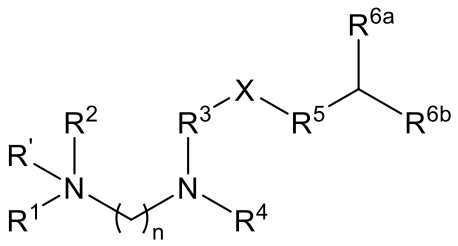
liposome or lipid membrane structure. Thus, uptake into cells is promoted and nucleic acids are delivered into cells.

Some widely used cationic lipids are CLinDMA, DLinDMA (DODAP), and DOTAP. These lipids have been employed for ribonucleic acid (siRNA or mRNA) delivery but suffer from sub-optimal delivery efficiency along with toxicity at higher doses. In view of the shortcomings of the current cationic lipids, there is a need in the field to provide lipid scaffolds that not only demonstrate enhanced efficacy along with reduced toxicity, but with improved pharmacokinetics and intracellular kinetics such as cellular uptake and nucleic acid release from the lipid carrier.

SUMMARY

The cationic lipids provided in the present disclosure comprise one hydrophobic tail containing a biodegradable group, and a hydrophobic tail that does not contain a biodegradable group. Some of the exemplary lipids provided in this disclosure comprise a hydrophobic tail that bifurcates at the terminal ends to form two branched aliphatic hydrocarbon chains, and a hydrophobic tail that does not bifurcate. The inventors have found that the cationic lipids of the present disclosure can be synthesized at satisfactory yield and purity. The inventors have also found that the cationic lipids of the present disclosure, when formulated as lipid nanoparticles (LNP) for carrying a therapeutic nucleic acid, provide sustained excellent and stable *in vivo* expression of the transgene insert within the nucleic acid and are well-tolerated. Moreover, without wishing to be bound by theory, the inventors believe that a delicate interplay between the length (*i.e.*, number of carbon atoms) of terminal branched aliphatic hydrocarbon chains in the bifurcated hydrophobic tails, the length of non-bifurcated hydrophobic tail, as well as the distance between the biodegradable group and the bifurcated hydrophobic tails, are important towards, *inter alia*, achieving excellent encapsulation efficiencies, expression levels, and *in vivo* tolerability of an LNP composition.

In a first aspect of the present disclosure, there is provided a lipid represented by Formula **I**:



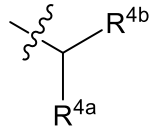
I

or a pharmaceutically acceptable salt thereof, wherein:

R' is absent, hydrogen, or C_1-C_3 alkyl; provided that when **R'** is hydrogen or C_1-C_3 alkyl, the nitrogen atom to which **R'**, $\mathbf{R^1}$, and $\mathbf{R^2}$ are all attached is protonated;

$\mathbf{R^1}$ and $\mathbf{R^2}$ are each independently hydrogen or C_1-C_3 alkyl;

$\mathbf{R^3}$ is C_3-C_{10} alkylene or C_3-C_{10} alkenylene;

$\mathbf{R^4}$ is C_1-C_{16} unbranched alkyl, C_2-C_{16} unbranched alkenyl, or 

; wherein:

$\mathbf{R^{4a}}$ and $\mathbf{R^{4b}}$ are each independently C_1-C_{16} unbranched alkyl or C_2-C_{16} unbranched alkenyl;

$\mathbf{R^5}$ is absent, C_1-C_8 alkylene, or C_2-C_8 alkenylene;

$\mathbf{R^{6a}}$ and $\mathbf{R^{6b}}$ are each independently C_7-C_{14} alkyl or C_7-C_{14} alkenyl;

X is -OC(=O)-, -SC(=O)-, -OC(=S)-, -C(=O)O-, -C(=O)S-, -S-S-, -C($\mathbf{R^a}$)=N-, -N=C($\mathbf{R^a}$)-, -C($\mathbf{R^a}$)=NO-, -O-N=C($\mathbf{R^a}$)-, -C(=O)N$\mathbf{R^a}$-, -N$\mathbf{R^a}$C(=O)-, -N$\mathbf{R^a}$C(=O)N$\mathbf{R^a}$-, -OC(=O)O-, -OSi($\mathbf{R^a}$)$_2$O-, -C(=O)(C$\mathbf{R^a}_2$)C(=O)O-, or OC(=O)(C$\mathbf{R^a}_2$)C(=O)-; wherein:

$\mathbf{R^a}$, for each occurrence, is independently hydrogen or C_1-C_6 alkyl; and

n is an integer selected from 1, 2, 3, 4, 5, and 6.

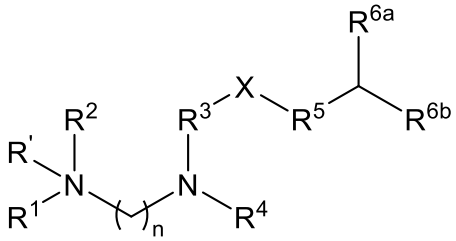
In another aspect of the present disclosure, there is provided a lipid nanoparticle (LNP) comprising the lipid as disclosed herein, or a pharmaceutically acceptable salt thereof; and a therapeutic nucleic acid.

In yet another aspect of the present disclosure, there is provided a pharmaceutical composition comprising the lipid as disclosed herein or the lipid nanoparticle as disclosed herein and a pharmaceutically acceptable excipient.

In a further aspect of the present disclosure, there is provided a use of a lipid nanoparticle as disclosed herein, or a pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating a genetic disorder in a subject.

In another aspect of the present disclosure, there is provided a lipid nanoparticle as disclosed herein, or a pharmaceutical composition as disclosed herein, for use in therapy.

Accordingly, in one example, provided herein are cationic lipids represented by Formula **I or Ia**:



I or Ia

as well as pharmaceutically acceptable salts thereof, wherein **R'**, $\mathbf{R^1}$, $\mathbf{R^2}$, $\mathbf{R^3}$, $\mathbf{R^4}$, $\mathbf{R^5}$, $\mathbf{R^{6a}}$, $\mathbf{R^{6b}}$, **X**, and **n** are as defined herein for each of Formula **I** or **Ia**, respectively.

Also provided are pharmaceutical compositions comprising a cationic lipid described herein, or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier.

Another example of the present disclosure relates to a composition comprising a lipid nanoparticle (LNP) comprising a cationic lipid described herein, or a pharmaceutically acceptable salt thereof, and a nucleic acid. In one example of any of the examples herein, the nucleic acid is encapsulated in the LNP. In a particular example, the nucleic acid is a closed-ended DNA (ceDNA).

A further example of the present disclosure relates to a method of treating a genetic disorder in a subject using a disclosed cationic lipid or composition described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the present disclosure, briefly summarized above and discussed in greater detail below, can be understood by reference to the illustrative embodiments of the disclosure depicted in the appended drawings. However, the appended drawings illustrate only typical embodiments of the disclosure and are therefore not to be considered limiting of scope, for the disclosure may admit to other equally effective embodiments.

FIG. 1 shows the Day 4 ceDNA-luciferase expression achieved by employing as delivery vehicles, lipid nanoparticles LNP 2, LNP 3, and LNP 4 that are each formulated with Lipid 6, compared to LNP 1 formulated with Reference Lipid A (positive control) and PBS (negative control), as observed in a preclinical study (dosage = 0.25 mg/kg).

FIG. 2A is a bar graph showing the Day 4 ceDNA-luciferase expression as measured by total flux, achieved by employing as delivery vehicles, lipid nanoparticles LNP 8, LNP 9, and LNP 10 that are respectively formulated with Lipid 7, Lipid 11, and Lipid 1, compared to LNP 5 formulated with Reference Lipid A (positive control), LNP 6 formulated with MC3, and LNP 7 formulated with Reference Lipid B (positive control), and PBS (negative control), as observed in a preclinical study (dosage = 0.5 mg/kg). **FIG. 2B** shows the Day 0 to Day 4 longitudinal body weight changes in the mice in the same study.

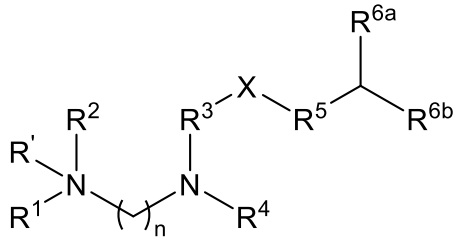
DETAILED DESCRIPTION

The present disclosure provides a lipid-based platform for delivering therapeutic nucleic acid (TNA) such as non-viral (*e.g.*, closed-ended DNA) or synthetic viral vectors,

CLAIMS

WHAT IS CLAIMED IS:

1. A lipid represented by Formula **I**:



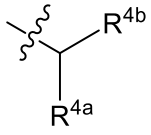
I

or a pharmaceutically acceptable salt thereof, wherein:

R' is absent, hydrogen, or C_1-C_3 alkyl; provided that when **R'** is hydrogen or C_1-C_3 alkyl, the nitrogen atom to which **R'**, $\mathbf{R^1}$, and $\mathbf{R^2}$ are all attached is protonated;

$\mathbf{R^1}$ and $\mathbf{R^2}$ are each independently hydrogen or C_1-C_3 alkyl;

$\mathbf{R^3}$ is C_3-C_{10} alkylene or C_3-C_{10} alkenylene;

$\mathbf{R^4}$ is C_1-C_{16} unbranched alkyl, C_2-C_{16} unbranched alkenyl, or  ; wherein:

$\mathbf{R^{4a}}$ and $\mathbf{R^{4b}}$ are each independently C_1-C_{16} unbranched alkyl or C_2-C_{16} unbranched alkenyl;

$\mathbf{R^5}$ is absent, C_1-C_8 alkylene, or C_2-C_8 alkenylene;

$\mathbf{R^{6a}}$ and $\mathbf{R^{6b}}$ are each independently C_7-C_{14} alkyl or C_7-C_{14} alkenyl;

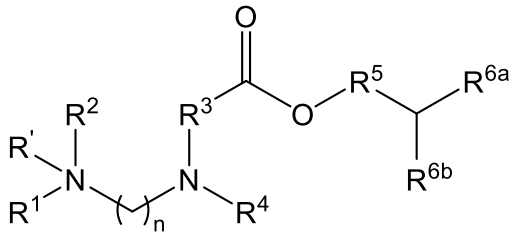
X is -OC(=O)-, -SC(=O)-, -OC(=S)-, -C(=O)O-, -C(=O)S-, -S-S-, -C($\mathbf{R^a}$)=N-, -N=C($\mathbf{R^a}$)-, -C($\mathbf{R^a}$)=NO-, -O-N=C($\mathbf{R^a}$)-, -C(=O)N$\mathbf{R^a}$-, -N$\mathbf{R^a}$C(=O)-, -N$\mathbf{R^a}$C(=O)N$\mathbf{R^a}$-, -OC(=O)O-, -OSi($\mathbf{R^a}$)$_2$O-, -C(=O)(C$\mathbf{R^a}_2$)C(=O)O-, or OC(=O)(C$\mathbf{R^a}_2$)C(=O)-; wherein:

$\mathbf{R^a}$, for each occurrence, is independently hydrogen or C_1-C_6 alkyl; and

n is an integer selected from 1, 2, 3, 4, 5, and 6.

2. The lipid according to claim 1, or a pharmaceutically acceptable salt thereof, wherein **X** is -OC(=O)-, -SC(=O)-, -OC(=S)-, -C(=O)O-, -C(=O)S-, or -S-S-.

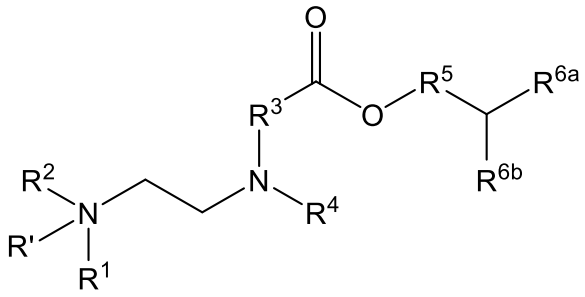
3. The lipid according to claim 1 or claim 2, wherein the lipid is represented by Formula **II**:



II

or a pharmaceutically acceptable salt thereof, wherein **n** is an integer selected from 1, 2, 3, and 4.

4. The lipid according to any one of claims 1 to 3, wherein the lipid is represented by Formula **III**:



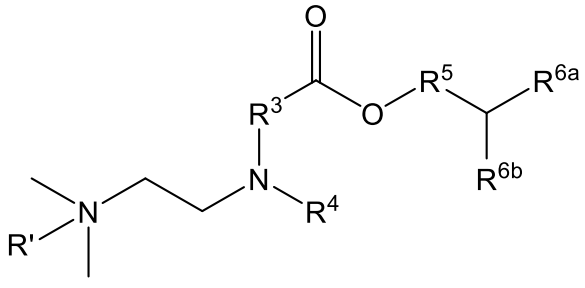
III

or a pharmaceutically acceptable salt thereof.

5. The lipid according to any one of claims 1 to 4, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^1}$ and $\mathbf{R^2}$ are each independently hydrogen, C_1-C_2 alkyl, or C_2-C_3 alkenyl.

6. The lipid according to any one of claims 1 to 5, or a pharmaceutically acceptable salt thereof, wherein **R'**, $\mathbf{R^1}$, and $\mathbf{R^2}$ are each independently hydrogen or C_1-C_2 alkyl.

7. The lipid according to any one of claims 1 to 6, wherein the lipid is represented by Formula **IV**:



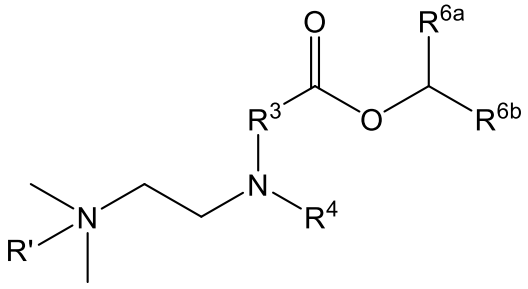
IV

or a pharmaceutically acceptable salt thereof.

8. The lipid according to any one of claims 1 to 7, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^5}$ is absent or C_1-C_8 alkylene.

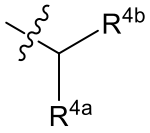
9. The lipid according to any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^5}$ is absent or C_2 alkylene.

10. The lipid according to any one of claims 1 to 9, wherein the lipid is represented by Formula **V**:



V

or a pharmaceutically acceptable salt thereof.

11. The lipid according to any one of claims 1 to 10, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^4}$ is C_1-C_{14} unbranched alkyl, C_2-C_{14} unbranched alkenyl, or 

; wherein $\mathbf{R^{4a}}$ and $\mathbf{R^{4b}}$ are each independently C_1-C_{12} unbranched alkyl or C_2-C_{12} unbranched alkenyl.

12. The lipid according to any one of claims 1 to 11, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^4}$ is C_2-C_{12} unbranched alkyl or C_2-C_{12} unbranched alkenyl.

13. The lipid according to any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^4}$ is C_5-C_{12} unbranched alkyl.

14. The lipid according to any one of claims 1 to 13, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^4}$ is C_6 unbranched alkyl, C_7 unbranched alkyl, C_8 unbranched alkyl, C_9 unbranched alkyl, C_{10} unbranched alkyl, C_{11} unbranched alkyl, or C_{12} unbranched alkyl.

15. The lipid according to any one of claims 1 to 14, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^4}$ is C_9 unbranched alkyl.

16. The lipid according to any one of claims 1 to 15, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^3}$ is C_3-C_8 alkylene or C_3-C_8 alkenylene.

17. The lipid according to any one of claims 1 to 16, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^3}$ is C_3-C_7 alkylene.

18. The lipid according to any one of claims 1 to 17, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^3}$ is C_7 alkylene or C_5 alkylene.

19. The lipid according to any one of claims 1 to 18, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^{6a}}$ and $\mathbf{R^{6b}}$ are each independently C_7-C_{12} alkyl or C_7-C_{12} alkenyl.

20. The lipid according to any one of claims 1 to 19, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^{6a}}$ and $\mathbf{R^{6b}}$ are each independently C_7 alkyl, C_8 alkyl, C_9 alkyl, C_{10} alkyl, C_{11} alkyl, C_{12} alkyl, C_8 alkenyl, C_{10} alkenyl, C_{11} alkenyl, or C_{12} alkenyl.

21. The lipid according to any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^{6a}}$ and $\mathbf{R^{6b}}$ are each independently C_7 alkyl, C_8 alkyl, C_9 alkyl, C_{10} alkyl, C_{11} alkyl, or C_{12} alkyl.

22. The lipid according to any one of claims 1 to 21, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^{6a}}$ and $\mathbf{R^{6b}}$ each contain the same number of carbon atoms.

23. The lipid according to any one of claims 1 to 22, or a pharmaceutically acceptable salt thereof, wherein $\mathbf{R^{6a}}$ and $\mathbf{R^{6b}}$ are the same.

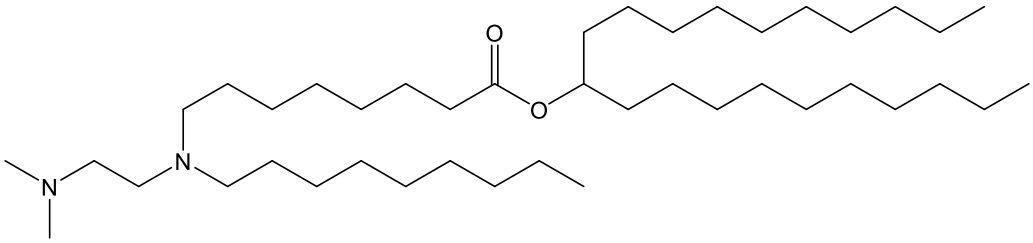
24. The lipid according to any one of claims 1 to 23, or a pharmaceutically acceptable salt thereof, wherein **R^{6a}** and **R^{6b}** are both C_7 alkyl, or C_8 alkyl, or C_9 alkyl, or C_{10} alkyl, or C_{11} alkyl, or C_{12} alkyl.

25. The lipid according to any one of claims 1 to 24, or a pharmaceutically acceptable salt thereof, wherein **R^{6a}** and **R^{6b}** are both C_8 alkyl, C_9 alkyl, C_{10} alkyl, C_{11} alkyl or C_{12} alkyl.

26. The lipid according to any one of claims 1 to 21, or a pharmaceutically acceptable salt thereof, wherein **R^{6a}** and **R^{6b}** each contain a different number of carbon atoms.

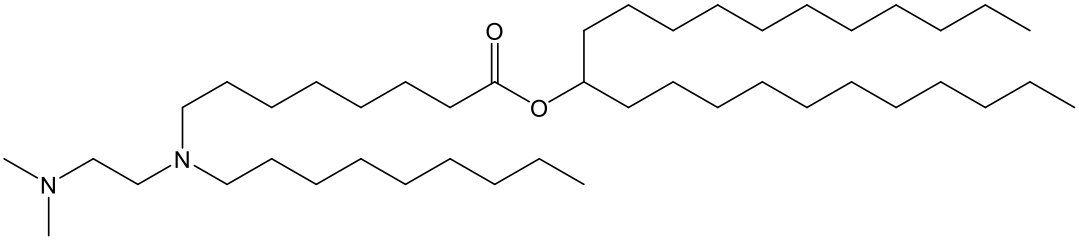
27. The lipid according to any one of claims 1 to 26, or a pharmaceutically acceptable salt thereof, wherein **R'** is absent.

28. The lipid according to claim 1, wherein the lipid is:



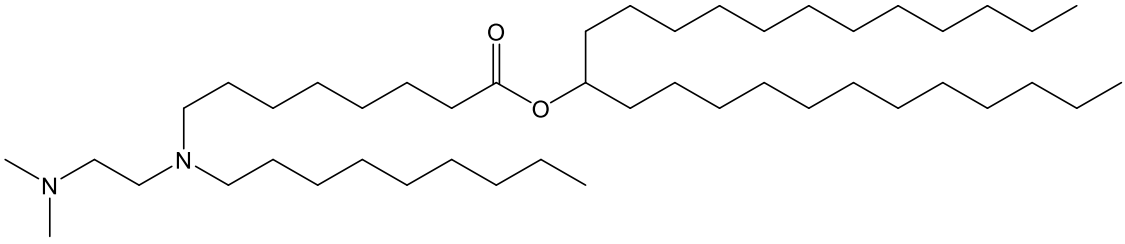
henicosan-11-yl 8-((2-(dimethylamino)ethyl)(nonyl)amino)octanoate

(**Lipid 1**);



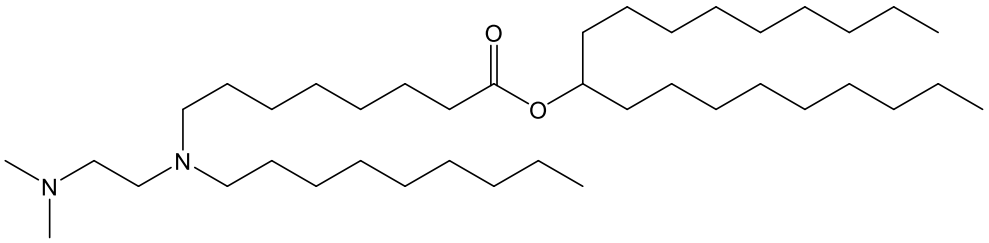
tricosan-12-yl 8-((2-(dimethylamino)ethyl)(nonyl)amino)octanoate

(**Lipid 2**);



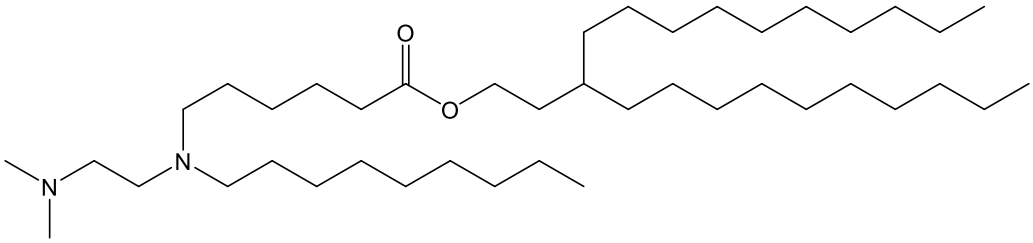
pentacosan-13-yl 8-((2-(dimethylamino)ethyl)(nonyl)amino)octanoate

(**Lipid 3**);



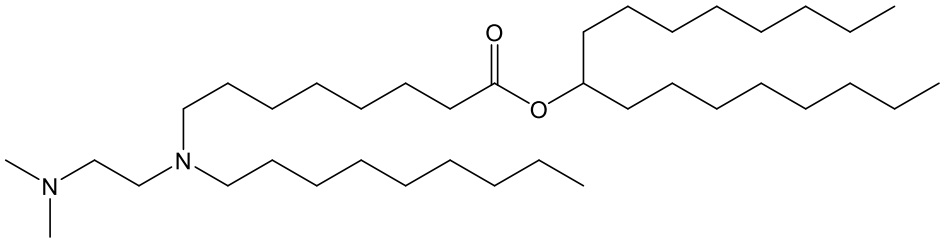
nonadecan-10-yl 8-((2-(dimethylamino)ethyl)(nonyl)amino)octanoate

(**Lipid 4**);



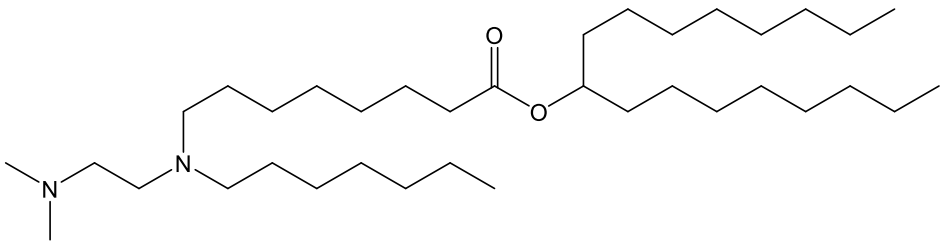
3-decyltridecyl 6-((2-(dimethylamino)ethyl)(nonyl)amino)hexanoate

(**Lipid 5**);



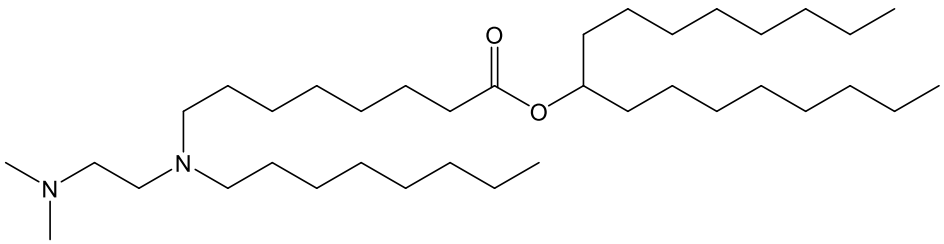
heptadecan-9-yl 8-((2-(dimethylamino)ethyl)(nonyl)amino)octanoate

(**Lipid 6**);



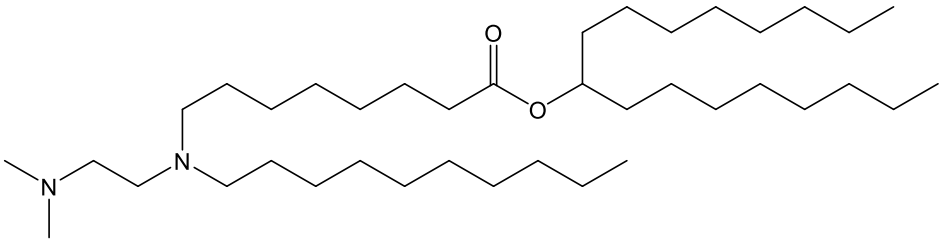
heptadecan-9-yl 8-((2-(dimethylamino)ethyl)(heptyl)amino)octanoate

(**Lipid 7**);



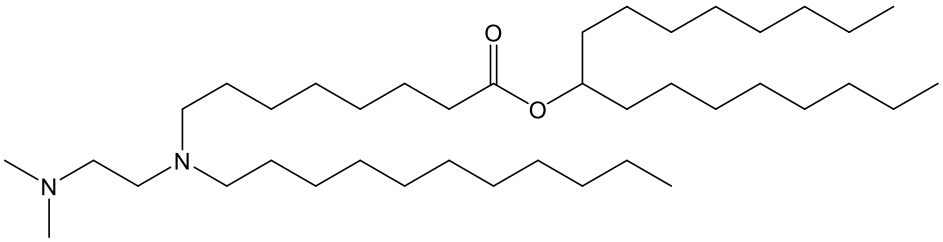
heptadecan-9-yl 8-((2-(dimethylamino)ethyl)(octyl)amino)octanoate

(**Lipid 8**);



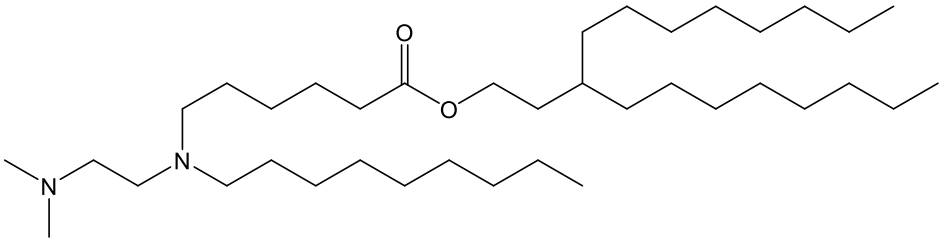
heptadecan-9-yl 8-(decyl(2-(dimethylamino)ethyl)amino)octanoate

(**Lipid 9**);



heptadecan-9-yl 8-((2-(dimethylamino)ethyl)(undecyl)amino)octanoate

(**Lipid 10**);



3-octylundecyl 6-((2-(dimethylamino)ethyl)(nonyl)amino)hexanoate

(**Lipid 11**);

or a pharmaceutically acceptable salt thereof.

29. A lipid nanoparticle (LNP) comprising the lipid according to any one of claims 1 to 28, or a pharmaceutically acceptable salt thereof; and a therapeutic nucleic acid.

30. The lipid nanoparticle according to claim 29, wherein the therapeutic nucleic acid is encapsulated in the lipid.

31. The lipid nanoparticle according to claim 29 or claim 30, wherein the therapeutic nucleic acid is selected from the group consisting of minigenes, plasmids, minicircles, small interfering RNA (siRNA), microRNA (miRNA), antisense oligonucleotides (ASO), ribozymes, ceDNA, ministring, doggybone™, protelomere closed ended DNA, or dumbbell linear DNA, dicer-substrate dsRNA, small hairpin RNA (shRNA), asymmetrical interfering

RNA (aiRNA), microRNA (miRNA), mRNA, tRNA, rRNA, DNA viral vectors, viral RNA vector, non-viral vector and any combination thereof.

32. The lipid nanoparticle according to any one of claims 29 to 31, wherein the therapeutic nucleic acid is mRNA.

33. The lipid nanoparticle according to any one of claims 29 to 31, further comprising a sterol.

34. The lipid nanoparticle according to claim 33, wherein the sterol is a cholesterol or beta-sitosterol.

35. The lipid nanoparticle according to any one of claims 29 to 34, further comprising a non-cationic lipid.

36. The lipid nanoparticle according to claim 35, wherein the non-cationic lipid is selected from the group consisting of distearoyl-sn-glycero-phosphoethanolamine (DSPE), distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), monomethyl-phosphatidylethanolamine (such as 16-O-monomethyl PE), dimethyl-phosphatidylethanolamine (such as 16-O-dimethyl PE), 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM), dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), distearoylphosphatidylglycerol (DSPG), dierucoylphosphatidylcholine (DEPC), palmitoyloleyolphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), 1,2-dilauroyl-sn-glycero-3 -pho sphoethanolamine (DLPE); 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (DPHyPE); lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg

sphingomyelin (ESM), cephalin, cardiolipin, phosphatidicacid,cerebrosides, dicetylphosphate, lysophosphatidylcholine, dilinoleoylphosphatidylcholine, and mixtures thereof.

37. The lipid nanoparticle according to claim 35 or claim 36, wherein the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).

38. The lipid nanoparticle according to any one of claims 29 to 37, further comprising at least one PEGylated lipid.

39. The lipid nanoparticle according to claim 38, wherein the at least one PEGylated lipid is selected from the group consisting of PEG-dilauryloxypropyl; PEG-dimyristyloxypropyl; PEG-dipalmityloxypropyl, PEG-distearyloxypropyl; l-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol-PEG (DMG-PEG); distearoyl-rac-glycerol-PEG (DSG-PEG); PEG-dilaurylglycerol; PEG-dipalmitoylglycerol; PEG-disterylglycerol; PEG-dilaurylglycamide; PEG-dimyristylglycamide; PEG-dipalmitoylglycamide; PEG-disterylglycamide; (l-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl] carbamoyl-[omega]-methyl-poly(ethylene glycol) (PEG-cholesterol); 3,4-ditetradecoxylbenzyl-[omega]- methyl-poly(ethylene glycol) ether (PEG-DMB), l,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N- [methoxy(polyethylene glycol) (DSPE-PEG), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-poly(ethylene glycol)-hydroxyl (DSPE-PEG-OH).

40. The lipid nanoparticle according to any one of claims 29 to 39, further comprising a tissue-specific targeting ligand.

41. The lipid nanoparticle according to claim 40, wherein the tissue-specific targeting ligand is N-acetylgalactosamine (GalNAc) or a GalNAc derivative.

42. The lipid nanoparticle according to claim 40 or claim 41, wherein the tissue-specific targeting ligand is covalently linked to the at least one PEGylated lipid to form a PEGylated lipid conjugate.

43. The lipid nanoparticle according to any one of claims 33 to 42, wherein the sterol is present in the lipid nanoparticle at a molar percentage of about 20% to about 50%; and/or wherein the non-cationic lipid is present at a molar percentage of about 2% to about 20%; and/or wherein the at least one PEGylated lipid is present at a molar percentage of about 2.1% to about 10%.

44. A pharmaceutical composition comprising the lipid according to any one of claims 1 to 28 or the lipid nanoparticle according to any one of claims 29 to 43 and a pharmaceutically acceptable excipient.

45. Use of a lipid nanoparticle according to any one of claims 29 to 43, or a pharmaceutical composition according to claim 44, in the manufacture of a medicament for treating a genetic disorder in a subject.

46. The use according to claim 45, wherein the subject is a human.

47. The use according to claim 45 or claim 46, wherein the genetic disorder is selected from the group consisting of sickle-cell anemia, melanoma, hemophilia A (clotting factor VIII (FVIII) deficiency) and hemophilia B (clotting factor IX (FIX) deficiency), cystic fibrosis (CFTR), familial hypercholesterolemia (LDL receptor defect), hepatoblastoma, Wilson disease, phenylketonuria (PKU), congenital hepatic porphyria, inherited disorders of hepatic metabolism, Lesch Nyhan syndrome, sickle cell anemia, thalassaemias, xeroderma pigmentosum, Fanconi's anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom's syndrome, retinoblastoma, a mucopolysaccharide storage disease (*e.g.*, Hurler syndrome (MPS Type I), Scheie syndrome (MPS Type I S), Hurler-Scheie syndrome (MPS Type I H-S), Hunter syndrome (MPS Type II), Sanfilippo Types A, B, C, and D (MPS Types III A, B, C, and D), Morquio Types A and B (MPS IVA and MPS IVB), Maroteaux-Lamy syndrome (MPS Type VI), Sly syndrome (MPS Type VII), hyaluronidase deficiency (MPS Type IX)), Niemann-Pick Disease Types A/B, C1 and C2, Fabry disease, Schindler disease, GM2-gangliosidosis Type II (Sandhoff Disease), Tay-Sachs disease, Metachromatic Leukodystrophy, Krabbe disease, Mucolipidosis Type I, II/III and IV, Sialidosis Types I and II, Glycogen Storage disease Types I and II (Pompe disease), Gaucher disease Types I, II and III, cystinosis, Batten disease, Aspartylglucosaminuria, Salla disease, Danon disease (LAMP-

2 deficiency), Lysosomal Acid Lipase (LAL) deficiency, neuronal ceroid lipofuscinoses (CLN1-8, INCL, and LINCL), sphingolipidoses, galactosialidosis, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, Huntington's disease, spinocerebellar ataxia, spinal muscular atrophy, Friedreich's ataxia, Duchenne muscular dystrophy (DMD), Becker muscular dystrophies (BMD), dystrophic epidermolysis bullosa (DEB), ectonucleotide pyrophosphatase 1 deficiency, generalized arterial calcification of infancy (GACI), Leber Congenital Amaurosis, Stargardt macular dystrophy (ABCA4), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, age-related macular degeneration (AMD), alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis (PFIC) type I (ATP8B1 deficiency), type II (ABCB11), type III (ABCB4), or type IV (TJP2), and Cathepsin A deficiency.

48. A lipid nanoparticle according to any one of claims 29 to 43, or a pharmaceutical composition according to claim 44, for use in therapy.