

LIPID NANOPARTICLES

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/589,691, filed
5 October 12, 2023, and U.S. Provisional Application No. 63/589,693, filed October 12, 2023,
the content of both is incorporated by reference in its entirety.

BACKGROUND

Conventional therapies using small molecules or antibodies transiently target
10 disease-related proteins. Such treatments may mitigate the symptoms of illness, but often
leave the underlying cause to perpetuate. In their wake, nucleic acids have arisen as a
popular form of therapeutics and prophylactics. As they can regulate gene expression and
target specific codes, nucleic acid-based drugs can treat diseases such as viral infections,
genetic disorders, and cancer by attacking them at the root. Such treatment promises to
15 provide thorough and potentially permanent relief.

At the core of dozens of drugs, particularly nucleic acid-based drugs, are molecules
that contain ionic residues and are highly hydrophilic, which precludes them from entering
cells, their obligate therapeutic environment. If left in the blood, naked therapeutics are
cleared via the kidneys and the liver. Thus, one of the most important issues facing nucleic
20 acid drugs is delivery and targeting.

To achieve the appropriate chemistry to prevent sequestration, opsonization,
immunoresponse and clearance, as well as promote payload carrying, delivery capacities,
and entry into the cell, lipid nanoparticles can be employed to encapsulate therapeutic
agents. Lipid nanoparticles are a type of drug carrier and delivery system mimicking
25 biological spherical vesicles comprised of a mixture of functional lipids. By incorporating
other molecules to create a heterogeneous composition, lipid nanoparticles can be designed
to direct therapeutic agents to the proper target organ, tissue, and/or cell. Once at the
location of interest, the amphipathic nature of lipid nanoparticles shields the hydrophilicity of
the therapeutic payload thus allowing it to enter and be delivered to the cell type of interest.

Lipid nanoparticles also protect the payload from degradation. As molecules pass
30 through the cell membrane, they are enveloped in a layer of membrane creating endosomes,
that can be directed to the lysosome for degradation. To prevent the digestion of the
therapeutic agent, the lipid nanoparticle can induce the destruction of the endosome prior to
its integration with the lysosome, thus safely releasing the payload into the cytosol. By
35 protecting the payload from destruction, lower doses of drugs can be administered with
similar or even better therapeutic results than naked therapeutic agents.

Though the development of lipid nanoparticles has come a need for structures that carry, protect, target, and release new and improving therapeutic agents.

SUMMARY

5 Provided herein is a lipid nanoparticle comprising:

(i) a PEG-conjugated phospholipid (PEG-phospholipid) having two or more C₁₂-C₃₀ aliphatic chains, at least one of which includes from 1-5 sites of unsaturation;

(ii) an ionizable lipid having at least two C₁₁-C₃₀ aliphatic chains and a head group that protonates to a cationic moiety having a pK_a of 5-7;

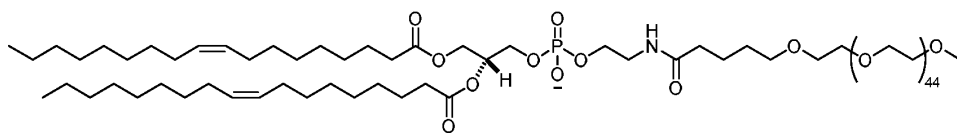
10 (iii) a non-cationic phospholipid having at least two C₁₁-C₃₀ aliphatic chains and a head group that remains a zwitterion or anion across a pH range of 6 to 8; and

(iv) a sterol, fat-soluble steroid, or vitamin.

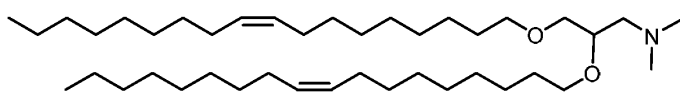
In an embodiment, (iv) is cholesterol.

In an embodiment, the lipid nanoparticle comprises

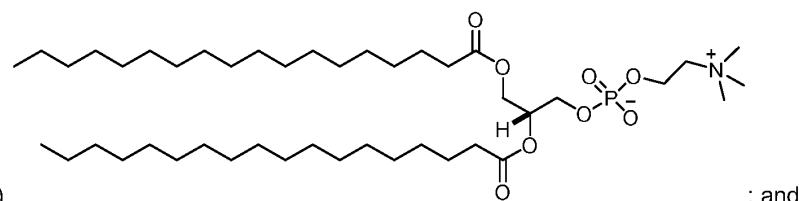
15 (i)



(ii)

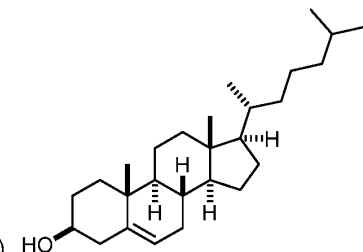


(iii)



; and

(iv)



20 This lipid nanoparticle is also referred to herein as "LNP-1."

The lipid nanoparticles of the present disclosure are useful in transporting active agents across lipid membranes to deliver the active agent to the target tissue, organ, or cell.

BRIEF DESCRIPTION OF FIGURES

Figure 1 shows an embodiment of the LNP-1 platform technology.

Figure 2 shows the lipid composition of LNP-1 formulations as confirmed at the expected values and for four lipid components' identity.

5 **Figure 3** shows confirmation of PEGylated L1A's synthesis, identity, purity, and integrity.

Figure 4 shows LNP-1 platform's high compatibility of PEG-conjugated phospholipid and flexibility with structural ionizable lipid component.

Figure 5 shows LNP-1 platform's flexibility with molar ratios of four lipid components.

10 **Figure 6** shows LNP-1 platform's flexibility with microfluid buffer for LNPs preparation.

Figure 7 shows RNA payload quantification method for LNP-1 LNPs formulations.

Figure 8 shows that LNP-1 formulations demonstrated increased stability at room temperature (25°C) when compared with other lipid nanoparticle formulations.

15 **Figure 9** shows that structural and functional stable PEGylated L1A is compatible with other ionizable lipids and PEGylated L1A LNPs have smaller sizes.

Figure 10 shows that eGFP mRNA/LNP-1s are stable for long-term storage at 2-8°C – the structural lipid composition and total LNP content of 9 months old formulations are comparable to that of freshly-formulated.

20 **Figure 11** shows that, when loaded with different RNA payloads, LNP-1 formulations are stable for long-term storage at different temperatures (2-8°C or 25°C).

Figure 12 shows that ApoB siRNA/LNP-1s are stable for long-term storage at different temperatures (2-8°C or 25°C).

25 **Figure 13** shows that different stock concentrations of ApoB siRNA/LNP-1s are stable for long-term storage at different temperatures (2-8°C or 25°C).

Figure 14 shows that ApoB siRNA/LNP-1s are stable for long-term storage at 40°C.

Figure 15 shows that, when loaded with different RNA payloads, LNP-1 formulations morphology and topology remain stable for long-term storage at 2-8°C.

30 **Figure 16** summarizes the payloads versatility and long-term stability of LNP-1 technology platform, which can encapsulate varied types and sizes of nucleic acids and remain stable for long-term storage at 2-8°C.

Figure 17 shows that eGFP mRNA/LNP-1s are functionally stable for long-term storage at different temperatures (2-8°C or 25°C) – the CAR-T generation potency of 9 months old formulations is comparable to that of freshly-formulated.

35 **Figure 18** shows that eGFP mRNA/LNP-1s are functionally stable for long-term storage at different temperatures (2-8°C or 25°C) – the gene delivery potency of 9 months old formulations is comparable to that of freshly-formulated.

Figure 19 shows that Cas9 mRNA + TRAC sgRNA/LNP-1s long-term storage at 2-8°C – the knockout potency of 3 months old formulations is comparable to that of freshly-formulated.

Figure 20 shows that ApoB siRNA/LNP-1s are functionally stable for long-term storage at different temperatures (2-8°C or 25°C) – the gene silencing potency of aged formulations is comparable to that of freshly-formulated (all had >70% knockdown efficiency).

Figure 21 shows LNP-1's potency using siRNA targeting mouse ApoB as the payload.

Figure 22 shows batch consistency of stable ApoB siRNA/LNP-1 formulations.

Figure 23 shows LNP-1's potency delivering eGFP mRNA to the liver, spleen and bone marrow.

Figure 24 shows LNP-1's potency delivering CAR (eGFP) mRNA to activated immune cells (e.g., antigen presenting cells (APC), monocytes/macrophages, and CD4 T cells).

Figure 25 shows LNP-1's CAR-T generation potency (ex vivo).

Figure 26 shows LNP-1's CAR-T generation potency (in vivo).

Figure 27 shows LNP-1's potency delivering eGFP mRNA to hematopoietic stem cells, e.g., bone marrow-derived erythroblast cells.

Figure 28 shows LNP-1's CRISPR gene editing potency.

Figure 29 shows LNP-1's potency delivering eGFP mRNA to human leukemia cells with persistent protein expression.

Figure 30 shows LNP-1's potency delivering eGFP mRNA to human lymphoma cells.

Figure 31 shows LNP-1's potency delivering eGFP mRNA to human cancer cells with persistent protein expression.

Figure 32 shows LNP-1's potency as vaccines, e.g., COVID-19 mRNA vaccines via intramuscular (IM) or subcutaneous (SC) delivery of Spike mRNA.

Figure 33 shows that, within the therapeutic window (e.g., 0.1-3 mg/kg), no in vivo toxicity is noted with LNP-1 formulations via i.v administration.

Figure 34 shows that LNP-1 formulations do not cause tissue toxicity in all major organs examined post i.v administration.

DETAILED DESCRIPTION

Provided herein is a lipid nanoparticle comprising a PEG-conjugated phospholipid (PEG-phospholipid) having two or more C₁₂-C₃₀ aliphatic chains, at least one of which includes from 1-5 sites of unsaturation, an ionizable lipid having at least two C₁₁-C₃₀ aliphatic chains and a head group that protonates to a cationic moiety having a pKa of 5-7, a non-

cationic phospholipid having at least two C₁₁-C₃₀ aliphatic chains at
remains a zwitterion or anion across a pH range of 6 to 8; and a sterol, fat-soluble steroid, or
vitamin. These lipid nanoparticles are useful for the delivery of a variety of therapeutic
agents, including but not limited to nucleic acid agents and small molecule drugs.

5

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the
same meaning as commonly understood to one of ordinary skill in the art to which this
disclosure pertains. Otherwise, certain terms used herein have the meanings as set forth in
the specification.

10

"A," "an," or "a(n)," is an indefinite article when used in reference to a group of
substituents or "substituent group" herein, mean at least one.

"About" when referring to a value includes the stated value +/- 10% of the stated
value. For example, about 50% includes a range of from 45% to 55%, while about 20 molar
equivalents includes a range of from 18 to 22 molar equivalents. Accordingly, when referring
to a range, "about" refers to each of the stated values +/- 10% of the stated value of each
end of the range. For instance, a ratio of from about 1 to about 3 (weight/weight) includes a
range of from 0.9 to 3.3.

15

"Composition" as used herein is intended to encompass a product that includes the
specified lipid nanoparticle, optionally an active agent, and pharmaceutically acceptable
excipients, carriers or diluents as described herein, which results from combination of
specific components.

20

As used herein, "polydispersity" refers a measure of broadness of molecular weight
distribution. The larger the polydispersity index (PDI), the broader the molecular weight. PDI
of a polymer is calculated as the ratio of weight average by number average molecular
weight.

25

The compounds described herein can be asymmetric (e.g., having one or more
stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended
unless otherwise indicated. Compounds of the present disclosure that contain
asymmetrically substituted carbon atoms can be isolated in optically active or racemic
forms. Methods on how to prepare optically active forms from optically inactive starting
materials are known in the art, such as by resolution of racemic mixtures or by
stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds and the
like can also be present in the compounds described herein, and all such stable isomers are
contemplated in the present disclosure. *Cis* and *trans* geometric isomers of the compounds
of the present disclosure are described and may be isolated as a mixture of isomers or as
separated isomeric forms.

30

35

The phrase “pharmaceutically acceptable” is used herein to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or
5 complication, commensurate with a reasonable benefit/risk ratio. In certain embodiments, a pharmaceutically acceptable formulation of a lipid nanoparticle of the present invention will be substantially pyrogen-free. For example, in certain preferred embodiments, the endotoxin concentration of the subject preparation, as assayed by the via the gel-clot method (as a limits test with comparison to the maximum allowed FDA limit, as stated in appendix E of the
10 Endotoxin Guidance), is less than 10 EU/mL or EU/single dosage formulation, and even more preferably less than 5, 1, or even 0.1 EU/mL or EU/single dosage formulation.

The phrase “pharmaceutically acceptable excipient,” as used herein, refers to any ingredient other than the compounds described herein (for example, a vehicle capable of suspending, complexing, or dissolving the active compound) and having the properties of
15 being substantially nontoxic and non-inflammatory in a patient. Excipients may include, for example: anti-adherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluent), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspending or dispersing agents, sweeteners, and waters of hydration. Exemplary excipients
20 include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl
25 pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E (alpha-tocopherol), vitamin C, xylitol, and other species disclosed herein.

As used herein, the term “polypeptide” or “polypeptide of interest” refers to a polymer
30 of amino acid residues typically joined by peptide bonds that can be produced naturally (e.g., isolated or purified) or synthetically.

The term “alkyl” or “aliphatic” employed alone or in combination with other terms, refers to a hydrocarbon group that may be straight-chained or branched. The term “C_{n-m} alkyl” or “C_{n-m} aliphatic” refers to an alkyl group having n to m carbon atoms. An alkyl group
35 formally corresponds to an alkane with one C-H bond replaced by the point of attachment of the alkyl group to the remainder of the compound. The alkyl and aliphatic groups herein may

be unsaturated or saturated when specified. An alkyl or aliphatic group has at least one double bond (sp^2 carbon atoms).

As used herein, the term "phospholipid" refers to a class of lipids whose molecule has a hydrophilic "head" containing a phosphate group ($-OPO_3$) and two hydrophobic "tails" derived from fatty acids, joined by an alcohol residue (usually a glycerol molecule). As used herein, the term "steroid" refers to a biologically active organic compound with four fused rings arranged in a specific molecular configuration. The steroid nucleus (core structure) is called gonane (cyclopentanoperhydrophenanthrene). It is typically composed of seventeen carbon atoms, bonded in four fused rings: three six-member cyclohexane rings (rings A, B and C) and one five-member cyclopentane ring (the D ring).

As used herein, the term "sterol" refers to a subgroup of steroids with a hydroxyl group at the 3-position of the A-ring.

As used herein, the term "vitamin" refers to a class of organic molecules (or a set of closely related molecules called vitamers) that are essential to an organism in small quantities for proper metabolic function.

As used herein, the terms "nucleic acid" as used herein refers to polymers of nucleotides of any length, including ribonucleotides, deoxyribonucleotides, analogs thereof, or mixtures thereof. This term refers to the primary structure of the molecule. Thus, the term includes triple-, double- and single-stranded deoxyribonucleic acid ("DNA"), as well as triple-, double- and single-stranded ribonucleic acid ("RNA"). It also includes modified, for example by alkylation, and/or by capping, and unmodified forms of the nucleic acid. More particularly, the term "nucleic acid" includes polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), including mRNA, shRNA and siRNA, whether spliced or unspliced, any other type of nucleic acid which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing normucleotidic backbones, for example, polyamide (e.g., peptide nucleic acids "PNAs") and polymorpholino polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA.

As used herein, the term "RNA" refers to a ribonucleic acid that may be naturally or non-naturally occurring. For example, an RNA may include modified and/or non-naturally occurring components such as one or more nucleobases, nucleosides, nucleotides, or linkers. An RNA may include a cap structure, untranslated regions (UTRs), a chain terminating nucleoside, a stem loop, a polyA sequence, and/or a polyadenylation signal. An RNA may have a nucleotide sequence encoding a polypeptide of interest. For example, an RNA may be a messenger RNA (mRNA). Translation of an mRNA encoding a particular polypeptide, for example, in vivo translation of an mRNA inside a mammalian cell, may

produce the encoded polypeptide. RNAs may be selected from the consisting of small interfering RNA (siRNA), asymmetrical interfering RNA (aiRNA), microRNA (miRNA), Dicer-substrate RNA (dsRNA), small hairpin RNA (shRNA), mRNA, circular RNA (circRNA), self-amplifying RNA (saRNA), long non-coding RNA (lncRNA), antisense RNA (asRNA), and mixtures thereof. In some aspects, the mRNA is a synthetic mRNA and comprises at least one unnatural nucleobase. In some aspects, all nucleobases of a certain class have been replaced with unnatural nucleobases (e.g., all uridines in a nucleic acid disclosed herein can be replaced with an unnatural nucleobase, e.g., 5-methoxyuridine). In some aspects, the nucleic acid (e.g., a synthetic RNA or a synthetic DNA) comprises only natural nucleobases, i.e., A, C, T and U in the case of a synthetic DNA, or A, C, T, and U in the case of a synthetic RNA.

As used herein, the term "DNA" refers to a deoxyribonucleic acid that may be naturally or non-naturally occurring. For example, DNA may include modified and/or non-naturally occurring components such as one or more nucleobases, nucleosides, or nucleotides. DNA includes cDNA, catalytic DNA, genomic DNA, and synthetic DNA. DNA can be double-stranded or single-stranded and, if single stranded, can be the coding strand or non-coding (anti-sense) strand. DNA can be used as an active drug agent in the lipid nanoparticles of the present disclosure.

As used herein, the term "gene therapy" refers to the introduction of genetic material into a recipient organism for healing purposes. Gene therapy can be used to obtain the desired therapeutic effect through different strategies, depending on the target disease. Gene therapy can be used to provide an absent gene in the receptor, or to complement or replace the defect in the function of a defective gene by inserting a normal copy of it into the cells. It is also possible to use gene therapy to obtain the opposite effect, that is, to inhibit or block the functioning of genes whose intervention contributes to the development of the disease. Alternatively, gene therapy can be used not to replace or inactivate the function of a gene, but to enter information that allows the cell to synthesize a protein with the desired therapeutic effect.

As used herein, the term "CRISPR" refers to a DNA modification technology. CRISPR utilizes short RNA sequences known as guide RNAs or gRNAs to direct a gene modification system to a specific sequence of DNA. Once bound to the target DNA, the enzyme(s) in the system modify the target DNA sequence. Multiple enzymes have been engineered to work with CRISPR technology such that DNA chains can be cut, nicked, extended, replaced, and/or otherwise edited to produce a sequence of interest. For example, CRISPR technology can be used for gene knock-out, gene knock-in, CRISPR interference (CRISPRi), CRISPR activation (CRISPRa), base editing, prime editing, CRISPR screens, or gene visualization. Alternatively, CRISPR technology can be used to modify

epigenomics – the genome-wide set of chemical groups that adorn histone packaging proteins, e.g., acetylating histone proteins at precise locations.

As used herein, the term “cell therapy” refers to therapy in which viable cells are injected, grafted or implanted into a patient in order to effectuate a medicinal effect. Cell therapy can be tissue transplantation therapy. Cell therapy can be allogeneic (the recipient will receive cells originated from another person), autologous (the recipient will receive cells originated from the patient’s own tissues), or xenogeneic (the recipient will receive cells from another species). The types of cells used for cell therapy can be pluripotent stem cells (e.g., embryonic stem cells, neural stem cells, mesenchymal stem cells, and hematopoietic stem cells). Cell therapy can also use differentiated or mature cells (e.g., cardiomyocytes, islet cells, and chondrocytes). The cells used for cell therapy can be unmodified or modified/reprogrammed (e.g., CAR-engineered, such as CAR-T cells, CAR-NK cells, CAR-dendritic cells, CAR-macrophages, CAR-monocytes, and CAR-mesenchymal stem cells).

As used herein, the term “CAR” refers to a cell engineering technology. By engineering chimeric antigen receptor (CAR) proteins to give immune cells such as T cells, NK cells, dendritic cells, macrophages, monocytes, and mesenchymal stem cells the new ability to target a specific antigen. The receptors are chimeric in that they combine both antigen-binding and cell activating functions into a single receptor.

“Patient” or “subject” refers to a living organism, which includes, but is not limited to a human subject suffering from or prone to a disease or condition that can be treated by administration of a pharmaceutical composition as provided herein. Further non-limiting examples may include, but are not limited to, humans or other mammals. In some embodiments, the subject or patient is a human.

As used herein, the term “treating” or “treatment” refers to inhibiting a disease; for example, inhibiting a disease, condition, or disorder in an individual who is experiencing or displaying the pathology or symptomology of the disease, condition, or disorder (i.e., arresting further development of the pathology and/or symptomology) or ameliorating the disease; for example, ameliorating a disease, condition, or disorder in an individual who is experiencing or displaying the pathology or symptomology of the disease, condition, or disorder (i.e., reversing the pathology and/or symptomology) such as decreasing the severity of the disease.

The term “prevent,” “preventing,” or “prevention” as used herein, comprises the prevention of at least one symptom associated with or caused by the state, disease or disorder being prevented. The term “pharmaceutical product” refers to a product that contains an active pharmaceutical ingredient that has been shown to be a safe and effective treatment of one or more indications as supported by findings from clinical trials regulated by

a governmental authority, e.g., the Food and Drug Administration of other countries.

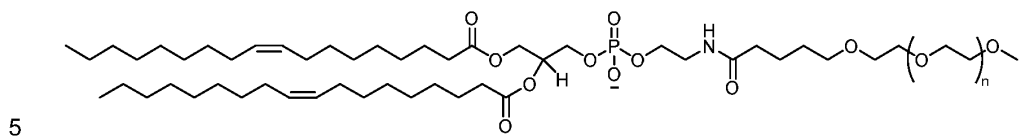
Lipid Nanoparticles

- 5 In an aspect, provided herein is a lipid nanoparticle comprising:
- (i) a PEG-conjugated phospholipid (PEG-phospholipid) having two or more C₁₂-C₃₀ aliphatic chains, at least one of which includes from 1-5 sites of unsaturation;
 - (ii) an ionizable lipid having at least two C₁₁-C₃₀ aliphatic chains and a head group that protonates to a cationic moiety having a pK_a of 5-7;
 - 10 (iii) a non-cationic phospholipid having at least two C₁₁-C₃₀ aliphatic chains and a head group that remains a zwitterion or anion across a pH range of 6 to 8; and
 - (iv) a sterol, fat-soluble steroid, or vitamin.
- In an embodiment, the PEG-phospholipid has a molecular weight in the range of 0.2-6 kDa.
- 15 In another aspect, provided herein is a lipid nanoparticle comprising:
- (i) a PEG-conjugated phospholipid (PEG-phospholipid) having two or more alkyl chains, at least one of which includes from 1-5 sites of unsaturation, and a head group substituted with a polyethylene glycol (PEG) polymer moiety, wherein the polyethylene glycol (PEG) polymer moiety has 2-50 polyethylene glycol subunits;
 - 20 (ii) a cationic lipid having a cationic head group and two or more C₁₁-C₃₀ aliphatic chains, at least one of which includes from 1-5 sites of unsaturation, wherein the aliphatic chains are attached to the headgroup through a biodegradable linker;
 - (iii) a non-cationic phospholipid that remains a zwitterion or anion across a pH range of 6 to 8, having two or more C₁₁-C₃₀ aliphatic chains; and
 - 25 (iv) a sterol, fat-soluble steroid, or vitamin.
- In an embodiment, the PEG-phospholipid has an average molecular weight in the range of 200 to 7500 amu.
- In another aspect, provided herein is a lipid nanoparticle comprising:
- (i) a PEG-conjugated phospholipid (PEG-phospholipid) having two or more alkyl
 - 30 chains, at least one of which includes from 1-5 sites of unsaturation, and a head group substituted with a polyethylene glycol (PEG) polymer moiety, wherein the polyethylene glycol (PEG) polymer moiety has 2-50 polyethylene glycol subunits;
 - (ii) a cationic lipid having a cationic head group and two or more C₁₂-C₃₀ aliphatic chains, at least one of which includes from 1-5 sites of unsaturation, wherein the aliphatic
 - 35 chains are attached to the headgroup through a biodegradable linker;
 - (iii) a non-cationic phospholipid that remains a zwitterion or anion across a pH range of 6 to 8, having two or more C₁₂-C₃₀ aliphatic chains; and

(iv) a sterol, fat-soluble steroid, or vitamin.

In an embodiment, the PEG-phospholipid has an average molecular weight in the range of 200 to 7500 amu.

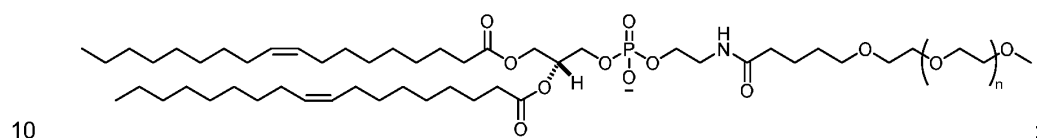
In an embodiment, (i) is



wherein n is an integer from 32 to 50.

In an embodiment n is 35-45. In another embodiment, n is 40, 41, 42, 43, 44, 45, 46, 47, or 48.

In an embodiment, (i) is

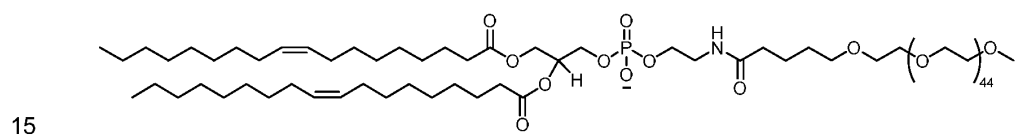


wherein n is an integer from 32 to 50.

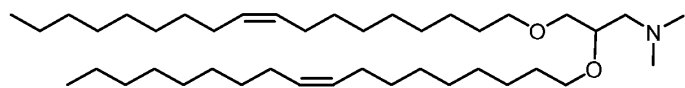
In an embodiment, n is 43. In an embodiment, n is 44.

In an embodiment, the lipid nanoparticles comprise:

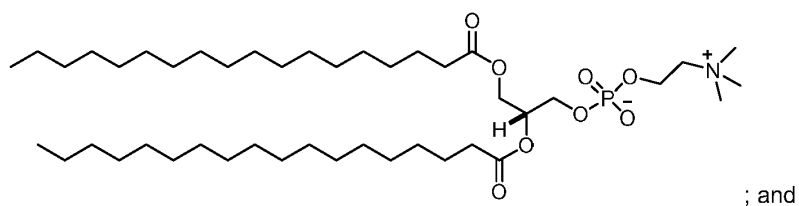
(i) a PEG-conjugated phospholipid having the structure



(ii) an ionizable lipid having the structure



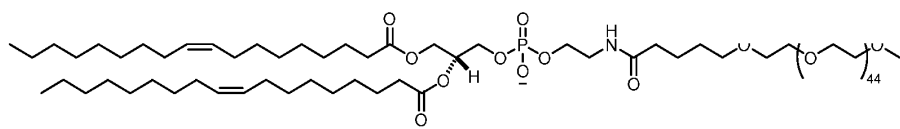
(iii) a non-cationic phospholipid having the structure



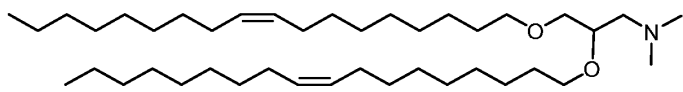
20 (iv) cholesterol.

In another embodiment, the lipid nanoparticles comprise:

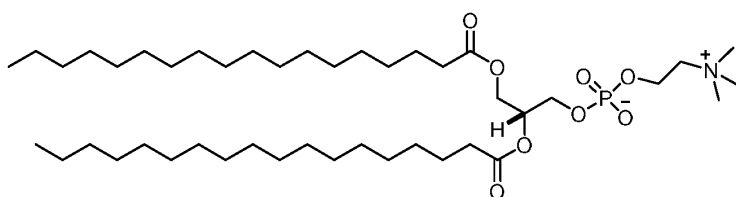
(i) a PEG-conjugated phospholipid having the structure



(ii) an ionizable lipid having the structure

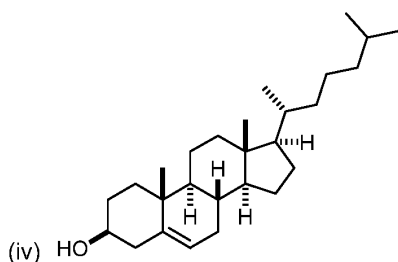


(iii) a non-cationic phospholipid having the structure



5

; and



(iv)

This lipid nanoparticle is also referred to herein as "LNP-1."

In an embodiment, the PEG-conjugated phospholipid is present in the lipid nanoparticle in an average amount of 0.25 to 10 mol% of the four components. In another embodiment, the ionizable lipid is present in the lipid nanoparticle in a relative amount of 5 to 60 mol%, the non-cationic phospholipid is present in the lipid nanoparticle in a relative amount of 5 to 55 mol%, and the cholesterol is present in the lipid nanoparticle in a relative amount of 30 to 60 mol%, with the proviso that the mol% of the four components totals 100 mol%.

In another embodiment, the lipid nanoparticles have a relative ratio of 50:10:39.25:0.75 for the ionizable lipid, the non-cationic phospholipid, cholesterol and the PEG-conjugated phospholipid.

In another embodiment, the lipid nanoparticle is stable at 2-8°C for at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, or 180 days. In another embodiment, the lipid nanoparticle is stable at 2-8°C for at least 180 days. In another embodiment, the lipid nanoparticle is stable at 2-8°C for at least 270 days. In another embodiment, the lipid nanoparticle is stable at 2-8°C for at least 360 days. In another embodiment, the lipid nanoparticle is stable at 2-8°C for at least 540 days. In another

embodiment, the lipid nanoparticle is stable at 2-8°C for at least 72
embodiment, the lipid nanoparticle is stable at 2-8°C for at least 900 days. In another
embodiment, the lipid nanoparticle is stable at 2-8°C for at least 1080 days. In another
embodiment, the lipid nanoparticle is stable at 2-8°C for at least 1260 days. In another
5 embodiment, the lipid nanoparticle is stable at 2-8°C for at least 1440 days.

In an embodiment, after storage for at least 180 days at 2-8°C the lipid nanoparticles
maintain a polydispersity within 10% of polydispersity measured at initial preparation of the
formulation. In another embodiment, after storage for at least 180 days at 2-8°C the lipid
nanoparticles maintain a polydispersity within 10% of polydispersity measured at initial
10 preparation of the formulation.

In yet another embodiment, the lipid nanoparticle is stable at about 25°C for at least
10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or 120 days. In another embodiment, the lipid
nanoparticle is stable at about 25°C for at least 120 days. the lipid nanoparticle is stable at
about 25°C for at least 150 days. In another embodiment, the lipid nanoparticle is stable at
15 about 25°C for at least 180 days. In another embodiment, the lipid nanoparticle is stable at
about 25°C for at least 270 days. In another embodiment, the lipid nanoparticle is stable at
about 25°C for at least 360 days. In another embodiment, the lipid nanoparticle is stable at
25°C for at least 540 days. In another embodiment, the lipid nanoparticle is stable at 25°C
for at least 720 days. In another embodiment, the lipid nanoparticle is stable at 25°C for at
20 least 900 days. In another embodiment, the lipid nanoparticle is stable at 25°C for at least
1080 days.

In still another embodiment, the lipid nanoparticle is stable at about 40°C for at least
1, 2, 3, 4, 5, 6, or 7 days. In another embodiment, the lipid nanoparticle is stable at about
40°C for at least 7 days. In another embodiment, the lipid nanoparticle is stable at about
25 40°C for at least 8 days. In another embodiment, the lipid nanoparticle is stable at about
40°C for at least 10 days. In another embodiment, the lipid nanoparticle is stable at about
40°C for at least 12 days. In another embodiment, the lipid nanoparticle is stable at about
40°C for at least 14 days. In another embodiment, the lipid nanoparticle is stable at about
40°C for at least 16 days. In another embodiment, the lipid nanoparticle is stable at about
30 40°C for at least 18 days.

In another embodiment, provided herein is a lipid nanoparticle formulation in which
the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at
formulation after at least 180 days at 2-8°C. In an embodiment, the lipid nanoparticle
maintains a polydispersity within 10% of its polydispersity at formulation after at least 270
35 days at 2-8°C. In another embodiment, the lipid nanoparticle maintains a polydispersity
within 10% of its polydispersity at formulation after at least 360 days at 2-8°C. In another
embodiment, the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity

at formulation after at least 540 days at 2-8°C. In another embodiment maintains a polydispersity within 10% of its polydispersity at formulation after at least 720 days at 2-8°C. In another embodiment, the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 900 days at 2-8°C. In another embodiment, the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 1080 days at 2-8°C. In another embodiment, the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 1260 days at 2-8°C. In another embodiment, the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 1440 days at 2-8°C.

In an embodiment, provided herein is a lipid nanoparticle formulation in which the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 60 days at 25°C. In another embodiment, provided herein is a lipid nanoparticle formulation in which the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 120 days at 25°C. In an embodiment, the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 180 days at 25°C. In another embodiment, the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 270 days at 25°C. In another embodiment, the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 360 days at 25°C. In another embodiment, the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 540 days at 25°C. In another embodiment, the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 720 days at 25°C. In another embodiment, the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 900 days at 25°C. In another embodiment, the lipid nanoparticle maintains a polydispersity within 10% of its polydispersity at formulation after at least 1080 days at 25°C.

In an embodiment, the PEG-phospholipid has a head group selected from the group consisting of phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid, phosphatidylglycerols, ethoxy, and methoxy.

In another embodiment, the PEG-phospholipid has a head group selected from the group consisting of phosphatidylethanolamine and phosphatidylcholine. In an embodiment, the PEG-phospholipid is present at 0.25-10 mol %. In an embodiment, the PEG-phospholipid is present at 0.25-5 mol %. In another embodiment, the PEG-phospholipid is present at 0.5-5 mol %. In yet another embodiment, the PEG-phospholipid is present at 0.75-2.5 mol %. In still another embodiment, the PEG-phospholipid is present at 0.75-1.0 mol %.

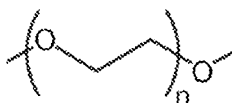
In certain preferred embodiments, the PEG-phospholipid has

In yet another embodiment, the two or more C₁₂-C₃₀ aliphatic chains of the PEG-phospholipid are C₁₄-C₁₈ aliphatic chains.

In still another embodiment, each of the C₁₄-C₁₈ aliphatic chains is unsaturated.

5 In an embodiment, each of the C₁₄-C₁₈ aliphatic chains is unsaturated at 1-3 positions.

In another embodiment, PEG polymer moiety is a linear polymer and represented in the formula



10 wherein n is an integer from 2-50. In an embodiment, n is an integer from 5-50. In yet another embodiment, n is an integer from 15-40. In still another embodiment, n is an integer from 20-50, or is an integer from 30-50, or is an integer from 40-50. In certain embodiments, n is 42, 43, 44, 45 or 46. In certain embodiments, n is 44. In an embodiment, n is an integer from 20 to 35. In an embodiment, n is an integer from 20 to 35.

15 In an embodiment, the PEG polymer moiety includes a ligand moiety that binds to a cognate binding partner expressed on the cell surface of cells in the target tissue and which, upon assembly of the lipid nanoparticle, is present on the surface of the nanoparticle.

The ionizable lipids of the present LNPs are lipids having headgroups which are capable of modulating their charge depending on the environmental pH.

20 In certain embodiments, the ionizable lipid is a cationic lipid having a headgroup which while neutral at serum pH becomes positively charged at the pH of an endosome.

In another embodiment, the cationic head group of the cationic lipid is an ionizable head group having a pKa less than 6.7. In another embodiment, the cationic lipid is present at 5-60 mol %. In another embodiment, the cationic lipid is present at 5-55 mol %. In certain 25 embodiments, the cationic lipid is present at 30-60 mol %. In certain embodiments, the cationic lipid is present at 40-55 mol %. In certain embodiments, the cationic lipid is present at about 50 mol %. In certain embodiments, the cationic lipid is present at 50 mol %.

In yet another embodiment, the ionizable head group includes a tertiary amine which has a pKa less than 6.7.

30 In still another embodiment, the pKa of the ionizable head group has a pKa in the range of 6.2 to 6.5.

In an embodiment, the cationic head group and its neighboring aliphatic chain are in a 1,2-relationship.

In another embodiment, the unsaturated C₁₁-C₃₀ aliphatic chains are C₁₈ aliphatic chains. In another embodiment, the unsaturated C₁₂-C₃₀ aliphatic chains are unsaturated C₁₄-C₁₈ aliphatic chains.

In yet another embodiment, the two or more aliphatic chains are, independently for each occurrence, selected from α -Linolenic acid, γ -Linolenic acid, Arachidonic acid, Cervonic acid, Dihomo- γ -linolenic acid, Docosatetraenoic acid, Eicosapentaenoic acid, Elaidic acid, Erucic acid, Gondoic acid, Linoleic acid, Linolelaidic acid, Mead acid, Nervonic acid, Oleic acid, Palmitoleic acid, Paullinic acid, Stearidonic acid, and Vaccenic acid.

In still another embodiment, the unsaturated C₁₁-C₃₀ aliphatic chains are unsaturated C₁₈ aliphatic chains. In still another embodiment, the unsaturated C₁₂-C₃₀ aliphatic chains are unsaturated C₁₈ aliphatic chains. In an embodiment, the unsaturated C₁₈ aliphatic chains are selected from oleic, linoleic and α -linoleic acids.

In another embodiment, the C₁₈ aliphatic chains are covalently linked to the head group through an ether, ester or amide bond, or other bond that can be hydrolyzed (e.g., by enzyme-mediated or by enzyme independent processes) in vivo to release the aliphatic chain.

In an embodiment, the cationic lipid can be one or a combination thereof selected from 1,2-dioleoyloxy-3-dimethylaminopropane or (2R)-N,N-dimethyl-2,3-bis[(9Z)-9-octadecen-1-yloxy]-1-propanamine (DODMA),

(N,N-dimethyl-2,3-bis[(9Z,12Z)-9,12-octadecadien-1-yloxy]-1-propanamine) (DLin-DMA),

(4-dimethylamino)-butanoic acid, (10Z,13Z)-1-(9Z,12Z)-9,12-octadecadien-1-yl-10,13-nonadecadien-1-yl ester (DLin-MC3-DMA),

N,N-dimethyl-2,2-di-(9Z,12Z)-9,12-octadecadien-1-yl-1,3-dioxolane-4-ethanamine (DLin-KC2-DMA),

(2-hexyl-decanoic acid, 1,1'-[[4-hydroxybutyl]imino]di-6,1-hexanediyl] ester (ALC-0315),

8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]-octanoic acid, 1-octylnonyl ester (SM-102),

1-1'-((2-(4-(2-((2-bis(2-hydroxydecyl)amino)ethyl)(2-hydroxydecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl]bis(decan-2-ol) (C10-200),

9,12-octadecadienoic acid, (9Z,12Z)-1,1'[2-[[[3-(diethylamino)propoxy]carbonyl]oxy]-1,3-propanediyl] ester (Ionizable Lipid 4, IL4),

5-(dimethylamino)-pentanoic acid, (6Z)-1,2-di-(4Z)-4-decen-1-yl-6-dodecen-1-yl ester (Lipid CL1),

- 4-methyl-1-piperazinepropanoic acid, 2-[di-(9Z,12Z)-9,12-oxo-ylamino]ethyl ester (Lipid 10),
- 9-[4-(dimethylamino)1-oxobutoxy]-heptadecanedioic acid, 1,17-di-3-decyn-1-yl ester (Lipid A6),
- 5 Di((Z)-octadec-9-en-1-yl) L-lysyl-L-glutamate, dihydrochloride (Lipid OA2 (hydrochloride)),
- 1-methyl-4-piperidinecarboxylic acid, 11-[(2-hexyl-1-oxodecyl)oxy]-5-[6-[(2hexyl-1-oxodecyl)oxy]hexyl]-5-hydroxyundecyl ester (CL15F6),
- Piperazine-1,4-diylbis(ethane-2,1-diyl) (9Z,9'Z,12Z,12'Z)-bis(octadeca-9,12-dienoate)
- 10 (AA3-DLin),
- 5-(((3dibutylamino)propyl)amino)methyl)-6-hydroxyundecane-1,11-diyl (9Z,9'Z,12,12'Z)-bis(octadeca-9,12-dienoate) (IR-117-17),
- 1-methyl-4,4-bis[9Z,12Z)-9,12-octadecadien-1-yloxy]-piperidine (YSK05),
- 9-[4-(dimethylamino)-1-oxobutoxy]-heptadecanoic acid, 1,17-di-(2Z)-2-nonen-1-yl
- 15 ester (L-319),
- 3,6-bis[4-[bis[(9Z,12Z)-2-hydroxy-9,12-octadecadien-1-yl]amino]butyl]-2,5-piperazinedione, (OF-02)9,12-octadecadienoic acid, (9Z,12Z)-1,1',1'',1'''-[3,6-dioxo-2,5-piperazinediyl]bis(4,1-butanediynitrilodi-4,1-butanediyl)] ester (OF-C4-Deg-Lin),
- 9Z,12Z-octadecadienoic acid, 1,1',1'',1'''-[3,6-dioxo-2,5-piperazinediyl]bis(4,1-butanediynitrilodi-2,1-ethanediyl)] ester (OF-Deg-Lin),
- 20 4,4'-[[[3-(dimethylamino)propyl]thio]carbonyl]imino]bis-butanoic acid, 1,1'-bis(1-heptyloctyl) ester (ATX-100),
- 1-[3-(dimethylamino)propyl]-5,5-di-(8Z)-8-heptadecen-1-yl-2,5-dihydro-1H-imidazole-2-carboxylic acid, ethyl ester (A12-Iso5-2DC18).
- 25 In yet another embodiment, the non-cationic phospholipid has a head group selected from phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid and phosphatidylglycerols. In still another embodiment, the non-cationic phospholipid is a phosphatidylethanolamine or phosphatidylcholine.
- 30 In an embodiment, the two or more C₁₁-C₃₀ aliphatic chains of the non-cationic phospholipid are C₁₄-C₁₈ aliphatic chains that, independently for each chain, are saturated or unsaturated at 1-3 positions.
- In another embodiment, the two or more C₁₁-C₃₀ aliphatic chains of the non-cationic lipid are saturated C₁₄-C₁₈ aliphatic chains.
- 35 In yet another embodiment, the non-cationic phospholipid can be one or a combination thereof selected from
- 1,2-diundecanoyl-sn-glycero-3-phosphatidylcholine (11:0 PC, DUPC),

- 1,2-dilauroyl-sn-glycero-3-phosphatidylcholine (12:0 PC),
1,2-ditridecanoyl-sn-glycero-3-phosphatidylcholine (13:0 PC),
1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (14:0 PC, DMPC),
1,2-dipentadecanoyl-sn-glycero-3-phosphatidylcholine (15:0 PC),
5 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (16:0 PC, DPPC),
1,2-diphytanoyl-sn-glycero-3-phosphatidylcholine (4ME 16:0 PC),
1,2-diheptadecanoyl-sn-glycero-3-phosphatidylcholine (17:0 PC),
1,2-distearoyl-sn-glycero-3-phosphatidylcholine (18:0 PC, DSPC),
1,2-Distearoyl-sn-glycero-3-Ethylphosphatidylcholine (Ethyl-DSPC)
10 1,2-dinonadecanoyl-sn-glycero-3-phosphatidylcholine (19:0 PC),
1,2-diarachidoyl-sn-glycero-3-phosphatidylcholine (20:0 PC),
1,2-dihenarachidoyl-sn-glycero-3-phosphatidylcholine (21:0 PC),
1,2-dibehenoyl-sn-glycero-3-phosphatidylcholine (22:0 PC),
1,2-ditricosanoyl-sn-glycero-3-phosphatidylcholine (23:0 PC),
15 1,2-dilignoceroyl-sn-glycero-3-phosphatidylcholine (24:0 PC),
1,2-dimyristoleoyl-sn-glycero-3-phosphatidylcholine (14:1 (49-Cis) PC),
1,2-dimyristelaidoyl-sn-glycero-3-phosphatidylcholine (14:1 (49-Trans) PC),
1,2-dipalmitoleoyl-sn-glycero-3-phosphatidylcholine (16:1 (49-Cis) PC),
1,2-dipalmitelaidoyl-sn-glycero-3-phosphatidylcholine (16:1 (49-Trans) PC),
20 1,2-dipetroselenoyl-sn-glycero-3-phosphatidylcholine (18:1 (46-Cis) PC),
1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (18:1 (49-Cis) PC, DOPC),
1,2-dielaidoyl-sn-glycero-3-phosphatidylcholine (18:1 (49-Trans) PC),
1,2-dilinoleoyl-sn-glycero-3-phosphatidylcholine (18:2 (Cis) PC, DLPC),
1,2-dilinolenoyl-sn-glycero-3-phosphatidylcholine (18:3 (Cis) PC, DLnPC),
25 1,2-dieicosenoyl-sn-glycero-3-phosphatidylcholine (20:1 (Cis) PC),
1,2-diarachidonoyl-sn-glycero-3-phosphatidylcholine (20:4 (Cis) PC, DAPC),
1,2-dierucoyl-sn-glycero-3-phosphatidylcholine (22:1 (Cis) PC),
1,2-didocosahexaenoyl-sn-glycero-3-phosphatidylcholine (22:6 (Cis) PC, DHAPC),
1,2-dinervonoyl-sn-glycero-3-phosphatidylcholine (24:1 (Cis) PC),
30 1,2-dihexanoyl-sn-glycero-3-phosphatidylethanolamine (06:0 PE),
1,2-dioctanoyl-sn-glycero-3-phosphatidylethanolamine (08:0 PE),
1,2-didecanoyl-sn-glycero-3-phosphatidylethanolamine (10:0 PE),
1,2-dilauroyl-sn-glycero-3-phosphatidylethanolamine (12:0 PE),
1,2-dimyristoyl-sn-glycero-3-phosphatidylethanolamine (14:0 PE),
35 1,2-dipentadecanoyl-sn-glycero-3-phosphatidylethanolamine (15:0 PE),
1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine (16:0 PE),
1,2-diphytanoyl-sn-glycero-3-phosphatidylethanolamine (4ME 16:0 PE),

- 1,2-diheptadecanoyl-sn-glycero-3-phosphatidylethanolamin
 1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine (18:0 PE, DSPE),
 1,2-dipalmitoleoyl-sn-glycero-3-phosphatidylethanolamine (16:1 PE),
 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine (18:1 (49-Cis) PE, DOPE),
 5 1,2-dielaideoyl-sn-glycero-3-phosphatidylethanolamine (18:1 (49-Trans) PE),
 1,2-dilinoeoyl-sn-glycero-3-phosphatidylethanolamine (18:2 PE, DLPE),
 1,2-dilinenoyl-sn-glycero-3-phosphatidylethanolamine (18:3 PE, DLnPE),
 1,2-diarachidonoyl-sn-glycero-3-phosphatidylethanolamine (20:4 PE, DAPE),
 1,2-didocosaheaxenoyl-sn-glycero-3-phosphatidylethanolamine (22:6 PE, DHAPE),
 10 1,2-di-O-octadecenyl-sn-glycero-3-phosphatidylcholine (18:0 Diether PC),
 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG),
 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphatidylcholine (14:0-16:0 PC, MPPC),
 1-myristoyl-2-stearoyl-sn-glycero-3-phosphatidylcholine (14:0-18:0 PC, MSPC),
 1-palmitoyl-2-acetyl-sn-glycero-3-phosphatidylcholine (16:0-02:0 PC),
 15 1-palmitoyl-2-myristoyl-sn-glycero-3-phosphatidylcholine (16:0-14:0 PC, PMPC),
 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphatidylcholine (16:0-18:0 PC, PSPC),
 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (16:0-18:1 PC, POPC),
 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphatidylcholine (16:0-18:2 PC, PLPC),
 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphatidylcholine (16:0-20:4 PC),
 20 1-palmitoyl-2-docosaheaxenoyl-sn-glycero-3-phosphatidylcholine (14:0-22:6 PC),
 1-stearoyl-2-myristoyl-sn-glycero-3-phosphatidylcholine (18:0-14:0 PC, SMPC),
 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphatidylcholine (18:0-16:0 PC, SPPC),
 1-stearoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (18:0-18:1 PC, SOPC),
 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphatidylcholine (18:0-18:2 PC),
 25 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphatidylcholine (18:0-20:4 PC),
 1-stearoyl-2-docosaheaxenoyl-sn-glycero-3-phosphatidylcholine (18:0-22:6 PC),
 1-oleoyl-2-myristoyl-sn-glycero-3-phosphatidylcholine (18:1-14:0 PC, OMPC),
 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphatidylcholine (18:1-16:0 PC, OPPC),
 1-oleoyl-2-stearoyl-sn-glycero-3-phosphatidylcholine (18:1-18:0 PC, OSPC),
 30 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine (16:0-18:1 PE, POPE),
 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphatidylethanolamine (16:0-18:2 PE),
 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphatidylethanolamine (16:0-20:4 PE),
 1-palmitoyl-2-docosaheaxenoyl-sn-glycero-3-phosphatidylethanolamine (16:0-22:6
 PE),
 35 1-stearoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine (18:0-18:1 PE),
 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphatidylethanolamine (18:0-18:2 PE),
 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphatidylethanolamine (18:0-20:4 PE),

1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphatidylethanolamine (PE),
 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphatidylcholine (OchemsPC),
 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE),
 5 dipentadecanoyl-phosphatidylcholine (DPDPC),
 dilauroylphosphatidylglycerol (DLPG),
 diarachidoylphosphatidylglycerol (DAPG),
 dimyristoylphosphatidylglycerol (DMPG),
 dipalmitoylphosphatidylglycerol (DPPG),
 10 distearoylphosphatidylglycerol (DSPG),
 dimyristoyl phosphatidic acid (DMPA),
 dipalmitoyl phosphatidic acid (DPPA),
 distearoyl phosphatidic acid (DSPA),
 diarachidoylphosphatidic acid (DAPA),
 15 dimyristoylphosphatidylethanolamine (DMPE),
 dipalmitoylphosphatidylethanolamine (DPPE),
 dimyristoyl phosphatidylserine (DMPS),
 diarachidoyl phosphatidylserine (DAPS),
 dipalmitoyl phosphatidylserine (DPPS),
 20 distearoylphosphatidylserine (DSPS),
 dioleoylphosphatidylserine (DOPS),
 dipalmitoyl sphingomyelin (DPSP),
 distearoylsphingomyelin (DSSP),
 dilauroyl-phosphatidylinositol (DLPI),
 25 diarachidoylphosphatidylinositol (DAPI),
 dimyristoylphosphatidylinositol (DMPI),
 dipalmitoylphosphatidylinositol (DPPI),
 distearoylphosphatidylinositol (DSPI), and
 dioleoyl-phosphatidylinositol (DOPI).
 30 In still another embodiment, the non-cationic lipid is selected from DSPC, DOPC,
 DPPC, DOPG, DPPG, DOPE, POPC, POPE, DPPE, DMPE, DSPE, SOPE, and DLPE, or a
 combination thereof.

In another embodiment, the non-cationic lipid is present at 5-55 mol %. In another
 embodiment, the non-cationic lipid is present at 5-15 mol %. In certain embodiments, the
 35 non-cationic lipid is present at 5-30 mol %. In certain embodiments, the non-cationic lipid is
 present at 5-20 mol %. In certain embodiments, the non-cationic lipid is present at about 10
 mol %. In certain embodiments, the non-cationic lipid is present at 9-12 mol %.

In an embodiment, (iv) is a sterol. In another embodiment, t
 an analog thereof. In yet another embodiment, the sterol is selected from 3-hydroxycholest-
 5-ene (Cholesterol), 9,10-secocholesta-5,7,10(19)-trien-3b-ol (Vitamin D3), ergocalciferol
 (Vitamin D2), calcipotriol, 24-ethyl-5,22-cholestadien-3b-ol (Stigmasterol), 22,23-
 5 Dihydrostigmasterol (β -Sitosterol), 3,28-Dihydroxy-lupeol (Betulin), Lupeol, Ursolic acid,
 Oleanolic acid, 24a-Methylcholesterol (Campesterol), 24-Ethylcholesta-5,24(28)E-dien-3b-ol
 (Fucosterol), 24-Methylcholesta-5,22-dien-3b-ol (Brassicasterol), (22E)-Ergosta-5,7,22-trien-
 3 β -ol (Ergosterol), 9,11-Dehydroergosterol, and Daucosterol, or any of the foregoing sterols
 modified with one or more amino acids.

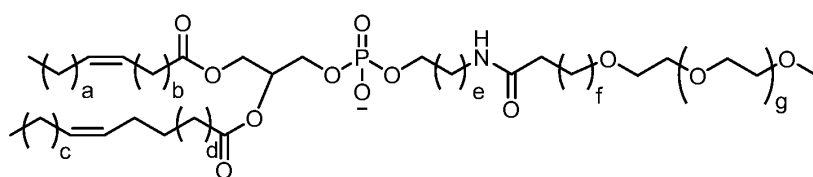
10 In still another embodiment, the sterol is a plant-derived sterol. In an embodiment,
 the plant-derived sterol is selected from β -sitosterol, β -sitostanol, stigmasterol, stigmasterol,
 campesterol, campestanol, ergosterol, avenasterol, and brassicasterol, or any combination
 thereof.

In another embodiment, (iv) is a fat-soluble vitamin. In yet another embodiment, the
 15 fat-soluble vitamin is selected from a vitamin A, a secosteroid, a tocopherol, a tocotrienol,
 and a menaquinone, or an analog or combination thereof. In still another embodiment, the
 fat-soluble vitamin is selected from retinol, retinal, retinyl esters, vitamin D2 (ergocalciferol),
 vitamin D3 (cholecalciferol), vitamin E, vitamin K1, and vitamin K2, or an analog or
 combination thereof.

20 In another embodiment, the sterol or fat-soluble vitamin is present at 30-60 mol %. In
 certain embodiments, the sterol or fat-soluble vitamin is present at 35-50 mol %. In certain
 embodiments, the sterol or fat-soluble vitamin is present at 35-45 mol %. In certain
 embodiments, the sterol or fat-soluble vitamin is present at 38-41 mol %. In certain
 embodiments, the sterol or fat-soluble vitamin is present at about 39 mol %.

25 In an embodiment, the sterol, fat-soluble steroid, or vitamin is PEGylated.

In another embodiment, (i) is



wherein

a is an integer between 1 and 10;
 30 b is an integer between 1 and 10;
 c is an integer between 1 and 10;
 d is an integer between 1 and 10;
 e is an integer between 1 and 5;

f is an integer between 1 and 5; and

g is an integer between 10 and 50.

In yet another embodiment,

a is an integer between 5 and 10;

5 b is an integer between 5 and 10;

c is an integer between 5 and 10;

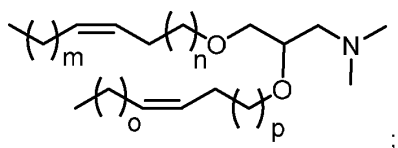
d is an integer between 1 and 5;

e is an integer between 1 and 3;

f is an integer between 1 and 5; and

10 g is an integer between 30 and 50.

In still another embodiment, (ii) is



wherein

m is an integer between 1 and 10;

15 n is an integer between 1 and 10;

o is an integer between 1 and 10; and

p is an integer between 1 and 10.

In an embodiment,

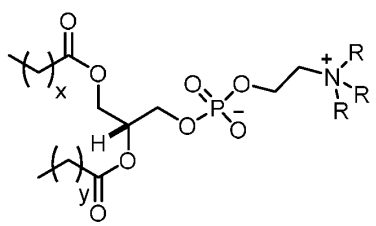
m is an integer between 5 and 10;

20 n is an integer between 5 and 10;

o is an integer between 5 and 10; and

p is an integer between 5 and 10

In another embodiment, (iii) is



25 wherein

x is an integer between 10 and 20;

y is an integer between 10 and 20; and

each R is independently C₁₋₆ alkyl.

In yet another embodiment,

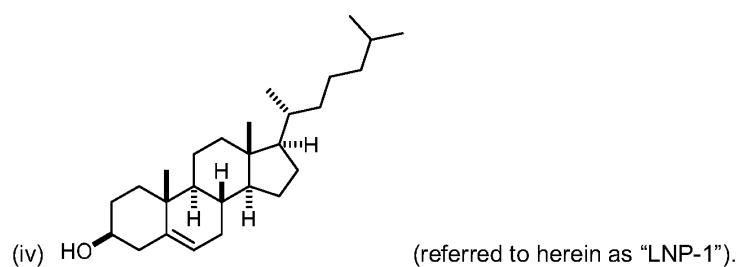
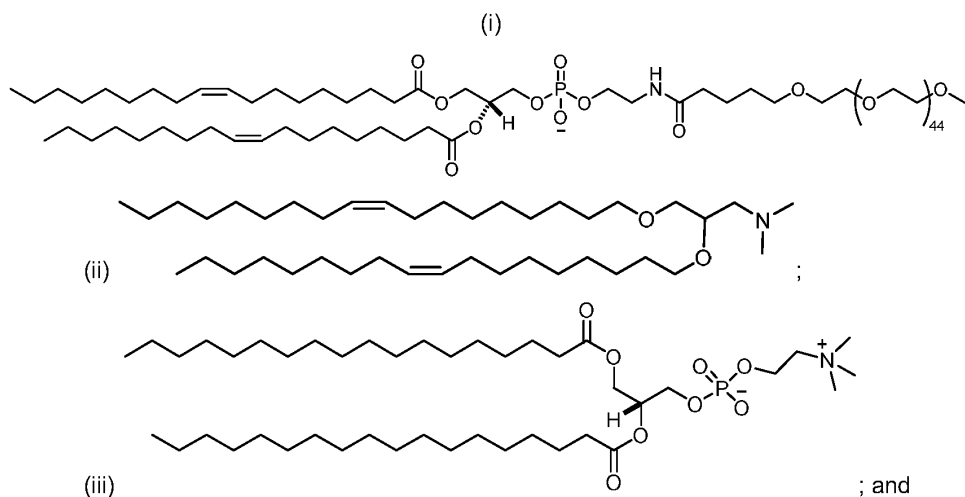
x is an integer between 15 and 20;

y is an integer between 15 and 20; and

each R is independently C₁₋₃ alkyl.

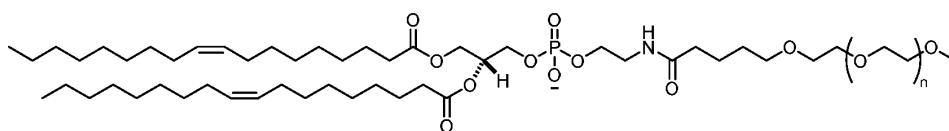
In still another embodiment, the lipid nanoparticle comprises

5



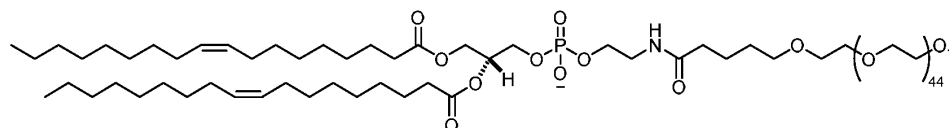
10

In an embodiment, (i) is



wherein n is an integer from 40 to 50.

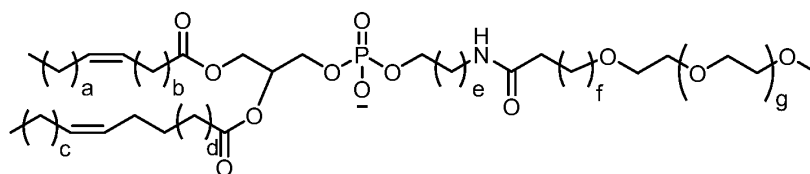
In an embodiment, (i) is



15

In another aspect, provided herein is a lipid nanoparticle comprising:

(i) a PEG-conjugated phospholipid (PEG-phospholipid) of Formula A:

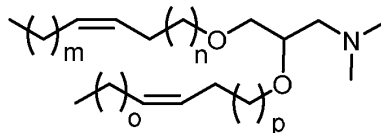


A;

wherein

- a is an integer between 1 and 10;
 b is an integer between 1 and 10;
 c is an integer between 1 and 10;
 d is an integer between 1 and 10;
 e is an integer between 1 and 5;
 f is an integer between 1 and 5; and
 g is an integer between 10 and 50;

(ii) an ionizable lipid of Formula B:

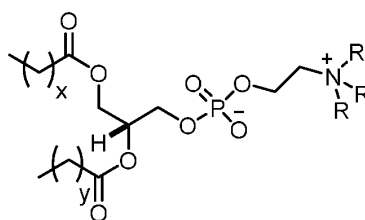


B;

wherein

- m is an integer between 1 and 10;
 n is an integer between 1 and 10;
 o is an integer between 1 and 10; and
 p is an integer between 1 and 10;

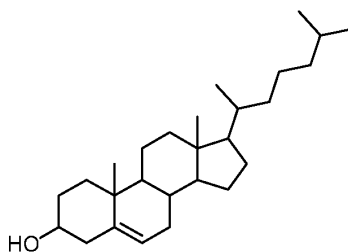
(iii) a non-cationic phospholipid of Formula C:



C;

wherein

- x is an integer between 10 and 20;
 y is an integer between 10 and 20; and
 each R is independently C₁₋₆ alkyl; and
 (iv) a sterol of Formula D:



D.

In an embodiment, in Formula A:

a is an integer between 5 and 10;

5 b is an integer between 5 and 10;

c is an integer between 5 and 10;

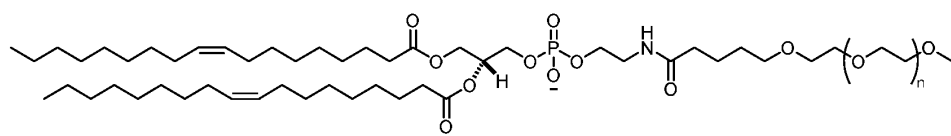
d is an integer between 1 and 5;

e is an integer between 1 and 3;

f is an integer between 1 and 5; and

10 g is an integer between 30 and 50.

In another embodiment, Formula A is



wherein n is an integer from 32 to 50.

In an embodiment of Formula A, n is 40, 41, 42, 43, 44, 45, 46, 47, or 48. In another
15 embodiment of Formula A, n is 44.

In yet another embodiment, in Formula B:

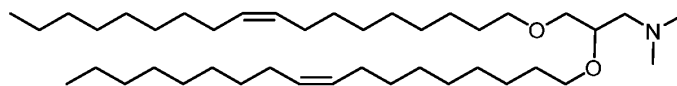
m is an integer between 5 and 10;

n is an integer between 5 and 10;

o is an integer between 5 and 10; and

20 p is an integer between 5 and 10.

In still another embodiment, Formula B is



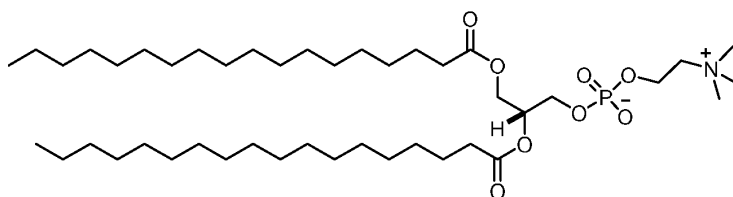
In an embodiment, in Formula C:

x is an integer between 15 and 20;

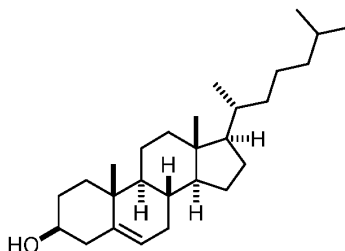
25 y is an integer between 15 and 20; and

each R is independently C₁₋₃ alkyl.

In another embodiment, Formula C is



In yet another embodiment, Formula D is



In an embodiment, provided herein is a pharmaceutical composition comprising the
 5 lipid nanoparticle and a pharmaceutically acceptable carrier.

In another embodiment, provided herein is a drug delivery system comprising the lipid nanoparticle and an active agent. In an embodiment, the drug delivery system is a nucleic acid delivery system comprising nucleic acids encapsulated by lipid nanoparticles.

10 Therapeutic Agents

The lipid nanoparticles provided herein are capable of carrying and delivering many different therapeutic agents. The therapeutic agents carried by lipid nanoparticles provided herein include, but are not limited to: nucleic acids (e.g., messenger RNA (mRNA)), small interfering RNA (siRNA), long non-coding RNA (lncRNA), antisense oligonucleotides,
 15 ribozymes, DNazymes, plasmids, self-amplifying RNA (saRNA), DNA aptamers, gene therapy, microRNAs, RNA decoys, anti-miRs, immunostimulatory RNA (isRNA), and circular RNAs); proteins and peptides (e.g., cyclosporine A, immunoglobulin G, insulin, alpha-lactalbumin, bovine serum albumin, adamantyltripeptides, anti-ovalbumin antibodies, basic fibroblast growth factor, calcitonin, enkephalin, epidermal growth factor receptor,
 20 erythropoietin, gonadorelin, haptides, hemoglobin, horseradish peroxidase, human gamma-globulin, human recombinant EPG, leishmania antigen, leridistim, leuprolide, lysozyme, nerve growth factor, octreotide, progenipoiectin, recombinant malaria protein antigens, streptavidin, superoxide dismutase, TAT peptide, and thymopentin); and chemotherapeutic agents (cisplatin, docetaxel, doxorubicin, etoposide, gemcitabine, mitoxantrone, paclitaxel, retinoic
 25 acid, sorafenib, and zoledronate). The siRNA therapeutic agents include but are not limited to TTR siRNA, ALAS1 siRNA, HAO1 siRNA, ANGPTL3 siRNA, PCSK9 siRNA, and AGT siRNA. The mRNA therapeutic agents include but are not limited to mRNA-1273 (also known

as Spikevax) and BNT162b2 (also known as Comirnaty), the coron
as CRISPR gene-editing therapies (wherein the guide RNA (gRNA) can be a single guide
RNA (sgRNA) or gRNA plasmid and the Cas protein can be a Cas protein, Cas plasmid, or
Cas mRNA).

- 5 In certain embodiments, the therapeutic agent carried by the lipid nanoparticle is an
anionic drug agent. Examples of the anionic pharmacologically active substance useful in
the present invention include bortezomib, methotrexate, olopatadine, tiotropium, ipratropium,
glycopyrronium, acridinium, umecclidinium, trospium, alendronic acid, ibandronic acid,
incadronic acid, pamidronic acid, risedronic acid, zoledronic acid, etidronic acid, clodronic
10 acid, tiludronic acid, olpadronic acid, neridronic acid, diclofenac, levocabastine,
indomethacin, ibuprofene, flurbiprofen, fenoprofen, ketoprofen, naproxene, diclofenac,
etodolac, sulindac, tolmetin, salicylic acid, diflunisal, oxaprozin, tiagabine, gabapentin,
ciprofloxacin, levofloxacin, fusidic acid, aminolevulinic acid, aminocaproic acid, isopropamide
iodide, trihexethyl chloride, cephalixin, aspirin, indoprofen, levodopa, methyl dopa,
15 zomepirac, cefamandole, alclofenac, mefenamic acid, flufenamic acid, lisinopril, enalapril,
enalaprilat, captopril, ramipril, fosinopril, benazepril, quinapril, temocapril, cilazapril,
valsartan, valproic acid, cromoglicic acid, tranilast, pantothenic acid, metiazinic acid,
fentiazac, fenbufen, pranoprofen, loxoprofen, dexibuprofen, alminoprofen, tiaprofenic acid,
aceclofenac, nalidixic acid, azelaic acid, mycophenolic acid, leucovorin, ethacrynic acid,
20 tranexamic acid, ursodeoxycholic acid, folic acid, meclofenamic acid, carbenicillin,
rebamipide, cetirizine, fexofenadine, letosteine, probenecid, hopantenic acid, baclofen,
furosemide, piretanide, methyl dopa, pravastatin, liothyronine, levothyroxine, minodronic acid,
P-aminosalicylic acid, gluconic acid, biotin, liraglutide, exenatide, taspoglutide, albiglutide,
lixisenatide, interferon alpha, interferon beta, interferon gamma, glucagon-like peptides,
25 adrenocorticotrophic hormone, insulin and insulin-like growth factors, parathyroid hormone
and its fragments, darbepoetin alpha, epoetin alpha, epoetin beta, epoetin delta, infliximab,
insulin, glucagon, glucagon-like peptides, thyrotropin hormone, thyroid stimulating hormone,
parathyroid hormone, calcitonin, adrenocorticotrophic hormone (ACTH), follicle stimulating
hormone, chorionic gonadotropin, gonadotropin releasing hormone, somatropin, GRF,
30 lyppressin, luteinizing hormone, interleukin, growth hormone, prostaglandin, platelet-derived
growth factors (PDGF), keratinocyte growth factors (KGF), fibroblast growth factors (FGF),
epidermal growth factors (EGF), transforming growth factor- α (TGF- α), transforming growth
factor- β (TGF- β), erythropoietin (EPO), insulin-like growth factor-I (IGF-I), insulin-like growth
factor-II (IGF-II), tumor necrosis factor- α (TNF- α), tumor necrosis factor- β (TNF- β), colony
35 stimulating factors (CSF), granulocyte colony stimulating factor (G-CSF), granulocyte
macrophage colony stimulating factor (GM-CSF), vascular cell growth factor (VEGF),
thrombopoietin (TPO), stromal cell-derived factors (SDF), placenta growth factor (PIGF),

hepatocyte growth factor (HGF), glial-derived neurotrophin factor (G factor (CNTF), bone growth factors, bone morphogenetic proteins (BMF), coagulation factors, human pancreas hormone releasing factor, analogues and derivative thereof, pharmaceutically acceptable salts thereof, and a combination thereof.

- 5 Preferably, the anionic pharmacologically active substance may be selected from the group consisting of bortezomib, methotrexate, olopatadine, liraglutide, exenatide, taspoglutide, albiglutide, lixisenatide, interferon alpha, interferon beta, interferon gamma, tiotropium, ipratropium, glycopyrronium, acridinium, umecclidinium, trospium, alendronic acid, ibandronic acid, incadronic acid, pamidronic acid, risedronic acid, zoledronic acid, etidronic
10 acid, clodronic acid, tiludronic acid, olpadronic acid, neridronic acid, glucagon-like peptides, adrenocorticotrophic hormone, insulin and insulin-like growth factors, parathyroid hormone and its fragments, darbepoetin alpha, epoetin alpha, epoetin beta, epoetin delta, diclofenac, levocabastine, indomethacin, ibuprofene, flurbiprofen, fenoprofen, ketoprofen, naproxene, diclofenac, etodolac, sulindac, tolmetin, salicylic acid, diflunisal, oxaprozin, tiagabine,
15 gabapentin, ciprofloxacin, levofloxacin, fusidic acid, aminolevulinic acid, a pharmaceutically acceptable salt thereof, and a combination thereof.

In certain embodiments, self-amplifying RNA (saRNA) may also be referred to as self-replicating RNA, replication-competent RNA, replicons or RepRNA. RepRNA, referred to as self-amplifying mRNA when derived from positive-strand viruses, is generated from a viral
20 genome lacking at least one structural gene; it can translate and replicate (hence, "self-amplifying") without generating infectious progeny virus. In certain embodiments, the RepRNA technology may be used to insert a gene cassette encoding a desired antigen of interest. For example, the alphaviral genome is divided into two open reading frames (ORFs): the first ORF encodes proteins for the RNA dependent RNA polymerase (replicase),
25 and the second ORF encodes structural proteins. In saRNA vaccine constructs, the ORF encoding viral structural proteins may be replaced with any antigen of choice, while the viral replicase remains an integral part of the vaccine and drives intracellular amplification of the RNA after immunization.

30 **Methods of Delivering Therapeutic Agents**

The present disclosure provides methods of delivering a therapeutic and/or prophylactic to a mammalian cell, tissue, or organ. Provided herein is a method of delivering a therapeutic and/or prophylactic to a cell by administering a nanoparticle composition including the therapeutic and/or prophylactic to a subject, where administration of the
35 composition involves contacting the cell with the composition. For example, a protein, cytotoxic agent, radioactive ion, chemotherapeutic agent, nucleic acid (such as an RNA, e.g., mRNA), or gene editing system may be delivered to a cell or organ. In the instance that

the therapeutic and/or prophylactic is a CRISPR system, the guide
guide RNA (sgRNA) or gRNA plasmid and the associated DNA modifying enzyme(s) can be
delivered as a protein, plasmid, or mRNA). If the therapeutic and/or prophylactic is an
mRNA, upon contacting a cell with the nanoparticle composition, a translatable mRNA may
5 be translated in the cell to produce a polypeptide of interest. However, mRNAs that are
substantially not translatable may also be delivered to cells. Substantially non-translatable
mRNAs may be useful as vaccines and/or may sequester translational components of a cell
to reduce expression of other species in the cell.

The compositions and methods of the present disclosure are used to treat a wide
10 variety of cell types, in vivo and in vitro. Suitable cells include, but are not limited to,
hematopoietic precursor (stem) cells, fibroblasts, keratinocytes, hepatocytes, endothelial
cells, skeletal and smooth muscle cells, osteoblasts, osteoclasts, pancreatic endocrine cells
(alpha cells, beta cells, PP cells, delta cells, and epsilon cells), pituitary endocrine cells,
neurons, quiescent or activated lymphocytes, terminally differentiated cells, slow or
15 noncycling primary cells, parenchymal cells, lymphoid cells, epithelial cells, bone cells, and
the like. In one embodiment, an nucleic acid, such as one or more nucleic acid molecules
(e.g., an interfering RNA (e.g., siRNA) or mRNA) is delivered to cancer cells such as, e.g.,
lung cancer cells, colon cancer cells, rectal cancer cells, anal cancer cells, bile duct cancer
cells, small intestine cancer cells, stomach (gastric) cancer cells, esophageal cancer cells,
20 gallbladder cancer cells, liver cancer cells, pancreatic cancer cells, appendix cancer cells,
breast cancer cells, ovarian cancer cells, cervical cancer cells, prostate cancer cells, renal
cancer cells, cancer cells of the central nervous system, glioblastoma tumor cells, skin
cancer cells, lymphoma cells, choriocarcinoma tumor cells, head and neck cancer cells,
osteogenic sarcoma tumor cells, and blood cancer cells.

25 In vivo delivery of lipid nanoparticles encapsulating one or more nucleic acid
molecules (e.g., interfering RNA (e.g., siRNA) or mRNA) is suited for targeting cells of any
cell type. The methods and compositions can be employed with cells of a wide variety of
vertebrates, including mammals, such as, e.g., canines, felines, equines, bovines, ovines,
caprines, rodents (e.g., mice, rats, and guinea pigs), lagomorphs, swine, and primates (e.g.,
30 monkeys, chimpanzees, and humans).

A nanoparticle composition including one or more therapeutic and/or prophylactic can
be administered by any route. In some embodiments, compositions, including prophylactic,
diagnostic, or imaging compositions including one or more nanoparticle compositions
described herein, are administered by one or more of a variety of routes, including oral,
35 intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, subcutaneous,
intraventricular, trans- or intra-dermal, interdermal, rectal, intravaginal, intraperitoneal,
intraocular, subretinal, intravitreal, topical (e.g., by powders, ointments, creams, gels, lotions,

and/or drops), mucosal, nasal, buccal, enteral, vitreal, intratumoral, intratracheal instillation, bronchial instillation, and/or inhalation; as an oral spray and/or powder, nasal spray, and/or aerosol, and/or through a portal vein catheter. In some embodiments, a composition may be administered intravenously, intramuscularly, 5 intradermally, intra-arterially, intratumorally, subcutaneously, intraocularly, subretinally, intravitreally, or by inhalation. However, the present disclosure encompasses the delivery or administration of compositions described herein by any appropriate route taking into consideration likely advances in the sciences of drug delivery. In general, the most appropriate route of administration will depend upon a variety of factors including the nature 10 of the nanoparticle composition including one or more therapeutic and/or prophylactic (e.g., its stability in various bodily environments such as the bloodstream and gastrointestinal tract), the condition of the patient (e.g., whether the patient is able to tolerate particular routes of administration), etc.

15 **Treating Disease with Therapeutic Agents Delivered by Lipid Nanoparticles**

The nanoparticle compositions provided herein can be useful for treating a disease, disorder, or condition. Such compositions can be useful in treating a disease, disorder, or condition characterized by missing or aberrant protein or polypeptide activity. Examples of such human diseases include X-linked severe combined immunodeficiency (X-SCID) and 20 adrenoleukodystrophy (X-ALD). X-SCID is caused by one or more mutations in the gene encoding the common gamma chain protein that is a component of the receptors for several interleukins that are involved in the development and maturation of B and T cells within the immune system. X-ALD is caused by one or more mutations in a peroxisomal membrane transporter protein gene called ABCD1. Individuals afflicted with X-ALD have very high 25 levels of long chain fatty acids in tissues throughout the body, which causes a variety of symptoms that may lead to mental impairment or death.

A nanoparticle composition comprising an mRNA encoding a missing or aberrant polypeptide may be administered or delivered to a cell. Subsequent translation of the mRNA may produce the polypeptide, thereby reducing or eliminating an issue caused by the 30 absence of or aberrant activity caused by the polypeptide. Because translation may occur rapidly, the methods and compositions may be useful in the treatment of acute diseases, disorders, or conditions such as sepsis, stroke, and myocardial infarction. A therapeutic and/or prophylactic included in a nanoparticle composition may also be capable of altering the rate of transcription of a given species, thereby affecting gene expression.

35 Diseases, disorders, and/or conditions characterized by dysfunctional or aberrant protein or polypeptide activity for which a composition may be administered include, but are not limited to, rare diseases, infectious diseases (as both vaccines and therapeutics), cancer

and proliferative diseases, genetic diseases (e.g., cystic fibrosis, W
hemophilia, phenylketonuria, and alpha-1-antitrypsin deficiency), autoimmune diseases,
diabetes, neurodegenerative diseases, cardio- and reno-vascular diseases, and metabolic
diseases. Multiple diseases, disorders, and/or conditions may be characterized by missing or
5 substantially diminished protein activity. Such proteins may not be present, or they may be
essentially non-functional. A specific example of a dysfunctional protein is the missense
mutation variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene,
which produce a dysfunctional protein variant of CFTR protein, which causes cystic fibrosis.

Proteins and mRNA can also be presented to treat or prevent endemic diseases,
10 such as those caused by bacteria and viruses. For example, lipid nanoparticle carriers
played an important role in delivering the coronavirus vaccines mRNA-1273 (also known as
Spikevax) and BNT162b2 (also known as Comirnaty). Once each of these treatments was
administered, the mRNA within the vaccine led to the production of the coronavirus spike
protein. Thus, the treatment here did not replace a missing protein, rather it led to the
15 production of a foreign protein which in turn triggered an adaptive immune response.

The present disclosure provides a method for treating such diseases, disorders,
and/or conditions in a subject by administering a nanoparticle composition including an RNA
and a lipid mixture including functional lipid components according to LNP-1, an ionizable
lipid that is unsaturated and cationic at low pH, an optionally unsaturated phospholipid, a
20 PEG lipid (such as L1A), and a structural lipid (such as sterol), wherein the RNA may be an
mRNA encoding a polypeptide that antagonizes or otherwise overcomes an aberrant protein
activity present in the cell of the subject.

The disclosure provides methods involving administering nanoparticle compositions
including one or more therapeutic and/or prophylactic agents and pharmaceutical
25 compositions including the same. The terms therapeutic and prophylactic can be used
interchangeably herein with respect to features and embodiments of the present disclosure.
Therapeutic compositions, or imaging, diagnostic, or prophylactic compositions thereof, may
be administered to a subject using any reasonable amount and any route of administration
effective for preventing, treating, diagnosing, or imaging a disease, disorder, and/or condition
30 and/or any other purpose. The specific amount administered to a given subject may vary
depending on the species, age, and general condition of the subject; the purpose of the
administration; the particular composition; the mode of administration; and the like.
Compositions in accordance with the present disclosure may be formulated in dosage unit
form for ease of administration and uniformity of dosage. It will be understood, however, that
35 the total daily usage of a composition of the present disclosure will be decided by an
attending physician within the scope of sound medical judgment. The specific therapeutically
effective, prophylactically effective, or otherwise appropriate dose level (e.g., for imaging) for

any particular patient will depend upon a variety of factors including of a disorder being treated, if any; the one or more therapeutic and/or prophylactic employed; the specific composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific pharmaceutical composition employed; the duration of the treatment; drugs used in combination or coincidental with the specific pharmaceutical composition employed; and like factors well known in the medical arts.

Nanoparticle compositions including one or more therapeutic and/or prophylactic may be used in combination with one or more other therapeutic, prophylactic, diagnostic, or imaging agents. By "in combination with," it is not intended to imply that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the present disclosure. For example, one or more nanoparticle compositions including one or more different therapeutic and/or prophylactic may be administered in combination. Compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In some embodiments, the present disclosure encompasses the delivery of compositions, or imaging, diagnostic, or prophylactic compositions thereof in combination with agents that improve their bioavailability, reduce and/or modify their metabolism, inhibit their excretion, and/or modify their distribution within the body.

It will further be appreciated that therapeutically, prophylactically, diagnostically, or imaging active agents utilized in combination may be administered together in a single composition or administered separately in different compositions. In general, it is expected that agents utilized in combination will be utilized at levels that do not exceed the levels at which they are utilized individually. In some embodiments, the levels utilized in combination may be lower than those utilized individually.

The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, a composition useful for treating cancer may be administered concurrently with a chemotherapeutic agent), or they may achieve different effects (e.g., control of any adverse effects, such as infusion related reactions).

A nanoparticle composition may be used in combination with an agent to increase the effectiveness and/or therapeutic window of the composition. Such an agent may be, for example, an anti-inflammatory compound, a steroid (e.g., a corticosteroid), a statin, an

estradiol, a BTK inhibitor, an S1P1 agonist, a glucocorticoid receptor, an antihistamine. In some embodiments, a nanoparticle composition may be used in combination with dexamethasone, methotrexate, acetaminophen, an H1 receptor blocker, or an H2 receptor blocker. In some embodiments, a method of treating a subject in need thereof or of delivering a therapeutic and/or prophylactic to a subject (e.g., a mammal) may involve pre-treating the subject with one or more agents prior to administering a nanoparticle composition. For example, a subject may be pre-treated with a useful amount (e.g., 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, or any other useful amount) of dexamethasone, methotrexate, acetaminophen, an H1 receptor blocker, or an H2 receptor blocker. Pre-treatment may occur 24 or fewer hours (e.g., 24 hours, 20 hours, 16 hours, 12 hours, 8 hours, 4 hours, 2 hours, 1 hour, 50 minutes, 40 minutes, 30 minutes, 20 minutes, or 10 minutes) before administration of the nanoparticle composition and may occur one, two, or more times in, for example, increasing dosage amounts.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the disclosure described herein. The scope of the present disclosure is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

Formulations

Formulations suitable for oral administration can consist of: (a) liquid solutions, such as an effective amount of a packaged therapeutic agent such as nucleic acid (e.g., interfering RNA, mRNA, or DNA) suspended in diluents such as water, saline, or PEG 400; (b) capsules, sachets, or tablets, each containing a predetermined amount of a therapeutic agent such as nucleic acid (e.g., interfering RNA, mRNA, or DNA), as liquids, solids, granules, or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise a therapeutic agent such as nucleic acid (e.g., interfering RNA, mRNA, or DNA) in a flavor, e.g., sucrose, as well as pastilles comprising the therapeutic agent in an inert base, such as gelatin and glycerin or sucrose and acacia emulsions, gels, and the like containing, in addition to the therapeutic agent, carriers known in the art.

In another example of their use, lipid nanoparticles can be incorporated into a broad range of topical dosage forms. For instance, a suspension containing nucleic acid-lipid

nanoparticles can be formulated and administered as gels, oils, emulsions, pastes, ointments, lotions, foams, mousses, and the like.

EXAMPLES

5 Exemplary Materials and Methods

Reagents: All chemicals, cell culture media, and commercial antibodies were purchased from Thermo Fisher Scientific (Waltham, MA), and cell culture consumables from CELLTREAT® Scientific Products (Shirley, MA) and Thermo Fisher Scientific, unless otherwise stated. All mRNAs and guide RNAs were synthesized by GenScript (Piscataway, NJ), except GFP mRNA from System Biosciences (Palo Alto, CA) and siRNA targeting mouse ApoB from Thermo Fisher Scientific. Phospholipids, polysorbates, and excipients were purchased from Avanti Polar Lipids Inc./CRODA (Alabaster, AL), Lysan Bio Inc. (Arab, AL), Cayman Chemical Company (Ann Arbor, MI), and NOF Corporation (White Plains, NY), unless otherwise specified. Sterol standard for clinical use was purchased from MP Biomedicals (Solon, OH).

L1A PEG-lipid synthesis: PEG-phospholipid L1A was synthesized at CodeBridgeBio Lab (Woburn, MA). Briefly, Starting Material 1 (SM1) (exemplary: 2-aminoethyl ((R)-2-3bis-(oleoyloxy)propyl) phosphate, DOPE) was dissolved in a solution of Starting Material 2 (SM2) (exemplary: mPEG-SVA Methoxy Poly(Ethylene Glycol) Succinimidyl Valerate, mPEG-SVA) in Chloroform at a mol ratio of 1.1:1, followed by the slow addition of Triethyl amine (10% mol, TEA). The resulting solution mixture was stirred gently overnight at 25°C. Thin-layer chromatography (TLC) with chloroform/methanol/water (100:10:2, v:v:v) was used to monitor the reaction. When the broad spot of the SM2 disappeared and subsequently a new polar well-defined spot appeared, the solvent was evaporated. Acetonitrile was added to dissolve the white-pale yellow powder, and the resulting solution mixture was kept at -20°C overnight (4°C can be also used). After centrifugation at 14,000 rpm (>5000 rpm can be also used) for 30 minutes, the upper solution without insoluble SM1 was collected and lyophilized at -80°C overnight. The lyophilized white powder was analyzed for identity, purity, and integrity by proton (¹H) NMR, LC-MS (ESI) and HPLC with ELSD High resolution mass spectroscopy negative ion mode method.

LNP-1 lipid nanoparticle preparation: PEG-phospholipid (using L1A as an exemplary presentation) and together with the other three complementary lipid components were used for engineering the LNP-1 LNP Platform Technology (e.g., four lipid components [Ionizable Lipid:Cholesterol:DSPC:L1A = 50:39.25:10:0.75 mol%]). In brief, LNPs were prepared by mixing an ethanol phase containing lipids with RNA in an aqueous phase in an Ignite NanoAssemblr microfluid mixing device (Precision Nanosystem Inc., Vancouver,

British Columbia, Canada) following the manufacturer's instructions prepared by solubilizing a mixture of ionizable lipid (e.g., DODMA, DODAP, DLin-DMA, DLin-MC3-DMA, ALC-0315, or SM-102), cholesterol, DSPC, and L1A at a predetermined molar ratio in ethanol. The aqueous phase was prepared in 25 mM citrate buffer (pH 3.75 ± 0.25) with RNA. Syringe pumps were used to mix the aqueous and ethanol phases at a ratio of 3:1. The resulting RNA-LNPs were first diafiltrated in citrate buffer (pH 4.0) to remove ethanol and strength the lipo-complex binding and then dialyzed twice against PBS (pH 7.4) for ≥12 hours and 4 hours, respectively. The final RNA-LNPs drug product formulation buffer solution (pH 7.4 ± 0.2, -18.7 ± 0.8 mV at 25°C) contains 10.14 mM Sodium Phosphate Dibasic, 1.76 mM Potassium Phosphate Monobasic, 2.7 mM Potassium Chloride, 137 mM Sodium Chloride, and Nuclease-free Water (not DEPC-treated).

For Figure 5: ApoB siRNA/LNPs were prepared as described above, except using varied molar ratios of four lipid components (DODMA:Cholesterol:DSPC:L1A).

For Figure 6: ApoB siRNA/LNPs were prepared as described above, except using different microfluid buffers (Exemplary: citrate buffer versus acetate buffer; and a fixed buffer at different concentrations (Exemplary: 25-100mM) to evaluate the effect of ionic strength on the making of LNPs). The buffers used here are listed in the bottom.

Encapsulation efficiency quantification of RNAs/ LNP-1 lipid nanoparticles: To quantify the RNAs loaded into LNP-1 LNPs and to determine the RNAs encapsulation efficiency, OD Nanodrop One spectroscopy for preparing stock solution at high concentration and the highly sensitive read-out Quant-iT RiboGreen RNA assay from Thermo Fisher Scientific were used according to the manufacturer's protocols. An exemplary RNA payload quantification method with technical details is shown in Figure 7.

Physical characterization of RNAs/LNP-1 lipid nanoparticles formulations: Size distribution and Zeta potential of RNAs/LNP-1 LNPs formulations were determined by Dynamic Light Scattering (DLS) using a Malvern Nano Zetasizer Ultra Red (Malvern Panalytical Inc, Westborough, MA). For size measurements, RNAs/LNP-1 LNPs were diluted 1:15 in PBS (pH 7.20). For Zeta potential (Charge) measurements, RNAs/LNP-1 LNPs were diluted 1:50 in Nuclease-Free Water (not DEPC-Treated).

Chemical characterization of RNAs/LNP-1 lipid nanoparticles formulations: Lipid composition of RNAs/LNP-1 LNPs formulations was determined by HPLC-ELSD/CAD; PEG-phospholipid L1A's identity was confirmed by 1D proton NMR spectrum and LC-MS (ESI), L1A's integrity was confirmed by HRMS negative ion mode method HPLC-ELSD, and L1A's purity was confirmed by HPLC-ELSD; all through external CRO services provided by Pace Analytical Life Sciences (Woburn, MA).

Morphology and lamellarity characterization of RNAs/L

formulations: This was analyzed using Cryo Transmission Electron Microscopy (Cryo-TEM) imaging through external CRO services provided by Nanolmaging Services Inc. (San Diego, CA).

5 **Cell culture:** Jurkat human T leukemia cells, THP-1 human monocytic leukemia cells and HCC1739BL human lymphoma cells were cultured in RPMI-1640 and HepG2 human liver cancer cells in EMEM, supplemented with 10% FBS, 1% penicillin-streptomycin, and were maintained in culture flasks at 37°C in a 5% CO₂ atmosphere at 100% humidity (cell culture incubator). For eGFP mRNA/LNP-1 LNPs studies, cells were seeded in a 96-well
10 plate and then incubated with eGFP mRNA/LNP-1 LNPs in a cell culture incubator. At the end of the study, intracellular GFP expression signal was evaluated by flow cytometry (for Jurkat, THP-1 and HCC1739BL cells) or by fluorescence microscopy (for HepG2 cells). Cells without LNPs treatment served as untreated controls.

Ex vivo CAR-T generation: T cells purified from human PBMCs were activated for 7
15 days in a cell culture incubator. On day 8, cells were transfected with CAR (eGFP) mRNA loaded LNP-1 LNPs for 48 hours, then CAR expression was examined as intracellular GFP signal by flow cytometry, in parallel with CAR-T cell viability assay.

In vivo CAR-T generation: NSG mice were reconstituted with 20*10⁶ of human
20 PBMCs, followed by intravenous (i.v) administration of CAR (eGFP) mRNA loaded LNP-1 LNPs (3 mg/kg) on day 7 post engraftment. Mouse blood was collected on days 2 and 4 post transfection and analyzed by flow cytometry for in vivo CAR expression (intracellular GFP signal) in human CD45+ cell populations.

Hematopoietic stem cells differentiation to erythroblast cells: C57BL6 mouse bone marrow cells were cultured in the presence of erythrocyte differentiation media
25 including cytokines (mIL-3/mEPO/mCSF) in a cell culture incubator for 7 days. Cells were then incubated with eGFP mRNA loaded LNP-1 LNPs for 48 hours, followed by flow cytometry analysis of intracellular GFP expression.

In vitro immune cell activation: Freshly purified mouse PBMCs incubated with
30 100ng (=1µg/ml) of eGFP mRNA loaded LNP-1 LNPs in the presence of cytokines (mM-CSF/mGM-CSF/mL-4) in a cell culture incubator for 48 hours, then gene delivery potency was examined as intracellular GFP expression detected by flow cytometry.

Tail vein injection of RNAs/LNP-1 lipid nanoparticles and tissue processing:
Eight to 12 weeks old C57BL/6 mice were intravenously (i.v) administrated with a single
dose of LNP-1 LNPs loaded with RNA payloads such as ApoB siRNA or eGFP mRNA at 10
35 µL/gram body weight with dosages ranging from 0.1 mg/kg to 10 mg/kg. RNAs/LNP-1 LNPs were slowly injected through the mouse tail vein over 30 seconds using a 28G needle. 48

hours later, potential target organs such as liver, spleen, kidneys, fi
and blood were harvested for subsequent analyses.

Assessment of ApoB siRNA/LNP-1 LNPs in vivo knockdown efficiency: Tissue
lysates were prepared from frozen livers as described above and then subjected for Western
5 Blotting of mouse ApoB proteins. Na/K ATPase, eRF1 or GAPDH served as the internal
loading control. Knockdown (KD) efficiency is calculated as the percentage (%) decrease of
ApoB protein levels in treated livers in contrast to untreated livers.

Visualization of GFP mRNA expression in LNP-1 LNPs target organs: Freshly
harvested bone marrow was made into single-cell suspensions and then dropped onto glass
10 slides, covered with coverslips, and examined under a fluorescence microscope. Frozen
sections of liver and spleen were examined for in situ intracellular GFP expression by
fluorescence microscopy.

Spike mRNA/LNP-1 COVID-19 vaccine production: COVID-19 coronavirus Spike
mRNA (benchmark Moderna mRNA-1273)/LNP-1 LNPs (5µg in 50µL PBS) were
15 intramuscularly (IM) or subcutaneously (SC) injected into C57BL/6 or BALB/c mice on days
0 (the prime dose) and 21 (the boost dose). On days 14 (Prime), 28 (Boost-d7) and 44
(Boost-d23) for C57BL/6 mice or on days 28 (Post-Boost 1w), 35 (Post-Boost 2w) and 49
(Post-Boost 4w) for BALB/c mice, mouse blood were collected and examined for anti-Spike
protein-RBD titres by ELISA. Mice received 50µL PBS served as the negative control
20 (labeled as PBS Ctrl or NC).

Example 1: An exemplary composition of the herein described lipid nanoparticle
gene delivery platform technology known as LNP-1 (Figure 1). In this embodiment, LNP-1
structural lipids include L1A (a PEG-phospholipid having two C18 aliphatic chains with one
unsaturation bond in the fatty acid chain), DODMA (an ionizable lipid having two C18
25 aliphatic chains with one unsaturated site and a head group that protonates to a cationic
moiety having a pKa of 5-7), DSPC (a non-cationic helper phospholipid having two saturated
C18 aliphatic chains and a head group that remains as a zwitterion or anion across a pH
range of 6 to 8), and cholesterol (a cell membrane stabilizer helper sterol lipid). An
exemplary formulation buffer solution for RNA-LNPs drug product is also shown.

30 **Example 2:** A working example of the lipid composition and identity of the LNP-1
platform (Figure 2). The lipid composition of LNP-1 formulations loaded with eGFP/mRNA
was successfully confirmed at the expected values – the assay mmol% of each lipid
component is comparable to the nominal value of their masses. Separation of four lipid
components of LNP-1 formulations is obtained by UPLC-ELSD method.

35 **Example 3:** Confirmation of PEG-phospholipid L1A's synthesis, identity, purity and
integrity (Figure 3). PEGylated L1A was successfully synthesized – TLC Plate indicates that
the Rxn of SM1 and SM2 took place and the PEGylated L1A spot was identified. PEGylated

L1A was successfully identified at the expected mass from proton (integrity confirmed with no impurity detected. RT shifts are predictable with sequential L1A PEGylation. L1A's PEGylation states (32 to 44 PEGs) was also confirmed.

Example 4: Working examples of LNP-1 platform's high compatibility of PEG-conjugated phospholipid and flexibility with structural ionizable lipid (IL) component (Figure 4). In vivo gene silencing potency of the exemplary LNP-1 formulation – [DODMA:Cholesterol:DSPC:L1A = 50:39.25:10:0.75 mol%] was compared to that of several other IL components clinical and commercially available with L1A, using ApoB siRNA as the payload. After 4 months storage at 2-8°C, all LNP formulations were intravenously (i.v) administrated (1 mg/kg) into C57BL/6 mice. 48 hours later, ApoB protein knockdown efficiency was evaluated by Western Blotting of liver tissue lysates. Livers from untreated mice served as controls for knockdown efficiency calculation – percentage (%) decrease of ApoB protein levels (including both monomeric and dimeric forms) in treated livers in contrast to untreated livers. GAPDH protein was used as the loading control. PEGylated L1A is highly compatible with DLin-DMA, DLin-MC3-DMA (Onpatro/Alnylam's IL component), ALC-0315 (Comirnaty/Pfizer-BioNTech's IL component), and SM-102 (Spikevax/Moderna's IL component) – all these new L1A LNP formulations achieved potent >70% knockdown efficiency similar to LNP1 (the exemplary LNP-1 formulation). The detailed lipid structures and formula as well as size and Zeta Potential measurements of all L1A LNP formulations are also shown.

Example 5: Working examples of LNP-1 platform's flexibility with molar ratios of four lipid components (Figure 5). The in vivo gene silencing potency of the LNP-1 delivery platform was tested using varied molar ratios of four lipid components for LNP preparation (e.g., using ApoB siRNA as the payload and liver as the target organ). In vivo ApoB protein knockdown efficiency was examined as described in Example 4. In the liver, new LNP formulations V1, V2 and V5 achieved >70% knockdown efficiency similar to LNP1 (the exemplary LNP-1 formulation); and V3, V6, V7 and V8 also achieved >30% knockdown efficiency. The exact molar ratios of four lipid components as well as size and Zeta Potential measurements for all LNP formulations are also listed.

Example 6: Working examples of LNP-1 platform's flexibility with microfluid buffer (Figure 6). In vivo gene silencing potency of the LNP-1 delivery platform was tested using different microfluid buffers as well as a fixed buffer at different concentrations to evaluate the effect of ionic strength on the making of LNP (e.g., using ApoB siRNA as the payload and liver as the target organ). In vivo ApoB protein knockdown efficiency was examined as described in Example 4. The microfluid buffers used for all LNP formulations are listed in the bottom. 1-GV Mix L1A (LNP23) achieved >70% knockdown efficiency similar to LNP1 (the

exemplary LNP-1 formulation); 2-GV Mix L1A (LNP24) and 3-GV M
>50% knockdown efficiency.

Example 7: Working examples of RNA payload assay for LNP-1 LNPs (Figure 7).

The fluorescent Quanti-iT RNA RiboGreen Quantification method was used. OD
5 measurements by NanoDrop One, Standard Curve formatting, and detailed technical
procedures are shown.

Example 8: LNP-1 formulations demonstrated increased stability at room
temperature (25°C) (Figure 8). The stability of the LNP-1 delivery formulation was compared
to that of Alnylam's IL component with L1A and Alnylam's ONPATTRO at room temperature
10 (25°C). Both combinations using Alnylam components precipitated out of solution, with
Alnylam's ONPATTRO crashing out more quickly (~7 months) than the combination Alnylam
IL component with L1A (~9 months). The LNP-1 formulation was able to remain in solution
for an extended period of time (>12 months) indicating that it is more stable than the other
two formulations.

15 **Example 9** shows that LNP-1 platform's PEGylated L1A is compatible with other
ionizable lipids, e.g., DLin-MC3-DMA in Alnylam's Onpattro LNP formulations (Figure 9).
PEGylated L1A LNPs have smaller sizes and are both structurally and functionally stable.
Whereas in contrast, the new LNPs combining Alnylam/Onpattro's PEG(2K)-DMG with
DODMA have no function and are only structurally stable.

20 **Example 10:** A working example of LNP-1's long-term structural lipids stability and
quantification (Figure 10). eGFP mRNA/LNP-1s are stable for long-term storage at 2-8°C –
the lipid composition and total lipid content of 9 months old formulations are comparable to
that of freshly-formulated.

Example 11: Working examples of LNP-1's long-term physicochemical stability
25 (Figure 11). When loaded with different RNA payloads, LNP-1 formulations are stable for
long-term storage at different temperatures (2-8°C or 25°C), as indicated by size distribution
and dispersity assessments by Z-Avg size spectra using the Zetasizer Dynamic Light
Scattering (DLS) system from Malvern Panalytical. LNP-1 platform's PDI (polydispersity
index, a leading indicator of shelf-life stability as well as in vivo stability and efficacy of
30 nucleic acid delivery) typically goes 0.01-0.07 (PDI<0.1). For other lipid nanoparticles clinical
and commercially available PDI goes 0.1-0.3 (PDI>0.1).

Example 12: A working example of LNP-1's long-term physicochemical stability
(Figure 12). ApoB siRNA/LNP-1s are stable for long-term storage at different temperatures
(2-8°C or 25°C), as indicated by the size homogeneity during the course of long-term
35 stability study.

Example 13: Different stock concentrations of ApoB siRNA/LNP-1s are stable for
long-term storage at different temperatures (Figure 13). ApoB siRNA/LNP-1 formulations

were prepared at four stock concentrations – 0.1, 0.3, 0.6 and 1.0 r
2-8°C or 25°C for long-term stability study. In vivo potency was first confirmed by Western
Blotting of livers from C57BL/6 mice received different dosages of ApoB siRNA/LNP-1s (a
single i.v injection; 1, 3, 6 or 10 mg/kg). These four LNPs stocks were also sampled at
5 various time points for size distribution and dispersity assessments by Z-Avg size spectra.
Data are shown as time course of size homogeneity at 2-8°C or 25°C.

Example 14: A working example of LNP-1's long-term physicochemical stability
(Figure 14). ApoB siRNA/LNP-1s are stable for long-term storage at 40°C, as indicated by
the size homogeneity and distribution.

10 **Example 15:** Working examples of LNP-1's long-term morphology stability (Figure
15). When loaded with different RNA payloads, LNP-1 formulations morphology remains
stable for long-term storage at 2-8°C, as indicated by the Cryo-TEM images.

Example 16: Summary of payloads versatility and long-term stability of the LNP-1
technology platform (Figure 16). LNP-1 formulations can encapsulate varied types and sizes
15 of nucleic acids payloads and remain stable for long-term storage at 2-8°C.

Example 17: A working example of LNP-1's long-term function stability (Figure 17).
eGFP mRNA/LNP-1s are functionally stable for long-term storage at different temperatures
(2-8°C or 25°C) – the CAR-T generation potency of 9 months old formulations is comparable
to that of freshly-formulated.

20 **Example 18:** A working example of LNP-1's long-term function stability (Figure 18).
eGFP mRNA/LNP-1s are functionally stable for long-term storage at different temperatures
(2-8°C or 25°C) – the gene delivery potency of 9 months old formulations is comparable to
that of freshly-formulated.

Example 19: A working example of LNP-1's long-term function stability (Figure 19).
25 LNP-1's gene editing potency using CRISPR-Cas9-mediated knockout of TCR α receptor in
Jurkat T cells was examined by flow cytometry. Cas9 mRNA + TRAC sgRNA/LNP-1s are
functionally stable for long-term storage at 2-8°C – the knockout potency of 3 months old
formulations is comparable to that of freshly-formulated.

Example 20: A working example of LNP-1's long-term function stability (Figure 20).
30 ApoB siRNA/LNP-1's in vivo gene silencing potency during long-term storage was evaluated
by Western Blotting of liver ApoB protein levels. ApoB siRNA/LNP-1s are functionally stable
for long-term storage at different temperatures (2-8°C or 25°C) – the gene silencing potency
of aged formulations is comparable to that of freshly-formulated (all achieved >70%
knockdown efficiency).

35 **Example 21:** Working examples of LNP-1's versatile therapeutic applications – gene
silencing (Figure 21). Marked in vivo ApoB protein knockdown efficiency was achieved in the
liver tissues from C57BL/6 mice i.v administrated with a single dose of ApoB siRNA/LNP-1s

(1, 3, 6, or 10 mg/kg). Mock siRNA/LNP-1s (1 mg/kg) served as the ATPase or eRF1 protein was used as the loading control.

Example 22: In vivo potency of different batches of stable ApoB siRNA/LNP-1 formulations are consistent (Figure 22). Five different batches of ApoB siRNA/LNP-1s were prepared and i.v administrated into C57BL/6 mice (1 mg/kg). 48 hours later, mouse livers were harvested, and knockdown (KD) efficiency was evaluated as detailed in Example 4. eRF1 protein was used as the loading control. All five batches of ApoB siRNA/LNP-1 achieved $\geq 70\%$ KD efficiency.

Example 23: A working example of LNP-1's versatile therapeutic applications – mRNA therapy and target organs (e.g., the liver, spleen and bone marrow) (Figure 23). C57BL/6 mice received a single i.v injection of 3 mg/kg GFP mRNA/LNP-1s. 48 hours later, GFP expression in different tissues were visualized by fluorescence microscopy. Tissues from untreated mice served as negative controls. The liver, spleen and bone marrow showed strong GFP signals. Specifically, strong GFP signal is seen in the liver parenchyma and areas around germinal center in the spleen. Also notably, typical evaluations of in vivo mRNA delivery potency by other LNP technologies are often using luciferase luminescence in vivo imaging, which is more sensitive than GFP fluorescent signal.

Example 24: A working example of LNP-1's versatile therapeutic applications – immune cell engineering (e.g., chimeric antigen receptor (CAR) expression) (Figure 24). LNP-1's potency delivering CAR (eGFP) mRNA to activated immune cells is demonstrated – 48 hours after treatment with 1 $\mu\text{g/ml}$ of CAR (eGFP) mRNA/LNP-1s, intracellular GFP signal is chiefly seen in antigen presenting cells (APC), monocytes/macrophages, and CD4 T cells.

Example 25: A working example of LNP-1's versatile therapeutic applications – ex vivo CAR-T cell engineering (Figure 25). High-quality transient CAR-T cells are ex vivo generated by CAR (eGFP) mRNA/LNP-1s – 80-90% transfection efficiency achieved in the 4-16 $\mu\text{g/ml}$ dose groups with over 80% CAR-T cell viability in all groups.

Example 26: A working example of LNP-1's versatile therapeutic applications – in vivo CAR-T cell engineering (Figure 26). Potent in vivo CAR-T cell generation is achieved by CAR (eGFP) mRNA/LNP-1s. Approximately 16-25% transfection efficiency is seen herein (whereas it's generally $<15\%$ in the literature); and persistent in vivo CAR expression is noted at day 4 post transfection.

Example 27: A working example of LNP-1's versatile therapeutic applications – stem cell engineering (e.g., hematopoietic stem cells) (Figure 27). eGFP mRNA is effectively and functionally delivered to bone marrow-derived erythroblast cells by LNP-1 formulations. 50-60% transfection efficiency is seen even in the lower dose ranges (i.e., 0.5-1 $\mu\text{g/ml}$), while cell viability remains $>95\%$ in all groups.

Example 28: A working example of LNP-1's versatile therapeutic applications – gene editing (Figure 28). >85% TCR α knockout efficiency is achieved within the dose ranges of 4-16 μ g/ml 4 days after Cas9 mRNA + TRAC sgRNA/LNP-1 transfection, while cell viability remains >95% in all groups. Such TCR α knockout efficiency is persistently maintained at the end of the study (day 14).

Example 29: A working example of LNP-1's versatile therapeutic applications – gene therapy for malignant cells (e.g., human leukemia cells) (Figure 29). eGFP mRNA is effectively and functionally delivered to human Jurkat T leukemia cells and human THP-1 monocytic leukemia cells. In Jurkat cells, even the lowest dose 12.5ng (=0.125 μ g/ml) eGFP mRNA/LNP-1s achieve 100% and strong delivery potency. 100ng (=1 μ g/ml) produce GFP signal beyond the highest detection range. Such GFP expression is persistent up to 10 days. Also notably, current evaluations of in vitro mRNA delivery potency by other LNP technologies are often using 100-200ng or higher doses.

Example 30: A working example of LNP-1's versatile therapeutic applications – gene therapy for malignant cells (e.g., human lymphoma cells) (Figure 30). eGFP mRNA is effectively and functionally delivered to HCC1739BL human lymphoma cells by LNP-1 formulations.

Example 31: A working example of LNP-1's versatile therapeutic applications – gene therapy for cancer cells (e.g., human liver cancer cells) (Figure 31). eGFP mRNA is effectively and functionally delivered to HepG2 human liver cancer cells by LNP-1 formulations and such GFP expression is persistent up to the study termination (day 7).

Example 32: Working examples of LNP-1's versatile therapeutic applications – vaccines (Figure 32). Spike mRNA-1273 loaded LNP-1s (5 μ g in 50 μ L PBS) were intramuscularly (IM) or subcutaneously (SC) administrated into C57BL6 or BALB/c mice on days 0 (the prime dose) and 21 (the boost dose). On days 14 (Prime), 28 (Boost-d7) and 44 (Boost-d23) for C57BL6 mice or on days 28 (Post-Boost 1w), 35 (Post-Boost 2w) and 49 (Post-Boost 4w) for BALB/c mice, mouse blood were collected and evaluated for Spike protein RBD-specific IgG titres by ELISA. Mice received 50 μ L PBS served as the negative control (PBS Ctrl or NC). LNP-1's potency as COVID-19 mRNA vaccines is demonstrated – all mice injected with Spike mRNA-1273/LNP-1s produced persistent high Spike RBD-specific IgG titres.

Example 33: Working examples of LNP-1's good safety profile (Figure 33). 48 hours after i.v administration of ApoB siRNA/LNP-1s (1, 3 or 6 mg/kg) into C57BL6 mice, blood chemistry analyses of liver enzymes (ALP, AST and ALT), kidney function (BUN and creatine kinase) and proteins levels (total protein, albumin and globulin) as well as liver

tissue histology were conducted. Within the therapeutic window (e.
no in vivo toxicity observed with LNP-1 formulations.

Example 34: A working example of LNP-1's good safety profile (Figure 34). 48 hours
after i.v administration of 3 mg/kg ApoB siRNA/LNP-1s into C57BL6 mice, major internal
5 organs including liver, heart, kidney, lungs, and spleen were harvested and subjected to
tissue histology assessment. LNP-1 formulations do not cause tissue toxicity in all organs
examined.

Various modifications of the invention, in addition to those described herein, will be
10 apparent to those skilled in the art from the foregoing description. Such modifications are
also intended to fall within the scope of the appended claims. Each reference, including
without limitation all patent, patent applications, and publications, cited in the present
application is incorporated herein by reference in its entirety.

CLAIMS

1. A lipid nanoparticle comprising:
 - (i) a PEG-conjugated phospholipid having two or more C₁₂-C₃₀ aliphatic chains,
5 at least one of which includes from 1-5 sites of unsaturation;
 - (ii) an ionizable lipid having at least two C₁₁-C₃₀ aliphatic chains and a head group that protonates to a cationic moiety having a pKa of 5-7;
 - (iii) a non-cationic phospholipid having at least two C₁₁-C₃₀ aliphatic chains and a head group that remains a zwitterion or anion across a pH range of 6 to 8; and
10 (iv) a sterol, fat-soluble steroid, or vitamin.
2. The lipid nanoparticle of claim 1, wherein the PEG-conjugated phospholipid has a molecular weight in the range of 0.2-6 kDa.
- 15 3. A lipid nanoparticle comprising:
 - (i) a PEG-conjugated phospholipid having two or more alkyl chains, at least one of which includes from 1-5 sites of unsaturation, and a head group substituted with a polyethylene glycol polymer, wherein the polyethylene glycol polymer has 2-50 polyethylene glycol subunits;
20 (ii) a cationic lipid having a cationic head group and two or more C₁₁-C₃₀ aliphatic chains, at least one of which includes from 1-5 sites of unsaturation, wherein the aliphatic chains are attached to the headgroup through a biodegradable linker;
 - (iii) a non-cationic phospholipid that remains a zwitterion or anion across a pH range of 6 to 8, having two or more C₁₁-C₃₀ aliphatic chains; and
25 (iv) a sterol, fat-soluble steroid, or vitamin.
4. The lipid nanoparticle of claim 3, wherein the PEG-conjugated phospholipid has an average molecular weight in the range of 200 to 7500 amu.
- 30 5. The lipid nanoparticle of any one of claims 1-4, wherein the lipid nanoparticle is stable at 2-8°C for at least 180 days.
6. The lipid nanoparticle of any one of claims 1-5, wherein the lipid nanoparticle is stable at 25°C for at least 60 days.
- 35 7. The lipid nanoparticle of any one of claims 1-6, wherein the lipid nanoparticle is stable at 40°C for at least 7 days.

8. The lipid nanoparticle of any one of claims 1-7, wherein the phospholipid has a head group selected from the group consisting of phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid, phosphatidylglycerols, ethoxy and methoxy.
- 5 9. The lipid nanoparticle of any one of claims 1-8, wherein the PEG-conjugated phospholipid has a head group selected from the group consisting of phosphatidylethanolamine and phosphatidylcholine.
- 10 10. The lipid nanoparticle of any one of claims 1-9, wherein the two or more C_{12} - C_{30} aliphatic chains of the PEG-conjugated phospholipid are C_{14} - C_{18} aliphatic chains.
11. The lipid nanoparticle of claim 10, wherein each of the C_{14} - C_{18} aliphatic chains is unsaturated.
- 15 12. The lipid nanoparticle of claim 10 or 11, wherein each of the C_{14} - C_{18} aliphatic chains is unsaturated at 1-3 positions.
13. The lipid nanoparticle of any one of claims 3-12, wherein PEG polymer is a linear polymer and represented in the formula
- 20
- $$\left(\text{O}-\text{CH}_2-\text{CH}_2-\text{O} \right)_n$$
- wherein n is an integer from 2-50.
14. The lipid nanoparticle of any one of claims 1-13, wherein PEG polymer includes a
- 25 ligand that binds to a cognate binding partner expressed on the cell surface of cells in the target tissue and which, upon assembly of the lipid nanoparticle, is present on the surface of the nanoparticle.
15. The lipid nanoparticle of any one of claims 1-14, wherein the cationic head group of
- 30 the cationic lipid is an ionizable head group having an acid-dissociation constant (pKa) less than 6.7.
16. The lipid nanoparticle of claim 15, wherein the ionizable head group includes a tertiary amine which has an acid-dissociation constant (pKa) less than 6.7.

17. The lipid nanoparticle of claim 15 or 16, wherein the pKa of the ionizable head group has a pKa in the range of 6.2 to 6.5.
- 5 18. The lipid nanoparticle of any one of claims 15-17, wherein the cationic head group and its neighboring aliphatic chain are in a 1,2-relationship.
19. The lipid nanoparticle of any one of claims 1-18, wherein the unsaturated C₁₁-C₃₀ aliphatic chains are unsaturated C₁₄-C₁₈ aliphatic chains.
- 10 20. The lipid nanoparticle of claim 19, wherein the two or more aliphatic chains are, independently for each occurrence, selected from α -Linolenic acid, γ -Linolenic acid, Arachidonic acid, Cervonic acid, Dihomo- γ -linolenic acid, Docosatetraenoic acid, Eicosapentaenoic acid, Elaidic acid, Erucic acid, Gondoic acid, Linoleic acid, Linolelaidic acid, Mead acid, Nervonic acid, Oleic acid, Palmitoleic acid, Paullinic acid, Stearidonic acid, and Vaccenic acid.
- 15 21. The lipid nanoparticle of claim 19, wherein the unsaturated C₁₁-C₃₀ aliphatic chains are unsaturated C₁₈ aliphatic chains.
- 20 22. The lipid nanoparticle of claim 21, wherein the unsaturated C₁₈ aliphatic chains are selected from oleic, linoleic and α -linoleic.
23. The lipid nanoparticle of claim 21, wherein the C₁₈ aliphatic chains are covalently
- 25 linked to the head group through an ether, ester or amide bond, or other bond that can be hydrolyzed (e.g., by enzyme-mediated or by enzyme independent processes) in vivo to release the aliphatic chain.
24. The lipid nanoparticle of any one of claims 1-23, wherein the ionizable phospholipid
- 30 can be one or a combination thereof selected from
- 1,2-dioleyloxy-3-dimethylaminopropane or (2R)-N,N-dimethyl-2,3-bis[(9Z)-9-octadecen-1-yloxy]-1-propanamine) (DODMA),
- (N,N-dimethyl-2,3-bis[(9Z,12Z)-9,12-octadecadien-1-yloxy]-1-propanamine) (DLin-DMA),
- 35 (4-dimethylamino)-butanoic acid, (10Z,13Z)-1-(9Z,12Z)-9,12-octadecadien-1-yl-10,13-nonadecadien-1-yl ester (DLin-MC3-DMA),

- N,N-dimethyl-2,2-di-(9Z,12Z)-9,12-octadecadien-1-yl-1,3-di
(DLin-KC2-DMA),
(2-hexyl-decanoic acid, 1,1'-[[4-hydroxybutyl]imino]di-6,1-hexanediyl] ester (ALC-
0315),
5 8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]-octanoic acid, 1-octylnonyl
ester (SM-102),
1-1'-((2-(4-(2-((2-(bis(2-hydroxydecyl)amino)ethyl)(2-
hydroxydecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(decan-2-ol) (C10-200),
9,12-octadecadienoic acid, (9Z, 12Z)-1,1'[2-[[[3-(diethylamino)
10 propoxy]carbonyl]oxy]-1,3-propanediyl] ester (Ionizable Lipid 4, IL4),
5-(dimethylamino)-pentanoic acid, (6Z)-1,2-di-(4Z)-4-decen-1-yl-6-dodecen-1-yl ester
(Lipid CL1),
4-methyl-1-piperazinepropanoic acid, 2-[di-(9Z,12Z)-9,12-octadecadien-1-
ylamino]ethyl ester (Lipid 10),
15 9-[4-(dimethylamino)1-oxobutoxy]-heptadecanedioic acid, 1,17-di-3-decyn-1-yl ester
(Lipid A6),
Di((Z)-octadec-9-en-1-yl) L-lysyl-L-glutamate, dihydrochloride (Lipid OA2
(hydrochloride)),
1-methyl-4-piperidinecarboxylic acid, 11-[(2-hexyl-1-oxodecyl)oxy]-5-[6-[(2hexyl-1-
20 oxodecyl)oxy]hexyl]-5-hydroxyundecyl ester (CL15F6),
Piperazine-1,4-diylbis(ethane-2,1-diyl) (9Z,9'Z,12Z,12'Z)-bis(octadeca-9,12-dienoate)
(AA3-DLin),
5-(((3dibutylamino)propyl)amino)methyl)-6-hydroxyundecane-1,11-diyl
(9Z,9'Z,12,12'Z)-bis(octadeca-9,12-dienoate) (IR-117-17),
25 1-methyl-4,4-bis[9Z,12Z)-9,12-octadecadien-1-yloxy]-piperidine (YSK05),
9-[4-(dimethylamino)-1-oxobutoxy]-heptadecanoic acid, 1,17-di-(2Z)-2-nonen-1-yl
ester (L-319),
3,6-bis[4-[bis[(9Z,12Z)-2-hydroxy-9,12-octadecadien-1-yl]amino]butyl]-2,5-
piperazinedione, (OF-02)9,12-octadecadienoic acid, (9Z,12Z)-1,1',1'',1'''-[3,6-dioxo-2,5-
30 piperazinediyl]bis(4,1-butanediylnitrilodi-4,1-butanediyl)] ester (OF-C4-Deg-Lin),
9Z,12Z-octadecadienoic acid, 1,1',1'',1'''-[(3,6-dioxo-2,5-piperazinediyl)bis(4,1-
butanediylnitrilodi-2,1-ethanediyl)] ester (OF-Deg-Lin),
4,4'-[[[3-(dimethylamino)propyl]thio]carbonyl]imino]bis-butanoic acid, 1,1'-bis(1-
heptyloctyl) ester (ATX-100),
35 1-[3-(dimethylamino)propyl]-5,5-di-(8Z)-8-heptadecen-1-yl-2,5-dihydro-1H-imidazole-
2-carboxylic acid, ethyl ester (A12-Iso5-2DC18).

25. The lipid nanoparticle of any one of claims 1-24, wherein the phospholipid has a head group selected from phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid and phosphatidylglycerols.
- 5
26. The lipid nanoparticle of any one of claims 1-25, wherein the non-cationic phospholipid is a phosphatidylethanolamine or phosphatidylcholine.
27. The lipid nanoparticle of any one of claims 1-26, wherein the two or more C₁₁-C₃₀ aliphatic chains of the non-cationic phospholipid are C₁₄-C₁₈ aliphatic chains that, independently for each chain, are saturated or unsaturated at 1-3 positions.
- 10
28. The lipid nanoparticle of any one of claims 1-27, wherein the two or more C₁₁-C₃₀ aliphatic chains of the non-cationic lipid are saturated C₁₄-C₁₈ aliphatic chains.
- 15
29. The lipid nanoparticle of any one of claims 1-28, wherein the non-cationic phospholipid can be one or a combination thereof selected from
- 1,2-diundecanoyl-sn-glycero-3-phosphatidylcholine (11:0 PC, DUPC),
- 1,2-dilauroyl-sn-glycero-3-phosphatidylcholine (12:0 PC),
- 20 1,2-ditridecanoyl-sn-glycero-3-phosphatidylcholine (13:0 PC),
- 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (14:0 PC, DMPC),
- 1,2-dipentadecanoyl-sn-glycero-3-phosphatidylcholine (15:0 PC),
- 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (16:0 PC, DPPC),
- 1,2-diphytanoyl-sn-glycero-3-phosphatidylcholine (4ME 16:0 PC),
- 25 1,2-diheptadecanoyl-sn-glycero-3-phosphatidylcholine (17:0 PC),
- 1,2-distearoyl-sn-glycero-3-phosphatidylcholine (18:0 PC, DSPC),
- 1,2-Distearoyl-sn-glycero-3-Ethylphosphatidylcholine (Ethyl-DSPC)
- 1,2-dinonadecanoyl-sn-glycero-3-phosphatidylcholine (19:0 PC),
- 1,2-diarachidoyl-sn-glycero-3-phosphatidylcholine (20:0 PC),
- 30 1,2-dihenearachidoyl-sn-glycero-3-phosphatidylcholine (21:0 PC),
- 1,2-dibehenoyl-sn-glycero-3-phosphatidylcholine (22:0 PC),
- 1,2-ditricosanoyl-sn-glycero-3-phosphatidylcholine (23:0 PC),
- 1,2-dilignoceroyl-sn-glycero-3-phosphatidylcholine (24:0 PC),
- 1,2-dimyristoleoyl-sn-glycero-3-phosphatidylcholine (14:1 (49-Cis) PC),
- 35 1,2-dimyristelaidoyl-sn-glycero-3-phosphatidylcholine (14:1 (49-Trans) PC),
- 1,2-dipalmitoleoyl-sn-glycero-3-phosphatidylcholine (16:1 (49-Cis) PC),
- 1,2-dipalmitelaidoyl-sn-glycero-3-phosphatidylcholine (16:1 (49-Trans) PC),

- 1,2-dipetroselenoyl-sn-glycero-3-phosphatidylcholine (18:1
 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (18:1 (49-Cis) PC, DOPC),
 1,2-dielaioyl-sn-glycero-3-phosphatidylcholine (18:1 (49-Trans) PC),
 1,2-dilinoleoyl-sn-glycero-3-phosphatidylcholine (18:2 (Cis) PC, DLPC),
 5 1,2-dilinolenoyl-sn-glycero-3-phosphatidylcholine (18:3 (Cis) PC, DLnPC),
 1,2-dieicosenoyl-sn-glycero-3-phosphatidylcholine (20:1 (Cis) PC),
 1,2-diarachidonoyl-sn-glycero-3-phosphatidylcholine (20:4 (Cis) PC, DAPC),
 1,2-dierucoyl-sn-glycero-3-phosphatidylcholine (22:1 (Cis) PC),
 1,2-didocosahexaenoyl-sn-glycero-3-phosphatidylcholine (22:6 (Cis) PC, DHAPC),
 10 1,2-dinervonoyl-sn-glycero-3-phosphatidylcholine (24:1 (Cis) PC),
 1,2-dihexanoyl-sn-glycero-3-phosphatidylethanolamine (06:0 PE),
 1,2-dioctanoyl-sn-glycero-3-phosphatidylethanolamine (08:0 PE),
 1,2-didecanoyl-sn-glycero-3-phosphatidylethanolamine (10:0 PE),
 1,2-dilauroyl-sn-glycero-3-phosphatidylethanolamine (12:0 PE),
 15 1,2-dimyristoyl-sn-glycero-3-phosphatidylethanolamine (14:0 PE),
 1,2-dipentadecanoyl-sn-glycero-3-phosphatidylethanolamine (15:0 PE),
 1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine (16:0 PE),
 1,2-diphytanoyl-sn-glycero-3-phosphatidylethanolamine (4ME 16:0 PE),
 1,2-diheptadecanoyl-sn-glycero-3-phosphatidylethanolamine (17:0 PE),
 20 1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine (18:0 PE, DSPE),
 1,2-dipalmitoleoyl-sn-glycero-3-phosphatidylethanolamine (16:1 PE),
 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine (18:1 (49-Cis) PE, DOPE),
 1,2-dielaioyl-sn-glycero-3-phosphatidylethanolamine (18:1 (49-Trans) PE),
 1,2-dilinoleoyl-sn-glycero-3-phosphatidylethanolamine (18:2 PE, DLPE),
 25 1,2-dilinolenoyl-sn-glycero-3-phosphatidylethanolamine (18:3 PE, DLnPE),
 1,2-diarachidonoyl-sn-glycero-3-phosphatidylethanolamine (20:4 PE, DAPE),
 1,2-didocosahexaenoyl-sn-glycero-3-phosphatidylethanolamine (22:6 PE, DHAPE),
 1,2-di-O-octadecenyl-sn-glycero-3-phosphatidylcholine (18:0 Diether PC),
 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG),
 30 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphatidylcholine (14:0-16:0 PC, MPPC),
 1-myristoyl-2-stearoyl-sn-glycero-3-phosphatidylcholine (14:0-18:0 PC, MSPC),
 1-palmitoyl-2-acetyl-sn-glycero-3-phosphatidylcholine (16:0-02:0 PC),
 1-palmitoyl-2-myristoyl-sn-glycero-3-phosphatidylcholine (16:0-14:0 PC, PMPC),
 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphatidylcholine (16:0-18:0 PC, PSPC),
 35 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (16:0-18:1 PC, POPC),
 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphatidylcholine (16:0-18:2 PC, PLPC),
 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphatidylcholine (16:0-20:4 PC),

- 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphatidylc
 1-stearoyl-2-myristoyl-sn-glycero-3-phosphatidylcholine (18:0-14:0 PC, SMPC),
 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphatidylcholine (18:0-16:0 PC, SPPC),
 1-stearoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (18:0-18:1 PC, SOPC),
 5 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphatidylcholine (18:0-18:2 PC),
 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphatidylcholine (18:0-20:4 PC),
 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphatidylcholine (18:0-22:6 PC),
 1-oleoyl-2-myristoyl-sn-glycero-3-phosphatidylcholine (18:1-14:0 PC, OMPC),
 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphatidylcholine (18:1-16:0 PC, OPPC),
 10 1-oleoyl-2-stearoyl-sn-glycero-3-phosphatidylcholine (18:1-18:0 PC, OSPC),
 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine (16:0-18:1 PE, POPE),
 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphatidylethanolamine (16:0-18:2 PE),
 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphatidylethanolamine (16:0-20:4 PE),
 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphatidylethanolamine (16:0-22:6
 15 PE),
 1-stearoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine (18:0-18:1 PE),
 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphatidylethanolamine (18:0-18:2 PE),
 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphatidylethanolamine (18:0-20:4 PE),
 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphatidylethanolamine (18:0-22:6
 20 PE),
 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphatidylcholine (OChemsPC),
 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE),
 dipentadecanoyl-phosphatidylcholine (DPDPC),
 dilauroylphosphatidylglycerol (DLPG),
 25 diarachidoylphosphatidylglycerol (DAPG),
 dimyristoylphosphatidylglycerol (DMPG),
 dipalmitoylphosphatidylglycerol (DPPG),
 distearoylphosphatidylglycerol (DSPG),
 dimyristoyl phosphatidic acid (DMPA),
 30 dipalmitoyl phosphatidic acid (DPPA),
 distearoyl phosphatidic acid (DSPA),
 diarachidoylphosphatidic acid (DAPA),
 dimyristoylphosphatidylethanolamine (DMPE),
 dipalmitoylphosphatidylethanolamine (DPPE),
 35 dimyristoyl phosphatidylserine (DMPS),
 diarachidoyl phosphatidylserine (DAPS),
 dipalmitoyl phosphatidylserine (DPPS),

distearoylphosphatidylserine (DSPS),
 dioleoylphosphatidylserine (DOPS),
 dipalmitoyl sphingomyelin (DPSP),
 distearoylsphingomyelin (DSSP),
 5 dilauroyl-phosphatidylinositol (DLPI),
 diarachidoylphosphatidylinositol (DAPI),
 dimyristoylphosphatidylinositol (DMPI),
 dipalmitoylphosphatidylinositol (DPPI),
 distearoylphosphatidylinositol (DSPI), and
 10 dioleoyl-phosphatidylinositol (DOPI).

30. The lipid nanoparticle of any one of claims 1-29, wherein the non-cationic lipid is selected from DSPC, DOPC, DPPC, DOPG, DPPG, DOPE, POPC, POPE, DPPE, DMPE, DSPE, SOPE, and DLPE, or a combination thereof.

15

31. The lipid nanoparticle of any one of claims 1-30, wherein (iv) is a sterol.

32. The lipid nanoparticle of claim 31, wherein the sterol is cholesterol or an analog thereof.

20

33. The lipid nanoparticle of claim 31, wherein the sterol is selected from 3-hydroxycholest-5-ene (Cholesterol), 9,10-secocholesta-5,7,10(19)-trien-3 β -ol (Vitamin D3), ergocalciferol (Vitamin D2), calcipotriol, 24-ethyl-5,22-cholestadien-3 β -ol (Stigmasterol), 22,23-Dihydrostigmasterol (β -Sitosterol), 3,28-Dihydroxy-lupeol (Betulin), Lupeol, Ursolic acid, Oleanolic acid, 24a-Methylcholesterol (Campesterol), 24-Ethylcholesta-5,24(28)E-dien-3 β -ol (Fucosterol), 24-Methylcholesta-5,22-dien-3b-ol (Brassicasterol), (22E)-Ergosta-5,7,22-trien-3 β -ol (Ergosterol), 9,11-Dehydroergosterol, and Daucosterol, or any of the foregoing sterols modified with one or more amino acids.

30 34. The lipid nanoparticle of claim 31, wherein the sterol is a plant-derived sterol.

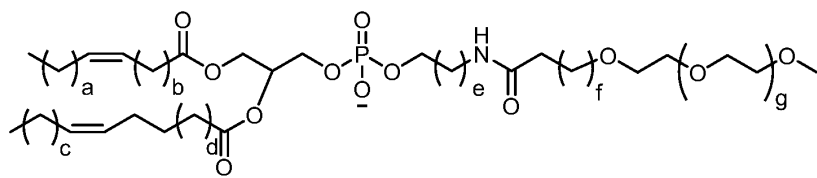
35. The lipid nanoparticle of claim 34, wherein the plant-derived sterol is selected from β -sitosterol, β -sitostanol, stigmasterol, stigmastanol, campesterol, campestanol, ergosterol, avenasterol, and brassicasterol, or any combination thereof.

35

36. The lipid nanoparticle of any one of claims 1-31, wherein (iv) is a fat-soluble vitamin.

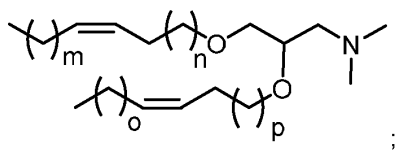
37. The lipid nanoparticle of claim 36, wherein the fat-soluble vitamin A, a secosteroid, a tocopherol, a tocotrienol, and a menaquinone, or an analog or combination thereof.
- 5 38. The lipid nanoparticle of claim 36, wherein the fat-soluble vitamin is selected from retinol, retinal, retinyl esters, vitamin D2 (ergocalciferol), vitamin D3 (cholecalciferol), vitamin E, vitamin K1, and vitamin K2, or an analog or combination thereof.
39. The lipid nanoparticle of any one of claims 1-38, wherein the sterol, fat-soluble
10 steroid, or vitamin is PEGylated.

40. The lipid nanoparticle of any one of claims 1-39, wherein (i) is



wherein

- 15 a is an integer between 1 and 10;
b is an integer between 1 and 10;
c is an integer between 1 and 10;
d is an integer between 1 and 10;
e is an integer between 1 and 5;
20 f is an integer between 1 and 5; and
g is an integer between 10 and 50.
41. The lipid nanoparticle of claim 40, wherein
a is an integer between 5 and 10;
25 b is an integer between 5 and 10;
c is an integer between 5 and 10;
d is an integer between 1 and 5;
e is an integer between 1 and 3;
f is an integer between 1 and 5; and
30 g is an integer between 30 and 50.
42. The lipid nanoparticle of any one of claims 1-41, wherein (ii) is

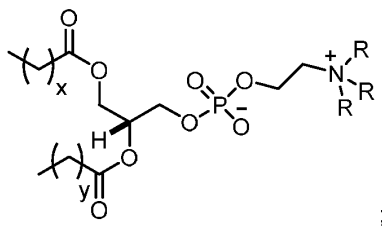


wherein

- 5 m is an integer between 1 and 10;
 n is an integer between 1 and 10;
 o is an integer between 1 and 10; and
 p is an integer between 1 and 10.

43. The lipid nanoparticle of claim 42, wherein
 m is an integer between 5 and 10;
 n is an integer between 5 and 10;
 o is an integer between 5 and 10; and
 p is an integer between 5 and 10

44. The lipid nanoparticle of any one of claims 1-43, wherein (iii) is



15

wherein

- x is an integer between 10 and 20;
 y is an integer between 10 and 20; and
 each R is independently C₁₋₆ alkyl.

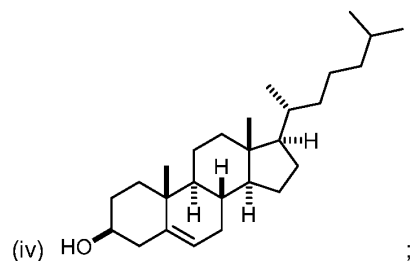
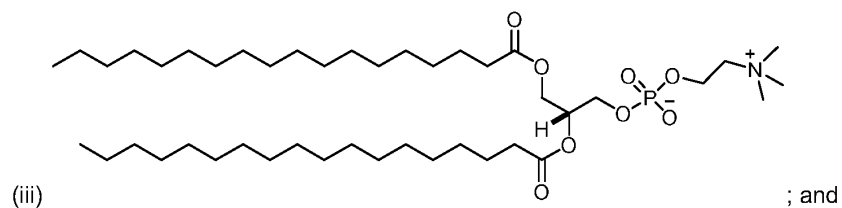
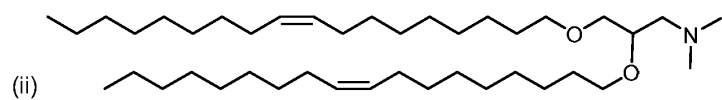
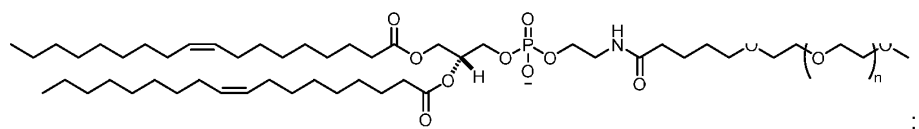
20

45. The lipid nanoparticle of claim 44, wherein
 x is an integer between 15 and 20;
 y is an integer between 15 and 20; and
 each R is independently C₁₋₃ alkyl.

25

46. The lipid nanoparticle of any one of claims 1-45, wherein the lipid nanoparticle comprises

(i)



5 wherein n is an integer from 32 to 50.

47. A pharmaceutical composition comprising the lipid nanoparticle of any one of claims 1-46 and a pharmaceutically acceptable carrier.

10 48. A drug delivery system comprising the lipid nanoparticle of any one of claims 1-46, or the pharmaceutical composition of claim 47, and an active agent.