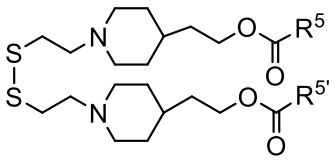
with reduced toxicity, but with improved pharmacokinetics and intracellular kinetics such as cellular uptake and nucleic acid release from the lipid carrier.

SUMMARY

In one aspect of the present disclosure, there is provided an ionizable lipid represented by the Formula (X):



(**X**);

or a pharmaceutically acceptable salt thereof, wherein R^5 is selected from the group consisting of $–(CH_2)_3C(O)O(CH_2)_8CH_3$, $–(CH_2)_5C(O)O(CH_2)_8CH_3$, $–(CH_2)_7C(O)O(CH_2)_8CH_3$, $–(CH_2)_7C(O)OCH[(CH_2)_7CH_3]_2$, $–(CH_2)_7$-C_3H_6-$(CH_2)_7CH_3$, $–(CH_2)_7CH_3$, $–(CH_2)_9CH_3$, $–(CH_2)_{16}CH_3$, $–(CH_2)_7CH=CH(CH_2)_7CH_3$, or $–(CH_2)_7CH=CHCH_2CH=CH(CH_2)_4CH_3$; and

wherein $R^{5'}$ is selected from the group consisting of $–(CH_2)_5C(O)OCH[(CH_2)_7CH_3]_2$, $–(CH_2)_7C(O)OCH[(CH_2)_7CH_3]_2$, $–(CH_2)_5C(O)OCH(CH_2)_2[(CH_2)_7CH_3]_2$, or $–(CH_2)_7C(O)OCH(CH_2)_2[(CH_2)_7CH_3]_2$.

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

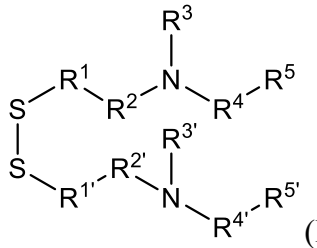
In a further aspect of the present disclosure, there is provided a pharmaceutical composition comprising the lipid nanoparticle disclosed herein or the ionizable lipid disclosed herein or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable excipient.

In another aspect of the present disclosure, there is provided a use of the lipid nanoparticle disclosed herein, or the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating a disease or disorder in a subject; optionally wherein the subject is a human; optionally wherein the disease or disorder is a genetic disorder; optionally wherein the genetic disorder is selected from the group consisting of melanoma, hemophilia A (clotting factor VIII (FVIII) deficiency), hemophilia B (clotting factor IX (FIX) deficiency), cystic fibrosis (CFTR defect), familial hypercholesterolemia (LDL receptor defect), hepatoblastoma, Wilson disease, phenylketonuria (PKU), congenital hepatic porphyria, an inherited disorder of hepatic metabolism, Lesch-Nyhan syndrome, sickle cell anemia, thalassaemia, xeroderma pigmentosum, Fanconi anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom syndrome, retinoblastoma, a mucopolysaccharide storage disease (*e.g.*, Hurler syndrome (MPS I), Scheie syndrome (MPS I S), Hurler-Scheie syndrome (MPS I H-S), Hunter syndrome (MPS II), Sanfilippo syndrome Type A (MPS IIIA), Sanfilippo syndrome Type B (MPS IIIB), Sanfilippo syndrome Type C (MPS IIIC), Sanfilippo syndrome Type D (MPS Type IIID),

Morquio syndrome Type A (MPS IVA), Morquio syndrome Type B (MPS IVB), Maroteaux-Lamy syndrome (MPS VI), Sly syndrome (MPS VII), hyaluronidase deficiency (MPS IX)), Niemann-Pick Disease Type A/B, Niemann-Pick Disease Type C1, Niemann-Pick Disease Type C2, Schindler disease, GM2-gangliosidosis Type II (Sandhoff Disease), Tay-Sachs disease, Metachromatic Leukodystrophy, Krabbe disease, Mucolipidosis Type I, Mucolipidosis Type II/III, Mucolipidosis Type IV, Sialidosis Type I, Sialidosis Type II, Glycogen Storage disease Type I, Glycogen Storage disease Type II (Pompe disease), Gaucher disease Type I, Gaucher disease Type II, Gaucher disease Type III, Fabry disease, 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 dystrophy (BMD), dystrophic epidermolysis bullosa (DEB), ectonucleotide pyrophosphatase 1 deficiency, generalized arterial calcification of infancy (GACI), Leber Congenital Amaurosis (LCA), Stargardt macular dystrophy (ABCA4 deficiency), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis (PFIC) type I (ATP8B1 deficiency), progressive familial intrahepatic cholestasis (PFIC) type II (ABCB11), progressive familial intrahepatic cholestasis (PFIC) type III (ABCB4), progressive familial intrahepatic cholestasis (PFIC) type IV (TJP2), and Cathepsin A deficiency.

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

In one example, provided herein are ionizable lipids having the Formula (**I**):



(**I**);

as well as pharmaceutically acceptable salts thereof, wherein R^1, $R^{1'}$, R^2, $R^{2'}$, R^3, $R^{3'}$, R^4, $R^{4'}$, $R^{5'}$, and $R^{5'}$, are as defined herein.

Also provided are pharmaceutical compositions comprising a disclosed ionizable lipid, 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 an ionizable lipid described herein, or a pharmaceutically acceptable salt thereof, and a nucleic acid. In one example, the nucleic acid is encapsulated in the ionizable lipid. In a particular example, the nucleic acid is a closed-ended DNA (ceDNA).

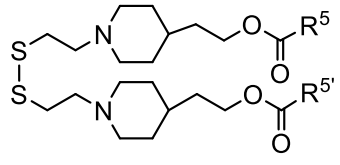
According to some examples herein, the LNP further comprises a sterol. According to some examples, the sterol can be a cholesterol, or beta-sitosterol.

According to some examples, the cholesterol is present at a molar percentage of about 20% to about 40%, for example about 20% to about 35%, about 20% to about 30%, about 20% to about 25%, about 25% to about 35%, about 25% to about 30%, or about 30% to about 35%, and the ionizable lipid is present at a molar percentage of about 80% to about 60%, for example about 80% to about 65%, about 80% to about 70%, about 80% to about 75%, about 75% to about 60%, about 75% to about 65%, about 75% to about 70%, about 70% to about 60%, or about 70% to about 60%. According to some examples, the cholesterol is present at a molar percentage of about 20% to about 40%, for example about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, or about 40%, and wherein the ionizable lipid is present at a molar percentage of about 80% to about 60%, for example about 80%, about 79%, about 78%, about 77%, about 76%, about 75%, about 74%, about

CLAIMS

What is Claimed is:

1. An ionizable lipid represented by the Formula (X):

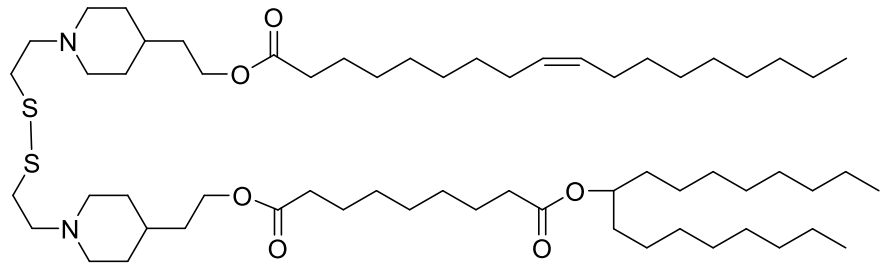


(**X**);

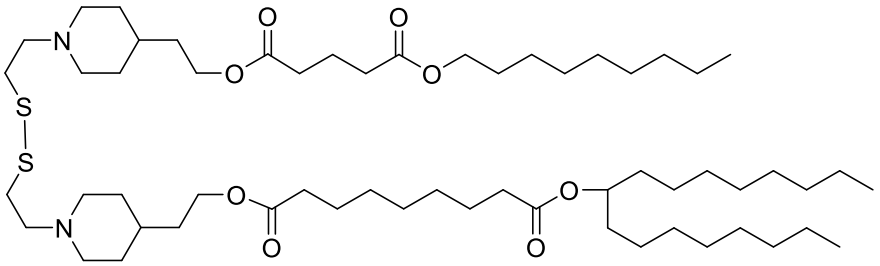
or a pharmaceutically acceptable salt thereof, wherein R^5 is selected from the group consisting of $–(CH_2)_3C(O)O(CH_2)_8CH_3$, $–(CH_2)_5C(O)O(CH_2)_8CH_3$, $–(CH_2)_7C(O)O(CH_2)_8CH_3$, $–(CH_2)_7C(O)OCH[(CH_2)_7CH_3]_2$, $–(CH_2)_7\text{-}C_3H_6\text{-}(CH_2)_7CH_3$, $–(CH_2)_7CH_3$, $–(CH_2)_9CH_3$, $–(CH_2)_{16}CH_3$, $–(CH_2)_7CH{=}CH(CH_2)_7CH_3$, or $–(CH_2)_7CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$; and

wherein $R^{5'}$ is selected from the group consisting of $–(CH_2)_5C(O)OCH[(CH_2)_7CH_3]_2$, $–(CH_2)_7C(O)OCH[(CH_2)_7CH_3]_2$, $–(CH_2)_5C(O)OCH(CH_2)_2[(CH_2)_7CH_3]_2$, or $–(CH_2)_7C(O)OCH(CH_2)_2[(CH_2)_7CH_3]_2$.

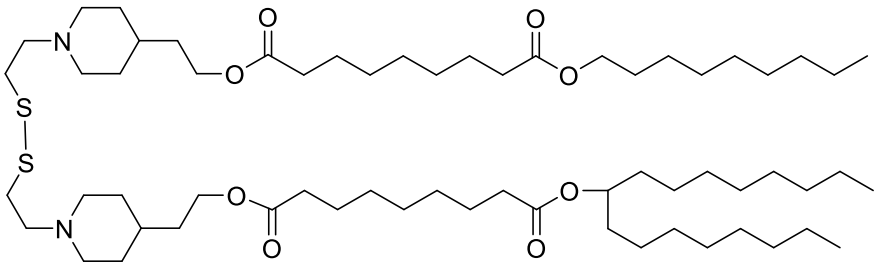
2. The ionizable lipid of claim 1, wherein the ionizable lipid is selected from the group consisting of:



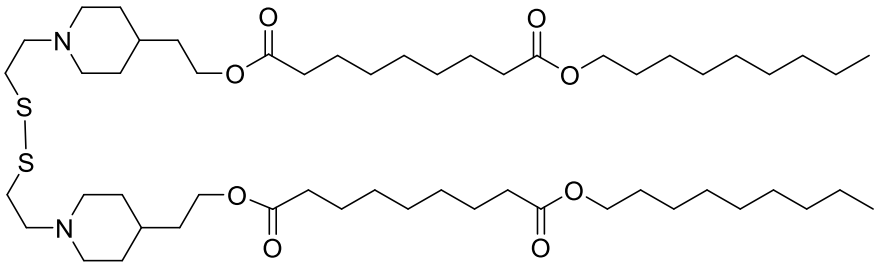
1-(heptadecan-9-yl) 9-(2-(1-(2-((2-(4-(2-(oleoyloxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethyl) nonanedioate;



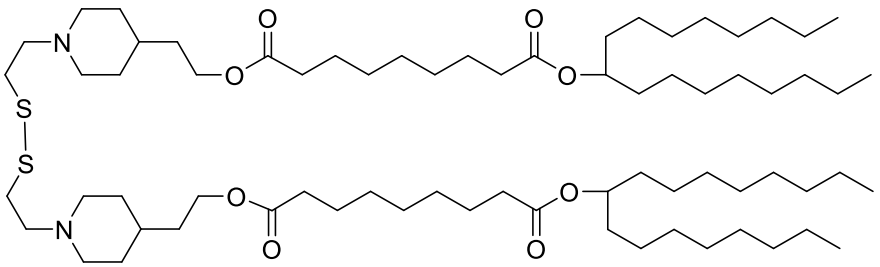
1-(heptadecan-9-yl) 9-(2-(1-(2-((2-(4-(2-((5-(nonyloxy)-5-oxopentanoyl)oxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethyl) nonanedioate;



1-(heptadecan-9-yl) 9-(2-(1-(2-((2-(4-(2-((9-(nonyloxy)-9-oxononanoyl)oxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethyl) nonanedioate;



O'1,O1-(((disulfanediylbis(ethane-2,1-diyl))bis(piperidine-1,4-diyl))bis(ethane-2,1-diyl)) 9,9'-dinonyl di(nonanedioate); and



O'1,O1-(((disulfanediylbis(ethane-2,1-diyl))bis(piperidine-1,4-diyl))bis(ethane-2,1-diyl)) 9,9'-di(heptadecan-9-yl) di(nonanedioate),

or a pharmaceutically acceptable salt thereof.

3. A lipid nanoparticle (LNP) comprising the ionizable lipid of claim 1 or 2, or a pharmaceutically acceptable salt thereof; and a nucleic acid.

4. The lipid nanoparticle of claim 3, wherein the nucleic acid is selected from the group consisting of a minigene, a plasmid, a minicircle, a small interfering RNA (siRNA), an antisense oligonucleotide (ASO), a ribozyme, a ceDNA, a ministring, a doggybone™, a protelomere closed ended DNA, a dumbbell linear DNA, a dicer-substrate dsRNA, a small hairpin RNA (shRNA), an asymmetrical interfering RNA (aiRNA), a microRNA (miRNA), an mRNA, a tRNA, an rRNA, a DNA viral vector, a viral RNA vector, a non-viral vector and any combination thereof.

5. The lipid nanoparticle of claim 3 or 4, further comprising at least one lipid selected from the group consisting of a sterol, a PEGylated lipid and a non-cationic lipid.

6. The lipid nanoparticle of claim 5, wherein the PEGylated lipid is present in the lipid nanoparticle at a molar percentage of 2.5% to 3%, and/or

(a) the sterol is present in the lipid nanoparticle at a molar percentage of 20% to 40% and wherein the ionizable lipid is present in the lipid nanoparticle at a molar percentage of 80% to 60%; or

(b) the sterol is present in the lipid nanoparticle at a molar percentage of about 40% and wherein the ionizable lipid is present in the lipid nanoparticle at a molar percentage of about 50%; or

(c) the non-cationic lipid is present in the lipid nanoparticle at a molar percentage of 2.5% to 12.5%.

7. The lipid nanoparticle of any one of claims 3 to 6, further comprising a cholesterol, a PEGylated lipid, and a non-cationic lipid.

8. The lipid nanoparticle of any one of claims 3 to 7, further comprising a tissue-specific targeting ligand.

9. The lipid nanoparticle of claim 8, wherein the tissue-specific targeting ligand is a peptide.

10. The lipid nanoparticle of claim 8, wherein the tissue specific targeting ligand is N-acetylgalactosamine (GalNAc) or a derivative thereof selected from mono-antennary GalNAc, tri-antennary GalNAc, and tetra-antennary GalNAc.

11. The lipid nanoparticle of claim 10, wherein the tissue-specific targeting ligand is conjugated to a PEGylated lipid.

12. The lipid nanoparticle of claim 11, wherein the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the lipid nanoparticle at a molar percentage of about 1.0%, about 0.9%, about 0.8%, about 0.7%, about 0.6%, about 0.5%, about 0.4%, about 0.3%, about 0.2%, or about 0.1%.

13. The lipid nanoparticle of any one of claims 3 to 12, wherein the lipid nanoparticle has a mean diameter ranging from 50 nm to 110 nm; or wherein the lipid nanoparticle has a mean diameter of less than 100 nm, less than 75 nm, less than 70 nm, or less than 65 nm.

14. A pharmaceutical composition comprising the lipid nanoparticle of any one of claims 3 to 13 or the ionizable lipid of claim 1 or 2, or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable excipient.

15. Use of the lipid nanoparticle of any one of claims 3 to 13, or the pharmaceutical composition according to claim 14, in the manufacture of a medicament for treating a disease or disorder in a subject; optionally wherein the subject is a human; optionally wherein the disease or disorder is a genetic disorder; optionally wherein the genetic disorder is selected from the group consisting of melanoma, hemophilia A (clotting factor VIII (FVIII) deficiency), hemophilia B (clotting factor IX (FIX) deficiency), cystic fibrosis (CFTR defect), familial hypercholesterolemia (LDL receptor defect), hepatoblastoma, Wilson disease, phenylketonuria (PKU), congenital hepatic porphyria, an inherited disorder of hepatic metabolism, Lesch-Nyhan syndrome, sickle cell anemia, thalassaemia, xeroderma pigmentosum, Fanconi anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom syndrome, retinoblastoma, a mucopolysaccharide storage disease (*e.g.*, Hurler syndrome (MPS I),

Scheie syndrome (MPS I S), Hurler-Scheie syndrome (MPS I H-S), Hunter syndrome (MPS II), Sanfilippo syndrome Type A (MPS IIIA), Sanfilippo syndrome Type B (MPS IIIB), Sanfilippo syndrome Type C (MPS IIIC), Sanfilippo syndrome Type D (MPS Type IIID), Morquio syndrome Type A (MPS IVA), Morquio syndrome Type B (MPS IVB), Maroteaux-Lamy syndrome (MPS VI), Sly syndrome (MPS VII), hyaluronidase deficiency (MPS IX)), Niemann-Pick Disease Type A/B, Niemann-Pick Disease Type C1, Niemann-Pick Disease Type C2, Schindler disease, GM2-gangliosidosis Type II (Sandhoff Disease), Tay-Sachs disease, Metachromatic Leukodystrophy, Krabbe disease, Mucolipidosis Type I, Mucolipidosis Type II/III, Mucolipidosis Type IV, Sialidosis Type I, Sialidosis Type II, Glycogen Storage disease Type I, Glycogen Storage disease Type II (Pompe disease), Gaucher disease Type I, Gaucher disease Type II, Gaucher disease Type III, Fabry disease, 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 dystrophy (BMD), dystrophic epidermolysis bullosa (DEB), ectonucleotide pyrophosphatase 1 deficiency, generalized arterial calcification of infancy (GACI), Leber Congenital Amaurosis (LCA), Stargardt macular dystrophy (ABCA4 deficiency), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis (PFIC) type I (ATP8B1 deficiency), progressive familial intrahepatic cholestasis (PFIC) type II (ABCB11), progressive familial intrahepatic cholestasis (PFIC) type III (ABCB4), progressive familial intrahepatic cholestasis (PFIC) type IV (TJP2), and Cathepsin A deficiency.

16. The lipid nanoparticle of any one of claims 3 to 13, or the pharmaceutical composition according to claim 14, for use in therapy.