COMPOSITIONS AND METHODS FOR TARGETED RNA DELIVERY

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 63/078,982 filed on September 16, 2020 and U.S. Provisional Application No. 62/984,866 filed on March 4, 2020, which are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 3, 2021, is named 53989706601_SL.txt and is 67,230 bytes in size.

FIELD OF THE DISCLOSURE

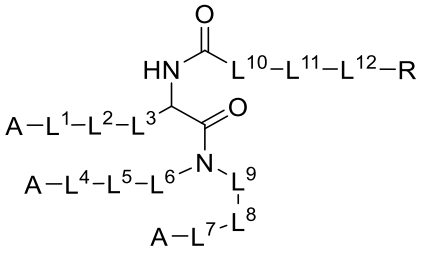
[0003] The instant disclosure relates to compositions and methods for targeted delivery of therapeutic agents such as CRISPR-guide RNA and other nucleic acid agents.

BACKGROUND

[0004] All publications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The following description includes information that may be useful in understanding the present disclosure. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

SUMMARY

[0004a] In an aspect of the present disclosure, there is provided a receptor targeting conjugate, comprising a compound of Formula (V):



Formula (V),

or a stereoisomer or salt thereof, wherein,

a plurality of the A groups collectively comprising a receptor targeting ligand;

each of L^1, L^2, L^3, L^4, L^5, L^6, L^7, L^8, L^9, L^{10} and L^{12} is independently substituted or unsubstituted C_1-C_{12} alkylene, substituted or unsubstituted C_1-C_{12} heteroalkylene, substituted or unsubstituted C_2-C_{12} alkenylene, substituted or unsubstituted C_2-C_{12} alkynylene, $-(CH_2CH_2O)_m-$, $-(OCH_2CH_2)_m-$, -O-, -S-, -S(=O)-, $-S(=O)_2-$, $-S(=O)(=NR^1)-$, -C(=O)-, $-C(=N-OR^1)-$, -C(=O)O-, -OC(=O)-, -C(=O)C(=O)-, $-C(=O)NR^1-$, $-NR^1C(=O)-$, $-OC(=O)NR^1-$, $-NR^1C(=O)O-$, $-NR^1C(=O)NR^1-$, $-C(=O)NR^1C(=O)-$, $-S(=O)_2NR^1-$, $-NR^1S(=O)_2-$, $-NR^1-$, or $-N(OR^1)-$;

L^{11} is substituted or unsubstituted $-(CH_2CH_2O)_n-$ or substituted or unsubstituted $-(OCH_2CH_2)_n-$;

each R^1 is independently H or substituted or unsubstituted C_1-C_6 alkyl;

R comprises a lipid, nucleic acid, amino acid, protein, or lipid nanoparticle;

m is an integer selected from 1 to 10; and

n is an integer selected from 1 to 200.

[0004b] In another aspect of the present disclosure, there is provided a formulation comprising a nanoparticle, wherein the nanoparticle comprises the receptor targeting conjugate disclosed herein.

[0004c] In a further aspect of the present disclosure, there is provided a use of a formulation disclosed herein, in the manufacture of a medicament for reducing the risk of coronary disease in a subject in need thereof.

[0004d] In another aspect of the present disclosure, there is provided a method of preparing a formulation disclosed herein, the method comprising:

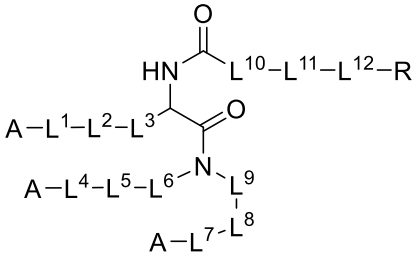
a. providing a first solution comprising the nucleic acid active agent in aqueous buffer;
b. providing a second solution comprising (i) the lipid excipient and (ii) at least a portion of the receptor targeting conjugate in a water-miscible organic solvent;
c. mixing the first solution and the second solution;
d. incubating a mixture of the first and second solutions to form nanoparticles; and
e. optionally carrying out one or more dilution, buffer exchange, concentration, filtration, and nanoparticle evaluation processes.

[0004e] In a further aspect of the present disclosure, there is provided a method of preparing a formulation disclosed herein, the method comprising:

a. providing a first solution comprising the nucleic acid active agent in an aqueous buffer;
b. providing a second solution comprising at least a portion of the lipid excipient in a water-miscible organic solvent;
c. providing a third solution comprising at least a portion of the receptor targeting conjugate;
d. mixing the first solution, the second solution, and the third solution in one or more mixing chambers wherein each solution is introduced separately via an inlet port to the one or more mixing chambers;
e. incubating a mixture of the first, second and third solutions to form nanoparticles; and
f. optionally carrying out one or more dilution, a buffer exchange, concentration, filtration, and nanoparticle evaluation processes.

[0004f] In another aspect of the present disclosure, there is provided a formulation disclosed herein, for use in therapy.

[0005] In one example, described herein is a receptor targeting conjugate, comprising a compound of Formula (V):



Formula (V)

wherein,

a plurality of the A groups collectively comprise a receptor targeting ligand;

each L^1, L^2, L^3, L^4, L^5, L^6, L^7, L^8, L^9, L^{10} and L^{12} is independently substituted or unsubstituted C_1-C_{12} alkylene, substituted or unsubstituted C_1-C_{12} heteroalkylene, substituted or unsubstituted C_2-C_{12} alkenylene, substituted or unsubstituted C_2-C_{12} alkynylene, -$(CH_2CH_2O)_m$-, -$(OCH_2CH_2)_m$-, -O-, -S-, -S(=O)-, -$S(=O)_2$-, -S(=O)(=NR^1)-, -C(=O)-, -C(=N-OR^1)-, -C(=O)O-, -OC(=O)-, -C(=O)C(=O)-, -C(=O)NR^1-, -NR^1C(=O)-, -OC(=O)NR^1-, -NR^1C(=O)O-, -NR^1C(=O)NR^1-, -C(=O)NR^1C(=O)-, -$S(=O)_2NR^1$-, -$NR^1S(=O)_2$-, -NR^1-, or -N(OR^1)-;

L^{11} is substituted or unsubstituted -$(CH_2CH_2O)_n$-, or substituted or unsubstituted -$(OCH_2CH_2)_n$-;

each R^1 is independently H or substituted or unsubstituted C_1-C_6alkyl;

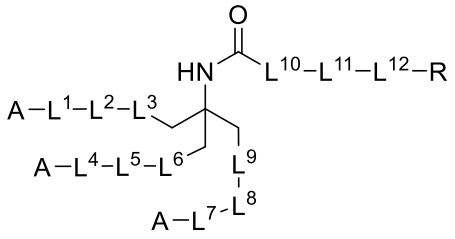
R is a lipid, nucleic acid, amino acid, protein, or lipid nanoparticle;

m is an integer selected from 1 to 10; and

n is an integer selected from 1 to 200.

[0006] In some examples, each L^1, L^4, and L^7 is independently substituted or unsubstituted C_1-C_{12} alkylene. In some examples, each L^1, L^4, and L^7 is independently substituted or unsubstituted C_2-C_6 alkylene. In some examples, each L^1, L^4, and L^7 is C_4 alkylene. In some examples, each L^2, L^5, and L^8 is independently -C(=O)NR^1-, -NR^1C(=O)-, -OC(=O)NR^1-, -NR^1C(=O)O-, -NR1C(=O)NR^1-, or -C(=O)NR^1C(=O)-. In some examples, each L^2, L^5, and L^8 is independently -C(=O)NR^1- or -NR^1C(=O)-. In some examples, each L^2, L^5, and L^8 is -C(=O)NH-. In some examples, each L^3, L^6, and L^9 is independently substituted or unsubstituted C_1-C_{12} alkylene. In some examples, each L^3 is substituted or unsubstituted C_2-C_6 alkylene. In some examples, L^3 is C_4 alkylene. In some examples, each L^6 and L^9 is independently substituted or unsubstituted C_2-C_{10} alkylene. In some examples, each L^6 and L^9 is independently substituted or unsubstituted C_2-C_6 alkylene. In some examples, each L^6 and L^9 is C_3 alkylene. In one example,

described herein is a receptor targeting conjugate, comprising a compound of Formula (VI):



Formula (VI)

wherein,

a plurality of the A groups collectively comprise a receptor targeting ligand;

each L^1, L^2, L^3, L^4, L^5, L^6, L^7, L^8, L^9,L^{10} and L^{12} is independently substituted or unsubstituted C_1-C_{12} alkylene, substituted or unsubstituted C_1-C_{12} heteroalkylene, substituted or unsubstituted C_2-C_{12} alkenylene, substituted or unsubstituted C_2-C_{12} alkynylene, -$(CH_2CH_2O)_m$-, -$(OCH_2CH_2)_m$-, -O-, -S-, -S(=O)-, -$S(=O)_2$-, -S(=O)(=NR^1)-, -C(=O)-, -C(=N-OR^1)-, -C(=O)O-, -OC(=O)-, -C(=O)C(=O)-, -C(=O)NR^1-, -NR^1C(=O)-, -OC(=O)NR^1-, -NR^1C(=O)O-, -NR^1C(=O)NR^1-, -C(=O)NR^1C(=O)-, -$S(=O)_2NR^1$-, -$NR^1S(=O)_2$-, -NR^1-, or -N(OR^1)-;

L^{11} is substituted or unsubstituted -$(CH_2CH_2O)_n$-, or substituted or unsubstituted -$(OCH_2CH_2)_n$-;

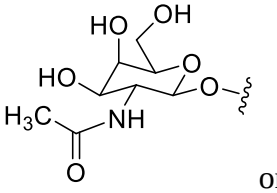
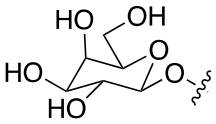
each R^1 is independently H or substituted or unsubstituted C_1-C_6alkyl;

R is a lipid, nucleic acid, amino acid, protein, or lipid nanoparticle;

m is an integer selected from 1 to 10; and

n is an integer selected from 1 to 200.

[0007] In some examples, each L^1, L^4, and L^7 is independently substituted or unsubstituted C_1-C_{12} alkylene or substituted or unsubstituted C_1-C_{12} heteroalkylene. In some examples, each L^1, L^4, and L^7 is independently substituted or unsubstituted C_1-C_{12} heteroalkylene. In some examples, each L^1, L^4, and L^7 is independently substituted or unsubstituted C_1-C_{12} heteroalkylene comprising 1-10 O atoms. In some examples, each L^1, L^4, and L^7 is independently -$(CH_2CH_2O)_{p1}$-$(CH_2)_{q1}$-; wherein p1 is 1-8; and q1 is 1-6. In some examples, each L^1, L^4, and L^7 is -$(CH_2CH_2O)_3$-$(CH_2)_2$-. In some examples, each L^1, L^4, and L^7 is independently substituted or unsubstituted C_1-C_{12} alkylene. In some examples, each L^1, L^4, and L^7 is independently substituted or unsubstituted C_2-C_6 alkylene. In some examples, each L^1, L^4, and L^7 is C_4 alkylene. In some examples, each L^2, L^5, and L^8 is independently -C(=O)NR^1-, -NR^1C(=O)-, -OC(=O)NR^1-, -NR^1C(=O)O-, -NR^1C(=O)NR^1-, or -C(=O)NR^1C(=O)-. In some examples, each L^2, L^5,

and L^8 is independently -C(=O)NR^1- or -NR^1C(=O)-. In some examples, each L^2, L^5, and L^8 is -NHC(=O)-. In some examples, each L^2, L^5, and L^8 is -C(=O)NH-. In some examples, each L^3, L^6, and L^9 is independently substituted or unsubstituted C_1-C_{12} heteroalkylene. In some examples, each L^3, L^6, and L^9 is independently substituted or unsubstituted C_1-C_{12} heteroalkylene comprising 1-10 O atoms. In some examples, each L^3, L^6, and L^9 is independently -$(CH_2CH_2O)_{p2}$-$(CH_2CH_2CH_2O)_{q2}$-; wherein p2 is 1-8; and q2 is 1-6. In some examples, each L^3, L^6, and L^9 is -(CH_2CH_2O)-$(CH_2CH_2CH_2O)$-. In some examples, each L^3, L^6, and L^9 is independently -$(CH_2CH_2CH_2O)_{q3}$-; wherein q3 is 1-8. In some examples, each L^3, L^6, and L^9 is -$(CH_2CH_2CH_2O)_2$-. In some examples, L^{10} is substituted or unsubstituted C_1-C_{12} alkylene. In some examples, L^{10} is substituted or unsubstituted C_1-C_4 alkylene. In some examples, L^{10} is C_2 alkylene. In some examples, L^{11} is -$(OCH_2CH_2)_n$-. In some examples, n is 1-100. In some examples, n is 2-50. In some examples, n is 2, 12, 37, or 45. In some examples, L^{12} is -O-, -C(=O)O-, -C(=O)NR^1-, -NR^1C(=O)-, or -NR^1C(=O)O-. In some examples, L^{12} is -C(=O)O- or -NR^1C(=O)O-. In some examples, L^{12} is -C(=O)O-. In some examples, L^{12} is -NHC(=O)O-. In some examples, L^{12} is -NHC(=O)-. In some examples, A binds to a lectin. In some examples, the lectin is an asialoglycoprotein receptor (ASGPR). In some examples, A is *N*-acetylgalactosamine (GalNAc) or a derivative  or a derivative thereof. A is *N*-acetylgalactosamine (GalNAc)  or a derivative thereof. In some examples, each R^1 is independently H or -CH_3. In some examples, each R^1 is H. In some examples, the R comprises one or more of fatty alcohols, fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, sterol lipids, and prenol lipids. In some examples, the R comprises one or more fatty alcohols. In some examples, each fatty alcohol is independently a saturated, monounsaturated, or polyunsaturated fatty alcohol. In some examples, the fatty alcohol comprises one or more a C_2-C_{26} fatty alcohol. In some examples, the fatty alcohol comprises two or more a C_2-C_{26} fatty alcohol. In some examples, each fatty alcohol is a C12, C14, C16, C18, C20, or C22 fatty alcohol. In some examples, each fatty alcohol is independently docosahexaenol, eicosapentaenol, oleyl

alcohol, stearyl alcohol, (9Z,12Z)-octadeca-9,12-dien-1-yl alcohol, (Z)-docos-13-en-1-yl alcohol, docosanyl alcohol, (E)-octadec-9-en-1-yl alcohol, icosanyl alcohol, (9Z,12Z,15Z)-octadeca-9,12,15-trien-1-yl alcohol, or palmityl alcohol. In some examples, each fatty alcohol is a stearyl alcohol. In some examples, the R comprises one or more sterol lipids. In some examples, the R comprises one or more of vitamins. In some examples, each vitamin is independently a vitamin A, vitamin D, vitamin E, or vitamin K. In some examples, the R comprises a nucleic acid. In some examples, the nucleic acid is a mRNA, guide RNA, siRNA, antisense oligonucleotide, aptamer, microRNA, immunostimulatory oligonucleotide, splice switching oligonucleotide, self-amplifying RNA, circular RNA or DNA, but not limited to the aforementioned. In some examples, the R comprises a protein. In some examples, the protein is a gene editor protein, an antibody, an antigen-binding antibody fragment or a peptide, but not limited to the aforementioned.

[0008] In one example, described herein is a receptor targeting conjugate, comprising a compound from Table 4.

[0009] In one example, described herein is a nanoparticle composition comprising: (a) one or more nucleic acid molecular entities; and (b) a receptor targeting conjugate as described herein. In some examples, the receptor targeting conjugate comprises from about 0.001 mol % to about 20 mol % of the total lipid content present in the nanoparticle composition. In some examples, the receptor targeting conjugate comprises from about 0.01 mol % to about 1 mol % of the total lipid content present in the nanoparticle composition. In some examples, comprising a sterol or a derivative thereof, comprising from 10 mol % to 70 mol % of the total lipid content present in the nanoparticle composition. In some examples, the sterol or the derivative thereof is cholesterol or a cholesterol derivative. In some examples, the cholesterol or the cholesterol derivative comprises from 20 mol % to 50 mol % of the total lipid content present in the nanoparticle composition. In some examples, the nanoparticle composition comprises a phospholipid, comprising from 1 mol % to 20 mol % of the total lipid content present in the nanoparticle composition. In some examples, the phospholipid comprises from about 5 mol % to about 15 mol % of the total lipid content present in said nanoparticle composition. In some examples, the phospholipid is selected from 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 2-oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (POPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), and sphingomyelin. In some examples, the phospholipid is DSPC. In some examples, the nanoparticle

composition comprises a stealth lipid, comprising from 0.1 mol % to 6 mol % of the total lipid content present in the nanoparticle composition. In some examples, the stealth lipid comprises about 2.0 mol % to about 2.5 mol % of the total lipid content present in said nanoparticle composition. In some examples, the stealth lipid is a PEG-lipid that has a number average molecular weight of from about 200 Da to about 5000 Da. In some examples, the nanoparticle composition comprises an amino lipid, comprising from about 10 mol % to about 60 mol % of the total lipid content present in the nanoparticle composition. In some examples, the nanoparticle composition comprises an antioxidant. In some examples, the antioxidant comprises ethylenediaminetetraacetic acid (EDTA). In some examples, the one or more nucleic acid molecular entities comprise a single guide RNA (sgRNA) or guide RNA (gRNA) targeting a disease causing gene of interest produced in the hepatocytes. In some examples, the one or more nucleic acid molecular entities comprise an mRNA that encodes a Cas nuclease. In some examples, at least one of the one or more nucleic acid molecular entities comprises a chemical modification. In some examples, the chemical modification is a 2'-F modification, a phosphorothioate internucleotide linkage modification, acyclic nucleotides, LNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-deoxy, 2'-fluoro, 2'-O-N-methylacetamido (2'-O-NMA), a 2'-O-dimethylaminoethoxyethyl (2'-0-DMAEOE), 2'-0-aminopropyl (2'-O-AP), 4'-O-methyl, or a 2'-ara-F modification. In some examples, the chemical modification is a 2'- O-methyl modification.

[0010] In one example, provided herein is a pharmaceutical composition comprising a herein described receptor targeting conjugate or a herein described nanoparticle composition, and an excipient or carrier. In some examples, the pharmaceutical composition comprises an mRNA encoding a gene editor nuclease. In some examples, the pharmaceutical composition comprises one or more guide RNA molecules. In some examples, the pharmaceutical composition comprises two or more guide RNA molecules. In some examples, the two or more guide RNA molecules target two or more genes of interest. In some examples, the mRNA encodes Cas9 nuclease. In some examples, the mRNA encodes a base editor nuclease. In some examples, the mRNA and the one or more guide RNA molecules are present in the same nanoparticle composition. In some examples, the mRNA and the one or more guide RNA molecules are present in different nanoparticle compositions. In some examples, a ratio of the gRNA molecules to the mRNA in the pharmaceutical composition is from about 0.01 to about 100 by weight or by mole. In some examples, a ratio of said gRNA molecules to said mRNA in said pharmaceutical

composition is about 50:1, about 40:1, about 30:1, about 20:1, about 18:1, about 16:1, about 14:1, about 12:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, or about 1:10 by weight or by mole.

[0011] In one example, provided herein is a pharmaceutical composition comprising: (a) a first receptor targeting conjugate or a first nanoparticle composition as described herein, and (b) a second receptor targeting conjugate or a second nanoparticle composition as described herein. In some examples, the first nanoparticle composition comprises a gene editor mRNA. In some examples, the second nanoparticle composition comprises one or more guide RNA molecules. In some examples, a ratio of guide RNA molecules to mRNA in said pharmaceutical composition is about 50:1, about 40:1, about 30:1, about 20:1, about 18:1, about 16:1, about 14:1, about 12:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, or about 1:10 by weight or by mole.

[0012] In one example, provided herein is a method of delivering a nucleic acid to a cell, the method comprising contacting the cell with a nanoparticle composition or a pharmaceutical composition as described herein, whereby the nucleic acid is delivered to said cell. In some examples, the cell is contacted *in vivo*, *ex vivo*, or *in intro*.

[0013] In one example, provided herein is a method of producing a polypeptide of interest in a cell, the method comprising contacting said cell with a nanoparticle composition or a pharmaceutical composition as described herein, whereby the nucleic acid is capable of being translated in said cell to produce the polypeptide.

[0014] In one example, provided herein is a method of treating a disease or condition in a subject, the method comprising administering to the subject a therapeutically effective amount of a pharmaceutical composition as described herein. In some examples, the disease or condition is a coronary disease. In some examples, the subject is low-density lipoprotein receptor (LDLR)-deficient. In some examples, the subject has heterozygous familial hypercholesterolemia (HeFH), homozygous familial hypercholesterolemia(HoFH) or clinical atherosclerotic cardiovascular disease (ASCVD). In some examples, the subject is at high risk of cardiovascular events. In some examples, the subject requires additional lowering of low-density lipoprotein cholesterol (LDL-C) despite maximally tolerated lipid-lowering therapy.

[0015] In one example, provided herein is a method of delivering a nucleic acid molecular entity to the liver of a subject, comprising administering to the subject a pharmaceutical

composition as described herein, thereby delivering the nucleic acid molecular entity.

[0016] In one example, described herein is a nucleotide conjugate comprising: (a) a nucleic acid, and (b) a targeting moiety connected to the nucleic acid in (a), wherein the targeting moiety comprises a structure of Table 1. In some examples, the targeting moiety further comprises a coupling sequence that hybridizes with the nucleic acid in (a). In one example, described herein is a nucleotide conjugate comprising: (a) a nucleic acid, and (b) a targeting moiety connected to the nucleic acid in (a), wherein the targeting moiety comprises a coupling sequence that hybridizes with the nucleic acid in (a). In some examples, the nucleic acid comprises a single stranded, double stranded, a partially double stranded, or a hairpin stem-loop nucleic acid, and wherein the targeting moiety is a receptor targeting moiety. In some examples, the targeting moiety binds to a lectin. In some examples, the lectin is an asialoglycoprotein receptor (ASGPR). In some examples, the targeting moiety comprises one or more *N*-acetylgalactosamine (GalNAc) or GalNAc derivatives. In some examples, the targeting moiety comprises one or more *N*-acetylgalactosamine (GalNAc) or GalNAc derivatives and a spacer. In some examples, the targeting moiety comprises one or more galactose or galactose derivatives. In some examples, the targeting moiety comprises one or more galactose or galactose derivatives and a spacer. In some examples, the spacer comprises polyethylene glycol, substituted or unsubstituted C_1-C_{12} alkylene, or both, wherein the polyethylene glycol has from 1 to 5 repeating units. In some examples, the targeting moiety is linked to one or more strands of the nucleic acid through one or more linkers. In some examples, the targeting moiety comprises a structure of Table 1. In some examples, the coupling sequence hybridizes with the nucleic acid in (a). In some examples, the coupling sequence hybridizes with an extension in the nucleic acid in (a). In some examples, the targeting moiety is attached to the 5’ end of the nucleic acid sequence, the 3’ end of the nucleic acid sequence, or the middle of the nucleic acid sequence. In some examples, the targeting moiety comprises at least two GalNAcs or GalNAc derivatives. In some examples, the targeting moiety comprises at least three GalNAcs or GalNAc derivatives. In some examples, the GalNAcs or GalNAc derivatives are connected to the nucleic acid in (a) via a linker in the targeting moiety, via hybridization of the coupling sequence in the targeting moiety that hybridizes with the nucleic acid in (a), or via a combination thereof. In some examples, the targeting moiety comprises at least two galactose or galactose derivatives. In some examples, the targeting moiety comprises at least three galactoses or galactose derivatives. In some examples, the galactoses or galactose derivatives are connected to the nucleic acid in (a)

via a linker in the targeting moiety, via hybridization of the coupling sequence in the targeting moiety that hybridizes with the nucleic acid in (a), or via a combination thereof. In some examples, the targeting moiety comprises at least two coupling sequences that hybridize with the nucleic acid in (a). In some examples, the at least two coupling sequences are identical. In some examples, the at least two coupling sequences are different. In some examples, the nucleotide conjugate further comprises a second targeting moiety. In some examples, the second targeting moiety binds to an asialoglycoprotein receptor (ASGPR). In some examples, the second targeting moiety is linked to one or more strands the nucleic acid through a spacer and/or through one or more linkers. In some examples, the second targeting moiety comprises a GalNAc or GalNAc derivative. In some examples, the second targeting moiety comprises at least three GalNAc moieties or GalNAc derivatives. In some examples, the GalNAc or GalNAc derivatives are connected to the nucleic acid in (a) via a linker in the targeting moiety, via hybridization of the coupling sequence in the targeting moiety that hybridizes with the nucleic acid in (a), or via a combination thereof. In some examples, the second targeting moiety comprises a galactose or galactose derivative. In some examples, the second targeting moiety comprises at least three galactose moieties or galactose derivatives. In some examples, the galactose or galactose derivatives are connected to the nucleic acid in (a) via a linker in the targeting moiety, via hybridization of the coupling sequence in the targeting moiety that hybridizes with the nucleic acid in (a), or via a combination thereof. In some examples, the second targeting moiety comprises a structure of Table 1. In some examples, the second targeting moiety comprises a coupling sequence that hybridizes with the nucleic acid. In some examples, the second targeting moiety is attached to the 5’ end of the nucleic acid, the 3’ end of the nucleic acid, or the middle of the nucleic acid. In some examples, the nucleic acid in (a) comprises RNA or DNA. In some examples, the coupling sequence comprises RNA, DNA, chemically modified RNA, chemically modified DNA, or a hybrid of DNA and RNA. In some examples, the coupling sequence comprises one or more of (a), (c), (g), (u), (A)n, (T)n, (U)n, (a)n, or (u)n, wherein n is an integer no less than 3, wherein a is 2’-O-methyladenosine (2’-OMe A), wherein c is 2’-O-methylacytidine (2’-OMe-C), wherein g is 2’-O-methylacytidine guanine (2’-OMe-G), and wherein u is 2’-O-methyluridine (2’-OMe-U). In some examples, the coupling sequence comprises one or more of (a), (c), (g), (u), (A)n, (T)n, (U)n, (a)n, or (u)n, wherein n is an integer no less than 3, wherein a is 2’-O-methyladenosine (2’-OMe A), wherein c is 2’-O-methylacytidine (2’-OMe-C), wherein g is 2’-O-methylacytidine guanine (2’-OMe-G), and

wherein u is 2’-O-methyluridine (2’-OMe-U). In some examples, the (a), (c), (g), or (u) is scattered along the nucleic acid or the coupling sequence. In some examples, the nucleic acid and the coupling sequence comprise one or more G-C base pairing within a hybridization duplex wherein the coupling sequence hybridizes with the nucleic acid and wherein said one or more G-C base pairing increases stability of the hybridization duplex. In some examples, the linker comprises a covalent linker. In some examples, the linker comprises a non-covalent linker. In some examples, the linker comprises a monovalent linker, a bivalent linker, a trivalent linker, or a combination thereof. In some examples, the linker comprises a biocleavable linker. In some examples, the linker comprises a non-biocleavable linker. In some examples, the linker comprises a phosphate, phosphorothioate, amide, ether, oxime, hydrazine or carbamate. In some examples, the linker is a phosphate or phosphorothioate. In some examples, the nucleic acid in (a) comprises a chemical modification. In some examples, the nucleic acid in (a) comprises a 2’-F modification, a phosphorothioate internucleotide linkage modification, acyclic nucleotides, LNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-deoxy, 2'-fluoro, 2'-O-N-methylacetamido (2'-O-NMA), a 2'-O-dimethylaminoethoxyethyl (2'-0-DMAEOE), 2'-0-aminopropyl (2'-O-AP), 4’-O-methyl, or a 2'-ara-F modification. In some examples, the nucleic acid comprises a 2'- O-methyl modification. In some examples, the nucleic acid comprises a phosphorothioate internucleotide linkage modification. In some examples, the nucleic acid is capable of hybridizing with a target sequence within a target gene of a genome. In some examples, the nucleic acid comprises a mRNA, siRNA, shRNA, antisense oligonucleotide, microRNA, anti-microRNA or antimir, supermir, antagomir, ribozyme, triplex-forming oligonucleotide, decoy oligonucleotide, splice-switching oligonucleotide, immunostimulatory oligonucleotide, RNA activator, Ul adaptor, guide RNA, or any combinations thereof. In some examples, the nucleic acid encodes a protein. In some examples, the nucleic acid is a CRISPR enzyme. In some examples, the nucleic acid is a guide RNA capable of forming a complex with a CRISPR enzyme. In some examples, the guide RNA is a single guide RNA or a dual guide RNA. In some examples, the CRISPR enzyme is selected from the group consisting of Cas9, Cpf1, CasX, CasY, C2c1, C2c3, and base editor fusion protein. In some examples, the nucleic acid further comprises a mRNA encoding the CRISPR enzyme. In some examples, the CRISPR enzyme results in an alteration in the target sequence. In some examples, the target gene is involved in a lipid metabolism pathway. In some examples, the target gene is selected from the group consisting of PCSK9, ANGPTL3,

APOC3, LPA, APOB, MTP, ANGPTL4, ANGPTL8, APOA5, APOE, LDLR, IDOL, NPC1L1, ASGR1, TM6SF2, GALNT2, GCKR, LPL, MLXIPL, SORT1, TRIB1, MARC1, ABCG5, and ABCG8. In some examples, the guide RNA comprises a sequence selected from sequences of Table 3. In some examples, the guide RNA comprises a sequence selected from sequences of Table 5.

[0017] In one example, described herein is a particle comprising the described nucleotide conjugate and the described CRISPR enzyme. In some examples, the particle is a lipid nanoparticle, a liposome, an inorganic nanoparticle, or an RNP.

[0018] In one example, described herein is a cell comprising the nucleotide conjugate of any one of the preceding claims. In some examples, the cell is a prokaryotic cell, a eukaryotic cell, a vertebrate cell, a mouse cell, a non-human primate cell, or a human cell.

[0019] In one example, described herein is a pharmaceutical composition comprising the described nucleotide conjugate, the particle, or the cell. In some examples, the pharmaceutical composition comprises a pharmaceutically acceptable adjuvant, diluent, carrier, preservative, excipient, buffer, stabilizer, or a combination thereof. In some examples, the carrier comprises solvents, dispersion media, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, lipids, lipidoids, polymers, lipoplexes, core-shell nanoparticles, hyaluronidase, nanoparticle mimics, or combinations thereof.

[0020] In one example, described herein is a kit comprising the described nucleotide conjugate.

[0021] In one example, described herein is a method for reducing the risk of coronary disease in a subject in need thereof, comprising administering to the subject an effective amount of the described nucleotide conjugate. In one example, described herein is a method for reducing the risk of coronary disease in a subject in need thereof, comprising administering to the subject an effective amount of a pharmaceutical composition, said pharmaceutical composition comprising (a) a nucleic acid, and (b) a targeting moiety connected to the nucleic acid in (a), wherein the targeting moiety comprises a structure of Table 1. In one example, described herein is a method of delivering a nucleic acid to the liver of a subject, comprising administering to the subject said nucleic acid connected to a targeting moiety, wherein the targeting moiety comprises a structure of Table 1. In some examples, the targeting moiety further comprises a coupling sequence that hybridizes with the nucleic acid in (a). In one example, described herein is a method for reducing the risk of coronary disease in a subject in need thereof, comprising administering to the subject an effective amount of a

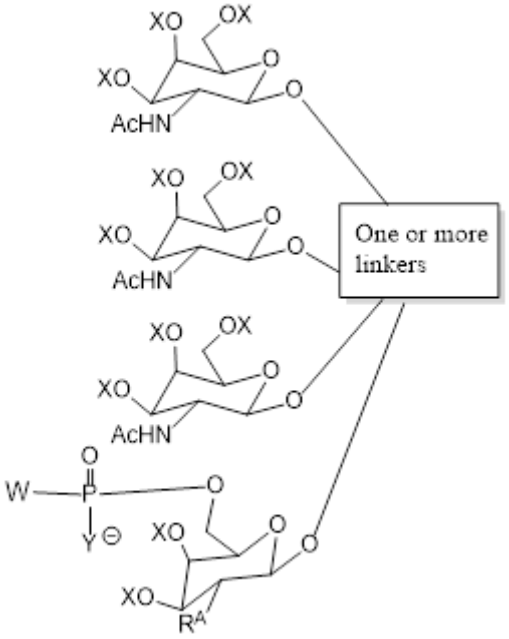
pharmaceutical composition, said pharmaceutical composition comprising (a) a nucleic acid, and (b) a targeting moiety connected to the nucleic acid in (a), wherein the targeting moiety comprises a coupling sequence that hybridizes with the nucleic acid in (a). In one example, described herein is a method of delivering a nucleic acid to the liver of a subject, comprising administering to the subject said nucleic acid connected to a targeting moiety, wherein the targeting moiety comprises a coupling sequence that hybridizes with the nucleic acid. In some examples, the nucleic acid comprises a single stranded, double stranded, a partially double stranded, or a hairpin stem-loop nucleic acid, and wherein the targeting moiety is a receptor targeting moiety. In some examples, the targeting moiety binds to a lectin. In some examples, the lectin is an asialoglycoprotein receptor (ASGPR). In some examples, the targeting moiety comprises one or more N-acetylgalactosamine (GalNAc) or GalNAc derivatives. In some examples, the targeting moiety comprises at least three GalNAc or GalNAc derivatives. In some examples, the targeting moiety comprises one or more galactose or galactose derivatives. In some examples, the targeting moiety comprises at least three galactose or galactose derivatives. In some examples, the nucleic acid comprises (i) a guide RNA and a nuclease mRNA or (ii) a guide RNA complexed in a nuclease RNP, and wherein the guide RNA is capable of directing the nuclease to a target sequence in a target gene. In some examples, the guide RNA comprises a single guide RNA or a dual guide RNA. In some examples, the nuclease is a CRISPR enzyme. In some examples, the CRISPR enzyme selected from the group consisting of Cas9, Cpf1, CasX, CasY, C2c1, C2c3, and base editor fusion protein. In some examples, the CRISPR enzyme results in an alteration in the target sequence. In some examples, the administration results in reduced expression of the target gene in the liver of the subject. In some examples, expression of the target gene in the liver of the subject is reduced by at least 1%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or greater than 99.99% as compared to a control tissue of the subject. In some examples, the target gene is associated with a coronary disease. In some examples, the target gene is selected from the group consisting of PCSK9, ANGPTL3, APOC3, LPA, APOB, MTP, ANGPTL4, ANGPTL8, APOA5, APOE, LDLR, IDOL, NPC1L1, ASGR1, TM6SF2, GALNT2, GCKR, LPL, MLXIPL, SORT1, TRIB1, MARC1, ABCG5, and ABCG8. In some examples, the coupling sequence comprises RNA, DNA, chemically modified RNA, chemically modified DNA, or a hybrid of DNA and RNA. In some examples, the coupling sequence comprises (A)n, (T)n, (U)n, (a)n, or (u)n,

wherein n is an integer no less than 3, wherein a is 2’-O-methyladenosine (2’-OMe A), and wherein u is 2’-O-methyluridine (2’-OMe-U). In some examples, the nucleic acid in (a) comprises (A)n, (T)n, (U)n, (a)n, or (u)n, wherein n is an integer no less than 3, wherein a is 2’-O-methyladenosine, and wherein u is 2’-O-methyluridine. In some examples, the targeting moiety is linked to the nucleic acid in (a) via a linker in the targeting moiety, via hybridization of the coupling sequence in the targeting moiety that hybridizes with the nucleic acid in (a), or via a combination thereof. In some examples, the linker comprises a covalent linker. In some examples, the linker comprises a phosphate, phosphorothioate, amide, ether, oxime, hydrazine or carbamate. In some examples, the linker is a phosphate or phosphorothioate. In some examples, the nucleic acid in (a) comprises a chemical modification. In some examples, the nucleic acid in (a) comprises a 2’-F modification, a phosphorothioate internucleotide linkage modification, acyclic nucleotides, LNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-deoxy, 2'-fluoro, 2'-O-N-methylacetamido (2'-O-NMA), a 2'-O-dimethylaminoethoxyethyl (2'-0-DMAEOE), 2'-0-aminopropyl (2'-O-AP), or a 2'-ara-F modification. In some examples, the nucleic acid comprises a 2'- O-methyl modification. In some examples, the nucleic acid comprises a phosphorothioate internucleotide linkage modification. In some examples, the level of the nucleic acid in the liver of the subject is at least 1.5, at least 2, at least 2.5, at least 3, at least 5, at least 10, at least 15, at least 20, at least 30, at least 40, at least 50 folds higher as compared to other tissues of the subject at least 1 hours, 2 hours, 6 hours, 12 hours, 24 hours, 2 days, 1 week, 2 weeks, 3 weeks, 6 weeks, or 8 weeks post delivery. In some examples, the effective amount is about 1 mg/kg to about 10 mg/kg. In some examples, the administration results in reduced blood triglycerides and/or reduced low-density lipo-protein cholesterol in the subject in need thereof. In some examples, the administration is performed intravenously, intrathecally, intramuscularly, intraventricularly, intracerebrally, intracerebellarly, intracerebroventricularly, intraperenchymally, subcutaneously, or a combination thereof.

[0022] In one example, described herein is a method for reducing the risk of coronary disease in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition comprising (a) (i) a single guide RNA and a nuclease mRNA , (ii)a dual guide RNA and a nuclease mRNA, (iii) a single guide RNA and an RNP, or (iv) a dual guide RNAs and an RNP; and (b) a asialoglycoprotein receptor (ASGPR) targeting moiety connected to the nucleic acid in (a), wherein the single guide RNA or the dual guide RNA comprises 4 or more 2’-O-methyl modifications and 2 or more phosphorothioate

internucleotide linkages, wherein the targeting moiety comprises a structure of Table 1, and wherein the guide RNA hybridizes with a PCSK9 gene. In one example, described herein is a method for reducing the risk of coronary disease in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition comprising (a) (i) a single guide RNA and a nuclease mRNA , (ii)a dual guide RNA and a nuclease mRNA, (iii) a single guide RNA and an RNP, or (iv) a dual guide RNAs and an RNP; and (b) a targeting moiety connected to the nucleic acid in (a), wherein the single guide RNA or the dual guide RNA comprises 4 or more 2'-O-methyl modifications and two or more phosphorothioate internucleotide linkages, wherein the targeting moiety comprises a coupling sequence that hybridizes with the single guide RNA in (a), and wherein the guide RNA hybridizes with a PCSK9 gene. In some examples, the nuclease mRNA and/or the single guide RNA comprises at least one chemical modification. In some examples, the chemical modification is selected from the group consisting of a 2'-F modification, phosphorothioate internucleotide linkage modification, acyclic nucleotides, LNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-deoxy, 2'-fluoro, 2'-O-N-methylacetamido (2'-O-NMA), a 2'-O-dimethylaminoethoxyethyl (2'-0-DMAEOE), 2'-0-aminopropyl (2'-O-AP), 4'-O-methyl, and a 2'-ara-F modification. In some examples, administrating of the nucleic acid conjugate results in a reduced level of immune response as compared to a control nucleic acid conjugate without said chemical modification.

[0023] In one example, described herein is a nucleotide conjugate comprising a structure of Formula (IV)



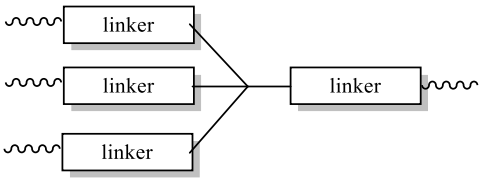
Formula (IV)

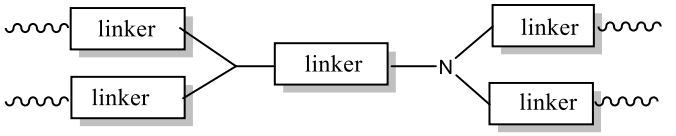
wherein each X is independently H or a protecting group, R^A is -OX or -NHAc, Y is O or S, and W represents

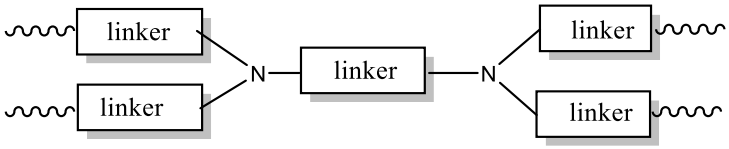
(a) (i) a single guide RNA and a nuclease mRNA , (ii) a dual guide RNA and a nuclease mRNA, (iii) a single guide RNA and an RNP, or (iv) a dual guide RNAs and an RNP; or

(b) a coupling sequence.

[0024] In some examples, the one or more linkers comprise a structure selected from the group consisting of:



,


,
and


. In some examples, each of the linkers independently has a structure of $–(L^1)_{k1}–(L^2)_{k2}–(L^3)_{k3}–(L^4)_{k4}–$, wherein each of k1, k2, k3, and k4 is independently 0, 1 or 2, and each of the L^1, L^2, L^3 and L^4 is independently selected from -O-, -S-, $S(=O)_{1-2}$-, -C(=O)-, -C(=S)-, $-NR^L$-, -OC(=O)-, -C(=O)O-, -OC(=O)O-, $-C(=O)NR^L$-, $-OC(=O)NR^L$-, $-NR^LC(=O)$-, $-NR^LC(=O)$ NR^L-, $-P(=O)R^L$-, $-NR^LS(=O)(=NR^L)$-, $-NR^LS(=O)_2$-, $-S(=O)_2NR^L$-, -N=N-, $-(CH_2-CH_2-O)_{1-6}$-, linear or branched C_{1-6} alkylene, linear or branched C_{2-6} alkenylene, linear or branched C_{2-6} alkynylene, $C_3–C_8$ cycloalkylene, $C_2–C_7$ heterocycloalkylene, $C_6–C_{10}$ arylene, and $C_5–C_9$ heteroarylene, wherein the alkylene, alkenylene, alkynylene, cycloalkylene, cycloalkylene, arylene, or heteroarylene is substituted or unsubstituted, and wherein each R^L is independently H, D, cyano, halogen, substituted or unsubstituted C_1-C_6 alkyl, $-CD_3$, $-OCH_3$, $-OCD_3$, substituted or unsubstituted C_1-C_6 haloalkyl, substituted or unsubstituted C_1-C_6 heteroalkyl, substituted or unsubstituted C_3-C_8 cycloalkyl, substituted or unsubstituted C_2-C_7 heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl. In some examples, each R^L is independently H, substituted or unsubstituted C_1-C_6 alkyl, $-OCH_3$, substituted or unsubstituted C_1-C_6 haloalkyl, substituted or unsubstituted C_1-C_6 heteroalkyl, substituted or unsubstituted C_3-C_8 cycloalkyl, or substituted or unsubstituted C_2-C_7 heterocycloalkyl. In some examples, the sum of k1, k2, k3, and k4 is 1, 2, or 3.

[0025] In one example, described herein is a method of preparing a formulation comprising

nanoparticles, wherein the nanoparticles comprise (i) one or more nucleic acid molecular entities, (ii) one or more lipids selected from a sterol or a derivative thereof, a phospholipid, a stealth lipid, and an amino lipid, and (iii) a receptor targeting conjugate, the method comprising: (a) providing a first solution comprising the one or more nucleic acid molecular entities;(b) providing a second solution comprising at least one of the one or more lipids; (c) mixing the first solution and the second solution, thereby producing a mixture comprising nanoparticles that comprise the one or more nucleic acid molecular entities and the one or more lipids; (d) mixing the receptor targeting conjugate with the nanoparticles produced in step (c); (e) incubating the nanoparticles; and (f) optionally carrying out a buffer exchange process. In some examples, the receptor targeting conjugate is combined with the one or more lipids after the mixing step in (c). In some examples, the receptor targeting conjugate is added in a dilution buffer, and wherein the dilution buffer is mixed with preformed nucleic acid-lipid nanoparticles coming out of an inline mixing chamber thereby forming the nanoparticles. In some examples, the receptor targeting conjugate is introduced after an addition of a dilution buffer to the mixture and holding the diluted mixture for a period of time. In some examples, the holding time is between 1 and 120 minutes. In some examples, the holding time is between 1 and 90 minutes, between 1 and 60 minutes, or between 10 and 40 minutes. In some examples, the holding time is about 30 minutes. In some examples, the receptor targeting conjugate is introduced to the nanoparticles after buffer exchange. In some examples, the receptor targeting conjugate is introduced to the nanoparticles after buffer exchange and concentration, but prior to storage. In some examples, the receptor targeting conjugate is introduced to the nanoparticles after storage and thawing, and prior to dosing or evaluation. In one example, described herein is a method of preparing a formulation comprising nanoparticles, wherein the nanoparticles comprise (i) one or more nucleic acid molecular entities, (ii) one or more lipids selected from a sterol or a derivative thereof, a phospholipid, a stealth lipid, and an amino lipid, and (iii) a receptor targeting conjugate, the method comprising: (a) providing a first solution comprising the one or more nucleic acid molecular entities; (b) providing a second solution comprising at least one of the one or more lipids; (c) inline mixing of the first solution and the second solution, thereby producing a mixture comprising nanoparticles that comprise the one or more nucleic acid molecular entities and the one or more lipids; (d) inline mixing of the receptor targeting conjugate to the mixture of step (c), thereby producing a mixture comprising nanoparticles that comprise the one or more nucleic acid molecular entities, the one or more lipids, and the receptor targeting conjugate; (e) diluting the mixture of step (d)

by adding a dilution buffer; and (f) optionally carrying out a buffer exchange process. In some examples, the inline mixing of step (c) and the inline mixing of step (d) are performed successively. In one example, described herein is a method of preparing a formulation comprising nanoparticles, wherein the nanoparticles comprise (i) one or more nucleic acid molecular entities, (ii) one or more lipids selected from a sterol or a derivative thereof, a phospholipid, a stealth lipid, and an amino lipid, and (iii) a receptor targeting conjugate, the method comprising: (a) providing a first solution comprising the one or more nucleic acid molecular entities; (b) providing a second solution comprising (i) at least one of the one or more lipids and (ii) at least a portion of the receptor targeting conjugate; (c) mixing the first solution and the second solution, thereby producing a mixture comprising nanoparticles; (d) optionally incubating the nanoparticles; and (e) optionally carrying out a buffer exchange process. In some examples, the second solution comprises all the receptor targeting conjugate. In one example, described herein is a method of preparing a formulation comprising nanoparticles, wherein the nanoparticles comprise (i) one or more nucleic acid molecular entities, (ii) one or more lipids selected from a sterol or a derivative thereof, a phospholipid, a stealth lipid, and an amino lipid, and (iii) a receptor targeting conjugate, the method comprising: (a) providing a first solution comprising the one or more nucleic acid molecular entities; (b) providing a second solution comprising at least one of the one or more lipids; (c) mixing the first solution and the second solution, thereby producing a mixture comprising nanoparticles that comprise the one or more nucleic acid molecular entities and the one or more lipids; (d) combining the receptor targeting conjugate with the one or more lipids, wherein at least a portion of the receptor targeting conjugate is combined with the one or more lipids prior to or concurrently with the mixing step; (e) optionally incubating the nanoparticles; and (f) optionally carrying out a buffer exchange process. In some examples, at least a portion of the receptor targeting conjugate is combined with the one or more lipids concurrently with the mixing step. In some examples, at least a portion of the receptor targeting conjugate is combined with the one or more lipids prior to the mixing step. In some examples, the receptor targeting conjugate is combined with the one or more lipids in the second solution. In some examples, a portion of the receptor targeting conjugate is combined with the one or more lipids in the second solution and a portion of the receptor targeting conjugate is combined with the one or more lipids after the mixing. In some examples, a portion of the receptor targeting conjugate is combined with the one or more lipids in the second solution and a portion of the receptor targeting conjugate is combined with the one or more lipids after the incubating step. In some examples, a

portion of the receptor targeting conjugate is combined with the one or more lipids in the second solution and a portion of the receptor targeting conjugate is combined with the one or more lipids after the buffer exchange step. In some examples, the method further comprises diluting the mixture produced by mixing the first and the second solutions by adding a dilution buffer. In some examples, the mixture is diluted inline. In some examples, the dilution buffer comprises at least a portion of the receptor targeting conjugate. In some examples, the dilution buffer comprises at least a portion of the stealth lipid. In some examples, the first solution comprises an aqueous buffer. In some examples, the second solution comprises ethanol. In some examples, the mixing comprises laminar mixing, vortex mixing, turbulent mixing, or a combination thereof. In some examples, the mixing comprises cross-mixing. In some examples, the mixing comprises inline mixing. In some examples, the mixing comprises introducing at least a portion of the first solution through a first inlet channel and at least a portion of the second solution through a second inlet channel, and wherein an angle between the first inlet channel and the second inlet channel is from about 15 to 180 degrees. In some examples, the mixing comprises introducing a portion of the first solution through a third inlet channel. In some examples, the buffer exchange comprises dialysis, chromatography, or tangential flow filtration (TFF). In some examples, the method further comprises a filtration step. In some examples, the receptor targeting conjugate comprises one or more N-acetylgalactosamine (GalNAc) or GalNAc derivatives. In some examples, the receptor targeting conjugate comprises one or more galactose or galactose derivatives. In some examples, the receptor targeting conjugate is selected from Table 4. In some examples, the receptor targeting conjugate is a targeting conjugate described herein. In some examples, the nanoparticles comprise a first nanoparticle composition described herein. In some examples, the formulation is a pharmaceutical composition described herein.

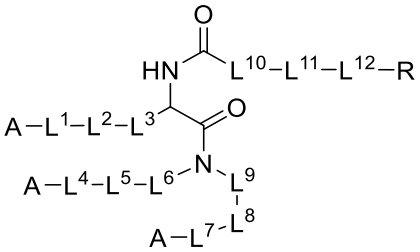
[0026] In one example, described herein is a pharmaceutical composition comprising nanoparticles, wherein the nanoparticles comprise (i) one or more nucleic acid molecular entities, (ii) one or more lipids selected from a sterol or a derivative thereof, a phospholipid, a stealth lipid, and an amino lipid, and (iii) a receptor targeting conjugate, wherein the formulation is prepared by a method described herein. In one example, described herein is a pharmaceutical composition comprising nanoparticles, wherein the nanoparticles comprise (i) one or more nucleic acid molecular entities, (ii) one or more lipids selected from a sterol or a derivative thereof, a phospholipid, a stealth lipid, and an amino lipid, and (iii) a receptor

targeting conjugate, wherein the formulation is prepared by a method comprising: (a) providing a first solution comprising the one or more nucleic acid molecular entities; (b) providing a second solution comprising at least one of the one or more lipids; (c) mixing the first solution and the second solution, thereby producing a mixture comprising nanoparticles that comprise the one or more nucleic acid molecular entities and the one or more lipids; (d) mixing the receptor targeting conjugate with the nanoparticles produced in step (c); (e) incubating the nanoparticles; and (f) optionally carrying out a buffer exchange process. In one example, described herein is a pharmaceutical composition comprising nanoparticles, wherein the nanoparticles comprise (i) one or more nucleic acid molecular entities, (ii) one or more lipids selected from a sterol or a derivative thereof, a phospholipid, a stealth lipid, and an amino lipid, and (iii) a receptor targeting conjugate, wherein the formulation is prepared by a method comprising: (a) providing a first solution comprising the one or more nucleic acid molecular entities; (b) providing a second solution comprising at least one of the one or more lipids; (c) mixing the first solution and the second solution, thereby producing a mixture comprising nanoparticles that comprise the one or more nucleic acid molecular entities and the one or more lipids; (d) combining the receptor targeting conjugate with the one or more lipids, wherein at least a portion of the receptor targeting conjugate is combined with the one or more lipids prior to or concurrently with the mixing step; (e) optionally incubating the nanoparticles; and (f) optionally carrying out a buffer exchange process. In one example, described herein is a pharmaceutical composition comprising nanoparticles, wherein the nanoparticles comprise (i) one or more nucleic acid molecular entities, (ii) one or more lipids selected from a sterol or a derivative thereof, a phospholipid, a stealth lipid, and an amino lipid, and (iii) a receptor targeting conjugate, wherein the formulation is prepared by a method comprising: (a) providing a first solution comprising the one or more nucleic acid molecular entities; (b) providing a second solution comprising at least one of the one or more lipids; (c) inline mixing of the first solution and the second solution, thereby producing a mixture comprising nanoparticles that comprise the one or more nucleic acid molecular entities and the one or more lipids; (d) inline mixing of the receptor targeting conjugate to the mixture of step (c), thereby producing a mixture comprising nanoparticles that comprise the one or more nucleic acid molecular entities, the one or more lipids, and the receptor targeting conjugate; (e) diluting the mixture of step (d) by adding a dilution buffer; and (f) optionally carrying out a buffer exchange process. In one example, described herein is a pharmaceutical composition comprising nanoparticles, wherein the nanoparticles comprise (i) one or more nucleic acid molecular entities, (ii) one or more lipids selected from a sterol or a

CLAIMS

WHAT IS CLAIMED IS:

1. A receptor targeting conjugate, comprising a compound of Formula (V):



Formula (V),

or a stereoisomer or salt thereof, wherein,

a plurality of the A groups collectively comprising a receptor targeting ligand;

each of L^1, L^2, L^3, L^4, L^5, L^6, L^7, L^8, L^9, L^{10} and L^{12} is independently substituted or unsubstituted C_1-C_{12} alkylene, substituted or unsubstituted C_1-C_{12} heteroalkylene, substituted or unsubstituted C_2-C_{12} alkenylene, substituted or unsubstituted C_2-C_{12} alkynylene, -$(CH_2CH_2O)_m$-, -$(OCH_2CH_2)_m$-, -O-, -S-, -S(=O)-, -$S(=O)_2$-, -S(=O)(=NR^1)-, -C(=O)-, -C(=N-OR^1)-, -C(=O)O-, -OC(=O)-, -C(=O)C(=O)-, -C(=O)NR^1-, -NR^1C(=O)-, -OC(=O)NR^1-, -NR^1C(=O)O-, -NR^1C(=O)NR^1-, -C(=O)NR^1C(=O)-, -$S(=O)_2NR^1$-, -$NR^1S(=O)_2$-, -NR^1-, or -N(OR^1)-;

L^{11} is substituted or unsubstituted -$(CH_2CH_2O)_n$- or substituted or unsubstituted -$(OCH_2CH_2)_n$-;

each R^1 is independently H or substituted or unsubstituted C_1-C_6 alkyl;

R comprises a lipid, nucleic acid, amino acid, protein, or lipid nanoparticle;

m is an integer selected from 1 to 10; and

n is an integer selected from 1 to 200.

2. The receptor targeting conjugate of claim 1, wherein:

each of L^1, L^4, and L^7 is independently substituted or unsubstituted C_2-C_6 alkylene;

each of L^2, L^5, and L^8 is independently -C(=O)N(R^1)- or -N(R^1)C(=O)-, wherein R^1 is H or -CH_3;

each of L^3, L^6, and L^9 is independently substituted or unsubstituted C_2-C_6 alkylene;

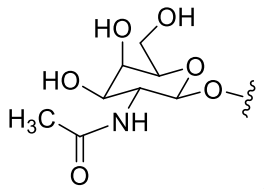
L^{10} is -$(CH_2)_{1\text{-}3}$-, -CH_2CH_2O- or -CH_2O-;

L^{11} is -$(CH_2CH_2O)_n$- or -$(OCH_2CH_2)_n$-; and n is an integer selected from 1 to 50;

L^{12} is -NHC(=O)O- or -NHC(=O)-; and

R comprises one or more of fatty alcohols, fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, sterol lipids, prenol lipids or vitamins.

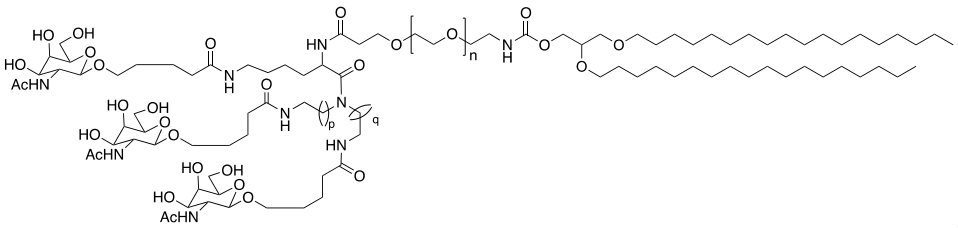
3. The receptor targeting conjugate of claim 1 or 2, wherein each of L^1, L^3, L^4, and L^7 is -$(CH_2)_4$-.
4. The receptor targeting conjugate of any one of claims 1-3, wherein each of L^2, L^5, and L^8 is -C(=O)NH.
5. The receptor targeting conjugate of any one of claims 1-4, wherein each of L^6 and L^9 is -$(CH_2)_3$-.
6. The receptor targeting conjugate of any one of claims 1-5, wherein L^{10} is -$(CH_2)_2$-.
7. The receptor targeting conjugate of any one of claims 1-6, wherein L^{11} is -$(OCH_2CH_2)_n$-.
8. The receptor targeting conjugate of any one of claims 1-7, wherein n is 33, 34, 35, 36, 37, 38, or 39.
9. The receptor targeting conjugate of any one of claims 1-8, wherein R comprises a glycerolipid.
10. The receptor targeting conjugate of any one of claims 1-9, wherein L^{12} is -NHC(=O)-.
11. The receptor targeting conjugate of claim 1, wherein
    a. the plurality of the A groups comprises a lectin receptor targeting ligand;
    b. each of L^1, L^3, L^4, and L^7 comprises -$(CH_2)_4$-;
    c. each of L^2, L^5, and L^8 comprises -C(=O)NH-;
    d. each of L^6 and L^9 comprises -$(CH_2)_3$-;
    e. L^{10} is -$(CH_2)_{1\text{-}3}$-, -CH_2CH_2O- or -CH_2O-;
    f. L^{11} is -$(CH_2CH_2O)_n$- or -$(OCH_2CH_2)_n$-, where n is an integer selected from 1 to 50;
    g. L^{12} is -NH(CO)O-; and
    h. R is selected from the group consisting of dialkylglycerol, diacylglycerol, sterol, n-alkyl comprising C_{10}-C_{30} carbon atoms, branched alkyl comprising C_{10}-C_{30} carbon atoms or tocopherol.
12. The receptor targeting conjugate of any one of claims 1-11, wherein each of the A groups is N-acetylgalactosamine



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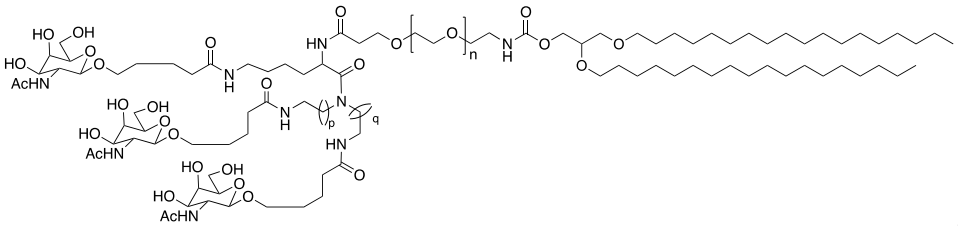
13. The receptor targeting conjugate of claim 1, wherein the receptor targeting conjugate is a conjugate selected from **1001-1019**, **1060**, **1065**, **1066** and **1075-1085** in Table 4.

14. The receptor targeting conjugate of claim 1, wherein the receptor targeting conjugate is



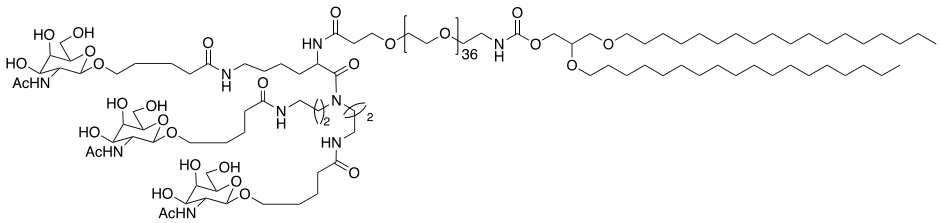
, wherein p and q are 2, and n is 34.

15. The receptor targeting conjugate of claim 1, wherein the receptor targeting conjugate is



, wherein p and q are 2, and n is 35.

16. The receptor targeting conjugate of claim 1, wherein the receptor targeting conjugate is



1004.

17. The receptor targeting conjugate of claim 1, wherein the R comprises one or more of fatty alcohols, fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, or sterols or derivatives thereof.

18. The receptor targeting conjugate of claim 1, wherein the R is a lipid nanoparticle that comprises one or more mRNA encoding one or more gene editor nuclease(s) or base editors and one or more guide RNAs.

19. A formulation comprising a nanoparticle, wherein the nanoparticle comprises the receptor targeting conjugate of any one of claims 1-17.

20. The formulation of claim 19, wherein the nanoparticle further comprises a lipid excipient selected from a sterol, a phospholipid, a stealth lipid, an amino lipid, or any combination thereof.

21. The formulation of claim 19 or 20, wherein the nanoparticle further comprises a nucleic acid active agent.
22. The formulation of claim 21, wherein the nucleic acid active agent comprises an mRNA encoding a gene editor and a guide RNA.
23. The formulation of any one of claims 19-22, wherein the amino lipid is an ionizable lipid.
24. The formulation of any one of claims 19-23, wherein the stealth lipid is a polyethylene glycol (PEG)-lipid.
25. The formulation of any one of claims 19-24, wherein the sterol is cholesterol.
26. The formulation of any one of claims 19-25, wherein the receptor targeting conjugate is present in the nanoparticle in an amount of up to 5 mol% of the total lipid content of the nanoparticle.
27. The formulation of any one of claims 19-26, wherein the receptor targeting conjugate is present in the nanoparticle in an amount of up to 2 mol% of the total lipid content of the nanoparticle.
28. The formulation of any one of claims 19-27, wherein the receptor targeting conjugate is present in the nanoparticle in an amount of up to 1 mol% of the total lipid content of the nanoparticle.
29. The formulation of any one of claims 19-28, wherein the receptor targeting conjugate is present in the nanoparticle in an amount of up to 0.5 mol% of the total lipid content of the nanoparticle.
30. The formulation of any one of claims 19-29, wherein the receptor targeting conjugate is present in the nanoparticle in an amount of up to 0.1 mol% of the total lipid content of the nanoparticle.
31. The formulation of any one of claims 19-30, wherein the receptor targeting conjugate is present in the nanoparticle in an amount of at least 0.001 mol% of the total lipid content of the nanoparticle.
32. The formulation of any one of claims 19-31, wherein the receptor targeting conjugate is present in the nanoparticle in an amount of at least 0.01 mol% of the total lipid content of the nanoparticle.
33. The formulation of any one of claims 19-32, wherein the receptor targeting conjugate is present in the nanoparticle in an amount of at least 0.05 mol% of the total lipid content of the nanoparticle.

34. The formulation of any one of claims 19-33, wherein the receptor targeting conjugate is present in the nanoparticle in an amount of at least 0.1 mol% of the total lipid content of the nanoparticle.
35. The formulation of any one of claims 19-34, wherein the receptor targeting conjugate is present in the nanoparticle in an amount of at least 0.25 mol% of the total lipid content of the nanoparticle.
36. The formulation of any one of claims 20-25,
    wherein the amino lipid comprises from 1 mol % to 65 mol % of the total lipid present in the nanoparticle,
    wherein the stealth lipid comprises from 0.1 mol % to 10 mol % of the total lipid present in the nanoparticle,
    wherein the sterol comprises from 20 mol % to 50 mol % of the total lipid present in the nanoparticle, and
    wherein the phospholipid comprises from 1 mol % to 20 mol % of the total lipid present in the nanoparticle.
37. The formulation of any one of claims 20-36,
    wherein the amino lipid comprises from 10 mol % to 60 mol % of the total lipid present in the nanoparticle,
    wherein the stealth lipid comprises from 0.1 mol% to 6 mol% of the total lipid present in the nanoparticle,
    wherein the sterol comprises about 25 mol %, about 26 mol %, about 27 mol %, about 28 mol %, about 29 mol %, about 30 mol %, about 31 mol %, about 32 mol %, about 33 mol %, about 34 mol %, about 35 mol %, about 36 mol %, about 37 mol %, about 38 mol %, about 39 mol %, about 40 mol %, about 41 mol %, about 42 mol %, about 43 mol %, about 44 mol %, about 45 mol %, about 46 mol %, about 47 mol %, about 48 mol %, or about 50 mol % of the total lipid present in the nanoparticle, and
    wherein the phospholipid comprises from 5 mol % to 15 mol % of the total lipid present in the nanoparticle.
38. The formulation of any one of claims 20-37,
    wherein the amino lipid comprises from 40 mol % to 65 mol % of the total lipid present in the nanoparticle,
    wherein the stealth lipid comprises from 0.5 mol% to 5 mol% of the total lipid present in the nanoparticle,

wherein the sterol comprises 30 mol %, about 31 mol %, about 32 mol %, about 33 mol %, about 34 mol %, about 35 mol %, about 36 mol %, about 37 mol %, about 38 mol %, about 39 mol %, about 40 mol %, about 41 mol %, about 42 mol %, about 43 mol %, about 44 mol %, about 45 mol %, about 46 mol %, about 47 mol %, about 48 mol %, or about 50 mol % of the total lipid present in the nanoparticle, and

wherein the phospholipid comprises from 8 mol % to 12 mol % of the total lipid present in the nanoparticle.

39. The formulation of any one of claims 20-38,

wherein the amino lipid comprises about 45 mol %, about 46 mol %, about 47 mol %, about 48 mol %, about 49 mol %, about 50 mol %, about 51 mol %, about 52 mol %, about 53 mol %, about 54 mol %, or about 55 mol % of the total lipid present in the nanoparticle,

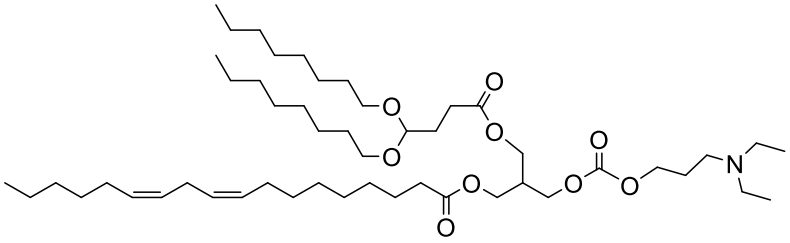
wherein the stealth lipid comprises from 1 mol % to 3 mol % of the total lipid present in the nanoparticle,

wherein the sterol comprises from about 35 mol %, about 36 mol %, about 37 mol %, about 38 mol %, about 39 mol %, about 40 mol %, about 41 mol %, about 42 mol %, about 43 mol %, about 44 mol %, about 45 mol %, about 46 mol %, about 47 mol %, about 48 mol %, or about 50 mol % of the total lipid present in the nanoparticle, and

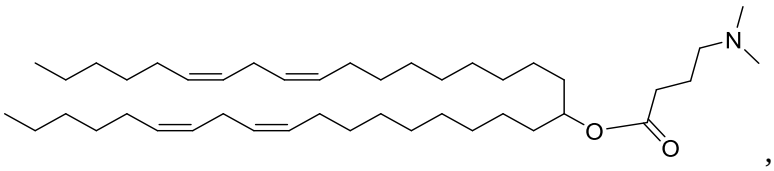
wherein the phospholipid comprises about 9 mol %, about 10 mol %, or about 11 mol % of the total lipid present in the nanoparticle.

40. The formulation of any one of claims 20-39,

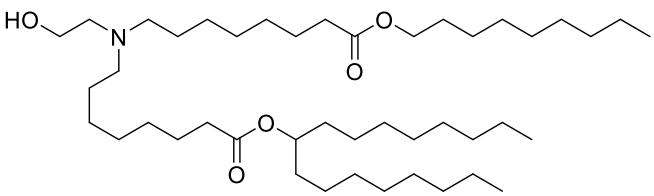
wherein the amino lipid is selected from the group consisting of



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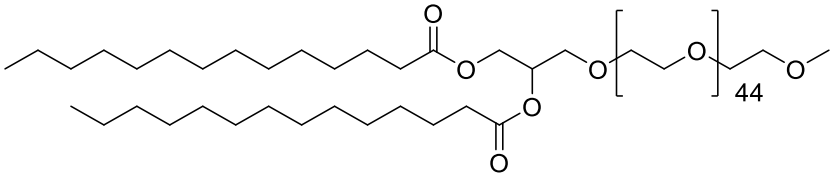


, and

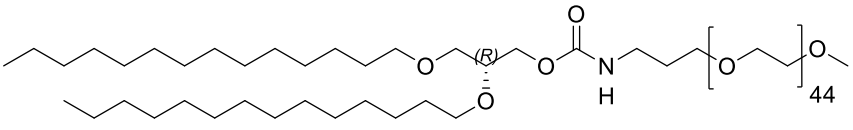
any combination thereof,

wherein the amino lipid comprises about 48 mol %, about 49 mol %, about 50 mol %, about 51 mol %, or about 52 mol % of the total lipid present in the nanoparticle,

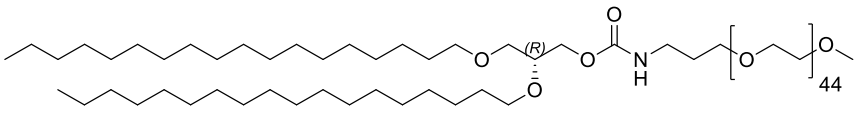
wherein the stealth lipid is selected from the group consisting of



,



,



, and

any combination thereof,

wherein the stealth lipid comprises from about 2.0 mol %, about 2.1 mol %, about 2.2 mol %, about 2.3 mol %, about 2.4 mol %, about 2.5 mol %, about 2.6 mol %, about 2.7 mol %, about 2.8 mol %, about 2.9 mol %, or about 3.0 mol % of the total lipid present in the nanoparticle,

wherein the sterol comprises about 35 mol %, about 36 mol %, about 37 mol %, about 38 mol %, about 39 mol %, about 40 mol %, or about 41 mol % of the total lipid present in the nanoparticle, and

wherein the phospholipid is 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), wherein the phospholipid comprises about 9 mol % of the total lipid present in the nanoparticle.

41. The formulation of any one of claims 22-40, wherein the ratio of the guide RNA to the mRNA is 10:1 to 1:10 by weight.

42. The formulation of any one of claims 22-40, wherein the ratio of the guide RNA to the mRNA is 5:1 to 1:5 by weight.

43. The formulation of any one of claims 22-40, wherein the ratio of the guide RNA to the mRNA is 3:1 to 1:3 by weight.

44. The formulation of any one of claims 22-40, wherein the ratio of the guide RNA to the mRNA is 2:1 to 1:2 by weight.

45. The formulation of any one of claims 22-40, wherein the ratio of the guide RNA to the mRNA is about 1:1 by weight.

46. The formulation of any one of claims 22-45, wherein the mRNA is an adenosine base editor and the guide RNA is complementary to (i) a segment of PCSK9 gene, (ii) a segment of ANGPTL3 gene, or both (i) and (ii).

47. The formulation of any one of claims 22-46,wherein the guide RNA is selected from guide RNA sequences of SEQ ID NOs: 121-125 of Table 5.

48. Use of a formulation of any one of claims 19-47, in the manufacture of a medicament for reducing the risk of coronary disease in a subject in need thereof.

49. The use of claim 48, wherein the nanoparticle comprising the receptor targeting conjugate provides an improved delivery in a LDLr deficient mammal compared to a corresponding nanoparticle without the receptor targeting conjugate.

50. The use of claim 49, wherein the improved delivery is at least 50% higher than the corresponding nanoparticle without the receptor targeting conjugate as determined by percent editingn

51. The use of claim 48, wherein the nanoparticle comprising the receptor targeting conjugate provides an improved delivery in a mammal that lacks ApoE compared to a corresponding nanoparticle without the receptor targeting conjugate .

52. The use of claim 51, wherein the improved delivery is at least 50% higher than the corresponding nanoparticle without the receptor targeting conjugate as determined by percent editing.

53. A method of preparing a formulation of any one of claims 21-45, the method comprising:

a. providing a first solution comprising the nucleic acid active agent in aqueous buffer;

b. providing a second solution comprising (i) the lipid excipient and (ii) at least a portion of the receptor targeting conjugate in a water-miscible organic solvent;

c. mixing the first solution and the second solution;

d. incubating a mixture of the first and second solutions to form nanoparticles; and

e. optionally carrying out one or more dilution, buffer exchange, concentration, filtration, and nanoparticle evaluation processes.

54. The method of claim 53, wherein the mixing is performed by an inline mixing apparatus having a first mixing chamber that includes a first port that separately introduces the first solution to the first mixing chamber and a second port that separately and simultaneously introduces the second solution into the first mixing chamber.

55. The method of claim 54, further comprising adding a second portion of the receptor targeting conjugate after the first solution and the second solution are mixed, wherein the addition of the second portion of the receptor targeting conjugate is pre-dissolved in a water miscible organic solvent and combined with an aqueous solution to form an aqueous dilution buffer that is mixed with the previously mixed first and second solutions in a second mixing chamber conjoined with and downstream from the first mixing chamber of the inline mixing apparatus prior to incubation.

56. The method of claim 55, further comprising a buffer exchange process after the second portion of the receptor targeting conjugate is added.

57. A method of preparing a formulation of any one of claims 21-45, the method comprising:

a. providing a first solution comprising the nucleic acid active agent in an aqueous buffer;

b. providing a second solution comprising at least a portion of the lipid excipient in a water-miscible organic solvent;

c. providing a third solution comprising at least a portion of the receptor targeting conjugate;
d. mixing the first solution, the second solution, and the third solution in one or more mixing chambers wherein each solution is introduced separately via an inlet port to the one or more mixing chambers;
e. incubating a mixture of the first, second and third solutions to form nanoparticles; and
f. optionally carrying out one or more dilution, a buffer exchange, concentration, filtration, and nanoparticle evaluation processes.

58. The method of claim 57, wherein the first solution comprises an aqueous buffer, and wherein the second solution and the third solution are each independently prepared from a water-miscible alcohol.

59. The method of claim 57, wherein the first solution, the second solution, and the third solution are introduced to a mixer simultaneously.

60. The method of claim 57, wherein the first solution, the second solution, and the third solution are introduced to a mixer sequentially prior to incubation.

61. The method of claim 57, wherein the first solution,the second solution, and the third solution are combined in an in-line mixer apparatus having a first mixing chamber conjoined to a second downstream mixing chamber, wherein the first and second solutions are pre-mixed in the first mixing chamber and immediately flow into a second mixing chamber and wherein the third solution is mixed with the first and second solution in the second mixing chamber; and wherein the third solution may optionally comprise a targeting conjugate and/or lipid excipients pre-dissolved in a water-miscible organic solvent mixed in or diluted with an aqueous dilution buffer.

62. The method of claim 57, wherein the water-miscible organic solvent is ethanol.

63. A formulation of any one of claims 19-47, for use in therapy.