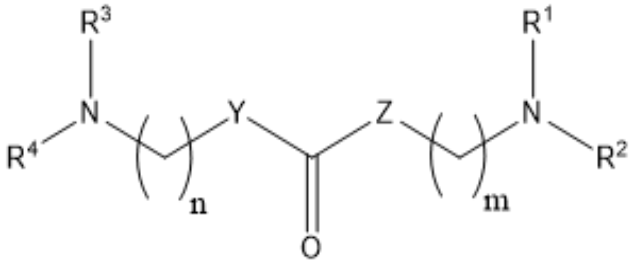
SUMMARY

The present disclosure 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 disclosure also provides the methods of chemical synthesis of these lipids, lipid nanoparticle preparation and formulations with nucleic acids.

In one aspect, the present disclosure refers to a lipid represented by the structure of Formula (I) or a salt thereof:



(I)

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

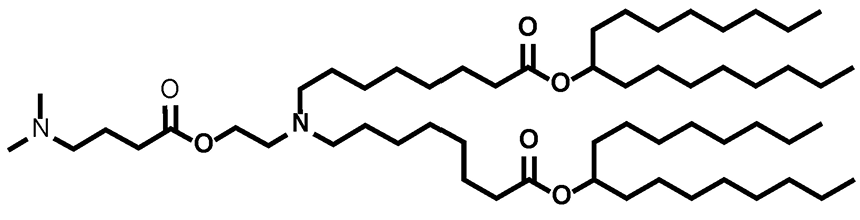
one of Y and Z is O and the other of Y and Z is NH;

each one of R^1 and R^2 is individually selected from the group consisting of C_{7-9} alkylene-CO_2-C_{13-17}alkyl and C_5 alkylene-CO_2-C_9alkenyl; or R^1 is C_{6-9} alkylene-O_2C -C_{9-16} alkyl or C_9 alkylene-CO_2-C_{12-18}alkyl, and R^2 is C_9 alkylene-CO_2-C_{12-18}alkyl;

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.

In one aspect, the present disclosure refers to a lipid that is

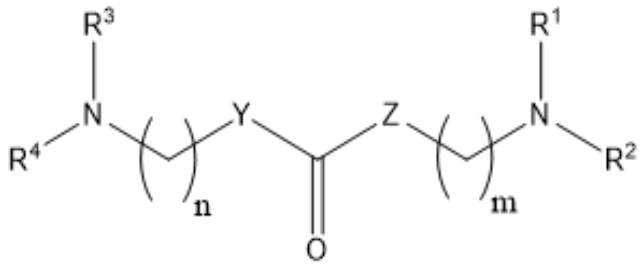


EA-524

,

or a salt thereof.

In one aspect, the present disclosure refers to 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;

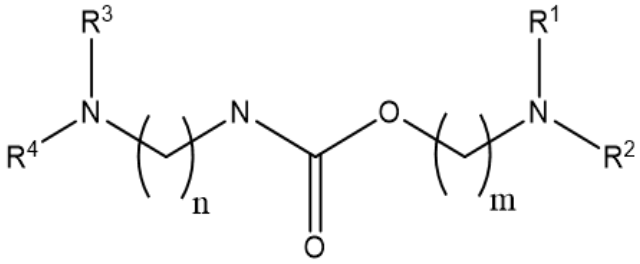
each one of R^1 and R^2 is individually selected from the group consisting of: $C_{10\text{-}20}$ alkyl, $C_{10\text{-}20}$ alkenyl, $C_{10\text{-}20}$ alkynyl, $C_{3\text{-}9}$ alkylene–CO_2–$C_{8\text{-}18}$ alkyl, $C_{3\text{-}9}$ alkylene–CO_2–$C_{8\text{-}18}$ alkenyl, $C_{3\text{-}9}$ alkenylene–CO_2–$C_{8\text{-}18}$ alkyl, $C_{3\text{-}9}$ alkenylene–CO_2–$C_{8\text{-}18}$ alkenyl, $C_{3\text{-}9}$ alkylene–O_2C–$C_{8\text{-}18}$ alkyl, $C_{3\text{-}9}$ alkylene–O_2C–$C_{8\text{-}18}$ alkenyl, $C_{3\text{-}9}$ alkenylene– O_2C–$C_{8\text{-}18}$ alkyl and $C_{3\text{-}9}$ alkenylene–O_2C–$C_{8\text{-}18}$ alkenyl; and

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

In some examples, the present disclosure relates to novel lipids, and formulations of such lipids with siRNA and pDNA.

The lipids of the present disclosure include a carbamate moiety, and were found to have increased hydrolytic and alcoholytic 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 disclosure can overcome this stability-activity barrier.

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



(V)

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;

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

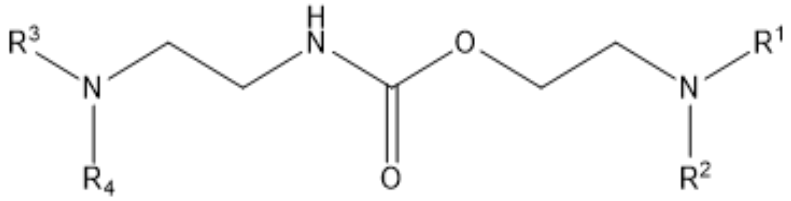
each one of R^3 and R^4 is individually, a C_{1-4} alkyl or wherein R^3 and R^4 together with the nitrogen atom to which they are bound form a 5-6 membered ring

According to some examples, n is 2, 3 or 4. Each possibility represents a separate example of the disclosure. According to some examples, n is 2.

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

According to some examples, m is 2, 3 or 4. Each possibility represents a separate example of the disclosure. According to some examples, m is 2.

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



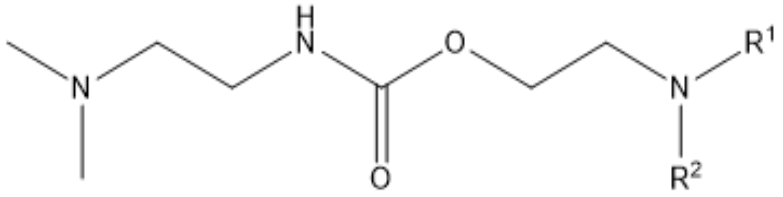
(III)

According to some examples, each one of R^3 and R^4 is individually, a C1-4 alkyl.

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

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

According to some examples, 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)

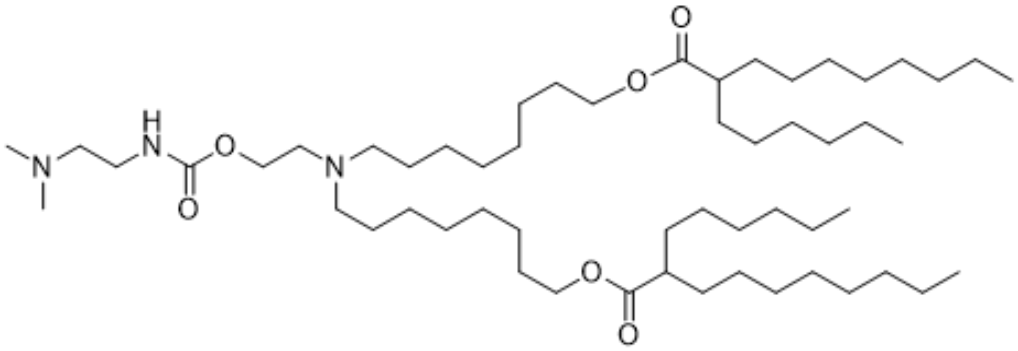
According to some examples, R^1 is selected from the group consisting of: C_{3-9} alkylene–CO_2–C_{8-18} alkyl, C_{3-9} alkylene–CO_2–C_{8-18} alkenyl, C_{3-9} alkenylene–CO_2–C_{8-18} alkyl, C_{3-9} alkylene–O_2C–C_{8-18} alkyl, C_{3-9} alkylene–O_2C–C_{8-18} alkenyl, C_{3-9} alkenylene–O_2C–C_{8-18}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

According to some examples, 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 examples, 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 examples, 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 examples, 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 examples, each one of R^1 and R^2 is C_{6-9} alkylene–CO_2–C_{14-18} alkyl.

According to some examples, each one of the alkenyl groups is unsubstituted. According to some examples, each one of the alkenylene groups is unsubstituted. According to some examples, each one of the alkyl and alkylene groups is unsubstituted.

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



EA-405C

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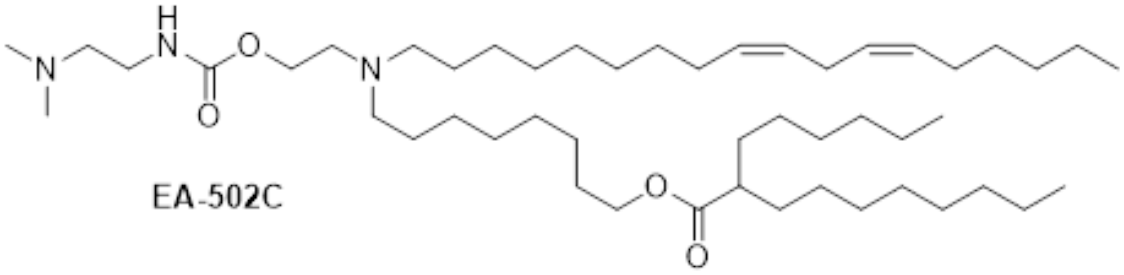

EA-502C

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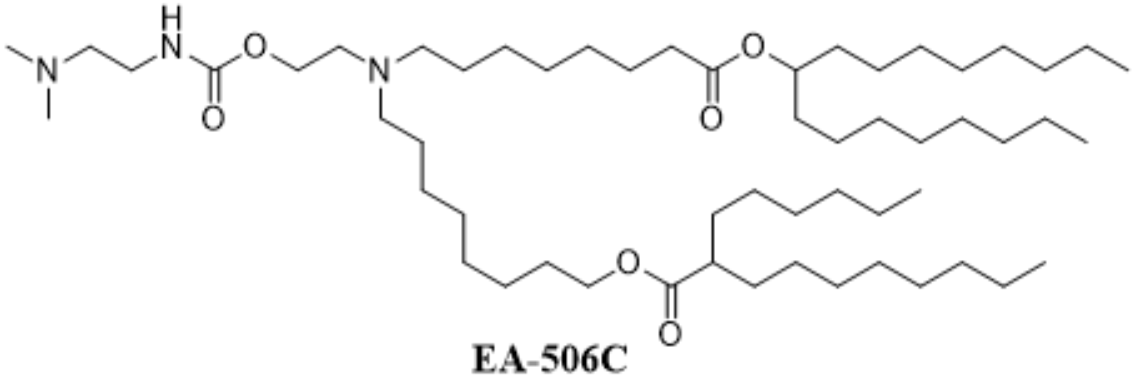

EA-506C

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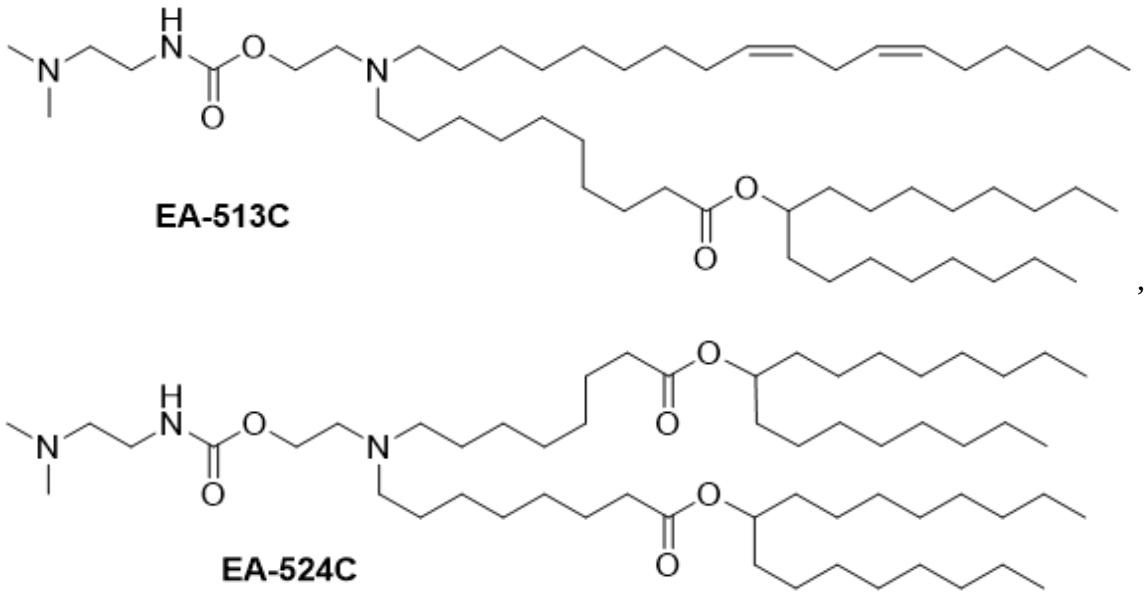


and salts thereof. Each possibility represents a separate example of the disclosure.

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

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

According to one aspect, there is provided particle comprising the lipid disclosed herein and a membrane stabilizing lipid.

According to some examples, the membrane stabilizing lipid is selected from the group consisting of cholesterol, phospholipids, cephalins, sphingolipids and glycoglycerolipids. Each possibility represents a separate example of the disclosure.

According to some examples, the membrane stabilizing lipid comprises cholesterol.

According to some examples, 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 example of the disclosure.

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

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

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

According to some examples, 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 example of the disclosure. According to some examples, 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.

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

According to some examples, 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 disclosure, and a pharmaceutically acceptable carrier, diluent or excipient.

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

According to some examples, 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 disclosure, and a therapeutic agent, and administering the composition to a subject in need thereof.

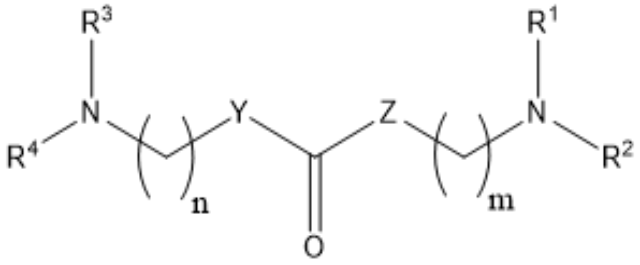
According to some examples, the method further comprises encapsulating the therapeutic agent within a particle comprising the lipid.

According to some examples, 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 examples, 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 disclosure and a pharmaceutically acceptable carrier, diluent or excipient.

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

According to some examples, 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 R^1 and R^2 is individually 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

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

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A–B depict the expression level of GFP by transfecting Z138 cells and primary T Cells with mRNA-NLPs. **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).

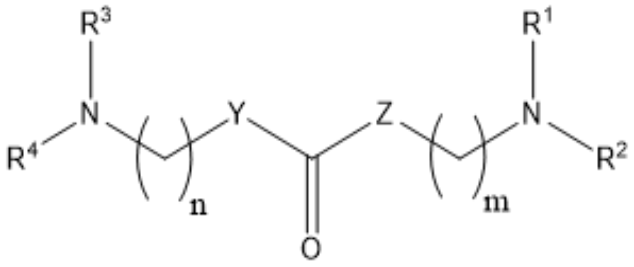
Figure 2A is a bar chart showing quantitative analysis of the GFP fluorescence intensity following Z138cells transfection of mRNA-LNPS wherein the lipids are EA–2C, EA–502C, EA–506C, and EA–405C. From left to right the different groups of lipids are at a concentration of 0.25 µg/ml, 0.5 µg/ml, 1 µg/ml, and 2 µg/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

CLAIMS

What is claimed is:

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



(I)

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

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

each one of R^1 and R^2 is individually selected from the group consisting of C_{7-9} alkylene-CO_2-C_{13-17}alkyl and C_5 alkylene-CO_2-C_9alkenyl; or R^1 is C_{6-9} alkylene-O_2C -C_{9-16} alkyl or C_9 alkylene-CO_2-C_{12-18}alkyl, and R^2 is C_9 alkylene-CO_2-C_{12-18}alkyl;

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.

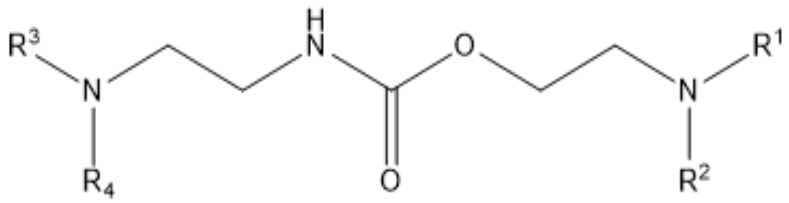
2. The lipid according to claim 1, wherein each one of R^1 and R^2 is individually selected from the group consisting of C_{7-9} alkylene-CO_2-C_{13-17}alkyl and C_5 alkylene-CO_2-C_9alkenyl.

3. The lipid according to claim 1, wherein R^1 is C_{6-9} alkylene-O_2C -C_{9-16} alkyl or C_9 alkylene-CO_2-C_{12-18}alkyl, and R^2 is C_9 alkylene-CO_2-C_{12-18}alkyl.

4. The lipid according to claim 1, wherein Y is NH and Z is O.

5. The lipid according to claim 1, wherein Y is O and Z is NH.

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:

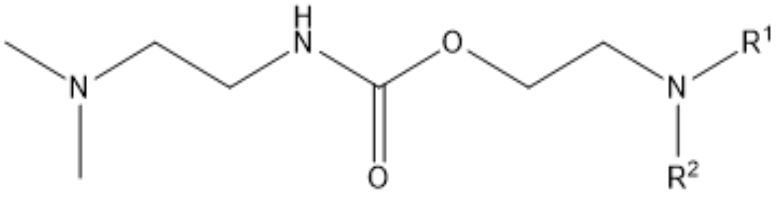


(III)

7. The lipid according to any one of claims 1 to 6, wherein each one of R^3 and R^4 is individually, a C1-4 alkyl.

8. The lipid according to claim 7, wherein R^3 is CH_3; and R^4 is CH_3.

9. The lipid according to claim 1, wherein 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)

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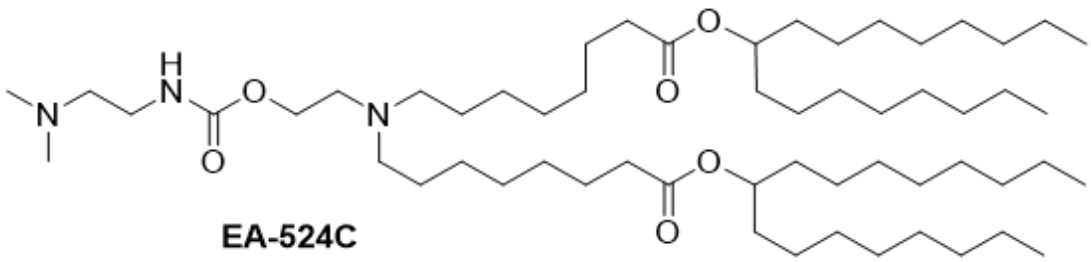
10. The lipid according to claim 1, which is selected from the group consisting of:



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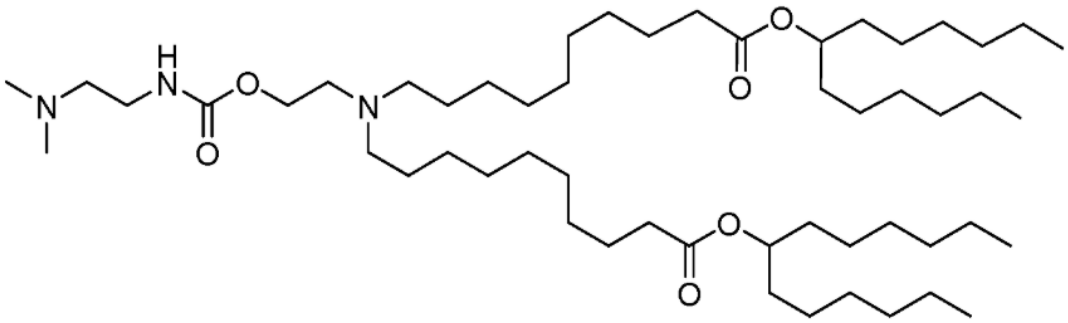

EA-402C

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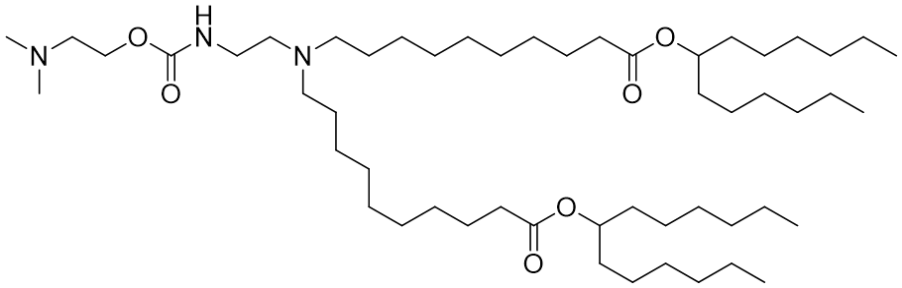

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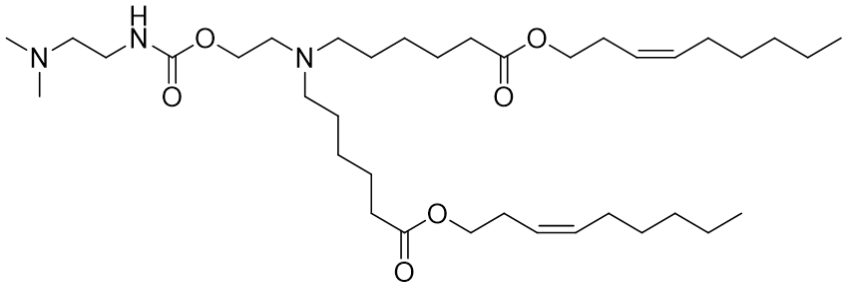
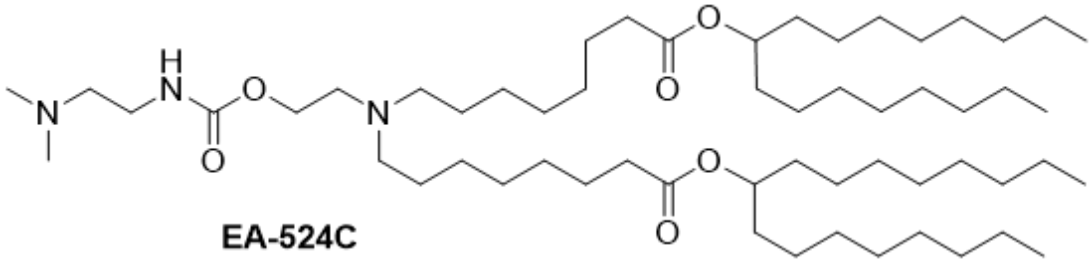


and salts thereof.

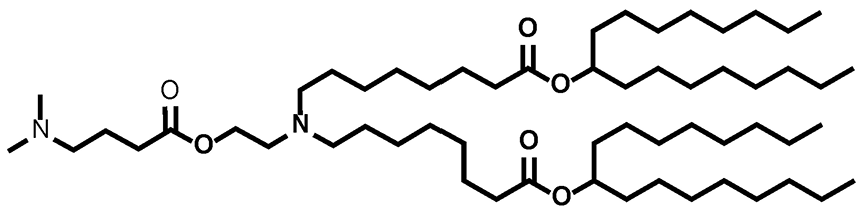
11. The lipid according to claim 1, which is



EA-524C

or a salt thereof.

12. A lipid that is

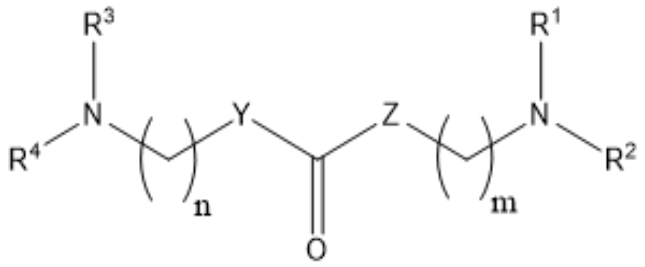


EA-524

,

or a salt thereof.

13. 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 R^1 and R^2 is individually selected from the group consisting of: $C_{10\text{-}20}$ alkyl, $C_{10\text{-}20}$ alkenyl, $C_{10\text{-}20}$ alkynyl, $C_{3\text{-}9}$ alkylene–CO_2–$C_{8\text{-}18}$ alkyl, $C_{3\text{-}9}$ alkylene–CO_2–$C_{8\text{-}18}$ alkenyl, $C_{3\text{-}9}$ alkenylene–CO_2–$C_{8\text{-}18}$ alkyl, $C_{3\text{-}9}$ alkenylene–CO_2–$C_{8\text{-}18}$ alkenyl, $C_{3\text{-}9}$ alkylene–O_2C–$C_{8\text{-}18}$ alkyl, $C_{3\text{-}9}$ alkylene–O_2C–$C_{8\text{-}18}$ alkenyl, $C_{3\text{-}9}$ alkenylene– O_2C–$C_{8\text{-}18}$ alkyl and $C_{3\text{-}9}$ alkenylene–O_2C–$C_{8\text{-}18}$ alkenyl; and

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

14. A particle comprising the lipid according to any one of claims 1 to 13 and a membrane stabilizing lipid.

15. The particle according to claim 14, wherein the membrane stabilizing lipid is selected from the group consisting of cholesterol, phospholipids, cephalins, sphingolipids and glycoglycerolipids.

16. The particle according to any one of claims 14 to 15, further comprising a nucleic acid encapsulated within a particle comprising the lipid, 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.