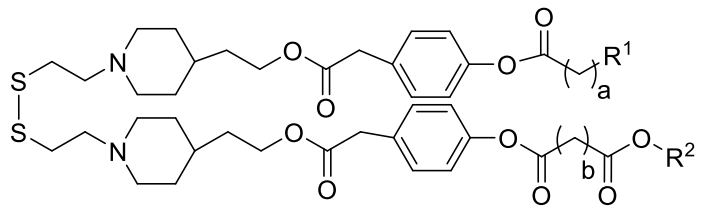
CLAIMS

What is Claimed is:

1. A lipid represented by the Formula (**I**):



(**I**);

or a pharmaceutically acceptable salt thereof, wherein:

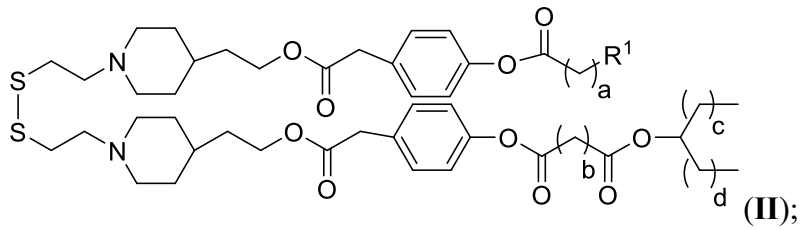
a is an integer ranging from 1 to 20;

b is an integer ranging from 2 to 10;

R^1 is selected from the group consisting of (C_2-C_{20})alkenyl, -C(O)O(C_2-C_{20})alkyl, and cyclopropyl substituted with (C_2-C_{20})alkyl; and

R^2 is (C_2-C_{20})alkyl.

2. The lipid of claim 1, wherein the lipid is represented by the Formula (**II**):



(**II**);

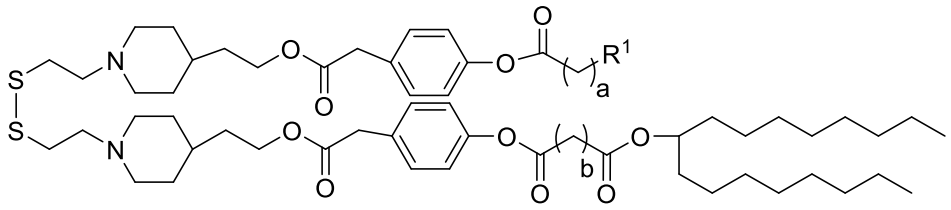
or a pharmaceutically acceptable salt thereof, wherein c and d are each independently integers ranging from 1 to 8.

3. The lipid of claim 2, or a pharmaceutically acceptable salt thereof, wherein c and d are each independently integers ranging from 2 to 8, or from 4 to 8, or from 6 to 8.

4. The lipid of claim 2, or a pharmaceutically acceptable salt thereof, wherein c and d are each independently 1, 3, 5, or 7.

5. The lipid of any one of claims 2 to 4, or a pharmaceutically acceptable salt thereof, wherein at least one of c and d is 7.

6. The lipid of any one of claims 1 to 5, wherein the lipid is represented by the Formula (**III**):



(**III**);

or a pharmaceutically acceptable salt thereof.

7. The lipid of any one of claims 1 to 6, or a pharmaceutically acceptable salt thereof, wherein b is an integer ranging from 3 to 9, or from 5 to 7.

8. The lipid of any one of claims 1 to 7, or a pharmaceutically acceptable salt thereof, wherein b is 5 or 7.

9. The lipid of any one of claims 1 to 8, or a pharmaceutically acceptable salt thereof, wherein a is an integer ranging from 2 to 18, or from 3 to 17, or from 6 to 18, or from 4 to 12, or from 2 to 5, or from 6 to 8, or from 16 to 18, or from 9 to 11.

10. The lipid of claim 9, or a pharmaceutically acceptable salt thereof, wherein a is 3, 7, 8, 10 or 17.

11. The lipid of any one of claims 1 to 10, or a pharmaceutically acceptable salt thereof, wherein R^1 is selected from the group consisting of (C_5-C_{15})alkenyl, -C(O)O(C_4-C_{18})alkyl, and cyclopropyl substituted with (C_4-C_{16})alkyl.

12. The lipid of any one of claims 1 to 11, or a pharmaceutically acceptable salt thereof, wherein R^1 is selected from the group consisting of (C_5-C_{12})alkenyl, -C(O)O(C_4-C_{12})alkyl, and cyclopropyl substituted with (C_4-C_{12})alkyl.

13. The lipid of any one of claims 1 to 12, or a pharmaceutically acceptable salt thereof, wherein R^1 is selected from the group consisting of (C_5-C_{10})alkenyl, -C(O)O(C_4-C_{10})alkyl, and cyclopropyl substituted with (C_4-C_{10})alkyl.

14. The lipid of any one of claims 1 to 13, or a pharmaceutically acceptable salt thereof, wherein R^1 is C_{10} alkenyl.

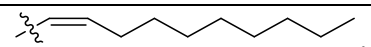
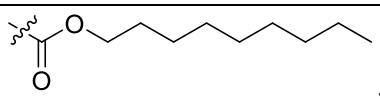
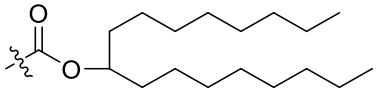
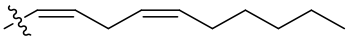
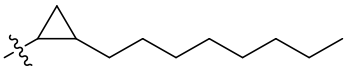
15. The lipid of any one of claims 1 to 13, or a pharmaceutically acceptable salt thereof, wherein R^1 is -C(O)O(C_2-C_{20})alkyl, -C(O)O(C_4-C_{18})alkyl, -C(O)O(C_4-C_{12})alkyl, or -C(O)O(C_4-C_{10})alkyl; and wherein the alkyl is an unbranched alkyl.

16. The lipid of claim 15, or a pharmaceutically acceptable salt thereof, wherein R^1 is -C(O)O(C_9 alkyl).

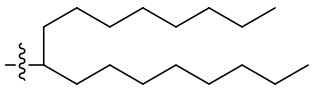
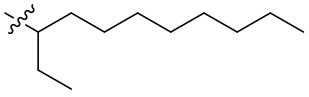
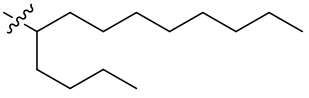
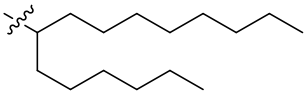
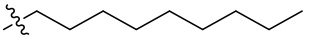
17. The lipid of any one of claims 11 to 13, or a pharmaceutically acceptable salt thereof, whereinR^1 is -C(O)O(C_4-C_{18})alkyl, -C(O)O(C_4-C_{12})alkyl, or -C(O)O(C_4-C_{10})alkyl; and wherein the alkyl is a branched alkyl.

18. The lipid of claim 17, or a pharmaceutically acceptable salt thereof, wherein R^1 is -C(O)O(C_{17} alkyl).

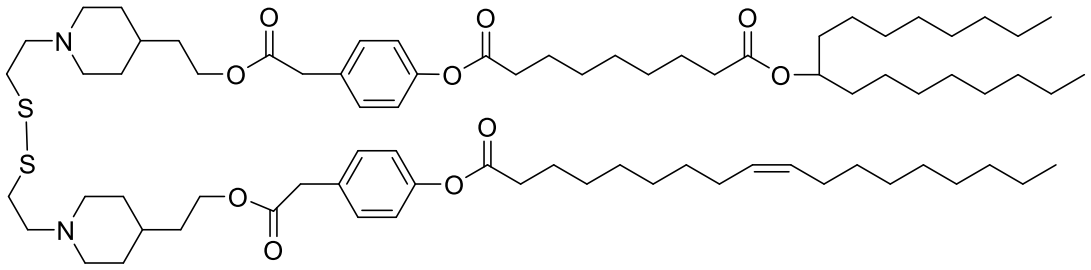
19. The lipid of any one of claims 1 to 10, or a pharmaceutically acceptable salt thereof, wherein R^1 is selected from the group consisting of:

 ,	 ,
 ,	 , and
 ;	

and/or wherein R^2 is selected from the group consisting of :

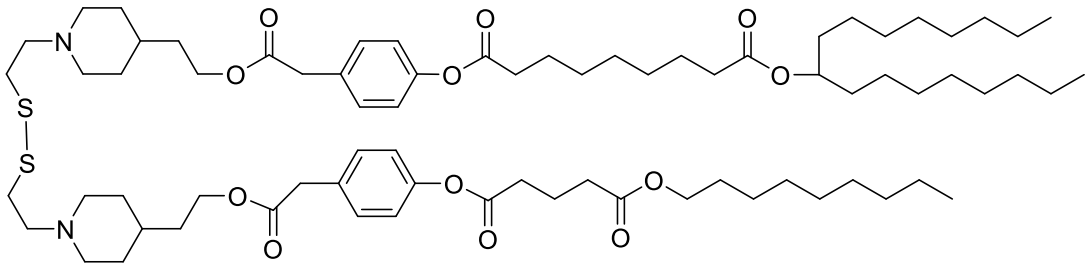
 ,	 ,
 ,	 , and
 .	

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



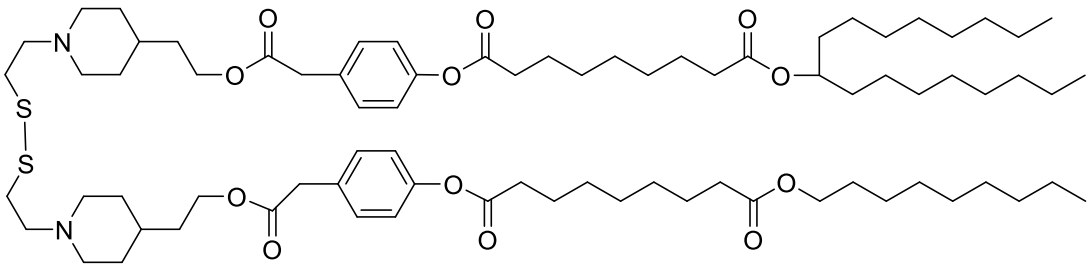
Lipid 1

1-(heptadecan-9-yl) 9-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-(oleoyloxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) nonanedioate,



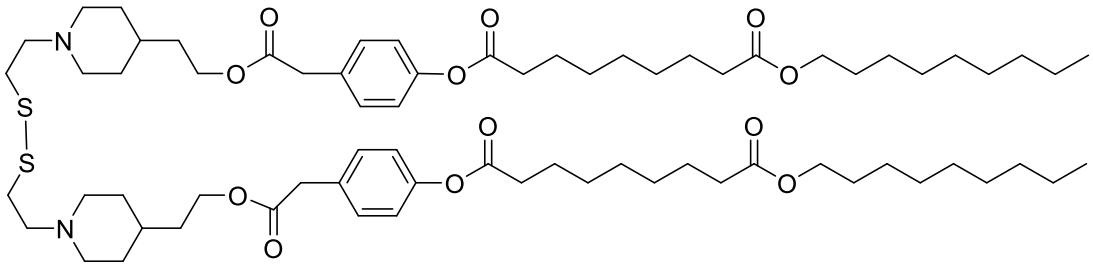
Lipid 2

1-(heptadecan-9-yl) 9-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-((5-(nonyloxy)-5-oxopentanoyl)oxy)phenyl)acetoxy)ethyl) piperidin-1-yl)ethyl)disulfaneyl)ethyl) piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) nonanedioate,



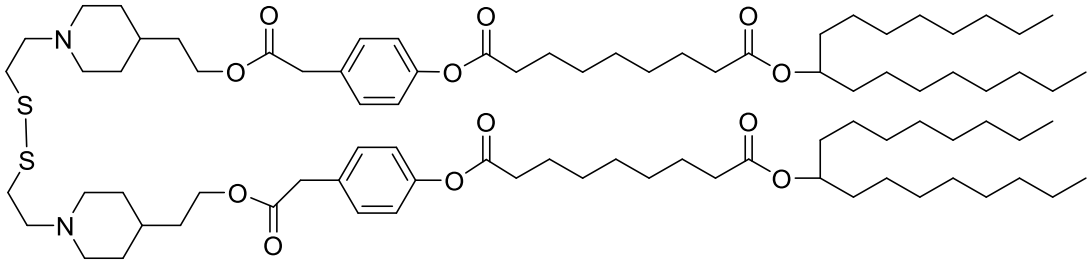
Lipid 3

1-(heptadecan-9-yl) 9-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-((9-(nonyloxy)-9-oxononanoyl)oxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) nonanedioate,



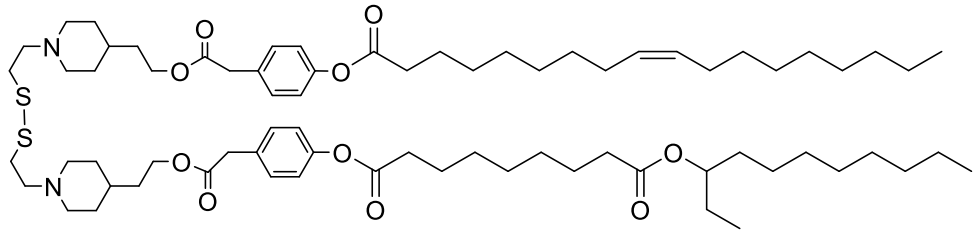
Lipid 4

1-(heptadecan-9-yl) 9-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-((5-(nonyloxy)-5-oxopentanoyl)oxy)phenyl)acetoxy)ethyl) piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) nonanedioate,



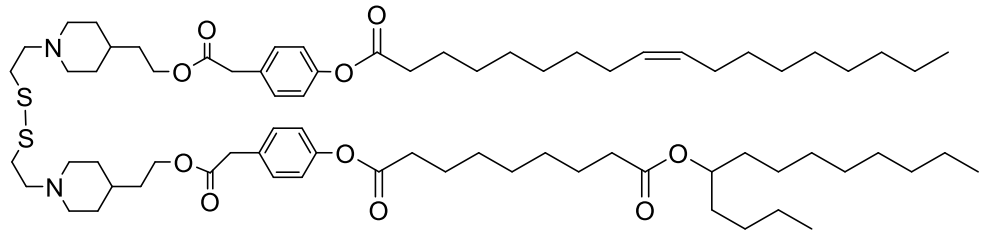
Lipid 5

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



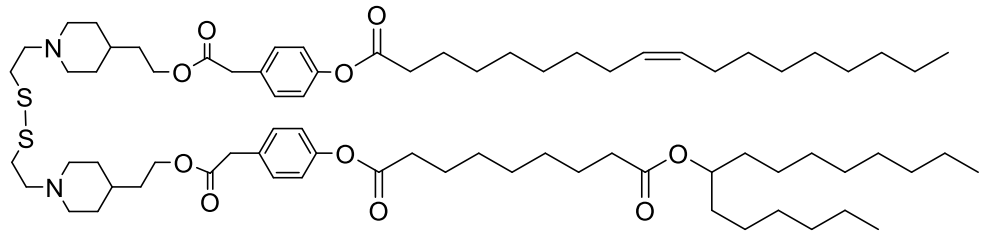
Lipid 6

1-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-(oleoyloxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) 9-(undecan-3-yl) nonanedioate,



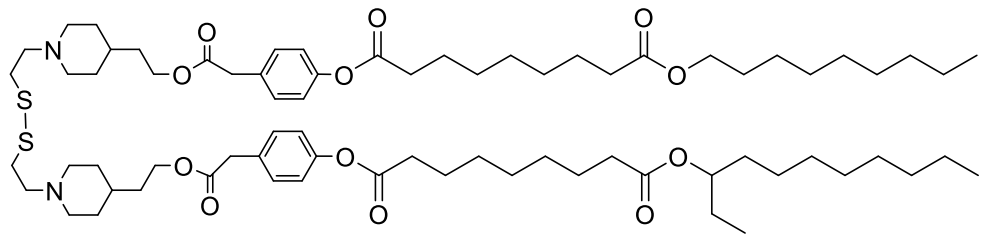
Lipid 7

1-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-(oleoyloxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) 9-(tridecan-5-yl) nonanedioate,



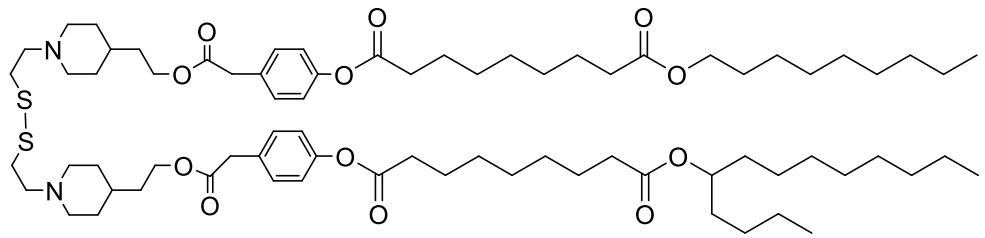
Lipid 8

1-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-(oleoyloxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) 9-(pentadecan-7-yl) nonanedioate,



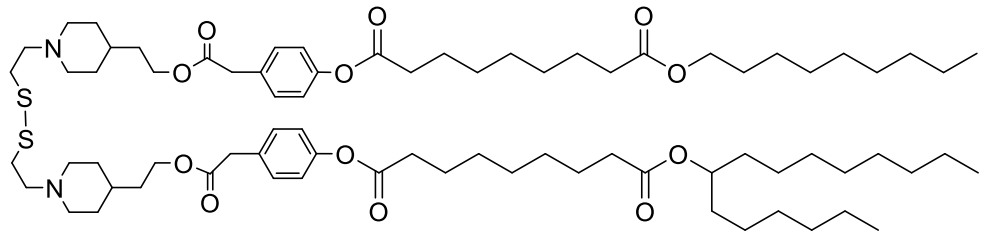
Lipid 9

1-nonyl 9-(4-(2-oxo-2-(2-(1-(2-((2-(4-(2-(2-(4-((9-oxo-9-(undecan-3-yloxy)nonanoyl)oxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)ethyl)phenyl) nonanedioate,



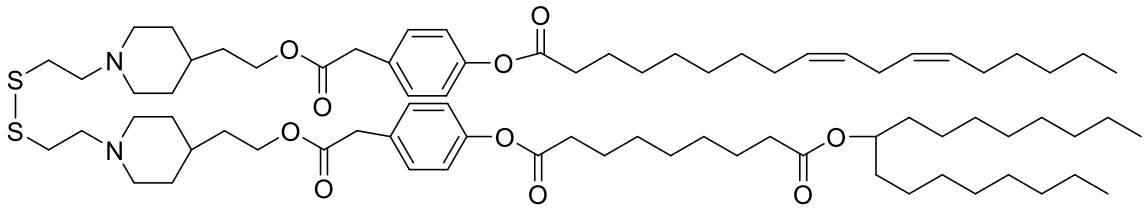
Lipid 10

1-nonyl 9-(4-(2-oxo-2-(2-(1-(2-((2-(4-(2-(2-(4-((9-oxo-9-(tridecan-5-yloxy)nonanoyl)oxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)ethyl)phenyl) nonanedioate,



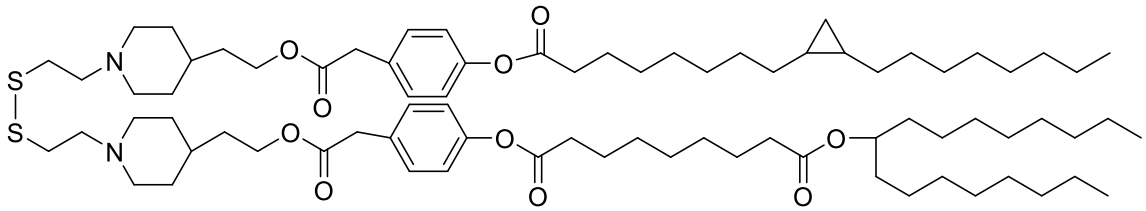
Lipid 11

1-nonyl 9-(4-(2-oxo-2-(2-(1-(2-((2-(4-(2-(2-(4-((9-oxo-9-(pentadecan-7-yloxy)nonanoyl)oxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)ethyl)phenyl) nonanedioate,



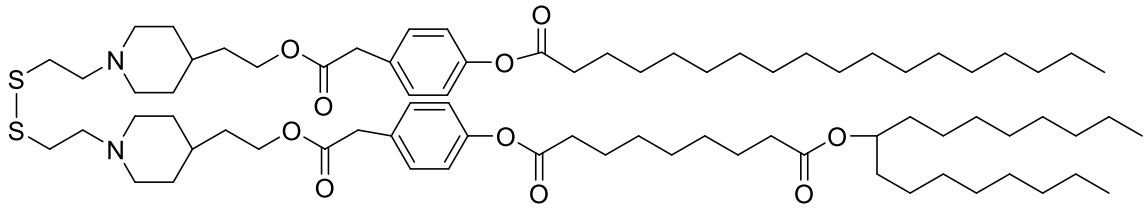
Lipid 12

1-(heptadecan-9-yl) 9-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-(((9Z,12Z)-octadeca-9,12-dienoyl)oxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) nonanedioate,



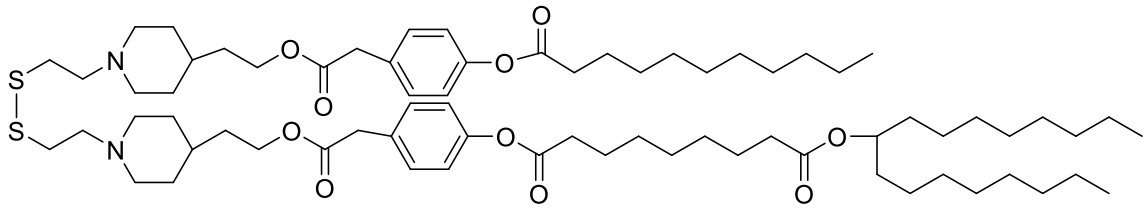
Lipid 13

1-(heptadecan-9-yl) 9-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-((8-(2-octylcyclopropyl)octanoyl)oxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) nonanedioate,



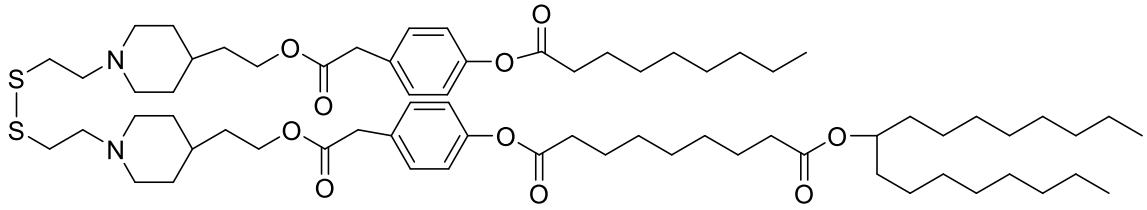
Lipid 14

1-(heptadecan-9-yl) 9-(4-(2-oxo-2-(2-(1-(2-((2-(4-(2-(2-(4-(stearoyloxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)ethyl)phenyl) nonanedioate,



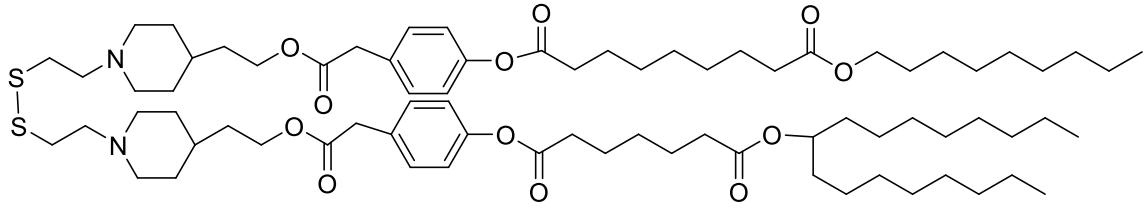
Lipid 15

1-(heptadecan-9-yl) 9-(4-(2-oxo-2-(2-(1-(2-((2-(4-(2-(2-(4-(undecanoyloxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)ethyl)phenyl) nonanedioate,



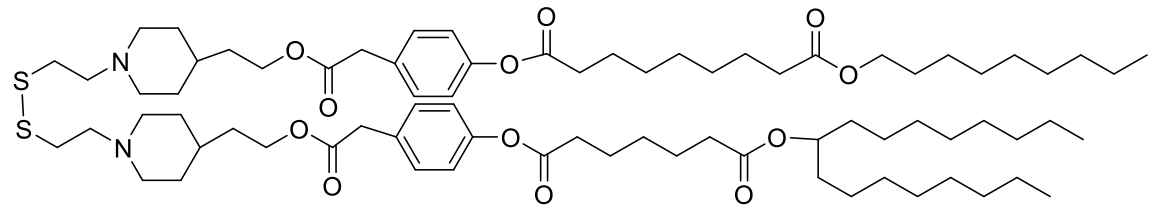
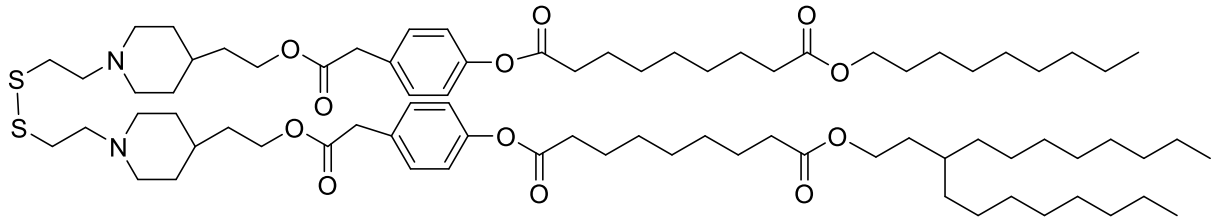
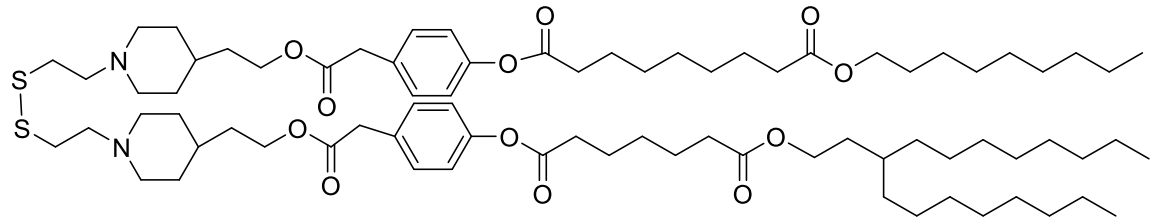
Lipid 16

1-(heptadecan-9-yl) 9-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-(nonanoyloxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) nonanedioate,



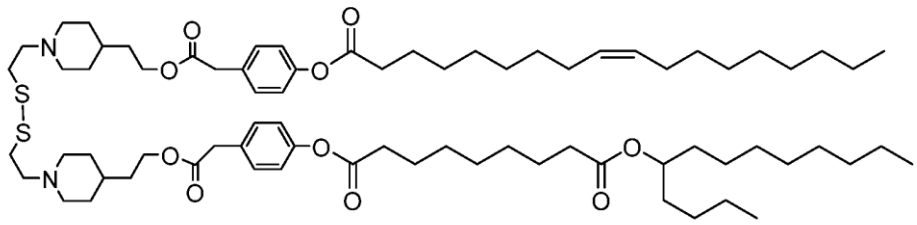
Lipid 17

1-nonyl 9-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-((9-((3-octylundecyl)oxy)-9-

oxononanoyl)oxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) nonanedioate,
 **Lipid 18** 1-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-((7-(heptadecan-9-yloxy)-7-oxoheptanoyl)oxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) 9-nonyl nonanedioate,
 **Lipid 19** 1-nonyl 9-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-((9-((3-octylundecyl)oxy)-9-oxononanoyl)oxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) nonanedioate, and
 **Lipid 20** 1-nonyl 9-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-((7-((3-octylundecyl)oxy)-7-oxoheptanoyl)oxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) nonanedioate,

or a pharmaceutically acceptable salt thereof; or

wherein the lipid is Lipid 7:



Lipid 7

1-(4-(2-(2-(1-(2-((2-(4-(2-(2-(4-(oleoyloxy)phenyl)acetoxy)ethyl)piperidin-1-yl)ethyl)disulfaneyl)ethyl)piperidin-4-yl)ethoxy)-2-oxoethyl)phenyl) 9-(tridecan-5-yl) nonanedioate , or

a pharmaceutically acceptable salt thereof.

21. A lipid nanoparticle (LNP) comprising the lipid of any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof; and a nucleic acid.

22. The lipid nanoparticle of claim 21 , 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.

23. The lipid nanoparticle of claim 21 or 22, further comprising at least one lipid selected from the group consisting of a sterol, a PEG-lipid conjugate, and a non-cationic lipid.

24. The lipid nanoparticle of any one of claims 21 to 23, wherein the lipid nanoparticle has a diameter ranging from 50 nm to 110 nm, or wherein the lipid nanoparticle is less than about 100 nm in diameter, less than about 70 nm in diameter or less than about 60 nm in diameter.

25. The lipid nanoparticle of any one of claims 21 to 24, further comprising a tissue specific targeting moiety.

26. The lipid nanoparticle of claim 25, wherein the tissue specific targeting moiety is a peptide or N-acetylgalactosamine (GalNAc).

27. The lipid nanoparticle of claim 26, wherein the GalNAc is linked to the PEG-lipid conjugate to form a GalNAc-linked PEG-lipid conjugate.

28. The lipid nanoparticle of claim 27,wherein the GalNAc-linked PEG-lipid conjugate is present in the nanoparticle at a molar percentage of about 1.5%, about 1.4%, about 1.3%, about 1.2%, about 1.1%, 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%.

29. A pharmaceutical composition comprising the lipid of any one of claims 1 to 20 or the lipid nanoparticle of any one of claims 21 to 28 and a pharmaceutically acceptable excipient.

30. Use of the lipid nanoparticle of any one of claims 21 to 28, or the pharmaceutical composition of claim 29 in the manufacture of a medicament for treating a genetic disorder in a subject in need thereof.

31. The use of claim 30, wherein the subject is a human.

32. The use of claim 30 or 31, 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, 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 dystrophies

(BMD), dystrophic epidermolysis bullosa (DEB), ectonucleotide pyrophosphatase 1 deficiency, generalized arterial calcification of infancy (GACI), Leber Congenital Amaurosis, Stargardt macular dystrophy (ABCA4 deficiency), ornithine transcarbamylase (OTC) deficiency, age-related macular degeneration, Usher syndrome, alpha-1 antitrypsin deficiency, progressive familial intrahepatic cholestasis (PFIC) type I (ATP8B1 deficiency), progressive familial intrahepatic cholestasis (PFIC) type II (ABCB11 deficiency), progressive familial intrahepatic cholestasis (PFIC) type III (ABCB4 deficiency), progressive familial intrahepatic cholestasis (PFIC) type IV (TJP2 deficiency), and Cathepsin A deficiency.

33. The use of claim 30 or 31, wherein the genetic disorder is selected from the group consisting of hemophilia A (clotting factor VIII (FVIII) deficiency), hemophilia B (clotting factor IX (FIX) deficiency), Wilson disease, phenylketonuria (PKU), retinitis pigmentosa, Hunter syndrome (MPS II), Glycogen Storage disease types I, Glycogen Storage disease Type II (Pompe disease), Gaucher disease Type I, Gaucher disease Type II, Gaucher disease Type III, Leber Congenital Amaurosis (e.g., LCA10), Stargardt macular dystrophy (ABCA4 deficiency), ornithine transcarbamylase (OTC) deficiency, age-related macular degeneration, Usher syndrome, alpha-1 antitrypsin deficiency, and progressive familial intrahepatic cholestasis (PFIC) type I (ATP8B1 deficiency).

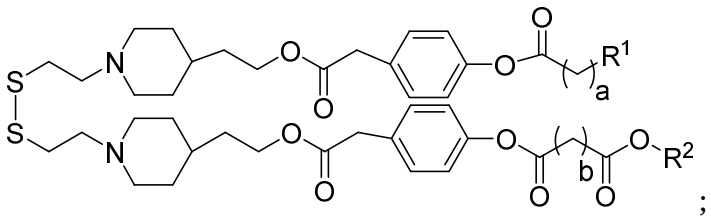
34. The use of claim 32, wherein the mucopolysaccharide storage disease is selected from the group consisting of Hurler syndrome (MPS I), Scheie syndrome (MPS I S), Hurler-Scheie syndrome (MPS I H-S), Hunter syndrome (MPS II), Sanfilippo syndrome Type A (MPS IIIA), Sanfilippo syndrome Type B (MPS IIIB), Sanfilippo syndrome Type C (MPS IIIC), Sanfilippo syndrome Type D (MPS IIID), Morquio syndrome Type A (MPS IVA), Morquio syndrome Type B (MPS IVB), Maroteaux-Lamy syndrome (MPS VI), Sly syndrome (MPS VII) and hyaluronidase deficiency (MPS IX).

35. The lipid nanoparticle of any one of claims 21 to 28, or the pharmaceutical composition of claim 29 for use in therapy.

Some widely used ionizable lipids are CLinDMA, DLinDMA (also known as DODAP), and cationic lipid such as DOTAP. Of note, these lipids have been employed for siRNA delivery to liver but suffer from non-optimal delivery efficiency along with liver 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

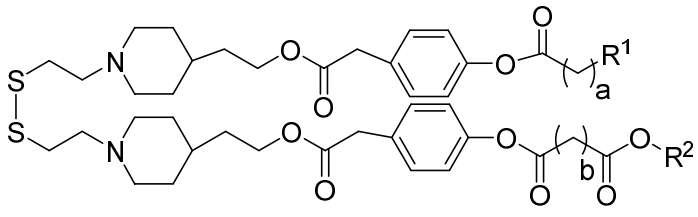
In one example, provided herein are ionizable lipids having the Formula (**I**):



;

as well as pharmaceutically acceptable salts thereof, wherein R^1, R^2, a, and b are as defined herein.

In one aspect, the present disclosure refers to a lipid represented by the Formula (**I**):



(**I**);

or a pharmaceutically acceptable salt thereof, wherein:

a is an integer ranging from 1 to 20;

b is an integer ranging from 2 to 10;

R^1 is selected from the group consisting of $(C_2\text{-}C_{20})$alkenyl, $-C(O)O(C_2\text{-}C_{20})$alkyl, and cyclopropyl substituted with $(C_2\text{-}C_{20})$alkyl; and

R^2 is $(C_2\text{-}C_{20})$alkyl.

Also provided are pharmaceutical compositions comprising a disclosed ionizable lipid, or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure refers to a lipid nanoparticle (LNP) comprising the lipid as disclosed herein, or a pharmaceutically acceptable salt thereof; and a nucleic acid.

According to some examples of any of the aspects or examples herein, the ITR is an ITR derived from an AAV serotype, derived from an ITR of goose virus, derived from a B19 virus ITR, a wild-type ITR from a parvovirus. According to some examples of any of the aspects or examples herein, the AAV serotype is selected from the group comprising of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12.

According to some examples of any of the aspects or examples herein, the ITR is a mutant ITR, and the ceDNA optionally comprises an additional ITR which differs from the first ITR. According to some examples of any of the aspects or examples herein, the ceDNA comprises two mutant ITRs in both 5' and 3' ends of the expression cassette, optionally wherein the two mutant ITRs are symmetric mutants. According to some examples of any of the aspects or examples herein, the ceDNA is a CELiD, DNA-based minicircle, a MIDGE, a ministering DNA, a dumbbell shaped linear duplex closed-ended DNA comprising two hairpin structures of ITRs in the 5' and 3' ends of an expression cassette, or a doggybone™ DNA. According to some examples of any of the aspects or examples herein, the pharmaceutical composition further comprises a pharmaceutically acceptable excipient.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising the lipid as disclosed herein or the lipid nanoparticle as disclosed herein and a pharmaceutically acceptable excipient. In yet another aspect, the present disclosure refers to the use of the lipid nanoparticle as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a genetic disorder in a subject in need thereof. In yet another aspect, the present disclosure refers to the lipid nanoparticle as disclosed herein, or the pharmaceutical composition as disclosed herein for use in therapy.

According to some examples, the disclosure provides a method of treating a genetic disorder in a subject, the method comprising administering to the subject an effective amount of the pharmaceutical composition according to any of the aspects or examples herein. According to some examples of any of the aspects or examples herein, the subject is a human. According to some examples of any of the aspects or examples herein, 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,