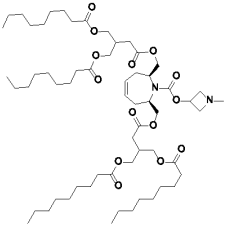
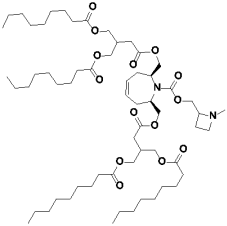
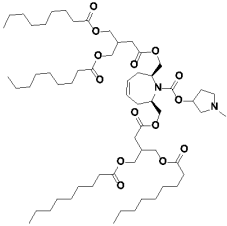
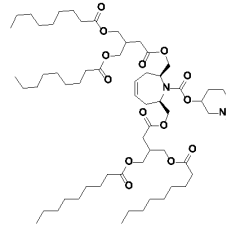
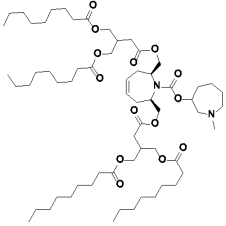
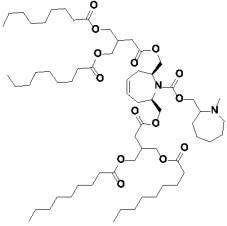
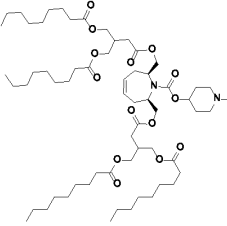
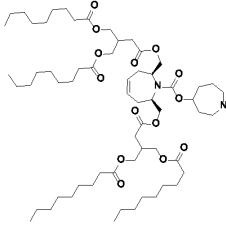
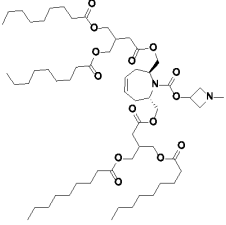
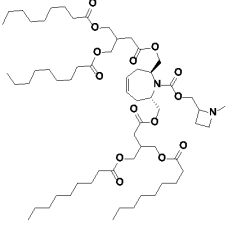
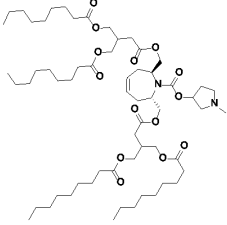
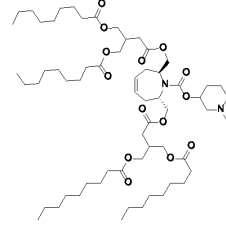
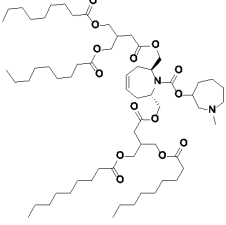
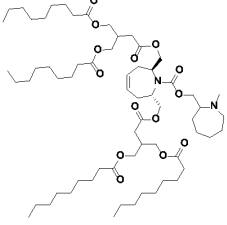
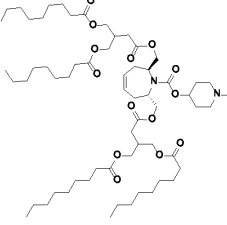
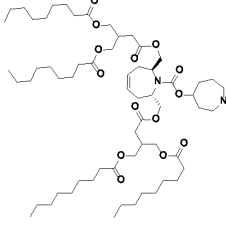
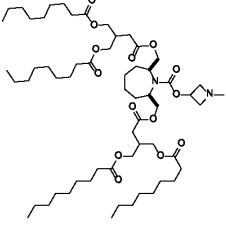
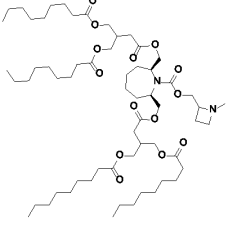
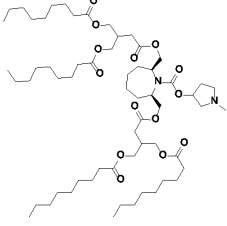
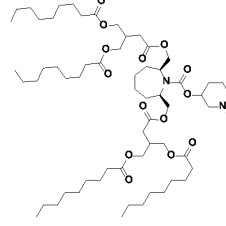
[00217] In some embodiments, the ionizable cationic lipid has a structure selected from the following:

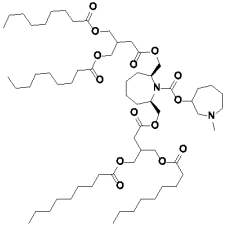
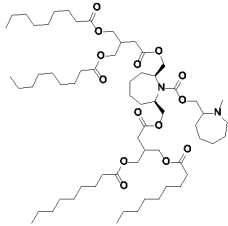
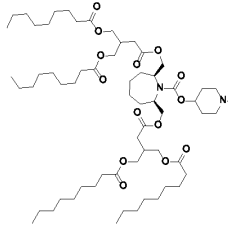
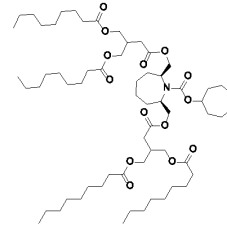
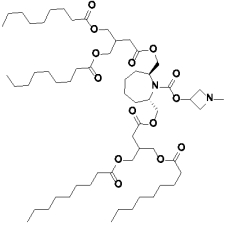
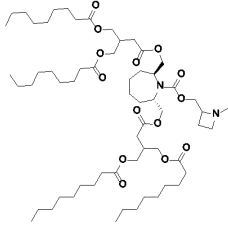
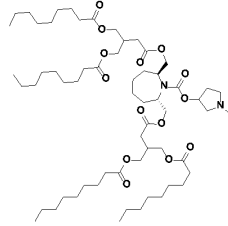
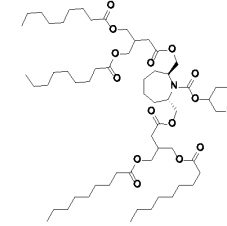
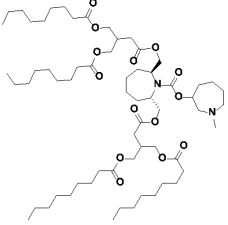
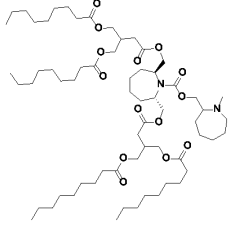
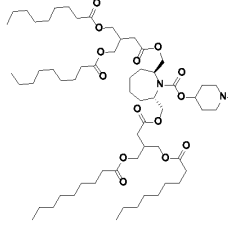
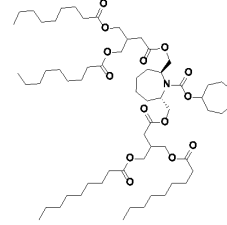
CICAL-221-91	CICAL-221-97	CICAL-221-102	CICAL-222-91
CICAL-222-92	CICAL-222-93	CICAL-222-95	CICAL-222-97
CICAL-222-98	CICAL-222-105	CICAL-224-91	CICAL-224-97

 C1CL-224-102	 C1CL-225-91	 C1CL-225-92	 C1CL-225-93
 C1CL-225-95	 C1CL-225-97	 C1CL-225-98	 C1CL-223-91
 C1CL-223-97	 C1CL-223-102	 C1CL-216-91	 C1CL-216-97
 C1CL-216-102	 C1CL-215-91	 C1CL-215-92	 C1CL-215-93
 C1CL-215-95	 C1CL-215-97	 C1CL-220-91	 C1CL-220-92
 C1CL-220-93	 C1CL-220-95	 C1CL-220-97	 C1CL-220-98

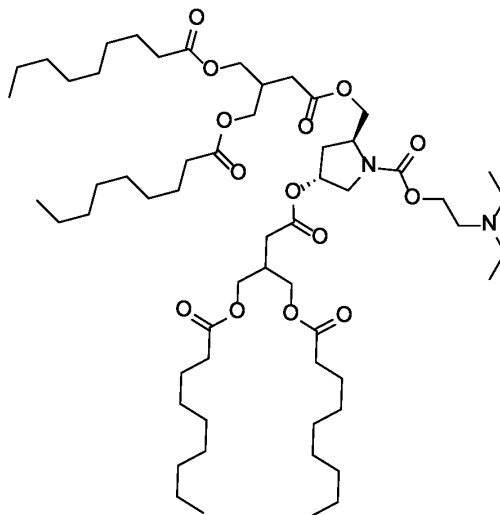
 C1CL-220-105	 C1CL-219-91	 C1CL-219-92	 C1CL-219-93
 C1CL-219-95	 C1CL-219-97	 C1CL-219-98	 C1CL-219-105
 C1CL-218-94	 C1CL-218-96	 C1CL-218-99	 C1CL-218-103
 C1CL-218-106	 C1CL-238-91	 C1CL-238-97	 C1CL-238-102
 C1CL-242-91	 C1CL-242-92	 C1CL-242-93	 C1CL-242-95
 C1CL-242-97	 C1CL-242-98	 C1CL-242-102	 C1CL-242-105

 C1CL-243-91	 C1CL-243-92	 C1CL-243-93	 C1CL-243-95
 C1CL-243-97	 C1CL-243-98	 C1CL-243-102	 C1CL-243-105
 C1CL-244-91	 C1CL-244-92	 C1CL-244-93	 C1CL-244-95
 C1CL-244-97	 C1CL-244-98	 C1CL-244-102	 C1CL-244-105
 C1CL-245-91	 C1CL-245-92	 C1CL-245-93	 C1CL-245-95
 C1CL-245-97	 C1CL-245-98	 C1CL-245-102	 C1CL-245-105

 C1CL-246-91	 C1CL-246-92	 C1CL-246-93	 C1CL-246-95
 C1CL-246-97	 C1CL-246-98	 C1CL-246-102	 C1CL-246-105
 C1CL-247-91	 C1CL-247-92	 C1CL-247-93	 C1CL-247-95
 C1CL-247-97	 C1CL-247-98	 C1CL-247-102	 C1CL-247-105
 C1CL-248-91	 C1CL-248-92	 C1CL-248-93	 C1CL-248-95

			
C1CL-248-97	C1CL-248-98	C1CL-248-102	C1CL-248-105
			
C1CL-249-91	C1CL-249-92	C1CL-249-93	C1CL-249-95
			
C1CL-249-97	C1CL-249-98	C1CL-249-102	C1CL-249-105

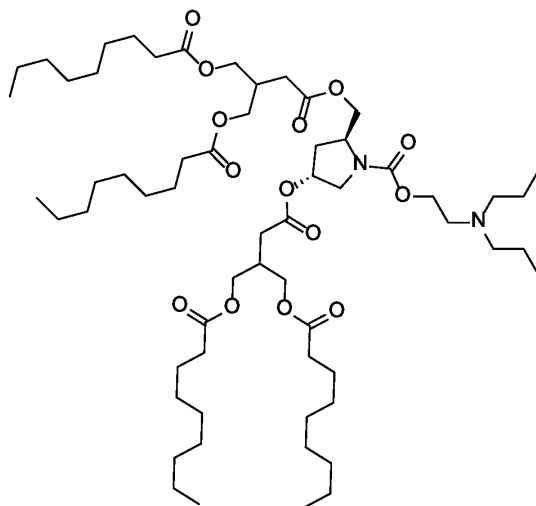
[00218] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-108:



[00219] C1CL-207-108 is an example of a lipid as disclosed herein in which A¹ and A⁴ are

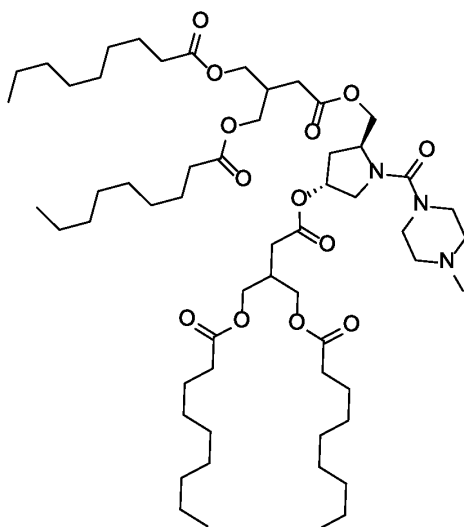
absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-108 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-108, and mixtures thereof.

[00220] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-109:



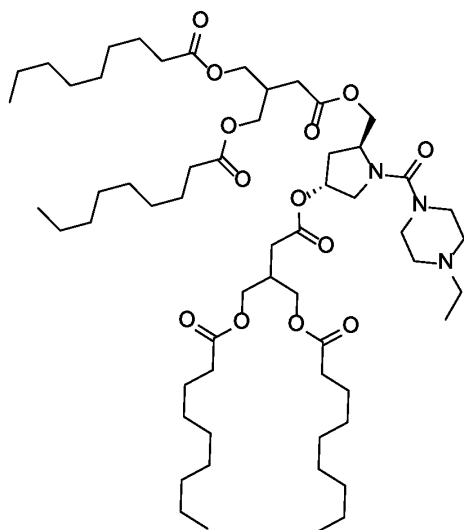
[00221] C1CL-207-109 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-109 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-109, and mixtures thereof.

[00222] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-110.



[00223] C1CL-207-110 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-110 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-110, and mixtures thereof.

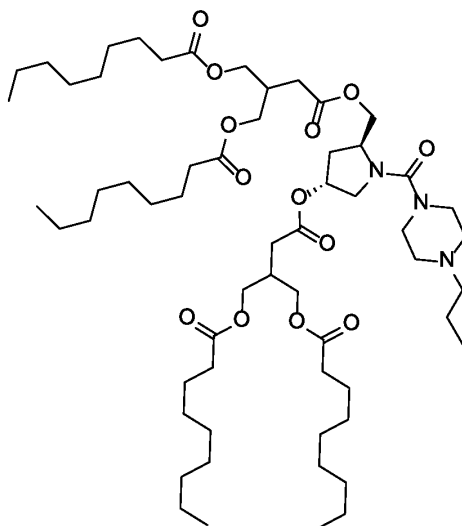
[00224] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-111:



[00225] C1CL-207-111 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to

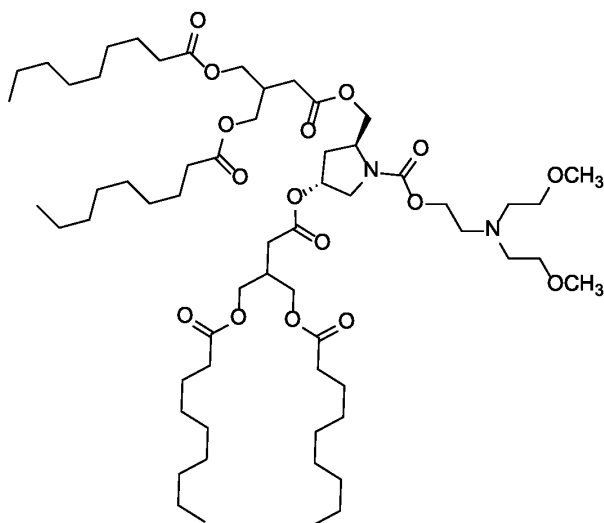
the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-111 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-111, and mixtures thereof.

[00226] In some embodiments, the ionizable cationic lipid has the structure C1CL-297-112:



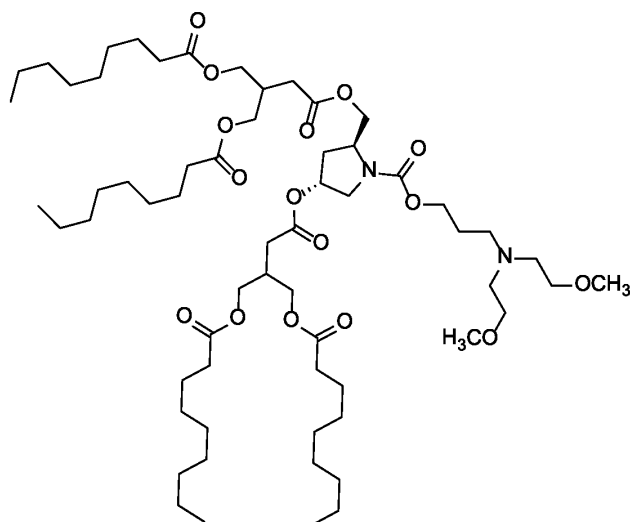
[00227] C1CL-207-112 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-112 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-112, and mixtures thereof.

[00228] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-113:



[00229] CICAL-207-113 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of CICAL-207-113 and mixtures of the enantiomers (*e.g.*, a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of CICAL-207-113, and mixtures thereof.

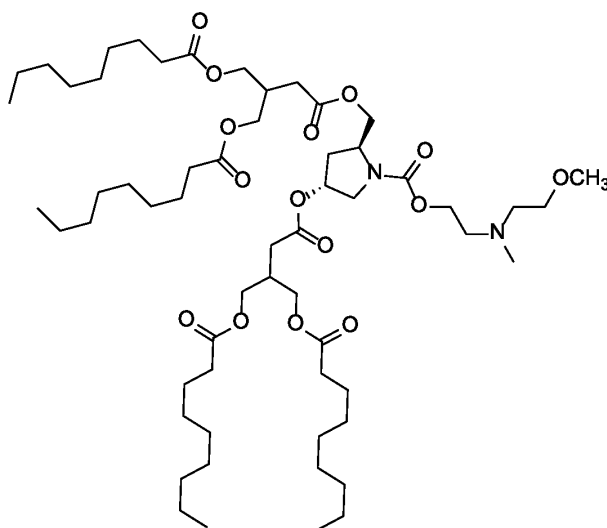
[00230] In some embodiments, the ionizable cationic lipid has the structure CICAL-207-114:



[00231] CICAL-207-114 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to

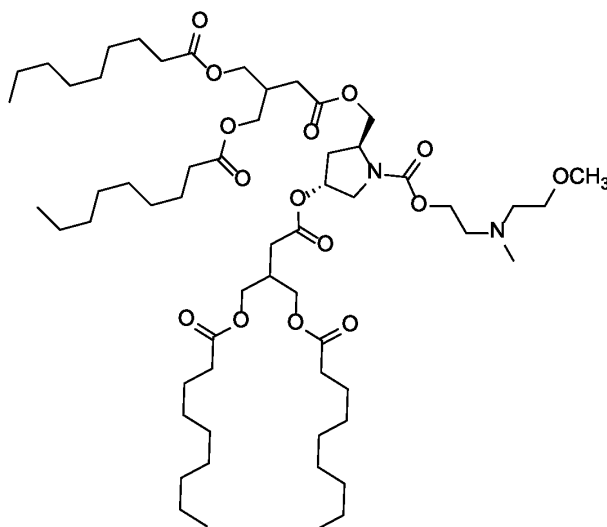
the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-114 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-114, and mixtures thereof.

[00232] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-115:



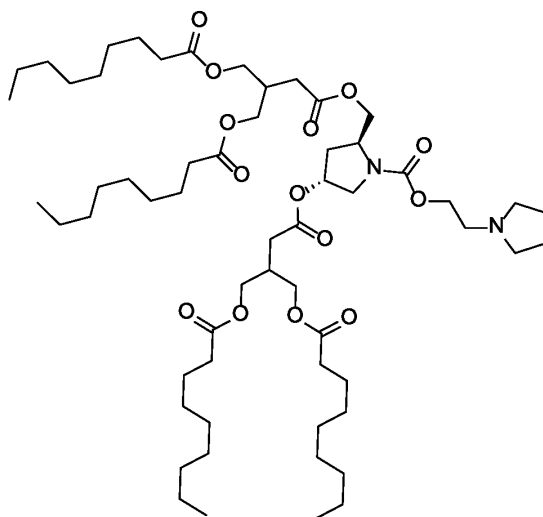
[00233] C1CL-207-115 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-115 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-115, and mixtures thereof.

[00234] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-116:



[00235] CICAL-207-116 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of CICAL-207-116 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of CICAL-207-116, and mixtures thereof.

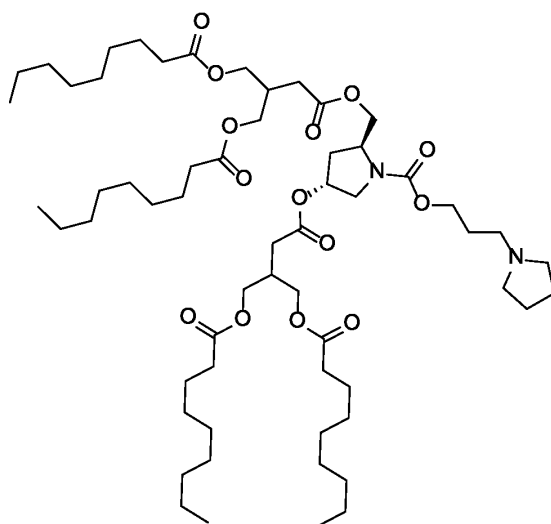
[00236] In some embodiments, the ionizable cationic lipid has the structure CICAL-207-117:



[00237] CICAL-207-117 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to

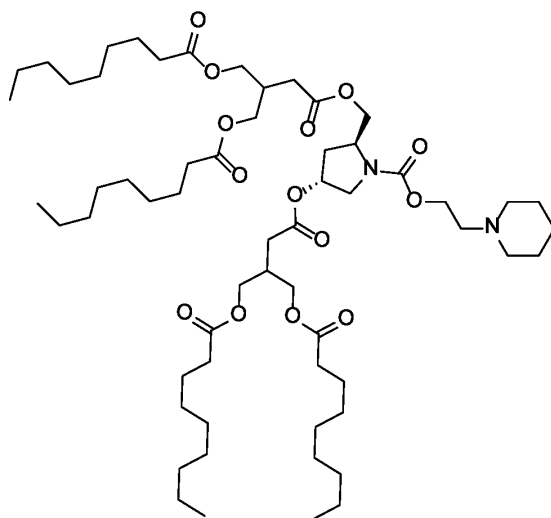
the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-117 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-117, and mixtures thereof.

[00238] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-118:



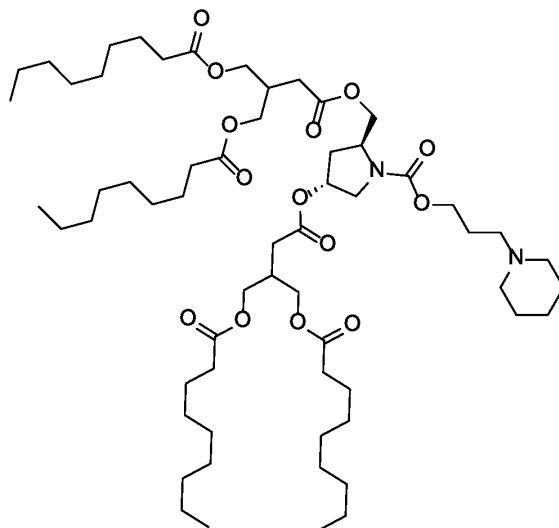
[00239] C1CL-207-118 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-118 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-118, and mixtures thereof.

[00240] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-119:



[00241] CICL-207-119 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of CICL-207-119 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of CICL-207-119, and mixtures thereof.

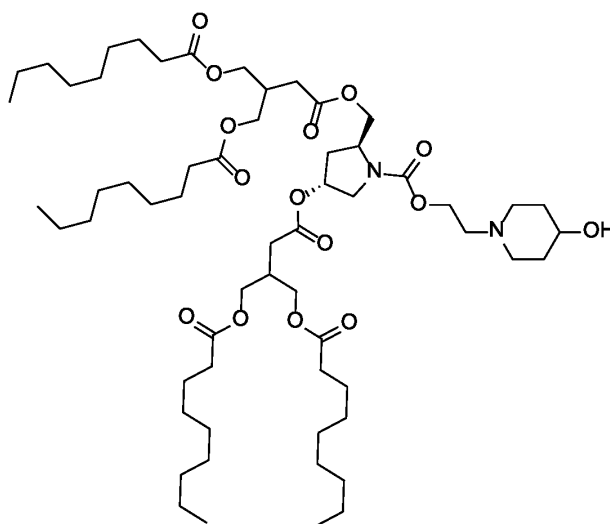
[00242] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-120:



[00243] C1CL-207-120 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to

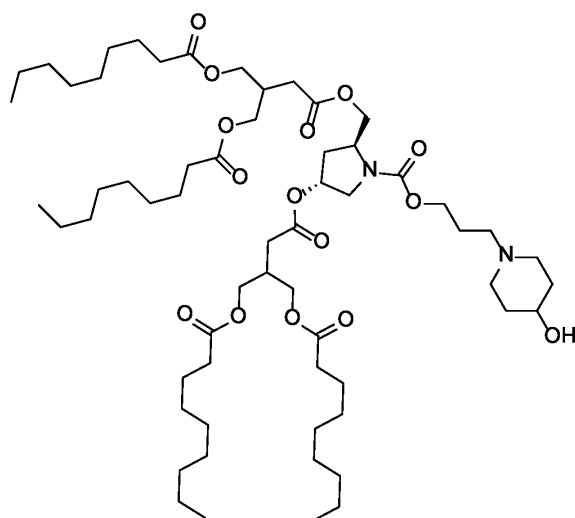
the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-120 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-120, and mixtures thereof.

[00244] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-121:



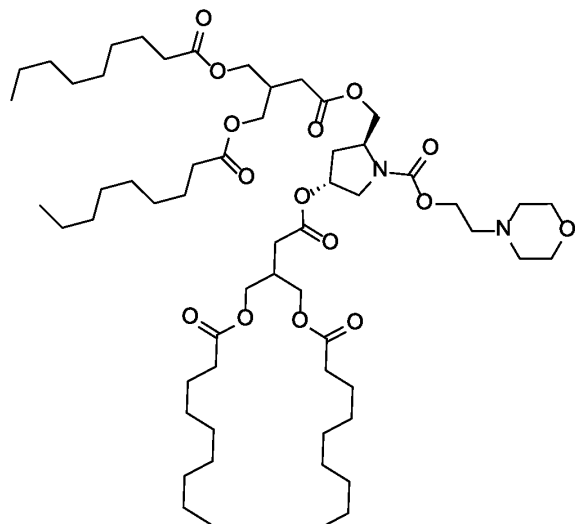
[00245] C1CL-207-121 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-121 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-121, and mixtures thereof.

[00246] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-122:



[00247] CICAL-207-122 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of CICAL-207-122 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of CICAL-207-122, and mixtures thereof.

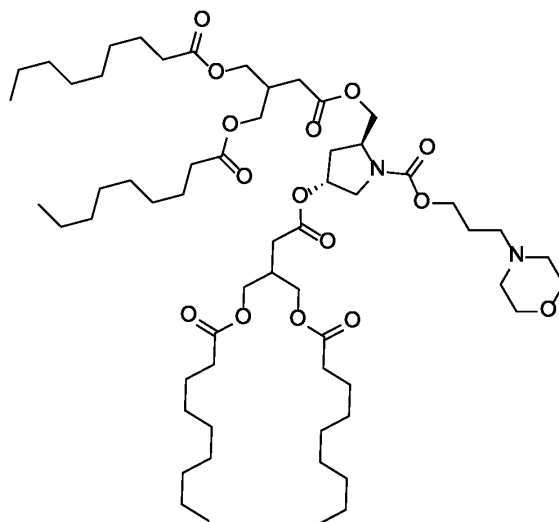
[00248] In some embodiments, the ionizable cationic lipid has the structure CICAL-207-123:



[00249] CICAL-207-123 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to

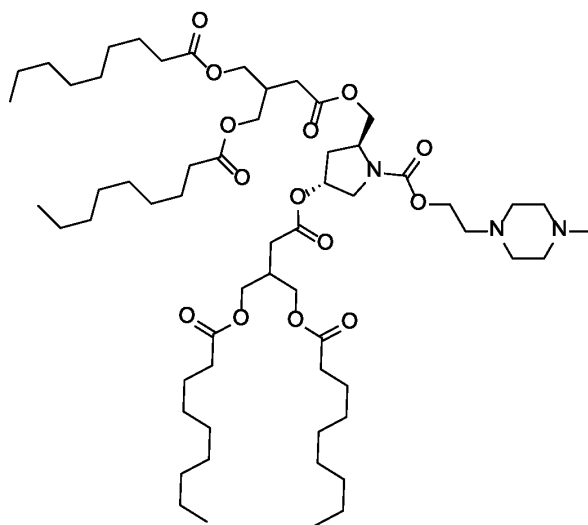
the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-123 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-123, and mixtures thereof.

[00250] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-124:



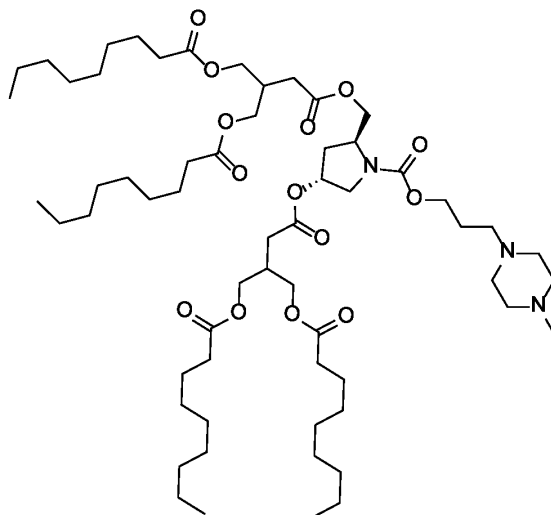
[00251] C1CL-207-124 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-124 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-124, and mixtures thereof.

[00252] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-125:



[00253] C1CL-207-125 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-125 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-125, and mixtures thereof.

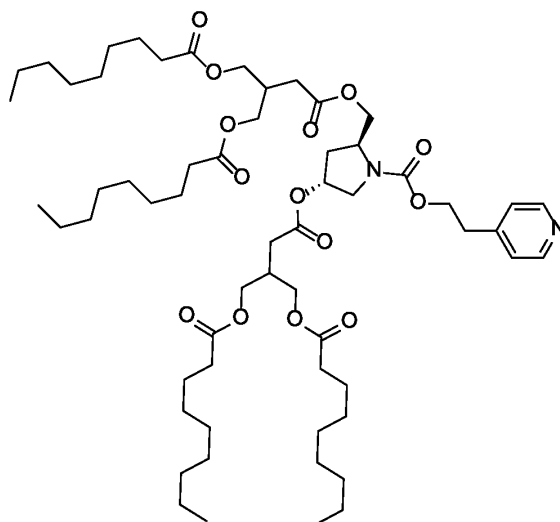
[00254] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-126:



[00255] C1CL-207-126 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute

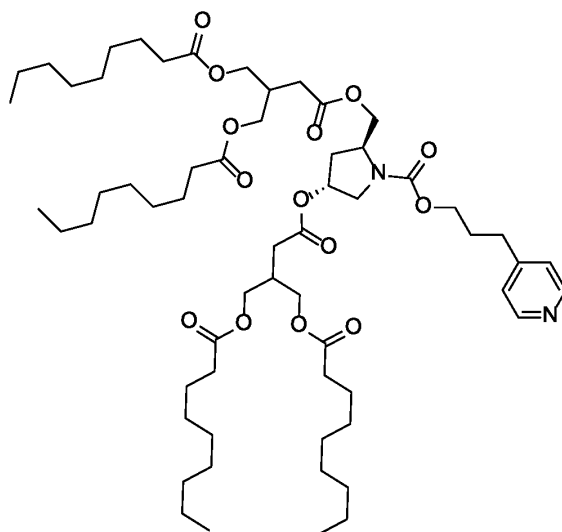
stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-126 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-126, and mixtures thereof.

[00256] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-127:



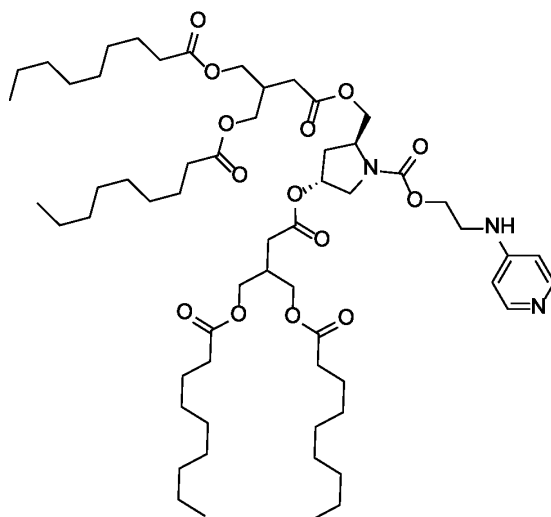
[00257] C1CL-207-127 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-127 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-127, and mixtures thereof.

[00258] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-128:



[00259] C1CL-207-128 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-128 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-128, and mixtures thereof.

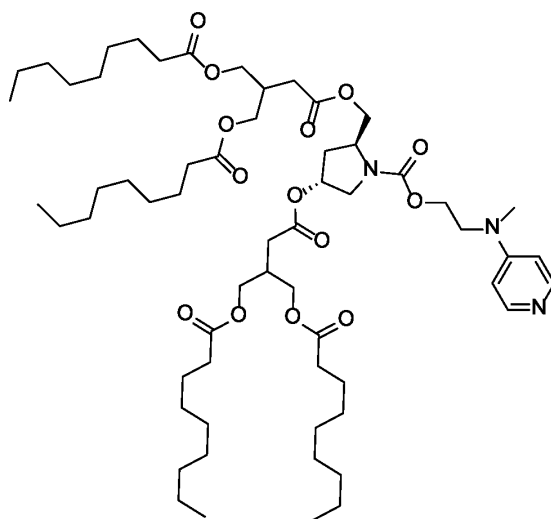
[00260] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-129:



[00261] C1CL-207-129 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to

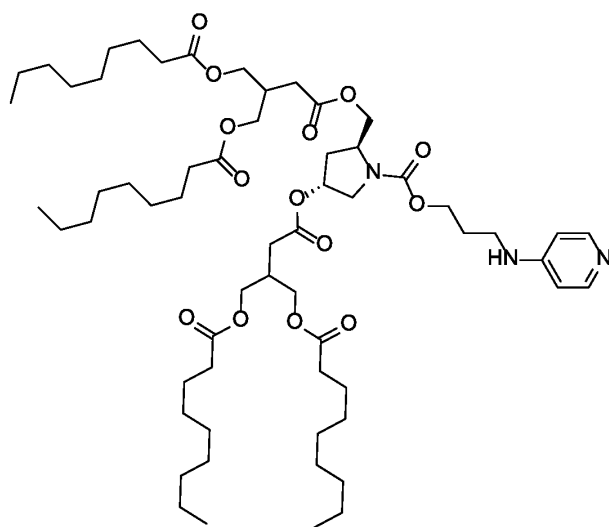
the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-129 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-129, and mixtures thereof.

[00262] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-130:



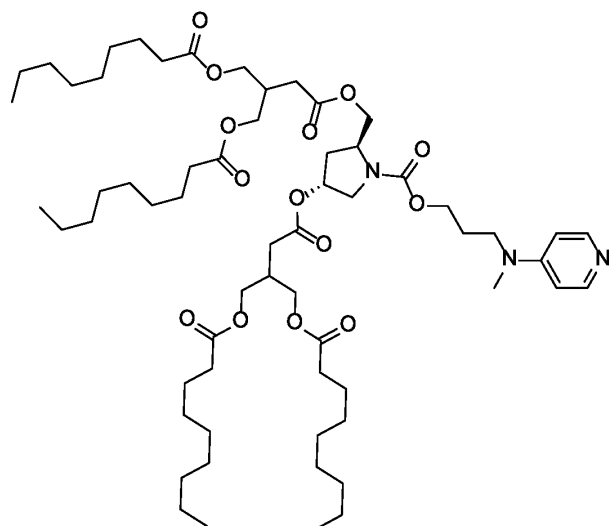
[00263] C1CL-207-130 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-130 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-130, and mixtures thereof.

[00264] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-131:



[00265] C1CL-207-131 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-131 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-131, and mixtures thereof.

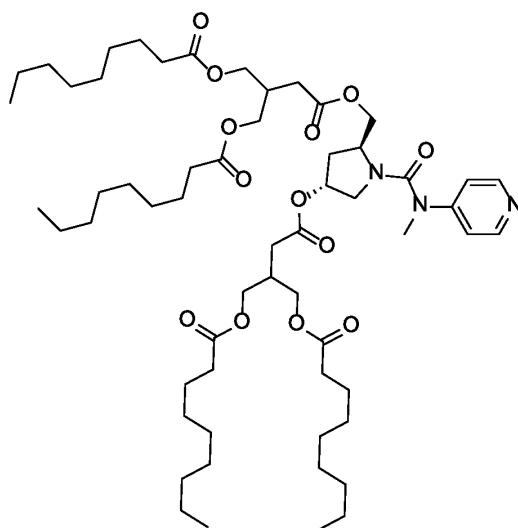
[00266] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-132:



[00267] C1CL-207-132 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to

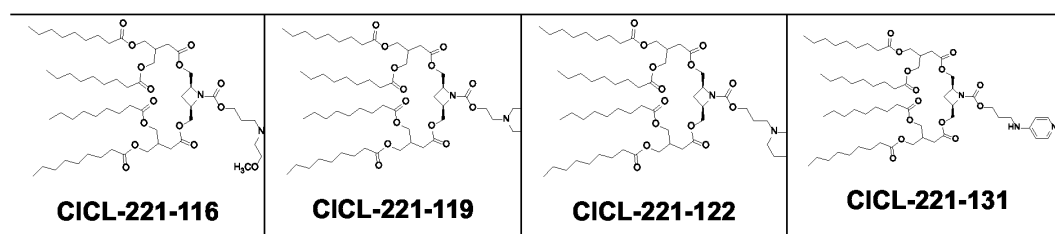
the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-132 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-132, and mixtures thereof.

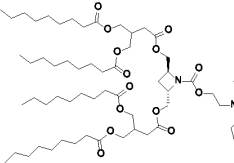
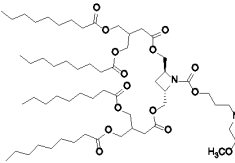
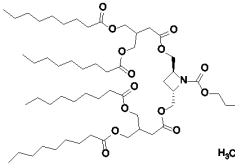
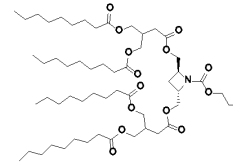
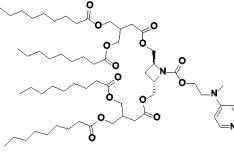
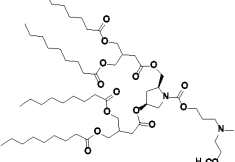
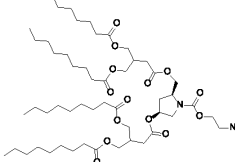
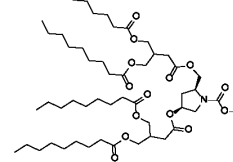
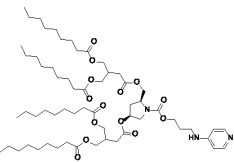
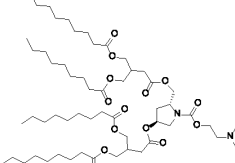
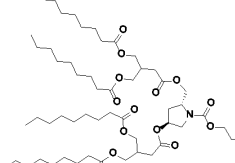
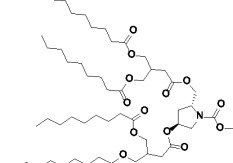
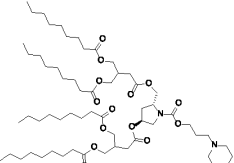
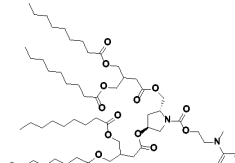
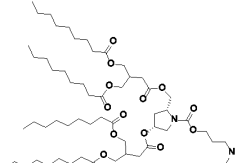
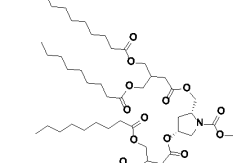
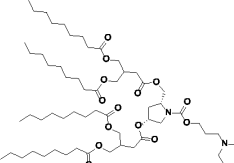
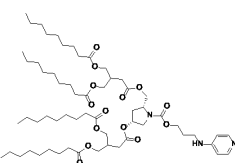
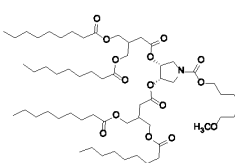
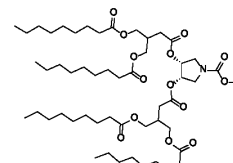
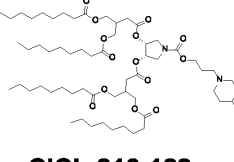
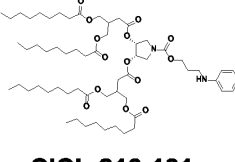
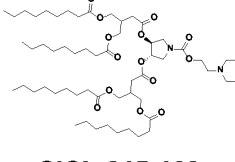
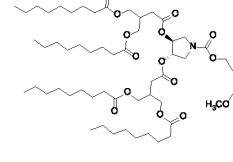
[00268] In some embodiments, the ionizable cationic lipid has the structure C1CL-207-133:

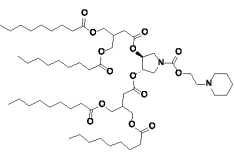
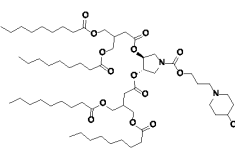
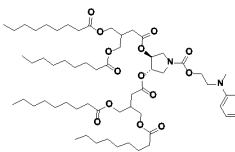
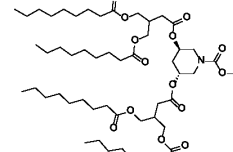
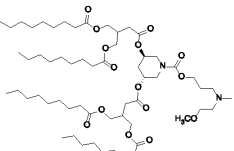
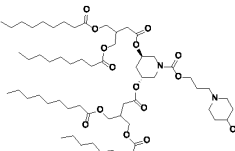
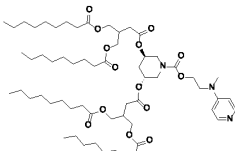
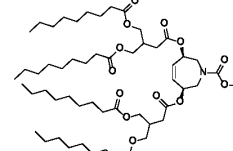
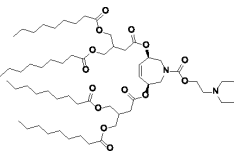
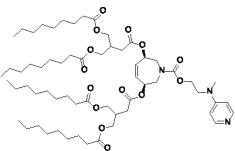
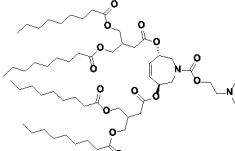
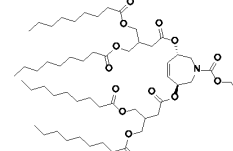
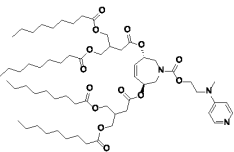
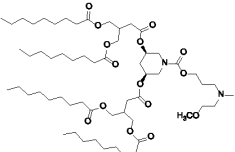
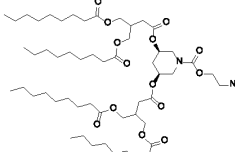
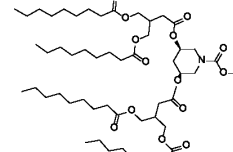
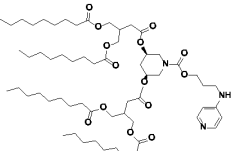
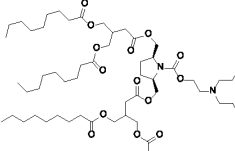
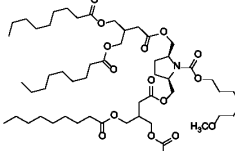
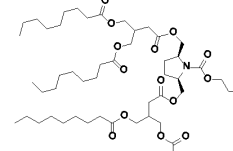
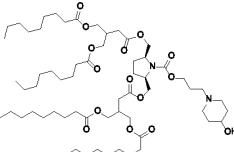
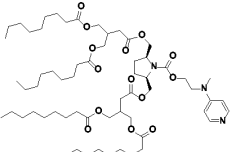
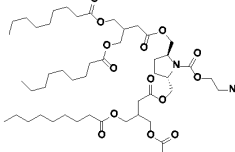
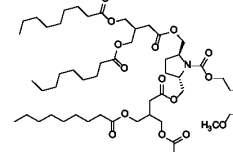


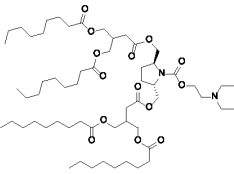
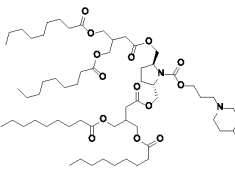
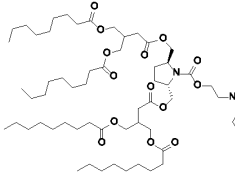
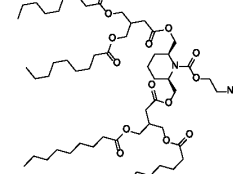
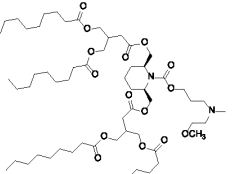
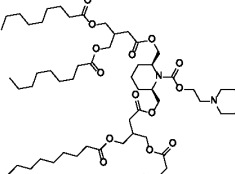
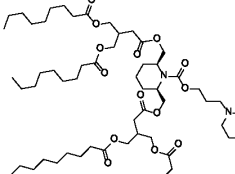
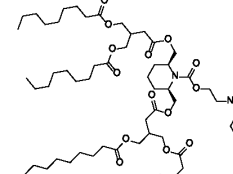
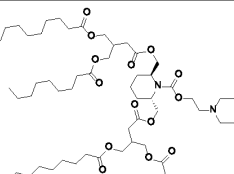
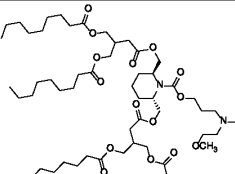
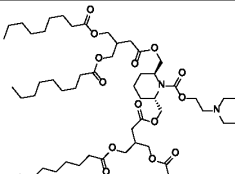
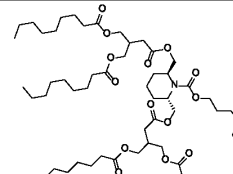
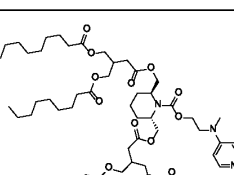
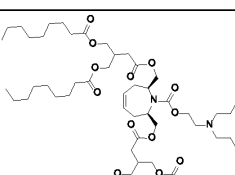
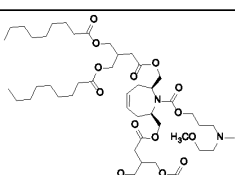
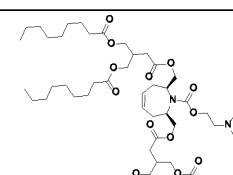
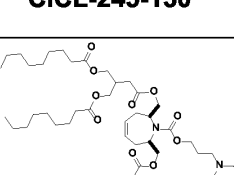
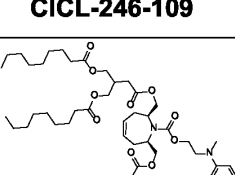
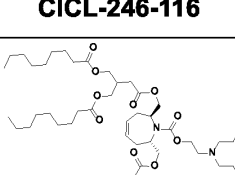
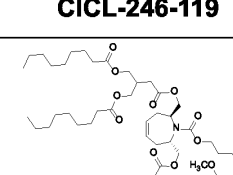
[00269] C1CL-207-133 is an example of a lipid as disclosed herein in which A¹ and A⁴ are absent, and A², A³, and A⁵ are CH₂, and the tail groups are asymmetrically placed relative to the ring nitrogen in a trans configuration. The structure drawn above indicates the absolute stereochemistry for this lipid. Other embodiments include the enantiomer of C1CL-207-133 and mixtures of the enantiomers (e.g., a racemate, or other mixtures with various ratios). Further embodiments include or are diastereomers of C1CL-207-133, and mixtures thereof.

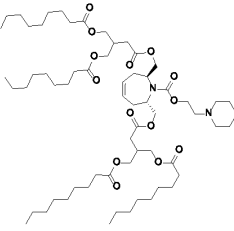
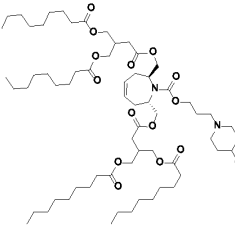
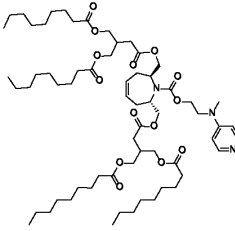
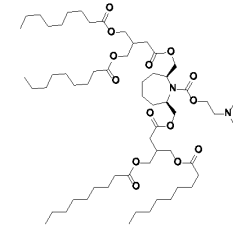
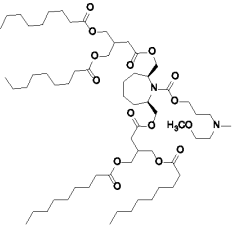
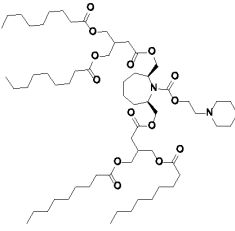
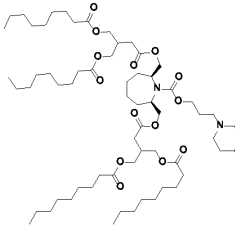
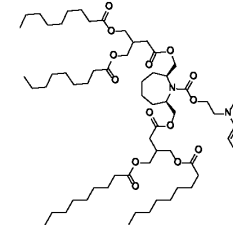
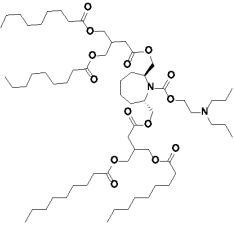
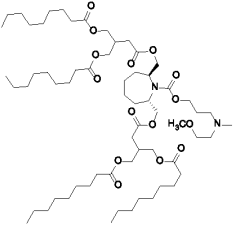
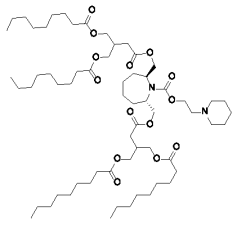
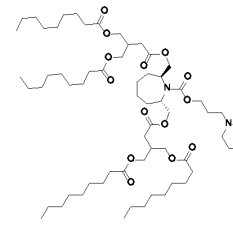
[00270] In some embodiments, the ionizable cationic lipid has a structure selected from the following:



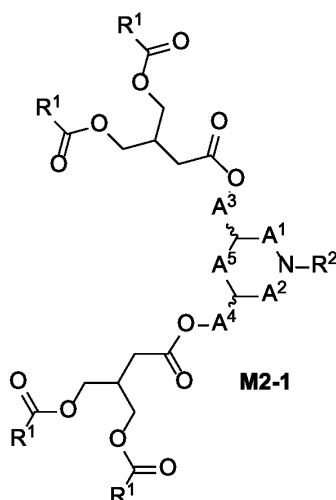
 C1CL-222-109	 C1CL-222-116	 C1CL-222-119	 C1CL-222-122
 C1CL-222-130	 C1CL-224-116	 C1CL-224-119	 C1CL-224-122
 C1CL-224-131	 C1CL-225-109	 C1CL-225-116	 C1CL-225-119
 C1CL-225-122	 C1CL-225-130	 C1CL-223-116	 C1CL-223-119
 C1CL-223-122	 C1CL-223-131	 C1CL-216-116	 C1CL-216-119
 C1CL-216-122	 C1CL-216-131	 C1CL-215-109	 C1CL-215-116

 C1CL-215-119	 C1CL-215-122	 C1CL-215-130	 C1CL-220-109
 C1CL-220-116	 C1CL-220-122	 C1CL-220-130	 C1CL-219-109
 C1CL-219-119	 C1CL-219-130	 C1CL-218-109	 C1CL-218-119
 C1CL-219-130	 C1CL-238-116	 C1CL-238-119	 C1CL-238-122
 C1CL-238-131	 C1CL-242-109	 C1CL-242-116	 C1CL-242-119
 C1CL-242-122	 C1CL-242-130	 C1CL-243-109	 C1CL-242-116

 C1CL-242-119	 C1CL-242-122	 C1CL-242-130	 C1CL-244-109
 C1CL-244-116	 C1CL-244-119	 C1CL-244-122	 C1CL-244-130
 C1CL-245-109	 C1CL-245-116	 C1CL-245-119	 C1CL-245-122
 C1CL-245-130	 C1CL-246-109	 C1CL-246-116	 C1CL-246-119
 C1CL-246-122	 C1CL-246-130	 C1CL-247-109	 C1CL-247-116

 C1CL-247-119	 C1CL-247-122	 C1CL-247-130	 C1CL-248-109
 C1CL-248-116	 C1CL-248-119	 C1CL-248-122	 C1CL-248-130
 C1CL-249-109	 C1CL-249-116	 C1CL-249-119	 C1CL-249-122

[00271] In other aspects of this disclosure are provided intermediate lipids of the ionizable cationic lipids disclosed herein. In certain aspects, the lipid (e.g., intermediate lipid) of this disclosure have a structure of formula M2-1:



wherein

each R^1 is independently selected from C_7 - C_{11} alkyl or C_7 - C_{11} alkenyl;

R^2 is H or a protecting group;

each A^1 , A^2 , A^3 , and A^4 is independently selected from $(CH_2)_0$ and $(CH_2)_1$,

A^5 is selected from $(CH_2)_{0-4}$, $CH=CH$, and $CH_2-CH=CH-CH_2$; and

wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom, or mixtures of stereo-configurations, can be assumed.

[00272] In various embodiments as described herein, R^2 is H.

[00273] In various embodiments as described herein, R^2 is a protecting group (i.e., PG_1). PG_1 can be selected from base labile or acid labile protecting groups as known in the art. For example, in some embodiments, R^2 is an acid labile protecting group such as t-butoxycarbonyl (BOC) or benzyloxycarbonyl (Cbz). In some other embodiments, R^2 is a base labile protecting group such as a trimethylsilylethoxycarbonyl moiety. In various embodiments of formula M2-1, R^1 , A^1 , A^2 , A^3 , A^4 , and A^5 are as otherwise described here.

[00274] For example, in various embodiments of formula M2-1, A^1 through A^4 are chosen so that there are only two main chain atoms between the ring nitrogen and each nearest ester oxygen in the nearest tail group.

[00275] In certain embodiments of formula M2-1, A^1 is $(CH_2)_0$, A^2 is $(CH_2)_0$, A^3 is $(CH_2)_1$, A^4 is $(CH_2)_1$, and A^5 is $(CH_2)_{1-4}$ or $CH_2-CH=CH-CH_2$.

[00276] In certain embodiments of formula M2-1, A^1 is $(CH_2)_0$, A^2 is $(CH_2)_1$, A^3 is $(CH_2)_1$, A^4

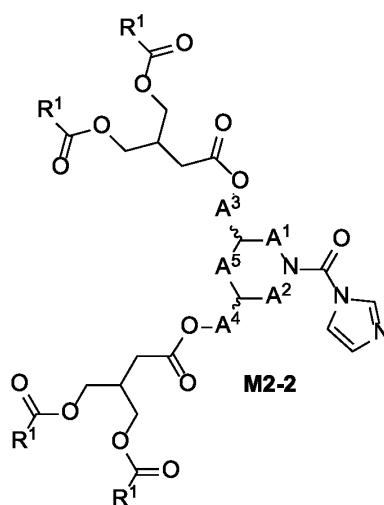
is $(\text{CH}_2)_0$, and A^5 is $(\text{CH}_2)_1$.

[00277] In certain embodiments of formula M2-1, A^1 is $(\text{CH}_2)_1$, A^2 is $(\text{CH}_2)_1$, A^3 is $(\text{CH}_2)_0$, A^4 is $(\text{CH}_2)_0$, and A^5 is $(\text{CH}_2)_0$.

[00278] In certain embodiments of formula M2-1, A^1 is $(\text{CH}_2)_1$, A^2 is $(\text{CH}_2)_1$, A^3 is $(\text{CH}_2)_0$, A^4 is $(\text{CH}_2)_0$, and A^5 is $(\text{CH}_2)_1$.

[00279] In certain embodiments of formula M2-1, A^1 is $(\text{CH}_2)_1$, A^2 is $(\text{CH}_2)_1$, A^3 is $(\text{CH}_2)_0$, A^4 is $(\text{CH}_2)_0$, and A^5 is $(\text{CH}_2)_2$ or $\text{CH}=\text{CH}$.

[00280] In certain aspects, the lipid (e.g., intermediate lipid) of this disclosure have a structure of formula M2-2:



each R^1 is independently selected from $\text{C}_7\text{-C}_{11}$ alkyl or $\text{C}_7\text{-C}_{11}$ alkenyl;

each A^1 , A^2 , A^3 , and A^4 is independently selected from $(\text{CH}_2)_0$ and $(\text{CH}_2)_1$,

A^5 is selected from $(\text{CH}_2)_{0-4}$, $\text{CH}=\text{CH}$, and $\text{CH}_2\text{-CH}=\text{CH-CH}_2$; and

wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom, or mixtures of stereo-configurations, can be assumed.

[00281] In various embodiments as described herein, R^1 , A^1 , A^2 , A^3 , A^4 , and A^5 are as otherwise described here.

[00282] For example, in various embodiments of formula M2-2, A^1 through A^4 are chosen so that there are only two main chain atoms between the ring nitrogen and each nearest ester

oxygen in the nearest tail group.

[00283] In certain embodiments of formula M2-2, A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is (CH₂)₁₋₄ or CH₂-CH=CH-CH₂.

[00284] In certain embodiments of formula M2-2, A¹ is (CH₂)₀, A² is (CH₂)₁, A³ is (CH₂)₁, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁.

[00285] In certain embodiments of formula M2-2, A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₀.

[00286] In certain embodiments of formula M2-2, A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁.

[00287] In certain embodiments of formula M2-2, A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₂ or CH=CH.

[00288] To promote biodegradability and minimize the accumulation of ionizable cationic lipids of this disclosure, the fatty acid tails are designed to comprise esters in a position that minimizes steric hindrance of ester cleavage. For example, while a single fatty acid tail will tend to extend away from the ester carbonyl to provide the most energetically favorable position, the presence of two tails leads to the tails extending in opposite directions to provide the most energetically favorable conformation. In certain other embodiments, the fatty acid tails can be in a less energetically favorable position. For example, in certain embodiments, one of the tails extends toward the carbonyl and sterically hinders cleavage of the ester. Accordingly, large branches immediately adjacent to the ester carbonyl were avoided in the design of the cationic lipids disclosed herein. Accordingly, large branches immediately adjacent to the ester carbonyl were avoided. Accordingly, in some embodiments, the ester carbonyls are not in an α position relative to the branch point, for example they are in a β position relative to the branch point. In positioning the ester(s) within the lipid, consideration was also given to potential degradation products to avoid the generation of toxic compounds, such as formaldehyde.

[00289] An advantage of relying, at least in part, on ionizable cationic lipids of this disclosure is that it can avoid the toxicity associated with quaternary ammonium cationic lipids. Accordingly, in various embodiments as described herein, a LNP or tLNP does not include a quaternary ammonium (e.g., a quaternary nitrogen group). Some LNPs containing such lipids, which are effectively permanently cationic, have displayed a fatal hyperacute toxicity in laboratory animals. The use of ionizable cationic lipids of this disclosure in an LNP obviates the need for quaternary ammonium cationic lipids and, thereby, can mitigate or avoid potential

LNP toxicity. In certain embodiments, use of an LNP or tLNP of this disclosure causes no detectable toxicity to cells or in a subject. In certain embodiments, use of an LNP or tLNP of this disclosure causes no more than mild toxicity to cells or in a subject that is asymptomatic or induces only mild symptoms that do not require intervention. In certain embodiments, use of an LNP or tLNP of this disclosure causes no more than moderate toxicity to cells or in a subject which can impair activities of daily living that requires only minimal, local, or non-invasive interventions.

[00290] The relationship between the efficacy and toxicity of a drug is generally expressed in terms of therapeutic window and therapeutic index. Therapeutic window is the dose range from the lowest dose that exhibits a detectable therapeutic effect up to the maximum tolerated dose (MTD); the highest dose that will the desired therapeutic effect without producing unacceptable toxicity. Most typically, therapeutic index is calculated as the ratio of LD50:ED50 when based on animal studies and TD50:ED50 when based on studies in humans (though this calculation could also be derived from animal studies and is sometimes called the protective index), where LD50, TD50, and ED50 are the doses that are lethal, toxic, and effective in 50% of the tested population, respectively. These concepts are applicable whether the toxicity is based on the active agent itself or some other component of the drug product, such as, for example, the LNP or its components. For any inherent level of toxicity of the disclosed lipids or LNPs themselves, an increase in the efficiency of delivering the nucleic acid into the cytoplasm improves the therapeutic window or index, as an effective amount of a biologically active payload (e.g., one or more species of nucleic acid molecule) would be deliverable with a smaller dosage of LNP (and its component lipids).

[00291] Toxicities and adverse events are sometimes graded according to a 5-point scale. A grade 1 or mild toxicity is asymptomatic or induces only mild symptoms; can be characterized by clinical or diagnostic observations only; and intervention is not indicated. A grade 2 or moderate toxicity can impair activities of daily living (such as preparing meals, shopping, managing money, using the telephone, etc.) but only minimal, local, or non-invasive interventions are indicated. Grade 3 toxicities are medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization is indicated; activities of daily living related to self-care (such as bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, and not being bedridden) can be impaired. Grade 4 toxicities are life-threatening and urgent intervention is indicated. Grade 5 toxicity produces an adverse event-related death. Thus, in various embodiments, by use of the disclosed LNP and tLNP a toxicity is confined to grade 2 or less, grade 1 or less, or produces no observation of the

toxicity.

Tolerability

[00292] Conventional LNPs deliver primarily to the liver. Liver toxicity has been the major dose limiting parameter observed with LNP-containing pharmaceuticals. For example, ONPATTRO®, comprising the ionizable lipid MC3, has a NOAEL (no observed adverse effect level) of only 0.3 mg/kg for multiple dosing in rats. A benchmark LNP comprising the ionizable cationic lipid ALC-0315, used in the SARS-CoV-2 vaccine COMIRNATY®, caused elevated levels of liver enzymes and acute phase proteins at single doses of ≥ 1 mg/kg in the rat. Merely attaching an antibody to the benchmark LNP partially reverses that elevation and the reversal is greater if the antibody directs the LNP to some other tissue (that is, a tLNP). However, use of a highly biodegradable ionizable cationic lipid, C1CL-1, the catabolism of which should be similar to those disclosed herein, reduced delivery to the liver and associated liver enzyme and acute phase protein levels to a greater extent for LNP, antibody-conjugated LNP, and tLNP. tLNP comprising C1CL-207 disclosed herein were generally well tolerated in rat at single doses of up to at least 8 mg/kg.

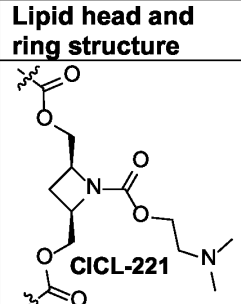
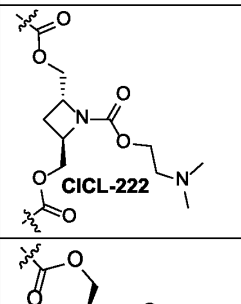
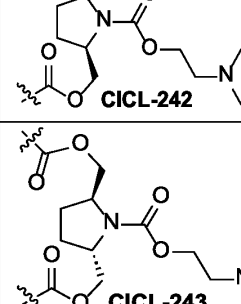
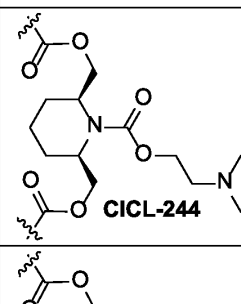
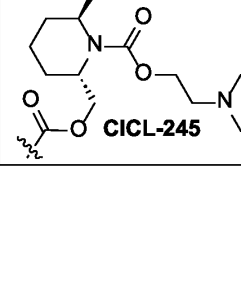
Methods of Making Ionizable Cationic Lipids

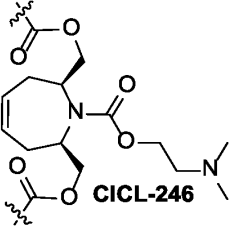
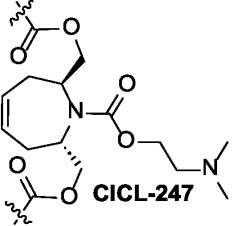
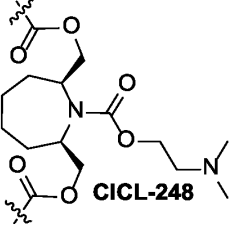
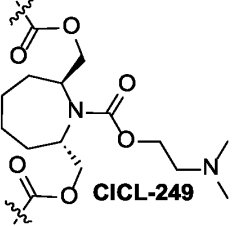
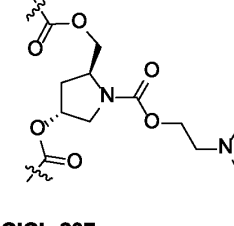
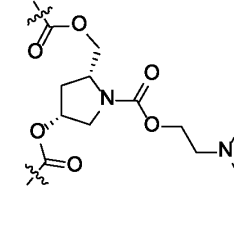
[00293] Structural symmetries and convergent rather than linear synthesis pathways can be used to simplify the synthesis of the ionizable lipids.

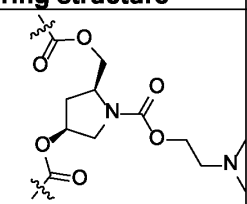
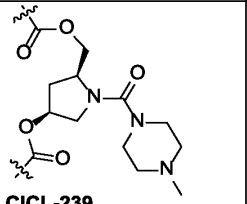
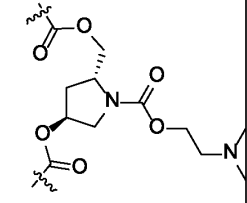
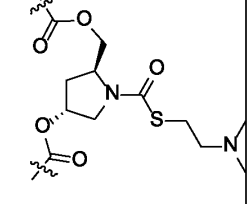
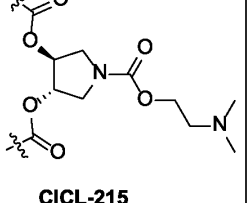
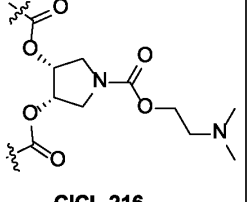
[00294] In certain aspects, this disclosure provides a method of synthesizing an ionizable cationic lipid of formula M2 (e.g., without limitation, C1CL-207, C1CL-215, C1CL-216, C1CL-217, C1CL-218, C1CL-219, C1CL-220, C1CL-221, C1CL-222, C1CL-223, C1CL-224, and C1CL-225).

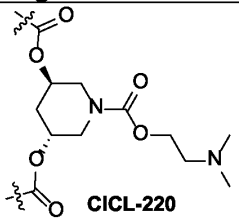
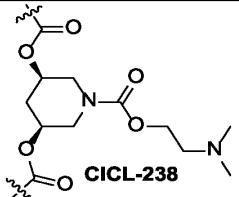
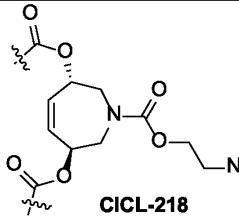
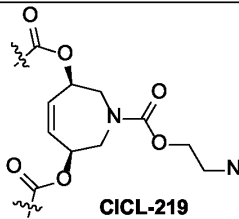
[00295] Table 2 provides a summary of substituents in formula M2 for the ring structures of C1CL-207, C1CL-215, C1CL-216, C1CL-217, C1CL-218, C1CL-219, C1CL-220, C1CL-221, C1CL-222, C1CL-223, C1CL-224, C1CL-225, C1CL-238, C1CL-239, C1CL-242, C1CL-243, C1CL-244, C1CL-245, C1CL-246, C1CL-247, C1CL-248, and C1CL-249.

Table 2. Substituents in formula M2 for the head and ring structures of C1CL-207, C1CL-215, C1CL-216, C1CL-217, C1CL-218, C1CL-219, C1CL-220, C1CL-221, C1CL-222, C1CL-223, C1CL-224, C1CL-225, C1CL-238, C1CL-239, C1CL-242, C1CL-243, C1CL-244, C1CL-245, C1CL-246, C1CL-247, C1CL-248, and C1CL-249

Lipid head and ring structure	A ¹	A ²	A ⁵	Ring size	A ³	A ⁴	Y
 C1CL-221	Absent	Absent	CH ₂	4	CH ₂	CH ₂	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-222	Absent	Absent	CH ₂	4	CH ₂	CH ₂	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-242	Absent	Absent	CH ₂ CH ₂	5	CH ₂	CH ₂	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-243	Absent	Absent	CH ₂ CH ₂	5	CH ₂	CH ₂	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-244	Absent	Absent	CH ₂ CH ₂ CH ₂	6	CH ₂	CH ₂	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-245	Absent	Absent	CH ₂ CH ₂ CH ₂	6	CH ₂	CH ₂	O-CH ₂ CH ₂ - N(CH ₃) ₂

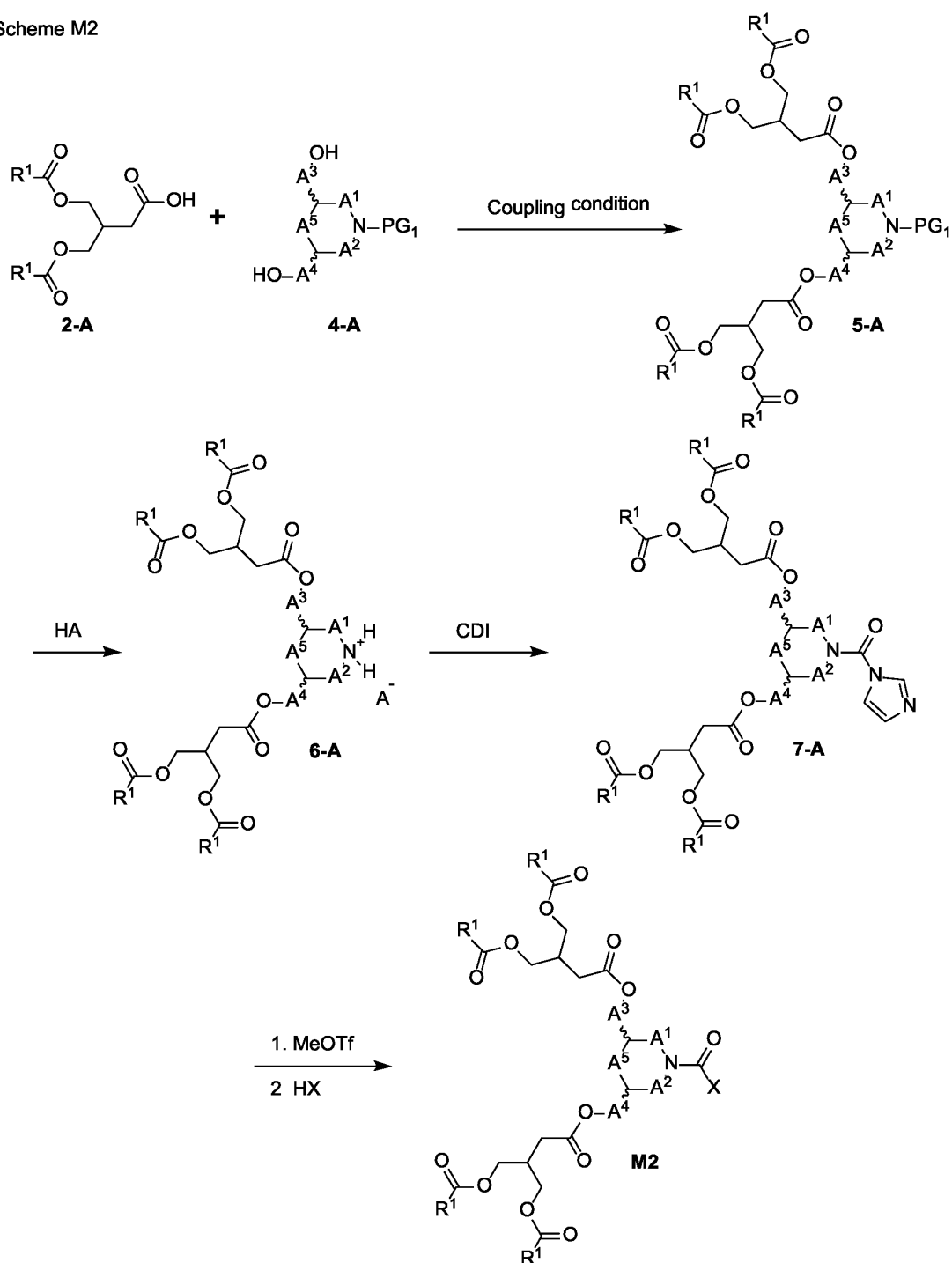
Lipid head and ring structure	A ¹	A ²	A ⁵	Ring size	A ³	A ⁴	Y
 C1CL-246	Absent	Absent	CH ₂ CH=CHCH ₂	7	CH ₂	CH ₂	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-247	Absent	Absent	CH ₂ CH=CHCH ₂	7	CH ₂	CH ₂	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-248	Absent	Absent	CH ₂ CH ₂ CH ₂ CH ₂	7	CH ₂	CH ₂	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-249	Absent	Absent	CH ₂ CH ₂ CH ₂ CH ₂	7	CH ₂	CH ₂	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-207	Absent	CH ₂	CH ₂	5	CH ₂	Absent	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-223	Absent	CH ₂	CH ₂	5	CH ₂	Absent	O-CH ₂ CH ₂ - N(CH ₃) ₂

Lipid head and ring structure	A ¹	A ²	A ⁵	Ring size	A ³	A ⁴	Y
 C1CL-224	Absent	CH ₂	CH ₂	5	CH ₂	Absent	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-239	Absent	CH ₂	CH ₂	5	CH ₂	Absent	N-CH ₂
 C1CL-225	Absent	CH ₂	CH ₂	5	CH ₂	Absent	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-217	Absent	CH ₂	CH ₂	5	CH ₂	Absent	S-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-215	CH ₂	CH ₂	Absent	5	Absent	Absent	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-216	CH ₂	CH ₂	Absent	5	Absent	Absent	O-CH ₂ CH ₂ - N(CH ₃) ₂

Lipid head and ring structure	A ¹	A ²	A ⁵	Ring size	A ³	A ⁴	Y
 C1CL-220	CH ₂	CH ₂	CH ₂	6	Absent	Absent	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-238	CH ₂	CH ₂	CH ₂	6	Absent	Absent	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-218	CH ₂	CH ₂	CH=CH	7	Absent	Absent	O-CH ₂ CH ₂ - N(CH ₃) ₂
 C1CL-219	CH ₂	CH ₂	CH=CH	7	Absent	Absent	O-CH ₂ CH ₂ - N(CH ₃) ₂

[00296] In certain embodiments, this disclosure provides a method of synthesizing an ionizable cationic lipid of formula M2 comprising the synthesis step as shown in **Scheme M2**, wherein A⁻ is an anion of an acid AH, and the rest of the substituents are defined the same as in formula M2. In certain embodiments, the method further comprises the synthesis step shown in **Scheme 4-A**. A particular stereoisomer of the ionizable cationic lipid of formula M2 can be prepared according to the synthesis method disclosed herein with starting materials and/or intermediates having the same stereochemistry.

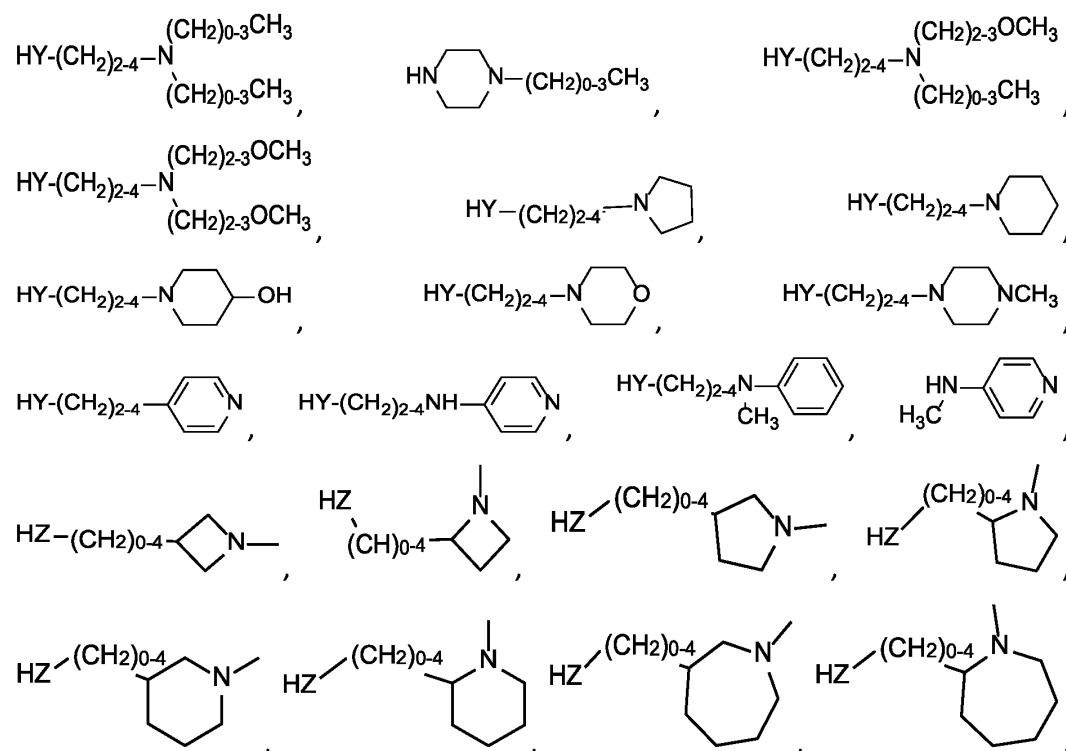
Scheme M2

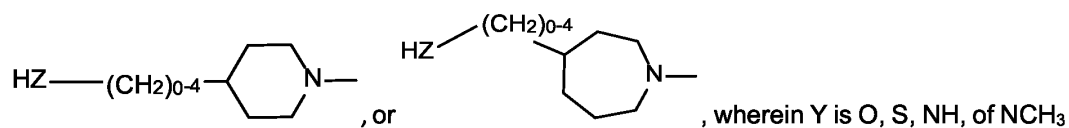


[00297] The synthesis step shown in **Scheme M2** comprises reacting a diester-amine **6-A** with 1,1'-carbonyldiimidazole (CDI) to provide an imidazolecarboxamide **7-A**; and after

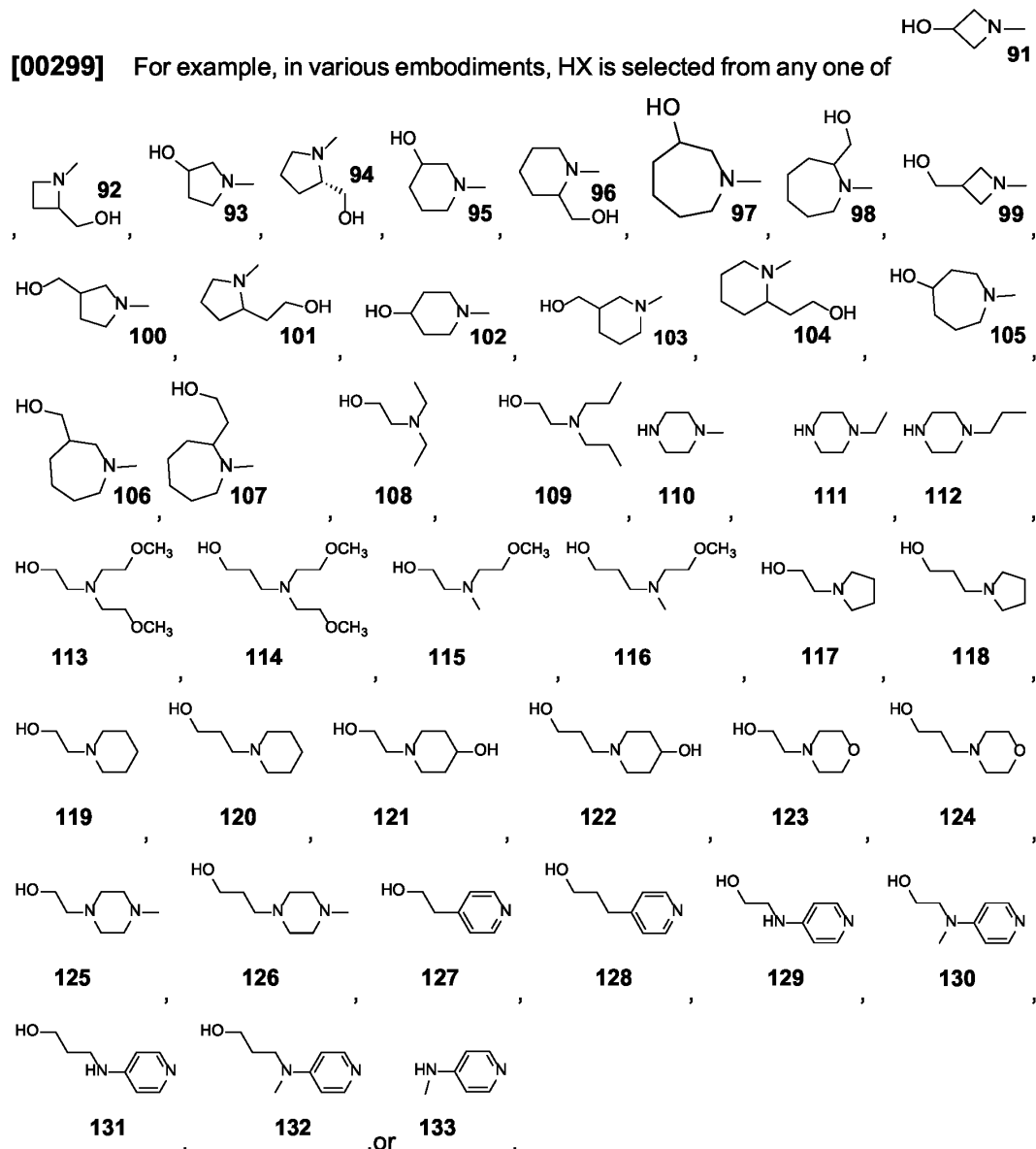
activation of the imidazolecarboxamide **7-A** it is coupled with a desired alcohol/thiol/amine (HX) to provide a corresponding carbamate/thiocarbamate/urea having the structure of formula **M2**, all substituents are defined the same as those in formula **M2**.

[00298] In certain embodiments, the imidazolecarboxamide **7-A** synthesis is carried out in an organic solvent (e.g., without limitation, CH_2Cl_2) in the presence of a basic catalyst (e.g., without limitation, trimethylamine). In certain embodiments, the carbamate/thiocarbamate/urea synthesis can comprise first reacting the imidazolecarboxamide **7-A** with trifluoromethanesulfonate (MeOTf), then reacting with the desired alcohol/thiol/amine (HX) in the presence of a base (e.g., trimethylamine). The reaction can be carried out in an organic solvent (e.g., acetonitrile). In certain embodiments as described herein, HX is an alcohol, wherein X is as described herein. In certain embodiments as described herein, HX is a thiol, wherein X is as described herein. In certain embodiments as described herein, HX is an amine, wherein X is as described herein. Accordingly, in some embodiments, HX is an alcohol/thiol/amine that provides that desired X group to the lipids of formula **M2**. For example, in various embodiments as described herein, HX is selected from





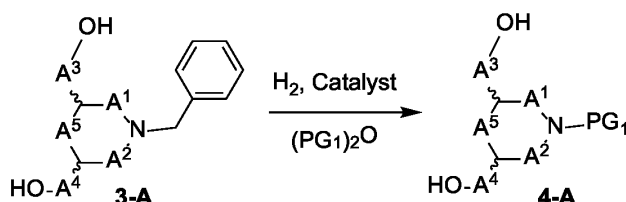
and Z is O, NH, NCH₃. The various HX compounds disclosed herein are commercially available, are known in the scientific literature, or can be made using procedures familiar to the person of ordinary skill in the art, provided from commercial sources, or the general procedures described in the Examples below.



[00300] In certain embodiments, the method can further comprise synthesis of the diester-amine **6-A** comprising deprotecting a protected diester-amine **5-A** to provide the unprotected diester-amine **6-A**. The deprotection can be carried out under acidic or basic conditions, depending on the protecting group (PG₁). For example, the deprotection step can be carried out under an acidic condition (e.g., in the presence of an acid AH, such as trifluoroacetic acid, TFA, when the protecting groups is an acid labile protecting group, e.g., t-butoxycarbonyl (BOC)) in an organic solution (e.g., CH₂Cl₂). Other PG₁ and deprotection steps can be used as known to the person of ordinary skill in the art. For example, PG₁ can be a benzyloxycarbonyl (Cbz) that can be removed in the presence of hydrogen and a Pd/C catalyst. A base labile PG₁, such as trimethylsilylethoxycarbonyl moiety, can also be used and be removed with nBuNH₄ or HF-pyridine.

[00301] In certain embodiments, the method further comprises synthesis of the protected diester-amine **5-A** comprising coupling an unprotected diester-acid **2-A** with a desired diol-amine protected by a first protecting group (PG₁) (diol-protected amine **4-A**) to form protected diester-amine **5-A**. In certain embodiments, the coupling reaction is carried out in an organic solvent (e.g., acetonitrile) in the presence of a nucleophilic catalyst (e.g., DMAP) and an acidic catalyst (e.g., 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC-HCl)). In certain embodiments, PG₁ can be -C(=O)-O-C(CH₃)₃.

[00302] In certain embodiments, the method further comprises synthesis of the diol-protected amine **4-A** as shown in **Scheme 4-A**, comprising reacting a benzyl diol-amine **3-A** with a dicarbonate (e.g., (PG₁)₂O such as di-tert-butyl dicarbonate (BOC₂O)) in the presence of a catalyst (e.g., Pd(OH)₂) and hydrogen to provide the diol-protected amine **4-A**.



Scheme 4-A

[00303] The syntheses are described using specific solvents, but in all cases alternative solvents are known to the person of skill in the art. THF can be substituted, for example, without limitation, by DMF, diethyl ether, methyl t-butyl ether, dioxane, or 2-methyl THF. Ethyl acetate can be substituted by, for example, without limitation, isopropyl acetate, THF, 2-methyl THF, dioxane, or methyl t-butyl ether. Dichloromethane can be substituted by, for example,

without limitation, ethyl acetate, isopropyl acetate, THF, methyl t-butyl ether, 2-methyl THF, dioxane, or heptane. Methanol can be substituted by, for example, without limitation, ethanol, or aqueous THF. Acetonitrile can be substituted by, for example, THF, 2-methyl THF, dichloromethane, ethyl acetate, isopropyl acetate, methyl t-butyl ether, or toluene.

[00304] Many general references providing commonly known chemical synthetic schemes and conditions useful for synthesizing materials/intermediates used in the synthesis of the disclosed compounds are available (see, e.g., Smith, *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, Eight Edition, Wiley-Interscience, 2019; or Furniss, Hannaford, Smith, Tatchell, *Vogel's Textbook of Practical Organic Chemistry, Including Qualitative Organic Analysis*, Fifth Edition, New York: Longman, 1989).

[00305] Compounds as described herein can be purified by any of the means known in the art, including chromatographic means, such as HPLC, preparative thin layer chromatography, flash column chromatography and ion exchange chromatography. Any suitable stationary phase can be used, including normal and reversed phases as well as ionic resins. Most typically, the disclosed compounds are purified via silica gel and/or alumina chromatography. See, e.g., Still, Kahn, Mitra, *J. Org. Chem.* **1978**, *43*, 2923-292, *Introduction to Modern Liquid Chromatography*, 2nd Edition, ed. L. R. Snyder and J. J. Kirkland, John Wiley and Sons, 1979; and *Thin Layer Chromatography*, ed E. Stahl, Springer-Verlag, New York, 1969.

[00306] During any of the processes for preparation of the subject compounds, it can be necessary and/or desirable to protect sensitive or reactive groups on any of the molecules concerned. This can be achieved by means of conventional protecting groups as described in standard works, such as J. F. W. McOmie, "Protective Groups in Organic Chemistry," Plenum Press, London and New York 1973, in T. P. G. M. Wuts, "Greene's Protective Groups in Organic Synthesis," Fifth edition, Wiley, New York 2014, in "The Peptides"; Volume 3 (editors: E. Gross and J. Meienhofer), Academic Press, London and New York 1981, in "Methoden der organischen Chemie," Houben-Weyl, 4th edition, Vol. 15/I, Georg Thieme Verlag, Stuttgart 1974, in H.-D. Jakubke and H. Jeschkeit, "Aminosäuren, Peptide, Proteine," Verlag Chemie, Weinheim, Deerfield Beach, and Basel 1982, and/or in Jochen Lehmann, "Chemie der Kohlenhydrate: Monosaccharide and Derivate," Georg Thieme Verlag, Stuttgart 1974. The protecting groups can be removed at a convenient subsequent stage using methods known from the art.

[00307] The intermediate compounds of the ionizable cationic lipids disclosed herein can be made using procedures familiar to the person of ordinary skill in the art and as

described herein. For example, compounds of structural formula M2 can be prepared according to Scheme M2, Scheme 4A, general procedures (see the Examples below), and/or analogous synthetic procedures. One of skill in the art can adapt the reaction sequences of Schemes M2 and 4A, general procedures, and Examples described to fit the desired target molecule. Of course, in certain situations one of skill in the art will use different reagents to affect one or more of the individual steps or to use protected versions of certain of the substituents. Additionally, one skilled in the art would recognize that compounds of the disclosure can be synthesized using different routes altogether.

Lipid Nanoparticles (LNPs) and Targeted LNPs (tLNPs)

[00308] In certain aspects, this disclosure provides an LNP comprising an ionizable cationic lipid of formula M2. In some embodiments, an LNP comprises an ionizable cationic lipid of formula M2 and a phospholipid, a sterol, a co-lipid, a PEGylated lipid, or a combination thereof. In certain embodiments, the PEG-lipids are not functionalized PEG-lipids. In other embodiments, the PEG-lipids are functionalized PEG-Lipids. In certain embodiments, the LNP comprises at least one PEG-lipid that is functionalized and at least one PEG-lipid that is not functionalized.

[00309] In further aspects, this disclosure provides a targeted lipid nanoparticle (tLNP) comprising an ionizable cationic lipid of formula M2. In some embodiments, the aforementioned tLNP can further comprise one or more of a phospholipid, a sterol, a co-lipid, and a PEG-lipid, or a combination thereof, and a functionalized PEG-lipid. As used herein, “functionalized PEG-lipid” refers to a PEG-lipid in which the PEG moiety has been derivatized with a chemically reactive group that can be used for conjugating a targeting moiety to the PEG-lipid. The functionalized PEG-lipid can be reacted with a binding moiety after the LNP is formed, so that the binding moiety is conjugated to the PEG portion of the lipid. The conjugated binding moiety can thus serve as a targeting moiety for the tLNP.

[00310] In various embodiments, a binding moiety of a LNP (or tLNP) comprises an antigen binding domain of an antibody, an antigen, a ligand-binding domain of a receptor, or a receptor ligand. In some embodiments, the binding moiety comprising an antigen binding domain of an antibody comprises a complete antibody, an F(ab)₂, an Fab, a minibody, a single-chain Fv (scFv), a diabody, a VH domain, or a nanobody, such as a VHH or single domain antibody. In some embodiments, the receptor ligand is a carbohydrate, for example, a carbohydrate comprising terminal galactose or N-acetylgalactosamine units, which are bound by the asialoglycoprotein receptor. These binding moieties constitute means for LNP targeting. Some

embodiments specifically include one or more of these binding moieties. Other embodiments specifically exclude one or more of these binding moieties.

LNP and tLNP Compositions

[00311] The LNP composition contributes to the formation of stable LNPs and tLNPs, efficient encapsulation of a payload, protection of a payload from degradation until it is delivered into a cell, and promotion of endosomal escape of a payload into the cytoplasm. These functions are primarily independent of the specificity of the binding moiety (or moieties) serving to direct or bias a tLNP to a particular cell type(s). Additional LNP and tLNP compositions are generally disclosed in PCT/US2024/032141, filed 31 May 2024 and entitled *Lipid Nanoparticle Formulations and Compositions*, which is incorporated by reference for all that it teaches about the design, formation, characterization, properties, and use of LNPs and tLNPs.

[00312] The LNPs and/or tLNPs can include the various components in amounts sufficient to provide a nanoparticle with a desired shape, fluidity, and bio-acceptability as described herein. With respect to LNPs or tLNPs of this disclosure, in some embodiments, the LNP (or tLNP) comprises at least one ionizable cationic lipid (e.g., as described herein) in an amount in the range of from about 35 to about 65 mol% or any integer bound sub-range thereof, e.g., in an amount of from about 40 to about 65 mol%, about 40 to about 60 mol% or about 40 mol% to about 62 mol%. In some embodiments, the LNP or tLNP comprises about 58 mol%, about 60 mol%, or 62 mol% ionizable cationic lipid. In some embodiments, the LNP (or tLNP) comprises a phospholipid in an amount in the range of from about 7 to about 30 mol% or any integer bound sub-range thereof, e.g., in an amount of from about 13 to about 30 mol%. In some embodiments, the LNP or tLNP comprises about 10 mol% phospholipid. In some embodiments, the LNP (or tLNP) comprises a sterol in an amount in the range of from about 20 to about 50 mol% or any integer bound sub-range thereof, e.g., in an amount in the range of from about 20 to about 45 mol%, or about 30 to about 50 mol%, or about 30 to about 45 mol%. In some embodiments, the LNP or tLNP comprises about 30.5, 26.5, or 23.5 mol% sterol. In some embodiments, the LNP (or tLNP) comprises at least one co-lipid in an amount in the range of from about 1 to about 30 mol%. In some embodiments, an LNP or tLNP comprises total PEG-lipid in an amount in the range of from about 1 mol% to about 5 mol% or any integer x 10⁻¹ bound sub-range thereof, e.g., in an amount in the range of from about 1 mol% to about 2 mol% total PEG-lipid. In some embodiments, the LNP (or tLNP) comprises at least one unfunctionalized PEG-lipid in an amount of from 0 to about 5 mol% or any integer x 10⁻¹ bound sub-range thereof, e.g., in the range of amount 0 to about 3 mol%, or about 0.1

to about 5 mol%, or about 0.5 to about 5 mol%, or about 0.5 to about 3 mol%. In some embodiments, the LNP or tLNP comprises about 1.4 mol% unfunctionalized PEG-lipid. In some embodiments, the LNP or tLNP comprises at least one functionalized PEG-lipid in an amount in the range of from about 0.1 to about 5 mol% or any integer $\times 10^{-1}$ bound sub-range thereof, e.g., in the range of from about 0.1 to 0.3 mol%. In certain embodiments, an LNP or tLNP comprises about 0.1 mol%, about 0.2 mol%, or about 0.3 mol% functionalized PEG-lipid. In some embodiments, the LNP or tLNP comprises about 0.1 mol% functionalized PEG-lipid. In some embodiments, the functionalized PEG-lipid is conjugated to a binding moiety. In certain instances, a tLNP is an LNP that further comprises an antibody (for example, a whole IgG) as the binding moiety which is present at an antibody:mRNA ratio (w/w) of about 0.3 to about 1.0.

[00313] In certain aspects, this disclosure provides an LNP or tLNP, wherein the LNP or tLNP comprises about 35 mol% to about 65 mol% of an ionizable cationic lipid, about 0.5 mol% to about 3 mol% of a PEG-lipid (including non-functionalized PEG-lipid and optionally a functionalized PEG-lipid), about 7 mol% to about 13 mol% of a phospholipid, and about 30 mol% to about 50 mol% of a sterol. In some embodiments, an LNP or tLNP comprises a payload with a net negative charge for example, a peptide, a polypeptide, a protein, a small molecule, or a nucleic acid molecule, and combinations thereof. A payload is generally encompassed by or in the interior of an LNP or tLNP. As disclosed herein dosages always refer to the amount of payload being provided. In some embodiments, a payload comprises one or more species of nucleic acid molecule. For tLNP encapsulating mRNA dosages are typically in the range of 0.05 to 5 mg/kg without regard for recipient species. In some embodiments, the dosage is in the range of 0.1 to 1 mg/kg.

[00314] With respect to LNPs or tLNPs of this disclosure, in some embodiments, the ratio of total lipid to nucleic acid is about 10:1 to about 50:1 on a weight basis. In some embodiments, the ratio of total lipid to nucleic acid is about 10:1, about 20:1, about 30:1, or about 40:1 to about 50:1, or 10:1 to 20:1, 30:1, 40:1 or 50:1, or any range bound by a pair of these ratios. The ratio of lipid to nucleic acid can also be reported as an N/P ratio, the ratio of positively chargeable lipid amine (N = nitrogen) groups to negatively-charged nucleic acid molecule phosphate (P) groups. In some embodiments, the N/P ratio is from about 3 to about 9, about 3 to about 7, about 3 to about 6, about 4 to about 6, about 5 to about 6, or about 6. In some embodiments, the N/P ratio is from 3 to 9, 3 to 7, 3 to 6, 4 to 6, 5 to 6, or 6. In certain embodiments as described herein, the LNP (or tLNP) comprises a binding moiety, wherein the binding moiety comprises an antigen binding domain of an antibody and wherein the

antibody is a whole antibody and the ratio of a lipid to nucleic acid is in the range of from about 0.3 to about 1.0 w/w.

[00315] Due to physiologic and manufacturing constraints LNP or tLNP, particles with a hydrodynamic diameter of about 50 to about 150 nm are desirable for *in vivo* use. Accordingly, in some embodiments, the LNP or tLNP has a hydrodynamic diameter of 50 to 150 nm and in some embodiments the hydrodynamic diameter is ≤ 120 , ≤ 110 , ≤ 100 , or ≤ 90 nm. Uniformity of particle size is also desirable with a polydispersity index (PDI) of ≤ 0.2 (on a scale of 0 to 1) being acceptable. Both hydrodynamic diameter and polydispersity index are determined by dynamic light scattering (DLS). Particle diameter as assessed from cryo-transmission electron microscopy (Cryo-TEM) can be smaller than the DLS-determined value.

Phospholipids

[00316] As described above, in various embodiments, the LNPs and tLNPs include a phospholipid. As would be understood by the person of ordinary skill in the art, phospholipids are amphiphilic molecules. Due to the amphiphilic nature of phospholipids, these molecules are known to form bilayers and by including them in the LNPs and tLNPs, as described herein, they can provide membrane formation, stability, and rigidity. As used herein, phospholipids include a hydrophilic head group including a functionalized phosphate group, and two hydrophobic tail groups derived from fatty acids. For example, in various embodiments as described herein, the phospholipids include a phosphate group functionalized with ethanolamine, choline, glycerol, serine, or inositol. As described above, the phospholipid includes two hydrophobic tail groups derived from fatty acids. These hydrophobic tail groups can be derived from unsaturated or saturated fatty acids. For example, the hydrophobic tail groups can be derived from a C₁₂-C₂₀ fatty acid. With respect to LNPs or tLNPs of this disclosure, in various embodiments, a phospholipid comprises dioleoylphosphatidyl ethanolamine (DOPE), dimyristoylphosphatidyl choline (DMPC), distearoylphosphatidylcholine (DSPC), dimyristoylphosphatidyl glycerol (DMPG), dipalmitoyl phosphatidylcholine (DPPC), or 1,2-diarachidoyl-sn-glycero-3-phosphocholine (DAPC), or a combination thereof. In various embodiments, the phospholipid is dioleoylphosphatidyl ethanolamine (DOPE), dimyristoylphosphatidyl choline (DMPC), distearoylphosphatidylcholine (DSPC), dimyristoylphosphatidyl glycerol (DMPG), dipalmitoyl phosphatidylcholine (DPPC), or 1,2-diarachidoyl-sn-glycero-3-phosphocholine (DAPC). In some embodiments, the phospholipid is distearoylphosphatidylcholine (DSPC). Phospholipids can contribute to formation of a membrane, whether monolayer, bilayer, or multi-layer, surrounding the core of the LNP or tLNP. Additionally, phospholipids such as DSPC, DMPC,

DPPC, DAPC impart stability and rigidity to membrane structure. Phospholipids, such as DOPE, impart fusogenicity. Further phospholipids, such as DMPG, which attain negative charge at physiologic pH, facilitates charge modulation. Thus, phospholipids constitute means for facilitating membrane formation, means for imparting membrane stability and rigidity, means for imparting fusogenicity, and means for charge modulation.

[00317] In some embodiments, an LNP or tLNP has about 7 mol% to about 13 mol% phospholipid, about 7 mol% to about 10 mol% phospholipid, or about 10 mol% to about 13 mol% phospholipid. In certain embodiments, an LNP has about 7 mol%, about 10 mol%, or about 13 mol% phospholipid. In certain instances, the phospholipid is DSPC. In certain instances, the phospholipid is DAPC.

Sterols

[00318] The disclosed LNP and tLNP comprise a sterol. Sterol refers to a subgroup of steroids that contain at least one hydroxyl (OH) group. More specifically, a gonane derivative with an OH group substituted for an H at position 3, or said differently, but equivalently, a steroid with an OH group substituted for an H at position 3. Examples of sterols include, without limitation, cholesterol, ergosterol, β -sitosterol, stigmasterol, stigmastanol, 20-hydroxycholesterol, 22-hydroxycholesterol, and the like. With respect to LNPs or tLNPs of this disclosure, in various embodiments, a sterol is cholesterol, 20-hydroxycholesterol, 22-hydroxycholesterol, or a phytosterol. In further embodiments, the phytosterol comprises campesterol, sitosterol, or stigmasterol, or combinations thereof. In preferred embodiments, the cholesterol is not animal-sourced but is obtained by synthesis using a plant sterol as a starting point. LNPs incorporating C-24 alkyl (such as methyl or ethyl) phytosterols have been reported to provide enhanced gene transfection. The length of the alkyl tail, the flexibility of the sterol ring, and polarity related to a retain C-3 -OH group are important to obtaining high transfection efficiency. While β -sitosterol and stigmasterol performed well, vitamin D2, D3 and calcipotriol, (analogs lacking intact body of cholesterol) and betulin, lupeol ursolic acid and olenolic acid (comprising a 5th ring) should be avoided. Sterols serve to fill space between other lipids in the LNP or tLNP and influence LNP or tLNP shape. Sterols also control fluidity of lipid compositions, reducing temperature dependence. Thus, sterols such as cholesterol, 20-hydroxycholesterol, 22-hydroxycholesterol, campesterol, fucosterol, β -sitosterol, and stigmasterol constitute means for controlling LNP shape and fluidity or sterol means for increasing transfection efficiency. In designing a lipid composition for a LNP or tLNP, in some embodiments, sterol content can be chosen to compensate for different amounts of other types of lipids, for example, ionizable cationic lipid or phospholipid.

[00319] In some embodiments, an LNP or tLNP has about 27 mol% or about 30 mol% to about 50 mol% sterol, or about 30 mol% to about 38 mol% sterol. In certain embodiments, an LNP or tLNP has about 30.5 mol%, about 33.5 mol%, or about 37.5 mol% sterol. In certain embodiments, an LNP or tLNP has 27 mol% or 30 mol% to 50 mol% sterol or 30 mol% to 38 mol% sterol. In further embodiments, an LNP or tLNP has 30.5 mol%, 33.5 mol%, or 37.5 mol% sterol. In certain instances, the sterol is cholesterol. In certain embodiments, the sterol is a mixture of sterols, for example, cholesterol and β -sitosterol or cholesterol and 20-hydroxycholesterol. In some instances, the sterol component is about 25 mol% 20-hydroxycholesterol and about 75 mol% cholesterol. In some instances, the sterol component is about 25 mol% β -sitosterol and about 75 mol% cholesterol. In some instances, the sterol component is about 50 mol% β -sitosterol and about 50 mol% cholesterol. In some instances, a sterol component is 25 mol% 20-hydroxycholesterol and 75 mol% cholesterol. In further instances, a sterol component is 25 mol% β -sitosterol and 75 mol% cholesterol. In still further instances, a sterol component is 50 mol% β -sitosterol and 50 mol% cholesterol.

Co-lipids

[00320] With respect to LNPs or tLNPs of this disclosure, in some embodiments, a co-lipid is absent or comprises an ionizable lipid, anionic or cationic. A co-lipid can be used to adjust various properties of an LNP or tLNP, such as surface charge, fluidity, rigidity, size, stability, and the like properties. In some embodiments, a co-lipid is an ionizable lipid, such as cholesterol hemisuccinate (CHEMS) or an ionizable lipid of this disclosure. In some embodiments, a co-lipid is a charged lipid, such as a quaternary ammonium headgroup containing lipid. In some embodiments, a quaternary ammonium headgroup containing lipid comprises 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium (DOTMA), or 3 β -(N-(N',N'-Dimethylaminoethane)carbamoyl)cholesterol (DC-Chol), or combinations thereof. In certain embodiments, these compounds a chloride, bromide, mesylate, or tosylate salt. As described above, when quaternary ammonium headgroup containing lipids are included in LNPs or tLNPs, fatal hyperacute toxicity in laboratory animals has been observed. Accordingly, when the co-lipid is a quaternary ammonium headgroup containing lipid, the quaternary ammonium headgroup containing lipid is present it makes up no more than 50 mol% of the total cationic lipid, for example, from 5 to 50% of the total cationic lipid. For illustration, if an LNP or tLNP were to have cationic lipid content of 70 mol% and 5 to 50 mol% of the total cationic lipid as quaternary ammonium lipid, the LNP or tLNP would have from 3.5 mol% quaternary ammonium lipid and 66.5 mol% ionizable cationic lipid to 35 mol% each of quaternary

ammonium lipid and ionizable cationic lipid.

[00321] When the disclosed ionizable lipids of formula M2 have a measured pKa between 6 and 7, they can contribute substantial endosomal release activity to an LNP or tLNP containing the ionizable lipid. More acidic or basic ionizable lipids of formula M2 can contribute surface charge and thus serve as a co-lipid as described immediately above. In such cases, it can be advantageous to incorporate another lipid with fusogenic activity into an LNP or tLNP of this disclosure. Surface charge is known to influence the tissue tropism of LNPs or tLNPs; for example, positively charged LNPs or tLNPs have shown a tropism for spleen and lung.

PEG-Lipids

[00322] With respect to an LNP or tLNP of this disclosure, a PEG-lipid is a lipid conjugated to a polyethylene glycol (PEG). In some embodiments as described herein, the PEG-lipid is a C₁₄-C₂₀ lipid conjugated with a PEG. For example, in various embodiments as described herein, the PEG-lipid is a C₁₄-C₂₀ lipid conjugated with a PEG, or a C₁₄-C₁₈ lipid conjugated with a PEG, or a C₁₄-C₁₆ lipid conjugated with a PEG. In certain embodiments as described herein, the PEG-lipid is a fatty acid conjugated with a PEG. The fatty acid of the PEG-lipid can have a variety of chain lengths. For each, in some embodiments, the PEG-lipid is a fatty acid conjugated with PEG, wherein the fatty acid chain length is in the range of C₁₄-C₂₀ (e.g., in the range of C₁₄-C₁₈, or C₁₄-C₁₆). PEG-lipids with fatty acid chain lengths less than C₁₄ are too rapidly lost from the LNP or tLNP while those with chain lengths greater than C₂₀ are prone to difficulties with formulation.

[00323] PEG can be made in a large range of sizes. In certain embodiments, the PEG of the disclosed LNP and tLNP is PEG-1000 to PEG-5000. It is to be understood that polyethylene preparations of these sizes are polydisperse and that the nominal size indicates an approximate average molecular weight of the distribution. Taking the molecular weight of an individual repeating unit of (OCH₂CH₂)_n to be 44, a PEG molecule with n=22 would have a molecular weight of 986, with n=45 a molecular weight of 1998, and with n=113 a molecular weight of 4990. n≈22 to 113 is used to represent PEG-lipids incorporating PEG moieties in the range of PEG-1000 to PEG-5000 such as PEG-1000, PEG-1500, PEG-2000, PEG-2500, PEG-3000, PEG-3500, PEG-4000, PEG-4500, and PEG-5000, although some molecules from preparations at the average molecular weight boundaries will have an n outside that range. For individual preparations n≈22 is used to represent PEG-lipids incorporating PEG moieties from PEG-1000, n≈45 is used to represent PEG-lipids incorporating PEG moieties from PEG-2000, n≈67 is used to represent PEG-lipids incorporating PEG moieties from PEG-3000, n≈90 is used to represent PEG-lipids incorporating PEG moieties from PEG-4000,

$n \approx 113$ is used to represent PEG-lipids incorporating PEG moieties from PEG-5000. Some embodiments incorporate PEG moieties in a range bounded by any pair of the foregoing values of n or average molecular weight. In some embodiments of the PEG-lipid, a PEG is of 500-5000 or 1000-5000 Da molecular weight (MW). For example, in some embodiments, the PEG of the PEG-lipid has a molecular weight in the range of 1500-5000 Da or 2000-5000 Da. In some embodiments as described herein, the PEG-lipid has a molecular weight in the range of 500-4000 Da, or 500-3000 Da, or 1000-4000 Da, or 1000-3000, or 1000-2500, or 1500-4000, or 1500-3000, or 1500-2500 Da. In some embodiments, the PEG moiety is PEG-500, PEG-1000, PEG-1500, PEG-2000, PEG-2500, PEG-3000, PEG-3500, PEG-4000, PEG-4500, and PEG-5000. In some embodiments, the PEG unit has a MW of 2000 Da (sometime abbreviated as PEG(2k)). Some embodiments incorporate PEG moieties of PEG-1000, PEG-2000, or PEG-5000. In some instances, the PEG moiety is PEG-2000. Certain embodiments comprise a DSG-PEG, for example, DSG-PEG-2000. Certain embodiments comprise a DSPE-PEG, for example, DSPE-PEG-2000. Certain embodiments comprise both DSG-PEG-2000 and/or DSPE-PEG2000.

[00324] Common PEG-lipids fall into two classes diacyl glycerols and diacyl phospholipids. Examples of diacyl glycerol PEG-lipids include DMG-PEG (1,2-dimyristoyl-glycero-3-methoxypolyethylene glycol), DPG-PEG (1,2-dipalmitoyl-glycero-3-methoxypolyethylene glycol), DSG-PEG (1,2-distearoyl-glycero-3-methoxypolyethylene glycol), and DOG-PEG (1,2-dioleoyl-glycero-3-methoxypolyethylene glycol). Examples of diacyl phospholipids include DMPE-PEG (1,2-dimyristoyl-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol), DPPE-PEG (1,2-dipalmitoyl-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol), DSPE-PEG (1,2-distearoyl-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol), and DOPE-PEG (1,2-dioleoyl-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol).

[00325] In some embodiments, the MW2000 PEG-lipid (e.g., a PEG-lipid comprising a PEG of a molecular weight of 2000 Da) comprises DMG-PEG2000 (1,2-dimyristoyl-glycero-3-methoxypolyethylene glycol-2000), DPG-PEG2000 (1,2-dipalmitoyl-glycero-3-methoxypolyethylene glycol-2000), DSG-PEG2000 (1,2-distearoyl-glycero-3-methoxypolyethylene glycol-2000), DOG-PEG2000 (1,2-dioleoyl-glycero-3-methoxypolyethylene glycol-2000), DMPE-PEG200 (1,2-dimyristoyl-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol-2000), DPPE-PEG2000 (1,2-dipalmitoyl-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol-2000), DSPE-PEG2000 (1,2-distearoyl-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol-

2000), DOPE-PEG2000 (1,2-dioleoyl-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol-2000), or combinations thereof. In some embodiments, the PEG unit has a MW of 2000 Da. In some embodiments, the MW2000 PEG-lipid comprises DMrG-PEG2000 (1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000), DPrG-PEG2000 (1,2-dipalmitoyl-rac-glycero-3-methoxypolyethylene glycol-2000), DSrG-PEG2000 (1,2-distearoyl-rac-glycero-3-methoxypolyethylene glycol-2000), DorG-PEG2000 (1,2-dioleoyl-glycero-3-methoxypolyethylene-rac-glycol-2000), DMPEr-PEG200 (1,2-dimyristoyl-rac-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol-2000), DPPEr-PEG2000 (1,2-dipalmitoyl-rac-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol-2000), DSPEr-PEG2000 (1,2-distearoyl-rac-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol-2000), DOPEr-PEG2000 (1,2-dioleoyl-rac-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol-2000), or combinations thereof. The glycerol in these lipids is chiral. Thus, in some embodiments, the PEG-lipid is racemic. Alternatively, optically pure antipodes of the glycerol portion can be employed, that is, the glycerol portion is homochiral. As used herein with respect to glycerol moieties, optically pure means $\geq 95\%$ of a single enantiomer (D or L). In some embodiments, the enantiomeric excess is $\geq 98\%$. In some embodiments, the enantiomeric excess is $\geq 99\%$. Additional PEG-lipids, including achiral PEG-lipids built on a symmetric dihydroxyacetone scaffold, a symmetric 2-(hydroxymethyl)butane-1,4-diol, or a symmetric glycerol scaffold, are disclosed in U.S. Provisional Application No. 63/362,502, filed on April 5, 2022, and PCT/US2023/017648 filed on April 5, 2023 (WO 2023/196445A1), both entitled *PEG-Lipids and Lipid Nanoparticles*, which are incorporated by reference in their entirety.

[00326] The above PEG-lipid examples are presented as methoxypolyethylene glycols, but the terminus need not necessarily be methoxyl. With respect to any of the PEG-lipids that have not been functionalized, in alternative embodiments, the PEG moiety of the PEG lipids can terminate with a methoxyl, a benzyloxyl, a 4-methoxybenzyloxyl, or a hydroxyl group (that is, an alcohol). The terminal hydroxyl facilitates functionalization. The methoxyl, benzyloxyl, and 4-methoxybenzyloxyl groups are advantageously provided for PEG-lipid that will be used as a component of the LNP without functionalization. However, all four of these alternatives are useful as the (non-functionalized) PEG-lipid component of LNPs. The 4-methoxybenzyloxyl group, often used as a protecting group during synthesis of the PEG-lipid, is readily removed to generate the corresponding hydroxyl group. Thus, the 4-methoxybenzyloxyl group offers a convenient path to the alcohol when it is not synthesized directly. The alcohol is useful for being functionalized, prior to incorporation of the PEG-lipid into a LNP, so that a binding moiety can be conjugated to it as a targeting moiety for the LNP

(making it a tLNP). As used herein, the terminus of the PEG moiety, and similar constructions, refers to the end of the PEG moiety that is not attached to the lipid.

[00327] A PEG-moiety provides a hydrophilic surface on the LNP, inhibiting aggregation or merging of LNP, thus contributing to their stability and reducing polydispersity, *i.e.* reducing the heterogeneity of a dispersion of LNPs. Additionally, a PEG moiety can impede binding by the LNP, including binding to plasma proteins. These plasma proteins include apoE which is understood to mediate uptake of LNP by the liver so that inhibition of binding can lead to an increase in the proportion of LNP reaching other tissues. These plasma proteins also include opsonins so that inhibition of binding reduces recognition by the reticuloendothelial system. The PEG-moiety can also be functionalized to serve as an attachment point for a targeting moiety. Conjugating a cell- or tissue-specific binding moiety to the PEG-moiety enables a tLNP to avoid the liver and bind to its target tissue or cell type, greatly increasing the proportion of LNP that reaches the targeted tissue or cell type. PEG-lipid can thus serve as means for inhibiting LNP binding, and PEG-lipid conjugated to a binding moiety can serve as means for LNP-targeting.

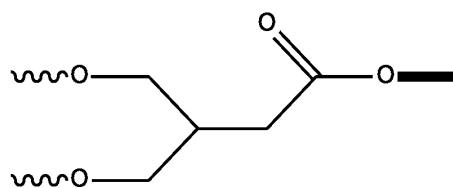
[00328] As used herein, the term “functionalized PEG-lipid” and similar constructions refer generally to both the unreacted and reacted entities. The lipid composition of a LNP can be described referencing the reactive species even after conjugation has taken place (forming a tLNP). For example, a lipid composition can be described as comprising DSPE-PEG-maleimide and can be said to further comprise a binding moiety without explicitly noting that upon reaction to form the conjugate the maleimide will have been converted to a succinimide (or hydrolyzed succinimide). Similarly, if the reactive group is bromomaleimide, after conjugation it will be maleimide. These differences of chemical nomenclature for the unreacted and reacted species are to be implicitly understood even when not explicitly stated. Certain embodiments comprise a DSG-PEG, for example, DSG-PEG-2000. Certain embodiments comprise a functionalized DSPE-PEG, for example, functionalized DSPE-PEG-2000. Certain embodiments comprise both DSG-PEG-2000 and functionalized DSPE-PEG-2000. In some instances, the functionalized PEG-lipid is functionalized with a maleimide moiety, for example, DSPE-PEG-2000-MAL.

[00329] In certain aspects, the LNP comprises one or more PEG-lipids and/or functionalized PEG-lipids; when both a functionalized and unfunctionalized PEG-lipid, the PEG-lipid present they can be the same or different; and one or more ionizable cationic lipids; the LNP can further comprise a phospholipid, a sterol, a co-lipid, or any combination thereof. The term “functionalized PEG-lipid” refers to a PEG-lipid in which the PEG moiety has been

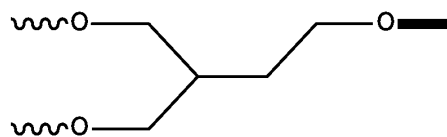
derivatized with a chemically reactive group that can be used for conjugating a targeting moiety to the PEG-lipid. The functionalized PEG-lipid can be reacted with a binding moiety so that the binding moiety is conjugated to the PEG portion of the lipid. The conjugated binding moiety can thus serve as a targeting moiety for the LNP to constitute a tLNP. In some embodiments, the binding moiety is conjugated to the functionalized PEG-lipid after an LNP comprising the functionalized PEG-lipid is formed. In other embodiments, the binding moiety is conjugated to the PEG-lipid and then the conjugate is inserted into a previously formed LNP.

[00330] In certain embodiments, the LNP is a tLNP comprising one or more functionalized PEG-lipids that has been conjugated to a binding moiety. In certain embodiments, the tLNP also comprises PEG-lipids not functionalized or conjugated with a binding moiety. In some embodiments, the functionalization is a maleimide. In some embodiments the functionalization is a bromomaleimide or bromomaleimide amide, alkynylamide, or alkynylimide moiety at the terminal hydroxyl end of the PEG moiety. In some embodiments, the binding moiety comprises an antibody or antigen binding portion thereof. In some embodiments, the binding moiety is a polypeptide comprising a binding domain and an N- or C-terminal extension comprising an accessible thiol group. In some embodiments, the conjugation linkage comprises a reaction product of a thiol in the binding moiety with a functionalized PEG-lipid. In some embodiments, the functionalization is a maleimide, azide, alkyne, dibenzocyclooctyne (DBCO), bromomaleimide or bromomaleimide amide, alkynylamide, or alkynylimide. In some embodiments, the binding moiety comprises an antibody or antigen binding portion thereof. In some embodiments, the binding moiety is a polypeptide comprising a binding domain and an N- or C-terminal extension comprising an accessible thiol group.

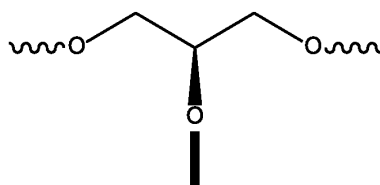
[00331] In certain embodiments, the PEG-lipid and/or functionalized PEG-lipid comprises a scaffold selected from Formula S1, Formula S2, Formula S3, or Formula S4:



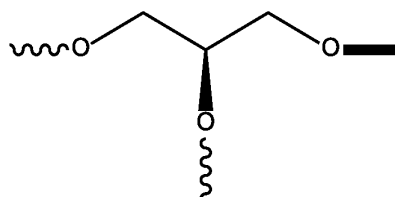
Formula S1



Formula S2



Formula S3



Formula S4

wherein  represents the points of ester connection with a fatty acid, and  represents the point of ester (S1) or ether (S2, S3, and S4) formation with the PEG moiety. In some embodiments, the fatty acid esters are C₁₄-C₂₀ straight-chain alkyl fatty acids. In some embodiments, the PEG moiety is functionalized and the fatty acid esters are C₁₆-C₂₀ straight-chain alkyl fatty acids. For example, the straight-chain alkyl fatty acid is C₁₄, C₁₅, C₁₆, C₁₇, C₁₈, C₁₉, or C₂₀. In some embodiments, the fatty acid esters are C₁₄-C₂₀ symmetric branched-chain alkyl fatty acids. For example, the branched-chain alkyl fatty acid is C₁₄, C₁₅, C₁₆, C₁₇, C₁₈, C₁₉, or C₂₀. By symmetric it is meant that each alkyl branch has the same number of carbons. In some embodiments, the branch is at the 3, 4, 5, 6, or 7 position of the fatty acid ester. The synthesis and use of PEG-lipids built on scaffolds S1-S4 is disclosed in WO2023/196445A1 which is incorporated by reference for all that it teaches about PEG-lipids and their use.

[00332] Some embodiments of the disclosed ionizable cationic lipids have head groups with small (<250 Da) PEG moieties. These lipids are not what is meant by the term PEG-lipid as used herein. These small PEG moieties are generally too small to impede binding to a similar extent as the larger PEG moieties of the PEG-lipids disclosed above, though they will impact the lipophilicity of ionizable cationic lipid. Moreover, the PEG-lipids are understood to be primarily located in an exterior facing lamella whereas much of the ionizable cationic lipid is in the interior of the LNP.

[00333] In certain embodiments, a functionalized PEG-lipid of a LNP (or tLNP) comprises one or more fatty acid tails, each that is no shorter than C₁₆ nor longer than C₂₀ for straight-chain fatty acids. For branched chain fatty acids, tails no shorter than C₁₄ fatty acids nor longer than C₂₀ are acceptable. In some embodiments, fatty acid tails are C₁₆. In some embodiments, the fatty acid tails are C₁₈. In some embodiments, the functionalized PEG-lipid comprises a dipalmitoyl lipid. In some embodiments, the functionalized PEG-lipid comprises a distearoyl lipid. The fatty acid tails serve as means to anchor the PEG-lipid in the tLNP to reduce or eliminate shedding of the PEG-lipid from the tLNP. This is a useful property for the PEG-lipid whether or not it is functionalized but has greater significance for the functionalized PEG-lipid

as it will have a targeting moiety attached to it and the targeting function could be impaired if the PEG-lipid (with the conjugated binding moiety) were shed from the tLNP.

[00334] In some embodiments, an LNP or tLNP comprises about 0.5 mol% to about 3 mol% or 0.5 mol% to 3 mol% PEG-lipid comprising functionalized and non-functionalized PEG-lipid. In certain embodiments, an LNP or tLNP comprises DSG-PEG. In other embodiments, an LNP or tLNP comprises DMG-PEG or DPG-PEG. In certain embodiments, an LNP or tLNP comprises DSPE-PEG. In some embodiments, the functionalized and non-functionalized PEG-lipids are not the same PEG-lipid, for example, the non-functionalized PEG-lipid can be a diacylglycerol and the functionalized PEG-lipid a diacyl phospholipid. tLNP with such mixtures have reduced expression in the liver, possibly due to reduced uptake. In certain embodiments the functionalized PEG-lipid is DSPE-PEG and the non-functionalized PEG-lipid is DSG-PEG. In some embodiments, an LNP or tLNP comprises about 0.4 mol% to about 2.9 mol% or about 0.9 mol% to about 1.4 mol% non-functionalized PEG lipid. In certain embodiments, an LNP or tLNP comprises about 1.4 mol% or 1.4 mol% non-functionalized PEG lipid. In some embodiments, an LNP or tLNP comprises about 0.1 mol% to about 0.3 mol% or 0.1 mol% to 0.3 mol% functionalized lipid. In some instances, the functionalized lipid is DSPE-PEG. In certain instances, an LNP or tLNP comprises about 0.1 mol%, about 0.2 mol%, or about 0.3 mol% DSPE-PEG. In certain instances, an LNP or tLNP comprises 0.1 mol%, 0.2 mol%, or 0.3 mol% DSPE-PEG. In certain instances, the functionalized PEG-lipid is conjugated to a binding moiety. As used herein, the phrase “is conjugated to” and similar constructions are meant to convey a state of being, that is, a structure, and not a process, unless context dictates otherwise.

Conjugation

[00335] Any suitable chemistry can be used to conjugate the binding moiety to the PEG of the PEG-lipid, including maleimide (see Parhiz et al., 2018, Journal of Controlled Release 291:106-115) and click (see Kolb et al., 2001, Angewandte Chemie International Edition 40(11):2004–2021; and Evans, 2007, Australian Journal of Chemistry 60(6):384–395) chemistries. Reagents for such reactions include lipid-PEG-maleimide, lipid-PEG-cysteine, lipid-PEG-alkyne, lipid, PEG- dibenzocyclooctyne (DBCO), and lipid-PEG-azide. Further conjugations reactions make use of lipid-PEG-bromo maleimide, lipid-PEG-alkynoic amide, PEG-alkynoic imide, and lipid-PEG-alkyne reactions, as disclosed in U.S. Provisional Application No. 63/362,502, filed on April 5, 2022, and PCT/US2023/017648 filed on April 5, 2023 (WO 2023/196445A1), both entitled *PEG-Lipids and Lipid Nanoparticles*, which are incorporated by reference in their entirety. On the binding moiety side of the reaction can be

used an existing cysteine sulfhydryl, or the protein derivatized by adding a sulfur containing carboxylic acid, for example, to the epsilon amino of a lysine to react with a maleimide, bromomaleimide, alkynoic amide, or alkynoic imide. In certain embodiments, to modify an epsilon amino of a binding moiety lysine to react with a maleimide functionalized PEG-lipid, the binding moiety (e.g., an antibody) can be reacted with N-succinimidyl S-acetylthioacetate (SATA). SATA is then deprotected, for example, using 0.5 M hydroxylamine followed by removal of the unreacted components by G-25 Sephadex Quick Spin Protein columns (Roche Applied Science, Indianapolis, IN). The reactive sulfhydryl group on the binding moiety is then conjugated to maleimide moieties on LNPs of the disclosure using thioether conjugation chemistry. Alternatively, an alkyne can be added to a sulfhydryl or an epsilon amino of a lysine to participate in a click chemistry reaction.

[00336] Purification can be performed using Sepharose CL-4B gel filtration columns (Sigma-Aldrich). tLNPs (LNPs conjugated with a targeting antibody) can be stored frozen, for example at -70 or -80°C until needed. Others have conjugated antibody to free functionalized PEG-lipid and then incorporated the conjugated lipid into pre-formed LNP. However, it was found that the present procedure is more controllable and produces more consistent results.

[00337] There are also several approaches to site-specific conjugation. Particularly but not exclusively suitable for truncated forms of antibody, C-terminal extensions of native or artificial sequences containing a particularly accessible cysteine residue are commonly used. Partial reduction of cysteine bonds in an antibody, for example, with tris(2-carboxy)phosphine (TCEP), can also generate thiol groups for conjugation which can be site-specific under defined conditions with an amenable antibody fragment. Alternatively, the C-terminal extension can contain a sortase A substrate sequence, LPXTG (SEQ ID NO: 6) which can then be functionalized in a reaction catalyzed by sortase A and conjugated to the PEG-lipid, including through click chemistry reactions (see, for example, Moliner-Morro *et al.*, Biomolecules 10(12):1661, 2020 which is incorporated by reference herein for all that it teaches about antibody conjugations mediated by the sortase A reaction and/or click chemistry). The use of click chemistry for the conjugation of a targeting moiety, such as various forms of antibody, is disclosed, for example, in WO2024/102,770 which is incorporated by reference in its entirety for all that it teaches about the conjugation of targeting moieties to LNPs that is not inconsistent with this disclosure.

[00338] For whole antibody and other forms comprising an Fc region, site-specific conjugation to either (or both) of two specific lysine residues (Lys248 and Lys288) can

be accomplished without any change to or extension of the native antibody sequence by use of one of the AJICAP® reagents (see, for example, Matsuda *et al.*, Mol. Pharmaceutics 18:4058, 2021 and Fujii *et al.*, Bioconjugate Chem. 34:728, 2023, which are incorporated by reference herein for all that they teach regarding conjugation of antibodies with AJICAP reagents). AJICAP reagents are modified affinity peptides that bind to specific loci on the Fc and react with an adjacent lysine residue to form an affinity peptide conjugate of the antibody. The peptide is then cleaved with base to leave behind a thiol-functionalized lysine residue which can then undergo conjugation through maleimide or haloamide reactions, for example). Functionalization with azide or dibenzocyclooctyne (DBCO) for conjugation by click chemistry is also possible. This and similar technology are further described in US 2020/0190165 (corresponding to WO 2018/199337), US 2021/0139549 (corresponding to WO 2019/240287) and US 2023/0248842 (corresponding to WO 2020/184944) which are incorporated by reference in their entirety for all that they teach about such modified affinity peptides and their use.

[00339] Accordingly, in some embodiments the binding moiety is conjugated to the PEG moiety of the PEG-lipid through a thiol modified lysine residue. In some embodiments, the conjugation is through a cysteine residue in a native or added antibody sequence. In some embodiments, a particular cysteine residue is preferentially or exclusively reacted, for example, a cysteine residue in an antibody hinge region. In further instances, a binding moiety with a conjugatable cysteine residue in an antibody hinge region is an Fab' or similar fragment. In other embodiments, the conjugation is through a sortase A substrate sequence. In still other embodiments, the conjugation is through a specific lysine residue (Lys248 or Lys288) in the Fc region.

Binding Moieties

[00340] The tLNP of the various disclosed aspects comprise a binding moiety, such as an antibody or antigen binding domain thereof or a cell surface receptor ligand. As used herein, a "binding moiety" or "targeting moiety" refers to a protein, polypeptide, oligopeptide or peptide, carbohydrate, nucleic acid, or combinations thereof capable of specifically binding to a target or multiple targets. A binding domain includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a biological molecule or another target of interest. Exemplary binding moieties of this disclosure include an antibody, a Fab', F(ab')₂, Fab, Fv, rIgG, scFv, hcAb (heavy chain antibody), a single domain antibody, VHH, VNAR, sdAb, nanobody, receptor ectodomain or ligand-binding portions thereof, or ligand (e.g., cytokines, chemokines). An "Fab" (antigen binding fragment) is the

part of an antibody that binds to antigens and includes the variable region and CH1 of the heavy chain linked to the light chain via an inter-chain disulfide bond. In other embodiments, a binding moiety comprises a ligand-binding domain of a receptor or a receptor ligand. In some embodiments, a binding moiety can have more than one specificity including, for example, bispecific or multispecific binders. A variety of assays are known for identifying binding moieties of this disclosure that specifically bind a particular target, including Western blot, ELISA, biolayer interferometry, and surface plasmon resonance. A binding moiety, such as a binding moiety comprising immunoglobulin light and heavy chain variable domains (e.g., scFv), can be incorporated into a variety of protein scaffolds or structures as described herein, such as an antibody or an antigen binding fragment thereof, a scFv-Fc fusion protein, or a fusion protein comprising two or more of such immunoglobulin binding domains.

[00341] The fundamental ability of the tLNP to deliver a payload into the cytoplasm of a cell is agnostic with respect to, and does not depend upon, a particular binding specificity. Of course, a binding moiety is a determinant of which cells a payload is delivered into. There are many known antibodies with specificity for one or another cell surface marker associated with particular cell type(s) that could be used as the target of the binding moiety on a disclosed tLNP and there are several sources that have compiled such information. An excellent source of information about antibodies for which an International Non-proprietary Name (INN) has been proposed or recommended is Wilkinson & Hale, *MAbs* 14(1):2123299, 2022, including its Supplementary Tables, which is incorporated by reference herein for all that it teaches about individual antibodies and the various antibody formats that can be constructed. US11,326,182 and especially its Table 9 Cancer, Inflammation and Immune System Antibodies, is a source of sequence and other information for a wide range of antibodies including many that do not have an INN and is incorporated herein by reference for all that it teaches about individual antibodies. Sequence information is not always readily available for antibodies mentioned in the art, even when commercially available. This is not necessarily an impediment to their use. Where the antibody or a cell line is commercially available or obtainable from its originator it can be used as the binding moiety of tLNP without any need for sequence information. Even where sequence information is needed, it is well within the capabilities of the skilled artisan to sequence the antibody protein (or have it done by a contract laboratory) so that the antibody's variable region can be incorporated into a scFv, a diabody, a minibody, or some other antibody format, or be humanized. In choosing among available antibodies in the art for the development of an agent to be used in humans, a human antibody is preferred to a humanized antibody is preferred to a non-human antibody, other factors being equal. Other factors can include stability and ease of production of the antibody,

affinity of the antibody, lack of binding to non-target extracellular and cell surface antigens, and cross-reactivity for the cognate antigen in model species to be used in product development.

[00342] In some embodiments, a binding moiety can be an antibody or an antigen-binding portion thereof; an antigen; a ligand-binding domain of a receptor; or a receptor ligand. In some embodiments, a binding moiety can have more than one specificity including, for example, bispecific or multispecific binders.

[00343] In some embodiments, a binding moiety comprises an antibody or an antigen-binding portion thereof. As used herein, “antibody” refers to a protein comprising an immunoglobulin domain having hypervariable regions determining the specificity with which the antibody binds antigen, termed complementarity determining regions (CDRs). The term antibody can thus refer to intact (*i.e.*, whole) antibodies as well as antibody fragments and constructs comprising an antigen binding portion of a whole antibody. While the canonical natural antibody has a pair of heavy and light chains, camelids (camels, alpacas, llamas, etc.) produce antibodies with both the canonical structure and antibodies comprising only heavy chains. The variable region of the camelid heavy chain-only antibody has a distinct structure with a lengthened CDR3 referred to as VHH or, when produced as a fragment, a nanobody. Antigen binding fragments and constructs of antibodies include F(ab')₂, F(ab'), F(ab), minibodies, Fv, single-chain Fv (scFv), diabodies, and VH. Such elements can be combined to produce bi- and multi-specific reagents, including various immune cell engagers, such as BiTEs (bi-specific T-cell engagers). The term “monoclonal antibody” arose out of hybridoma technology but is now used to refer to any singular molecular species of antibody regardless of how it was originated or produced. Antibodies can be obtained through immunization, selection from a naïve or immunized library (for example, by phage display), alteration of an isolated antibody-encoding sequence, or any combination thereof. Numerous antibodies that can be used as binding moieties are known in the art. An excellent source of information about antibodies for an International Non-proprietary Name (INN) has been proposed or recommended, including sequence information, is Wilkinson & Hale, 2022, *MAbs* 14(1):2123299, including its Supplementary Tables, which is incorporated by reference herein for all that it teaches about individual antibodies and the various antibody formats that can be constructed. U.S. Patent No. 11,326,182 and especially its Table 9 entitled “Cancer, Inflammation and Immune System Antibodies,” is a source of sequence and other information for a wide range of antibodies including many that do not have an INN and is incorporated herein by reference for all that it teaches about individual antibodies and the antigens they

bind. WO2024040195A1 is also a source of sequence and other information for a wide range of antibodies with specificity for various cell surface antigens of immune system cells and cancer cells and is incorporated herein by reference for all that it teaches about individual antibodies and the antigens they bind.

[00344] An antibody or other binding moiety (or a fusion protein thereof) “specifically binds” a target if it binds the target with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of 1/Molar or 1/M) equal to or greater than 10^5 M^{-1} , while not significantly binding other components present in a test sample. Binding domains (or fusion proteins thereof) can be classified as “high affinity” binding domains (or fusion proteins thereof) and “low affinity” binding domains (or fusion proteins thereof). “High affinity” binding domains refer to those binding domains with a K_a of at least 10^8 M^{-1} , at least 10^9 M^{-1} , at least 10^{10} M^{-1} , at least 10^{11} M^{-1} , at least 10^{12} M^{-1} , or at least 10^{13} M^{-1} , preferably at least 10^8 M^{-1} or at least 10^9 M^{-1} . “Low affinity” binding domains refer to those binding domains with a K_a of up to 10^8 M^{-1} , up to 10^7 M^{-1} , up to 10^6 M^{-1} , up to 10^5 M^{-1} . Alternatively, affinity can be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (*e.g.*, 10^{-5} M to 10^{-13} M). Affinities of binding domain polypeptides and fusion proteins according to this disclosure can be readily determined using conventional techniques (*see, e.g.*, Scatchard et al., 1949, Ann. N.Y. Acad. Sci. 51:660; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

[00345] A diabody is a type of scFv dimer in which each chain consists of the V_H and V_L regions connected by a small peptide linker that is too short to allow pairing between the two domains of the same chain. This arrangement forces the V_H of one chain to pair with the V_L of a second chain thereby forming a bivalent, and often bispecific, dimer. A BiTE is a fusion protein having two scFvs of different antibodies, usually an antibody for a tumor-associated antigen and antibody for CD3, on a single peptide chain, thus forming a cytolytic synapse between T cells and target antigen-bearing cells. The term “antigen-binding portion” can refer to a portion of an antibody as described that possesses the ability to specifically recognize, associate, unite, or combine with a target molecule. An antigen-binding portion includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a specific antigen. Thus, antibodies and antigen-binding portions thereof constitute means for binding to the surface molecule on a cell. In various embodiments, the cell can be an immune cell, a leukocyte, a lymphocyte, a monocyte, a stem cell, an HSC or an MSC, according to the specificity of the antibody.

[00346] In some embodiments, the antibody or antigen-binding portion thereof can be derived from a mammalian species, for example, mice, rats, or human. Antibody variable regions can be those arising from one species, or they can be chimeric, containing segments of multiple species possibly further altered to optimize characteristics such as binding affinity or low immunogenicity. For human applications, it is desirable that the antibody has a human sequence. In the cases where the antibody or antigen-binding portion thereof is derived from a non-human species, the antibody or antigen-binding portion thereof can be humanized to reduce immunogenicity in a human subject. For example, if a human antibody of the desired specificity is not available, but such an antibody from a non-human species is, the non-human antibody can be humanized, e.g., through CDR grafting, in which the CDRs from the non-human antibody are placed into the respective positions in a framework of a compatible human antibody. Less preferred is an antibody in which only the constant region of the non-human antibody is replaced with human sequence. Such antibodies are commonly referred to as chimeric antibodies in distinction to humanized antibodies.

[00347] In some embodiments, the antibody or antigen-binding portion thereof is non-immunogenic. In some embodiments, the antibody can be modified in its Fc region to reduce or eliminate secondary functions, such as FcR engagement, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and/or complement-dependent cytotoxicity (CDC); this is often referred to as an Fc silenced antibody.

[00348] A binding moiety density on the LNP (or tLNP) can be defined according to the ratio of antibody (binder) to mRNA (w/w) either based on the amount of antibody input in the conjugation reaction or as measured in the LNP or (tLNP) after conjugation. For an intact antibody (e.g., whole IgG), in some embodiments, preferred ratios are about 0.3 to about 1.0, about 0.3 to about 0.7, about 0.3 to about 0.5, about 0.5 to about 1.0, and about 0.5 to about 0.7 for either the input or final measured binder ratio. In certain embodiments, a LNP (or tLNP) has an antibody ratio of 0.3 to 1.0, 0.3 to 0.7, 0.3 to 0.5, 0.5 to 1.0, and 0.5 to 0.7 for either the input or final measured binder ratio. In some embodiments, if the binder is different in size from an intact antibody (for example a scFv, diabody, or minibody, etc.) the w/w ratio is adjusted for the different size of the binder.

[00349] In certain embodiments, a LNP (or tLNP) comprises a binding moiety derived from an anti-CD40[±] antibody, an anti-LRRC15[±] antibody, an anti-CTSK antibody, an anti-ADAM12[±] antibody, an anti-ITGA11 antibody, an anti-FAP[±] antibody, an anti-NOX4 antibody, an anti-SGCD antibody, an anti-SYNDIG1 antibody, an anti-CDH11[±] antibody, an anti-PLPP4 antibody, an anti-SLC24A2 antibody, an anti-PDGFRB[±] antibody, an anti-THY1[±]

antibody, an anti-ANTXR1[‡] antibody, an anti-GAS1 antibody, an anti-CALHM5 antibody, an anti-SDC1^{*‡} antibody, an anti-HER2^{*‡} antibody, an anti-TROP2^{*‡} antibody, an anti-MSLN^{*‡} antibody, an anti-Nectin4[‡] antibody, or an anti-MUC16^{*‡} antibody. In further embodiments, a LNP (or tLNP) comprises a binding moiety specific for an immune cell antigen selected from CD1, CD2^{*‡}, CD3^{*‡}, CD4^{*‡}, CD5[‡], CD7[‡], CD8[‡], CD11b[‡], CD14[‡], CD16, CD25[‡], CD26^{*‡}, CD27^{*‡}, CD28^{*‡}, CD30^{*‡}, CD32^{*}, CD38^{*‡}, CD39[‡], CD40^{*‡}, CD40L (CD154)^{*‡}, CD44^{*‡}, CD45[‡], CD64^{*‡}, CD62[‡], CD68, CD69[‡], CD73[‡], CD80^{*‡}, CD83[‡], CD86^{*‡}, CD95[‡], CD103[‡], CD119[‡], CD126[‡], CD137 (4-1BB)[‡], CD150[‡], CD153[‡], CD161[‡], CD166[‡], CD183 (CXCR3)[‡], CD183 (CXCR5)[‡], CD223 (LAG-3)^{*‡}, CD254[‡], CD275[‡], CD45RA, CTLA-4^{*‡}, DEC205, OX40[‡], PD-1^{*‡}, GITR[‡], TIM-3^{*‡}, FasL^{*‡}, IL18R1, ICOS (CD278)[‡], Ieu-12, TCR[‡], TLR1, TLR2[‡], TLR3^{*‡}, TLR4[‡], TLR6, TREM2[‡], NKG2D[‡], CCR, CCR1 (CD191)[‡], CCR2 (CD192)^{*‡}, CCR4(CD194)^{*‡}, CCR6(CD196)[‡], CCR7[‡], low affinity IL-2 receptor[‡], IL-7 receptor[‡], IL-12 receptor[‡], IL-15 receptor[‡], IL-18 receptor[‡], and IL-21 receptor[‡]. In further embodiments, a tLNP comprises a binding moiety specific for an HSC surface molecule selected from CD117[‡], CD34^{*‡}, CD44^{*‡}, CD90 (Thy1)[‡], CD105[‡], CD133[‡], BMPR2[‡], and Sca-1; or specific for an MSC surface molecules selected from CD70^{*‡}, CD105[‡], CD73[‡], Stro-1[‡], SSEA-3[‡], SSEA-4[‡], CD271[‡], CD146[‡], GD2^{*‡}, SUSD2, Stro-4, MSCA-1, CD56[‡], CD200^{*‡}, PODXL[‡], CD13[‡], CD29^{*‡}, CD44^{*‡}, and CD10[‡]. In various embodiments, a binding moiety is an antibody or antigen-binding portion thereof. (* indicates that exemplary antibodies with the indicated specificity from which a binding moiety could be derived can be found in US Patent 11,326,182B2 Table 9 or 10. † indicates that exemplary antibodies with the indicated specificity from which a binding moiety could be derived can be found in Wilkinson & Hale, 2022. Both references cited and incorporated by reference above. ‡ indicates that exemplary antibodies with the indicated specificity from which a binding moiety could be derived can be found in the Therapeutic Antibody Database (TABS) at tabs.craic.com.). Other suitable antibodies can be found in Appendix A or WO2024040195A1 each of which is incorporated herein by reference for all that it teaches about individual antibodies and the antigens they bind.

[00350] The following paragraphs provide non-exhaustive examples of known antibodies that bind to cell surface markers/antigens on immune cells (lymphocytes and monocytes) and stem cells (HSC and MSC). These antibodies or the antigen binding domains thereof can be used as binding moieties to target the disclosed LNP. Similarly, these antibodies can contribute their antigen binding domains to immune cell reprogramming agents such as CARs and ICEs. While typically an immune cell reprogramming agent is expressed in an immune cell, one can also express a biological response modifier (conditioning agent) or an immune cell reprogramming agent, such as an ICE, in a tumor cell. The immune and

stem cell surface markers that can serve as a targeted antigen of a tLNP can also usefully be a target of an immune cell reprogramming agent when the cell expressing that antigen has a role in the pathology of some disease or condition. Collectively these antibodies and polypeptides comprising the antigen binding domains thereof constitute means for binding cell surface markers or means for binding immune and stem cells.

[00351] In some embodiments, CD2 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD2 antibody. CD2 contains three well-characterized epitopes (T11.1, T11.2, and T11.3/CD2R). T11.3/CD2R are membrane proximal and exposure is increased upon T cell activation and CD2 clustering. Accordingly, in some such embodiments, the anti-CD2 antigen binding domain is derived from, RPA-2.10; OKT11, UMCD2, 0.1, and 3T4-8B5 (T11.1 epitope); 9.6 and 1OLD2-4C1 (T11.2 epitope); 1Mono2A6 (T11.3 epitope), sipilizumab (T11.2/T11.3 epitope), HuMCD2, TS2/18, TS1/8, AB75, LT-2, T6.3, MEM-65, OTI4E4, or an antigen-binding portion thereof. Additionally, the ligand of CD2, CD58 (LFA-3) can be used as a CD2 binding moiety as can alefacept, a CD58-Fc fusion. Each of these constitutes a means for binding CD2 (Li *et al.*, 1996, *J Mol Biol.* 263:209-26; Binder *et al.*, 2020, *Front Immunol.* 9:11:1090).

[00352] In some embodiments, CD3 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD3 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from muromonab-CD3 (OKT3), teplizumab, oteplizumab, visilizumab, cevostamab, teclistamab, elranatamab pavurutamab, vibecotamab, odronextamab, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD3.

[00353] In some embodiments, CD4 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD4 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from ibalizumab, inezetamab, semzuvolimab, zanolimumab, tregalizumab, UB-421, priliximab, MTRX1011A, cedelizumab, clenoliximab, keliximab, M-T413, TRX1, hB-F5, MAX.16H5, IT208, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD4.

[00354] In some embodiments, CD5 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD5 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from 5D7, UCHT2, L17F12, H65, HE3, OKT1, MAT304, as well as those disclosed in WO1989006968, WO2008121160, US8,679,500, WO2010022737, WO2019108863, WO2022040608, or WO2022127844, each of which is incorporated by reference for all that they teach about anti-CD5 antibodies and

their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD5.

[00355] In some embodiments, CD7 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD7 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from TH-69, 3A1E, 3A1F, Huly-m2, WT1, YTH3.2.6, T3-3A1, grisonlimab, as well as those disclosed in US10,106,609, WO2017213979, WO2018098306, US11447548, WO2022136888, WO2020212710, WO2021160267, WO2022095802, WO2022095803, WO2022151851, or WO2022257835 each of which is incorporated by reference for all that they teach about anti-CD7 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD7.

[00356] In some embodiments, CD8 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD8 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from crefmirlimab (IAB22M), 3B5, SP-16, LT8, 17D8, MEM-31, MEM-87, RIV11, UCHT4, YTC182.20, RPA-T8, OKT8, SK1, 51.1, TRX2, MT807-R1, HIT8 α , C8/144B, RAVB3, SIDI8BEE, BU88, EPR26538-16, 2ST8.5H7, as well as those disclosed in US10,414,820, WO2015184203, WO2017134306, WO2019032661, WO2020060924, US10,730,944, WO2019033043, WO2021046159, WO2021127088, WO2022081516, US11,535,869, or WO2023004304 each of which is incorporated by reference for all that they teach about anti-CD8 antibodies and their properties, or an antigen-binding portion thereof. Additionally, humanized anti-CD8 antibodies are described in U.S. Provisional Patent Application Number 63/610,917, filed on December 15, 2023, and U.S. Provisional Patent Application Number (Atty Docket No. 23-1742-US-PRO2), filed on May 31, 2024, each of which is incorporated by reference for all that it teaches about these humanized anti-CD8 antibodies and their properties, or an antigen-binding portion thereof. Each of the foregoing anti-CD8 antibodies constitutes a means for binding CD8.

[00357] In some embodiments, a tLNP is targeted to CD10 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD10 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from the one produced by the hybridoma represented by Accession No. NITE BP-02489 (disclosed in WO2018235247 which is incorporated by reference for all that they teach about anti-CD10 antibodies and their properties), FR4D11, or REA877, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD10.

[00358] In some embodiments, CD11b is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD11b antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from ASD141 or MAB107 as well as those disclosed in US20150337039, US10,738,121, WO2016197974, US10,919,967, or WO2022147338 each of which is incorporated by reference for all that they teach about anti-CD11b antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD11b.

[00359] In some embodiments, CD13 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD13 antibody. CD13 is also known as aminopeptidase N (APN). Accordingly, in some such embodiments, the antigen binding domain is derived from MT95-4 or Nbl57 (disclosed in WO2021072312 which is incorporated by reference for all that they teach about anti-CD13 antibodies and their properties), as well as those disclosed in WO2023037015 which is incorporated by reference for all that it teaches about anti-CD13 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD13.

[00360] In some embodiments, CD14 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD14 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from atibuclimab or r18D11 as well as those disclosed in WO2018191786 or WO2015140591 each of which is incorporated by reference for all that they teach about anti-CD14 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD14.

[00361] In some embodiments, CD16a is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD16a antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from AFM13, sdA1, sdA2, or hu3G8-5.1-N297Q as well as those disclosed in US11535672, WO2018158349, WO2007009065, US10385137, WO2017064221, US10,758,625, WO2018039626, WO2018152516, WO2021076564, WO2022161314, or WO2023274183 each of which is incorporated by reference for all that they teach about anti-CD16A antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD16a.

[00362] In some embodiments, CD25 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD25 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from daclizumab,

basiliximab, camidanlumab, tesirine, inolimomab, RO7296682, HuMax-TAC, CYT-91000, STI-003, RTX-003, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD25.

[00363] In some embodiments, CD28 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD28 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from GN1412, acazicolcept, lulizumab, prezalumab, theralizumab, FR104CD, and davoceticept, as well as those disclosed in US8,454,959, US8,785,604, US11,548,947, US11,530,268, US11,453,721, US11,591,401, WO2002030459, WO2002047721, US20170335016, US20200181260, US11608376, WO2020127618, WO2021155071, or WO2022056199 each of which is incorporated by reference for all that they teach about anti-CD28 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD28.

[00364] In some embodiments, CD29 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD29 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from OS2966, 6D276, 12G10, REA1060, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD29.

[00365] In some embodiments, CD32A is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD32A antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from VIB9600, humanized IV.3, humanized AT-10, or MDE-8 as well as those disclosed in US9,688,755, US9,284,375, US9,382,321, US11306145, or WO2022067394 each of which is incorporated by reference for all that they teach about anti-CD32A antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD32A.

[00366] In some embodiments, CD34 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD34 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from h4C8, 9C5, 2E10, 5B12, REA1164, C5B12, C2e10, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD34.

[00367] In some embodiments, CD40 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD40 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from cifurtilimab,

sotigalimab, iscalimab, dacetuzumab, selicrelumab, bleseelumab, lucatumumab, or mitazalimab as well as those disclosed in US10633444, each of which is incorporated by reference for all that they teach about anti-CD40 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD40.

[00368] In some embodiments, CD44 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD44 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from RO5429083, VB6-008, PF-03475952, or RG7356, as well as those disclosed in WO2008144890, US8,383,117, WO2008079246, US20100040540, WO2015076425, US9,220,772, US20140308301, WO2020159754, WO2021160269, WO2021178896, WO2022022749, WO2022022720, or WO2022243838, each of which is incorporated by reference for all that they teach about anti-CD44 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD44.

[00369] In some embodiments, CD45 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD45 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from apamistamab, BC8-B10, as well as those disclosed in WO2023183927, WO2023235772, US7,825,222, WO2017009473, WO2021186056, US9,701,756, US9,701,756, WO2020092654, WO2022040088, WO2022040577, WO2022064191, WO2022063853, or WO2024064771, each of which is incorporated by reference for all that they teach about anti-CD45 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD45.

[00370] In some embodiments, CD56 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD56 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from lorvotuzumab, adcitmer, or promiximab, as well as those disclosed in WO2012138537, US10,548,987, US10,730,941, or US20230144142, each of which is incorporated by reference for all that they teach about anti-CD56 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD56.

[00371] In some embodiments, CD64 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD64 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from HuMAb 611 or H22 as well as those disclosed in US7,378,504, WO2014083379, US20170166638, or WO2022155608 each of which is incorporated by reference for all that they teach about anti-

CD64 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD64.

[00372] In some embodiments, CD68 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD68 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from KI-M7, PG-M1, 514H12, ABM53F5, 3F7C6, 3F7D3, Y1/82A, EPR20545, CDLA68-1, LAMP4-824, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD68.

[00373] In some embodiments, CD70 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD70 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from cusatuzumab, vorsetuzumab, MDX-1203, MDX-1411, AMG-172, SGN-CD70A, ARX305, PRO1160, as well as those disclosed in US9,765,148, US8,124,738, IS10,266,604, WO2021138264, US9,701,752, US10,108,123, WO2014158821, US10,689,456, WO2017062271, US11,046,775, US11,377,500, WO2021055437, WO2021245603, WO2022002019, WO2022078344, WO2022105914, WO2022143951, WO2023278520, WO2022226317, WO2022262101, US11,613,584, or WO2023072307, each of which is incorporated by reference for all that they teach about anti-CD70 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD70.

[00374] In some embodiments, CD73 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD73 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from oleclumab, uliledlimab, mupadolimab, AK119, IBI325, BMS-986179, NZV930, JAB-BX102, Sym024, TB19, TB38, HBM1007, 3F7, mAb19, Hu001-MMAE, IPH5301, or INCA00186, as well as those disclosed in US9,938,356, US10,584,169, WO2022083723, WO2022037531, WO2021213466, WO2022083049, US10,822,426, WO2021259199, US10,100,129, US11,312,783, US11,174,319, US11,634,500, WO2021138467, WO2017118613, US9,388,249, WO2020216697, US11180554, US11,530,273, WO2019173692, WO2019170131, US11,312,785, WO2020098599, WO2020143836, WO2020143710, US11,034,771, US11,299,550, WO2020253568, WO2021017892, WO2021032173, WO2021032173, WO2021097223, WO2021205383, WO2021227307, WO2021241729, WO2022096020, WO2022105881, WO2022179039, WO2022214677, or WO2022242758, each of which is incorporated by reference for all that they teach about anti-CD73 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD73.

[00375] In some embodiments, CD90 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD90 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from REA897, OX7, 5E10, K117, L127, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD90.

[00376] In some embodiments, CD105 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD105 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from carotuximab, TRC205, or huRH105, as well as those disclosed in US8,221,753, US9,926,375, WO2010039873, WO2010032059, WO2012149412, WO2015118031, WO2021118955, US20220233591, or US20230075244, each of which is incorporated by reference for all that they teach about anti-CD105 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD105.

[00377] In some embodiments, CD117 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD117 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from briquilimab, barzolvolimab, CDX-0158, LOP628, MGTA-117, NN2101, CK6, JSP191, Ab85, 104D2, or SR1, as well as those disclosed in US7,915,391, WO2022159737, US9540443, WO2015050959, US9,789,203, US8,552,157, US10,406,179, US9,932,410, WO2019084067, WO2020219770, US10,611,838, WO2020076105, WO2021107566, US11,208,482, WO2021044008, WO2021099418, WO2022187050, or WO2023026791, WO2021188590, each of which is incorporated by reference for all that they teach about anti-CD117 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD117.

[00378] In some embodiments, CD133 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD133 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from AC133, 293C3, CMab-43, or RW03, as well as those disclosed in WO2018045880, US8,722,858, US9,249,225, WO2014128185, US10,711,068, US10,106,623, WO2018072025, or WO2022022718, each of which is incorporated by reference for all that they teach about anti-CD133 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD133.

[00379] In some embodiments, CD137 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD137 antibody. CD137 is

also known as 4-1BB. Accordingly, in some such embodiments, the antigen binding domain is derived from YH004, urelumab (BMS-663513), utomilumab (PF-05082566), ADG106, LVGN6051, PRS-343, as well as those disclosed in WO2005035584, WO2012032433, WO2017123650, US11,203,643, US11,242,395, US11,555,077, US20230067770, US11,535,678, US11,440,966, WO2019092451, US10,174,122, US11,242,385, US10,716,851, WO2020011966, WO2020011964, or US11,447,558, each of which is incorporated by reference for all that they teach about CD137 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD137.

[00380] In some embodiments, CD146 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD146 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from imaprelimab, ABX-MA1, huAA98, M2H, or IM1-24-3, as well as those disclosed in US10,407,506, US10,414,825, US6,924,360, US9,447,190, WO2014000338, US9,782,500, WO2018220467, US11,427,648, WO2019133639, WO2019137309, WO2020132190, or WO2022082073, each of which is incorporated by reference for all that they teach about CD146 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD146.

[00381] In some embodiments, CD166 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD166 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from praluzatamab, AZN-L50, REA442, or AT002, as well as those disclosed in US10,745,481, US11,220,544, or WO2008117049, each of which is incorporated by reference for all that they teach about CD166 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD166.

[00382] In some embodiments, CD200 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD200 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from samalizumab, OX-104, REA1067, B7V3V2, HPAB-0260-YJ, or TTI-CD200, as well as those disclosed in WO2007084321 or WO2019126536, each of which is incorporated by reference for all that they teach about CD200 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD200.

[00383] In some embodiments, CD205 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD205 antibody. CD205 is also known as DEC205. Accordingly, in some such embodiments, the antibody comprises

3G9-2D2 (a component of CDX-1401) or LY75_A1 (a component of MEN1309) as well as those disclosed in US8,236,318, US10,081,682, or US11,365,258, each of which is incorporated by reference for all that they teach about anti-CD205 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD205.

[00384] In some embodiments, CD271 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CD271 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from REA844 or REAL709 as well as those disclosed in WO2022166802 which is incorporated by reference for all that it teaches about anti-CD271 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding CD271.

[00385] In some embodiments, BMPR2 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-BMPR2 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from TAB-071CL (Creative Biolabs catalog no.) as well as those disclosed in US11,292,846 or WO2021174198, each of which is incorporated by reference for all that they teach about anti-BMPR2 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding BMPR2.

[00386] In some embodiments, claudin 18.2 (CLDN 18.2) is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-claudin 18.2 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from zolbetuximab, osemitamab, RC118, IBI-343, AZD0901, M108, SYSA1801, TORL-2-307-ADC, LM-302, ASKB589, gresonitamab, SPX-101, SKB315, Q-1802, GIVASTOMIG, LCAR-C18S, SOT102, CT041 as well as those disclosed in WO2013167259, WO2021032157, WO2021254481, WO2022007808, WO2021008463, WO2022111616, WO2018006882, WO2020147321, WO2019219089, US20200040101, WO2020025792, WO2020139956, WO2020135201, US20240228610, WO2021218874, WO2021027850, WO2021129765, WO2022068854, WO2021111003, each of which is incorporated by reference for all that it teaches about anti-claudin 18.2 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding claudin 18.2.

[00387] In some embodiments, CTLA-4 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-CTLA-4 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from botensilimab, ipilimumab, nurulimab, quavonlimab, tremelimumab, zalifrelimab, ADG116,

ADG126, ADU-1604, AGEN1181, BCD-145, BMS-986218, BMS-986249, BT-007, CS1002, GIGA-564, HBM4003, IBI310 JK08, JMW-3B3, JS007, KD6001, KN044, ONC-392, REGN4659, TG6050, XTX101, YH001, or an antigen-binding portion thereof. Each of these constitutes a means for binding CTLA-4.

[00388] In some embodiments, GD2 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-GD2 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from dinutuximab, ganglidximab, naxitamab, nivatrotamab, EMD 273063, hu14.18k322A, MORAb-028, 3F8BiAb, BCD-245, KM666, ATL301, Ektomab, as well as those disclosed in US9,777,068, US9,315,585, WO2004055056, US9,617,349, US9,493,740, US20210002384, US8507657, WO2001023573, WO2012071216, WO2018010846, US8,951,524, WO2023280880, US9,856,324, WO2015132604, WO2017055385, WO2019059771, WO2020020194, or an antigen-binding portion thereof. Each of these constitutes a means for binding GD2.

[00389] In some embodiments, GITR is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-GITR antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from ragifilimab, TRX518, MK-4166, AMG 228, MEDI1873, BMS-986156, REGN6569, ASP1951, MK-1248, FRA154, GWN323, JNJ-64164711, ATOR-1144, or an antigen-binding portion thereof. Each of these constitutes a means for binding GITR.

[00390] In some embodiments, a low affinity IL-2 receptor is a targeted cell surface antigen (CD122 and/or CD132) and a binding moiety comprises the antigen binding domain of an anti-IL-2 receptor antibody. Accordingly, in some such embodiments, the antiCD122 antibody comprises ANV419, FB102, MiK-Beta-1 and the anti CD122 antibodies disclosed in WO2011127324, WO2017021540, WO2022212848, WO2022221409, WO2023078113, US20230272090, WO2024073723, or an antigen-binding portion thereof. Accordingly, in some such embodiments, the anti-CD132 antibody comprises REGN7257 and the anti-CD132 antibodies disclosed in WO2020160242, WO2017021540, WO2022212848, WO2023078113, US20230272089, or an antigen-binding portion thereof. Each of these constitutes a means for binding the low affinity IL-2 receptor (CD122 or CD132, as appropriate),

[00391] In some embodiments, a high affinity IL-2 receptor is a targeted cell surface antigen (CD25) and a binding moiety comprises the antigen binding domain of an anti-IL-2 receptor antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from daclizumab, basiliximab, camidanlumab, vopitug, inolimomab, HuMAx-TAC,

Xenopax, STI-003, RA8, RTX-003, and the anti-CD25 antibodies disclosed in WO2023031403, WO2006108670, WO2019175223, WO2019175215, WO2019175226, WO2004045512, WO2022104009, WO2020102591, or an antigen-binding portion thereof. Each of these constitutes a means for binding the high affinity IL-2 receptor (CD25).

[00392] In some embodiments, IL-7 receptor (CD127) is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-IL-7 receptor antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from PF-06342674, GSK2618960, OSE-127, lusvertikimab, bempikibart, and the anti-CD127 antibodies disclosed in WO2011104687, WO2011094259, WO2013056984, WO2015189302, WO2017062748, WO2020154293, WO2020254827, WO2021222227, WO2023201316, or an antigen-binding portion thereof. Each of these constitutes a means for binding the CD127.

[00393] In some embodiments, IL-12 receptor is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-IL-12 receptor antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from CBY-10413, REA333, or an antigen-binding portion thereof. Each of these constitutes a means for binding the IL-12 receptor.

[00394] In some embodiments, IL-15 receptor α is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-IL-15 receptor α antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from MAB1472-100, MAB5511, JM7A4, 5E3E1, JM7A4, 2639B, or an antigen-binding portion thereof. Each of these constitutes a means for binding the IL-15 receptor α .

[00395] In some embodiments, IL-18 receptor α is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-IL-18 receptor α antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from H44, or an antigen-binding portion thereof. Each of these constitutes a means for binding the IL-18 receptor α .

[00396] In some embodiments, IL-21 receptor is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-IL-21 receptor antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from 1D1C2, 19F5, 18A5, REA233, or an antigen-binding portion thereof. Each of these constitutes a means for binding the IL-21 receptor α .

[00397] In some embodiments, LAG-3 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-LAG-3 antibody. Accordingly,

in some such embodiments, the antigen binding domain is derived from relatlimab, tebotelimab, favezelimab, fianlimab, miptenlimab, HLX26, ieramlimab, GSK2831781, INCAGN2385, RO7247669, encelimab, FS118, SHR-1802, Sym022, IBI110, IBI323, bavunlimab, EMB-02, ABL501, INCA32459, AK129, or an antigen-binding portion thereof. Each of these constitutes a means for binding LAG-3.

[00398] In some embodiments, MSCA-1 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti- MSCA-1 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from REAL219, W8B2, X9C3, or an antigen-binding portion thereof. Each of these constitutes a means for binding MSCA-1.

[00399] In some embodiments, OX40 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-OX40 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from MEDI6469, ivuxolimab, rocatinlimab, GSK3174998, BMS-986178, vonlerizumab, INCAGN1949, tavolimab, BGB-A445, INBRX-106, BAT6026, telazorlimab, ATOR-1015, MEDI6383, cudarolimab, FS120, HFB301001, EMB-09, HLX51, Hu222, ABM193, or an antigen-binding portion thereof. Each of these constitutes a means for binding OX40.

[00400] In some embodiments, PD-1 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-PD-1 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from nivolumab, pembrolizumab, camrelizumab, torpalimab, sintilimab, tislelizumab, cemiplimab, spartalizumab, serplulimab, cadonilimab, penpulimab, dostarlimab, zimberelimab, retifanlimab, pucotenlimab, pidilizumab, pidilizumab, balstilimab, ezabenlimab, AK112, geptanolimab, cetrelimab, prolgolimab, tebotelimab, sasanlimab, SG001, vudalimab, MEDI5752, rulonilimab, peresolimab, IBI318, budigalimab, MEDI0680, pimivalimab, QL1706, AMG 404, RO7121661, lorigerlimab, nofazinlimab, sindelizumab, or an antigen-binding portion thereof. Each of these constitutes a means for binding PD-1.

[00401] In some embodiments, PODXL is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-PODXL antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from MAI1738, HPAB-3334LY, HPAB-MO612-YC, REA246, REA157, or an antigen-binding portion thereof. Each of these constitutes a means for binding PODXL.

[00402] In some embodiments, Sca-1 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-Sca-1 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from CPP32 4-1-18, 2D4-C9-F1, AMM22070N, or an antigen-binding portion thereof. Each of these constitutes a means for binding SCA-1.

[00403] In some embodiments, SSEA-3 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-SSEA-3 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from MC631, 2A9, 8A7, ND-742, 3H420, as well as those disclosed in US11,643,456 or WO2021138378, each of which is incorporated by reference for all that they teach about anti-SSEA-3 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding SSEA-3.

[00404] In some embodiments, SSEA-4 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-SSEA-4 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from ch28/11, REA101, MC-813-70, ND-942-80, as well as those disclosed in US11,446,379, US10,273,295, US11,643,456, WO2019190952, or WO2021044039, each of which is incorporated by reference for all that they teach about anti-SSEA-4 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding SSEA-4.

[00405] In some embodiments, Stro-1 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-Stro-1 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from STRO-1, TUSP-2, as well as those disclosed in US20130122022, which is incorporated by reference for all that it teaches about anti-Stro-1 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding Stro-1.

[00406] In some embodiments, Stro-4 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-Stro-4 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from STRO-4, efungumab, 4C5, as well as those disclosed in US7,722,869, US20110280881, US9,115,192, US10,273,294, US10,457,726, WO2023091148, each of which is incorporated by reference for all that they teach about anti-Stro-4 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding Stro-4 (also known as heat shock protein-90).

[00407] In some embodiments, SUSD2 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-SUSD2 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from REA795, CBXS-3571, CBXS-1650, CBXS-1989, CBXS-1671, CBXS1990, CBXS-3676, 1279B, EPR8913(2), W5C5, or an antigen-binding portion thereof. Each of these constitutes a means for binding SUSD2.

[00408] In some embodiments, TIM-3 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-TIM-3 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from TQB2618, sabatolimab, cobolimab, RO7121661, INCAGN02390, AZD7789, surzebiclimab, LY3321367, Sym023, BMS-986258, SHR-1702, LY3415244, LB1410, or an antigen-binding portion thereof. Each of these constitutes a means for binding TIM-3.

[00409] In some embodiments, TREM2 is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-TREM2 antibody. Accordingly, in some such embodiments, the antigen binding domain is derived from PI37012 as well as those disclosed in US10,508,148, US10,676,525, WO2017058866, US11,186,636, US11,124,567, WO2020055975, US11,492,402, WO2020121195, WO2023012802, WO2021101823, WO2023047100, WO2022032293, WO2022241082, WO2023039450, or WO2023039612, each of which is incorporated by reference for all that they teach about anti-TREM2 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding TREM2.

[00410] In some embodiments, G protein-coupled receptor, class C, group 5, member D (GPCR5D) is a targeted cell surface antigen and a binding moiety comprises the antigen binding domain of an anti-GPCR5D antibody. Accordingly, in some such embodiments, the antigen binding domain of an anti-GPCR5D antibody is derived from talquetamab, forimtamig, BMS-986393, IBI-3003, QLS32015, SIM0500, or EPR28376-41, or is disclosed in WO2018017786, WO2016090329, WO2022174813, WO2023236889, WO2018147245, WO2024079015, WO2019154890, WO2021018859, WO2021018925, WO2020092854, WO2024031091, WO2020148677, WO2022175255, WO2022222910, WO2022247804, WO2022247756, WO2023078382, WO2023125728, WO2023143537, WO2024046239, or WO2024131962 each of which is incorporated by reference for all that they teach about anti-GPCR5D antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding GPCR5D.

[00411] In some embodiments, FCRL5 (CD307E) is a targeted cell surface antigen and binding moiety comprises the antigen binding domain of an anti-FCRL5 antibody.

Accordingly, in some such embodiments, the antigen binding domain of an anti-FCRL5 antibody is derived from cevostamab, 2A10H7, 307307, 2A10D6, EPR27365-87, EPR26948-19, or EPR26948-67, or is disclosed in WO2016090337, WO2017096120, WO2022263855, or WO2024047558 each of which is incorporated by reference for all that they teach about anti-FCRL5 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding FCRL5.

[00412] In some embodiments, LRRC15 is a targeted cell surface antigen and binding moiety comprises the antigen binding domain of an anti-LRRC15 antibody. Accordingly, in some such embodiments, the antigen binding domain of an anti-LRRC15 antibody is derived from samrotamab or DUNP19 or is disclosed in WO2005037999, WO2021022304, WO2024081729, WO2021102332, WO2021202642, WO2022157094, or WO2024158047, each of which is incorporated by reference for all that they teach about anti-LRRC15 antibodies and their properties, or an antigen-binding portion thereof. Each of these constitutes a means for binding LRRC15.

[00413] In still further embodiments, a tLNP is targeted to a tumor cell. In some embodiments, the tumor cell expresses one of the antigens described above and the tLNP is targeted to antigen expressing tumors using the same means as described above. In other embodiments the tLNP is targeted to some other tumor antigen, such as those enumerated in U.S. Provisional Application No. 63/371,742, filed on August 17, 2022, entitled CONDITIONING FOR IN VIVO IMMUNE CELL ENGINEERING which is incorporated by reference for all that it teaches about the delivery of nucleic acids into tumor cells using tLNP that is not inconsistent with the present disclosure.

Nucleic Acid

[00414] In certain embodiments, the disclosed LNP and tLNP comprise a payload comprising or consisting of one or more nucleic acid species. In some embodiments, the LNP or tLNP payload comprises only one nucleic acid species while in other embodiments the LNP or tLNP payload comprises multiple nucleic acid species, for example, 2, 3, or 4 nucleic acid species. For example, in embodiments in which the payload comprises a nucleic acid encoding a CAR or immune cell engager (ICE), the payload can comprise or consist of 1) a single nucleic acid species encoding a single species of CAR or ICE, 2) a single nucleic acid species encoding 2 or more species of CAR or ICE (or a mixture of CAR and ICE) such as a bicistronic or multicistronic mRNA in which each CAR and/or ICE has specificity for a same target antigen, 3) a single nucleic acid species encoding 2 or more species of CAR or ICE (or a mixture of CAR and ICE) such as a bicistronic or multicistronic mRNA in which at

least one CAR and/or ICE has specificity for a different target antigen than the other(s), 4) two or more nucleic acid species encoding 2 or more species of CAR or ICE (or a mixture of CAR and ICE) in which each CAR and/or ICE has specificity for a same target antigen, 5) two or more nucleic acid species encoding 2 or more species of CAR or ICE (or a mixture of CAR and ICE) in which at least one CAR and/or ICE has specificity for a different target antigen than the other(s). When two or more CAR and/or ICE have specificity for a same target antigen, they can have specificity for same or different epitopes of the same target antigen. Further variations will be apparent to one of skill in the art (e.g., multiple bi- or multicistronic nucleic acids, nucleic acids encoding a TCR and the like). The nucleic acid can be RNA or DNA. The nucleic acid can be multicistronic, for example, bicistronic.

[00415] In some embodiments, LNPs or tLNPs of this disclosure further comprise a nucleic acid payload. In various embodiments, a nucleic acid is an mRNA, a self-replicating RNA, a circular RNA, a siRNA, a miRNA, DNA, a gene editing component (for example, a guide RNA, a tracr RNA, an sgRNA), a gene writing component, an mRNA encoding a gene or base editing protein, a zinc-finger nuclease, a TALEN, a CRISPR nuclease, such as Cas9, a DNA molecule to be inserted or serve as a template for repair), and the like, or a combination thereof. In some embodiments, the nucleic acid comprises small interfering RNA (siRNA), microRNA (miRNA), antisense oligonucleotide (ASO). In some embodiments, the nucleic acid comprises a self-replicating RNA or a circular RNA. In some embodiments, the mRNA encodes a reprogramming agent or comprises or encodes a conditioning agent. In some embodiments, the mRNA (linear, circular, or self-replicating) comprises an miRNA binding site. In some embodiments, an mRNA encodes a chimeric antigen receptor (CAR). In other embodiments, an mRNA encodes a gene-editing or base-editing or gene writing protein. In some embodiments, a nucleic acid is a guide RNA. In some embodiments, an LNP or tLNP comprises both a gene- or base-editing or gene writing protein-encoding mRNA and one or more guide RNAs. CRISPR nucleases can have altered activity, for example, modifying the nuclease so that it is a nickase instead of making double-strand cuts or so that it binds the sequence specified by the guide RNA but has no enzymatic activity. Base-editing proteins are often fusion proteins comprising a deaminase domain and a sequence-specific DNA binding domain (such as an inactive CRISPR nuclease).

[00416] In some embodiments, the reprogramming agent comprises an immune receptor (for example, a chimeric antigen receptor or a T cell receptor) or an immune cell engager (for example, a bispecific T cell engager (BiTE), a bispecific killer cell engager (BiKE), a trispecific kill cell engager (TriKE), a dual affinity retargeting antibody (DART), a TRIDENT

(linking two DART units or a DART unit and a Fab domain), a macrophage engager (e.g., BiME), an innate cell engager, and the like).

[00417] In some embodiments, the nucleic acid is an RNA, for example, mRNA, and the RNA comprises at least one modified nucleoside. In some embodiments, the modified nucleoside is pseudouridine, N¹-methylpseudouridine, 5-methylcytosine, 5-methyluridine, N⁶-methyladenosine, 2'-O-methyluridine, or 2-thiouridine. In certain embodiments, all of the uridines are substituted with a modified nucleoside. Further disclosure of modified nucleosides and their use can be found in U.S. Patent No. 8,278,036 which is incorporated herein by reference for those teachings.

[00418] In some embodiments, the reprogramming agent encodes or is a gene/genome editing component. In some embodiments, the gene/genome editing component is a guide RNA for an RNA-directed nuclease or other nucleic acid editing enzyme, clustered regularly interspaced short palindromic repeat RNA (crisprRNA), a trans-activating clustered regularly interspaced short palindromic repeat RNA (tracrRNA). In some embodiments, the gene/genome editing component is a nucleic acid-encoded enzyme, such as RNA-guided nuclease, a gene or base editing protein, a zinc finger nuclease (ZFN), a transcription activator-like effector nuclease (TALEN), a meganuclease, a transposase, or a CRISPR nuclease (e.g., Cas9 or Cas 12, etc.). In some embodiments, the gene/genome editing component is DNA to be inserted or that serves as a template in gene or genome editing for example a template for repair of a double-strand break.

[00419] In some embodiments comprising multiple agents, the nucleic acid can be multicistronic. In other embodiments comprising multiple agents or components, each agent or component is encoded or contained is a separate nucleic acid species. In some embodiments involving multiple payload nucleic acid species, two or more nucleic acid species are packaged together in a single LNP species. In other embodiments, a subset of the payload nucleic acid species to be delivered, (e.g., a single nucleic acid species) is packaged in one LNP or tLNP species while another subset of the nucleic acid species is packaged in another LNP or tLNP species. The different (t)LNP species can differ by only the payload they contain. The different (t)LNP species can be combined in a single formulation or pharmaceutical composition for administration.

Methods of Making an LNP or tLNP

[00420] In some aspects, the present disclosure provides a method of making a LNP or

tLNP comprising mixing of an aqueous solution of a nucleic acid (or other negatively charged payload) and an alcoholic solution of the lipids in proportions disclosed herein. In particular embodiments, the mixing is rapid.

[00421] The aqueous solution is buffered at pH of about 3 to about 5, for example, without limitation, with citrate or acetate. In various embodiments, the alcohol can be ethanol, isopropanol, t-butanol, or a combination thereof. In some embodiments, rapid mixing is accomplished by pumping the two solutions through a T-junction or with an impinging jet mixer. Microfluidic mixing through a staggered herringbone mixer (SHM) or a hydrodynamic mixer (microfluidic hydrodynamic focusing), microfluidic bifurcating mixers, and microfluidic baffle mixers can also be used. After the LNPs are formed they are diluted with buffer, for example phosphate, HEPES, or Tris, in a pH range of 6 to 8.5 to reduce the alcohol (ethanol) concentration. The diluted LNPs are purified either by dialysis or ultrafiltration or diafiltration using tangential flow filtration (TFF) against a buffer in a pH range of 6 to 8.5 (for example, phosphate, HEPES, or Tris) to remove the alcohol. Alternatively, one can use size exclusion chromatography. Once the alcohol is completely removed the buffer is exchanged with like buffer containing a cryoprotectant (for example, glycerol or a sugar such as sucrose, trehalose, or mannose). The LNPs are concentrated to a desired concentration, followed by 0.2 μ m filtration through, for example, a polyethersulfone (PES) or modified PES filter and filled into glass vials, stoppered, capped, and stored frozen. In alternative embodiments, a lyoprotectant is used and the LNP lyophilized for storage instead of as a frozen liquid. Further methodologies for making LNP can be found, for example, in U.S. Patent Application Publication Nos. US2020/0297634, US2013/0115274, and International Patent Application Publication No. WO2017/048770, each of which is incorporated by reference for all that they teach about the production of LNP.

[00422] Some aspects are a method of making a tLNP comprising rapid mixing of an aqueous solution of a nucleic acid (or other negatively charged payload) and an alcoholic solution of the lipids as disclosed for LNP. In some embodiments, the lipid mixture includes functionalized PEG-lipid, for later conjugation to a targeting moiety. As used herein, functionalized PEG-lipid refers to a PEG-lipid in which the PEG moiety has been derivatized with a chemically reactive group (such as, maleimide, N-hydroxysuccinimide (NHS) ester, Cys, azide, alkyne, and the like) that can be used for conjugating a targeting moiety to the PEG-lipid, and thus, to the LNP comprising the PEG-lipid. In other embodiments, the functionalized PEG-lipid is inserted into an LNP subsequent to initial formation of an LNP from other components. In either type of embodiment, the targeting moiety is conjugated to functionalized

PEG-lipid after the functionalized PEG-lipid containing LNP is formed. Protocols for conjugation can be found, for example, in Parhiz et al., 2018, *J. Controlled Release* 291:106-115 and Tombacz et al., 2021, *Molecular Therapy* 29(11):3293-3304, each of which is incorporated by reference for all that it teaches about conjugation of PEG-lipids to binding moieties. Alternatively, the targeting moiety can be conjugated to the PEG-lipid prior to insertion into pre-formed LNP.

[00423] In certain embodiments of the preparation methods of tLNP, the method comprises:

- i). forming an initial LNP by mixing all components of the tLNP, in proportions disclosed herein, except for the one or more functionalized PEG-lipids and the one or more targeting moieties;
- ii). forming a pre-conjugation tLNP by mixing the initial LNP with the one or more functionalized PEG-lipids; and
- iii). forming the tLNP by conjugating the pre-conjugation tLNP with the one or more targeting moieties.

[00424] In certain embodiments of the preparation methods of tLNP, the method comprises:

- i). forming a pre-conjugation tLNP by mixing all components of the tLNP, in proportions disclosed herein, including the one or more functionalized PEG-lipids, except for the one or more targeting moieties; and
- ii). forming the tLNP by conjugating the pre-conjugation tLNP with the one or more targeting moieties.

[00425] In certain embodiments of the preparation methods of tLNP, the method comprises:

- i). forming one or more conjugated functionalized PEG-lipids by conjugating the one or more functionalized PEG-lipids with the one or more targeting moieties; and
- ii) forming the tLNP by mixing all components of the tLNP, in proportions disclosed herein, including the one or more conjugated functionalized PEG-lipids.

[00426] In certain embodiments of the preparation methods of tLNP, the method comprises:

- i). forming one or more conjugated functionalized PEG-lipids by conjugating the one or more functionalized PEG-lipids with the one or more targeting moieties;
- ii) forming an LNP by mixing all components of the tLNP, except the one or more conjugated functionalized PEG-lipids; and
- iii) forming the tLNP by mixing the initial LNP with the one or more conjugated functionalized PEG-lipids.

[00427] After the conjugation the tLNPs are purified by dialysis, tangential flow filtration, or size exclusion chromatography, and stored, as disclosed above for LNPs.

[00428] The encapsulation efficiency of the nucleic acid by the LNP or tLNP is typically determined with a nucleic acid binding fluorescent dye added to intact and lysed aliquots of the final LNP or tLNP preparation to determine the amounts of unencapsulated and total nucleic acid, respectively. Encapsulation efficiency is typically expressed as a percentage and calculated as $100 \times (T-U)/T$ where T is the total amount of nucleic acid and U is the amount of unencapsulated nucleic acid. In various embodiments, the encapsulation efficiency is $\geq 80\%$, $\geq 85\%$, $\geq 90\%$, or $\geq 95\%$.

Methods of Delivering a Payload into a Cell

[00429] In other aspects, disclosed herein are methods of delivering a nucleic acid (or other negatively charged payload) into a cell comprising contacting the cell with LNP or tLNP as disclosed herein. Accordingly, each of the herein disclosed genera, subgenera, and or species of LNP or tLNP disclosed herein including those based on the inclusion or exclusion of particular lipids, particular lipid compositions, particular payloads, and/or particular targeting moieties can be used in defining the scope of the methods of delivering a payload to a cell. In some embodiments, the contacting takes place *ex vivo*. In some embodiments, the contacting takes place extracorporeally. In some embodiments, the contacting takes place *in vivo*. In some embodiments, an LNP or tLNP is contacted with target cells *in vivo*, by systemic or local administration. In some embodiments, the *in vivo* contacting comprises intravenous, intramuscular, subcutaneous, intralesional, intranodal or intralymphatic administration. In some embodiments, administration is by intravenous or subcutaneous infusion or injection. In some embodiments, administration is by intraperitoneal or intralesional infusion injection. In further instances, transfection of hepatocytes is reduced as compared to tLNPs comprising a conventional ionizable cationic lipid, such as ALC-0315. In some embodiments, an LNP or tLNP is administered 1-3 times a week for 1, 2, 3, or 4 weeks. In some embodiments, toxicity is confined (or largely confined) to grades of 0 or 1 or 2, as discussed above.

[00430] The herein disclosed LNP and tLNP compositions and formulations have reduced toxicity as compared to widely used prior art LNP compositions such as those containing ALC-0315. In various embodiments the toxicity can be described as an observable toxicity, a substantial toxicity, a severe toxicity, or an acceptable toxicity, or a dose-limiting toxicity (such as but not limited to a maximum tolerated dose (MTD)). By an observable toxicity it is meant that while a change is observed the effect is negligible or mild. By substantial toxicity it is meant that there is a negative impact on the patient's overall health or quality of life. In some instances, a substantial toxicity can be mitigated or resolved with other ongoing medical intervention. By a severe toxicity it is meant that the effect requires acute medical intervention and/or dose reduction or suspension of treatment. The acceptability of a toxicity will be influenced by the particular disease being treated and its severity and the availability of mitigating medical intervention. In some embodiments, toxicity is confined (or largely confined) to an observable toxicity. In some embodiments, toxicity is confined (or largely confined) to grades of 0 or 1 or 2.

[00431] In some embodiments, the payload is a nucleic acid and the method of delivering is a method of transfecting. In some embodiments, the nucleic acid payload comprises an mRNA, circular RNA, self-amplifying RNA, or guide RNA. Nucleic acid structures and especially mRNA structures, as well as individual RNA molecules encoding particular polypeptides, that are well-adapted to delivery by LNP or tLNP are disclosed in U.S. Provisional Patent Application Number. 63/595,753 filed November 2, 2023, U.S. Provisional Patent Application Number. 63/611,092 filed December 15, 2023, and U.S. Provisional Patent Application Number (Attorney Docket Number 23-1871-US-PRO3), filed May 31, 2024, each of which is incorporated by reference for all that it teaches about nucleic acid payloads for *in vivo* transfection and their design.

[00432] In some embodiments, the payload comprises a nucleic acid encoding an immune receptor or immune cell engager and the method of delivering is also a method of reprogramming an immune cell. In some embodiments, the payload comprises a nucleic acid that encodes, or is, a BRM and the method of delivering is also a method of providing a conditioning agent. In various embodiments, the BRM or conditioning agent is a gamma chain receptor cytokine such as IL-2, IL-7, IL-15, IL-15/15Ralpha, IL-21; an immune modulating cytokine such as IL-12, IL-18; a chemokine such as RANTES, IP10, MIG; or another BRM such as Flt3, GM-CSF, and G-CSF.

[00433] In some embodiments, the payload comprises a nucleic acid encoding a gene/genome editing enzyme and/or a guide RNA or other component of a gene/genome

editing system and the method of delivering is also a method of reprogramming a cell. In some instances, the cell is an immune cell. In some instances, the cell is an HSC. In some instances, the cell is an MSC.

[00434] In certain embodiments comprising delivering the payload into an immune cell, the binding moiety binds to a lymphocyte surface molecule or a monocyte surface molecule. Lymphocyte surface molecules include CD2, CD3, CD4, CD5, CD7, CD8, CD28, 4-1BB (CD137), CD166, CTLA-4, OX40, PD-1, GITR, LAG-3, TIM-3, CD25, low affinity IL-2 receptor, IL-7 receptor, IL-12 receptor, IL-15 receptor, IL-18 receptor, and IL-21 receptor. Monocyte surface molecules include CD5, CD14, CD16a, CD32, CD40, CD11b (Mac-1), CD64, DEC205, CD68, and TREM2. Exemplary antibodies that can provide antigen binding domains to bind these surface molecules are disclosed herein. Such antibodies, individually and collectively, constitute means for binding to an immune cell (or leukocyte) – or to a lymphocyte or monocyte, as indicated.

[00435] In certain embodiments comprising delivering the payload into a stem cell, the binding moiety binds to a HSC surface molecule or a MSC surface molecule. HSC surface molecules include CD117, CD34, CD44, CD90 (Thy1), CD105, CD133, BMPR2, and Sca-1. MSC surface molecules include CD70, CD105, CD73, Stro-1, SSEA-4, CD271, CD146, GD2, SSEA-3, SUSD2, Stro-4, MSCA-1, CD56, CD200, PODXL, CD13, CD29, CD44, and CD10. Exemplary antibodies that can provide antigen binding domains to bind these surface molecules are disclosed herein above. Such antibodies, individually and collectively, constitute means for binding to a stem cell – or to an HSC or MSC, as indicated.

Methods of Treatment

[00436] In certain aspects, this disclosure provides methods of treating a disease or disorder comprising administering a tLNP of this disclosure to a subject in need thereof. Each of the herein disclosed genera, subgenera, and or species of LNP or tLNP disclosed herein including those based on the inclusion or exclusion of particular lipids, particular lipid compositions, particular payloads, and/or particular targeting moieties can be used in defining the scope of the methods of treatment.

[00437] In some embodiments, a subject is a human. In some embodiments, a tLNP is administered systemically. In some embodiments, a tLNP is administered by intravenous or subcutaneous infusion or injection. In some embodiments, a tLNP is administered locally. In some embodiments, a tLNP is administered by intraperitoneal or intralesional infusion injection.

[00438] In further embodiments, a tLNP can be administered in combination with the standard of care for a particular indication, such as corticosteroids (e.g., prednisone) for management of myositis or lupus nephritis. In certain cases, myositis is also treated with methotrexate, which can be combined with immunosuppressive agents (e.g., azathioprine, mycophenolate mofetil, tacrolimus), which are usually required in addition to corticosteroids. For membranous nephropathy, cyclical steroids and cyclophosphamide might be used in combination with tLNPs of this disclosure. In other cases, an anti-IL-6, such as tocilizumab, can also be used as a pretreatment or in combination with tLNPs of this disclosure. These combinations can be administered concurrently or sequentially.

[00439] In some embodiments, the disease or disorder is an autoimmune disease. Examples of autoimmune disease include, without limitation, myocarditis, acute idiopathic thrombocytopenic purpura, chronic idiopathic thrombocytopenic purpura, dermatomyositis, Sydenham's chorea, myasthenia gravis, systemic lupus erythematosus, fibrosing alveolitis, multiple sclerosis, rheumatic fever, polyglandular syndromes, agranulocytosis, autoimmune hemolytic anemias, bullous pemphigoid, Wegener's granulomatosis, membranous nephropathy, amyotrophic lateral sclerosis, tabes dorsalis, giant cell arteritis/polymyalgia, pernicious anemia, rapidly progressive glomerulonephritis, IgA nephropathy, polyarteritis nodosa, ankylosing spondylitis, allergic responses, insulin-resistant diabetes, psoriasis, diabetes mellitus, Addison's disease, Grave's disease, diabetes, endometriosis, celiac disease, Crohn's disease, Henoch-Schonlein purpura, ulcerative colitis, Goodpasture's syndrome, thromboangitis obliterans, Sjögren's syndrome, aplastic anemia, rheumatoid arthritis, sarcoidosis, scleritis, a T cell-mediated autoimmunity or a B cell-mediated autoimmunity, a B cell-mediated (antibody-mediated) autoimmune disease, necrotizing myopathy, chronic inflammatory demyelinating polyneuropathy (CIDP), neuromyelitis optica (NMO) myositis, neuromyelitis optica spectrum disorders, pemphigus vulgaris, systemic sclerosis, antisynthetase syndrome (idiopathic inflammatory myopathy), lupus nephritis, membranous nephropathy, Fanconi anemia, and vasculitis.

[00440] In some embodiments, the autoimmune disease is a T cell-mediated autoimmunity or a B cell-mediated autoimmunity. In some instances, the B cell-mediated autoimmune disease is myositis (such as anti-synthetase myositis), lupus nephritis, membranous nephropathy, systemic lupus erythematosus, anti-neutrophilic cytoplasmic antibody (ANCA) vasculitis, neuromyelitis optica spectrum disorder (NMOSD), myasthenia gravis, pemphigus vulgaris, rheumatoid arthritis, dermatomyositis, immune mediated necrotizing myopathy (IMNM), anti-synthetase syndrome, polymyositis, systemic sclerosis, diffuse cutaneous

systemic sclerosis, limited cutaneous systemic sclerosis, anti-synthetase syndrome (idiopathic inflammatory myopathy), stiff person syndrome, myeloid oligodendrocyte glycoprotein autoantibody associated disease (MOGAD), amyloid light-chain amyloidosis, multiple sclerosis, relapsing-remitting multiple sclerosis, secondary progressive multiple sclerosis, primary progressive multiple sclerosis, non-active secondary progressive multiple sclerosis, Sjörger's syndrome, IgA nephropathy, IgG4-related disease, or Fanconi anemia. In certain embodiments, the B cell-mediated autoimmune disease is myositis, lupus nephritis, membranous neuropathy, scleroderma, systemic lupus erythematosus, myasthenia gravis, ANCA vasculitis, multiple sclerosis, or pemphigus vulgaris. In certain embodiments, the B cell-mediated autoimmune disease is myositis, lupus nephritis, membranous neuropathy, or scleroderma. In certain embodiments, the B cell-mediated autoimmune disease is myositis. In some instances, the myositis is anti-synthetase myositis. In certain embodiments, the B cell-mediated autoimmune disease is systemic lupus erythematosus, myasthenia gravis, ANCA vasculitis, multiple sclerosis, or pemphigus vulgaris.

[00441] In some embodiments, the disease or disorder is rejection of an allogeneic organ or tissue graft. Pre-existing antibodies and/or B cells, in their role as antigen presenting cells, can facilitate rapid immune rejection through known mechanisms hence depleting a large number of B cells can help prevent allograft rejection.

[00442] In some embodiments, the disease or disorder is a cancer. Examples of cancers include, without limitation, carcinomas, sarcomas, and hematologic cancers. In some embodiments, the hematologic cancer is a lymphoma, leukemia, or myeloma. In some instances, the hematologic cancer is a B lineage or T lineage cancer. In some instances, the B lineage cancer is multiple myeloma, diffuse large B cell lymphoma, acute myeloid leukemia, Mantle Cell lymphoma, follicular lymphoma, B acute lymphoblastic leukemia, chronic lymphocytic leukemia, or myelodysplastic syndrome. In some embodiments, the cancer is a sarcoma. In some embodiments, the cancer is a carcinoma, such as breast cancer, colon cancer, ovarian cancer, lung cancer, testicular cancer, or pancreatic cancer. In some embodiments, the cancer is melanoma.

[00443] In some embodiments, the disease or disorder is a genetic disease or disorder such as a monogenic genetic disease. In some instances, the genetic disease or disorder is a hemoglobinopathy, for example, sickle cell disease or β -thalassemia.

[00444] In some embodiments, the disease or disorder is a fibrotic disease or disorder. In some instances, the fibrotic disease is cardiac fibrosis, arthritis, idiopathic pulmonary fibrosis,

and nonalcoholic steatohepatitis (also known as metabolic dysfunction-associated steatohepatitis). In other instances, the disorder involves tumor-associated fibroblasts.

[00445] In some embodiments, a tLNP of this disclosure comprises a nucleic acid encoding a chimeric antigen receptor (CAR). The receptors are chimeric because they combine both antigen-binding and T cell activating functions into a single receptor. In some embodiments, a nucleic acid encoding a CAR refers to one or more nucleic acid species encoding one or more CARs; for example, a single or multiple species of nucleic acid encoding a single CAR species, or multiple species of nucleic acid encoding multiple CAR species. In some instances, these multiple CAR species have a same specificity while in other instances they have multiple specificities. In some embodiments, a CAR of this disclosure is multispecific, for example, bispecific, comprising multiple antigen binding moieties each specific for separate antigens. For example, the CAR in LCAR-AIO targets three antigens — CD19, CD20 and CD22 (see, *Blood* (2021) 138 (Supplement 1): 1700). In some embodiments, a CAR can comprise an extracellular binding domain that specifically binds a target antigen, a transmembrane domain, and one or more intracellular signaling domains. In some embodiments, a CAR can further comprise one or more additional elements, including one or more signal peptides, one or more extracellular hinge domains, or one or more intracellular costimulatory domains. Domains can be directly adjacent to one another, or there can be one or more amino acids linking the domains. The signal peptide can be derived from an antibody, a TCR, CD8 or other type 1 membrane proteins, preferably a protein expressed in a T or other immune cell. The transmembrane domain can be one associated with any of the potential intracellular domains or from another type 1 membrane protein, such as TCR alpha, beta, or zeta chain, CD3 epsilon, CD4, CD8, or CD28, amongst other possibilities known in the art. The transmembrane domain can further comprise a hinge domain located between the extracellular binding domain and the hydrophobic membrane-spanning region of the transmembrane domain. In some but not all embodiments, the hinge domain and transmembrane domain are contiguous sequences in the same source protein. In some instances, the hinge and membrane-spanning domains are derived from CD28. In other instances, the hinge and membrane-spanning domains are derived from CD8 α . The intracellular signaling domain can be derived from the CD3 zeta chain, DAP10, DAP12, Fc γ RIII, Fc ϵ RI, or an immunoreceptor tyrosine-based activation motif (ITAM) bearing cytoplasmic domain, amongst other possibilities known in the art. The intracellular costimulatory domain can be derived from CD27, CD28, 4-1BB, OX40, or ICOS, amongst other possibilities known in the art.

[00446] In certain embodiments, CARs are used to treat a disease or condition associated with a target cell that expresses the antigen targeted by the CAR. For example, in some embodiments, an anti-CD19 or anti-CD20 CAR can be used to target and treat B cell malignancies or B cell-mediated autoimmune conditions or diseases (e.g., having an immune cell targeting moiety, such as an anti-CD8 antibody). In other embodiments, an anti-FAP CAR can be used to target and treat solid tumors or fibrosis (e.g., cardiac fibrosis, cancer-associated fibroblasts), which can also have an immune cell targeting moiety, such as an anti-CD8 antibody. Examples of CARs that can be used in accordance with the embodiments described herein include those disclosed in US 7,446,190, US 9,328,156, US 11,248,058, US20190321404, WO2019119822, WO2019159193, WO2020011706, WO2022125837, and WO2024086190 (anti-CD19), US 10,287,35 (anti-CD19), US 10,442,867 and US2021/0363245 (anti-CD19 and anti-CD20), US 10,543,263 (anti-CD22), WO2016149578 (anti-CD19 and anti-CD22), US 10,316,101, US 11,623,961 WO2015052538, WO2016166630, WO2017130223, WO2017173256, WO2019085102, WO2019241426, WO2020065330, WO2020038146, WO2020190737, WO2021091945 (anti-BCMA), WO2016130598 (anti-BCMA and syndecan-1), US 10,426,797 (anti-CD33), US 10,844,128 (anti-CD123), US 10,428,141, US 10,752,684, US 11,723,925, WO2016187216, WO2017156479, WO2018197675, WO2020014366, and WO2020198531 (anti-ROR1), WO2022247756, WO2020148677, WO2020092854, & US20230331872 (anti-GPRC5D), WO2016090337, WO2022263855, & WO2024047558 (anti-FCRL5), and US2021/0087295 (anti-FAP), each of which is incorporated by reference for all that it teaches about CAR structure and function generically and with respect to the CAR's antigenic specificity and target indications to the extent that it is not inconsistent with the present disclosure. Each CAR constitutes means for targeting an immune cell, for example, a T cell, to the indicated antigen.

[00447] Exemplary target antigens against which a CAR, TCR, or ICE can have specificity include, but are not limited to, B cell maturation agent (BCMA)^{†‡}, CA9^{†‡}, CD4^{†‡}, CD5^{†‡}, CD19^{*†‡}, CD20 (MS4A1)^{*†‡}, CD22^{*†‡}, FCRL5^{†‡}, GPRC5D^{†‡}, CD23^{*†‡}, CD30 (TNFRSF8)^{*†‡}, CD33^{*†‡}, CD38^{*†‡}, CD44^{*†‡}, CD70^{*†‡}, CD133[‡], CD174, CD274 (PD-L1)^{*†‡}, CD276 (B7-H3)^{†‡}, CEACAM5^{*†‡}, CLL1[‡], CSPG4^{*†‡}, EGFR^{*†‡}, EGFRvIII^{*}, EPCAM^{*†‡}, EPHA2^{*†‡}, ERBB2^{*†‡}, FAP^{*†‡}, FOLH1, FOLR1^{*†‡}, GD2^{*†‡}, GPC3^{*†‡}, GPNMB^{*†‡}, IL1RAP^{†‡}, IL3RA^{*†‡}, IL13RA2^{*†‡}, Kappa^{*}, KDR (VEGFR2)^{*†‡}, CD171 (L1CAM)^{*†‡}, Lambda^{*}, MET^{*†‡}, MSLN (mesothelin)^{* †‡}, MUC1^{*†‡}, NCAM1 (CD56)^{*†‡}, PD-1 (CD279)^{†‡}, PSCA[‡], ROR1^{†‡}, CD138 (SDC1)^{*†‡}, CD319 (SLAMF7)^{*†‡}, CD248 (TEM1)[‡], ULBP1, ULBP2, and G-protein coupled receptor family C group 5 member D (GPRC5D)^{†‡} (associated with leukemias); CD319 (SLAMF7)^{*†‡}, CD38^{*†‡}, CD138^{†‡}, GPRC5D^{†‡}, CD267 (TACI)[‡], and BCMA^{†‡} (associated with myelomas); and GD2^{*†‡},

GPC3^{*†}, HER2^{*†}, EGFR^{*†}, EGFRvIII^{*}, CD276 (B7H3)[†], PSMA^{*†}, PSCA[‡], CAIX (CA9)[†], CD171 (L1-CAM)^{*†}, CEA^{*†}, CSPG4^{*†}, EPHA2^{*†}, FAP^{*†}, LRRC15[†], FOLR1^{*†}, IL-13R α ^{*†}, Mesothelin^{*†}, MUC1^{*†}, MUC16^{*†}, TROP2^{*†}, claudin 18.2[†], and ROR1[†] (associated with solid tumors). (* indicates that exemplary antibodies with the indicated specificity from which a binding moiety could be derived can be found in US Patent 11,326,182B2 Table 9 or 10. † indicates that exemplary antibodies with the indicated specificity from which a binding moiety could be derived can be found in Wilkinson & Hale, 2022. Both references cited and incorporated by reference above. ‡ indicates that exemplary antibodies with the indicated specificity from which a binding moiety could be derived can be found in the Therapeutic Antibody Database (TABS) at tabs.craic.com. Other suitable antibodies can be found in Appendix A which is incorporated herein by reference for all that it teaches about individual antibodies and the antigens they bind. Many of these target antigens are themselves receptors that could bind to their ligand if expressed on an immune cell. Accordingly, in some embodiments, the extracellular binding domain of the CAR comprises a ligand of a receptor expressed on the target cell. In still further embodiments, the extracellular binding domain of the CAR comprises a ligand binding domain of a receptor for a ligand expressed on the target cell. The advantages of the aspects and embodiments disclosed herein are independent of the specificity of the binding moiety. As such, the disclosed aspects and embodiments are generally agnostic to binding specificity. In certain embodiments, a particular binding specificity can be required.

[00448] In some embodiments, the tLNP comprises a nucleic acid encoding an anti-CD19 chimeric antigen receptor (CAR). In some embodiments, the nucleic acid comprises mRNA. Examples of anti-CD19 CARs include those incorporating a CD19 binding moiety derived from the human antibody 47G4 or the mouse antibody FMC63. FMC63 and the derived scFv have been described in Nicholson et al., Mol. Immun. 34(16-17):1157-1165 (1997) and PCT Application Publication Nos. WO 2018/213337 and WO 2015/187528, the entire contents of each of which are incorporated by reference herein for all that they teach about anti-CD19 CARs and their use. CAR based on 47G4 are disclosed in United States Patent No. 10,287,350 which is incorporated by reference herein for all that it teaches about anti-CD19 CARs and their use. In some instances, the anti-CD19 CAR is the CAR found in tisagenlecleucel, lisocabtagene maraleucel, axicabtagene ciloleucel, or brexucabtagene autoleucel. Each of these CARs constitutes means for targeting an immune cell, for example, a T cell, to CD19. The entire contents of each of foregoing references in this paragraph are incorporated by reference for all that they teach about the design, structure, and activity of anti-CD19 CARs. In any of the aforementioned tLNP embodiments, certain embodiments

include tLNPs encapsulating a CD19 CAR payload encoded by RNA and having a T cell targeting moiety, such as an anti-CD8 antibody.

[00449] In some embodiments, the tLNP comprises a nucleic acid encoding an anti-CD20 chimeric antigen receptor (CAR). CD20 is an antigen found on the surface of B cells as early as the pro-B phase and progressively at increasing levels until B cell maturity, as well as on the cells of most B-cell neoplasms. CD20 positive cells are also sometimes found in cases of Hodgkin's disease, myeloma, and thymoma. In some embodiments, the nucleic acid comprises mRNA. Examples of anti-CD20 CARs include those incorporating a CD20 binding moiety derived from an antibody specific to CD20, including, for example, Leu16, IF5, 1.5.3, rituximab, obinutuzumab, ibritumomab, ofatumumab, tositumumab, odronextamab, veltuzumab, ublituximab, and ocrelizumab. In some embodiments, the anti-CD20 CAR is derived from a CAR specific to CD20, including, for example, MB-106 (Fred Hutchinson Cancer Research Center, see Shadman et al., *Blood* 134(Suppl.1):3235 (2019)) UCART20 (Cellestis, www.cellbiomedgroup.com), or C-CAR066 (Cellular Biomedicine Group, see Liang et al., *J. Clin. Oncol.* 39(15) suppl:2508 (2021)). In some embodiments, the extracellular binding domain of the anti-CD20 CAR comprises an scFv derived from the Leu16 monoclonal antibody, which comprises the heavy chain variable region (V_H) and the light chain variable region (V_L) of Leu16 connected by a linker. See Wu et al., *Protein Engineering*. 14(12):1025-1033 (2001). Each of these CARs constitutes means for targeting an immune cell, for example, a T cell, to CD20. The entire contents of each of foregoing references in this paragraph are incorporated by reference for all that they teach about the design, structure, and activity of anti-CD20 CARs. In any of the aforementioned tLNP embodiments, certain embodiments include tLNPs encapsulating a CD20 CAR payload encoded by RNA and having a T cell targeting moiety, such as an anti-CD8 antibody.

[00450] In some embodiments, the tLNP comprises a nucleic acid encoding an anti-BCMA chimeric antigen receptor (CAR). BCMA is a tumor necrosis family receptor (TNFR) member expressed on cells of the B cell lineage, with the highest expression on terminally differentiated B cells or mature B lymphocytes. BCMA is involved in mediating the survival of plasma cells for maintaining long-term humoral immunity. The expression of BCMA has been recently linked to a number of cancers, such as multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma, various leukemias, and glioblastoma. In some embodiments, the nucleic acid comprises mRNA. Examples of anti-BCMA CARs include those incorporating a BCMA binding moiety derived from C11D5.3, a Mouse monoclonal antibody as described in Carpenter et al., *Clin. Cancer Res.* 19(8):2048-2060 (2013). See also PCT Application Publication No.

WO 2010/104949. In some embodiments, the extracellular binding domain of the BCMA CAR comprises an scFv derived from another Mouse monoclonal antibody, C12A3.2, as described in Carpenter *et al.*, Clin. Cancer Res. 19(8):2048-2060 (2013) and PCT Application Publication No. WO2010104949. In some embodiments, the extracellular binding domain of the BCMA CAR comprises an scFv derived from a Mouse monoclonal antibody with high specificity to human BCMA, referred to as BB2121 in Friedman *et al.*, Hum. Gene Ther. 29(5):585-601 (2018). See also, PCT Application Publication No. WO2012163805. In some embodiments, the extracellular binding domain of the BCMA CAR comprises single variable fragments of two heavy chains (VHH) that can bind to two epitopes of BCMA as described in Zhao *et al.*, J. Hematol. Oncol. 11(1):141 (2018), also referred to as LCAR-B38M. See also, PCT Application Publication No. WO 2018/028647. In some embodiments, the extracellular binding domain of the BCMA CAR comprises a fully human heavy-chain variable domain (FHVH) as described in Lam *et al.*, Nat. Commun. 11(1):283 (2020), also referred to as FHVH33. See also, PCT Application Publication No. WO 2019/006072. In some embodiments, the extracellular binding domain of the BCMA CAR comprises an scFv derived from CT103A (or CAR0085) as described in U.S. Patent No. 11,026,975 B2. Further anti-BCMA CARs are disclosed in U.S. Application Publication Nos. 2020/0246381 and 2020/0339699. Further anti-BCMA CARs include Allo-605 (described in U.S. Patent Publication No. 20200261503), CT053 (described in U.S. Patent No. US11,525,006), Descartes-08 (described in U.S. Patent No. 10,934,337), LCAR-B38M (described in U.S. Patent No. 10,934,363), PersonGen anti-BCMA CAR (described in CN114763383), Pregene Bio anti-BCMA CAR (described in U.S. Patent Publication No. US20220218746), the CAR in ciltacabtagene autoleucel (binding moiety described in US20170051068), and the CAR in idecabtagene vicleucel (described in U.S. Patent No. 10,383,929). Each of these CARs constitutes means for targeting an immune cell, for example, a T cell, to BCMA. Further antibodies comprising an anti-BCMA antigen binding domains that can be used in construction a CAR include AMG224 (described with other anti-BCMA antibodies in U.S. Patent No. 9,243,058), EMB-06 (described with other anti-BCMA antibodies in U.S. Patent Publication No. US20230002489), HPN217 (described in U.S. Patent No. 11,136,403), MEDI2228 (described in U.S. Patent No. 10,988,546), REGN5459 (described in U.S. Patent No. 11,384,153), SAR445514 (described in U.S. Patent Publication No. 20240034816), SEA-BCMA (described in U.S. Patent No. 11,078,291), TNB-383B (described in U.S. Patent No. 11,505,606), TQB2934 (described in U.S. Patent Publication No. 20230193292), WV078 (described in U.S. Patent No. 11,492,409), alnuctamab (described in U.S. Patent No. 10,683,369), belantamab (described in U.S. Patent No. 9,273,141), elranatamab (described in U.S. Patent No. 11,814,435), ispectamab (described in U.S. Patent

Publication No. 20210130483), linvoseltamab (described in U.S. Patent No. 11,919,965), pavurutamab (described in U.S. Patent No. 11,419,933), and teclistamab (described in U.S. Patent No. 10,072,088). A bispecific anti-BCMA, anti-CD19 CAR is described in WO2022007650. The entire contents of each of foregoing references in this paragraph are incorporated by reference for all that they teach about the design, structure, and activity of anti-BCMA CARs and anti-BCMA antibodies that can provide an antigen binding domain for a CAR or immune cell engager. In any of the aforementioned tLNP embodiments, certain embodiments include tLNPs encapsulating a BCMA CAR payload encoded by RNA and having a T cell targeting moiety, such as an anti-CD8 antibody.

[00451] In some embodiments, the tLNP comprises a nucleic acid encoding an anti-GPRC5D chimeric antigen receptor (CAR). GPRC5D is a G protein-coupled receptor without known ligands and of unclear function in human tissue. However, this receptor is expressed in myeloma cell lines and in bone marrow plasma cells from patients with multiple myeloma. GPRC5D has been identified as an immunotherapeutic target in multiple myeloma and Hodgkin lymphomas. Examples of anti-GPRC5D CARs include those incorporating a GPRC5D binding moiety such as MCARH109 (Mailankody *et al.*, N Engl J Med. 387(13): 1196-1206 (2022)), BMS-986393, or OriCAR-017 (Rodriguez-Otero *et al.*, Blood Cancer J. 14(1): 24 (2024)). Examples of anti-GPRC5D CARs include those incorporating a GPRC5D binding moiety derived from an antibody specific to GPRC5D, for example, talquetamab (Pillariseti *et al.*, Blood 135:1232-43 (2020)), or forimtamig. In some embodiments, the extracellular binding domain of the anti-GPRC5D CAR comprises an scFv derived from a 6D9 Mouse antibody with specificity to human GPRC5D (see creative-biolabs.com/car-t/anti-gprc5d-6d9-h-41bb-cd3-car-pcdcar1-26380.htm). In some embodiments, the extracellular binding domain of the GPRC5D CAR comprises an scFv of anti-GPRC5D antibody linked to 4-1BB or CD28 costimulatory domain and CD3 ζ signaling domain as described in Mailankody *et al.*, N Engl J Med. 387(13): 1196-1206 (2022); creative-biolabs.com/car-t/anti-gprc5d-6d9-h-41bb-cd3-car-pcdcar1-26380.htm; and Rodriguez-Otero *et al.*, Blood Cancer J. 14(1): 24 (2024). The entire contents of each of foregoing references in this paragraph are incorporated by reference for all that they teach about the design, structure, and activity of anti-GPRC5D CARs and anti-GPRC5D antibodies that can provide an antigen binding domain for a CAR or immune cell engager, and each example constitutes a means for binding GPRC5D. In any of the aforementioned tLNP embodiments, certain embodiments include tLNPs encapsulating an anti-GPRC5D CAR payload encoded by RNA and having a T cell targeting moiety, such as an anti-CD8 antibody.

[00452] In some embodiments, the tLNP comprises a nucleic acid encoding an anti-FCRL5 chimeric antigen receptor (CAR). FCRL5 (Fc receptor-like 5), also known as FCRH5, BXMAS1, CD307, CD307E, and IRTA2, is a protein marker expressed on the surface of plasma cells in patients with multiple myeloma. Furthermore, contact with FCRL5 stimulates B-cell proliferation; thus, FCRL5 has been identified as an immunotherapeutic target for this disease. Examples of anti-FCRL5 CARs include those incorporating an FCRL5 binding moiety, such as those described in WO2016090337, WO2017096120, WO2022263855, and WO2024047558. In some embodiments, the extracellular binding domain of the anti-FCRL5 CAR comprises an scFv with specificity to FCRL5, such as ET200-31, ET200-39, ET200-69, ET200-104, ET200-105, ET200-109, or ET200-117. In some embodiments, the extracellular binding domain of the anti-FCRL5 CAR comprises an scFv derived from a mouse antibody with specificity to human FCRL5. Such antibodies include 7D11, F25, F56, and F119, as described in Polson *et al.*, *Int. Immunol.*, 18(9): 1363-1373 (2006); Franco *et al.*, *J. Immunol.* 190(11): 5739-5746 (2013); Ise *et al.*, *Clin. Cancer Res.* 11(1): 87-96 (2005); and Ise *et al.*, *Clin. Chem. Lab. Med.* 44(5): 594-602 (2006), all of which are incorporated by reference herein. In some embodiments, the extracellular binding domain of the anti-FCRL5 CAR comprises a binding moiety derived from the antigen binding domain of an anti-FCRL5 antibody or nanobody, including cevostamab, 2A10H7, 307307, 2A10D6, 13G9, 10A8, 509f6, EPR27365-87, EPR26948-19, or EPR26948-67, or as disclosed in WO2016090337, WO2017096120, WO2022263855, or WO2024047558. In some embodiments, the extracellular binding domain of the anti-FCRL5 CAR comprises a binding moiety derived from an antibody-drug conjugate targeting FCRL5, such as those described in Elkins *et al.*, *Mol. Cancer Ther.* 11(10): 2222-2232 (2012). In some embodiments, the extracellular binding domain of the anti-FCRL5 CAR is linked to a costimulatory domain, such as a 4-1BB or CD28 costimulatory domain, and a signaling domain, such as a CD3 ζ signaling domain. The entire contents of each of the foregoing references in this paragraph are incorporated by reference for all that they teach about the design, structure, properties, and activity of anti-FCRL5 CARs and anti-FCRL5 antibodies that can provide an antigen binding domain for a CAR or immune cell engager. Each example constitutes a means for binding FCRL5. In any of the aforementioned tLNP embodiments, certain embodiments include tLNPs encapsulating a FCRL5 CAR payload encoded by RNA and having a T cell targeting moiety, such as an anti-CD8 antibody.

[00453] In some embodiments, the tLNP comprises a nucleic acid(s) encoding one or more CARs that target multiple antigens. In some embodiments, the tLNP comprises distinct mRNAs that are encapsulated together in a single tLNP, with each mRNA encoding one

monospecific CAR. For examples, the tLNP can comprise an mRNA encoding an anti-CD19 CAR and an mRNA encoding an anti-CD20 CAR, an mRNA encoding an anti-CD19 CAR and an mRNA encoding an anti-BCMA CAR, an mRNA encoding an anti-GPRC5D CAR and an mRNA encoding an anti-BCMA CAR, or an mRNA encoding an anti-FCRL5 CAR and an mRNA encoding an anti-BCMA CAR. In some embodiments, the tLNP comprises a single mRNA encoding a bicistronic mRNA encoding two monospecific CARs. For example, the bicistronic mRNA can encode an anti-CD19 CAR and an anti-CD20 CAR, an anti-CD19 CAR and an anti-BCMA CAR, an anti-GPRC5D CAR and an anti-BCMA CAR, or an anti-FCRL5 CAR and an anti-BCMA CAR. In some embodiments, the tLNP comprises a single mRNA encoding an mRNA encoding a multispecific CAR. In some embodiments, the tLNP comprises a single mRNA encoding an mRNA encoding a bispecific CAR. For example, the mRNA can encode an anti-CD19 and anti-CD20 bispecific CAR, an anti-CD19 and anti-BCMA bispecific CAR, an anti-GPRC5D and anti-BCMA bispecific CAR, or an anti-FCRL5 and anti-BCMA bispecific CAR. In some embodiments, multiple tLNPs can be co-formulated in a combination with each tLNP comprising one mRNA. In some instances, the one mRNA encodes one monospecific CAR. For examples, two tLNPs can be co-formulated with one tLNP comprising an mRNA encoding an anti-CD19 CAR and the other tLNP comprising an mRNA encoding an anti-CD20 CAR, one tLNP comprising an mRNA encoding an anti-C19 CAR and the other tLNP comprising an mRNA encoding an anti-BCMA CAR, one tLNP comprising an mRNA encoding an anti-GPRC5D CAR and the other tLNP comprising an mRNA encoding an anti-BCMA CAR, or one tLNP comprising an mRNA encoding an anti-FCRL5 CAR and the other tLNP comprising an mRNA encoding an anti-BCMA CAR. In some embodiments, multiple tLNPs can be co-administered in a combination, either simultaneously or sequentially, wherein each comprises one mRNA. In some instances, the one mRNA encodes one monospecific CAR. For examples, two tLNPs can be co-administered in a combination, either simultaneously or sequentially, with one tLNP comprising an mRNA encoding an anti-CD19 CAR and the other tLNP comprising an mRNA encoding an anti-CD20 CAR, one tLNP comprising an mRNA encoding an anti-C19 CAR and the other tLNP comprising an mRNA encoding an anti-BCMA CAR, one tLNP comprising an mRNA encoding an anti-GPRC5D CAR and the other tLNP comprising an mRNA encoding an anti-BCMA CAR, or one tLNP comprising an mRNA encoding an anti-FCRL5 CAR and the other tLNP comprising an mRNA encoding an anti-BCMA CAR. The targeting can be mediated by any of the CARs described herein. In addition to combinations of two specificities, higher order combinations are also possible, especially with the use of bi- and tri-specific CARs. Following these patterns, further embodiments are constituted *mutatis mutandis* by other tLNP or combinations of tLNPs

comprising a nucleic acid(s) encoding one or more CARs that target multiple antigens involving these and other CAR specificities disclosed herein.

[00454] Cellular therapy involving the administration of genetically engineered cells to a patient has generally required depleting or ablative conditioning to facilitate engraftment of the engineered cells (for example, T cells or HSC). In the context of *in vivo* engineering and reprogramming such conditioning would be counterproductive as the conditioning would eliminate the very cells that are to be engineered. Instead, one can utilize activating and/or adjuvant conditioning to increase the number of cells amenable to engineering, to mobilize them to the locus of pathology, to make the locus of pathology (for example, a tumor microenvironment) more susceptible to treatment, to augment the therapeutic effect, etc., as appropriate for the particular disease and primary treatment. Conditioning agents include biological response modifiers (BRMs) that can be delivered directly to a subject or encoded in nucleic acid molecules, including as mRNA, and delivered to a subject using the LNP and tLNP compositions and formulations disclosed herein.

[00455] Accordingly, certain aspects are methods of conditioning a subject who receives an engineering agent comprising providing a tLNP comprising a nucleic acid molecule encoding a conditioning agent to the subject prior to, concurrently with, or subsequent to administration of the engineering agent. In various embodiments, an encoded conditioning agent comprises a γ -chain receptor agonist, an inflammatory chemokine, a pan-activating cytokine, an antigen presenting cell activity enhancer, an immune checkpoint inhibitor, or an anti-CCR4 antibody. In some embodiments, the γ -chain receptor cytokine comprises IL-15, IL-2, IL-7, or IL-21. In some embodiments, the immune checkpoint inhibitor comprises an anti-CTLA-4, anti-PD-1, anti-PD-L1, anti-Tim-3, or anti-LAG-3 antibody. In some embodiments, the inflammatory chemokine comprises CCL2, CCL3, CCL4, CCL5, CCL11, CXCL1, CXCL2, CXCL-8, CXCL9, CXCL10, or CXCL11. In some embodiments, the antigen presenting cell activity enhancer comprises Flt-3 ligand, gm-CSF, or IL-18. In some embodiments, a pan-activating cytokine comprises IL-12 or IL 18. In certain embodiments, a conditioning agent comprises a transcription factor, for example, one selected from the group consisting of nuclear factor of activated T-cells (NFAT), NF- κ B, T-bet, signal transducer and activator of transcription 4 (STAT4), Blimp-1, c-Jun, and Eomesodermin (Eomes) and the tLNP is targeted to a T cell. In some embodiments, a tLNP encapsulating the nucleic acid-encoded conditioning agent is administered systemically, for example, by intravenous or subcutaneous infusion or injection. In other embodiments, the tLNP is administered locally, for example, by intralesional or intraperitoneal injection or infusion. In some embodiments, nucleic acid molecules encoding

the conditioning agent and the engineering agent are encapsulated in the same tLNP while in other embodiments they are encapsulated in separate tLNPs. These two modes of delivery of conditioning agents are described in greater detail in PCT application PCT/US 2023/072426, which is incorporated by reference for all that it teaches about conditioning agents and their delivery of LNPs or tLNPs that is not inconsistent with the present disclosure. In some embodiments, the nucleic acid comprises mRNA.

[00456] The term “treating” or “treatment” broadly includes any kind of treatment activity, including the mitigation, cure or prevention of disease, or aspect thereof, in man or other animals, or any activity that otherwise affects the structure or any function of the body of man or other animals. Treatment activity includes the administration of the medicaments, dosage forms, and pharmaceutical compositions described herein to a patient, especially according to the various methods of treatment disclosed herein, whether by a healthcare professional, the patient his/herself, or any other person. Treatment activities include the orders, instructions, and advice of healthcare professionals such as physicians, physician’s assistants, nurse practitioners, and the like, that are then acted upon by any other person including other healthcare professionals or the patient him/herself. In some embodiments, the orders, instructions, and advice aspect of treatment activity can also include encouraging, inducing, or mandating that a particular medicament, or combination thereof, be chosen for treatment of a condition - and the medicament is actually used - by approving insurance coverage for the medicament, denying coverage for an alternative medicament, including the medicament on, or excluding an alternative medicament, from a drug formulary, or offering a financial incentive to use the medicament, as might be done by an insurance company or a pharmacy benefits management company, and the like. In some embodiments, treatment activity can also include encouraging, inducing, or mandating that a particular medicament be chosen for treatment of a condition - and the medicament is actually used - by a policy or practice standard as might be established by a hospital, clinic, health maintenance organization, medical practice or physicians group, and the like. All such orders, instructions, and advice are to be seen as conditioning receipt of the benefit of the treatment on compliance with the instruction. In some instances, a financial benefit is also received by the patient for compliance with such orders, instructions, and advice. In some instances, a financial benefit is also received by the healthcare professional for compliance with such orders, instructions, and advice.

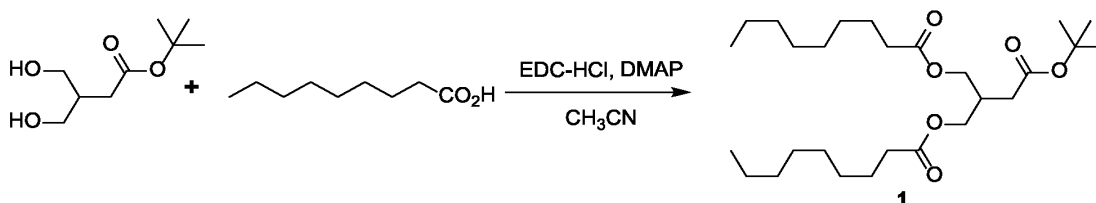
[00457] Some embodiments of these methods of treatment comprise administration of an effective amount of a compound or a composition disclosed herein. Some instances relate to

a therapeutically (or prophylactically) effective amount. A therapeutically effective amount is not necessarily a clinically effective amount, that is, while there can be therapeutic benefit as compared to no treatment, a method of treatment may not be equivalent or superior to a standard of care treatment existing at some point in time. Other instances relate to a pharmacologically effective amount, that is an amount or dose that produces an effect that correlates with or is reasonably predictive of therapeutic (or prophylactic) utility. As used herein, the term “therapeutically effective amount” is synonymous with “therapeutically effective dose” and means at least the minimum dose of a compound or composition disclosed herein necessary to achieve the desired therapeutic or prophylactic effect. Similarly, a pharmacologically effective dose means at least the minimum dose of a compound or composition disclosed herein necessary to achieve the desired pharmacologic effect. Some embodiments refer to an amount sufficient to prevent or disrupt a disease process, or to reduce the extent or duration of pathology. Some embodiments refer to a dose sufficient to reduce a symptom associated with the disease or condition being treated.

[00458] The following examples are intended to illustrate various embodiments. As such, the specific embodiments discussed are not to be constructed as limitations on the scope of this disclosure. It is apparent to one skilled in the art that various equivalents, changes, and modifications can be made without departing from the scope of this disclosure, and it is understood that such equivalent embodiments are to be included herein. Further, all references cited in the disclosure are hereby incorporated by reference in their entirety, as if fully set forth herein.

EXAMPLES

Example 1: Synthesis of 2-(2-(tert-butoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (1)

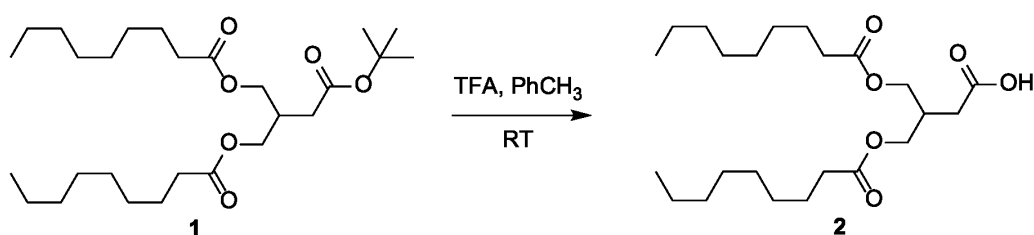


[00459] To a solution of tert-butyl 4-hydroxy-3-(hydroxymethyl)butanoate (Org. Proc. Res. Dev. 2011, 15, 515; 44.0g, 0.231mol), in acetonitrile (900mL), cooled in an ice water bath under nitrogen, was added nonanoic acid (76.86g, 0.486mol), followed by the addition of DMAP (28.22g, 0.231mol) and EDC-HCl (97.8g, 0.513mol). The mixture was stirred for 1 hour, then was allowed to warm to room temperature and was stirred for 12 hours. The solution was cast into n-heptane (1.40L) and water (0.9L) and the organic phase was separated. The

organic phase was washed twice with MeOH:10% aq. citric acid (0.90L), followed by washing twice with a mixture of MeOH:H₂O:triethyl amine (0.90L, 3:1:0.1). The organic phase was then washed with 10% aq. NaCl, dried over Na₂SO₄, filtered, and concentrated in vacuo to provide 2-(2-(tert-butoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate 1 (90.20g, 96.3% purity by HPLC, 0.185mol, 80% yield) as a pale yellow viscous liquid.

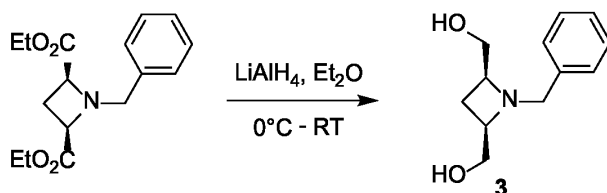
[00460] ¹H-NMR (300MHz, CDCl₃): δ = 4.12 (m, 4H), 2.53 (m, 1H), 2.29-2.34 (6H), 1.52-1.64 (4H), 1.46 (s, 9H), 1.16-1.37 (24H), 0.89 (t, J = 7.0Hz, 6H); **LCMS**: RT = 1.748, calcd. for C₂₇H₅₀O₆ minus t-butyl + H⁺: 415.31. Found: 415.20.

Example 2: Synthesis of 4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoic acid (2)



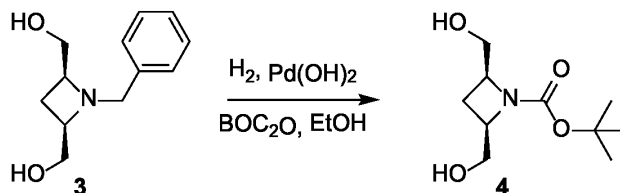
[00461] To a solution of 1 (90.0g, 96.3% purity, 0.184mol) in toluene (0.41L), cooled in an ice-water bath under nitrogen, was added TFA (208.46g, 1.83mol, 140mL) over a period of 30 minutes. After the addition was complete the mixture was warmed to 15° and the mixture was stirred for 18 hours. The chilled solution was cast into n-heptane (1.50L) and the resulting solution was extracted with 5% aq. Potassium phosphate (2.0L) and the aqueous phase was collected. The organic phase was extracted with MeOH:H₂O:triethyl amine (2.0L, 5:1:0.1), and the combined aq. phases were cast into n-heptane (1.80L) and 1.2M aq. HCl (1.0L). The organic layer was separated, washed with MeOH:water (1.0L, 1:1), dried over Na₂SO₄, filtered and concentrated in vacuo to afford acid 2 (69.0g, 96.1% purity by HPLC, 0.177mol, 96% yield) as a pale yellow, viscous oil.

[00462] ¹H-NMR (300MHz, CDCl₃): δ = 4.13 (m, 4H), 2.58 (m, 1H), 2.48 (m, 2H), 2.32 (m, 4H), 1.63 (m, 4H), 1.20-1.37 (24H), 0.89 (t, J = 7.0Hz, 6H); **LCMS**: RT = 1.723, Calcd. for C₂₃H₄₂O₆+H⁺ 415.31. Found 415.30.

Example 3: Synthesis of *rel*-(2S,4R)-1-benzylazetidine-2,4-diyl)dimethanol (3)

[00463] To a solution of commercially available diethyl *rel*-(2S,4R)-1-benzylazetidine-2,4-dicarboxylate (10.0g, 34.32mmol) in ether (200mL), cooled in an ice-water bath under nitrogen, was added LiAlH₄ (2.74g, 74.08mmol) in portions over a period of 15 minutes. The cooling bath was removed, and the mixture was allowed to warm to room temperature and was stirred for 5 hours. The mixture was cooled in an ice-water bath and the reaction was carefully quenched by the of ice-water (200mL). The organic phase was separated, the aqueous phase was extracted with EtOAc (3 x 300mL), the combined organic phases were washed with brine (2 x 300mL), dried over Na₂SO₄, filtered and concentrated in vacuo to provide crude 3 (4.00g, 83.3% HPLC purity, 16.1mmol, 47% yield) as a yellow viscous liquid.

[00464] ¹H-NMR (300MHz, CDCl₃): δ = 7.26-7.33 (5H), 4.03 (m, 1H), 3.62-3.77 (3H), 3.49-3.63 (4H), 2.26 (brs, 2H), 2.13 (m, 2H); **LCMS**: RT = 0.91, Calcd. for C₁₂H₁₇NO₂ + H⁺ 208.13. Found 208.10.

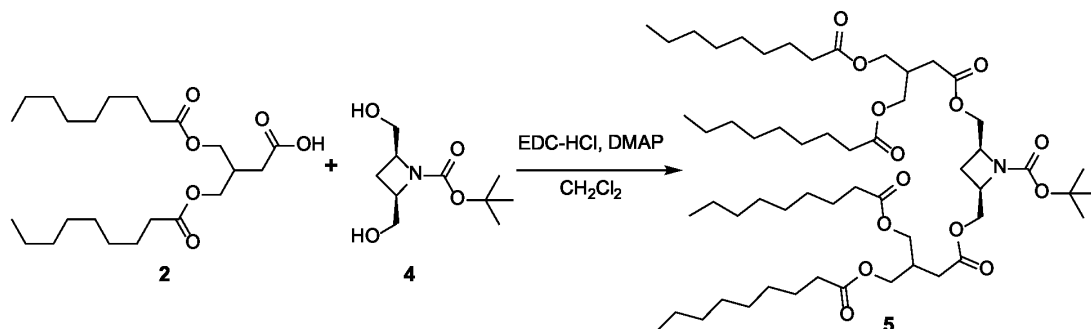
Example 4: Synthesis of *tert*-butyl *rel*-(2S,4R)-2,4-bis(hydroxymethyl)azetidine-1-carboxylate (4)

[00465] To a solution of 3 (4.00g, 16.1mmol) in EtOH (200mL) was added Pd(OH)₂ (0.80g, 20% w/w and BOC₂O (5.27g, 2.15mmol), and the mixture was placed under 30psi of hydrogen. The mixture was shaken under 30psi of hydrogen for 20hours, then the catalyst was removed by filtration through a pad of Celite, the filter cake was rinsed with EtOH (50mL) and the combined filtrates were concentrated in vacuo to give crude 4 as a yellow oil. Crude 4 was purified by chromatography on a column of silica gel (250g, 100-200 mesh), packed and eluted with petroleum ether-EtOA (4:1). Fractions containing 4 were pooled and concentrated in vacuo to furnish 4 (2.10g, HPLC purity 97.9%, 9.20mmol, 57% yield) as a clear, pale yellow,

oil.

[00466] $^1\text{H-NMR}$ (300MHz, CDCl_3): δ = 4.31 (m, 2H), 3.80 (dd, J = 11.7, 2.7Hz, 2H), 3.67 (dd, J = 11.7, 5.7Hz, 2H), 2.93 (brs, 2H), 2.22 (m, 1H), 1.94 (m, 1H), 1.49 (s, 9H).

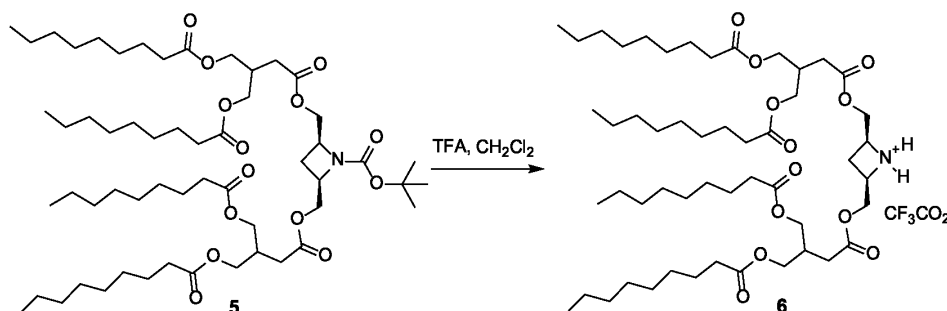
Example 5: Synthesis of ((((*rel*-(2S,4R)-1-(tert-butoxycarbonyl)azetidine-2,4-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (5)



[00467] To solution of 4 (2.00g, 9.205mmol) in dichloromethane (100mL), at room temperature under nitrogen, was added in order: 2 (8.01g, 19.331mmol), DMAP (1.12g, 9.205mmol) and EDC-HCl (4.41g, 23.012mmol). The reaction mixture was stirred for 18 hours at room temperature, then was concentrated in vacuo and the residue was dissolved in n-heptane (500mL). The resulting solution was washed with MeOH/water (10:1, 2 x 200mL) and the organic phase was concentrated in vacuo to provide crude 5. Crude 5 was purified by chromatography on a column of silica gel (500g, 100-200 mesh, packed with n-heptane, eluted with a gradient of n-heptane:EtOAc 100:0 to 90:10). Qualified fractions were combined and concentrated in vacuo to give 5 (7.00g, HPLC purity 97.33%, 6.93mmol, 75.3% yield) as a light-yellow oil.

[00468] $^1\text{H-NMR}$ (400MHz, CDCl_3): δ = 4.25-4.37 (6H), 4.18 (m, 8H), 2.62 (m, 2H), 2.50 (m, 4H), 2.42 (m, 1H), 2.33 (t, J = 7.0Hz, 8H), 1.83 (m, 1H), 1.52-1.60 (8H), 1.45 (s, 9H), 1.18-1.37 (40H), 0.89 (t, J = 7.9Hz, 12H); **LCMS**: RT = 2.24, Calcd. for $\text{C}_{56}\text{H}_{99}\text{NO}_{14} + \text{Na}^+$ 1032.70, Found 1032.70.

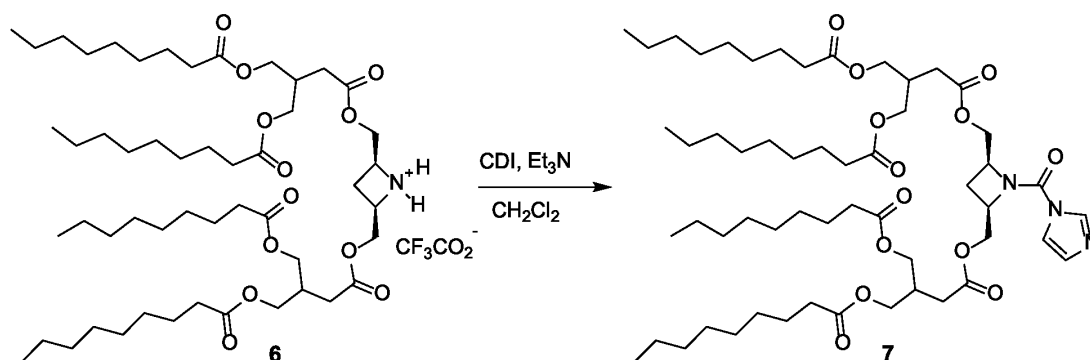
Example 6: Synthesis of *Rel*-(2*S*,4*R*)-2,4-bis(((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)methyl)azetidin-1-ium trifluoroacetate (6)



[00469] To a solution of 5 (7.00g, 6.928mmol) in dichloromethane (35mL), at room temperature under nitrogen, was added trifluoroacetic acid (14mL, 20.85g, 0.183mol) over a period of 10 minutes. The mixture was allowed to stir for 3 hours at room temperature, then was concentrated in vacuo to afford crude 6. Ammonium salt 6 was dissolved in n-heptane (500mL) and solution was washed with brine (2 x 200mL), water (200mL), then the solution was concentrated in vacuo to afford 6 as a light-yellow oil (6.80g, HPLC purity 91.82%).

[00470] ¹H-NMR (400MHz, CDCl₃): δ = 4.77 (brs, 2H), 4.44 (m, 4H), 4.02-4.25 (9H), 2.72 (brm, 1H), 2.38-2.5 (8H), 2.33 (m, 8H), 1.51-1.62 (8H), 1.20-1.37 (40H), 0.88 (m, 12H); LCMS: RT = 1.72, Calcd. for C₅₁H₉₂NO₁₂ 910.55. Found 910.70.

Example 7: Synthesis of ((((*rel*-(2*S*,4*R*)-1-(1H-imidazole-1-carbonyl)azetidine-2,4-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (7)

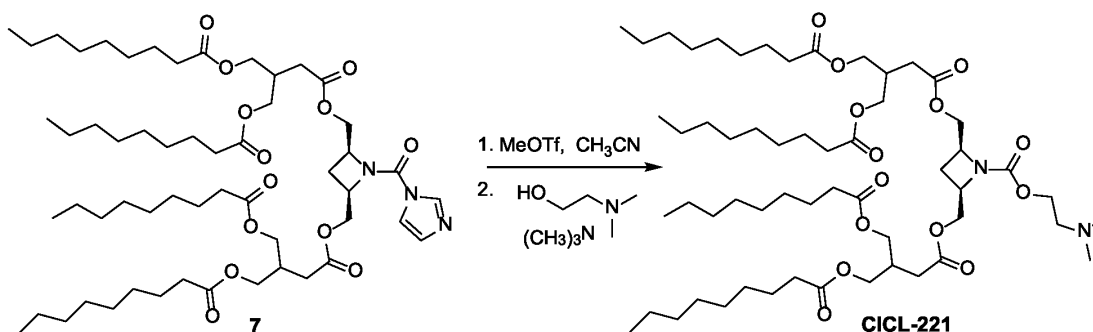


[00471] To a solution of 6 (6.50g, 91.82% pure by HPLC) in dichloromethane (180mL), cooled in an ice-water bath under nitrogen, was added CDI (10.44g, 64.44mmol) and Et₃N (3.26g, 32.27mmol), the mixture was allowed to stir for 1 hour, then was warmed to room

temperature and stirred for 18 hours. The reaction was quenched with 0.5M aq. HCl (150mL) and the organic phase was separated. The aqueous layer was extracted with dichloromethane (2 x 300mL) and the combined organic phases were concentrated in vacuo and the residual oil was dissolved in n-heptane (300mL). The n-heptane solution was washed with MeOH/water (5:1, 2 x 200mL) and the solvent was removed in vacuo to provide 7 (6.00g, HPLC purity 97.24%, 5.97mmol, 92% yield over 2 steps) as a light-yellow oil.

[00472] ¹H-NMR (400MHz, CDCl₃): δ = 8.09 (s, 1H), 7.39 (s, 1H), 7.11 (s, 1H), 4.52 (m, 2H), 4.44 (m, 4H), 4.12 (m, 8H), 2.75 (m, 1H), 2.57 (m, 2H), 2.43 (m, 4H), 2.28 (m, 8H), 2.07 (m, 1H), 1.52-1.64 (8H), 1.18-1.32 (40H), 0.88 (t, J = 7.20Hz, 12H); LCMS: RT = 1.74, Calcd. for C₅₅H₉₃N₃O₁₃ + H⁺ 1004.68, Found 1004.70.

Example 8: Synthesis of ((((*rel*-(2S,4R)-1-((2-(dimethylamino)ethoxy)carbonyl)azetidine-2,4-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-221)

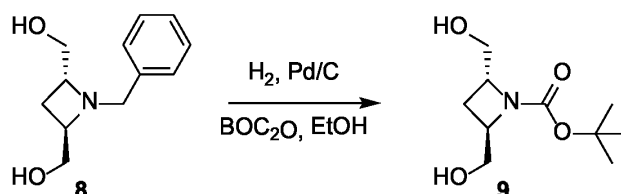


[00473] To a solution of 7 (5.80g, 5.775mmol), in CH₃CN (120mL), cooled in an ice-water bath under nitrogen was added methyl trifluoromethanesulfonate (MeOTf, 1.14g, 6.947mmol) over a period of 5 minutes. The mixture was allowed to stir for 2 hours at 0°C, then a solution of trimethylamine in CH₃CN (2.4M, 11.55mL, 27.7mmol) was added over a period of 10 minutes followed by the addition of 2-dimethylaminoethanol (0.77g, 8.638mmol) in one portion. The mixture was stirred for 1 hour at 0°C, then, was warmed to 60°C and stirred for 120 hours. The reaction mixture was concentrated in vacuo and the residue was dissolved in n-heptane (400mL). The solution was washed with MeOH/water (5:1, 2 x 150mL), and the organic phase was dried over Na₂SO₄. Filtration afforded a heptane solution of crude CICL-221 to which was added silica gel (15g, type: ZCX-2, 100-200 mesh). The solvent was removed in vacuo and the impregnated silica gel was placed atop a combi-flash column of silica gel (150g, type: ZCX-2, 100-200 mesh). The column was eluted with a gradient of n-heptane:ethyl acetate from 100:0 to 35:65. Qualified fractions (eluted at 50:50) were combined

and concentrated in vacuo to provide C1CL-221 (1.876g, 1.83mmol, 32%) as a pale yellow oil.

[00474] ¹H-NMR (400MHz, CDCl₃): δ = 4.27 (m, 8H), 4.13 (m, 8H), 2.58 (m, 4H), 2.40-2.52 (5H), 2.23-2.34 (14H), 1.89 (m, 1H), 1.60 (m, 8H), 1.18-1.38 (40H), 0.88 (t, *J* = 6.90Hz, 12H); HPLC (RT = 20.11) 96.57% purity; LCMS: RT = 1.215, Calcd. for C₅₆H₁₀₀N₂O₁₄ + H⁺ 1025.73. Found 1025.70.

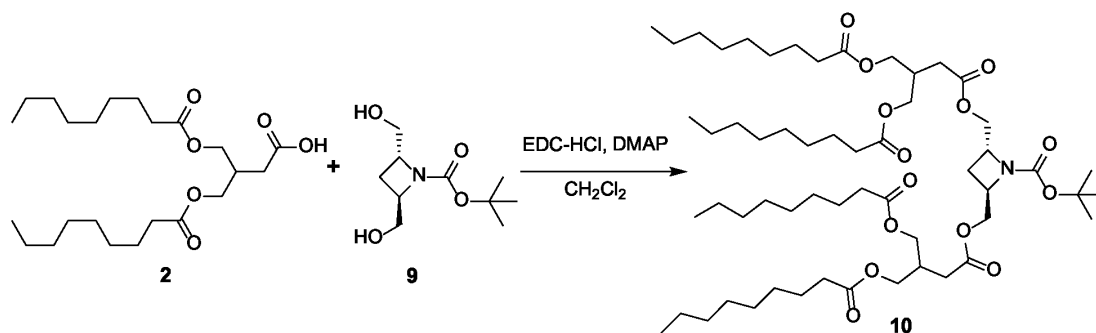
Example 9: Synthesis of tert-butyl *rel*-(2R,4R)-2,4-bis(hydroxymethyl)azetidine-1-carboxylate (9)



[00475] To a solution of 8 (7.90g, 38.11mmol, Tetrahedron Asymmetry 2001, 12, 605) in EtOH (240mL) was added 10% Pd/C (1.60g, 20% w/w and BOC₂O (12.48g, 57.16mmol), and the mixture was placed under 30psi of hydrogen. The mixture was shaken under 30psi of hydrogen for 30hours, then the catalyst was removed by filtration through a pad of Celite, the filter cake was rinsed with EtOH (100mL) and the combined filtrates were concentrated in vacuo to give crude 9 as a yellow oil. Crude 9 was purified by chromatography on a column of silica gel (300g, 100-200 mesh), packed and eluted with petroleum ether-EtOA (4:1). Fractions containing 9 were pooled and concentrated in vacuo to furnish 9 (6.90g, HPLC purity 98.05%, 31.63mmol, 83% yield) as a clear, pale yellow, oil.

[00476] ¹H-NMR (300MHz, CDCl₃): δ = 4.58–4.23 (3H), 3.93–3.62 (4H), 2.32 (brs, 1H), 2.00 (brm, 2H), 1.47 (s, 9H); LCMS: RT = 0.897, Calcd. for C₁₀H₁₉NO₄ + H⁺ 218.14. Found 218.10.

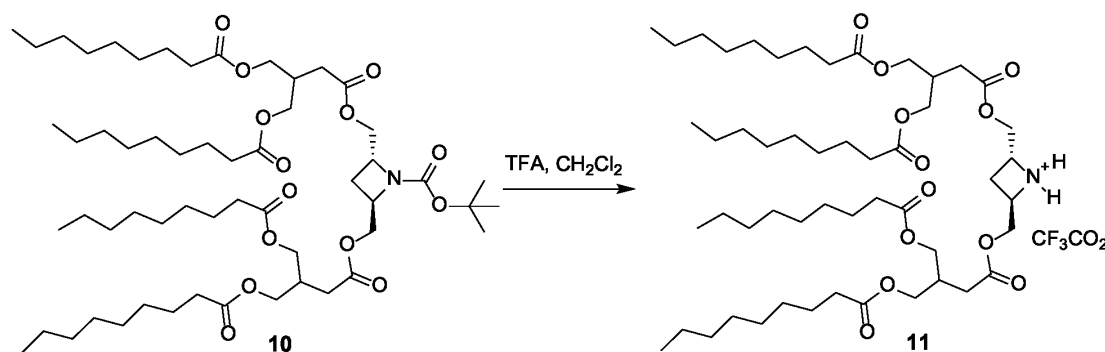
Example 10: Synthesis of ((((*rel*-(2R,4R)-1-(tert-butoxycarbonyl)azetidine-2,4-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propene-2,1,3-triyl) tetranonanoate (10)



[00477] To solution of 9 (2.00g, 9.205mmol) in dichloromethane (100mL), at room temperature under nitrogen, was added in order: 2 (8.01g, 19.331mmol), DMAP (1.12g, 9.205mmol) and EDC-HCl (4.41g, 23.012mmol). The reaction mixture was stirred for 18 hours at room temperature, then was concentrated in vacuo and the residue was dissolved in n-heptane (500mL). The resulting solution was washed with MeOH/water (10:1, 2 x 200mL) and the organic phase was concentrated in vacuo to provide crude 10. Crude 10 was purified by chromatography on a column of silica gel (500g, 100-200 mesh, packed with n-heptane, eluted with a gradient of n-heptane:EtOAc 100:0 to 90:10). Qualified fractions were combined and concentrated in vacuo to give 10 (7.00g, HPLC purity 95.94%, 6.93mmol, 75.3% yield) as a light-yellow oil.

[00478] ¹H-NMR (300MHz, CDCl₃): δ = 4.20-4.52 (5H), 4.13 (m, 8H), 2.60 (m, 2H), 2.46 (m, 4H), 2.30 (t, *J* = 7.5Hz, 8H), 2.19 (m, 2H), 1.52-1.65 (9H), 1.40 (s, 9H), 1.18-1.30 (40H), 0.88 (m, 12H); LCMS: RT = 2.235, Calcd. for C₅₆H₉₉NO₁₄ + Na⁺ 1032.70, Found 1032.80.

Example 11: Synthesis of *Rel*-(2R,4R)-2,4-bis(((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)methyl)azetidin-1-ium trifluoroacetate (11)

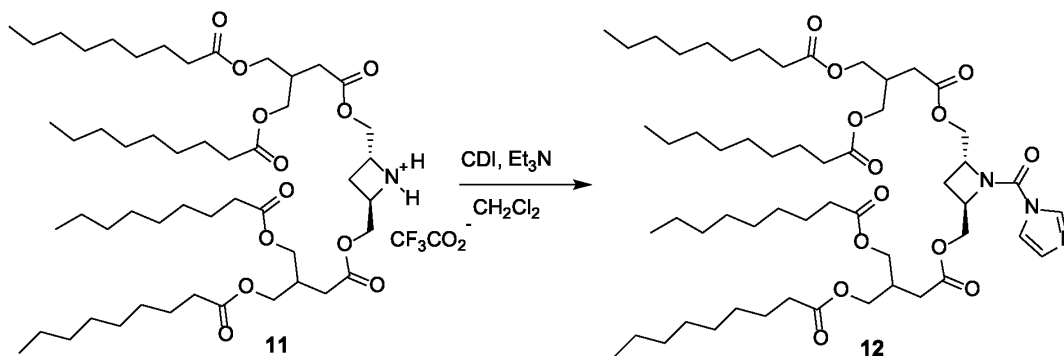


[00479] To a solution of 10 (7.00g, 6.928mmol) in dichloromethane (35mL), at room temperature under nitrogen, was added trifluoroacetic acid (14mL, 20.85g, 0.183mol) over a

period of 10 minutes. The mixture was allowed to stir for 3 hours at room temperature, then was concentrated in vacuo to afford crude 11. Ammonium trifluoroacetate salt 11 was dissolved in n-heptane (500mL) and solution was washed with brine (2 x 200mL), water (200mL), then the solution was concentrated in vacuo to afford 6 as a light-yellow oil (6.80g, HPLC purity 91.63%).

[00480] ¹H-NMR (300MHz, CDCl₃): δ = 4.66 (brm, 2H), 4.46 (m, 4H), 4.16 (m, 8H), 2.42-2.65 (9H), 2.30 (t, *J* = 7.4Hz, 8H), 1.55-1.65 (9H), 1.20-1.40 (40H), 0.88 (m, 12H); **LCMS**: RT = 1.724, Calcd. for C₅₁H₉₂NO₁₂ 910.66, Found 910.70.

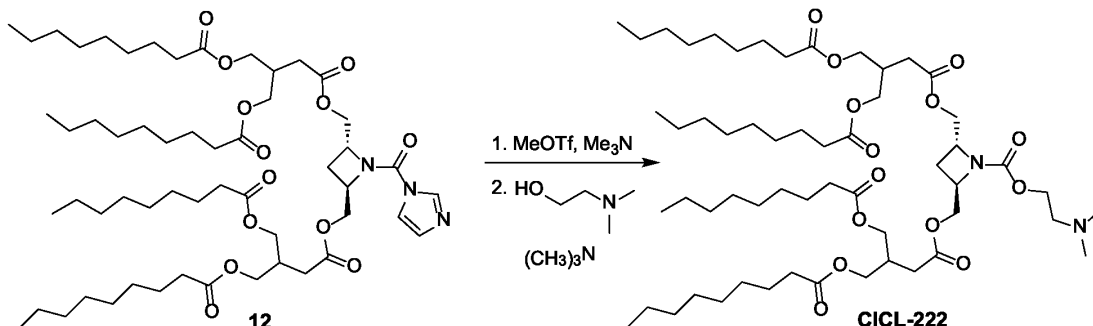
Example 12: Synthesis of ((((*rel*-(2R,4R)-1-(1H-imidazole-1-carbonyl)azetidine-2,4-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (12)



[00481] To a solution of 11 (6.50g, 91.63% pure by HPLC) in dichloromethane (180mL), cooled in an ice-water bath under nitrogen, was added CDI (10.44g, 64.44mmol) and Et₃N (3.26g, 32.27mmol), the mixture was allowed to stir for 1 hour, then was warmed to room temperature and stirred for 18 hours. The reaction was quenched with 0.5M aq. HCl (150mL) and the organic phase was separated. The aqueous layer was extracted with dichloromethane (2 x 300mL) and the combined organic phases were concentrated in vacuo and the residual oil was dissolved in n-heptane (300mL). The n-heptane solution was washed with MeOH/water (5:1, 2 x 200mL) and the solvent was removed in vacuo to provide 12 (6.00g, HPLC purity 96.67%, 5.97mmol, 93% yield over 2 steps) as a light-yellow oil.

[00482] ¹H-NMR (300MHz, CDCl₃): δ = 0.87 (s, 1H), 7.32 (s, 1H), 7.12 (s, 1H), 4.85 (brm, 2H), 4.08-4.30 (12H), 2.57 (m, 2H), 2.26-2.51 (14H) 1.62 (m, 8H), 1.23-1.40 (40H), 0.89 (t, *J* = 6.9Hz, 12H); **LCMS**: RT 1.743, Calcd. for C₅₅H₉₃N₃O₁₃ + H⁺ 1004.68, Found 1004.70.

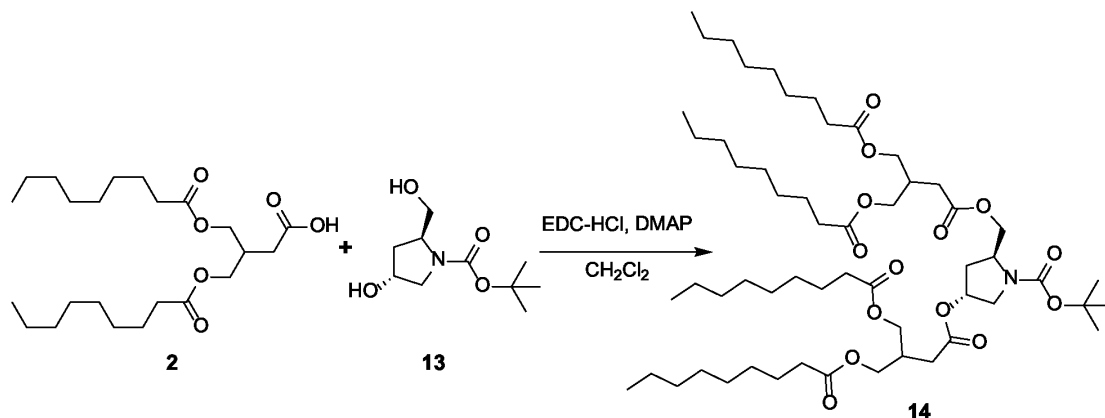
Example 13: Synthesis of (((*rel*-(2S,4R)-1-((2-(dimethylamino)ethoxy)carbonyl)azetidine-2,4-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-222)



[00483] A solution of 12 (5.80g, 5.78mmol), in acetonitrile (120mL) under nitrogen, was cooled in an ice water bath, then MeOTf (1.14g, 6.95mmol) was added over a period of 5 minutes. The mixture was stirred at 0°C for 2 hours, then trimethylamine (11.55mL, 2M in THF, 23.10mmol) was added over a period of 5 minutes, followed by the addition of 2-dimethylaminoethanol (0.77g, 8.64mmol) in one portion. The resulting solution was stirred for 1 hour at 0°C, then was warmed to room temperature, followed by heating in a 75°C oil bath for 8 days. After cooling to room temperature, the solvent was removed in vacuo and the residue was dissolved in n-heptane (400mL). The n-heptane solution was washed with MeOH/water (5:1, 2 x 150mL), and the organic phase was dried over Na₂SO₄. Filtration and concentration in vacuo yielded crude CICL-222, which was dissolved in CH₃CN (10mL), and purified by preparative reverse phase HPLC (XB-phenyl column 19x250mm, 5μm; Mobile Phase A: water/0.1% TFA; Mobile Phase B: CH₃CN; flow rate 20mL/min; Gradient 50% B to 90% B in 18 minutes; Wave Length 200nm). Qualified fractions were concentrated in vacuo, to remove CH₃CN, and the pH of the aq. Residue was adjusted to 8.0 with 2% aq. Na₂CO₃. The aq. phase was extracted with n-heptane (3 x 100mL), the combined organic phases were then washed with MeOH/water (5:1, 2 x 100mL), water (200mL), and dried (Na₂SO₄). Filtration and concentration in vacuo gave CICL-222 (1.26g, 1.23mmol, 97% purity by HPLC, 21% yield) as a clear, colorless, viscous oil.

[00484] ¹H-NMR (300MHz, CDCl₃): δ = 4.31-4.57 (5H), 4.08-4.22 (11H), 2.61 (brm, 4H), 2.49 (m, 4H), 2.22-2.35 (16H), 1.63 (m, 8H), 1.20-1.40 (40H), 0.90 (t, J = 6.6Hz, 12H); LCMS: RT 1.588, Calcd. for C₅₆H₁₀₀N₂O₁₄ + H⁺ 1025.73. Found 1025.70.

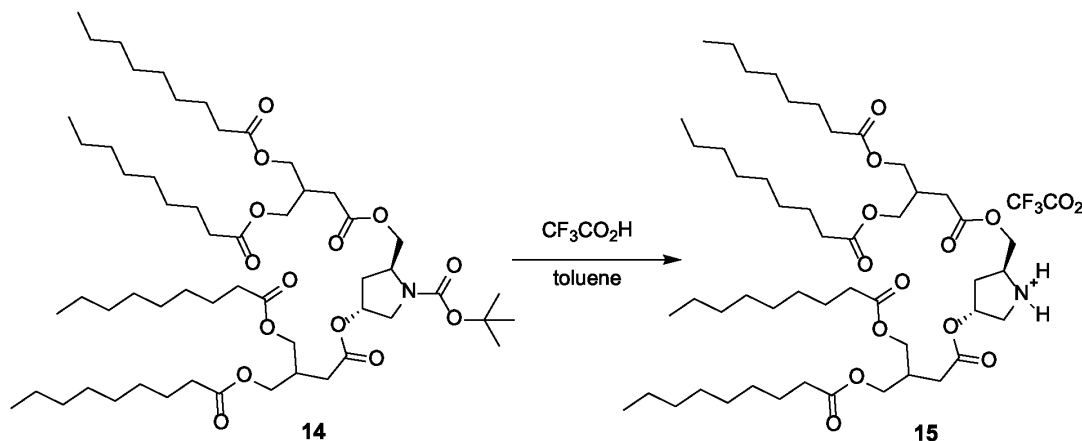
Example 14: Synthesis of 2-(2-(((2S,4R)-1-(tert-butoxycarbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (14)



[00485] To a solution of 13 (3.10g, 14.27mmol, TCI America #B3662) in CH₃CN (60mL), at room temperature under nitrogen, was added in order: 2 (12.0g, 28.94mmol), DMAP (1.80g, 14.73mmol), and EDC-HCl (7.00g, 36.70mmol). The mixture was stirred for 18 hours at room temperature, then was concentrated in vacuo to afford crude 14. Crude 14 was dissolved in n-heptane (60mL), washed with MeOH/10% aq. citric acid (5:1, 2 x 60mL), and water (60mL). The organic phase was concentrated in vacuo and the residue was dissolved in CH₂Cl₂ (20mL). To the solution of crude 14 was added silica gel (25g, type ZCX-2, 200-300 mesh) and the solvent was removed in vacuo to furnish silica gel impregnated with crude 14. This silica gel was placed atop a combi-flash column of silica gel (210g, type ZCX-2, 200-300 mesh) and the column was eluted with a gradient of CH₂Cl₂/MeOH (100:0 to 90:10). Qualified fractions were combined and concentrated in vacuo to yield 14 (5.80g, 5.82mmol, 41%) as a viscous, pale-yellow oil.

[00486] ¹H-NMR (300MHz, CDCl₃): δ = 5.28 (brm, 1H), 4.04-4.37 (8H), 3.61 (brm, 1H), 2.55 (m, 2H), 2.42 (m, 4H), 2.32 (t, J = 7.5Hz, 8H), 2.18 (brm, 2H), 1.60-1.75 (10H), 1.5 (9H), 1.22-1.38 (40H), 0.88 (m, 12H); **LCMS**: RT 2.017, Calcd. for C₅₅H₉₇NO₁₄ + Na⁺ 1032.70. Found 1032.60.

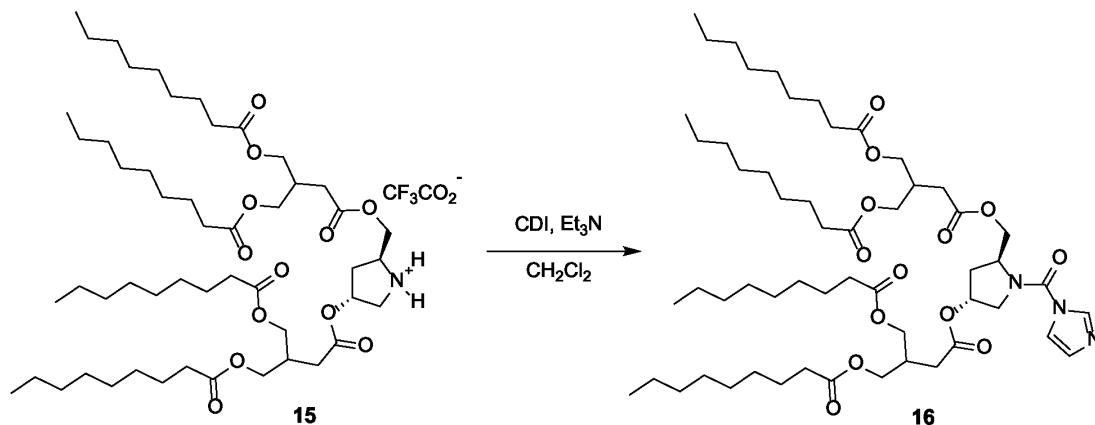
Example 15: Synthesis of (2S,4R)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)-2-(((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)methyl)pyrrolidin-1-ium trifluoroacetate (15)



[00487] To a solution of 14 (5.80g, 5.74mmol) in toluene (36mL), cooled to 20°C under nitrogen, was added TFA (12mL, 17.87g, 157mmol) over a period of 15 minutes. After the addition was complete, the mixture was stirred at 20°C for 2 hours, then was concentrated in vacuo to give crude 15. Crude ammonium trifluoroacetate salt 15 was dissolved in n-heptane (60mL) and the resulting solution was washed with 10% aq. K₂HPO₄ (30mL) and water (2 x 60mL). The organic layer was concentrated in vacuo to give 15 (5.50g, 85% HPLC purity, 5.37mmol, 94% yield) as a viscous, yellow oil.

[00488] ¹H-NMR (300MHz, CDCl₃): δ = 5.50 (m, 1H), 4.62 (m, 1H), 4.26-4.36 (3H), 4.08-4.24 (8H), 3.89 (m, 1H), 3.72 (m, 1H), 2.61 (m, 2H), 2.25-2.49 (15H), 1.63 (m, 8H), 1.20-1.43 (40H), 0.90 (t, *J* = 6.6Hz, 12H); **LCMS**: RT 1.679, Calcd. for C₅₁H₉₂NO₁₂ 910.66, Found 910.60.

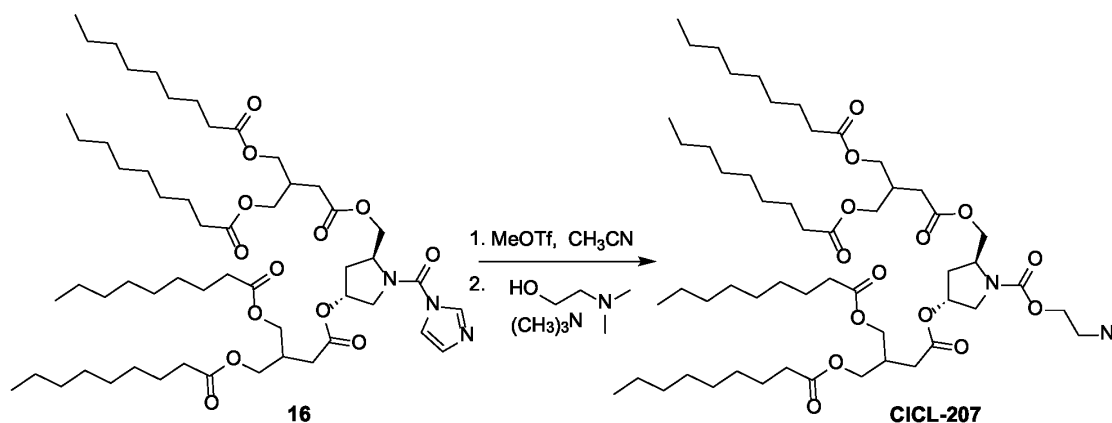
Example 16: Synthesis of 2-(2-(((2S,4R)-1-(1H-imidazole-1-carbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (16)



[00489] To a solution of 15 (5.50g, 5.37mmol) in CH_2Cl_2 (60mL), cooled to 20°C under nitrogen, was added in order CDI (3.48g, 21.48mmol) and Et_3N (1.09g, 10.74mmol). The solution was warmed to room temperature after the additions were complete, and the mixture was allowed to stir for 20 hours. The solution was cast into 1M aq. HCl (70mL), the organic phase was separated. The aq. layer was extracted with CH_2Cl_2 (3 x 70mL) and the combined organic phases were concentrated in vacuo to afford crude 16. Crude 16 was dissolved in n-heptane (150mL) and the solution was washed with MeOH/water (2 x 150mL), brine (150mL), and dried (Na_2SO_4). Filtration and concentration in vacuo provided 16 (4.80g, 4.78mmol, 89% yield) as a pale-yellow oil.

[00490] $^1\text{H-NMR}$ (300MHz, CDCl_3): δ = 8.75 (s, 1H), 7.56 (s, 1H), 7.32 (s, 1H), 5.37 (m, 1H), 4.80 (m, 1H), 4.68 (m, 2H), 3.99-4.28 (10H), 3.75 (d, J = 12Hz, 1H), 2.38-2.60 (4H), 2.25-2.37 (11H), 1.62 (m, 8H), 1.24-1.40 (40H), 0.90 (t, J = 6.6Hz, 12H); **LCMS**: RT 1.709, Calcd. for $\text{C}_{55}\text{H}_{93}\text{N}_3\text{O}_{13} + \text{H}^+$ 1004.68. Found 1004.60.

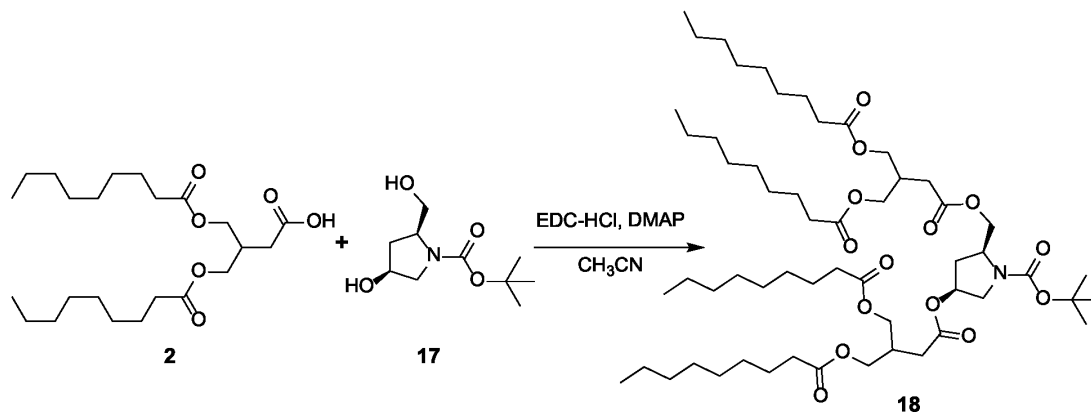
Example 17: Synthesis of 2-(2-(((2S,4R)-1-((2-(dimethylamino)ethoxy)carbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (CICL-207)



[00491] To a solution of 16 (4.80g, 4.78mmol), in CH₃CN (50mL), cooled in an ice-water bath under nitrogen was added MeOTf (0.862g, 5.25mmol) over a period of 5 minutes. The mixture was allowed to stir for 2 hours at 0°C, then a solution of trimethylamine in THF (2.0M, 7.20mL, 14.40mmol) was added over a period of 10 minutes followed by the addition of 2-dimethylaminoethanol (0.638g, 7.16mmol) in one portion. The mixture was stirred for 1 hour at 0°C, then, was warmed to 60°C and stirred for 120 hours. The reaction mixture was concentrated in vacuo and the residue was dissolved in n-heptane (100mL). The solution was washed with MeOH/water (5:1, 2 x 100mL), MeOH/5.6% aq. citric acid (10:1). The organic phase was separated, the aq. Citric acid / MeOH phase was extracted with n-heptane (5 x 50mL). To the MeOH / aq. citric acid phase was added n-heptane (200mL) and 4.6% aq. Na₂CO₃ (100mL), and the organic phase was separated, washed with MeOH/water (5:1, 100mL). The organic phase was separated, dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo to yield CICAL-207 (4.00g, 84.1% purity by HPLC) which was further purified by reverse phase prep-HPLC. CICAL-207 was dissolved in CH₃CN (10mL), and purified by chromatography (Xselect column CSH Phenyl-Hexyl 19x250mm, 5μM; Mobile Phase A: water/0.1% TFA; Mobile Phase B: CH₃CN; flow rate 20mL/min; Gradient 65% B to 85% B in 10 minutes; Wave Length 200nm; RT (min): 9.5). Qualified fractions were concentrated in vacuo, to remove CH₃CN, and the pH of the aq. Residue was adjusted to 7.0 with satd. Aq. Na₂CO₃. The aq. Phase was extracted with n-heptane (3 x 100mL), and the combined organic phases were dried over Na₂SO₄. Filtration and concentration in vacuo gave CICAL-207 (2.11g, 2.06mmol, 99% purity by HPLC, 43% yield) as a clear, colorless, viscous oil.

[00492] ¹H-NMR (300MHz, CDCl₃): δ = 5.29 (m, 1H), 4.18-4.40 (5H), 4.11 (m, 8H), 3.60 (m, 2H), 2.48-2.70 (4H), 2.02-2.45 (20H), 1.61 (m, 8H), 1.19-1.39 (40H), 0.88 (m, 12H); LCMS: RT 1.127, Calcd. for C₅₆H₁₀₀N₂O₁₄ + H⁺ 1025.73. Found 1025.80.

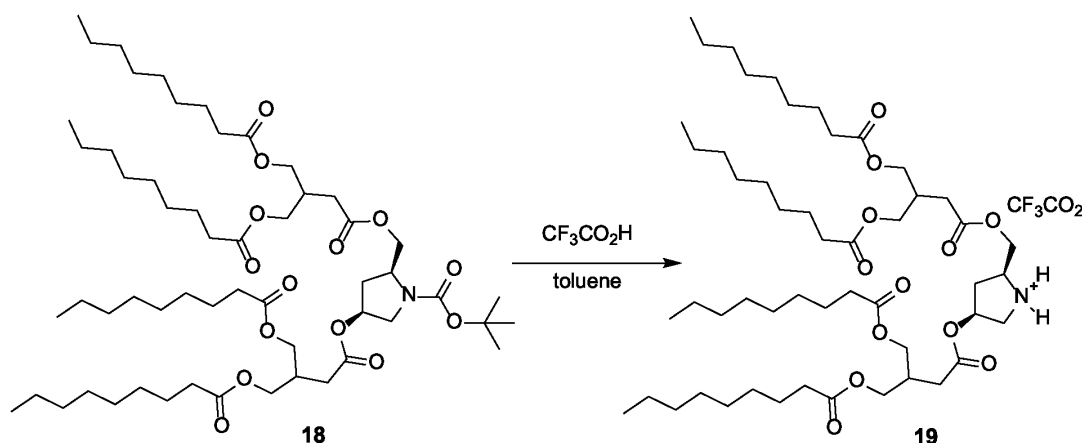
Example 18: Synthesis of 2-(2-(((2S,4S)-1-(tert-butoxycarbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (18)



[00493] To a solution of 17 (2.00g, 9.21mmol, ACS Med. Chem. Lett. 2014, 5, 56) in CH₃CN (115mL), at room temperature under nitrogen, was added in order: 2 (7.63g, 18.42mmol), DMAP (1.14g, 9.33mmol), and EDC-HCl (3.89g, 20.40mmol). The mixture was stirred for 18 hours at room temperature, then the reaction was quenched by the addition of water (115mL). The resulting mixture was cast into n-heptane (200mL) and the organic phase was separated. The organic layer was washed with MeOH/10% aq. Citric acid (75:25, 2 x 200mL), MeOH/water/Et₃N (70:22:8, 2 x 100mL), MeOH/water (75:25, 21x 100mL), brine (100mL), and dried over Na₂SO₄. Filtration and concentration in vacuo gave 18 (6.65g, 6.58mmol, 93% HPLC purity, 71.5% yield) as a pale-yellow oil.

[00494] ¹H-NMR (400MHz, CDCl₃): δ = 5.33 (m, 1H), 4.18-4.40 (2H), 4.02-4.18 (10H), 3.72 (m, 1H), 3.43 (m, 1H), 2.58 (m, 2H), 2.43 (m, 4H), 2.20-2.37 (10H), 1.62 (m, 8H), 1.50 (m, 8H), 1.22-1.40 (40H), 0.89 (t, J = 6.8Hz, 12H); **LCMS**: RT 1.997, Calcd. for C₅₆H₉₉NO₁₄ + Na⁺ 1032.70. Found 1032.70.

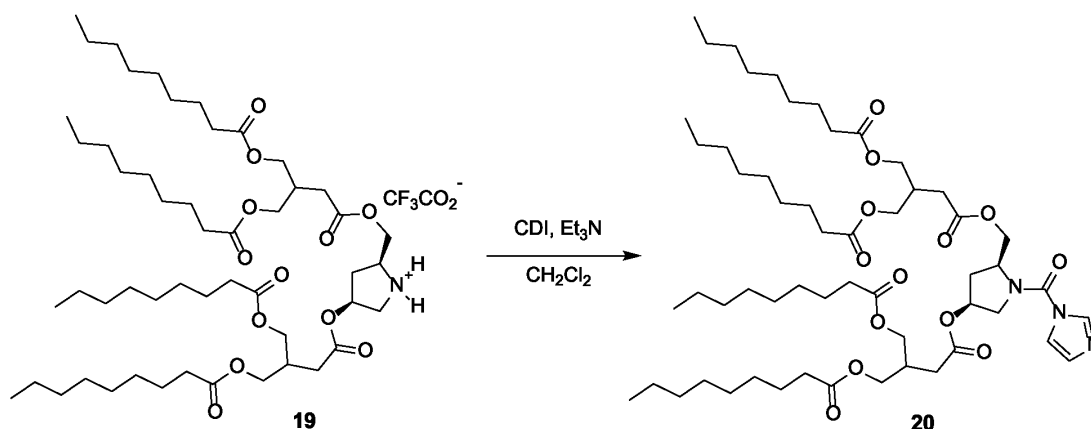
Example 19: Synthesis of (2S,4S)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)-2-(((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)methyl)pyrrolidin-1-ium trifluoroacetate (19)



[00495] To a solution of 18 (6.50g, 6.43mmol) in toluene (65mL), at room temperature under nitrogen, was added TFA (20mL, 29.78g, 261mmol) over a period of 15 minutes. After the addition was complete, the mixture was stirred at room temperature for 18 hours, then was concentrated in vacuo to give crude 19. Crude ammonium trifluoroacetate salt 19 was dissolved in n-heptane (200mL) and the resulting solution was washed with brine (2 x 200mL) and dried over Na₂SO₄. Filtration and concentration in vacuo provided 19 (6.30g, 93% HPLC purity, 6.24mmol, 97% yield) as a viscous, yellow oil.

[00496] ¹H-NMR (400MHz, CDCl₃): δ = 10.44 (brs, 1H), 9.98 (brs, 1H), 5.31 (m, 1H), 4.42 (m, 4H), 4.02-4.21 (8H), 3.60 (m, 2H), 2.37-2.60 (4H), 2.35 (m, 8H), 2.10 (m, 1H), 1.62 (m, 8H), 1.21-1.38 (40H), 0.89 (t, *J* = 6.8Hz, 12H); **LCMS**: RT 1.622, Calcd. for C₅₁H₉₂NO₁₂ 910.66. Found 910.60.

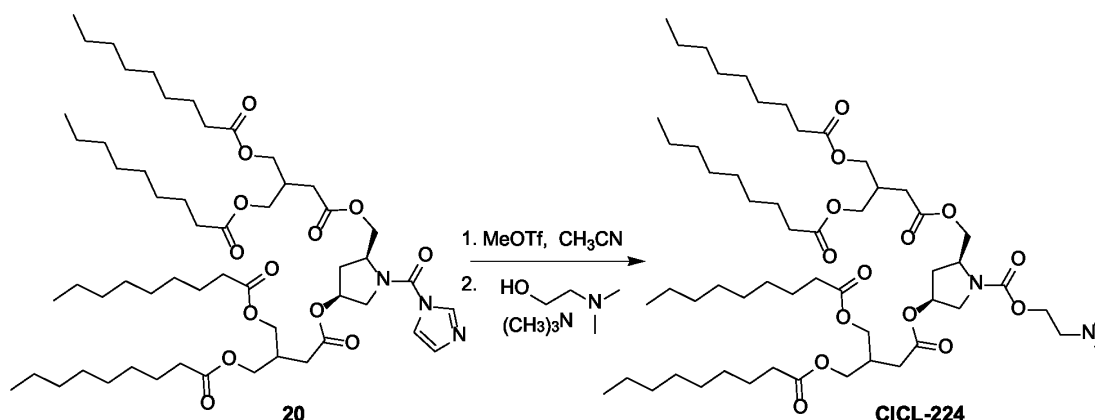
Example 20: Synthesis of 2-(2-(((2S,4S)-1-(1H-imidazole-1-carbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (20)



[00497] To a solution of 19 (6.20g, 6.20mmol) in CH_2Cl_2 (100mL), at room temperature under nitrogen, was added CDI (9.96g, 61.50mmol) and Et_3N (3.11g, 30.80mmol). The resulting mixture was stirred for 18 hours at room temperature, then was cast into 0.5M aq. HCl (100mL). The organic phase was separated, then was washed with 0.5M aq. HCl (100mL), brine (2 x 100mL), and dried over Na_2SO_4 . Filtration and concentration in vacuo afforded crude 20 which was dissolved in CH_3CN and purified by reverse phase prep-HPLC (Column: XB Phenyl 50x250mm, 10m; Mobile Phase A: water (0.1% $\text{CF}_3\text{CO}_2\text{H}$), Mobile Phase B: CH_3CN ; Flow Rate: 90mL/min; Gradient: 50% B to 85% B over 10 minutes, 85% collected; Wave Length: 220nm). Qualified fractions were pooled, the acetonitrile was removed in vacuo and n-heptane (150mL) was added to the resulting mixture. The organic phase was separated, washed with 5% aq. NaHCO_3 (200mL) and dried (Na_2SO_4). Filtration and concentration in vacuo gave 20 (4.95g, 4.93mmol, 95% purity by HPLC, 80% yield) as a light-yellow oil.

[00498] ¹H-NMR (400MHz, CDCl₃): δ = 8.07 (s, 1H), 7.36 (s, 1H), 7.12 (s, 1H), 5.31 (m, 1H), 4.72 (m, 1H), 4.33 (m, 2H), 4.22 (m, 1H), 4.08-4.18 (7H), 4.02 (m, 1H), 3.78 (m, 1H), 2.58 (m, 2H), 2.34-2.51 (5H), 2.26-2.31 (8H), 2.08 (m, 1H), 1.61 (m, 8H), 1.21-1.37 (40H), 0.89 (t, *J* = 6.6Hz, 12H); **LCMS**: RT 1.735, Calcd. for C₅₅H₉₃N₃O₁₃ + H⁺ 1004.68. Found 1004.60.

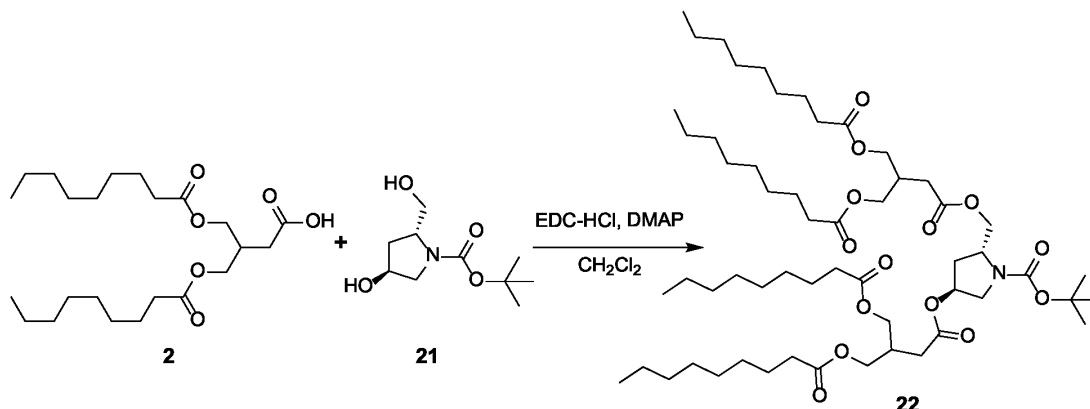
Example 21: Synthesis of 2-(2-(((2S,4S)-1-((2-(dimethylamino)ethoxy)carbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (CICL-224)



[00499] To a solution of 20 (4.00g, 4.00mmol), in CH₃CN (80mL), cooled in an ice-water bath under nitrogen was added MeOTf (0.784g, 4.80mmol) over a period of 5 minutes. The mixture was allowed to stir for 2 hours at 0°C, then a solution of trimethylamine in THF (2.0M, 6.00mL, 12.00mmol) was added over a period of 10 minutes followed by the addition of 2-dimethylaminoethanol (0.532g, 6.00mmol) in one portion. The mixture was stirred for 1 hour at 0°C, then, was warmed to 60°C and stirred for 72 hours. The reaction mixture was cast into n-heptane (160mL) and water (160mL). The organic phase was separated, washed with MeOH/water (4:1, 3 x 100mL), MeOH/5% aq. Citric acid (10:1). The organic phase was separated, the aq. citric acid / MeOH phase was diluted with 10% aq. NaCl (320mL) and extracted with n-heptane (5 x 160mL). The combined organic phases were washed with 5% aq. NaHCO₃ (2 x 100mL), and the organic phase was separated and washed with water (2 x 100mL). The organic phase was separated, dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo and the residue was dissolved in CH₃CN (10mL), and the crude product was purified by preparative reverse phase HPLC (XB Phenyl column CSH Phenyl-Hexyl 50x250mm, 10µM; Mobile Phase A: water/0.1% TFA; Mobile Phase B: CH₃CN; flow rate 90mL/min; Gradient 60% B to 90% B in 10 minutes; Wave Length: 200nm; RT (min): 12). Qualified fractions were concentrated in vacuo, to remove CH₃CN, and the pH of the aq. residue was adjusted to 9.0 with 5% aq. NaHCO₃. The aq. phase was extracted with n-heptane (200mL), and the organic phase was washed with MeOH/water (5:1, 100mL), water (100mL) and dried (Na₂SO₄). Filtration and concentration in vacuo gave C1C2-224 (1.50g, 1.46mmol, 97% purity by HPLC, 37% yield) as a clear, colorless, viscous oil.

[00500] ¹H-NMR (400MHz, CDCl₃): δ = 5.32 (m, 1H), 4.03-4.38 (13H), 3.78 (brm, 1H), 3.48 (brd, *J* = 12.8Hz, 1H), 2.55 (m, 4H), 2.43 (m, 4H), 2.20-2.37 (14H), 2.07 (m, 2H), 1.61 (m, 8H), 1.21-1.36 (40H), 0.88 (m, 12H); **LCMS:** RT 1.588; Calcd. for C₅₈H₁₀₀N₂O₁₄ + H⁺ 1025.73. Found 1025.60.

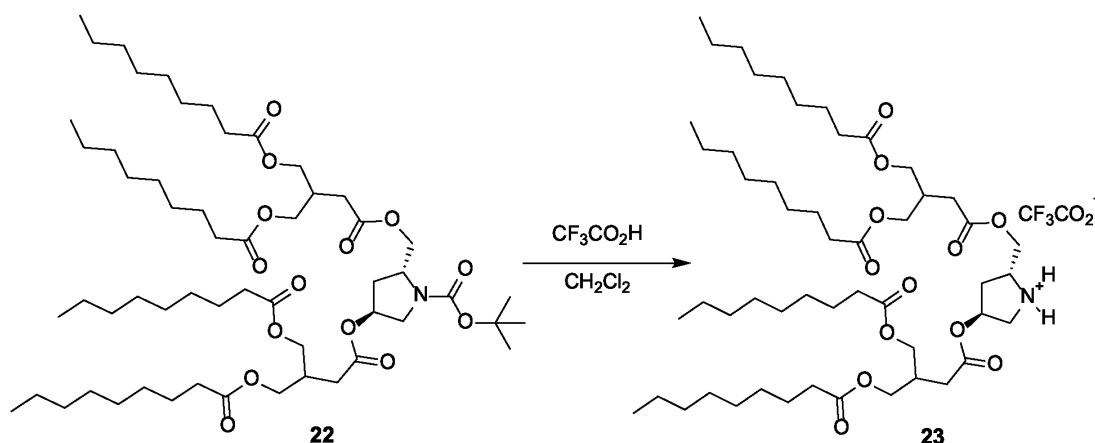
Example 22: Synthesis of 2-(2-(((2R,4S)-1-(tert-butoxycarbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (22)



[00501] To a solution of 21 (2.20g, 10.10mmol, ACS Med. Chem. Lett. 2014, 5, 56) in CH_2Cl_2 (160mL), at room temperature under nitrogen, was added in order: 2 (8.40g, 20.30mmol), DMAP (1.25g, 10.10mmol), and EDC-HCl (4.28g, 22.30mmol). The mixture was stirred for 18 hours, then was concentrated in vacuo. The residue was dissolved in n-heptane (500mL), then the n-heptane solution was washed with MeOH/Water and dried over Na_2SO_4 (4:1, 3 x 150mL). Filtration afforded a solution of crude 22 to which was added silica gel (25g, Type: ZCX-2, 100-200 mesh) and the solvent was then removed in vacuo (temperature < 35°C). The resulting 22 impregnated silica gel was placed atop a combi-flash column charged with silica gel (250g, Type: ZCX-2, 100-200 mesh), and the combi-flash column was eluted with a gradient of n-heptane/ethyl acetate (100:0 to 90:10). Qualified fractions were combined and concentrated in vacuo to furnish 22 (8.30g, 8.21mmol, HPLC purity 98.5%, 81% yield as a pale-yellow oil

[00502] $^1\text{H-NMR}$ (400MHz, CDCl_3): δ = 5.26 (m, 1H), 4.00-4.30 (11H), 3.54 (m, 1H), 2.54 (m, 2H), 2.40 (m, 4H), 2.25-2.38 (7H), 2.17 (m, 2H), 1.63 (m, 8H), 1.50 (m, 8H), 1.21-1.38 (40H), 0.88 (m, 12H); **LCMS**: RT 2.478, Calcd. for $\text{C}_{56}\text{H}_{99}\text{NO}_{14} + \text{Na}^+$ 1032.70. Found 1032.70.

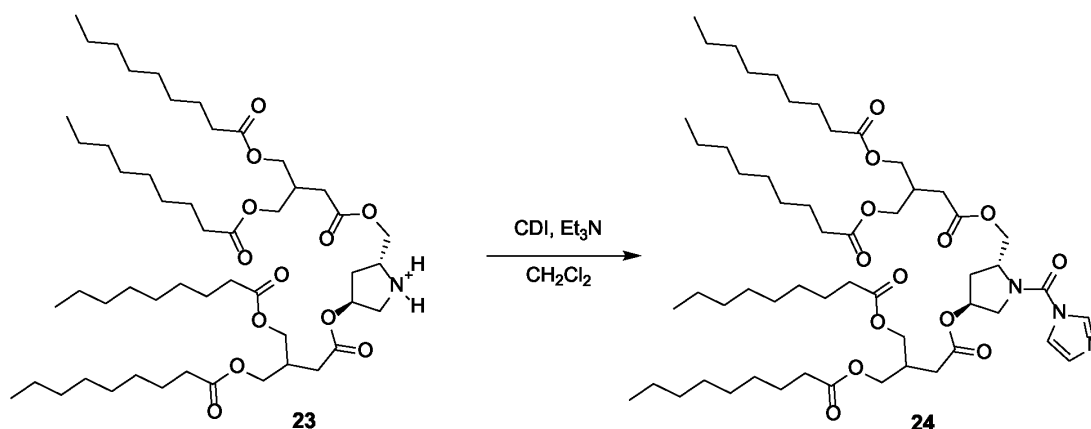
Example 23: Synthesis of (2R,4S)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)-2-(((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)methyl)pyrrolidin-1-ium trifluoroacetate (23)



[00503] To a solution of 22 (8.30g, 8.22mmol) in dichloromethane (50mL), at room temperature under nitrogen, was added TFA (16mL, 23.82g, 209mmol) over a period of 15 minutes. After the addition was complete, the mixture was stirred at room temperature for 3 hours, then was concentrated in vacuo to give crude 23. Crude ammonium trifluoroacetate salt 23 was dissolved in n-heptane (400mL) and the resulting solution was washed with brine (2 x 200mL) and dried over Na₂SO₄. Filtration and concentration in vacuo provided 23 (8.10g, 92% HPLC purity, 7.90mmol, 96% yield) as a viscous, light-yellow oil.

[00504] ¹H-NMR (400MHz, CDCl₃): δ = 10.44 (brs, 1H), 9.98 (brs, 1H), 5.40 (m, 1H), 4.65 (m, 1H), 4.37 (m, 1H), 4.13 (m, 8H), 3.78 (m, 1H), 3.49 (m, 1H), 2.40-2.62 (6H), 2.24-2.37 (10H), 1.61 (m, 8H), 1.21-1.34 (40H), 0.88 (m, 12H); **LCMS**: RT 1.612, Calcd. for C₅₁H₉₂NO₁₂ 910.66. Found 910.60.

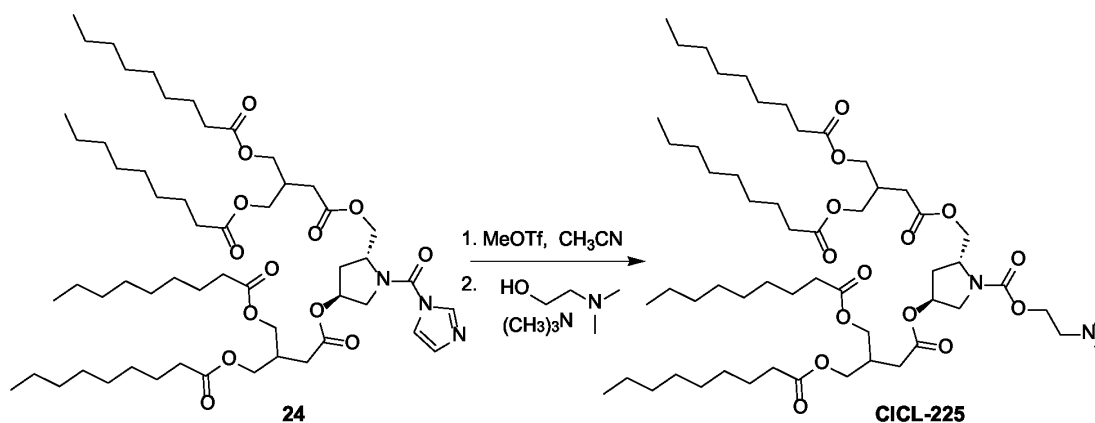
Example 24: Synthesis of 2-(2-(((2R,4S)-1-(1H-imidazole-1-carbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (24)



[00505] To a solution of 23 (8.10g, 8.03mmol) in CH_2Cl_2 (250mL), at room temperature under nitrogen, was added CDI (13.03g, 80.33mmol) and Et_3N (4.06g, 40.17mmol). The resulting mixture was stirred for 18 hours at room temperature, then was cast into 0.5M aq. HCl (300mL). The organic phase was separated, then was washed with 0.5M aq. HCl (300mL), brine (2 x 300mL), and dried over Na_2SO_4 . Filtration and concentration in vacuo afforded crude 24 which was dissolved in CH_3CN (30mL) and purified by reverse phase prep-HPLC (Column: XB Phenyl 50x250mm, 10m; Mobile Phase A: water (0.1% $\text{CF}_3\text{CO}_2\text{H}$), Mobile Phase B: CH_3CN ; Flow Rate: 90mL/min; Gradient: 50% B to 85% B over 10 minutes, 85% collected; Wave Length: 220nm). Qualified fractions were pooled, the acetonitrile was removed in vacuo and n-heptane (150mL) was added to the resulting mixture. The organic phase was separated, washed with 5% aq. NaHCO_3 (150mL) and dried (Na_2SO_4). Filtration and concentration in vacuo gave 24 which was dissolved in n-heptane (400mL) and the solution was extracted with MeOH/water (4:1, 150mL), and dried over Na_2SO_4 . Filtration and concentration in vacuo provided 24 (8.00g, 7.96mmol, 97.6% purity by HPLC, 99% yield) as a pale-yellow, viscous oil.

[00506] ¹H-NMR (300MHz, CDCl₃): δ = 8.14 (s, 1H), 7.37 (s, 1H), 7.14 (s, 1H), 5.36 (m, 1H), 4.54-4.75 (2H), 4.01-4.27 (9H), 3.90 (m, 1H), 3.76 (m, 1H), 2.57 (m, 2H), 2.20-2.49 (14H), 1.61 (m, 8H), 1.22-1.38 (40H), 0.90 (t, *J* = 7.20Hz, 12H); **LCMS**: RT 1.878, Calcd. for C₅₅H₉₃N₃O₁₃ + H⁺ 1004.68. Found 1004.60.

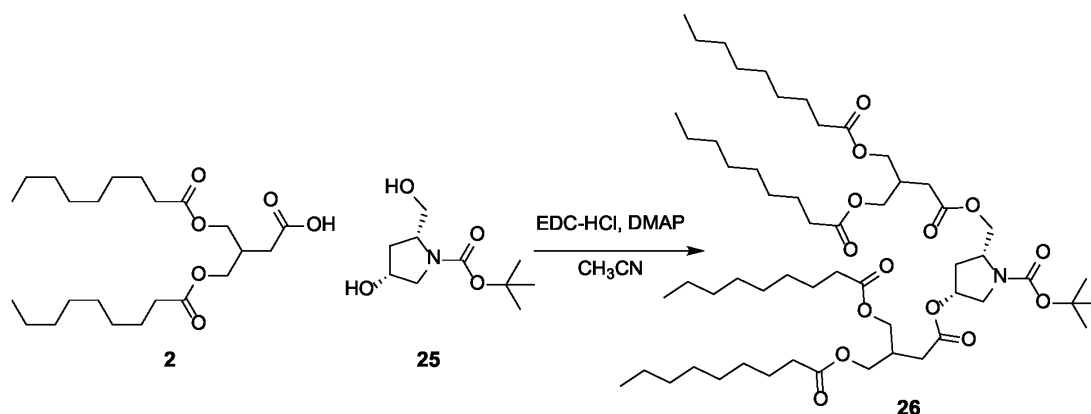
Example 25: Synthesis of 2-(2-(((2R,4S)-1-((2-(dimethylamino)ethoxy)carbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (CICL-225)



[00507] To a solution of 24 (8.00g, 7.97mmol), in CH₃CN (80mL), cooled in an ice-water bath under nitrogen was added MeOTf (1.57g, 9.57mmol) over a period of 5 minutes. The mixture was allowed to stir for 2 hours at 0°C, then a solution of trimethylamine in THF (2.0M, 12.00mL, 24.00mmol) was added over a period of 10 minutes followed by the addition of 2-dimethylaminoethanol (1.06g, 11.91mmol) in one portion. The mixture was stirred for 1 hour at 0°C, then, was warmed to 60°C and stirred for 120 hours. The reaction mixture was cast into n-heptane (400mL) and washed with MeOH/water (5:1, 2 x 150mL), the organic phase was then dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo and the residue was dissolved in CH₃CN (15mL), and the crude product was purified by preparative reverse phase HPLC (XB Phenyl column, 50x250mm, 10μM; Mobile Phase A: water/0.1% TFA; Mobile Phase B: CH₃CN; flow rate 90mL/min; Gradient 60% B to 90% B in 10 minutes; Wave Length: 200nm; RT (min): 12 Qualified fractions were concentrated in vacuo, to remove CH₃CN, and the pH of the aq. residue was adjusted to 9.0 with 5% aq. NaHCO₃. The aq. phase was extracted with n-heptane (300mL), and the organic phase was washed with MeOH/water (5:1, 200mL), water (200mL) and dried (Na₂SO₄). Filtration and concentration in vacuo gave C1CL-225 (2.017g, 1.97mmol, 97% purity by HPLC, 25% yield) as a clear, pale-yellow, viscous oil.

[00508] ¹H-NMR (400MHz, CDCl₃): δ = 5.30 (brm, 1H), 4.19-4.40 (5H), 4.12 (m, 8H), 3.62 (brm, 2H), 2.50-2.68 (4H), 2.42 (m, 4H), 2.27-2.38 (14H), 2.21 (brm, 2H), 1.63 (m, 8H), 1.20-1.38 (40H), 0.90 (t, *J* = 6.9Hz, 12H); **LCMS**: Calcd. for C₅₈H₁₀₀N₂O₁₄ + H⁺ 1025.73. Found 1025.60.

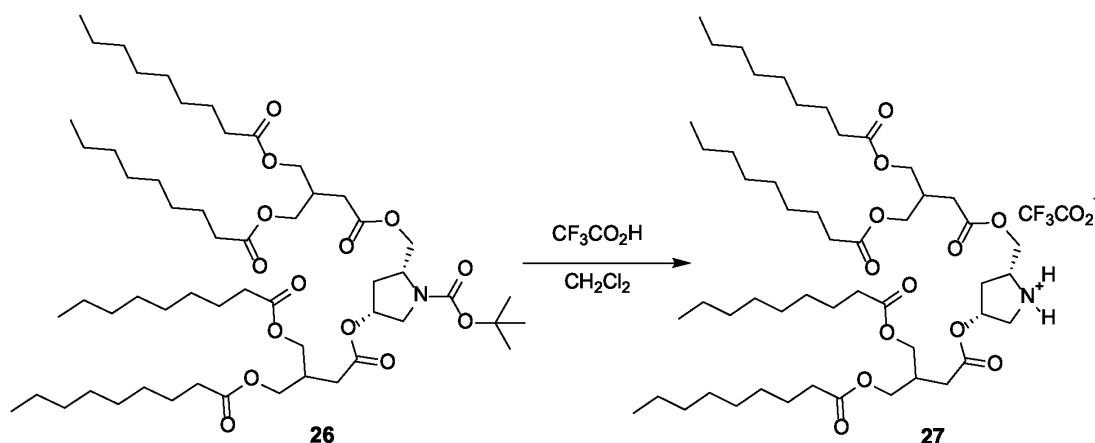
Example 26: Synthesis of 2-(2-(((2R,4R)-1-(tert-butoxycarbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (26)



[00509] To a solution of 25 (1.83g, 8.42mmol, ACS Med. Chem. Lett. 2014, 5, 56) in CH₃CN (700mL), at room temperature under nitrogen, was added in order: 2 (7.00g, 16.88mmol), DMAP (1.04g, 8.51mmol), and EDC-HCl (3.56g, 18.67mmol). The mixture was stirred for 18 hours, then the reaction was quenched by the addition of water (140mL). The mixture was extracted with n-heptane (140mL), and the organic phase was separated. The organic layer was washed with MeOH/10% aq. citric acid (3:1, 2 x 70mL), brine (70mL) and dried over Na₂SO₄. Filtration and concentration in vacuo provided 26 (5.65g, 5.59mmol, 92.7% purity by HPLC, 66% yield, as a pale-yellow oil.

[00510] ¹H-NMR (400MHz, CDCl₃): δ = 5.33 (m, 1H), 4.30 (m, 1H), 4.06-4.21 (12H), 3.75 (m, 1H), 3.41 (m, 1H), 2.62 (m, 2H), 2.43 (m, 4H), 2.24 (m, 1H), 2.32 (m, 8H), 1.62 (m, 8H), 1.51 (m, 8H), 1.25-1.40 (40H), 0.89 (m, 12H); LCMS: RT 1.608, Calcd. for C₅₆H₉₉NO₁₄ + Na⁺ 1032.70. Found 1032.70.

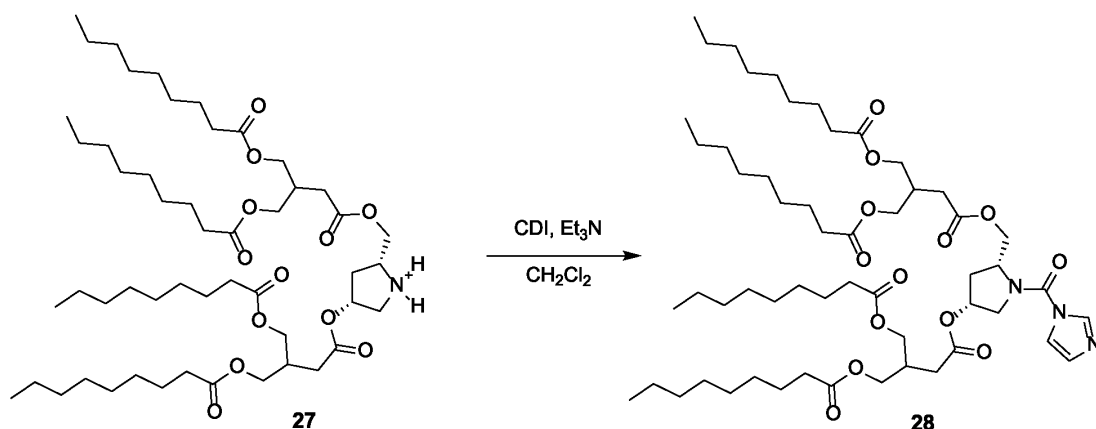
Example 27: Synthesis of (2R,4R)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)-2-(((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)methyl)pyrrolidin-1-ium trifluoroacetate (27)



[00511] To a solution of 26 (5.60g, 5.54mmol) in dichloromethane (35mL), at room temperature under nitrogen, was added TFA (11mL, 16.38g, 144mmol) over a period of 15 minutes. After the addition was complete, the mixture was stirred at room temperature for 18 hours, then was concentrated in vacuo to give crude 27. Crude ammonium trifluoroacetate salt 23 was dissolved in n-heptane (200mL) and the resulting solution was washed with brine (2 x 100mL) and dried over Na₂SO₄. Filtration and concentration in vacuo provided 27 (5.55g, 88% HPLC purity, 5.42mmol, 98% yield) as a viscous, light-yellow oil.

[00512] ¹H-NMR (400MHz, CDCl₃): δ = 10.21 (brs, 1H), 9.28 (brs, 1H), 5.45 (m, 1H), 4.38-4.52 (2H), 4.08-4.25 (9H), 3.50 (brs, 2H), 2.70 (m, 1H), 2.58 (m, 2H), 2.28-2.45 (12H), 2.14 (m, 1H), 1.63 (m, 8H), 1.22-1.37 (40H), 0.90 (m, 12H); **LCMS**: RT 1.602, Calcd. for C₅₁H₉₂NO₁₂ 910.66. Found 910.60.

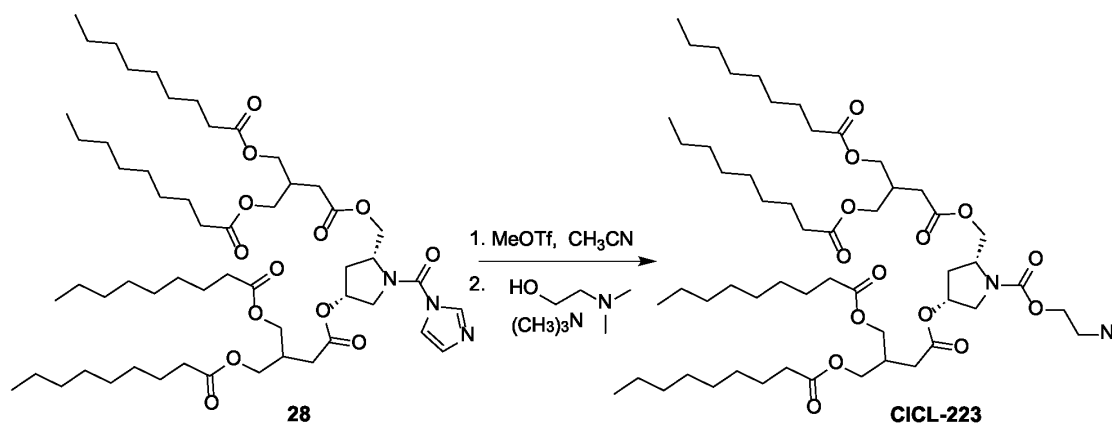
Example 28: Synthesis of 2-(2-(((2R,4R)-1-(1H-imidazole-1-carbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (28)



[00513] To a solution of 27 (5.55g, 5.42mmol) in CH_2Cl_2 (55mL), at room temperature under nitrogen, was added CDI (8.92g, 55.01mmol) and Et_3N (1.67g, 16.50mmol). The resulting mixture was stirred for 2 hours at room temperature, then was cast into 0.5M aq. HCl (40mL). The organic phase was separated, then was washed with 0.5M aq. HCl (2 x 40mL), water (3 x 20mL), and dried over Na_2SO_4 . Filtration and concentration in vacuo afforded crude 28 which was dissolved in CH_3CN (30mL) and purified by reverse phase prep-HPLC (Column: XB Phenyl 50x250mm, 10m; Mobile Phase A: water (0.1% $\text{CF}_3\text{CO}_2\text{H}$), Mobile Phase B: CH_3CN ; Flow Rate: 90mL/min; Gradient: 50% B to 85% B over 10 minutes, 85% collected; Wave Length: 220nm). Qualified fractions were pooled, the acetonitrile was removed in vacuo and n-heptane (150mL) was added to the resulting mixture. The organic phase was separated, washed with 5% aq. NaHCO_3 (150mL), water (150mL), and dried (Na_2SO_4). Filtration and concentration in vacuo gave 28 (3.75g, 3.73mmol, 96.5% purity by HPLC, 69% yield) as a pale-yellow, viscous oil.

[00514] $^1\text{H-NMR}$ (300MHz, CDCl_3): δ = 8.07 (s, 1H), 7.36 (s, 1H), 7.12 (s, 1H), 5.31 (m, 1H), 4.72 (m, 1H), 4.33 (m, 2H), 4.22 (m, 1H), 4.08-4.18 (7H), 4.02 (m, 1H), 3.78 (m, 1H), 2.58 (m, 2H), 2.34-2.51 (5H), 2.26-2.31 (8H), 2.08 (m, 1H), 1.61 (m, 8H), 1.21-1.37 (40H), 0.89 (t, J = 6.6Hz, 12H); **LCMS**: RT 1.868, Calcd. for $\text{C}_{55}\text{H}_{93}\text{N}_3\text{O}_{13} + \text{H}^+$ 1004.68. Found 1004.60.

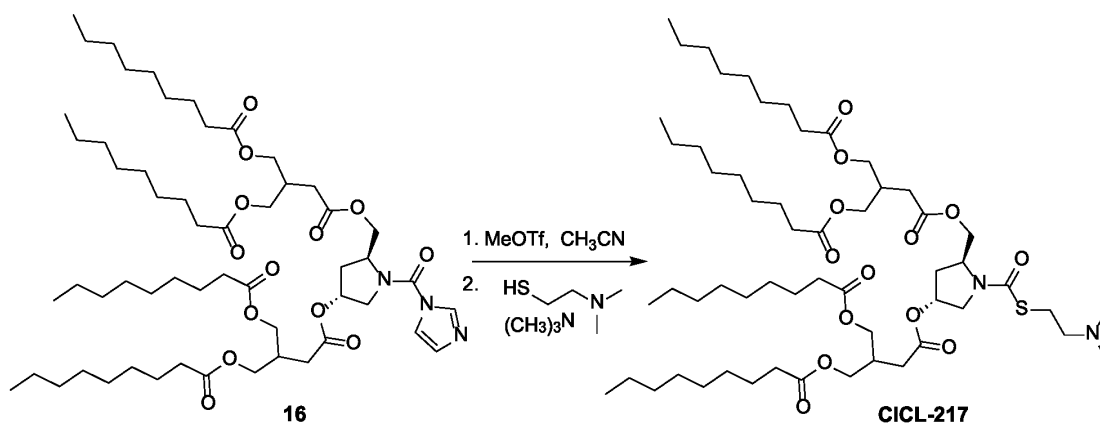
Example 29: Synthesis of 2-(2-(((2R,4R)-1-((2-(dimethylamino)ethoxy)carbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (CICL-223)



[00515] To a solution of 28 (3.75g, 3.73mmol), in CH₃CN (75mL), cooled in an ice-water bath under nitrogen was added MeOTf (0.73g, 4.45mmol) over a period of 5 minutes. The mixture was allowed to stir for 2 hours at 0°C, then a solution of trimethylamine in THF (2.0M, 5.60mL, 11.2mmol) was added over a period of 10 minutes followed by the addition of 2-dimethylaminoethanol (0.50g, 5.61mmol) in one portion. The mixture was stirred for 1 hour at 0°C, then, was warmed to 60°C and stirred for 100 hours. The reaction mixture was cast into n-heptane (200mL) and washed with 10% aq. Citric acid (100mL), water (300mL), brine (300mL), the organic phase was then dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo and the residue was dissolved in i-PrOH (5mL), and the crude product was purified by preparative reverse phase HPLC (XB Phenyl column, 50x250mm, 10µM; Mobile Phase A: water/0.1% TFA; Mobile Phase B: CH₃CN; flow rate 90mL/min; Gradient 55% B to 95% B in 32 minutes; Wave Length: 205nm; RT (min): 17). Qualified fractions were concentrated in vacuo, to remove CH₃CN, and the pH of the aq. residue was adjusted to 9.0 with 2% aq. Na₂CO₃. The aq. phase was extracted with n-heptane (3 x 300mL), and the organic phases were washed with MeOH/water (5:1, 2 x 300mL), water (300mL) and dried (Na₂SO₄). Filtration and concentration in vacuo gave C1C1-223 (1.38g, 1.35mmol, 98% purity by HPLC, 36% yield) as a clear, pale-yellow, viscous oil.

[00516] ¹H-NMR (300MHz, CDCl₃): δ = 5.33 (brm, 1H), 4.03-4.40 (13H), 3.79 (brm, 1H), 3.50 (brd, *J* = 13.2Hz, 1H), 2.52-2.68 (4H), 2.46 (m, 4H), 2.20-2.34 (14H), 2.07 (brm, 2H), 2.63 (m, 8H), 1.21-1.40 (40H), 0.90 (m, 12H); **LCMS**: RT 0.905, Calcd. for C₅₈H₁₀₀N₂O₁₄ + H⁺ 1025.73. Found 1025.60.

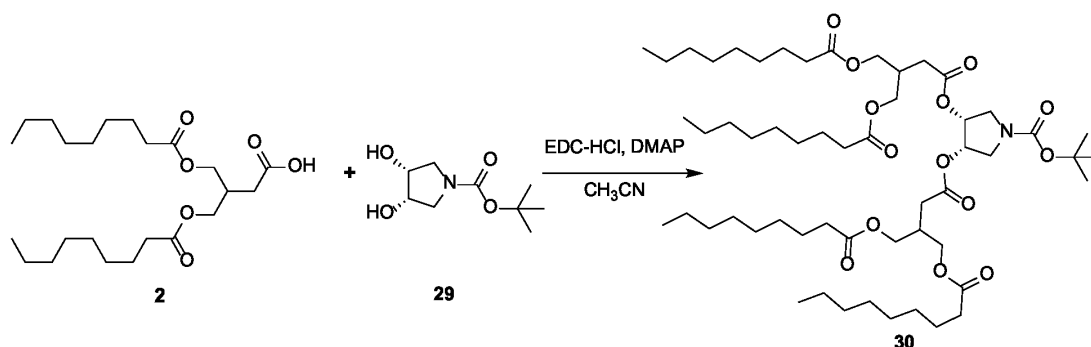
Example 30: Synthesis of 2-(2-(((2S,4R)-1-(((2-(dimethylamino)ethyl)thio)carbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (CICL-217)



[00517] To a solution of 16 (7.00g, 6.83mmol), in CH₃CN (70mL), cooled in an ice-water bath under nitrogen was added MeOTf (1.37g, 8.35mmol) over a period of 5 minutes. The mixture was allowed to stir for 2 hours at 0°C, then a solution of trimethylamine in THF (2.0M, 10.50mL, 21.00mmol) was added over a period of 10 minutes followed by the addition of 2-dimethylaminoethane thiol (1.10g, 10.46mmol) in one portion. The mixture was stirred for 1 hour at 0°C, then, was warmed to room temperature and was stirred for 18 hours. The reaction mixture concentrated in vacuo, the residue was dissolved with n-heptane (400mL) and the resulting solution was washed with MeOH/water (5:1, 2 x 150mL), the organic phase was then dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo and the residue was dissolved in CH₃CN (15mL), and the crude product was purified by preparative reverse phase HPLC (XB Phenyl column, 50x250mm, 10μm; Mobile Phase A: water/0.1% TFA; Mobile Phase B: CH₃CN; flow rate 90mL/min; Gradient 50% B to 90% B in 18 minutes; Wave Length: 200nm). Qualified fractions were concentrated in vacuo, to remove CH₃CN, and the pH of the aq. residue was adjusted to 8.0 with 2% aq. Na₂CO₃. The aq. phase was extracted with n-heptane (300mL), and the organic phase was washed with MeOH/water (5:1, 200mL), water (200mL) and dried (Na₂SO₄). Filtration and concentration in vacuo gave C1CL-217 (2.11g, 2.03mmol, 98% purity by HPLC, 30% yield) as a clear, pale-yellow, viscous oil.

[00518] ¹H-NMR (300MHz, CDCl₃): δ = 5.35 (m, 1H), 4.42 (m, 2H), 4.26 (m, 1H), 4.11 (m, 8H), 3.66 (brm, 2H), 3.13 (m, 2H), 2.49-2.71 (4H), 2.18-2.48 (20H), 1.63 (m, 8H), 1.20-1.39 (40H), 0.88 (t, J = 6.9Hz, 12H); LCMS: RT 1.638, Calcd. for C₅₆H₁₀₀N₂O₁₃S + H⁺ 1041.70. Found 1041.60.

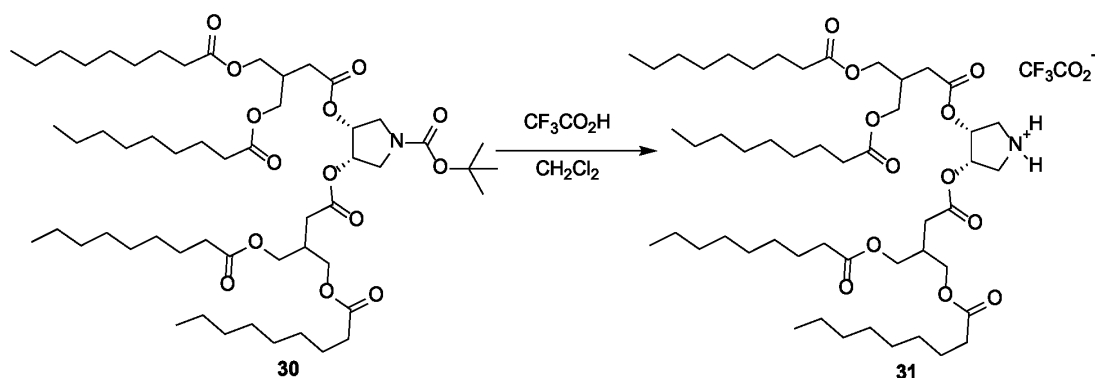
Example 31: Synthesis of (((*rel*-(3S,4R)-1-(tert-butoxycarbonyl)pyrrolidine-3,4-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (30)



[00519] To a solution of 29 (Nature Communications 2019, 10, 21, 2.20g, 10.84mmol), in CH₃CN (44mL) under nitrogen at room temperature, was added in order 2 (9.20g, 22.22mmol), DMAP (1.32g, 10.84mmol), and EDC-HCl (5.20g, 27.09mmol). The mixture was stirred for 18 hours at room temperature after the additions were complete, then the solvent was removed in vacuo. The residue was dissolved in n-heptane (440mL) and the solution was washed with MeOH/water (8:1, 2 x 110mL). The solvent was removed in vacuo to provide crude 30 which was purified by reverse phase prep-HPLC (Column: XB Phenyl 50x250mm, 10m; Mobile Phase A: water (0.1% CF₃CO₂H), Mobile Phase B: CH₃CN; Flow Rate: 90mL/min; Gradient: 50% B to 95% B over 10 minutes, 85% collected; Wave Length: 205nm). Qualified fractions were pooled, the acetonitrile was removed in vacuo and the resulting solution was extracted with n-heptane (440mL). The organic phase was washed with MeOH/water (4:1, 2 x 110mL), water (110mL), and dried (Na₂SO₄). Filtration and concentration in vacuo gave 30 (6.80g, 6.82mmol, HPLC purity 97%, 63% yield) as a viscous, colorless oil.

[00520] ¹H-NMR (400MHz, CDCl₃): δ = 5.37 (brn, 2H), 4.13 (m, 8H), 3.73 (m, 2H), 3.28-3.51 (2H), 2.58 (m, 2H), 2.45 (m, 4H), 2.32 (t, J = 6.0Hz, 8H), 1.63 (m, 8H), 1.49 (s, 9H), 1.21-1.40 (40H), 0.90 (t, J = 6.8Hz, 12H); LCMS: RT 1.991, Calcd. for C₅₅H₉₇NO₁₄ + Na⁺ 1018.68. Found 1018.60.

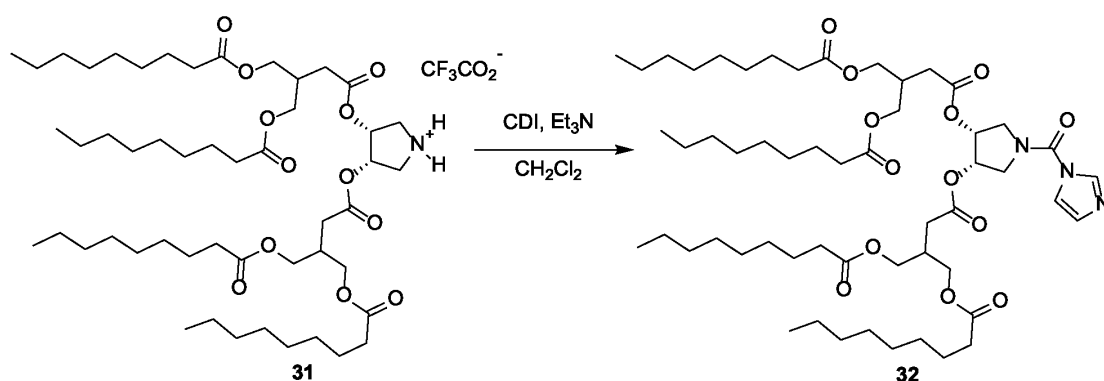
Example 32: Synthesis of *Rel*-(3*S*,4*R*)-3,4-bis((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-1-ium trifluoroacetate (31)



[00521] To a solution of 30 (6.60g, 6.63mmol), in CH_2Cl_2 (33mL) under nitrogen at room temperature, was added $\text{CF}_3\text{CO}_2\text{H}$ (13.2mL, 19.65g, 172mmol) over a period of 10 minutes. The reaction mixture was allowed to stir for 2 hours after the addition was complete, and then was concentrated in vacuo. The residue was dissolved in n-heptane (660mL) and the solution was extracted with brine (2 x 110mL). The organic phase was concentrated in vacuo to afford 31 (6.20g, 6.14mmol, HPLC purity 97%, 93% yield) as a viscous, light-yellow oil.

[00522] **¹H-NMR (400MHz, CDCl₃):** δ = 5.48 (brm, 2H), 4.10-4.23 (8H), 3.72 (m, 2H), 3.49 (m, 2H), 2.56 (m, 2H), 2.47 (m, 4H), 2.32 (m, 8H), 1.63 (m, 8H), 1.23-1.37 (40H), 0.90 (t, *J* = 6.8Hz, 12H); **LCMS:** RT 1.601, Calcd. for C₅₀H₉₀NO₁₂ 896.65. Found 896.60.

Example 33: Synthesis of (((*Rel*-(3*S*,4*R*)-1-(1*H*-imidazole-1-carbonyl)pyrrolidine-3,4-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (32)

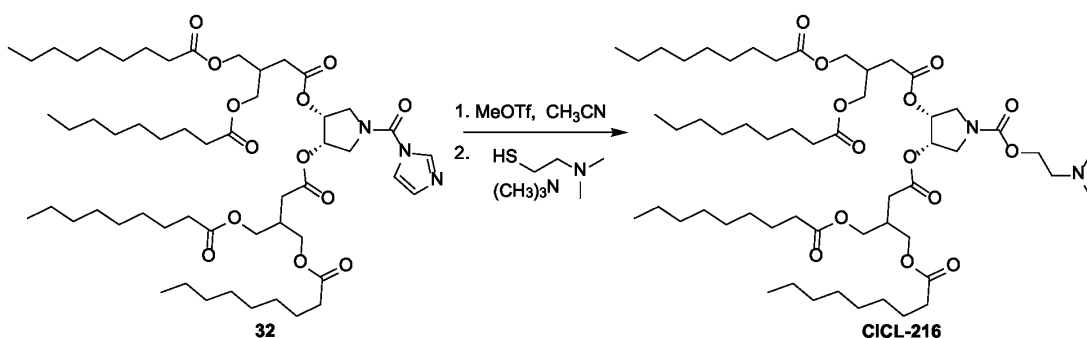


[00523] To a solution of 31 (6.00g, 6.04mmol) in dichloromethane (120mL), at room temperature under nitrogen, was added in order: CDI (9.78g, 60.36mmol) and Et₃N (4.88g, 48.29mmol). The reaction mixture was stirred for 18 hours at room temperature, then was cast

into 0.5M aq. HCl (200mL). The organic phase was separated, the aq. phase was extracted with dichloromethane (2 x 240mL) and the combined organic layers were concentrated in vacuo to yield crude 32. Crude 32 was dissolved in ne-heptane (600mL) and the resulting solution was extracted with MeOH/water (5:1, 2 x 240mL) and water (240mL). The solvent was removed in vacuo to afford 32 (5.60g, 5.65mmol, HPLC purity 96%, 94% yield) as a light-yellow oil.

[00524] ¹H-NMR (400MHz, CDCl₃): δ = 8.27 (brn, 1H), 7.44 (brs, 1H), 7.18 (brs, 1H), 5.48 (brn, 2H), 3.92-4.23 (10H), 3.82 (brn, 2H), 2.57 (m, 2H), 2.46 (m, 4H), 2.32 (m, 8H), 1.63 (m, 8H), 1.23-1.37 (40H), 0.90 (t, *J* = 6.8Hz, 12H); **LCMS**: RT 1.760, Calcd. for C₅₄H₉₁N₃O₁₃ + H⁺ 990.66. Found 990.50.

Example 34: Synthesis of (((*Rel*-(3*S*,4*R*)-1-((2-(dimethylamino)ethoxy)carbonyl)pyrrolidine-3,4-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-216)

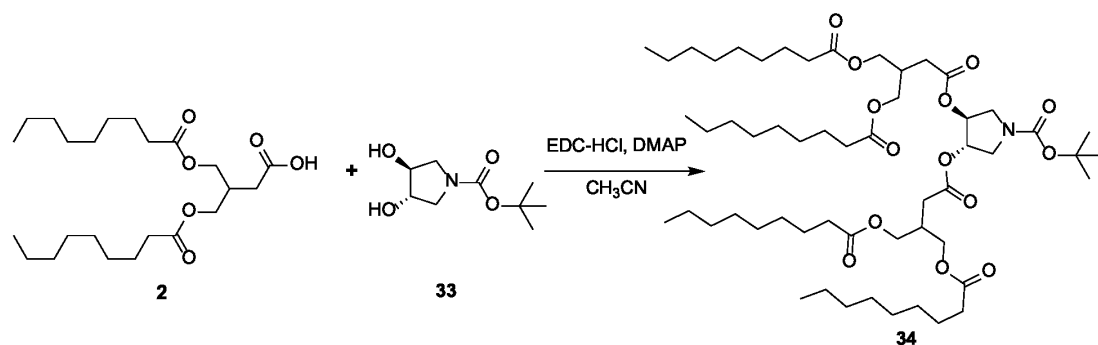


[00525] To a solution of 32 (5.50g, 5.55mmol), in CH₃CN (55mL), cooled in an ice-water bath under nitrogen was added MeOTf (1.09g, 6.66mmol) over a period of 5 minutes. The mixture was allowed to stir for 2 hours at 0°C, then a solution of trimethylamine in THF (1.0M, 22.20mL, 22.20mmol) was added over a period of 10 minutes followed by the addition of 2-dimethylaminoethanol (0.743g, 8.33mmol) in one portion. The mixture was stirred for 1 hour at 0°C, then, was warmed in a 60°C oil bath and was stirred for 120 hours. The reaction mixture concentrated in vacuo, the residue was dissolved with n-heptane (550mL) and the resulting solution was washed with MeOH/water (5:1, 2 x 100mL), acetonitrile/water (5:1, 2 x 100mL), then the organic phase was then dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo and the residue was dissolved in CH₃CN (15mL), and the crude product was purified by preparative reverse phase HPLC (XB Phenyl column, 50x250mm, 10μM;

Mobile Phase A: water/0.1% TFA; Mobile Phase B: CH₃CN; flow rate 90mL/min; Gradient 55% B to 90% B in 12 minutes; Wave Length: 205nm). Qualified fractions were concentrated in vacuo, to remove CH₃CN, and the pH of the aq. residue was adjusted to 9.0 with 2% aq. Na₂CO₃. The aq. phase was extracted with n-heptane (2 x 275mL), and the combined organic phases were washed with MeOH/water (4:1, 3 x 110mL), water (145mL) and dried (Na₂SO₄). Filtration and concentration in vacuo gave C1CL-216 (2.22g, 2.20mmol, 98% purity by HPLC, 40% yield) as a clear, colorless, viscous oil.

[00526] ¹H-NMR (400MHz, CDCl₃): δ = 5.37 (m, 2H), 4.20 (m, 2H), 4.11 (m, 8H), 3.78 (m, 2H), 3.48 (m, 2H), 2.50-2.65 (4H), 2.42 (m, 4H), 2.25-2.35 (14H), 1.59 (m, 8H), 1.20-1.38 (40H), 0.88 (m, 12H); **LCMS**: RT 1.575, Calcd. for C₅₅H₉₈N₂O₁₄ + H⁺ 1011.71. Found 1011.70.

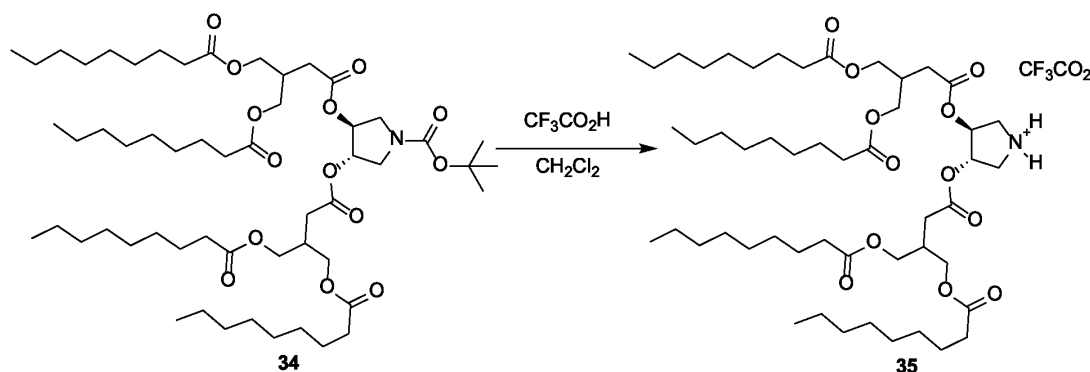
Example 35: Synthesis of (((((3S,4S)-1-(tert-butoxycarbonyl)pyrrolidine-3,4-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (34)



[00527] To a solution of 33 (Combi-Blocks #QE-0378, 2.35g, 11.56mmol) in acetonitrile (94mL), at room temperature under nitrogen, was added in order: 2 (10.00g, 24.12mmol), DMAP (1.42g, 11.62mmol), and EDC-HCl (5.55g, 29.10mmol). The reaction mixture was stirred for 16 hours at room temperature then was concentrated in vacuo. The residue was dissolved in n-heptane (200mL) and the resulting solution was extracted with MeOH/water (10:1, 2 x 100mL). The solvent was removed in vacuo and the residue was dissolved in dichloromethane (25mL) and silica gel (25g, Type: ZCX-2, 100-200 mesh) was added. The solvent was then removed in vacuo (temperature < 35°C). The resulting 34 impregnated silica gel was placed atop a combi-flash column charged with silica gel (120g, Type: ZCX-2, 100-200 mesh), and the combi-flash column was eluted with a gradient of n-heptane/ethyl acetate (40:1 to 25:1). Qualified fractions were combined and concentrated in vacuo to furnish 34 (8.20g, 8.23mmol, HPLC purity 95%, 71% yield as a pale-yellow oil.

[00528] $^1\text{H-NMR}$ (300MHz, CDCl_3): δ = 5.20 (brn, 2H), 4.13 (m, 8H), 3.73 (m, 2H), 3.40-3.62 (2H), 2.58 (m, 2H), 2.42 (m, 4H), 2.37 (m, 8H), 1.62 (m, 8H), 1.50 (s, 9H), 1.24-1.38 (40H), 0.90 (t, J = 6.8Hz, 12H); **LCMS**: RT 2.144, Calcd. for $\text{C}_{55}\text{H}_{97}\text{NO}_{14}$ + Na^+ 1018.68. Found 1018.80.

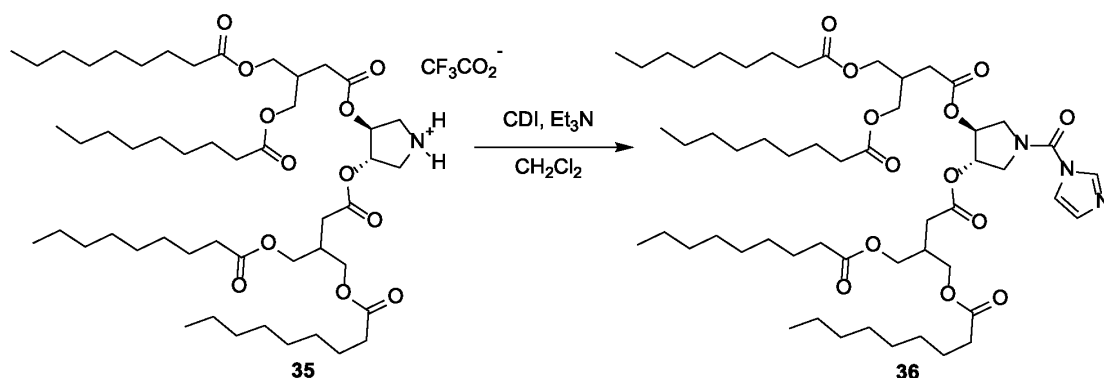
Example 36: Synthesis (3S,4S)-3,4-bis((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-1-ium trifluoroacetate (35)



[00529] To a solution of 34 (8.00g, 8.03mmol), in CH_2Cl_2 (80mL) under nitrogen at room temperature, was added $\text{CF}_3\text{CO}_2\text{H}$ (20mL, 29.78g, 261mmol) over a period of 15 minutes. The reaction mixture was allowed to stir for 3 hours after the addition was complete, then the solution was cast into 6.5% aq. K_2HPO_4 (160mL). The organic phase was separated, washed with brine (2 x 80mL), and dried over Na_2SO_4 . Filtration and concentration in vacuo provided 35 (7.60g, 7.52mmol, HPLC purity 94%, 94% yield) as a light-yellow oil.

[00530] $^1\text{H-NMR}$ (300MHz, CDCl_3): δ = 10.48 (brs, 2H), 5.35 (M, 2H), 4.18 (m, 8H), 3.75 (m, 2H), 3.62 (m, 2H), 2.42-2.62 (6H), 2.35 (m, 8H), 1.62 (m, 8H), 1.21-1.33 (40H), 0.90 (t, J = 6.8Hz, 12H); **LCMS**: RT 1.59, Calcd. for $\text{C}_{50}\text{H}_{90}\text{NO}_{12}$ 895.65. Found 896.6.

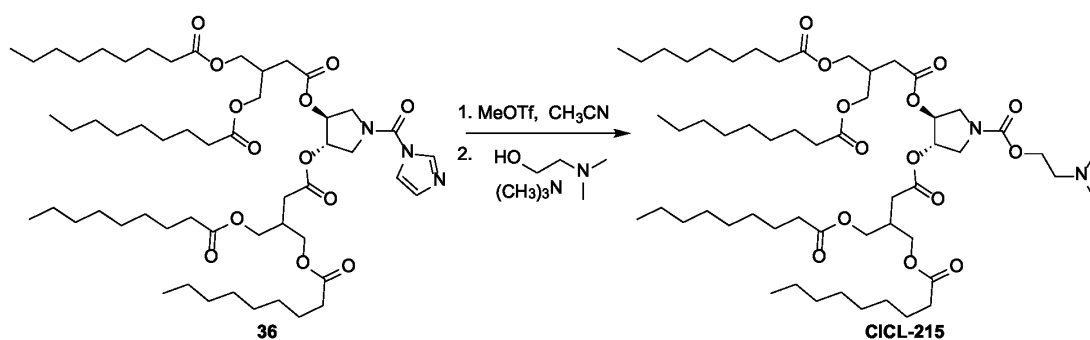
Example 37: Synthesis (((3S,4S)-1-(1H-imidazole-1-carbonyl)pyrrolidine-3,4-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl)bis(propane-2,1,3-triyl) tetranonanoate (36)



[00531] To a solution of 35 (7.50g, 7.42mmol) in dichloromethane (150mL), at room temperature under nitrogen, was added in order: CDI (12.22g, 75.36mmol) and Et₃N (6.10g, 60.28mmol). The reaction mixture was stirred for 16 hours at room temperature, then was cast into 0.5M aq. HCl (150mL). The organic phase was separated, washed with brine (150mL) and dried (Na₂SO₄). Filtration and concentration in vacuo afforded 36 which was dissolved in n-heptane (300mL), the organic phase was washed with MeOH/water (4:1, 75mL), water (75mL), and dried (Na₂SO₄). Filtration and concentration in vacuo gave 36 (7.00g, 7.07mmol, 93% purity by HPLC, 95% yield) as a pale-yellow oil.

[00532] ¹H-NMR (400MHz, CDCl₃): δ = 8.11 (brs, 1H), 7.42 (brs, 1H), 7.13 (brs, 1H), 5.29 (m, 2H), 4.06-4.25 (10H), 3.82 (m, 2H), 2.60 (m, 2H), 2.42 (m, 4H), 2.33 (m, 8H), 1.62 (m, 8H), 1.23-1.38 (40H), 0.90 (t, J = 6.9Hz, 12H); **LCMS**: RT 1.755, Calcd. for C₅₄H₉₁N₃O₁₃ + H⁺ 990.66. Found 990.60.

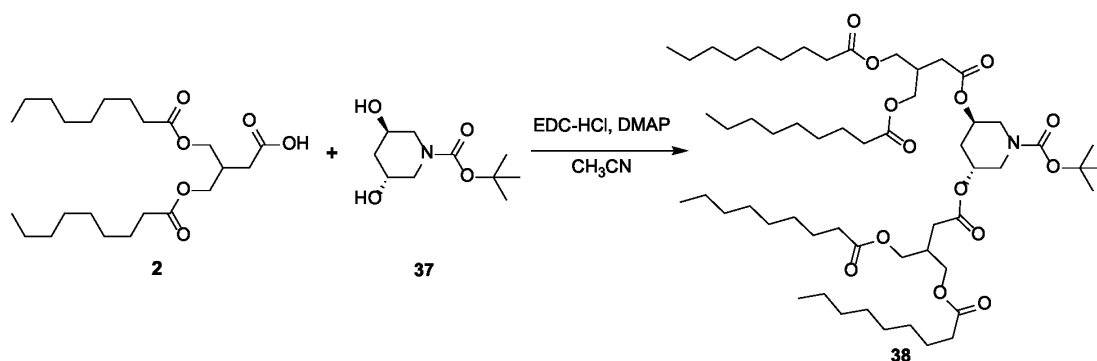
Example 38: Synthesis of (((3S,4S)-1-((2-(dimethylamino)ethoxy)carbonyl)pyrrolidine-3,4-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-215)



[00533] To a solution of 36 (5.00g, 5.05mmol), in CH₃CN (100mL), cooled in an ice-water bath under nitrogen was added MeOTf (0.91g, 5.55mmol) over a period of 5 minutes. The mixture was allowed to stir for 2 hours at 0°C, then a solution of trimethylamine in THF (2.0M, 7.57mL, 22.20mmol) was added over a period of 10 minutes followed by the addition of 2-dimethylaminoethanol (0.81g, 9.09mmol) in one portion. The mixture was stirred for 1 hour at 0°C, then, was warmed in a 70°C oil bath, and was stirred for 48 hours. The reaction mixture was concentrated in vacuo, the residue was dissolved with n-heptane (550mL) and the resulting solution was washed with MeOH/water (5:1, 2 x 100mL), then the organic phase was dried over Na₂SO₄, and filtered. The filtrate was concentrated in vacuo and the residue was dissolved in CH₃CN (15mL), and the crude product was purified by preparative reverse phase HPLC (XB Phenyl column, 50x250mm, 10μM; Mobile Phase A: water/0.1% TFA; Mobile Phase B: CH₃CN; flow rate 90mL/min; Gradient 55% B to 90% B in 12 minutes; Wave Length: 205nm). Qualified fractions were concentrated in vacuo, to remove CH₃CN, and the pH of the aq. residue was adjusted to 9.0 with 2% aq. Na₂CO₃. The aq. phase was extracted with n-heptane (2 x 275mL), and the combined organic phases were washed with MeOH/water (4:1, 3 x 110mL), water (145mL) and dried (Na₂SO₄). Filtration and concentration in vacuo gave C1CL-215 (2.02g, 2.00mmol, 97% purity by HPLC, 40% yield) as a clear, colorless, viscous oil.

[00534] ¹H-NMR (300MHz, CDCl₃): δ = 5.22 (m, 2H), 4.22 (m, 2H), 4.11 (m, 8H), 3.76 (dd, J = 12.9, 4.2Hz, 2H), 3.59 (m, 2H), 2.49-2.64 (4H), 2.43 (m, 4H), 2.28-2.38 (14H), 1.62 (m, 8H), 1.21-1.39 (40H), 0.90 (m, 12H); LCMS: RT 1.575, Calcd. for C₅₅H₉₈N₂O₁₄ + H⁺ 1011.71. Found 1011.60.

Example 39: (((3R,5R)-1-(tert-butoxycarbonyl)piperidine-3,5-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propene-2,1,3-triyl) tetranonanoate (38)

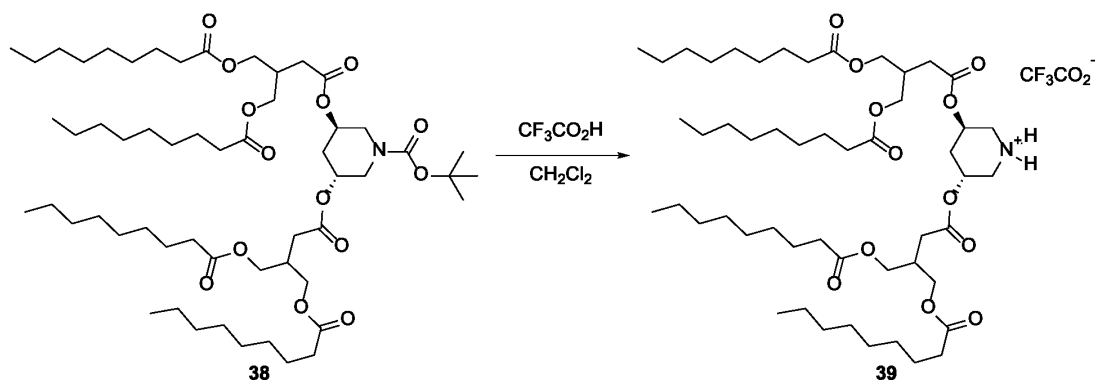


[00535] To a solution of 37 (Tetrahedron 2011, 67, 1485, 2.20g, 10.14mmol) in acetonitrile (44mL), at room temperature under nitrogen, was added in order: 2 (8.60g, 20.78mmol), DMAP (1.24g, 10.14mmol), and EDC-HCl (4.87g, 23.34mmol). The reaction mixture was stirred for 18 hours at room temperature then was concentrated in vacuo. The residue was dissolved in n-heptane (440mL) and the resulting solution was extracted with MeOH/water (10:1, 2 x 110mL). The solvent was removed in vacuo and the residue was dissolved in dichloromethane (44mL) and silica gel (20g, Type: ZCX-2, 100-200 mesh) was added. The solvent was then removed in vacuo (temperature < 35°C). The resulting 38 impregnated silica gel was placed atop a combi-flash column charged with silica gel (120g, Type: ZCX-2, 100-200 mesh), and the combi-flash column was eluted with a gradient of n-heptane/ethyl acetate (100:0 to 90:10). Qualified fractions were combined and concentrated in vacuo to furnish 38 which was further purified by reverse phase prep-HPLC (Column: XB Phenyl 50x250mm, 10m; Mobile Phase A: water (0.1% CF₃CO₂H), Mobile Phase B: CH₃CN; Flow Rate: 90mL/min; Gradient: 50% B to 95% B over 10 minutes; Wave Length: 205nm). Qualified fractions were pooled, the acetonitrile was removed in vacuo and the resulting solution was extracted with n-heptane (440mL). The organic phase was washed with MeOH/water (4:1, 2 x 110mL), water (110mL), and dried (Na₂SO₄). Filtration and concentration in vacuo gave 38 (7.10g, 7.02mmol, HPLC purity 98%, 69% yield) as a viscous, colorless oil.

[00536] ¹H-NMR (300MHz, CDCl₃): δ = 5.05 (brs, 2H), 4.12 (m, 8H), 3.30-3.75 (4H), 2.58 (m, 2H), 2.40 (m, 4H), 2.34 (m, 8H), 2.00 (brm, 2H), 1.62 (m, 8H), 1.49 (s, 9H), 1.20-1.37 (40H), 0.88 (t, J = 6.8Hz, 12H); LCMS: RT 2.017, Calcd. for C₅₆H₉₉NO₁₄ + Na⁺ 1032.70. Found 1032.60.

Example 40: Synthesis of (3R,5R)-3,5-bis((4-(nonanoyloxy)-3-

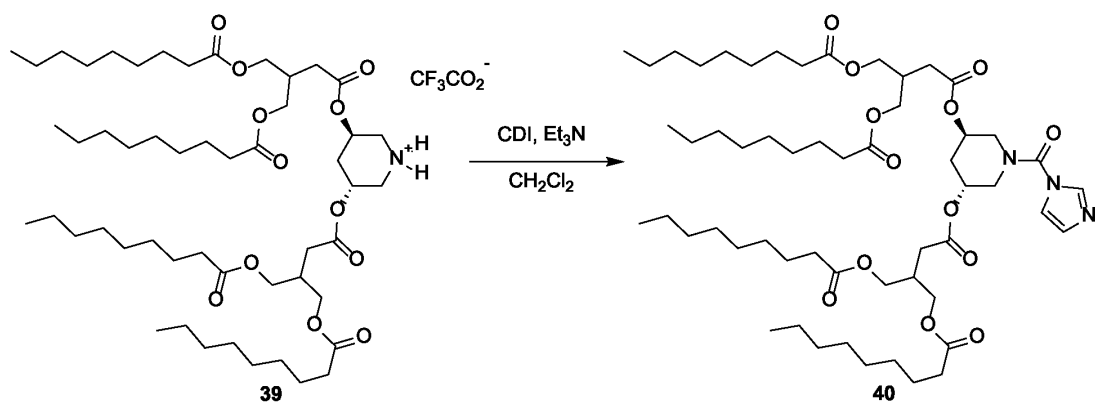
((nonanoyloxy)methyl)butanoyl)oxy)piperidin-1-ium trifluoroacetate (39)



[00537] To a solution of 38 (6.00g, 5.94mmol), in CH_2Cl_2 (30mL) under nitrogen, cooled to 20°C , was added $\text{CF}_3\text{CO}_2\text{H}$ (12mL, 17.87g, 157mmol) over a period of 15 minutes. The reaction mixture was allowed to stir for 3 hours at 20°C after the addition was complete, then the solution was concentrated in vacuo and the residue was dissolved in n-heptane (600mL). The resulting solution was washed with brine (2 x 120mL), then the organic phase was concentrated in vacuo to provide 39 (5.40g, 5.27mmol, HPLC purity 97%, 89% yield) as a pale-yellow oil.

[00538] $^1\text{H-NMR}$ (400MHz, CDCl_3): δ = 5.30 (m, 2H), 4.11 (m, 8H), 3.38 (dd, J = 13.6, 3.6Hz, 2H), 3.23 (m, 2H), 2.40-2.58 (6H), 2.38 (m, 8H), 2.18 (m, 2H), 1.63 (m, 8H), 1.23-1.38 (40H), 0.90 (t, J = 7.2Hz, 12H); **LCMS**: RT 1.611, Calcd. for $\text{C}_{51}\text{H}_{92}\text{NO}_{12}$ 910.66. Found 910.60.

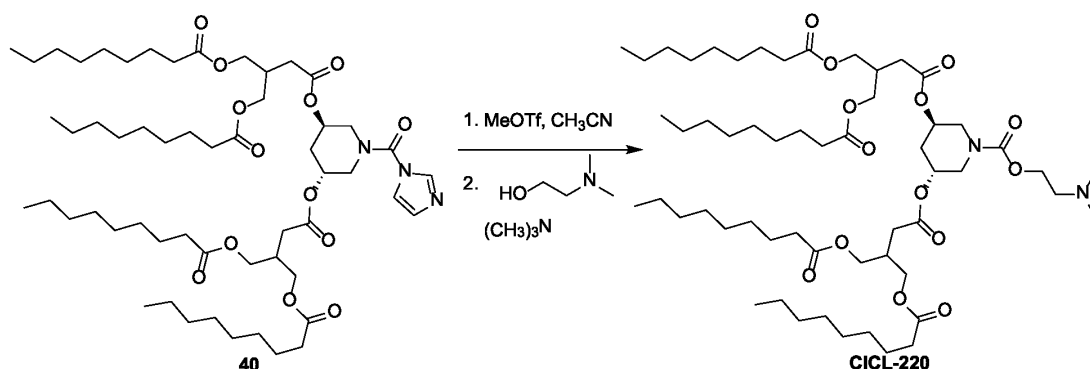
Example 41: Synthesis of (((3R,5R)-1-(1H-imidazole-1-carbonyl)piperidine-3,5-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (40)



[00539] To a solution of 39 (5.40g, 5.33mmol) in dichloromethane (108mL), at room temperature under nitrogen, was added in order: CDI (8.64g, 53.60mmol) and Et₃N (4.33g, 42.86mmol). The reaction mixture was stirred for 18 hours at room temperature, then was cast into 0.5M aq. HCl (125mL). The aq. layer was extracted with dichloromethane (2 x 270mL) and the combined organic phases were concentrated in vacuo to afford crude 40 which was dissolved in n-heptane (675mL). The organic phase was washed with MeOH/water (5:1, 2 x 270mL), and was concentrated in vacuo, providing 40 (4.90g, 4.68mmol, 94% purity by HPLC, 88% yield) as a pale-yellow oil.

[00540] ¹H-NMR (400MHz, CDCl₃): δ = 7.98 (brs, 1H), 7.30 (s, 1H), 7.14 (s, 1H), 5.18 (m, 2H), 4.10 (m, 8H), 3.80 (m, 2H), 3.62 (m, 2H), 2.54 (m, 2H), 2.46 (m, 4H), 2.32 (m, 8H), 2.14 (m, 2H), 1.62 (m, 8H), 1.22-1.38 (40H), 0.89 (t, J = 6.8Hz, 12H); LCMS: RT 1.800, Calcd. for C₅₅H₉₃N₃O₁₃ + H⁺ 1004.68. Found 1004.60.

Example 42: Synthesis of (((((3R,5R)-1-((2-(dimethylamino)ethoxy)carbonyl)piperidine-3,5-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-220)

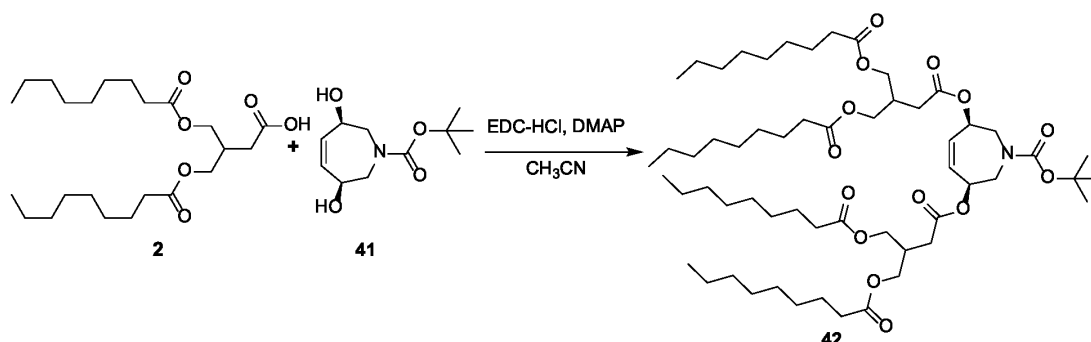


[00541] A solution of 40 (5.50g, 6.57mmol) in acetonitrile (75mL), under nitrogen, was cooled in an ice-water bath. To this cooled solution was added MeOTf (1.08g, 6.57mmol) over a period of 5 minutes. The mixture was stirred for 2 hours, then Me₃N (2M in THF, 10.9mL, 21.8mmol) was added over a period of 10 minutes, this was followed by the addition of 2-dimethylaminoethanol (0.73g, 8.22mmol) in one portion. The reaction mixture was stirred for 1 hour at 0°C, then it was placed in a 60°C oil bath and stirred for 120 hours. After cooling to room temperature, the mixture was concentrated in vacuo and the residue was dissolved in

n-heptane (550mL). The solution was washed with MeOH/water (5:1, 2 x 100mL), and the organic phase was dried over Na₂SO₄. Filtration and concentration in vacuo provided crude C1CL-220 (4.80g) which was purified by preparative reverse phase HPLC (Waters C18 column; mobile phase A (0.1% TFA in water), mobile phase B (acetonitrile); with a gradient of 50% to 90% B over 12 minutes; UV 205nm). Qualified fractions were combined, and the acetonitrile was removed in vacuo. The resulting mixture was adjusted to pH 8 with 2% aq. Na₂CO₃ and extracted with n-heptane (2 x 275mL). The combined organic phases were washed with MeOH/water (4:1, 3 x 110mL), water (110mL), and dried (Na₂SO₄). Filtration and concentration in vacuo afforded C1CL-220 (2.35g, 2.29mmol, 97% purity by HPLC, 35% yield) as a clear, colorless oil.

[00542] ¹H-NMR (400MHz, CDCl₃): δ = 5.06 (brm, 2H), 4.22 (m, 2H), 4.10 (m, 8H), 3.73 (brm, 1H), 3.58 (brm, 2H), 3.41 (brm, 1H), 2.58 (m, 4H), 2.41 (m, 4H), 2.24-2.34 (14H), 1.99 (brm, 2H), 1.62 (m, 8H), 1.20-1.37 (40H), 0.87 (t, J = 6.6Hz, 12H); LCMS: RT 1.588, Calcd. for C₅₆H₁₀₀N₂O₁₄ + H⁺ 1025.73. Found 1025.70.

Example 43: Synthesis of (((*Rel*-(3R,6S)-1-(tert-butoxycarbonyl)-2,3,6,7-tetrahydro-1H-azepine-3,6-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propene-2,1,3-triyl) tetranonanoate (42)

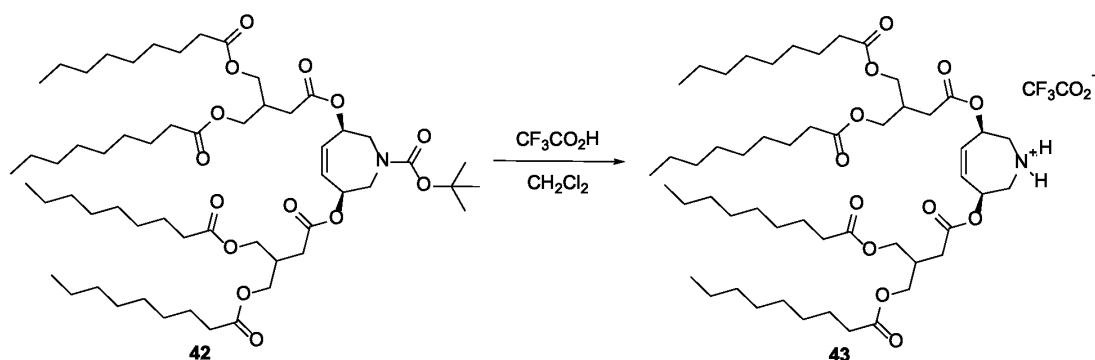


[00543] To a solution of 41 (Nature Communications 2019, 10, 21, 1.42g, 6.20mmol) in acetonitrile (50mL), at room temperature under nitrogen, was added in order: 2 (5.26g, 12.68mmol), DMAP (0.76g, 6.20mmol), and EDC-HCl (2.98g, 15.50mmol). The reaction mixture was stirred for 18 hours at room temperature then was concentrated in vacuo. The residue was dissolved in n-heptane (200mL) and the resulting solution was extracted with MeOH/water (10:1, 2 x 100mL). The solvent was removed in vacuo and the residue was dissolved in dichloromethane (15mL) and silica gel (20g, Type: ZCX-2, 100-200 mesh) was

added. The solvent was then removed in vacuo (temperature < 35°C). The resulting 42 impregnated silica gel was placed atop a combi-flash column charged with silica gel (120g, Type: ZCX-2, 100-200 mesh), and the combi-flash column was eluted with a gradient of n-heptane/ethyl acetate (40:1 to 25:1). Qualified fractions were combined and concentrated in vacuo to furnish 42 (5.20g, 5.09mmol, HPLC purity 92%, 84% yield) as a pale-yellow oil.

[00544] ¹H-NMR (400MHz, CDCl₃): δ = 5.69 (s, 2H), 5.48 (m, 2H), 4.12 (m, 8H), 4.08 (m, 1H), 3.92 (m, 1H), 3.17 (m, 2H), 2.59 (m, 2H), 2.48 (m, 4H), 2.31 (m, 8H), 1.63 (m, 8H), 1.50 (s, 9H), 1.22-1.35 (40H), 0.89 (m, 12H); **LCMS**: RT 2.230, Calcd. for C₅₇H₉₉NO₁₄ + Na⁺ 1044.70. Found 1044.80.

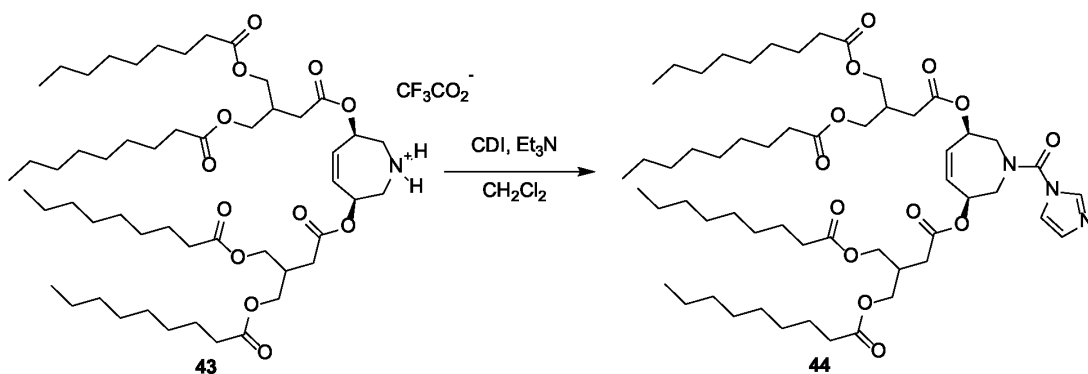
Example 44: Synthesis of *Rel*-(3R,6S)-3,6-bis((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)-2,3,6,7-tetrahydro-1H-azepin-1-ium trifluoroacetate (43)



[00545] To a solution of 42 (5.10g, 5.10mmol), in CH₂Cl₂ (75mL) under nitrogen, at room temperature, was added CF₃CO₂H (25mL, 37.23g, 326mmol) over a period of 15 minutes. The reaction mixture was allowed to stir for 18 hours at room temperature after the addition was complete, then the solution was concentrated in vacuo and the residue was dissolved in n-heptane (500mL). The resulting solution was washed with brine (2 x 200mL), then the organic phase was concentrated in vacuo to provide 43 (5.03g, 4.85mmol, HPLC purity 93%, 95% yield) as a pale-yellow oil.

[00546] ¹H-NMR (400MHz, CDCl₃): δ = 6.00 (s, 2H), 5.74 (m, 2H), 4.13 (m, 8H), 3.52 (m, 4H), 2.57 (m, 2H), 2.46 (m, 4H), 2.32 (m, 8H), 1.62 (m, 8H), 1.20-1.37 (40H), 0.89 (m, 12H); **LCMS**: RT 1.77, Calcd. for C₅₂H₉₂NO₁₂ 922.66. Found 922.8.

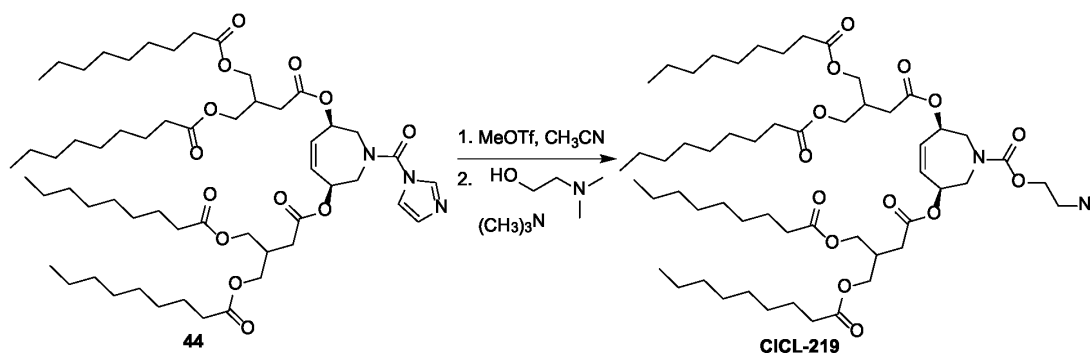
Example 45: Synthesis of (((*Rel*-(3*R*,6*S*)-1-(1*H*-imidazole-1-carbonyl)-2,3,6,7-tetrahydro-1*H*-azepine-3,6-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (44)



[00547] To a solution of 43 (5.00g, 4.82mmol) in dichloromethane (100mL), at room temperature under nitrogen, was added in order: CDI (7.94g, 49.00mmol) and Et₃N (3.97g, 39.20mmol). The reaction mixture was stirred for 18 hours at room temperature, then was cast into 0.5M aq. HCl (125mL). The aq. layer was extracted with dichloromethane (2 x 200mL) and the combined organic phases were concentrated in vacuo to afford crude 44 which was dissolved in n-heptane (300mL). The organic phase was washed with MeOH/water (8:1, 2 x 200mL), and was concentrated in vacuo, providing 44 (4.30g, 4.23mmol, 96% purity by HPLC, 88% yield) as a yellow oil.

[00548] ¹H-NMR (300MHz, CDCl₃): δ = 8.01 (s, 1H), 7.28 (s, 1H), 7.15 (s, 1H), 5.85 (m, 2H), 5.61 (m, 2H), 4.00-4.22 (10H), 3.61-3.73 (2H), 2.48 (m, 2H), 2.45 (m, 4H), 2.27-2.38 (8H), 1.61 (m, 8H), 1.20-1.39 (40H), 0.89 (m, 12H); LCMS: RT 1.777, Calcd. for C₅₆H₉₃N₃O₁₃ + H⁺ a 1016.68. Found 1016.70.

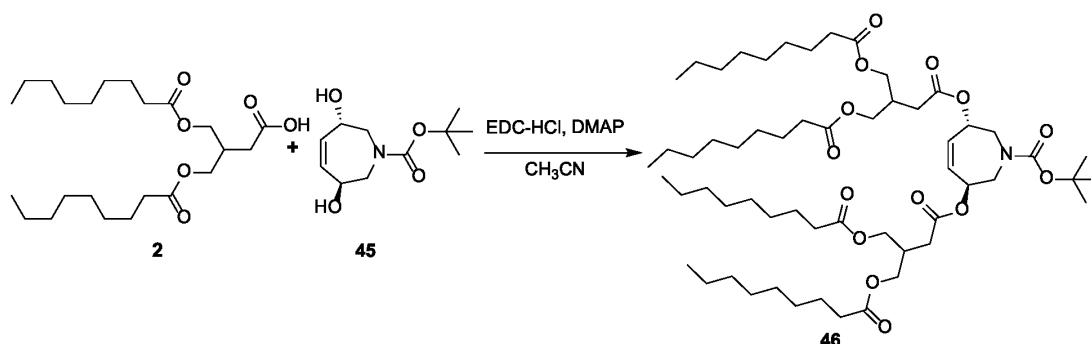
Example 46: Synthesis of (((*Rel*-(3*R*,6*S*)-1-((2-(dimethylamino)ethoxy)carbonyl)-2,3,6,7-tetrahydro-1*H*-azepine-3,6-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-219)



[00549] A solution of 44 (4.20g, 4.13mmol) in acetonitrile (60mL), under nitrogen, was cooled in an ice-water bath. To this cooled solution was added MeOTf (0.81g, 5.00mmol) over a period of 5 minutes. The mixture was stirred for 2 hours, then Me₃N (2M in THF, 6.20mL, 12.40mmol) was added over a period of 10 minutes, and this was followed by the addition of 2-dimethylaminoethanol (0.55g, 6.20mmol) in one portion. The reaction mixture was stirred for 1 hour at 0°C, then it was placed in a 60°C oil bath and stirred for 48 hours. After cooling to room temperature, the mixture was concentrated in vacuo and the residue was dissolved in n-heptane (300mL). The solution was washed with MeOH/water (5:1, 2 x 50mL), and the organic phase was dried over Na₂SO₄. Filtration and concentration in vacuo provided crude CICAL-219 which was dissolved in dichloromethane (20mL), and silica gel (20g, Type: ZCX-2, 100-200 mesh) was added. The solvent was then removed in vacuo (temperature < 35°C). The resulting CICAL-219 impregnated silica gel was placed atop a combi-flash column charged with silica gel (120g, Type: ZCX-2, 100-200 mesh), and the combi-flash column was eluted with a gradient of n-heptane/ethyl acetate (100:0 to 40:70). Qualified fractions were combined and concentrated in vacuo to furnish CICAL-219 which was dissolved in n-heptane (300mL), washed with MeOH/water (5:1, 2 x 50mL), water (2 x 50mL), and dried (Na₂SO₄). Filtration and concentration in vacuo provided CICAL-219 (1.49g, 1.44mmol, 98% purity by HPLC, 35% yield) as a pale-yellow oil.

[00550] ¹H-NMR (400MHz, CDCl₃): δ = 5.69 (s, 2H), 5.52 (brn, 2H), 4.27 (m, 2H), 3.98-4.20 (10H), 3.23 (m, 2H), 2.62 (m, 4H), 2.45 (m, 4H), 2.28-2.38 (14H), 1.64 (m, 8H), 1.21-1.39 (40H), 0.90 (m, 12H); LCMS: RT 1.652, Calcd. for C₅₇H₁₀₀N₂O₁₄ + H⁺ 1037.72. Found 1037.60.

Example 47: Synthesis of (((*Rel*-(3*S*,6*S*)-1-(tert-butoxycarbonyl)-2,3,6,7-tetrahydro-1*H*-azepine-3,6-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propene-2,1,3-triyl) tetranonanoate (46)



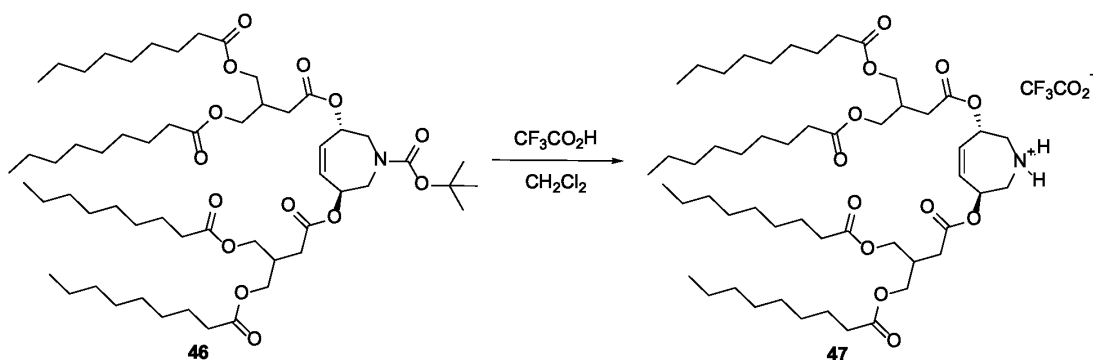
[00551] To a solution of 45 (Nature Communications 2019, 10, 21, 1.45g, 6.32mmol) in acetonitrile (50mL), at room temperature under nitrogen, was added in order: 2 (5.37g, 13.00mmol), DMAP (0.78g, 6.30mmol), and EDC-HCl (3.04g, 15.80mmol). The reaction mixture was stirred for 18 hours at room temperature then was concentrated in vacuo. The residue was dissolved in n-heptane (100mL) and the resulting solution was extracted with MeOH/water (10:1, 2 x 100mL). The solvent was removed in vacuo and the residue was dissolved in dichloromethane (15mL) and silica gel (20g, Type: ZCX-2, 100-200 mesh) was added. The solvent was then removed in vacuo (temperature < 35°C). The resulting 46 impregnated silica gel was placed atop a combi-flash column charged with silica gel (120g, Type: ZCX-2, 100-200 mesh), and the combi-flash column was eluted with a gradient of n-heptane/ethyl acetate (40:1 to 25:1). Qualified fractions were combined and concentrated in vacuo to furnish 46 (5.32g, 5.20mmol, HPLC purity 95%, 82% yield) as a pale-yellow oil.

[00552] ¹H-NMR (400MHz, CDCl₃): δ = 5.78-5.88 (2H), 5.52 (brm, 1H), 5.42 (brm, 1H), 4.13 (m, 8H), 3.90 (m, 1H), 3.82 (m, 1H), 3.49 (m, 1H), 3.40 (m, 1H), 2.59 (m, 2H), 2.44 (m, 4H), 2.33 (m, 8H), 1.62 (m, 8H), 1.49 (s, 9H), 1.21-1.38 (40H), 0.89 (t, J = 6.8Hz, 12H); LCMS: RT 2.037, Calcd. for C₅₇H₉₉NO₁₄ + Na⁺ 1044.70. Found 1044.70.

Example 48: Synthesis of *Rel*-(3*S*,6*S*)-3,6-bis((4-(nonanoyloxy)-3-

((nonanoyloxy)methyl)butanoyl)oxy)-2,3,6,7-tetrahydro-1H-azepin-1-ium

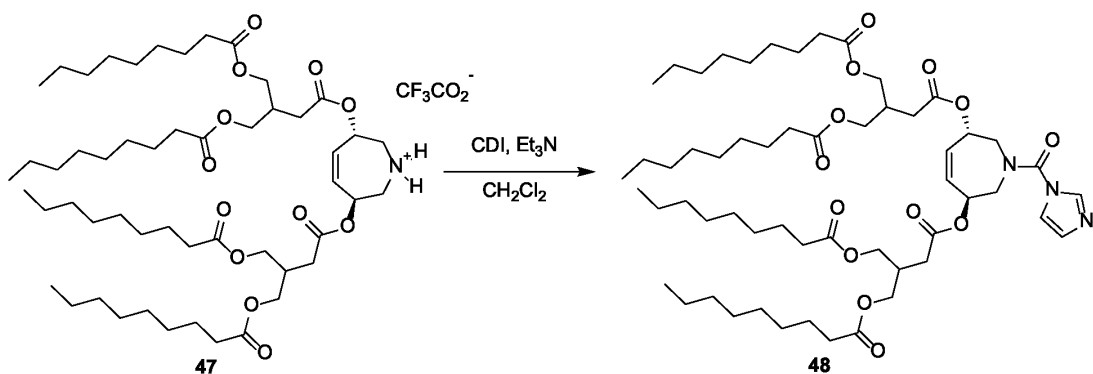
trifluoroacetate (47)



[00553] To a solution of 46 (5.20g, 5.10mmol), in CH_2Cl_2 (75mL) under nitrogen, at room temperature, was added $\text{CF}_3\text{CO}_2\text{H}$ (25mL, 37.23g, 326mmol) over a period of 15 minutes. The reaction mixture was allowed to stir for 18 hours at room temperature after the addition was complete, then the solution was concentrated in vacuo and the residue was dissolved in n-heptane (500mL). The resulting solution was washed with brine (2 x 200mL), then the organic phase was concentrated in vacuo to provide 47 (5.16g, 4.98mmol, HPLC purity 90%, 98% yield) as a pale-yellow oil.

[00554] $^1\text{H-NMR}$ (300MHz, CDCl_3): δ = 6.10-6.18 (2H), 5.62 (m, 2H), 5.31 (s, 2H), 4.10 (m, 8H), 3.54 (m, 4H), 2.47-2.62 (6H), 2.30 (m, 8H), 1.63 (m, 8H), 1.20-1.37 (40H), 0.90 (m, 12H); **LCMS**: RT 1.767, Calcd. for $\text{C}_{52}\text{H}_{92}\text{NO}_{12}$ 922.66. Found 922.8.

Example 49: Synthesis of (((*Rel*-(3*S*,6*S*)-1-(1*H*-imidazole-1-carbonyl)-2,3,6,7-tetrahydro-1*H*-azepine-3,6-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (48)

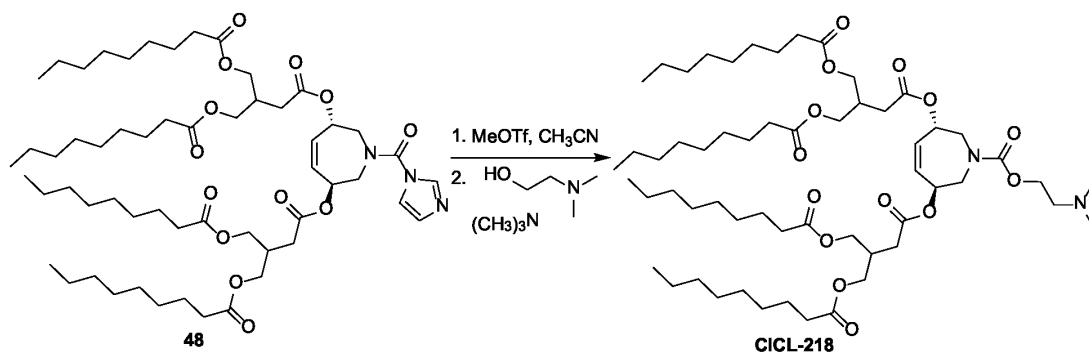


[00555] To a solution of 47 (5.10g, 4.92mmol) in dichloromethane (100mL), at room

temperature under nitrogen, was added in order: CDI (8.10g, 50.00mmol) and Et₃N (4.05g, 40.00mmol). The reaction mixture was stirred for 18 hours at room temperature, then was cast into 0.5M aq. HCl (125mL). The aq. layer was extracted with dichloromethane (2 x 300mL) and the combined organic phases were concentrated in vacuo to afford crude 48 which was dissolved in n-heptane (300mL). The organic phase was washed with MeOH/water (8:1, 2 x 200mL), water (200mL), and was concentrated in vacuo, providing 48 (4.30g, 4.23mmol, 95% purity by HPLC, 86% yield) as a yellow oil.

[00556] ¹H-NMR (300MHz, CDCl₃): δ = 7.99 (s, 1H), 7.31 (s, 1H), 7.14 (s, 1H), 5.80-5.90 (2H), 5.68 (m, 2H), 4.12 (m, 8H), 3.95 (m, 2H), 3.82 (m, 2H), 2.57 (m, 2H), 2.41 (m, 4H), 2.32 (m, 8H), 1.62 (m, 8H), 1.20-1.40 (40H), 0.89 (m, 12H); **LCMS**: RT 1.945, Calcd. for C₅₆H₉₃N₃O₁₃ + H⁺ a 1016.68. Found 1016.70.

Example 50: Synthesis of (((*Re*)-(3*S*,6*S*)-1-((2-(dimethylamino)ethoxy)carbonyl)-2,3,6,7-tetrahydro-1*H*-azepine-3,6-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-218)

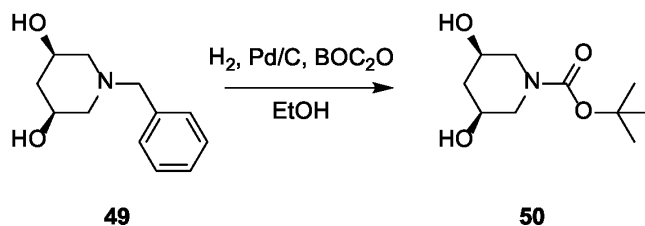


[00557] A solution of 48 (4.20g, 4.13mmol) in acetonitrile (60mL), under nitrogen, was cooled in an ice-water bath. To this cooled solution was added MeOTf (0.81g, 5.00mmol) over a period of 5 minutes. The mixture was stirred for 2 hours, then Me₃N (2M in THF, 6.20mL, 12.40mmol) was added over a period of 10 minutes, and this was followed by the addition of 2-dimethylaminoethanol (0.55g, 6.20mmol) in one portion. The reaction mixture was stirred for 1 hour at 0°C, then it was placed in a 60°C oil bath and stirred for 48 hours. After cooling to room temperature, the mixture was concentrated in vacuo and the residue was dissolved in n-heptane (300mL). The solution was washed with MeOH/water (5:1, 2 x 50mL), and the organic phase was dried over Na₂SO₄. Filtration and concentration in vacuo provided crude CICL-218 which was dissolved in n-heptane (300mL). The organic phase was washed with

MeOH/water (5:1, 2 x 50mL), water (50mL), and dried (Na_2SO_4). Filtration and concentration in vacuo gave crude C1CL-218 which was dissolved with dichloromethane (20mL), and silica gel (20g, Type: ZCX-2, 100-200 mesh) was added. The solvent was then removed in vacuo (temperature $< 35^\circ\text{C}$). The resulting C1CL-218 impregnated silica gel was placed atop a combi-flash column charged with silica gel (120g, Type: ZCX-2, 100-200 mesh), and the combi-flash column was eluted with a gradient of n-heptane/ethyl acetate (100:0 to 40:70). Qualified fractions were combined and concentrated in vacuo to furnish C1CL-218 which was dissolved in n-heptane (300mL), washed with MeOH/water (5:1, 2 x 50mL), water (2 x 50mL), and dried (Na_2SO_4). Filtration and concentration in vacuo provided C1CL-218 (1.16g, 1.12mmol, 97% purity by HPLC, 27% yield) as a pale-yellow oil.

[00558] $^1\text{H-NMR}$ (400MHz, CDCl_3): δ = 5.77-5.89 (2H), 5.50 (m, 2H), 4.27 (m, 1H), 4.07-4.20 (9H), 3.83 (m, 2H), 3.64 (m, 1H), 3.53 (m, 1H), 2.60 (m, 4H), 2.42 (m, 4H), 2.24-2.33 (14H), 1.62 (m, 8H), 1.21-1.37 (40H), 0.89 (m, 12H); **LCMS**: RT 1.652, Calcd. for $\text{C}_{57}\text{H}_{100}\text{N}_2\text{O}_{14} + \text{H}^+$ 1037.72. Found 1037.70.

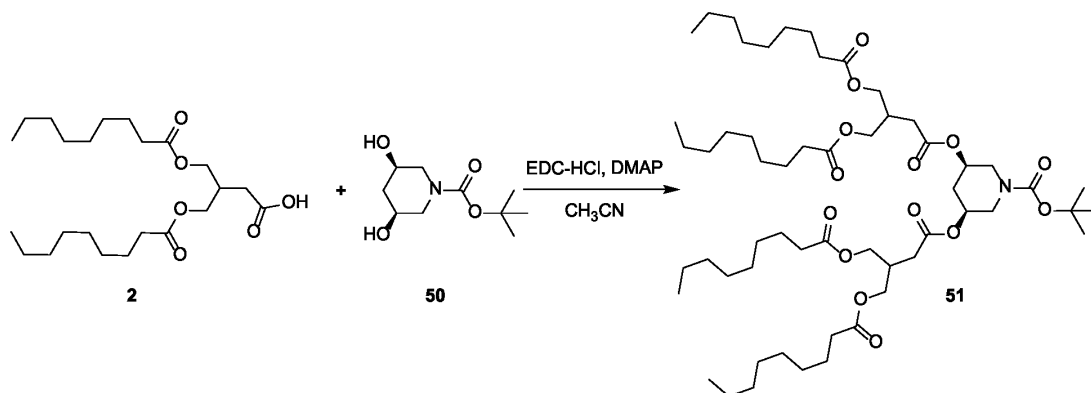
Example 51: Synthesis of tert-Butyl *Rel*-(3*R*,5*S*)-3,5-dihydroxypiperidine-1-carboxylate (50)



[00559] To a solution of 49 (WO2013149362, 8.00g, 38.6mmol) in EtOH (160mL) was added BOC_2O (12.64g, 57.9mmol) and Pd/C (1.60g, 20wt%). The resulting solution was placed under hydrogen (15atm) at room temperature for 24 hours. The mixture was filtered through a pad of Celite®, the filter cake rinsed with EtOH (3 x 50mL) and the combined filtrates were concentrated in vacuo to provide crude 50 as a pale yellow oil. Crude 50 was dissolved in THF (30mL) and purified by reverse phase preparative HPLC (Column: XB Phenyl, 50x250 mm, 10 μm ; Mobile Phase A: water (0.1 % TFA), Mobile Phase B: acetonitrile; Flow rate: 90mL/min; Gradient: 0 % B to 50 % B in 10 min, 50 % collected; Wave Length: 200 nm). Qualified fractions were pooled and concentrated in vacuo to yield 50 (4.20g, 19.33mmol, 50% yield), as a clear, colorless oil.

[00560] $^1\text{H-NMR}$ (300MHz, CDCl_3): δ = 4.31 (brt, J = 5.00Hz, 1H), 4.17 (brs, 2H), 3.91-4.20 (2H), 3.41-3.63 (3H), 2.35 (m, 1H), 1.89 (brd, J = 14.1Hz, 1H).

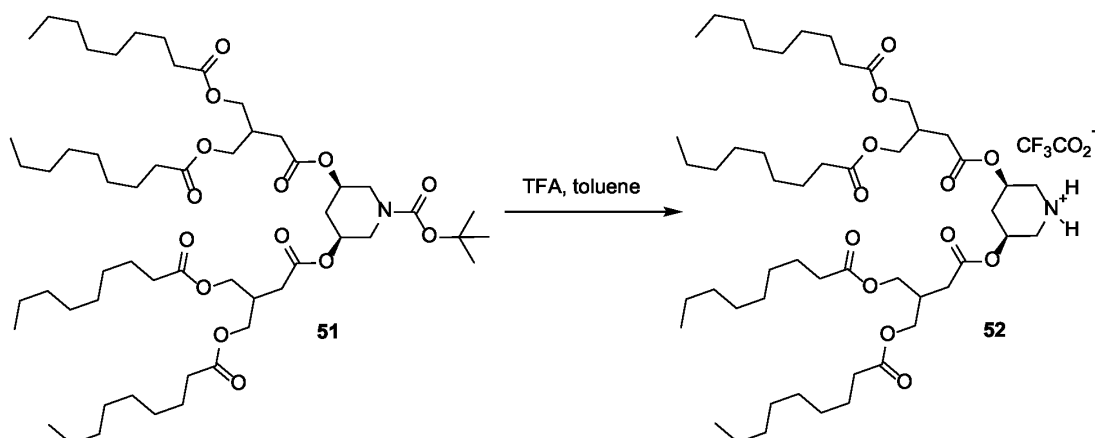
Example 52: Synthesis of (((*Rel*-(3R,5S)-1-(tert-Butoxycarbonyl)piperidine-3,5-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (51)



[00561] To a solution of **50** (2.50g, 11.50mmol) in acetonitrile (200mL), at room temperature under nitrogen, was added in order **2** (10.00g, 24.12mmol), DMAP (1.40g, 11.50mmol), and EDC-HCl (5.53g, 28.85mmol). The resulting solution was allowed to stir for 18h at room temperature, then was concentrated *in vacuo*. The residue was treated with n-heptane (500mL) and MeOH/H₂O (150mL, 4:1). The n-heptane phase was separated, washed with MeOH/H₂O (2 x 150mL, 4:1) and then dried over Na₂SO₄. The mixture was filtered through a sintered glass funnel, the filter cake was rinsed with n-heptane (50mL) and the combined filtrates were concentrated *in vacuo* to afford crude **51** as a viscous, yellow oil. Crude **51** was dissolved in acetonitrile (30mL) and purified by reverse phase preparative HPLC (Column: XB Phenyl, 50x250 mm, 10μm; Mobile Phase A: water (0.1 % TFA), Mobile Phase B: acetonitrile; Flow rate: 90mL/min; Gradient: 60 % B to 95 % B in 10 min, 50 % collected; Wave Length: 200 nm). Qualified fractions eluted at 95% B and were concentrated *in vacuo*. The residue was extracted with n-heptane (500mL). The n-heptane phase was then extracted with MeOH/H₂O (4:1, 200mL), H₂O (200mL), and dried over Na₂SO₄. Filtration and concentration *in vacuo* gave **51** (10.50g, 10.39mmol, 90% yield) as a pale yellow, viscous, oil.

[00562] ¹H-NMR (300MHz, CDCl₃): δ = 5.30 (brm, 1H), 3.98-4.42 (8H), 3.73 (brm, 1H), 3.42 (brm, 1H), 2.56 (m, 2H), 2.42 (m, 4H), 2.20-2.37 (12H), 2.00 (m, 1H), 1.53-1.68 (8H), 1.49 (s, 9H), 1.20-1.38 (40H), 0.88 (m, 12H); LCMS: RT 2.004, Calcd. for C₅₆H₉₉NO₁₄ + Na⁺ 1032.70. Found 1032.60.

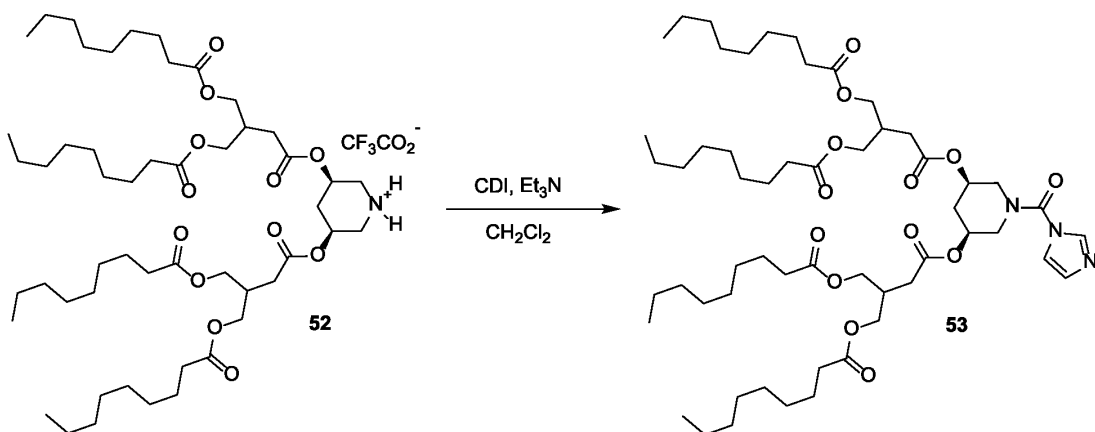
Example 53: *Rel*-(3R,5S)-3,5-bis((4-(Nonanoyloxy)-3 ((nonanoyloxy)methyl) butanoyl)oxy)piperidin-1-ium trifluoroacetate (52)



[00563] To a solution of 51 (10.50g, 10.30mmol) in toluene (47mL), at room temperature under nitrogen, was added TFA (16mL, 23.84g, 0.21mol) over a period of 10 minutes. The mixture was stirred for 18h at room temperature, then was diluted with n-heptane (105mL). The resulting solution was washed with 10% aq. K_2HPO_4 (200mL), MeOH/H₂O (5:1, 2 x 100mL), brine (200mL), and dried (Na_2SO_4). Filtration and concentration *in vacuo* afforded crude 52 (9.80g, 9.57mmol, 89% HPLC purity, 73% yield) as a pale yellow, viscous, oil.

[00564] ¹H-NMR (300MHz, $CDCl_3$): δ = 5.38 (brm, 1H), 4.40 (brm, 2H), 3.90-4.20 (9H), 3.49-3.62 (2H), 2.40-2.70 (7H), 2.31 (m, 8H), 2.07 (m, 1H), 1.56 (m, 8H), 1.19-1.37 (40H), 0.88 (m, 12H); LCMS: RT 1.610, Calcd. for $C_{51}H_{91}NO_{12} + Na^+$ 932.64. Found 932.60.

Example 54: (((*Rel*-(3*R*,5*S*)-1-(1*H*-imidazole-1-carbonyl)piperidine-3,5-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (53)

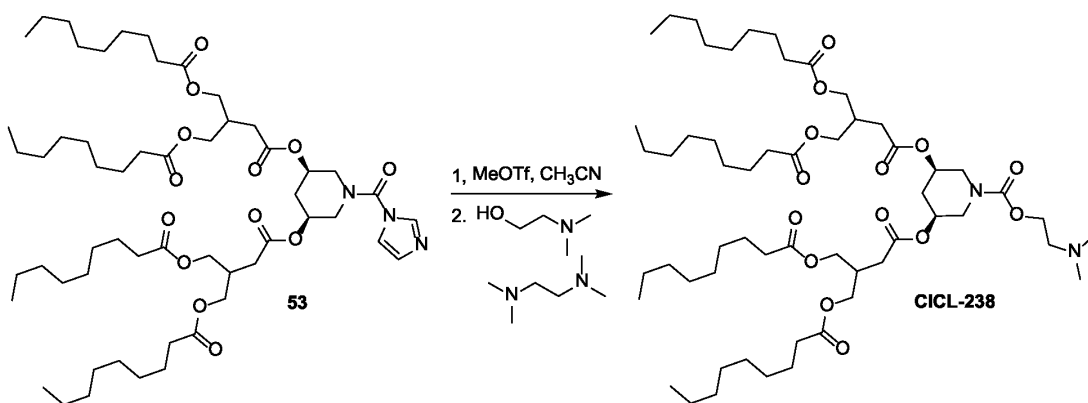


[00565] To a solution of 52 (9.80g, 9.57mmol) in CH_2Cl_2 (200mL), cooled in an ice-water bath under nitrogen, was added CDI (15.75g, 97.13mmol) followed by Et₃N (4.84 g,

47.9mmol). The mixture was stirred for 1 hour at 0°C, then was warmed to room temperature and stirred for 18 hours. The solution was washed with 0.5M aq. HCl (2 x 200mL), brine (200mL), and dried (Na₂SO₄). Filtration, and concentration *in vacuo* afforded crude **53** which was dissolved in n-heptane (400mL), and the resulting solution was washed with MeOH/H₂O (5:1, 2 x 100mL), and the organic phase was dried over Na₂SO₄. Filtration and concentration *in vacuo* afforded crude **53** (9.30g, 9.26mmol, 92% HPLC purity, 96% yield) as a pale yellow oil.

[00566] ¹H-NMR (300MHz, CDCl₃): δ = 8.06 (brs, 1H), 7.35 (brs, 1H), 7.11 (brs, 1H), 5.27 (m, 1H), 4.70 (m, 1H), 3.90-4.37 (12H), 2.37-2.57 (7H), 2.29 (m, 8H), 2.03 (m, 1H), 1.62 (m, 8H), 1.20-1.37 (40H), 0.88 (m, 12H); **LCMS**: RT 1.746, Calcd. for C₅₅H₉₃N₃O₁₃ + H⁺ 1004.67. Found 1004.60.

Example 55: (((*Rel*-(3R,5S)-1-((2-(dimethylamino)ethoxy)carbonyl)piperidine-3,5-diyl)bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (**CICL-238**)

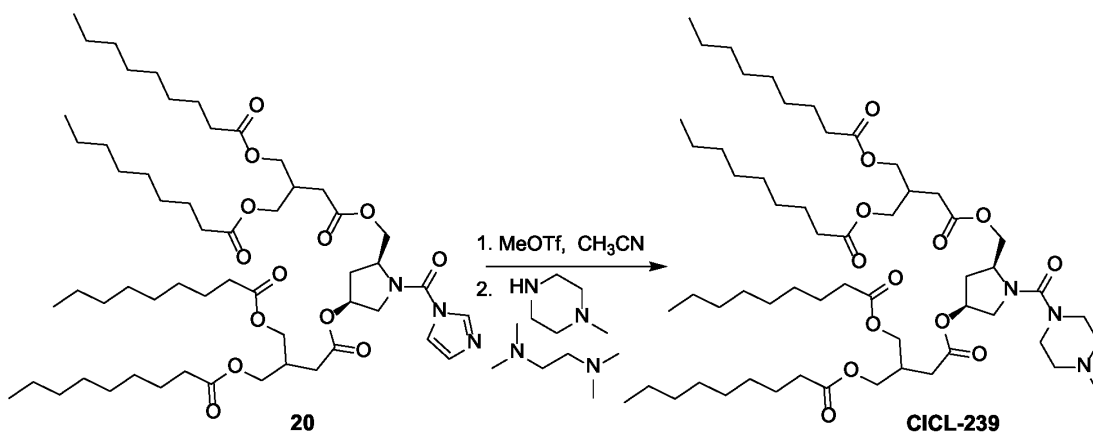


[00567] To a solution of **53** (6.00g, 5.97mmol) in CH₃CN (60mL), cooled in an ice-water bath under nitrogen, was added MeOTf (1.18g, 7.19mmol) over a period of 5 minutes. The mixture was allowed to stir for 2 hours after the addition was complete, then tetramethylethylenediamine (2.10g, 18.1mmol) and dimethylamino-ethanol (0.798g, 8.95mmol) were added in order. The mixture was stirred for 1 hour at 0°C then was warmed to room temperature and stirred for an additional 18 hours. The solvent was removed *in vacuo* and the residue was dissolved in n-heptane (500mL) and the resulting solution was washed with MeOH/H₂O (5:1, 2 x 150mL), and dried over Na₂SO₄. Filtration and concentration *in vacuo* afforded crude **CICL-238** which was purified by reverse phase preparative HPLC (Column: XB Phenyl, 50x250 mm, 10μm; Mobile Phase A: water (0.1 % TFA), Mobile Phase B: acetonitrile; Flow rate: 90mL/min; Gradient: 50 % B to 90 % B in 10 min, 90 % collected; Wave 197

Length: 200 nM). Qualified fractions were pooled, and the acetonitrile was removed *in vacuo* to give **CICL-238** which was dissolved in n-heptane (400mL), and the aqueous phase treated at pH 8 with saturated aq. Na₂CO₃. The organic phase was separated, washed with MeOH/H₂O (5:1, 2 x 100mL), water (200mL), and dried (Na₂SO₄). Filtration and concentration *in vacuo* afforded **CICL-238** (2.04g, 1.99mmol, HPLC purity 97%, 33% yield) as a pale, yellow oil.

[00568] ¹H-NMR (300MHz, CDCl₃): δ = 5.33 (brm, 1H), 4.01-4.41 (13H), 3.77 (brm, 1H), 3.49 (brd, *J* = 12.6Hz, 1H), 2.57 (m, 4H), 2.42 (m, 4H), 2.18-2.39 (15H), 2.04 (brm, 1H), 1.62 (m, 8H), 1.18-1.39 (40H), 0.89 (m, 12H); **LCMS**: RT 0.835, Calcd. for C₅₆H₁₀₀N₂O₁₄ + H⁺ 1025.73. Found 1025.60.

Example 56: 2-(2-(((2S,4S)-1-(4-methylpiperazine-1-carbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate (CICL-239)

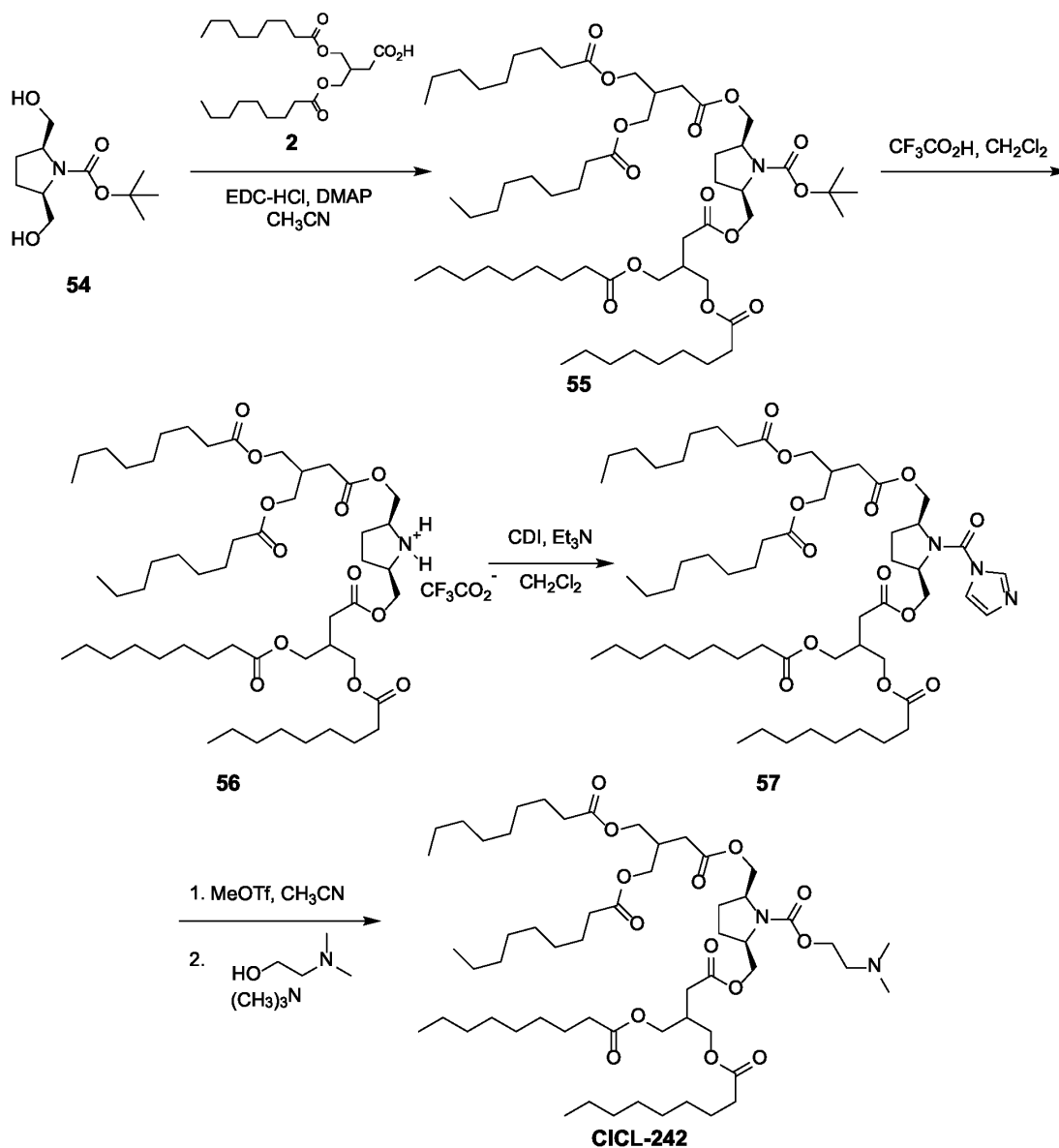


[00569] To a solution of **20** (6.00g, 5.97mmol), in acetonitrile (60mL) cooled in an ice-water bath under nitrogen, was added MeOTf (1.18g, 7.19mmol) in one portion. The solution was allowed to stir for 2 hours at 0°C, then tetramethylethylenediamine (2.10g, 18.1mmol) and N-methylpiperazine (0.90g, 9.00mmol) were added in order. The mixture was stirred for 1 hour at 0°C, then warmed to room temperature and stirring was continued for an additional 18 hours. The solvent was removed *in vacuo*, the residue was dissolved in n-heptane (500mL), and the resulting solution was washed with MeOH/H₂O (5:1, 2 x 150mL), then the organic phase was dried over Na₂SO₄. Filtration and concentration *in vacuo* provided crude **CICL-239**, which was purified by reverse phase preparative HPLC (Column: XB Phenyl, 50x250 mm, 10μm; Mobile Phase A: water (0.1 % TFA), Mobile Phase B: acetonitrile; Flow rate: 90mL/min; Gradient: 50 % B to 90 % B in 10 min, 90 % collected; Wave Length: 200 nM). Qualified

fractions were pooled, and the acetonitrile was removed *in vacuo* to give **CICL-239** which was dissolved in n-heptane (400mL), the aqueous phase was treated at pH 8 with saturated aq. Na₂CO₃. The organic phase was separated, washed with MeOH/H₂O (5:1, 2 x 100mL), water (200mL), and dried (Na₂SO₄). Filtration and concentration *in vacuo* afforded **CICL-239** (2.06g, 1.99mmol, HPLC purity 96%, 33% yield) as a pale, yellow oil.

[00570] ¹H-NMR (300MHz, CDCl₃): δ = 5.13 (m, 1H), 4.52 (m, 1H), 4.04-4.17 (10H), 3.72 (m, 1H), 3.14-3.47 (5H), 2.55 (m, 2H), 2.24-2.50 (20H), 1.81 (m, 1H), 1.63 (m, 8H), 1.18-1.39 (40H), 0.89 (m, 12H); **LCMS**: RT 1.582, Calcd. for C₅₇H₁₀₁N₃O₁₃ + H⁺ 1036.74. Found 1036.60.

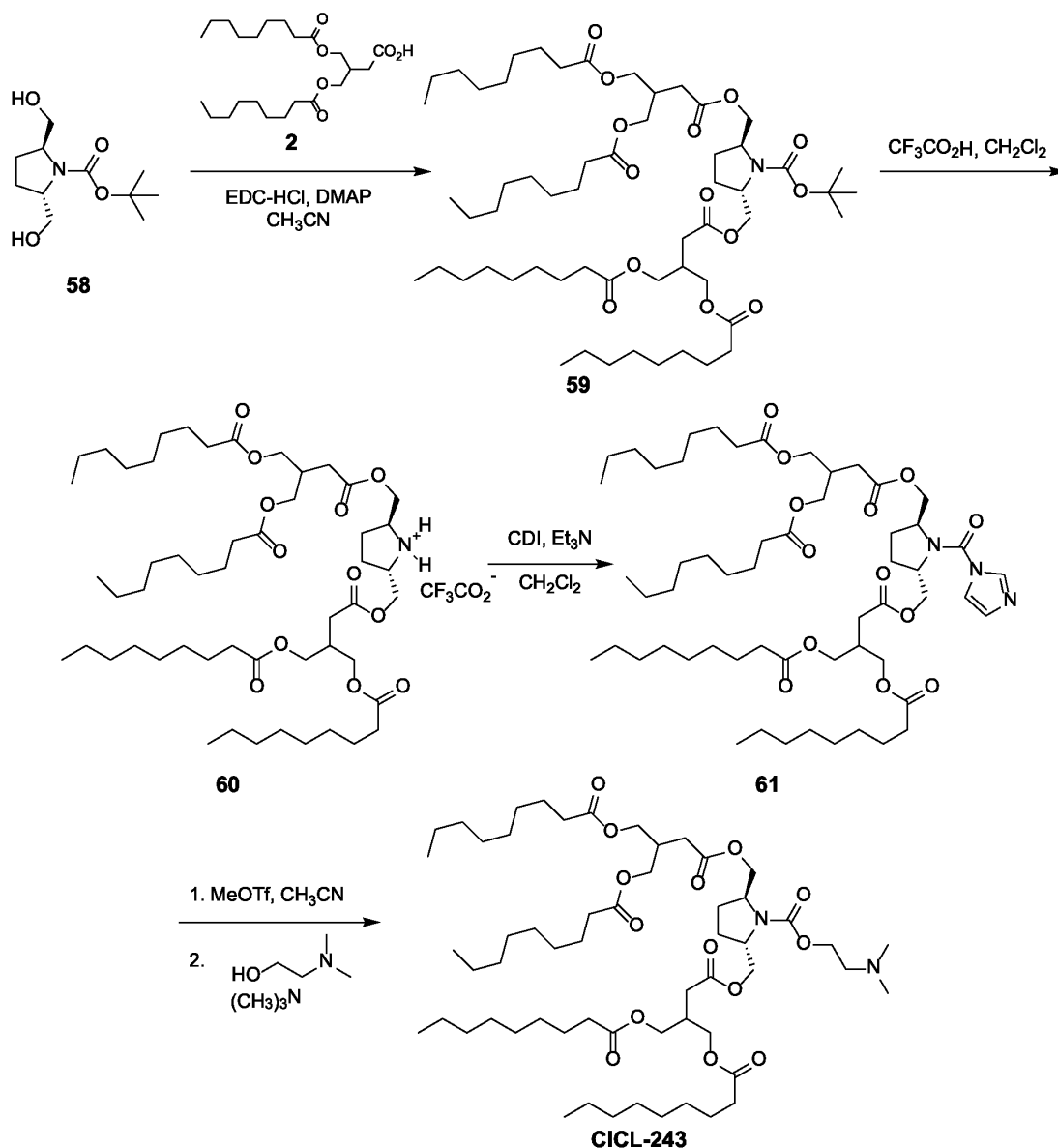
Example 57: Synthesis of ((((*Rel*-(2*R*,5*S*)-1-((2-dimethylamino)ethoxy)carbonyl)pyrrolidine-2,5-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-242)



[00571] tert-Butyl *Rel*-(2*R*,5*S*)-2,5-bis(hydroxymethyl)pyrrolidine-1-carboxylate **54** (*Synthesis* 2013, 45, 2966-2970) is coupled with **2** utilizing EDC-HCl and DMAP in CH₃CN to give **55**. Deprotection of the BOC-amine in **55** with CF₃CO₂H in CH₂Cl₂, provides ammonium

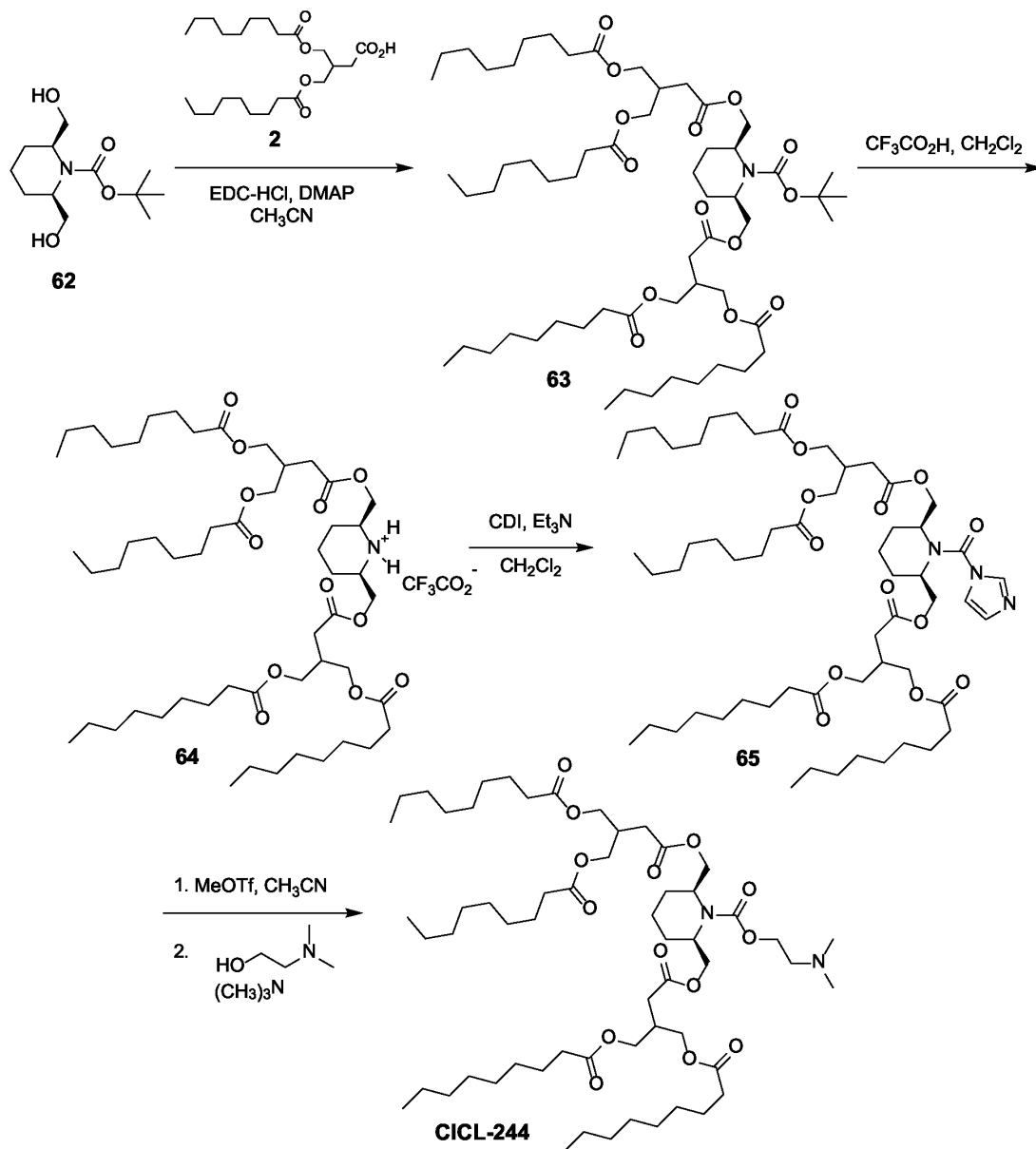
trifluoroacetate salt **56**. Treatment of **56** with carbonyl-di-imidazole and Et₃N, in CH₂Cl₂, then gives acylimidazole **57**. Alkylation of **57** with MeOTf in CH₃CN, followed by the reaction of the derived acylimidazolium salt with 2-dimethylaminoethanol and (CH₃)₃N results in **CICL-242**.

Example 58: Synthesis of (((((2S,5S)-1-((2-(dimethylamino)ethoxy)carbonyl)pyrrolidine-2,5-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-243**)**



[00572] tert-Butyl (2S,5S)-2,5-bis(hydroxymethyl)pyrrolidine-1-carboxylate **58** (WO2023141570, *Tetrahedron Lett.* **1989**, 29, 3805) is coupled with **2** utilizing EDC-HCl and DMAP in CH₃CN to give **59**. Deprotection of the BOC-amine in **59** with CF₃CO₂H in CH₂Cl₂, provides ammonium trifluoroacetate salt **60**. Treatment of **60** with carbonyl-di-imidazole and Et₃N in CH₂Cl₂, then gives acylimizadole **61**. Alkylation of **61** with MeOTf in CH₃CN, followed by the reaction of the derived acylimidazolium salt with 2-dimethylaminoethanol and (CH₃)₃N results in **CICL-243**.

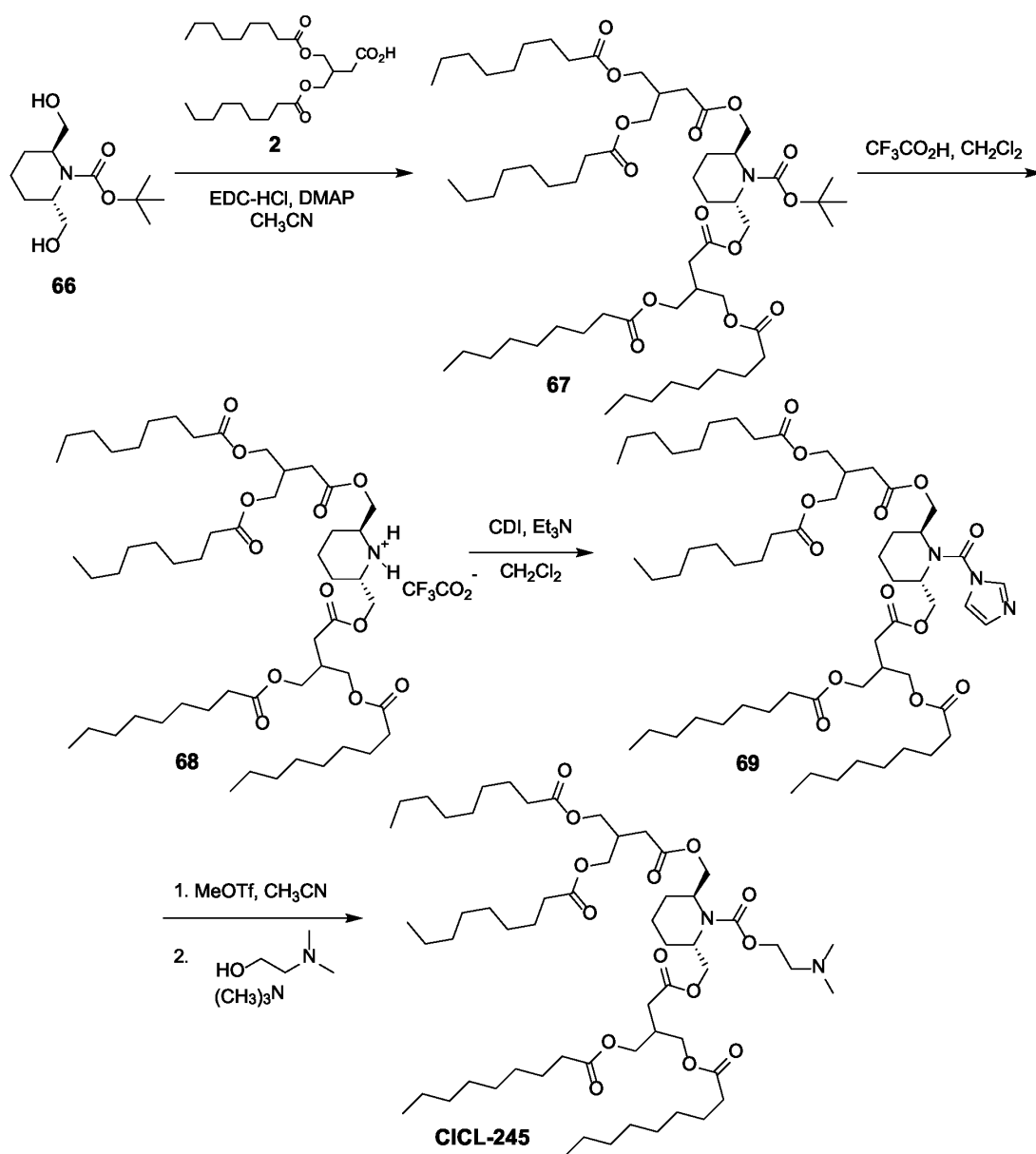
Example 59: Synthesis of (((*Rel*-(2*S*,6*R*)-1-((2-(dimethylamino)ethoxy)carbonyl)piperidine-2,6-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-244)



[00573] tert-Butyl *Rel*-(2*S*,6*R*)-2,6-bis(hydroxymethyl)piperidine-1-carboxylate **62** (*Bioorg. Med. Chem. Lett.* **2010**, *20*, 3584) is coupled with **2** utilizing EDC-HCl and DMAP in CH₃CN to give **63**. Deprotection of the BOC-amine in **63** with CF₃CO₂H in CH₂Cl₂, provides ammonium trifluoroacetate salt **64**. Treatment of **64** with carbonyl-di-imidazole and Et₃N in CH₂Cl₂, gives

acylimizadole **65**. Alkylation of **65** with MeOTf in CH₃CN, followed by the reaction of the derived acylimidazolium salt with 2-dimethylaminoethanol and (CH₃)₃N results in **CICL-244**.

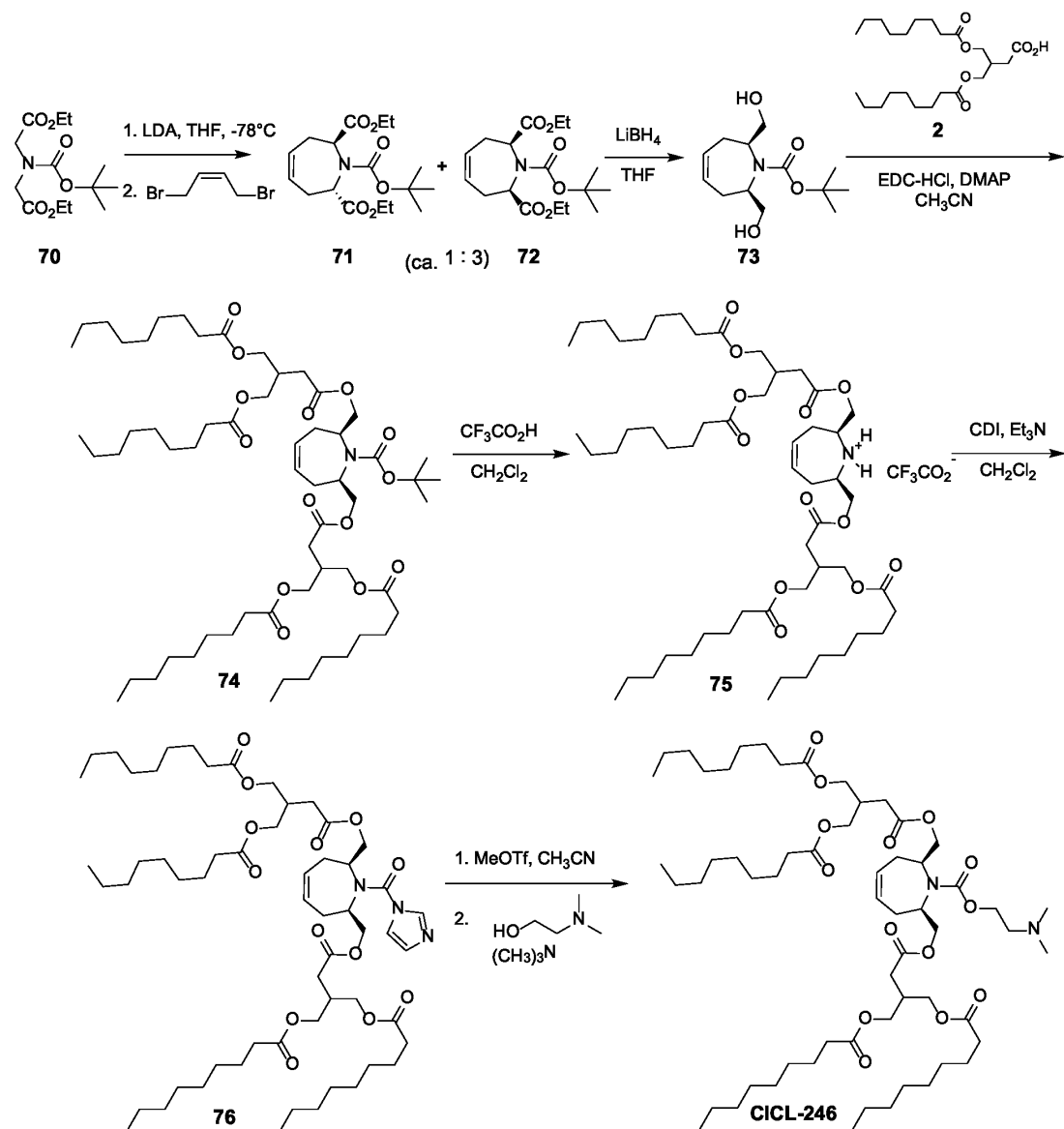
Example 60: Synthesis of (((*Rel*-(2S,6S)-1-((2-(dimethylamino)ethoxy)carbonyl)piperidine-2,6-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-245**)**



[00574] tert-Butyl *Rel*-(2S,6S)-2,6-bis(hydroxymethyl)piperidine-1-carboxylate **66** (*Tetrahedron Lett.* **1989**, 30, 6077) is coupled with **2** utilizing EDC-HCl and DMAP in CH₃CN

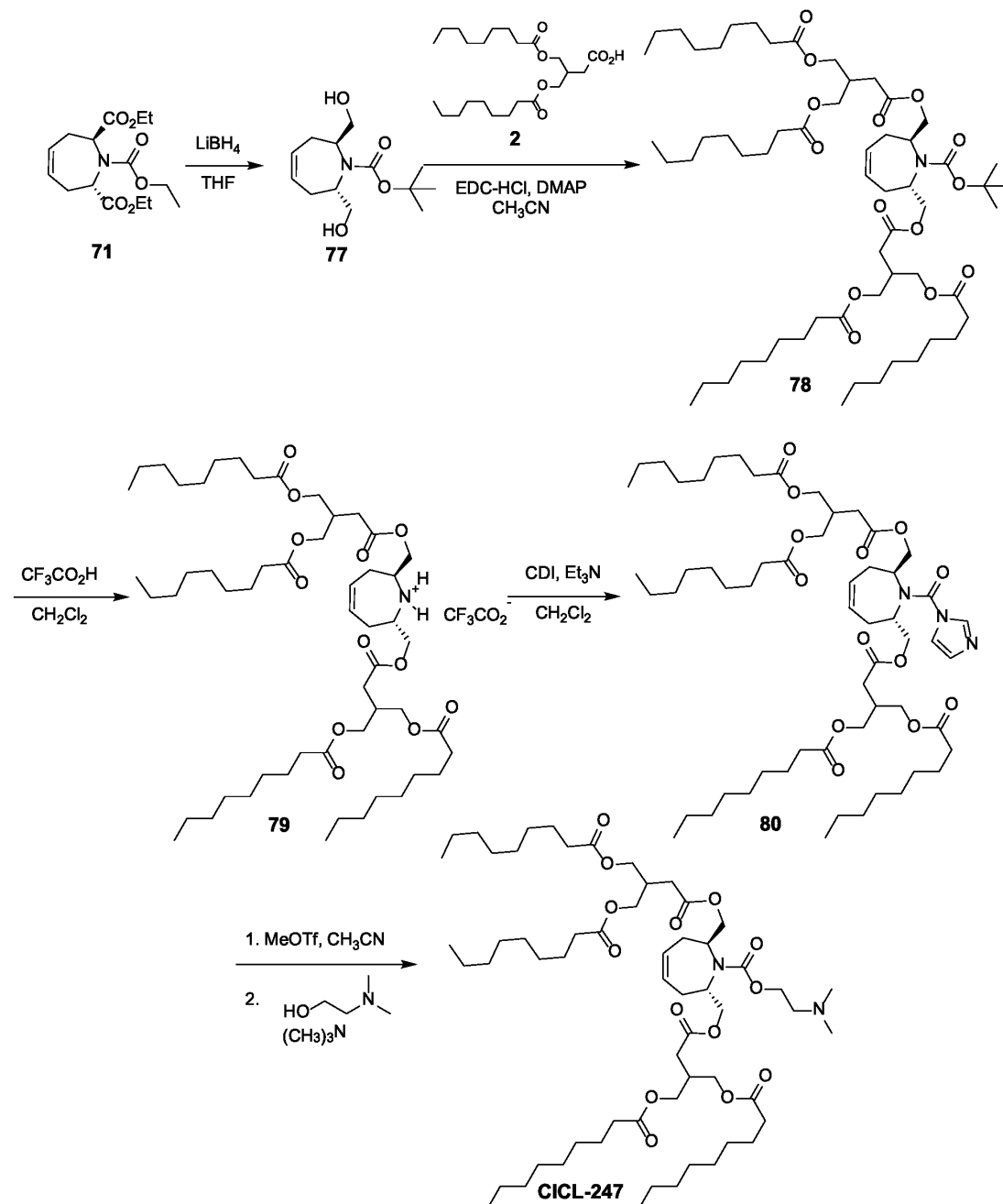
to give **67**. Deprotection of the BOC-amine in **67** with $\text{CF}_3\text{CO}_2\text{H}$ in CH_2Cl_2 , provides ammonium trifluoroacetate salt **68**. Treatment of **68** with carbonyl-di-imidazole and Et_3N in CH_2Cl_2 , then gives acylimidazole **69**. Alkylation of **69** with MeOTf in CH_3CN , followed by the reaction of the derived acylimidazolium salt with 2-dimethylaminoethanol and $(\text{CH}_3)_3\text{N}$ results in **CICL-245**.

Example 61: Synthesis of ((((*Rel*-(2*R*,7*S*)-1-((2-(dimethylamino)ethoxy)carbonyl)-2,3,6,7-tetrahydro-1*H*-azepine-2,7-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-246)



[00575] As described in *Synlett* **1994**, 1093, alkylation of the dianion of **70** with Z-1-4-dibromo-2-butene (*J. Org. Chem.* **2020**, *85*, 5787) leads to a mixture of diesters **71** and **72** in approximately a 1:3 ratio. Separation of the diesters, and reduction of **72** (LiBH₄, THF) leads to diol **73**. Diol **73** is coupled with **2** utilizing EDC-HCl and DMAP in CH₃CN to give **74**. Deprotection of the BOC-amine in **74** with CF₃CO₂H in CH₂Cl₂, provides ammonium trifluoroacetate salt **75**. Treatment of **75** with carbonyl-di-imidazole and Et₃N in CH₂Cl₂, then gives acylimizadole **76**. Alkylation of **76** with MeOTf in CH₃CN, followed by the reaction of the derived acylimidazolium salt with 2-dimethylaminoethanol and (CH₃)₃N results in **CICL-246**.

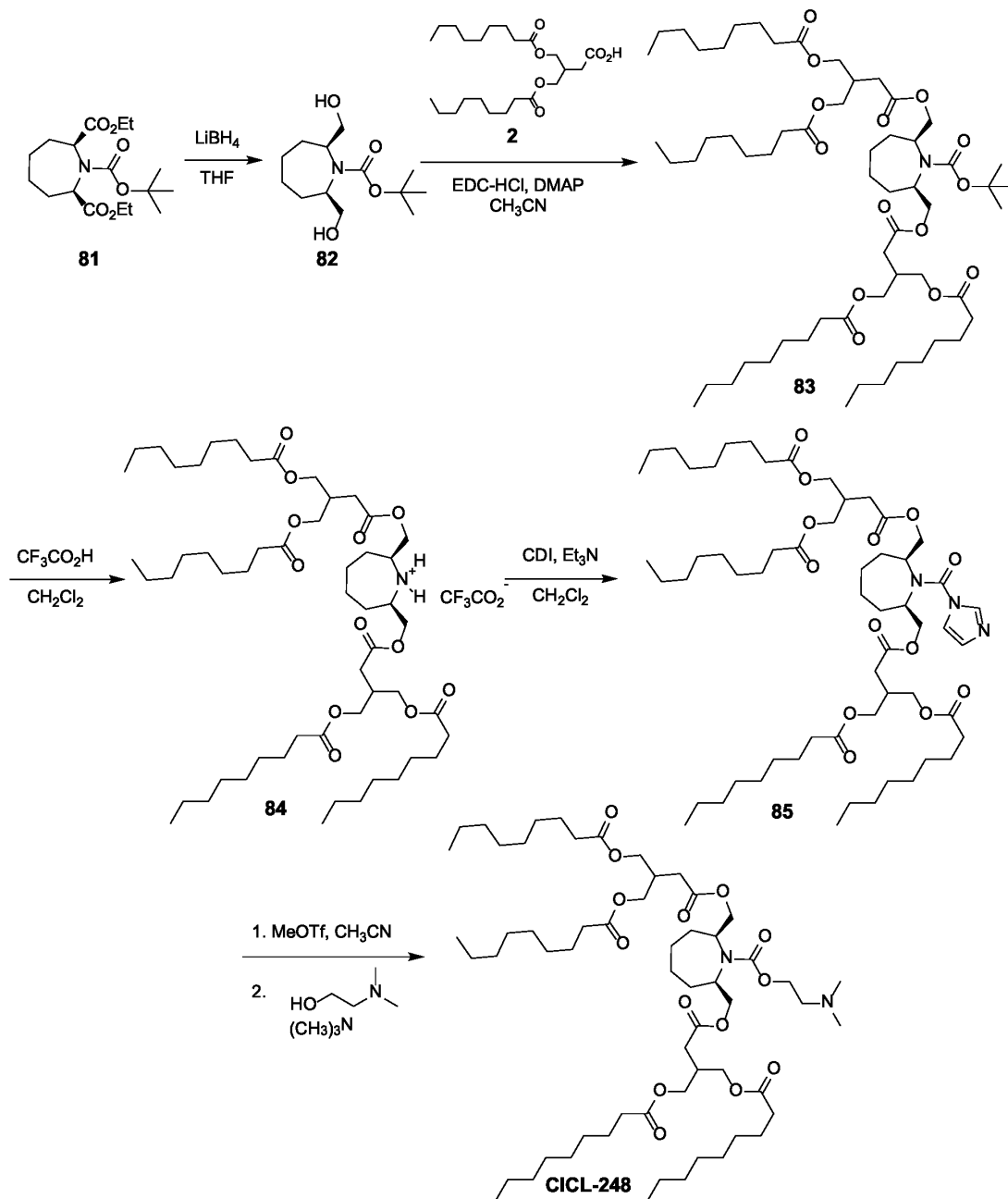
Example 62: Synthesis of ((((*Rel*-(2*S*,7*S*)-1-((2-(dimethylamino)ethoxy)carbonyl)-2,3,6,7-tetrahydro-1*H*-azepine-2,7-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-247)



[00576] Reduction of diester **71**, prepared as described in Example 62, with LiBH_4 in THF, leads to diol **77**. Diol **77** is coupled with **2** utilizing EDC-HCl and DMAP in CH_3CN to give **78**.

Deprotection of the BOC-amine in **78** with $\text{CF}_3\text{CO}_2\text{H}$ in CH_2Cl_2 , provides ammonium trifluoroacetate salt **79**. Treatment of **79** with carbonyl-di-imidazole and Et_3N in CH_2Cl_2 , then gives acylimizadole **80**. Alkylation of **80** with MeOTf in CH_3CN , followed by the reaction of the derived acylimidazolium salt with 2-dimethylaminoethanol and $(\text{CH}_3)_3\text{N}$ results in **CICL-247**.

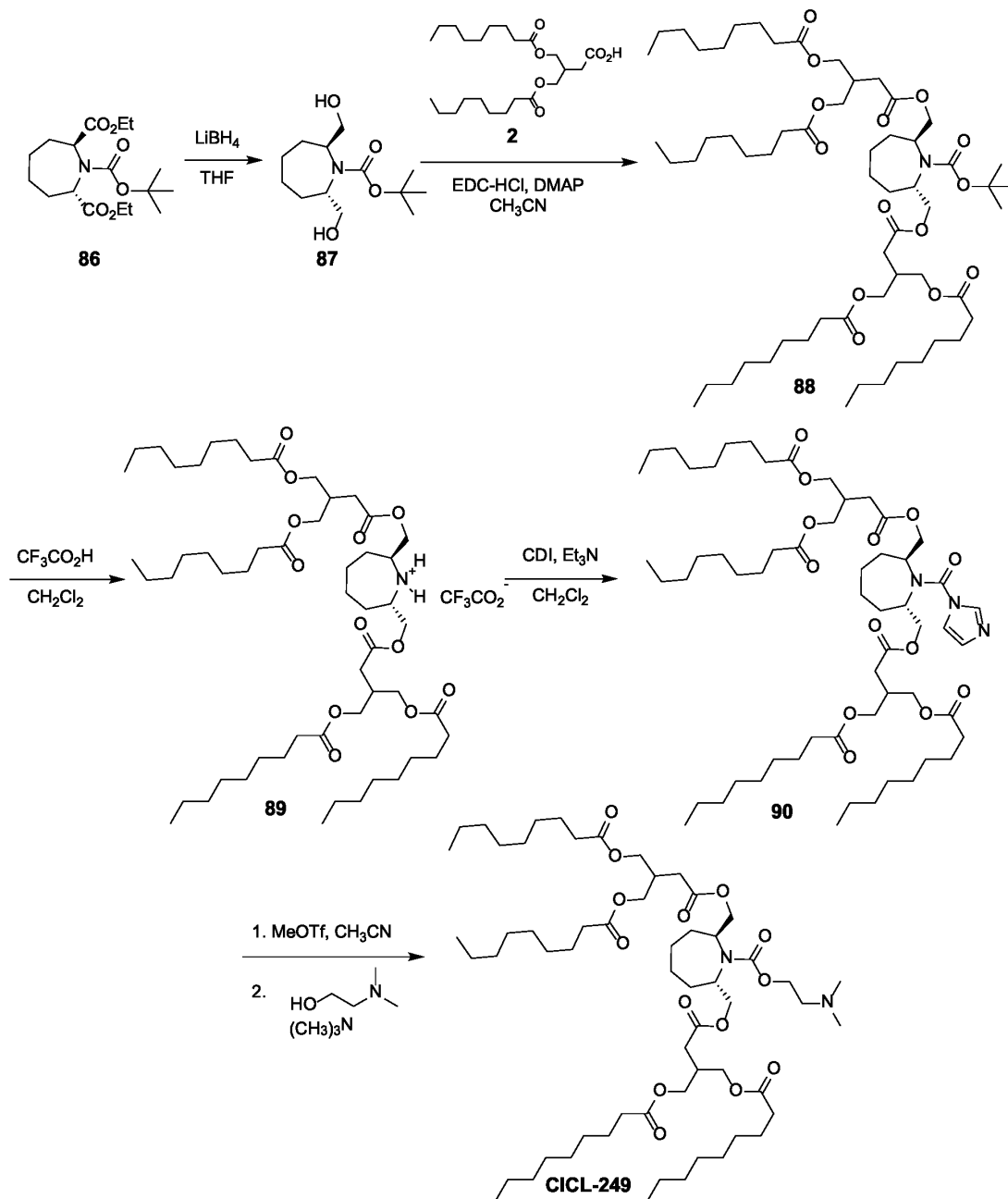
Example 63: Synthesis of ((((*Rel*-(2*S*,7*R*)-1-((2-(dimethylamino)ethoxy)carbonyl)azepane-2,7-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-248)



[00577] 1-(tert-Butyl) 2,7-diethyl *Rel*-(2*S*,7*R*)-azepane-1,2,7-tricarboxylate **81** (*Synlett* **1994**, 1023) is reduced with LiBH_4 in THF, to give diol **82**. Diol **82** is coupled with **2** utilizing

EDC-HCl and DMAP in CH₃CN to give **83**. Deprotection of the BOC-amine in **83** with CF₃CO₂H in CH₂Cl₂, provides ammonium trifluoroacetate salt **84**. Treatment of **84** with carbonyl-diimidazole and Et₃N in CH₂Cl₂, then gives acylimizadole **85**. Alkylation of **85** with MeOTf in CH₃CN, followed by the reaction of the derived acylimidazolium salt with 2-dimethylaminoethanol and (CH₃)₃N results in **CICL-248**.

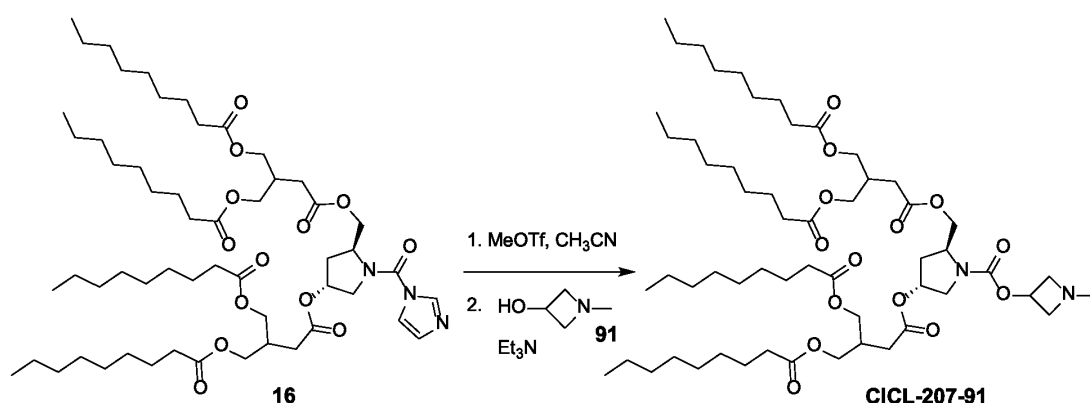
Example 64: Synthesis of ((((*Rel*-(2*S*,7*S*)-1-((2-(dimethylamino)ethoxy)carbonyl)azepane-2,7-diyl)bis(methylene))bis(oxy))bis(2-oxoethane-2,1-diyl))bis(propane-2,1,3-triyl) tetranonanoate (CICL-249)



[00578] 1-(tert-Butyl) 2,7-diethyl *Rel*-(2*S*,7*S*)-azepane-1,2,7-tricarboxylate **86** (*Synlett* **1994**, 1023) is reduced with LiBH_4 in THF, to give diol **87**. Diol **87** is coupled with **2** utilizing

EDC-HCl and DMAP in CH₃CN to give **88**. Deprotection of the BOC-amine in **88** with CF₃CO₂H in CH₂Cl₂, provides ammonium trifluoroacetate salt **89**. Treatment of **89** with carbonyl-diimidazole and Et₃N in CH₂Cl₂, then gives acylimidazole **90**. Alkylation of **90** with MeOTf in CH₃CN, followed by the reaction of the derived acylimidazolium salt with 2-dimethylaminoethanol and (CH₃)₃N results in **CICL-249**.

Example 65: Synthesis of 2-(2-(((2S,4R)-1-(((1-methylazetidin-3-yl)oxy)carbonyl)-4-((4-(nonanoyloxy)-3-((nonanoyloxy)methyl)butanoyl)oxy)pyrrolidin-2-yl)methoxy)-2-oxoethyl)propane-1,3-diyl dinonanoate: CICL-207-91

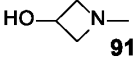
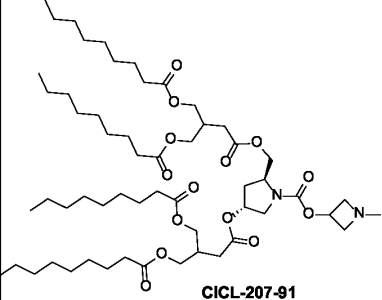
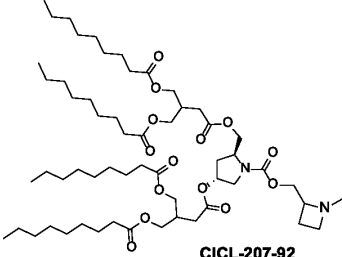
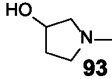
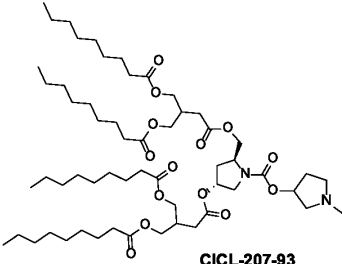
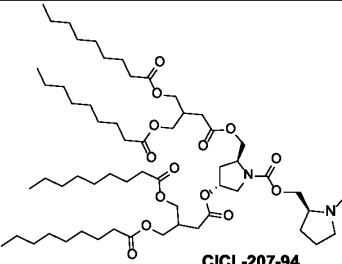
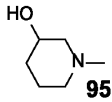
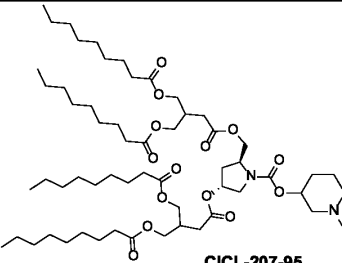


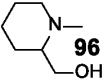
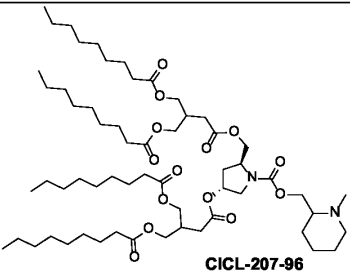
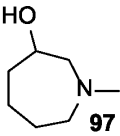
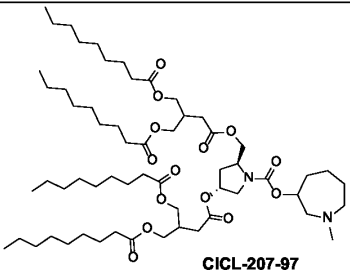
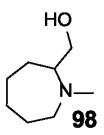
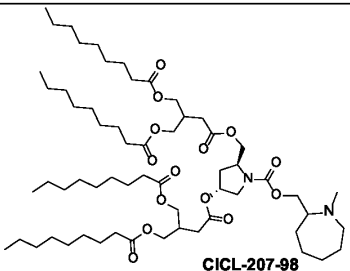
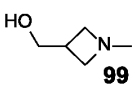
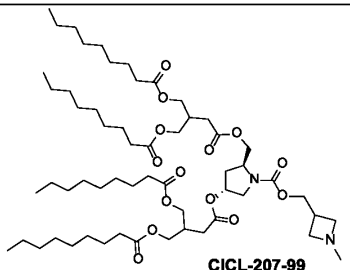
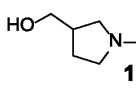
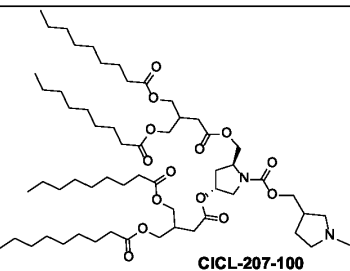
[00579] Acylimidazolidine **16**, when reacted with MeOTf provides an activated acylimidazolium species. The reaction of the activated acylimidazolium species with alcohol **91** (CombiBlocks # JL-5330), in the presence of triethyl amine leads to **CICL-207-91**.

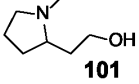
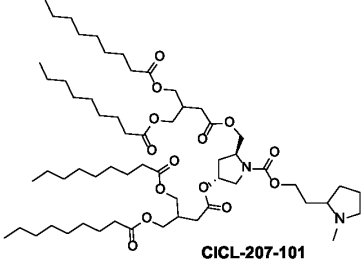
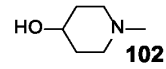
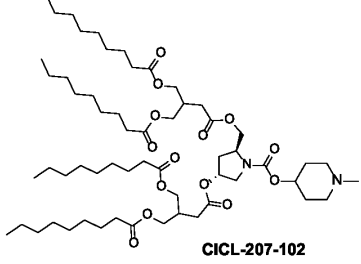
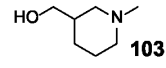
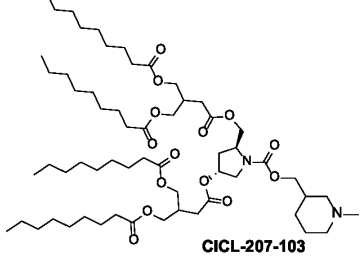
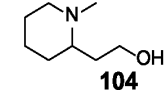
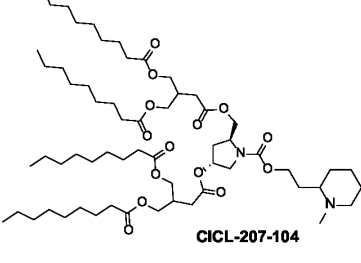
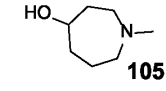
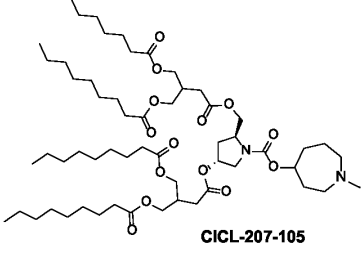
[00580] The numbering paradigm utilized for cyclic-amino-alcohol head groups is based on an ionizable cationic lipid described above that was generated from an acylimidazolidine, in this case **16**, when it was reacted with 2-dimethylaminoethanol, with the number of the head group-forming alcohol appended, in this case **91**. Acylimidazolidine **16** was converted to lipid **CICL-207** in Example 17.

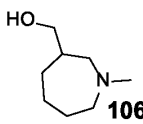
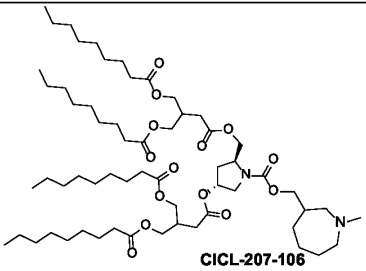
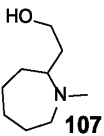
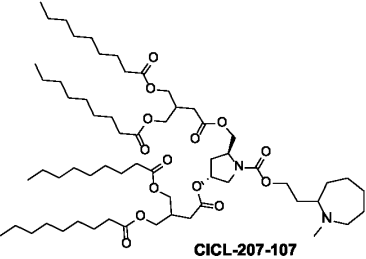
[00581] The utilization of the chemistry described in this Example 65 to generate the active imidazolium species, enables the synthesis of a selection of lipids containing cyclic head groups by substitution of the alcohol **91** (vide supra) with the alcohols appearing in Table 3.

Table 3: CICL-207-91 and Substitution of Alcohol 91 with Alcohols 92-107 to Prepare Lipids CICL-207-92 to CICL-207-107

Alcohol	Source	Lipid	c-pKa*
 91	CombiBlocks # JL-5330	 C1CL-207-91	8.39
 92	CombiBlocks # JL-5446	 C1CL-207-92	8.62
 93	CombiBlocks # AM-2015	 C1CL-207-93	8.70
 94	CombiBlocks # OR-0903	 C1CL-207-94	9.47
 95	<i>J. Med. Pharm. Chem.</i> 1960, 2, 107	 C1CL-207-95	8.83

Alcohol	Source	Lipid	c-pKa*
 96	CombiBlocks # OR-1501	 C1CL-207-96	9.24
 97	CombiBlocks #ST-4233 WO2008022979 <i>J. Med. Pharm. Chem.</i> 1960, 2, 107	 C1CL-207-97	8.44
 98	CombiBlocks #JA-4992 <i>J. Med. Pharm. Chem.</i> 1960, 2, 107	 C1CL-207-98	8.88
 99	CombiBlocks # QV-6461	 C1CL-207-99	9.23
 100	CombiBlocks # QH-5249	 C1CL-207-100	9.83

Alcohol	Source	Lipid	c-pKa*
 101	CombiBlocks # ST-1090	 C1CL-207-101	10.28
 102	CombiBlocks # AM-2246	 C1CL-207-102	8.03
 103	CombiBlocks # OR-1504	 C1CL-207-103	9.20
 104	CombiBlocks # QZ-3821	 C1CL-207-104	9.75
 105	CombiBlocks # SS-2386	 C1CL-207-105	8.81

Alcohol	Source	Lipid	c-pKa*
 106	Enamine #EN300-147890 <i>Med. Pharm. Chem.</i> 1960 , 2, 107	 C1CL-207-106	9.34
 107	Enamine #EN300-202111 <i>Med. Pharm. Chem.</i> 1960 , 2, 107	 C1CL-207-107	9.52

[00582] In a similar fashion, utilizing the chemistry of this Example 65, the various constrained core acylimidazolides: 7, 12, 20, 24, 28, 32, 36, 40, 44, 48, 53, 57, 61, 65, 69, 76, 80, 85 and 90 are reacted with alcohols 91-107 to create the congeners having the various cyclic head groups on each of the constrained cores described above. Given a desired range of measured pKa of from 6-7, a restriction of the range of calculated pKa (c-pKa) is likely needed to achieve that target measured pKa range. The alcohols which are appended to acylimidazolide 16 to provide lipids C1CL-207-91 to C1CL-207-107, which result in a calculated pKa (c-pKa) of ca. 8 to 9 (Table 3), are utilized to form lipids with the constrained core acylimidazolides 7, 12, 20, 24, 28, 32, 36, 40, 44, 48, 53, 57, 61, 65, 69, 76, 80, 85 and 90. The alcohols thus selected are: 91, 92, 93, 95, 97, 98, 102, and 105.

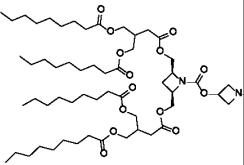
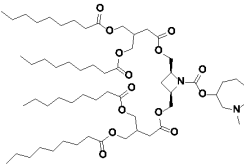
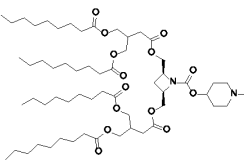
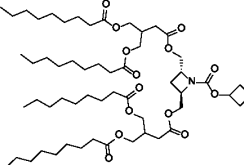
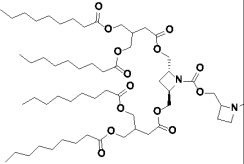
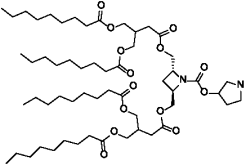
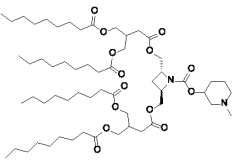
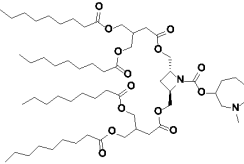
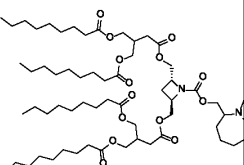
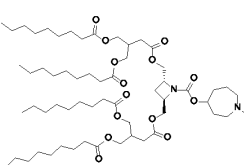
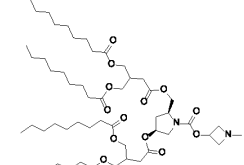
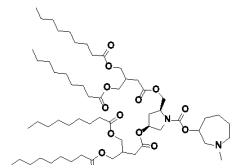
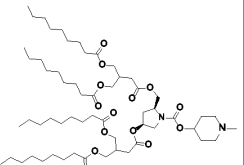
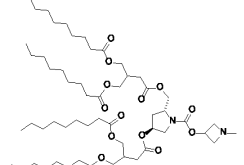
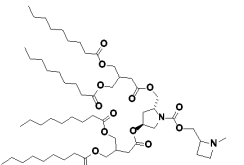
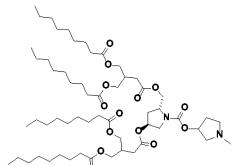
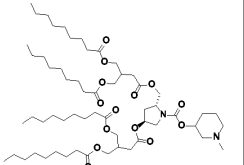
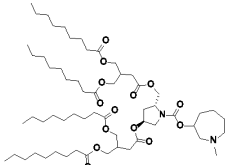
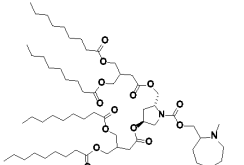
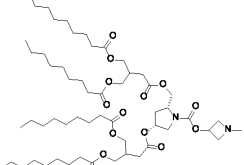
[00583] To obtain lipids conferring advantageous *in vivo* transfection performance on tLNPs incorporating the lipid, the alcohols should be selected to alter the measured pKa values for the core imidazolides appended with N,N-dimethylamino ethanol in Table 10 toward the mid-point of the target range of 6-7. To maintain or reduce the basicity of the lipids prepared from acylimidazolide core structure 7 (used in synthesizing C1CL-221), alcohols 91, 97, and 102 are selected to generate C1CL-221-91, C1CL-221-97, and C1CL-221-102. Core acylimidazolide 12 (used in synthesizing C1CL-222) is combined with alcohols 91, 92, 93, 95, 97, 98, and 105 to maintain or raise measured pKa as described to generate congeners of C1CL-222. Core acylimidazolide 20 (used in synthesizing C1CL-224) is combined with alcohols

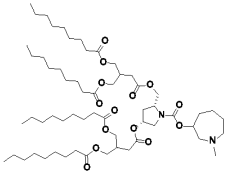
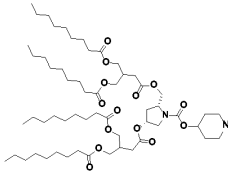
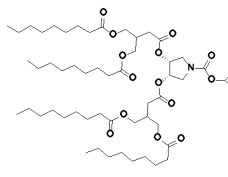
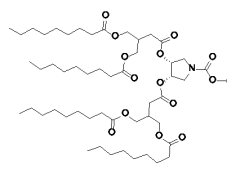
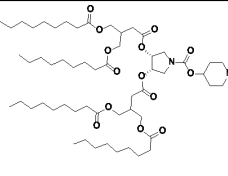
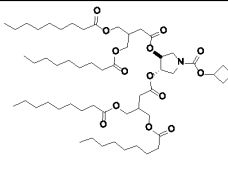
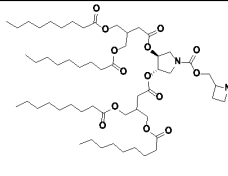
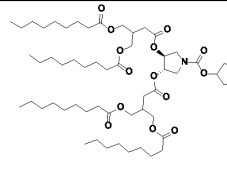
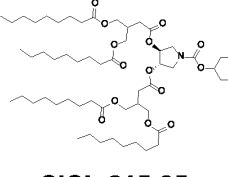
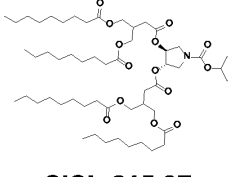
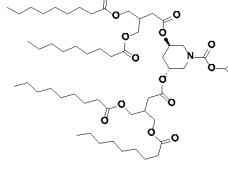
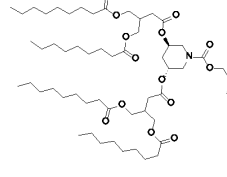
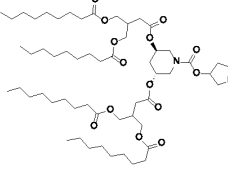
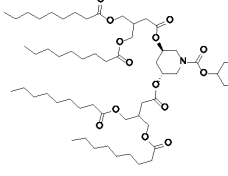
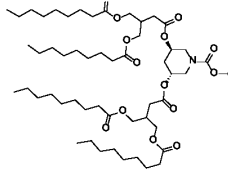
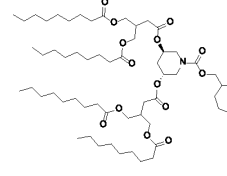
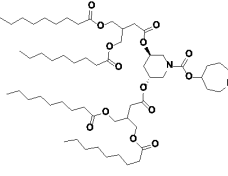
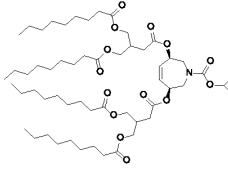
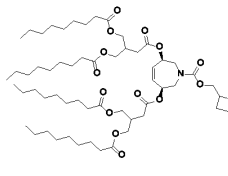
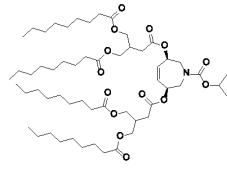
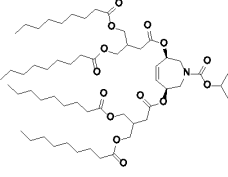
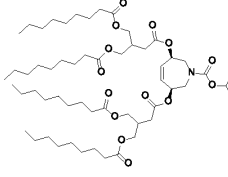
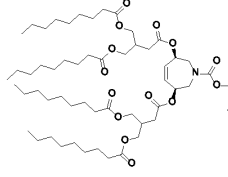
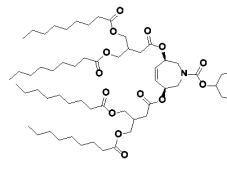
91, 97, and 102 to maintain or reduce measured pKa toward the target range and generate CICAL-224-91, CICAL-224-97, and CICAL-224-102. The combination of core acylimidazolide 24 (used in synthesizing CICAL-225) with alcohols 91, 92, 93, 95, 97, and 98 is selected to maintain or raise measured pKa toward the desired target range to generate congeners of CICAL-225. Acylimidazolide 28, (used in synthesizing CICAL-223) when combined with alcohols 91, 97, and 102, results in lipids (CICAL-223-91, CICAL-223-97, and CICAL-223-102) that approach the target measured pKa values by maintaining or reducing basicity. Acylimidazolide 32 (used in synthesizing CICAL-216) is combined with alcohols 91, 97, and 102 to afford lipids (CICAL-216-91, CICAL-216-97, and CICAL-216-102) in the target pKa range by maintaining or reducing basicity. Core acylimidazolide 36 (used in synthesizing CICAL-215) is combined with alcohols 91, 92, 93, 95, and 97 to create lipids (congeners of CICAL-215) with similar basicity and a pKa in the target pKa range. Acylimidazolide 40, (used in synthesizing CICAL-220) when combined with alcohols 91, 92, 93, 95, 97, 98, and 105 gives lipids (congeners of CICAL-220) with a maintained or raised basicity moving pKa toward the desired target range. Similarly, the combination of acylimidazolide 44 (used in synthesizing CICAL-219) with alcohols 91, 92, 93, 95, 97, 98, and 105 provides lipids (congeners of CICAL-219) with a maintained or raised basicity moving pKa toward the desired target pKa range. The reaction of acylimidazolide 48 (used in synthesizing CICAL-218) with alcohols 94, 96, 99, 103, and 106 provides lipids (congeners of CICAL-218) with a maintained or raised basicity moving pKa toward the target pKa range. The combination of acylimidazolide 53 (used in synthesizing CICAL-238) with alcohols 91, 97, and 102 leads to lipids (CICAL-238-91, CICAL-238-97, and CICAL-238-102) with maintained or reduced basicity and measured pKa values, within the target zone. The concatenation of acylimidazolide 57 (used in synthesizing CICAL-242), which is not associated with a measured pKa value in Table 10, with alcohols 91, 92, 93, 95, 97, 98, 102, and 105 affords lipids (congeners of CICAL-242) targeted for measured pKa values within the desired range by having a similar basicity to CICAL-207. Similarly, the reaction of acylimidazolides 61, 65, 69, 76, 80, 85, and 90 (used in synthesizing CICAL-243, CICAL-244, CICAL-245, CICAL-246, CICAL-247, CICAL-248, and CICAL-249, respectively) with alcohols 91, 92, 93, 95, 97, 98, 102, and 105 provides lipids targeted for measured pKa values within the desired range by having a similar basicity to CICAL-207.

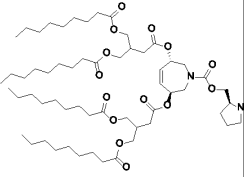
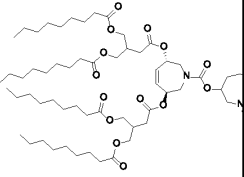
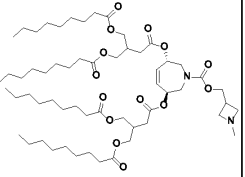
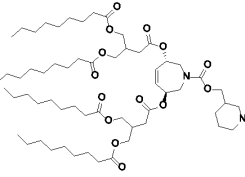
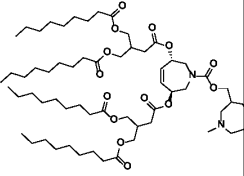
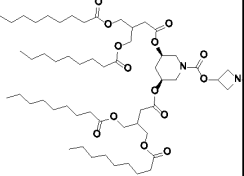
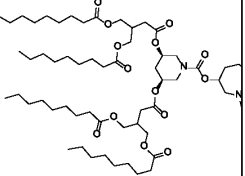
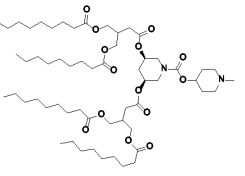
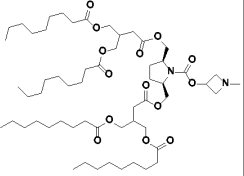
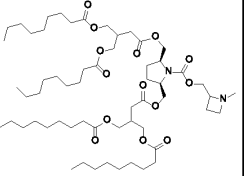
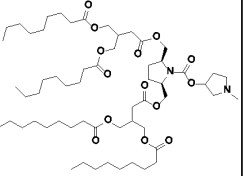
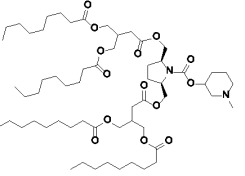
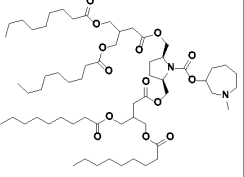
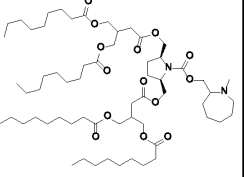
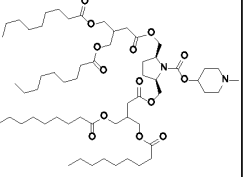
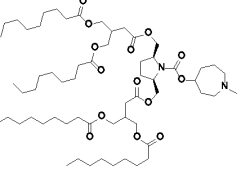
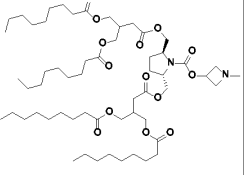
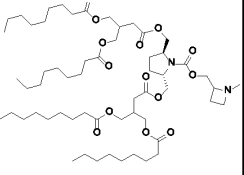
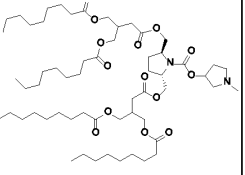
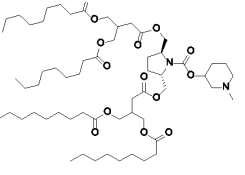
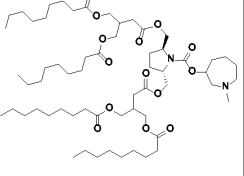
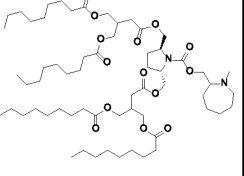
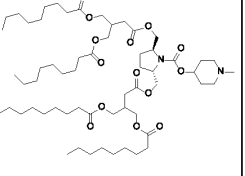
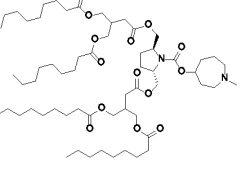
[00584] Table 4 presents the structures that result from the combination of acylimidazolide core structures: 7, 12, 20, 24, 28, 32, 36, 40, 44, 48, 53, 57, 61, 65, 69, 76, 80, 85 and 90 with the above mentioned alcohols (91, 92, 93, 95, 97, 98, 102, and 105) which are expected to result in lipids with calculated pKa values in the range of 8-9. The numbering convention applied to the structures in Table 4, as described above, is taken from the initial lipid structure

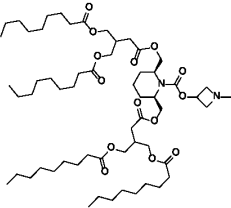
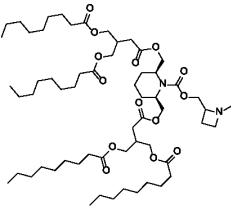
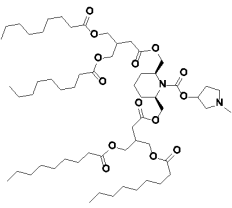
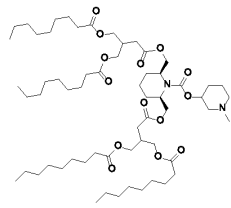
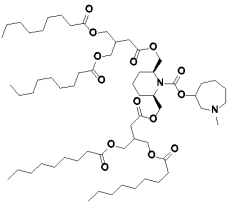
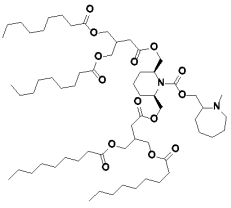
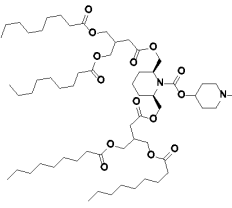
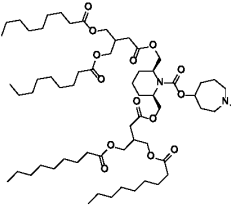
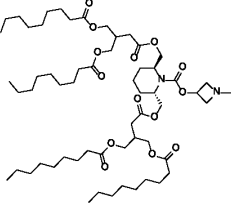
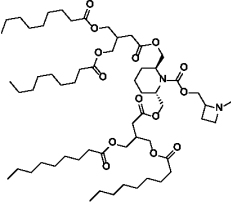
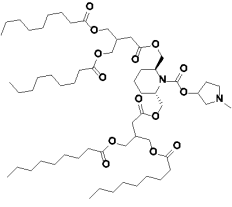
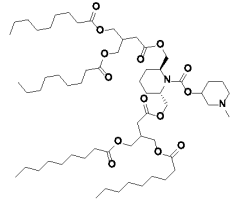
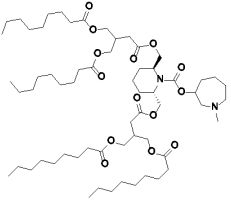
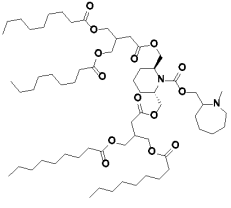
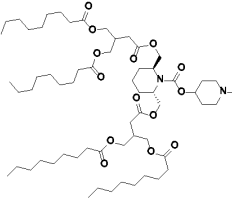
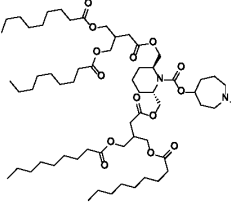
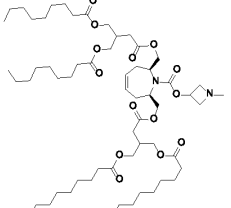
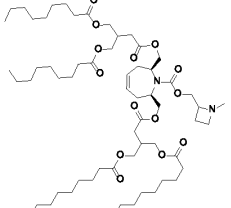
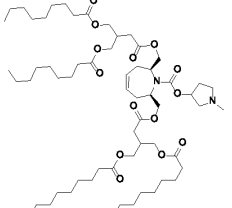
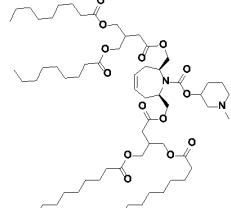
created from the listed core acylimidazolide, now combined with the selected alcohols listed above, e.g. 7 leading to C1CL-221, when 7 might be combined with alcohol 91, the number appearing in Table 4 is listed as: C1CL-221-91.

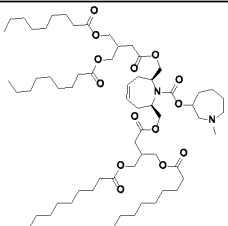
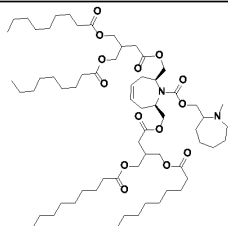
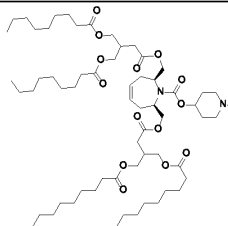
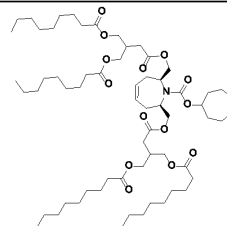
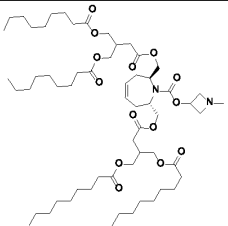
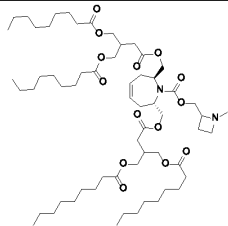
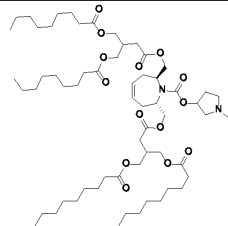
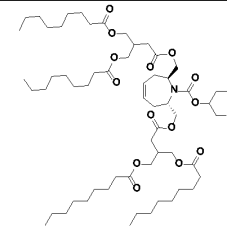
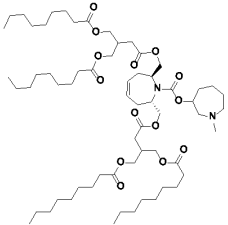
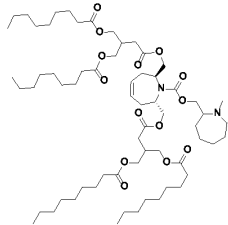
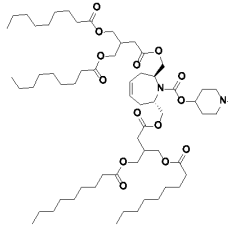
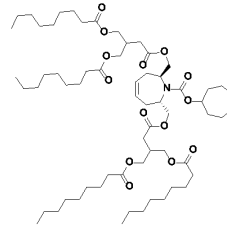
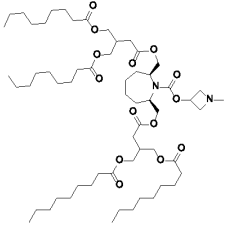
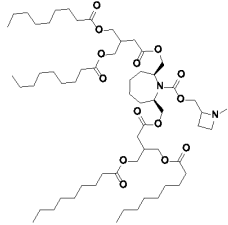
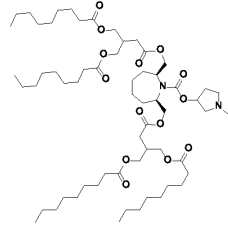
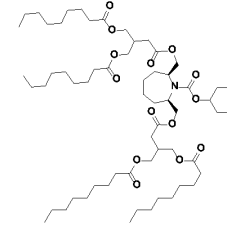
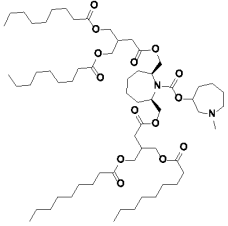
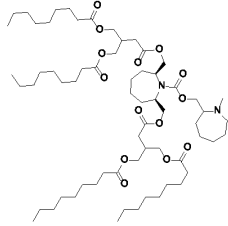
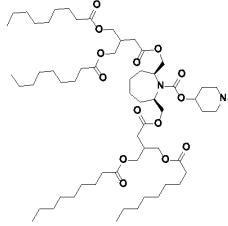
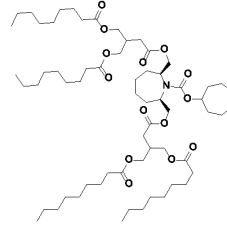
Table 4. Lipids formed from the Combination of Core Acylimidazolides 7, 12, 20, 24, 28, 32, 36, 40, 44, 48, 53, 57, 61, 65, 69, 76, 80, 85 and 90 with Alcohols selected from 91, 92, 93, 94, 95, 97, 98, 99, 102, 103, 105, and 106.

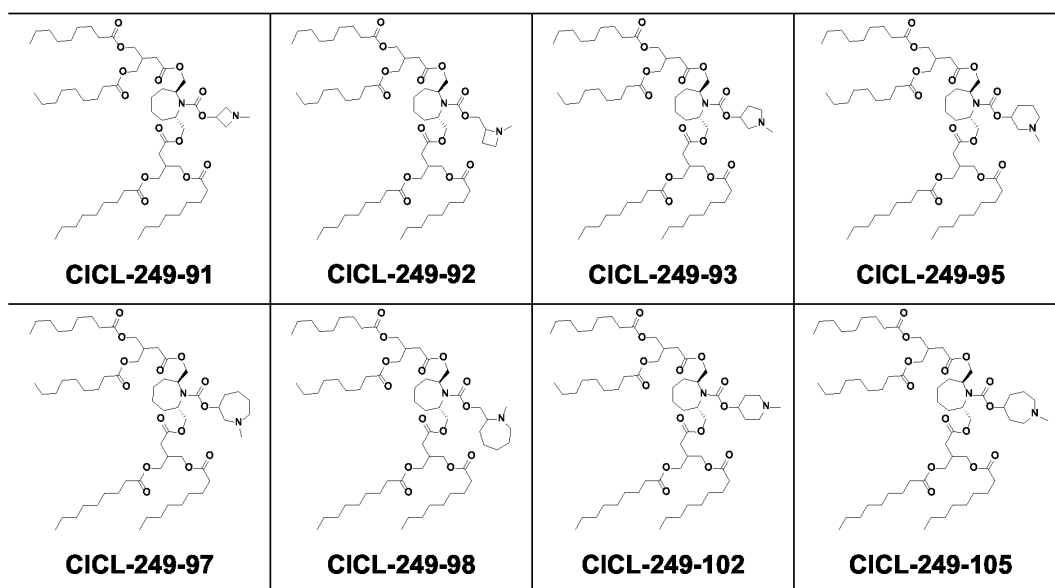
			
C1CL-221-91	C1CL-221-97	C1CL-221-102	C1CL-222-91
			
C1CL-222-92	C1CL-222-93	C1CL-222-95	C1CL-222-97
			
C1CL-222-98	C1CL-222-105	C1CL-224-91	C1CL-224-97
			
C1CL-224-102	C1CL-225-91	C1CL-225-92	C1CL-225-93
			
C1CL-225-95	C1CL-225-97	C1CL-225-98	C1CL-223-91

 C1CL-223-97	 C1CL-223-102	 C1CL-216-91	 C1CL-216-97
 C1CL-216-102	 C1CL-215-91	 C1CL-215-92	 C1CL-215-93
 C1CL-215-95	 C1CL-215-97	 C1CL-220-91	 C1CL-220-92
 C1CL-220-93	 C1CL-220-95	 C1CL-220-97	 C1CL-220-98
 C1CL-220-105	 C1CL-219-91	 C1CL-219-92	 C1CL-219-93
 C1CL-219-95	 C1CL-219-97	 C1CL-219-98	 C1CL-219-105

 C1CL-218-94	 C1CL-218-96	 C1CL-218-99	 C1CL-218-103
 C1CL-218-106	 C1CL-238-91	 C1CL-238-97	 C1CL-238-102
 C1CL-242-91	 C1CL-242-92	 C1CL-242-93	 C1CL-242-95
 C1CL-242-97	 C1CL-242-98	 C1CL-242-102	 C1CL-242-105
 C1CL-243-91	 C1CL-243-92	 C1CL-243-93	 C1CL-243-95
 C1CL-243-97	 C1CL-243-98	 C1CL-243-102	 C1CL-243-105

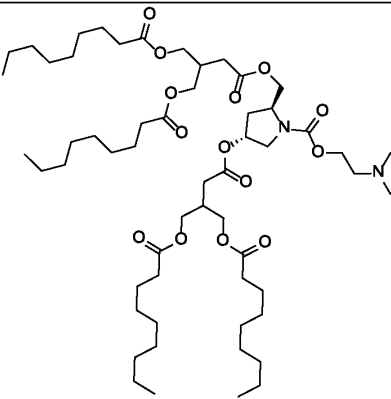
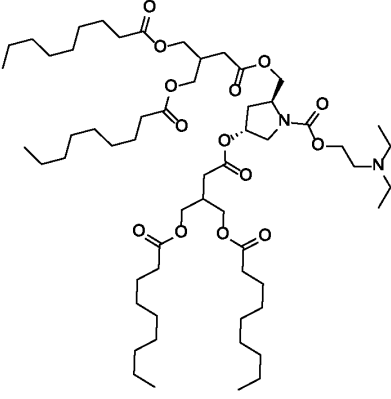
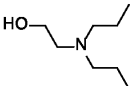
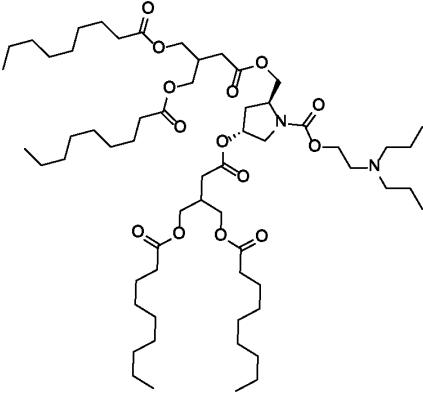
			
C1CL-244-91	C1CL-244-92	C1CL-244-93	C1CL-244-95
			
C1CL-244-97	C1CL-244-98	C1CL-244-102	C1CL-244-105
			
C1CL-245-91	C1CL-245-92	C1CL-245-93	C1CL-245-95
			
C1CL-245-97	C1CL-245-98	C1CL-245-102	C1CL-245-105
			
C1CL-246-91	C1CL-246-92	C1CL-246-93	C1CL-246-95

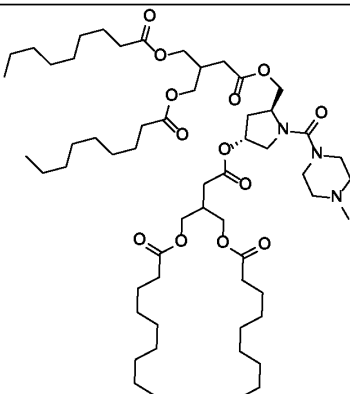
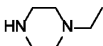
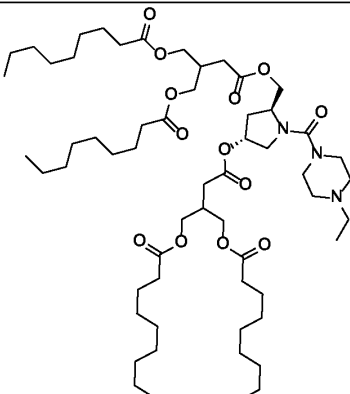
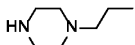
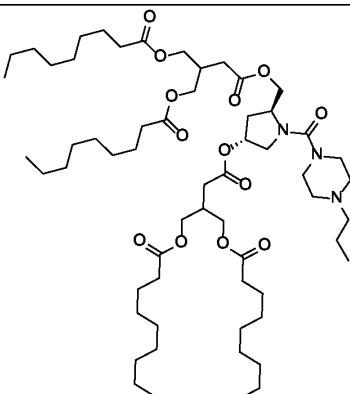
 CICAL-246-97	 CICAL-246-98	 CICAL-246-102	 CICAL-246-105
 CICAL-247-91	 CICAL-247-92	 CICAL-247-93	 CICAL-247-95
 CICAL-247-97	 CICAL-247-98	 CICAL-247-102	 CICAL-247-105
 CICAL-248-91	 CICAL-248-92	 CICAL-248-93	 CICAL-248-95
 CICAL-248-97	 CICAL-248-98	 CICAL-248-102	 CICAL-248-105

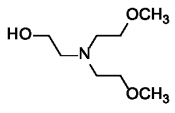
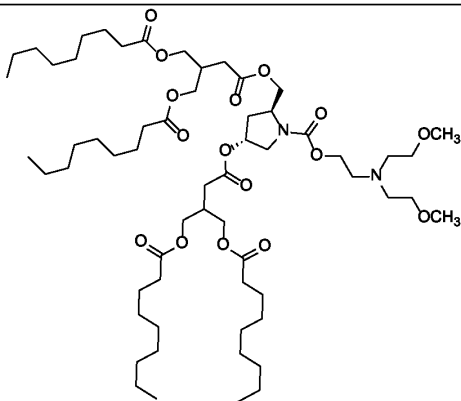
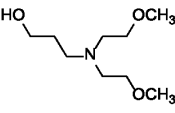
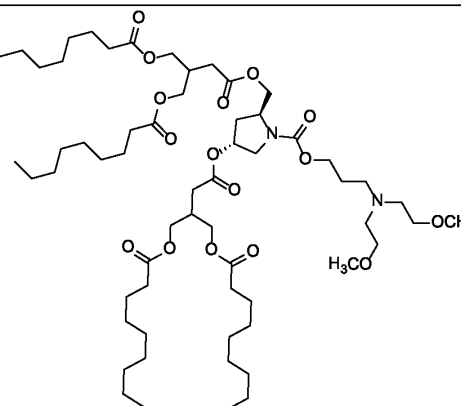
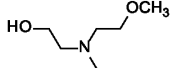
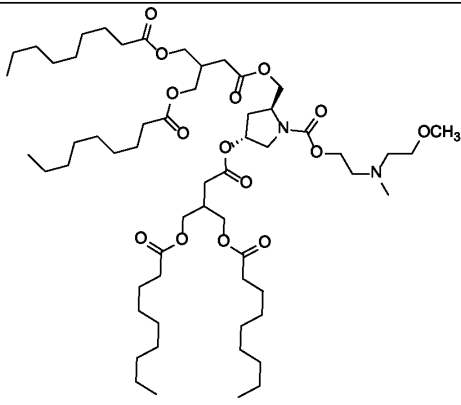


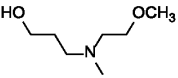
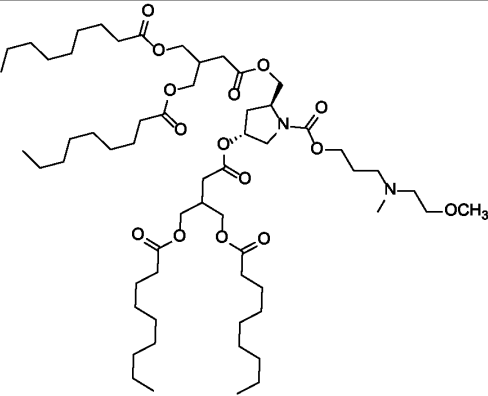
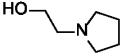
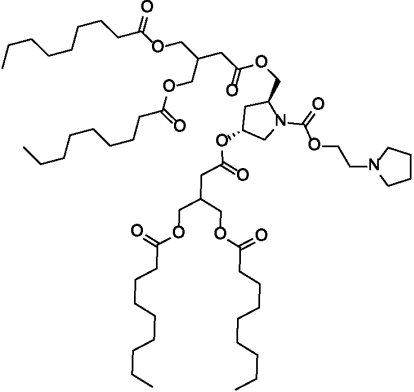
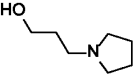
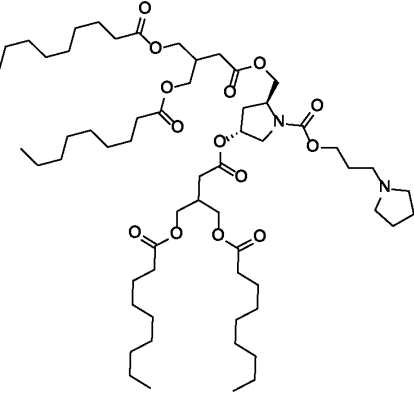
[00585] The data presented in Tables 8, 9, and 10 illustrates the impact of the structure of the core acylimidazolidine on the measured pKa in formulation of lipids as tLNPs all constructed by the addition of N,N-dimethylamino ethanol to form the basic head group. The measured pKa values range from 6.11 (CICAL-218) to 7.09 (CICAL-238). As described above, cyclic head groups were appended to the core acylimidazolidine 16 (used in the synthesis of CICAL-207) and calculated (c-pKa) values were determined. Alcohols were selected to move the measured pKa values for alternate core structures associated with the data of Table 10, toward the midpoint of the target range of 6-7 (Table 4). In the description of Formula M2 (above) the definition of groups X includes further substituents which can be appended to a core structure to form ionizable lipids M2. The examination of the connection of a subset of the groups X, when appended to acylimidazolidine 16, instructs the selection of groups X to be appended to the core acylimidazolidines 7, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 53, 57, 61, 65, 69, 76, 80, 85 and 90 in order to target the midpoint of the measured pKa range of 6-7 for the construction of advantageous ionizable cationic lipids. Table 5 illustrates the products of the combination of acylimidazolidine 16 with head groups 108-133 (selected from the definition of X in the definition of Formula M2) to form lipids CICAL-207-108 through CICAL-207-133, with associated c-pKa data.

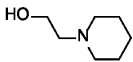
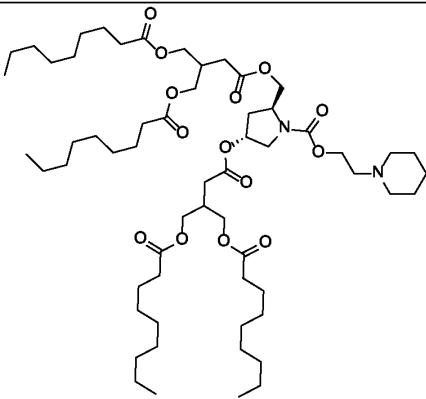
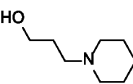
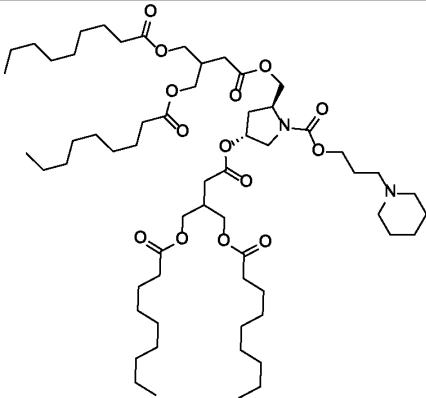
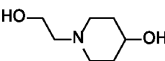
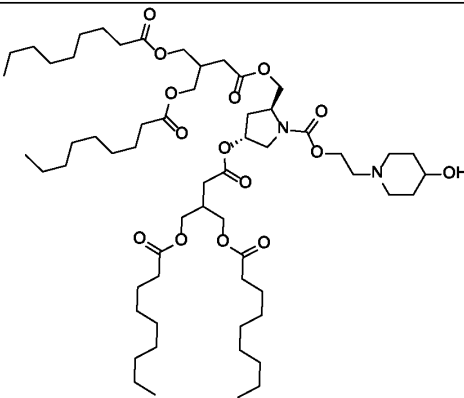
Table 5. Lipids formed from the Combination of Core Acylimidazolidine 7 with head groups selected from 108 through 133.

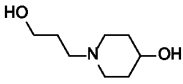
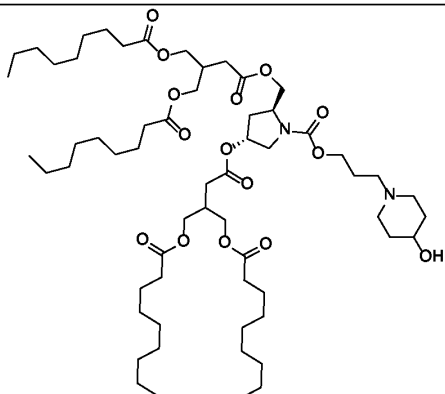
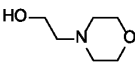
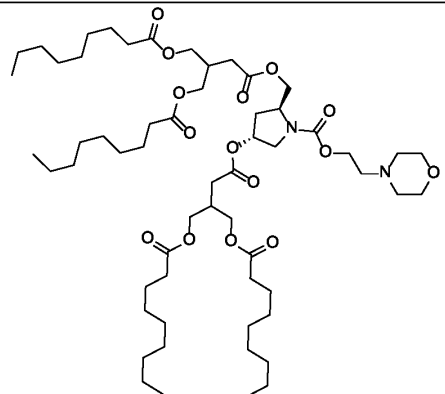
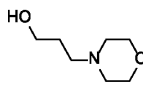
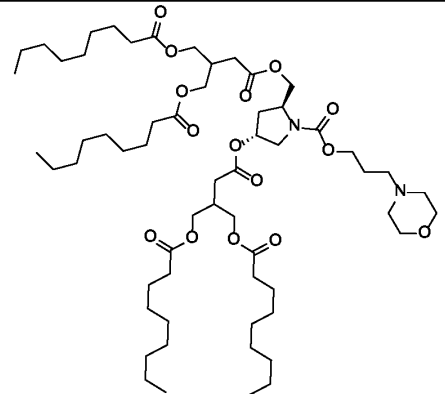
X Precursor	Lipid	c-pKa
	 C1CL-207	8.42
 108	 C1CL-207-108	9.39
 109	 C1CL-207-109	8.58

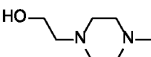
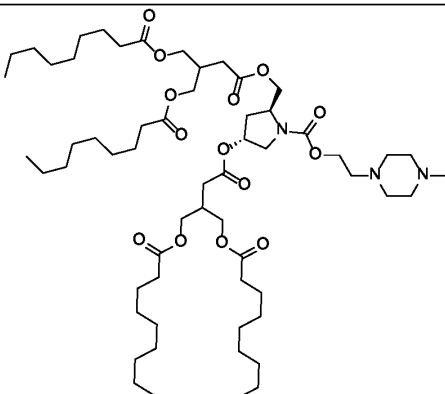
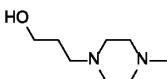
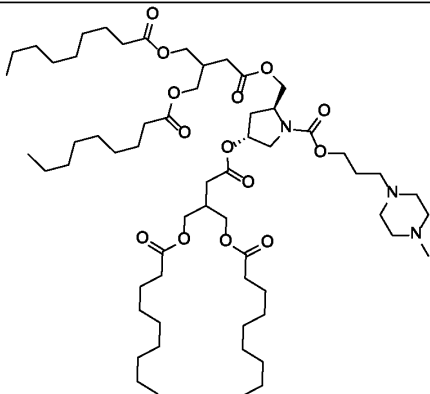
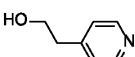
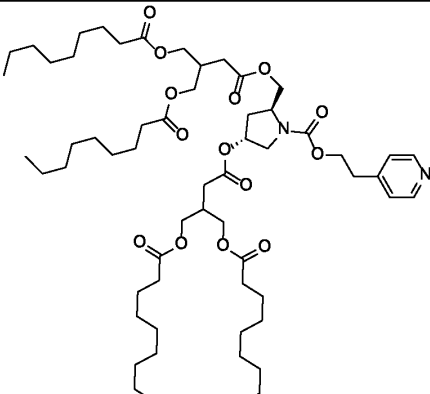
X Precursor	Lipid	c-pKa
 110	 C1CL-207-110	7.16
 111	 C1CL-207-111	7.65
 112	 C1CL-207-112	7.59

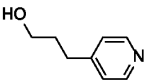
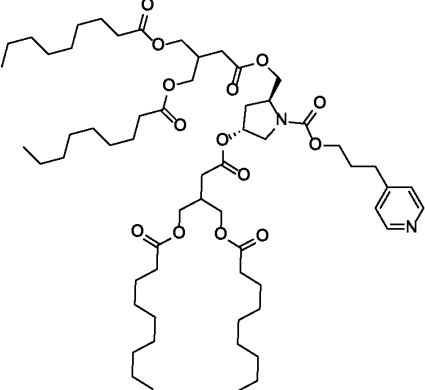
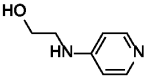
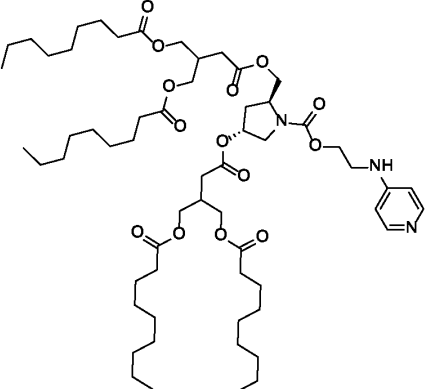
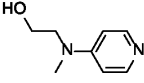
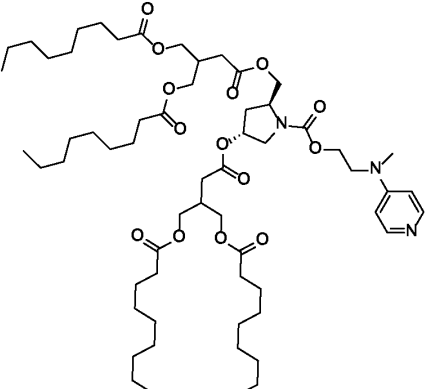
X Precursor	Lipid	c-pKa
 113	 C1CL-207-113	6.68
 114	 C1CL-207-114	7.50
 115	 C1CL-207-115	7.55

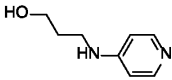
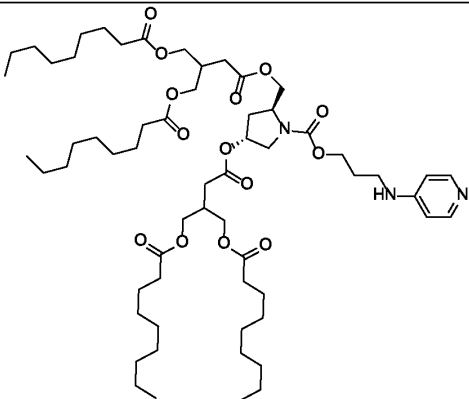
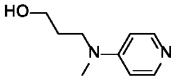
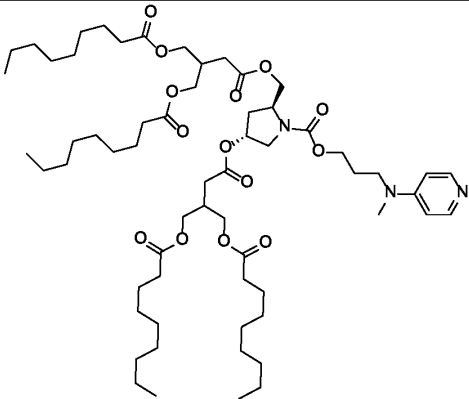
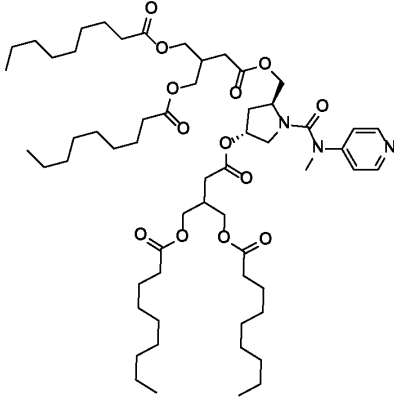
X Precursor	Lipid	c-pKa
 116	 C1CL-207-116	8.37
 117	 C1CL-207-117	9.43
 118	 C1CL-207-118	10.12

X Precursor	Lipid	c-pKa
 119	 C1CL-207-119	8.44
 120	 C1CL-207-120	9.21
 121	 C1CL-207-121	7.77

X Precursor	Lipid	c-pKa
 122	 C1CL-207-122	8.38
 123	 C1CL-207-123	6.17
 124	 C1CL-207-124	7.04

X Precursor	Lipid	c-pKa
 125	 C1CL-207-125	7.56
 126	 C1CL-207-126	7.64
 127	 C1CL-207-127	5.64

X Precursor	Lipid	c-pKa
 128	 C1CL-207-128	5.93
 129	 C1CL-207-129	7.90
 130	 C1CL-207-130	8.65

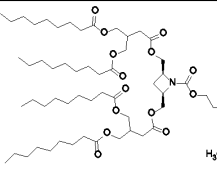
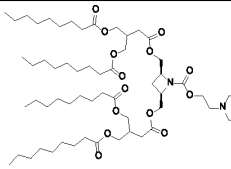
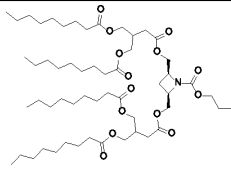
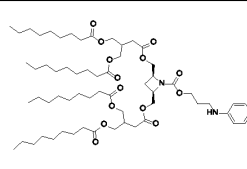
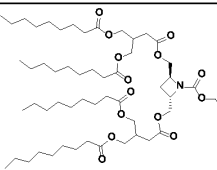
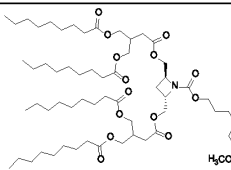
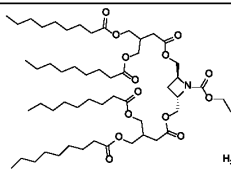
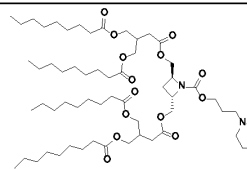
X Precursor	Lipid	c-pKa
 131	 C1CL-207-131	8.14
 132	 C1CL-207-132	9.18
 133	 C1CL-207-133	7.33

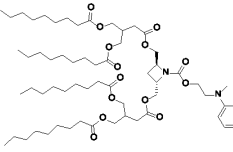
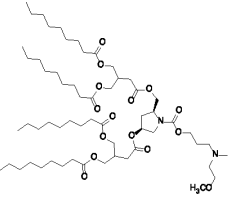
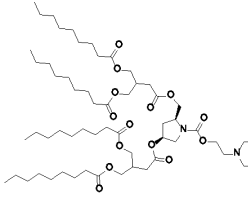
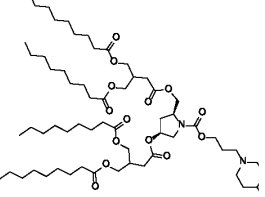
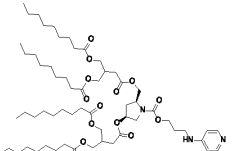
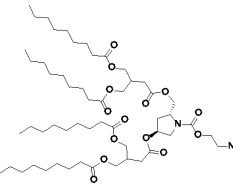
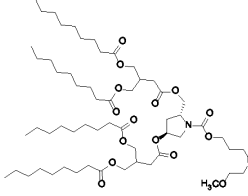
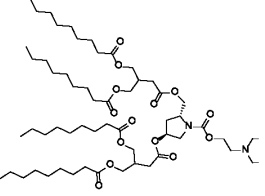
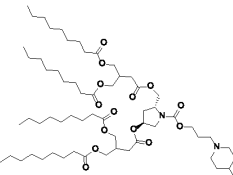
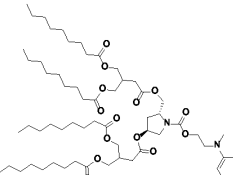
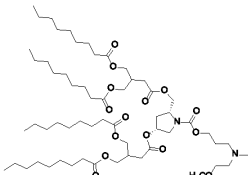
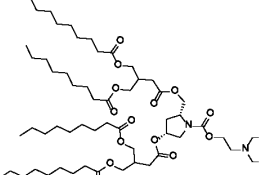
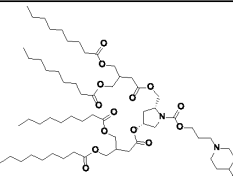
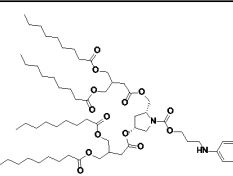
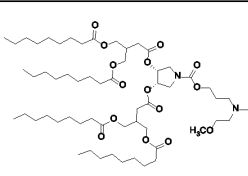
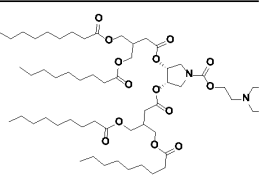
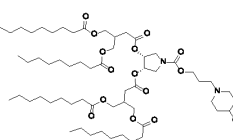
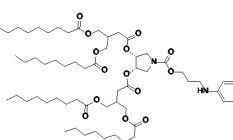
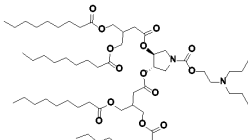
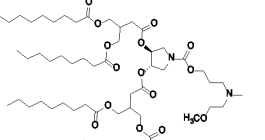
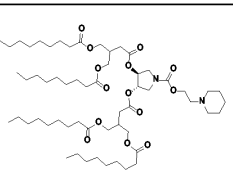
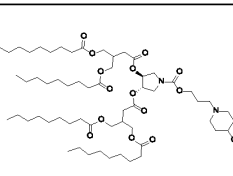
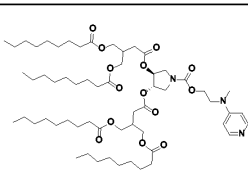
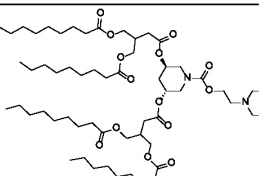
[00586] The measured pKa data associated with C1CL-207 in Table 9 and the calculated pKa data for C1CL-207-108 through C1CL-207-133 enables the prediction that the

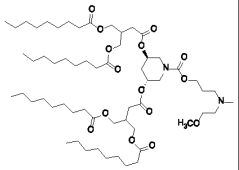
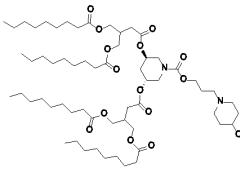
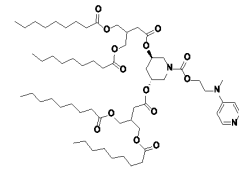
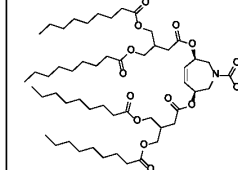
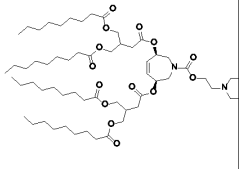
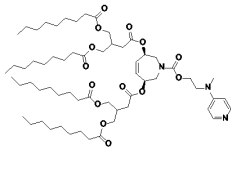
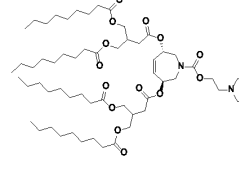
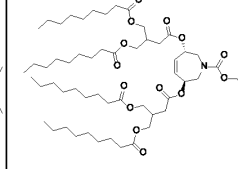
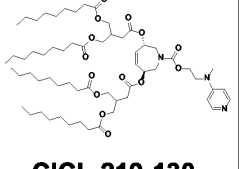
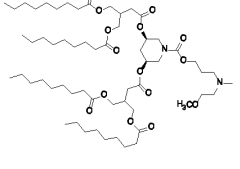
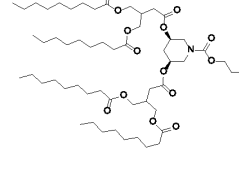
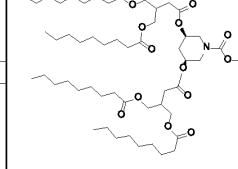
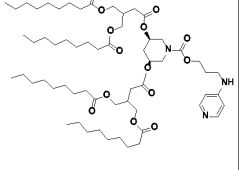
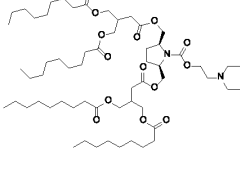
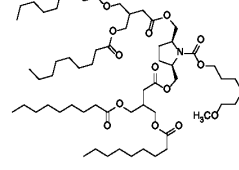
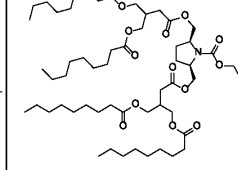
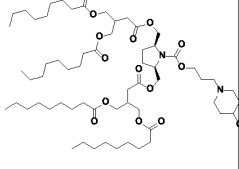
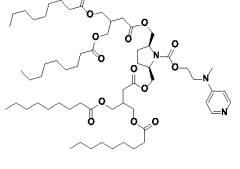
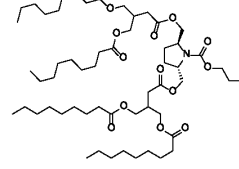
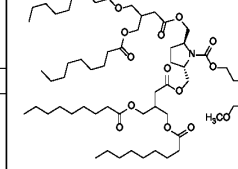
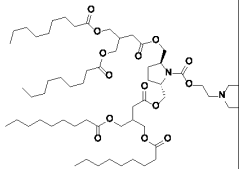
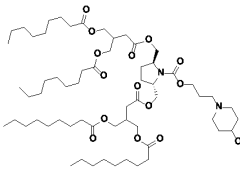
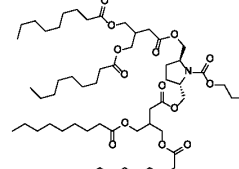
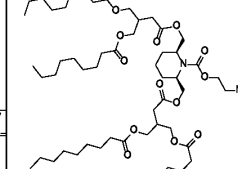
combinations CICL-207-109, CICL-207-116, CICL-207-119, CICL-207-122 and CICL-207-130 should have measured pKa data in formulation close to the desired pKa = 6-7 midpoint.

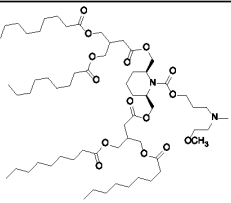
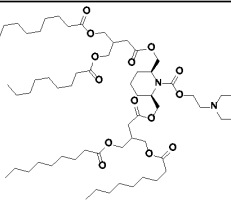
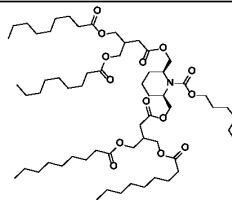
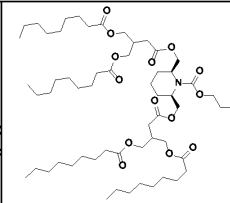
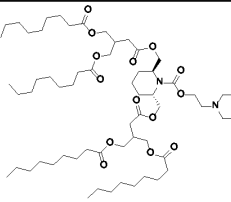
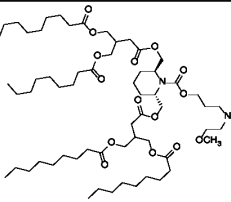
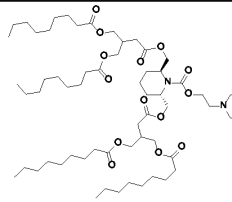
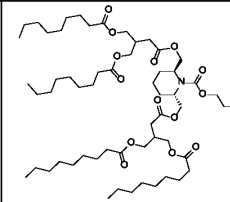
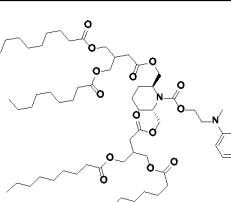
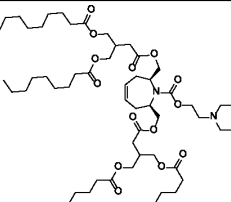
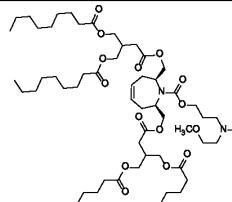
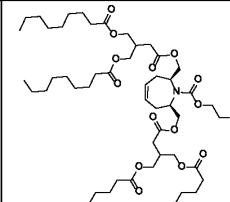
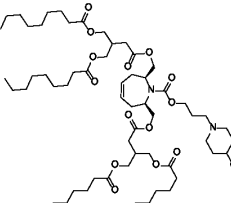
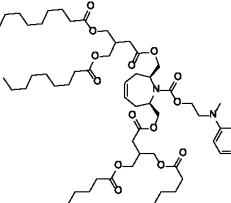
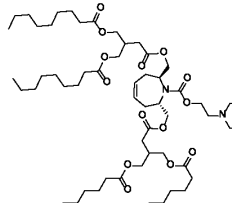
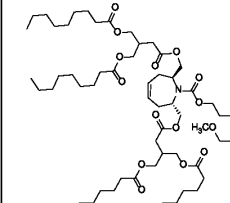
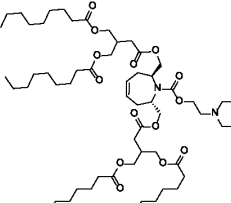
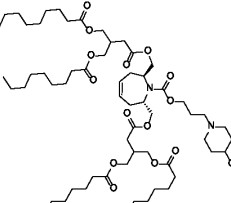
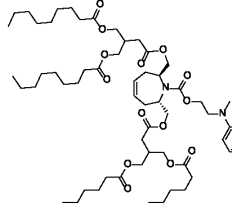
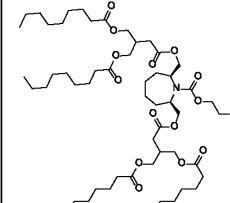
[00587] In order to move the measured pKa of lipids which result from the combination of acylimidazolides 7, 12, 20, 24, 28, 32, 36, 40, 44, 48, 53 to target the midpoint of the measured pKa range of 6-7 when combined with the X groups 108-133, the c-pKa from Table 5 and the measured pKa data from Tables 8, 9, and 10 will inform the choices made. Acylimidazolides 7, 20, 28, 32, and 53 (used in the synthesis of CICL-221, CICL-224, CICL-223, CICL-216, and CICL-238, respectively) will be combined with X groups: 116, 119, 122, and 131. Acylimidazolides 12, 24, and 36 (used in the synthesis of CICL-222, CICL-225, and CICL-215, respectively) will approach the pKa 6-7 midpoint when combined with X groups 109, 116, 119, 122, and 130. The combination of acylimidazolidine 40 (used in the synthesis of CICL-220) with X groups 109, 116, 122, and 130 will approach the mid-point target, as will the concatenation of acylimidazolides 44 and 48 (used in the synthesis of CICL-219 and CICL-218, respectively) with X groups: 109, 119, and 130. Lipids previously derived from acylimidazolides 57, 61, 65, 69, 76, 80, 85 have not been associated with measured pKa values. These acylimidazolides will be combined with head groups 109, 116, 119, 122, and 130 to target mid-range (pKa 6-7) lipids. The structures of the ionizable cationic lipids that result from the combinations cited above are presented in Table 6.

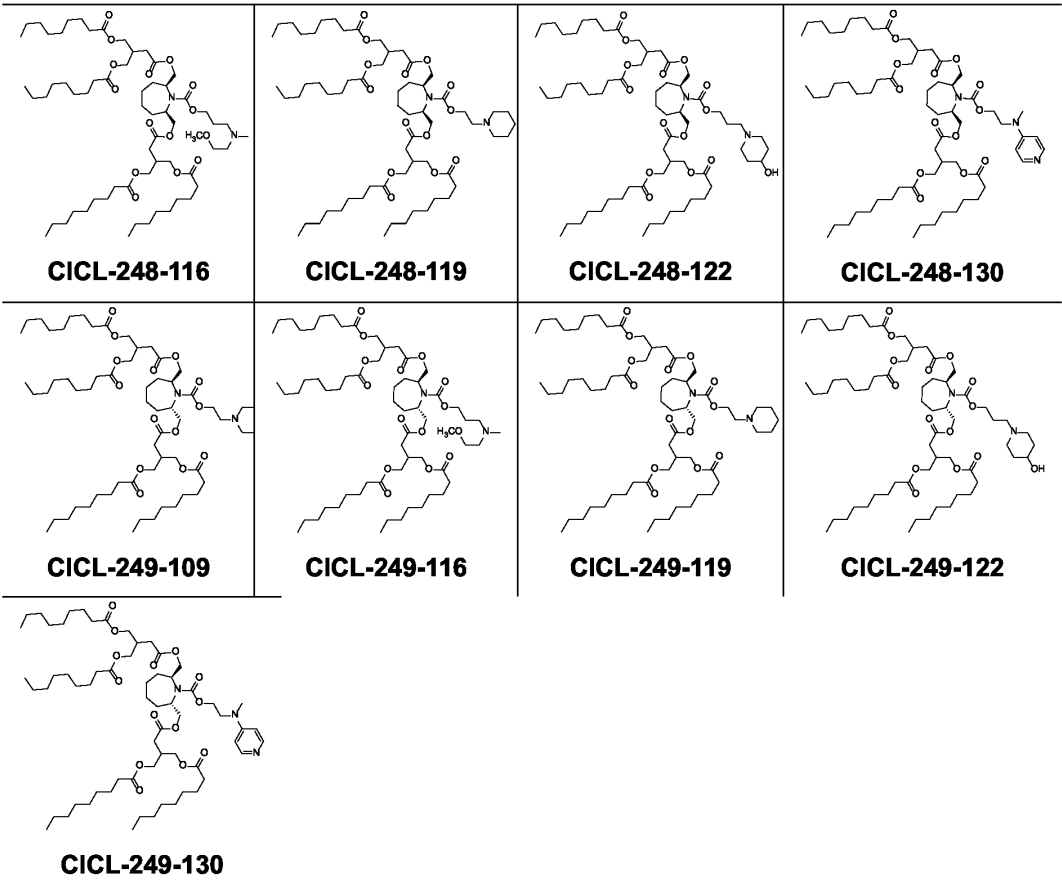
Table 6. Lipids formed from the Combination of Core Acylimidazolidine 7, 12, 20, 24, 28, 32, 36, 40, 44, 48, 53 with head groups selected from 108 through 133.

			
CICL-221-116	CICL-221-119	CICL-221-122	CICL-221-131
			
CICL-222-109	CICL-222-116	CICL-222-119	CICL-222-122

 C1CL-222-130	 C1CL-224-116	 C1CL-224-119	 C1CL-224-122
 C1CL-224-131	 C1CL-225-109	 C1CL-225-116	 C1CL-225-119
 C1CL-225-122	 C1CL-225-130	 C1CL-223-116	 C1CL-223-119
 C1CL-223-122	 C1CL-223-131	 C1CL-216-116	 C1CL-216-119
 C1CL-216-122	 C1CL-216-131	 C1CL-215-109	 C1CL-215-116
 C1CL-215-119	 C1CL-215-122	 C1CL-215-130	 C1CL-220-109

 C1CL-220-116	 C1CL-220-122	 C1CL-220-130	 C1CL-219-109
 C1CL-219-119	 C1CL-219-130	 C1CL-218-109	 C1CL-218-119
 C1CL-219-130	 C1CL-238-116	 C1CL-238-119	 C1CL-238-122
 C1CL-238-131	 C1CL-242-109	 C1CL-242-116	 C1CL-242-119
 C1CL-242-122	 C1CL-242-130	 C1CL-243-109	 C1CL-242-116
 C1CL-242-119	 C1CL-242-122	 C1CL-242-130	 C1CL-244-109

 C1CL-244-116	 C1CL-244-119	 C1CL-244-122	 C1CL-244-130
 C1CL-245-109	 C1CL-245-116	 C1CL-245-119	 C1CL-245-122
 C1CL-245-130	 C1CL-246-109	 C1CL-246-116	 C1CL-246-119
 C1CL-246-122	 C1CL-246-130	 C1CL-247-109	 C1CL-247-116
 C1CL-247-119	 C1CL-247-122	 C1CL-247-130	 C1CL-248-109



[00588] The following table provides the specific lipid compositions of the LNPs and tLNPs used in the following Examples (Examples 66, 67, 68, 69, 70, 71, and 72).

Table 7. Compositions of LNPs and tLNPs used in Examples 66-72

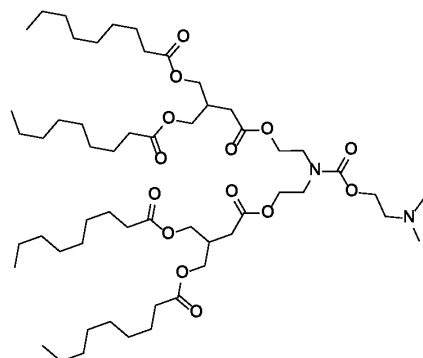
Composition	Composition Code
ALC-0315:DSPC:CHOL:DMG-PEG(2k):DSPE-PEG(2k)-MAL [50:10:38.5:1.4:0.1]	BF1
CICL-1:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F9
CICL-1:DSPC:CHOL:DMG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F30
CICL-207:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F50
CICL-207:DSPC:CHOL:DMG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F51

Composition	Composition Code
CICL-207:DSPC:CHOL:DMG-PEG(2k):DSPE-PEG(2k)-MAL [50:10:38.5:1.4:0.1]	F52
CICL-207:DSPC:CHOL: DSG-PEG(2k):DSPE-PEG(2k)-MAL [62:10:26.5:1.4:0.1]	F53
CICL-215:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F54
CICL-216:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F55
CICL-217:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F56
CICL-218:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F57
CICL-219:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F58
CICL-220:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F59
CICL-221:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F60
CICL-222:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F61
CICL-223:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F62
CICL-224:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F63
CICL-225:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F64
CICL-225:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [62:10:26.5:1.4:0.1]	F65
CICL-225:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [65:10:23.5:1.4:0.1]	F66
CICL-238:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F67
CICL-239:DSPC:CHOL:DSG-PEG(2k):DSPE-PEG(2k)-MAL [58:10:30.5:1.4:0.1]	F68

Example 66. *In Vivo* Delivery of mRNA by tLNP Incorporating CICL-207 as the Ionizable Cationic Lipid

[00589] To assess the ability of a constrained ionizable cationic lipids to facilitate *in vivo* transfection of T cells with mCherry mRNA (SEQ ID NO. 5), tLNP incorporating CICL-207, or for comparison, CICL-1, and conjugated to an anti-mouse CD5 antibody were prepared and administered to C57BL/6 mice. The tLNP comprised either CICL-207 or CICL-1, DSPC, cholesterol, either DMG-PEG(2000) or DSG-PEG(2000), and DSPE-PEG(2000)-maleimide in

the proportions indicated in Table 7 (below) and an N/P ratio of 6. C1CL-1 has the structure:



C1CL-1

[00590] Briefly, to prepare the tLNP, N1-methylpseudouridine (m1ψ)-substituted mCherry mRNA was encapsulated in LNP using a self-assembly process in which an aqueous solution of mRNA at pH 3.5 was rapidly mixed with a solution of lipids dissolved in ethanol, then followed by stepwise phosphate and Tris buffer dilution and tangential flow filtration (TFF) purification. Then an anti-CD5 mAb was conjugated to the above LNP to generate tLNP. Purified rat anti-mouse CD5 antibody, clone 53-7.3 (BioLegend), was coupled to LNP via N-succinimidyl S-acetylthioacetate (SATA)–maleimide conjugation chemistry. Briefly, LNPs with DSPE-PEG(2000)-maleimide incorporated were formulated and stored at 4°C on the day of conjugation. The antibody was modified with SATA (Sigma-Aldrich) to introduce sulfhydryl groups at accessible lysine residues allowing conjugation to maleimide. SATA was deprotected using 0.5 M hydroxylamine followed by removal of the unreacted components by G-25 Sephadex Quick Spin Protein columns (Roche Applied Science, Indianapolis, IN). The reactive sulfhydryl group on the antibody was then conjugated to maleimide moieties on the LNPs using thioether conjugation chemistry. Purification was performed using Sepharose CL-4B gel filtration columns (Sigma-Aldrich). tLNPs (LNPs conjugated with a targeting antibody) were frozen at -80°C.

[00591] The particle size (hydrodynamic diameter) and polydispersity index of the targeted lipid nanoparticles were determined using dynamic light scattering (DLS) on a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). Size measurement was carried out in pH 7.4 Tris buffer at 25°C in disposable capillary cells. A non-invasive back scatter system (NIBS) with a scattering angle of 173° was used for size measurements. mRNA content was determined using a Quant-iT™ RiboGreen RNA assay kit (Invitrogen™). Encapsulation efficiency was calculated by determining the unencapsulated mRNA content by measuring the fluorescence intensity (Fi) upon the addition of RiboGreen® reagent to the LNP and comparing this value to the total fluorescence intensity (Ft) of the RNA content that

is obtained upon lysis of the LNPs by 1% Triton X-100, where % encapsulation = $(F_t - F_i)/F_t \times 100$). After conjugation, tLNP antibody to mRNA weight ratio (binder density) was determined with the BCA (bicinchoninic acid) total protein assay and Ribogreen® assay of mRNA content.

Table 8. Physicochemical properties of the tLNP

Composition	Size (nm)	PDI	Encapsulation Efficiency (%)	Ab:mRNA Ratio (wt:wt) by BCA
F51	91	0.09	96	0.76
F50	85	0.11	96	0.71
F52	75	0.14	96	0.65
F30	81	0.07	97	0.50

[00592] As seen in Table 8, all of these tLNP compositions had hydrodynamic diameters and polydispersity indices within the acceptable ranges of 50-150 nm and ≤ 0.2 for PDI. Encapsulation efficiency is acceptable at $\geq 80\%$ although $\geq 85\%$ and $\geq 90\%$ are preferred. Binder density (Ab:mRNA ratio (wt:wt)) is acceptable at ratios of 0.3 to 1.0. Additionally, the stability of these tLNPs at -20°C was found to be superior to that of tLNPs incorporating a similar lipid lacking a constraining ring.

[00593] The apparent or measured pKa of ionizable lipid in the lipid nanoparticle was determined using 6-(p-toluidino)-2-naphthalenesulfonic acid sodium salt (TNS salt, Toronto Research Chemicals, Toronto, ON, Canada). Lipid nanoparticles were diluted in 1xDulbecco's PBS to a concentration of 1 mM total lipids. TNS salt was prepared as a 1 mg/mL stock solution in DMSO and then further diluted using distilled water to a working solution of 60 $\mu\text{g/mL}$ (179 mM). Diluted lipid nanoparticle samples were further diluted to 90 μM total lipids in 165 μL of buffered solution containing 10 mM HEPES, 10 mM MES, 10 mM ammonium acetate, 130 mM NaCl, and final TNS concentration of 1.33 $\mu\text{g/mL}$ (4 μM) with the pH ranging from 3.5 to 12.2. Following pipette mixing and incubation at room temperature in the dark for 15 min, fluorescence intensity was measured at room temperature in a BioTek Synergy H1 plate reader using excitation and emission wavelengths of 321 and 445 nm, respectively. The

fluorescence signal was blank subtracted and plotted as a function of the pH, then analyzed using a nonlinear (Boltzmann) regression analysis with the apparent pKa determined as the pH giving rise to half maximal fluorescence intensity as calculated by the Henderson–Hasselbalch equation.

[00594] The mice were administered tLNPs containing 10 µg of mRNA by tail vein injection and tissue was harvested 24 hours later. As seen in Figure 1A, tLNP comprising DMG-PEG(2000) and either C1CL-207 or C1CL-1 had similar performance in splenic T cells for both transfection rate and expression level (measured as molecular equivalent of soluble fluorochrome (MESF)). Reducing the amount of C1CL-207 from 58% to 50% decreased performance, while replacing DMG-PEG(2000) with DSG-PEG(2000) increased performance, with respect to both transfection rate and expression. These same trends have been observed with tLNP comprising C1CL-1 as the ionizable lipid (data not shown). The tLNP comprising 58% ionizable cationic lipid had similarly low transfection rates (<4%) for CD45⁺ liver cells (hepatocytes)(Figure 1B). The tLNP incorporating C1CL-207 had similarly low levels in liver Kupffer cells (CD45⁺/CD11⁺ liver cells) although the tLNP incorporating C1CL-1 (and DMG-PEG) had a transfection rate that was somewhat higher (~6%) for Kupffer cells (Figure 1C).

[00595] The best performing composition was the one comprising C1CL-207 (58%) and DSG-PEG(2000) – that is high transfection rate and expression level in splenic T cells and low transfection rate and expression level in liver. However, there were no tLNP prepared with C1CL-1 and DSG-PEG(2000) for a head-to-head comparison. Therefore, tLNP with 58% ionizable cationic lipid, 10% DSPC, 30.5% cholesterol, 1.4% DSG-PEG(2000), and 0.1% DSPE-PEG(2000)-MAL were prepared as described above. As seen in Table 9, their physicochemical properties were within acceptable ranges.

Table 9. Physicochemical properties of the tLNP

Composition	Size (nm)	PDI	Encapsulation Efficiency (%)	Ab:mRNA Ratio (wt:wt) by BCA	Measured pKa (TNS Assay)
F9	89	0.10	98	0.69	6.58
F50	82	0.05	99	0.52	6.51

[00596] The performance of these tLNP differing only in the ionizable lipid component was

similar in all three cell types assessed (Figures 2A-2C). In all cases the tLNP comprising C1CL-207 had somewhat more desirable transfection rates (higher in splenic T cells, lower in liver cells) and somewhat more desirable expression levels in splenic T cells and CD45⁺ liver cells. Accordingly, tLNP comprising the constrained ionizable cationic lipid C1CL-207 as the ionizable cationic lipid performed similarly well to tLNP comprising C1CL-1 instead.

Example 67. *In Vivo* Delivery of mRNA by LNP Incorporating Further Ionizable Cationic Lipids

[00597] Further ionizable cationic lipids according to Formula M2 were incorporated into tLNP and the ability of the tLNP to transfect cells *in vivo* assessed essentially as described in the preceding Example. Their physicochemical properties as determined in various experiments are compiled in Table 10.

Table 10. Physicochemical properties of the tLNP

Composition	Size (nm)	PDI	Encapsulation Efficiency (%)	Ab:mRNA Ratio (wt:wt) by BCA	Measured pKa (TNS Assay)
F54	88	0.02	99	0.72	6.61
F55	115	0.01	99	0.78	7.05
F56	87	0.04	97	0.69	5.87
F57	92	0.04	97	0.71	6.11
F58	86	0.04	98	0.55	6.20
F59	84	0.06	98	0.55	6.34
F60	99	0.07	95	0.61	6.97
F61	82	0.05	98	0.58	6.40
F62	110	0.01	96	0.6	6.96

Composition	Size (nm)	PDI	Encapsulation Efficiency (%)	Ab:mRNA Ratio (wt:wt) by BCA	Measured pKa (TNS Assay)
F63	112	0.03	93	0.79	6.99
F64	88	0.07	93	0.70	6.47
F67	104	0.03	97	0.57	7.09
F68	87	0.03	95	0.52	6.26

[00598] Size, PDI, encapsulation efficiency and antibody:mRNA ratio were all within acceptable limits. Measured pKa for tLNP comprising three of these lipids, C1CL-216, -217, and -238, fell outside the range of 6 to 7. Nonetheless, all of these ionizable cationic lipids were able to form tLNP and deliver mRNA into cells. For purposes of comparison, transfection rate and expression level (as mean equivalents of soluble fluorochrome) were normalized to results obtained with tLNP incorporating C1CL-207 as the ionizable cationic lipid which was used as a control in each experiment (Figures 3A-3B). The tLNPs comprising C1CL-216, -217, or -238 were 3 of the 4 poorest performing tLNP.

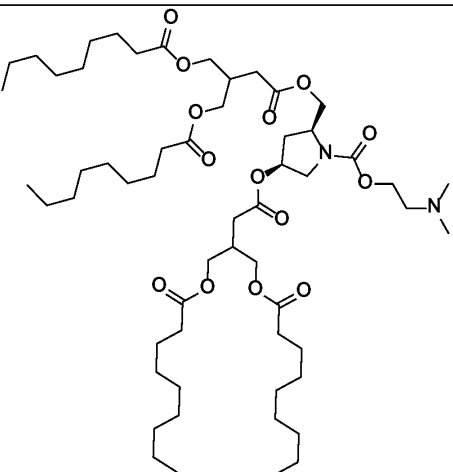
[00599] A plot of representative data points for each of these ionizable cationic lipids for transfection rate of splenic T cells versus measured pKa (Figure 4A) showed increasing transfection rate as pKa approached about 6.6, which then fell as pKa approached and exceeded 7.0. Liver cells, both CD45⁻ (hepatocytes) and CD45⁺/CD11⁺ (Kupffer cells), had generally low transfection rates, under 5% for the CD45⁻ cells and, with the exception of tLNP that incorporated C1CL-216, under 10% for the CD45⁺/CD11⁺ cells. Transfection rates for the liver cells trended upward with increasing pKa even as measured pKa exceeded 7.0 (Figures 4B-4C).

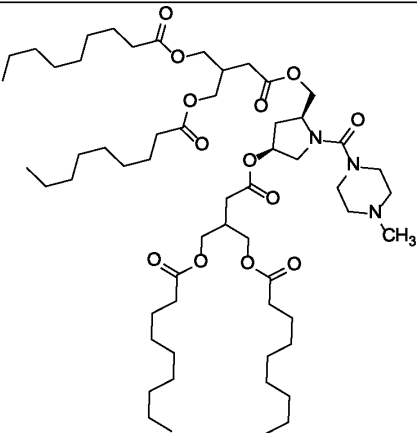
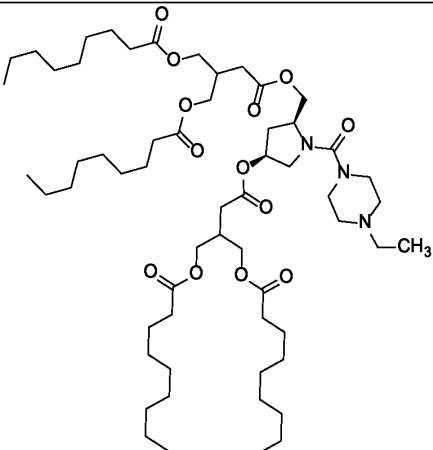
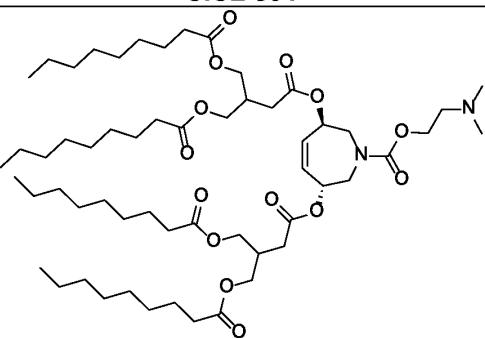
[00600] Transfection rate and expression level tended to increase together in splenic T cells and liver CD45⁺/CD11⁺ (Kupffer) cells (Figures 5A and 5C, respectively), but there was no clear correlation between transfection rate and expression level in liver CD45⁻ cells (hepatocytes; Figure 5B).

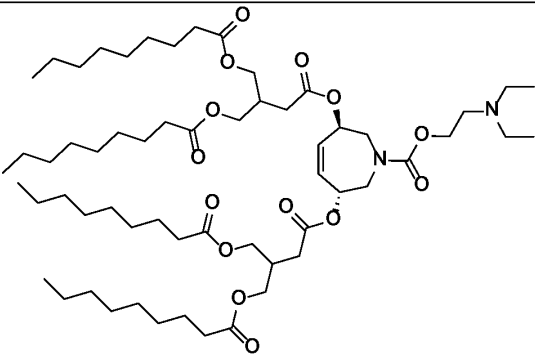
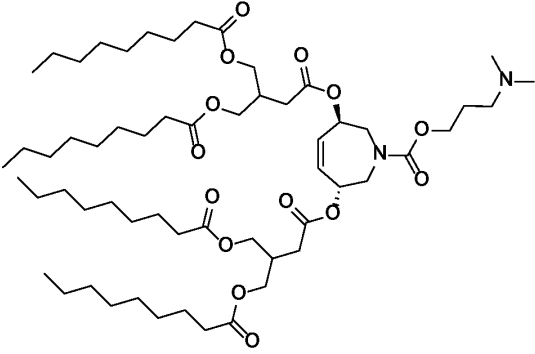
[00601] The data of Figure 4A indicated that a transfection rate in spleen T cells of ca. 50% was realized at a measured pKa in tLNP formulation of ca. pKa = 6.6. At more basic measured

pKa, as in the case of **CICL-224** (measured pKa ca. 7.0), the transfection rate fell below 40%. At less basic measured pKa, as in the case of **CICL-218** (measured pKa ca. 6.20), the transfection rate fell to ca. 20%. Alterations in the basic head group were designed to lower or increase calculated basicity of some of the ionizable cationic lipids, with attendant changes in measured pKa in tLNPs to move the lipid toward a more preferred measured pKa of ca. 6.6. Table 11 presents modifications to **CICL-224** and **CICL-218** which modulated calculated pKa as well as measured pKa for some of these lipids. **CICL-224** has a calculated pKa (**c-pKa**) of 8.42 and a measured pKa (in tLNP formulation) of 6.98. Changing the basic head group to N-methylpiperazine (**CICL-239**) lowered the calculated pKa to 7.16 and measured pKa to 6.25. The measured pKa values for **CICL-224** and **CICL-239** bracketed the pKa = ca. 6.60 value of the lipids with the highest transfection rates shown in Figure 4A. Changing the basic head group to N-ethylpiperazine for **CICL-305** resulted in a c-pKa of 7.65, between of the c-pKa's of **CICL-224** and **CICL-239** which should move measured pKa toward the 6.6 value associated with higher transfection rates. The measured pKa for **CICL-218** (c-pKa = 8.49, pKa = 6.07) is lower than associated with optimal performance. **CICL-306** and **CICL-307** were designed to raise pKa by added a CH₂ group between the ester O and basic N if the dimethylamine group or by altering the basic dimethylamine group to a diethylamine and indeed these lipids have c-pKa values of 9.45 and 9.27, respectively. All of the lipids in Table 11 exhibit cLogD values in an acceptable range of 13-15. As cLogD is increased for any set value of c-pKa the measured pKa of the tLNP will tend to decrease.

Table 11. Modifications of CICL-224 and CICL-218 Modulating pKa

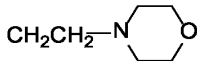
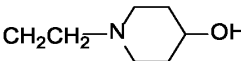
Structure	cLogD	c-pKa	Measured pKa
 CICL-224	13.75	8.42	6.98

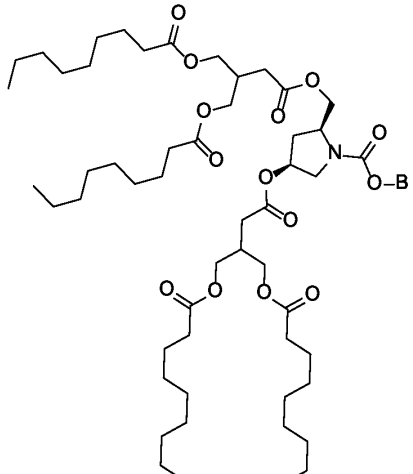
Structure	cLogD	c-pKa	Measured pKa
 CICL-239	14.77	7.16	6.25
 CICL-304	14.79	7.65	NT
 CICL-218	13.71	8.49	6.07

Structure	cLogD	c-pKa	Measured pKa
 C1CL-305	13.84	9.45	NT
 C1CL-306	13.18	9.27	NT

[00602] Further modifications of **C1CL-224** and **C1CL-218** were designed by altering their basic head groups to lower and raise pKa, respectively, toward a measured pKa = ca. 6.6 and c-pKa calculated as shown in Table 12.

Table 12. Further modifications of C1CL-224 and C1CL-218 to Modulate pKa

Structure	B	c-pKa
	 CH₂CH₂-N for C1CL-224-123	6.17
	 CH₂CH₂-N for C1CL-224-121	7.77

Structure	B	c-pKa
	$\text{CH}_2\text{CH}_2\text{NH}-\text{C}_5\text{H}_4\text{N}$ For C1CL-224-129	7.90
	$\text{CH}_2\text{CH}_2-\text{N}$ for C1CL-218-117	9.42
	$\text{CH}_2\text{CH}_2\text{CH}_2-\text{N}$ for C1CL-218-120	9.24
	$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{N}$ for C1CL-218-134	9.61

Example 68. Evaluation of tLNP with Increased Ionizable Cationic Lipid Content

[00603] As seen in Example 60 (above), increasing the content of ionizable cationic lipid in tLNPs tended to increase transfection rate and expression level. To further assess this trend tLNP containing 62% C1CL-207 or C1CL 225, or 65% C1CL-225 were made. Their physicochemical properties as determined in various experiments are compiled in Table 13.

Table 13. Physicochemical properties of the tLNP

Composition	Size (nm)	PDI	Encapsulation Efficiency (%)	Ab:mRNA Ratio (wt:wt) by BCA	Measured pKa (TNS Assay)
F50	81.87	0.05	98.73	0.52	6.51
F53	95.19	0.06	97.58	0.64	6.36
F64	88	0.07	93	0.7	6.47
F65	95.11	0.02	96.24	0.65	6.56
F66	105	0.00	85.13	0.66	6.40

[00604] Increasing the ionizable cationic lipid content of the tLNPs to 62% increased transfection rate in all three cell types tested (Figures 6A-6C). Increasing the ionizable cationic lipid content of the tLNPs further to 65% decreased transfection rate in splenic T cells and liver Kupffer cells indicating that the optimal content of ionizable cationic lipid for this LNP composition had been exceeded. In contrast, increasing the ionizable cationic lipid content of the tLNPs further to 65% further increased transfection rate for CD45⁺ liver cells (hepatocytes), though it remained less than 5%. In this data set, the increase in transfection rate associated with raising ionizable cationic lipid content from 58% to 62% was greater for C1CL-207 than for C1CL-225 in splenic T cells while the increased transfection rate for C1CL-225 was greater in both types of liver cells. In some embodiments, 58% ionizable cationic lipid can be preferred over 62% in order to minimize transfection of liver cells.

Example 69: Assessment of F9 tLNP and F50 tLNP in NCG Mice

[00605] LNPs with the F9 and F50 composition were prepared as described in Example 66, except tLNPs were generated by conjugating a humanized anti-CD8 antibody through use of an AJICAP reagent. Specifically, an antibody with the CBD1033 heavy and light chains (SEQ ID NOs: 1 and 2, respectively). The tLNP encapsulated N1-methylpseudouridine-substituted mRNA encoding an anti-CD19 CAR (SEQ ID NO: 3).

[00606] Female NCG mice (~10 weeks old, Charles River Laboratories) were injected

intravenously via the tail vein with 10 million human PBMCs. After 14 days of T cell engraftment mice were injected intravenously with the indicated tLNPs. 6 hours after injection, mice were sacrificed, and blood and spleen were analyzed for CAR expression and B cell depletion by flow cytometry.

[00607] The F9 and F50 tLNPs decorated with the anti-CD8 antibodies achieved similar transfection rates in CD8⁺ blood and spleen T cells. Both tLNPs showed comparable CAR engineering rates (Figure 7A-B) and CAR expression (as MFI; Figure 7C-D) in CD8⁺ blood and spleen T cells, as well as the comparable CAR molecules/cell (Figure 7E-F). Both tLNPs also induced complete B cell depletion 6-hours after administration (Figure 7G-H).

Example 70: Assessment of BF1 LNP, BF1 tLNP, F50 LNP, F50 tLNP by luciferase

biodistribution

[00608] LNP and tLNP with the F9 and F50 composition were prepared as example 66 described, except the payload consisted of mRNA encoding luciferase (SEQ ID NO. 4).

[00609] Eight-week-old female C57Bl/6 mice (Charles River Laboratories) were intravenously injected with BF1 or F50 LNPs or CD5-targeted tLNPs encapsulating mRNA encoding luciferase at a dose of 2 µg mRNA/animal via the tail vein. At 6 hours post-injection, prone and supine bioluminescence images were collected from all mice (Figures 8A-8B). Following whole-animal imaging, the mice were sacrificed, and the following tissues were collected: liver, spleen, lung, kidneys (both), heart, bone marrow (femur), lymph nodes (left and right inguinal), and brain. Tissues were placed into luciferin pre-filled black polystyrene plates with spacing between tissues, and bioluminescent images were collected at 6 hours and 24 hours post-injection (Figures 8C-8D, respectively). Quantitative results (Figure 8E) show that use of CICL-207 reduced bioluminescence in the liver about 2 logs compared to the BF1 compositions yet for the tLNP incorporating CICL-207 bioluminescence in the spleen was similar or greater than the BF1 compositions and about 1 log greater than in the liver.

[00610] These compositions displayed the general pattern that bioluminescence in spleen > lymph node > lung > bone marrow > kidney > heart > brain, with the CICL-207 containing tLNP composition generally producing the least bioluminescence in each of the non-target organs.

Example 71: Delivery of CRISPR Gene-Editing Components In Vivo

[00611] To assess the ability of the disclosed tLNP to deliver gene-editing components to

human hematopoietic stem cells (CD34⁺ CD117⁺) *in vivo*, female NCG mice engrafted with human CD34⁺ cells (~25-29 weeks after engraftment, Charles River Laboratories) were intravenously administered CD117-targeted tLNPs encapsulating mRNA payload encoding the mCherry reporter protein (SEQ ID NO. 5). Both F9 and F50 tLNP were able to deliver mCherry payload to all analyzed subsets of CD34⁺ cells (Figures 9-10).

[00612] Female NCG mice engrafted with human CD34⁺ cells (~25-29 weeks after engraftment Charles River Laboratories) were intravenously administered CD117-targeted tLNPs. The tLNP used the F50 lipid composition with and overall N/P ratio of 6 and a 1:1 (w/w) SpCas9:sgRNA payload. sgRNA targeted the B2M locus and successful gene editing was measured as loss of B2M expression. Eight days after infusion, bone marrow was collected and analyzed by flow cytometry for B2M knockout on indicated populations of CD34⁺ cells. The results are presented in Figure 11 and demonstrate effective delivery of SpCas9 and the sgRNA into CD34⁺ cells *in vivo*. Editing of bone marrow CD34⁺ or CD34⁺CD38^{low} cells in the humanized CD34⁺ NCG mice by F50 tLNP was approximately 10%, for both 30ug and 60ug dose, with a single dose of CD117-targeted tLNPs.

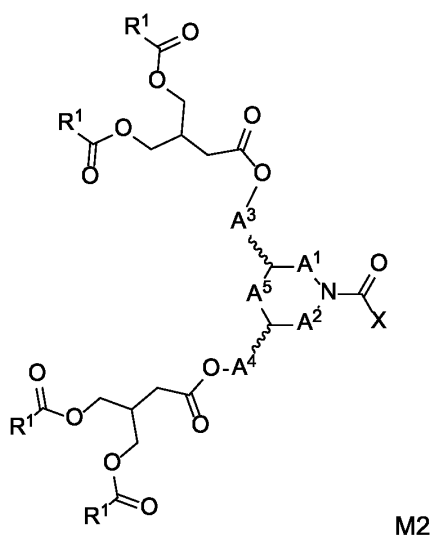
Example 72: Tolerability of F50 LNP

[00613] A study in which various dosages of undecorated F50 LNP encapsulating mCherry mRNA (up to 8 mg/kg) were administered intravenously to 8-week-old male Sprague Dawley rats (Charles River Laboratories). Liver enzymes in serum were determined (Figures 12A-B). Acute phase protein (Figures 12C-E) and cytokine levels (Figures 12F-H) were assessed in plasma by Luminex assay. There were only minimal increases in liver enzyme levels. The acute phase proteins were mildly elevated at 24 hours. The cytokine elevations were transient and resolved by 24 hours. None of these led to any clinical observations. There is a lack of dose responsiveness in the elevations of acute phase proteins and cytokines suggesting that it is a general response to LNP infusion and does not indicate a lack of tolerability of the LNP components. These data demonstrate that even at these high doses, LNPs incorporating C1CL-207 were well tolerated. Prior experience also indicates that addition of an antibody to an LNP, especially one that targets a non-liver tissue as in a tLNP, leads to reduction in any elevation of liver enzymes.

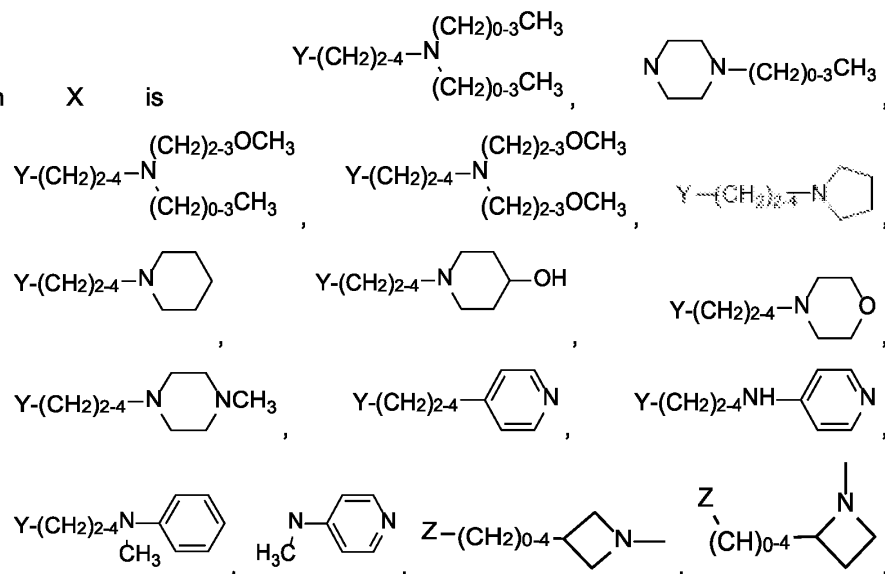
[00614] Additional aspects of the disclosure are provided by the following enumerated embodiments, which can be combined in any number and in any combination not technically or logically inconsistent. This is not an exhaustive listing of the embodiments disclosed which include similar embodiments directed to different species and genera than those exemplified

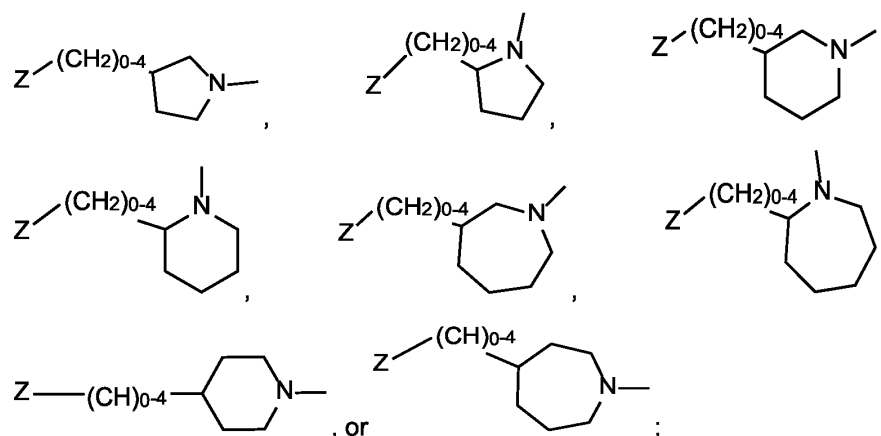
here. Furthermore, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and enumerated below.

Embodiment 1. An ionizable cationic lipid having a structure of formula M2,



wherein X is





Y is O, S, NH, or NCH₃;

Z is O, NH, or NCH₃;

each R¹ is independently selected from C₇-C₁₁ alkyl or C₇-C₁₁ alkenyl; and

A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is (CH₂)₁₋₄ or CH₂-CH=CH-CH₂; or

A¹ is (CH₂)₀, A² is (CH₂)₁, A³ is (CH₂)₁, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁; or

A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₀; or

A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁; or

A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₂ or CH=CH, and

wherein the wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom, or mixtures of stereo-configurations, can be assumed.

Embodiment 2. The ionizable cationic lipid of embodiment 1 wherein A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is (CH₂)₁₋₄ or CH₂-CH=CH-CH₂.

Embodiment 3. The ionizable cationic lipid of embodiment 2, wherein A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is (CH₂)₁.

Embodiment 4. The ionizable cationic lipid of embodiment 2, wherein A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is (CH₂)₂.

Embodiment 5. The ionizable cationic lipid of embodiment 2, wherein A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is (CH₂)₃.

Embodiment 6. The ionizable cationic lipid of embodiment 2, wherein A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is (CH₂)₄.

Embodiment 7. The ionizable cationic lipid of embodiment 2, wherein A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is CH₂-CH=CH-CH₂.

Embodiment 8. The ionizable cationic lipid of embodiment 1 wherein A¹ is (CH₂)₀, A² is (CH₂)₁, A³ is (CH₂)₁, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁.

Embodiment 9. The ionizable cationic lipid of embodiment 1 wherein A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₀.

Embodiment 10. The ionizable cationic lipid of embodiment 1 wherein A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁.

Embodiment 11. The ionizable cationic lipid of embodiment 1 wherein A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₂ or CH=CH.

Embodiment 12. The ionizable cationic lipid of embodiment 11, wherein A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₂.

Embodiment 13. The ionizable cationic lipid of embodiment 11, wherein A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is CH=CH.

Embodiment 14. The ionizable cationic lipid of any one of embodiments 1 to 13, wherein each R¹ is independently selected from C₇-C₁₀ alkyl or C₇-C₉ alkyl.

Embodiment 15. The ionizable cationic lipid of any one of embodiments 1 to 13, wherein each R¹ is independently selected from a linear C₇-C₁₁ alkyl, e.g., a linear C₇-C₁₀ alkyl, or a linear C₇-C₉ alkyl.

Embodiment 16. The ionizable cationic lipid of any one of embodiments 1 to 13, wherein each R¹ is independently selected from (CH₂)₆₋₈CH₃.

Embodiment 17. The ionizable cationic lipid of any one of embodiments 1 to 13, wherein R¹ is (CH₂)₇CH₃.

Embodiment 18. The ionizable cationic lipid of any one of embodiments 1 to 13, wherein each R¹ is independently selected from a linear C₇-C₁₁ alkenyl, e.g., a linear C₇-C₁₀ alkenyl, or a linear C₇-C₉ alkenyl.

Embodiment 19. The ionizable cationic lipid of any one of embodiments 1 to 13, wherein each R¹ is a linear C₈ alkenyl.

Embodiment 20. The ionizable cationic lipid of any one of embodiments 1 to 13, wherein each R^1 is independently selected from a branched C_7 - C_{11} alkyl, e.g., C_7 - C_{10} alkyl, or C_7 - C_9 alkyl.

Embodiment 21. The ionizable cationic lipid of any one of embodiments 1 to 13, wherein each R^1 is a branched C_8 alkyl.

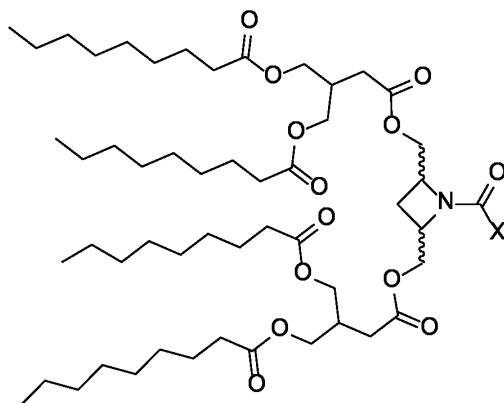
Embodiment 22. The ionizable cationic lipid of any one of embodiments 1 to 13, wherein each R^1 is independently selected from a branched C_7 - C_{11} alkenyl, e.g., C_7 - C_{10} alkenyl, or C_7 - C_9 alkenyl.

Embodiment 23. The ionizable cationic lipid of any one of embodiments 1 to 13, wherein each R^1 is a branched C_8 alkenyl.

Embodiment 24. The ionizable cationic lipid of any one of embodiments 1 to 13, wherein R^1 is a branched alkyl or alkenyl, the branch point is positioned so that ester carbonyls are not in an α position relative to the branch point, for example they are in a β position relative to the branch point.

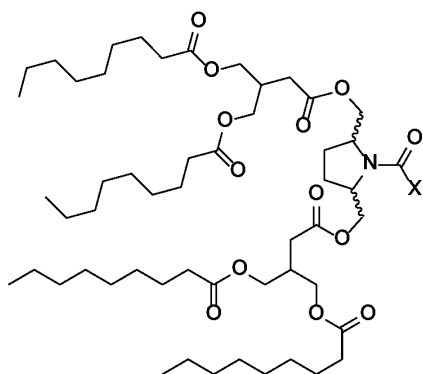
Embodiment 25. The ionizable cationic lipid of any one of embodiments 1 to 24, wherein each R^1 is the same.

Embodiment 26. The ionizable cationic lipid of any one of embodiments 1 to 25 having the structure:



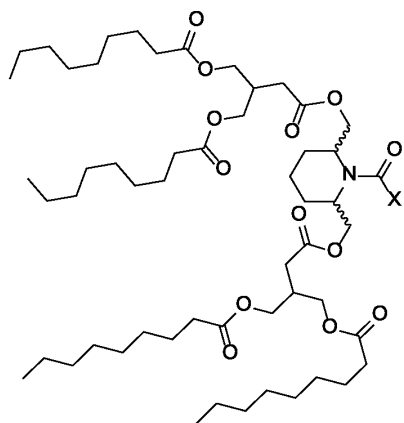
wherein wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

Embodiment 27. The ionizable cationic lipid of any one of embodiment 1 to 25 having the structure:



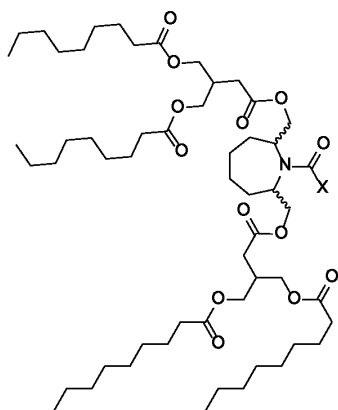
wherein wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom, or mixtures of stereo-configurations, can be assumed.

Embodiment 28. The ionizable cationic lipid of any one of embodiments 1 to 25 having the structure:



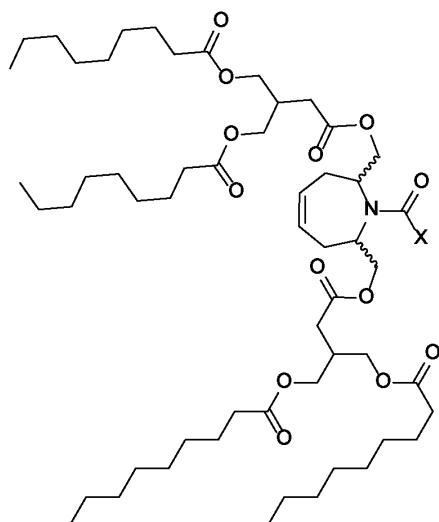
wherein wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom, or mixtures of stereo-configurations, can be assumed.

Embodiment 29. The ionizable cationic lipid of any one of embodiments 1 to 25 having the structure:



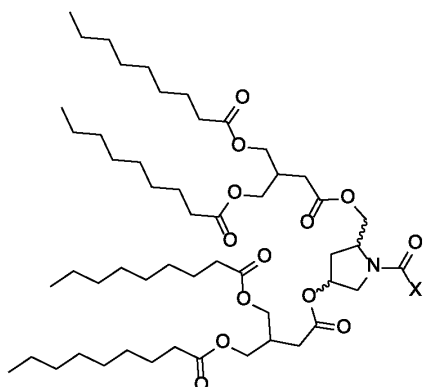
wherein wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom, or mixtures of stereo-configurations, can be assumed.

Embodiment 30. The ionizable cationic lipid of any one of embodiments 1 to 25 having the structure:



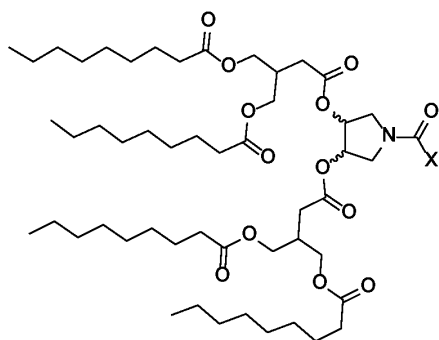
wherein wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom, or mixtures of stereo-configurations, can be assumed.

Embodiment 31. The ionizable cationic lipid of any one of embodiments 1 to 25 having the structure:



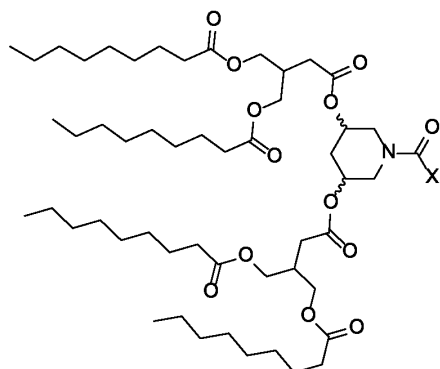
wherein wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom, or mixtures of stereo-configurations, can be assumed.

Embodiment 32. The ionizable cationic lipid of any one of embodiments 1 to 25 having the structure:



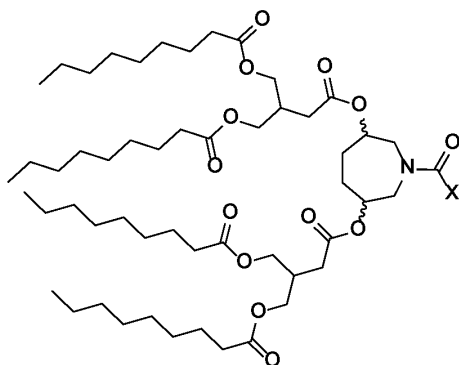
wherein wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom, or mixtures of stereo-configurations, can be assumed.

Embodiment 33. The ionizable cationic lipid of any one of embodiments 1 to 25 having the structure:



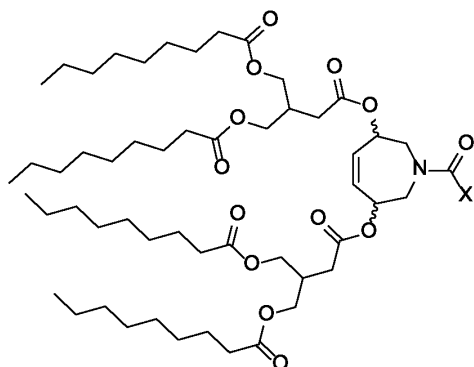
wherein wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom, or mixtures of stereo-configurations, can be assumed.

Embodiment 34. The ionizable cationic lipid of any one of embodiments 1 to 25 having the structure:



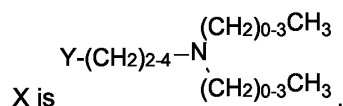
wherein wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom, or mixtures of stereo-configurations, can be assumed.

Embodiment 35. The ionizable cationic lipid of any one of embodiments 1 to 25 having the structure:

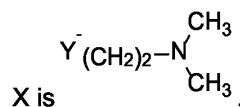


wherein wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom, or mixtures of stereo-configurations, can be assumed.

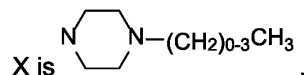
Embodiment 36. The ionizable cationic lipid of any one of embodiments 1 to 35 wherein



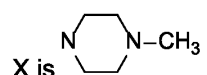
Embodiment 37. The ionizable cationic lipid of any one of embodiments 1 to 35 wherein



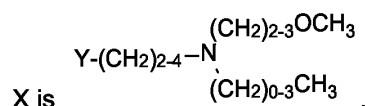
Embodiment 38. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



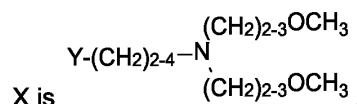
Embodiment 39. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



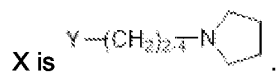
Embodiment 40. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



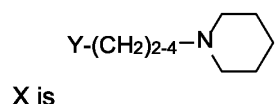
Embodiment 41. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



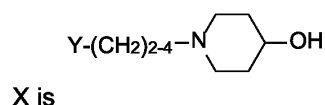
Embodiment 42. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



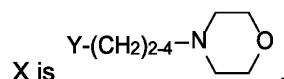
Embodiment 43. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



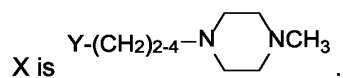
Embodiment 44. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



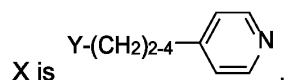
Embodiment 45. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



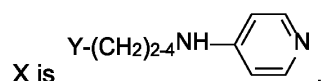
Embodiment 46. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



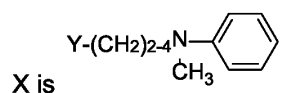
Embodiment 47. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



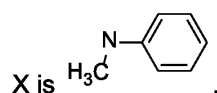
Embodiment 48. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



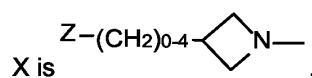
Embodiment 49. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



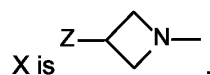
Embodiment 50. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



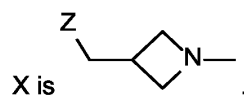
Embodiment 51. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



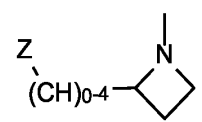
Embodiment 52. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



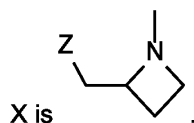
Embodiment 53. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



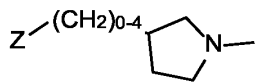
Embodiment 54. The ionizable cationic lipid of embodiment 1 to 35, wherein X is



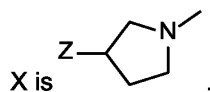
Embodiment 55. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



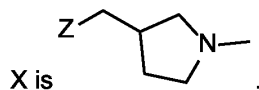
Embodiment 56. The ionizable cationic lipid of any of embodiments 1 to 35, wherein X is



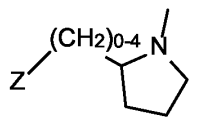
Embodiment 57. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



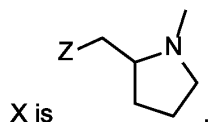
Embodiment 58. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



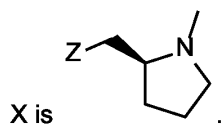
Embodiment 59. The ionizable cationic lipid of any of embodiments 1 to 35, wherein X is



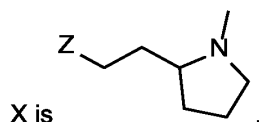
Embodiment 60. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



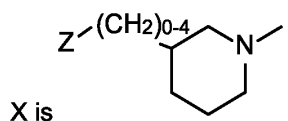
Embodiment 61. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



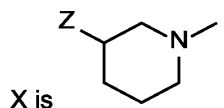
Embodiment 62. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



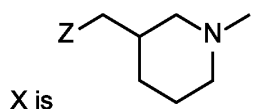
Embodiment 63. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



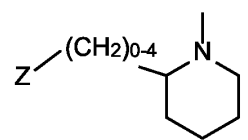
Embodiment 64. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



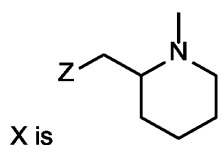
Embodiment 65. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



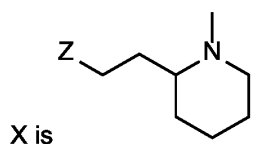
Embodiment 66. The ionizable cationic lipid of any of embodiments 1 to 35, wherein X is



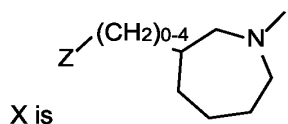
Embodiment 67. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



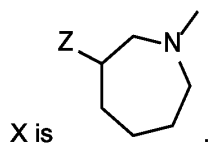
Embodiment 68. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



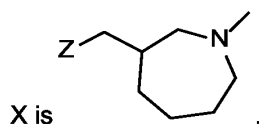
Embodiment 69. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



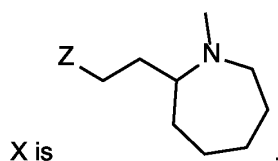
Embodiment 70. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



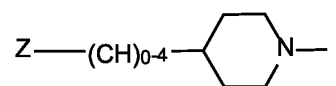
Embodiment 71. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



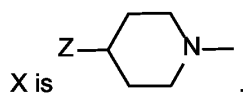
Embodiment 72. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



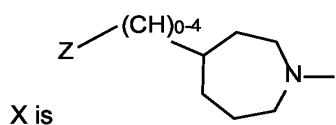
Embodiment 73. The ionizable cationic lipid of any of embodiments 1 to 35, wherein X is



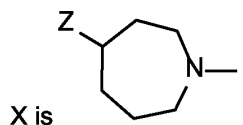
Embodiment 74. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



Embodiment 75. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein



Embodiment 76. The ionizable cationic lipid of any one of embodiments 1 to 35, wherein

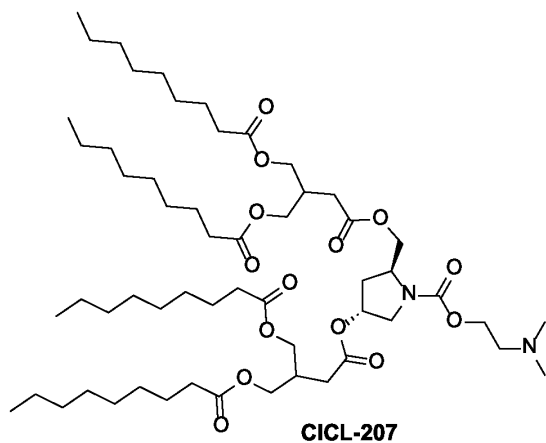


Embodiment 77. The ionizable cationic lipid of any one of embodiments 1 to 49 wherein
Y is O.

Embodiment 78. The ionizable cationic lipid of any one of embodiments 1 to 49 wherein
Y is S.

Embodiment 79. The ionizable cationic lipid of any one of embodiments 1 to 35 or 51-76, wherein Z is O.

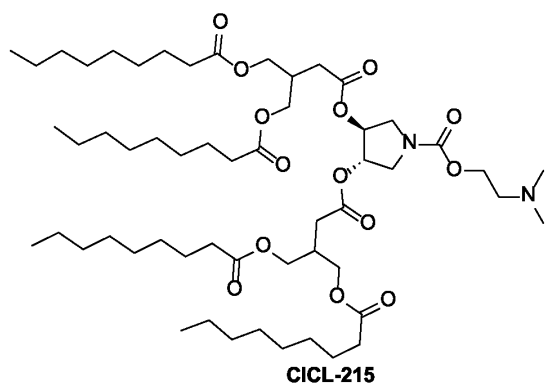
Embodiment 80. The ionizable cationic lipid of embodiment 1 having the structure CICL-207:



or a racemate or other mixture

comprising CICAL-225.

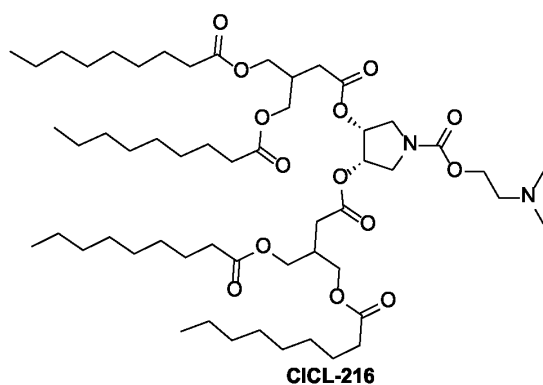
Embodiment 81. The ionizable cationic lipid of embodiment 1 having the structure CICAL-215:



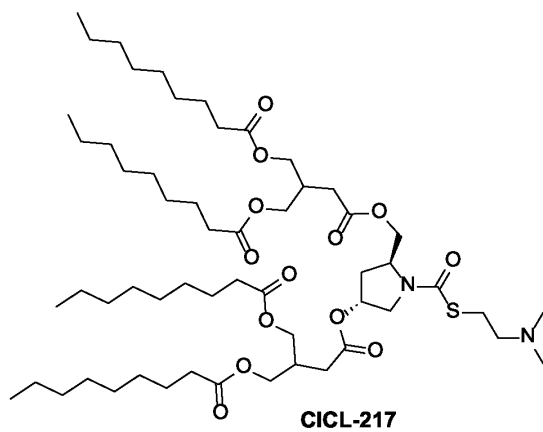
, its enantiomer or a racemate, or

other mixture comprising CICAL-216.

Embodiment 82. The ionizable cationic lipid of embodiment 1 having the structure CICAL-216:

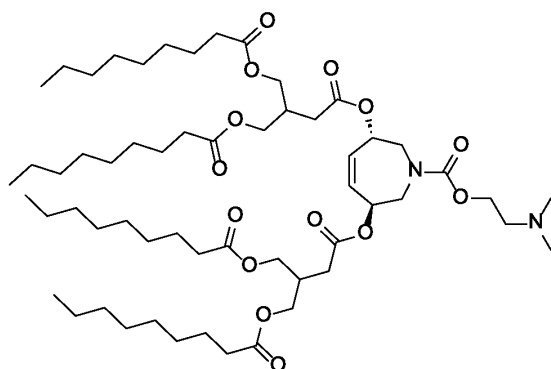


Embodiment 83. The ionizable cationic lipid of embodiment 1 having the structure C1CL-217:



, its enantiomer, a racemate or any other mixture thereof.

Embodiment 84. The ionizable cationic lipid of embodiment 1 having the structure C1CL-218:

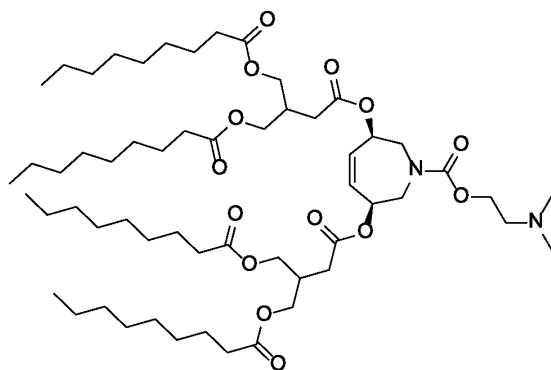


CICL-218

, as a racemate, any other mixture of

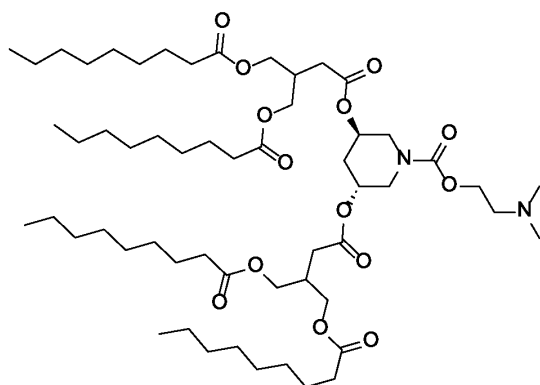
enantiomers, or either individual enantiomer.

Embodiment 85. The ionizable cationic lipid of embodiment 1 having the structure C1CL-219:



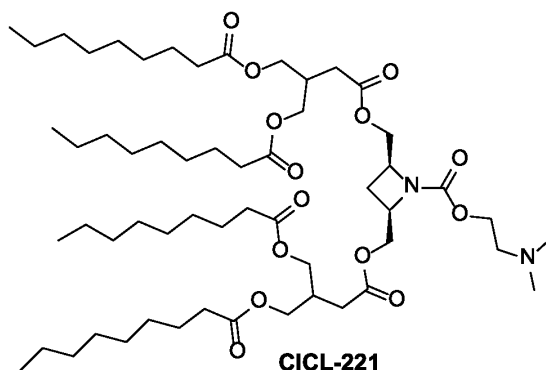
CICL-219

Embodiment 86. The ionizable cationic lipid of embodiment 1 having the structure C1CL-220:

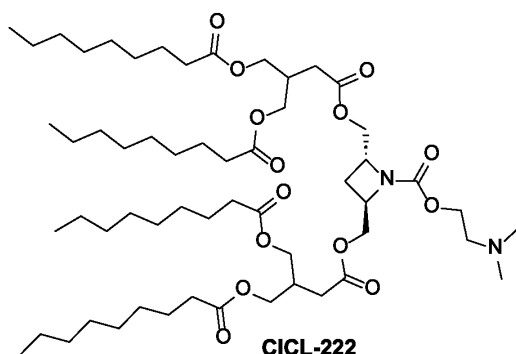
**C1CL-220**

, its enantiomer, a racemate, any other mixture of enantiomers.

Embodiment 87. The ionizable cationic lipid of embodiment 1 having the structure C1CL-221:

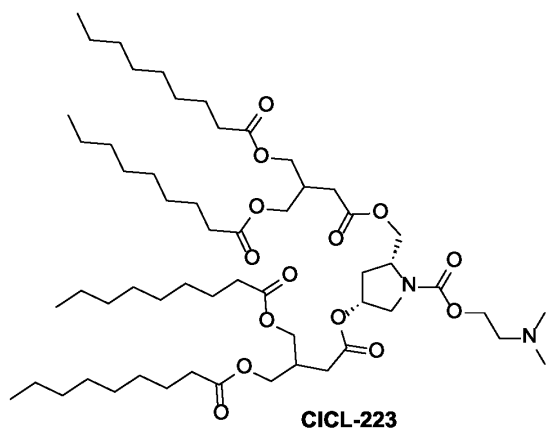
**C1CL-221**

Embodiment 88. The ionizable cationic lipid of embodiment 1 having the structure C1CL-222:

**C1CL-222**

, as a racemate, any other mixture of enantiomers, or either individual enantiomer.

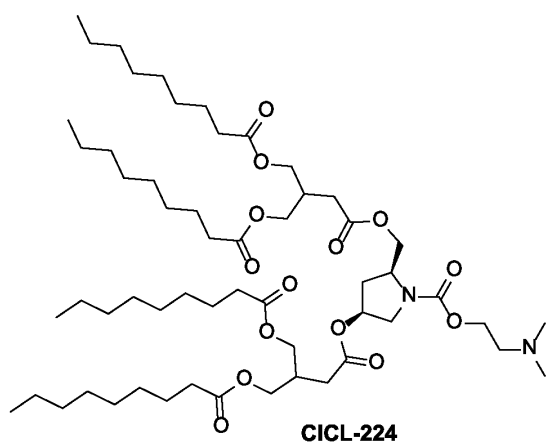
Embodiment 89. The ionizable cationic lipid of embodiment 1 having the structure C1CL-223:



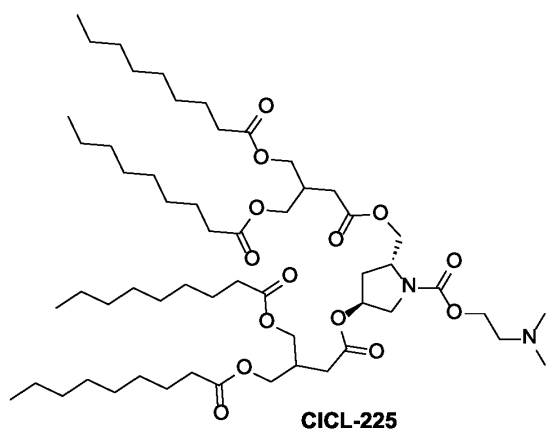
, or a racemate or other mixture

comprising C1CL-224.

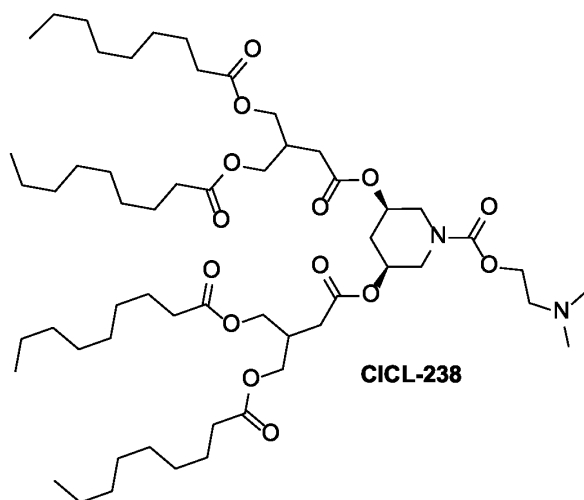
Embodiment 90. The ionizable cationic lipid of embodiment 1 having the structure C1CL-224:



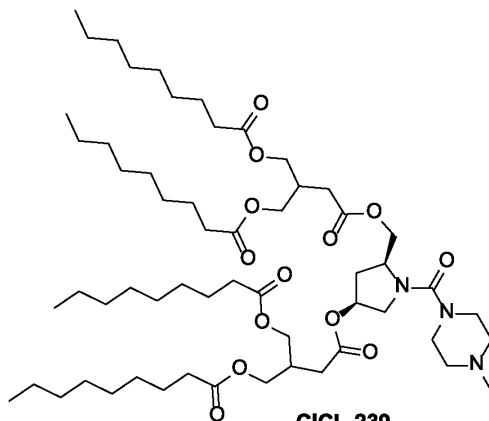
Embodiment 91. The ionizable cationic lipid of embodiment 1 having the structure C1CL-225:



Embodiment 92. The ionizable lipid of embodiment 1 having the structure C1CL-238:

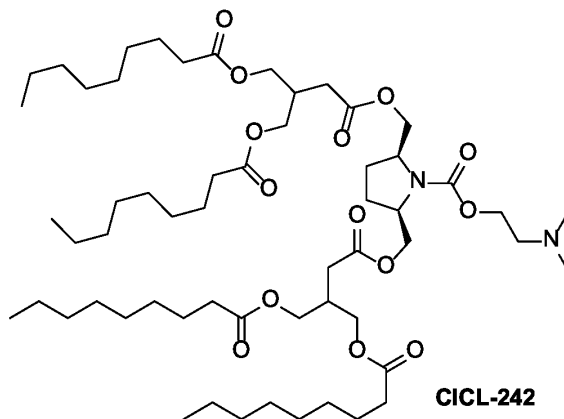


Embodiment 93. The ionizable cationic lipid of embodiment 1 having the structure C1CL-



239: C1CL-239, its enantiomer, or a racemate or any other mixture thereof.

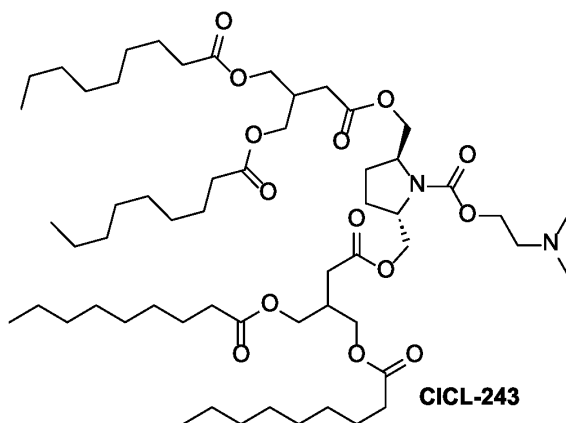
Embodiment 94. The ionizable cationic lipid of embodiment 1 having the structure C1CL-



242:

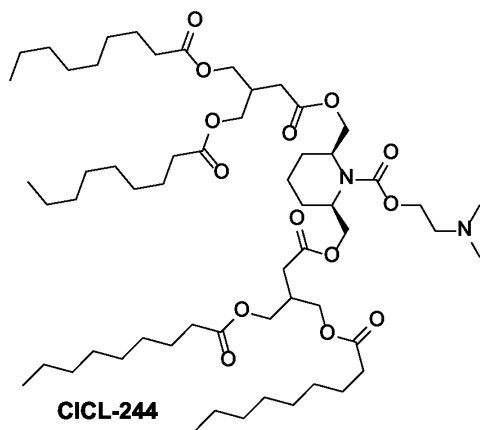
C1CL-242

Embodiment 95. The ionizable cationic lipid of embodiment 1 having the structure CICL-



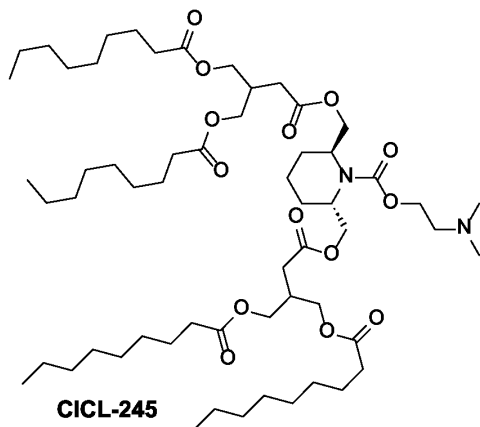
243: **C1CL-243**, its enantiomer, or a racemate or any other mixture thereof.

Embodiment 96. The ionizable cationic lipid of embodiment 1 having the structure C1CL-



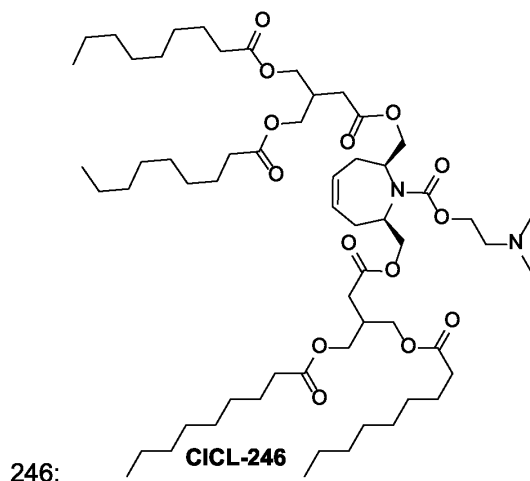
244: **C1CL-244**.

Embodiment 97. The ionizable lipid of embodiment 1 having the structure C1CL-245:



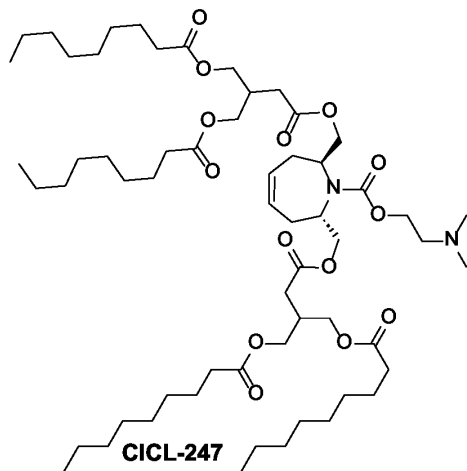
, a racemate, any other mixture of enantiomers, or either individual enantiomer.

Embodiment 98. The ionizable cationic lipid of embodiment 1 having the structure C1CL-



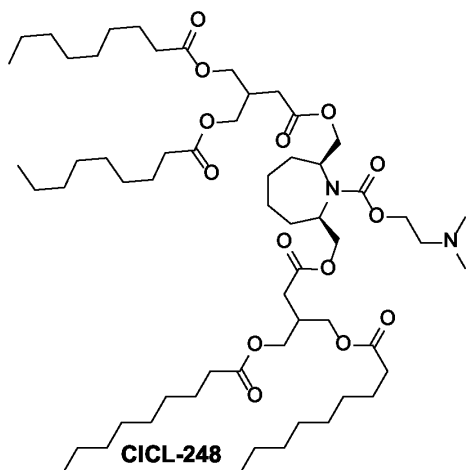
246:

Embodiment 99. The ionizable cationic lipid of embodiment 1 having the structure C1CL-



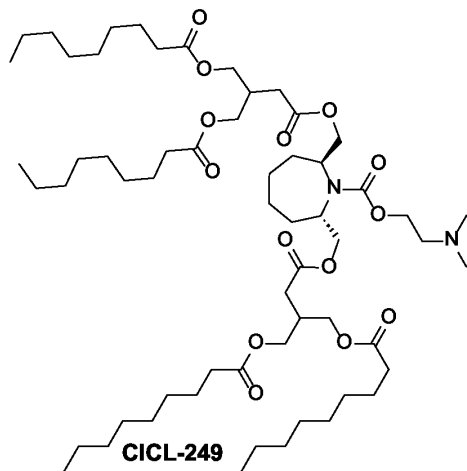
247: , a racemate, any other mixture of enantiomers, or either individual enantiomer.

Embodiment 100. The ionizable cationic lipid of embodiment 1 having the structure C1CL-



248: .

Embodiment 101. The ionizable cationic lipid of embodiment 1 having the structure C1CL-



249: , a racemate, any other mixture of enantiomers, or either individual enantiomer.

Embodiment 102. The ionizable cationic lipid of embodiment 1 having a structure as shown in Table 4.

Embodiment 103. The ionizable cationic lipid of embodiment 1 having a structure as shown in Table 5.

Embodiment 104. The ionizable cationic lipid of embodiment 1 having a structure as shown in Table 6.

Embodiment 105. The ionizable cationic lipid of embodiment 1 having a structure as shown in Table 11.

Embodiment 106. The ionizable cationic lipid of embodiment 1 having a structure as shown in Table 12.

Embodiment 107. The ionizable cationic lipid of any one of embodiments 1 to 106 having a -pKa (calculated pKa) in the range of from about 6, 7, or 8 to about 9, 10, or 11.

Embodiment 108. The ionizable cationic lipid of any one of embodiments 1 to 106 having a c-pKa ranging from about 6 to about 10, about 7 to about 10, about 8 to about 10, about 8 to about 9, 6 to 10, 7 to 10, 8 to 10, or 8 to 9.

Embodiment 109. The ionizable cationic lipid of any one of embodiments 1 to 106 having a c-pKa ranging from about 8.2 to about 9.0 or from 8.2 to 9.0.

Embodiment 110. The ionizable cationic lipid of any one of embodiments 1 to 106 having a c-pKa ranging from about 8.4 to about 8.7 or from 8.4 to 8.7.

Embodiment 111. The ionizable cationic lipid of any one of embodiments 1 to 110 having a cLogD ranging from about 9 to about 18, for example, ranging from about 10 to about 18, or about 10 to about 16, to about 10 to about 14, or about 11 to about 18, or about 11 to about 15, or about 11 to about 14.

Embodiment 112. The ionizable cationic lipid of any one of embodiments 1 to 110 having a cLogD ranging from 9 to 18, for example, ranging from 10 to 18, or 10 to 16, to 10 to 14, or 11 to 18, or 11 to 15, or 11 to 14.

Embodiment 113. The ionizable cationic lipid of any one of embodiments 1 to 110 having a cLogD ranging from about 13.6 to about 14.4 or from 13.6 to 14.4.

Embodiment 114. The ionizable cationic lipid of any one of embodiments 1 to 110 having a c-pKa ranging from about 8 to about 11 or from 8 to 11 and a cLogD ranging from about 9 to about 18 or from 9 to 18.

Embodiment 115. The ionizable cationic lipid of any of embodiments 1 to 110 having a c-pKa ranging from about 8.4 to about 8.7 or from 8.4 to 8.7 and cLogD ranging from about 13.6 to about 14.4 or from 13.6 to 14.4.

Embodiment 116. The ionizable cationic lipid of any one of embodiments 1 to 110 having a cLogD is about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, or about 18.

Embodiment 117. A lipid nanoparticle (LNP) or targeted lipid nanoparticle (tLNP), comprising at least one ionizable cationic lipid of any one of embodiments 1-116.

Embodiment 118. The LNP or tLNP of embodiment 117, further comprising one or more of a phospholipid, a sterol, a co-lipid, a PEG-lipid, or combinations thereof.

Embodiment 119. The LNP or tLNP of embodiment 118, comprising an unfunctionalized PEG-lipid.

Embodiment 120. The LNP or tLNPOf embodiment 118 or embodiment 119, comprising a functionalized PEG-lipid.

Embodiment 121. The tLNP of embodiment 120, wherein the functionalized PEG-lipid has been conjugated with a binding moiety.

Embodiment 122. The tLNP of embodiment 120 or 121, wherein the binding moiety comprises an antigen binding domain of an antibody.

Embodiment 123. The tLNP of embodiment 120 or 121, wherein the binding moiety comprises an antigen, a ligand-binding domain of a receptor, or a receptor ligand.

Embodiment 124. The LNP or tLNP of any one of embodiments 118-123, comprising at least one phospholipid, wherein the phospholipid comprises dioleoylphosphatidyl ethanolamine (DOPE), dimyristoylphosphatidyl choline (DMPC), distearoylphosphatidylcholine (DSPC), dimyristoylphosphatidyl glycerol (DMPG), dipalmitoyl phosphatidylcholine (DPPC), or 1,2-diarachidoyl-sn-glycero-3-phosphocholine (DAPC), or a combination thereof.

Embodiment 125. The LNP or tLNP of any one of embodiments 118-124, comprising at least one sterol, wherein the sterol comprises cholesterol, campesterol, sitosterol, stigmasterol, or combinations thereof.

Embodiment 126. The LNP or tLNP of any one of embodiments 118-125, comprising at least one co-lipid, wherein the co-lipid comprises cholesterol hemisuccinate (CHEMS) or a quaternary ammonium headgroup containing lipid.

Embodiment 127. The LNP or tLNP of embodiment 126, wherein the quaternary ammonium headgroup containing lipid comprises 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium (DOTMA), or 3 β -(N-(N',N'-Dimethylaminoethane)carbamoyl)cholesterol (DC-Chol), or combinations thereof.

Embodiment 128. The LNP or tLNP of any one of embodiments 119-127, comprising at least one functionalized or unfunctionalized PEG-lipid, wherein the functionalized or unfunctionalized PEG-lipid comprises a PEG moiety of 1000-5000 Da molecular weight (MW).

Embodiment 129. The LNP or tLNP of any one of embodiments 119-128, comprising at least one functionalized or unfunctionalized PEG-lipid, wherein the functionalized or unfunctionalized PEG-lipid comprises fatty acids with a fatty acid chain length of C₁₄-C₁₈.

Embodiment 130. The LNP or tLNP of any one of embodiments 119-129, comprising at least one functionalized or unfunctionalized PEG-lipid, wherein the functionalized or unfunctionalized PEG-lipid comprises DMG-PEG2000 (1,2-dimyristoyl-glycerol-3-methoxypolyethylene glycol-2000), DPG-PEG2000 (1,2-dipalmitoyl-glycerol-3-methoxypolyethylene glycol-2000), DSG-PEG2000 (1,2-distearoyl-glycerol-3-methoxypolyethylene glycol-2000), DOG-PEG2000 (1,2-dioleoyl-glycerol-3-

methoxypolyethylene glycol-2000), DMPE-PEG200 (1,2-dimyristoyl-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol-2000), DPPE-PEG2000 (1,2-dipalmitoyl-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol-2000), DSPE-PEG2000 (1,2-distearoyl-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol-2000), DOPE-PEG2000 (1,2-dioleoyl-glycero-3-phosphoethanolamine-3-methoxypolyethylene glycol-2000), or combinations thereof.

Embodiment 131. The LNP or tLNP of any one of embodiments 117-130, wherein the at least one ionizable cationic lipid is present in an amount in the range from 40 to 65 mol%.

Embodiment 132. The LNP or tLNP of any one of embodiments 124-131, comprising a phospholipid in an amount in the range from 7 to 30 mol%.

Embodiment 133. The LNP or tLNP of any one of embodiments 125-132, comprising a sterol in an amount in the range from 20 to 45 mol%.

Embodiment 134. The LNP or tLNP of any one of embodiments 126-133, comprising at least one co-lipid in an amount in the range from 1 to 30 mol%.

Embodiment 135. The LNP or tLNP of any one of embodiments 118, or 121-134, comprising at least one unfunctionalized PEG-lipid in an amount in the range from 0.1 to 5 mol%.

Embodiment 136. The LNP or tLNP of any one of embodiments 118-135, comprising at least one functionalized PEG-lipid in an amount in the range from 0.1 to 5 mol%.

Embodiment 137. The LNP or tLNP of any one of embodiments 117-136, further comprising a biologically active payload nucleic acid.

Embodiment 138. The LNP or tLNP of embodiment 137, wherein the weight ratio of total lipid to nucleic acid is 10:1 to 50:1.

Embodiment 139. The LNP or tLNP of embodiment 137, wherein the N/P ratio is from 3 to 9.

Embodiment 140. The LNP or tLNP of any one of embodiments 137-139, wherein the nucleic acid payload is mRNA.

Embodiment 141. The tLNP of any one of embodiments 120-122 or 124-140, wherein the binding moiety is a whole antibody and the ratio of antibody to nucleic acid is from about 0.3 to about 1.0 (w/w).

Embodiment 142. The tLNP of any one of embodiment 118-141, wherein the tLNP is targeted to a T cell.

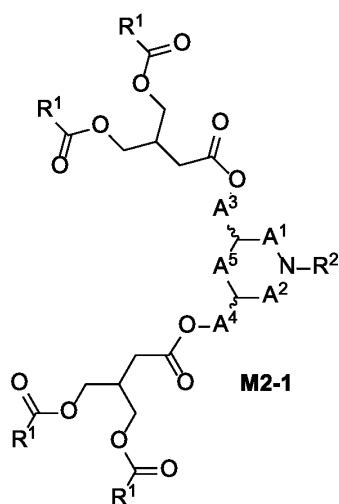
Embodiment 143. The tLNP of any one of embodiments 118-141, wherein the tLNP is targeted to a CD8+ T cell.

Embodiment 144. The tLNP of any one of embodiments 118-141, wherein the tLNP is targeted to an HSC.

Embodiment 145. The tLNP of any one of embodiments 118-141, wherein the tLNP is targeted to a CD117+ cell.

Embodiment 146. A method of delivering a nucleic acid into a cell comprising contacting the cell with the LNP or tLNP of any one of embodiments 137 to 145.

Embodiment 147. A lipid having the structure of formula M2-1,



wherein

each R¹ is independently selected from C₇-C₁₁ alkyl or C₇-C₁₁ alkenyl;

R² is H of a protecting group;

each A¹, A², A³, and A⁴ is independently selected from (CH₂)₀ and (CH₂)₁,

A⁵ is selected from (CH₂)₀₋₄, CH=CH, and CH₂-CH=CH-CH₂; and

wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

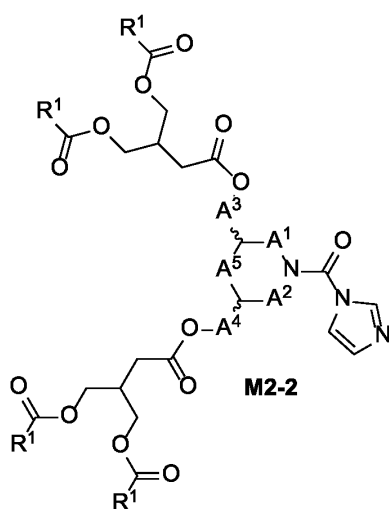
Embodiment 148. The lipid of embodiment 147, wherein R^1 is as described in any one of embodiments 14-25.

Embodiment 149. The lipid of embodiments 147 or 148, wherein A^1 , A^2 , A^3 , A^4 , and A^5 are as described in any one of embodiments 2 to 13.

Embodiment 150. The lipid of any one of embodiments 147 to 149, wherein R^2 is H.

Embodiment 151. The lipid any one of embodiments 147 to 150, wherein R^2 is a protecting group (e.g., t-butoxycarbonyl (BOC), benzyloxycarbonyl (Cbz), or a trimethylsilylethoxycarbonyl moiety).

Embodiment 152. A lipid having the structure of formula M2-2,



each R^1 is independently selected from C_7 - C_{11} alkyl or C_7 - C_{11} alkenyl;

each A^1 , A^2 , A^3 , and A^4 is independently selected from $(CH_2)_0$ and $(CH_2)_1$,

A^5 is selected from $(CH_2)_{0-4}$, $CH=CH$, and $CH_2-CH=CH-CH_2$; and

wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

Embodiment 153. The lipid of embodiment 152, wherein R^1 is as described in any one of embodiments 14-25.

Embodiment 154. The lipid of embodiments 152 or 153, wherein A^1 , A^2 , A^3 , A^4 , and A^5 are as described in any one of embodiments 2-13.

patent, and patent application was specifically and individually indicated to be incorporated by reference.

[00616] While some embodiments have been illustrated and described in detail in the appended drawings and the foregoing description, such illustration and description are to be considered illustrative and not restrictive. Other variations to the disclosed embodiments can be understood and effected in practicing the claims, from a study of the drawings, the disclosure, and the appended claims. The mere fact that certain measures or features are recited in mutually different dependent claims does not indicate that the combination of these measures or features cannot be used. Any reference signs in the claims should not be construed as limiting the scope.

A₄ and A⁴ are intended to represent the same variable moiety wherever it is used herein

APPENDIX A

Antibody	Antigen/ Target	Commercial Source(s) (if available)		Publication Information
		Product Name	Supplier and Catalog Number	
1.5.3	CD20			Gazit-Bornstein et al., 2010, Invest. New Drugs 28: 561-574.
1C5	CD117	c-Kit / CD117 Antibody (1C5)	ThermoFisher, #MA5-15894	
1D1C2	IL-21R α	IL21R Monoclonal Antibody (1D1C2)	ThermoFisher, #66319-1-IG	
1F5	CD20	Anti-CD20 [1F5]	Absolute Antibody, #Ab01133	
1mono2A6 (T113)	CD2	Ellis Reinherz, Dana-Farber Cancer Institute, Boston, MA		
1OLD2-4C1 (T112)	CD2	Ellis Reinherz, Dana-Farber Cancer Institute, Boston, MA		US 2022/0073639 (SEQ ID NO. 51 & 52)
11B1H1B4	CD150	CD150 Monoclonal Antibody (11B1H1B4), Invitrogen™	Fisher Scientific, #16384654	
158-4D3	CD45	CD45 Monoclonal Antibody (158-4D3)	ThermoFisher, #MA1-7636	
190-2F2.5	CD45RO	CD45RO (T-Cell Marker) Monoclonal Antibody (190-2F2.5)	ThermoFisher, #5788-MSM12-P0	
2.1.2	CD20	Anti-Human MS4A1 Recombinant Antibody (2.1.2)	Creative Biolabs, #TAB-1645CL	US 2007/0014720
22HCLC	CD117	Phospho-c-Kit (Tyr703) Recombinant Polyclonal Antibody (22HCLC), Invitrogen™	Fisher Scientific, #PI710762	
2A9	SSEA-3	Anti-SSEA-3/GalGb4 Antibody (2A9)	AntibodySystem, #RGK27501	
2A10D6	FCRL5	CD307e (FcRL5) Monoclonal Antibody	ThermoFisher, #MA5-48789	
2A10H7	FCRL5	CD307e (FcRL5) Monoclonal Antibody	ThermoFisher, #MA5-38483	

2A11D6	CD43	CD43 Monoclonal Antibody (2A11D6)	ThermoFisher, #66224-1-IG	
2B8	CD117	CD117 (c-Kit) Monoclonal Antibody (2B8), eBioscience™	ThermoFisher, #14-1171-82	
2B8/BM	CD117	CD117 (c-Kit) Monoclonal Antibody (2B8/BM)	ThermoFisher, #MA5-28270	
2D4-C9-F1	Sca-1	Mouse anti-Casp3-1(138-157) (2D4-C9-F1)	Biocat, #130-10623-20-RB	
2E10	CD34	<i>available from multiple sources, including:</i>		
		Anti-CD34 [2E10]	Absolute Antibody, #Ab01129	
		Human Anti-CD34 Recombinant Antibody (clone 2E10)	Creative Biolabs, #FAMAB-1049CQ	
2ST8.5H7	CD8β	<i>available from multiple sources, including:</i>		
		CD8 Beta antibody 2ST8.5H7	Bio-Rad, #MCA1723	
		CD8 Antibody (2ST8.5H7)	Novus Biologicals, #NB100-65928	
307307	FCRL5	CD307e (FcRL5) Monoclonal Antibody	ThermoFisher, #MA5-24020	
3A1E	CD7	CD7 Chimeric Recombinant Rabbit Monoclonal Antibody (3A1E)	ThermoFisher, #MHCD0800	
3A1F	CD7	Anti-CD7 [3A1F]	Absolute Antibody, #Ab03052	
3B5	CD8	<i>available from multiple sources, including:</i>		
		CD8 Monoclonal Antibody (3B5)	ThermoFisher, #MHCD0800	
			Aviva Systems Biology, #OAAI00153	

3B8-2C2	CD48	CD48 Monoclonal Antibody (3B8-2C2)	ThermoFisher, #H00000962-M01	
3F7	CD73			Qiao et al., 2019, Int. J. Mol. Sci. 20 (5): 1057.
3F7C6	CD68	CD68 monoclonal antibody, clone 3F7C6	Abnova, #MAB12263	
3F7D3	CD68	<i>available from multiple sources, including:</i>		
		CD68 monoclonal antibody, clone 3F7D3	Abnova, #MAB12260	
		Anti-CD68 antibody [3F7D3]	Abcam, #ab201973	
3F8BiAb	GD2 CD3			Yankelevich et al., 2012, Pediatr. Blood Cancer 59(7): 1198–1205.
3G9-2D2	CD205			Cheong et al., 2010, Blood 116 (19): 3828–3838.
3H420	SSEA-3	SSEA-3 Antibody (3H420)	Santa Cruz Biotechnology, #sc-73066	
3T4-8B5 (T111)	CD2	Ellis Reinherz, Dana-Farber Cancer Institute, Boston, MA		
4C5	Stro-4			Thomaidou and Patsavoudi, 1993, Neuroscience 53: 813–827.
4G5	FAP			WO 2021/061708; WO 2021/061778
4G7	CD19			Meeker et al., 1984, Hybridoma 3: 305-320.
4KB5	CD45RA	Anti-CD45RA antibody [4KB5]	Abcam, #ab755	
47G4	CD19			US 2010/0104509
51.1	CD8	Anti-CD8 [51.1]	Absolute Antibody, #Ab04357	
53H8L29	CD117	Phospho-c-Kit (Tyr703) Recombinant Rabbit Monoclonal Antibody (53H8L29)	ThermoFisher, #700135	

5B4G1	CD150	SLAM/CD150 Monoclonal Antibody (5B4G1)	ThermoFisher, #60043-1-IG	
5B12	CD34	<i>available from multiple sources, including:</i>		
		Anti-CD34 [5B120	Absolute Antibody, #Ab00422	
		Human Anti-CD34 Recombinant Antibody (clone 5B12)	Creative Biolabs, #FAMAB-1047CQ	
5D7	CD5	CD5 Monoclonal Antibody (CD5-5D7), APC	ThermoFisher, #MHCD0505	
5E3E1	IL-15R α	IL-15RA Monoclonal Antibody (5E3E1)	ThermoFisher, #MHCD0505	
5E10	CD90	<i>available from multiple sources, including:</i>		
		CD90 (Thy-1) Monoclonal Antibody (eBio5E10 (5E10)), eBioscience™	ThermoFisher, #14-0909-82	
		Anti-Human CD90 Antibody, Clone 5E10	Stemcell Technologies, #60045	
509F6	FCRL5	CD307e (FcRL5) Monoclonal Antibody (509F6), eFluor™ 660, eBioscience™	ThermoFisher, #50-3078-42	
6D276	CD29 CD49b	Anti-CD49b, CD29 Complex Mouse Monoclonal Antibody [clone: 6D276]	VWR, #USBIC2404-07W	
6D9	GPRC5D	Anti-GPRC5D (6D9) h(41BB-CD3 ζ) CAR, pCDCAR1	Creative Biolabs, #CAR-LY1362	
6F2	CD117	KIT Monoclonal Antibody (6F2)	ThermoFisher, #H00003815-M02	
7D11	FCRL5	Anti-FcRH5 (clone 7D11)-SMCC-DM1 ADC	Creative Biolabs, #ADC-161CL	
7E1	CD48	CD48 Recombinant Rabbit Monoclonal Antibody (7E1)	ThermoFisher, #MA5-38301	
8A7	SSEA-3	Anti-SSEA-3/GalGb4 Antibody (8A7)	AntibodySystem, #RGK27504	

8D7	CD117	c-Kit Monoclonal Antibody (8D7)	ThermoFisher, #MA5-15327	
9.1	CD2	Peter Linsley, Benaroya Research Institute, Seattle, WA		Connelly et al., 1998, Int. Immunol., 10 (12): 1863–1872.
9.6	CD2	Peter Linsley, Benaroya Research Institute, Seattle, WA		Connelly et al., 1998, Int. Immunol., 10 (12): 1863–1872.
9C5	CD34			US 8,399,249
9D1	CD150	CD150 Monoclonal Antibody (9D1), PE, eBioscience™	ThermoFisher, #12-1501-82	
10A8	FCRL5	Anti-FcRL5 (10A8)-SPDB-DM4 ADC	Creative Biolabs, #ADC-117LCT	
12G10	CD29	<i>available from multiple sources, including:</i>		
		CD29 (Stem Cell Marker) Monoclonal Antibody (12G10)	ThermoFisher, #3688-MSM2-P0	
		CD29 antibody 12G10	Bio-Rad, #MCA2028	
13G9	FCRL5	Mouse Anti-FCRL5 Recombinant Antibody	Creative Biolabs, #MOB-057CQ	
17D8	CD8	CD8 Monoclonal Antibody (17D8)	ThermoFisher, #MA1-12027	
18A5	IL-21Rα	Human Anti-IL21R Recombinant Antibody	Creative Biolabs, #HPAB-0668-CN	
84-3C1	CD43	CD43 Monoclonal Antibody (PE, eBioscience™)	ThermoFisher, #12-0439-42	
104D2	CD117	<i>available from multiple sources, including:</i>		
		Purified anti-human CD117 (c-kit) Antibody	BioLegend, #313201	
		Anti-Human CD117 (c-Kit) Antibody, Clone 104D2	Stemcell Technologies, #60087	

		MOUSE ANTI HUMAN CD117	Bio-Rad, #MCA1841	
		c-Kit Monoclonal Antibody (104D2)	ThermoFisher, #MA1-10072	
131-I- apamistamab	CD45			Seropian et al., 2023, Blood 142 (Supplement 1): 2159.
131-I- omburtamab (131-I-8H9)	B7H3			Kramer et al., 2022, J. Hematol. Oncol. 15: 165.
177-Lu-DTPA- omburtamab	B7H3			Khatua et al., 2021, Ann. Oncol. 32 (Suppl. 5): S528- S529.
156-4H9	CD48	CD48 Monoclonal Antibody (eBio156- 4H9 (156-4H9)), eBioscience™	ThermoFisher, #14-0489-82	
293C3	CD133	<i>available from multiple sources, including:</i>		
		Anti-CD133 antibody [293C3]	Abcam, #ab252553	
		CD133/2 Antibody, anti-human, pure	Miltenyi Biotec, #130-090-851	
341	CD48	CD48 Recombinant Rabbit Monoclonal Antibody (341)	ThermoFisher, #MA5-29675	
514H12	CD68	CD68 Monoclonal Antibody (514H12)	ThermoFisher, #MA1-80133	
1279B	SUSD2	SUSD2 Antibody (1279B)	Novus Biologicals, #MAB90564	
2639B	IL-15Rα	Human IL-15R alpha Antibody	R&D Systems, #MAB10900	
A7R34	CD127	CD127 Monoclonal Antibody (A7R34), eBioscience™	ThermoFisher, #14-1271-82	
A12 (7D4)	CD150	CD150 Monoclonal Antibody (A12 (7D4)), PE, eBioscience™	ThermoFisher, #12-1509-42	
AB75	CD2	<i>available from multiple sources, including:</i>		
		CD2 Monoclonal Antibody (AB75)	ThermoFisher, #MA5-11373	

		Anti-CD2 Mouse Monoclonal Antibody [clone: AB75]	Avantor (VWR), #GTX75367 (89365-772)	
Ab85	CD117			WO 2019/084053
ABL501	LAG-3 PD-L1			Park et al., 2021, Cancer Res. 81 (13_Supplement): 1633.
ABM53F5	CD68	Monoclonal antibody to CD68 (Clone: ABM53F5)	Abeomics, #10-4171	
ABX-MA1	CD146	Anti-CD146 [ABX-MA1 (3.19.1)]	Absolute Antibody, #Ab02820	
AC133	CD133	CD133/1 Antibody, anti-human, pure	Miltenyi Biotec, #130-090-422	
acazicolcept	CD28	Acazicolcept	MedChemExpress, #HY-P99420	
ACK2	CD117	CD117 (c-Kit) Monoclonal Antibody (ACK2), APC, eBioscience™	ThermoFisher, #17-1172-82	
ACK4	CD117	c-Kit Monoclonal Antibody (ACK4)	ThermoFisher, #MA1-70079	
Additmer®	CD56			Esnault et al., 2022, Br. J. Dermatol. 186 (2): 295-306.
ADG106	CD137			Ma et al., 2024, Cell Rep. Med. 5 (2): 101414.
ADG116	CTLA-4			Park et al., 2022, J. Immunother. Cancer 10 (Suppl 2): A1–A1603.
ADG126	CTLA-4			Richardson et al., 2022, Ann. Oncol. 33 (suppl 7): S882-S883.
ADU-1604	CTLA-4			De Velasco Oria de Rueda et al., 2023, J. Immunother. Cancer 11 (Suppl 1): A1–A1731.
AFM13	CD16a	Anti-FcγR3a / CD16a (AFM13)	Selleckchem, #A2750	

AGEN1181	CTLA-4			El-Khoueiry, et al., 2021, J. Immunother. Cancer 9 (Suppl 2): A1–A1054.
AK112	PD-1 VEGF	Research Grade Ivonescimab	AntibodySystem, #DHD12605	
AK119	CD73	Research Grade Dresbuxelimab	Cell Sciences, #DHD46603A	
AK129	LAG-3			Huang et al., 2022, Cancer Res. 82 (12_Supplement): 5520.
alefacept	CD2	Alefacept	MedChemExpress, #HY-P99429	
AlexaFluor647 anti-hlgG Fc detection antibody	IgG	<i>available from multiple sources, including:</i>		
		Alexa Fluor® 647 AffiniPure™ Goat Anti-Human IgG, Fcy fragment specific	Jackson ImmunoResearch Laboratories, #109-605-098	
		Human IgG Alexa Fluor® 647- conjugated Antibody	R&D Systems, #FAB110R	
alirocumab	PCSK9	<i>available from multiple sources, including:</i>		
		Alirocumab	MedChemExpress, #HY-P9928	
		Alirocumab Biosimilar – Anti-PCSK9 mAb	Proteogenix, #PX-TA1287	
		Alirocumab (anti- PCSK9)	SelleckChem, #A2047	
alnuctumab	BCMA			US 10,683,369
AMG-172	CD70			Massard et al., 2019, Cancer Chemother. Pharmacol. 83(6):1057-63.
AMG 224	BCMA			US 9,243,058

AMG 228	GITR			Tran et al., 2017, J. Clin. Oncol. 35 (15 suppl): 2521.
AMG 404	PD-1	Zelvalimab	MedChemExpress, #HY-P99957	
AMM22070N	Sca-1	Caspase-3 Mouse mAb	Leading Biology, #AMM22070N	
anti-BCMA CARs	BCMA			US 2020/0246381; US 2020/0339699
anti-CD3 FITC antibody conjugate	CD3	BD Pharmingen™ FITC mouse anti-human CD3ε	BD Biosciences, #556611	
anti-CD3 SP34-2 Alexa Fluor 700 conjugate antibody	CD3	BD Pharmingen™ Alexa Fluor® 700 mouse anti-human CD3	BD Biosciences, #557917	
anti-CD3 V450	CD3	<i>available from multiple sources, including:</i>		
		BD Horizon™ V450 Mouse Anti-Human CD3	BD Biosciences, #560366	
		violetFluor™ 450 Anti-Human CD3 (UCHT1)	Cytex, #75-0038-T025	
anti-CD4 (PerCP-Cy5.5)	CD4	<i>available from multiple sources, including:</i>		
		BD Pharmingen™ PerCP-Cy™5.5 Mouse Anti-Human CD4	BD Biosciences, #560650	
		CD4 Monoclonal Antibody (RM4-5), PerCP-Cyanine5.5, eBioscience™	ThermoFisher, #45-0042-82	
anti-CD4 BV 421 antibody conjugate	CD4	Brilliant Violet 421™ anti-human CD4 Antibody	BioLegend, #317434	
anti-CD4 OKT4 BV650 conjugate antibody	CD4	Brilliant Violet 650™ anti-human CD4 Antibody	BioLegend, #317436	
anti-CD8a (APC-H7)	CD8α	BD Pharmingen™ APC-H7 Mouse anti-Human CD8	BD Biosciences, #560179	

anti-claudin 18.2 antibodies	CLDN18.2			WO 2013/167259, WO 2021/032157, WO 2021/254481, WO 2022/007808, WO 2021/008463, WO 2022/111616, WO 2018/006882, WO 2020/147321, WO 2019/219089, US 2020/0040101, WO 2020/025792, WO 2020/139956, WO 2020/135201, US 2024/0228610, WO 2021/218874, WO 2021/027850, WO 2021/129765, WO 2022/068854, WO 2021/111003
anti-FAP CARs	FAP			WO 2021/061778
anti-FCRL5 CARs and ADCs	FCRL5			WO 2016/090337, WO 2017/096120, WO 2022/263855, WO 2024/047558, Elkins et al., Mol. Cancer Ther. 11(10): 2222-2232 (2012)
anti-G4S PE antibody	G4S	G4S Linker (E7O2V) Rabbit mAb (PE Conjugate)	Cell Signaling Technology, #38907	
anti-human Fc secondary antibody (conjugated to BV421 fluorophore)	Fc	Brilliant Violet 421™ anti-human IgG Fc Antibody	BioLegend, #410704	
anti-scFv linker PE antibody	scFv linker	MonoRab™ Rabbit Anti-scFv Cocktail [PE]	GenScript, #A02285	
anti-siglec-15 antibody	Siglec-15			US 8,575,531
anti-Whitlow linker PE antibody		Whitlow/218 Linker (E3U7Q) Rabbit mAb (PE Conjugate)	Cell Signaling Technology, #62405	
ANV419	CD122			Joerger et al., 2023, J. Immunother. Cancer 11 (11): e007784.

ARC0672	CD127	CD127 Recombinant Rabbit Monoclonal Antibody (ARC0672)	ThermoFisher, #MA5-35152	
ARX305	CD70			Skidmore et al., 2023, Ann. Oncol. 34 (Suppl. 2): S208.
ASD141	CD11b			Chang et al., 2021, J. Immunother. Cancer 9 (Suppl. 2): A1-A1054.
ASKB589	CLDN18.2			Zhang et al., 2023, J. Clin. Oncol. 41 (4 suppl.), 397.
ASP1951	GITR			Seidel-Dugan et al., 2018, J. Immunother. Cancer 6 (115).
AT002	CD166	<i>available from multiple sources, including:</i>		
		Recombinant Anti-CD166 antibody	Antibodies.com, #A323924	
		Research Grade Anti-Human CD166/ALCAM	AntibodySystem, #DHG64002	
atibuclimab	CD14	<i>available from multiple sources, including:</i>		
		Atibuclimab	MedChemExpress, #HY-P99008	
		Atibuclimab (Anti-CD14)	SelleckChem, #A2566	
ATL301	GD2	Research Grade Anti-Ganglioside GD2 (ATL301)	AntibodySystem, #DHK07801	
ATOR-1015	OX40			Veitonmäki et al., 2018, Cancer Res. 78 (13_Supplement): 3623.
ATOR-1144	GITR			Fritzell et al., 2019, Cancer Res. 79 (13_Supplement): 4077.
axicabtagene ciloleucel	CD19			Neelapu et al., 2017, N. Engl. J. Med. 377: 2531-44.

AZD0901	CLDN18.2			Raufi et al., 2024, J. Clin. Oncol. 42 (16 Suppl.), TPS3163.
AZD7789	TIM-3			Clancy-Thompson et al., J. Immunother. Cancer 10 (Suppl. 2): A1-A1603.
AZN-L50	CD166	<i>available from multiple sources, including:</i>		
		CD166, Human, mAB AZN-L50	Hycult Biotech, #HM2002	
		CD166 antibody [AZN-L50]	GeneTex, #GTX18159	
B4	CD19			Freedman et al., 1987, Blood 70: 418-427.
B4 HB12b	CD19			Kansas & Tedder, 1991, J. Immunol. 147: 4094-4102; Yazawa et al., 2005, PNAS 102: 15178-15183; Herbst et al., 2010, J. Pharmacol. Exp. Ther. 335: 213-222.
B7V3V2	CD200	Anti-Human CD200 Antibody (B7V3V2)	AntibodySystem, #FHE30310	
B43	CD19			Bejcek et al., 1995, Cancer Res. 55 (11):2346-2351.
balstilimab	PD-1			Moore et al., 2018, J. Clin. Oncol. 36 (15 suppl.): 3086.
barzolvolimab	CD117	CDX 0159	MedChemExpress, #HY-P99462	
basiliximab (STI-003)	CD25	Basiliximab Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-41768	Onrust et al., 1999, Drugs 57, 207-213.
BAT6026	OX40			Liang et al., 2023, Front. Oncol., 13: 1211759.
bavunalimab	LAG-3	<i>available from multiple sources, including:</i>		
		Bavunalimab	MedChemExpress, #HY-P99354	

		Bavunlimab	ChemScene, #CS-0621488	
BB2121	BCMA			WO 2012/163805
BC8-B10	CD45			Li et al., 2018, PLoS One 13(10): e0205135.
BCD-145	CTLA-4	<i>available from multiple sources, including:</i>		
		Nurulimab	MedChemExpress, #HY-P99760	
		Nurulimab	AbMole, #M24967	
BCD-245	GD2			Clinical Trial ID: NCT05782959
belantamab	BCMA			US 9,273,141
BGB-A445	OX40			Jiang et al., 2023, Front. Med. 17(6): 1170-1185.
bleselumab	CD40	<i>available from multiple sources, including:</i>		
		Bleselumab	MedChemExpress, #HY-P99257	
		Bleselumab	SelleckChem, #A2634	
BL-178-12C7	CD45	Anti-CD45 antibody	Abcam, #ab243869	
BLY3	CD19			Bejcek et al., 1995, Cancer Res. 55 (11): 2346-2351.
BMS-986156	GITR			Siu et al., 2017, J. Clin. Oncol. 35 (15_suppl): 104.
BMS-986178	OX40			Gutierrez et al., 2021, Clin. Cancer Res. 27 (2): 460- 472.
BMS-986179	CD73			Siu et al., 2018, Cancer Res. 78(13_Suppl): CT180.
BMS-986218	CTLA-4			Engelhardt et al., 2020, Cancer Res. 80 (16_Supplement): 4552.

BMS-986249	CTLA-4			Gutierrez et al., 2020, J. Clin. Oncol. 38 (15 suppl): 3058.
BMS-986258	TIM-3			Clinical Trial ID: NCT03446040
BMS-986393	GPRC5D			Bal et al. (2023). Blood 142 (Suppl. 1): 219.
bococizumab	PCSK9	<i>available from multiple sources, including:</i>		
		Bococizumab	MedChemExpress, #HY-P99187	
		Bococizumab (Anti-PCSK9)	SelleckChem, #A2788	
		Bococizumab Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-42019	
bococizumab NEI	PCSK9			Dyson et al. (2020). MAbs 12: 1829335.
botensilimab	CTLA-4			Wilky et al., 2022, J. Immunother. Cancer, 10 (Suppl. 2): A1-A1603.
BP-02489	CD10	Accession No. NITE BP-02489 (JP)		WO 2018/235247
brexucabtagene autoleucl	CD19			Anderson et al., 2022, Ann. Pharmacother. 56(5): 609-619.
briquilimab (JSP-191)	CD117	<i>available from multiple sources, including:</i>		
		Briquilimab	MedChemExpress, #HY-P99488	
		Research Grade Briquilimab	Cell Sciences, #DHC83102A	
BTI-322	CD2	Anti-Human CD2 Recombinant Antibody (BTI-322)	Creative Biolabs, #TAB-110CL	
BU12	CD19			Callard et al., 1992, J. Immunology, 148(10): 2983-2987
Bu88	CD8	Anti-CD8 alpha Antibody	Abcam, #ab316355	

budigalimab	PD-1	Budigalimab	MedChemExpress, #HY-P99489	
C2e10	CD34	<i>available from multiple sources, including:</i>		
		Mouse Anti-CD34 Recombinant Antibody (clone C2e10); scFv Fragment	Creative Biolabs, #HPAB-1044-FY-F(E)	
		Anti-CD34 [2E10], Human IgG1, Kappa	Absolute Antibody, #Ab01129	
C5B12	CD34	<i>available from multiple sources, including:</i>		
		Human Anti-CD34 Recombinant Antibody (clone C5B12); Fab Fragment	Creative Biolabs, #HPAB-1043-FY-S(P)	
		Anti-CD34 [5B12], Human IgG1, Kappa	Absolute Antibody, #Ab00422	
C8/144B	CD8 α	<i>available from multiple sources, including:</i>		
		CD8 alpha Monoclonal Antibody (C8/144B)	ThermoFisher, #MA5-13473	
		Anti-CD8 alpha antibody [C8/144B]	Abcam, #ab17147	
C11D5.3	BCMA			WO 2010/104949
C12A3.2				
C363.16A	CD45RB	CD45RB Monoclonal Antibody (PE, eBioscience™)	ThermoFisher, #12-0455-82	
cadonilimab	PD-1			Pang et al., 2023, MAbs 15(1): 2180794.
camidanlumab tesirine	CD25			Puzanov et al., 2020, Ann. Oncol. 31(suppl. 4), S710-S711.
camrelizumab	PD-1	Camrelizumab (anti-PD-1)	SelleckChem, #A2016	
carotuximab	CD105	<i>available from multiple sources, including:</i>		

		Carotuximab	MedChemExpress, #HY-P99494	
		Carotuximab (Anti-Endoglin / CD105)	SelleckChem, #A2514	
CBXS-1620	SUSD2	Rabbit Anti-SUSD2 Recombinant Antibody (CBXS-1620)	Creative Biolabs, #CBMAB-S4469-CQ	
CBXS-1671	SUSD2	Rabbit Anti-SUSD2 Recombinant Antibody (CBXS-1671)	Creative Biolabs, #CBMAB-S0094-CQ	
CBXS-1989	SUSD2	Mouse Anti-SUSD2 Recombinant Antibody (CBXS-1989)	Creative Biolabs, #CBMAB-S4758-CQ	
CBXS-1990	SUSD2	Mouse Anti-SUSD2 Recombinant Antibody (CBXS-1990)	Creative Biolabs, #CBMAB-S0156-CQ	
CBXS-3571	SUSD2	Human Anti-SUSD2 Recombinant Antibody (CBXS-3571)	Creative Biolabs, #CBMAB-S0386-CQ	
CBXS-3676	SUSD2	Rabbit Anti-SUSD2 Recombinant Antibody (CBXS-3676)	Creative Biolabs, #CBMAB-S0214-CQ	
CBYY-I0413	IL-12R	Mouse Anti-IL12RB2 Recombinant Antibody (CBYY-I0683)	Creative Biolabs, #CBMAB-I1506-YY	
C-CAR066 Leu16 and 2.1.2	CD20			Liang et al., 2021, J. Clin. Oncol. 39(15) suppl:2508)
CD58 (LFA-3)	CD2	Recombinant Human CD58/LFA-3 His-tag Protein	R&D Systems, #1689-CD	
CDLA68-1	CD68	CD68 Antibody [clone CDLA68-1]	NSJ Bioreagents, #V7045	
CDX-0158	CD117	Anti-SCFR / c-Kit / CD117 Reference Antibody (CDX-0158)	BiOrbyt, #orb1817461	
cedelizumab	CD4	<i>available from multiple sources, including:</i>		
		Cedelizumab	MedChemExpress, #HY-P99495	
		Cedelizumab (CD4)	ProSci, #10-093	
cemiplimab	PD-1			Burova et al., 2017, Mol. Cancer Ther. 16 (5), 861-870.

cetrelimab	PD-1	Cetrelimab	MedChemExpress, #HY-P99499	
cetuximab	EGFR	<i>available from multiple sources, including:</i>		
		EGF R antibody C225	BioRad, #MCA6102	
		Cetuximab	MedChemExpress, #HY-P9905	
		Cetuximab (anti-EGFR)	SelleckChem, #A2000	
cevostamab	CD3	Cevostamab	MedChemExpress, #HY-P99601	
ch28/11	SSEA-4	Chimeric (Mouse/Human) Anti-SSEA-4 Recombinant Antibody (clone ch28/11)	Creative Biolabs, #PABJ-0121	
cifurtilimab	CD40	Cifurtilimab; SEA-CD40	MedChemExpress, #HY-P99603	
ciltacabtagene autoleucel	BCMA			Berdeja et al., 2021, Lancet 398 (10297): 314-324.
CK6	CD117			Lebron et al., 2014, Cancer Biol. Ther. 15 (9): 1208–1218.
CL1659	CD117	CD117/c-kit Antibody (CL1657)	Novus Biologicals, #NBP2-52975	
CLB-CD19	CD19			De Rie, 1989, Cell. Immunol. 118:368-381
clenoliximab	CD4	Clenoliximab	Novus Biologicals, #NBP2-52681H	
Cmab-43	CD133	Recombinant Anti-CD133 antibody	Abcam, #AB264538	
CO.44B8	CD43	CD43 Monoclonal Antibody (CO.44B8)	ThermoFisher, #MA5-28371	
cobolimab	TIM-3	Cobolimab	MedChemExpress, #HY-P99827	

CPP32 4-1-18	Sca-1	Caspase 3 Monoclonal Antibody (CPP32 4-1-18)	ThermoFisher, MA1-16843	
crefmirlimab	CD8α	<i>available from multiple sources, including:</i>		Farwell et al., J Nucl Med 63(5):720-726 (2021); US 10,414,820
		Crefmirlimab	MedChemExpress, #HY-P99834	
		Crefmirlimab Biosimilar – Anti-CD8A mAb – Research Grade	ProteGenix, #PX-TA1828-100	
CS1002	CTLA-4			Bishnoi et al., 2021, Ann. Oncol. 32, (Suppl. 5): S840.
CT041	CLDN18.2			Qi et al., 2022, Nat. Med. 28, 1189-1198.
CT103A (CAR0085)	BCMA			US 11,026,975
cudarolimab	OX40	Cudarolimab	MedChemExpress, #HY-P99836	Kuang, Z et al. Cancer Immunol Immunother. 2020 Jun;69(6):939-950.
cusatuzumab	CD70	Cusatuzumab	MedChemExpress, #HY-P99014	Riether, C et al. Nat Med. 2020 Sep;26(9):1459-1467.
CYT-91000	CD25	Research Grade Anti-Human CD25/IL2RA (CYT-91000)	AntibodySystem, #DHB95805	
dacetuzumab	CD40	Dacetuzumab	MedChemExpress, #HY-P99015	Hussein, M et al. Haematologica. 2010 May;95(5):845-8.
daclizumab	CD25	Daclizumab	MedChemExpress, #HY-108738	Cohan, S. L. et al. Biomedicines. 2019 Mar 11;7(1):18.
daratumumab	CD38	Daratumumab (anti-CD38)	SelleckChem, #A2027	
davoceticept	CD28	Davoceticept	MedChemExpress, #HY-P99844	Patnaik, A. et al. JCO 40, TPS2683-TPS2683(2022).
DF-T1	CD43	CD43 Antibody (DF-T1)	Santa Cruz Biotechnology, #sc-6256	
dinutuximab	GD2	Dinutuximab	ThermoFisher, #MA5-42000	Mohd, A.B. et al. J Res Med Sci. 2023 Sep 29;28:71.

dostarlimab	PD-1	Dostarlimab	SelleckChem, #A2835	Mirza, M.R. et al. N Engl J Med. 2023 Jun 8;388(23):2145-2158.
DS-7300A	B7H3			Yamato et al., 2022, Mol. Cancer Ther. 21(4): 635–646.
EasySep™		EasySep™ Human T Cell Isolation Kit	Stemcell Technologies, #100-0695	
efungumab	Stro-4	Efungumab	MedChemExpress, #HY-P9962	Karwa, R. et al. Ann Pharmacother. 2009 Nov;43(11):1818-23.
ektomab	GD2			Ruf, P. et al. J Transl Med. 2012 Nov 7;10:219.
ELISA MAX™ Deluxe Set Human TNF-α kit	TNF-α	ELISA MAX™ Deluxe Set Human TNF-α kit	BioLegend, #430204	
elranatamab	CD3	Research Grade Elranatamab	AntibodySystem, #DHF92406	Lesokhin, A. M. et al. Nat Med. 2023 Sep;29(9):2259-2267.
EMB-02	LAG-3 PD-1			Day et al., 2023, Ann. Oncol. 34 (Suppl. 2): S625.
EMB-06	BCMA			US 2023/0002489
EMB-09	OX40 PD-L1			Li et al., 2023, Antib. Ther. 6 (Suppl 1): tbad014.008.
EMD 273063	GD2	Anti-GD2 (EMD 273063)	SelleckChem, #A2916	
encelimumab	LAG-3	Encelimumab	MedChemExpress, #HY-P99922	Perez-Santos, M. et al. Expert Opin Drug Discov. 2022 Dec;17(12):1341-1355.
enoblituzumab	B7H3	Anti-Human CD276 Recombinant Antibody	Creative Biolabs, #TAB-424CQ	
EOS-448	TIGIT			Cuende et al., 2022, Cancer Res. 82 (12_Supplement): LB189.

EP322Y	CD45	Anti-CD45 antibody [EP322Y]	Abcam, #ab40763	
EPR8913(2)	SUSD2	Anti-SUSD2 antibody [EPR8913(2)]	Abcam, #ab182147	
EPR20545	CD68	Anti-CD68 antibody [EPR20545]	Abcam, #ab213363	
EPR26538-16	CD8 β	Recombinant Anti-CD8 beta antibody [EPR26538-16]	Abcam, #ab300067	
EPR26948-19	FCRL5	Anti-FCRL5 antibody [EPR26948-19] - BSA and Azide free (Detector)	Abcam, #ab307569	
EPR26948-67	FCRL5	Anti-FCRL5 antibody [EPR26948-67] - BSA and Azide free (Capture)	Abcam, #ab307568	
EPR27365-87		Anti-FCRL5 antibody [EPR27365-87]	Abcam, #ab307730	
epratuzumab	CD22	Anti-Human CD22 Recombinant Antibody	Creative Biolabs, #TAB-176	
ET200-31	FCRL5			US 10,913,796
ET200-39				
ET200-69				
ET200-104				
ET200-105				
ET200-109				
ET200-117				
etigilimab	TIGIT			Mettu et al., 2022, Clin. Cancer. Res. 28(5): 882-892.
ezabenlimab	PD-1	Ezabenlimab	MedChemExpress, #HY-P99610	Zetti, M. et al. Oncoimmunology. 2022 Jun 16;11(1):2080328.
F10-89-4	CD45	Anti-CD45 antibody [F10-89-4] - Hematopoietic Stem Cell Marker	Abcam, #ab30470	
F119	FCRL5			Ise et al. (2005) Clin. Cancer Res. 11(1): 87-96.

F25	FCRL5	Anti-FcRH5 Antibody, clone F25	Millipore Sigma, #MABF2103	
F26	FCRL5			Franco et al. (2013) J. Immunol. 190(11): 5739-5746.
favezelimab	LAG-3	Favezelimab	MedChemExpress, #HY-P99613	Gareth, P. et al. Cancer Res 1 July 2019; 79 (13_Supplement): CT106.
felzartamab	CD38	Felzartamab	MedChemExpress, #HY-P99616	
FHVH33	BCMA			WO 2019/006072
fianlimab	LAG-3	Fianlimab	MedChemExpress, #HY-P99617	Omid, H. et al. JCO 39, 9515-9515 (2021).
FMC63	CD19			Nicholson et al., 1997, Mol. Immun. 34(16-17):1157-1165; WO 2018/213337, WO 2015/187528
forimtamig	GPRC5D			Harrison et al. (2023). Clin. Lymphoma Myeloma Leuk. 23 (Suppl. 2): S3-4.
FR4D11	CD10	Anti-Human CD10 Antibody, Clone FR4D11	Stemcell Technologies, #60149	
FR104CD	CD28	Recombinant Anti-CD28 Antibody [FR104]	Antibodies.com, #A323936	
FS118	LAG-3			Kraman et al., 2020, Clin. Cancer Res. 26 (13): 3333–3344.
FS120	OX40 CD137			Gaspar et al., 2020, Cancer Immunol. Res. 8 (6): 781–793.
G.813.2	CD117	Phospho-c-Kit (Tyr703) Monoclonal Antibody (G.813.2)	ThermoFisher, #MA5-14830	
ganglidximab	GD2			Eger et al., 2016, PLoS One 11 (3): e0150479.

GEN-3014	CD38			Hiemstra et al., 2023, EBioMedicine 93: 104663.
geptanolimab	PD-1	Geptanolimab	AntibodySystem, #DHH02224	Shi, Y. et al. Clin Cancer Res. 2020 Dec 15;26(24):6445-6452. doi: 10.1158/1078-0432.CCR-20-2819. Epub 2020 Oct 12. PMID: 33046518.
GIGA-564	CTLA-4			Stone, E. et al. Journal for ImmunoTherapy of Cancer, 2022;10.
givastomig	CLDN18.2			Gao et al., 2023, J. Immunother. Cancer 11(6): e006704.
GN1412	CD28	Theralizumab	MedChemExpress, #HY-P9975	Hussain, K. et al. Blood. 2015 Jan 1;125(1):102-10.
gresonitamab	CLDN18.2	Gresonitamab	MedChemExpress, #HY-P99350	
grisnilimab	CD7	Grisnilimab	MedChemExpress, #HY-P99650	Scharnhorst, V, et al. Gene. 2001 Aug 8;273(2):141-61. doi: 10.1016/s0378-1119(01)00593-5. PMID: 11595161.
GSK2831781	LAG-3			Clinical Trial ID: NCT03893565
GSK3174998	OX40			Infante, J. R. et al. JCO 34, TPS3107-TPS3107 (2016).
GWN323	GITR			Piha-Paul, S. A. et al. Journal for ImmunoTherapy of Cancer 2021;9:e002863.
h4C8	CD34	Anti-Human CD34 Recombinant Antibody Fab Fragment (h4C8)	Creative Biolabs, #TAB-149LC-F(E)	

H22	CD64			Hristodorov, D. et al. MAbs. 2014;6(5):1283-9. doi: 10.4161/mabs.32182. PMID: 25517313; PMCID: PMC4622438.
H44	IL-18R α	APC anti-human CD218a (IL-18R α) Antibody	Biolegend, #313814	
H65	CD5	CD5 Antibody	Novus Biologicals, #NB100-2667	
hB-F5	CD4	NeuroMab™ Anti-CD4 BBB Shuttle Antibody, Clone hB-F5	Creative Biolabs, #NRZP-1022-ZP3717	
HBM1007	CD73			Gan et al., 2020, 2020 AACR Poster #6056.
HBM4003	CTLA-4			Shanzhi, G. et al. JCO 40, 2641-2641 (2022).
HC34LC14	CD117	c-Kit Recombinant Rabbit Monoclonal Antibody (HC34LC14)	ThermoFisher, #701494	
HCD27.15	CD27			US 9,527,916
HD37	CD19			Pezutto et al., 1987, J. Immunol. 138(9): 2793-2799
HE3	CD5			US 5,770,196
HFB301001	OX40			Fulton, R. et al. Cancer Res 1 July 2021; 81 (13_Supplement): 1882.
HI30	CD45	Purified anti-human CD45 Antibody	BioLegend, #304001	
HIT8a	CD8 α	Purified anti-human CD8a Antibody	BioLegend, #300901	
HLX26	LAG-3			Rujiao, L. et al. JCO 41, e14671-e14671 (2023).
HLX51	OX40			Clinical Trial ID: NCT05788107
HM48-1	CD48	CD48 Monoclonal Antibody (HM48-1), APC, eBioscience™	ThermoFisher, #17-0481-82	
HPAB-0260-YJ	CD200	Human Anti-CD200 Recombinant Antibody	Creative Biolabs, #HPAB-0260-YJ-F(E)	

HPAB-3334LY	PODXL	Human Anti-PODXL Recombinant Antibody	Creative Biolabs, #HPAB-3334LY	
HPAB-MO612-YC	PODXL	Human Anti-LAM Recombinant Antibody	Creative Biolabs, #HPAB-M0561-YC	
HPN217	BCMA			US 11,136,403
Hu001-MMAE	CD73			Jin et al., 2020, Mol. Cancer Ther. 19 (11): 2340-2352.
hu3G8-5.1-N297Q	CD16a			US 7,351,803
hu14.18k322A	GD2	Anti-Human GD2 Recombinant Antibody (hu14.18K322A)	Creative Biolabs, #TAB-304CL	
Hu222	OX40			US 9,006,399
huAA98	CD146	Anti-Human MCAM Recombinant Antibody (huAA98)	Creative Biolabs, #TAB-444MZ	
HuLy-m2	CD7	CD7 (T-Cell Leukemia Marker) Monoclonal Antibody (HuLy-m2)	ThermoFisher, #924-MSM2-P1ABX	
HuMab 611	CD64			WO 2006/002438
Human IgG1 poly-specificity control antibody	IgG1	Human IgG1 Poly-Specificity Control Antibody	Medna Scientific, #H1308	
Human IgG4 isotype control antibody	IgG4	Human IgG4 Isotype Control Antibody	Medna Scientific, #H1314	
humanized AT-10	CD32A	CD32 Antibody	Biorad, #MCA1075	
humanized IV.3	CD32A	Anti-Human CD32 Antibody, Clone IV.3	StemCell Technologies, #60012	
HuMax-TAC	CD25	Camidanlumab	MedChemExpress, #HY-P99233	
huRH105	CD105	Anti-CD105 [huRH105-1]	Absolute Antibody, #Ab04017	
IAB22M	CD8			US 11,254,744
IAb_CysDb3b_CD8	CD8			US 10,414,820
IAb_M1b_CD8	CD8			
IAb_M2b_CD8	CD8			
ibalizumab	CD4	Ibalizumab	MedChemExpress, #HY-P99028	

IBI110	LAG-3			Xu et al., 2022, J. Clin. Oncol. 40 (16_suppl): 2650.
IBI310	CTLA-4			Zhou et al., 2022, J. Clin. Oncol. 40 (4_suppl): 421.
IBI318	PD-1	Reozalimab (LY3434172)	MedChemExpress, #HY-P9996	
IBI323	LAG-3			Ni et al., 2020, Cancer Res. 80 (16_Supplement): 3270.
IBI325	CD73			Zhou et al., 2023, Int. J. Biol. Macromol. 229:158-167.
IBI343	CLDN18.2			Yu et al., 2024, J. Clin. Oncol. 42 (16 Suppl.), 3037.
ibritumomab	CD20	Ibritumomab Biosimilar - Anti-MS4A1(CD20,MS4A-1) mAb - Research Grade	ProteoGenix, #PX-TA1620	
idecabtagene vicleucel	BCMA			Munshi et al., 2021, N. Engl. J. Med. 384: 705-716.
ieramilimab	LAG-3	Ieramilimab	MedChemExpress, #HY-P99027	Schöffski, P et al. J Immunother Cancer. 2022 Feb;10(2):e003776.
IL-A116	CD45RO	CD45RO Monoclonal Antibody (IL-A116)	ThermoFisher, #MA5-28403	
imaprelimab	CD146	Imaprelimab	MedChemExpress, #HY-P99658	Pelletier, J. P. R. Et al. Immunologic Concepts in Transfusion Medicine, Elsevier, 2020, 251-348.
INBRX-106	OX40			Clinical Trial ID: NCT04198766
INCA00186	CD73			Stewart, S. et al. Cancer Res 1 July 2021; 81 (13_Supplement): LB174.
INCA32459	LAG-3			Clinical Trial ID: NCT05577182

INCAGN1949	OX40			Davis, E. J. et al. J Immunother Cancer. 2022 Oct;10(10):e004235. doi: 10.1136/jitc-2021-004235. PMID: 36316061; PMCID: PMC9628691.
INCAGN2385	LAG-3			Clinical Trial ID: NCT03538028
INCAGN02390	TIM-3			Clinical Trial ID: NCT03652077
inezetamab	CD4	Inezetamab	MedChemExpress, #HY-P99663	
inolimomab	CD25	Research Grade Inolimomab	Antibody System, #DHB95804	
inotuzumab	CD22	Humanized Anti-CD22 Recombinant Antibody (clone Inotuzumab)	Creative Biolabs, #TAB-198	
IPH5301	CD73			Gonçalves et al., 2022, Immuno-Oncology and Technology 16 (S1): 199TiP.
ipilimumab	CTLA-4	Ipilimumab	MedChemExpress, #HY-P9901	
IPO-3	CD150	Anti-SLAM / CD150 antibody [IPO-3]	Abcam, #ab2604	
isatuximab	CD38	Isatuximab (anti-CD38)	SelleckChem, #A2039	
iscalimab	CD40	Iscalimab	MedChemExpress, #HY-P99670	
ispectamab	BCMA			US 2021/0130483
IT1208	CD4			Shitara, K et al. J Immunother Cancer. 2019 Jul 24;7(1):195.
ivuxolimab	OX40	Ivuxolimab	MedChemExpress (MCE), #HY-P99159	Diab, A et al. Clin Cancer Res. 2022 Jan 1;28(1):71-83.
JAB-BX102	CD73			Clinical Trial ID: NCT05174585
JK08	CTLA-4			Kotecki, et al., 2023, Ann. Oncol. 34(S2), pg. S635.

JM1-24-3	CD146			Feng et al., 2020, J. Exp. Clin. Cancer Res. 39: 273.
JM7A4	IL-15R α	<i>available from multiple sources, including:</i>		
		Anti-IL-15RA antibody [JM7A4]	Abcam, #ab91270	
		IL-15R alpha Antibody (JM7A4) - BSA Free	Novus Biologicals, #NBP1-43238	
JMW-3B3	CTLA-4	Afuco™ Anti-Human CTLA4 ADCC Recombinant Antibody (JMW-3B3), ADCC Enhanced	Creative Biolabs, #AFC-185CL	
JNJ-64164711	GITR	Research Grade Anti-Human CD357/TNFRSF18/GITR	AntibodySystem, #DHJ89808	Holland et al., 2018, Cancer Res. 78 (13_Supplement): 3813.
JS007	CTLA-4			Guan et al., 2023, MAbs 15(1): 2153409.
JTX-2011	ICOS	Vopratelimab	MedChemExpress, #HY-P99382	
K45	CD117	c-Kit Monoclonal Antibody (K45)	ThermoFisher, #MA5-12944	
K117	CD90	Anti-CD90 Antibody, Purified	RayBiotech, #136-00048	
KD6001	CTLA-4			Chen et al., 2024, Cancer Res. 84 (6_Suppl.): 1350.
keliximab	CD4	<i>available from multiple sources, including:</i>		
		Keliximab	MedChemExpress, #HY-P99680	
		Keliximab (CD4) - Research Grade Biosimilar	ProSci, #10-178	
Ki-M7	CD68	<i>available from multiple sources, including:</i>		
		CD68 antibody Ki-M7	Bio-Rad, #MCA2375F	

		CD68 Monoclonal Antibody (Ki-M7), FITC	ThermoFisher, #MA1-82715	
kit2c75	CD117	CD117 (c-Kit) Monoclonal Antibody (kit2c75), PE	ThermoFisher, #MA5-28805	
KM666	GD2			Nakamura et al., 2001, Cancer Immunol. Immunother. 50, 275-284.
KN044	CTLA-4			Clinical Trial ID: NCT04126590
L17F12	CD5	<i>available from multiple sources, including:</i>		
		Purified anti-human CD5 Antibody	BioLegend, #364001	
		CD5 Antibody (L17F12)	Santa Cruz Biotechnology, #sc-18898	
LAMP4-824	CD68	<i>available from multiple sources, including:</i>		
		CD68 Monoclonal Mouse Antibody (LAMP4/824)	Biotium, #0824	
		CD68 Monoclonal Antibody (LAMP4, 824)	ThermoFisher, #968-MSM4-P1	
LB1410	TIM-3			Liu, 2023, J. Clin. Oncol. 41 (16_suppl): TPS2663.
LCAR-B38M	BCMA			Zhao et al., 2018, J. Hematol. Oncol. 11(1):141; WO 2018/028647
LCAR-C18S (LB1908)	CLDN18.2			Zhen et al., 2023, J. Clin. Oncol. 41 (4 suppl.), TPS480.
Leu16	CD20			Wu et al., 2001, Protein Engineering. 14(12):1025-1033
linvoseltamab	BCMA			US 11,919,965

lisocabtagene maraleucel	CD19			Abramson et al., 2020, Lancet 396: 839-852.
LM-302	CLDN18.2			Huang et al., 2022, Eur. J. Cancer 174 (Suppl. 1): S41-S42.
LOP628	CD117	Anti-cKit-maitansine ADC (LOP628)	Creative Biolabs, #ADC-L039	
lorigerlimab	PD-1			Luke et al., J. Clin. Oncol. 41 (6 suppl): 155.
lorvotuzumab	CD56	<i>available from multiple sources, including:</i>		
		Lorvotuzumab	MedChemExpress, #HY-P99372	
		Lorvotuzumab Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-41708	
LT-2	CD2	CD2 Monoclonal Antibody (LT2)	ThermoFisher, #MA1-10131	
LT-8	CD8	<i>available from multiple sources, including:</i>		
		Mouse monoclonal [LT8] antibody to CD8 (PE)	Antibodies.com, #A242891	
		Monoclonal Anti-CD8 antibody	Millipore Sigma, #SAB4701064	
lucatumumab	CD40	<i>available from multiple sources, including:</i>		
		Lucatumumab	MedChemExpress, #HY-P99167	
		Anti-CD40 Recombinant Antibody (Lucatumumab)	Creative Biolabs, #TAB-759	
lulizumab	CD28	<i>available from multiple sources, including:</i>		
		Lulizumab Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-42032	

		Humanized Anti-CD28 Recombinant Antibody (clone Lulizumab)	Creative Biolabs, #TAB-H46	
LVGN6051	CD137			Qi et al., 2019, Nat. Commun. 10(1): 2141.
LY75_A1	CD205	LY75/DEC-205 Protein (AA 28-1667) (His tag)	Antibodies Online, #ABIN7275222	
LY3321367	TIM-3			Harding et al., 2021, Clin. Cancer Res. 27(8): 2168-2178.
LY3415244	TIM-3			Hellmann et al., 2021, Clin. Cancer Res. 27(10): 2773-2781.
M108 (FG-M108)	CLDN18.2			Jin et al., 2024, J. Clin. Oncol. 42 (16 suppl.), 4142.
M971	CD22	Anti-Siglec-2 / CD22 (NCI m971)	SelleckChem, #A3057	
mAb19	CD73			Wurm et al., 2021, Mol. Cancer Ther. 20(11): 2250-2261
MAB107	CD11b	<i>available from multiple sources, including:</i>		
		Anti-CD11b/CD18 Antibody, clone mAb107	Millipore Sigma, #MABF2085	
		Anti-human IgE mAb (107), unconjugated	Mabtech, #3810-3	
MAB1472-100	IL-15R α	<i>available from multiple sources, including:</i>		
		Human IL-15R alpha Antibody	R&D Systems, #MAB1472	
		IL-15R alpha Antibody (1020456) [Unconjugated]	Novus Biologicals, #MAB1472	
MAB5511	IL-15R α	Mouse IL-15R alpha Antibody	R&D Systems, #MAB5511	
MAI1738	PODXL	Research Grade Anti-Human PODXL (MAI1738)	AntibodySystem, #DHA16801	
MAT304	CD5	Research Grade Anti-Human CD5 (MAT 304)	AntibodySystem, #DHC18804	

MAX.16H5	CD4			Guse et al., 1994, J. Chromatogr. A 661: 13-23.
MB-106	CD20	Fred Hutchinson Cancer Research Center		Shadman et al., 2019, Blood 134(Suppl.1):3235
MC631	SSEA-3	<i>available from multiple sources, including:</i>		
		Anti-SSEA3 antibody [MC631]	Abcam, #ab16286	
		Anti-Mouse SSEA-3 Antibody, Clone MC-631	StemCell Technologies, #60061.1	
MC-813-70	SSEA-4	<i>available from multiple sources, including:</i>		
		SSEA4 Monoclonal Antibody (MC-813-70)	ThermoFisher, #MA1-021	
		Anti-Human SSEA-4 Antibody, Clone MC-813-70	StemCell Technologies, #60062	
MCARH109	GPRC5D			Mailankody et al., N Engl J Med. 387(13): 1196-1206 (2022)
MDE-8	CD32A	Anti-CD32 [MDE-8]	Absolute Antibodies, #Ab02072	
MDX-1203	CD70			Owonikoko et al., 2014, J. Clin. Oncol. 32:5s,(suppl; abstr 2558).
MDX-1411	CD70	Anti-CD70 monoclonal antibody (MDX-1411)	Creative Biolabs, #Gly-008CL	
MEDI0680	PD-1			Naing et al., 2019, J. Immunother. Cancer 7: 225.
MEDI1873	GITR			Tigue et al., 2017, Oncoimmunology 6(3): e1280645.
MEDI2228	BCMA			US 10,988,546
MEDI5752	PD-1			Dovedi et al., 2021, Cancer Discov. 11(5): 1100-1117.

MEDI6383	OX40			Hammond et al., 2015, J. Clin. Oncol. 33 (15_suppl): 3056.
MEDI6469	OX40			Leidner et al., 2015, J. Clin. Oncol. 33 (15_suppl): TPS6083.
MEM-28	CD45	Anti-CD45 antibody [MEM-28]	Abcam, #ab8216	
MEM-31	CD8	<i>available from multiple sources, including:</i>		
		CD8 Monoclonal Antibody (MEM-31)	ThermoFisher, #MA1-19082	
		Monoclonal Anti-CD8 antibody produced in mouse	SigmaAldrich, #SAB4700084	
MEM-55	CD45RB	Anti-CD45RB antibody [MEM-55]	Abcam, #ab8218	
MEM-59	CD43	CD43 Monoclonal Antibody (MEM-59)	ThermoFisher, #MA1-19009	
MEM-65	CD2	<i>available from multiple sources, including:</i>		
		CD2 Monoclonal Antibody (MEM-65)	ThermoFisher, #MA1-19070	
		Anti-CD2 Antibody [MEM-65]	Antibodies.com, #A86387	
MEM-87	CD8	<i>available from multiple sources, including:</i>		
		Anti-CD8 Antibody (MEM-87)	Antibodies.com, #A85681	
		Monoclonal Anti-CD8 antibody produced in mouse	SigmaAldrich, #SAB4700089	
MEM-102	CD48	CD48 Monoclonal Antibody (MEM-102)	ThermoFisher, #MA1-19119	
mezagitamab	CD38	Mezagitamab	MedChemExpress, #HY-P99730	
MGC018	B7H3			Scribner et al., 2020, Mol. Cancer Ther. 19(11): 2235-2244.

MGTA-117	CD117			Westervelt et al., 2022, Blood 140 (Supplement 1): 2117–2119.
MIK-Beta-1	CD122	CD122 Monoclonal Antibody (MIK-Beta 1)	ThermoFisher, #MA1-35896	
miptenlimab	LAG-3	Miptenlimab	MedChemExpress, #HY-P99736	
mitazalimab	CD40			Calvo et al., 2019, J. Clin. Oncol. 37 (15 suppl): 2527.
MK-1248	GITR			Geva et al., 2020, Cancer 126(22): 4926-4935.
MK-4166	GITR			Sukumar et al., 2017, Cancer Res. 77 (16): 4378–4388.
MORAb-028	GD2	MORAb-028 Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-41810	
moxetumomab	CD22	Anti-CD22 (Moxetumomab pasudotox)-SMCC-DM1 ADC	Creative Biolabs, #ADC-W-800	
mShad150	CD150	CD150 Monoclonal Antibody (mShad150), PE, eBioscience™	ThermoFisher, #12-1502-82	
M-T413	CD4			Rieber et al., 1992, PNAS 89(22): 10792-6.
MT95-4	CD13			WO 2021/072312
MRTX1011A	CD4			Scheerens et al., 2011, Arthritis Res. Ther. 13 (5): R177.
MT807-R1	CD8α	Anti-CD8 alpha (MT807R1)	NIH Nonhuman Primate Reagent Resources, #AB_2716320	Nakamura-Hoshi et al., 2024, Mol. Ther. 32 (7): 1-12.
mupadolimab	CD73	Mupadolimab	MedChemExpress, #HY-P99181	
My10	CD34	Anti-CD34 antibody [My10]	Abcam, #ab245689	
naxitamab	GD2	Naxitamab	MedChemExpress, #HY-P99206	
Nbl57	CD13			WO 2021/072312

ND-742	SSEA-3	Anti-SSEA-3 monoclonal antibody	Creative Diagnostics, #DMAB12907	
nivatrotamab	GD2	Nivatrotamab	MedChemExpress, #HY-P99757	
nivolumab	PD-1	<i>available from multiple sources, including:</i>		
		Nivolumab (anti-PD-1)	SelleckChem, #A2002	
		Nivolumab	Evidentic, #8006657	
NN2101	CD117			Kim et al., 2020, Int. J. Biol. Macromol. 159: 66-78.
nofazinlimab	PD-1	Nofazinlimab	MedChemExpress, #HY-P99758	
nurulimab	CTLA-4	<i>available from multiple sources, including:</i>		
		Nurulimab	MedChemExpress, #HY-P99760	
		Nurulimab (Anti-CTLA-4 / CD152)	SelleckChem, #A2859	
NZV930	CD73			Fu et al., 2022, Cancer Res. 82(12_Suppl), CT503.
obinutuzumab	CD20	<i>available from multiple sources, including:</i>		
		Obinutuzumab	MedChemExpress, #HY-P9910	
		Obinutuzumab (anti-CD20)	SelleckChem, #A2023	
		Obinutuzumab Monoclonal Antibody (16B7)	ThermoFisher, #A01946-40	
ocrelizumab	CD20	<i>available from multiple sources, including:</i>		
		Ocrelizumab	MedChemExpress, #HY-P9960	

		Ocrelizumab (Anti-CD20)	SelleckChem, #A2561	
		Ocrelizumab Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-41829	
odronextamab	CD20 CD3	<i>available from multiple sources, including:</i>		
		Odronextamab	MedChemExpress, #HY-P99038	
		Odronextamab Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-42283	
ofatumumab	CD20	<i>available from multiple sources, including:</i>		
		Ofatumumab	MedChemExpress, #HY-P9961	
		Ofatumumab Biosimilar – Anti-MS4A1, CD20 mAb – Research Grade	ProteoGenix, #PX-TA1146	
		Ofatumumab Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-41813	
OKT1	CD5	CD5 Unconjugated OKT1 IgG1k (RUO)	Biohippo, #BHA19900487	
OKT3 (muromonab-CD3)	CD3	CD3 Monoclonal Antibody (OKT3), eBioscience™	ThermoFisher, #14-0037-82	
OKT4	CD4	Brilliant Violet 650™ anti-human CD4 Antibody	BioLegend, #317436	
OKT8	CD8α	CD8α Monoclonal Antibody (OKT8)	ThermoFisher, #14-0086-80	
OKT11	CD2	<i>available from multiple sources, including:</i>		US 4,364,937
		OKT11	ATCC, #CRL-8027	
			MilliporeSigma, #86050802	
oleclumab	CD73	Oleclumab	MedChemExpress, #HY-P99039	

ONC-392	CTLA-4			Rolfo et al., 2020, J. Clin. Oncol. 38 (15_suppl): TPS3159.
OriCAR-017	GPRC5D			Rodriguez-Otero et al., Blood Cancer J. 14(1): 24 (2024)
OS2966	CD29			Nigim et al., 2019, Target. Oncol. 14: 479-489.
osemitamab	CLDN18.2			Qian et al., 2023, Ann. Oncol. 34 (Suppl. 2), S873, 1560P.
otelixizumab	CD3	Otelixizumab	MedChemExpress, #HY-P99211	
OTI14B1	CD117	KIT Mouse anti-Human, Clone: OTI14B1, lyophilized, TrueMAB™	Fisher Scientific, #50-166-9129	
OTI1B6		KIT Monoclonal Antibody (OTI1B6), TrueMAB™	ThermoFisher, #CF808721	
OTI1E2		CD117 Mouse Monoclonal Antibody [Clone ID: OTI1E2]	OriGene, #TA801037	
OTI1F6		KIT Monoclonal Antibody (OTI1F6), TrueMAB™	ThermoFisher, #CF801131	
OTI2B12		CD117 Mouse Monoclonal Antibody [Clone ID: OTI2B12]	OriGene, #CF808739	
OTI2B5		KIT Monoclonal Antibody (OTI2B5), TrueMAB™	ThermoFisher, #TA801047	
OTI2C1D5		KIT Monoclonal Antibody (OTI2C1D5), TrueMAB™	ThermoFisher, #CF801043	
OTI2C1H4		KIT Monoclonal Antibody (OTI2C1H4), TrueMAB™	ThermoFisher, #CF805183	

OTI2E3		Monoclonal Mouse anti-Human c-Kit / CD117 Antibody (Carrier-free, aa546-976, IHC, WB)	LSBio, #LS-C789071-100	
OTI3F9	CD117	KIT Monoclonal Antibody (OTI3F9), TrueMAB™	ThermoFisher, #TA801045	
OTI4E4	CD2	<i>available from multiple sources, including:</i>		
		CD2 Monoclonal Antibody (OTI4E4)	ThermoFisher, #TA500404	
		CD2 Mouse Monoclonal Antibody [Clone ID: OTI4E4]	Cambridge Bioscience, #TA500404	
OTI4G8	CD43	ASPDH Monoclonal Antibody (OTI4G8)	ThermoFisher, #MA5-27041	
OTI6A4	CD43	PRRX1 Monoclonal Antibody (OTI6A4)	ThermoFisher, #MA5-26579	
OTI6F8	CD117	KIT Monoclonal Antibody (OTI6F8), TrueMAB™	ThermoFisher, #CF808746	
OTI9A11	CD117	CD117 Mouse Monoclonal Antibody [Clone ID: OTI9A11]	OriGene, #CF801205	
OX7	CD90	<i>available from multiple sources, including:</i>		
		CD90 Monoclonal Antibody (OX-7)	ThermoFisher, #MA1-81491	
		Thy-1/CD90 Antibody (OX7)	Santa Cruz Biotechnology, #sc-53116	
OX-45	CD48	CD48 Monoclonal Antibody (OX-45), PE	ThermoFisher, #MA5-17528	
OX-104	CD200	<i>available from multiple sources, including:</i>		

		CD200 Monoclonal Antibody (OX104), eBioscience™	ThermoFisher, #14-9200-82	
		CD200 antibody OX-104	Bio-Rad, #MCA1960	
pavurutamab	CD3	Pavurutamab	MedChemExpress, #HY-P99814	
PD7/26	CD45RB	CD45RB Monoclonal Antibody (PD7/26), eBioscience™	ThermoFisher, #14-9458-82	
pembrolizumab	PD-1	<i>available from multiple sources, including:</i>		
		Pembrolizumab	MedChemExpress, #HY-P9902	
			Evidentic, #6302603D04	
penpulimab	PD-1	<i>available from multiple sources, including:</i>		
		Penpulimab	AbMole, #M25087	
		Penpulimab (Anti-PDCD1 / PD-1 / CD279)	Selleckchem, #A3034	
peresolimab	PD-1	Peresolimab	MedChemExpress, #HY-P9993	
PF-03475952	CD44			Runnels et al., 2010, Adv. Ther. 27(3): 168-180.
PG-M1	CD68	<i>available from multiple sources, including:</i>		
		Anti-CD68 antibody [PG-M1]	Abcam, #ab783	
		CD68 Monoclonal Antibody (PG-M1)	ThermoFisher, #MA5-12407	
PI37012	TREM2			WO 2019/118513

pidilizumab	PD-1	Anti-Human PD-1/PDCD1/CD279 Recombinant Antibody (Pidilizumab)	Creative Biolabs, #TAB-900	
pimivalimab	PD-1	Pimivalimab	MedChemExpress, #HY-P99887	
pinatuzumab	CD22	Pinatuzumab	MedChemExpress, #HY-P99230	
praluzatamab	CD166			Boni et al., 2022, Clin. Cancer Res. 28(10): 2020–2029.
prezalumab	CD28	Prezalumab	MedChemExpress, #HY-P99414	
priliximab	CD4	<i>available from multiple sources, including:</i>		
		Priliximab	MedChemExpress, #HY-P99790	
		Anti-Human CD4 Recombinant Antibody (Priliximab)	Creative Biolabs, #TAB-164	
PRO1160	CD70			Jonasch et al., 2023, Oncologist 28(Suppl 1): S14–S15.
prolgolimab	PD-1	Prolgolimab	MedChemExpress, #HY-P99807	
promiximab	CD56	Human Anti-NCAM1 Recombinant Antibody	Creative Biolabs, #HPAB-N0187-YC	
PRS-343	CD137			Hinner et al., 2016, Cancer Immunol. Res. 4 (11_Supplement): B016.
pucotenlimab	PD-1	Pucotenlimab	MedChemExpress, #HY-P99938	
Q-1802	CLDN18.2			Yk et al., 2023, J. Clin. Oncol. 41 (4 suppl.), 382.
QBEND/10	CD34	CD34 Monoclonal Antibody (QBEND/10), PE	ThermoFisher, #MA1-10205	
QL1706	PD-1 CTLA-4			Zhang et al., 2021, Cancer Res. 81(13_Suppl): CT119.
quavonlimab	CTLA-4	Quavonlimab	MedChemExpress, #HY-P99809	

r18D11	CD14	Anti-Human CD14 Recombinant Antibody (18D11)	Creative Biolabs, #TAB-1583CL	
ragifilimab	GITR	Ragifilimab	MedChemExpress, #HY-P99812	
RAVB3	CD8	<i>available from multiple sources, including:</i>		
		CD8 alpha Monoclonal Antibody (RAVB3)	ThermoFisher, #MA5-17048	
		CD8 (RAVB3)	Santa Cruz Biotechnology, #sc-32812	
RC118	CLDN18.2			Liu et al., 2024, Ann. Oncol. 35 (Suppl. 2), S903, 1456P.
REA101	SSEA-4	SSEA-4 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotec, #130-122-914	
REA157	PODXL	TRA-1-60 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotec, #130-122-921	
REA246	PODXL	TRA-1-81 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotec, #130-123-293	
REA233	IL-21Rα	CD360 (IL-21R) Antibody, anti-human, PE, REAfinity™	Miltenyi Biotec, #130-126-094	
REA333	IL-12R	IL-12R β2 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotec, #130-120-068	
REA442	CD166	CD166 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotec, #130-118-349	
REA795	SUSD2	SUSD2 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotec, #130-111-641	
REA844	CD271	CD271 (LNGFR) Antibody, anti-human, PE, REAfinity™	Miltenyi Biotec, #130-112-601	
REA877	CD10	CD10 Antibody, anti-human, pure, REAfinity™	Miltenyi Biotec, #130-124-312	
REA897	CD90	CD90 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotec, #130-114-860	

REA1060	CD29	CD29 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotec, #130-118-121	
REA1067	CD200	CD200 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotec, #130-118-129	
REA1164	CD34	CD34 Antibody, anti-human, PE, REAfinity™	Miltenyi Biotec, #130-120-515	
REAL219	MSCA-1	MSCA-1 Antibody, anti-human, PE, REAlease®	Miltenyi Biotec, #130-119-917	
REAL709	CD271	CD271 (LNGFR) Antibody, anti-human, PE, REAlease®	Miltenyi Biotec, #130-125-053	
REGN5459	BCMA			US 11,384,153
REGN4659	CTLA-4			Burova et al., 2018, Cancer Res. 78 (13_Supplement): 3824.
REGN6569	GITR			Lakhani et al., 2022, IOTECH 16 (S1): 196TiP.
REGN7257	CD132			Le Floch-Ramondou et al., 2022, Blood 140 (Supplement 1): 473–474.
relatlimab	LAG-3	Relatlimab	MedChemExpress, #HY-P99156	
retifanlimab	PD-1	Retifanlimab	MedChemExpress, #HY-P99941	
RG7356	CD44	Anti-Human CD44 Recombinant Antibody (RG7356)	Creative Biolabs, #TAB-128CL	
rituximab	CD20	<i>available from multiple sources, including:</i>		
		BD Pharmingen™ Purified NA/LE Anti-Human CD20 Rituximab Biosimilar	BD Biosciences, #569496	
		HUMAN ANTI CD20 (RITUXIMAB BIOSIMILAR)	BioRad, #MCA6091	

		Rituximab	MedChemExpress, #HY-P9913	
RIV-11	CD8	<i>available from multiple sources, including:</i>		
		Anit-CD8 Antibody (RIV-11)	Antibodies.com, #A115691	
		CD8 Recombinant Antibody(RIV-11)	Aviva Systems Biology, #OAAV00971	
RM2051	CD45	Anti-CD45 antibody [RM2051]	Abcam, #ab318154	
RO5429083	CD44			Perez et al., 2012, Cancer Res. 72 (8 Suppl.): 2521.
RO7121661	PD-1 TIM-3			Clinical Trial ID: NCT04785820
RO7247669	PD-1 LAG-3			Clinical Trial ID: NCT04785820
RO7296682	CD25	Research Grade Anti-Human CD25/IL2RA (RO7296682)	AntibodySystem, #DHB95808	
rocatinlimab	OX40	Rocatinlimab	MedChemExpress, #HY-P99955	
RPA-2.10	CD2	<i>available from multiple sources, including:</i>		
		Purified anti-human CD2 antibody	BioLegend, #300202	
		CD2 Monoclonal Antibody (RPA-2.10)	ThermoFisher, #14-0029-82	
		Anti-Human CD2 Antibody, Clone RPA-2.10	Stemcell Technologies, #60007	
RPA-T8 (CT8)	CD8 α	<i>available from multiple sources, including:</i>		
		MOUSE ANTI HUMAN CD8	Bio-Rad, #MCA4609T	
		CD8a Monoclonal Antibody (RPA-T8), APC	ThermoFisher, #17-0088-42	
RTX-003	CD25			"Nature-inspired, antibody discovery driven by AI." Nature, Dec. 2020, B44.

rulonilimab	PD-1	Rulonilimab	MedChemExpress, #HY-P99895	
RW03	CD133			Vora et al., 2020, Cell Stem Cell 26(6): 832-844.e6
sabatolimab	TIM-3	Sabatolimab	MedChemExpress, #HY-P99044	
samalizumab	CD200	Samalizumab	MedChemExpress, #HY-P99400	
SAR445514	BCMA			US 2024/0034816
sasanlimab	PD-1	Sasanlimab (Anti-PDCD1 / PD-1 / CD279)	Selleckchem, #A3038	
sdA1	CD16a			WO 2018/039626
sdA2	CD16a			WO 2018/039626
SEA-BCMA	BCMA			US 11,078,291
selicrelumab	CD40	Selicrelumab	MedChemExpress, #HY-P99046	
semzuvolimab	CD4	Semzuvolimab	MedChemExpress, #HY-P9998	
serplulimab	PD-1	Serplulimab (Anti-PDCD1 / PD-1 / CD279)	Selleckchem, #A2381	
SG001	PD-1			Clinical Trial ID: NCT04886700
SGN-CD70A	CD70			Sandall et al., 2014, Cancer Res. 74 (19_Supplement): 2647.
SHR-1702	TIM-3			Clinical Trial ID: NCT03871855
SHR-1802	LAG-3			Deng et al., 2023, Ther. Adv. Med. Oncol. 15: 17588359231186025.
SIDI8BEE	CD8 β	<i>available from multiple sources, including:</i>		
		CD8b Monoclonal Antibody (SIDI8BEE)	ThermoFisher, #14-5273-82	

		Mouse Anti-CD8B Recombinant Antibody (SID18BEE)	CreativeBiolabs, #CBMAB-C10732-LY	
sindelizumab	PD-1			Li et al., 2022, Front. Pharmacol. 13: 918709.
sintilimab	PD-1	Sintilimab	MedChemExpress, #HY-P99048	
siplizumab	CD2	<i>available from multiple sources, including:</i>		
		Siplizumab	Medchem Express, #HY-P99904	
		Anti-Human CD2 Recombinant Antibody	Creative Biolabs, #TAB-104	
SJ25C1	CD19			Bejcek et al., 1995, Cancer Res. 55:2346-2351
SK1	CD8 α	<i>available from multiple sources, including:</i>		
		Purified Anti-human CD8 Antibody *SK1*	AAT Bioquest, #10081000	
		Anti-CD8 Mouse Monoclonal Antibody [clone: SK1]	Avantor (VWR), #344702-BL	
SKB315	CLDN18.2			Clinical Trial ID: NCT05367635
SLAM.4	CD150	CD150 Monoclonal Antibody (SLAM.4)	ThermoFisher, #MA5-46198	
SM03	CD22			Wong et al., 2022, J. Immunol. 208(12): 2726-2737.
SOT102	CLDN18.2			Spisek, 2023, ESMO Open 8(1), Suppl. 2, 101196, 2P.
sotigalimab	CD40	Sotigalimab	MedChemExpress, #HY-P99049	
SP-16	CD8	<i>available from multiple sources, including:</i>		

		Anti-CD8 monoclonal antibody (SP-16)	ThermoFisher, #MA5-14548	
			Novus Biologicals, #NBP2-26484	
SP34-2	CD3	BD Pharmingen™ APC-Cy™ 7 Mouse Anti-Human CD3	BD Biosciences, #557757	
SP55	CD43	CD43 Monoclonal Antibody (SP55)	ThermoFisher, #MA5-16339	
spartalizumab	PD-1	Spartalizumab	MedChemExpress, #HY-P9972	
SPM504	CD45RA	Anti-CD45RA antibody [SPM504]	Abcam, #ab231426	
SPX-101	CLDN18.2			Zhu et al., 2020, Cancer Res. 80 (16_Supplement): 3361.
SR1	CD117	Anti-Human KIT Recombinant Antibody (SR1)	Creative Biolabs, #TAB-467LC	
ST04-99	CD117	CD117/c-kit Rabbit anti-Human, Clone: ST04-99, Novus Biologicals™	Fisher Scientific, #NBP267531	
STRO-1	Stro-1	STRO-1 Monoclonal Antibody (STRO-1)	ThermoFisher, #39-8401	
STRO-4	Hsp90			Gronthos et al., 2009, Stem Cells Dev. 18(9): 1253-62.
surzebiclimab	TIM-3	Surzebiclimab	MedChemExpress, #HY-P99962	
Sym022	LAG-3	Research Grade Anti-Human CD223/LAG3 (Sym022)	AntibodySystem, #DHD30423	
Sym023	TIM-3			Lindsted et al., 2018, Cancer Res. 78 (13_Supplement): 5629.
Sym024	CD73			Jakobsen et al., 2021, Cancer Res. 81 (13_Supplement): 1797.
SYSA180	CLDN18.2			Wang et al., 2023, J. Clin. Oncol. 41 (16 suppl.), 3016.

T200/797	CD45RO	Anti-CD45RO antibody [T200/797]	Abcam, #ab216024	
T3-3A1	CD7	CD7 (T-Cell Leukemia Marker) Monoclonal Antibody (T3-3A1)	ThermoFisher, #924-MSM5-P0	
T6.3	CD2	<i>available from multiple sources, including:</i>		
		Mouse Anti-CD2 Recombinant Antibody (clone T6.3)	Creative Biolabs, #ZG-0903F	
		Monoclonal Mouse anti-Human CD2 Antibody (clone T6.3)	LS Bio, #LS-C140259	
T595	CD117	CD117 Mouse Monoclonal Antibody [Clone ID: T595]	OriGene, #TA355261	
TAB-071CL	BMPR2	Anti-Human BMPR Recombinant Antibody	Creative Biolabs, #TAB-071CL	
talquetamab	GPRC5D			Pillarisetti et al., Blood 135:1232-43 (2020)
tavolimab	OX40	Tavolimab Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-42300	
TB19	CD73	InVivoMAb Anti-Human CD73/NT5E Antibody (TB19)	AntibodySystem, #VHD46601	
TB38	CD73	InVivoMAb Anti-Human CD73/NT5E Antibody (TB38)	AntibodySystem, #VHD46602	
tebotelimab	LAG-3 PD-1	Tebotelimab	MedChemExpress, #HY-P99573	
teclistamab	CD3	Teclistamab	MedChemExpress, #HY-P99392	
telazorlimab	OX40	Telazorlimab	MedChemExpress, #HY-P99570	
teplizumab	CD3	Teplizumab	MedChemExpress, #HY-P99222	
TG6050	CTLA-4			Marchand et al., 2023, Cancer Res. 83 (7_Supplement): 694.
TH-69	CD7	Anti-CD7 [TH69]	Absolute Antibody, #Ab03054	

theralizumab	CD28	Theralizumab	MedChemExpress, #HY-P9975	
tifcemalimab	BTLA			Song et al., 2023, Blood 142 (Supplement 1): 4458.
tiragolumab	TIGIT			Kim et al., 2023, JAMA Oncol. 9(11):1574-1582.
tisagenlecleucel	CD19			Mueller et al., 2018, Clin. Cancer Res. 24 (24): 6175–6184.
tislelizumab	PD-1	Tislelizumab	MedChemExpress, #HY-P99052	
TNB-383B	BCMA			US 11,505,606
tocilizumab	IL-6R	<i>available from multiple sources, including:</i>		
		IL6R antibody rhPM-1	BioRad, #MCA6106	
		Tocilizumab	MedChemExpress, #HY-P9917	
		Tocilizumab Recombinant Human Monoclonal Antibody (rhPM-1 (Tocilizumab))	ThermoFisher, #MA5-48061	
toripalimab	PD-1	Toripalimab	MedChemExpress, #HY-P9978	
TORL-2-307-ADC	CLDN18.2			Clinical Trial ID: NCT05156866
tositumomab	CD20	<i>available from multiple sources, including:</i>		
		Human CD20 (Research Grade Tositumomab Biosimilar) Alexa Fluor® 647-conjugated Antibody	R&D Systems, #FAB10440R	

		Tositumomab Recombinant Mouse Monoclonal Antibody	ThermoFisher, #MA5-41763	
TQB2618	TIM-3	Research Grade Anti- Human CD366/HAVCR2/TIM- 3 (TQB2618)	AntibodySystem, #DHJ28406	
TQB2934	BCMA			US 2023/0193292
TRC205	CD105			US 9,926,375
tregalizumab	CD4	Tregalizumab	MedChemExpre ss, #HY-P99327	
tremelimumab	CTLA-4	Tremelimumab	MedChemExpre ss, #HY-P9918	
TRX1	CD4	Anti-Human CD4 Recombinant Antibody (TRX1)	Creative Biolabs, #TAB-168LC	
TRX2	CD8	Oxford Therapeutic Antibody Centre, Oxford University, Oxford, United Kingdom		
TRX518	GITR	Research Grade Anti- Human CD357/TNFRSF18/GI TR (TRX518)	AntibodySystem, #DHJ89807	
TS1/8	CD2	<i>available from multiple sources, including:</i>		
		CD2 Monoclonal Antibody (TS1/8)	ThermoFisher, #MA5-44080	
		Purified anti-human CD2 Antibody	BioLegend, #309202	
TS2/18	CD2	<i>available from multiple sources, including:</i>		
		CD2 Monoclonal Antibody (TS2/18)	ThermoFisher, #MA0200	
		CD2 Antibody (TS2/18.1.1)	Santa Cruz Bio, #sc-19640	
TTI-CD200	CD200			Rastogi et al., 2021, Br. J. Haematol. 193, 155–159.
TUSP-2	Stro-1	Anti-STRO-1 monoclonal antibody [Alexa Fluor® 647]	Creative Diagnostics, #DMAB18656	

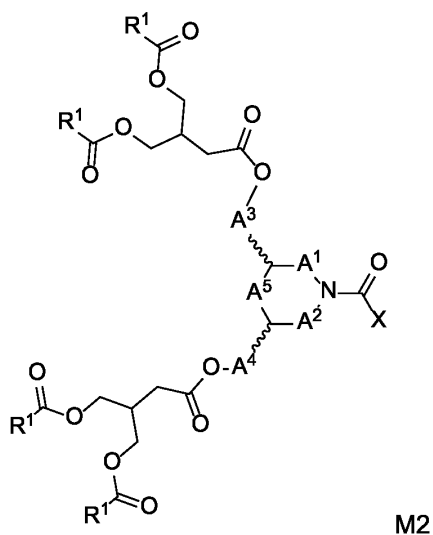
UB-421	CD4	Research Grade Semzumolimab	AntibodySystem, #DHB95908	
ublrituximab	CD20	<i>available from multiple sources, including:</i>		
		Ublrituximab	MedChemExpress, #HY-P99538	
		Ublrituximab (Anti-CD20)	SelleckChem, #A2807	
		Ublrituximab Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-41938	
UCART20	CD20			Kang et al., 2024, J. Hem. Oncol. 17:29.
UCHL1	CD45RO	Anti-CD45RO antibody [UCH-L1]	Abcam, #ab23	
UCHT2	CD5	CD5 Monoclonal Antibody (UCHT2), eBioscience™	ThermoFisher, #14-0059-82	
UCHT4	CD8	<i>available from multiple sources, including:</i>		
		CD8 Monoclonal Antibody (UCHT4)	ThermoFisher, #65204-1-IG	
		Anti-CD8 [UCHT4]	Absolute Antibody, #Ab00694-2.0	
uliledlimab	CD73	Uliledlimab	MedChemExpress, #HY-P99169	
UMAB216	CD117	KIT Monoclonal Antibody (UMAB216), UltraMAB™	ThermoFisher, #UM800108CF	
UMCD2	CD2	<i>available from multiple sources, including:</i>		
		anti-human CD2	Biohippo, #BHA14700014	
		CD2 Monoclonal Mouse Antibody (UMCD2)	Biotium, #1235	
urelumab (BMS-663513)	CD137	Urelumab	MedChemExpress, #HY-P99055	

ustekinumab	IL-12 IL-23	<i>available from multiple sources, including:</i>		
		Ustekinumab Biosimilar – Anti-Human IL-12 IL-23 mAb – Research Grade	Proteogenix, #PX-TA1011	
		Ustekinumab (anti-IL-12/IL-23)	SelleckChem, #A2024	
utomilumab (PF-05082566)	CD137	Utomilumab	MedChemExpress, #HY-P99056	
varlilumab	CD27	Varlilumab Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-42030	
VB6-008	CD44			US 8,383,117
veltuzumab	CD20	<i>available from multiple sources, including:</i>		
		Veltuzumab	MedChemExpress, #HY-P99224	
		Veltuzumab Biosimilar – Anti- CD20 receptors mAb – Research Grade	Proteogenix, #PX-TA1616	
		Veltuzumab Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-42301	
VIB9600	CD32A			Chen et al., 2019, Ann. Rheum. Dis. 78(2): 228–237.
vibecotamab	CD3	Vibecotamab Recombinant Human Monoclonal Antibody, Invitrogen™	Fisher Scientific, #PIMA542248	
vibostolimab (MK-7684)	TIGIT			Niu et al., 2022, Ann. Oncol. 33(2): 169-180.
visilizumab	CD3	Anti-CD3E Recombinant Antibody (Visilizumab)	Creative Biolabs, #TAB-714	
vonlerizumab	OX40	Vonlerizumab (Anti-TNFRSF4 / OX40 / CD134)	SelleckChem, #A2325	
vorsetuzumab	CD27 CD70	Vorsetuzumab	MedChemExpress, #HY-P99273	
vudalimab	PD-1	Research Grade Vudalimab	AntibodySystem, #DHH02222	

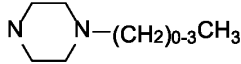
vunakizumab	CD27	Anti-Human IL17A Recombinant Antibody (Vunakizumab)	Creative Biolabs, #TAB-458CQ	
W3/13HLK	CD43	CD43 Monoclonal Antibody (W3/13HLK), PE	ThermoFisher, #MA5-17386	
W5C5	SUSD2	Purified anti-human SUSD2 Antibody	BioLegend, #327401	
W8B2	MSCA-1	MSCA-1 Antibody, anti-human	Miltenyi Biotec, #130-093-595	
WT1	CD7	Mouse Anti-CD7 Recombinant Antibody (WT1)	Creative Biolabs, #CBMAB-C10581-LY	
WV078	BCMA			US 11,492,409
X9C3	MSCA-1	Mouse anti-Human MSCA-1 monoclonal antibody, PE	Creative Diagnostics, #CABT-B12374	
XTX101	CTLA-4			Jenkins et al., 2023, J. Immunother. Cancer 11(12): e007785.
Y1/82A	CD68	CD68 antibody Y1/82A	Bio-Rad, #MCA6014GA	
YB5.B8	CD117	CD117 (c-Kit) Monoclonal Antibody (YB5.B8), eBioscience™	ThermoFisher, #14-1179-82	
YH001	CTLA-4			Ganju et al., 2021, J. Clin. Oncol. 39 (15 suppl): 2577.
YTC182.20	CD8	Anti-CD8 alpha antibody [YTC182.20]	Abcam, #ab60076	
YTH3.2.6	CD7	Anti-CD7 [YTH3.2.6]	Absolute Antibody, #Ab01019	
zalifrelimab	CTLA-4	Zalifrelimab	MedChemExpress, #HY-P99514	
zanolimumab	CD4	Zanolimumab Recombinant Human Monoclonal Antibody	ThermoFisher, #MA5-41819	
zimberelimab	PD-1	Zimberelimab	MedChemExpress, #HY-P99109	
zolbetuximab (IMAB362)	CLDN18.2			Sahin et al., 2018, Eur. J. Cancer 100, 17-26.

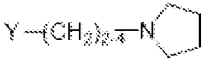
CLAIMS

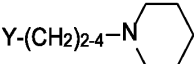
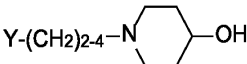
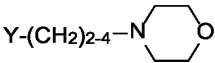
1. An ionizable cationic lipid having a structure of formula M2,

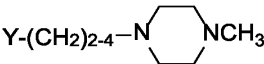
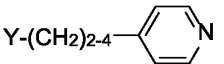
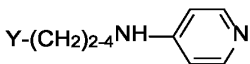


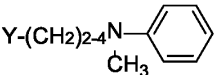
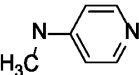
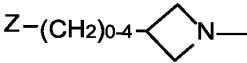
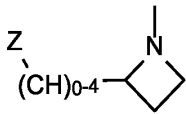
wherein X is

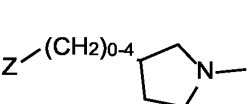
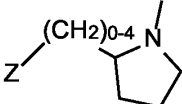
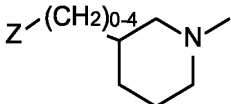
$\text{Y}-(\text{CH}_2)_{2-4}-\text{N}\begin{matrix} (\text{CH}_2)_{0-3}\text{CH}_3 \\ (\text{CH}_2)_{0-3}\text{CH}_3 \end{matrix}$, ,

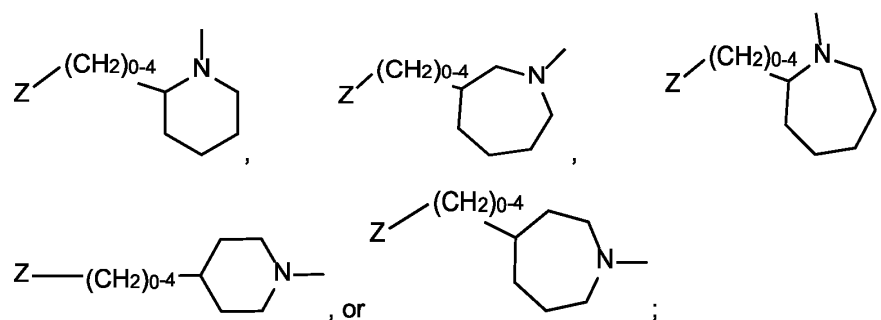
$\text{Y}-(\text{CH}_2)_{2-4}-\text{N}\begin{matrix} (\text{CH}_2)_2\text{OCH}_3 \\ (\text{CH}_2)_0\text{CH}_3 \end{matrix}$, $\text{Y}-(\text{CH}_2)_{2-4}-\text{N}\begin{matrix} (\text{CH}_2)_2\text{OCH}_3 \\ (\text{CH}_2)_2\text{OCH}_3 \end{matrix}$, ,

, , ,

, , ,

, , , ,

, , 



Y is O, S, NH, or NCH₃;

Z is O, NH, or NCH₃;

each R¹ is independently selected from C₇-C₁₁ alkyl or C₇-C₁₁ alkenyl; and

A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is (CH₂)₁₋₄ or CH₂-CH=CH-CH₂; or

A¹ is (CH₂)₀, A² is (CH₂)₁, A³ is (CH₂)₁, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁; or

A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₀; or

A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁; or

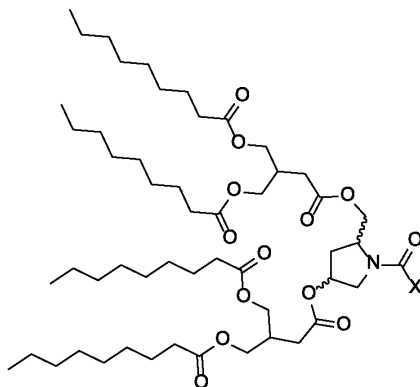
A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₂ or CH=CH, and

wherein a wavy bond indicates that any relative or absolute stereo-configuration of the corresponding ring atom, or a mixture of stereo-configurations, can be assumed.

2. The ionizable cationic lipid of claim 1, wherein A¹ is (CH₂)₀, A² is (CH₂)₀, A³ is (CH₂)₁, A⁴ is (CH₂)₁, and A⁵ is (CH₂)₁₋₄ or CH₂-CH=CH-CH₂.
3. The ionizable cationic lipid of claim 1, wherein A¹ is (CH₂)₀, A² is (CH₂)₁, A³ is (CH₂)₁, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁.
4. The ionizable cationic lipid of claim 1, wherein A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₀.
5. The ionizable cationic lipid of claim 1, wherein A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₁.
6. The ionizable cationic lipid of claim 1, wherein A¹ is (CH₂)₁, A² is (CH₂)₁, A³ is (CH₂)₀, A⁴ is (CH₂)₀, and A⁵ is (CH₂)₂ or CH=CH.

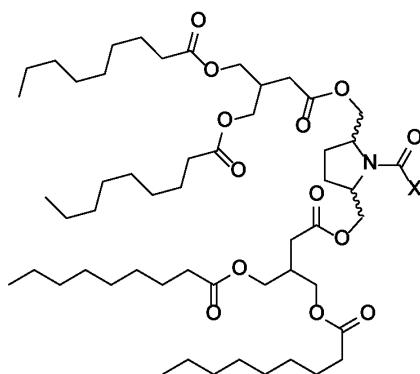
7. The ionizable cationic lipid of any one of claims 1 to 6, wherein R^1 is $(CH_2)_7CH_3$.

8. The ionizable cationic lipid of claim 1, having the structure:



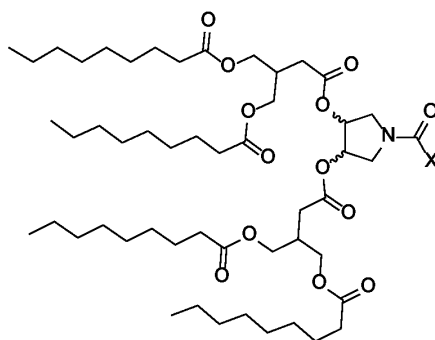
wherein the wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

9. The ionizable cationic lipid of claim 1, having the structure:



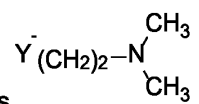
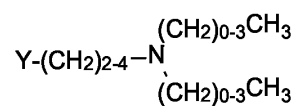
wherein the wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

10. The ionizable cationic lipid of claim 1, having the structure:



wherein the wavy bonds indicate that any relative or absolute stereo-configuration of the corresponding ring atom can be assumed.

11. The ionizable cationic lipid of any one of claims 1 to 10, wherein X is



12. The ionizable cationic lipid of claim 11, wherein X is
13. The ionizable cationic lipid of any one of claims 1 to 12, wherein Y is O.
14. The ionizable cationic lipid of any one of claims 1 to 11, wherein Z is O.
15. A lipid nanoparticle (LNP), comprising at least one ionizable cationic lipid of any one of claims 1-14.
16. A targeted lipid nanoparticle (tLNP), comprising at least one ionizable cationic lipid of any one of claims 1-14 and a functionalized PEG-lipid, wherein the functionalized PEG-lipid has been conjugated with a binding moiety.
17. The LNP of claim 15 or tLNP of 16, further comprising one or more of a phospholipid, a sterol, a co-lipid, an unfunctionalized PEG-lipid, or combinations thereof.
18. The LNP or tLNP of claim 17, comprising at least one phospholipid, wherein the phospholipid comprises dioleoylphosphatidyl ethanolamine (DOPE), dimyristoylphosphatidyl choline (DMPC), distearoylphosphatidylcholine (DSPC), dimyristoylphosphatidyl glycerol (DMPG), dipalmitoyl phosphatidylcholine (DPPC), or 1,2-diarachidoyl-sn-glycero-3-phosphocholine (DAPC), or a combination thereof.
19. The LNP or tLNP of claim 17, comprising at least one sterol, wherein the sterol comprises cholesterol, campesterol, sitosterol, stigmasterol, or combinations thereof.

20. The LNP or tLNP of any one of claims 17-19, wherein the PEG-lipid comprises a PEG moiety of 1000-5000 Da molecular weight (MW).
21. The LNP or tLNP of any one of claims 17-20, wherein the PEG-lipid comprises fatty acids with a fatty acid chain length of C₁₄-C₁₈.
22. The LNP or tLNP of any one of claims 17-21, wherein the at least one ionizable cationic lipid is present in an amount in the range from about 40 to about 65 mol%.
23. The LNP or tLNP of any one of claims 17-22, comprising a phospholipid in an amount in the range from about 7 to about 30 mol%, a sterol in an amount in the range from about 20 to about 45 mol%, at least one co-lipid in an amount in the range from about 1 to about 30 mol%, at least one unfunctionalized PEG-lipid in an amount in the range from about 0.1 to about 5 mol%, or at least one functionalized PEG-lipid in an amount in the range from about 0.1 to about 5 mol%, or any combination thereof.
24. The LNP of any one of claims 17-23, comprising a functionalized PEG-lipid.
25. The tLNP of any one of claims 17-23, wherein the binding moiety comprises an antigen, a ligand-binding domain of a receptor, a receptor ligand, or an antigen binding domain of an antibody or an antigen binding fragment thereof.
26. The LNP of any one of claims 15-24 or the tLNP of any one of claims 17-23 or 25, further comprising a biologically active payload nucleic acid.
27. A method of delivering a nucleic acid into a cell comprising contacting the cell with the LNP or tLNP of claim 26.