POLYNUCLEIC ACID MOLECULES FOR INHIBITING EXPRESSION OF LP(A) OR APOC3, PHARMACEUTICAL COMPOSITIONS, AND USES THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/549,870, filed on February 5, 2024, and U.S. Provisional Application No. 63/703,100, filed on October 3, 2024, each of which is incorporated herein by references in its entirety.

BACKGROUND OF THE DISCLOSURE

[0002] The discovery of RNA interference (RNAi) as a cellular mechanism that selectively degrades mRNAs allows for both the targeted manipulation of cellular phenotypes in cell culture and the potential for development of directed therapeutics (Behlke, 2006, Mol. Ther. 13, 644-670; Xie et al., 2006, Drug Discov. Today 11, 67-73).

[0003] Lipoprotein(a) (Lp(a)) is a lipoprotein composed of an LDL (low-density lipoprotein) lipid core, apolipoprotein B (apo B), and a unique apolipoprotein, apolipoprotein(a) (apo(a)). Lp(a) is considered one of the most common independent genetically inherited causal risk factors for cardiovascular disease (CVD). Accordingly, there is a need for developing an effective Lp(a) inhibitor. The polynucleic acid molecules, conjugates thereof, and methods described herein satisfy this need and provide related advantages.

[0004] Apolipoprotein C3 (APOC3) is a protein encoded by the APOC3 gene. APOC3 may be secreted by the liver as well as the small intestine and may play an important role in inhibiting hepatic uptake of triglyceride-rich particles. Therefore, high plasma APOC3 correlates with high glyceride and an increased risk of developing cardiovascular diseases. Accordingly, there is a need for developing an effective APOC3 inhibitor without cytotoxicity. The polynucleic acid molecules, conjugates thereof, and methods described herein satisfy this need and provide related advantages.

INCORPORATION BY REFERENCE

[0005] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. To the extent publications and patents or patent applications incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

SUMMARY OF THE DISCLOSURE

[0006] Disclosed herein, in certain aspects, is a polynucleic acid molecule for modulating expression of lipoprotein(a) (Lp(a)) gene, comprising a guide strand and a passenger strand, wherein the guide strand comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from UCUCUGUCCAUUACCGUGGUAGC (SEQ ID NO: 1) or UUGCGUCUGAGCAUUGUGUCAGG (SEQ ID NO: 2). In some instances, the guide strand comprises a nucleotide analogue selected from a group consisting of the nucleotide analogue is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f); 2'-deoxythymidine-3'-phosphate (dT), and 2'-deoxy-2-thiothymidine-3'-phosphate (dT3). In some cases, the guide strand comprises a nucleic acid sequence selected from SEQ ID NOs: 1-13.

[0007] In some cases, the passenger strand comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 14-20. In some embodiments, the passenger strand comprises a nucleic acid sequence selected from SEQ ID NOs: 14-20. In some embodiments, the passenger strand comprises a nucleic acid sequence of UACCACGGUAAUGGACAGAGA (SEQ ID NO: 14) and a guide strand comprises a nucleic acid sequence of UCUCUGTCCAUUACCGUGGUAGC (SEQ ID NO: 6) or UCUCUGUCCAUUACCGUGGUAGC (SEQ ID NO: 1).

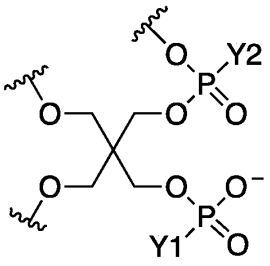
[0008] In some cases, the nucleotide analogue is located at the seed region of the guide strand (positions 2-8), or positions 11-13, from the 5' end of the guide strand. In some cases, the nucleotide analogue is located at any one of positions 4-8, or position 12, from the 5' end of the guide strand. In some cases, the nucleotide analogue is located at any one of positions 5-8, or position 12, from the 5' end of the guide strand. In some instances, the guide strand comprises a nucleic acid sequence selected from SEQ ID NOs: 21-71. In some instances, the guide strand comprises a nucleic acid sequence selected from SEQ ID NOs: 26-27.

[0009] In some embodiments, the passenger strand comprises 2-amino-2'-O-methyladenosine-3'-phosphate substituting at least one of adenine nucleotides. In some cases, the passenger strand comprises 2-amino-2'-O-methyladenosine-3'-phosphate at the location complementary to the seed region of the guide strand. In some cases, the 2-amino-2'-O-methyladenosine-3'-phosphate is

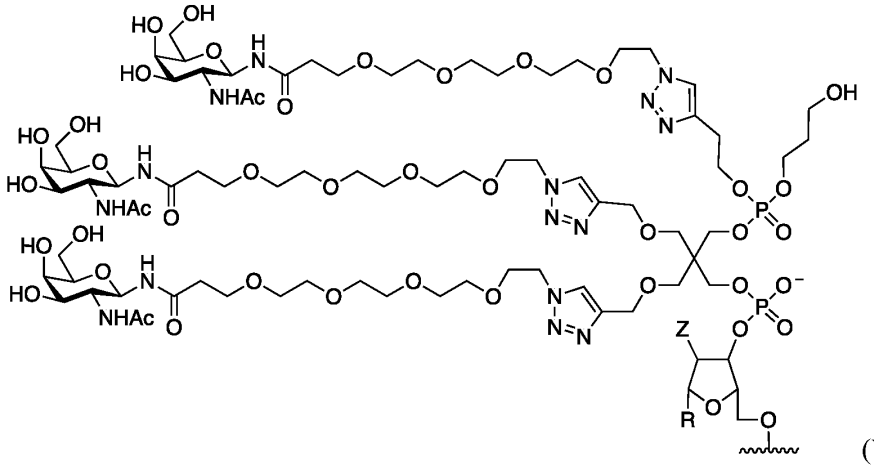
located at any one of positions 4-8 form the 3' end of the passenger strand. In some cases, the passenger strand comprises a nucleic acid sequence selected from SEQ ID NOs: 72-91.

[0010] In some instances, the guide strand comprises a nucleic acid sequence of usCfsucug(T-T)ccauUfaCfcGfugguasgsc (SEQ ID NO: 26) and a passenger strand comprises a nucleic acid sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72). In some instances, the guide strand comprises a nucleic acid sequence of usCfsucugUfccauu3aCfcGfugguasgsc (SEQ ID NO: 27) and a passenger strand comprises a nucleic acid sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72).

[0011] In another aspect, provided herein is a polynucleic acid molecule conjugate for modulating expression of lipoprotein(a) gene (Lp(a)), wherein the polynucleic acid molecule conjugate comprises a polynucleic acid molecule described herein and an asialoglycoprotein receptor targeting moiety. In some cases, the asialoglycoprotein receptor targeting moiety comprises N-Acetylgalactosamine (GalNAc) or galactose. In some cases, the GalNAc comprises an anomeric carbon bonded to trivalent, tetravalent linker, pentavalent, or hexavalent linker, wherein the anomeric carbon is part of a hemiaminal group.

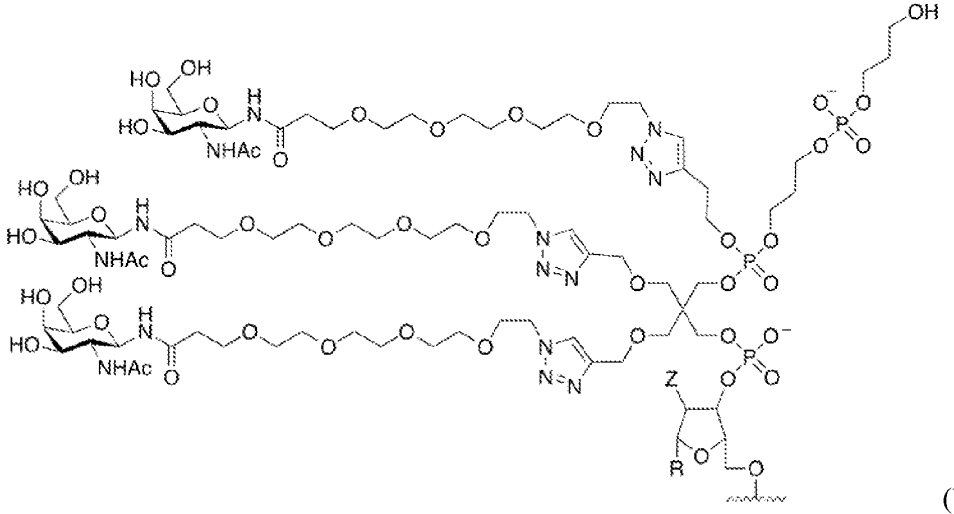
[0012] In some cases, the polynucleic acid molecule and the asialoglycoprotein receptor targeting moiety is coupled via a linker. In some cases, the linker is a cleavable linker. In some cases, the linker comprises formula (IV) below,  wherein at least one of Y1 and Y2 is a nucleotide in the polynucleic acid molecule. In some cases, the Y1 is the last nucleotide on the 3' end of the sense strand of the polynucleic acid molecule.

[0013] In some cases, the linker and the asialoglycoprotein receptor targeting moiety with the last nucleotide on the 3' end of the sense strand of the polynucleic acid molecule are shown in Formula (V'), Formula (V''''), Formula (V'''''), or Formula (V''''''):



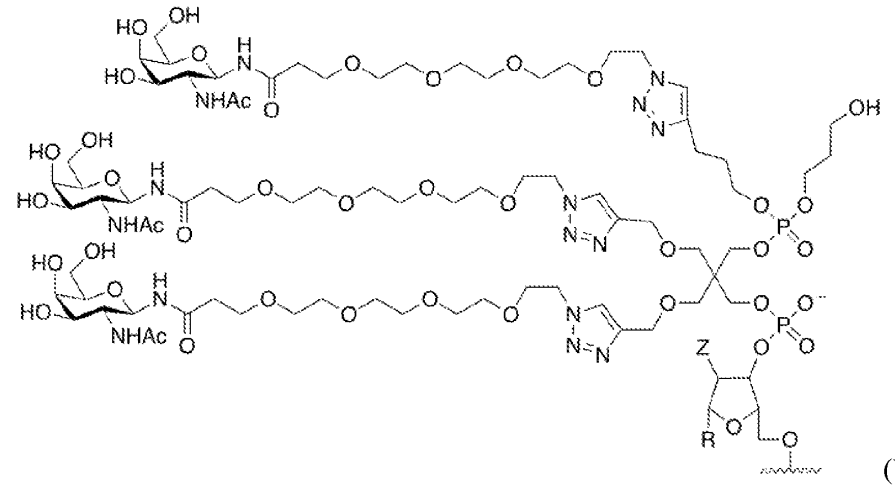
(V'),

wherein Z in formula (V') is -H, -OH, -O-Methyl, -F, or -O-methoxyethyl and R in formula (V') is adenine, uracil, guanine, cytosine, thymine, abasic, or others;



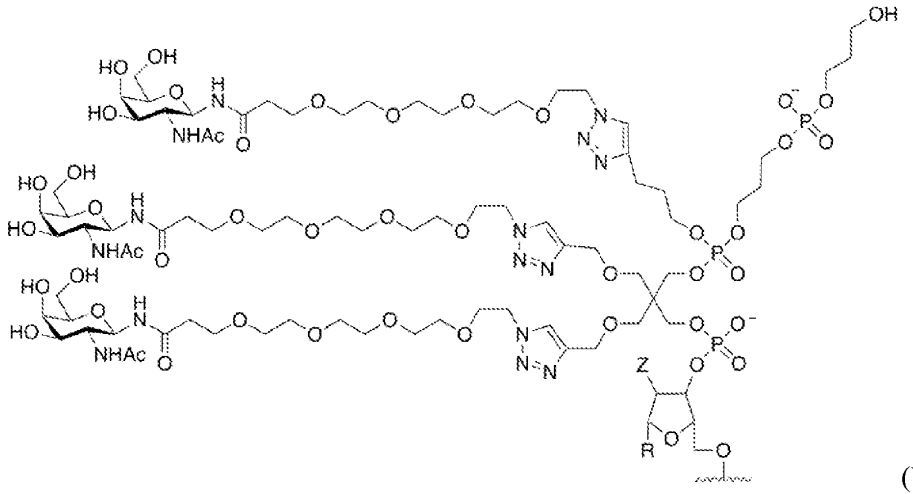
(V''''),

wherein Z in formula (V'''') is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V'''') is adenine, uracil, guanine, cytosine, thymine, abasic, or others;



(V’’’’’),

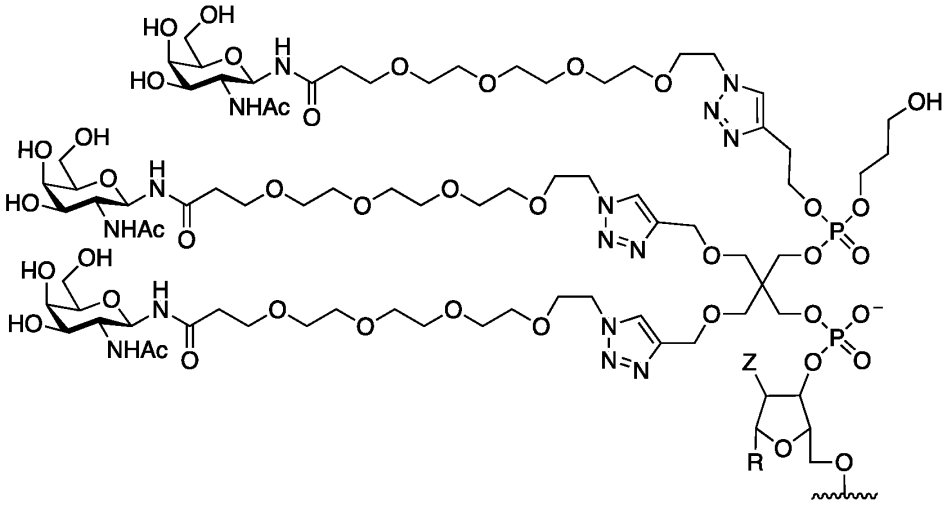
wherein Z in formula (V’’’’’) is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V’’’’’) is adenine, uracil, guanine, cytosine, thymine, abasic, or others; or



(V’’’’’’),

wherein Z in formula (V’’’’’’) is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V’’’’’’) is adenine, uracil, guanine, cytosine, thymine, abasic, or others.

[0014] In some cases, the linker and the asialoglycoprotein receptor targeting moiety with the last nucleotide on the 3’ end of the sense strand of the polynucleic acid molecule are shown in Formula (V’):



(V'), wherein Z in formula (V') is -H, -OH, -O-Methyl, -F, or -O-methoxyethyl and R in formula (V') is adenine, uracil, guanine, cytosine, thymine, abasic, or others.

[0015] In another aspect, provided herein is a pharmaceutical composition comprising a polynucleic acid molecule described herein or a polynucleic acid molecule conjugate described herein, and a pharmaceutically acceptable excipient. In some cases, the pharmaceutical composition is formulated for intravenous, subcutaneous, parenteral, oral, intranasal, buccal, rectal, transdermal, or intrathecal administration.

[0016] In another aspect, provided herein is method of modulating expression of lipoprotein(a) (Lp(a)) gene in a subject in need thereof, comprising administering to the subject a polynucleic acid molecule described herein, a polynucleic acid molecule conjugate described herein, or a pharmaceutical composition described herein, thereby modulating the expression of Lp(a) gene in the subject in need thereof.

[0017] In another aspect, provided herein is a method for treating or preventing a cardiovascular disease or a lipid disorder, comprising administering to a subject having or being diagnosed with a cardiovascular disease or a lipid disorder, or suffering from a symptom of a cardiovascular disease or a lipid disorder, or being suspected to develop a cardiovascular disease or a lipid disorder or a symptom thereof, a polynucleic acid molecule described herein, a polynucleic acid molecule conjugate described herein, or a pharmaceutical composition described herein, thereby treating or preventing a cardiovascular disease or a lipid disorder in the subject. In some cases, the cardiovascular disease is coronary artery disease, acute myocardial infarction, asymptomatic carotid atherosclerosis, stroke, atrial fibrillation, hypercholesterolemia, or peripheral artery occlusive disease. In some cases, the lipid disorder is hyperlipidemia or hypercholesterolemia.

[0018] In some cases, the polynucleic acid molecule is administered at a dose sufficient to decrease the expression of Lp(a) gene in a cell of said subject or to decrease plasma Lp(a) level of said

subject by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to a control.

[0019] Also provided herein, in certain aspect, is a polynucleic acid molecule for modulating expression of apolipoprotein C3 (APOC3) gene, comprising a guide strand and a passenger strand, wherein the guide strand comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 101-103. In some cases, the guide strand comprises a nucleotide analogue selected from a group consisting of acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn). In some cases, the guide strand comprises a nucleic acid sequence selected from SEQ ID NOs: 104-106.

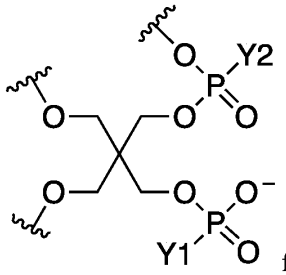
[0020] In some embodiments, the passenger strand comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 107-112. In some cases, the passenger strand comprises a nucleic acid sequence selected from SEQ ID NOs: 107-112. In some cases, the passenger strand comprises a nucleic acid sequence selected from SEQ ID NOs: 110-112.

[0021] In some cases, the nucleotide analogue is located at the seed region of the guide strand (positions 2-8) from the 5' end. In some cases, the nucleotide analogue is located at any one of positions 4-8 from the 5' end of the guide strand. In some cases, the nucleotide analogue is located at any one of positions 5-8 from the 5' end of the guide strand. In some cases, the guide strand comprises a nucleic acid sequence selected from SEQ ID NOs: 113-121.

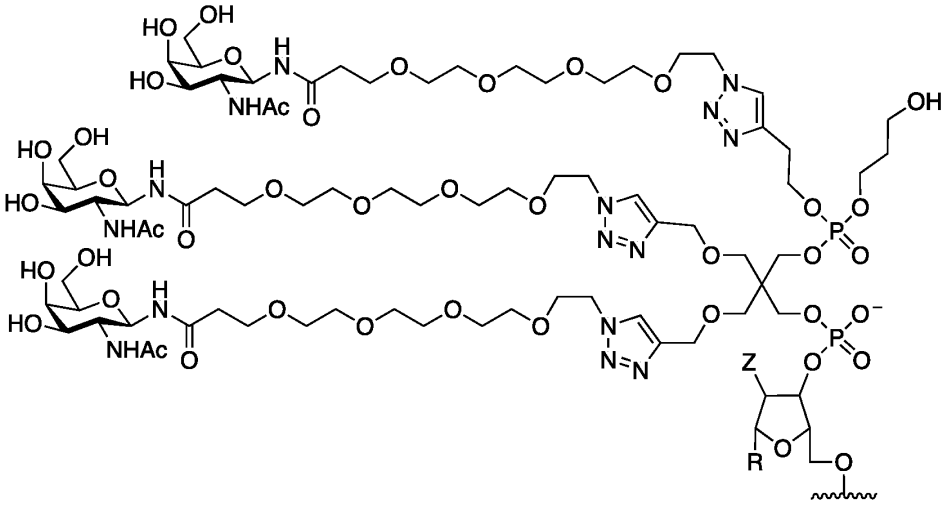
[0022] In some cases, the passenger strand comprises 2-Amino-2'-O-methyladenosine-3'-phosphate substituting at least one of adenine nucleotides. In some cases, the passenger strand comprises 2-Amino-2'-O-methyladenosine-3'-phosphate at the location complementary to the seed region of the guide strand. In some instances, the 2-Amino-2'-O-methyladenosine-3'-phosphate is located at any one of positions 4-8 form the 3' end of the passenger strand. In some cases, the passenger strand comprises a nucleic acid sequence selected from SEQ ID NOs: 122-127.

[0023] In another aspect, provided herein is a polynucleic acid molecule conjugate for modulating expression of apolipoprotein C3 (APOC3) gene, wherein the polynucleic acid molecule conjugate comprises a polynucleic acid molecule described herein and an asialoglycoprotein receptor targeting moiety. In some cases, the asialoglycoprotein receptor targeting moiety comprises N-Acetylgalactosamine (GalNAc) or galactose.

[0024] In some cases, the polynucleic acid molecule and the asialoglycoprotein receptor targeting moiety is coupled via a linker. In some cases, the linker comprises formula (IV) below,

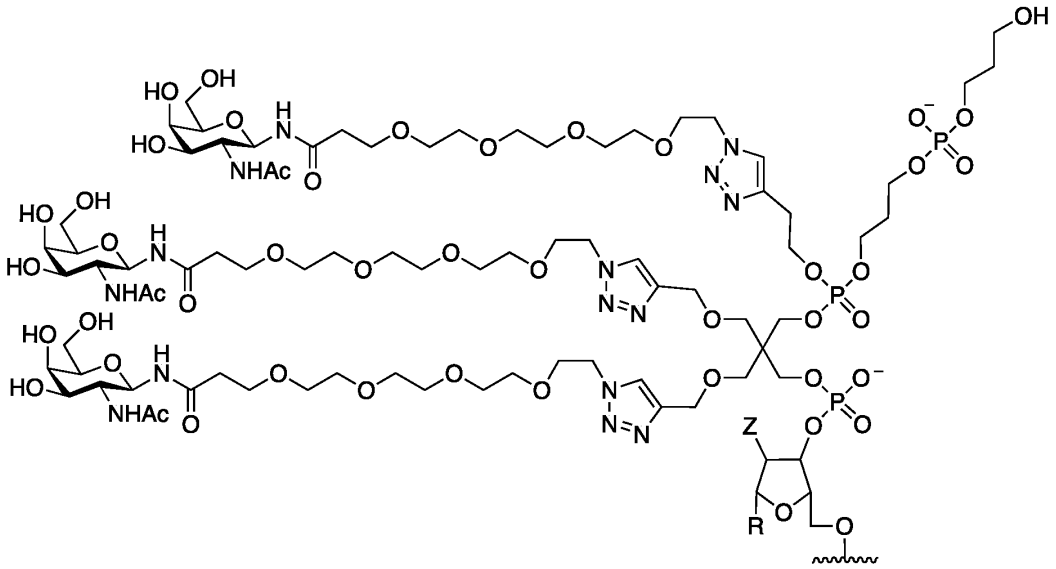


formula (IV), wherein at least one of Y1 and Y2 is a nucleotide in the polynucleic acid molecule. In some cases, the Y1 is the last nucleotide on the 3' end of the sense strand of the polynucleic acid molecule or the Y1 and Y2 are two consecutive nucleotides in the polynucleic acid molecule. In some instances, the linker and the asialoglycoprotein receptor targeting moiety with the last nucleotide on the 3' end of the sense strand of the polynucleic acid molecule are shown in Formula (V'):



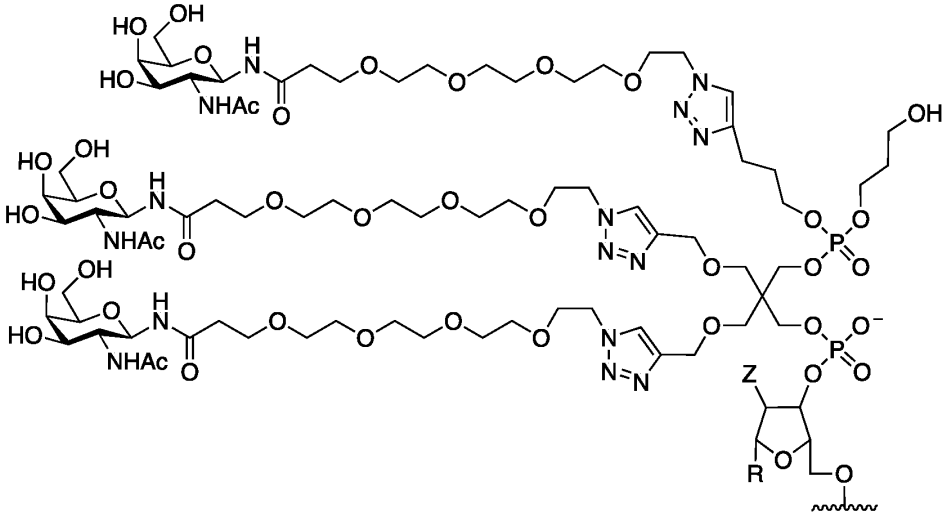
(V'), wherein Z in formula (V') is -H, -OH, -O-Methyl, -F, or -O-methoxyethyl and R in formula (V') is adenine, uracil, guanine, cytosine, thymine, abasic, or others.

[0025] In some instances, the linker and the asialoglycoprotein receptor targeting moiety with the last nucleotide on the 3' end of the sense strand of the polynucleic acid molecule are shown in Formula (V''''):



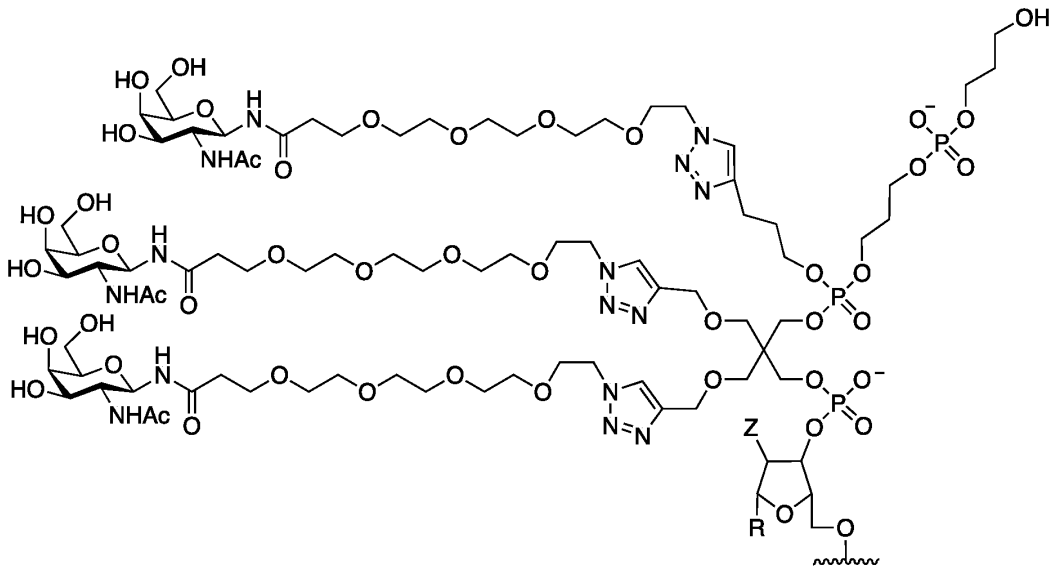
(V’’’’), wherein Z in formula (V’’’’) is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V’’’’) is adenine, uracil, guanine, cytosine, thymine, abasic, or others.

[0026] In some instances, the linker and the asialoglycoprotein receptor targeting moiety with the last nucleotide on the 3’ end of the sense strand of the polynucleic acid molecule are shown in Formula (V’’’’’):



(V’’’’’), wherein Z in formula (V’’’’’) is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V’’’’’) is adenine, uracil, guanine, cytosine, thymine, abasic, or others.

[0027] In some instances, the linker and the asialoglycoprotein receptor targeting moiety with the last nucleotide on the 3' end of the sense strand of the polynucleic acid molecule are shown in Formula (V''''''):



(V''''''), wherein Z in formula (V''''''') is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V''''''') is adenine, uracil, guanine, cytosine, thymine, abasic, or others.

[0028] In another aspect, provided herein is a pharmaceutical composition comprising a polynucleic acid molecule described herein or a polynucleic acid molecule conjugate described herein, and a pharmaceutically acceptable excipient.

[0029] In some cases, the pharmaceutical composition is formulated as a nanoparticle formulation. In some cases, the pharmaceutical composition is formulated for intravenous, subcutaneous, parenteral, oral, intranasal, buccal, rectal, transdermal, or intrathecal administration.

[0030] In another aspect, provided herein is a method of modulating expression of apolipoprotein C3 (APOC3) gene in a subject, comprising administering to the subject a polynucleic acid molecule described herein or a polynucleic acid molecule conjugate described herein, or a pharmaceutical composition described herein, thereby modulating the expression of APOC3 gene in the subject.

[0031] In another aspect, provided herein is a method of modulating triglyceride level in a subject in need thereof, comprising administering to the subject a polynucleic acid molecule described herein or a polynucleic acid molecule conjugate described herein, or a pharmaceutical composition described herein, thereby modulating triglyceride level in the subject.

[0032] In another aspect, provided herein is a method for treating or preventing a cardiovascular disease or a lipid disorder, comprising administering to a subject, having or being diagnosed with a

cardiovascular disease or a lipid disorder, or suffering from a symptom of a cardiovascular disease or a lipid disorder, or being suspected to develop a cardiovascular disease or a lipid disorder or a symptom thereof, a polynucleic acid molecule described herein or a polynucleic acid molecule conjugate described herein, or a pharmaceutical composition described herein, thereby treating or preventing a cardiovascular disease or a lipid disorder.

[0033] In some cases, the polynucleic acid molecule is administered at a dose sufficient to decrease the expression of APOC3 gene in a cell of said subject or to decrease plasma APOC3 level of said subject by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to a control.

[0034] In some cases, the subject in need thereof is diagnosed, suffers from, or having a symptom of cardiovascular disease, wherein the cardiovascular disease is coronary artery disease, acute myocardial infarction, asymptomatic carotid atherosclerosis, stroke, atrial fibrillation, hypercholesterolemia, or peripheral artery occlusive disease. In some instances, the lipid disorder is hyperlipidemia, hypertriglyceridemia, or hypercholesterolemia.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] Various aspects of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative aspects, in which the principles of the disclosure are utilized, and the accompanying drawings below.

[0036] FIG. 1 depicts the in vivo efficacy of modified siRNAs conjugated with a GalNAc targeting Lp(a) mRNA in cynomolgus monkeys at a dosage of 1 mg/kg. The results are shown as % change in serum Lp(a) protein levels relative to the pre-dose (day 1) timepoint. The results correspond to the data in **Table 6**.

[0037] FIG. 2 depicts the in vivo efficacy of modified siRNAs conjugated with a GalNAc targeting Lp(a) mRNA in cynomolgus monkeys at a dosage of 0.5 mg/kg. The results are shown as % change in serum Lp(a) protein levels relative to the pre-dose (day 1) timepoint. The results correspond to the data in **Table 7.**

[0038] FIG. 3 depicts the in vivo efficacy of modified siRNAs conjugated with a GalNAc targeting Lp(a) mRNA in cynomolgus monkeys at a dose of 0.5 mg/kg. The results are shown as % change in serum Lp(a) protein levels relative to the pre-dose (day 1) timepoint. The results correspond to the data in **Table 8**.

[0039] FIG. 4 depicts the in vivo efficacy of modified siRNAs targeting APOC3 mRNA in transgenic mice at a dosage of 0.5 mg/kg. The results are shown as plasma APOC3 protein levels relative to the vehicle group. The results correspond to the data in **Table 9**.

[0040] FIG. 5 depicts the in vivo efficacy of modified siRNAs conjugated with a GalNAc targeting Lp(a) mRNA in cynomolgus monkeys. The results are shown as % change in serum Lp(a) protein levels relative to the pre-dose (day 1) timepoint. The results correspond to the data in **Table 10**.

[0041] FIG. 6 depicts volcano plots of differentially expressed genes (DEGs) obtained from in vitro RNAseq results upon siRNAs treatment.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0042] Lp(a) gene (LPA) is located on chromosome 6q26-q27 is the major gene locus for Lp(a) concentrations in all populations (F. Kronenberg et al., J Intern Med, 273 (1) (2013)). LPA gene is one of the strongest monogenic risk factors for CVD (S. Tsimikas, J Am Coll Cardiol, 69 (6) (2017)). The LPA gene is highly expressed in liver with dramatic decrease as well as increase in Lp(a) observed after liver transplant (A.A. Damluji et al., Journal of clinical lipidology, 10 (2) (2016)).

[0043] Lipoprotein(a) (Lp(a)) is a low-density lipoprotein-like particle formed by the association of apo(a) with apolipoprotein B (apo B). The apo(a) protein is covalently linked to apo B in the assembled Lp(a) particle via a disulfide bond. Lp(a) carries atherosclerosis-causing cholesterol and binds atherogenic pro-inflammatory oxidized phospholipids as a preferential carrier of oxidized phospholipids in human plasma, which facilitate inflammation reactions.

[0044] The plasma level of Lp(a) is primarily determined by the LPA gene encoding apo(a) (S. Tsimikas, J Am Coll Cardiol, 69 (6) (2017). The Lp(a) level varies between individuals and is directly proportional to CVD risk. Elevated plasma levels of Lp(a) are associated with increased risk for atherosclerosis and its manifestations, which may include hypercholesterolemia (Seed et al., N. Engl. J. Med., 1990, 322, 1494-1499), myocardial infarction (Sandkamp et al., Clin. Chem., 1990, 36, 20-23), and thrombosis (Nowak-Gottl et al., Pediatrics, 1997, 99, E11). Therefore, inhibition of LPA is viewed as a potential therapeutic strategy to treat CVD.

[0045] APOC3 is a small protein with 79 amino acid residues that contains two amphipathic helices (*see e.g.*, Gangabadage et al., J Biol Chem. 2008;283(25):17416–17427). APOC3 is found to be on circulating lipoproteins including high-density lipoproteins (HDL), low-density lipoprotein (LDL), and triglyceride-rich lipoproteins (TRLs) such as chylomicrons (CM) and very low-density lipoprotein (VLDL).

[0046] APOC3 is an important regulator in lipid (e.g., triglyceride) metabolism and cardiovascular diseases (pathological processes involved in atherosclerosis). Specifically, studies have shown that impaired catabolism of TRLs is linked to increased levels of plasma APOC3 (*see e.g.*, Boren et al., Arterioscler Thromb Vasc Biol. 2015;35(10):2218–2224). In addition to effects on lipid metabolism, APOC3 has been shown to directly influence development of atherosclerosis.

[0047] A lifelong deficiency of APOC3 may be cardioprotective. A reduction of plasma triglycerides by inhibition of APOC3 might be a promising strategy in management of severe hypertriglyceridemia or those with elevated plasma triglyceride.

[0048] Described herein are polynucleic acid molecules for modulating expression of Lp(a) gene and polynucleic acid molecules for modulating expression of APOC3 gene. In some instances, polynucleic acid molecules for modulating expression of Lp(a) gene comprise a nucleic acid sequence selected from Table 1, Table 2, Table 3, or Table 4. In some instances, polynucleic acid molecules for modulating expression of APOC3 gene comprise a nucleic acid sequence disclosed in Table 5.

[0049] Also described herein is a method of modulating expression of Lp(a) mRNA or protein in a subject. Described further herein is a method of modulating LDL and/or cholesterol in a subject in need thereof. Also described herein is a method of modulating expression of APOC3 mRNA or protein in a subject. Described further herein is a method of modulating triglyceride in a subject in need thereof.

Definitions

[0050] The singular form “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes one or more cells, including mixtures thereof. “A and/or B” is used herein to include all of the following alternatives: “A”, “B”, “A or B”, and “A and B.”

[0051] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

[0052] Certain ranges are presented herein with numerical values being preceded by the term "about." The term "about" is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number.

[0053] "Percent (%) sequence identity" or "Percent (%) identity" with respect to the nucleic acid sequences identified herein is defined as the percentage of nucleic acid in a candidate sequence that are identical with the nucleic acid sequence being compared, after aligning the sequences considering any conservative substitutions as part of the sequence identity.

[0054] All ranges disclosed herein also encompass any and all possible sub-ranges and combinations of sub-ranges thereof. Any listed range can be recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, and so forth. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, and the like. As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like include the number recited and refer to ranges which can be subsequently broken down into sub-ranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 articles refers to groups having 1, 2, or 3 articles. Similarly, a group having 1-5 articles refers to groups having 1, 2, 3, 4, or 5 articles, and so forth.

[0055] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the polynucleic acid molecules, the polynucleic acid molecule conjugates, the pharmaceutical compositions, the methods, and other aspects belong.

[0056] As used herein, the term "complementary" indicates a sufficient degree of complementarity between two nucleic acid molecules that bind stably and specifically to avoid nonspecific binding.

[0057] As used herein, the term "polynucleic acid" and the term "polynucleotide" are interchangeably used to refer a chain of nucleotides. The term "nucleotide" includes a sequence "G," "C," "A," "T" and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base. In some instances, the "nucleotide" can refer to a modified nucleotide (e.g., with modified sugar moiety, modified base, modified internucleotide linkage, or combination thereof, including, but not limited to 2'-modified nucleotide, LNA, ENA, BNA, UNA, GNA etc.) In some instances, the "nucleotide" can refer to a modified nucleotide with a non-

canonical base (e.g., including, but not limited to, 2-thiouridine, 2-thiothymidine, inosine, 2-aminopurine, 2,6-diaminopurine, dihydrouridine, 4-thiouridine, 4-thiothymidine, 2-thiocytidine).

[0058] As used herein, a "subject" can be any mammal, including a human and a non-human primate.

[0059] A "subject in need thereof," refers to a subject having a Lp(a) related disorder or symptoms thereof, including but not limited to, CVD or a lipid disorder, or a subject having an increased risk of developing Lp(a) related disorder or symptoms thereof, including but not limited to, a CVD or lipid disorder relative to the population at large. In some instances, a subject in need thereof has coronary heart disease or atherosclerosis, or symptoms thereof. In some instances, a subject in need thereof has hyperlipidemia or hypercholesterolemia, or symptoms thereof. In some instances, a subject in need thereof is being administered or has been administered a drug different from the polynucleic acid molecules or conjugates disclosed herein, for treating or preventing a CVD or lipid disorder. For example, a subject in need thereof is being administered or has been administered atorvastatin.

[0060] The term "condition," as used herein, includes diseases, disorders, and susceptibilities. In some cases, the condition is an Lp(a) related disorder, atherosclerotic vascular disease, or symptoms thereof. In some cases, the condition is a hypertriglyceridemia or symptoms thereof.

[0061] The term "atherosclerosis" or "atherosclerotic vascular disease," as used herein, refers to a disease in which the inside of an artery narrows due to the buildup of plaque. In some instances, it may result in coronary artery disease, stroke, peripheral artery disease, or kidney problems.

[0062] The term "cardiovascular disease" or "CVD" refers to all types of diseases that affect the heart or blood vessels, including coronary heart disease (clogged arteries), which can cause heart attacks, stroke, congenital heart defects and peripheral artery disease. A CVD or symptoms thereof can include myocardial infarction, stroke, atrial fibrillation, or calcific aortic valve stenosis. A CVD or symptoms thereof include cardiac arrest or peripheral arterial disease.

[0063] The term "lipid disorder" refers to a disorder or a condition that increase levels of LDLs, triglycerides, or both. For example, the lipid disorder can be hyperlipidemia or hypercholesterolemia.

[0064] The term "low-density lipoprotein (LDL)," as used herein, refers to a microscopic blob made up of an outer rim of lipoprotein and a cholesterol center. LDL can have a highly hydrophobic core composed of a polyunsaturated fatty acid known as linoleate and hundreds to thousands esterified and unesterified cholesterol molecules. The core of LDL can also carry triglycerides and other fats and can be surrounded by a shell of phospholipids and unesterified cholesterol.

[0065] As used herein, the term "treat," "treating" or "treatment" of any disease or disorder refers, in one instance, to ameliorating the disease or disorder (i.e., slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In another instance, "treat," "treating" or "treatment" refers to alleviating or ameliorating at least one physical parameter including those which may not be discernible by the patient. In yet another instance, "treat," "treating" or "treatment" refers to modulating the disease or disorder, either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both.

[0066] The terms "prevent," "preventing," and "prevention," as used herein, refer to a decrease in the occurrence of pathology of a condition in a subject, who does not have, but is at risk of or susceptible to developing a disease or condition. The prevention may be complete, e.g., the total absence of pathology of a condition in a subject. The prevention may also be partial, such that the occurrence of pathology of a condition in a subject is less than that which would have occurred without the present disclosure.

[0067] "Administering" and its grammatical equivalents as used herein can refer to providing pharmaceutical compositions described herein to a subject or a patient. Conventional methods, known to those of ordinary skill in the art of medicine, can be used to administer the composition to the subject, depending upon the type of disease to be treated or the site of the disease. For example, the composition can be administered, *e.g.*, orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally, via an implanted reservoir, or via infusion. One or more such routes can be employed.

[0068] The terms "pharmaceutical composition" and its grammatical equivalents as used herein can refer to a mixture or solution comprising a therapeutically effective amount of an active pharmaceutical ingredient together with one or more pharmaceutically acceptable excipients, carriers, and/or a therapeutic agent to be administered to a subject, *e.g.*, a human in need thereof.

[0069] The term "pharmaceutically acceptable" and its grammatical equivalents as used herein can refer to an attribute of a material which is useful in preparing a pharmaceutical composition that is generally safe, non–toxic, and neither biologically nor otherwise undesirable and is acceptable for veterinary as well as human pharmaceutical use. "Pharmaceutically acceptable" can refer a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively nontoxic, *i.e.*, the material may be administered to a subject without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the pharmaceutical composition in which it is contained.

[0070] A "pharmaceutically acceptable excipient" refers to an excipient that can be administered to a subject, together with an agent, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the agent.

[0071] The term "therapeutic agent" can refer to any agent that, when administered to a subject, has a therapeutic, diagnostic, and/or prophylactic effect and/or elicits a desired biological and/or pharmacological effect. Therapeutic agents can also be referred to as "actives" or "active agents." Such agents include, but are not limited to, cytotoxins, radioactive ions, chemotherapeutic agents, small molecule drugs, proteins, and nucleic acids.

[0072] It is appreciated that certain features of the polynucleic acid molecules, and/or polynucleic acid molecule conjugates, pharmaceutical composition comprising the polynucleic acid molecules or the polynucleic acid molecule conjugates, methods, and other aspects, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the polynucleic acid molecules, and/or polynucleic acid molecule conjugates, pharmaceutical composition comprising the polynucleic acid molecules or the polynucleic acid molecule conjugates, methods, and other aspects, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments are specifically embraced by the present disclosure and are disclosed herein just as if each and every combination was individually and explicitly disclosed, to the extent that such combinations embrace operable processes and/or compositions. In addition, all sub-combinations listed in the embodiments describing such variables are also specifically embraced by the present polynucleic acid molecules, and/or polynucleic acid molecule conjugates, pharmaceutical composition comprising the polynucleic acid molecules or the polynucleic acid molecule conjugates, methods and other aspects and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0073] As used herein, the term "sense strand" can be interchangeably used with the term "passenger strand," and the tern "antisense strand" can be interchangeably used with the term "guide strand."

[0074] As used herein, the term "consecutive sequence" refers to a sequence contains a number of consecutive nucleotides from a reference sequence. For example, if a reference sequence is $N_1N_2N_3N_4N_5N_6N_7$, a consecutive sequence can be $N_1N_2N_3N_4$ or $N_3N_4N_5N_6$, but a sequence of $N_1N_3N_4N_5$ or $N_3N_4N_7$ cannot be a consecutive sequence.

[0075] As used herein, the term "negative control" refers to a subject or a cell receiving no treatment or placebo.

Polynucleic Acid Molecules

Target Regions in Lp(a) mRNA by Polynucleic Acid Molecules

[0076] Described herein is a polynucleic acid molecule for modulating expression of Lp(a) gene. In some instances, the polynucleic acid molecule is a single-stranded nucleic acid molecule. In some instances, the polynucleic acid molecule is a double-stranded nucleic acid molecule.

[0077] In some aspects, the polynucleic acid molecule described herein hybridizes to certain regions of human Lp(a) mRNA. In some instances, the polynucleic acid molecule comprises a sense strand and an antisense strand, and wherein the antisense strand hybridizes to a certain region of Lp(a) mRNA.

[0078] In some aspects, the polynucleic acid molecule described herein hybridizes to the 5' UTR region of human Lp(a) mRNA. In some aspects, the polynucleic acid molecule described herein hybridizes to the coding region of human Lp(a) mRNA. In some aspects, the polynucleic acid molecule described herein hybridizes to the 3' UTR region of human Lp(a) mRNA. In some aspects, the polynucleic acid molecule described herein hybridizes to a portion of exon 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, or 39 of human Lp(a) mRNA. In some aspects, the polynucleic acid molecule described herein hybridizes to a coding region of human Lp(a) mRNA (NCBI Reference Sequence: NM_005577.4).

[0079] In some aspects, the starting position of the binding site of the polynucleic acid molecule described herein on human Lp(a) mRNA (NCBI Reference Sequence: NM_005577.4) is between position 190-210, 250-270; 360-390; 400-450; 460-520, 530-550, 1100-1200, 2600-2700, 2700-2800, 2800-2900, 3000-3300; 3300-3600, 3600-4000, 4000-4200, 4400-4700, 4700-5000, 5000-5200, or 5500-6000.

[0080] The starting position of the binding site of the polynucleic acid molecule described herein on human Lp(a) mRNA (NCBI Reference Sequence: NM_005577.4) can be between position 200-210, 380-390, 410-420, 1100-1200, 2700-2800, 3200-3300, or 3300-3400.

[0081] The starting position of the binding site of the polynucleic acid molecule described herein on human Lp(a) mRNA (NCBI Reference Sequence: NM_005577.4) can be between position 200-210, 250-260, 380-390, 410-430, 430-440, 460-470, 490-500, 500-510, 540-550, 1150-1180, 2650-2700, 2700-2750, 2760-2770, 2850-2900, 3200-3250, 3250-3300, 3300-3310, 3700-3750, 3750-3800, 3950-4000, 4800-4850, 5100-5150, 5500-5550, 5850-5900, or 2750-2800.

Target Regions in APOC3 mRNA by Polynucleic Acid Molecules

[0082] Described herein is a polynucleic acid molecule for modulating expression of APOC3 gene. In some instances, the polynucleic acid molecule comprises a single-stranded nucleic acid molecule that hybridizes to certain regions of mRNA. In some instances, the polynucleic acid molecule is a double-stranded nucleic acid molecule. Also described herein is a polynucleic acid molecule for modulating expression of APOC3 gene, wherein the polynucleic acid molecule is a double-stranded nucleic acid molecule, which comprises a sense strand and an antisense strand, and the antisense strand hybridizes to certain regions of APOC3 mRNA.

[0083] In some aspects, the polynucleic acid molecule described herein hybridizes to certain regions of human APOC3 mRNA. In some instances, the human APOC3 mRNA is NM_000040.3. In some aspects, the polynucleic acid molecule described herein hybridizes to certain regions of non-human APOC3 mRNA.

[0084] In some aspects, the polynucleic acid molecule described herein hybridizes to the 5' UTR region of human APOC3 mRNA. In some aspects, the polynucleic acid molecule described herein hybridizes to the coding region of human APOC3 mRNA. In some aspects, the polynucleic acid molecule described herein hybridizes to a portion of exon 1 of human APOC3 mRNA. In some aspects, the polynucleic acid molecule described herein hybridizes to a portion of exon 2 of human APOC3 mRNA. In some aspects, the polynucleic acid molecule described herein hybridizes to a portion of exon 3 of human APOC3 mRNA. In some aspects, the polynucleic acid molecule described herein hybridizes to a portion of exon 4 of human APOC3 mRNA. In some aspects, the polynucleic acid molecule described herein hybridizes to the 3' UTR region of human APOC3 mRNA.

[0085] In some aspects, the target region that the polynucleic acid molecule described herein hybridizes to is determined by APOC3 silencing effectiveness and possible off-target effects. In some instances, the start of the target region falls between positions 1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, or 91-100 of NM_000040.3. In some instances, the start of the target region falls between positions 101-110, 111-120, 121-130, 131-140, 141-150, 151-160, 161-170, 171-180, 181-190, or 191-200 of NM_000040.3. In some instances, the start of the target region falls between positions of 201-210, 211-220, 221-230, 231-240, 241-250, 251-260, 261-270, 271-280, 281-290, or 291-300 of NM_000040.3. In some instances, the start of the target region falls between positions 301-310, 311-320, 321-330, 331-340, 341-350, 351-360, 361-370, 371-380, 381-390, or 391-400 of NM_000040.3. In some instances, the start of the target region falls between positions 401-410, 411-420, 421-430, 431-440, 441-450, 451-460, 461-470, 471-480, 481-

490, or 491-500 of NM_000040.3. In some instances, the start of the target region falls between positions 501-510, 511-520, 521-530, or 531-535 of NM_000040.3.

Structure of Double-stranded Polynucleic Acid Molecules

Lp(a) targeting small inhibitory RNA (siRNA)

[0086] In some instances, the polynucleic acid molecule is a double-stranded nucleic acid molecule comprising a sense strand and an antisense strand, and wherein the antisense strand is at least partially reverse complementary to a target region of Lp(a) mRNA.

[0087] In some aspects, the antisense strand described herein is not 100% complementary to a target region of Lp(a) mRNA. Accordingly, in some instances, the antisense strand described herein is 100% complementary to a target region of Lp(a) mRNA. In some aspects, the antisense strand described herein is about 95% complementary to a target region of Lp(a) mRNA. In some aspects, the antisense strand described herein is about 90% complementary to a target region of Lp(a) mRNA. In some aspects, the antisense strand described herein is about 85% complementary to a target region of Lp(a) mRNA. In some aspects, the antisense strand described herein is about 80% complementary to a target region of Lp(a) mRNA. In some aspects, the antisense strand described herein is about 75% complementary to a target region of Lp(a) mRNA. In some aspects, the antisense strand described herein is about 70% complementary to a target region of Lp(a) mRNA.

[0088] In some aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence in Table 1, Table 2, Table 3, or Table 4. In other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a sequence in Table 1, Table 2, Table 3, or Table 4. In some instances, the sense strand described herein comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 14-20. In some instances, the antisense strand described herein comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 1-13. In some instances, the sense strand described herein comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 14-20. In some instances, the antisense strand described herein comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, identical to a nucleic acid sequence selected from SEQ ID NOs: 1-13. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising 15 consecutive sequences of the sequences in Table 1, Table 2, Table 3, or Table 4 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the sense strand described herein

comprises a nucleic acid sequence comprising at least 15 consecutive sequences from SEQ ID NOs: 14-20 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 15 consecutive sequences from SEQ ID NOs: 1-13 with no more than 1, 2, 3, or 4 mismatches. In yet still other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising at least 16 consecutive sequences out of the sequences in Table 1, Table 2, Table 3, or Table 4 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 16 consecutive sequences from SEQ ID NOs: 14-20 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 16 consecutive sequences from SEQ ID NOs: 1-13 with no more than 1, 2, 3, or 4 mismatches. In yet still other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising at least 17 consecutive sequences from the sequences in Table 1, Table 2, Table 3, or Table 4 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 17 consecutive sequences from SEQ ID NOs: 14-20 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 17 consecutive sequences from SEQ ID NOs: 1-13 with no more than 1, 2, 3, or 4 mismatches. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising at least 18 consecutive sequences from the sequences in Table 1, Table 2, Table 3, or Table 4 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 18 consecutive sequences from SEQ ID NOs: 14-20 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 18 consecutive sequences from SEQ ID NOs: 1-13 with no more than 1, 2, 3, or 4 mismatches. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising at least 19 consecutive sequences from the sequences in Table 1, Table 2, Table 3, or Table 4 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 19 consecutive sequences from SEQ ID NOs: 14-20 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 19 consecutive sequences from SEQ ID NOs: 1-13 with no more than 1, 2, 3, or 4 mismatches. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising at least 20 consecutive sequences from the sequences in Table 1, Table 2, Table 3, or Table 4 with no more than 1, 2, 3, or 4 mismatches. In

specific aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 20 consecutive sequences from SEQ ID NOs: 14-20 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 20 consecutive sequences from SEQ ID NOs: 1-13 with no more than 1, 2, 3, or 4 mismatches. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising at least 21 consecutive sequences from the sequences in Table 1, Table 2, Table 3, or Table 4 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 21 consecutive sequences from SEQ ID NOs: 14-20 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 21 consecutive sequences from SEQ ID Nos: 1-13 with no more than 1, 2, 3, or 4 mismatches. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising at least 22 consecutive sequences from the sequences in Table 1, Table 2, Table 3, or Table 4 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 22 consecutive sequences from SEQ ID NOs: 1-13 with no more than 1, 2, 3, or 4 mismatches. In specific aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 23 consecutive sequences from SEQ ID NOs: 1-13 with no more than 1, 2, 3, or 4 mismatches. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising at least 23 consecutive sequences from the sequences in Table 1, Table 2, Table 3, or Table 4 with no more than 1, 2, 3, or 4 mismatches. As used herein, the term “consecutive sequence” refers to a sequence contains a number of consecutive nucleotides from a reference sequence.

[0089] In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 15 consecutive sequences of SEQ ID NOs: 14-20 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 15 consecutive sequences of SEQ ID NOs: 1-13 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 16 consecutive sequences of SEQ ID NOs: 14-20 with no more than 1, 2, 3 or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 16 consecutive sequences of SEQ ID NOs: 1-13 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 17 consecutive sequences of SEQ ID NOs: 14-20 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein

comprises a nucleic acid sequence comprising at least 17 consecutive sequences of SEQ ID NOs: 1-13 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 18 consecutive sequences of SEQ ID NOs: 14-20 with no more than 1, 2 or 3 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 18 consecutive sequences of SEQ ID NOs: 1-13 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 19 consecutive sequences of SEQ ID NOs: 14-20 with no more than 1, 2 or 3 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 19 consecutive sequences of SEQ ID NOs: 1-13 with no more than 1, 2 or 3 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 20 consecutive sequences from SEQ ID NOs: 14-20 with no more than 1, 2 or 3 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 20 consecutive sequences of SEQ ID NOs: 1-13 with no more than 1, 2 or 3 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising at least 21 consecutive sequences of SEQ ID NOs: 14-20 with no more than 1, 2 or 3 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 21 consecutive sequences of SEQ ID NOs: 1-13 with no more than 1, 2 or 3 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 22 consecutive sequences of SEQ ID NOs: 14-20 with no more than 1, 2 or 3 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising at least 23 consecutive sequences of SEQ ID NOs: 1-13 with no more than 1, 2 or 3 mismatches.

[0090] In some aspects, the polynucleic acid molecule described herein comprises a sense and an antisense strand of at least 10, 11, 12, 13, 14, or 15 nucleotides in length. In some aspects, the polynucleic acid molecule described herein comprises a sense and an antisense strand of about 15-30, 16-30, 17-30, 18-30, 18-27, 18-25, 18-23, 19-23, 20-23, or 21-23 nucleotides in length. In some aspects, the polynucleic acid molecule described herein comprises a sense and an antisense strand of about 15, 16, 17, 18, 19, 20 nucleotides long. In some aspects, the polynucleic acid molecule described herein comprises a sense and an antisense strand of about 21, 22, 23, 24, 25 nucleotides long. In some aspects, the polynucleic acid molecule described herein comprises a sense and an antisense strand of about 26, 27, 28, 29, 30 nucleotides long. In some aspects, the polynucleic acid molecule described herein comprises a sense strand of 19 nucleotides long, and an antisense strand of about 21 nucleotides long. In some aspects, the polynucleic acid molecule described herein

comprises a sense strand of 21 nucleotides long, and an antisense strand of about 23 nucleotides long.

[0091] In some aspects, the sense strand and the antisense strand described herein are reverse complementary to each other and form a duplex with a 3' overhang on the antisense strand. In some aspects, the sense strand and the antisense strand described herein are reverse complementary to each other and form a duplex with a 5' overhang on the antisense strand. In some aspects, the sense strand and the antisense strand described herein are reverse complementary to each other and form a duplex with a 3' overhang on the sense strand. In some aspects, the sense strand and the antisense strand described herein are reverse complementary to each other and form a duplex with a 5' overhang on the sense strand.

[0092] In some aspects, the polynucleic acid molecule provided herein comprises a sense strand comprising a nucleic acid sequence selected from SEQ ID NOs: 14-20 and an antisense strand comprising a nucleic acid sequence selected from SEQ ID NOs: 1-13. In other aspects, the polynucleic acid molecule provided herein comprises a sense strand comprising a nucleic acid sequence selected from SEQ ID NOs: 14-20, an antisense strand comprising a nucleic acid sequence selected from SEQ ID NOs: 1-13, and wherein the sense and/or antisense strand is modified as described herein.

[0093] Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 80% identical to a nucleic acid sequence selected from SEQ ID NOs: 72-91. Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 85% identical to a nucleic acid sequence selected from SEQ ID NOs: 72-91. Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 90% identical to a nucleic acid sequence selected from SEQ ID NOs: 72-91. Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 72-91.

[0094] Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 80% identical to a nucleic acid sequence selected from SEQ ID NOs: 21-71. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 85% identical to a nucleic acid sequence selected from SEQ ID NOs: 21-71. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 90% identical to a nucleic acid sequence selected from SEQ ID NOs: 21-71. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic

acid sequence that is at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 21-71.

[0095] In some aspects, the polynucleic acid molecule provided herein comprises a sense strand comprising a nucleic acid sequence selected from SEQ ID NOs: 72-91, and an antisense strand comprising a nucleic acid sequence selected from SEQ ID NOs: 21-71.

APOC3 targeting small inhibitory RNA (siRNA)

[0096] Further described herein is a polynucleic acid molecule for modulating expression of APOC3 gene, wherein the polynucleic acid molecule is a double-stranded molecule that comprises a sense strand and an antisense strand, and the antisense strand is reverse complementary to the target region of APOC3 mRNA .

[0097] In some aspects, the antisense strand described herein is 100% complementary to the target region of APOC3 mRNA. In other aspects, the antisense strand described herein is not 100% complementary to the target region of APOC3 mRNA. Accordingly, in some instances, the antisense strand described herein is about 95% complementary to the target region of APOC3 mRNA. In some aspects, the antisense strand described herein is about 90% complementary to the target region of APOC3 mRNA. In some aspects, the antisense strand described herein is about 85% complementary to the target region of APOC3 mRNA. In some aspects, the antisense strand described herein is about 80% complementary to the target region of APOC3 mRNA. In some aspects, the antisense strand described herein is about 75% complementary to the target region of APOC3 mRNA. In some aspects, the antisense strand described herein is about 70% complementary to the target region of APOC3 mRNA.

[0098] In some aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence in Table 5. In other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a sequence in Table 5. In some instances, the sense strand described herein comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 107-112. In some instances, the antisense strand described herein comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 101-106. In some instances, the sense strand described herein comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 107-112. In some instances, the antisense strand described herein comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 101-106.

[0099] In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising 14 consecutive sequences out of the sequences in Table 5 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 14 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 14 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising 15 consecutive sequences out of the sequences in Table 5 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 15 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 15 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In yet still other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising 16 consecutive sequences out of the sequences in Table 5 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 16 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 16 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In yet still other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising 17 consecutive sequences out of the sequences in Table 5 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 17 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 17 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising 18 consecutive sequences out of the sequences in Table 5 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 18 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 18 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising 19 consecutive sequences out of the sequences in Table 5 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the

sense strand described herein comprises a nucleic acid sequence comprising 19 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 19 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising 20 consecutive sequences out of the sequences in Table 5 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 20 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 20 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising 21 consecutive sequences out of the sequences in Table 5 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 21 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 21 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In yet other aspects, the polynucleic acid molecule described herein comprises a nucleic acid sequence comprising 22 consecutive sequences out of the sequences in Table 5 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 22 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 22 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches.

[0100] In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 15 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 15 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 16 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 16 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 17 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid

sequence comprising 17 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 18 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 18 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 19 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 19 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 20 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 20 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 21 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 21 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the sense strand described herein comprises a nucleic acid sequence comprising 22 consecutive sequences of SEQ ID NOs: 107-112 with no more than 1, 2, 3, or 4 mismatches. In some aspects, the antisense strand described herein comprises a nucleic acid sequence comprising 22 consecutive sequences of SEQ ID NOs: 101-106 with no more than 1, 2, 3, or 4 mismatches.

[0101] In some aspects, the polynucleic acid molecule described herein comprises a sense and an antisense strand of at least 10, 11, 12, 13, 14, or 15 nucleotides in length. In some aspects, the polynucleic acid molecule described herein comprises a sense and an antisense strand of about 15-30, 16-30, 17-30, 18-30, 18-27, 18-25, 18-23, 19-23, 20-23, or 21-23 nucleotides in length. In some aspects, the polynucleic acid molecule described herein comprises a sense and an antisense strand of about 15, 16, 17, 18, 19, 20 nucleotides long. In some aspects, the polynucleic acid molecule described herein comprises a sense and an antisense strand of about 21, 22, 23, 24, 25 nucleotides long. In some aspects, the polynucleic acid molecule described herein comprises a sense and an antisense strand of about 26, 27, 28, 29, 30 nucleotides long. In some aspects, the polynucleic acid molecule described herein comprises a sense strand of 19 nucleotides long, and an antisense strand of about 21 nucleotides long. In some aspects, the polynucleic acid molecule described herein

comprises a sense strand of 21 nucleotides long, and an antisense strand of about 23 nucleotides long.

[0102] In some aspects, the sense strand and the antisense strand described herein are reverse complementary to each other and form a duplex with a 3' overhang on the antisense strand. In some aspects, the sense strand and the antisense strand described herein are reverse complementary to each other and form a duplex with a 5' overhang on the antisense strand. In some aspects, the sense strand and the antisense strand described herein are reverse complementary to each other and form a duplex with a 3' overhang on the sense strand. In some aspects, the sense strand and the antisense strand described herein are reverse complementary to each other and form a duplex with a 5' overhang on the sense strand.

[0103] Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 80% identical to a nucleic acid sequence selected from SEQ ID NOs: 122-127. Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 85% identical to a nucleic acid sequence selected from SEQ ID NOs: 122-127. Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 90% identical to a nucleic acid sequence selected from SEQ ID NOs: 122-127. Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 122-127.

[0104] Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 80% identical to a nucleic acid sequence selected from SEQ ID NOs: 113-121. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 85% identical to a nucleic acid sequence selected from SEQ ID NOs: 113-121. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 90% identical to a nucleic acid sequence selected from SEQ ID NOs: 113-121. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 113-121.

Modifications of Polynucleic Acid Molecules

[0105] In some aspects, described herein is the polynucleic acid molecule described herein with one or more modifications. In some aspects, the modifications described herein occurs one or more different structures of the polynucleic acid molecule described herein (*e.g.*, modifications on sugar ring(s), backbone(s) or internucleotide linkage(s), base(s)). In some instances, the modifications

described herein comprise incorporations of one or more nucleotide analogue described herein to the polynucleic acid molecule. In some aspects, the modifications described herein comprise substitutions of one or more nucleotide in the polynucleic acid molecule described herein. In some aspects, different percentages of the polynucleic acid molecule described herein comprise the modifications described herein. In some aspects, different positions of the polynucleic acid molecule described herein comprise the modifications described herein. WO/2018/035380 is herein incorporated by reference in its entirety.

Nucleotide Analogue

[0106] In some aspects, the present disclosure provides a polynucleic acid molecule incorporating a nucleotide analogue. In some instances, the present disclosure provides a polynucleic acid molecule substituting one or more nucleotide in the polynucleic acid molecule with a nucleotide analogue described herein.

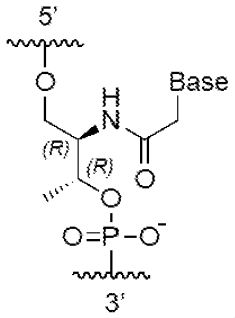
[0107] In some instances, modifications of the nucleotide with the nucleotide analogue described herein can alter base pairings and structural changes of the inhibitory polynucleic acid molecule. In some instances, the nucleotide analogue can be incorporated into the polynucleic acid molecule, thereby suppressing off-target effects. In some instances, the nucleotide analogue can be incorporated into the polynucleic acid molecule, thereby improving stability and efficacy of the polynucleic acid molecule. In some instances, the nucleotide analogue described herein can be incorporated into a guide strand, a passenger strand, or a combination thereof.

[0108] In some instances, the nucleotide analogue can be placed in the polynucleic acid molecule or be a substitute for a nucleotide in the polynucleic acid molecule, thereby suppressing the off-target effects. In some instances, the nucleotide analogue can be substituted for a nucleotide in the polynucleic acid molecule, thereby improving stability and/or efficacy of the polynucleic acid molecule. In some instances, the nucleotide analogue described herein can be placed in a guide strand, a passenger strand, or both.

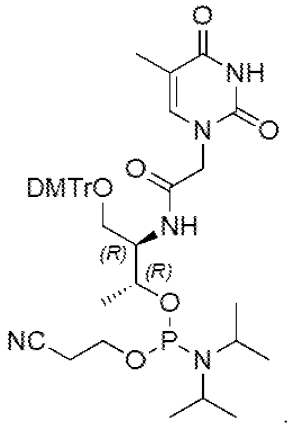
[0109] In some aspects, the present disclosure provides a polynucleic acid molecule comprising a passenger strand (sense strand) and a guide strand (antisense strand), wherein the guide strand comprises a nucleotide analogue as described herein. In some instances, the nucleotide analogue comprises acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-

phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), 2'-deoxyadenosine-3-phosphate (dA), 2'-deoxycytidine-3'-phosphate (dC), 2'-deoxyguanosine-3'-phosphate (dG), or 2'-deoxythymidine-3'-phosphate (dT). In some instance, the nucleotide analogue is selected from a group consisting of the nucleotide analogue selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), 2'-deoxyadenosine-3-phosphate (dA), 2'-deoxycytidine-3'-phosphate (dC), 2'-deoxyguanosine-3'-phosphate (dG), and 2'-deoxythymidine-3'-phosphate (dT). In some instances, the nucleotide analogue is selected from a group consisting of the nucleotide analogue selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), and 2'-fluoro-2-thiouridine-3'-phosphate (U3f).

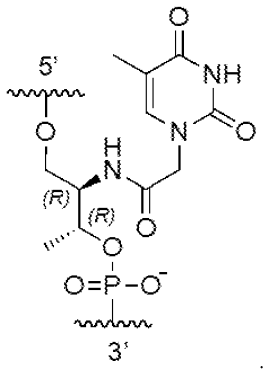
[0110] In some instances, the acyclic L-threoninol nucleic acid- thymine-3'-phosphate (T-T) has a generic representation shown as below, where the base can be any suitable base or modified base that can make Watson-Crick binding with the base on the opposite strand:



[0111] In some instances, the amidite structure of acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T) is shown as below:

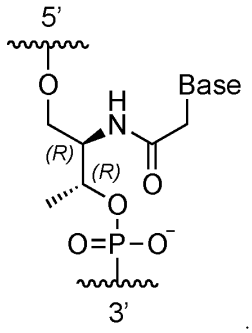


[0112] In some instances, the acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T) is incorporated into the polynucleic acid molecule. In some instances, the acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T) is incorporated into the siRNA. In some instances, the acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T) is incorporated into a guide strand. In some instances, the acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T) is incorporated into a passenger strand. In some instances, an incorporated acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T) has structure shown as below:

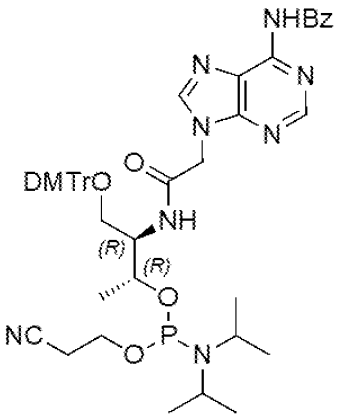


[0113] In some instances, the acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T) is placed in the polynucleic acid molecule. In some instances, the acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T) substitutes one or more nucleotide in siRNA. In some instances, the acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T) substitutes one or more nucleotide in a passenger strand and/or a guide strand. In some instances, the acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T) substitutes one or more nucleotide in a passenger strand. In some instances, the acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T) substitutes one or more nucleotide in a guide strand.

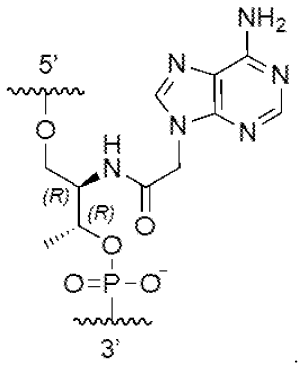
[0114] In some instances, the acyclic L-threoninol nucleic acid- adenine-3'-phosphate (T-A) has a generic representation shown as below, where the base can be any suitable base or modified base that can make Watson-Crick binding with the base on the opposite strand:



[0115] In some instances, the amidite structure of acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A) is shown as below:

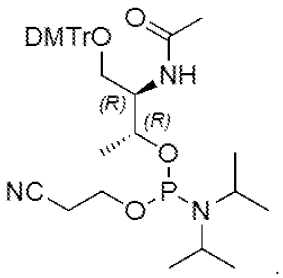


[0116] In some instances, the acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A) is incorporated into the polynucleic acid molecule. In some instances, the acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A) is incorporated into the siRNA. In some instances, the acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A)is incorporated into a guide strand. In some instances, the acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A) is incorporated into a passenger strand. In some instances, an incorporated acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A) has structure shown as below:

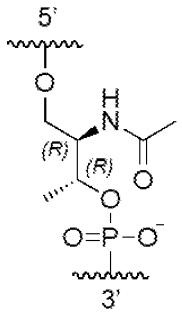


[0117] In some instances, the acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A) is placed in the polynucleic acid molecule. In some instances, the acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A) substitutes one or more nucleotide in siRNA. In some instances, the acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A) substitutes one or more nucleotide in a passenger strand and/or a guide strand. In some instances, the acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A) substitutes one or more nucleotide in a passenger strand. In some instances, the acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A) substitutes one or more the nucleotide in a guide strand.

[0118] In some instances, the amidite structure of acyclic N-Acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc) is shown as below:

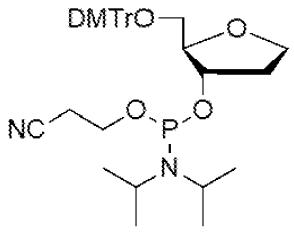


[0119] In some instances, the acyclic N-Acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc) is incorporated into the polynucleic acid molecule. In some instances, the acyclic N-Acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc) is incorporated into the siRNA. In some instances, the acyclic N-Acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc) is incorporated into a guide strand. In some instances, the acyclic N-Acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc) is incorporated into a passenger strand. In some instances, an incorporated acyclic N-Acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc) has structure shown as below:

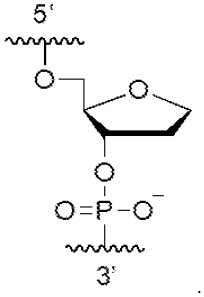


[0120] In some instances, the acyclic N-Acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc) is placed in the polynucleic acid molecule. In some instances, the acyclic N-Acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc) substitutes one or more nucleotide in siRNA. In some instances, the acyclic N-Acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc) substitutes one or more nucleotide in a passenger strand and/or a guide strand. In some instances, acyclic N-Acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc) substitutes one or more nucleotide in a passenger strand. In some instances, the acyclic N-Acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc) substitutes one or more nucleotide in a guide strand.

[0121] In some instances, the amidite structure of 1',2'-Dideoxyribose-3'-phosphate (dAB) is shown as below:

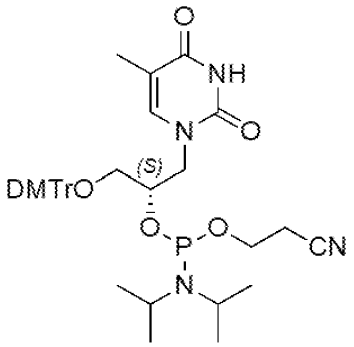


[0122] In some instances, the 1',2'-Dideoxyribose-3'-phosphate (dAB) is incorporated into the polynucleic acid molecule. In some instances, the 1',2'-Dideoxyribose-3'-phosphate (dAB) is incorporated into the siRNA. In some instances, the 1',2'-Dideoxyribose-3'-phosphate (dAB) is incorporated into a guide strand. In some instances, the 1',2'-Dideoxyribose-3'-phosphate (dAB) is incorporated into a passenger strand. In some instances, an incorporated 1',2'-Dideoxyribose-3'-phosphate (dAB) has structure shown as below:

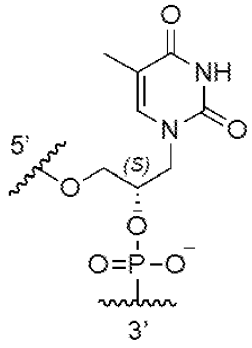


[0123] In some instances, the 1',2'-Dideoxyribose-3'-phosphate (dAB) is placed in the polynucleic acid molecule. In some instances, the 1',2'-Dideoxyribose-3'-phosphate (dAB) substitutes one or more nucleotide in siRNA. In some instances, the 1',2'-Dideoxyribose-3'-phosphate (dAB) substitutes one or more nucleotide in a passenger strand and/or a guide strand. In some instances, the 1',2'-Dideoxyribose-3'-phosphate (dAB) substitutes one or more nucleotide in a passenger strand. In some instances, the 1',2'-Dideoxyribose-3'-phosphate (dAB) substitutes one or more nucleotide in a guide strand.

[0124] In some instances, the amidite structure of thymidine-glycol nucleic acid (GNA) S-isomer (Tgn) is shown as below:



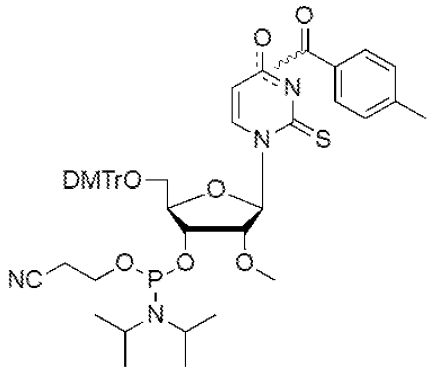
[0125] In some instances, the thymidine-glycol nucleic acid (GNA) S-isomer (Tgn) is incorporated into the polynucleic acid molecule. In some instances, the thymidine-glycol nucleic acid (GNA) S-isomer (Tgn) is incorporated into the siRNA. In some instances, the thymidine-glycol nucleic acid (GNA) S-isomer (Tgn) is incorporated into a guide strand. In some instances, the thymidine-glycol nucleic acid (GNA) S-isomer (Tgn) is incorporated into a passenger strand. In some instances, an incorporated thymidine-glycol nucleic acid (GNA) S-isomer (Tgn) has structure shown as below:



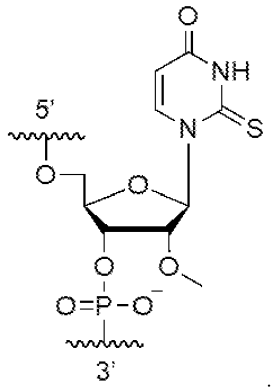
[0126] In some instances, the thymidine-glycol nucleic acid (GNA) S-isomer (Tgn) is placed in the polynucleic acid molecule. In some instances, the thymidine-glycol nucleic acid (GNA) S-isomer (Tgn) substitutes one or more nucleotide in siRNA. In some instances, the thymidine-glycol nucleic acid (GNA) S-isomer (Tgn) substitutes one or more nucleotide in a passenger strand and/or a guide

strand. In some instances, the thymidine-glycol nucleic acid (GNA) S-isomer (Tgn) substitutes one or more nucleotide in a passenger strand. In some instances, the thymidine-glycol nucleic acid (GNA) S-isomer (Tgn) substitutes one or more nucleotide in a guide strand.

[0127] In some instances, the amidite structure of 2'-O-methyl-2-thiouridine-3'-phosphate (u3) is shown as below:

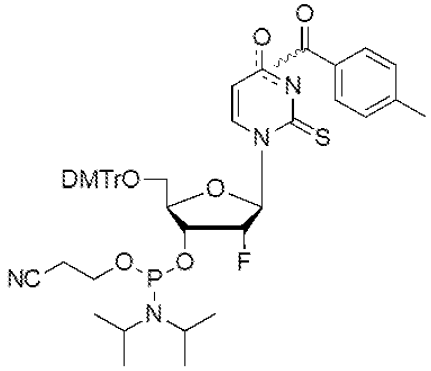


[0128] In some instances, the 2'-O-methyl-2-thiouridine-3'-phosphate (u3) is incorporated into the polynucleic acid molecule. In some instances, the 2'-O-methyl-2-thiouridine-3'-phosphate (u3) is incorporated into the siRNA. In some instances, the 2'-O-methyl-2-thiouridine-3'-phosphate (u3) is incorporated into a guide strand. In some instances, the 2'-O-methyl-2-thiouridine-3'-phosphate (u3) is incorporated into a passenger strand. In some instances, an incorporated 2'-O-methyl-2-thiouridine-3'-phosphate (u3) has structure shown as below:

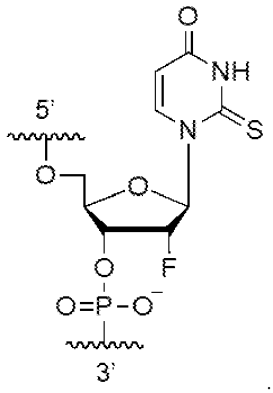


[0129] In some instances, the 2'-O-methyl-2-thiouridine-3'-phosphate (u3) is placed in the polynucleic acid molecule. In some instances, the 2'-O-methyl-2-thiouridine-3'-phosphate (u3) substitutes one or more nucleotide in siRNA. In some instances, the 2'-O-methyl-2-thiouridine-3'-phosphate (u3) substitutes one or more nucleotide in a passenger strand and/or a guide strand. In some instances, the 2'-O-methyl-2-thiouridine-3'-phosphate (u3) substitutes one or more nucleotide in a passenger strand. In some instances, the 2'-O-methyl-2-thiouridine-3'-phosphate (u3) substitutes one or more nucleotide in a guide strand.

[0130] In some instances, the amidite structure of 2'-fluoro-2-thiouridine-3'-phosphate (U3f) is shown as below:

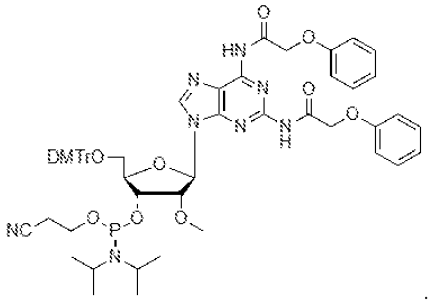


[0131] In some instances, the 2'-fluoro-2-thiouridine-3'-phosphate (U3f) is incorporated into the polynucleic acid molecule. In some instances, the 2'-fluoro-2-thiouridine-3'-phosphate (U3f) is incorporated into the siRNA. In some instances, the 2'-fluoro-2-thiouridine-3'-phosphate (U3f) is incorporated into a guide strand. In some instances, the 2'-fluoro-2-thiouridine-3'-phosphate (U3f) is incorporated into a passenger strand. In some instances, an incorporated 2'-fluoro-2-thiouridine-3'-phosphate (U3f) has structure shown as below:

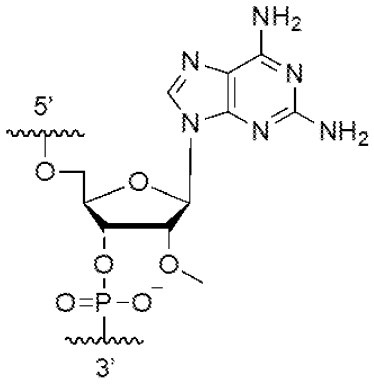


[0132] In some instances, the 2'-fluoro-2-thiouridine-3'-phosphate (U3f) is placed in the polynucleic acid molecule. In some instances, the 2'-fluoro-2-thiouridine-3'-phosphate (U3f) substitutes one or more nucleotide in siRNA. In some instances, the 2'-fluoro-2-thiouridine-3'-phosphate (U3f) substitutes one or more nucleotide in a passenger strand and/or a guide strand. In some instances, the 2'-fluoro-2-thiouridine-3'-phosphate (U3f) substitutes one or more the nucleotide in a passenger strand. In some instances, the 2'-fluoro-2-thiouridine-3'-phosphate (U3f) substitutes one or more the nucleotide in a guide strand.

[0133] In some instances, the amidite structure of 2-amino-2'-O-methyladenosine-3'-phosphate (a1) is shown as below:

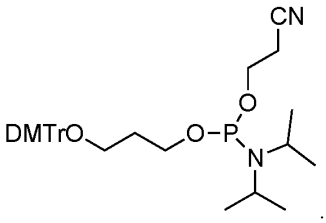


[0134] In some instances, the 2-amino-2'-O-methyladenosine-3'-phosphate (a1) is incorporated into the polynucleic acid molecule. In some instances, the 2-amino-2'-O-methyladenosine-3'-phosphate (a1) is incorporated into the siRNA. In some instances, the 2-amino-2'-O-methyladenosine-3'-phosphate (a1) is incorporated into a guide strand. In some instances, the 2-amino-2'-O-methyladenosine-3'-phosphate (a1) is incorporated into a passenger strand. In some instances, an incorporated 2-amino-2'-O-methyladenosine-3'-phosphate (a1) has structure shown as below:

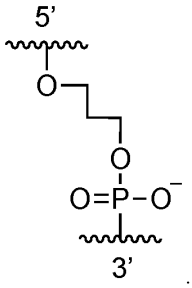


[0135] In some instances, the 2-amino-2'-O-methyladenosine-3'-phosphate (a1) is placed in the polynucleic acid molecule. In some instances, the 2-amino-2'-O-methyladenosine-3'-phosphate (a1) substitutes one or more nucleotide in siRNA. In some instances, the 2-amino-2'-O-methyladenosine-3'-phosphate (a1) substitutes one or more nucleotide in a passenger strand and/or a guide strand. In some instances, the 2-amino-2'-O-methyladenosine-3'-phosphate (a1) substitutes one or more nucleotide in a passenger strand. In some instances, the 2-amino-2'-O-methyladenosine-3'-phosphate (a1) substitutes one or more nucleotide in a guide strand.

[0136] In some instances, the amidite structure of C3-spacer (Pr) is shown as below:

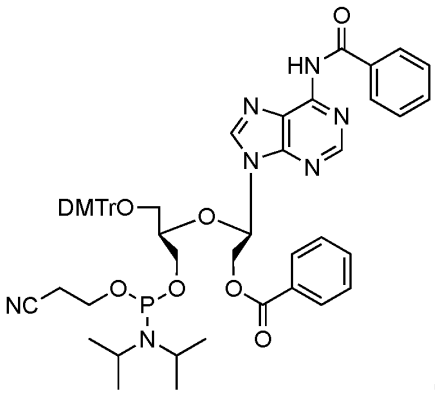


[0137] In some instances, the C3-spacer (Pr) is incorporated into the polynucleic acid molecule. In some instances, the C3-spacer (Pr) is incorporated into the siRNA. In some instances, the C3-spacer (Pr) is incorporated into a guide strand. In some instances, the C3-spacer (Pr) is incorporated into a passenger strand. In some instances, an incorporated C3-spacer (Pr) has structure shown as below:

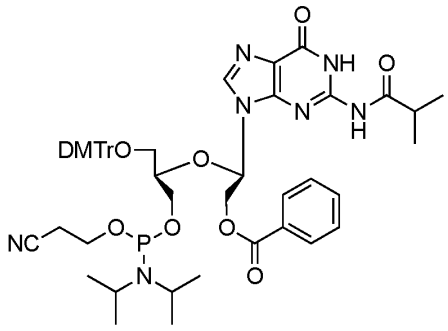


[0138] In some instances, the C3-spacer (Pr) is placed in the polynucleic acid molecule. In some instances, the C3-spacer (Pr) substitutes one or more nucleotide in siRNA. In some instances, the C3-spacer (Pr) substitutes one or more nucleotide in a passenger strand and/or a guide strand. In some instances, the C3-spacer (Pr) substitutes one or more nucleotide in a passenger strand. In some instances, the C3-spacer (Pr) substitutes one or more nucleotide in a guide strand.

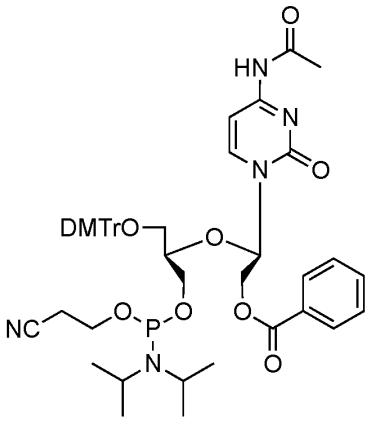
[0139] In some instances, the amidite structures of 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2) are shown as below:



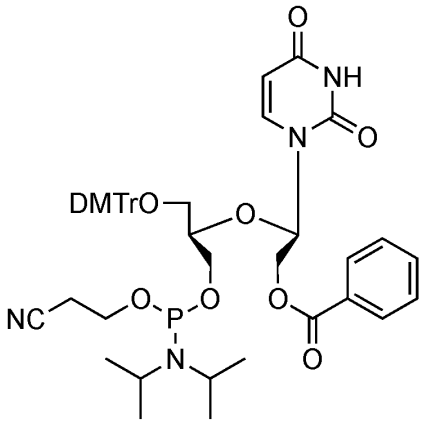
(A2),



(G2),

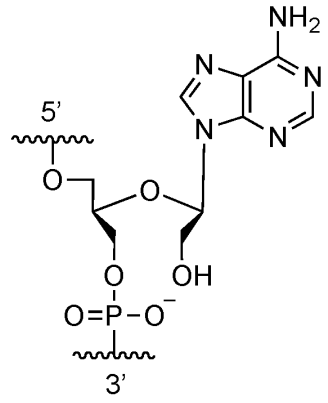


(C2),

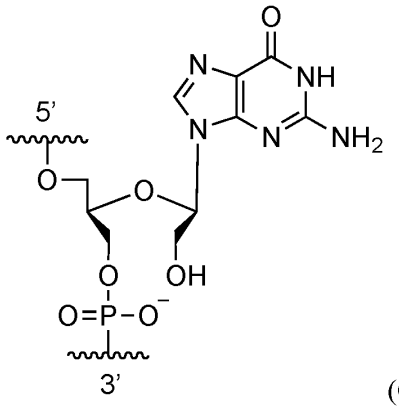


(U2).

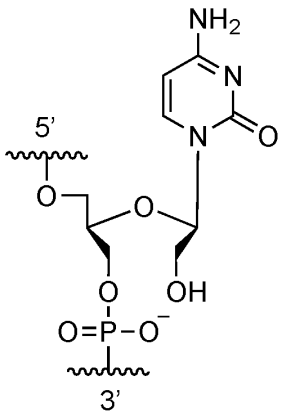
[0140] In some instances, the A2, G2, C2, or U2 is incorporated into the polynucleic acid molecule. In some instances, the A2, G2, C2, or U2 is incorporated into the siRNA. In some instances, the A2, G2, C2, or U2 is incorporated into a guide strand. In some instances, the A2, G2, C2, or U2 is incorporated into a passenger strand. In some instances, an incorporated A2, G2, C2, or U2 has structure shown as below:



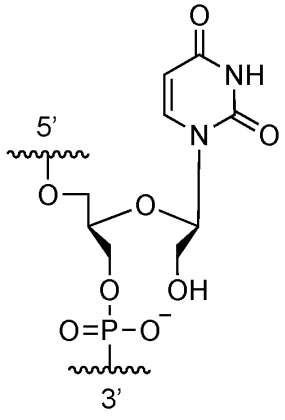
(A2),



(G2),



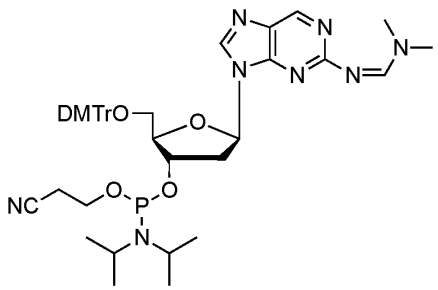
(C2),



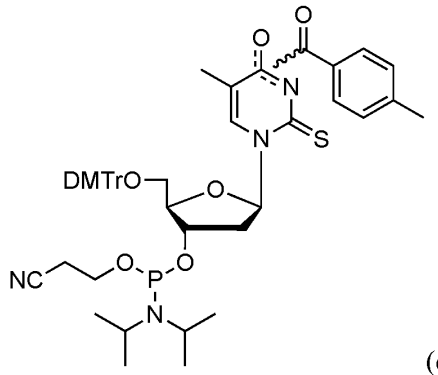
(U2).

[0141] In some instances, the A2, G2, C2, or U2 is placed in the polynucleic acid molecule. In some instances, the A2, G2, C2, or U2 substitutes one or more nucleotide in siRNA. In some instances, the A2, G2, C2, or U2 substitutes one or more nucleotide in a passenger strand and/or a guide strand. In some instances, the A2, G2, C2, or U2 substitutes one or more nucleotide in a passenger strand. In some instances, the A2, G2, C2, or U2 substitutes one or more nucleotide in a guide strand.

[0142] In some instances, the amidite structures of 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3) and 2'-deoxy-2-thiothymidine-3'-phosphate (dT3) are shown as below:

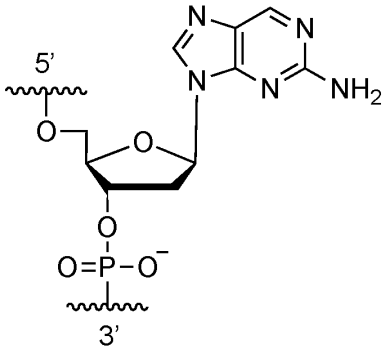


(dA3),

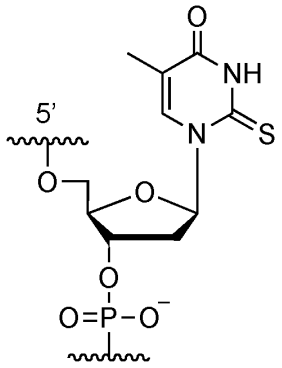


(dT3).

[0143] In some instances, the 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3) or 2'-deoxy-2-thiothymidine-3'-phosphate (dT3) is incorporated into the polynucleic acid molecule. In some instances, the dA3 or dT3 is incorporated into the siRNA. In some instances, the dA3 or dT3 is incorporated into a guide strand. In some instances, the dA3 or dT3 is incorporated into a passenger strand. In some instances, an incorporated dA3 or dT3 has structure shown as below:



(dA3),



(dT3).

[0144] In some instances, the dA3 or dT3 is placed in the polynucleic acid molecule. In some instances, the dA3 or dT3 substitutes one or more nucleotide in siRNA. In some instances, the dA3 or dT3 substitutes one or more nucleotide in a passenger strand and/or a guide strand. In some instances, the dA3 or dT3 substitutes one or more nucleotide in a passenger strand. In some instances, the dA3 or dT3 substitutes one or more nucleotide in a guide strand.

[0145] In some instances, the 2'-deoxyadenosine-3-phosphate (dA), 2'-deoxycytidine-3'-phosphate (dC), 2'-deoxyguanosine-3'-phosphate (dG), or 2'-deoxythymidine-3'-phosphate (dT) is incorporated into the polynucleic acid molecule. In some instances, the 2'-deoxyadenosine-3-phosphate (dA), 2'-deoxycytidine-3'-phosphate (dC), 2'-deoxyguanosine-3'-phosphate (dG), or 2'-deoxythymidine-3'-phosphate (dT) is incorporated into the siRNA. In some instances, the 2'-deoxyadenosine-3-phosphate (dA), 2'-deoxycytidine-3'-phosphate (dC), 2'-deoxyguanosine-3'-phosphate (dG), or 2'-deoxythymidine-3'-phosphate (dT) is incorporated into a guide strand. In some instances, the 2'-deoxyadenosine-3-phosphate (dA), 2'-deoxycytidine-3'-phosphate (dC), 2'-deoxyguanosine-3'-phosphate (dG), or 2'-deoxythymidine-3'-phosphate (dT) is incorporated into a passenger strand.

[0146] In some instances, the dA, dC, dG, or dT is placed in the polynucleic acid molecule. In some instances, the dA, dC, dG, or dT substitutes one or more nucleotide in siRNA. In some instances, the dA, dC, dG, or dT substitutes one or more nucleotide in a passenger strand and/or a guide strand. In some instances, the dA, dC, dG, or dT substitutes one or more nucleotide in a passenger strand. In some instances, the dA, dC, dG, or dT substitutes one or more nucleotide in a guide strand.

[0147] In some instances, the nucleotide analogue comprises hypoxanthine nucleobase-containing nucleoside (e.g., inosine).

[0148] In some aspects, the polynucleic acid molecule is an siRNA comprising a guide strand and a passenger strand, and the polynucleic acid molecule is modified with a nucleotide analogue described herein by incorporating the nucleotide analogue in a seed region of the guide strand. As

described herein, "seed region" refers to a region on the polynucleic acid molecule that comprises sequence that is essential for the binding of the polynucleic acid molecule described herein to the target RNA, e.g., mRNA. In some instances, the seed region refers the positions 2-8 from 5' end of the guide strand.

[0149] In some instances, the polynucleic acid molecule is modified with a nucleotide analogue described herein at positions 3-8, positions 4-8, positions 5-8, positions 6-8, or positions 7-8 from the 5' end of the guide strand. In some instances, the polynucleic acid molecule is modified with a nucleotide analogue described herein at position 2, position 3, position 4, position 5, position 6, position 7, position 8, or combination thereof, from the 5' end of the guide strand. In some instances, the polynucleic acid molecule is modified with one, two, three, four, five, six, or seven nucleotide analogues. In some instances, two or more consecutive positions in the guide strand of the polynucleic acid molecule are modified with nucleotide analogues. In some instances, two or more nucleotide analogue modifications can be placed in the polynucleic acid molecule at alternative positions (e.g., positions 3 and 5, positions 4 and 6, positions 5 and 7, etc.).

[0150] In some instances, the polynucleic acid molecule comprises an inhibitory polynucleic acid molecule. In some instances, the polynucleic acid molecule is an siRNA comprising a guide (or antisense) strand and a passenger (or sense) strand. In some instances, the guide strand comprises one or more nucleotide analogue described herein. In some instances, the passenger strand comprises one or more nucleotide analogue described herein. In some instances, the nucleotide analogue is located at the seed region of the guide strand at positions 2-8 from the 5' end. In some instances, the nucleotide analogue is located at positions 3-8 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at positions 4-8 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at positions 5-8 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at positions 6-8 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at positions 6-7 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at positions 7-8 from the 5' end of the guide strand.

[0151] In some instances, the nucleotide analogue is located at position 1 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 2 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 3 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 4 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 5 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 6 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 7

from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 8 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 9 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 10 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 11 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 12 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 13 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 14 from the 5' end of the guide strand.

[0152] In some instances, the nucleotide analogue is located at position 3 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 3 from the 5' end of the guide strand, and the guide strand further comprises 2-thiouridine-3'-phosphate nucleotide. In some instances, the nucleotide analogue located at position 3 from the 5' end of the guide strand, and the guide strand further comprises 2'-O-methyl-2-thiouridine-3'-phosphate (u3). In some instances, the nucleotide analogue is located at position 3 from the 5' end of the guide strand, and the guide strand further comprises at least one, at least two, at least three, or at least four 2'-F modified nucleotides. In some instances, the nucleotide analogue is located at position 3 from the 5' end of the guide strand, and the guide strand further comprises 2'-F modified nucleotides at at least one of positions 2, 7, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 3 from the 5' end of the guide strand, and the guide strand further comprises 2'-F modified nucleotides at positions 2, 7, 12, 14, and 16 from the 5' end.

[0153] In some instances, the passenger strand comprises one or more nucleotide analogue at locations opposite to the seed region of the guide strand. In some instances, the passenger strand comprises one or more nucleotide analogue at locations 12-22 from the 5' end. In some instances, the passenger strand comprises one or more nucleotide analogue at locations 2-10 from the 3' end. In some instances, the passenger strand comprises one or more 2-amino-2'-O-methyladenosine-3'-phosphate (a1). In some instances, the passenger strand comprises 2-amino-2'-O-methyladenosine-3'-phosphate (a1) at location 11, 12, 13, 14, 15, 16, or 17 from the 5' end. In some instances, the passenger strand comprises 2-amino-2'-O-methyladenosine-3'-phosphate (a1) at location 13 from the 5' end. In some instances, the passenger strand comprises 2-amino-2'-O-methyladenosine-3'-phosphate (a1) at location 14 from the 5' end. In some instances, the passenger strand comprises 2-amino-2'-O-methyladenosine-3'-phosphate (a1) at location 15 from the 5' end. In some instances, the passenger strand comprises 2-amino-2'-O-methyladenosine-3'-phosphate (a1) at location 16 from the 5' end. In some instances, the passenger strand comprises 2-amino-2'-O-methyladenosine-3'-phosphate (a1) at location 17 from the 5' end.

[0154] In some aspects, the guide strand comprises a nucleotide analogue selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), or thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), and inosine, 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), c3 spacer (Pr), A2, C2, U2, or G2. In some instances, the guide strand comprises a nucleotide analogue selected from a group consisting of acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), and 1',2'-Dideoxyribose-3'phosphate (dAB).

Types of modifications

[0155] In some aspects, the polynucleic acid molecule described herein comprises one or more sugar-modified nucleotide. In some instances, the one or more sugar-modified nucleotide comprises a 2'-fluoro modified nucleotide. In some instances, the 2'-fluoro modified nucleotide comprises thio-modified base containing nucleotide, e.g., 2'-fluoro-2-thiouridine-3'-phosphate (U3f). In some instances, the sugar-modified nucleotide includes, but is not limited to, a modification at a 2' hydroxyl group of the ribose moiety. In some instances, the sugar-modified nucleotide includes modification with an H, OR, R, halo, SH, SR, NH2, NHR, NR2, or CN, wherein R is an alkyl moiety. In some aspects, the sugar-modified nucleotide is a 2'-fluoro modified nucleotide. In some aspects, the sugar-modified nucleotide is a 2'-O-methyl modified nucleotide or a 2'-alkoxy modified nucleotide (*e.g.*, 2'-methoxy modified nucleotide). In some instances, 2' hydroxyl group modification includes 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O- dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA). In some instances, the alkyl moiety comprises a hetero substitution. In some instances, the carbon of the heterocyclic group is substituted by a nitrogen, oxygen, or sulfur. In some instances, the heterocyclic substitution includes morpholino, imidazole, and pyrrolidino.

[0156] In some instances, 2' hydroxyl group of the ribose moiety includes, but is not limited to, a locked or bridged ribose modification (e.g., LNA), an unlocked ribose modification (e.g., UNA), or ethylene nucleic acids (ENA).

[0157] In some aspects, the sugar-modified nucleotide is a 2'- amino modified nucleotide. In some aspects, the sugar-modified nucleotide is a 2'- azido modified nucleotide. In some aspects, the sugar-modified nucleotide is a 2'- deoxy modified nucleotide. In some aspects, the sugar-modified

nucleotide is a 2'-O-methoxythyl (2'-MOE). In some aspects, the sugar-modified nucleotide is a locked nucleic acid (LNA). In some aspects, the sugar-modified nucleotide is an ethylene-bridged nucleic acid (ENA). In some aspects, the sugar-modified nucleotide is a (S)-constrained ethyl (cEt). In some aspects, the sugar-modified nucleotide is a tricyclo-DNA (tcDNA). In some aspects, the sugar-modified nucleotide is a 2'-NH2 nucleic acid.

[0158] In some aspects, the polynucleotide acid molecule described herein comprises one or more sugarphosphate-modified nucleotide. In some aspects, the modified sugarphosphate is phosphorodiamidate morpholino (PMO). In some aspects, the modified sugarphosphate is phosphoramidate. In some aspects, the modified sugarphosphate is thiophosphoramidate. In some aspects, the modified sugarphosphate is peptide nucleic acid (PNA).

[0159] In some aspects, the polynucleic acid molecule described herein comprises one or more backbone-modified nucleotide. In some aspects, the modified backbone is phosphorothioate. In some aspects, the modified backbone is methylphosphonate. In some aspects, the modified backbone is guanidinopropyl phosphoramidate. In some aspects, the modified backbone is a mesyl-phosphoramidate (MsPA) linkages. In some instances, the modified backbone comprises one or more of phosphorodithioates, methylphosphonates, 5'- alkylenephosphonates, 5'-methylphosphonate, 3'-alkylene phosphonates, borontrifluoridates, borano phosphate esters and selenophosphates of 3'-5' linkage or 2'-5' linkage, phosphotriesters, thionoalkylphosphotriesters, hydrogen phosphonate linkages, alkyl phosphonates, alkylphosphonothioates, arylphosphonothioates, phosphoroselenoates and phosphoramidates.

[0160] In some aspects, the modified nucleotide comprises a modified guanine (e.g., inosine) or one or more of any types of unnatural nucleic acids.

[0161] In some aspects, the modified backbone is phosphorothioate, and the phosphorothioate is a stereochemically enriched phosphorothioate. In certain aspects, the strand contains at least one stereochemically enriched phosphorothioate. In some aspects, the strand comprises at least 1, 2, 3 stereochemically enriched phosphorothioates. In some aspects, the strand comprises only 1, 2, 3, or 4 stereochemically enriched phosphorothioates. In further aspects, at least one (*e.g.*, one or two) stereochemically enriched phosphorothioate is disposed between two consecutive nucleosides that are two of six 5'-terminal nucleosides of the strand. In yet further aspects, at least one (*e.g.*, one or two) stereochemically enriched phosphorothioate is disposed between two consecutive nucleosides that are two of six 3'-terminal nucleosides of the strand. In still further aspects, one stereochemically enriched phosphorothioate is covalently bonded to the first nucleoside and the second nucleoside from the 5' end within the strand. In some aspects, one stereochemically enriched phosphorothioate is covalently bonded to the twenty first nucleoside and the twenty

second nucleoside from the 5' end within the strand. In certain aspects, one stereochemically enriched phosphorothioate is covalently bonded to the twenty second nucleoside and the twenty third nucleoside from the 5' end within the strand. In particular aspects, the stereochemically enriched phosphorothioate has R_P stereochemical identity. In certain aspects, the stereochemically enriched phosphorothioate has S_P stereochemical identity.

[0162] In some aspects, the polynucleic acid molecules described herein comprises one or more (e.g., from 1 to 20, from 1 to 10, or from 1 to 5) stereochemically enriched (e.g., internucleoside) phosphorothioates (e.g., having diastereomeric excess of at least 10%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90%, e.g., up to about 99%, for the P-stereogenic center). In some instances, the polynucleic acid molecules described herein comprises one or more (e.g., from 1 to 20, from 1 to 10, or from 1 to 5; e.g., internucleoside) phosphorodithioates. The phosphorodithioates may be non-P-stereogenic in the polynucleic acid molecules described herein. In some instances, phosphorothioates and phosphorodithioates may enhance the stability of the polynucleic acid molecules described herein to exonuclease activity of serum. Non-P-stereogenic phosphorodithioates may simplify the synthesis of the polynucleic acid molecule described herein by reducing the number of possible diastereomers. Typically, the phosphorothioate or phosphorodithioate may connect two contiguous nucleosides within the six 3'-terminal nucleosides and the six 5'-terminal nucleosides of the polynucleic acid molecules described herein. In some aspects, the stereochemically enriched phosphorothioate (e.g., R_P-enriched phosphorothioate) may be covalently bonded to the first nucleoside (e.g., the 3'-carbon atom of the first nucleoside) and the second nucleoside (e.g., the 5'-carbon atom of the second nucleoside) from the 5' end of the antisense strand. Additionally, or alternatively, the stereochemically enriched phosphorothioate (e.g., S_P-enriched phosphorothioate) may be covalently bonded to the 21st nucleoside (e.g., the 3'-carbon atom of the 21st nucleoside) from the 5' end and the 22nd nucleoside (e.g., the 5'-carbon atom of the 22nd nucleoside) of the antisense strand. Further, additionally or alternatively, the stereochemically enriched phosphorothioate (e.g., S_P-enriched phosphorothioate or Rp-enriched phosphorothioate) may be covalently bonded to the 22nd nucleoside (e.g., the 3'-carbon atom of the 22nd nucleoside) and the 23rd nucleoside (e.g., the 5'-carbon atom of the 23rd nucleoside) from the 5' end of the antisense strand.

[0163] Combinations of a 5' R_P-enriched phosphorothioate (e.g., R_P-enriched phosphorothioate covalently bonded to the first nucleoside (e.g., the 3'-carbon atom of the first nucleoside) and the second nucleoside (e.g., the 5'-carbon atom of the second nucleoside) from the 5' end and a 3' S_P-enriched phosphorothioate (e.g., S_P-enriched phosphorothioate covalently bonded to the 21st nucleoside (e.g., the 3'-carbon atom of the 21st nucleoside) and the 22nd nucleoside (e.g., the 5'-

carbon atom of the 22nd nucleoside) from the 5' end in an antisense strand can produce superior efficacy and/or duration of action, e.g., as measured by the reduction in the activity of the target relative to a reference guide strand that lacks the combination of a 5' *R*P-enriched phosphorothioate and a 3' *S*P-enriched phosphorothioate, or a 5' Rp-enriched phosphorothioate and a 3' Sp and Rp-enriched phosphorothioate. In some embodiments, the stereochemically enriched phosphorothioate may comprise $R_pR_pS_pS_p$ (R_pR_p at the positions 1 and 2 of the guide strand and S_pS_p at the positions 21 and 22 of the guide strand) or $R_pR_pS_pR_p$ (R_pR_p at the positions 1 and 2 of the guide strand and S_pR_p at the positions 21 and 22 of the guide strand). In some aspects, the polynucleic acid molecules described herein comprise four stereochemically enriched phosphorothioates: (1) a Rp-enriched phosphorothioate covalently bonded to the 1st nucleoside (e.g., the 3'-carbon atom of the 1st nucleoside) and the 2nd nucleoside (e.g., the 5'-carbon atom of the 2nd nucleoside) from the 5' end of the antisense strand; (2) a Rp-enriched phosphorothioate covalently bonded to the 2nd nucleoside (e.g., the 3'-carbon atom of the 2nd nucleoside) and the 3rd nucleoside (e.g., the 5'-carbon atom of the 3rd nucleoside) from the 5' end of the antisense strand; (3) a Sp-enriched phosphorothioate covalently bonded to the 21st nucleoside (e.g., the 3'-carbon atom of the 21st nucleoside) and the 22th nucleoside (e.g., the 5'-carbon atom of the 22th nucleoside) from the 5' end of the antisense strand; and (4) a Sp-enriched phosphorothioate covalently bonded to the 22th nucleoside (e.g., the 3'-carbon atom of the 22th nucleoside) and the 23rd nucleoside (e.g., the 5'-carbon atom of the 23rd nucleoside) from the 5' end of the antisense strand. In some aspects, the polynucleic acid molecules described herein comprises four stereochemically enriched phosphorothioates: (1) a Rp-enriched phosphorothioate covalently bonded to the 1st nucleoside (e.g., the 3'-carbon atom of the 1st nucleoside) and the 2nd nucleoside (e.g., the 5'-carbon atom of the 2nd nucleoside) from the 5' end of the antisense strand; (2) a Rp-enriched phosphorothioate covalently bonded to the 2nd nucleoside (e.g., the 3'-carbon atom of the 2nd nucleoside) and the 3rd nucleoside (e.g., the 5'-carbon atom of the 3rd nucleoside) from the 5' end of the antisense strand; (3) a Sp-enriched phosphorothioate covalently bonded to the 21st nucleoside (e.g., the 3'-carbon atom of the 21st nucleoside) and the 22th nucleoside (e.g., the 5'-carbon atom of the 22th nucleoside) from the 5' end of the antisense strand; and (4) a Rp-enriched phosphorothioate covalently bonded to the 22th nucleoside (e.g., the 3'-carbon atom of the 22th nucleoside) and the 23rd nucleoside (e.g., the 5'-carbon atom of the 23rd nucleoside) from the 5' end of the antisense strand.

[0164] In some aspects, the polynucleic acid molecule described herein comprises one or more purine modification. In some aspects, the purine modification described herein is 2,6-diaminopurine. In some aspects, the purine modification described herein is 3-deaza-adenine. In

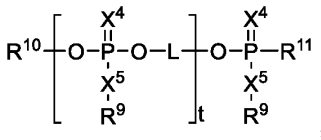
some aspects, the purine modification described herein is 7-deaza-guanine. In some aspects, the purine modification described herein is 8-azido-adenine.

[0165] In some aspects, the polynucleic acid molecule described herein comprises one or more pyrimidine modification. In some aspects, the pyrimidine modification described herein is 2-thio-thymidine. In some aspects, the pyrimidine modification described herein is 5-carboxamide-uracil. In some aspects, the pyrimidine modification described herein is 5-methyl-cytosine. In some aspects, the pyrimidine modification described herein is 5-ethynyl uracil.

[0166] In some cases, the polynucleic acid molecule described herein comprises an abasic substitution. In those cases where a hybridized polynucleotide construct is contemplated for use as siRNA, a reduction of miRNA-like off-target effects is desirable. The inclusion of one or more (e.g., one or two) abasic substitutions in the hybridized polynucleotide constructs may reduce or even eliminate miRNA-like off-target effects, as the abasic substitutions lack nucleobases that are capable of engaging in base-pairing interactions and alleviate steric hindrance. Thus, the polynucleic acid molecule disclosed herein may comprises one or more (e.g., one or two) abasic substitutions. In some aspects, abasic substitution is at the 5^{th} nucleotide from the 5' end of the antisense strand described herein. In some aspects, abasic substitution is at the 7^{th} nucleotide from the 5' end of the antisense strand described herein.

[0167] When the polynucleic acid molecule disclosed herein includes, but is not limited to, two or more of the abasic substitutions, their structures may be same or different. In certain aspects, a sense strand contains one abasic substitution (e.g., an antisense strand may be free of abasic substitutions). In other aspects, an antisense strand contains one abasic substitution (e.g., a sense strand may be free of abasic substitutions). In yet other aspects, an antisense strand contains one abasic substitution, and a sense strand contains one abasic substitution. In further aspects, a sense strand includes, but is not limited to, an abasic substitution between a nucleoside number (x) and a nucleoside number (x+1), where x is an integer from 2 to 7. In yet further aspects, an antisense strand includes, but is not limited to, an abasic substitution between a nucleoside number (x) and a nucleoside number (x+1), where x is an integer from 2 to 7.

[0168] The abasic substitution may be of formula (III):



(III)

where

L is a sugar analogue, or is substituted with a heteroacyl from A, U ,C, G, or is any other substituted nucleic acid (e.g., locked or unlocked nucleic acid, glycol nucleic acid, etc.);

each X^4 is independently O or S;

each X^5 is independently O, S, NH, or a bond;

each R^9 is independently H, optionally substituted C_{1-6} alkyl, optionally substituted C_{2-6} alkenyl, optionally substituted C_{2-6} alkynyl, optionally substituted (C_{1-9} heterocyclyl)-C_{1-6}-alkyl, optionally substituted (C_{6-10} aryl)-C_{1-6}-alkyl, optionally substituted (C_{3-8} cycloalkyl)-C_{1-6}-alkyl, –LinkA(–T)$_p$, or a conjugation moiety;

each LinkA is independently a multivalent linker (e.g., including –C(O)–N(H)–);

each T is independently an auxiliary moiety;

R^{10} is a bond to a 3'-carbon atom of a nucleoside (x) in the strand;

R^{11} is a bond to a 5'-oxygen atom of a nucleoside (x+1) in the strand;

p is an integer from 1 to 6; and

t is an integer from 1 to 6.

[0169] In some aspects, the abasic substitution described herein is attached to the antisense strand of the polynucleic acid molecule described herein. In particular aspects, an abasic substitution (e.g., an internucleotide, abasic spacer of formula (III) in which t is 1) may be included, but is not limited to, in the antisense strand described herein (e.g., within the seed region of the guide strand). In some aspects, an abasic substitution (e.g., an internucleotide, abasic spacer of formula (III) in which t is 1) may be bonded to the 3' carbon atom of the second, third, fourth, or fifth nucleoside from the 5' end of the antisense strand described herein. In certain aspects, an abasic substitution (e.g., an internucleotide, abasic spacer of formula (III) in which t is 1) may be bonded to the 3' carbon atom of the thirteenth, fourteenth, fifteenth, or sixteenth nucleoside from the 5' end of the antisense strand described herein. In some aspects, an abasic substitution fourth, fifth, sixth, seventh, eighth, and/or ninth nucleoside from the 5' end of the antisense strand described herein.

[0170] The polynucleic acid molecule described herein may contain a strand, e.g., guide strand, including a seed region including, but not limiting to, a hypoxanthine nucleobase-containing nucleoside (e.g., inosine).

[0171] In certain aspects, the hypoxanthine nucleobase-containing nucleoside is a second nucleoside from the 5' end of the strand, e.g., guide strand. In further aspects, the hypoxanthine nucleobase-containing nucleoside is a third nucleoside from the 5' end of the strand, e.g., guide strand. In yet further aspects, the hypoxanthine nucleobase-containing nucleoside is a fourth nucleoside from the 5' end of the strand, e.g., guide strand. In still further aspects, the hypoxanthine

nucleobase-containing nucleoside is a fifth nucleoside from the 5' end of the strand, e.g., guide strand. In particular aspects, the hypoxanthine nucleobase-containing nucleoside is a sixth nucleoside from the 5' end of the strand, e.g., guide strand. In particular aspects, the hypoxanthine nucleobase-containing nucleoside is a seventh nucleoside from the 5' end of the strand, e.g., guide strand.

The Amount and Location of Modifications

[0172] In some aspects, the polynucleic acid molecule described herein comprises one or more type of modifications as described above. Accordingly, in some aspects, about 10% of the nucleotides from the polynucleic acid molecule described herein are modified with one or more type of modifications as described above. In other aspects, about 20% of the nucleotides from the polynucleic acid molecule described herein are modified with one or more type of modifications as described above. In other aspects, about 30% of the nucleotides from the polynucleic acid molecule described herein are modified with one or more type of modifications as described above. In other aspects, about 40% of the nucleotides from the polynucleic acid molecule described herein are modified with one or more type of modifications as described above. In other aspects, about 50% of the nucleotides from the polynucleic acid molecule described herein are modified with one or more type of modifications as described above. In other aspects, about 60% of the nucleotides from the polynucleic acid molecule described herein are modified with one or more type of modifications as described above. In other aspects, about 70% of the nucleotides from the polynucleic acid molecule described herein are modified with one or more type of modifications as described above. In other aspects, about 80% of the nucleotides from the polynucleic acid molecule described herein are modified with one or more type of modifications as described above. In other aspects, about 90% of the nucleotides from the polynucleic acid molecule described herein are modified with one or more type of modifications as described above. In other aspects, 100% of the nucleotides from the polynucleic acid molecule described herein are modified with one or more type of modifications as described above.

[0173] In some aspects, the one or more types of modifications described herein occurs in the seed region within the polynucleic acid molecule described herein. In some aspects, the one or more types of modifications described herein occurs at different positions within the polynucleic acid molecule described herein. In specific aspects, the one or more types of modifications described herein occurs at 3' end of the polynucleic acid molecule described herein. In specific aspects, the one or more types of modifications described herein occurs at 5' end of the polynucleic acid molecule described herein. In specific aspects, the one or more types of modifications described

herein occurs dispersedly within the polynucleic acid molecule described herein. In specific aspects, the one or more types of modifications described herein occurs in clusters within the polynucleic acid molecule described herein.

[0174] In some aspects, the guide strand comprises a nucleotide analogue at one of positions 2-8 from the 5' end, and further comprises a 2'-fluoro modified nucleotide at position 2 from the 5' end. In some aspects, the guide strand comprises a nucleotide analogue at one of positions 2-8 from the 5' end, and further comprises a 2'-fluoro modified nucleotide at position 7 from the 5' end. In some aspects, the guide strand comprises a nucleotide analogue at one of positions 2-8 from the 5' end, and further comprises 2'-fluoro modified nucleotides at position 12 from the 5' end. In some aspects, the guide strand comprises a nucleotide analogue at one of positions 2-8 from the 5' end, and further comprises a 2'-fluoro modified nucleotide at position 14 from the 5' end. In some aspects, the guide strand comprises a nucleotide analogue at one of positions 2-8 from the 5' end, and further comprises a 2'-fluoro modified nucleotide at position 16 from the 5' end. In other aspects, the guide strand comprises a nucleotide analogue at one of positions 2-8 from the 5' end, and further comprises a 2'-fluoro modified nucleotide at positions 2, 12, 14, 16, or combination thereof from the 5' end. In other aspects, the guide strand comprises a nucleotide analogue at one of positions 2-8 from the 5' end, and further comprises a 2'-fluoro modified nucleotide at positions 2, 7, 12, 14, 16, or combination thereof from the 5' end. In other aspects, the guide strand comprises a nucleotide analogue at one of positions 2-8 from the 5' end, and further comprises a 2'-fluoro modified nucleotide at at least three of positions 2, 12, 14, and 16 from the 5' end. In other aspects, the guide strand comprises a nucleotide analogue at one of positions 2-8 from the 5' end, and further comprises a 2'-fluoro modified nucleotide at at least three of positions 2, 7, 12, 14, and 16 from the 5' end.

[0175] In some instances, the nucleotide analogue is located at position 6 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 6 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at at least one of positions 2, 7, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 6 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at at least two of positions 2, 7, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 6 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at at least three of positions 2, 7, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 6 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at positions 2, 7, 12, 14, and 16 from the 5' end.

[0176] In some instances, the nucleotide analogue is located at position 7 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 7 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at at least one of positions 2, 6, 8, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 7 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at at least one of positions 2, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 7 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at at least two of positions 2, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 7 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at at least three of positions 2, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 7 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at positions 2, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 7 from the 5' end of the guide strand, and the guide strand comprises 2'-F modified nucleotides at positions 2, 6, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 7 from the 5' end of the guide strand, and the guide strand comprises 2'-F modified nucleotides at positions 2, 8, 12, 14, and 16 from the 5' end.

[0177] In some instances, the nucleotide analogue is located at position 8 from the 5' end of the guide strand. In some instances, the nucleotide analogue is located at position 8 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at at least one of positions 2, 7, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 8 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at at least two of positions 2, 7, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 8 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at at least three of positions 2, 7, 12, 14, and 16 from the 5' end. In some instances, the nucleotide analogue is located at position 8 from the 5' end of the guide strand, and the guide strand further comprises 2'-fluoro (2'-F) modified nucleotides at positions 2, 7, 12, 14, and 16 from the 5' end.

Specific Modification Patterns

[0178] In some aspects, described herein are modified polynucleic acid molecules comprising a passenger strand and a guide strand. In some instances, the guide strand comprises 2'-O-methyl modified nucleotide, 2'-F modified nucleotide, and/or nucleotide analogue described herein.

[0179] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmXFmmmmFmFmFmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-fluoro (2'-F) modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT). In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmXFmmmmFmFmFmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-fluoro (2'-F) modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn).

[0180] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmmXmmmmFmFmFmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT). In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmmXmmmmFmFmFmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn).

[0181] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmFXmmmmFmFmFmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is

2’-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3’-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2’-O-methyl-2-thiouridine-3’-phosphate (u3), 2’-fluoro-2-thiouridine-3’-phosphate (U3f), 2-amino-2’-O-methyladenosine-3’-phosphate (a1), C3-spacer (Pr), 2’,3’-seco-adenosine-3’-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2’,3’-seco-uridine-3’-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3’-phosphate (dA3), 2’-deoxy-2-thiothymidine-3’-phosphate (dT3), and 2’-deoxythymidine-3’-phosphate (dT). In some instances, the guide strand comprises a nucleic acid sequence of 5’- mFmmmFXmmmmFmFmFmmmmmmmm-3’, wherein m is 2’-O-methyl modified nucleotide, F is 2’-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3’-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn).

[0182] In some instances, the guide strand comprises a nucleic acid sequence of 5’-mFmmmmXFmmmFmFmFmmmmmmmm-3’, wherein m is 2’-O-methyl modified nucleotide, F is 2’-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3’-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2’-O-methyl-2-thiouridine-3’-phosphate (u3), 2’-fluoro-2-thiouridine-3’-phosphate (U3f), 2-amino-2’-O-methyladenosine-3’-phosphate (a1), C3-spacer (Pr), 2’,3’-seco-adenosine-3’-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2’,3’-seco-uridine-3’-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3’-phosphate (dA3), 2’-deoxy-2-thiothymidine-3’-phosphate (dT3), and 2’-deoxythymidine-3’-phosphate (dT). In some instances, the guide strand comprises a nucleic acid sequence of 5’- mFmmmmXFmmmFmFmFmmmmmmmm-3’, wherein m is 2’-O-methyl modified nucleotide, F is 2’-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3’-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn).

[0183] In some instances, the guide strand comprises a nucleic acid sequence of 5’-mFmmmmFXmmmFmFmFmmmmmmmm-3’, wherein m is 2’-O-methyl modified nucleotide, F is 2’-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3’-phosphate (T-A), acyclic N-acetyl L-

threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT). In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmmFXmmmFmFmFmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn).

[0184] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmmFmmmmXmFmFmmmmmmm -3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT). In some instances, the guide strand comprises a nucleic acid sequence of 5'- mFmmmmFmmmmXmFmFmmmmmmm -3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), and 2'-O-methyl-2-thiouridine-3'-phosphate (u3).

[0185] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmmXmmmmmmFmFmmmmmmm -3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB),

thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT). In some instances, the guide strand comprises a nucleic acid sequence of 5'- mFmmmmXmmmmmmFmFmmmmmmmm -3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), and 2'-O-methyl-2-thiouridine-3'-phosphate (u3).

[0186] In some instances, the guide strand comprises 2'-O-methyl modified nucleotide, 2'-F modified nucleotide, nucleotide analogue described herein, or phosphorothioate internucleotide linkage. In some instances, the guide strand comprises one or more phosphorothioate modified internucleotide linkages at the 5' end and one or more phosphorothioate modified internucleotide linkages at the 3' end. In some instances, the guide strand comprises two phosphorothioate modified internucleotide linkages at the 5' end and two phosphorothioate modified internucleotide linkages at the 3' end.

[0187] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmXFmmmmFmFmFmmmmmsmsm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, s is phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0188] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmmXmmmmFmFmFmmmmmsmsm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, s is phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-

adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0189] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmmFXmmmFmFmFmmmmmsmsm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, s is phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0190] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmmFmmmmYmFmFmmmmmsmsm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and Y is selected from 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2'-deoxythymidine-3'-phosphate (dT), and 2'-deoxy-2-thiothymidine-3'-phosphate (dT3).

[0191] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmIFmmmmYmFmFmmmmmsmsm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, I stands for 2'-O-methylinosine-3'-phosphate (i), and Y is selected from 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2'-deoxythymidine-3'-phosphate (dT), and 2'-deoxy-2-thiothymidine-3'-phosphate (dT3).

[0192] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmmXmmmmmmFmFmmmmmsmsm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-

thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0193] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmmXmmmmYmFmFmmmmmsmsm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT), and Y is selected from 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2'-deoxythymidine-3'-phosphate (dT), and 2'-deoxy-2-thiothymidine-3'-phosphate (dT3). In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'-msFsmmmmXmmmmYmFmFmmmmmsmsm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), and Y is selected from 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2'-deoxythymidine-3'-phosphate (dT), and 2'-deoxy-2-thiothymidine-3'-phosphate (dT3).

[0194] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmFXmmmmFmFmFmmmmmsmsm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a

phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0195] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmmXFmmmFmFmFmmmmmsmsm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'-phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0196] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmmFmmmmFmFmFmmmmmsmsm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, and s stands for a phosphorothioate internucleotide linkage.

[0197] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5' – msFsmmmmFmmmmXmFmFmmmmmsmsm – 3' where m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-

phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0198] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5' – msFsmmmYFmmmmXmFmFmmmmmsmsm – 3' where m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, Y stands for 2'-O-methylinosine-3'-phosphate (i), and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0199] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5' – msFsmmmmXmmmmXmFmFmmmmmsmsm – 3' where m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0200] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5' – msFsmmmmXXmmmFmFmFmmmmmsmsm – 3' where m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate

(dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0201] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5' – msFsmmmXXmmmmFmFmFmmmmmsmsm – 3' where m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0202] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5' – msFsmmmXFXmmmFmFmFmmmmmsmsm – 3' where m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0203] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5' – msFsmmXXFmmmmFmFmFmmmmmsmsm – 3' where m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-

thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0204] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5' – msFsmmmXFmmmmXmFmFmmmmmsmsm – 3' where m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0205] In some instances, the nucleotide analogue is located at at least one of positions 2-12 from the 5' end of the guide strand, and the nucleotide analogue comprises 2'-O-methyl-2-thiouridine-3'-phosphate (u3) or 2'-fluoro-2-thiouridine-3'-phosphate (U3f). In some instances, the nucleotide analogue that is located at position 12 of the 5' end of the guide strand, and the nucleotide analogue consists of 2'-fluoro-2-thiouridine-3'-phosphate (U3f). In some instances, the nucleotide analogue is located at position 3 of the 5' end of the guide strand, and the nucleotide analogue comprises 2'-O-methyl-2-thiouridine-3'-phosphate (u3). In some instances, the nucleotide analogue is located at position 3 of the 5' end of the guide strand, and the nucleotide analogue consists of 2'-O-methyl-2-thiouridine-3'-phosphate (u3).

[0206] In some instances, the nucleotides of the passenger strand comprises DNA nucleotide or RNA nucleotide. As described herein, in some instances, the DNA nucleotide comprises an unmodified DNA nucleotide comprising: an unmodified adenine nucleotide (A), an unmodified guanine nucleotide (G), an unmodified thymine nucleotide (T), or an unmodified cytosine nucleotide (C). As described herein, in some instances, the RNA nucleotide comprises an

unmodified RNA nucleotide comprising: an unmodified adenine nucleotide (A), an unmodified guanine nucleotide (G), an unmodified uracil nucleotide (U), or an unmodified cytosine nucleotide (C).

[0207] In some instances, the nucleotides of the passenger strand comprises the DNA nucleotide, RNA nucleotide, nucleotide analogue, 2'-F modified nucleotide, or 2'-O-alkyl modified nucleotide. In some instances, the 2'-O-alkly modified nucleotide comprises 2'-O-methyl modified nucleotides. In some instances, the nucleotides of the passenger strand that are not the nucleotide analogue or 2'-F modified nucleotide are selected from 2′-O-alkyl modified nucleotide, 2′-alkoxy modified nucleotide, 2′-alkyl modified nucleotide, 2′-halo modified nucleotide, DNA nucleotide, RNA nucleotide, ENA, BNA, LNA, and UNA. In some instances, the nucleotides in the passenger strand that are not 2'-F modified nucleotide are 2'-O-methyl modified nucleotides.

[0208] In some instances, the passenger strand comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, or at least eight 2'-fluoro (2'-F) modified nucleotides. In some instances, the passenger strand comprises at least two, at least three, or at least four 2'-F modified nucleotides. In some instances, the passenger strand comprises at most one, at most two, at most three, at most four, at most five, at most six, at most seven, or at most eight 2'-fluoro (2'-F) modified nucleotides. In some instances, the passenger strand comprises from one to eight, from two to eight, from three to eight, from four to eight, from five to eight, from six to eight, or from seven to eight 2'-fluoro (2'-F) modified nucleotides. In some instances, the passenger strand comprises one, two, three, four, five, six, seven, or eight 2'-fluoro (2'-F) modified nucleotides. In some instances, the passenger strand comprises one 2'-F modified nucleotides. In some instances, the passenger strand comprises two 2'-F modified nucleotides. In some instances, the passenger strand comprises three 2'-F modified nucleotides. In some instances, the passenger strand comprises four 2'-F modified nucleotides. In some instances, the passenger strand comprises five 2'-F modified nucleotides.

[0209] In some aspects, the passenger strand comprises a 2'-fluoro modified nucleotide at position 7 from the 5' end. In some aspects, the passenger strand comprises a 2'-fluoro modified nucleotide at position 9 from the 5' end. In some aspects, the passenger strand comprises 2'-fluoro modified nucleotides at position 11 from the 5' end. In some aspects, the passenger strand comprises a 2'-fluoro modified nucleotide at at least one of positions 7, 9, and 11 from the 5' end. In some aspects, the passenger strand comprises a 2'-fluoro modified nucleotide at positions 7, 9, 11, or combination thereof from the 5' end. In some aspects, the passenger strand comprises a 2'-fluoro modified nucleotide at positions 7, 9, and 11 from the 5' end.

[0210] In some instances, the passenger strand comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, or at least eight phosphorothioate modified internucleotide linkages. In some instances, the passenger strand comprises at least two, at least three, or at least four phosphorothioate modified internucleotide linkages. In some instances, the passenger strand comprises at least one phosphorothioate modified internucleotide linkage. In some instances, the passenger strand comprises at most one, at most two, at most three, at most four, at most five, at most six, at most seven, or at most eight phosphorothioate modified internucleotide linkages. In some instances, the passenger strand comprises one, two, three, four, five, six, seven, or eight phosphorothioate modified internucleotide linkages. In some instances, the passenger strand comprises from 1 to 8, from 2 to 8, from 3 to 8, from 4 to 8, from 5 to 8, or from 6 to 8 phosphorothioate modified internucleotide linkages. In some instances, the passenger strand comprises from 1 to 4, from 2 to 4, or from 3 to 4 phosphorothioate modified internucleotide linkages.

[0211] In some instances, the passenger strand comprises at least one, at least two, at least three, or at least four phosphorothioate modified internucleotide linkage at the 5' end. In some instances, the passenger strand comprises at least one phosphorothioate modified internucleotide linkage at the 5' end. In some instances, the passenger strand comprises at least one, at least two, at least three, or at least four phosphorothioate modified internucleotide linkage at the 3' end. In some instances, the passenger strand comprises at least one phosphorothioate modified internucleotide linkage at the 3' end.

[0212] In some instances, the passenger strand comprises one phosphorothioate modified internucleotide linkage at the 5' end. In some instances, the passenger strand comprises two phosphorothioate modified internucleotide linkage at the 5' end. In some instances, the passenger strand comprises three phosphorothioate modified internucleotide linkage at the 5' end. In some instances, the passenger strand comprises four phosphorothioate modified internucleotide linkage at the 5' end.

[0213] In some instances, the passenger strand comprises one phosphorothioate modified internucleotide linkage at the 3' end. In some instances, the passenger strand comprises two phosphorothioate modified internucleotide linkage at the 3' end. In some instances, the passenger strand comprises three phosphorothioate modified internucleotide linkage at the 3' end. In some instances, the passenger strand comprises four phosphorothioate modified internucleotide linkage at the 3' end.

[0214] In some instances, the passenger strand comprises one phosphorothioate modified internucleotide linkages at the 5' end and one phosphorothioate modified internucleotide linkages

at the 3’ end. In some instances, the passenger strand comprises two phosphorothioate modified internucleotide linkages at the 5’ end and two phosphorothioate modified internucleotide linkages at the 3’ end. In some instances, the passenger strand comprises three phosphorothioate modified internucleotide linkages at the 5’ end and three phosphorothioate modified internucleotide linkages at the 3’ end. In some instances, the passenger strand comprises four phosphorothioate modified internucleotide linkages at the 5’ end and four phosphorothioate modified internucleotide linkages at the 3’ end.

[0215] In some aspects, described herein is a modified polynucleic acid molecule comprising a guide strand and a passenger strand, and the passenger strand comprises 5’-msmsmmmmFmFmFmmmmmmmmmm-3’, 5’-msmsmmmmFmFmFmmX’mmmmmmm-3’, 5’-msmsmmmmFmFmFmmmX’mmmmmm -3’, 5’- msmsmmmmFmFmFmmmmX’mmmmm-3’, or 5’- msmsmmmmFmFmFmmmmmX’mmmm -3’, wherein m stands for a 2’-O-methyl modified nucleotide, F stands for a 2’-F modified nucleotide, “s” stands for a phosphorothioate internucleotide linkage, and X’ is 2-amino-2’-O-methyladenosine-3’-phosphate (a1).

[0216] In some aspects, described herein is modified double-stranded polynucleic nucleic acid molecule comprising a passenger strand and a guide strand. In some instances, the guide strand comprises a nucleic acid sequence of 5’-mFmmmXFmmmmFmFmFmmmmmmm-3’, and the passenger strand comprises 5’-mmmmmmFmFmFmmmmmmmmm-3’, wherein m is 2’-O-methyl modified nucleotide, F is 2’-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3’-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), or thymidine-glycol nucleic acid (GNA) S-isomer (Tgn).

[0217] In some instances, the guide strand comprises a nucleic acid sequence of 5’-mFmmmXFmmmmFmFmFmmmmmmm-3’, and the passenger strand comprises 5’-mmmmmmFmFmFmmmmmmmmm-3’, wherein m is 2’-O-methyl modified nucleotide, F is 2’-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3’-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn).

[0218] In some instances, the guide strand comprises a nucleic acid sequence of 5’-mFmmmXFmmmmFmFmFmmmmmmm-3’, and the passenger strand comprises 5’-mmmmmmFmFmFmmX’mmmmmmm-3’, wherein m is 2’-O-methyl modified nucleotide, F is 2’-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3’-phosphate (T-A), acyclic N-acetyl L-

threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), and X' is 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0219] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmXFmmmmFmFmFmmmmmmmm-3', and the passenger strand comprises 5'-mmmmmmFmFmFmmmX'mmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), and X' is 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0220] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmXFmmmmFmFmFmmmmmsmsm-3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0221] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmXFmmmmFmFmFmmmmmsmsm-3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmX'mmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-Nac), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-

aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT), and X' is 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0222] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmXFmmmmFmFmFmmmmmsmsm-3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmmX'mmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-Nac), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT), and X' is 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0223] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmmXmmmmFmFmFmmmmmmm-3', and the passenger strand comprises 5'-mmmmmmFmFmFmmmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), or thymidine-glycol nucleic acid (GNA) S-isomer (Tgn).

[0224] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmmXmmmmFmFmFmmmmmmm -3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmX'mmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), and X' is 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0225] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmmXmmmmFmFmFmmmmmmm -3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmmX'mmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-

threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), and X' is 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0226] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmmXmmmmFmFmFmmmmmsmsm-3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, s is phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0227] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmmXmmmmFmFmFmmmmmsmsm -3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmX'mmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT), and X' is 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0228] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmmXmmmmFmFmFmmmmmsmsm-3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmmX'mmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate

(u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT), and X' is 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0229] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmmFXmmmFmFmFmmmmmmm-3', and the passenger strand comprises 5'-mmmmmmFmFmFmmmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), or 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0230] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmmFXmmmFmFmFmmmmmmm-3', and the passenger strand comprises 5'-mmmmmmFmFmFmmmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn).

[0231] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmmFXmmmFmFmFmmmmmmm-3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmX'mmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), and X' is 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0232] In some instances, the guide strand comprises a nucleic acid sequence of 5'-mFmmmmFXmmmFmFmFmmmmmmm-3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmmX'mmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and

thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), and X' is 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0233] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmmFXmmmFmFmFmmmmmsmsm-3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, s is phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0234] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmmFXmmmFmFmFmmmmmsmsm-3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmmmmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, s is phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0235] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmmFXmmmFmFmFmmmmmsmsm-3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmX'mmmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate

(a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT), and X' is 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0236] In some instances, the guide strand comprises a nucleic acid sequence of 5'-msFsmmmmFXmmmFmFmFmmmmmsmsm-3', and the passenger strand comprises 5'-msmsmmmmFmFmFmmmX'mmmmmm-3', wherein m is 2'-O-methyl modified nucleotide, F is 2'-F modified nucleotide, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT), and X' is 2-amino-2'-O-methyladenosine-3'-phosphate (a1).

[0237] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmmFmmmmYmFmFmmmmmsmsm -3' and the passenger strand comprises 5'- msmsmmmmFmFmFmmmmmmmmmm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, Y is selected from 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2'-deoxythymidine-3'-phosphate (dT), and 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn).

[0238] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmIFmmmmYmFmFmmmmmsmsm -3' and the passenger strand comprises 5'- msmsmmmmFmFmFmmmmmmmmmm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, I stands for 2'-O-methylinosine-3'-phosphate (i), and Y is selected from 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2'-deoxythymidine-3'-phosphate (dT), and 2'-deoxy-2-thiothymidine-3'-phosphate (dT3).

[0239] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmmXmmmmmmFmFmmmmmsmsm -3' and the passenger strand comprises 5'- msmsmmmmFmFmFmmmmmmmmmm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0240] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmmXmmmmYmFmFmmmmmsmsm -3' and the passenger strand comprises 5'- msmsmmmmFmFmFmmmmmmmmmm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, Y is selected from 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2'-deoxythymidine-3'-phosphate (dT), and 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), and thymidine-glycol nucleic acid (GNA) S-isomer (Tgn).

[0241] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmFXmmmmFmFmFmmmmmsmsm -3' and the passenger strand comprises 5'- msmsmmmmFmFmFmmmmmmmmmm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-

aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

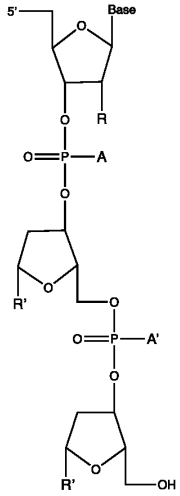
[0242] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmmXFmmmFmFmFmmmmmsmsm -3' and the passenger strand comprises 5'- msmsmmmmFmFmFmmmmmmmmmm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, s stands for a phosphorothioate internucleotide linkage, and X is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2-amino-2'-O-methyladenosine-3'-phosphate (a1), C3-spacer (Pr), 2',3'-seco-adenosine-3'-phosphate (A2), 2',3'-seco-guanosine-3'-phosphate (G2), 2',3'-seco-cytidine-3'-phosphate (C2), 2',3'-seco-uridine-3'-phosphate (U2), 2'-deoxy-2-aminopurine riboside-3'-phosphate (dA3), 2'-deoxy-2-thiothymidine-3'-phosphate (dT3), and 2'-deoxythymidine-3'-phosphate (dT).

[0243] In some aspects, described herein is a specific modification pattern, wherein the guide strand comprises 5'- msFsmmmmFmmmmFmFmFmmmmmsmsm -3' and the passenger strand comprises 5'- msmsmmmmFmFmFmmmmmmmmmm -3' wherein m stands for a 2'-O-methyl modified nucleotide, F stands for a 2'-F modified nucleotide, and s stands for a phosphorothioate internucleotide linkage.

[0244] In some aspects, described herein is a modified polynucleic acid having a guide strand and a passenger strand, wherein the passenger strand comprises about three 2'-fluoro modified nucleotides and about eighteen 2'-O-methyl modified nucleotides, with one or more inverted deoxy-nucleotides on the 3' end as an overhang.

[0245] In some instances, the passenger strand comprises 5'-mmmmmmFmFmFmmmmmmmmmm-invdN-invdN -3', wherein "F" stands for a 2'-fluoro modified nucleotide, wherein "m" stands for a 2'-O-methyl modified nucleotide, and "invdN" stands for an inverted deoxy-nucleotide. In some instances, the invdN is an inverted deoxyl-thymine. In some aspects, the linker conjugated with one or more targeting moieties as shown in Formula (IV'' or IV''') is added to the first nucleic acid on the 5' end. In some aspects, the linker conjugated with one or more GalNAc as shown in Formula (V'') or (V''') is added to the first nucleic acid on the 5' end. In some aspects, the modification pattern comprises one or more phosphorothioate linkages. In some aspects, the modification pattern is shown in Formula (VII). In

some aspects, the 5' end modification known in the art is applied to the one or more inverted nucleotides.



(VII), wherein R is a moiety that corresponds to the sugar modification described herein, in some instances, R is -O-methyl; wherein R' is thymine, abasic, or others; wherein A is -O or -S; and wherein A' is -O or -S.

[0246] In some aspects, the polynucleic acid molecule provided herein comprises a sense strand comprising a nucleic acid sequence selected from SEQ ID NOs: 14-20, and/or antisense strand comprising a nucleic acid sequence selected from SEQ ID NOs: 1-13, and wherein the sense strand is modified with modification pattern as described in the preceding paragraph. In some aspects, the polynucleic acid molecule provided herein comprises a sense strand comprising a nucleic acid sequence selected from SEQ ID NOs: 107-122, and/or antisense strand comprising a nucleic acid sequence selected from SEQ ID NOs: 101-106, and wherein the sense strand is modified with modification pattern as described in the preceding paragraph.

[0247] Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 80% identical to a nucleic acid sequence selected from a nucleic acid sequence selected from SEQ ID NOs: 72-91. Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 85% identical to a nucleic acid sequence selected from a nucleic acid sequence selected from SEQ ID NOs: 72-91. Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 90% identical to a nucleic acid sequence selected from SEQ ID NOs: 72-91. Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 72-91.

[0248] Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 80% identical to a nucleic acid sequence selected from a nucleic acid

sequence selected from SEQ ID NOs: 122-127. Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 85% identical to a nucleic acid sequence selected from a nucleic acid sequence selected from SEQ ID NOs: 122-127. Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 90% identical to a nucleic acid sequence selected from SEQ ID NOs: 122-127. Described herein is a polynucleic acid molecule, whose sense strand comprises a nucleic acid sequence that is at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 122-127.

[0249] Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 80% identical to a nucleic acid sequence selected from SEQ ID NOs: 21-71. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 85% identical to a nucleic acid sequence selected from SEQ ID NOs: 21-71. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 90% identical to a nucleic acid sequence selected from SEQ ID NOs: 21-71. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 21-71. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 80% identical to a nucleic acid sequence selected from SEQ ID NOs: 113-121. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 85% identical to a nucleic acid sequence selected from SEQ ID NOs: 113-121. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 90% identical to a nucleic acid sequence selected from SEQ ID NOs: 113-121. Described herein is a polynucleic acid molecule, which antisense strand comprises a nucleic acid sequence that is at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 113-121.

[0250] A polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence selected from SEQ ID NOs: 1-13 and a sense strand comprising the nucleotide sequence selected from SEQ ID NOs: 14-20.

[0251] A polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of UUGCGUUGAGCAUUGUGUCAGG (SEQ ID NO: 3) and a sense strand comprising the nucleotide sequence of UGACACAAUGCUCAAACGCAA (SEQ ID NO: 16).

[0252] A polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of

UUGCGCUGAGCAUUGUGUCAGG (SEQ ID NO: 4) and a sense strand comprising the nucleotide sequence of UGACACAAUGCUCAGGCGCAA (SEQ ID NO: 17).

[0253] A polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of UUGCGUCGAGCAUUGUGUCAGG (SEQ ID NO: 5) and a sense strand comprising the nucleotide sequence of UGACACAAUGCUCGGACGCAA (SEQ ID NO: 18).

[0254] A polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of UCUCUGTCCAUUACCGUGGUAGC (SEQ ID NO: 6) and a sense strand comprising the nucleotide sequence of UACCACGGUAAUGGACAGAGA (SEQ ID NO: 14).

[0255] A polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of UCUCUGUCCAUUACCGUGGUAGC (SEQ ID NO: 1) and a sense strand comprising the nucleotide sequence of UACCACGGUAAUGGACAGAGA (SEQ ID NO: 14).

[0256] In some instances, the passenger strand (sense strand) comprises a nucleic acid sequence of UACCACGGUAAUGGACAGAGA (SEQ ID NO: 14) and a guide strand (antisense strand) comprises a nucleic acid sequence of UCUCUGTCCAUUACCGUGGUAGC (SEQ ID NO: 6) or UCUCUGUCCAUUACCGUGGUAGC (SEQ ID NO: 1).

[0257] A polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of UCUCUGUCCAUTACCGUGGUAGC (SEQ ID NO: 7) and a sense strand comprising the nucleotide sequence of UACCACGGUAAUGGACAGAGA (SEQ ID NO: 14).

[0258] A polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of UCUCUGTCCAUTACCGUGGUAGC (SEQ ID NO: 8) and a sense strand comprising the nucleotide sequence of UACCACGGUAAUGGACAGAGA (SEQ ID NO: 14).

[0259] A polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence selected from SEQ ID NOs: 21-71 and a sense strand comprising the nucleotide sequence selected from SEQ ID NOs: 14-20. In some instances, the guide strand (antisense strand) comprises a nucleic acid sequence selected from SEQ ID NOs: 26-27.

[0260] In some aspects, provided herein is a polynucleic acid molecule for modulating expression of lipoprotein(a) (Lp(a)) gene, comprising a guide strand and a passenger strand, wherein the guide

strand comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from UCUCUGUCCAUUACCGUGGUAGC (SEQ ID NO: 1) or UUGCGUCUGAGCAUUGUGUCAGG (SEQ ID NO: 2), wherein the guide strand comprises a nucleotide analogue selected from a group consisting of the nucleotide analogue is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2'-deoxythymidine-3'-phosphate (dT); and 2'-deoxy-2-thiothymidine-3'-phosphate (dT3).

[0261] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usUfsgcgu(T-NAc)ugagCfaUfuGfugucasgsg (SEQ ID NO: 23) and a sense strand comprising the nucleotide sequence of usgsacacAfaUfgCfucaaacgcaa (SEQ ID NO: 74), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "(T-NAc)" refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate; and "s" refers to 3'-phosphorothioate.

[0262] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usUfsgcgu(T-NAc)ugagCfaUfuGfugucasgsg (SEQ ID NO: 23) and a sense strand comprising the nucleotide sequence of usgsacacAfaUfgCfucaa1acgcaa (SEQ ID NO: 75), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "(T-NAc)" refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate; "a1" refers to 2-Amino-2'-O-methyladenosine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0263] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usUfsgcg(T-NAc)CfugagCfaUfuGfugucasgsg (SEQ ID NO: 24) and a sense strand comprising the nucleotide sequence of usgsacacAfaUfgCfucaggcgcaa (SEQ ID NO: 76), wherein

"a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "(T-NAc)" refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate; and "s" refers to 3'-phosphorothioate.

[0264] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usUfsgcguCf(T-NAc)gagCfaUfuGfugucasgsg (SEQ ID NO: 25) and a sense strand comprising the nucleotide sequence of usgsacacAfaUfgCfucggacgcaa (SEQ ID NO: 77), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "(T-NAc)" refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate; and "s" refers to 3'-phosphorothioate.

[0265] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usCfsucug(T-T)ccauUfaCfcGfugguasgsc (SEQ ID NO: 26) and a sense strand comprising the nucleotide sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "(T-T)" refers to acyclic L-threoninol nucleic acid-thymine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0266] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usCfsucug(T-T)ccauUfaCfcGfugguasgsc (SEQ ID NO: 26) and a sense strand comprising the nucleotide sequence of usasccacGfgUfaAfugga1cagaga (SEQ ID NO: 78), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "(T-T)"

refers to acyclic L-threoninol nucleic acid-thymine-3'-phosphate; "a1" refers to 2-Amino-2'-O-methyladenosine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0267] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usCfsucugUfccauu3aCfcGfugguasgsc (SEQ ID NO: 27) and a sense strand comprising the nucleotide sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "u3" refers to 2'-O-methyl-2-thiouridine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0268] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usCfsucugUfccaudT3aCfcGfugguasgsc (SEQ ID NO: 28) and a sense strand comprising the nucleotide sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "dT3" refers to 2'-deoxy-2-thiothymidine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0269] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usCfsucugUfccauU3faCfcGfugguasgsc (SEQ ID NO: 29) and a sense strand comprising the nucleotide sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "U3f" refers to 2'-fluoro-2-thiouridine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0270] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usCfsucuiUfccauU3faCfcGfugguasgsc (SEQ ID NO: 30) and a sense strand comprising the nucleotide sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate;

"c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "U3f" refers to 2'-fluoro-2-thiouridine-3'-phosphate; "i" refers to 2'-O-methylinosine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0271] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usCfsucug(T-T)ccauuaCfcGfugguasgsc (SEQ ID NO: 31) and a sense strand comprising the nucleotide sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "(T-T)" refers to acyclic L-threoninol nucleic acid-thymine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0272] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usCfsucug(T-T)ccaudTaCfcGfugguasgsc (SEQ ID NO: 32) and a sense strand comprising the nucleotide sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "(T-T)" refers to acyclic L-threoninol nucleic acid-thymine-3'-phosphate; "dT" refers to 2'-deoxythymidine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0273] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usCfsucuGf(T-T)ccauUfaCfcGfugguasgsc (SEQ ID NO: 33) and a sense strand comprising the nucleotide sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "(T-T)"

refers to acyclic L-threoninol nucleic acid-thymine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0274] Further provided herein is a polynucleic acid molecule for modulating expression of Lp(a) gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usCfsucug(T-T)CfcauUfaCfcGfugguasgsc (SEQ ID NO: 34) and a sense strand comprising the nucleotide sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "(T-T)" refers to acyclic L-threoninol nucleic acid-thymine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0275] A polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence selected from SEQ ID NOs: 101-106 and a sense strand comprising the nucleotide sequence selected from SEQ ID NOs: 107-112.

[0276] A polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence selected from SEQ ID NOs: 113-121 and a sense strand comprising the nucleotide sequence selected from SEQ ID NOs: 122-127.

[0277] A polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of UCUGAGAAUACUGUCCCUUUGAA (SEQ ID NO: 103) and a sense strand comprising the nucleotide sequence of CAAAGGGACAGUAUUCUCAGA (SEQ ID NO: 109).

[0278] A polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of UGAAUACTGUCCCUUUUAAGCAA (SEQ ID NO: 104) and a sense strand comprising the nucleotide sequence of GCUUAAAAGGGACAGUAUUCA (SEQ ID NO: 107).

[0279] A polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of UGAAUACGUCCCUUUUAAGCAA (SEQ ID NO: 105) and a sense strand comprising the nucleotide sequence of GCUUAAAAGGGACAGUAUUCA (SEQ ID NO: 107).

[0280] A polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of

UGAAUACGUCCCUUUUAAGCAA (SEQ ID NO: 105) and a sense strand comprising the nucleotide sequence of GCUUAAAAGGGACGGUAUUCA (SEQ ID NO: 110).

[0281] A polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of UGAAUCUGUCCCUUUUAAGCAA (SEQ ID NO: 106) and a sense strand comprising the nucleotide sequence of GCUUAAAAGGGACAGAAUUCA (SEQ ID NO: 111).

[0282] A polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of UGAAUCUGUCCCUUUUAAGCAA (SEQ ID NO: 106) and a sense strand comprising the nucleotide sequence of GCUUAAAAGGGACAGGAUUCA (SEQ ID NO: 112).

[0283] Further provided herein is a polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usCfsugaGf(T-A)auacUfgUfcCfcuuugsasa (SEQ ID NO: 116) and a sense strand comprising the nucleotide sequence of csasaaggGfaCfaGfuauucucaga (SEQ ID NO: 124), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "(T-A)" refers to acyclic L-threoninol nucleic acid-adenine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0284] Further provided herein is a polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usCfsugag(T-A)AfuacUfgUfcCfcuuugsasa (SEQ ID NO: 117) and a sense strand comprising the nucleotide sequence of csasaaggGfaCfaGfuauucucaga (SEQ ID NO: 124), wherein "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "(T-A)" refers to acyclic L-threoninol nucleic acid-adenine-3'-phosphate; and "s" refers to 3'-phosphorothioate.

[0285] Further provided herein is a polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usGfsaauaCf(T-T)gucCfcUfuUfuaagcsasa (SEQ ID NO: 118) and a sense strand comprising the nucleotide sequence of gscsuuaaAfaGfgGfacaguauuca (SEQ ID NO: 122),

wherein “a” refers to 2’-O-methyladenosine-3’-phosphate; “Af” refers to 2’-fluoroadenosine-3’-phosphate; “c” refers to 2’-O-methylcytidine-3’-phosphate; “Cf” refers to 2’-fluorocytidine-3’-phosphate; “g” refers to 2’-O-methylguanosine-3’-phosphate; “Gf” refers to 2’-fluoroguanosine-3’-phosphate; “u” refers to 2’-O-methyluridine-3’-phosphate; “Uf” refers to 2’-fluorouridine-3’-phosphate; “(T-T)” refers to acyclic L-threoninol nucleic acid-thymine-3’-phosphate; and “s” refers to 3’-phosphorothioate.

[0286] Further provided herein is a polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usGfsaauaCf(T-NAc)gucCfcUfuUfuaagcsasa (SEQ ID NO: 119) and a sense strand comprising the nucleotide sequence of gscsuuaaAfaGfgGfacaguauuca (SEQ ID NO: 122), wherein “a” refers to 2’-O-methyladenosine-3’-phosphate; “Af” refers to 2’-fluoroadenosine-3’-phosphate; “c” refers to 2’-O-methylcytidine-3’-phosphate; “Cf” refers to 2’-fluorocytidine-3’-phosphate; “g” refers to 2’-O-methylguanosine-3’-phosphate; “Gf” refers to 2’-fluoroguanosine-3’-phosphate; “u” refers to 2’-O-methyluridine-3’-phosphate; “Uf” refers to 2’-fluorouridine-3’-phosphate; “(T-NAc)” refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate; and “s” refers to 3’-phosphorothioate.

[0287] Further provided herein is a polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usGfsaauaCf(T-NAc)gucCfcUfuUfuaagcsasa (SEQ ID NO: 119) and a sense strand comprising the nucleotide sequence of gscsuuaaAfaGfgGfacgguauuca (SEQ ID NO: 125), wherein “a” refers to 2’-O-methyladenosine-3’-phosphate; “Af” refers to 2’-fluoroadenosine-3’-phosphate; “c” refers to 2’-O-methylcytidine-3’-phosphate; “Cf” refers to 2’-fluorocytidine-3’-phosphate; “g” refers to 2’-O-methylguanosine-3’-phosphate; “Gf” refers to 2’-fluoroguanosine-3’-phosphate; “u” refers to 2’-O-methyluridine-3’-phosphate; “Uf” refers to 2’-fluorouridine-3’-phosphate; “(T-NAc)” refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate; and “s” refers to 3’-phosphorothioate.

[0288] Further provided herein is a polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usGfsaau(T-A)CfugucCfcUfuUfuaagcsasa (SEQ ID NO: 120) and a sense strand comprising the nucleotide sequence of gscsuuaaAfaGfgGfacaguauuca (SEQ ID NO: 122), wherein “a” refers to 2’-O-methyladenosine-3’-phosphate; “Af” refers to 2’-fluoroadenosine-3’-phosphate; “c” refers to 2’-O-methylcytidine-3’-phosphate; “Cf” refers to 2’-fluorocytidine-3’-phosphate; “g” refers to 2’-O-methylguanosine-3’-phosphate; “Gf” refers to 2’-fluoroguanosine-3’-phosphate; “u” refers to 2’-O-methyluridine-3’-phosphate; “Uf” refers to 2’-fluorouridine-3’-

phosphate; “(T-A)” refers to acyclic L-threoninol nucleic acid-adenine-3’-phosphate; and “s” refers to 3’-phosphorothioate.

[0289] Further provided herein is a polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usGfsaau(T-NAc)CfugucCfcUfuUfuaagcsasa (SEQ ID NO: 121) and a sense strand comprising the nucleotide sequence of gscsuuaaAfaGfgGfacagaauuca (SEQ ID NO: 126), wherein “a” refers to 2’-O-methyladenosine-3’-phosphate; “Af” refers to 2’-fluoroadenosine-3’-phosphate; “c” refers to 2’-O-methylcytidine-3’-phosphate; “Cf” refers to 2’-fluorocytidine-3’-phosphate; “g” refers to 2’-O-methylguanosine-3’-phosphate; “Gf” refers to 2’-fluoroguanosine-3’-phosphate; “u” refers to 2’-O-methyluridine-3’-phosphate; “Uf” refers to 2’-fluorouridine-3’-phosphate; “(T-NAc)” refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate; and “s” refers to 3’-phosphorothioate.

[0290] Further provided herein is a polynucleic acid molecule for modulating expression of APOC3 gene, wherein polynucleic acid molecule comprises an antisense strand comprising the nucleotide sequence of usGfsaau(T-NAc)CfugucCfcUfuUfuaagcsasa (SEQ ID NO: 121) and a sense strand comprising the nucleotide sequence of gscsuuaaAfaGfgGfacaggauuca (SEQ ID NO: 127), wherein “a” refers to 2’-O-methyladenosine-3’-phosphate; “Af” refers to 2’-fluoroadenosine-3’-phosphate; “c” refers to 2’-O-methylcytidine-3’-phosphate; “Cf” refers to 2’-fluorocytidine-3’-phosphate; “g” refers to 2’-O-methylguanosine-3’-phosphate; “Gf” refers to 2’-fluoroguanosine-3’-phosphate; “u” refers to 2’-O-methyluridine-3’-phosphate; “Uf” refers to 2’-fluorouridine-3’-phosphate; “(T-NAc)” refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate; and “s” refers to 3’-phosphorothioate.

Conjugation

Targeting Moiety

[0291] In certain aspects, the polynucleic acid molecule described herein is coupled or conjugated with one or more targeting moieties to form a polynucleotide-targeting moiety conjugate molecule. In some instances, a targeting moiety is selected based on its ability to target the conjugate molecule described herein to a desired cell population, tissue, or an organ selectively or preferably. In some instances, the targeting moiety targets the cell, tissue, or an organ that expresses the corresponding binding partner (e.g., either the corresponding receptor or ligand) of the targeting moiety. For example, the polynucleic acid molecule described herein could be targeted to hepatocytes expressing asialoglycoprotein (ASGP-R) by selecting a targeting moiety containing N-acetyl galactosamine (GalNAc) as the targeting moiety. Any suitable GalNAc molecules that are

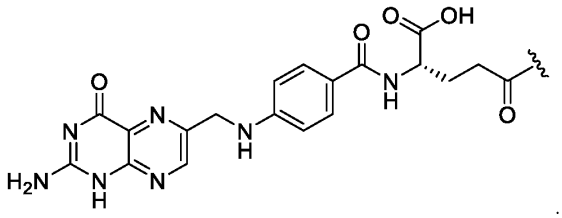
known in the art to be used as a targeting moiety are contemplated. Exemplary GalNAc molecule includes a triantennary GalNAc (e.g., L96). A further example of the targeting moiety is galactose. The targeting moiety can also be a lipid, peptide, or small molecule.

[0292] A targeting moiety (i.e., an intracellular targeting moiety) that targets a desired site within the cell (e.g., endoplasmic reticulum, Golgi apparatus, nucleus, or mitochondria) may be included in the hybridized polynucleotide constructs disclosed herein. Non-limiting examples of the intracellular targeting moieties are provided in WO 2015/069932 and in WO 2015/188197; the disclosure of the intracellular targeting moieties in WO 2015/069932 and in WO 2015/188197 is incorporated herein by reference.

[0293] The polynucleic acid molecule described herein, thus, may include one or more targeting moieties selected from the group consisting of intracellular targeting moieties, extracellular targeting moieties, and combinations thereof. Thus, the inclusion of one or more targeting moieties (e.g., extracellular targeting moieties including targeting moieties independently selected from the group consisting of folate, mannose, N-acetyl galactosamine, and prostate specific membrane antigen) and one or more intracellular targeting moiety (e.g., a moiety targeting endoplasmic reticulum, Golgi apparatus, nucleus, or mitochondria) in the polynucleic acid molecule described herein can facilitate the delivery of the polynucleotides to a specific site within the specific cell population. In some aspects, the targeting moiety contains one or more mannose carbohydrates. Mannose targets the mannose receptor, which is a 175 KDa membrane-associated receptor that is expressed on sinusoidal liver cells and antigen presenting cells (e.g., macrophages and dendritic cells). It is a highly effective endocytotic/recycling receptor that binds and internalizes mannosylated pathogens and proteins (Lennartz et. al. J. Biol. Chem. 262:9942-9944,1987; Taylor et. al. J. Biol. Chem. 265:12156-62, 1990).

[0294] Some of the targeting moieties are described herein. In some aspects, the targeting moiety contains or specifically binds to a protein selected from the group including insulin, insulin-like growth factor receptor 1 (IGF1R), IGF2R, insulin-like growth factor (IGF; e.g., IGF 1 or 2), mesenchymal epithelial transition factor receptor (c-met; also known as hepatocyte growth factor receptor (HGFR)), hepatocyte growth factor (HGF), epidermal growth factor receptor (EGFR), epidermal growth factor (EGF), heregulin, fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), platelet-derived growth factor (PDGF), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor (VEGF), tumor necrosis factor receptor (TNFR), tumor necrosis factor alpha (TNF-α), TNF-β, folate receptor (FOLR), folate, transferrin, transferrin receptor (TfR), mesothelin, Fc receptor, c-kit receptor, c-kit, an integrin (e.g., an α4 integrin or a β-1 integrin), P-selectin, sphingosine-1-phosphate receptor-1

(S1PR), hyaluronate receptor, leukocyte function antigen-1 (LFA-1), CD4, CD11, CD18, CD20, CD25, CD27, CD52, CD70, CD80, CD85, CD95 (Fas receptor), CD106 (vascular cell adhesion molecule 1 (VCAM1), CD166 (activated leukocyte cell adhesion molecule (ALCAM)), CD178 (Fas ligand), CD253 (TNF-related apoptosis-inducing ligand (TRAIL)), ICOS ligand, CCR2, CXCR3, CCR5, CXCL12 (stromal cell-derived factor 1 (SDF-1)), interleukin 1 (IL-1), IL-1ra, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, CTLA-4, MART-1, gp100, MAGE-1, ephrin (Eph) receptor, mucosal addressing cell adhesion molecule 1 (MAdCAM-1), carcinoembryonic antigen (CEA), LewisY, MUC-1, epithelial cell adhesion molecule (EpCAM), cancer antigen 125 (CA125), prostate specific membrane antigen (PSMA), TAG-72 antigen, and fragments thereof. In further aspects, the targeting moiety contains erythroblastic leukemia viral oncogene homolog (ErbB) receptor (e.g., ErbB1 receptor; ErbB2 receptor; ErbB3 receptor; and ErbB4 receptor). In some aspects, the targeting moiety contains one or more (e.g., from 1 to 6) N-acetyl galactosamines (GalNAc). In some aspects, the targeting moiety contains one or more (e.g., from 1 to 6) galactose. In certain aspects, the targeting moiety contains one or more (e.g., from 1 to 6) mannoses. In other aspects, the targeting moiety contains a folate ligand. The folate ligand has the structure:



Certain targeting moieties may include bombesin, gastrin, gastrin-releasing peptide, tumor growth factors (TGF) (e.g., TGF-α or TGF-β), or vaccinia virus growth factor (VVGF). Non-peptidyl targeting moieties can also be used in the targeting moieties and may include, for example, steroids, carbohydrates, vitamins, and lectins. Some targeting moieties may include a polypeptide, such as somatostatin or somatostatin analog (e.g., octreotide or lanreotide), bombesin, or an antibody or antigen-binding fragment thereof. Antibodies may be of any recognized class or subclass, e.g., IgG, IgA, IgM, IgD, or IgE. Typical are those antibodies which fall within the IgG class. The antibodies can be derived from any species according to techniques known in the art. Typically, however, the antibody is of human, murine, or rabbit origin. In addition, the antibody may be polyclonal or monoclonal, but is typically monoclonal. Human or chimeric (e.g., humanized) antibodies may be used in targeting moieties. Targeting moieties may include an antigen-binding fragment of an antibody. Such antibody fragments may include, for example, the Fab,' F(ab')2, Fv, or Fab fragments, single domain antibody, ScFv, or other antigen-binding fragments. Fc fragments may also be employed in targeting moieties. Such antibody fragments can

be prepared, for example, by proteolytic enzyme digestion, for example, by pepsin or papain digestion, reductive alkylation, or recombinant techniques. The materials and methods for preparing antibody fragments are well-known to those skilled in the art. See, e.g., Parham, J. Immunology, 131:2895, 1983; Lamoyi et al., J. Immunological Methods, 56:235, 1983.

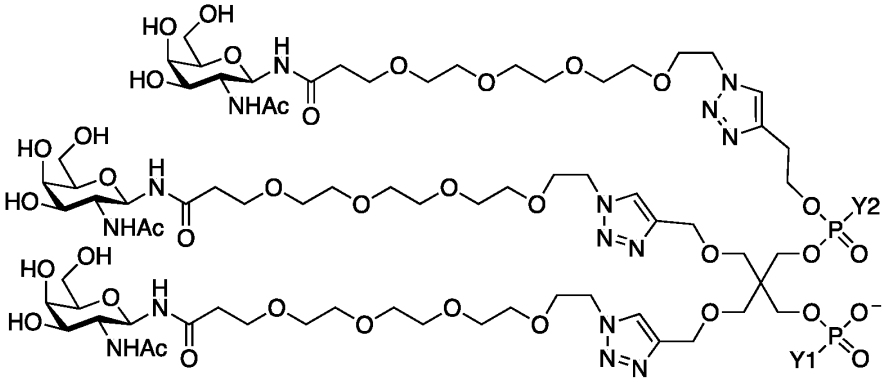
[0295] Other peptides for use as a targeting auxiliary moiety in polynucleic acid molecule described herein can be selected from KiSS peptides and analogs, urotensin II peptides and analogs, GnRH I and II peptides and analogs, depreotide, vapreotide, vasoactive intestinal peptide (VIP), cholecystokinin (CCK), RGD-containing peptides, melanocyte-stimulating hormone (MSH) peptide, neurotensin, calcitonin, glutathione, YIGSR (leukocyte-avid peptides, e.g., P483H, which contains the heparin-binding region of platelet factor-4 (PF-4) and a lysine-rich sequence), atrial natriuretic peptide (ANP), β-amyloid peptides, delta-opioid antagonists (such as ITIPP(psi)), annexin-V, endothelin, leukotriene B4 (LTB4), chemotactic peptides (e.g., N-formyl-methionyl-leucyl-phenylalanine-lysine (fMLFK), GP IIb/IIIa receptor antagonists (e.g., DMP444), human neutrophil elastase inhibitor (EPI-HNE-2 and EPI-HNE-4), plasmin inhibitor, antimicrobial peptides, apticide (P280 and P274), thrombospondin receptor (including analogs such as TP-1300), bitistatin, pituitary adenylyl cyclase type I receptor (PAC1), fibrin α-chain, peptides derived from phage display libraries, and conservative substitutions thereof.

[0296] One or more (e.g., from 1 to 6) targeting moieties can be linked to MOIETY or to X2 in formula (V', V'', V''', V'''', V''''', V''''''') through –LinkA–.

[0297] In some aspects, the targeting moiety includes one or more (e.g., from 1 to 6 or from 1 to 3) asialoglycoprotein receptor ligands (e.g., GalNAc). In some aspects, an asialoglycoprotein receptor ligand (e.g., GalNAc) is attached to –LinkA– through an anomeric carbon (e.g., where the anomeric carbon is the carbon atom in an acetal or a hemiaminal). An asialoglycoprotein receptor ligand (e.g., GalNAc) attached to a linker through a hemiaminal may produce a hybridized polynucleotide construct having superior efficacy in gene silencing as compared to hybridized polynucleotide constructs having the asialoglycoprotein receptor ligand (e.g., GalNAc) attached to a linker through an acetal.

[0298] In some aspects, the linker and three asialoglycoprotein receptor targeting moieties, each of which comprises GalNAc, are as shown in Formula (V). In some instances, the conjugate described herein only comprises one asialoglycoprotein receptor targeting moiety, so the conjugate comprises a structure of Formula (V) with any two of the targeting moieties removed. In some instances, the conjugate described herein only comprises two asialoglycoprotein receptor targeting moieties, so the conjugate described herein comprises a structure of Formula (V) with any one of the targeting moieties removed.

[0299] In some instances, the structure of Formula (V) is shown below:

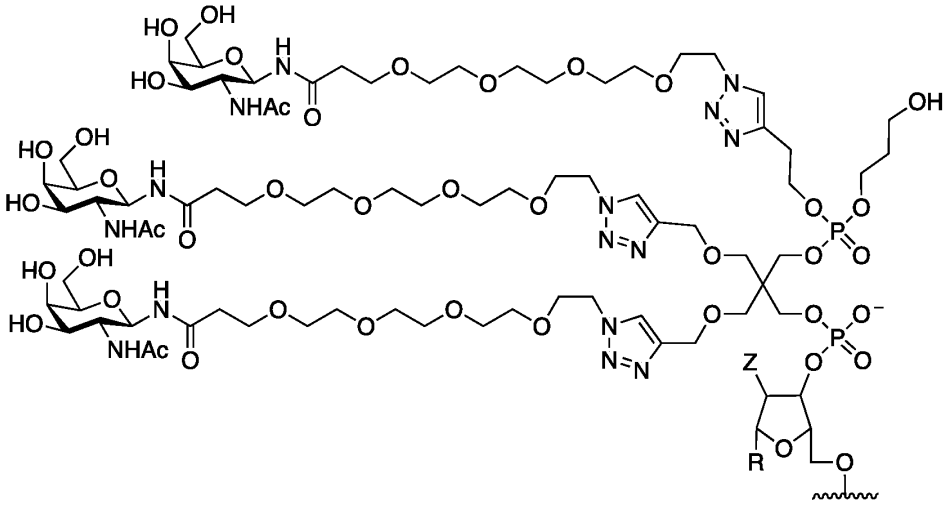


(V),

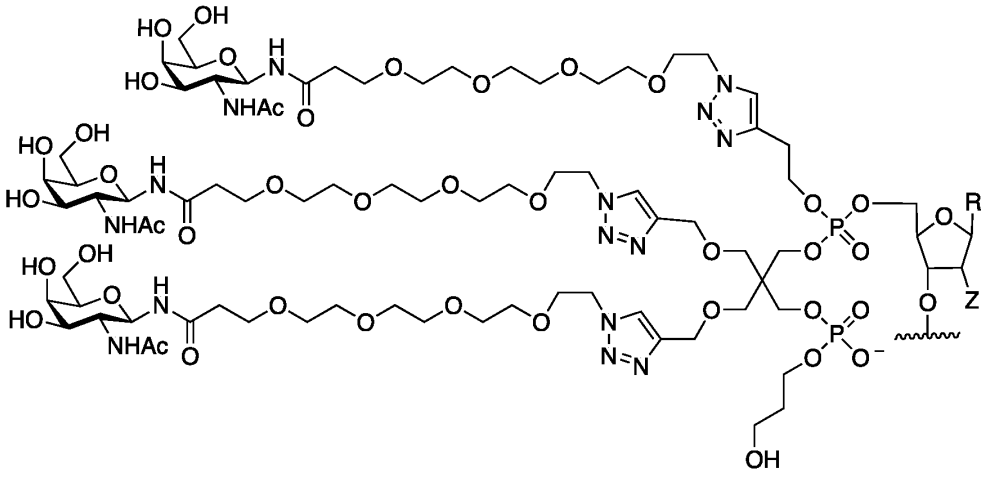
wherein one of Y1 and Y2 is nucleotide, or wherein both Y1 and Y2 are nucleotides and Y1 and Y2 are consecutive or neighboring nucleotides from the polynucleic acid molecule described herein.

[0300] In some aspects, the linker and the targeting moieties described herein are conjugated to 3' end of the sense strand (e.g., as shown in Formula (V', V'''', V''''', V''''''')). In some aspects, the linker and the targeting moieties described herein are conjugated to 5' end of the sense strand (e.g., as shown in Formula (V'') or (V''')). In some aspects, the linker and the targeting moieties described herein are conjugated to 3' end of the antisense strand(e.g., as shown in Formula (V'), (V''''), (V''''')), (V''''''')). In some aspects, the linker and the targeting moieties described herein are conjugated to 5' end of the antisense strand (e.g., as shown in Formula (V'') or (V''')).

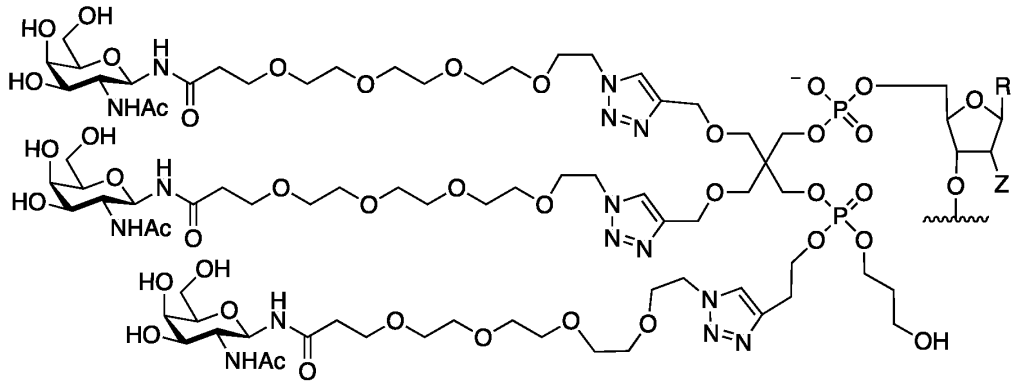
[0301] In some instances, the structure of Formula (V'), Formula (V''), Formula (V'''), Formula (V''''), Formula (V'''''), or Formula (V''''''') is shown below:



(V’) , wherein Z in formula (V’) is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V’) is adenine, uracil, guanine, cytosine, thymine, abasic, or others;

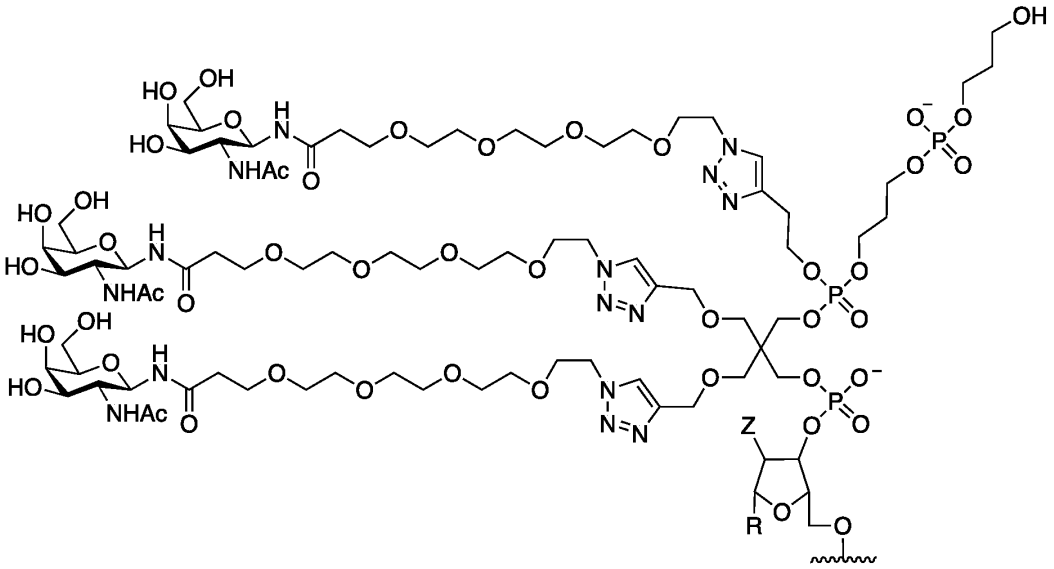


(V’’), wherein Z in formula (V’’) is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V’’) is adenine, uracil, guanine, cytosine, thymine, abasic, or others;



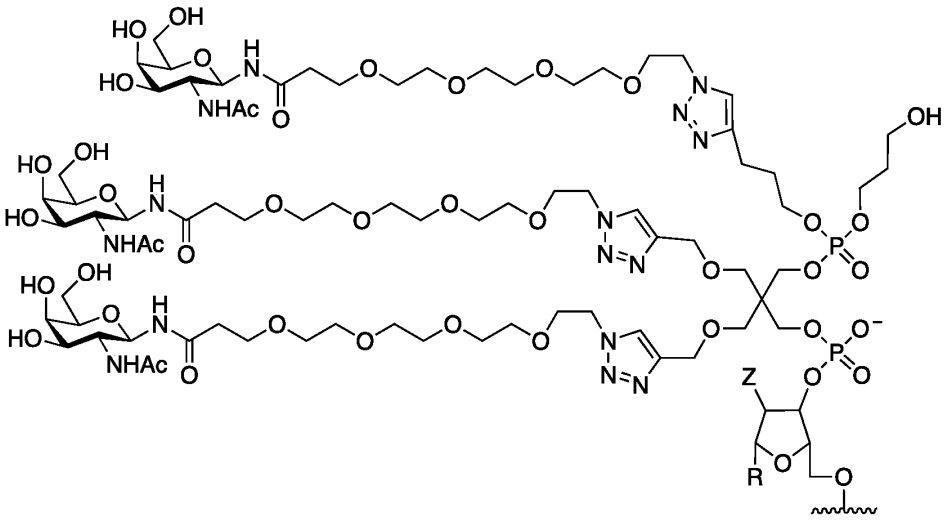
(V’’’),

wherein Z in formula (V’’’) is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V’’’) is adenine, uracil, guanine, cytosine, thymine, abasic, or others;



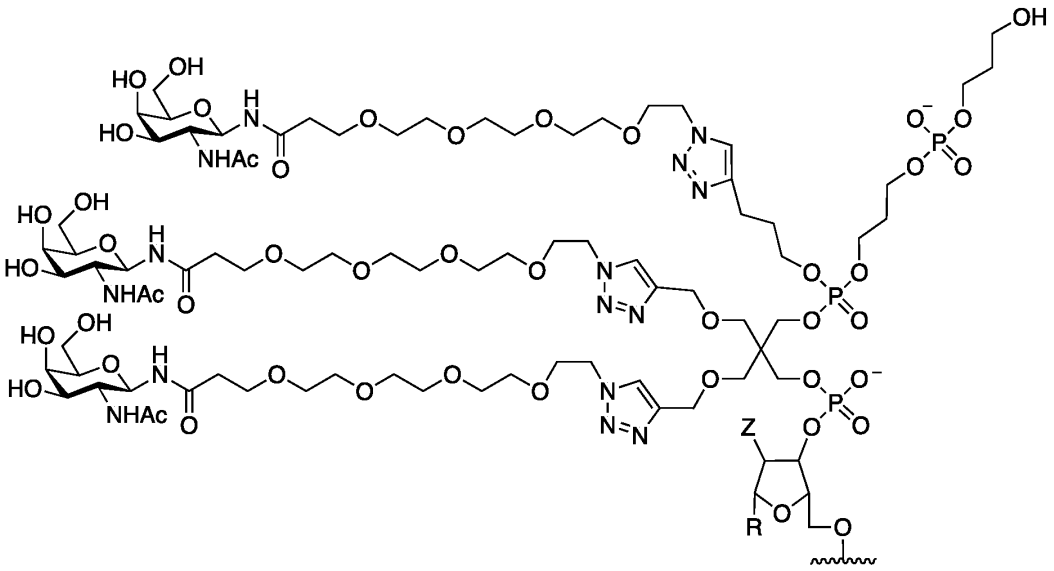
(V’’’’),

wherein Z in formula (V’’’’) is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V’’’’) is adenine, uracil, guanine, cytosine, thymine, abasic, or others;



(V’’’’’),

wherein Z in formula (V’’’’’) is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V’’’’’) is adenine, uracil, guanine, cytosine, thymine, abasic, or others;



(V’’’’’’),

wherein Z in formula (V’’’’’’) is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V’’’’’’) is adenine, uracil, guanine, cytosine, thymine, abasic, or others.

[0302] In some instances, the 3’ end of passenger/sense strand from Table 1, Table 2, Table 3, Table 4, or Table 5 is conjugated with X2-GalNAc (*see* Formula (V), (V’), (V’’’’), (V’’’’’), (V’’’’’’)). In some instances, the 5’ end of passenger/sense strand from Table 1, Table 2, Table 3, Table 4, or Table 5 is conjugated with X2-GalNAc (*see* Formula (V), (V’’), or (V’’’)). In some

instances, a nucleic acid within passenger/sense strand (not at the 5' or 3' end) from Table 1, Table 2, Table 3, Table 4, or Table 5 is conjugated with X2-GalNAc (*see* Formula (V)). In some instances, the 3' end of guide/antisense strand from Table 1, Table 2, Table 3, Table 4, or Table 5 is conjugated with X2-GalNAc (*see* Formula (V), (V'), (V''''), (V'''''), (V''''''')). In some instances, the 5' end of guide/antisense strand from Table 1, Table 2, Table 3, Table 4, or Table 5 is conjugated with X2-GalNAc (*see* Formula (V), (V''), or (V''')). In some instances, a nucleic acid within guide/antisense strand (not at the 5' or 3' end) from Table 1, Table 2, Table 3, Table 4, or Table 5 is conjugated with X2-GalNAc (*see* Formula (V)).

[0303] In some instances, one or more endosomal escape moieties (e.g., from 1 to 6 or from 1 to 3) can be attached to a polynucleotide construct or a hybridized polynucleotide construct disclosed herein as an auxiliary moiety. Exemplary endosomal escape moieties include chemotherapeutics (e.g., quinolones such as chloroquine); fusogenic lipids (e.g., dioleoylphosphatidyl-ethanolamine (DOPE)); and polymers such as polyethylenimine (PEI); poly(beta-amino ester)s; polypeptides, such as polyarginines (e.g., octaarginine) and polylysines (e.g., octalysine); proton sponges, viral capsids, and peptide transduction domains as described herein. For example, fusogenic peptides can be derived from the M2 protein of influenza A viruses; peptide analogs of the influenza virus hemagglutinin; the HEF protein of the influenza C virus; the transmembrane glycoprotein of filoviruses; the transmembrane glycoprotein of the rabies virus; the transmembrane glycoprotein (G) of the vesicular stomatitis virus; the fusion protein of the Sendai virus; the transmembrane glycoprotein of the Semliki forest virus; the fusion protein of the human respiratory syncytial virus (RSV); the fusion protein of the measles virus; the fusion protein of the Newcastle disease virus; the fusion protein of the visna virus; the fusion protein of murine leukemia virus; the fusion protein of the HTL virus; and the fusion protein of the simian immunodeficiency virus (SIV). Other moieties that can be employed to facilitate endosomal escape are described in Dominska et al., Journal of Cell Science, 123(8):1183-1189, 2010. Specific examples of endosomal escape moieties including moieties suitable for conjugation to the hybridized polynucleotide constructs disclosed herein are provided, e.g., in WO 2015/188197; the disclosure of these endosomal escape moieties is incorporated by reference herein.

[0304] One or more endosomal escape moieties (e.g., from 1 to 6 or from 1 to 3) can be attached to a MOIETY or X2 in formula (V', V'', V''', V'''', V''''', or V''''''') through –LinkA–, as described herein.

[0305] One or more cell penetrating peptides (CPP) (e.g., from 1 to 6 or from 1 to 3) can be attached to a polynucleotide construct or a hybridized polynucleotide construct disclosed herein as an auxiliary moiety. The CPP can be linked to the hybridized polynucleotide bioreversibly through

a disulfide linkage, as disclosed herein. Thus, upon delivery to a cell, the CPP can be cleaved intracellularly, e.g., by an intracellular enzyme (e.g., protein disulfide isomerase, thioredoxin, or a thioesterase) and thereby release the polynucleotide.

[0306] CPPs are known in the art (e.g., TAT or Arg8) (Snyder and Dowdy, 2005, Expert Opin. Drug Deliv. 2, 43-51). Specific examples of CPPs including moieties suitable for conjugation to the hybridized polynucleotide constructs disclosed herein are provided, e.g., in WO 2015/188197; the disclosure of these CPPs is incorporated by reference herein.

[0307] CPPs are positively charged peptides that are capable of facilitating the delivery of biological cargo to a cell. It is believed that the cationic charge of the CPPs is essential for their function. Moreover, the transduction of these proteins does not appear to be affected by cell type, and these proteins can efficiently transduce nearly all cells in culture with no apparent toxicity (Nagahara et al., Nat. Med. 4:1449-52, 1998). In addition to full-length proteins, CPPs have also been used successfully to induce the intracellular uptake of DNA (Abu-Amer, supra), antisense polynucleotides (Astriab-Fisher et al., Pharm. Res, 19:744-54, 2002), small molecules (Polyakov et al., Bioconjug. Chem. 11:762-71, 2000) and even inorganic 40 nm iron particles (Dodd et al., J. Immunol. Methods 256:89-105, 2001; Wunderbaldinger et al., Bioconjug. Chem. 13:264-8, 2002; Lewin et al., Nat. Biotechnol. 18:410-4, 2000; Josephson et al., Bioconjug. Chem. 10:186-91, 1999) suggesting that there is considerable flexibility in particle size in this process.

[0308] In one case, a CPP useful in the methods and compositions as described herein includes a peptide featuring substantial alpha-helicity. It has been discovered that transfection is optimized when the CPP exhibits significant alpha-helicity. In another case, the CPP includes a sequence containing basic amino acid residues that are substantially aligned along at least one face of the peptide. A CPP described herein may be a naturally occurring peptide or a synthetic peptide.

[0309] One or more cell penetrating peptides (e.g., from 1 to 6 or from 1 to 3) can be attached to a MOIETY or X2 in formula (I) through –LinkA–, as described herein.

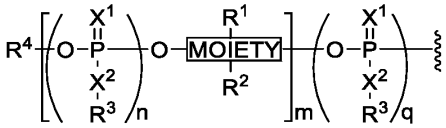
[0310] The polynucleotide constructs and the hybridized polynucleotide constructs disclosed herein can also include covalently attached neutral polymer-based auxiliary moieties. Neutral polymers include poly(C1-6 alkylene oxide), e.g., poly(ethylene glycol) and poly(propylene glycol) and copolymers thereof, e.g., di- and triblock copolymers. Other examples of polymers include esterified poly(acrylic acid), esterified poly(glutamic acid), esterified poly(aspartic acid), poly(vinyl alcohol), poly(ethylene-co-vinyl alcohol), poly(N-vinyl pyrrolidone), poly(ethyloxazoline), poly(alkylacrylates), poly(acrylamide), poly(N-alkylacrylamides), poly(N-acryloylmorpholine), poly(lactic acid), poly(glycolic acid), poly(dioxanone), poly(caprolactone), styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolide) copolymer, divinyl ether-maleic anhydride

copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyurethane, N-isopropylacrylamide polymers, and poly(N,N-dialkylacrylamides). Exemplary polymer auxiliary moieties may have molecular weights of less than 100, 300, 500, 1000, or 5000 Da (e.g., greater than 100 Da). Other polymers are known in the art.

[0311] One or more polymers (e.g., from 1 to 6 or from 1 to 3) can be attached to a MOIETY or X2 in formula (V', V'', V''', V'''', V''''', V'''''') through –LinkA–, as described herein.

Conjugation Linkers

[0312] In some aspects, the polynucleic acid molecules described herein comprises a sense or antisense strand bonded to at least one group of formula (I)



(I)

or a salt thereof, or a stereoisomer thereof,

where

each X^1 is independently O or S;

each X^2 is independently O, S, NH, or a bond;

MOIETY is optionally substituted C_{2-10} alkane-tetrayl or a group $–M^1–M^2–M^3–$, wherein each M^1 and each M^3 is independently absent or optionally substituted C_{1-6} alkylene, and M^2 is optionally substituted C_{3-9} heterocycle-tetrayl, optionally substituted C_{6-10} arene-tetrayl, or optionally substituted C_{3-8} cycloalkane-tetrayl;

each R^1 and each R^2 is independently H, optionally substituted C_{1-16} alkyl, optionally substituted C_{2-16} heteroalkyl, a conjugation moiety, or $–LinkA(–T)_p$, provided that at least one R^1 or at least one R^2 is a conjugation moiety or $–LinkA(–T)_p$;

each R^3 is independently H, optionally substituted C_{1-16} alkyl, optionally substituted C_{2-16} heteroalkyl, optionally substituted C_{2-16} alkenyl, optionally substituted C_{2-16} alkynyl, optionally substituted (C_{1-9} heterocyclyl)-C_{1-6}-alkyl, optionally substituted (C_{6-10} aryl)-C_{1-6}-alkyl, optionally substituted (C_{3-8} cycloalkyl)-C_{1-6}-alkyl, a conjugation moiety, or $–LinkA(–T)_p$;

R^4 is H, optionally substituted C_{1-6} alkyl, $–LinkA(–T)_p$, or –Sol;

each LinkA is independently a multivalent linker (e.g., including –C(O)–N(H)– (e.g., at least one multivalent linker including –C(O)–N(H)– bonded to T));

each T is independently an auxiliary moiety;

Sol is solid support;

m is an integer from 1 to 6;

each n is independently 0 or 1;

each p is independently an integer from 1 to 6; and

q is an integer from 0 to 3.

The at least one group of formula (I) may be bonded to a 5' end, 3' end, internucleoside phosphate, internucleoside phosphorothioate, or internucleoside phosphorodithioate of the polynucleotide. When the at least one group of formula (I) is bonded to the internucleoside phosphate, internucleoside phosphorothioate, or internucleoside phosphorodithioate, q is 0. The polynucleotide construct contains no more than one Sol.

[0313] Group –LinkA– can include from 0 to 3 multivalent monomers (e.g., optionally substituted C1-6 alkane-triyl, optionally substituted C1-6 alkane-tetrayl, or trivalent nitrogen atom) and one or more divalent monomers (e.g., from 1 to 40), where each divalent monomer is independently optionally substituted C1-6 alkylene; optionally substituted C2-6 alkenylene; optionally substituted C2-6 alkynylene; optionally substituted C3-8 cycloalkylene; optionally substituted C3-8 cycloalkenylene; optionally substituted C6-14 arylene; optionally substituted C1-9 heteroarylene having 1 to 4 heteroatoms selected from N, O, and S; optionally substituted C1-9 heterocyclylene having 1 to 4 heteroatoms selected from N, O, and S; imino; optionally substituted N; O; or S(O)m, wherein m is 0, 1, or 2. In some aspects, each monomer is independently optionally substituted C1-6 alkylene; optionally substituted C3-8 cycloalkylene; optionally substituted C3-8 cycloalkenylene; optionally substituted C6-14 arylene; optionally substituted C1-9 heteroarylene having 1 to 4 heteroatoms selected from N, O, and S; optionally substituted C1-9 heterocyclylene having 1 to 4 heteroatoms selected from N, O, and S; imino; optionally substituted N; O; or S(O)m, where m is 0, 1, or 2 (e.g., m is 2). In certain aspects, each monomer is independently optionally substituted C1-6 alkylene; optionally substituted C3-8 cycloalkylene; optionally substituted C3-8 cycloalkenylene; optionally substituted C6-14 arylene; optionally substituted C1-9 heteroarylene having 1 to 4 heteroatoms selected from N, O, and S; optionally substituted C1-9 heterocyclylene having 1 to 4 heteroatoms selected from N, O, and S; optionally substituted N; O; or S(O)m, where m is 0, 1, or 2 (e.g., m is 2). The non-bioreversible linker connecting the auxiliary moiety to the conjugating moiety or to the reaction product thereof can include from 2 to 500 (e.g., from 2 to 300 or from 2 to 200) of such monomers. Group –LinkA– may include a poly(alkylene oxide) (e.g., polyethylene oxide, polypropylene oxide, poly(trimethylene oxide), polybutylene oxide, poly(tetramethylene oxide), and diblock or triblock co-polymers thereof). In some aspects, the non-bioreversible linker

includes polyethylene oxide (e.g., poly(ethylene oxide) having a molecular weight of less than 1 kDa).

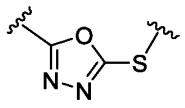
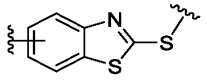
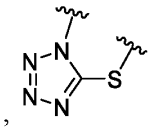
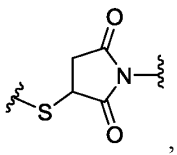
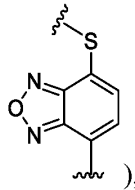
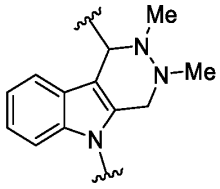
[0314] Group –LinkA(–T)p in formula (I) may be prepared by a process described in the sections below. In some instances, –LinkA(–T)p is of formula (II):

$$–Q^1–Q^2([–Q^3–Q^4–Q^5]_s–Q^6–T)_p,$$

(II)

where

each s is independently an integer from 0 to 20 (e.g., from 0 to 10), where the repeating units are the same or different;

Q^1 is a conjugation linker (e.g., $[–Q^3–Q^4–Q^5]_s–Q^C–$, where Q^C is optionally substituted C_{2-12} heteroalkylene (e.g., a heteroalkylene containing –C(O)–N(H)–, –N(H)–C(O)–, –S(O)$_2$–N(H)–, or –N(H)–S(O)$_2$–), optionally substituted C_{1-12} thioheterocyclylene (e.g., , , , , or ), optionally substituted C_{1-12} heterocyclylene (e.g., 1,2,3-triazole-1,4-diyl or ), cyclobut-3-ene-1,2-dione-3,4-diyl, or pyrid-2-yl hydrazone);

Q^2 is a linear group (e.g., $[–Q^3–Q^4–Q^5]_s–$), if p is 1, or a branched group (e.g., $[–Q^3–Q^4–Q^5]_s–Q^7([–Q^3–Q^4–Q^5]_s–(Q^7)_{p1})_{p2}$, where p1 is 0 or 1, p2 is 0, 1, 2, or 3), if p is an integer from 2 to 6;

each Q^3 and each Q^6 is independently absent, –CO–, –NH–, –O–, –S–, –SO$_2$–, –OC(O)–, –COO–, –NHC(O)–, –C(O)NH–, –CH$_2$–, –CH$_2$NH–, –NHCH$_2$–, –CH$_2$O–, or –OCH$_2$–;

each Q^4 is independently absent, optionally substituted C_{1-12} alkylene, optionally substituted C_{2-12} alkenylene, optionally substituted C_{2-12} alkynylene, optionally substituted C_{2-12} heteroalkylene, optionally substituted C_{6-10} arylene, optionally substituted C_{1-9} heteroarylene, or optionally substituted C_{1-9} heterocyclylene;

each Q^5 is independently absent, –CO–, –NH–, –O–, –S–, $–SO_2–$, $–CH_2–$, –C(O)O–, –OC(O)–,

–C(O)NH–, –NH–C(O)–, –NH–CH(R^a)–C(O)–, or –C(O)–CH(R^a)–NH–;

each Q^7 is independently optionally substituted C_{1-6} alkane-triyl, optionally substituted C_{1-6} alkane-tetrayl, optionally substituted C_{2-6} heteroalkane-triyl, or optionally substituted C_{2-6} heteroalkane-tetrayl; and

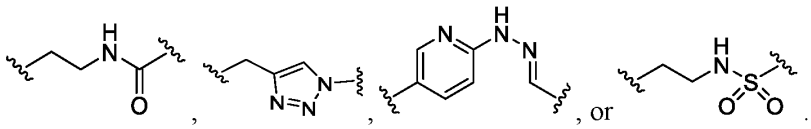
each R^a is independently H or an amino acid side chain;

provided that at least one of Q^3, Q^4, and Q^5 is present.

[0315] In some aspects, each Q^4 is independently absent, optionally substituted C_{1-12} alkylene, optionally substituted C_{2-12} alkenylene, optionally substituted C_{2-12} alkynylene, optionally substituted C_{2-12} heteroalkylene, or optionally substituted C_{1-9} heterocyclylene. In certain aspects, s is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

[0316] Thus, in formula (II), LinkA may include a single branching point, if each p1 is 0, or multiple branching points, if at least one p1 is 1.

[0317] In formula (II), Q^1 may be $–O–Q^L–Q^C–$, where Q^L is optionally substituted C_{2-12} heteroalkylene, optionally substituted C_{1-12} alkylene, or –(optionally substituted C_{1-6} alkylene)–(optionally substituted C_{6-10} arylene)–. In some aspects, Q^L is optionally substituted C_{2-12} heteroalkylene or optionally substituted C_{1-12} alkylene. In formula (II), Q^C may be:



[0318] In formula (II), Q^2 may be a linear group of formula $[–Q^3–Q^4–Q^5]_s–$, where Q^3, Q^4, and Q^5 are as defined for formula (II). Alternatively, Q^2 may be a branched group $[–Q^3–Q^4–Q^5]_s–Q^7([–Q^3–Q^4–Q^5]_s–(Q^7)_{p1})_{p2}$, where each Q^7 is independently optionally substituted C_{1-6} alkane-triyl, optionally substituted C_{1-6} alkane-tetrayl, optionally substituted C_{2-6} heteroalkane-triyl, or optionally substituted C_{2-6} heteroalkane-tetrayl;

where

p1 is 0 or 1;

p2 is 0, 1, 2, or 3;

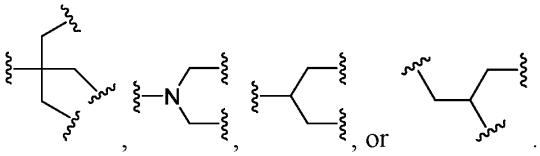
where,

when p1 is 0, LinkA is a trivalent or tetravalent linker, and,

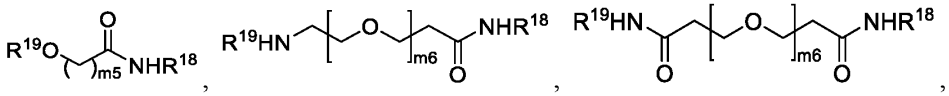
when p1 is 1, LinkA is a tetravalent, pentavalent, or hexavalent linker.

In certain aspects, p1 is 0.

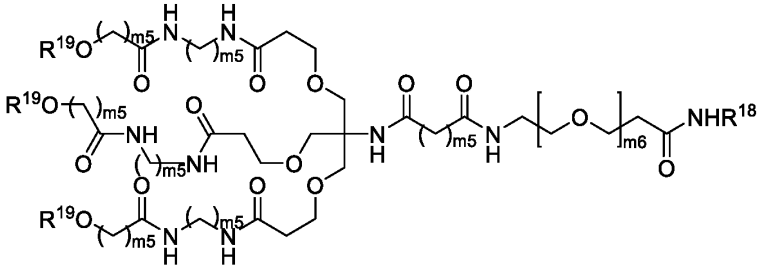
In some aspects, Q^7 is:



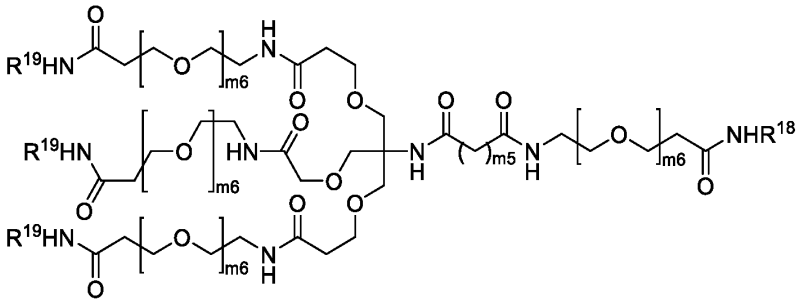
[0319] Compounds that may be used in the preparation of group –LinkA(–T)p in formula (I) are described herein as well as in WO 2015/188197. Non-limiting examples of –LinkA include:



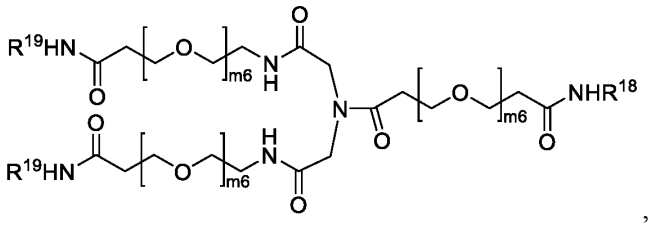
(i) (ii) (iii)



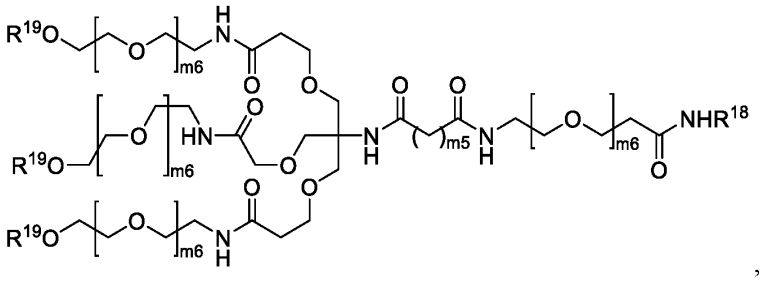
(iv)



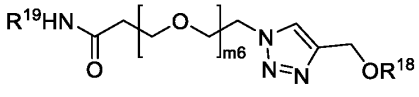
(v)



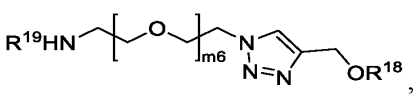
(vi)



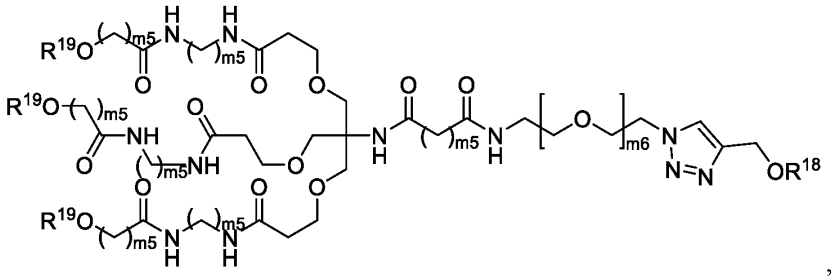
(vii)



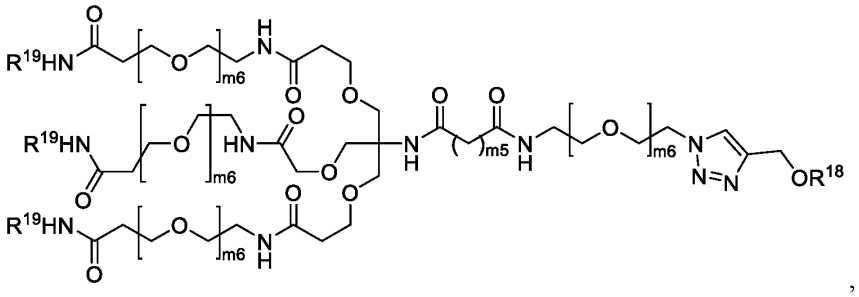
(viii)



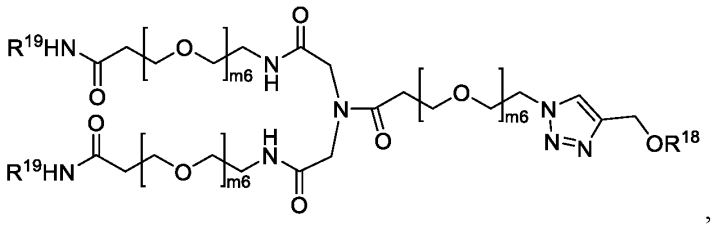
(ix)



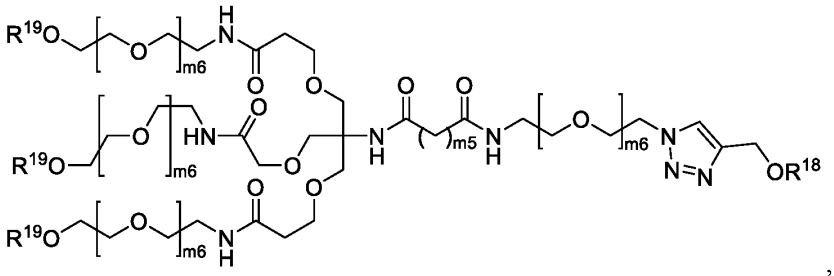
(x)



(xi)

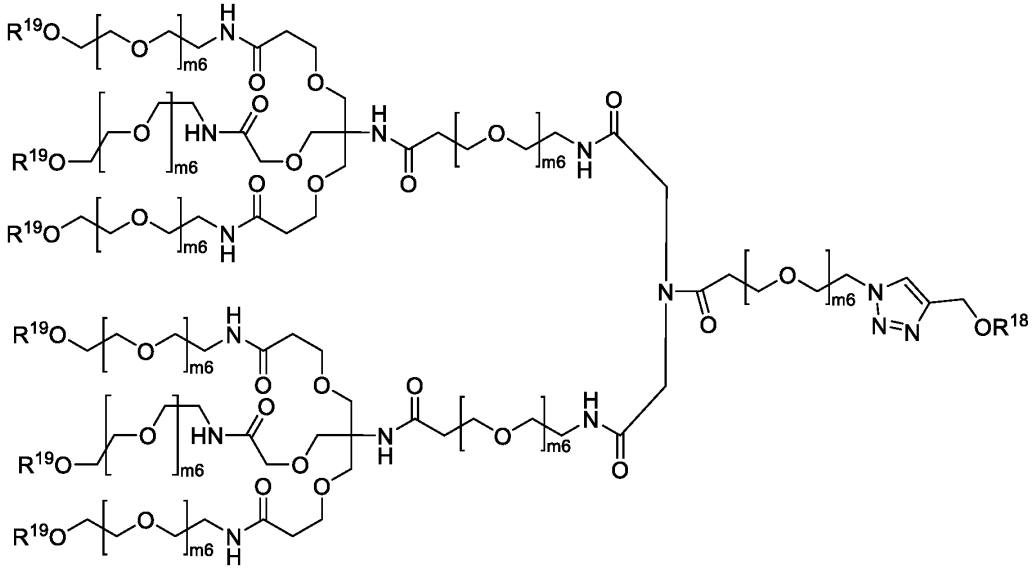


(xii)



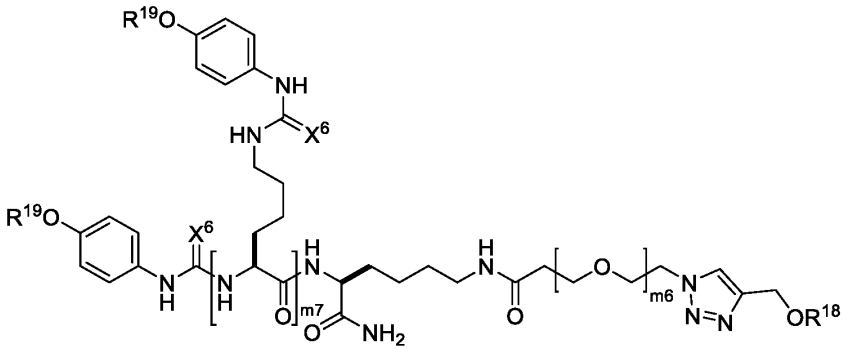
,

(xiii)



, and

(xiv)



(xv)

where

R^{18} is a bond to MOIETY,

each R^{19} is independently a bond to auxiliary moiety,

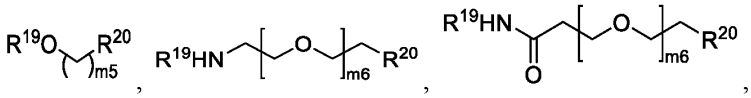
each m5 is independently an integer from 1 to 20,

each m6 is independently an integer from 1 to 10,

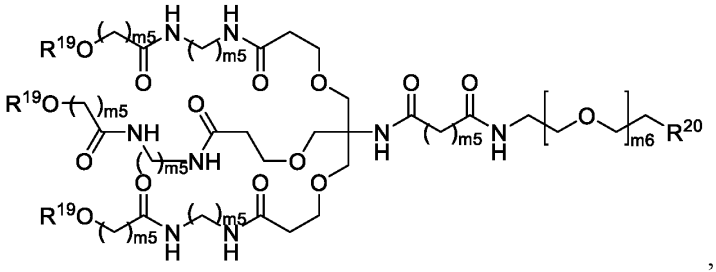
m7 is an integer from 1 to 6, and

each X^6 is independently O or S.

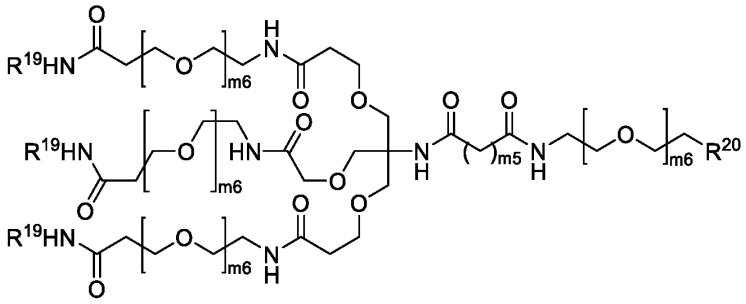
In formula (II), when the conjugation linker is of formula $[–Q^3–Q^4–Q^5]_s–Q^C–$, $–Q^2([–Q^3–Q^4–Q^5]_s–Q^6–T)_p$ may be:



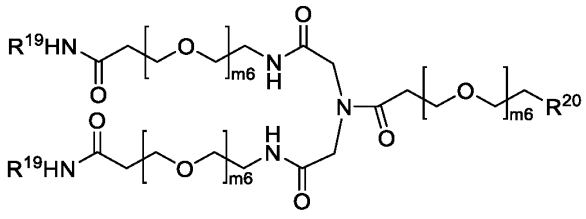
(xvi) (xvii) (xviii)



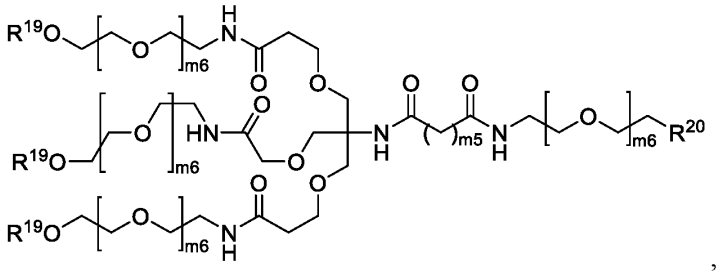
(xix)



(xx)

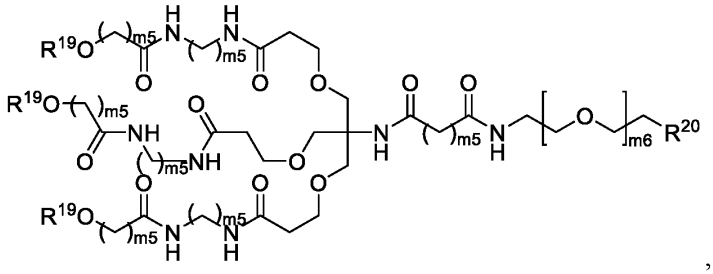


(xxi)



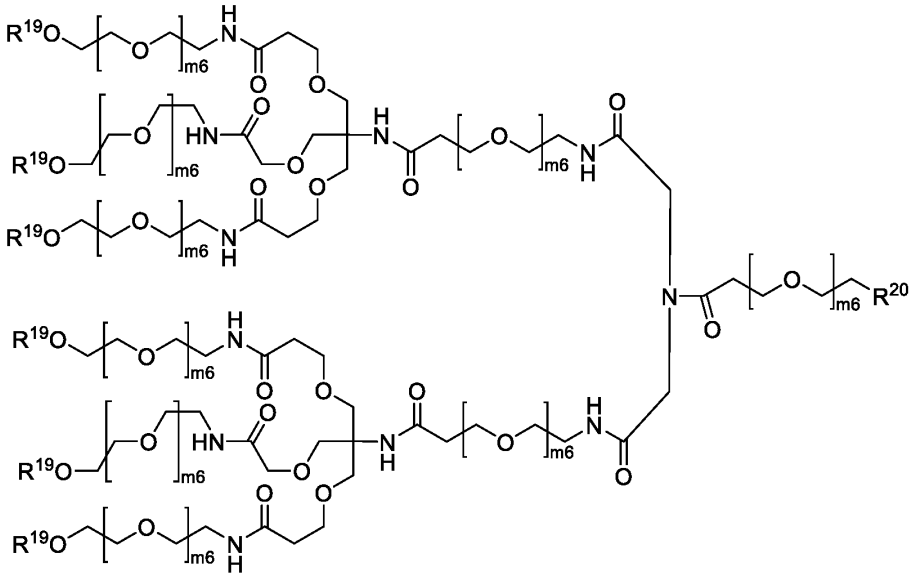
,

(xxii)



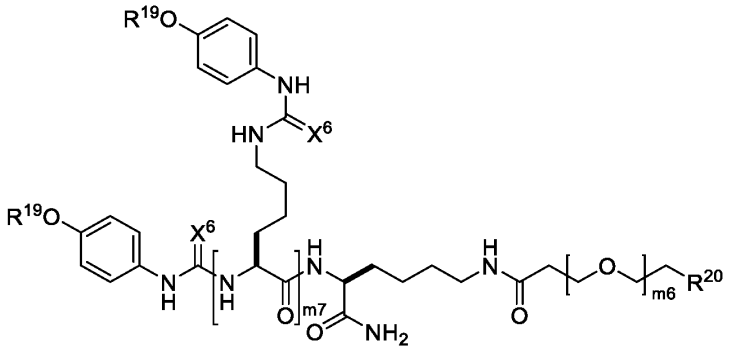
,

(xxiii)



, or

(xxiv)



(xxv)

where

R^{20} is a bond to Q^C in Q^1,

each R^{19} is independently a bond to an auxiliary moiety,

each m5 is independently an integer from 1 to 20,

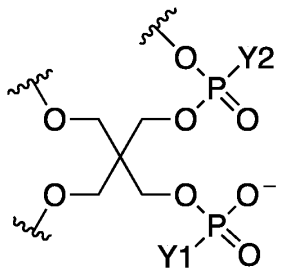
each m6 is independently an integer from 1 to 10,

m7 is an integer from 1 to 6, and

each X^6 is independently O or S.

[0320] In some aspects, the linker described herein is cleavable. In some aspects, the linker described herein is non-cleavable.

[0321] In some aspects, the polynucleic acid molecule described herein comprises a sense or antisense strand bonded to at least one group of formula (IV),



(IV),

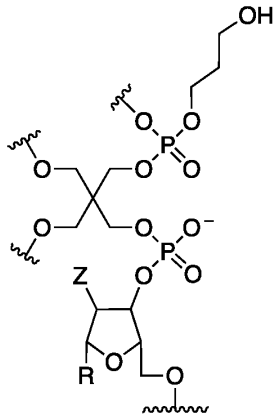
wherein at least one of Y1 and Y2 is a nucleotide from the polynucleic acid molecule.

[0322] In some instances, the Y1 is the last nucleotide on the 3' end or the first nucleotide on the 5' terminus of one of the strands of the polynucleic acid molecule. In some instances, the Y1 is the last nucleotide on the 3' end or the first nucleotide on the 5' terminus of the sense strand of the polynucleic acid molecule . In some instances, the Y1 is the last nucleotide on the 3' end or the first nucleotide on the 5' terminus of the sense strand of the polynucleic acid molecule, and the Y2 is a 3-hydroxy-propoxy group. In some instances, the Y2 is the first nucleotide on the 5' end or the last nucleotide on the 3' end of one of the strands of the polynucleic acid molecule. In some

instances, the Y2 is the first nucleotide on the 5' end or the last nucleotide on the 3' end of the sense strand of the polynucleic acid molecule. In some instances, the Y2 is the first nucleotide or the last nucleotide on the 3' end on the 5' end of the sense strand of the polynucleic acid molecule, and the Y1 is a 3-hydroxy-propoxy group. In other instances, the Y1 and Y2 are two consecutive nucleotides in one of the strands of the polynucleic acid molecule.

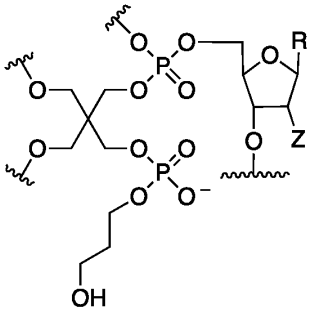
[0323] In some aspects, the targeting moiety described herein is conjugated to 3' end of the sense strand (e.g., formula (IV') or (IV'''')). In some aspects, the targeting moiety described herein is conjugated to 5' end of the sense strand (e.g., formula (IV'') or (IV''')). In some aspects, the targeting moiety described herein is conjugated to 3' end of the antisense strand (e.g., formula (IV') or (IV'''')). In some aspects, the targeting moiety described herein is conjugated to 5' end of the antisense strand (e.g., formula (IV'') or (IV''')).

[0324] In some instances, the structure of formula (IV'), formula (IV''), formula (IV'''), or formula (IV'''') is shown below:



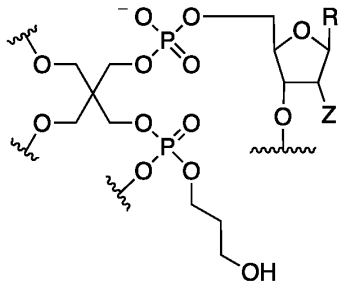
(IV'),

wherein Z in formula (IV') is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (IV') is adenine, uracil, guanine, cytosine, thymine, abasic, or others;



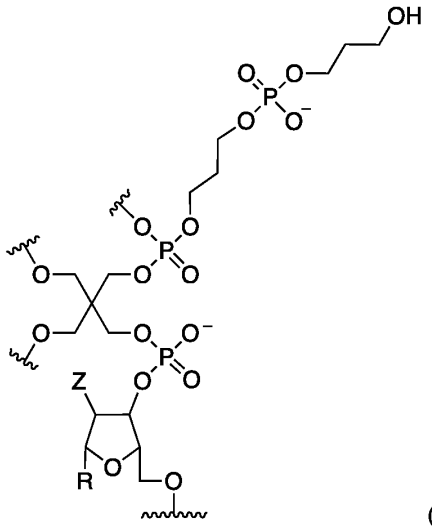
(IV''),

wherein Z in formula (IV'') is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (IV'') is adenine, uracil, guanine, cytosine, thymine, abasic, or others;



(IV'''),

wherein Z in formula (IV''') is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (IV''') is adenine, uracil, guanine, cytosine, thymine, abasic, or others;



(IV''''),

wherein Z in formula (IV'''') is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (IV'''') is adenine, uracil, guanine, cytosine, thymine, abasic, or others.

[0325] In some aspects, the linker conjugated with one or more targeting moieties as shown in Formula (IV'') or (IV''') is added to the first nucleotide on the 5' end. In some aspects, the linker conjugated with one or more GalNAc as shown in Formula (V'') or (V''') is added to the first nucleotide on the 5' end. In some aspects, the modification pattern comprises one or more phosphorothioate modified internucleotide linkages. In some aspects, the modification pattern is shown in Formula (VII). In some aspects, the 5' end modification known in the art is applied to the one or more inverted nucleotides.

Pharmaceutical Compositions

[0326] Delivery of the polynucleic acid molecules described herein can be achieved by contacting a cell with the polynucleic acid molecules using a variety of methods. In particular aspects, the polynucleic acid molecule described herein is formulated with various excipients, vehicles, and carriers, as described more fully elsewhere herein.

[0327] A pharmaceutical composition described herein can be prepared to include a hybridized polynucleotide construct disclosed herein, into a form suitable for administration to a subject using carriers, excipients, and vehicles. Frequently used excipients include magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as sterile water, alcohols, glycerol, and polyhydric alcohols. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial, anti-oxidants, chelating agents, and inert gases. Other pharmaceutically acceptable vehicles include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers, and the like, as described, for instance, in Remington: The Science and Practice of Pharmacy, 21st Ed., Gennaro, Ed., Lippencott Williams & Wilkins (2005), and The United States Pharmacopeia: The National Formulary (USP 36 NF31), published in 2013. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. See Goodman and Gilman's, The Pharmacological Basis for Therapeutics.

[0328] The pharmaceutical compositions described herein may be administered locally or systemically. The therapeutically effective amounts will vary according to factors, such as the degree of infection in a subject, the age, sex, and weight of the individual. Dosage regimes can be adjusted to provide the optimum therapeutic response. For example, several divided doses can be administered daily, or the dose can be proportionally reduced as indicated by the exigencies of the therapeutic situation.

[0329] The pharmaceutical composition can be administered in a convenient manner, such as by injection (e.g., subcutaneous, intravenous, intraorbital, and the like), oral administration, ophthalmic application, inhalation, topical application, or rectal administration. Depending on the route of administration, the pharmaceutical composition can be coated with a material to protect the pharmaceutical composition from the action of enzymes, acids, and other natural conditions that may inactivate the pharmaceutical composition. The pharmaceutical composition can also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof, and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

[0330] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The composition will typically be sterile and fluid to the extent that easy syringability exists. Typically, the composition will be stable under the conditions of manufacture and storage and preserved against the contaminating action of microorganisms, such as bacteria and fungi. The vehicle can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size, in the case of dispersion, and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, isotonic agents, for example, sugars, polyalcohols, such as mannitol, sorbitol, or sodium chloride are used in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

[0331] Sterile injectable solutions can be prepared by incorporating the pharmaceutical composition in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the pharmaceutical composition into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above.

[0332] It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein, refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of pharmaceutical composition is calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The specification for the dosage unit forms is related to the characteristics of the pharmaceutical composition and the particular therapeutic effect to be achieve. The principal pharmaceutical composition is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable vehicle in an acceptable dosage unit. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the ingredients.

[0333] The pharmaceutical composition can be orally administered, for example, in a carrier, e.g., in an enteric-coated unit dosage form. The pharmaceutical composition and other ingredients can

also be enclosed in a hard or soft-shell gelatin capsule or compressed into tablets. For oral therapeutic administration, the pharmaceutical composition can be incorporated with excipients and used in the form of ingestible tablets, troches, capsules, pills, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations can, of course, be varied and can conveniently be between about 5% to about 80% of the weight of the unit. The tablets, troches, pills, capsules, and the like can also contain the following: a binder, such as gum tragacanth, acacia, corn starch, or gelatin; excipients such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid, and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin, or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it can contain, in addition to materials of the above type, a liquid carrier. Various other materials can be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules can be coated with shellac, sugar, or both. A syrup or elixir can contain the agent, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye, and flavoring, such as cherry or orange flavor. Any material used in preparing any dosage unit form should be of pharmaceutically acceptable purity and substantially non-toxic in the amounts employed. In addition, the pharmaceutical composition can be incorporated into sustained-release preparations and formulations.

[0334] The pharmaceutical composition described herein may comprise one or more permeation enhancer that facilitates bioavailability of the polynucleic acid molecule described herein. WO 2000/67798, Muranishi, 1990, Crit. Rev. Ther. Drug Carrier Systems, 7, 1, Lee *et al.*, 1991, Crit. Rev. Ther. Drug Carrier Systems, 8, 91 are herein incorporated by reference in its entirety. In some aspects, the permeation enhancer is intestinal. In some aspects, the permeation enhancer is transdermal. In some aspects, the permeation enhancer is to facilitate crossing the brain-blood barrier. In some aspects, the permeation enhancer improves the permeability in the oral, nasal, buccal, pulmonary, vaginal, or corneal delivery model. In some aspects, the permeation enhancer is a fatty acid or a derivative thereof. In some aspects, the permeation enhancer is a surfactant or a derivative thereof. In some aspects, the permeation enhancer is a bile salt or a derivative thereof. In some aspects, the permeation enhancer is a chelating agent or a derivative thereof. In some aspects, the permeation enhancer is a non-chelating non-surfactant or a derivative thereof. In some aspects, the permeation enhancer is an ester or a derivative thereof. In some aspects, the permeation enhancer is an ether or a derivative thereof. In some aspects, the permeation enhancer is arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin,

glyceryl 1-monocaprate, 1- dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof. In one specific aspect, the permeation enhancer is sodium caprate (C10). In some aspects, the permeation enhancer is chenodeoxycholic acid (CDCA), ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate or sodium glycodihydrofusidate. In some aspects, the permeation enhancer is polyoxyethylene-9-lauryl ether, or polyoxyethylene-20-cetyl ether.

[0335] For the polynucleic acid molecule described herein, suitable pharmaceutically acceptable salts include (i) salts formed with cations such as sodium, potassium, ammonium, magnesium, calcium, polyamines such as spermine and spermidine, etc.; (ii) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, and the like; and (iii) salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like.

[0336] While the hybridized polynucleotide constructs described herein may not require the use of excipients for delivery to the target cell, the use of excipients may be advantageous in some aspects. Thus, for delivery to the target cell, the hybridized polynucleic acid molecule described herein can non-covalently bind an excipient to form a complex. The excipient can be used to alter biodistribution after delivery, to enhance uptake, to increase half-life or stability of the strands in the hybridized polynucleotide constructs (e.g., improve nuclease resistance), and/or to increase targeting to a particular cell or tissue type.

[0337] Exemplary excipients include a condensing agent (e.g., an agent capable of attracting or binding a nucleic acid through ionic or electrostatic interactions); a fusogenic agent (e.g., an agent capable of fusing and/or being transported through a cell membrane); a protein to target a particular cell or tissue type (e.g., thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, or any other protein); a lipid; a lipopolysaccharide; a lipid micelle or a liposome (e.g., formed from phospholipids, such as phosphotidylcholine, fatty acids, glycolipids, ceramides, glycerides, cholesterols, or any combination thereof); a nanoparticle (e.g., silica, lipid, carbohydrate, or other pharmaceutically-acceptable polymer nanoparticle); a polyplex formed from cationic polymers and an anionic agent (e.g., a CRO), where exemplary cationic polymers include polyamines (e.g., polylysine, polyarginine, polyamidoamine, and polyethylene imine); cholesterol; a dendrimer (e.g.,

a polyamidoamine (PAMAM) dendrimer); a serum protein (e.g., human serum albumin (HSA) or low-density lipoprotein (LDL)); a carbohydrate (e.g., dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, or hyaluronic acid); a lipid; a synthetic polymer, (e.g., polylysine (PLL), polyethylenimine, poly-L-aspartic acid, poly-L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolic) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacrylic acid), N-isopropylacrylamide polymer, pseudopeptide-polyamine, peptidomimetic polyamine, or polyamine); a cationic moiety (e.g., cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or alpha helical peptide); a multivalent sugar (e.g., multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine, multivalent mannose, or multivalent fucose); a vitamin (e.g., vitamin A, vitamin E, vitamin K, vitamin B, folic acid, vitamin B12, riboflavin, biotin, or pyridoxal); a cofactor; or a drug to disrupt cellular cytoskeleton to increase uptake (e.g., taxol, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin).

[0338] Other therapeutic agents as described herein may be included in a pharmaceutical composition described herein in combination with a polynucleic acid molecule described herein.

Methods of Treatment

[0339] In some aspects, described herein is a method of modulating expression of Lp(a) gene in a subject, comprising administering to the subject a polynucleic acid molecule described herein, a polynucleic acid molecule conjugate described herein, or a pharmaceutical composition described herein, thereby modulating the expression of Lp(a) gene in the subject.

[0340] In some aspects, the method described herein reduces expression of Lp(a) gene (e.g., expression of Lp(a) mRNA) in a subject by about or at least 10% compared to a control. The term "control" as used herein refers to a subject or a cell receiving no treatment or placebo. In some aspects, the method described herein reduces expression of Lp(a) gene in a subject by about or at least 20% compared to a control. In some aspects, the method described herein reduces expression of Lp(a) gene in a subject by about or at least 30% compared to a control. In some aspects, the method described herein reduces expression of Lp(a) gene in a subject by about or at least 40% compared to a control. In some aspects, the method described herein reduces expression of Lp(a) gene in a subject by about or at least 50% compared to a control. In some aspects, the method described herein reduces expression of Lp(a) gene in a subject by about or at least 60% compared to a control. In some aspects, the method described herein reduces expression of Lp(a) gene in a

subject by about or at least 70% compared to a control. In some aspects, the method described herein reduces expression of Lp(a) gene in a subject by about or at least 80% compared to a control. In some aspects, the method described herein reduces expression of Lp(a) gene in a subject by about or at least 90% compared to a control. In some aspects, the method described herein reduces expression of Lp(a) gene in a subject by about 100% compared to a control.

[0341] In some aspects, described herein is a method of modulating expression of APOC3 gene in a subject, comprising administering to the subject a polynucleic acid molecule described herein, a polynucleic acid molecule conjugate described herein, or a pharmaceutical composition described herein, thereby modulating the expression of APOC3 gene in the subject.

[0342] In some aspects, the method described herein reduces expression of APOC3 gene in a subject by about or at least 10% compared to a negative control. In some aspects, the method described herein reduces expression of APOC3 gene in a subject by about or at least 20% compared to a negative control. In some aspects, the method described herein reduces expression of APOC3 gene in a subject by about or at least 30% compared to a negative control. In some aspects, the method described herein reduces expression of APOC3 gene in a subject by about or at least 40% compared to a negative control. In some aspects, the method described herein reduces expression of APOC3 gene in a subject by about or at least 50% compared to a negative control. In some aspects, the method described herein reduces expression of APOC3 gene in a subject by about or at least 60% compared to a negative control. In some aspects, the method described herein reduces expression of APOC3 gene in a subject by about or at least 70% compared to a negative control. In some aspects, the method described herein reduces expression of APOC3 gene in a subject by about or at least 80% compared to a negative control. In some aspects, the method described herein reduces expression of APOC3 gene in a subject by about or at least 90% compared to a negative control. In some aspects, the method described herein reduces expression of APOC3 gene in a subject by about 100% compared to a negative control.

[0343] In some aspects, the method described herein achieves an IC50 value of about 5nM. In some aspects, the method described herein achieves an IC50 value of about 10nM. In some aspects, the method described herein achieves an IC50 value of about 15nM. In some aspects, the method described herein achieves an IC50 value of about 20nM. In some aspects, the method described herein achieves an IC50 value of about 25nM. In some aspects, the method described herein achieves an IC50 value of about 30nM. In some aspects, the method described herein achieves an IC50 value of about 35nM. In some aspects, the method described herein achieves an IC50 value of about 40nM. In some aspects, the method described herein achieves an IC50 value of about 45nM. In some aspects, the method described herein achieves an IC50 value of about 50nM. In some

aspects, the method described herein achieves an IC50 value of about 55nM. In some aspects, the method described herein achieves an IC50 value of about 60nM. In some aspects, the method described herein achieves an IC50 value of about 65nM. In some aspects, the method described herein achieves an IC50 value of about 70nM. In some aspects, the method described herein achieves an IC50 value of about 75nM. In some aspects, the method described herein achieves an IC50 value of about 80nM. In some aspects, the method described herein achieves an IC50 value of about 85nM. In some aspects, the method described herein achieves an IC50 value of about 90nM. In some aspects, the method described herein achieves an IC50 value of about 95nM. In some aspects, the method described herein achieves an IC50 value of about 100nM.

[0344] In some aspects, the method described herein achieves an IC50 value of about 1 μM. In some aspects, the method described herein achieves an IC50 value of about 1.1 μM. In some aspects, the method described herein achieves an IC50 value of about 1.2 μM. In some aspects, the method described herein achieves an IC50 value of about 1.3 μM. In some aspects, the method described herein achieves an IC50 value of about 1.4 μM. In some aspects, the method described herein achieves an IC50 value of about 1.5 μM. In some aspects, the method described herein achieves an IC50 value of about 2 μM. In some aspects, the method described herein achieves an IC50 value of about 4 μM. In some aspects, the method described herein achieves an IC50 value of about 6 μM. In some aspects, the method described herein achieves an IC50 value of about 8 μM. In some aspects, the method described herein achieves an IC50 value of about 10 μM. In some aspects, the method described herein achieves an IC50 value of about 12 μM. In some aspects, the method described herein achieves an IC50 value of about 13 μM. In some aspects, the method described herein achieves an IC50 value of about 14 μM. In some aspects, the method described herein achieves an IC50 value of about 15 μM. In some aspects, the method described herein achieves an IC50 value of about 30 μM. In some aspects, the method described herein achieves an IC50 value of about 35 μM. In some aspects, the method described herein achieves an IC50 value of about 40 μM. In some aspects, the method described herein achieves an IC50 value of about 50 μM. In some aspects, the method described herein achieves an IC50 value of about 60 μM. In some aspects, the method described herein achieves an IC50 value of about 80 μM. In some aspects, the method described herein achieves an IC50 value of about 100 μM. In some aspects, the method described herein achieves an IC50 value of about 120 μM. In some aspects, the method described herein achieves an IC50 value of about 160 μM.

[0345] In some aspects, described herein is a method of modulating plasma Lp(a) level in a subject in need thereof, comprising administering to the subject a polynucleic acid molecule described herein, a polynucleic acid molecule conjugate described herein, or a pharmaceutical composition

described herein, wherein the polynucleic acid molecule described herein, the polynucleic acid molecule conjugate described herein, or the pharmaceutical composition described herein reduces the plasma Lp(a) level in the subject.

[0346] In some aspects, the method described herein reduces plasma Lp(a) level in a subject by about or at least 10% compared to a control (e.g., untreated subject or a subject before the treatment). In some aspects, the method described herein reduces plasma Lp(a) level in a subject by about or at least 20% compared to a control. In some aspects, the method described herein reduces plasma Lp(a) level in a subject by about or at least 30% compared to a control. In some aspects, the method described herein reduces plasma Lp(a) level in a subject by about or at least 40% compared to a control. In some aspects, the method described herein reduces plasma Lp(a) level in a subject by about or at least 50% compared to a control. In some aspects, the method described herein reduces plasma Lp(a) level in a subject by about or at least 60% compared to a control. In some aspects, the method described herein reduces plasma Lp(a) level in a subject by about or at least 70% compared to a control. In some aspects, the method described herein reduces plasma Lp(a) level in a subject by about or at least 80% compared to a control. In some aspects, the method described herein reduces plasma Lp(a) level in a subject by about or at least 90% compared to a control. In some aspects, the method described herein reduces plasma Lp(a) level in a subject by about 100% compared to a control.

[0347] In some aspects, described herein is a method for treating or preventing a Lp(a) related disorder or symptoms thereof, cardiovascular disease or a lipid disorder, comprising administering to the subject a polynucleic acid molecule as disclosed herein, a polynucleic acid molecule conjugate as disclosed herein, or a pharmaceutical composition as disclosed herein. In some instances, the subject has or is diagnosed with a cardiovascular disease or a lipid disorder, or is suffering from a symptom of a cardiovascular disease or a lipid disorder, or is suspected to develop a cardiovascular disease or a lipid disorder or a symptom thereof.

[0348] In some aspects, provided herein is a method for treating or preventing a cardiovascular disease or a lipid disorder, comprising: administering to a subject having or being diagnosed with a cardiovascular disease or a lipid disorder, or suffering from a symptom of a cardiovascular disease or a lipid disorder, or being suspected to develop a cardiovascular disease or a lipid disorder or a symptom thereof, a polynucleic acid molecule described herein, a polynucleic acid molecule conjugate described herein, or a pharmaceutical composition described herein, thereby treating or preventing a cardiovascular disease or a lipid disorder in the subject.

[0349] In some aspects, the Lp(a) related disorder or cardiovascular disease includes, but is not limited to, coronary artery disease, acute myocardial infarction, asymptomatic carotid

atherosclerosis, stroke, atrial fibrillation, or peripheral artery occlusive disease. In some instances, the cardiovascular disease is coronary artery disease, acute myocardial infarction, asymptomatic carotid atherosclerosis, stroke, atrial fibrillation, hypercholesterolemia, or peripheral artery occlusive disease. In some aspects, the cardiovascular disease is hypercholesterolemia. In some aspects, the cardiovascular disease is myocardial infarction. In some aspects, the cardiovascular disease is the stroke. In some aspects, the cardiovascular disease is calcific aortic valve stenosis. In some aspects, the cardiovascular disease is cardiac arrest. In some aspects, the cardiovascular disease is peripheral arterial disease. In some aspects, the lipid disorder is hyperlipidemia or hypercholesterolemia.

[0350] In some aspects, described herein is a method of modulating triglyceride in a subject in need thereof, comprising administering to the subject a polynucleic acid molecule described herein, a polynucleic acid molecule conjugate described herein, or a pharmaceutical composition described herein, wherein the polynucleic acid molecule described herein, the polynucleic acid molecule conjugate described herein, or the pharmaceutical composition described herein reduces the expression of APOC3 gene in the subject.

[0351] In some aspects, the method described herein reduces triglyceride level in a subject by about or at least 10% compared to a negative control. In some aspects, the method described herein reduces triglyceride level in a subject by about or at least 20% compared to a negative control. In some aspects, the method described herein reduces triglyceride level in a subject by about or at least 30% compared to a negative control. In some aspects, the method described herein reduces triglyceride level in a subject by about or at least 40% compared to a negative control. In some aspects, the method described herein reduces triglyceride level in a subject by about or at least 50% compared to a negative control. In some aspects, the method described herein reduces triglyceride level in a subject by about or at least 60% compared to a negative control. In some aspects, the method described herein reduces triglyceride level in a subject by about or at least 70% compared to a negative control. In some aspects, the method described herein reduces triglyceride level in a subject by about or at least 80% compared to a negative control. In some aspects, the method described herein reduces triglyceride level in a subject by about or at least 90% compared to a negative control. In some aspects, the method described herein reduces triglyceride level in a subject by about 100% compared to a negative control.

[0352] In some aspects, the subject receiving the method described herein suffers from severe hypertriglyceridemia. In some specific case, the subject receiving the method described herein suffers from familial chylomicron syndrome (FCS). In other aspects, the subject receiving the method described herein suffers from high fasting triglycerides on restricted low-fat diet. In other

aspects, the subject receiving the method described herein suffers from overweight or obese. In other aspects, the subject receiving the method described herein suffers from type 2 diabetes. In other aspects, the subject receiving the method described herein suffers from dyslipidaemia. In other aspects, the subject receiving the method described herein suffers a cardiovascular disease. In other aspects, the subject receiving the method described herein suffers from atherosclerosis. In other aspects, the subject receiving the method described herein suffers from stroke. In other aspects, the subject receiving the method described herein suffers from acute pancreatitis. In other aspects, the subject receiving the method described herein suffers from atherosclerotic cardiovascular disease. In other aspects, the subject receiving the method described herein suffers from atrial fibrillation. In other aspects, the subject receiving the method described herein suffers from myocardial infarction. In other aspects, the subject receiving the method described herein suffers from calcific aortic valve stenosis. In other aspects, the subject receiving the method described herein suffers from cardiac arrest. In other aspects, the subject receiving the method described herein suffers from peripheral arterial disease.

Combination Therapy

[0353] In some instances, the methods as disclosed herein comprises administering a polynucleic acid molecule as disclosed herein, a polynucleic acid molecule conjugate as disclosed herein, or a pharmaceutical composition as disclosed herein to a subject (e.g., a human patient) who is on a therapeutic regime for the treatment of Lp(a) related disorder or CVD at the time of, or just prior to, administration of the polynucleic acid molecule as disclosed herein, a polynucleic acid molecule conjugate as disclosed herein, or a pharmaceutical composition as disclosed herein. For example, a patient who has previously been diagnosed with atherosclerosis may have been prescribed and is taking a stable therapeutic regimen of another drug prior to and/or concurrent with administration of a polynucleic acid molecule as disclosed herein, a polynucleic acid molecule conjugate as disclosed herein, or a pharmaceutical composition as disclosed herein. The prior or concurrent therapeutic regimen can comprise, for example, (1) an agent which induces a cellular depletion of cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase, such as a statin (e.g., cerivastatin, atorvastatin, simvastatin, pitavastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, etc.); (2) an agent which inhibits cholesterol uptake and or bile acid re-absorption; (3) an agent which increase lipoprotein catabolism (such as niacin); and/or (4) activators of the LXR transcription factor that plays a role in cholesterol elimination such as 22-hydroxycholesterol.

Patient Population

[0354] The methods disclosed herein are useful for reducing plasma Lp(a) levels in subjects that exhibit an elevated level of plasma Lp(a). The subject can be human or non-human primates. In some instances, the subject is otherwise healthy except for exhibiting elevated serum Lp(a). For example, the subject may not exhibit any other risk factor of cardiovascular, thrombotic, or other diseases or disorders at the time of treatment. In other instances, however, the subject is selected on the basis of being diagnosed with, or at risk of developing, a disease or disorder that is caused by or correlated with elevated plasma Lp(a). For instance, at the time of, or prior to administration of the polynucleic acid molecule as disclosed herein, the polynucleic acid molecule conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, the subject may be diagnosed with or identified as being at risk of developing a cardiovascular disease or disorder, such as, e.g., coronary artery disease, acute myocardial infarction, asymptomatic carotid atherosclerosis, stroke, atrial fibrillation, peripheral artery occlusive disease, etc. The cardiovascular disease or disorder, in some instances, is hypercholesterolemia. For example, a subject may be selected for treatment with the methods as disclosed herein if the subject is diagnosed with or identified as being at risk of developing a hypercholesterolemia condition such as, e.g., heterozygous Familial Hypercholesterolemia (heFH), homozygous Familial Hypercholesterolemia (hoFH), as well as incidences of hypercholesterolemia that are distinct from Familial Hypercholesterolemia (nonFH). The subject may be diagnosed with or identified as being at risk of developing a lipid disease or disorder, such as, e.g., hyperlipidemia and hypercholesterolemia.

[0355] In some instances, at the time of, or prior to administration of the polynucleic acid molecule as disclosed herein, the polynucleic acid molecule conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein, the subject may be diagnosed with or identified as being at risk of developing a thrombotic occlusive disease or disorder, such as, e.g., pulmonary embolism, central retinal vein occlusion, etc. The patient can be selected on the basis of being diagnosed with or at risk of developing a combination of two or more of the abovementioned diseases or disorders. For example, at the time of, or prior to administration of the pharmaceutical composition of the present invention, the subject may be diagnosed with or identified as being at risk of developing coronary artery disease and pulmonary embolism. Other diagnostic combinations (e.g., atherosclerosis and central retinal vein occlusion, heFH and stroke, etc.) are also included in the definition of the patient populations that are treatable by the methods as disclosed herein.

[0356] In some instances, the subject to be treated with the methods as disclosed herein is selected on the basis of one or more factors selected from the group consisting of age (e.g., older than 40,

45, 50, 55, 60, 65, 70, 75, or 80 years), race, gender (male or female), exercise habits (e.g., regular exerciser, non-exerciser), other preexisting medical conditions (e.g., type-II diabetes, high blood pressure, etc.), and current medication status (e.g., currently taking statins (e.g., cerivastatin, atorvastatin, simvastatin, pitavastatin, rosuvastatin, fluvastatin, lovastatin, pravastatin, etc.), beta blockers, niacin, etc.). The present disclosure also provides methods for reducing plasma Lp(a) levels in patients who are intolerant of, non-responsive to, or inadequately responsive to conventional statin therapy. Potential patients can be selected/screened on the basis of one or more of these factors (e.g., by questionnaire, diagnostic evaluation, etc.) before being treated with the methods as disclosed herein.

Dosage

[0357] The amount of the polynucleic acid molecule as disclosed herein, the polynucleic acid molecule conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein administered to a subject according to the methods as disclosed herein is, generally, a therapeutically effective amount. As used herein, the term "therapeutically effective amount" refers to a dose of the polynucleic acid molecule as disclosed herein, the polynucleic acid molecule conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein that results in a detectable reduction in plasma Lp(a). For example, a therapeutically effective amount of the polynucleic acid molecule as disclosed herein, the polynucleic acid molecule conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein includes, for example, an amount of the polynucleic acid molecule as disclosed herein, the polynucleic acid molecule conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein that causes a reduction of at least 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, or more in plasma Lp(a) levels when administered to a subject. In some instances, a therapeutically effective amount of the polynucleic acid molecule as disclosed herein can a dose that can cause a reduction of at least 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, or more in Lp(a) mRNA levels when administered to a subject.

Regimens

[0358] The polynucleic acid molecule as disclosed herein, the polynucleic acid molecule conjugate as disclosed herein, or the pharmaceutical composition as disclosed herein can be administered to a subject over a defined time course. The methods disclosed herein can comprise sequentially administering to a subject multiple doses of the polynucleic acid molecule as disclosed herein, the polynucleic acid molecule conjugate as disclosed herein, or the pharmaceutical composition as

disclosed herein. As used herein, "sequentially administering" means that each dose of the polynucleic acid molecule, the polynucleic acid molecule conjugate, or the pharmaceutical composition is administered to the subject at a different point in time, e.g., on different days separated by a predetermined interval (e.g., hours, days, weeks, or months). The present disclosure provides methods which comprise sequentially administering to the patient a single initial dose of the polynucleic acid molecule, the polynucleic acid molecule conjugate, or the pharmaceutical composition, followed by one or more secondary doses of the polynucleic acid molecule, the polynucleic acid molecule conjugate, or the pharmaceutical composition, and optionally followed by one or more tertiary doses of the polynucleic acid molecule, the polynucleic acid molecule conjugate, or the pharmaceutical composition.

[0359] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the polynucleic acid molecule, the polynucleic acid molecule conjugate, or the pharmaceutical composition. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses can all contain the same amount of the polynucleic acid molecule, the polynucleic acid molecule conjugate, or the pharmaceutical composition, but will generally differ from one another in terms of frequency of administration. In some instances, the amount of the polynucleic acid molecule, the polynucleic acid molecule conjugate, or the pharmaceutical composition contained in the initial, secondary and/or tertiary doses will vary from one another (e.g., adjusted up or down as appropriate) during the course of treatment.

[0360] In some instances, each secondary and/or tertiary dose is administered 1 to 30 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more) days after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose of the polynucleic acid molecule, the polynucleic acid molecule conjugate, or the pharmaceutical composition which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

[0361] The methods as disclosed herein can comprise administering to a patient any number of secondary and/or tertiary doses of the polynucleic acid molecule, the polynucleic acid molecule conjugate, or the pharmaceutical composition. For example, in some instances, only a single secondary dose is administered to the patient. In other instances, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. In some instances, only a single tertiary

dose is administered to the patient. In other instances, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0362] Where multiple secondary doses are administered, each secondary dose can be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 29 days after the immediately preceding dose. Similarly, where multiple tertiary doses are administered, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 1 to 60 days after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

EXAMPLES

[0363] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein. For all of the sequences presented below, oligonucleotide structure representation reads from left to right (5' to 3'). Monomer codes present in the oligonucleotide code are linked by 5'-3' phosphodiester bonds unless specified (succeeded by 3' internucleotide linkage reading left to right). Abbreviations of nucleotide monomers used in oligonucleotide structure representation are as follows. “A” stands for Adenosine-3'-phosphate; “a” stands for 2'-O-methyladenosine-3'-phosphate; “Af” stands for 2'-fluoroadenosine-3'-phosphate; “dA” stands for 2'-deoxyadenosine-3'-phosphate; "a1" refers to 2-Amino-2'-O-methyladenosine-3'-phosphate; “C” stands for Cytidine-3'-phosphate; “c” stands for 2'-O-methylcytidine-3'-phosphate; “Cf” stands for 2'-fluorocytidine-3'-phosphate; “dC” stands for 2'-deoxycytidine-3'-phosphate; “G” stands for Guanosine-3'-phosphate; “g” stands for 2'-O-methylguanosine-3'-phosphate; “Gf” stands for 2'-fluoroguanosine-3'-phosphate; “dG” stands for 2'-deoxyguanosine-3'-phosphate; “U” stands for Uridine-3'-phosphate; “u” stands for 2'-O-methyluridine-3'-phosphate; “Uf” stands for 2'-fluorouridine-3'-phosphate; "u3" refers to 2'-O-methyl-2-thiouridine-3'-phosphate; "U3f" refers to 2'-fluoro-2-thiouridine-3'-phosphate; “dU” stands for 2'-deoxyuridine-3'-phosphate; “T” stands for 5-methyluridine-3'-phosphate; “t” stands for 2'-O-methyl-5-methyluridine-3'-phosphate; “Tf” stands for 2'-fluoro-5-methyluridine-3'-phosphate; “dT” stands for thymidine-3'-phosphate; “s” stands for 3'-phosphorothioate; “(T-T)” refers to acyclic L-threoninol nucleic acid-thymine-3’-phosphate; “(T-A)” refers to acyclic L-threoninol nucleic acid-adenine-3’-phosphate; “(T-NAc)” refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3’phosphate; "i" refers to 2'-O-

methylinosine-3'-phosphate; "(dAB)" refers to 1',2'-Dideoxyribose-3'-phosphate; "(Tgn)" refers to thymidine-glycol nucleic acid (GNA) S-isomer; "(Pr)" refers to C3-spacer; "A2" refers to 2',3'-seco-adenosine-3'-phosphate; "G2" refers to 2',3'-seco-guanosine-3'-phosphate; "C2" refers to 2',3'-seco-cytidine-3'-phosphate; "U2" refers to 2',3'-seco-uridine-3'-phosphate; "dA3" refers to 2'-deoxy-2-aminopurine riboside-3'-phosphate; and "dT3" refers to 2'-deoxy-2-thiothymidine-3'-phosphate.

Example 1 - Testing Lp(a) siRNAs in Non-human Primates

[0364] Eight (8) Lp(a) siRNAs (denoted as "SRS-000016, SRS-000018, SRS-001955, SRS-001958, SRS-002041, SRS-002042, SRS-002064, and SRS-002065" from **Table 1**) were tested in a non-human primate study. For each siRNA, the 3' end of the passenger/sense strand was conjugated with a GalNAc via X2 (*see* Formula (V')). The Lp(a) siRNAs had sequences which were cross reactive with the cynomolgus monkey Lp(a) gene. Male cynomolgus monkeys (n=4 per treatment group/siRNA) were administered a single 1 mg/kg subcutaneous injection of Lp(a) siRNA constructs from **Table 1,** or saline. Animals were fasted overnight prior to collection of blood samples on day -15, -8, and 1 (pre-dose) and on days 4, 8, 11, 15, 22, 29, 36, 43, and 50. Additional blood samples were collected on day 57, 64, 71, 78, 85, 99, 113, 127, 141, 155, and 169 for the SRS-000018, SRS-00016, SRS-002064, and SRS-002065 treated groups. Lp(a) circulating protein levels in all serum samples were analyzed using an Lp(a) ELISA assay (Abcam, catalog # ab212165). Results for each individual animal were expressed as the % change of circulating Lp(a) protein relative to the pre-dose (day 1) timepoint. The mean % change for each treatment group along with standard deviation is plotted in **FIG. 1** and **Table 6.**

[0365] Additional sequences of Lp(a) siRNA as shown in **Table 4** were designed and synthesized.

Example 2 - Testing Lp(a) siRNAs in Non-human Primates

[0366] In a similar study described in example 1, eight (8) Lp(a) siRNAs (denoted as "SRS-000016, SRS-002064, SRS-002081, SRS-002082, SRS-002096, SRS-002328, SRS-002333, and SRS-002335 from **Table 2**) were tested in cynomolgus monkeys. For each siRNA, the 3' end of the passenger/sense strand was conjugated with a GalNAc via X2 (*see* Formula (V')). The Lp(a) siRNAs had sequences which were cross reactive with the cynomolgus monkey LPA gene. Male cynomolgus monkeys (n=4 per treatment group/siRNA) were administered a single 0.5 mg/kg subcutaneous injection of each Lp(a) siRNA. Animals were fasted overnight prior to collection of blood samples on day -15, -8, and 1 (pre-dose) and on days 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 99, and 113. Lp(a) circulating protein levels in all serum samples were analyzed using an Lp(a)

ELISA assay (Abcam, catalog # ab212165). Results for each individual animal were expressed as the % change of circulating Lp(a) protein relative to the pre-dose (day 1) timepoint. The mean % change for each treatment group along with standard deviation is plotted in **FIG. 2** and listed in **Table 7**.

Example 3 – Testing Lp(a) siRNAs in Non-human Primates

[0367] In a similar study described in example 1, three (3) Lp(a) siRNAs (denoted as SRS-002064, SRS-002378, and SRS-002379 from **Table 3**) were tested in cynomolgus monkeys. For each siRNA, the 3' end of the passenger/sense strand was conjugated with a GalNAc via X2 (*see* Formula (V')). The Lp(a) siRNAs had sequences which were cross reactive with the cynomolgus monkey LPA gene. Male cynomolgus monkeys (n=4 per treatment group/siRNA) were administered a single 0.5 mg/kg subcutaneous injection of each Lp(a) siRNA. Animals were fasted overnight prior to collection of blood samples on day -15, -8, and 1 (pre-dose) and on days 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, and 78. Lp(a) circulating protein levels in all serum samples were analyzed using an Lp(a) ELISA assay (Abcam, catalog # ab212165). Results for each individual animal were expressed as the % change of circulating Lp(a) protein relative to the pre-dose (day 1) timepoint. The mean % change for each treatment group along with standard deviation is plotted in **FIG. 3** and listed in **Table 8**.

Example 4 - Testing APOC3 siRNAs in transgenic mice

[0368] siRNAs of sequences listed in **Table 5** were administered to APOC3 transgenic mice (The Jackson Laboratory, B6;CBA-Tg(APOC3)3707/Bres/J, strain #006907) at a single 0.5 mg/kg dose by subcutaneous injection. The 3' end of the passenger strand of each siRNA was conjugated with a triantennary GalNAc moiety (X2-GalNAc as in Formula (V')). Plasma from all groups was collected on days -7, 0 (pre-dose), and on days 7, 14, 21, 28, and 35. Plasma APOC3 protein levels were measured using an ELISA assay (Abcam, catalog #: ab154131). For normalization, the APOC3 level for each animal at each timepoint was divided by the pre-dose (day 0) level of that animal. Then, for each individual at a specific timepoint, the pre-dose normalized value was normalized to the vehicle control group's mean pre-dose normalized value. The mean results along with standard deviation are plotted in **FIG. 4** and is listed in **Table 9**. The majority of seed region modified siRNAs utilizing TNA backbone chemistry still resulted in reduced APOC3 protein expression at a dose of 0.5 mg/kg.

Example 5 - Testing Lp(a) siRNAs in Non-human Primates

[0369] Two Lp(a) siRNAs, SRS-002064 and SRS-002081, were tested in cynomolgus monkeys. The 3' end of the passenger strand of each siRNA was conjugated with a GalNAc via X2 linker (see Formula (V')). The Lp(a) siRNAs had sequences which were cross reactive with the cynomolgus monkey LPA gene. SRS-002064 was administered via subcutaneous injection to male cynomolgus monkeys (n=4 per group) at single doses of 1 mg/kg, 3 mg/kg, or 10 mg/kg. SRS-002081 was administered via subcutaneous injection to male cynomolgus monkeys (n=3 per group) at single doses of 1 mg/kg or 3 mg/kg. Animals were fasted overnight prior to collection of blood samples on day -15, -8, and 1 (pre-dose) and on days 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 99, and 113. Additional collections for SRS-002064 treated groups occurred on days 127 and 141, and the group treated with 10 mg/kg SRS-002064 had additional collections on days 155 and 169. Lp(a) circulating protein levels in all serum samples were analyzed using an Lp(a) ELISA assay (Abcam, catalog # ab212165). Results for each individual animal were expressed as the % change of circulating Lp(a) protein relative to the pre-dose (day 1) timepoint. The mean % change for each treatment group along with standard deviation is plotted in **FIG. 5** and listed in **Table 10**.

Example 6 - Specificity Evaluation of Modified siRNAs Targeting Lp(a)

[0370] To evaluate the ability of siRNAs modified with nucleotide analogs to mitigate off-target effects, RNA sequencing was utilized to assess global transcriptional dysregulation following transfection of siRNAs in primary human hepatocytes (PHHs). Two siRNAs modified with nucleotide analogs, SRS-002064 and SRS-002081, were selected for the experiment and compared against an siRNA with standard nucleotide modifications (SRS-000016).

[0371] Briefly, SRS-000016, SRS-002064, and SRS-002081 were each transfected (Lipofectamine RNAiMAX) in primary human hepatocytes at a concentration of 10nM, in triplicate, and a transfection reagent/mock control was tested in parallel. After incubation for 72 hours, the media was removed and the cells were harvested for RNA isolation. Total RNA comprising mRNA from the cells were isolated and purified according to the manufacturer's (Qiagen-217604, miRNeasy tissue/cells advanced mini kit) protocol. The RNA integrity number (RIN) was analyzed (Agilent 2100 Bioanalyzer, Agilent RNA 6000 Nano Kit, cat#5067-1511), and all values were determined to be greater than 9.1. The total RNA concentration was measured. The sequencing library was prepared according to the manufacturer's protocol (VAHTS Universal V10 RNA-seq Library Prep Kit for Illumina). The concentration of libraries was measured (Thermo Fisher Scientific-Qubit Flex Fluorometer\ Thermo Fisher Scientific-Q33231_Qubit 1X dsDNA HS Assay Kit). Fragment size of libraries was analyzed according to the manufacturer's protocol (Qseq400 Bio-Fragment

Analyzer (Bioptic)\ Bioptic-C405101_S2 Cartridge (Standard Cartridge)). Sequencing was performed using an Illumina-NovaSeq 6000 with an Illumina NovaSeq 6000 S4 Reagent kit, according to the manufacturer's protocol (~50 million reads per sample, Q30 greater than 94% for all samples). Sequencing quality was assessed using FastQC and MultiQC software and reads were trimmed. Reads were aligned (STAR version 2.7.10a), counted, and assigned (HTSeq-count version 0.11.3) to genes. Differentially expressed genes (DEGs) were analyzed by comparing siRNA treated samples with RNAiMAx control samples. Significant DEGs were defined using the criterion that |Log2 Fold Change| was greater than or equal to 1 and the adjusted p value was less than 0.05.

[0372] The results of the DEG analysis are shown in **Table 11** and plotted in **FIG. 6**. Results demonstrate that the nucleotide analogs incorporated into SRS-002064 and SRS-002081 show better specificity. For example, the siRNA without nucleotide analogs, SRS-000016, resulted in 88 DEGs following treatment in PHHs, whereas SRS-002064, which comprises an acyclic L-threoninol nucleic acid, e.g., acyclic L-threoninol nucleic acid-thymine-3'-phosphate, specifically and significantly downregulated the expression of LPA mRNA.

[0373] Overall, results show that siRNAs modified with nucleotide analogs show better specificity to downregulate LPA mRNA expression while maintaining the efficacy.

[0374] While preferred aspects of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such aspects are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the aspects of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Table 1. Sequence Information for Modified Lp(a) siRNAs Evaluated in Non-Human Primates

Duplex ID	Target Position	SEQ ID No	Guide/Antisense Structure (5'-3')	SEQ ID No	Guide/Antisense Base Sequence (5'-3')	SEQ ID No	Passenger/Sense Structure Code (5'-3')	SEQ ID No	Passenger/Sense Base Sequence (5'-3')
SRS-000016	1172	21	usCfsucugUfccauUfaCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-000018	2760	22	usUfsgcguCfugagCfaUfuGfugucasgsg	2	UUGCGUCUGAGCAUUGUGUCAGG	73	usgsacacAfaUfgCfucagacgcaa	15	UGACACAAUGCUCAGACGCAA
SRS-001955	2760	23	usUfsgcgu(T-NAc)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	74	usgsacacAfaUfgCfucaaacgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001958	2760	23	usUfsgcgu(T-NAc)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	75	usgsacacAfaUfgCfucaa1acgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-002041	2760	24	usUfsgcgg(T-NAc)CfugagCfaUfuGfugucasgsg	4	UUGCGCUGAGCAUUGUGUCAGG	76	usgsacacAfaUfgCfucagcgcgcaa	17	UGACACAAUGCUCAGGCGCAA
SRS-002042	2760	25	usUfsgcguCf(T-NAc)gagCfaUfuGfugucasgsg	5	UUGCGUCGAGCAUUGUGUCAGG	77	usgsacacAfaUfgCfucggacgcaa	18	UGACACAAUGCUCGGACGCAA
SRS-002064	1172	26	usCfsucug(T-T)ccauUfaCfcGfugguasgsc	6	UCUCUGTCCAUUACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002065	1172	26	usCfsucug(T-T)ccauUfaCfcGfugguasgsc	6	UCUCUGTCCAUUACCGUGGUAGC	78	usasccacGfgUfaAfugga1cagag a	14	UACCACGGUAAUGGACAGAGA

Note: the target position is relative to human transcript NM_005577.4; the corresponding sequence of a certain SEQ ID NO. is located on its right column of the same row. "A" refers to adenosine-3'-phosphate; "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "dA" refers to 2'-deoxyadenosine-3'-phosphate; "a1" refers to 2-Amino-2'-O-methyladenosine-3'-phosphate; "C" refers to cytidine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "dC" refers to 2'-deoxycytidine-3'-phosphate; "G" refers to guanosine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "dG" refers to 2'-deoxyguanosine-3'-phosphate; "U" refers to uridine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "u3" refers to 2'-O-methyl-2-thiouridine-3'-phosphate; "U3f" refers to 2'-fluoro-2-thiouridine-3'-phosphate; "T" refers to 5-methyluridine-3'-phosphate; "t" refers to 2'-O-methyl-5-methyluridine-3'-phosphate; "Tf" refers to 2'-fluoro-5-methyluridine-3'-phosphate; "dT" refers to 2'-deoxythymidine-3'-phosphate; "s" refers to 3'-phosphorothioate; "(T-T)" refers to acyclic L-threoninol nucleic acid-thymine-3'-phosphate; "(T-A)" refers to acyclic L-threoninol nucleic acid-adenine-3'-phosphate; "(T-NAc)" refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate; "(dAB)" refers to 1',2'-Dideoxyribose-3'phosphate; "(1gn)" refers to thymidine-glycol nucleic acid (GNA) S-isomer; Each modified sense sequence is coupled to a targeting moiety (e.g., GalNAc or galactose) at its 3' end via a linker (e.g., Formula V', V'''', V''''', or V''''''').

Table 2. Sequence Information for Modified Lp(a) siRNAs Evaluated in Non-Human Primates

Duplex ID	Target Position	SEQ ID No	Guide/Antisense Structure (5'-3')	SEQ ID No	Guide/Antisense Base Sequence (5'-3')	SEQ ID No	Passenger/sense Structure Code (5'-3')	SEQ ID No	Passenger/sense Base Sequence (5'-3')
SRS-000016	1172	21	usCfsucugUfccauUfaCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002064	1172	26	usCfsucug(T-Tp)ccauUfaCfcGfugguasgsc	6	UCUCUGUCCAUUACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002081	1172	27	usCfsucugUfccauu3aCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002082	1172	28	usCfsucugUfccaudT3aCfcGfugguasgsc	7	UCUCUGUCCAUTACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002096	1172	29	usCfsucugUfccauU3faCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002328	1172	30	usCfsucuiUfccauU3faCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002333	1172	31	usCfsucug(T-Tp)ccauuaCfcGfugguasgsc	6	UCUCUGUCCAUUACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002335	1172	32	usCfsucug(T-Tp)ccaudTaCfcGfugguasgsc	8	UCUCUGUCCAUTACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA

Note: the target position is relative to human transcript NM_005577.4; the corresponding sequence of a certain SEQ ID NO. is located on its right column of the same row. "A" refers to adenosine-3'-phosphate; "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "dA" refers to 2'-deoxyadenosine-3'-phosphate; "a1" refers to 2-Amino-2'-O-methyladenosine-3'-phosphate; "C" refers to cytidine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "dC" refers to 2'-deoxycytidine-3'-phosphate; "G" refers to guanosine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "dG" refers to 2'-deoxyguanosine-3'-phosphate; "i" refers to 2'-O-methylinosine-3'-phosphate; "U" refers to uridine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "u3" refers to 2'-O-methyl-2-thiouridine-3'-phosphate; "U3f" refers to 2'-fluoro-2-thiouridine-3'-phosphate; "T" refers to 5-methyluridine-3'-phosphate; "t" refers to 2'-O-methyl-5-methyluridine-3'-phosphate; "Tf" refers to 2'-fluoro-5-methyluridine-3'-phosphate; "dT" refers to 2'-deoxythymidine-3'-phosphate; "dT3" refers to 2'-deoxy-2-thiothymidine-3'-phosphate; "s" refers to 3'-phosphorothioate; "(T-T)" refers to acyclic L-threoninol nucleic acid-thymine-3'-phosphate; "(T-A)" refers to acyclic L-threoninol nucleic acid-adenine-3'-phosphate; "(T-NAc)" refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate; "(Tgn)" refers to thymidine-glycol nucleic acid (GNA) S-isomer; Each modified sense sequence is coupled to a targeting moiety (e.g., GalNAc or galactose) at its 3' end via a linker (e.g., Formula V', V'''', V''''', or V''''''').

Table 3. Sequence Information for Modified Lp(a) siRNAs Evaluated in Non-Human Primates

Duplex ID	Target Position	SEQ ID No	Guide/Antisense Structure (5'-3')	SEQ ID No	Guide/Antisense Base Sequence (5'-3')	SEQ ID No	Passenger/Sense Structure Code (5'-3')	SEQ ID No	Passenger/Sense Base Sequence (5'-3')
SRS-002064	1172	26	usCfsucug(T-T)ccauUfaCfcGfugguasgsc	6	UCUCUGTCCAUUACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002378	1172	33	usCfsucuGf(T-T)ccauUfaCfcGfugguasgsc	6	UCUCUGTCCAUUACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002379	1172	34	usCfsucug(T-T)CfcauUfaCfcGfugguasgsc	6	UCUCUGTCCAUUACCGUGGUAGC	72	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA

Note: the target position is relative to human transcript NM_005577.4; the corresponding sequence of a certain SEQ ID NO. is located on its right column of the same row. "A" refers to adenosine-3'-phosphate; "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "dA" refers to 2'-deoxyadenosine-3-phosphate; "a1" refers to 2-Amino-2'-O-methyladenosine-3'-phosphate; "C" refers to cytidine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "dC" refers to 2'-deoxycytidine-3'-phosphate; "G" refers to guanosine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "dG" refers to 2'-deoxyguanosine-3'-phosphate; "i" refers to 2'-O-methylinosine-3'-phosphate; "U" refers to uridine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "u3" refers to 2'-O-methyl-2-thiouridine-3'-phosphate; "U3f" refers to 2'-fluoro-2-thiouridine-3'-phosphate; "T" refers to 5-methyluridine-3'-phosphate; "t" refers to 2'-O-methyl-5-methyluridine-3'-phosphate; "Tf" refers to 2'-fluoro-5-methyluridine-3'-phosphate; "dT" refers to 2'-deoxythymidine-3'-phosphate; "dT3" refers to 2'-deoxy-2-thiothymidine-3'-phosphate; "s" refers to 3'-phosphorothioate; "(T-T)" refers to acyclic L-threoninol nucleic acid-thymine-3'-phosphate; "(T-A)" refers to acyclic L-threoninol nucleic acid-adenine-3'-phosphate; "(T-NAc)" refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate; "(dAB)" refers to 1',2'-Dideoxyribose-3'phosphate; "(Tgn)" refers to thymidine-glycol nucleic acid (GNA) S-isomer; Each modified sense sequence is coupled to a targeting moiety (e.g., GalNAc or galactose) at its 3' end via a linker (e.g., Formula V', V'''', V''''', or V''''''').

Table 4. Sequence Information for Additional Synthesized Modified Lp(a) siRNAs

Duplex ID	Target Position	SEQ ID No	Guide/Antisense Structure (5'-3')	SEQ ID No	Guide/Antisense Base Sequence (5'-3')	SEQ ID No	Passenger/Sense Structure Code (5'-3')	SEQ ID No	Passenger/Sense Base Sequence (5'-3')
SRS-001956	2760	23	usUfsgcgu(T-NAc)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	79	usgsacacAfaUfgCfuca1aacgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001957	2760	23	usUfsgcgu(T-NAc)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	80	usgsacacAfaUfgCfucaaa1cgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001959	2760	35	usUfsgcgu(Pr)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	74	usgsacacAfaUfgCfucaaacgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001960	2760	35	usUfsgcgu(Pr)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	79	usgsacacAfaUfgCfuca1aacgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001961	2760	35	usUfsgcgu(Pr)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	80	usgsacacAfaUfgCfucaaa1cgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001969	1172	27	usCfsucugUfccauu3aCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	81	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-001970	1172	36	usCfsucug(Pr)ccauUfaCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	81	usasccacGfgUfaAfuggacagaga	14	UACCACGGUAAUGGACAGAGA

Duplex ID	Target Position	SEQ ID No	Guide/Antisense Structure (5'-3')	SEQ ID No	Guide/Antisense Base Sequence (5'-3')	SEQ ID No	Passenger/Sense Structure Code (5'-3')	SEQ ID No	Passenger/Sense Base Sequence (5'-3')
SRS-001971	1172	37	usCfsucug(Pr)ccauu3aCfcGfuguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-001972	2760	35	usUfsgcgu(Pr)ugagCfaUfuGfugucasgsg	2	UUGCGUCUGAGCAUUGUGUCAGG	82	usgsacacAfaUfgCfucaaacgcaa	15	UGACACAAUGCUCAGACGCAA
SRS-001973	2760	35	usUfsgcgu(Pr)ugagCfaUfuGfugucasgsg	2	UUGCGUCUGAGCAUUGUGUCAGG	83	usgsacacAfaUfgCfuca1aacgcaa	15	UGACACAAUGCUCAGACGCAA
SRS-001974	2760	35	usUfsgcgu(Pr)ugagCfaUfuGfugucasgsg	2	UUGCGUCUGAGCAUUGUGUCAGG	84	usgsacacAfaUfgCfucaaa1cgcaa	15	UGACACAAUGCUCAGACGCAA
SRS-001975	2760	38	usUfsgcgu(Pr)u3gagCfaUfuGfugucasgsg	2	UUGCGUCUGAGCAUUGUGUCAGG	82	usgsacacAfaUfgCfucaaacgcaa	15	UGACACAAUGCUCAGACGCAA
SRS-001976	2760	39	usUfsgcgu3(Pr)ugagCfaUfuGfugucasgsg	2	UUGCGUCUGAGCAUUGUGUCAGG	82	usgsacacAfaUfgCfucaaacgcaa	15	UGACACAAUGCUCAGACGCAA
SRS-001977	2760	39	usUfsgcgu3(Pr)ugagCfaUfuGfugucasgsg	2	UUGCGUCUGAGCAUUGUGUCAGG	83	usgsacacAfaUfgCfuca1aacgcaa	15	UGACACAAUGCUCAGACGCAA
SRS-001978	2760	38	usUfsgcgu(Pr)u3gagCfaUfuGfugucasgsg	2	UUGCGUCUGAGCAUUGUGUCAGG	84	usgsacacAfaUfgCfucaaa1cgcaa	15	UGACACAAUGCUCAGACGCAA
SRS-001983	2760	40	usUfsgcgu(dAB)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	82	usgsacacAfaUfgCfucaaacgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001984	2760	40	usUfsgcgu(dAB)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	83	usgsacacAfaUfgCfuca1aacgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001985	2760	40	usUfsgcgu(dAB)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	84	usgsacacAfaUfgCfucaaa1cgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001986	2760	41	usUfsgcgu(dAB)u3gagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	82	usgsacacAfaUfgCfucaaacgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001987	2760	42	usUfsgcgu3(dAB)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	82	usgsacacAfaUfgCfucaaacgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001988	2760	42	usUfsgcgu3(dAB)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	83	usgsacacAfaUfgCfuca1aacgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001989	2760	41	usUfsgcgu(dAB)u3gagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	84	usgsacacAfaUfgCfucaaa1cgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-001990	2760	43	usUfsgcg(dAB)CfugagCfaUfuGfugucasgsg	4	UUGCGCUGAGCAUUGUGUCAGG	85	usgsacacAfaUfgCfucaggcgcaa	17	UGACACAAUGCUCAGGCGCAA
SRS-001991	2760	43	usUfsgcg(dAB)CfugagCfaUfuGfugucasgsg	4	UUGCGCUGAGCAUUGUGUCAGG	86	usgsacacAfaUfgCfucal ggcgca a	17	UGACACAAUGCUCAGGCGCAA
SRS-001992	2760	44	usUfsgcg(dAB)Cfu3gagCfaUfuGfugucasgsg	4	UUGCGCUGAGCAUUGUGUCAGG	85	usgsacacAfaUfgCfucagcgcaa	17	UGACACAAUGCUCAGGCGCAA
SRS-001993	2760	45	usUfsgcg(Pr)CfugagCfaUfuGfugucasgsg	4	UUGCGCUGAGCAUUGUGUCAGG	85	usgsacacAfaUfgCfucaggcgcaa	17	UGACACAAUGCUCAGGCGCAA
SRS-001994	2760	45	usUfsgcg(Pr)CfugagCfaUfuGfugucasgsg	4	UUGCGCUGAGCAUUGUGUCAGG	86	usgsacacAfaUfgCfucal ggcgca a	17	UGACACAAUGCUCAGGCGCAA
SRS-001995	2760	46	usUfsgcg(Pr)Cfu3gagCfaUfuGfugucasgsg	4	UUGCGCUGAGCAUUGUGUCAGG	85	usgsacacAfaUfgCfucaggcgcaa	17	UGACACAAUGCUCAGGCGCAA
SRS-001996	1172	47	usCfsucu(Pr)UfccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA

Duplex ID	Target Position	SEQ ID No	Guide/Antisense Structure (5'-3')	SEQ ID No	Guide/Antisense Base Sequence (5'-3')	SEQ ID No	Passenger/Sense Structure Code (5'-3')	SEQ ID No	Passenger/Sense Base Sequence (5'-3')
SRS-001997	1172	47	usCfsucu(Pr)UfccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	87	usasccacGfgUfa.Afugga1cagag a	14	UACCACGGUAAUGGACAGAGA
SRS-001998	1172	47	usCfsucu(Pr)UfccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	88	usasccacGfgUfa.Afuggaca1gag a	14	UACCACGGUAAUGGACAGAGA
SRS-001999	1172	48	usCfsucu(Pr)u3ccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002000	1172	49	usCfsucu3(Pr)UfccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002001	1172	49	usCfsucu3(Pr)UfccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	87	usasccacGfgUfa.Afugga1cagag a	14	UACCACGGUAAUGGACAGAGA
SRS-002002	1172	48	usCfsucu(Pr)u3ccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	88	usasccacGfgUfa.Afuggaca1gag a	14	UACCACGGUAAUGGACAGAGA
SRS-002003	1172	50	usCfsucu(dAB)UfccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002004	1172	50	usCfsucu(dAB)UfccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	87	usasccacGfgUfa.Afugga1cagag a	14	UACCACGGUAAUGGACAGAGA
SRS-002005	1172	50	usCfsucu(dAB)UfccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	88	usasccacGfgUfa.Afuggaca1gag a	14	UACCACGGUAAUGGACAGAGA
SRS-002006	1172	51	usCfsucu(dAB)u3ccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002007	1172	52	usCfsucu3(dAB)UfccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002008	1172	52	usCfsucu3(dAB)UfccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	87	usasccacGfgUfa.Afugga1cagag a	14	UACCACGGUAAUGGACAGAGA
SRS-002009	1172	51	usCfsucu(dAB)u3ccauUfaCfcGfugguasgsc	9	UCUCUUCCAUUACCGUGGUAGC	88	usasccacGfgUfa.Afuggaca1gag a	14	UACCACGGUAAUGGACAGAGA
SRS-002010	1172	53	usCfsucuG2UfccauUfaCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002011	1172	53	usCfsucuG2UfccauUfaCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	87	usasccacGfgUfa.Afugga1cagag a	14	UACCACGGUAAUGGACAGAGA
SRS-002012	1172	53	usCfsucuG2UfccauUfaCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	88	usasccacGfgUfa.Afuggaca1gag a	14	UACCACGGUAAUGGACAGAGA
SRS-002013	1172	54	usCfsucuG2u3ccauUfaCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002014	1172	55	usCfsucu3G2UfccauUfaCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002015	1172	55	usCfsucu3G2UfccauUfaCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	87	usasccacGfgUfa.Afugga1cagag a	14	UACCACGGUAAUGGACAGAGA
SRS-002016	1172	54	usCfsucuG2u3ccauUfaCfcGfugguasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	88	usasccacGfgUfa.Afuggaca1gag a	14	UACCACGGUAAUGGACAGAGA
SRS-002017	1172	56	usCfsucug(dAB)ccauUfaCfcGfugguasgsc	10	UCUCUGCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002018	1172	57	usCfsucug(dAB)ccauu3aCfcGfugguasgsc	10	UCUCUGCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA

Duplex ID	Target Position	SEQ ID No	Guide/Antisense Structure (5'-3')	SEQ ID No	Guide/Antisense Base Sequence (5'-3')	SEQ ID No	Passenger/Sense Structure Code (5'-3')	SEQ ID No	Passenger/Sense Base Sequence (5'-3')
SRS-002019	1172	58	usCfsucugU2ccauUfaCfcGfuggsasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002020	1172	59	usCfsucugU2ccauu3aCfcGfuggsasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002021	2760	40	usUfsgcgu(d.AB)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	74	usgsacacAfaUfgCfucaaacgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-002022	2760	40	usUfsgcgu(d.AB)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	79	usgsacacAfaUfgCfuca1aacgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-002023	2760	40	usUfsgcgu(d.AB)ugagCfaUfuGfugucasgsg	3	UUGCGUUGAGCAUUGUGUCAGG	80	usgsacacAfaUfgCfucaaa1cgcaa	16	UGACACAAUGCUCAAACGCAA
SRS-002036	2760	60	usUfsgcguCf(dAB)gagCfaUfuGfugucasgsg	5	UUGCGUCGAGCAUUGUGUCAGG	89	usgsacacAfaUfgCfucggacgcaa	18	UGACACAAUGCUCGGACGCAA
SRS-002037	2760	61	usUfsgcguCf(Pr)gagCfaUfuGfugucasgsg	5	UUGCGUCGAGCAUUGUGUCAGG	89	usgsacacAfaUfgCfucggacgcaa	18	UGACACAAUGCUCGGACGCAA
SRS-002038	1172	62	usCfsucugU(Pr)cauUfaCfcGfuggsasgsc	11	UCUCUGUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002039	1172	63	usCfsucugU(f.AB)cauUfaCfcGfuggsasgsc	11	UCUCUGUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002040	1172	64	usCfsucugUfC2cauUfaCfcGfuggsasgsc	1	UCUCUGUCCAUUACCGUGGUAGC	81	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002043	2760	43	usUfsgcg(d.AB)CfugagCfaUfuGfugucasgsg	4	UUGCGCUGAGCAUUGUGUCAGG	76	usgsacacAfaUfgCfucagcgcaa	17	UGACACAAUGCUCAGGCGCAA
SRS-002044	2760	65	usUfsgcguCf(dAB)gagCfaUfuGfugucasgsg	5	UUGCGUCGAGCAUUGUGUCAGG	77	usgsacacAfaUfgCfucggacgcaa	18	UGACACAAUGCUCGGACGCAA
SRS-002329	1172	66	usCfsucugUfucauU3faCfcGfuggsasgsc	12	UCUCUGUUCAUUACCGUGGUAGC	90	usasccacGfgUfa.Afugaacagaga	19	UACCACGGUAAUGAACAGAGA
SRS-002330	1172	67	usCfsucudA3UfcauU3faCfcGfuggsasgsc	13	UCUCUAUCCAUUACCGUGGUAGC	91	usasccacGfgUfa.Afuggauagaga	20	UACCACGGUAAUGGAUAGAGA
SRS-002334	1172	68	usCfsucug(T-T)ccauu3aCfcGfuggsasgsc	6	UCUCUGTCCAUUACCGUGGUAGC	72	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002336	1172	69	usCfsucug(T-T)ccauudT3aCfcGfuggsasgsc	8	UCUCUGTCCAUTACCGUGGUAGC	72	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002530	1172	70	u(Rps)Cf(Rps)ucug(T-T)ccauUfaCfcGfuggua(Sps)gsc	6	UCUCUGTCCAUUACCGUGGUAGC	72	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA
SRS-002531	1172	71	u(Rps)Cf(Rps)ucugUfccauu3aCfcGfuggua(Sps)gsc	1	UCUCUGUCCAUUACCGUGGUAGC	72	usasccacGfgUfa.Afuggacagaga	14	UACCACGGUAAUGGACAGAGA

Note: the target position is relative to human transcript NM_005577.4; the corresponding sequence of a certain SEQ ID NO. is located on its right column of the same row. "A" refers to adenosine-3'-phosphate; "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "dA" refers to 2'-deoxyadenosine-3-phosphate; "a1" refers to 2-Amino-2'-O-methyladenosine-3'-phosphate; "C" refers to cytidine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "dC" refers to 2'-deoxycytidine-3'-phosphate; "G" refers to guanosine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "dG" refers to 2'-deoxyguanosine-3'-phosphate; "i" refers to 2'-O-methylinosine-3'-phosphate; "U" refers to uridine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "U3f" refers to 2'-fluoro-2-thiouridine-3'-phosphate; "T" refers to 5-methyluridine-3'-phosphate; "t" refers to 2'-O-methyl-5-methyluridine-3'-phosphate; "Tf" refers to 2'-fluoro-5-methyluridine-3'-phosphate; "dT" refers to 2'-deoxythymidine-3'-phosphate; "dT3" refers to 2'-deoxy-2-thiothymidine-3'-phosphate; "s" refers to 3'-phosphorothioate; "(T-T)" refers to acyclic L-threoninol nucleic acid-thymine-3'-phosphate; "(T-A)" refers to acyclic L-threoninol nucleic acid-adenine-3'-phosphate; "(T-NAc)" refers to acyclic

Duplex ID	Target Position	SEQ ID No	Guide/Antisense Structure (5'-3')	SEQ ID No	Guide/Antisense Base Sequence (5'-3')	SEQ ID No	Passenger/Sense Structure Code (5'-3')	SEQ ID No	Passenger/Sense Base Sequence (5'-3')
N-acetyl L-threoninol abasic nucleic acid-3'phosphate; "(dAB)" refers to 1',2'-Dideoxyribose-3'phosphate; "(Tgn)" refers to thymidine-glycol nucleic acid (GNA) S-isomer; Each modified sense sequence of SEQ ID NOs: 72, 74, 76-77, 79-80, and 90-91 is coupled to a targeting moiety (e.g., GalNAc or galactose) at its 3' end via a linker (e.g., Formula V', V'''', V''''', or V'''''').									

Table 5. Sequence Information for Modified APOC3 siRNAs Evaluated in Transgenic Mice

Duplex ID	Target Position	SEQ ID No	Guide/Antisense Structure (5'-3')	SEQ ID No	Guide/Antisense Base Sequence (5'-3')	SEQ ID No	Passenger/Sense Structure Code (5'-3')	SEQ ID No	Passenger/Sense Base Sequence (5'-3')
SRS-000228	431	113	usGfsaauaCfugucCfcUfuUfuaagcsasa	101	UGAAUACUGUCCCUUUUAAGCAA	122	gscsuuaaAfaGfgGfacaguauuca	107	GCUUAAAAGGGACAGUAUUCA
SRS-000231	435	114	usCfsugagAfauacUfgUfcCfcuuuusasa	102	UCUGAGAAUACUGUCCCUUUUAA	123	asasaaggGfaCfaGfuauucucaga	108	AAAAGGGACAGUAUUCUCAGA
SRS-001862	435	115	usCfsugagAfauacUfgUfcCfcuuugsasa	103	UCUGAGAAUACUGUCCCUUUGAA	124	csasaaggGfaCfaGfuauucucaga	109	CAAAGGGACAGUAUUCUCAGA
SRS-002514	435	116	usCfsugaGf(T-A)auacUfgUfcCfcuuugsasa	103	UCUGAGAAUACUGUCCCUUUGAA	124	csasaaggGfaCfaGfuauucucaga	109	CAAAGGGACAGUAUUCUCAGA
SRS-002515	435	117	usCfsugag(T-A)AfuacUfgUfcCfcuuugsasa	103	UCUGAGAAUACUGUCCCUUUGAA	124	csasaaggGfaCfaGfuauucucaga	109	CAAAGGGACAGUAUUCUCAGA
SRS-002516	431	118	usGfsaauaCf(T-T)gucCfcUfuUfuaagcsasa	104	UGAAUACTGUCCCUUUUAAGCAA	122	gscsuuaaAfaGfgGfacaguauuca	107	GCUUAAAAGGGACAGUAUUCA
SRS-002517	431	119	usGfsaauaCf(T-NAc)gucCfcUfuUfuaagcsasa	105	UGAAUACGUCCCUUUUAAGCAA	122	gscsuuaaAfaGfgGfacaguauuca	107	GCUUAAAAGGGACAGUAUUCA
SRS-002518	431	119	usGfsaauaCf(T-NAc)gucCfcUfuUfuaagcsasa	105	UGAAUACGUCCCUUUUAAGCAA	125	gscsuuaaAfaGfgGfacgguauuca	110	GCUUAAAAGGGACGGUAUUCA
SRS-002519	431	120	usGfsaau(T-A)CfugucCfcUfuUfuaagcsasa	101	UGAAUACUGUCCCUUUUAAGCAA	122	gscsuuaaAfaGfgGfacaguauuca	107	GCUUAAAAGGGACAGUAUUCA
SRS-002520	431	121	usGfsaau(T-NAc)CfugucCfcUfuUfuaagcsasa	106	UGAAUCUGUCCCUUUUAAGCAA	126	gscsuuaaAfaGfgGfacagaauuca	111	GCUUAAAAGGGACAGAAUUCA
SRS-002521	431	121	usGfsaau(T-NAc)CfugucCfcUfuUfuaagcsasa	106	UGAAUCUGUCCCUUUUAAGCAA	127	gscsuuaaAfaGfgGfacaggauuca	112	GCUUAAAAGGGACAGGAUUCA
Note: the target position is relative to human transcript NM_000040.3; the corresponding sequence of a certain SEQ ID NO. is located on its right column of the same row. "A" refers to adenosine-3'-phosphate; "a" refers to 2'-O-methyladenosine-3'-phosphate; "Af" refers to 2'-fluoroadenosine-3'-phosphate; "dA" refers to 2'-deoxyadenosine-3-phosphate; "a1" refers to 2-Amino-2'-O-methyladenosine-3'-phosphate; "C" refers to cytidine-3'-phosphate; "c" refers to 2'-O-methylcytidine-3'-phosphate; "Cf" refers to 2'-fluorocytidine-3'-phosphate; "dC" refers to 2'-deoxycytidine-3'-phosphate; "G" refers to guanosine-3'-phosphate; "g" refers to 2'-O-methylguanosine-3'-phosphate; "Gf" refers to 2'-fluoroguanosine-3'-phosphate; "dG" refers to 2'-deoxyguanosine-3'-phosphate; "i" refers to 2'-O-methylinosine-3'-phosphate; "U" refers to uridine-3'-phosphate; "u" refers to 2'-O-methyluridine-3'-phosphate; "Uf" refers to 2'-fluorouridine-3'-phosphate; "u3" refers to 2'-O-methyl-2-thiouridine-3'-phosphate; "U3f" refers to 2'-fluoro-2-thiouridine-3'-phosphate; "T" refers to 5-methyluridine-3'-phosphate; "t" refers to 2'-O-methyl-5-methyluridine-3'-phosphate; "Tf" refers to 2'-fluoro-5-methyluridine-3'-phosphate; "dT" refers to 2'-deoxythymidine-3'-phosphate; "dT3" refers to 2'-deoxy-2-thiothymidine-3'-phosphate; "s" refers to 3'-phosphorothioate; "(T-T)" refers to acyclic L-threoninol nucleic acid-thymine-3'-phosphate; "(T-A)" refers to acyclic L-threoninol nucleic acid-adenine-3'-phosphate; "(T-NAc)" refers to acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate; "(dAB)" refers to 1',2'-Dideoxyribose-3'phosphate; "(Tgn)" refers to thymidine-glycol nucleic acid (GNA) S-isomer; Each modified sense sequence is coupled to a targeting moiety (e.g., GalNAc or galactose) at its 3' end via a linker (e.g., Formula V', V'''', V''''', or V'''''').									

Table 6. Percent Change in Serum LP(a) Relative to Day 1 Pre-Dose

Study Day	Dose	Statistic	Study Day																			
			1	4	8	11	15	22	29	36	43	50	57	64	71	78	85	99	113	127	141	155	169
Saline	1 mg/kg	Mean	0.0	-8.9	-15.8	-12.4	-23.7	-6.0	1.7	-29.8	-3.4	9.5	11.0	-0.7	-16.1	-34.5	-33.5	-15.1	-38.4	-39.1	-14.7	-28.4	-19.4
		SD	0.0	12.8	20.0	29.2	24.3	22.3	16.8	37.0	32.3	49.6	48.5	36.0	14.7	4.8	13.4	18.3	12.5	24.0	10.5	18.6	29.4
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-000018	1 mg/kg	Mean	0.0	-29.5	-49.1	-62.8	-83.6	-91.6	-94.9	-96.3	-96.3	-96.0	-95.5	-93.5	-93.4	-93.2	-91.8	-87.2	-82.0	-81.4	-68.0	-68.9	-67.4
		SD	0.0	4.6	8.0	6.1	3.2	2.5	1.8	2.1	2.5	3.3	3.6	5.1	4.9	5.7	6.8	10.7	14.6	16.7	27.0	25.3	21.9
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-001955	1 mg/kg	Mean	0.0	-21.6	-21.5	-27.9	-48.5	-50.9	-47.2	-56.2	-48.2	-34.2											
		SD	0.0	4.4	12.8	14.0	11.4	17.3	20.8	17.6	22.8	34.2											
		N	4	4	4	4	4	4	4	4	4	4											
SRS-001958	1 mg/kg	Mean	0.0	-12.4	-12.2	-28.4	-41.1	-44.6	-34.1	-40.8	-32.6	-24.5											
		SD	0.0	2.5	9.1	2.1	9.7	10.3	13.8	6.2	11.2	8.7											
		N	4	4	4	4	4	4	4	4	4	4											
SRS-000016	1 mg/kg	Mean	0.0	-21.1	-27.2	-50.5	-68.8	-82.6	-88.5	-92.4	-93.7	-93.1	-92.9	-91.9	-90.7	-90.5	-89.0	-83.4	-82.9	-79.7	-76.9	-77.3	-69.9
		SD	0.0	6.4	10.5	10.1	11.0	7.0	5.1	3.5	1.8	2.7	2.4	2.8	4.1	5.0	5.0	11.8	9.6	16.4	13.4	12.3	12.4
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-002041	1 mg/kg	Mean	0.0	-21.8	-17.1	-19.1	-31.7	-30.5	-20.5	-35.5	-34.6	-20.9											
		SD	0.0	19.1	27.4	36.9	25.9	39.6	41.5	26.5	29.9	35.0											
		N	4	4	4	4	4	4	4	4	4	4											
SRS-002042	1 mg/kg	Mean	0.0	-15.4	-12.9	-18.2	-29.9	-33.0	-34.4	-35.9	-31.1	-33.4											
		SD	0.0	7.2	21.4	13.1	17.5	21.9	22.3	20.8	15.9	20.6											
		N	4	4	4	4	4	4	4	4	4	4											
SRS-002064	1 mg/kg	Mean	0.0	-12.7	-22.1	-39.4	-58.9	-73.5	-80.9	-84.8	-86.4	-86.2	-85.9	-85.9	-83.8	-84.8	-82.0	-77.1	-74.2	-74.3	-65.2	-63.2	-57.4
		SD	0.0	6.8	7.0	25.3	19.6	15.2	12.1	11.1	10.0	10.1	9.6	8.3	8.1	9.0	9.1	11.1	9.2	8.4	11.1	13.2	7.8

Study Day	Dose	Statistic	Study Day																			
			1	4	8	11	15	22	29	36	43	50	57	64	71	78	85	99	113	127	141	155	169
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-002065	**1 mg/kg**	**Mean**	**0.0**	**-20.0**	**-20.7**	**-33.8**	**-41.7**	**-60.9**	**-69.3**	**-71.9**	**-74.8**	**-72.4**	**-75.8**	**-71.8**	**-71.9**	**-71.9**	**-69.0**	**-61.3**	**-63.4**	**-59.4**	**-54.8**	**-49.9**	**-46.8**
		SD	0.0	8.5	9.2	12.1	19.5	18.6	14.5	17.4	15.9	21.5	17.0	18.4	15.6	15.8	15.6	20.7	14.7	21.2	16.5	18.1	20.4
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

Table 7. Percent Change in Serum LP(a) Relative to Day 1 Pre-Dose

Study Day	Dose	Statistic	Study Day														
			1	8	15	22	29	36	43	50	57	64	71	78	85	99	113
SRS-000016	**0.5 mg/kg**	**Mean**	**0.0**	**-22.9**	**-46.7**	**-68.8**	**-75.3**	**-78.6**	**-80.2**	**-80.3**	**-81.4**	**-81.5**	**-79.6**	**-75.2**	**-71.0**	**-68.5**	**-62.9**
		SD	0.0	3.8	7.5	7.7	10.3	8.5	9.1	8.1	6.0	5.5	7.2	7.2	9.4	8.1	8.1
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-002064	**0.5 mg/kg**	**Mean**	**0.0**	**-13.0**	**-38.4**	**-49.2**	**-50.9**	**-61.9**	**-64.5**	**-64.4**	**-65.5**	**-64.7**	**-62.0**	**-54.6**	**-47.8**	**-42.3**	**-48.1**
		SD	0.0	9.9	10.1	14.1	18.2	13.9	10.9	13.2	11.8	9.3	9.3	14.6	16.0	7.2	12.0
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-002081	**0.5 mg/kg**	**Mean**	**0.0**	**-22.2**	**-51.5**	**-72.0**	**-75.9**	**-80.4**	**-82.0**	**-82.7**	**-84.2**	**-84.7**	**-81.6**	**-79.0**	**-75.0**	**-72.7**	**-71.2**
		SD	0.0	10.2	2.3	3.0	4.2	2.0	1.9	4.9	3.7	2.9	3.6	4.0	3.1	6.8	5.3
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-002082	**0.5 mg/kg**	**Mean**	**0.0**	**-18.5**	**-44.2**	**-60.3**	**-67.9**	**-69.6**	**-73.5**	**-74.5**	**-66.7**	**-62.6**	**-64.8**	**-61.3**	**-49.8**	**-39.7**	**-32.0**
		SD	0.0	13.5	12.0	6.0	1.7	4.7	3.8	5.9	10.0	12.4	11.3	14.3	20.4	27.6	39.0
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-002096	**0.5 mg/kg**	**Mean**	**0.0**	**-14.7**	**-43.7**	**-67.7**	**-72.5**	**-78.1**	**-76.9**	**-79.2**	**-72.1**	**-69.3**	**-71.1**	**-63.1**	**-59.7**	**-51.7**	**-50.6**
		SD	0.0	7.8	3.4	7.2	5.9	2.7	5.4	3.4	7.0	11.5	8.5	12.9	15.7	14.5	13.8
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-002328	**0.5 mg/kg**	**Mean**	**0.0**	**-22.5**	**-48.1**	**-70.8**	**-75.4**	**-79.2**	**-81.7**	**-82.6**	**-83.6**	**-82.0**	**-80.7**	**-76.6**	**-75.6**	**-72.3**	**-66.9**
		SD	0.0	9.7	5.9	7.9	8.4	6.0	6.3	6.5	3.2	5.0	5.2	4.6	5.2	6.0	5.8
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-002333	**0.5 mg/kg**	**Mean**	**0.0**	**0.6**	**-22.7**	**-47.5**	**-50.9**	**-57.6**	**-58.2**	**-56.7**	**-47.3**	**-43.8**	**-46.8**	**-44.8**	**-40.7**	**-39.2**	**-42.5**
		SD	0.0	11.1	21.3	10.7	11.8	10.2	8.2	7.7	12.0	11.4	9.8	10.7	8.4	11.1	12.9

Study Day	Dose	Statistic	Study Day														
			1	**8**	**15**	**22**	**29**	**36**	**43**	**50**	**57**	**64**	**71**	**78**	**85**	**99**	**113**
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-002335	**0.5 mg/kg**	**Mean**	**0.0**	**-20.5**	**-38.8**	**-58.9**	**-64.5**	**-68.4**	**-67.6**	**-70.0**	**-59.6**	**-57.8**	**-56.9**	**-56.7**	**-59.4**	**-53.3**	**-47.6**
		SD	0.0	10.2	13.6	11.9	12.3	7.2	11.8	7.0	15.6	15.9	18.4	7.9	7.9	14.8	21.3
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

Table 8. Percent Change in Serum LP(a) Relative to Day 1 Pre-Dose

Study Day	Dose	Statistic	Study Day														
			1	8	15	22	29	36	43	50	57	64	71	78	85	99	113
SRS-002064	**0.5 mg/kg**	**Mean**	**0.0**	**-14.8**	**-40.1**	**-59.6**	**-74.4**	**-79.8**	**-80.7**	**-79.4**	**-77.3**	**-73.6**	**-63.3**	**-69.8**	**-62.7**	**-57.6**	**-48.1**
		SD	0.0	13.8	7.8	8.5	7.5	5.3	5.1	4.6	5.8	5.9	11.4	8.4	7.3	12.2	12.9
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-002378	**0.5 mg/kg**	**Mean**	**0.0**	**-3.9**	**-29.1**	**-53.1**	**-64.7**	**-68.8**	**-70.4**	**-66.4**	**-62.9**	**-62.0**	**-67.1**	**-55.1**	**-43.1**	**-41.0**	**-35.6**
		SD	0.0	15.3	10.8	10.0	6.6	5.6	8.0	8.0	12.3	10.2	15.2	13.5	14.2	16.7	17.4
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
SRS-002379	**0.5 mg/kg**	**Mean**	**0.0**	**1.6**	**-31.4**	**-54.6**	**-64.2**	**-70.4**	**-70.8**	**-70.3**	**-67.1**	**-68.4**	**-55.5**	**-66.1**	**-56.0**	**-55.2**	**-53.5**
		SD	0.0	14.5	20.4	26.9	21.0	15.2	23.1	19.7	25.6	22.5	32.5	24.8	30.4	28.8	31.8
		N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

Table 9. APOC3 Protein Expression Normalized to Pre-Dose and Control

Study Day	Dose	Statistic	Study Day				
			7	**14**	21	28	35
1x PBS	**0.5 mg/kg**	**Mean**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
		SD	0.18	0.14	0.06	0.18	0.25
		N	5	5	5	5	5
SRS-000231	**0.5 mg/kg**	**Mean**	**0.56**	**0.71**	**0.83**	**0.82**	**0.84**
		SD	0.20	0.22	0.18	0.27	0.16

Study Day	Dose	Statistic	Study Day				
			7	14	21	28	35
SRS-001862	0.5 mg/kg	N	5	5	5	5	5
		Mean	**0.40**	**0.38**	**0.28**	**0.54**	**0.63**
		SD	0.08	0.09	0.16	0.12	0.15
SRS-002514	0.5 mg/kg	N	5	5	5	5	5
		Mean	**0.67**	**0.73**	**0.72**	**0.92**	**0.84**
		SD	0.13	0.20	0.19	0.18	0.33
SRS-002515	0.5 mg/kg	N	5	5	5	5	5
		Mean	**0.54**	**0.59**	**0.66**	**0.85**	**0.91**
		SD	0.14	0.17	0.16	0.21	0.30
SRS-000228	0.5 mg/kg	N	5	5	5	5	5
		Mean	**0.40**	**0.48**	**0.57**	**0.57**	**0.73**
		SD	0.06	0.05	0.25	0.11	0.26
SRS-002516	0.5 mg/kg	N	5	5	5	5	5
		Mean	**0.47**	**0.41**	**0.50**	**0.54**	**0.38**
		SD	0.04	0.14	0.17	0.08	0.12
SRS-002517	0.5 mg/kg	N	5	5	5	5	5
		Mean	**0.56**	**0.56**	**0.44**	**0.66**	**0.54**
		SD	0.13	0.11	0.11	0.14	0.03
SRS-002518	0.5 mg/kg	N	4	4	4	4	4
		Mean	**0.57**	**0.60**	**0.62**	**0.93**	**0.53**
		SD	0.07	0.11	0.14	0.20	0.14
SRS-002519	0.5 mg/kg	N	5	5	5	5	5
		Mean	**0.59**	**0.62**	**0.42**	**1.02**	**0.54**
		SD	0.08	0.17	0.16	0.30	0.13
SRS-002520	0.5 mg/kg	N	5	5	5	5	5
		Mean	**0.78**	**1.05**	**0.87**	**1.28**	**0.80**
		SD	0.12	0.24	0.28	0.33	0.14
SRS-002521	0.5 mg/kg	N	5	5	5	4	4
		Mean	**0.53**	**0.73**	**1.17**	**0.98**	**0.62**

Study Day	Dose	Statistic	Study Day				
			7	14	21	28	35
		SD	0.26	0.19	0.12	0.23	0.05
		N	4	4	4	4	4

Table 10. Percent Change in Serum Lp(a) Relative to Pre-Dose (Day 1)

Group		Day 1	Day 8	Day 15	Day 22	Day 29	Day 36	Day 43	Day 50	Day 57	Day 64	Day 71	Day 78	Day 85	Day 99	Day 113	Day 127	Day 141	Day 155	Day 169
SRS-002064, 1 mg/kg	**Mean**	**0.0**	**-18.9**	**-54.9**	**-72.4**	**-81.5**	**-86.8**	**-88.0**	**-90.8**	**-89.2**	**-88.3**	**-88.2**	**-88.3**	**-85.7**	**-82.4**	**-82.7**	**-76.5**	**-72.5**		
	SD	0.0	13.8	13.3	9.0	8.0	6.6	6.5	5.3	5.9	6.6	7.7	7.8	11.2	11.9	12.8	22.7	26.7		
SRS-002064, 3 mg/kg	**Mean**	**0.0**	**-19.6**	**-65.5**	**-83.0**	**-89.4**	**-93.9**	**-94.8**	**-96.7**	**-95.5**	**-95.9**	**-94.8**	**-94.2**	**-91.3**	**-89.9**	**-87.1**	**-81.6**	**-75.6**		
	SD	0.0	25.2	15.0	10.0	7.7	4.9	4.2	2.5	4.6	3.8	5.0	5.0	6.8	7.4	8.0	13.5	15.8		
SRS-002064, 10 mg/kg	**Mean**	**0.0**	**-39.9**	**-77.2**	**-90.5**	**-95.2**	**-97.5**	**-98.0**	**-98.6**	**-98.5**	**-98.4**	**-98.2**	**-97.9**	**-97.4**	**-97.2**	**-97.2**	**-96.6**	**-94.7**	**-90.8**	**-88.0**
	SD	0.0	15.4	11.1	6.0	2.9	2.2	1.4	1.0	1.0	1.2	0.9	1.2	1.4	2.1	2.4	2.9	5.3	9.4	12.5
SRS-002081, 1 mg/kg	**Mean**	**0.0**	**-32.9**	**-65.4**	**-82.1**	**-86.8**	**-92.8**	**-94.4**	**-95.9**	**-95.1**	**-95.5**	**-94.9**	**-94.5**	**-92.4**	**-91.1**	**-88.7**				
	SD	0.0	9.5	7.0	7.2	4.7	2.9	2.5	1.7	2.0	1.7	2.2	2.0	3.9	5.1	6.6				
SRS-002081, 3 mg/kg	**Mean**	**0.0**	**-34.3**	**-64.4**	**-83.6**	**-86.2**	**-95.0**	**-96.4**	**-97.6**	**-97.6**	**-97.1**	**-96.7**	**-96.1**	**-95.4**	**-94.1**	**-93.8**				
	SD	0.0	7.2	3.5	3.1	3.2	1.6	1.0	0.5	0.6	1.0	1.3	0.9	1.4	1.9	3.1				

Table 11. Differentially Expressed Genes (DEG) by RNAseq

Test Article	Concentration (nM)	Qty of significant DEGs (Excluding LPA)
SRS-000016	10	88
SRS-002081	10	55
SRS-002064	10	0

CLAIMS

WHAT IS CLAIMED IS:

1. A polynucleic acid molecule for modulating expression of lipoprotein(a) (Lp(a)) gene, comprising a guide strand and a passenger strand, wherein the guide strand comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from UCUCUGUCCAUUACCGUGGUAGC (SEQ ID NO: 1) or UUGCGUCUGAGCAUUGUGUCAGG (SEQ ID NO: 2), wherein the guide strand comprises a nucleotide analogue selected from a group consisting of the nucleotide analogue is selected from acyclic L-threoninol nucleic acid-thymine-3'-phosphate (T-T), acyclic L-threoninol nucleic acid-adenine-3'-phosphate (T-A), acyclic N-acetyl L-threoninol abasic nucleic acid-3'phosphate (T-NAc), 1',2'-Dideoxyribose-3'-phosphate (dAB), thymidine-glycol nucleic acid (GNA) S-isomer (Tgn), 2'-O-methyl-2-thiouridine-3'-phosphate (u3), 2'-fluoro-2-thiouridine-3'-phosphate (U3f), 2'-deoxythymidine-3'-phosphate (dT), and 2'-deoxy-2-thiothymidine-3'-phosphate (dT3).

2. The polynucleic acid molecule of claim 1, wherein the guide strand comprises a nucleic acid sequence selected from SEQ ID NOs: 1-13.

3. The polynucleic acid molecule of claim 1 or claim 2, wherein the passenger strand comprises a nucleic acid sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to a nucleic acid sequence selected from SEQ ID NOs: 14-20.

4. The polynucleic acid molecule of any one of claims 1-3, wherein the passenger strand comprises a nucleic acid sequence selected from SEQ ID NOs: 14-20.

5. The polynucleic acid molecule of any one of claims 1-4, wherein the passenger strand comprises a nucleic acid sequence of UACCACGGUAAUGGACAGAGA (SEQ ID NO: 14) and a guide strand comprises a nucleic acid sequence of UCUCUGTCCAUUACCGUGGUAGC (SEQ ID NO: 6) or UCUCUGUCCAUUACCGUGGUAGC (SEQ ID NO: 1).

6. The polynucleic acid molecule of any one of claims 1-5, wherein the nucleotide analogue is located at the seed region of the guide strand (positions 2-8), or positions 11-13, from the 5' end of the guide strand.

7. The polynucleic acid molecule of any one of claims 1-6, wherein the nucleotide analogue is located at any one of positions 4-8, or position 12, from the 5’ end of the guide strand.

8. The polynucleic acid molecule of any one of claims 1-7, wherein the nucleotide analogue is located at any one of positions 5-8, or position 12, from the 5’ end of the guide strand.

9. The polynucleic acid molecule of any one of claims 1-8, wherein the guide strand comprises a nucleic acid sequence selected from SEQ ID NOs: 21-71.

10. The polynucleic acid molecule of any one of claims 1-8, wherein the guide strand comprises a nucleic acid sequence selected from SEQ ID NOs: 26-27.

11. The polynucleic acid molecule of any one of claims 1-10, wherein the passenger strand comprises 2-amino-2'-O-methyladenosine-3'-phosphate substituting at least one of adenine nucleotides.

12. The polynucleic acid molecule of claim 11, wherein the passenger strand comprises 2-amino-2'-O-methyladenosine-3'-phosphate at the location complementary to the seed region of the guide strand.

13. The polynucleic acid molecule of claim 11 or claim 12, wherein the 2-amino-2'-O-methyladenosine-3'-phosphate is located at any one of positions 4-8 from the 3’ end of the passenger strand.

14. The polynucleic acid molecule of any one of claims 1-13, wherein the passenger strand comprises a nucleic acid sequence selected from SEQ ID NOs: 72-91.

15. The polynucleic acid molecule of any one of claims 1-14, wherein the guide strand comprises a nucleic acid sequence of usCfsucug(T-T)ccauUfaCfcGfugguasgsc (SEQ ID NO: 26) and a passenger strand comprises a nucleic acid sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72).

16. The polynucleic acid molecule of any one of claims 1-14, wherein the guide strand comprises a nucleic acid sequence of usCfsucugUfccauu3aCfcGfugguasgsc (SEQ ID NO: 27) and a passenger strand comprises a nucleic acid sequence of usasccacGfgUfaAfuggacagaga (SEQ ID NO: 72).

17. A polynucleic acid molecule conjugate for modulating expression of lipoprotein(a) gene (Lp(a)), wherein the polynucleic acid molecule conjugate comprises a polynucleic acid molecule of any one of claims 1-16 and an asialoglycoprotein receptor targeting moiety.

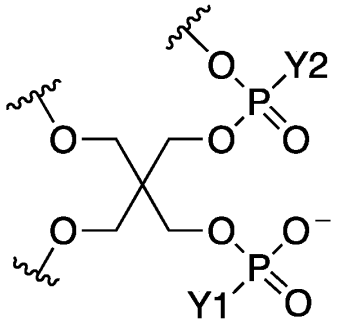
18. The polynucleic acid molecule conjugate of claim 17, wherein the asialoglycoprotein receptor targeting moiety comprises N-Acetylgalactosamine (GalNAc) or galactose.

19. The polynucleic acid molecule conjugate of one of claims 17-18, wherein the polynucleic acid molecule and the asialoglycoprotein receptor targeting moiety is coupled via a linker.

20. The polynucleic acid molecule conjugate of claim 19, wherein the linker is a cleavable linker.

21. The polynucleic acid molecule conjugate of any one of claims 18-20, wherein the GalNAc comprises an anomeric carbon bonded to trivalent, tetravalent linker, pentavalent, or hexavalent linker, wherein the anomeric carbon is part of a hemiaminal group.

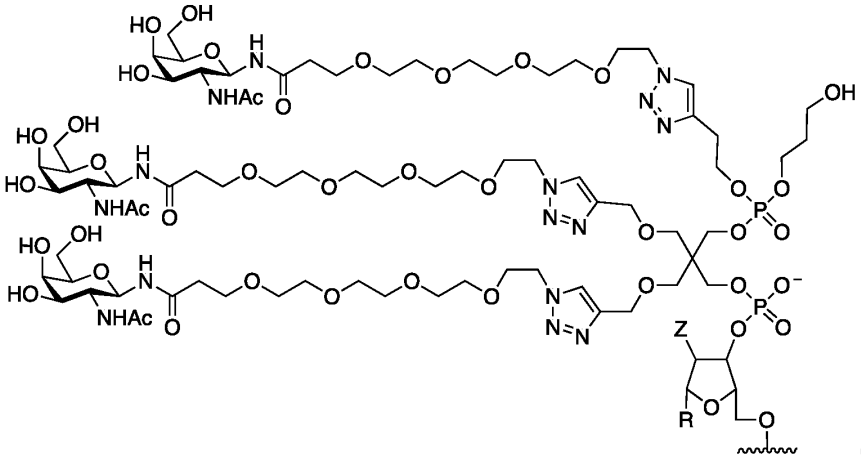
22. The polynucleic acid molecule conjugate of any one of claims 19-21, wherein the linker comprises formula (IV) below,



, wherein at least one of Y1 and Y2 is a nucleotide in the polynucleic acid molecule.

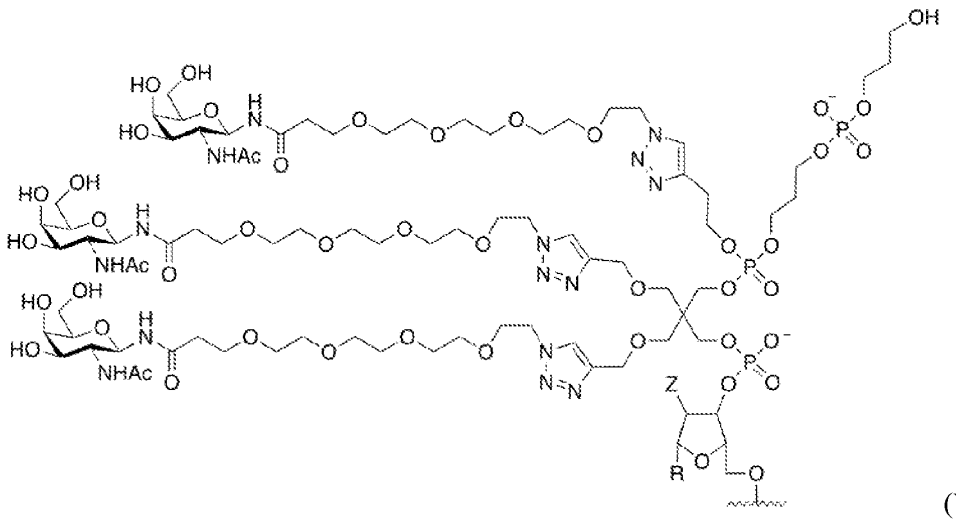
23. The polynucleic acid molecule conjugate of claim 22, wherein the Y1 is the last nucleotide on the 3' end of the sense strand of the polynucleic acid molecule.

24. The polynucleic acid molecule conjugate of any one of claims 18-23, wherein the linker and the asialoglycoprotein receptor targeting moiety with the last nucleotide on the 3' end of the sense strand of the polynucleic acid molecule are shown in Formula (V'), Formula (V''''), Formula (V'''''), or Formula (V'''''''):



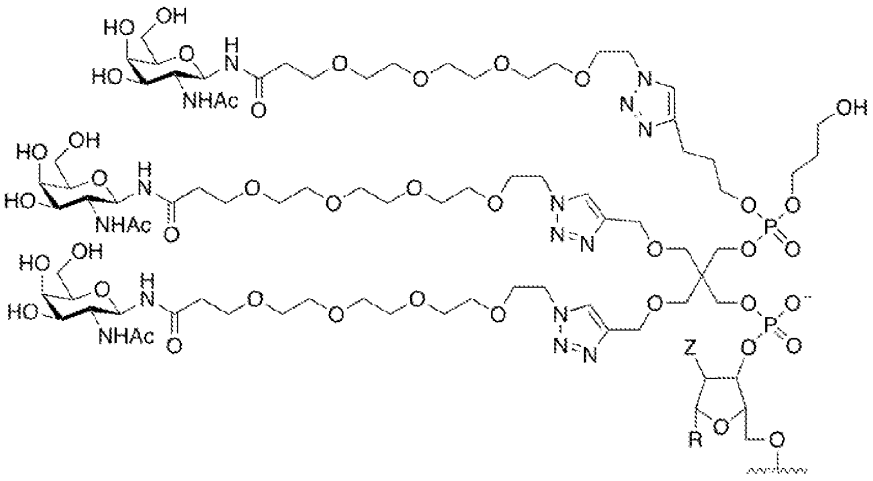
(V'),

wherein Z in formula (V') is -H, -OH, -O-Methyl, -F, or -O-methoxyethyl and R in formula (V') is adenine, uracil, guanine, cytosine, thymine, abasic, or others;



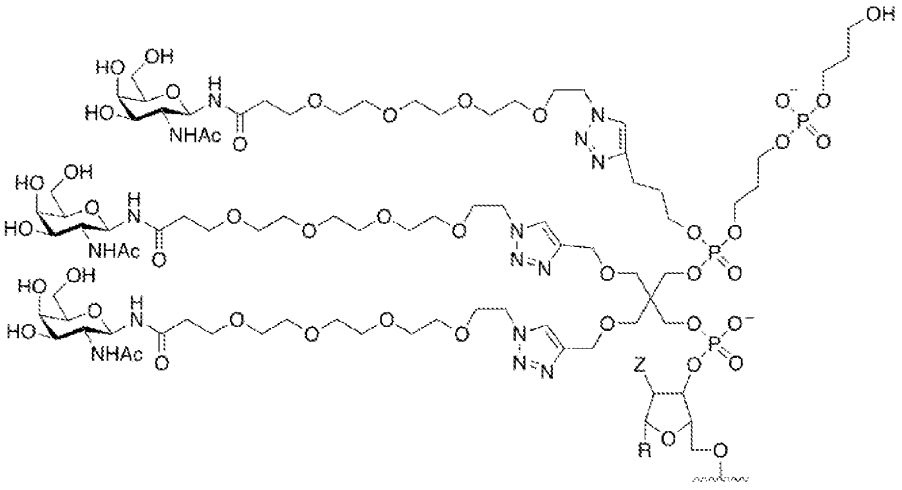
(V''''),

wherein Z in formula (V'''') is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V'''') is adenine, uracil, guanine, cytosine, thymine, abasic, or others;



(V’’’’’),

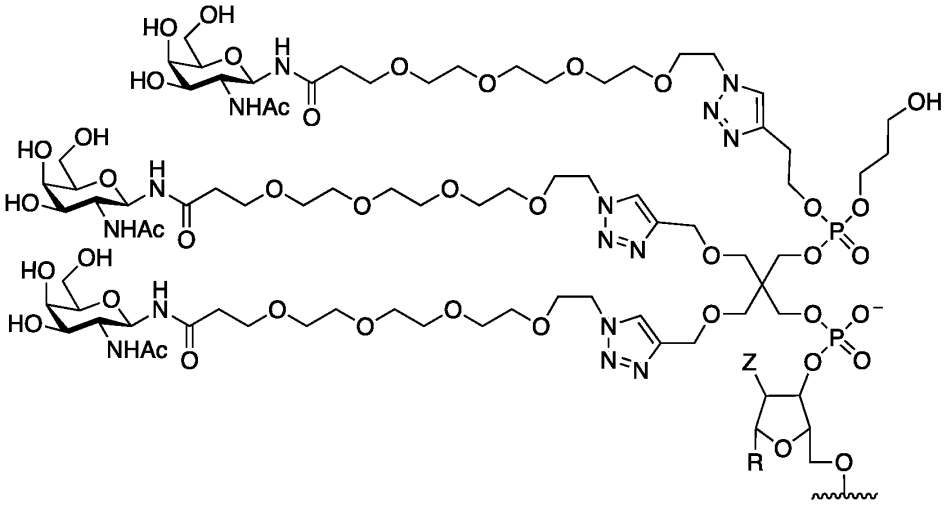
wherein Z in formula (V’’’’’) is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V’’’’’) is adenine, uracil, guanine, cytosine, thymine, abasic, or others; or



(V’’’’’’),

wherein Z in formula (V’’’’’’) is a moiety that corresponds to one of the sugar modifications described herein (e.g., -H, -OH, -O-Methyl, -F, or -O-methoxyethyl) and R in formula (V’’’’’’) is adenine, uracil, guanine, cytosine, thymine, abasic, or others.

25. The polynucleic acid molecule conjugate of claim 24, wherein the linker and the asialoglycoprotein receptor targeting moiety with the last nucleotide on the 3’ end of the sense strand of the polynucleic acid molecule are shown in Formula (V’):



(V'), wherein Z in formula (V') is -H, -OH, -O-Methyl, -F, or -O-methoxyethyl and R in formula (V') is adenine, uracil, guanine, cytosine, thymine, abasic, or others.

26. A pharmaceutical composition comprising a polynucleic acid molecule of any one of claims 1-16 or a polynucleic acid molecule conjugate of any one of claims 17-25, and a pharmaceutically acceptable excipient.

27. The pharmaceutical composition of claim 26, wherein the pharmaceutical composition is formulated for intravenous, subcutaneous, parenteral, oral, intranasal, buccal, rectal, transdermal, or intrathecal administration.

28. A method of modulating expression of lipoprotein(a) (Lp(a)) gene in a subject in need thereof, comprising:

administering to the subject a polynucleic acid molecule of any one of claims 1-16, a polynucleic acid molecule conjugate of any one of claims 17-25, or a pharmaceutical composition of any one of claims 26-27, thereby modulating the expression of Lp(a) gene in the subject in need thereof.

29. A method for treating or preventing a cardiovascular disease or a lipid disorder, comprising:

administering to a subject having or being diagnosed with a cardiovascular disease or a lipid disorder, or suffering from a symptom of a cardiovascular disease or a lipid disorder, or being suspected to develop a cardiovascular disease or a lipid disorder or a symptom thereof, a polynucleic acid molecule of any one of claims 1-16, a polynucleic acid molecule conjugate of any one of claims 17-25, or a pharmaceutical composition of any one of claims

26-27, thereby treating or preventing a cardiovascular disease or a lipid disorder in the subject.

30. The method of any one of claims 28-29, wherein the polynucleic acid molecule is administered at a dose sufficient to decrease the expression of Lp(a) gene in a cell of said subject or to decrease plasma Lp(a) level of said subject by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to a control.

31. The method of any one of claims 29-30, wherein the cardiovascular disease is coronary artery disease, acute myocardial infarction, asymptomatic carotid atherosclerosis, stroke, atrial fibrillation, hypercholesterolemia, or peripheral artery occlusive disease.

32. The method of any one of claims 29-30, wherein the lipid disorder is hyperlipidemia or hypercholesterolemia.