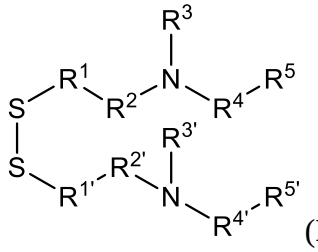
with reduced toxicity, but with improved pharmacokinetics and intracellular kinetics such as cellular uptake and nucleic acid release from the lipid carrier.

SUMMARY

In one aspect of the present disclosure, there is provided an ionizable lipid of the Formula (**I**):



(**I**);

or a pharmaceutically acceptable salt thereof, wherein:

R^1 and $R^{1'}$ are each independently (C_1-C_6)alkylene optionally substituted with one or more groups selected from R^a;

R^2 and $R^{2'}$ are each independently (C_1-C_2)alkylene;

R^3 and $R^{3'}$ are each independently (C_1-C_6)alkyl optionally substituted with one or more groups selected from R^b;

or alternatively, R^2 and R^3 and/or $R^{2'}$ and $R^{3'}$ are taken together with their intervening N atom to form a 4- to 7-membered heterocyclyl;

R^4 and $R^{4'}$ are each independently a (C_2-C_6)alkylene interrupted by –C(O)O-;

R^5 and $R^{5'}$ are each independently a (C_2-C_{30})alkyl or (C_2-C_{30})alkenyl, each of which are optionally interrupted with –C(O)O- or (C_3-C_6)cycloalkyl; and

R^a and R^b are each halo or cyano.

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.

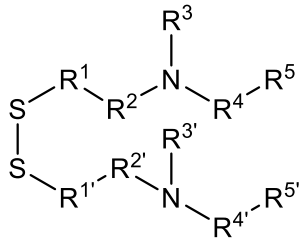
In a further aspect of the present disclosure, there is provided a pharmaceutical composition comprising the lipid nanoparticle disclosed herein or the ionizable lipid

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 genetic disorder in a subject; optionally wherein the subject is a human; optionally 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, mucopolysaccharide storage diseases (*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, 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, 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 (LCA), Stargardt macular dystrophy (ABCA4), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, 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.

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

73%, about 72%, about 71%, about 70%, about 69%, about 68%, about 67%, about 66%, about 65%, about 64%, about 63%, about 62%, about 61%, or about 60%. According to some examples, the cholesterol is present at a molar percentage of about 40%, and wherein the ionizable lipid is present at a molar percentage of about 50%. According to some examples herein, the composition further comprises a cholesterol, a PEGylated lipid, and a non-cationic lipid. According to some examples, the PEGylated lipid is present at a molar percentage of about 1.5% to about 4% or about 1.5% to about 3%, for example about 1.5% to about 2.75%, about 1.5% to about 2.5%, about 1.5% to about 2.25%, about 1.5% to about 2%, about 2% to about 3%, about 2% to about 2.75%, about 2% to about 2.5%, about 2% to about 2.25%, about 2.25% to about 3%, about 2.25% to about 2.75%, or about 2.25% to about 2.5%. According to some examples, the PEGylated lipid is present at a molar percentage of about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, or about 3%. According to some examples, the cholesterol is present at a molar percentage of about 30% to about 50%, for example about 30% to about 45%, about 30% to about 40%, about 30% to about 35%, about 35% to about 50%, about 35% to about 45%, about 35% to about 40%, about 20% to about 40%, about 40% to about 50%, or about 45% to about 50%. According to some examples, the cholesterol is present at a molar percentage of about 30%, about 31%, about 32%, about 33%, about 34%, about 35%, about 36%, about 37%, about 38%, about 39%, about 40%, about 41%, about 42%, about 43%, about 44%, about 45%, about 46%, about 47%, about 48%, about 49%, or about 50%.

According to some examples, the PEGylated lipid in a composition described herein is l-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG). According to some examples herein, the LNP further comprises a non-cationic lipid. According to some examples, the non-cationic lipid is selected from the group consisting of distearoyl-sn-glycero-phosphoethanolamine, 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, or mixtures thereof. According to some examples, the non-cationic lipid is selected from the group consisting of dioleoylphosphatidylcholine (DOPC), distearoylphosphatidylcholine (DSPC), and dioleoyl-phosphatidylethanolamine (DOPE).

According to some examples, the ionizable lipid is present at a molar percentage of about 42.5% to about 62.5%. According to some examples, the ionizable lipid is present at a molar percentage of about 42.5%, about 43%, about 43.5%, about 44%, about 44.5%, about 45%, about 45.5%, about 46%, about 46.5%, about 47%, about 47.5%, about 48%, about 48.5%, about 49%, about 49.5%, about 50%, about 50.5%, about 51%, 51.5%, about 52%, about 52.5%, about 53%, about 53.5%, about 54%, about 54.5%, about 55%, about 55.5%, about 56%, about 56.5%, about 57%, 57.5%, about 58%, about 58.5%, about 59%, about 59.5%, about 60%, about 60.5%, about 61%, about 61.5%, about 62%, or about 62.5%. According to some examples herein, the non-cationic lipid is present at a molar percentage of about 2.5% to about 12.5%. According to some examples herein, the cholesterol is present at a molar percentage of about 40%, the ionizable lipid is present at a molar percentage of about 52.5%, the non-cationic lipid is present at a molar percentage of about 7.5%, and wherein the PEGylated lipid is present at a molar percentage of about 3%.

According to some examples herein, the LNP further comprises a tissue-specific targeting ligand. The tissue targeting moiety can be a peptide, oligosaccharide or the like, which can be used for the delivery of the LNP to one or more specific tissues such as cancer, the liver, the CNS, or the muscle. According to some examples, the tissue-specific targeting ligand is a ligand for liver specific receptors.

According to one example, the ligand of liver specific receptors used for liver targeting is an oligosaccharide such as N-Acetylgalactosamine (GalNAc) or a GalNAc derivative such as mono-, bi-, tri-, or tetra-antennary GalNAc. According to some examples herein, the tissue-specific targeting ligand is conjugated to a PEGylated lipid. According to some examples, the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the lipid nanoparticle at a molar percentage of 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%. According to some examples, the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the LNP at a molar percentage of 0.2%. According to some examples, the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the LNP at a molar percentage of 0.3%. According to some examples, the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the LNP at a molar percentage of 0.4%. According to some examples, the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the LNP at a molar percentage of 0.5%. According to some examples, the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the LNP at a molar percentage of 0.6%. According to some examples, the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the LNP at a molar percentage of 0.7%. According to some examples, the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the LNP at a molar percentage of 0.8%. According to some examples, the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the LNP at a molar percentage of 0.9%. According to some examples, the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the LNP at a molar percentage of 1.0%. According to some examples, the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the LNP at a molar percentage of about 1.5%. According to some examples, the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the LNP at a molar percentage of 2.0%.

According to some examples herein, the LNP composition further comprises dexamethasone palmitate.

According to some examples herein, the LNP is in a size ranging from about 50 nm to about 110 nm in mean diameter, for example about 50 nm to about 100 nm, about 50 nm to about 95 nm, about 50 nm to about 90 nm,

about 50 nm to about 85 nm, about 50 nm to about 80 nm, about 50 nm to about 75 nm, about 50 nm to about 70 nm, about 50 nm to about 65 nm, about 50 nm to about 60 nm, about 50 nm to about 55 nm, about 60 nm to about 110 nm, about 60 nm to about 100 nm, about 60 nm to about 95 nm, about 60 nm to about 90 nm, about 60 nm to about 85 nm, about 60 nm to about 80 nm, about 60 nm to about 75 nm, about 60 nm to about 70 nm, about 60 nm to about 65 nm, about 70 nm to about 110 nm, about 70 nm to about 100 nm, about 70 nm to about 95 nm, about 70 nm to about 90 nm, about 70 nm to about 85 nm, about 70 nm to about 80 nm, about 70 nm to about 75 nm, about 80 nm to about 110 nm, about 80 nm to about 100 nm, about 80 nm to about 95 nm, about 80 nm to about 90 nm, about 80 nm to about 85 nm, about 90 nm to about 110 nm, or about 90 nm to about 100 nm. According to some examples herein, the LNP is less than about 100 nm in mean size, for example less than about 105 nm, less than about 100 nm, less than about 95 nm, less than about 90 nm, less than about 85 nm, less than about 80 nm, less than about 75 nm, less than about 70 nm, less than about 65 nm, less than about 60 nm, less than about 55 nm, less than about 50 nm, less than about 45 nm, less than about 40 nm, less than about 35 nm, less than about 30 nm, less than about 25 nm, less than about 20 nm, less than about 15 nm, or less than about 10 nm in size. According to some examples, the LNP is less than about 70 nm in mean size., for example less than about 65 nm, less than about 60 nm, less than about 55 nm, less than about 50 nm, less than about 45 nm, less than about 40 nm, less than about 35 nm, less than about 30 nm, less than about 25 nm, less than about 20 nm, less than about 15 nm, or less than about 10 nm in size. According to some examples, the LNP is less than about 60 nm in mean size, for example less than about 55 nm, less than about 50 nm, less than about 45 nm, less than about 40 nm, less than about 35 nm, less than about 30 nm, less than about 25 nm, less than about 20 nm, less than about 15 nm, or less than about 10 nm in size. According to some examples herein, the LNP composition has a total lipid to nucleic acid ratio of about 10:1. According to some examples herein, the LNP composition has a total lipid to nucleic acid ratio of about 20:1. According to some examples herein, the composition has a total lipid to nucleic acid ratio of about 30:1. According to some examples herein, the composition has a total lipid to nucleic acid ratio of about 40:1. According to some examples herein, the composition has a total lipid to nucleic acid ratio of about 50:1.

According to some examples herein, the LNP composition is prepared in a buffer such as malic acid. In some examples, the composition is prepared in about 10 mM to about 30 mM malic acid, for example about 10 mM to about 25 mM, about 10 mM to about 20 mM, about 10 mM to about 15 mM, about 15 mM to about 25 mM, about 15 mM to about 20 mM, about 20 mM to about 25 mM. According to some examples herein, the composition is prepared in about 10 mM malic acid, about 11 mM malic acid, about 12 mM malic acid, about 13 mM malic acid, about 14 mM malic acid, about 15 mM malic acid, about 16 mM malic acid, about 17 mM malic acid, about 18 mM malic acid, about 19 mM malic acid, about 20 mM malic acid, about 21 mM malic acid, about 22 mM malic acid, about 23 mM malic acid, about 24 mM malic acid, about 25 mM malic acid, about 26 mM malic acid, about 27 mM malic acid, about 28 mM malic acid, about 29 mM malic acid, or about 30 mM malic acid. According to some examples, the composition comprises about 20 mM malic acid.

According to some examples herein, the LNP composition is prepared in a solution having about 30 mM to about 50 mM NaCl, for example about 30 mM to about 45 mM NaCl, about 30 mM to about 40 mM NaCl, about 30 mM to about 35 mM NaCl, about 35 mM to about 45 mM NaCl, about 35 mM to about 40 mM NaCl, or about 40 mM to about 45 mM NaCl. According to some examples herein, the LNP composition is prepared in a solution having about 30 mM NaCl, about 35 mM NaCl, about 40 mM NaCl, or about 45 mM NaCl. According to some examples, the LNP composition is prepared in a solution having about 40 mM NaCl.

According to some examples, the LNP composition is prepared in a solution having about 20 mM to about 100 mM $MgCl_2$, for example about 20 mM to about 90 mM $MgCl_2$, about 20 mM to about 80 mM $MgCl_2$, about 20 mM to about 70 mM $MgCl_2$, about 20 mM to about 60 mM $MgCl_2$, about 20 mM to about 50 mM $MgCl_2$, about 20 mM to about 40 mM $MgCl_2$, about 20 mM to about 30 mM $MgCl_2$, about 320 mM to about 90 mM $MgCl_2$, about 30 mM to about 80 mM $MgCl_2$, about 30 mM to about 70 mM $MgCl_2$, about 30 mM to about 60 mM $MgCl_2$, about 30 mM to about 50 mM $MgCl_2$, about 30 mM to about 40 mM $MgCl_2$, about 40 mM to about 90 mM $MgCl_2$, about 40 mM to about 80 mM $MgCl_2$, about 40 mM to about 70 mM $MgCl_2$, about 40 mM to about 60 mM $MgCl_2$, about 40 mM to about 50 mM $MgCl_2$, about 50 mM to about 90 mM $MgCl_2$, about 50 mM to about 80 mM $MgCl_2$, about 50 mM to about 70 mM $MgCl_2$, about 50 mM to about 60 mM $MgCl_2$, about 60 mM to

about 90 mM $MgCl_2$, about 60 mM to about 80 mM $MgCl_2$, about 60 mM to about 70 mM $MgCl_2$, about 70 mM to about 90 mM $MgCl_2$, about 70 mM to about 80 mM $MgCl_2$, or about 80 mM to about 90 mM $MgCl_2$.

According to some examples herein, the ceDNA is closed-ended linear duplex DNA. According to some examples herein, the ceDNA comprises an expression cassette comprising a promoter sequence and a transgene.

According to some examples, the ceDNA comprises expression cassette comprising a polyadenylation sequence.

According to some examples herein, the ceDNA comprises at least one inverted terminal repeat (ITR) flanking either 5' or 3' end of said expression cassette. According to some examples, the expression cassette is flanked by two ITRs, wherein the two ITRs comprise one 5' ITR and one 3' ITR. According to some examples, the expression cassette is connected to an ITR at 3' end (3' ITR). According to some examples, the expression cassette is connected to an ITR at 5' end (5' ITR). According to some examples, at least one of 5' ITR and 3' ITR is a wild-type AAV ITR. According to some examples, at least one of 5' ITR and 3' ITR is a modified ITR. According to some examples, the ceDNA further comprises a spacer sequence between a 5' ITR and the expression cassette.

According to some examples, the ceDNA further comprises a spacer sequence between a 3' ITR and the expression cassette. According to some examples, the spacer sequence is at least 5 base pairs long in length. According to some examples, the spacer sequence is 5 to 100 base pairs long in length. According to some examples, the spacer sequence is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 base pairs long in length. According to some examples, the spacer sequence is 5 to 500 base pairs long in length. According to some examples, the spacer sequence is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, or 495 base pairs long in length.

According to some examples herein, the ceDNA has a nick or a gap.

According to some examples, 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, 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, the ITR is a mutant ITR, and the ceDNA optionally comprises an additional ITR which differs from the first ITR. According to some examples, 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 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 herein, the pharmaceutical composition further comprises a pharmaceutically acceptable excipient.

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 examples herein. According to some examples, the subject is a human. According to some examples, 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, mucopolysaccharide storage diseases (*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, 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, 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 (LCA), Stargardt macular dystrophy (ABCA4), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, 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. According to some examples, the genetic disorder is Leber congenital amaurosis (LCA). According to some examples, the LCA is LCA10. According to some examples, the genetic disorder is Niemann-Pick disease. According to some examples, the genetic disorder is Stargardt macular dystrophy. According to some examples, the genetic disorder is glucose-6-phosphatase (G6Pase) deficiency (glycogen storage disease type I) or Pompe disease (glycogen storage disease type II). According to some examples, the genetic disorder is hemophilia A (Factor VIII deficiency). According to some examples, the genetic disorder is hemophilia B (Factor IX deficiency). According to some examples, the genetic disorder is hunter syndrome (Mucopolysaccharidosis II). According to some examples, the genetic disorder is cystic fibrosis. According to some examples, the genetic disorder is Usher syndrome. According to some examples, the genetic disorder is dystrophic epidermolysis bullosa (DEB). According to some examples, the genetic disorder is phenylketonuria (PKU). According to some examples, the genetic disorder is progressive familial intrahepatic cholestasis (PFIC). According to some examples, the genetic disorder is Wilson disease. According to some examples, the genetic disorder is Gaucher disease Type I, II or III.

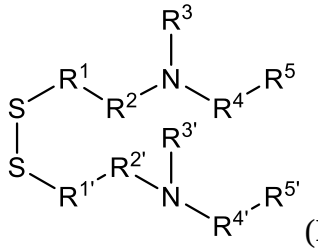
BRIEF DESCRIPTION OF DRAWING

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 drawing. However, the appended drawing illustrates 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.

CLAIMS

What is Claimed is:

1. An ionizable lipid of the Formula (**I**):



(**I**);

or a pharmaceutically acceptable salt thereof, wherein:

R^1 and $R^{1'}$ are each independently $(C_1\text{-}C_6)$alkylene optionally substituted with one or more groups selected from R^a;

R^2 and $R^{2'}$ are each independently $(C_1\text{-}C_2)$alkylene;

R^3 and $R^{3'}$ are each independently $(C_1\text{-}C_6)$alkyl optionally substituted with one or more groups selected from R^b;

or alternatively, R^2 and R^3 and/or $R^{2'}$ and $R^{3'}$ are taken together with their intervening N atom to form a 4- to 7-membered heterocyclyl;

R^4 and $R^{4'}$ are each independently a $(C_2\text{-}C_6)$alkylene interrupted by –C(O)O-;

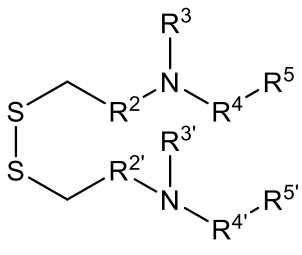
R^5 and $R^{5'}$ are each independently a $(C_2\text{-}C_{30})$alkyl or $(C_2\text{-}C_{30})$alkenyl, each of which are optionally interrupted with –C(O)O- or $(C_3\text{-}C_6)$cycloalkyl; and

R^a and R^b are each halo or cyano.

2. The ionizable lipid of claim 1, or a pharmaceutically acceptable salt thereof, wherein R^1 and $R^{1'}$ are each independently $(C_1\text{-}C_6)$alkylene.

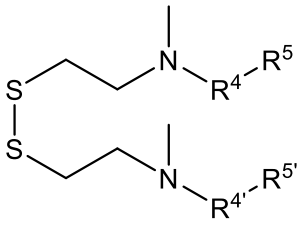
3. The ionizable lipid of claim 1 or 2, or a pharmaceutically acceptable salt thereof, wherein R^1 and $R^{1'}$ are each independently $(C_1\text{-}C_3)$alkylene.

4. The ionizable lipid of any one of claims 1 to 3, wherein the lipid is of the Formula (**II**):

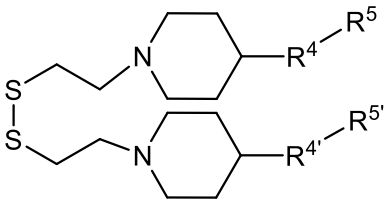


(**II**);

or a pharmaceutically acceptable salt thereof; or wherein the lipid is of the Formula (**III**) or (**IV**):

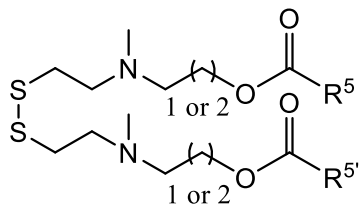


(**III**); or

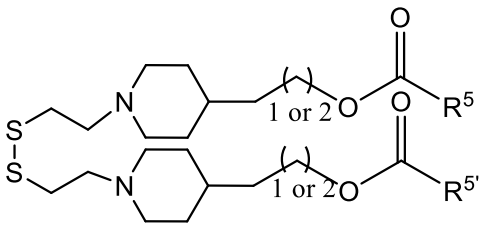


(**IV**);

or a pharmaceutically acceptable salt thereof; or wherein the lipid is of the Formula (**V**) or (**VI**):

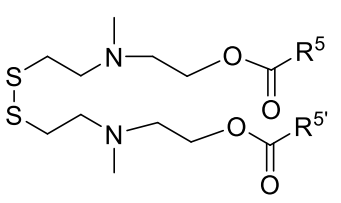
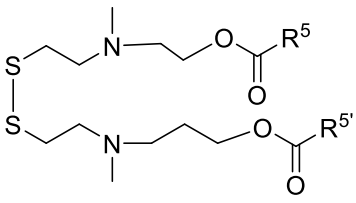
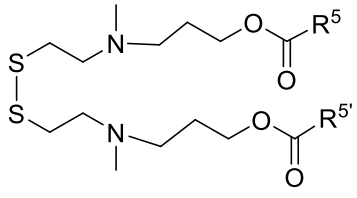
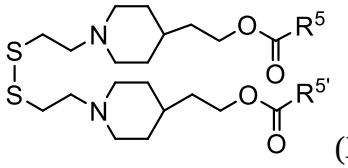


(**V**); or



(**VI**);

or a pharmaceutically acceptable salt thereof; or wherein the lipid is of the Formula (**VII**) or (**VIII**):


(**VII**);

(**VIII**);

(**IX**); or

(**X**);

or a pharmaceutically acceptable salt thereof.

5. The ionizable lipid of any one of claims 1 to 4, or a pharmaceutically acceptable salt thereof, wherein R^5 is a (C_6-C_{26})alkyl or (C_6-C_{26})alkenyl, each of which are optionally interrupted with –C(O)O- or (C_3-C_6)cycloalkyl; or wherein R^5 is a (C_6-C_{24})alkyl or (C_6-C_{24})alkenyl, each of which are optionally interrupted with –C(O)O- or cyclopropyl; or wherein R^5 is a (C_8-C_{24})alkyl or (C_8-C_{24})alkenyl, wherein said (C_8-C_{24})alkyl is optionally interrupted with –C(O)O- or cyclopropyl; or wherein R^5 is a (C_8-C_{10})alkyl; or wherein R^5 is a (C_{14}-C_{16})alkyl interrupted with cyclopropyl; or wherein R^5 is a (C_{10}-C_{24})alkyl interrupted with –C(O)O-; or wherein R^5 is a (C_{16}-C_{18})alkenyl; or wherein R^5 is $–(CH_2)_3C(O)O(CH_2)_8CH_3$, $–(CH_2)_5C(O)O(CH_2)_8CH_3$, $–(CH_2)_7C(O)O(CH_2)_8CH_3$, $–(CH_2)_7C(O)OCH[(CH_2)_7CH_3]_2$, $–(CH_2)_7$-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 $–(CH_2)_7CH=CHCH_2CH=CH(CH_2)_4CH_3$.

6. The ionizable lipid of any one of claims 1 to 5, or a pharmaceutically acceptable salt thereof, wherein $R^{5'}$ is a (C_{15}-C_{28})alkyl interrupted with –C(O)O-; or wherein $R^{5'}$ is a (C_{20}-C_{26})alkyl interrupted with –C(O)O-; or wherein $R^{5'}$ is a (C_{22}-C_{24})alkyl interrupted with –C(O)O-; or wherein $R^{5'}$ is $–(CH_2)_5C(O)OCH[(CH_2)_7CH_3]_2$, $–(CH_2)_7C(O)OCH[(CH_2)_7CH_3]_2$, $–(CH_2)_5C(O)OCH(CH_2)_2[(CH_2)_7CH_3]_2$, or $–(CH_2)_7C(O)OCH(CH_2)_2[(CH_2)_7CH_3]_2$.

7. A lipid nanoparticle (LNP) comprising the ionizable lipid of any one of claims 1 to 6, or a pharmaceutically acceptable salt thereof; and a nucleic acid.

8. The lipid nanoparticle of claim 7, wherein the nucleic acid is selected from the group consisting of minigenes, plasmids, minicircles, small interfering RNA (siRNA), 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.

9. The lipid nanoparticle of claim 7 or 8, further comprising at least one lipid selected from the group consisting of a sterol, a PEGylated lipid and a non-cationic lipid.

10. The lipid nanoparticle of claim 9, wherein the PEGylated lipid is present at a molar percentage of about 4%, and/or

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

wherein the sterol is present at a molar percentage of about 40% and wherein the ionizable lipid is present at a molar percentage of about 50%; or

wherein the non-cationic lipid is present at a molar percentage of about 2.5% to about 12.5%.

11. The lipid nanoparticle of any one of claims 7 to 10, further comprising a cholesterol, a PEGylated lipid, and a non-cationic lipid.

12. The lipid nanoparticle of any one of claims 7 to 11, further comprising a tissue-specific targeting ligand.

13. The lipid nanoparticle of claim 12, wherein the tissue-specific targeting ligand is conjugated to a PEGylated lipid and is N-acetylgalactosamine (GalNAc) or a derivative thereof selected from mono-antennary GalNAc, tri-antennary GalNAc, and tetra-antennary GalNAc; optionally wherein the PEGylated lipid having the tissue-specific targeting ligand conjugated thereto is present in the particle 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%.

14. The lipid nanoparticle of any one of claims 7 to 13, wherein the nanoparticle has a mean diameter ranging from about 50 nm to about 110 nm; or wherein the nanoparticle is less than about 100 nm in size, or less than about 75 nm in size, or less than about 70 nm in size, or less than about 65 nm in size.

15. A pharmaceutical composition comprising the lipid nanoparticle of any one of claims 7 to 14 or the ionizable lipid of any one of claims 1 to 6 or a pharmaceutically acceptable salt thereof; and a pharmaceutically acceptable excipient.

16. Use of the lipid nanoparticle of any one of claims 7 to 14, or the pharmaceutical composition according to claim 15, in the manufacture of a medicament for treating a genetic disorder in a subject; optionally wherein the subject is a human; optionally 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, mucopolysaccharide storage diseases (*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, 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, 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 (LCA), Stargardt macular dystrophy (ABCA4), ornithine transcarbamylase (OTC) deficiency, Usher syndrome, 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.

17. The lipid nanoparticle of any one of claims 7 to 14, or the pharmaceutical composition according to claim 15, for use in therapy.