

BIODEGRADABLE CARBAMATE LIPIDS WITH INCREASED STABILITY

FIELD OF THE INVENTION

The present invention provides lipids and lipid nanoparticle formulations comprising these lipids, alone or in combination with other lipids. These lipid nanoparticles may be formulated with nucleic acids to facilitate their intracellular delivery both *in vitro* and for *in vivo* therapeutic applications. The lipids of the present invention are characterized as particularly hydrolytically and alcoholytically stable, while maintaining their biological activity.

BACKGROUND OF THE INVENTION

Therapeutic nucleic acids including small interfering RNA (siRNA), micro RNA (miRNA), antisense oligonucleotides, messenger RNA (mRNA), ribozymes, pDNA and immune stimulating nucleic acids act via a variety of mechanisms. Specific proteins can be downregulated by siRNA or miRNA through RNA interference (RNAi). Hematopoietic cells, such as leukocytes in general, and primary T lymphocytes and B-cells in particular, are notoriously hard to transfect with small interfering RNAs (siRNAs). Modulating immune cells function, such as T cells and B cells, by downregulating specific genes using RNA interference (RNAi) holds tremendous potential in advancing targeted therapies in many immune-related disorders including cancer, inflammation, autoimmunity and viral infections. The therapeutic applications of RNAi are extremely broad, since siRNA and miRNA constructs can be synthesized with any nucleotide sequence directed against a target protein. To date, siRNA constructs have shown the ability to specially silence target proteins in both *in vitro* and *in vivo* models. These are currently being evaluated in clinical studies.

Messenger RNA (mRNA) is the family of large RNA molecules which transport the genetic information from DNA to ribosome. Some nucleic acids, such as mRNA or plasmids, can be used to effect expression of specific cellular products. Such nucleic acids would be useful in the treatment to the of diseases related deficiency of a protein or enzyme. However, there are many problems associated with nucleic acids in therapeutic contexts. One of the major problems with therapeutic nucleic acids is the stability of the phosphodiester inter nucleotide link and its susceptibility to nucleases. Apart from that these nucleic acids have limited ability to cross the cell membrane.

Various lipids, e.g., cationic lipids, have proved to be excellent carriers of nucleic acids to treat different diseases in gene therapy applications. Lipid nanoparticles formed from cationic lipids and other co-lipids such as cholesterol, DSPC and PEGylated lipids encapsulated oligonucleotides which protect them from degradation and facilitate the cellular uptake.

WO 2014007398 discloses a cationic lipid that facilitates introduction of a nucleic acid into a cell, wherein the nucleic acid has an activity of suppressing the expression of a target gene by utilizing RNA interference (RNAi).

WO 2018087753 discloses a cationic lipid comprising a functional group represented by the structure: $-W-(T=O)_m-X-(CH_2)_z-Y$, wherein X and Y are each independently O, N or NH, wherein X and Y cannot both be O; W is a bond, O, NH or S; T is C or S; m is 0 or 1; and z is 0 or 2, wherein said functional group is linked to at least one saturated or unsaturated fatty acid residue.

WO 2022168085 discloses lipids for lipid nanoparticles (LNPs) preparation. These lipid nanoparticles protect nucleic acids from degradation and/or clearance from circulation and intracellular release.

However, although some of the mentioned prior art LNPs have retained their physicochemical properties, their transfection efficiency was, in some cases, compromised due to head group cleavage via hydrolysis or alcoholysis.

Biodegradable lipids and nanoparticles containing the same, show reverse correlation between lipid and particle stability and their activity in delivering active agents, such as nucleic acids to the target sites. Once the stability reaches a certain threshold, they become inactive as vesicles. The challenge to balance between stability and degradability is one of the key limitations of such nanocarriers.

Therefore, there remains a need in the art for suitable, efficient LNP platforms for the delivery of oligonucleotides exhibiting better hydrolytic and alcoholytic stability, while retaining their properties in delivering agents to various organs.

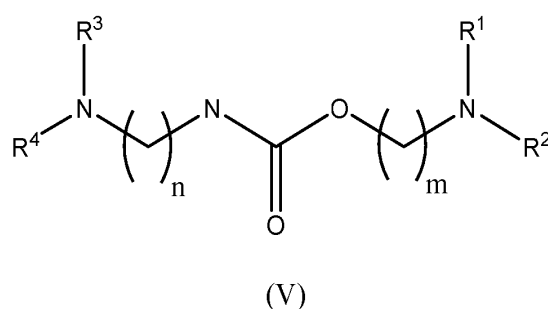
SUMMARY OF THE INVENTION

The present invention relates to novel lipids which can be used in lipid nanoparticle preparation. These lipid nanoparticles protect nucleic acids from degradation, clearance from circulation and intracellular release. In addition, the nucleic acid encapsulated lipid nanoparticles advantageously are well-tolerated and provide an adequate therapeutic index, such that patient treatment at an effective dose of the nucleic acid is not associated with unacceptable toxicity and/or risk to the patient. The present invention also provides the methods of chemical synthesis of these lipids, lipid nanoparticle preparation and formulations with nucleic acids.

10 In some embodiments, the present invention relates to novel lipids, and formulations of such lipids with siRNA and pDNA.

The lipids of the present invention include a carbamate moiety, and were found to have increased hydrolytic and alcoholic stability from degradation compared to counterparts, which lack the carbamate moiety. Moreover, it was surprisingly found that the present carbamate lipids, despite the increase in stability do not suffer a decrease in their biological activity. Specifically, in the typical case in the art, once the stability of lipids within an LNP reaches a certain threshold, they become inactive in delivering agents, such as RNA, to various organs. It was surprisingly found that lipids according to the present invention can overcome this stability-activity barrier.

20 According to some embodiments, there is lipid represented by the structure of Formula (V) or a salt thereof:



each one of n and m is individually selected from the group consisting of: 1, 2, 3, 4 and 5;

R^1 is selected from the group consisting of: C_{3-9} alkylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkylene- CO_2 - C_{8-18} alkenyl, C_{3-9} alkenylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkenylene- CO_2 - C_{8-18} alkenyl, C_{3-9} alkylene- O_2C - C_{8-18} alkyl, C_{3-9} alkylene- O_2C - C_{8-18} alkenyl, C_{3-9} alkenylene- O_2C - C_{8-18} alkyl and C_{3-9} alkenylene- O_2C - C_{8-18} alkenyl;

- 5 R^2 is selected from the group consisting of: C_{10-20} alkyl, C_{10-20} alkenyl, C_{10-20} alkynyl, C_{3-9} alkylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkylene- CO_2 - C_{8-18} alkenyl, C_{3-9} alkenylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkenylene- CO_2 - C_{8-18} alkenyl, C_{3-9} alkylene- O_2C - C_{8-18} alkyl, C_{3-9} alkylene- O_2C - C_{8-18} alkenyl, C_{3-9} alkenylene- O_2C - C_{8-18} alkyl and C_{3-9} alkenylene- O_2C - C_{8-18} alkenyl; and

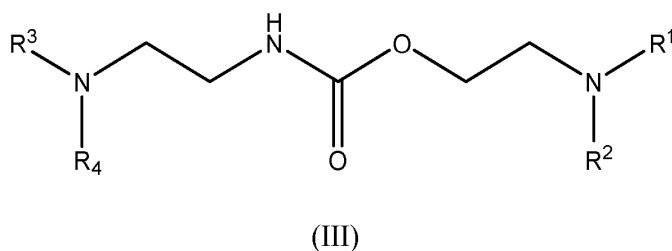
- 10 each one of R^3 and R^4 is individually, a C_{1-4} alkyl or wherein R^3 and R^4 together with the nitrogen atom to which they are bound form a 5-6 membered ring

According to some embodiments, n is 2, 3 or 4. Each possibility represents a separate embodiment of the invention. According to some embodiments, n is 2.

The lipid according to any one of claims 1 to 3, wherein m is 2, 3 or 4.

- 15 According to some embodiments, m is 2, 3 or 4. Each possibility represents a separate embodiment of the invention. According to some embodiments, m is 2.

According to some embodiments, each one of n and m is 2, and the lipid is represented by the structure of Formula (III) or a salt thereof:

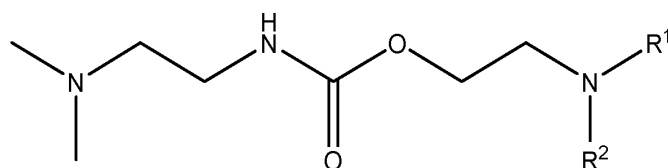


- 20 According to some embodiments, each one of R^3 and R^4 is individually, a C_{1-4} alkyl.

According to some embodiments, R^3 is a C_{1-2} alkyl. According to some embodiments, R^3 is CH_3 .

According to some embodiments, R^4 is a C_{1-2} alkyl. According to some embodiments, R^4 is CH_3 .

According to some embodiments, each one of n and m is 2, each one of R^3 and R^4 is individually CH_3 , and the lipid is represented by the structure of Formula (IV) or a salt thereof:



(IV)

According to some embodiments, R^1 is selected from the group consisting of: C_{3-9} alkylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkylene- CO_2 - C_{8-18} alkenyl, C_{3-9} alkenylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkylene- O_2C - C_{8-18} alkyl, C_{3-9} alkylene- O_2C - C_{8-18} alkenyl, C_{3-9} alkenylene- O_2C - C_{8-18} alkyl. According to some embodiments, R^1 is selected from the group consisting of: C_{3-9} alkylene- CO_2 - C_{8-18} alkyl and C_{3-9} alkylene- O_2C - C_{8-18} alkyl. According to some embodiments, R^1 is selected from the group consisting of: C_{6-9} alkylene- CO_2 - C_{14-18} alkyl, C_{6-9} alkylene- O_2C - C_{14-18} alkyl. According to some embodiments, wherein R^1 is C_{6-9} alkylene- CO_2 - C_{14-18} alkyl.

According to some embodiments, R^2 is selected from the group consisting of: C_{10-20} alkenyl, C_{3-9} alkylene- CO_2 - C_{8-18} alkyl and C_{3-9} alkylene- O_2C - C_{8-18} alkyl. According to some embodiments, wherein R^2 is selected from the group consisting of: C_{16-20} alkenyl, C_{6-9} alkylene- CO_2 - C_{14-18} alkyl and C_{6-9} alkylene- O_2C - C_{14-18} alkyl.

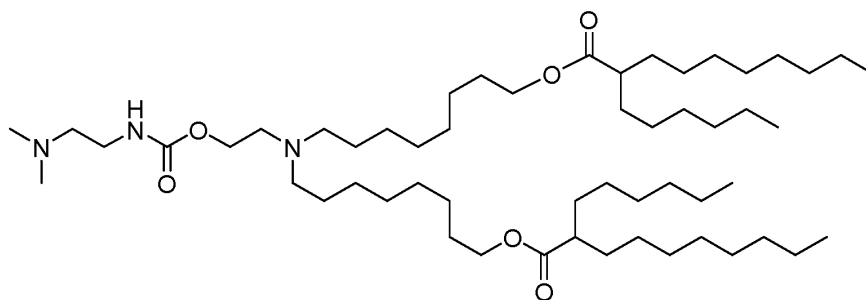
According to some embodiments, each one of R^1 and R^2 is individually selected from the group consisting of: C_{3-9} alkylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkylene- CO_2 - C_{8-18} alkenyl, C_{3-9} alkenylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkylene- O_2C - C_{8-18} alkyl, C_{3-9} alkylene- O_2C - C_{8-18} alkenyl, C_{3-9} alkenylene- O_2C - C_{8-18} alkyl. According to some embodiments, each one of R^1 and R^2 is individually selected from the group consisting of: C_{3-9} alkylene- CO_2 - C_{8-18} alkyl and C_{3-9} alkylene- O_2C - C_{8-18} alkyl. According to some embodiments, each one of R^1 and R^2 is C_{6-9} alkylene- CO_2 - C_{14-18} alkyl.

According to some embodiments, each one of the alkenyl groups is unsubstituted.

According to some embodiments, each one of the alkenylene groups is unsubstituted.

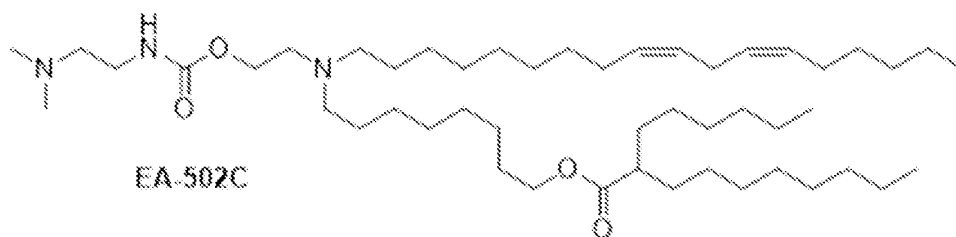
According to some embodiments, each one of the alkyl and alkylene groups is unsubstituted.

According to some embodiments, the lipid is selected from the group consisting of:

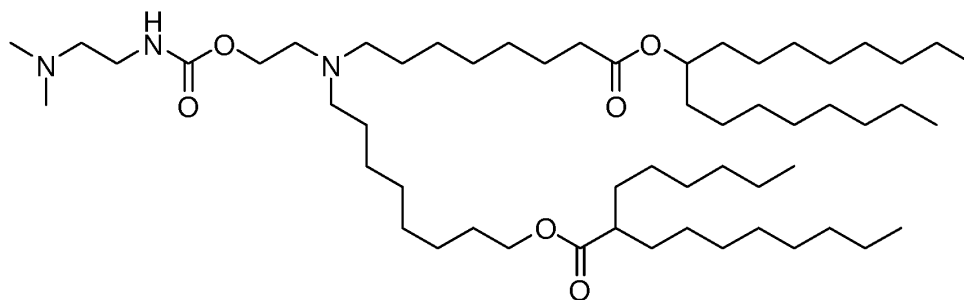


EA-405C

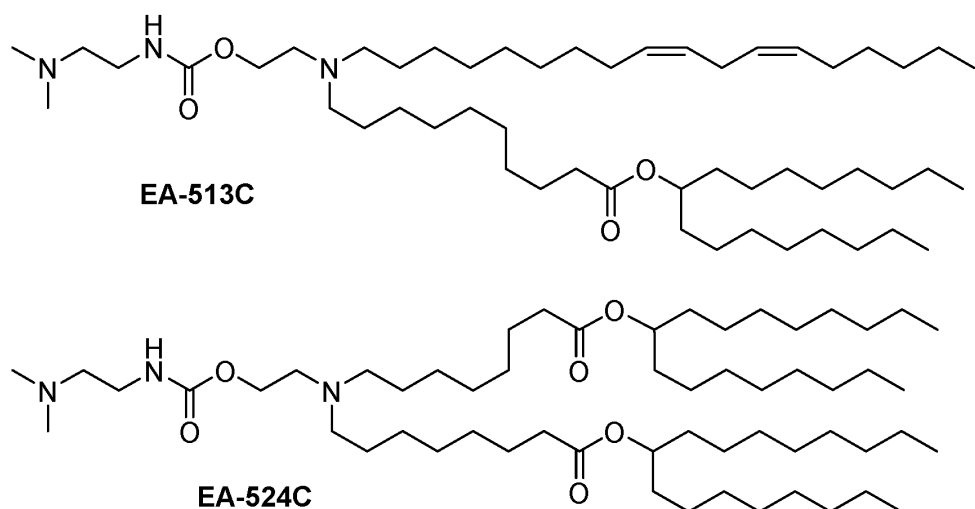
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EA-502C



EA-506C



and salts thereof. Each possibility represents a separate embodiment of the invention.

- 5 According to some embodiments, the lipid is selected from the group consisting of: EA-405C, EA-506C, EA-513C, EA-524C and salts thereof. According to some embodiments, the lipid is EA-524C or a salt thereof.

According to some embodiments, the lipid is hydrolytically stable, alcoholytically stable or both, for at least one week.

- 10 According to some embodiments, there is provided particle comprising the lipid disclosed herein and a membrane stabilizing lipid.

According to some embodiments, the membrane stabilizing lipid is selected from the group consisting of cholesterol, phospholipids, cephalins, sphingolipids and glycolipids. Each possibility represents a separate embodiment of the invention.

- 15 According to some embodiments, the membrane stabilizing lipid comprises cholesterol.

According to some embodiments, the particle further comprises one or more additional components selected from the group consisting of a PEG-lipid conjugate, a neutral lipid and a charged lipid. Each possibility represents a separate embodiment of the invention.

According to some embodiments, the additional component comprises 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC).

According to some embodiments, the additional component comprises 1,2-Dimyristoyl-sn-glyceryl-methoxy polyethylene glycol (DMG-PEG).

- 5 According to some embodiments, the particle comprises the lipid, cholesterol, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-Dimyristoyl-sn-glyceryl-methoxy polyethylene glycol (DMG-PEG).

According to some embodiments, the particle further comprises a nucleic acid encapsulated within a particle comprising the lipid.

- 10 According to some embodiments, the nucleic acid is selected from the group consisting of small interfering RNA (siRNA), micro RNA (miRNA), antisense oligo nucleotides, messenger RNA (mRNA), ribozymes, pDNA, CRISPR mRNA, gRNA, circular RNA and immune stimulating nucleic acids. Each possibility represents a separate embodiment of the invention. According to some embodiments, the nucleic acid is RNA comprising an open
15 reading frame encoding a polypeptide that comprises a SARS-CoV-2 spike protein or an immunogenic fragment or variant thereof.

According to some embodiments, there is provided a composition comprising a plurality of particles as disclosed herein and a pharmaceutically acceptable carrier, diluent or excipient. According to some embodiments, the composition is a liposomal composition.

- 20 According to some embodiments, there is provided a method of gene silencing, the method comprising the step of contacting a cell with a composition comprising a plurality of particles according to the present invention, and a pharmaceutically acceptable carrier, diluent or excipient.

According to some embodiments, the cell is a cancer cell.

- 25 According to some embodiments, there is provided a method for administering a therapeutic agent, the method comprising the step of preparing a composition comprising a lipid according to the present invention, and a therapeutic agent, and administering the composition to a subject in need thereof.

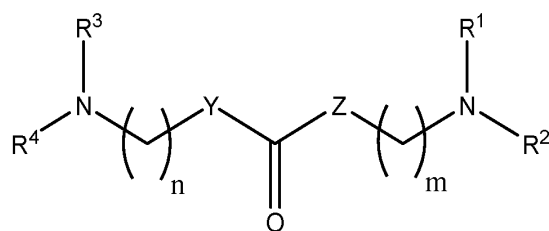
According to some embodiments, the method further comprises encapsulating the therapeutic agent within a particle comprising the lipid.

According to some embodiments, the therapeutic agent is RNA comprising an open reading frame encoding a polypeptide that comprises a SARS-CoV-2 spike protein or an immunogenic fragment or variant thereof.

According to some embodiments, there is provided a method of treating a leukocyte associated condition, the method comprising the step of administering to a subject in need thereof a composition comprising a plurality of particles according to the present invention and a pharmaceutically acceptable carrier, diluent or excipient.

According to some embodiments, the leukocyte associated condition is selected from the group consisting of cancer, infection, autoimmune diseases, neurodegenerative diseases and inflammation.

According to some embodiments, there is provided a lipid represented by the structure of Formula (I) or a salt thereof:



(I)

wherein one of Y and Z is O and the other of Y and Z is NH;

each one of n and m is individually selected from the group consisting of: 1, 2, 3, 4 and 5;

each one of R¹ and R² is individually selected from the group consisting of: C₁₀₋₂₀ alkyl, C₁₀₋₂₀ alkenyl, C₁₀₋₂₀ alkynyl, C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkyl, C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkenyl, C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkyl, C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkenyl, C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkyl, C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkenyl, C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkyl and C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkenyl; and

each one of R^3 and R^4 is individually, a C_{1-4} alkyl or wherein R^3 and R^4 together with the nitrogen atom to which they are bound form a 5-6 membered ring;

wherein the lipid is hydrolytically stable for at least 2 hours.

Further embodiments and the full scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A–B depict the expression level of GFP by transfecting Z138 cells and primary T Cells with mRNA-LNPs. **Figure 1A** is a bar chart showing quantitative analysis of the GFP fluorescence intensity following transfection of mRNA-LNPs, wherein the LNPs are EA–519, EA–402, EA–506C, EA–405 and EA–506CN. **Figure 1B** is a line chart showing quantitative analysis of the percentage of Z138 expressing GFP following the transfection of mRNA-LNP, EA–519 (broken line + squares), EA–402 (solid line + empty diamonds), EA–506C (broken line + triangles), EA–405 (broken line + circles) and EA–506CN (solid line + full diamonds).

Figure 2A is a bar chart showing quantitative analysis of the GFP fluorescence intensity following Z138cells transfection of mRNA-LNPS wherein the lipids are EA–2C, EA–502C, EA–506C, and EA–405C. From left to right the different groups of lipids are at a concentration of 0.25 $\mu\text{g/ml}$, 0.5 $\mu\text{g/ml}$, 1 $\mu\text{g/ml}$, and 2 $\mu\text{g/ml}$. **Figure 2B** is a line chart showing quantitative analysis of the percentage of Z138 cells expressing GFP following the transfection of mRNA-LNP; EA–2C (solid line + triangle facing down), EA–502C (solid line + triangle facing up), EA–506C (solid line + circles), and EA–405C (solid line + triangle facing up).

Figure 3 is a bar graph showing quantitative analysis of LUC expression in HeLa human cells after being transfected with the lipids EA–506 (clear frame), EA–506C (horizontal lines), EA–2 (vertical lines), and EA2–C (solid fill). as described in Example 2. Left group

of bars are lipids at a concentration of 0.25 µg/ml, middle group of bars are lipids at a concentration of 0.125 µg/ml, and right group of bars are lipids at a concentration of 0.065 µg/ml.

Figure 4A is a bar chart showing quantitative analysis of the GFP fluorescence intensity following primary T-cells transfection of mRNA-LNPs comprising EA2C, EA-502C, EA-506C, and EA-405C lipids. From left to right the different groups of lipids are at a concentration of 0.25 µg/ml, 0.5 µg/ml, 1 µg/ml, 2 µg/ml, and 4 µg/ml. **Figure 4B** is a line chart showing quantitative analysis of the percentage of primary T-cells expressing GFP following the transfection of mRNA-LNP comprising EA-2C (broken line – triangles facing down), EA-502C (broken line – circles), EA-506C (solid line – circles), and EA-405C (solid line – squares).

Figure 5 is a bar graph showing quantitative analysis of LUC expression in a murine model (mice) after intramuscularly administration of lipids comprising EA-506 (no fill), EA-506C (vertical lines), EA-2 (horizontal lines), and EA2-C (dots) from left to right. Detailed procedures are described in Example 5.

Figure 6 shows the chemical structures of the lipids EA-506, EA-2, EA-519, EA-402, EA-506CN and EA-405.

Figure 7A shows GFP mean fluorescence and percentage of GFP⁺ cells analyzed by flow cytometry of LNPs comprising the lipids EA2C (full circles), EA502C (full squares), EA506C (full triangles), EA405C (empty triangles), EA513C (empty circles) and EA524C (empty squares), upon transfected at concentrations 0.25µg/ml, 0.5µg/ml, 1µg/ml and 2µg/ml.

Figure 7B shows the GFP mean fluorescence intensities of LNPs comprising the lipids EA2C, EA502C (large squares), EA506C (horizontal lines), EA405C (vertical lines), EA513C (diagonal lines) and EA524C (small squares), upon transfected at concentrations 0.25µg/ml (left block), 0.5µg/ml (second to left block), 1µg/ml (second to right block) and 2µg/ml (second to right block).

Figure 8 shows the GFP mean fluorescence intensities of LNPs comprising the lipids EA2C, EA502C (large squares), EA506C (horizontal lines), EA405C (vertical lines),

EA513C (diagonal lines) and EA524C (small squares), upon transfection of HCC cells at concentrations 0.25µg/ml (left block), 0.5µg/ml (second to left block), 1µg/ml (second to right block) and 2µg/ml (second to right block).

Figure 9 shows the GFP mean fluorescence intensities of LNPs comprising the lipids EA2C, EA502C (large squares), EA506C (horizontal lines), EA405C (vertical lines), EA513C (diagonal lines) and EA524C (small squares), upon transfection of Ovar8 cells at concentrations 0.25µg/ml (left block), 0.5µg/ml (second to left block), 1µg/ml (second to right block) and 2µg/ml (second to right block).

Figure 10 shows the GFP mean fluorescence intensities of LNPs comprising the lipids EA2C, EA502C (large squares), EA506C (horizontal lines), EA405C (vertical lines), EA513C (diagonal lines) and EA524C (small squares), upon transfection of Detroit562 pharyngeal cancer cells at concentrations 0.25µg/ml (left block), 0.5µg/ml (second to left block), 1µg/ml (second to right block) and 2µg/ml (second to right block).

Figure 11 is a heat map depicting cancer cell lines: ovar8, Detroit562, HepG2 and Z138 versus LNPs expressed therein, the LNPs comprising the lipids EA2C, EA502C, EA506C, EA405C, EA513C and EA524C. Optimized concentration is shown for each cell line.

Figure 12A shows GFP mean fluorescence and percentage of GFP⁺ Primary T cells analyzed by flow cytometry of LNPs comprising the lipids EA2C (full circles), EA502C (full squares), EA506C (full triangles), EA405C (empty triangles), EA513C (empty circles) and EA524C (empty squares), upon transfected at concentrations 0.25µg/ml, 0.5µg/ml, 1µg/ml and 2µg/ml.

Figure 12B shows the GFP mean fluorescence intensities of LNPs comprising the lipids EA2C, EA502C (large squares), EA506C (horizontal lines), EA405C (vertical lines), EA513C (diagonal lines) and EA524C (small squares), upon transfection of Primary T cells at concentrations 0.25µg/ml (left block), 0.5µg/ml (second to left block), 1µg/ml (second to right block) and 2µg/ml (second to right block).

Figure 13A shows % Indel mutations upon gene editing in HCC cells with sgHPRT-LNPs and sgNCAPG-LNPs.

Figure 13B shows cellular viability measured via XTT upon gene editing in HCC cells with sgHPRT-LNPs and sgNCAPG-LNPs.

Figure 14A shows fluorescence imaging of femurs 4h after LNPs injection for LNPs comprising the lipids EA506C and EA405C.

5 **Figure 14B** shows fluorescence imaging of femurs 4h after injection to MCL bearing mice LNPs comprising the lipids EA506C and EA405C.

Figure 14C shows Relative Total Flux [p/s] of liver, spleen, kidneys, heart and lungs and femurus normalized to the mock after injection to MCL bearing mice LNPs comprising the lipids EA506C and EA405C.

10 **Figure 15A** shows Total Flux [p/s] of the femurs normalized to the mock after injection to MCL bearing mice LNPs comprising the lipids EA506C and EA405C.

Figure 15B shows Relative Total Flux [p/s] of liver, spleen, kidneys, heart and lungs and femurus normalized to the mock after injection to MCL bearing mice LNPs comprising the lipid EA524C.

15 **Figure 15C** shows Total Flux [p/s] of the femurs normalized to the mock after injection to MCL bearing mice LNPs comprising the lipid EA524C.

Figure 16 is a bar chart of % gene editing using EA524-LNPs and EA524C-LNPs formulations encapsulated with sgSOX11 and with sgGFP.

20 **Figures 17A-17D** relative decomposition measured as %purity over 6 days at room temperature for lipid EA-524 in ethanol (**Figure 17A**), lipid EA-524 in ethanol:water 3:1 v/v (**Figure 17B**), lipid EA-524C in ethanol (**Figure 17C**), and lipid EA-524C in ethanol:water 3:1 v/v (**Figure 17D**).

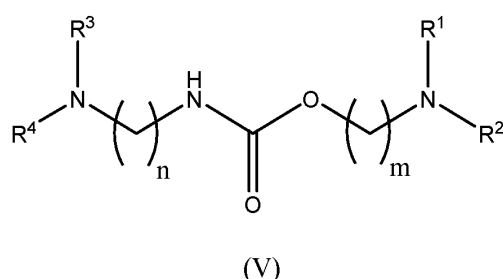
DETAILED DESCRIPTION

25 The present invention based on the discovery of hydrolytically and alcoholytically stable lipids useful in preparing lipid nanoparticles to deliver active agents in vitro and in vivo. The lipids of the present invention are useful in delivery of nucleic acids such as siRNA, miRNA and mRNA etc.

According to some embodiments, the present invention provides carbamate lipids. According to some embodiments, the lipids are ionizable lipids. According to some embodiments, the lipid of the present invention is a cationic lipid. The terms “cationic lipid” and “ionizable lipid” are as defined herein below.

5 Carbamate lipids – Formula (V)

As contemplated herein, the present invention relates to carbamate lipid(s) represented by the structure of Formula (V):



including salts, hydrates, solvates, polymorphs, optical isomers, geometrical isomers,
10 enantiomers, diastereomers, and mixtures thereof.

According to some embodiments, n is selected from the group consisting of: 0, 1, 2, 3, 4, 5, 6, 7 and 8. Each possibility represents a separate embodiment of the invention. According to some embodiments, n is selected from the group consisting of: 1, 2, 3, 4, 5, 6 and 7. According to some embodiments, n is selected from the group consisting of: 1, 2, 3, 4 and
15 5. According to some embodiments, n is selected from the group consisting of: 1, 2 and 3. According to some embodiments, n is 2.

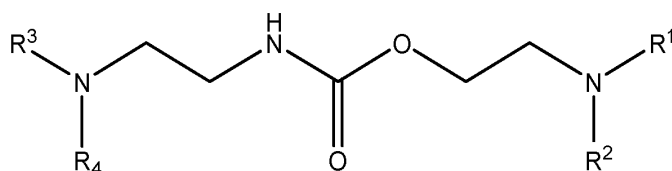
According to some embodiments, m is selected from the group consisting of: 0, 1, 2, 3, 4, 5, 6, 7 and 8. Each possibility represents a separate embodiment of the invention. According to some embodiments, m is selected from the group consisting of: 1, 2, 3, 4, 5, 6 and 7.
20 According to some embodiments, m is selected from the group consisting of: 1, 2, 3, 4 and 5. According to some embodiments, m is selected from the group consisting of: 1, 2 and 3. According to some embodiments, m is 2.

According to some embodiments, n equals to m. According to some embodiments, each one of n and m is individually selected from the group consisting of: 0, 1, 2, 3, 4, 5, 6, 7 and 8.

Each possibility represents a separate embodiment of the invention. According to some embodiments, each one of n and m is individually selected from the group consisting of: 1, 2, 3, 4, 5, 6 and 7. According to some embodiments, each one of n and m is individually selected from the group consisting of: 1, 2, 3, 4 and 5. According to some embodiments, each one of n and m is individually selected from the group consisting of: 1, 2 and 3. According to some embodiments, each one of n and m is individually 2.

According to some embodiments, each one of n and m is individually selected from the group consisting of: 1, 2, 3, 4 and 5.

According to some embodiments, each one of n and m is 2, and the lipid is represented by the structure of Formula (III) or a salt thereof:



(III)

According to some embodiments, R^1 is selected from the group consisting of: C_{3-9} alkylene- CO_2-C_{8-18} alkyl, C_{3-9} alkylene- CO_2-C_{8-18} alkenyl, C_{3-9} alkenylene- CO_2-C_{8-18} alkyl, C_{3-9} alkenylene- CO_2-C_{8-18} alkenyl, C_{3-9} alkylene- O_2C-C_{8-18} alkyl, C_{3-9} alkylene- O_2C-C_{8-18} alkenyl, C_{3-9} alkenylene- O_2C-C_{8-18} alkyl and C_{3-9} alkenylene- O_2C-C_{8-18} alkenyl. Each possibility represents a separate embodiment of the invention. As detailed herein, unless specified otherwise, the terms “alkyl”, “alkylene”, “alkenyl” and “alkenylene” relate to both substituted and unsubstituted groups. Also as detailed herein, unless specified otherwise, the terms “alkyl”, “alkylene”, “alkenyl” and “alkenylene” relate to both linear and branched chains. According to some embodiments, the alkyl is a branched alkyl. According to some embodiments, the alkyl is a straight alkyl. According to some embodiments, the alkyl is an unsubstituted alkyl. According to some embodiments, the alkylene is a straight alkylene. According to some embodiments, the alkylene is an unsubstituted alkylene. According to some embodiments, the alkenyl is a straight alkenyl. As also detailed herein, the terms “alkenyl” and “alkenylene” relate to fragments, which include one or more double carbon-

carbon bonds, and thus include dienes, trienes, tetraenes and the like. According to some embodiments, the alkenyl is an unsubstituted alkenyl. According to some embodiments, the alkenylene is a straight alkenylene. According to some embodiments, the alkenyl includes more than one C=C bond. According to some embodiments, the alkenyl is a dienyl.

- 5 According to some embodiments, the alkenylene is an unsubstituted alkenylene.

Specifically, it was surprisingly found that ester groups within the terminal lipidic substituent (R^1 and/or R^2) do not cause a substantial reduction of hydrolytic or alcoholic stability of the ionizable lipid. This, whereas an ester core group of the ionizable lipid results in a substantially less stable lipid than its counterpart that includes a core carbamate group.

- 10 According to some embodiments, R^1 is C_{3-9} alkylene-CO₂-C₈₋₁₈ alkyl. According to some embodiments, R^1 is C_{3-9} alkylene-CO₂-C₈₋₁₈ alkenyl. According to some embodiments, R^1 is C_{3-9} alkenylene-CO₂-C₈₋₁₈ alkyl. According to some embodiments, R^1 is C_{3-9} alkenylene-CO₂-C₈₋₁₈ alkenyl. According to some embodiments, R^1 is C_{3-9} alkylene-O₂C-C₈₋₁₈ alkyl. According to some embodiments, R^1 is C_{3-9} alkylene-O₂C-C₈₋₁₈ alkenyl.
- 15 According to some embodiments, R^1 is C_{3-9} alkenylene-O₂C-C₈₋₁₈ alkyl. According to some embodiments, R^1 is C_{3-9} alkenylene-O₂C-C₈₋₁₈ alkenyl. According to some embodiments, R^1 is selected from the group consisting of: C_{3-9} alkylene-CO₂-C₈₋₁₈ alkyl, C_{3-9} alkylene-CO₂-C₈₋₁₈ alkenyl, C_{3-9} alkenylene-CO₂-C₈₋₁₈ alkyl, C_{3-9} alkenylene-CO₂-C₈₋₁₈ alkenyl, C_{3-9} alkylene-O₂C-C₈₋₁₈ alkyl, C_{3-9} alkylene-O₂C-C₈₋₁₈ alkenyl, C_{3-9} alkenylene-O₂C-C₈₋₁₈ alkyl.
- 20 According to some embodiments, R^1 is selected from the group consisting of: C_{3-9} alkylene-CO₂-C₈₋₁₈ alkyl and C_{3-9} alkylene-O₂C-C₈₋₁₈ alkyl. According to some embodiments, R^1 is selected from the group consisting of: C_{6-9} alkylene-CO₂-C₁₄₋₁₈ alkyl, C_{6-9} alkylene-O₂C-C₁₄₋₁₈ alkyl.

According to some embodiments, R^1 is C_{6-9} alkylene-CO₂-C₁₄₋₁₈ alkyl. According to some

- 25 According to some embodiments, R^1 is C_{7-8} alkylene-CO₂-C₁₅₋₁₇ alkyl. According to some embodiments, R^1 is -C₇H₁₄-CO₂-CH(C₈H₁₇)C₈H₁₇.

According to some embodiments, R^1 is C_{6-9} alkylene-O₂C-C₁₄₋₁₈ alkyl. According to some embodiments, R^1 is C_{7-8} alkylene-O₂C-C₁₅₋₁₇ alkyl. According to some embodiments, R^1 is -C₈H₁₆-O₂C-CH(C₆H₁₃)C₈H₁₇.

According to some embodiments, R^2 is selected from the group consisting of: C_{10-20} alkyl, C_{10-20} alkenyl, C_{10-20} alkynyl, C_{3-9} alkylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkylene- CO_2 - C_{8-18} alkenyl, C_{3-9} alkenylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkenylene- CO_2 - C_{8-18} alkenyl, C_{3-9} alkylene- O_2C - C_{8-18} alkyl, C_{3-9} alkylene- O_2C - C_{8-18} alkenyl, C_{3-9} alkenylene- O_2C - C_{8-18} alkyl and C_{3-9} alkenylene- O_2C - C_{8-18} alkenyl. Each possibility represents a separate embodiment of the invention. As detailed herein, unless specified otherwise, the terms “alkyl”, “alkylene”, “alkenyl” and “alkenylene” relate to both substituted and unsubstituted groups. Also as detailed herein, unless specified otherwise, the terms “alkyl”, “alkylene”, “alkenyl” and “alkenylene” relate to both linear and branched chains. According to some embodiments, the alkyl is a branched alkyl. According to some embodiments, the alkyl is a straight alkyl. According to some embodiments, the alkyl is an unsubstituted alkyl. According to some embodiments, the alkylene is a straight alkylene. According to some embodiments, the alkylene is an unsubstituted alkylene. According to some embodiments, the alkenyl is a straight alkenyl. As also detailed herein, the terms “alkenyl” and “alkenylene” relate to fragments, which include one or more double carbon-carbon bonds, and thus include dienes, trienes, tetraenes and the like. According to some embodiments, the alkenyl is an unsubstituted alkenyl. According to some embodiments, the alkenylene is a straight alkenylene. According to some embodiments, the alkenyl includes more than one $C=C$ bond. According to some embodiments, the alkenyl is a dienyl. According to some embodiments, the alkenylene is an unsubstituted alkenylene. According to some embodiments, the alkenyl is an unsubstituted alkenyl. According to some embodiments, the alkenylene is a straight alkenylene. According to some embodiments, the alkenylene is an unsubstituted alkenylene.

According to some embodiments, R^2 is selected from the group consisting of: C_{10-20} alkenyl, C_{3-9} alkylene- CO_2 - C_{8-18} alkyl and C_{3-9} alkylene- O_2C - C_{8-18} alkyl. According to some embodiments, R^2 is selected from the group consisting of: C_{16-20} alkenyl, C_{6-9} alkylene- CO_2 - C_{14-18} alkyl and C_{6-9} alkylene- O_2C - C_{14-18} alkyl.

According to some embodiments, R^2 is C_{6-9} alkylene- CO_2 - C_{14-18} alkyl. According to some embodiments, R^2 is C_{7-8} alkylene- CO_2 - C_{15-17} alkyl. According to some embodiments, R^1 is $-C_7H_{14}-CO_2-CH(C_8H_{17})C_8H_{17}$.

According to some embodiments, R^2 is C_{6-9} alkylenes- O_2C-C_{14-18} alkyl. According to some embodiments, R^2 is C_{7-8} alkylenes- O_2C-C_{15-17} alkyl. According to some embodiments, R^2 is $-C_8H_{16}-O_2C-CH(C_6H_{13})C_8H_{17}$.

- According to some embodiments, R^2 is C_{10-20} alkenyl. According to some embodiments, R^2 is C_{15-20} alkenyl. According to some embodiments, R^2 is C_{17-19} alkenyl. According to some embodiments, R^2 is C_{18} alkenyl. According to some embodiments, R^2 is C_{18} dienyl. According to some embodiments, R^2 is $-C_8H_{16}-CH=CH-CH_2-CH=CH-C_5H_{11}$. According to some embodiments, each one of the carbon-carbon double bonds within R^2 has a *cis* configuration.
- 10 According to some embodiments, each one of R^1 and R^2 is individually selected from the group consisting of: C_{3-9} alkylenes- CO_2-C_{8-18} alkyl, C_{3-9} alkylenes- CO_2-C_{8-18} alkenyl, C_{3-9} alkenylenes- CO_2-C_{8-18} alkyl, C_{3-9} alkylenes- O_2C-C_{8-18} alkyl, C_{3-9} alkylenes- O_2C-C_{8-18} alkenyl, C_{3-9} alkenylenes- O_2C-C_{8-18} alkyl. Each possibility represents a separate embodiment of the invention. According to some embodiments, each one of R^1 and R^2 is individually selected from the group consisting of: C_{3-9} alkylenes- CO_2-C_{8-18} alkyl and C_{3-9} alkylenes- O_2C-C_{8-18} alkyl. According to some embodiments, each one of R^1 and R^2 is C_{6-9} alkylenes- CO_2-C_{14-18} alkyl.

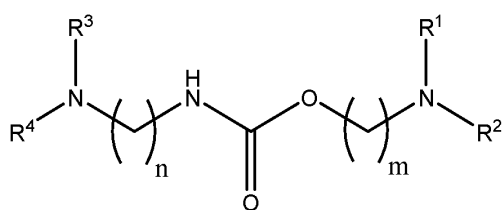
- According to some embodiments, R^3 is a C_{1-4} alkyl. As detailed herein, unless specified otherwise, the term alkyl relates to both substituted and unsubstituted alkyls. Also as detailed herein, unless specified otherwise, the term alkyl relates to both linear and branched alkyls. According to some embodiments, R^3 is a C_{1-3} alkyl. According to some embodiments, R^3 is a C_{1-2} alkyl. According to some embodiments, R^3 is a C_1 alkyl. According to some embodiments, the alkyl is a straight alkyl. According to some embodiments, the alkyl is an unsubstituted alkyl. According to some embodiments, R^3 is CH_3 or CH_2CH_3 . According to some embodiments, R^3 is CH_3 .

- According to some embodiments, R^4 is a C_{1-4} alkyl. As detailed herein, unless specified otherwise, the term alkyl relates to both substituted and unsubstituted alkyls. Also as detailed herein, unless specified otherwise, the term alkyl relates to both linear and branched alkyls. According to some embodiments, R^4 is a C_{1-3} alkyl. According to some embodiments, R^4 is a C_{1-2} alkyl. According to some embodiments, R^4 is a C_1 alkyl.

According to some embodiments, the alkyl is a straight alkyl. According to some embodiments, the alkyl is an unsubstituted alkyl. According to some embodiments, R^4 is CH_3 or CH_2CH_3 . According to some embodiments, R^4 is CH_3 .

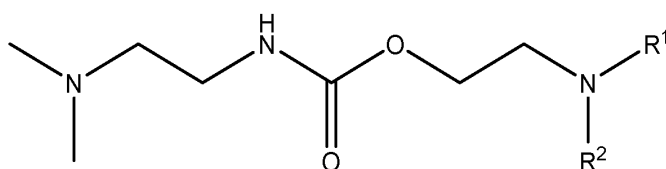
- According to some embodiments, R^3 is the same as R^4 . According to some embodiments, each one of R^3 and R^4 is individually, a C_{1-4} alkyl or wherein R^3 and R^4 together with the nitrogen atom to which they are bound form a 5-6 membered ring. According to some embodiments, R^3 and R^4 together with the nitrogen atom to which they are bound form a 5-6 membered ring. According to some embodiments, each one of R^3 and R^4 individually is a C_{1-3} alkyl. According to some embodiments, each one of R^3 and R^4 individually is a C_{1-2} alkyl. According to some embodiments, each one of R^3 and R^4 is individually a C_1 alkyl. According to some embodiments, each one of R^3 and R^4 is individually CH_3 or CH_2CH_3 . According to some embodiments, each one of R^3 and R^4 is individually CH_3 .

According to some embodiments, each one of n and m is 2, and the lipid is represented by the structure of Formula (IVa) or a salt thereof:



(IVa)

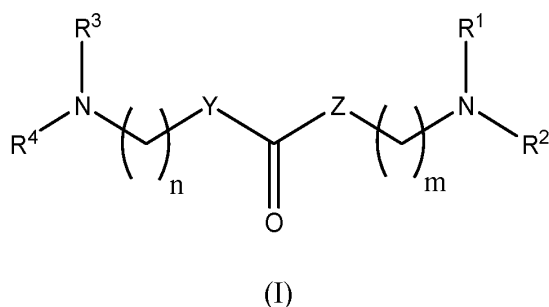
According to some embodiments, each one of n and m is 2, each one of R^3 and R^4 is individually CH_3 , and the lipid is represented by the structure of Formula (IV) or a salt thereof:



(IV)

Carbamate lipids – Formula (I)

According to some embodiments, there is provided a lipid represented by the structure of Formula (I) or a salt thereof:



5 wherein one of Y and Z is O and the other of Y and Z is NH;

each one of n and m is individually selected from the group consisting of: 1, 2, 3, 4 and 5;

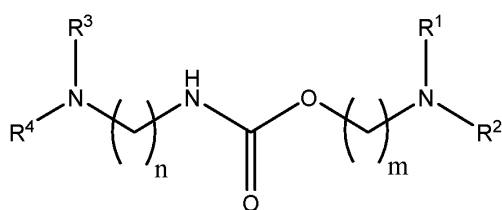
each one of R¹ and R² is individually selected from the group consisting of: C₁₀₋₂₀ alkyl, C₁₀₋₂₀ alkenyl, C₁₀₋₂₀ alkynyl, C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkyl, C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkenyl, C₃₋₉ alkenylene-CO₂-C₈₋₁₈ alkyl, C₃₋₉ alkenylene-CO₂-C₈₋₁₈ alkenyl, C₃₋₉ alkenylene-O₂C-C₈₋₁₈ alkyl, C₃₋₉ alkenylene-O₂C-C₈₋₁₈ alkenyl, C₃₋₉ alkenylene-O₂C-C₈₋₁₈ alkyl and C₃₋₉ alkenylene-O₂C-C₈₋₁₈ alkenyl; and

each one of R³ and R⁴ is individually, a C₁₋₄ alkyl or wherein R³ and R⁴ together with the nitrogen atom to which they are bound form a 5-6 membered ring.

According to some embodiments, the lipid is hydrolytically stable for at least 2 hours.

15 According to some embodiments, Y is NH and Z is O. According to some embodiments, Z is NH and Y is O.

According to some embodiments, Y is NH, Z is O and the lipid is represented by the structure of Formula (Ia) or a salt thereof:

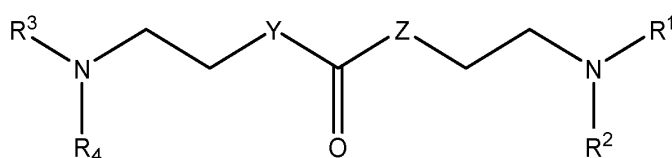


(Ia)

According to some embodiments, n is 2, 3 or 4. Each possibility represents a separate embodiment of the invention. According to some embodiments, n is 2.

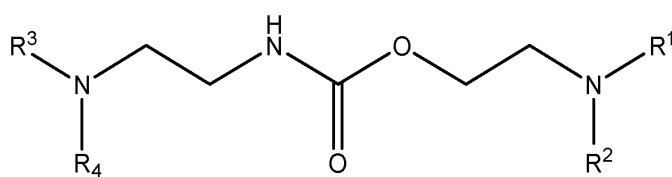
According to some embodiments, m is 2, 3 or 4. Each possibility represents a separate embodiment of the invention. According to some embodiments, m is 2.

According to some embodiments, each one of n and m is 2, and the lipid is represented by the structure of Formula (II) or a salt thereof:



(II)

According to some embodiments, each one of n and m is 2, Y is NH and Z is O, and the lipid is represented by the structure of Formula (III) or a salt thereof:

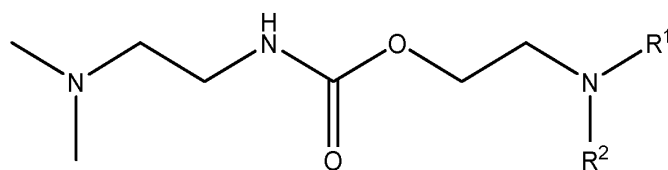


(III)

According to some embodiments, each one of R³ and R⁴ is individually, a C₁₋₄ alkyl. According to some embodiments, R³ is a C₁₋₂ alkyl. According to some embodiments, R³ is

CH₃. According to some embodiments, R⁴ is a C₁₋₂ alkyl. According to some embodiments, R⁴ is CH₃.

According to some embodiments, each one of n and m is 2, Y is NH and Z is O, each one of R³ and R⁴ is individually CH₃, and the lipid is represented by the structure of Formula
 5 (IV) or a salt thereof:



(IV)

According to some embodiments, R¹ is selected from the group consisting of: C₁₀₋₂₀ alkenyl, C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkyl, C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkyl and C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkenyl. According to some embodiments, R¹ is selected from the group consisting of:
 10 C₁₄₋₁₈ alkenyl, C₆₋₉ alkylene-CO₂-C₁₂₋₁₈ alkyl, C₆₋₉ alkylene-O₂C-C₉₋₁₆ alkyl and C₄₋₆ alkylene-CO₂-C₈₋₁₀ alkenyl.

According to some embodiments, R¹ contains an ester group. According to some embodiments, R¹ is selected from the group consisting of: C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkyl, C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkenyl, C₃₋₉ alkenylene-CO₂-C₈₋₁₈ alkyl, C₃₋₉ alkenylene-CO₂-C₈₋₁₈ alkenyl, C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkyl, C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkenyl, C₃₋₉ alkenylene-O₂C-C₈₋₁₈ alkyl and C₃₋₉ alkenylene-O₂C-C₈₋₁₈ alkenyl
 15

According to some embodiments, R² contains an ester group. According to some embodiments, R² is selected from the group consisting of: C₁₀₋₂₀ alkenyl, C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkyl, C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkyl and C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkenyl.
 20 According to some embodiments, R² is selected from the group consisting of: C₁₄₋₁₈ alkenyl, C₆₋₉ alkylene-CO₂-C₁₂₋₁₈ alkyl, C₆₋₉ alkylene-O₂C-C₉₋₁₆ alkyl and C₄₋₆ alkylene-CO₂-C₈₋₁₀ alkenyl.

According to some embodiments, at least one of R¹ and R² contains an ester group. According to some embodiments, each one of R¹ and R² contains an ester group. According

to some embodiments, each one of R^1 and R^2 is individually selected from the group consisting of: C_{10-20} alkenyl, C_{3-9} alkylene-CO₂- C_{8-18} alkyl, C_{3-9} alkylene-O₂C- C_{8-18} alkyl and C_{3-9} alkylene-CO₂- C_{8-18} alkenyl. According to some embodiments, each one of R^1 and R^2 is individually selected from the group consisting of: C_{14-18} alkenyl, C_{6-9} alkylene-CO₂- C_{12-18} alkyl, C_{6-9} alkylene-O₂C- C_{9-16} alkyl and C_{4-6} alkylene-CO₂- C_{8-10} alkenyl.

According to some embodiments, each one of R^1 and R^2 is individually selected from the group consisting of: C_{18} alkenyl, C_5 alkylene-CO₂- C_9 alkenyl; C_{7-9} alkylene-CO₂- C_{13-17} alkyl and C_{7-8} alkylene-O₂C- C_{9-15} alkyl.

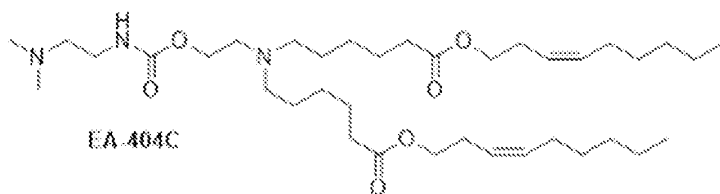
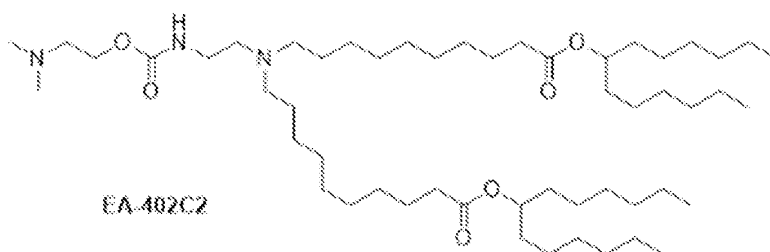
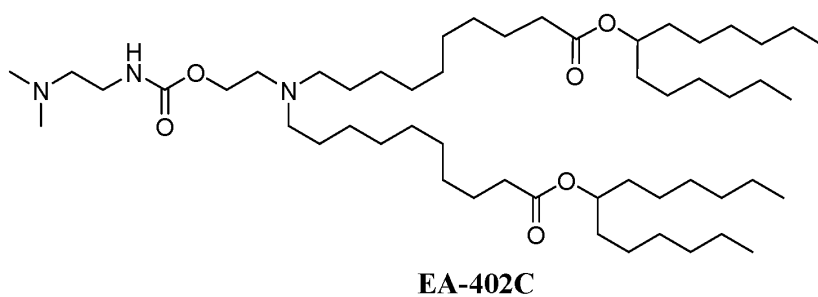
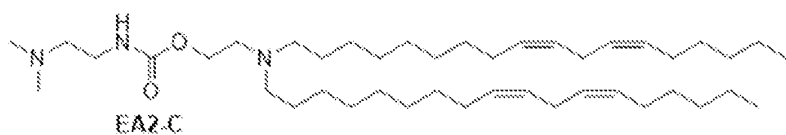
According to some embodiments, each one of the alkyl, alkenyl, alkynyl, alkylene and alkenylene groups within Formula 1 is unsubstituted. According to some embodiments, each one of the alkyl, alkenyl, alkynyl, alkylene and alkenylene groups is straight chain or branched. Each possibility represents a separate embodiment of the invention. According to some embodiments, each one of the alkyl and alkenyl groups is unsubstituted. According to some embodiments, each one of the alkyl and alkenyl groups is straight-chain or branched.

According to some embodiments, Y is NH, Z is O, R^1 is selected from the group consisting of: C_{3-9} alkylene-CO₂- C_{8-18} alkyl, C_{3-9} alkylene-CO₂- C_{8-18} alkenyl, C_{3-9} alkenylene-CO₂- C_{8-18} alkyl, C_{3-9} alkenylene-CO₂- C_{8-18} alkenyl, C_{3-9} alkylene-O₂C- C_{8-18} alkyl, C_{3-9} alkylene-O₂C- C_{8-18} alkenyl, C_{3-9} alkenylene-O₂C- C_{8-18} alkyl and C_{3-9} alkenylene-O₂C- C_{8-18} alkenyl; R^2 is selected from the group consisting of: C_{10-20} alkyl, C_{10-20} alkenyl, C_{10-20} alkynyl, C_{3-9} alkylene-CO₂- C_{8-18} alkyl, C_{3-9} alkylene-CO₂- C_{8-18} alkenyl, C_{3-9} alkenylene-CO₂- C_{8-18} alkyl, C_{3-9} alkenylene-CO₂- C_{8-18} alkenyl, C_{3-9} alkylene-O₂C- C_{8-18} alkyl, C_{3-9} alkylene-O₂C- C_{8-18} alkenyl, C_{3-9} alkenylene-O₂C- C_{8-18} alkyl and C_{3-9} alkenylene-O₂C- C_{8-18} alkenyl; and the lipid is the carbamate lipid represented by the structure of Formula (V). Formula (V) is shown and described herein.

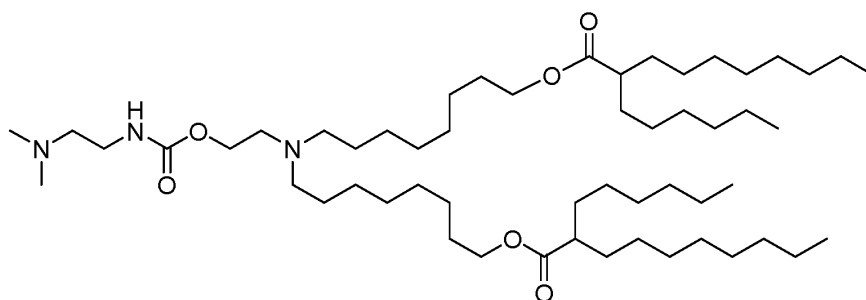
25 Exemplary lipids

As contemplated herein, the present invention relates to a lipid represented by the structure of any one of formulas (I), (Ia), (II), (III), (IV), (IVa) or (V) as described above. Each possibility represents a separate embodiment of the invention, including salts, hydrates, solvates, polymorphs, optical isomers, geometrical isomers, enantiomers, diastereomers, and mixtures thereof.

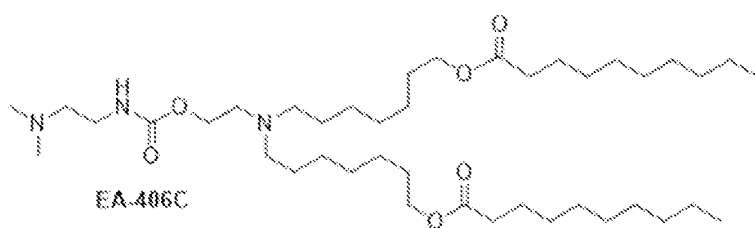
According to some embodiments, the lipid of the present invention is selected from the group consisting of:



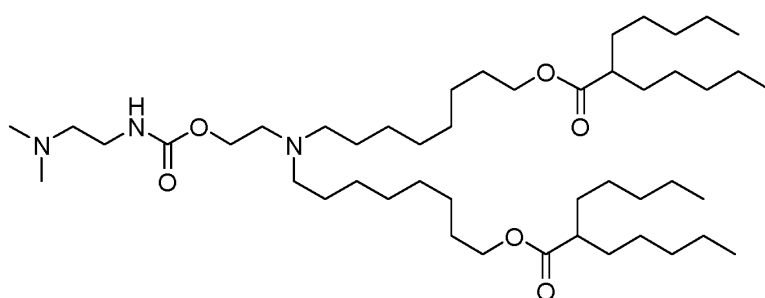
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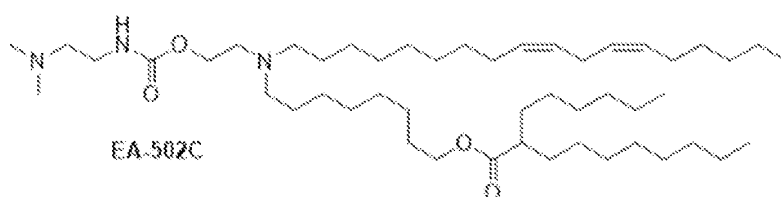
EA-405C



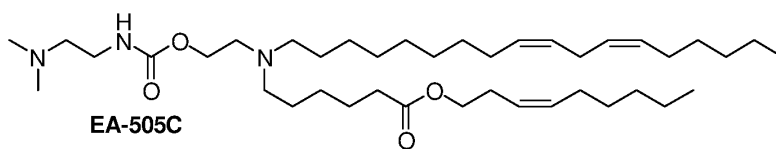
EA-406C



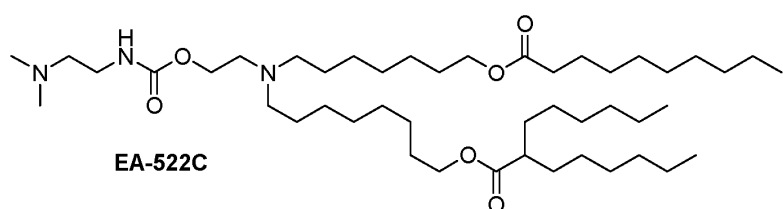
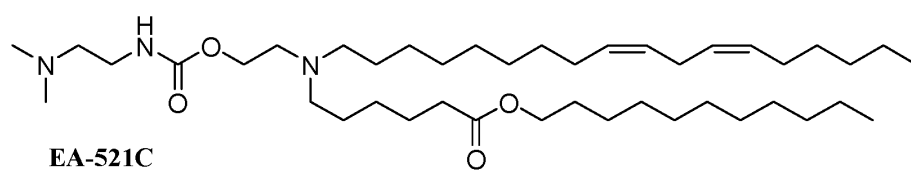
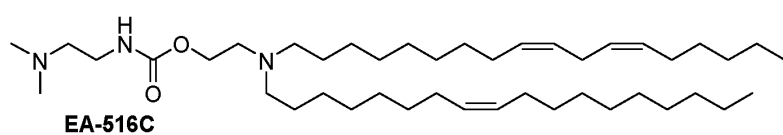
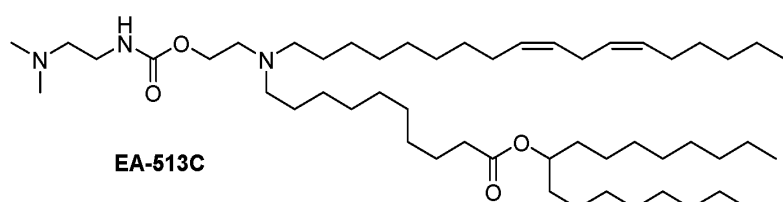
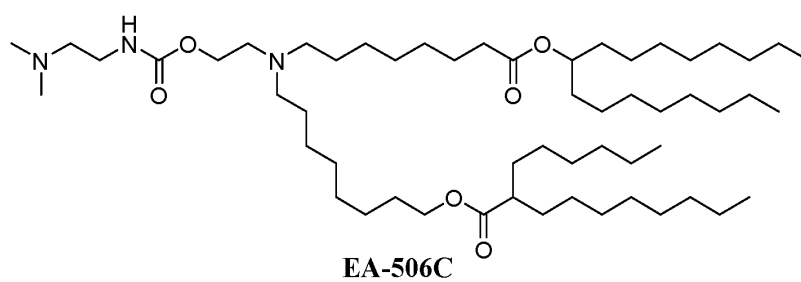
EA-408C



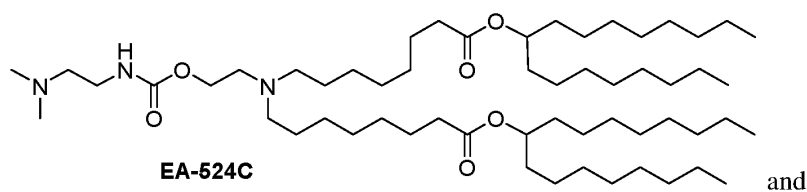
EA-502C

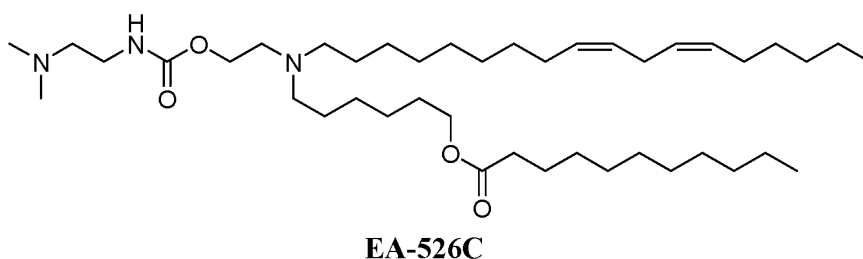


EA-505C



5





or salts thereof. Each possibility represents a separate embodiment of the invention.

According to some embodiments, the lipid of Formula (I) is selected from the group consisting of: EA-2C, EA-402C, EA-402C2, EA-404C, EA-405C, EA-406C, EA-408C,
 5 EA-502C, EA-505C, EA-506C, EA-513C, EA-516C, EA-521C, EA-522C, EA-524C
 EA-526C and salts thereof. Each possibility represents a separate embodiment of the invention.

According to some embodiments, the lipid of the present invention is selected from the group consisting of: EA-402C, EA-404C, EA-405C, EA-406C, EA-408C, EA-502C, EA-
 10 505C, EA-506C, EA-513C, EA-521C, EA-522C, EA-524C, EA-526C and salts thereof.
 According to some embodiments, the lipid of Formula (V) is selected from the group consisting of: EA-402C, EA-404C, EA-405C, EA-406C, EA-408C, EA-502C, EA-505C, EA-506C, EA-513C, EA-521C, EA-522C, EA-524C, EA-526C and salts thereof. Each possibility represents a separate embodiment of the invention.

15 According to some embodiments, the lipid of the present invention is selected from the group consisting of: EA-402C, EA-405C, EA-406C, EA-408C, EA-502C, EA-505C, EA-506C, EA-513C, EA-521C, EA-522C, EA-524C, EA-526C and salts thereof. According to some embodiments, the lipid of Formula (V) is selected from the group consisting of: EA-402C, EA-405C, EA-406C, EA-408C, EA-502C, EA-505C, EA-506C, EA-513C, EA-
 20 521C, EA-522C, EA-524C, EA-526C and salts thereof.

According to some embodiments, the lipid of the present invention is selected from the group consisting of: EA-405C, EA-502C, EA-506C, EA-513C, EA-524C and salts thereof. According to some embodiments, the lipid of Formula (V) is selected from the group consisting of: EA-405C, EA-502C, EA-506C, EA-513C, EA-524C and salts thereof.

According to some embodiments, the lipid of the present invention is selected from the group consisting of: EA-405C, EA-506C, EA-513C, EA-524C and salts thereof. According to some embodiments, the lipid of Formula (V) is selected from the group consisting of: EA-405C, EA-506C, EA-513C, EA-524C and salts thereof.

- 5 According to some embodiments, the lipid of the present invention is selected from the group consisting of: EA-405C, EA-513C, EA-524C and salts thereof. According to some embodiments, the lipid of Formula (V) is selected from the group consisting of: EA-405C, EA-513C, EA-524C and salts thereof.

- 10 According to some embodiments, the lipid of the present invention is selected from the group consisting of: EA-506C, EA-513C, EA-524C and salts thereof. According to some embodiments, the lipid of Formula (V) is selected from the group consisting of: EA-506C, EA-513C, EA-524C and salts thereof.

- 15 According to some embodiments, the lipid of the present invention is selected from the group consisting of: EA-405C, EA-506C, EA-524C and salts thereof. According to some embodiments, the lipid of Formula (V) is selected from the group consisting of: EA-405C, EA-506C, EA-524C and salts thereof.

- 20 According to some embodiments, the lipid of the present invention is selected from the group consisting of: EA-506C, EA-524C and salts thereof. According to some embodiments, the lipid of the present invention is selected from the group consisting of: EA-405C, EA-524C and salts thereof. According to some embodiments, the lipid of the present invention is EA-524C or a salt thereof.

Chemical Stability

- 25 According to some embodiments, the carbamate lipid of the present invention is hydrolytically stable, alcoholytically stable or both for at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 10 hours, at least 12 hours, at least 24 hours, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 10 days, at least 15 days, at least

20 days and/or at least 30 days. Each possibility represents a separate embodiment of the invention.

The term “hydrolytic stability” refers to the material's ability to resist hydrolysis. A material is considered “hydrolytically stable” if it does not undergo substantial hydrolysis upon exposure to hydrolytic conditions. Hydrolytic conditions include immersion of the material at room temperature in a solution having physiological pH (e.g., upon immersion in PBS containing solutions) for a predetermined time period. The level of hydrolysis can be determined e.g., using HPLC, to monitor the decomposition of the starting material upon exposure to the hydrolytic conditions. The term “substantial hydrolysis” refers to hydrolysis of at least 10%, at least 5%, at least 3%, at least 2% or at least 1%. Each possibility represents a separate embodiment of the invention. In the context of the present invention, lipids, such as cationic lipids undergo gradual hydrolysis upon exposure to hydrolytic conditions. Without wishing to be bound by any theory of mechanism of action, the gradual hydrolysis results in dissociation of ester and/or carbamate groups contained within the lipid.

The term “alcoholytic stability” refers to the material's ability to resist alcoholysis. A material is considered “alcoholytically stable” if it does not undergo substantial alcoholysis upon exposure to alcoholytic conditions. Alcoholytic conditions include immersion of the material at room temperature in an alcohol for a predetermined time period. The level of hydrolysis can be determined e.g., using HPLC, to monitor the decomposition of the starting material upon exposure to the alcoholytic conditions. The term “substantial alcoholysis” refers to alcoholysis of at least 10%, at least 5%, at least 3%, at least 2% or at least 1%. Each possibility represents a separate embodiment of the invention. In the context of the present invention, lipids, such as ionizable lipids undergo gradual alcoholysis upon exposure to alcoholysis conditions. Without wishing to be bound by any theory of mechanism of action, the gradual alcoholysis results in dissociation of ester and/or carbamate groups contained within the lipid.

As specified herein, the present lipids may form lipid nanoparticles formulated with nucleic acids to facilitate their intracellular delivery both *in vitro* and for *in vivo* therapeutic

applications. Thus, the lipids of the present are required to be hydrolytically stable for time frames relevant for such applications.

According to some embodiments, alcoholytic stability entails stability to ethanolysis upon immersion of the material at room temperature in ethanol for a predetermined time period.

- 5 According to some embodiments, the lipid is hydrolytically stable for at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 10 hours, at least 12 hours, at least 24 hours, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 10 days, at least 15 days, at least 20 days and/or at least 30 days. Each possibility represents a separate embodiment of the invention.
- 10 According to some embodiments, the lipid is alcoholytically stable for at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 10 hours, at least 12 hours, at least 24 hours, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 10 days, at least 15 days, at least 20 days and/or at least 30 days. Each possibility represents a separate embodiment of the invention.
- 15 According to some embodiments, the lipid is hydrolytically stable and alcoholytically stable upon immersion at room temperature in aqueous ethanol for at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 10 hours, at least 12 hours, at least 24 hours, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 10 days, at least 15 days, at least 20 days and/or at least 30 days. Each possibility represents a separate embodiment of
- 20 the invention.

According to some embodiments, the lipid of the present invention is hydrolytically stable for at least 24 hours, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 10 days, at least 15 days, at least 20 days and/or at least 30 days. Each possibility represents a separate embodiment of the invention.

- 25 According to some embodiments, the lipid of the present invention is alcoholytically stable for at least 24 hours, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 10 days, at least 15 days, at least 20 days and/or at least 30 days. Each possibility represents a separate embodiment of the invention.

According to some embodiments, the lipid of the present invention is hydrolytically stable and alcoholytically stable upon immersion at room temperature in aqueous ethanol for at least 24 hours, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 10 days, at least 15 days, at least 20 days and/or at least 30 days. Each possibility represents a separate
5 embodiment of the invention

As specified herein, the present lipids may form lipid nanoparticles formulated with nucleic acids to facilitate their intracellular delivery both *in vitro* and for *in vivo* therapeutic applications. Thus, the lipids of the present are required to be hydrolytically stable for time frames relevant for such applications. Furthermore, ionizable lipids are prepared in batches
10 and are typically stored in ethanol until use. Therefore, their alcoholytic stability is important.

According to some embodiments, no more than 10% of the lipid of the present invention decomposes in ethanol at RT after 15 days. According to some embodiments, no more than 15% of the lipid of the present invention decomposes in ethanol at RT after 1 day. According
15 to some embodiments, no more than 20% of the lipid of the present invention decomposes in ethanol at RT after 15 days. According to some embodiments, no more than 25% of the lipid of the present invention decomposes in ethanol at RT after 1 day. According to some embodiments, no more than 30% of the lipid of the present invention decomposes in ethanol at RT after 15 days. According to some embodiments, no more than 35% of the lipid of the
20 present invention decomposes in ethanol at RT after 1 day.

According to some embodiments, no more than 20% of the lipid of the present invention decomposes in ethanol at RT after 30 days. According to some embodiments, no more than 25% of the lipid of the present invention decomposes in ethanol at RT after 2 days. According to some embodiments, no more than 30% of the lipid of the present invention
25 decomposes in ethanol at RT after 30 days. According to some embodiments, no more than 35% of the lipid of the present invention decomposes in ethanol at RT after 2 days. According to some embodiments, no more than 40% of the lipid of the present invention decomposes in ethanol at RT after 30 days. According to some embodiments, no more than 45% of the lipid of the present invention decomposes in ethanol at RT after 2 days.

- According to some embodiments, no more than 25% of the lipid of the present invention decomposes in ethanol at RT after 90 days. According to some embodiments, no more than 30% of the lipid of the present invention decomposes in ethanol at RT after 3 days. According to some embodiments, no more than 35% of the lipid of the present invention decomposes in ethanol at RT after 90 days. According to some embodiments, no more than 40% of the lipid of the present invention decomposes in ethanol at RT after 3 days. According to some embodiments, no more than 45% of the lipid of the present invention decomposes in ethanol at RT after 90 days. According to some embodiments, no more than 50% of the lipid of the present invention decomposes in ethanol at RT after 3 days.
- 10 According to some embodiments, no more than 30% of the lipid of the present invention decomposes in ethanol at RT after 1 year. According to some embodiments, no more than 35% of the lipid of the present invention decomposes in ethanol at RT after 4 days. According to some embodiments, no more than 40% of the lipid of the present invention decomposes in ethanol at RT after 1 year. According to some embodiments, no more than 45% of the lipid of the present invention decomposes in ethanol at RT after 4 days. According to some embodiments, no more than 50% of the lipid of the present invention decomposes in ethanol at RT after 1 year. According to some embodiments, no more than 55% of the lipid of the present invention decomposes in ethanol at RT after 4 days.
- 20 According to some embodiments, no more than 20% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 15 days. According to some embodiments, no more than 25% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 15 days. According to some embodiments, no more than 30% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 15 days. According to some embodiments, no more than 35% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 15 days. According to some embodiments, no more than 40% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 15 days.
- 30 According to some embodiments, no more than 30% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 30 days. According to some embodiments, no more than 35% of the lipid of the present invention decomposes in 75%

ethanol 25% v/v water solution at RT after 30 days. According to some embodiments, no more than 40% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 30 days. According to some embodiments, no more than 45% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 30 days. According to some embodiments, no more than 50% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 30 days. According to some embodiments, no more than 55% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 30 days.

According to some embodiments, no more than 50% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 1 year. According to some embodiments, no more than 60% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 1 year. According to some embodiments, no more than 65% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 1 year. According to some embodiments, no more than 70% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 1 year. According to some embodiments, no more than 75% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 1 year. According to some embodiments, no more than 80% of the lipid of the present invention decomposes in 75% ethanol 25% v/v water solution at RT after 1 year.

Chemical Definitions

The term “ionizable lipid”, as used herein refers to lipid species that carries a charge at a selected pH. Selected pH values include, but not limited to physiological pH, pH=7 and the like. It is to be understood by the person having ordinary skill in the art that the lipids of Formulae (I), (Ia), (II), (III), (IV), (IVa) and (V) may be considered as ionizable lipids.

The term “cationic lipid”, as used herein refers to lipid species that carries a net positive charge at a selected pH. Selected pH values include, but not limited to physiological pH, pH=7 and the like. It is to be understood by the person having ordinary skill in the art that the lipids of the present invention may be considered as cationic lipids, since they bear nitrogen atoms that are typically basic and protonizable at the selected pH, so that the

compound may carry a net positive charge. According to some embodiments, the lipid of the present invention is a cationic lipid.

The term "carbamate lipid", as used herein refers to any lipid, which contains a carbamate moiety.

- 5 An "alkyl" group refers to any saturated aliphatic hydrocarbon, including straight-chain and branched-chain alkyl groups. The alkyl group may be unsubstituted or substituted by one or more groups selected from halogen, hydroxy, alkoxy carbonyl, amido, alkylamido, dialkylamido, nitro, amino, alkylamino, dialkylamino, carboxyl, thio and thioalkyl. The term " C_{n-m} alkyl", refers to an alkyl group having n to m carbon atoms. For example, an
- 10 unsubstituted C_{17} alkyl may refer to the straight chain $n-C_{17}$, or to any branched alkyl have the appropriate number of carbon atoms, e.g., $-CH(C_8C_{17})_2$. An alkyl group 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. Examples of alkyl moieties include, but are not limited to, chemical groups such as methyl, ethyl, n -propyl, isopropyl, n -butyl, tert-butyl,
- 15 isobutyl, sec-butyl; higher homologs such as 2-methyl-1-butyl, 3-pentyl, hexyl, 1,2,2-trimethylpropyl and the like. The term "alkylene," employed alone or in combination with other terms, refers to a divalent alkyl linking group. An alkylene group formally corresponds to an alkane with two C-H bonds replaced by points of attachment of the alkylene group to the remainder of the compound. The term " C_{n-m} alkylene" refers to an alkylene group having
- 20 n to m carbon atoms. Examples of alkylene groups include, but are not limited to, ethan-1,2-diyl, ethan-1,1-diyl, propan-1,3-diyl, propan-1,2-diyl, propan-1,1-diyl, butan-1,4-diyl, butan-1,3-diyl, butan-1,2-diyl, 2-methyl-propan-1,3-diyl and the like. It is to be understood that such C_{n-m} ranges include any sub range thereof. for example, C_{4-14} alkyl may include and/or be directed to: C_{4-8} alkyl, C_{8-14} alkyl, C_{6-12} alkyl, C_9 alkyl etc.
- 25 An "alkenyl" group refers to an aliphatic hydrocarbon group containing at least one carbon-carbon double bond including straight-chain, branched-chain and cyclic alkenyl groups. Exemplary alkenyl groups include ethenyl, propenyl, n -butenyl, i -butenyl, 3-methylbut-2-enyl, n -pentenyl, heptenyl, octenyl, cyclohexyl-butenyl and decenyl. The alkenyl group can be unsubstituted or substituted through available carbon atoms with one or more groups
- 30 defined hereinabove for alkyl. Alkenyls according to the present invention may include

- more than one carbon-carbon double bond. Thus, dienes (see e.g., compound EA-513C, substituent R²) and trienes are within the definition of alkenyl. According to some embodiments, the alkenyl is a dienyl. The term "C_{n-m} alkenyl", refers to an alkyl group having n to m carbon atoms. The term "C_{n-m} alkenyl", refers to an alkenyl group having n to m carbon atoms. For example, an unsubstituted C₁₈ alkenyl may refer to the straight chain alkenyls, such as $-\text{CH}_2(\text{CH}_2)_7-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_4\text{CH}_3$ and $-\text{CH}_2(\text{CH}_2)_{10}-\text{CH}=\text{CH}-(\text{CH}_2)_4\text{CH}_3$, or to branched alkenyls, such as $-\text{CH}_2(\text{CH}_2)_7-\text{C}(\text{CH}_3)=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_3\text{CH}_3$. An alkenyl group formally corresponds to an alkene with one C-H bond replaced by the point of attachment of the alkenyl group to the remainder of the compound. Examples of alkenyl moieties include, but are not limited to, chemical groups such as ethenyl, propenyl, isopropenyl, n- butenyl, sec-butenyl the like. The term "alkenylene," employed alone or in combination with other terms, refers to a divalent alkenyl linking group. An alkenylene group formally corresponds to an alkane with two C-H bonds replaced by points of attachment of the alkenylene group to the remainder of the compound. The term "C_{n-m} alkenylene" refers to an alkenylene group having n to m carbon atoms. In various sections of the present application ranges of alkenyl chains are presented, e.g., C₂₋₈ alkenyl, C₄₋₂₀ alkenyl etc. It is to be understood that such ranges include any sub range thereof. for example, C₄₋₁₄ alkenyl may include and/or be directed to: C₄₋₈ alkenyl, C₈₋₁₄ alkenyl, C₆₋₁₂ alkenyl, C₉ alkenyl etc.
- One or more of the lipids of the invention, may be present as a salt. The term "salt" encompasses both basic and acid addition salts, including but not limited to, carboxylate salts or salts with amine nitrogen atoms, and include salts formed with the organic and inorganic anions and cations discussed below. Furthermore, the term includes salts that form by standard acid-base reactions with basic groups (such as amino groups) and organic or inorganic acids. Such acids include hydrochloric, hydrofluoric, trifluoroacetic, sulfuric, phosphoric, acetic, succinic, citric, lactic, maleic, fumaric, palmitic, cholic, pamoic, mucic, D-glutamic, D-camphoric, glutaric, phthalic, tartaric, lauric, stearic, salicylic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic, and like acids. Each possibility represents a separate embodiment of the invention.

The term "organic or inorganic cation" refers to counter-ions for the anion of a salt. The counter-ions include, but are not limited to, alkali and alkaline earth metals (such as lithium, sodium, potassium, barium, aluminum and calcium); ammonium and mono-, di- and tri-alkyl amines such as trimethylamine, cyclohexylamine; and the organic cations, such as
5 dibenzylammonium, benzylammonium, 2-hydroxyethylammonium, bis(2-hydroxyethyl)ammonium, phenylethylbenzylammonium, dibenzylethylenediammonium, and like cations. See, for example, Berge et al., *J. Pharm. Sci.* (1977), **66**:1-19, which is incorporated herein by reference.

Particles, Compositions and Uses

10 According to some embodiments, the present invention provides a particle comprising the lipid according to the present invention. According to some embodiments, the present invention provides a particle comprising the lipid according to the present invention and at least one membrane stabilizing lipid. Thus, according to some embodiments, the present invention provides a composition comprising a lipid according to any one of formulae (I),
15 (Ia), (II), (III), (IV), (IVa) and (V), e.g., any one of lipids EA-402C, EA-404C, EA-405C, EA-406C, EA-408C, EA-502C, EA-505C, EA-506C, EA-513C, EA-521C, EA-522C, EA-524C, EA-526C or a salt thereof and a pharmaceutically acceptable excipient.

According to some embodiments, there is provided a composition comprising a plurality of particles as discloses herein and a pharmaceutically acceptable carrier, diluent or excipient.

20 According to some embodiments, the composition is a liposomal composition. According to some embodiments, the particles of the present invention are in the form of liposomes.

According to some embodiments, the ratio between the various lipids in the particle may vary. In some embodiments, the ratio is a molar ratio. In some embodiments, the ratio is a weight ratio. In some embodiments, each of the lipid groups may be at molar ratio/a weight
25 ratio of about 1%-99%, including each value and sub-range within the specified range. According to some embodiments, the particle comprises 10% to 70% mol% of the lipid according to the present invention. According to some embodiments, the particle comprises 20% to 70% mol% of the lipid according to the present invention. According to some embodiments, the particle comprises 30% to 65% mol% of the lipid according to the present
30 invention. According to some embodiments, the particle comprises 40% to 60% mol% of

the lipid according to the present invention. According to some embodiments, the particle comprises 45% to 55% mol% of the lipid according to the present invention. According to some embodiments, the particle comprises about 50 mol% of the lipid according to the present invention.

- 5 According to some embodiments, the molar percentage of the lipid is at least 15 mol% of the particle. According to some embodiments, the molar percentage of the lipid is at least 25 mol% of the particle. According to some embodiments, the molar percentage of the lipid is at least 35 mol% of the particle. According to some embodiments, the molar percentage of the lipid is at least 45 mol% of the particle. According to some embodiments, the molar percentage of the lipid is no more than 75 mol% of the particle. According to some
10 embodiments, the molar percentage of the lipid is no more than 65 mol% of the particle. According to some embodiments, the molar percentage of the lipid is no more than 60 mol% of the particle. According to some embodiments, the molar percentage of the lipid is no more than 55 mol% of the particle.
- 15 It is to be understood that by the phrase “the molar percentage of the lipid is at least x mol% of the particle” it is meant that at least x% of the particle molecules are of the lipid. The same terminology is reflected with other components of the present particle. Similarly, the phrase “the molar percentage of the lipid is no more than x mol% of the particle” it is meant that no more than x% of the particle molecules are of the lipid. The unit “mol%” is also
20 sometimes referred as “mol:mol” or “% mol:mol”.

According to some embodiments, the composition further comprises one or more components selected from the group consisting of a neutral lipid, a charged lipid, a steroid, and a polymer-conjugated lipid. Each possibility represents a separate embodiment of the present invention.

- 25 According to some embodiments, the membrane stabilizing lipid is selected from the group consisting of a sterol, a phospholipid, a cephalin, a sphingolipid and a glycolipid. Each possibility represents a separate embodiment of the invention. According to some embodiments, the membrane stabilizing lipid comprises a sterol. According to some embodiments, the membrane stabilizing lipid comprises cholesterol.

According to some embodiments, the lipid nanoparticle formulation comprises 15 to 65 mol% of the sterol. According to some embodiments, the lipid nanoparticle formulation comprises 20 to 60 mol% of the sterol. According to some embodiments, the lipid nanoparticle formulation comprises 25 to 55 mol% of the sterol. According to some
5 embodiments, the lipid nanoparticle formulation comprises 30 to 50 mol% of the sterol. According to some embodiments, the lipid nanoparticle formulation comprises 35 to 45 mol% of the sterol. According to some embodiments, the lipid nanoparticle formulation comprises about 38 mol% of the sterol. According to some embodiments, the lipid nanoparticle formulation comprises at least 20 mol% of the sterol. According to some
10 embodiments, the lipid nanoparticle formulation comprises at least 30 mol% of the sterol. According to some embodiments, the lipid nanoparticle formulation comprises at least 35 mol% of the sterol. According to some embodiments, the lipid nanoparticle formulation comprises no more than 80 mol% of the sterol. According to some embodiments, the lipid nanoparticle formulation comprises no more than 70 mol% of the sterol. According to some
15 embodiments, the lipid nanoparticle formulation comprises no more than 60 mol% of the sterol. According to some embodiments, the lipid nanoparticle formulation comprises no more than 50 mol% of the sterol.

According to some embodiments, the lipid nanoparticle formulation comprises 15 to 65 mol% cholesterol. According to some embodiments, the lipid nanoparticle formulation
20 comprises 20 to 60 mol% cholesterol. According to some embodiments, the lipid nanoparticle formulation comprises 25 to 55 mol% cholesterol. According to some embodiments, the lipid nanoparticle formulation comprises 30 to 50 mol% cholesterol. According to some embodiments, the lipid nanoparticle formulation comprises 35 to 45 mol% cholesterol. According to some embodiments, the lipid nanoparticle formulation
25 comprises about 38 mol% cholesterol. According to some embodiments, the lipid nanoparticle formulation comprises at least 20 mol% cholesterol. According to some embodiments, the lipid nanoparticle formulation comprises at least 30 mol% cholesterol. According to some embodiments, the lipid nanoparticle formulation comprises at least 35 mol% cholesterol. According to some embodiments, the lipid nanoparticle formulation
30 comprises no more than 80 mol% cholesterol. According to some embodiments, the lipid nanoparticle formulation comprises no more than 70 mol% cholesterol. According to some

embodiments, the lipid nanoparticle formulation comprises no more than 60 mol% cholesterol. According to some embodiments, the lipid nanoparticle formulation comprises no more than 50 mol% cholesterol.

- In some embodiments, the membrane stabilizing lipids may be selected from, but not limited to: cholesterol, phospholipids (such as, for example, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, diphosphatidylglycerols), cephalins, sphingolipids (sphingomyelins and glycosphingolipids), glycosphingolipids, and combinations thereof. Each possibility represents a separate embodiment of the present invention.
- 10 In some embodiments, the phosphatidylethanolamines may be selected from, but not limited to: 1,2-dilauroyl-L-phosphatidyl-ethanolamine (DLPE), 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-Diphytanoyl-sn-glycero-3-phosphoethanolamine (DPPE) 1,3-Dipalmitoyl-sn-glycero-2-phosphoethanolamine (1,3-DPPE), 1-Palmitoyl-3-oleoyl-sn-glycero-2-phosphoethanolamine (1,3-POPE), Biotin-Phosphatidylethanolamine,
- 15 1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE), Dipalmitoylphosphatidylethanolamine (DPPE), 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) or combinations thereof. In some embodiments, the Phosphatidylethanolamines may be conjugated to a PEG-Amine derivative. Each possibility represents a separate embodiment of the present invention.
- 20 According to some embodiments, the particle further comprising one or more additional components selected from the group consisting of a PEG-lipid conjugate, a neutral lipid and a charged lipid. According to some embodiments, the additional component comprises 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC). According to some embodiments, the additional component comprises 1,2-Dimyristoyl-sn-glyceryl-methoxy polyethylene glycol
- 25 (DMG-PEG).

According to some embodiments, the particle comprises 4 to 35 mol% DSPC. According to some embodiments, the particle comprises 5 to 30 mol% DSPC. According to some embodiments, the particle comprises 5 to 20 mol% DSPC. According to some embodiments, the particle comprises 6 to 15 mol% DSPC. According to some embodiments, the particle

comprises 8 to 12 mol% DSPC. According to some embodiments, the particle comprises about 10 mol% DSPC.

According to some embodiments, the particle comprises at least 2 mol% DSPC. According to some embodiments, the particle comprises at least 3 mol% DSPC. According to some
5 embodiments, the particle comprises at least 5 mol% DSPC. According to some
embodiments, the particle comprises at least 7 mol% DSPC. According to some
embodiments, the particle comprises at least 9 mol% DSPC.

According to some embodiments, the particle comprises no more than 40 mol% DSPC. According to some embodiments, the particle comprises no more than 30 mol% DSPC.
10 According to some embodiments, the particle comprises no more than 25 mol% DSPC. According to some embodiments, the particle comprises no more than 20 mol% DSPC. According to some embodiments, the particle comprises no more than 15 mol% DSPC.

According to some embodiments, the particle further comprises a PEGylated lipid.

The term “PEG” refers to polyethylene glycol. The term “PEGylated lipid” means a lipid
15 that is bonded to PEG.

According to some embodiments, the PEGylated lipid comprises a PEG moiety having a molecular weight in the range of 1000 gr/mol to 3000 gr/mol, including each value and sub-range within the specified range. According to some embodiments, the PEGylated lipid comprises a PEG moiety having a molecular weight in the range of 1000 gr/mol to 2000
20 gr/mol. According to some embodiments, the PEG moiety has a molecular weight of about 2000 gr/mol.

According to some embodiments, the PEGylated lipid comprises DMG-PEG. According to some embodiments, the PEGylated lipid comprises DMG-PEG-2000.

The term “DMG-PEG” means 1,2-Dimyristoyl-sn-glycero-3-methoxypolyethylene glycol, or ,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol. The term “DMG-PEG-2000”
25 means 1,2-Dimyristoyl-sn-glycero-3-methoxypolyethylene glycol, or ,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol, wherein the polyethylene glycol has a molecular weight of about 2000 gr/mol.

- According to some embodiments, the particle comprises 1 to 5 mol% PEGylated lipid.
- According to some embodiments, the particle comprises 1 to 4 mol% PEGylated lipid.
- According to some embodiments, the particle comprises 1 to 3 mol% PEGylated lipid.
- According to some embodiments, the particle comprises 1.5 to 2.5 mol% PEGylated lipid.
- 5 According to some embodiments, the particle comprises about 2 mol% PEGylated lipid.
- According to some embodiments, the particle comprises at least 1 mol% PEGylated lipid.
- According to some embodiments, the particle comprises no more than 10 mol% PEGylated lipid. According to some embodiments, the particle comprises no more than 5 mol% PEGylated lipid.
- 10 According to some embodiments, the particle comprises the lipid of the present invention, cholesterol, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-Dimyristoyl-sn-glyceryl-methoxy polyethylene glycol (DMG-PEG). According to some embodiments, particle comprises the lipid according to the present invention, a membrane stabilizing lipid, an additional phospholipid and PEG-lipid conjugate.
- 15 According to some embodiments, the particle comprises 40%-60% of the present lipid, 30-50% mol% cholesterol, 5-15% DSPC and 0.5-5% of the PEG-lipid conjugate.
- According to some embodiments, the particles of the present intention are nanoparticles.
- According to some embodiments, the lipidic particles of the present intention are lipid nanoparticles.
- 20 According to some embodiments, the particle has average nanoparticle size (Z_{average}) in the range of 10 to 500 nanometers. According to some embodiments, the particle has average nanoparticle size in the range of 25 to 400 nanometers. According to some embodiments, the particle has nanoparticle size (Z_{average}) in the range of 50 to 200 nanometers. According to some embodiments, the particle has nanoparticle size (Z_{average}) in the range of 50 to 100
- 25 nanometers. According to some embodiments, the particle has nanoparticle size (Z_{average}) in the range of 60 to 95 nanometers. According to some embodiments, the particle has nanoparticle size (Z_{average}) in the range of 65 to 90 nanometers. According to some embodiments, the particle has nanoparticle size (Z_{average}) in the range of 70 to 85 nanometers.

In some embodiments, the particles (including any nucleic acid, therapeutic agent and the like encapsulated within and any targeting moiety conjugated thereto) have a particle size (diameter) in the range of about 10 to about 500 nm. In some embodiments, the particles have a particle size (diameter) in the range of about 10 to about 350 nm. In some
5 embodiments, the particles have a particle size (diameter) in the range of about 40 to about 270 nm. In some embodiments, the particles have a particle size (diameter) of over about 10 nm. In some embodiments, the particles have a particle size (diameter) of over about 20 nm. In some embodiments, the particles have a particle size (diameter) of over about 30 nm. In some embodiments, the particles have a particle size (diameter) of
10 over about 40 nm. In some embodiments, the particles have a particle size (diameter) of over about 45 nm. In some embodiments, the particles have a particle size (diameter) of over about 50 nm. In some embodiments, the particles have a particle size (diameter) of over about 60 nm. In some embodiments, the particles have a particle size (diameter) of over about 70 nm. In some embodiments, the particles have a particle size (diameter) of not
15 more than about 500 nm. In some embodiments, the particles have a particle size (diameter) of not more than about 250 nm. In some embodiments, the particles have a particle size (diameter) of not more than about 150 nm. In some embodiments, the particles have a particle size (diameter) of not more than about 100 nm. In some embodiments, the particles have a particle size (diameter) of not more than about 90 nm. In some embodiments, the
20 size is a hydrodynamic diameter.

According to some embodiments, the particle has Zeta potential of at least 1mV. According to some embodiments, the particle has Zeta potential of at least 1.5mV. According to some embodiments, the particle has Zeta potential of at least 2mV. According to some
25 embodiments, the particle has Zeta potential of at least 2.5mV. According to some embodiments, the particle has Zeta potential of at least 2.9mV.

According to some embodiments, the particle has Zeta potential of no more than 25mV. According to some embodiments, the particle has Zeta potential of no more than 15mV. According to some embodiments, the particle has Zeta potential of no more than 12mV. According to some embodiments, the particle has Zeta potential of no more than 10mV.
30 According to some embodiments, the particle has Zeta potential of no more than 8mV.

According to some embodiments, the particle has Zeta potential in the range of 2 to 14 mV, including each value and sub-range within the specified range. According to some embodiments, the particle has Zeta potential in the range of 2.8 to 7.2 mV. According to some embodiments, the particle has Zeta potential in the range of 2.8 to 6.5 mV.

- 5 The term “zeta potential” refers to a physical measurement of a colloidal system by electrophoresis. It gives the value of the potential (in mV) of a colloid in a suspension at the boundary between the Stern layer and the diffuse layer. In other words, the zeta potential in a colloidal system is the difference in potential between the immovable layer attached to the surface of the dispersed phase and the dispersion medium. The zeta potential is related to
10 stability of suspensions of particles. Zeta potential may be adjusted, in part, for example, by adjusting the concentration of an electrolyte in the buffer system.

- According to some embodiments, the particle has polydispersity index (PDI) of no more than 0.75. According to some embodiments, the particle has PDI of no more than 0.5. According to some embodiments, the particle has PDI of no more than 0.25. According to
15 some embodiments, the particle has PDI of no more than 0.2. According to some embodiments, the particle has PDI of no more than 0.1.

- According to some embodiments, the particle further comprises a nucleic acid. According to some embodiments, the nucleic acid is encapsulated within a particle comprising the lipid. According to some embodiments, the nucleic acid is selected from the group consisting of
20 small interfering RNA (siRNA), micro RNA (miRNA), antisense oligo nucleotides, messenger RNA (mRNA), ribozymes, pDNA, CRISPR mRNA, gRNA, circular RNA and immune stimulating nucleic acids. In some embodiments, the composition may further comprise a nucleic acid. Examples of nucleic acids include small interfering RNA (siRNA), micro RNA (miRNA), antisense oligo nucleotides, messenger RNA (mRNA), ribozymes,
25 pDNA, CRISPR mRNA, gRNA, circular RNA and immune stimulating nucleic acids. Each possibility represents a separate embodiment of the present invention.

- According to some embodiments, the weight ratio between the nucleic acid and the lipid mixture may be adjusted so as to achieve maximal biological effect by the nucleic acid on the target site. In some embodiments, the ratio between the nucleic acid and the lipid phase
30 may be 1:1. For example, the weight ratio between the nucleic acid and the lipid phase may

be 1:2. For example, the weight ratio between the nucleic acid and the lipid phase may be 1:5. For example, the weight ratio between the nucleic acid and the lipid phase may be 1:10. For example, the weight ratio between the nucleic acid and the lipids phase may be 1:16. For example, the weight ratio between the nucleic acid and the lipid phase may be 1:20. In
5 some embodiments, the weight ratio between the nucleic acid and the lipid phase is about 1:1 to 1:20 (w:w).

According to some embodiments, the particle further comprises a therapeutic agent. According to some embodiments, the therapeutic agent is encapsulated within a particle comprising the lipid.

10 According to some embodiments, the therapeutic agent is RNA comprising an open reading frame encoding a polypeptide that comprises a SARS-CoV-2 spike protein or an immunogenic fragment or variant thereof. Each possibility represents a separate embodiment of the invention. According to some embodiments, the therapeutic agent is RNA comprising an open reading frame encoding a polypeptide that comprises a SARS-
15 CoV-2 spike protein, an immunogenic fragment of SARS-CoV-2 or a SARS-CoV-2 variant. Each possibility represents a separate embodiment of the invention. According to some embodiments, the therapeutic agent is RNA comprising an open reading frame encoding a polypeptide that comprises a SARS-CoV-2 spike protein.

According to some embodiments, there is provided a method of gene editing, the method
20 comprising the step of contacting a cell with a composition comprising a plurality of particles according to the present invention and a pharmaceutically acceptable carrier, diluent or excipient. In some embodiments, the present invention provides a method of gene editing, comprising the step of contacting a cell with a composition comprising a lipid of the present invention. In some embodiments, the cell is a cancer cell.

25 According to some embodiments, there is provided a method of gene silencing, comprising the step of contacting a cell with a composition comprising a plurality of particles according to the present invention and a pharmaceutically acceptable carrier, diluent or excipient. In some embodiments, the present invention provides a method of gene silencing, comprising the step of contacting a cell with a composition comprising a lipid of the present invention.

30 In some embodiments, the cell is a cancer cell.

In other embodiments, the compositions of the present invention may be used as a delivery system to administer a therapeutic agent to its target location in the body. Thus, in some embodiments, the present invention relates to a method for administering a therapeutic agent, by preparing a composition comprising a lipid as described herein and a therapeutic agent, and administering the composition to a subject in need thereof. According to some
5 embodiments, the present invention relates to a method for administering a therapeutic agent, by preparing a particle as described herein comprising a therapeutic agent, and administering the composition to a subject in need thereof. According to some embodiments, the method further comprises encapsulating the therapeutic agent within a
10 particle comprising the lipid. According to some embodiments, the therapeutic agent is RNA comprising an open reading frame encoding a polypeptide that comprises a SARS-CoV-2 spike protein, an immunogenic fragment of SARS-CoV-2 or a SARS-CoV-2 variant. Each possibility represents a separate embodiment of the invention.

In particular embodiments, the present invention provides novel lipids that enable the
15 formulation of improved compositions for the in vitro and in vivo delivery of IVT- mRNA and/or other oligonucleotides.

In some embodiments, these lipid nanoparticle compositions are useful for expression of protein encoded by mRNA.

In other embodiments, these improved lipid nanoparticles compositions are useful for
20 upregulation of endogenous protein expression by delivering miRNA inhibitors targeting one specific miRNA or a group of miRNA regulating one target mRNA or several mRNA.

In other embodiments, these improved lipid nanoparticle compositions are useful for down-regulating (e.g., silencing) the protein levels and/or mRNA levels of target genes.

In some other embodiments, the lipid nanoparticles are also useful for delivery of mRNA
25 and plasmids for expression of transgenes.

In yet other embodiments, the lipid nanoparticle compositions are useful for inducing a pharmacological effect resulting from expression of a protein, e.g., increased production of red blood cells through the delivery of a suitable erythropoietin mRNA, or protection against infection through delivery of mRNA encoding for a suitable antibody.

According to some embodiments, the lipid may be in the form of nanoparticles and administered as is. In some embodiments, the nanoparticles may be administered in a solution. In some embodiments, the nanoparticles may be formulated to a suitable pharmaceutical composition to be administered by any desired route of administration.

- 5 Exemplary routes of administration include such routes as, but not limited to: topical, oral or parenteral. Depending on the intended mode of administration, the compositions used may be in the form of solid, semi-solid or liquid dosage forms, such, as for example, tablets, suppositories, pills, capsules, powders, liquids, suspensions, or the like, preferably in unit dosage forms suitable for single administration of precise dosages. The pharmaceutical
10 compositions may include the particles, a pharmaceutical acceptable excipient, and, optionally, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, and the like. It is preferred that the pharmaceutically acceptable carrier be one which is inert to the nucleic acid encapsulated within the particles and which has no detrimental side effects or toxicity under the conditions of use. In some embodiments, the administration is
15 localized. In some embodiments, the administration is systemic.

- In some embodiments, injectable formulations for parenteral administration can be prepared as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like. In addition, if desired, the pharmaceutical
20 compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, and the like. Aqueous injection suspensions may also contain substances that increase the viscosity of the suspension, including, for example, sodium carboxymethylcellulose, sorbitol, and/or
25 dextran. Optionally, the suspension may also contain stabilizers. The parenteral formulations can be present in unit dose or multiple dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, such as, for example, water, for injections immediately prior to use. In some embodiments, parenteral administration includes intravenous
30 administration.

In other embodiments, for oral administration, a pharmaceutically acceptable, non-toxic composition may be formed by the incorporation of any of the normally employed excipients, such as, for example, mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, sodium crosscarmellose, glucose, gelatin, sucrose, magnesium carbonate, and the like. Such compositions include solutions, suspensions, tablets, dispersible tablets, pills, capsules, powders, sustained release formulations and the like. Formulations suitable for oral administration can consist of liquid solutions such as effective amounts of the compound(s) dissolved in diluents such as water, saline, or orange juice; sachets, lozenges, and troches, each containing a predetermined amount of the active ingredient as solids or granules; powders, suspensions in an appropriate liquid; and suitable emulsions. Liquid formulations may include diluents such as water and alcohols, (such as, for example ethanol, benzyl alcohol, and the polyethylene alcohols), either with or without the addition of a pharmaceutically acceptable surfactant, suspending agents, or emulsifying agents.

In determining the dosages of the particles to be administered, the dosage and frequency of administration may be selected in relation to the pharmacological properties of the specific nucleic acids encapsulated within the particles.

The lipids of the present invention can be used alone or in combination with other lipid components such as neutral lipids, charged lipids, steroids (including, for example, sterols) and/or their analogs, and/or polymer conjugated lipids to form lipid nanoparticles for the delivery of therapeutic agents. In some instances the lipid nanoparticles are used to deliver nucleic acids for the treatment of various diseases or conditions, in particular leukocyte associated conditions such as cancer.

Thus, in some embodiments, the present invention relates to a method of treating a leukocyte associated condition, the method comprising the step of administering to a subject in need thereof a composition according to the present invention. The leukocyte associated condition may be selected from the group consisting of cancer, infection, autoimmune diseases, neurodegenerative diseases and inflammation. According to some embodiments, there is provided a method of treating a leukocyte associated condition, the method comprising the step of administering to a subject in need thereof a composition comprising

a plurality of particles according to the present invention and a pharmaceutically acceptable carrier, diluent or excipient.

- In some representative embodiments, the particle comprises a nucleic acid, such as, for example, siRNA, miRNA, shRNA, anti-sense RNA, and the like, which may be used in the treatment of various leukocyte-associated conditions, depending on the identity of the nucleic acid, the specific target leukocyte, and the like. In some embodiments, the nucleic acid encapsulated within the particles may be a nucleic acid capable of inducing silencing of a target gene. In some embodiments, the target gene may be any gene, the expression of which is related to the condition to be treated. In some embodiments, the target gene may be a gene selected from, but not limited to: growth factors (such as EGFR, PDGFR), genes related to angiogenesis pathways (such as VEGF, Integrins), genes involved in intracellular signaling pathways and cell cycle regulation (such as PI3K/AKT/mTOR, Ras/Raf/MAPK, PDK1, CHK1, PLK1, Cyclins). In some embodiments, a combination of nucleic acids, each having one or more targets may be encapsulated within the particles.
- According to some embodiments, exemplary leukocyte-associated conditions that may be treated by the targeted particles may be selected from, but not limited to: various types of cancer, various infections (such as, for example, viral infection, bacterial infection, fungal infection, and the like), autoimmune diseases, neurodegenerative diseases, inflammations, and the like.
- In some representative embodiments, the targeted particles comprising a nucleic acid (such as, siRNA or miRNA, shRNA, anti-sense RNA, or the like), may be used for the treatment of cancer.

- In some embodiments, cancer is a disorder in which a population of cells has become, in varying degrees, unresponsive to the control mechanisms that normally govern proliferation and differentiation. In some embodiments, the cancer is a blood cancer. Non-limiting examples of blood cancers are lymphoma, leukemia and myeloma. Lymphomas may be divided into two categories: Hodgkin lymphoma and non-Hodgkin lymphoma. Most non-Hodgkin lymphomas are B-cell lymphomas, that grow quickly (high-grade) or slowly (low-grade). There are 14 types of B-cell non-Hodgkin lymphomas, such as mantle cell lymphoma (MCL). The others are T-cell lymphomas.

In some representative embodiments, the nucleic acid that may be used for the treatment of cancer is directed against a target gene, which is involved in the regulation of cell cycle. In some representative embodiments, the target gene may be Polo-like Kinase 1 (PLK), Cyclin D1, CHK1, Notch pathway genes.

- 5 According to some exemplary embodiments, the plurality of lipids of the lipid particles may be of natural or synthetic source and may be selected from, but not limited to: cationic lipids, phosphatidylethanolamines, ionized lipids, membrane stabilizing lipids, phospholipids, and the like, or combinations thereof. Each possibility represents a separate embodiment of the present invention.
- 10 According to some embodiments, the particle further comprises a targeting moiety connected to a component of the composition. According to some embodiments, the particle is conjugated to a targeting moiety. According to some embodiments, the targeting moiety may be conjugated to any one of the lipids included in the present particle.

- According to some embodiments, the particles may be comprised of any one or more of the lipids of the present invention, a phospholipid (e.g. DSPC), a membrane stabilizing lipid (e.g. cholesterol), a PEG-lipid conjugate (e.g. DMG-PEG); at various mol:mol ratios, and further conjugated to a targeting moiety, wherein the targeting moiety is conjugated, linked or attached to any one of the particle's components.
- 15

Definitions

- 20 To facilitate an understanding of the present invention, a number of terms and phrases are defined below. It is to be understood that these terms and phrases are for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.
- 25 As referred to herein, the terms "nucleic acid", "nucleic acid molecules" "oligonucleotide", "polynucleotide", and "nucleotide" may interchangeably be used herein. The terms are directed to polymers of deoxy ribonucleotides (DNA), ribonucleotides (RNA), and modified forms thereof in the form of a separate fragment or as a component of a larger construct, linear or branched, single stranded, double stranded, triple stranded, or hybrids thereof. The

term also encompasses RNA/DNA hybrids. The polynucleotides may include sense and antisense oligonucleotide or polynucleotide sequences of DNA or RNA. The DNA or RNA molecules may be, for example, but not limited to: complementary DNA (cDNA), genomic DNA, synthesized DNA, recombinant DNA, or a hybrid thereof or an RNA molecule such as, for example, mRNA, shRNA, siRNA, miRNA, Antisense RNA, and the like. Each possibility represents a separate embodiment of the present invention. The terms further include oligonucleotides composed of naturally occurring bases, sugars, and covalent inter nucleoside linkages, as well as oligonucleotides having non-naturally occurring portions, which function similarly to respective naturally occurring portions.

10 The terms “polypeptide,” “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

The term “construct”, as used herein, refers to an artificially assembled or isolated nucleic acid molecule which may include one or more nucleic acid sequences, wherein the nucleic acid sequences may include coding sequences (that is, sequence which encodes an end product), regulatory sequences, non-coding sequences, or any combination thereof. The term construct includes, for example, vector but should not be seen as being limited thereto.

“Expression vector” refers to constructs that have the ability to incorporate and express heterologous nucleic acid fragments (such as, for example, DNA), in a foreign cell. In other words, an expression vector comprises nucleic acid sequences/fragments (such as DNA, mRNA, tRNA, rRNA), capable of being transcribed. Many prokaryotic and eukaryotic expression vectors are known and/or commercially available. Selection of appropriate expression vectors is within the knowledge of those having skill in the art. In some representative embodiments, the expression vector may encode for a double stranded RNA molecule in the target site.

The term “expression”, as used herein, refers to the production of a desired end-product molecule in a target cell. The end-product molecule may include, for example an RNA molecule; a peptide or a protein; and the like; or combinations thereof.

As used herein, the terms “introducing” and “transfection” may interchangeably be used and refer to the transfer of molecules, such as, for example, nucleic acids, polynucleotide molecules, vectors, and the like into a target cell(s), and more specifically into the interior of a membrane-enclosed space of a target cell(s). The molecules can be “introduced” into the target cell(s) by any means known to those of skill in the art, for example as taught by Sambrook et al. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York (2001), the contents of which are incorporated by reference herein. Means of “introducing” molecules into a cell include, for example, but are not limited to: heat shock, calcium phosphate transfection, PEI transfection, electroporation, lipofection, transfection reagent(s), viral-mediated transfer, and the like, or combinations thereof. The transfection of the cell may be performed on any type of cell, of any origin, such as, for example, human cells, animal cells, plant cells, virus cell, and the like. The cells may be selected from isolated cells, tissue cultured cells, cell lines, cells present within an organism body, and the like.

As used herein, the term “gene editing,” refers to genetic engineering in which one or more nucleotides are inserted, replaced, or removed from a genome. Gene editing can be performed using a nuclease (e.g., a natural-existing nuclease or an artificially engineered nuclease), such as Cas9 and the like. Gene editing includes, *inter alia*, gene silencing.

The term “silencing” as used herein refers to suppression of expression of a (target) gene. Gene silencing may be achieved via reduction of transcription, and may additionally or alternatively include post-transcriptional suppression of gene expression. The degree of gene silencing can be complete, i.e., substantially abolishing production of the encoded gene product (yielding a null phenotype), although in general partial silencing of the gene expression is achieved, with some degree of expression remaining (yielding an intermediate phenotype). The term should not therefore be taken to require complete “silencing” of expression.

The term “treating” and “treatment” as used herein refers to abrogating, inhibiting, slowing or reversing the progression of a disease or condition, ameliorating clinical symptoms of a disease or condition or preventing the appearance of clinical symptoms of a disease or

condition. The term “preventing” is defined herein as barring a subject from acquiring a disorder or disease or condition.

The term "treatment of cancer" is directed to include one or more of the following: a decrease in the rate of growth of the cancer (i.e. the cancer still grows but at a slower rate);
5 cessation of growth of the cancerous growth, i.e., stasis of the tumor growth, and, the tumor diminishes or is reduced in size. The term also includes reduction in the number of metastases, reduction in the number of new metastases formed, slowing of the progression of cancer from one stage to the other and a decrease in the angiogenesis induced by the cancer. In most preferred cases, the tumor is totally eliminated. Additionally included in this
10 term is lengthening of the survival period of the subject undergoing treatment, improving the well-being of the subject undergoing treatment, lengthening the time of diseases progression, tumor regression, and the like. In some embodiments, the cancer is a blood cancer.

The term “Leukocytes” is directed to white blood cells (WBCs), produced and derived from
15 a multipotent, hematopoietic stem cell in the bone marrow. The white blood cells have nuclei, and types of white blood cells can be classified in into five main types, including, neutrophils, eosinophils, basophils, lymphocytes, and monocytes, based on functional or physical characteristics. The main types may be classified into subtypes. For example, lymphocytes include B cells, T cells, and NK cells. B-cells, for example, release antibodies
20 and assist activation of T cells. T cells, for example, can be classified to several subtypes, including: T-helper cells (CD4+ Th) which activate and regulate T and B cells; cytotoxic T cells (CD8+) that can target and kill virus-infected cells and tumor cells; Gamma-delta T cells ($\gamma\delta$ T cells) which can bridge between innate and adaptive immune responses and be involved in phagocytosis; and Regulatory (suppressor) T cells which modulate the immune
25 system, maintain tolerance to self-antigens, and abrogate autoimmune conditions.

Examples

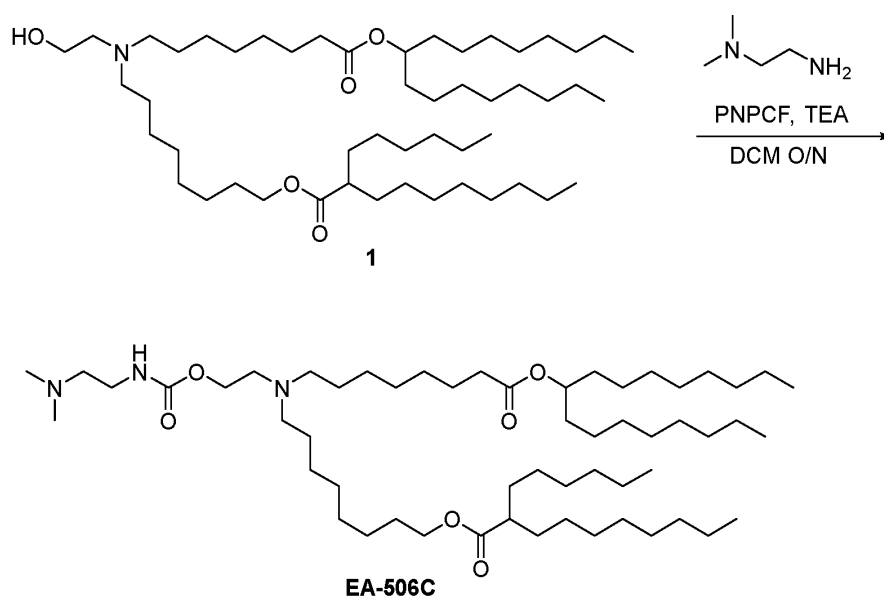
EXAMPLE 1: Synthesis of hydrolytically stable lipids

Precursor **1** was synthesized according to previously described procedures [Ramishetti S, Hazan-Halevy I, Palakuri R, Chatterjee S, Naidu Gonna S, Dammes N, Freilich I, Kolik

Shmuel L, Danino D, and Peer D “A Combinatorial Library of Lipid Nanoparticles for RNA Delivery to Leukocytes”. *Adv Mater.* (2020) 32, 1906128].

Synthesis of EA-506C:

The carbamate moiety was introduced via p-Nitrophenyl chloroformate (PNPCF) activation of the alcohol and concomitant reaction with a nucleophile, namely N,N-dimethylethylenediamine. The reaction was performed in one pot with adequate yields over two steps (70 – 85%) with EA-506C at 77% yield. ¹H NMR (400 MHz, CDCl₃): δ 0.85 (12H, m), 1.23 (71H, m), 2.19 (6H, s), 2.22-2.30 (4H, 2.25 (t, *J* = 7.54 Hz)), 2.34-2.45 (7H, 2.40 (m), 2.42 (t, *J* = 7.58 Hz)), 2.64 (2H, t, *J* = 5.96 Hz), 3.23 (2H, q, *J* = 5.69 Hz), 4.03 (5H, m), 4.84 (1H, i, *J* = 6.21 Hz), 5.20-5.25 (1H, br s). ESI-MS: *m/z* 922.9 [M+1]⁺.



Synthesis of EA-2C:

Synthesis of 2-(di((9Z,12Z)-octadeca-9,12-dien-1-yl) amino) ethyl (2-(dimethylamino) ethyl) carbamate (EA-2C)

To a stirred solution of 2-(di((9Z,12Z)-octadeca-9,12-dien-1-yl) amino) ethan-1-ol **1** (250 mg, 0.448mmol, 1.0 equiv.) in dry THF (15 mL) was added CDI (290 mg, 1.792 mmol, 4.0 equiv.) under argon atmosphere and stirred for 18h at room temperature. Then *N, N*-

dimethyl ethylene diamine (0.1mL, 0.896 mmol, 2.0 equiv.) and followed by DMAP (10 mg, 0.089 mmol, 0.2 equiv.) were added and stirred for 16 h at room temperature. Then the reaction was quenched with water followed by extracted with EtOAc (3x15 mL) and washed with brine solution and dried over with anhydrous Na₂SO₄. The solvent was evaporated, and the residue was purified by column chromatography using 0-10% IPA in CHCl₃ to bestow EA-2C (273 mg, 90.69%) as pale-yellow oil.

¹H NMR (400 MHz, CDCl₃): δ 5.41-5.30 (8 H, m), 5.25 (1 H, brs), 4.13 (2 H, brs), 3.25 (2 H, d, *J* = 5.2 Hz), 2.77 (4 H, t, *J* = 6.4 Hz), 2.71 (2 H, brs), 2.54-2.38 (6 H, m), 2.23 (6 H, s), 2.05 (8 H, q, *J* = 6.8 Hz), 1.44-1.28 (36 H, m), 0.89 (6 H, t, *J* = 6.8 Hz). ESI-MS: *m/z* 672.9 [M+1]⁺; 337.2 [M/2+1]⁺.

Synthesis of EA-405C:

Synthesis of 10-(8-((2-hexyldecanoyl) oxy) octyl)-2-methyl-6-oxo-7-oxa-2,5,10-triazaoctadecan-18-yl 2-hexyldecanoate (EA-405C)

To a stirred solution of ((2-hydroxyethyl) azanediyl) bis(octane-8,1-diyl) bis(2-hexyldecanoate) 2 (250 mg, 0.314 mmol, 1.0 equiv.) in dry THF (15 mL) was added CDI (204 mg, 1.259 mmol, 4.0 equiv.) under argon atmosphere and stirred for 18h at room temperature. Then N, N-dimethyl ethylene diamine (0.067mL, 0.629 mmol, 2.0 equiv.) and followed by DMAP (7 mg, 0.0629 mmol, 0.2 equiv.) were added and stirred for 16 h at room temperature. Then the reaction was quenched with water followed by extracted with EtOAc (3x15 mL) and washed with brine solution and dried over with anhydrous Na₂SO₄. The solvent was evaporated, and the residue was purified by column chromatography using 0-10% IPA in CHCl₃ to bestow EA-405C (190 mg, 66.66%) as pale-yellow oil.

¹H NMR (400 MHz, CDCl₃): δ 5.30 (1 H, brs), 4.15 (2 H, brs), 4.06 (4 H, t, *J* = 6.8 Hz), 3.27 (2 H, q, *J* = 5.2 Hz), 2.74 (2 H, brs), 2.57-2.39 (6 H, m), 2.35-2.28 (4 H, m), 2.26 (6H, s), 1.63-1.53 (10 H, m), 1.51-1.37 (10 H, m), 1.36-1.24 (50 H, m), 0.87 (12 H, t, *J* = 6.8 Hz). ESI-MS: *m/z* 909.2 [M+1]⁺; 455.6 [M/2+1]⁺.

Synthesis of EA-502C:

Synthesis of 2-methyl-10-((9Z,12Z)-octadeca-9,12-dien-1-yl)-6-oxo-7-oxa-2,5,10-triazaoctadecan-18-yl 2-hexyldecanoate (EA-502C)

- To a stirred solution of 8-((2-hydroxyethyl) ((9Z,12Z)-octadeca-9,12-dien-1-yl) amino) octyl 2-hexyldecanoate 4 (318 mg, 0.470 mmol, 1.0 equiv.) in dry THF (18 mL) was added CDI (304 mg, 1.881 mmol, 4.0 equiv.) under argon atmosphere and stirred for 18h at room temperature. Then N, N-dimethyl ethylene diamine (0.103 mL, 0.940 mmol, 2.0 equiv.) and followed by DMAP (11 mg, 0.094 mmol, 0.2 equiv.) were added and stirred for 16 h at room temperature. Then the reaction was quenched with water followed by extracted with EtOAc (3x15 mL) and washed with brine solution and dried over with anhydrous Na₂SO₄. The solvent was evaporated, and the residue was purified by column chromatography using 0-10% IPA in CHCl₃ to bestow EA-502C (304 mg, 81.94%) as pale-yellow oil.
- ¹H NMR (400 MHz, CDCl₃): δ 5.41-5.30 (4 H, m), 5.26 (1 H, brs), 4.13 (2 H, brs), 4.06 (2 H, t, *J* = 6.8 Hz), 3.26 (2 H, q, *J* = 5.6 Hz), 2.77 (2 H, t, *J* = 6.4 Hz), 2.72 (2 H, brs), 2.49 (4 H, t, *J* = 6.8 Hz), 2.41 (2 H, brs), 2.33-2.27 (2 H, m), 2.23 (6 H, s), 2.05 (4 H, q, *J* = 6.8 Hz), 1.66-1.52 (5 H, m), 1.49-1.25 (48 H, m), 0.89 (3 H, t, *J* = 6.8 Hz), 0.88 (6 H, t, *J* = 7.2 Hz). ESI-MS: *m/z* 791.05 [M+1]⁺; 396.36 [M/2+1]⁺.

15 Synthesis of EA-513C

Synthesis of heptadecan-9-yl 2-methyl-10-((9Z,12Z)-octadeca-9,12-dien-1-yl)-6-oxo-7-oxa-2,5,10-triazaoctadecan-18-oate (EA-513C)

- To a stirred solution of heptadecan-9-yl 8-((2-hydroxyethyl) (9Z,12Z)-octadeca-9,12-dien-1-yl) amino) octanoate 6 (455 mg, 0.659 mmol, 1.0 equiv.) in dry THF (20 mL) was added CDI (427 mg, 2.636 mmol, 4.0 equiv.) under argon atmosphere and stirred for 18 h at room temperature. Then N, N-dimethyl ethylene diamine (0.14 mL, 1.318 mmol, 2.0 equiv.) and followed by DMAP (16 mg, 0.1318 mmol, 0.2 equiv.) were added and stirred for 16 h at room temperature. Then the reaction was quenched with water followed by extracted with EtOAc (3 x 20 mL) and washed with brine solution and dried over with anhydrous Na₂SO₄.
- The solvent was evaporated, and the residue was purified by column chromatography using 0-10% IPA in CHCl₃ to bestow EA-513C (482mg, 91%) as pale-brown oil.

¹H NMR (400 MHz, CDCl₃): δ 5.42-5.29 (4 H, m), 5.26 (1 H, brs), 4.86 (1 H, quint, *J* = 6.4 Hz), 4.12 (2 H, brs), 3.26 (2 H, q, *J* = 5.2 Hz), 2.77 (2 H, t, *J* = 6.4 Hz), 2.71 (2 H, brs), 2.48 (4 H, brs), 2.41 (2 H, brs), 2.29-2.25 (4 H, m), 2.23 (6 H, s), 2.05 (4 H, q, *J* = 6.8 Hz), 1.65-

1.40 (12 H, m), 1.38-1.25 (42 H, m), 0.89 (3 H, t, $J = 6.8$ Hz), 0.88 (6 H, t, $J = 7.2$ Hz). ESI-MS: m/z 805.10 $[M+1]^+$; 403.43 $[M/2+1]^+$.

Synthesis of EA-402C:

5 Synthesis of heptadecan-9-yl 2-methyl-10-(9Z,12Z)-octadeca-9,12-dien-1-yl)-6-oxo-7-oxa-2,5,10-triazaoctadecan-18-oate (EA-402C)

To a stirred solution of di(tridecan-7-yl) 10,10'-((2-hydroxyethyl) azanediyl) bis(decanoate) 7 (600 mg, 0.782 mmol, 1.0 equiv.) in dry THF (30 mL) was added CDI (507 mg, 3.131 mmol, 4.0 equiv.) under argon atmosphere and stirred for 18 h at room temperature. Then N, N-dimethyl ethylene diamine (0.17 mL, 1.565 mmol, 2.0 equiv.) and followed by DMAP 10 (19 mg, 0.156 mmol, 0.2 equiv.) were added and stirred for 16 h at room temperature. Then the reaction was quenched with water followed by extracted with EtOAc (3 x 25 mL) and washed with brine solution and dried over with anhydrous Na₂SO₄. The solvent was evaporated, and the residue was purified by column chromatography using 0-10% IPA in CH₂Cl₂ to bestow EA-402C (566 mg, 82.14%) as pale-yellow oil.

15 ¹H NMR (400 MHz, CDCl₃): δ 5.26 (1 H, brs), 4.86 (2 H, quint, $J = 6.4$ Hz), 4.13 (2 H, brs), 3.25 (2 H, q, $J = 5.6$ Hz), 2.71 (2 H, brs), 2.48 (4 H, t, $J = 7.2$ Hz), 2.41 (2 H, t, $J = 5.6$ Hz), 2.30-2.25 (5 H, m), 2.23 (6 H, s), 1.63-1.42 (19 H, m), 1.35-1.25 (48 H, m), 0.8 (12 H, t, $J = 7.2$ Hz). ESI-MS: m/z 881.1 $[M+1]^+$; 441.6 $[M/2+1]^+$.

Synthesis of EA-406C:

20 Synthesis of 10-(7-(decanoyloxy) heptyl)-2-methyl-6-oxo-7-oxa-2,5,10-triazaheptadecan-17-yl decanoate (EA-406 C)

To a stirred solution of ((2-hydroxyethyl) azanediyl) bis(heptane-7,1-diyl) bis(decanoate) 12 (520 mg, 0.869 mmol, 1.0 equiv.) in dry THF (30 mL) was added CDI (563 mg, 3.478 mmol, 4.0 equiv.) under argon atmosphere and stirred for 18 h at room temperature. Then 25 N, N-dimethyl ethylene diamine (0.19 mL, 1.739 mmol, 2.0 equiv.) and followed by DMAP (21 mg, 0.173 mmol, 0.2 equiv.) were added and stirred for 16 h at room temperature. Then the reaction was quenched with water followed by extracted with EtOAc (3 x 25 mL) and washed with brine solution and dried over with anhydrous Na₂SO₄. The solvent was

evaporated and the residue was purified by column chromatography using 0-10% IPA in CH₂Cl₂ to bestow EA-406C (527mg, 85.13%) as pale-yellow oil.

¹H NMR (400 MHz, CDCl₃): δ 5.24 (1 H, brs), 4.11 (2 H, t, J = 6.0 Hz), 4.05 (4 H, t, J = 6.8 Hz), 3.25 (2 H, q, J = 5.6 Hz), 2.69 (2 H, t, J = 6.0 Hz), 2.46 (4 H, t, J = 7.6 Hz), 2.40 (2 H, t, J = 6.0 Hz), 2.28 (4 H, t, J = 7.6 Hz), 2.25 (6 H, s), 1.65-1.57 (8 H, m), 1.47-1.40 (4 H, m), 1.37-1.25 (36 H, m), 0.87 (6 H, t, J = 6.8 Hz). ESI-MS: m/z 713.0 [M+1]⁺; 357.3 [M/2+1]⁺.

Synthesis of EA-516C:

Synthesis of 2-(((Z)-octadec-8-en-1-yl) ((9Z,12Z)-octadeca-9,12-dien-1-yl) amino) ethyl
10 (2-(dimethylamino) ethyl) carbamate (EA-516 C)

To a stirred solution of 2-(((Z)-octadec-8-en-1-yl) ((9Z,12Z)-octadeca-9,12-dien-1-yl) amino) ethan-1-ol 13 (276 mg, 0.492 mmol, 1.0 equiv.) in dry THF (15 mL) was added CDI (319 mg, 1.971 mmol, 4.0 equiv.) under argon atmosphere and stirred for 18 h at room temperature. Then N, N-dimethyl ethylene diamine (0.10 mL, 0.985 mmol, 2.0 equiv.) and
15 followed by DMAP (12 mg, 0.098 mmol, 0.2 equiv.) were added and stirred for 16 h at room temperature. Then the reaction was quenched with water followed by extracted with EtOAc (3 x 15 mL) and washed with brine solution and dried over with anhydrous Na₂SO₄. The solvent was evaporated, and the residue was purified by column chromatography using 0-10% IPA in CHCl₃ to bestow EA-516C (263 mg, 79.21%) as colorless oil.

20 ¹H NMR (400 MHz, CDCl₃): δ 5.41-5.30 (6 H, m), 5.25 (1 H, brs), 4.13 (2 H, t, J = 5.8 Hz), 3.26 (2 H, q, J = 5.6 Hz), 2.77 (2 H, t, J = 6.4 Hz), 2.71 (2 H, brs), 2.49 (4 H, t, J = 7.6 Hz), 2.41 (2 H, t, J = 5.8 Hz), 2.23 (6 H, s), 2.07-1.98 (8 H, m), 1.37-1.25 (42 H, m), 0.89 (3 H, t, J = Hz), 0.88 (3 H, t, J = 7.2 Hz). ESI-MS: m/z 675.0 [M+1]⁺; 338.4 [M/2+1]⁺.

25 Synthesis of EA-505C:

Synthesis of (Z)-non-3-en-1-yl 2-methyl-10-((9Z,12Z)-octadeca-9,12-dien-1-yl)-6-oxo-7-oxa-2,5,10-triazahexadecan-16-oate (EA-505 C)

To a stirred solution of (Z)-non-3-en-1-yl 6-((2-hydroxyethyl) ((9Z,12Z)-octadeca-9,12-dien-1-yl) amino) hexanoate 14 (150 mg, 0.273 mmol, 1.0 equiv.) in dry THF (10 mL) was

added CDI (177 mg, 1.094 mmol, 4.0 equiv.) under argon atmosphere and stirred for 18 h at room temperature. Then N, N-dimethyl ethylene diamine (0.06 mL, 0.547 mmol, 2.0 equiv.) and followed by DMAP (6.5 mg, 0.0547mmol, 0.2 equiv.) were added and stirred for 16 h at room temperature. Then the reaction was quenched with water followed by
5 extracted with EtOAc (3 x 10 mL) and washed with brine solution and dried over with anhydrous Na₂SO₄. The solvent was evaporated, and the residue was purified by column chromatography using 0-10% IPA in CH₂Cl₂ to bestow EA-505C (150 mg, 82.87%) as colorless oil.

¹H NMR (400 MHz, CDCl₃): δ 5.53-5.46 (1 H, m), 5.41-5.29 (5 H, m), 5.27 (1 H, brs), 4.11
10 (2 H, t, *J* = 6.0 Hz), 4.05 (2 H, t, *J* = 6.8 Hz), 3.26 (2 H, q, *J* = 5.6 Hz), 2.77 (2 H, t, *J* = 6.4 Hz), 2.69 (2 H, t, *J* = 5.6 Hz), 2.49-2.27 (10 H, m), 2.23 (6 H, s), 2.03 (6 H, quint, *J* = 6.4 Hz), 1.63 (2 H, quint, *J* = 7.6 Hz), 1.49-1.25 (28 H, m), 0.89 (3 H, t, *J* = 6.4 Hz). 0.89 (3 H, t, *J* = 7.2 Hz). ESI-MS: *m/z* 662.9 [M+1]⁺; 332.2 [M/2+1]⁺.

EXAMPLE 2: Preparation and characterization of LNPs comprising carbamate lipids

15 Lipids were synthesized as described in Example 1. Cholesterol, DSPC (1,2- distearoyl-sn-glycero-3-phosphocholine), polyethylene glycol (PEG)-DMG (1,2-dimyristoyl-rac-glycerol), were purchased from Avanti Polar Lipids Inc. Briefly, one volume of lipid mixture (ionizable cationic lipid, Cholesterol, DSPC and PEG-DMG at 50:38.5:10:1.5 molar ratio) in absolute ethanol and three volumes of LUC mRNA (1:9 mole N to P ratio
20 RNA to ionizable lipid) in a 25mM Citrate buffer were injected into a NanoAssemblr microfluidic mixing device (Precision Nanosystems Inc.) at a combined flow rate of 12 mL min⁻¹. After LNPs generation, the particles were dialyzed against PBS (pH 7.4) for 24 hours with two buffer exchanges to remove ethanol.

The prepared LNPs comprising different lipids of the present invention were characterized
25 using dynamic light scattering (DLS) and zeta potential measurements. **Table 1** represents the measured hydrodynamic diameter (Z-ave; d.nm), polydispersity index (PDI) and zeta potential (mV) of various LNPs (structures of comparative lipids are show in Figure 7). The resultant LNPs were spherical and uniformly distributed with a hydrodynamic diameter size

of ~50 – 260 nm, depending on the lipid in use. Additionally, LNP surface potentials were negative for some of the measured LNPs.

Table 1: Physicochemical characterization of LNPs made of different lipids

	Size (nm)	PDI	ζ Potential (mV)	% Encapsulation
EA-519	148.8±1.7	0.05	1.54±0.3	97.75
EA-402	126.9±0.6	0.05	-0.74±0.1	97.14
EA-506C	108.27±0.9	0.06	1.47±0.2	97.85
EA-405	122.4±2.6	0.03	-4.7±0.7	97.74
EA-405C	108.4±2.0	0.08	3.95±0.6	97.76
EA-506CN	67.83±0.2	0.14	-4.53±0.9	96.37
EA-502C	78.7±1.8	0.07	7.89±0.5	98.14
EA-2C	90.36±1.2	0.1	14.77±0.4	97.66

- 5 To find the most efficient LNP formulation for mRNA delivery into the different cells, we designed LNPs to encapsulate GFP mRNA (Fig. 1.A). The LNPs consist of an ionizable lipid, DSPC, cholesterol, and PEG-DMG (50:10:38:2 molar ratio). For the screening, we chose six different ionizable lipids from a novel ionizable amino lipid library (Fig. 1.B). Following preparation, the LNPs were ~70-80 nm, were highly uniform with low polydispersity index (PDI) of 0.04 to 0.13 and had ζ potential of 2.9 to 15.4 mV, as measured by DLS. All LNP formulations showed high RNA encapsulation efficiency (>96%) as measured by RiboGreen (Fig 1.C).
- 10

EXAMPLE 3: GFP expression by mRNA-LNP in Z138 cells

- Z138 cells were seeded in a 24-well plate, at a concentration of 200,000 cells/mL. The cells were transfected with different amounts of 6 LNP formulations, which were added to the 1.5mL media already in the plate. 48 hours post transfection, 750uL cells of each treatment were washed and taken for GFP measurement via flow cytometry. 300uL cells of each treatment were re-seeded in a 96-well plate in 3 wells of 100uL each and were measured for cellular viability via XTT at 72 hours post transfection.
- 15

LNPs comprising EA-519, EA-402, EA-506C, EA-506CN, EA-405 lipids were prepared according to Example 2 and were encapsulated with mRNA encoding and were used to transfect Z138 cells. The level of GFP expression was measured by flow cytometry, showing improved efficiency for GFP expression by the use of EA-506C compared to the other mRNA-LNPs evaluated in this disclosure (**Figures 1A-1B**).

EXAMPLE 4: GFP expression by mRNA-LNP

LNPs comprising EA-2C, EA502-C, EA-506C and EA-405C lipids were prepared according to Example 2 and were encapsulated with mRNA encoding GFP and were used to transfect cells. The level of GFP expression was measured showing improved efficiency for GFP expression by the use of EA-506C compared to the other mRNA-LNPs evaluated (**Figures 2A-2B**).

EXAMPLE 5: In-vitro transfection in HeLa cells

In vitro transfection of EA-506, EA-506C, EA-2, EA-2C was performed in HeLa cells according to Example 2. **Figure 3** shows that the lipid EA-506C has an improved transfection efficiency compared to the other tested lipids.

EXAMPLE 6: GFP expression by mRNA-LNPs in primary T-cells

Messenger RNA encoding GFP were encapsulated in LNPs comprising EA-2C, EA-502C, EA-506C and EA-405C lipids Primary T cells

Human PBMCs were activated with anti-CD3 and anti-CD28 antibodies together with IL2, IL15 and IL7. 72 hours post activation, primary T-cells, which are the only cells remaining in the culture, were collected, and re-seeded without the addition of anti-CD3 and anti-CD28. The day after, the cells were collected and reseeded in a 24-well plate, at a concentration of 200,000 cells/mL. The cells were transfected with different amounts of 6 LNP formulations, which were added to the 1.5mL media already in the plate. 48 hours post transfection, 750uL cells of each treatment were washed and taken for GFP measurement via flow cytometry. 300uL cells of each treatment were re-seeded in a 96-well plate in 3 wells of 100uL each and were measured for cellular viability via XTT at 72 hours post transfection. The level of GFP expression was measured, showing EA-506C as having an

increased efficiency for GFP expression among the current disclosure mRNA-LNPs (Figures 4A-4B.)

EXAMPLE 7: In vivo transfection in a murine model

All animal protocols were approved by Tel Aviv University Institutional Animal Care and Usage Committee and in accordance with current regulations and standards of the Israel Ministry of Health. The mice were housed and maintained in laminar flow cabinets under specific pathogen-free conditions. To evaluate LNPs activity. Six- to eight-week-old female C57BLj6 mice, (Envigo, Rehovot, Israel) mice were injected with either LNPs at 1mg per kg body weight to the retroorbital sinus or at 1mg per kg body weight to the quadriceps. At the indicated time (2, 6 or 24hrs) the mice were injected intraperitoneally with 15mg of XenoLight D-Luciferin (122799, PerkinElmer Inc.) and imaged by IVIS bioluminescence imaging system (IVIS SpectrumCT, PerkinElmer Inc.) under isoflurane anesthesia.

LNPs comprising EA-506, EA-506C, EA-2, EA-2C lipids were administered intramuscularly to mice, and the organs were excised and imaged to test their transfection rate in-vivo. **Figure 5** shows that the lipid EA-506C has an improved transfection efficiency compared to the other tested LNPs.

EXAMPLE 8: LNPs screening using Carbamate ionizable lipids

EXAMPLE 8A: LNPs Formulation and Characterization

To find an efficient LNP (lipid nanoparticle) formulation for mRNA delivery into the different cells, LNPs were designed to encapsulate GFP mRNA (Green Fluorescent Protein messenger ribonucleic acid). The LNPs include an ionizable lipid, DSPC, cholesterol, and PEG-DMG (50:10:38:2 molar ratio). For the screening, six different ionizable lipids were chosen (EA2C, EA502C, EA405C, EA506C, EA513C and EA524C, the structures of which are shown herein). Following preparation, the LNP sizes were ~70-80 nm, and highly uniform with low polydispersity index (PDI) of 0.04 to 0.13. The LNPs had ζ potential of 2.9 to 15.4 mV, as measured by Dynamic Light Scattering (DLS). All LNP formulations showed high RNA encapsulation efficiency (>96%) as measured by RiboGreen.

Table 2 represents the measured hydrodynamic diameter (Z-ave; d.nm), polydispersity index (PDI) and zeta potential (mV) of the LNPs.

Table 2: Physicochemical characterization of LNPs made of different lipids

	Size (nm)	PDI	ζ Potential (mV)	% Encapsulation
EA-2C	72.6±1.2	0.13	15.4±0.6	99.02
EA-502C	71.5±0.2	0.07	7.2±0.5	98.58
EA-506C	75.3±0.7	0.04	4.6±0.7	97.92
EA-405C	85.3±1.1	0.05	6.5±0.2	98.19
EA-513C	78.0±0.6	0.08	6.7±0.0	98.58
EA-524C	83.4±0.3	0.04	2.9±0.8	98.94

EXAMPLE 8B: *In-Vitro* Screening for mRNA Delivery to Different Cell Types

Next, the *in vitro* transfection efficiency of the different LNP formulations of Example 8A was evaluated, following transfection of five different cell types; Z138 Mantle Cell Lymphoma cell line, HepG2 Hepatocellular Carcinoma cell line, Ovarc8 ovarian cancer cell line, Detroit562 pharyngeal cancer cell line, and Primary T cells (Examples 8C-F and Example 8H).

EXAMPLE 8C: Mantle Cell Lymphoma cell line

Z138 cells were seeded in a 24-well plate, at a concentration of 200,000 cells/mL. The cells were transfected with increasing doses (0.25-2µg/mL) of each LNP formulation, which were added to the 1.5mL media already in the plate. 48 hours post transfection, 750µL cells of each treatment were washed and taken for GFP measurement via flow cytometry. 300µL cells of each treatment were re-seeded in a 96-well plate in 3 wells of 100µL each and were measured for cellular viability via XTT at 72 hours post transfection.

Figure 7A shows GFP mean fluorescence and percentage of GFP⁺ cells analyzed by flow cytometry of LNPs comprising the lipids EA2C (full circles), EA502C (full squares), EA506C (full triangles), EA405C (empty triangles), EA513C (empty circles) and EA524C (empty squares), upon transfected at concentrations 0.25µg/ml, 0.5µg/ml, 1µg/ml and 2µg/ml.

Figure 7B shows the GFP mean fluorescence intensities of LNPs comprising the lipids EA2C, EA502C (large squares), EA506C (horizontal lines), EA405C (vertical lines),

EA513C (diagonal lines) and EA524C (small squares), upon transfection of MCL cells at concentrations 0.25µg/ml (left block), 0.5µg/ml (second to left block), 1µg/ml (second to right block) and 2µg/ml (second to right block).

EXAMPLE 8D: HepG2 Hepatocellular Carcinoma cell line

- 5 HepG2 cells were pre-seeded in a 24-well plate, at a concentration of 150,000 cells/well. 24 hours post seeding, the cells were transfected with increasing doses (0.05-0.5µg/mL) of each LNP formulation, which were added to the 1mL media already in the plate. 48 hours post transfection, cells were collected with Trypsin and neutralized by media at a final volume of 1mL. 300µL of the cells were taken and re-seeded in a 96-well plate in 3 wells of 100µL each, and the rest were washed and taken for GFP measurement via flow cytometry. Cellular viability was measured in the 96-well plate via XTT at 72 hours post transfection.

Figure 8 shows the GFP mean fluorescence intensities of LNPs comprising the lipids EA2C, EA502C (large squares), EA506C (horizontal lines), EA405C (vertical lines), EA513C (diagonal lines) and EA524C (small squares), upon transfection of HCC cells at concentrations 0.25µg/ml (left block), 0.5µg/ml (second to left block), 1µg/ml (second to right block) and 2µg/ml (second to right block).

EXAMPLE 8E: Ovc8 ovarian cancer cell line

- Ovc8 cells were pre-seeded in a 24-well plate, at a concentration of 40,000 cells/well. 24 hours post seeding, the cells were transfected with increasing doses (0.025-0.5µg/mL) of each LNP formulation, which were added to the 1mL media already in the plate. 48 hours post transfection, cells were collected with Trypsin and neutralized by media at a final volume of 1mL. 300µL of the cells were taken and re-seeded in a 96-well plate in 3 wells of 100µL each, and the rest was washed and taken for GFP measurement via flow cytometry. Cellular viability was measured in the 96-well plate via XTT at 72 hours post transfection.

Figure 9 shows the GFP mean fluorescence intensities of LNPs comprising the lipids EA2C, EA502C (large squares), EA506C (horizontal lines), EA405C (vertical lines), EA513C (diagonal lines) and EA524C (small squares), upon transfection of Ovc8 cells at concentrations 0.25µg/ml (left block), 0.5µg/ml (second to left block), 1µg/ml (second to right block) and 2µg/ml (second to right block).

EXAMPLE 8F: Detroit562 pharyngeal cancer cell line

Detroit562 cells were pre-seeded in a 24-well plate, at a concentration of 50,000 cells/well. 24 hours post seeding, the cells were transfected with increasing doses (0.025-0.5µg/mL) of each LNP formulation, which were added to the 1mL media already in the plate. 48 hours post transfection, cells were collected with Trypsin and neutralized by media at a final volume of 1mL. 300µL of the cells were taken and re-seeded in a 96-well plate in 3 wells of 100µL each, and the rest was washed and taken for GFP measurement via flow cytometry. Cellular viability was measured in the 96-well plate via XTT at 72 hours post transfection.

Figure 10 shows the GFP mean fluorescence intensities of LNPs comprising the lipids EA2C, EA502C (large squares), EA506C (horizontal lines), EA405C (vertical lines), EA513C (diagonal lines) and EA524C (small squares), upon transfection of Detroit562 pharyngeal cancer cells at concentrations 0.25µg/ml (left block), 0.5µg/ml (second to left block), 1µg/ml (second to right block) and 2µg/ml (second to right block).

EXAMPLE 8G: mRNA Delivery to Different Cell Types - summary

The results of the different screens shown in Examples 8C-8F are summarized in the heatmap of Figure 11.

Figure 11 is a heat map depicting cancer cell lines: ovc8, Detroit562, HepG2 and Z138 versus LNPs expressed therein, the LNPs comprising the lipids EA2C, EA502C, EA506C, EA405C, EA513C and EA524C. Optimized concentration is shown for each cell line.

EXAMPLE 8H: Primary T cells

In addition, human PBMCs were activated with anti-CD3 and anti-CD28 antibodies together with IL2, IL15 and IL7. 72 hours post activation, primary T-cells, which are the only cells remaining in the culture, were collected, and re-seeded without the addition of anti-CD3 and anti-CD28. The day after, the cells were collected and reseeded in a 24-well plate, at a concentration of 200,000 cells/mL. The cells were transfected with increasing doses (0.25-4µg/mL) of four out of the six LNP formulations, which were added to the 1.5mL media already in the plate. 48 hours post transfection, 750µL cells of each treatment were washed and taken for GFP measurement via flow cytometry. 300µL cells of each

treatment were re-seeded in a 96-well plate in 3 wells of 100 μ L each and were measured for cellular viability via XTT at 72 hours post transfection.

Figure 12A shows GFP mean fluorescence and percentage of GFP⁺ Primary T cells analyzed by flow cytometry of LNPs comprising the lipids EA2C (full circles), EA502C (full squares), EA506C (full triangles), EA405C (empty triangles), EA513C (empty circles) and EA524C (empty squares), upon transfected at concentrations 0.25 μ g/ml, 0.5 μ g/ml, 1 μ g/ml and 2 μ g/ml.

Figure 12B shows the GFP mean fluorescence intensities of LNPs comprising the lipids EA2C, EA502C (large squares), EA506C (horizontal lines), EA405C (vertical lines), EA513C (diagonal lines) and EA524C (small squares), upon transfection of Primary T cells at concentrations 0.25 μ g/ml (left block), 0.5 μ g/ml (second to left block), 1 μ g/ml (second to right block) and 2 μ g/ml (second to right block).

EXAMPLE 9A: LNPs Formulation and Characterization

Following the ionizable lipid screening, and the identification of EA513C as an efficient lipid for HepG2 transfection, LNPs were formulation with Cas9 mRNA and sgRNAs targeting either *HPRT* or *NCAPG*. The LNPs consist of the EA513C ionizable lipid, DSPC, cholesterol, and PEG-DMG (50:10.5:38:1.5 molar ratio). Following preparation, the LNPs were ~104-115 nm and were highly uniform with low polydispersity index (PDI) of 0.04 to 0.07 as measured by DLS. Both LNP formulations showed high RNA encapsulation efficiency (>96%) as measured by RiboGreen.

Table 2 represents the measured hydrodynamic diameter (Z-ave; d.nm), polydispersity index (PDI) and zeta potential (mV) of the LNPs.

Table 2: Physicochemical characterization of LNPs made of different lipids

	Size (nm)	PDI	% Encapsulation
sgHPRT-LNPs	115.5 $\pm$ 0.2	0.04	96.42
sgNCAPG-LNPs	104.4 $\pm$ 0.6	0.07	97.50

EXAMPLE 9B: *In-Vitro* Evaluation of Gene Editing in HCC Cells

HepG2 cells were seeded at 150,000 cells per well in 1ml media. 24 hours post seeding, cells were transfected with 0.25µg/mL of LNP (Example 9A) added directly to the media.

5 72 hours post transfection, cells were collected with trypsin and media in a final volume of 1mL, 300ul of which was reseeded as previously described for XTT measurement, while the rest of the cells were taken to DNA extraction. DNA was extracted and the HPRT and NCAPG loci were amplified in PCR. The resulting amplicons were sent for sanger sequencing and the resulting data was analyzed in the ICE algorithm to determine editing efficiency via the frequency of Insertions and Deletions mutations (Indels) in the treated

10 samples when compared to a non-treated control, highlighting high levels of editing in both target genes (Figure 13A). XTT was performed at 96 hours post transfection, showing no toxicity for either LNP (Figure 13B). HepG2 cells were transfected with CRISPR-LNPs targeting either NCAPG or HPRT. 72 hours post transfection, cells were reseeded for XTT

15 and harvested for DNA. DNA was amplified via PCR and sequenced, and sequencing results were analyzed via the ICE algorithm to determine editing efficiency in the form of Indel mutations as shown in Figure 13A. Cellular viability was measured via XTT 96 hours post transfection with treated samples normalized to an UT as shown in Figure 13B.

Figure 13A shows % Indel mutations upon gene editing in HCC cells with sgHPRT-LNPs and sgNCAPG-LNPs.

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Figure 13B shows cellular viability measured via XTT upon gene editing in HCC cells with sgHPRT-LNPs and sgNCAPG-LNPs.

EXAMPLE 10: *In-Vivo* Biodistribution of CRISPR-Cy5 LNPs in MCL-Bearing Mice

To evaluate the therapeutic potential of the different LNP formulations in treating MCL, their biodistribution was evaluated to the bone marrow of MCL-bearing mice. LNPs comprising lipids EA405C and EA506C were designed to encapsulate Cas9 mRNA and a Cy5-fluorescently labeled siRNA in 1:1 weight ratio. R2G2 mice were injected with 1.5×10^6 Z138 cells that constitutively express luciferase (Z138-Luc), through the caudal artery of immunodeficient mice. The mice were monitored twice a week by weight measurement and

25

luciferase expression measurement by IVIS, indicating the engraftment levels of the cancer cells. As determined by the intensity of luciferase signal, on day 24, mice were mock-treated or injected retro-orbitally with either EA506C or EA405C CRISPR-Cy5 LNPs.

5 The liver, spleen, kidneys, heart, lungs, and femurs were extracted 4h post injection and analyzed by IVIS live imaging. Localization of Cy5 signal in the femurs of the treated mice was evident (Figures 14A-C).

Figure 14A shows fluorescence imaging of femurs 4h after injection to MCL bearing mice LNPs comprising the lipids EA506C and EA405C.

10 **Figure 14B** shows Relative Total Flux [p/s] of liver, spleen, kidneys, heart and lungs and femurus normalized to the mock after injection to MCL bearing mice LNPs comprising the lipids EA506C and EA405C.

Figure 14C shows Total Flux [p/s] of the femurs normalized to the mock after injection to MCL bearing mice LNPs comprising the lipids EA506C and EA405C.

15 In a following experiment, R2G2 mice were injected with 1.5×10^6 Z138-Luc cells, through the caudal artery. The mice were monitored twice a week by weight measurement and luciferase expression measurement by IVIS. As determined by the intensity of luciferase signal, on day 33, mice were mock-treated or injected retro-orbitally with EA524C CRISPR-Cy5 LNPs. The liver, spleen, kidneys, heart, lungs, and femurs were extracted 4h post injection and analyzed by IVIS live imaging. Localization of Cy5 signal in the femurs
20 of the treated mice was evident (Figures 15A-C).

Figure 15A shows fluorescence imaging of femurs 4h after injection to MCL bearing mice LNPs comprising the lipid EA524C.

Figure 15B shows Relative Total Flux [p/s] of liver, spleen, kidneys, heart and lungs and femurus normalized to the mock after injection to MCL bearing mice LNPs comprising the lipid EA524C.

25 **Figure 15C** shows Total Flux [p/s] of the femurs normalized to the mock after injection to MCL bearing mice LNPs comprising the lipid EA524C.

EXAMPLE 11: In vivo gene editing in MCL R2G2 mouse model - comparison study EA524 vs. EA524C.

Following the ionizable lipid screening, and the identification of EA524C as a highly efficient lipid for MCL transfection, LNPs were formulated with Cas9 mRNA and sgRNAs targeting SOX11. The LNPs included the EA524C ionizable lipid, DSPC, cholesterol, and PEG-DMG (50:10.5:38:1.5 molar ratio) or with EA524 (which has a similar structure to the EA524C, but with a core ester group instead of carbamate, see Figure 6). Following preparation, the LNPs were ~89-96nm and were highly uniform with low polydispersity index (PDI) of 0.04 as measured by DLS. Both LNP formulations showed high RNA encapsulation efficiency (>96%) as measured by RiboGreen.

Table 3 represents the measured hydrodynamic diameter (Z-ave; d.nm), polydispersity index (PDI) and zeta potential (mV) of the LNPs.

Table 3: Physicochemical characterization of LNPs made of EA524C and EA524 lipids

	Size (nm)	ζ Potential (mV)	Size	% Encapsulation
EA524-LNPs	89.5 ± 5.1	-3.1 ± 0.3	0.04 ± 0.01	96.1%
EA-524C- LNPs	96.2 ± 7.1	-3.6 ± 0.5	0.04 ± 0.02	97.3%

LNPs formulations (EA524 or EA524C) with sgGFP (as a negative control) and with sgSOX11 were injected i.v. into R2G2 MCL-Bearing Mice (as detailed above) at day 33 from tumor implantation. 72 h post injection, MCL cells were isolated from the BM and sorted via FACS sorting (CD38+, CD138+) and subjected to NGS. All doses were at 0.5mg/Kg as a single dose.

While specific editing of SOX11 was observed with EA524-LNPs formulation (17.1%), with EA524C-LNPs we observed a specific SOX11 editing of 42%. Without being bound by theory, these surprising results might be because the carbamate linker provided increased stability to the lipids within the LNPs (Figure 16).

Figure 16 is a bar chart of % gene editing using EA524-LNPs and EA524C-LNPs formulations encapsulated with sgSOX11 and with sgGFP.

EXAMPLE 12: Hydrolysis stability of hydrolytically stable lipids

Hydrolysis stability was tested by dissolving at RT the lipids EA-524 (structure in **Figure 6**) and EA-524C in ethanol and in an ethanol:water mixture (3:1) and analyzing their integrity via HPLC. The results shown in **Figures 1A-1D**. The indicate that EA-524C, which bears the carbamate moiety according to Formulas (I) to (V) shows an improved hydrolytic stability (**Figures 1C-1D**), compared to EA-524 which bears the ester bond (**Figures 1A-1B**). EA-524 underwent significant hydrolysis already after 1 day as shown in **Figures 1A-B**, and almost complete hydrolysis in aqueous ethanol after 6 days, as shown in **Figure 1B**. In contrast, EA-524C remained stable to hydrolysis throughout the 6-day period as shown in **Figures 1C-D**.

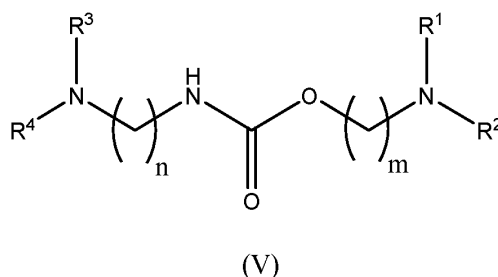
The foregoing examples of the related art and limitations related therewith are intended to be illustrative and not exclusive. Other limitations of the related art will become apparent to those of skill in the art upon a reading of the specification and a study of the figures.

While the present invention has been particularly described, persons skilled in the art will appreciate that many variations and modifications can be made. Therefore, the invention is not to be construed as restricted to the particularly described embodiments, and the scope and concept of the invention will be more readily understood by reference to the claims, which follow.

CLAIMS

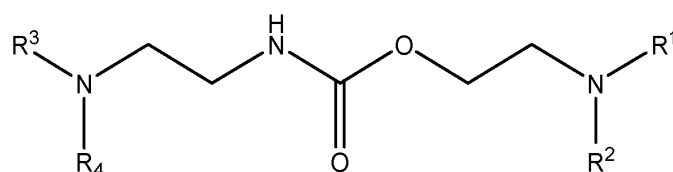
What is claimed is:

1. A lipid represented by the structure of Formula (V) or a salt thereof:



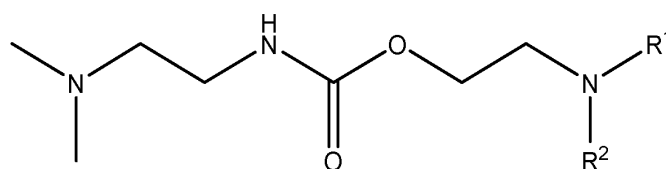
- 5 each one of n and m is individually selected from the group consisting of: 1, 2, 3, 4 and 5;
- R^1 is selected from the group consisting of: C_{3-9} alkylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkylene- CO_2 - C_{8-18} alkenyl, C_{3-9} alkenylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkenylene- CO_2 - C_{8-18} alkenyl, C_{3-9} alkylene- O_2C - C_{8-18} alkyl, C_{3-9} alkylene- O_2C - C_{8-18} alkenyl, C_{3-9} alkenylene- O_2C - C_{8-18} alkyl and C_{3-9} alkenylene- O_2C - C_{8-18} alkenyl;
- 10 R^2 is selected from the group consisting of: C_{10-20} alkyl, C_{10-20} alkenyl, C_{10-20} alkynyl, C_{3-9} alkylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkylene- CO_2 - C_{8-18} alkenyl, C_{3-9} alkenylene- CO_2 - C_{8-18} alkyl, C_{3-9} alkenylene- CO_2 - C_{8-18} alkenyl, C_{3-9} alkylene- O_2C - C_{8-18} alkyl, C_{3-9} alkylene- O_2C - C_{8-18} alkenyl, C_{3-9} alkenylene- O_2C - C_{8-18} alkyl and C_{3-9} alkenylene- O_2C - C_{8-18} alkenyl; and
- 15 each one of R^3 and R^4 is individually, a C_{1-4} alkyl or wherein R^3 and R^4 together with the nitrogen atom to which they are bound form a 5-6 membered ring.
2. The lipid according to claim 1, wherein n is 2, 3 or 4.
3. lipid according to claim 2, wherein n is 2.
- 20 4. The lipid according to any one of claims 1 to 3, wherein m is 2, 3 or 4.
5. The lipid according to claim 5, wherein m is 2.

6. The lipid according to any one of claims 1 to 5, wherein each one of n and m is 2, and the lipid is represented by the structure of Formula (III) or a salt thereof:



(III)

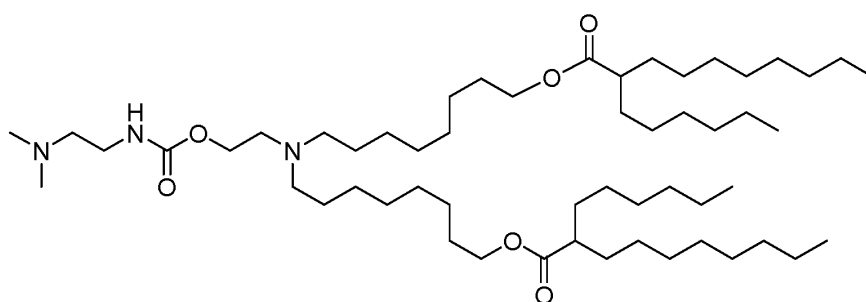
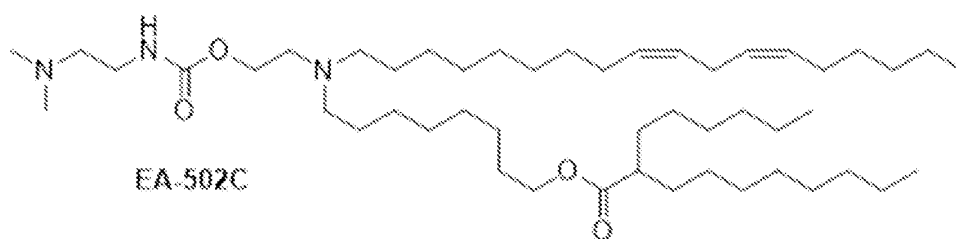
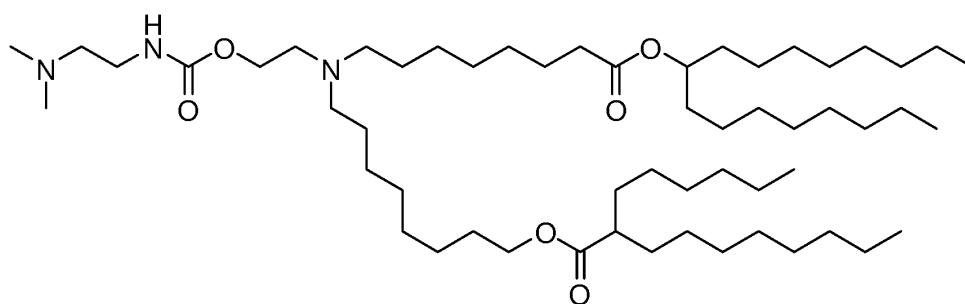
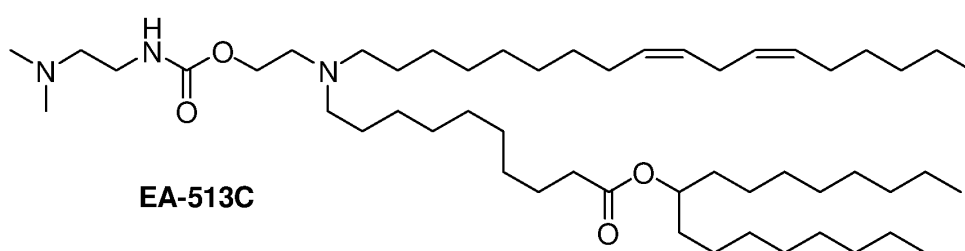
7. The lipid according to any one of claims 1 to 6, wherein each one of R³ and R⁴ is individually, a C₁₋₄ alkyl.
8. The lipid according to any one of claims 1 to 7, wherein R³ is a C₁₋₂ alkyl.
9. The lipid according to claim 8, wherein R³ is CH₃.
10. The lipid according to any one of claims 1 to 9, wherein R⁴ is a C₁₋₂ alkyl.
11. The lipid according to claim 10, wherein R⁴ is CH₃.
12. The lipid according to claim 1, wherein each one of n and m is 2, each one of R³ and R⁴ is individually CH₃, and the lipid is represented by the structure of Formula (IV) or a salt thereof:

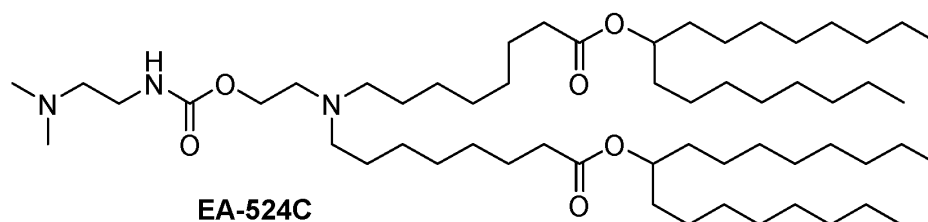


(IV)

13. The lipid according to any one of claims 1 to 12, wherein R¹ is selected from the group consisting of: C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkyl, C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkenyl, C₃₋₉ alkenylene-CO₂-C₈₋₁₈ alkyl, C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkyl, C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkenyl, C₃₋₉ alkenylene-O₂C-C₈₋₁₈ alkyl.
14. The lipid according to claim 13, wherein R¹ is selected from the group consisting of: C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkyl and C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkyl.

15. The lipid according to claim 13, wherein R^1 is selected from the group consisting of:
 C_{6-9} alkylene-CO₂-C₁₄₋₁₈ alkyl, C_{6-9} alkylene-O₂C-C₁₄₋₁₈ alkyl.
16. The lipid according to claim 13, wherein R^1 is C_{6-9} alkylene-CO₂-C₁₄₋₁₈ alkyl.
17. The lipid according to any one of claims 1 to 16, wherein R^2 is selected from the
5 group consisting of: C₁₀₋₂₀ alkenyl, C_{3-9} alkylene-CO₂-C₈₋₁₈ alkyl and C_{3-9}
alkylene-O₂C-C₈₋₁₈ alkyl.
18. The lipid according to claim 17, wherein R^2 is selected from the group consisting of:
C₁₆₋₂₀ alkenyl, C_{6-9} alkylene-CO₂-C₁₄₋₁₈ alkyl and C_{6-9} alkylene-O₂C-C₁₄₋₁₈ alkyl.
19. The lipid according to any one of claims 1 to 19, wherein each one of R^1 and R^2 is
10 individually selected from the group consisting of: C_{3-9} alkylene-CO₂-C₈₋₁₈ alkyl,
 C_{3-9} alkylene-CO₂-C₈₋₁₈ alkenyl, C_{3-9} alkenylene-CO₂-C₈₋₁₈ alkyl, C_{3-9} alkylene-
O₂C-C₈₋₁₈ alkyl, C_{3-9} alkylene-O₂C-C₈₋₁₈ alkenyl, C_{3-9} alkenylene-O₂C-C₈₋₁₈
alkyl.
20. The lipid according to claim 20, wherein each one of R^1 and R^2 is individually
15 selected from the group consisting of: C_{3-9} alkylene-CO₂-C₈₋₁₈ alkyl and C_{3-9}
alkylene-O₂C-C₈₋₁₈ alkyl.
21. The lipid according to any claim 21, wherein each one of R^1 and R^2 is C_{6-9} alkylene-
CO₂-C₁₄₋₁₈ alkyl.
22. The lipid according to any one of claims 1 to 13 or 17 to 19, wherein each one of the
20 alkenyl groups is unsubstituted.
23. The lipid according to any one of claims 1 to 13, 19 or 22, wherein each one of the
alkenylene groups is unsubstituted.
24. The lipid according to any one of claims 1 to 23, wherein each one of the alkyl and
alkylene groups is unsubstituted.
25. The lipid according to claim 1, which is selected from the group consisting of:

**EA-405C****EA-502C****EA-506C****EA-513C**

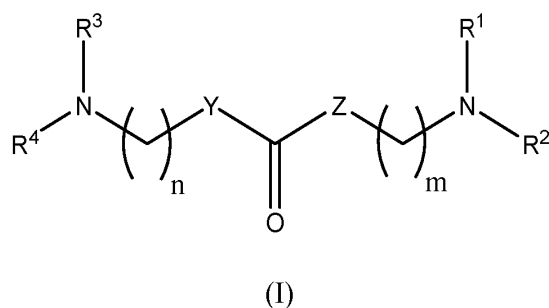


and salts thereof.

26. The lipid according to claim 25, which is selected from the group consisting of: EA-405C, EA-506C, EA-513C, EA-524C and salts thereof.
27. The lipid according to claim 25, which is EA-524C or a salt thereof.
28. The lipid according to claim 1, which is hydrolytically stable, alcoholytically stable or both, for at least one week.
29. A particle comprising the lipid according to any one of claims 1 to 28 and a membrane stabilizing lipid.
30. The particle according to claim 29, wherein the membrane stabilizing lipid is selected from the group consisting of cholesterol, phospholipids, cephalins, sphingolipids and glycolipids.
31. The particle according to any one of claims 29 to 31, wherein the membrane stabilizing lipid comprises cholesterol.
32. The particle according to any one of claims 29 to 31, further comprising one or more additional components selected from the group consisting of a PEG-lipid conjugate, a neutral lipid and a charged lipid.
33. The particle according to claim 32, wherein the additional component comprises 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC).
34. The particle according to any one of claims 32 to 33, wherein the additional component comprises 1,2-Dimyristoyl-sn-glyceryl-methoxy polyethylene glycol (DMG-PEG).

35. The particle according to any one of claims 29 to 34, comprising the lipid, cholesterol, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-Dimyristoyl-sn-glyceryl-methoxy polyethylene glycol (DMG-PEG).
- 5 36. The particle according to any one of claims 29 to 35, further comprising a nucleic acid encapsulated within a particle comprising the lipid.
37. The particle according to claim 36, wherein the nucleic acid is selected from the group consisting of small interfering RNA (siRNA), micro RNA (miRNA), antisense oligo nucleotides, messenger RNA (mRNA), ribozymes, pDNA, CRISPR mRNA, gRNA, circular RNA and immune stimulating nucleic acids.
- 10 38. The particle according to claim 37, wherein the nucleic acid is RNA comprising an open reading frame encoding a polypeptide that comprises a SARS-CoV-2 spike protein or an immunogenic fragment or variant thereof.
39. A composition comprising a plurality of particles according to any one of claims 29 to 38 and a pharmaceutically acceptable carrier, diluent or excipient.
- 15 40. The composition according to claim 39, being a liposomal composition.
41. A method of gene silencing, the method comprising the step of contacting a cell with a composition comprising a plurality of particles according to any one of claims 29 and 37 and a pharmaceutically acceptable carrier, diluent or excipient.
42. The method according to claim 41, wherein the cell is a cancer cell.
- 20 43. A method for administering a therapeutic agent, the method comprising the step of preparing a composition comprising a lipid according to any one of claims 1 to 28, and a therapeutic agent, and administering the composition to a subject in need thereof.
44. The method according to claim 43, further comprising encapsulating the therapeutic agent within a particle comprising the lipid.
- 25 45. The method of any one of claims 43 to 44, wherein the therapeutic agent is RNA comprising an open reading frame encoding a polypeptide that comprises a SARS-CoV-2 spike protein or an immunogenic fragment or variant thereof.

46. A method of treating a leukocyte associated condition, the method comprising the step of administering to a subject in need thereof a composition comprising a plurality of particles according to any one of claims 29 and 37 and a pharmaceutically acceptable carrier, diluent or excipient.
- 5 47. The method according to claim 46, wherein the leukocyte associated condition is selected from the group consisting of cancer, infection, autoimmune diseases, neurodegenerative diseases and inflammation.
48. A lipid represented by the structure of Formula (I) or a salt thereof:



- 10 wherein one of Y and Z is O and the other of Y and Z is NH;
- each one of n and m is individually selected from the group consisting of: 1, 2, 3, 4 and 5;
- each one of R¹ and R² is individually selected from the group consisting of: C₁₀₋₂₀ alkyl, C₁₀₋₂₀ alkenyl, C₁₀₋₂₀ alkynyl, C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkyl, C₃₋₉ alkylene-CO₂-C₈₋₁₈ alkenyl, C₃₋₉ alkenylene-CO₂-C₈₋₁₈ alkyl, C₃₋₉ alkenylene-CO₂-C₈₋₁₈ alkenyl, C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkyl, C₃₋₉ alkylene-O₂C-C₈₋₁₈ alkenyl, C₃₋₉ alkenylene-O₂C-C₈₋₁₈ alkyl and C₃₋₉ alkenylene-O₂C-C₈₋₁₈ alkenyl; and
- 15 each one of R³ and R⁴ is individually, a C₁₋₄ alkyl or wherein R³ and R⁴ together with the nitrogen atom to which they are bound form a 5-6 membered ring;
- 20 wherein the lipid is hydrolytically stable for at least 2 hours.