CHIRAL DESIGN

Cross-Reference to Related Applications

[0001] This application claims priority to United States Provisional Application Serial No. 61/928,405, filed January 16, 2014, and 62/063,359, filed October 13, 2014, the entirety of each of which is incorporated herein by reference.

Background of the Invention

[0002] Oligonucleotides are useful in therapeutic, diagnostic, research and nanomaterials applications. The use of naturally occurring nucleic acids (e.g., unmodified DNA or RNA) for therapeutics can be limited, for example, because of their instability against extra- and intracellular nucleases and/or their poor cell penetration and distribution. Additionally, *in vitro* studies have shown that properties of antisense oligonucleotides such as binding affinity, sequence specific binding to the complementary RNA (Cosstick and Eckstein, 1985; LaPlanche *et al.,* 1986; Latimer *et al.,* 1989; Hacia *et al.,* 1994; Mesmaeker *et al.,* 1995), and stability to nucleases can be affected by the absolute stereochemical configurations of the phosphorus atoms (Cook, *et al*. US005599797A). Therefore, there is a need for new and improved oligonucleotides and oligonucleotide compositions, such as, e.g., new antisense and siRNA oligonucleotides and oligonucleotide compositions.

Summary of the Invention

[0003] Among other things, the present invention encompasses the recognition that stereorandom oligonucleotide preparations contain a plurality of distinct chemical entities that differ from one another in the stereochemical structure of individual backbone chiral centers within the oligonucleotide chain. Moreover, the present invention encompasses the insight that it is typically unlikely that a stereorandom oligonucleotide preparation will include every possible stereoisomer of the relevant oligonucleotide. Thus, among other things, the present invention provides new chemical entities that are particular stereoisomers of oligonucleotides of interest. That is, the present invention provides substantially pure preparations of single oligonucleotide compounds, where a particular oligonucleotide compound may be defined by its base sequence, its length, its pattern of backbone linkages, and its pattern of backbone chiral centers.

[0004] The present invention demonstrates, among other things, that individual stereoisomers of a particular oligonucleotide can show different stability and/or activity from each other. Moreover, the present disclosure demonstrates that stability improvements achieved through inclusion and/or location of particular chiral structures within an oligonucleotide can be comparable to, or even better than those achieved through use of certain modified backbone linkages, bases, and/or sugars (e.g., through use of certain types of modified phophates, 2'-modifications, base modifications, etc.).

[0005] Among other things, the present invention recognizes that properties and activities of an oligonucleotide can be adjusted by optimizing its pattern of backbone chiral centers. In some embodiments, the present invention provides compositions of oligonucleotides, wherein the oligonucleotides have a common pattern of backbone chiral centers which, unexpectedly, greatly enhances the stability and/or biological activity of the oligonucleotides. In some embodiments, a pattern of backbone chiral centers provides increased stability. In some embodiments, a pattern of backbone chiral centers provides surprisingly increased activity. In some embodiments, a pattern of backbone chiral centers provides increased stability and activity. In some embodiments, when an oligonucleotide is utilized to cleave a nucleic acid polymer, a pattern of backbone chiral centers of the oligonucleotide, surprisingly by itself, changes the cleavage pattern of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers effectively prevents cleavage at secondary sites. In some embodiments, a pattern of backbone chiral centers creates new cleavage sites. In some embodiments, a pattern of backbone chiral centers minimizes the number of cleavage sites. In some embodiments, a pattern of backbone chiral centers minimizes the number of cleavage sites so that a target nucleic acid polymer is cleaved at only one site within the sequence of the target nucleic acid polymer that is complementary to the oligonucleotide. In some embodiments, a pattern of backbone chiral centers enhances cleavage efficiency at a cleavage site. In some embodiments, a pattern of backbone chiral centers of the oligonucleotide improves cleavage of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers increases selectivity. In some embodiments, a pattern of backbone chiral centers minimizes off-target effect. In some embodiments, a pattern of backbone chiral centers increase selectivity, *e.g.*, cleavage selectivity between two target sequences differing only by a single nucleotide polymorphism (SNP).

[0006] All publications and patent documents cited in this application are incorporated herein by reference in their entirety.

Definitions

[0007] *Aliphatic*: The term "aliphatic" or "aliphatic group", as used herein, means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation, or a monocyclic hydrocarbon or bicyclic or polycyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic (also referred to herein as "carbocycle" "cycloaliphatic" or "cycloalkyl"), that has a single point of attachment to the rest of the molecule. In some embodiments, aliphatic groups contain 1-50 aliphatic carbon atoms. Unless otherwise specified, aliphatic groups contain 1-10 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-6 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-5 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-4 aliphatic carbon atoms. In still other embodiments, aliphatic groups contain 1-3 aliphatic carbon atoms, and in yet other embodiments, aliphatic groups contain 1-2 aliphatic carbon atoms. In some embodiments, "cycloaliphatic" (or "carbocycle" or "cycloalkyl") refers to a monocyclic or bicyclic C_3-C_{10} hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule. In some embodiments, "cycloaliphatic" (or "carbocycle" or "cycloalkyl") refers to a monocyclic C_3–C_6 hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule. Suitable aliphatic groups include, but are not limited to, linear or branched, substituted or unsubstituted alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

[0008] *Alkylene*: The term "alkylene" refers to a bivalent alkyl group. An "alkylene chain" is a polymethylene group, i.e., $–(CH_2)_n–$, wherein n is a positive integer, preferably from 1 to 6, from 1 to 4, from 1 to 3, from 1 to 2, or from 2 to 3. A substituted alkylene chain is a polymethylene group in which one or more methylene hydrogen atoms are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

[0009] *Alkenylene*: The term "alkenylene" refers to a bivalent alkenyl group. A substituted alkenylene chain is a polymethylene group containing at least one double bond in which one or more hydrogen atoms are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

[0010] *Animal:* As used herein, the term "animal" refers to any member of the animal kingdom. In some embodiments, "animal" refers to humans, at any stage of development. In some embodiments, "animal" refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, and/or worms. In some embodiments, an animal may be a transgenic animal, a genetically-engineered animal, and/or a clone.

[0011] *Approximately:* As used herein, the terms "approximately" or "about" in reference to a number are generally taken to include numbers that fall within a range of 5%, 10%, 15%, or 20% in either direction (greater than or less than) of the number unless otherwise stated or otherwise evident from the context (except where such number would be less than 0% or exceed 100% of a possible value). In some embodiments, use of the term "about" in reference to dosages means ± 5 mg/kg/day.

[0012] *Aryl:* The term "aryl" used alone or as part of a larger moiety as in "aralkyl," "aralkoxy," or "aryloxyalkyl," refers to monocyclic and bicyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic and wherein each ring in the system contains three to seven ring members. The term "aryl" may be used interchangeably with the term "aryl ring." In certain embodiments of the present invention, "aryl" refers to an aromatic ring system which includes, but not limited to, phenyl, biphenyl, naphthyl, anthracyl and the like, which may bear one or more substituents. Also included within the scope of the term "aryl," as it is used herein, is a group in which an aromatic ring is fused to one or more non–aromatic rings, such as indanyl, phthalimidyl, naphthimidyl, phenanthridinyl, or tetrahydronaphthyl, and the like.

[0013] *Characteristic portion*: As used herein, the phrase a "characteristic portion" of a protein or polypeptide is one that contains a continuous stretch of amino acids, or a collection of continuous stretches of amino acids, that together are characteristic of a protein or polypeptide.

Each such continuous stretch generally will contain at least two amino acids. Furthermore, those of ordinary skill in the art will appreciate that typically at least 5, 10, 15, 20 or more amino acids are required to be characteristic of a protein. In general, a characteristic portion is one that, in addition to the sequence identity specified above, shares at least one functional characteristic with the relevant intact protein.

[0014] *Characteristic sequence*: A "characteristic sequence" is a sequence that is found in all members of a family of polypeptides or nucleic acids, and therefore can be used by those of ordinary skill in the art to define members of the family.

[0015] *Characteristic structural element:* The term "characteristic structural element" refers to a distinctive structural element (e.g., core structure, collection of pendant moieties, sequence element, etc) that is found in all members of a family of polypeptides, small molecules, or nucleic acids, and therefore can be used by those of ordinary skill in the art to define members of the family.

[0016] *Comparable:* The term "comparable" is used herein to describe two (or more) sets of conditions or circumstances that are sufficiently similar to one another to permit comparison of results obtained or phenomena observed. In some embodiments, comparable sets of conditions or circumstances are characterized by a plurality of substantially identical features and one or a small number of varied features. Those of ordinary skill in the art will appreciate that sets of conditions are comparable to one another when characterized by a sufficient number and type of substantially identical features to warrant a reasonable conclusion that differences in results obtained or phenomena observed under the different sets of conditions or circumstances are caused by or indicative of the variation in those features that are varied.

[0017] *Dosing regimen:* As used herein, a "dosing regimen" or "therapeutic regimen" refers to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regime comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some

embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount.

[0018] *Equivalent agents:* Those of ordinary skill in the art, reading the present disclosure, will appreciate that the scope of useful agents in the context of the present invention is not limited to those specifically mentioned or exemplified herein. In particular, those skilled in the art will recognize that active agents typically have a structure that consists of a core and attached pendant moieties, and furthermore will appreciate that simple variations of such core and/or pendant moieties may not significantly alter activity of the agent. For example, in some embodiments, substitution of one or more pendant moieties with groups of comparable three-dimensional structure and/or chemical reactivity characteristics may generate a substituted compound or portion equivalent to a parent reference compound or portion. In some embodiments, addition or removal of one or more pendant moieties may generate a substituted compound equivalent to a parent reference compound. In some embodiments, alteration of core structure, for example by addition or removal of a small number of bonds (typically not more than 5, 4, 3, 2, or 1 bonds, and often only a single bond) may generate a substituted compound equivalent to a parent reference compound. In many embodiments, equivalent compounds may be prepared by methods illustrated in general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional or provided synthesis procedures. In these reactions, it is also possible to make use of variants, which are in themselves known, but are not mentioned here.

[0019] *Equivalent Dosage:* The term "equivalent dosage" is used herein to compare dosages of different pharmaceutically active agents that effect the same biological result. Dosages of two different agents are considered to be "equivalent" to one another in accordance with the present invention if they achieve a comparable level or extent of the biological result. In some embodiments, equivalent dosages of different pharmaceutical agents for use in accordance with the present invention are determined using *in vitro* and/or *in vivo* assays as described herein. In some embodiments, one or more lysosomal activating agents for use in accordance with the present invention is utilized at a dose equivalent to a dose of a reference lysosomal activating agent; in some such embodiments, the reference lysosomal activating agent for such purpose is

selected from the group consisting of small molecule allosteric activators (e.g., pyrazolpyrimidines), imminosugars (e.g., isofagomine), antioxidants (e.g., n-acetyl-cysteine), and regulators of cellular trafficking (e.g., Rab1a polypeptide).

[0020] *Heteroaliphatic*: The term "heteroaliphatic" refers to an aliphatic group wherein one or more units selected from C, CH, CH_2, or CH_3 are independently replaced by a heteroatom. In some embodiments, a heteroaliphatic group is heteroalkyl. In some embodiments, a heteroaliphatic group is heteroalkenyl.

[0021] *Heteroaryl*: The terms "heteroaryl" and "heteroar–," used alone or as part of a larger moiety, e.g., "heteroaralkyl," or "heteroaralkoxy," refer to groups having 5 to 10 ring atoms, preferably 5, 6, or 9 ring atoms; having 6, 10, or 14 π electrons shared in a cyclic array; and having, in addition to carbon atoms, from one to five heteroatoms. The term "heteroatom" refers to nitrogen, oxygen, or sulfur, and includes any oxidized form of nitrogen or sulfur, and any quaternized form of a basic nitrogen. Heteroaryl groups include, without limitation, thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, indolizinyl, purinyl, naphthyridinyl, and pteridinyl. The terms "heteroaryl" and "heteroar–," as used herein, also include groups in which a heteroaromatic ring is fused to one or more aryl, cycloaliphatic, or heterocyclyl rings, where the radical or point of attachment is on the heteroaromatic ring. Nonlimiting examples include indolyl, isoindolyl, benzothienyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4*H*–quinolizinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, and pyrido[2,3–b]–1,4–oxazin–3(4H)–one. A heteroaryl group may be mono– or bicyclic. The term "heteroaryl" may be used interchangeably with the terms "heteroaryl ring," "heteroaryl group," or "heteroaromatic," any of which terms include rings that are optionally substituted. The term "heteroaralkyl" refers to an alkyl group substituted by a heteroaryl, wherein the alkyl and heteroaryl portions independently are optionally substituted.

[0022] *Heteroatom*: The term "heteroatom" means one or more of oxygen, sulfur, nitrogen, phosphorus, boron, selenium, or silicon (including, any oxidized form of nitrogen, boron, selenium, sulfur, phosphorus, or silicon; the quaternized form of any basic nitrogen or; a

substitutable nitrogen of a heterocyclic ring, for example N (as in 3,4-dihydro-2*H*-pyrrolyl), NH (as in pyrrolidinyl) or NR^+ (as in N-substituted pyrrolidinyl)).

[0023] *Heterocycle*: As used herein, the terms "heterocycle," "heterocyclyl," "heterocyclic radical," and "heterocyclic ring" are used interchangeably and refer to a stable 3– to 7–membered monocyclic or 7–10–membered bicyclic heterocyclic moiety that is either saturated or partially unsaturated, and having, in addition to carbon atoms, one or more, preferably one to four, heteroatoms, as defined above. When used in reference to a ring atom of a heterocycle, the term "nitrogen" includes a substituted nitrogen. As an example, in a saturated or partially unsaturated ring having 0–3 heteroatoms selected from oxygen, sulfur or nitrogen, the nitrogen may be N (as in 3,4–dihydro–2*H*–pyrrolyl), NH (as in pyrrolidinyl), or $^+$NR (as in *N*–substituted pyrrolidinyl).

[0024] A heterocyclic ring can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted. Examples of such saturated or partially unsaturated heterocyclic radicals include, without limitation, tetrahydrofuranyl, tetrahydrothiophenyl pyrrolidinyl, piperidinyl, pyrrolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, dioxanyl, dioxolanyl, diazepinyl, oxazepinyl, thiazepinyl, morpholinyl, and quinuclidinyl. The terms "heterocycle," "heterocyclyl," "heterocyclyl ring," "heterocyclic group," "heterocyclic moiety," and "heterocyclic radical," are used interchangeably herein, and also include groups in which a heterocyclyl ring is fused to one or more aryl, heteroaryl, or cycloaliphatic rings, such as indolinyl, 3*H*–indolyl, chromanyl, phenanthridinyl, or tetrahydroquinolinyl, where the radical or point of attachment is on the heterocyclyl ring. A heterocyclyl group may be mono– or bicyclic. The term "heterocyclylalkyl" refers to an alkyl group substituted by a heterocyclyl, wherein the alkyl and heterocyclyl portions independently are optionally substituted.

[0025] *Intraperitoneal:* The phrases "intraperitoneal administration" and "administered intraperitonealy" as used herein have their art-understood meaning referring to administration of a compound or composition into the peritoneum of a subject.

[0026] *In vitro*: As used herein, the term "*in vitro*" refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, *etc.*, rather than within an organism (*e.g.*, animal, plant, and/or microbe).

[0027] *In vivo:* As used herein, the term "*in vivo*" refers to events that occur within an organism (*e.g.*, animal, plant, and/or microbe).

[0028] *Lower alkyl:* The term "lower alkyl" refers to a C_{1-4} straight or branched alkyl group. Exemplary lower alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tert-butyl.

[0029] *Lower haloalkyl:* The term "lower haloalkyl" refers to a C_{1-4} straight or branched alkyl group that is substituted with one or more halogen atoms.

[0030] *Optionally substituted:* As described herein, compounds of the invention may contain "optionally substituted" moieties. In general, the term "substituted," whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this invention are preferably those that result in the formation of stable or chemically feasible compounds. The term "stable," as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

[0031] Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group are independently halogen; $-(CH_2)_{0-4}R°$; $-(CH_2)_{0-4}OR°$; $-O(CH_2)_{0-4}R^o$, $-O-(CH_2)_{0-4}C(O)OR°$; $-(CH_2)_{0-4}CH(OR°)_2$; $-(CH_2)_{0-4}SR°$; $-(CH_2)_{0-4}Ph$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; $-CH=CHPh$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}$-pyridyl which may be substituted with R°; $-NO_2$; $-CN$; $-N_3$; $-(CH_2)_{0-4}N(R°)_2$; $-(CH_2)_{0-4}N(R°)C(O)R°$; $-N(R°)C(S)R°$; $-(CH_2)_{0-4}N(R°)C(O)NR°_2$; $-N(R°)C(S)NR°_2$; $-(CH_2)_{0-4}N(R°)C(O)OR°$; $-N(R°)N(R°)C(O)R°$; $-N(R°)N(R°)C(O)NR°_2$; $-N(R°)N(R°)C(O)OR°$; $-(CH_2)_{0-4}C(O)R°$; $-C(S)R°$; $-(CH_2)_{0-4}C(O)OR°$; $-(CH_2)_{0-4}C(O)SR°$; $-(CH_2)_{0-4}C(O)OSiR°_3$; $-(CH_2)_{0-4}OC(O)R°$; $-OC(O)(CH_2)_{0-4}SR$, $-SC(S)SR°$; $-(CH_2)_{0-4}SC(O)R°$; $-(CH_2)_{0-4}C(O)NR°_2$; $-C(S)NR°_2$; $-C(S)SR°$; $-SC(S)SR°$, $-(CH_2)_{0-4}OC(O)NR°_2$; $-C(O)N(OR°)R°$; $-C(O)C(O)R°$; $-C(O)CH_2C(O)R°$; $-C(NOR°)R°$; $-(CH_2)_{0-4}SSR°$; $-(CH_2)_{0-4}S(O)_2R°$; $-(CH_2)_{0-4}S(O)_2OR°$;

$-(CH_2)_{0-4}OS(O)_2R°$; $-S(O)_2NR°_2$; $-(CH_2)_{0-4}S(O)R°$; $-N(R°)S(O)_2NR°_2$; $-N(R°)S(O)_2R°$; $-N(OR°)R°$; $-C(NH)NR°_2$; $-P(O)_2R°$; $-P(O)R°_2$; $-OP(O)R°_2$; $-OP(O)(OR°)_2$; $-SiR°_3$; $-(C_{1-4}$ straight or branched alkylene)$O-N(R°)_2$; or $-(C_{1-4}$ straight or branched alkylene)$C(O)O-N(R°)_2$, wherein each R° may be substituted as defined below and is independently hydrogen, C_{1-6} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, $-CH_2$-(5-6 membered heteroaryl ring), or a 5-6 membered saturated, partially unsaturated, or aryl ring having 0−4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3-12 membered saturated, partially unsaturated, or aryl mono− or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

[0032] Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $-(CH_2)_{0-2}R^{\bullet}$, $-(haloR^{\bullet})$, $-(CH_2)_{0-2}OH$, $-(CH_2)_{0-2}OR^{\bullet}$, $-(CH_2)_{0-2}CH(OR^{\bullet})_2$; -O(haloR•), −CN, $-N_3$, $-(CH_2)_{0-2}C(O)R^{\bullet}$, $-(CH_2)_{0-2}C(O)OH$, $-(CH_2)_{0-2}C(O)OR^{\bullet}$, $-(CH_2)_{0-2}SR^{\bullet}$, $-(CH_2)_{0-2}SH$, $-(CH_2)_{0-2}NH_2$, $-(CH_2)_{0-2}NHR^{\bullet}$, $-(CH_2)_{0-2}NR^{\bullet}_2$, $-NO_2$, $-SiR^{\bullet}_3$, $-OSiR^{\bullet}_3$, -C(O)SR•, $-(C_{1-4}$ straight or branched alkylene)$C(O)OR^{\bullet}$, or $-SSR^{\bullet}$ wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5-6 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

[0033] Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: =O, =S, $=NNR^*_2$, $=NNHC(O)R^*$, $=NNHC(O)OR^*$, $=NNHS(O)_2R^*$, $=NR^*$, $=NOR^*$, $-O(C(R^*_2))_{2-3}O-$, or $-S(C(R^*_2))_{2-3}S-$, wherein each independent occurrence of R^* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: $-O(CR^*_2)_{2-3}O-$, wherein each independent occurrence of R^* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5–6–membered

saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0034] Suitable substituents on the aliphatic group of R^* include halogen, $-R^\bullet$, -(halo$R^\bullet$), -OH, $-OR^\bullet$, -O(halo$R^\bullet$), −CN, −C(O)OH, $-C(O)OR^\bullet$, $-NH_2$, $-NHR^\bullet$, $-NR^\bullet{}_2$, or $-NO_2$, wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5-6 membered saturated, partially unsaturated, or aryl ring having 0−4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0035] Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include $-R^\dagger$, $-NR^\dagger{}_2$, $-C(O)R^\dagger$, $-C(O)OR^\dagger$, $-C(O)C(O)R^\dagger$, $-C(O)CH_2C(O)R^\dagger$, $-S(O)_2R^\dagger$, $-S(O)_2NR^\dagger{}_2$, $-C(S)NR^\dagger{}_2$, $-C(NH)NR^\dagger{}_2$, or $-N(R^\dagger)S(O)_2R^\dagger$; wherein each $R^\dagger$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted –OPh, or an unsubstituted 5-6 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^\dagger$, taken together with their intervening atom(s) form an unsubstituted 3-12 membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0036] Suitable substituents on the aliphatic group of $R^\dagger$ are independently halogen, $-R^\bullet$, -(halo$R^\bullet$), −OH, $-OR^\bullet$, -O(halo$R^\bullet$), −CN, −C(O)OH, $-C(O)OR^\bullet$, $-NH_2$, $-NHR^\bullet$, $-NR^\bullet{}_2$, or $-NO_2$, wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5-6 membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[0037] *Oral:* The phrases "oral administration" and "administered orally" as used herein have their art-understood meaning referring to administration by mouth of a compound or composition.

[0038] *Parenteral:* The phrases "parenteral administration" and "administered parenterally" as used herein have their art-understood meaning referring to modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular,

intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticulare, subcapsular, subarachnoid, intraspinal, and intrasternal injection and infusion.

[0039] *Partially unsaturated:* As used herein, the term "partially unsaturated" refers to a ring moiety that includes at least one double or triple bond. The term "partially unsaturated" is intended to encompass rings having multiple sites of unsaturation, but is not intended to include aryl or heteroaryl moieties, as herein defined.

[0040] *Pharmaceutical composition:* As used herein, the term "pharmaceutical composition" refers to an active agent, formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

[0041] *Pharmaceutically acceptable:* As used herein, the phrase "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0042] *Pharmaceutically acceptable carrier:* As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being

compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

[0043] *Pharmaceutically acceptable salt:* The term "pharmaceutically acceptable salt", as used herein, refers to salts of such compounds that are appropriate for use in pharmaceutical contexts, i.e., salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, et al. describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977). In some embodiments, pharmaceutically acceptable salt include, but are not limited to, nontoxic acid addition salts, which are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. In some embodiments, pharmaceutically acceptable salts include, but are not limited to, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate,

pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. In some embodiments, pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl having from 1 to 6 carbon atoms, sulfonate and aryl sulfonate.

[0044] *Prodrug:* A general, a "prodrug," as that term is used herein and as is understood in the art, is an entity that, when administered to an organism, is metabolized in the body to deliver an active (e.g., therapeutic or diagnostic) agent of interest. Typically, such metabolism involves removal of at least one "prodrug moiety" so that the active agent is formed. Various forms of "prodrugs" are known in the art. For examples of such prodrug moieties, see:

a) *Design of Prodrugs*, edited by H. Bundgaard, (Elsevier, 1985) and *Methods in Enzymology*, 42:309-396, edited by K. Widder, *et al. (Academic* Press, 1985);

b) *Prodrugs and Targeted Delivery,* edited by by J. Rautio (Wiley, 2011);

c) *Prodrugs and Targeted Delivery,* edited by by J. Rautio (Wiley, 2011);

d) A Textbook of Drug Design and Development, edited by Krogsgaard-Larsen;

e) Bundgaard, Chapter 5 "Design and Application of Prodrugs", by H. Bundgaard, p. 113-191 (1991);

f) Bundgaard, *Advanced Drug Delivery Reviews*, 8:1-38 (1992);

g) Bundgaard, *et al., Journal of Pharmaceutical Sciences*, 77:285 (1988); and

h) Kakeya, *et al., Chem. Pharm. Bull.*, 32:692 (1984).

[0045] As with other compounds described herein, prodrugs may be provided in any of a variety of forms, e.g., crystal forms, salt forms etc. In some embodiments, prodrugs are provided as pharmaceutically acceptable salts thereof.

[0046] *Protecting group:* The term "protecting group," as used herein, is well known in the art and includes those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of which is incorporated herein by reference. Also included are those protecting groups specially adapted for nucleoside and nucleotide chemistry described in *Current Protocols in Nucleic Acid Chemistry*, edited by Serge L. Beaucage *et al.* 06/2012, the entirety of Chapter 2 is incorporated herein by reference. Suitable amino–protecting groups include methyl carbamate, ethyl

carbamante, 9–fluorenylmethyl carbamate (Fmoc), 9–(2–sulfo)fluorenylmethyl carbamate, 9–(2,7–dibromo)fluoroenylmethyl carbamate, 2,7–di–*t*–butyl–[9–(10,10–dioxo–10,10,10,10–tetrahydrothioxanthyl)]methyl carbamate (DBD–Tmoc), 4–methoxyphenacyl carbamate (Phenoc), 2,2,2–trichloroethyl carbamate (Troc), 2–trimethylsilylethyl carbamate (Teoc), 2–phenylethyl carbamate (hZ), 1–(1–adamantyl)–1–methylethyl carbamate (Adpoc), 1,1–dimethyl–2–haloethyl carbamate, 1,1–dimethyl–2,2–dibromoethyl carbamate (DB–*t*–BOC), 1,1–dimethyl–2,2,2–trichloroethyl carbamate (TCBOC), 1–methyl–1–(4–biphenylyl)ethyl carbamate (Bpoc), 1–(3,5–di–*t*–butylphenyl)–1–methylethyl carbamate (*t*–Bumeoc), 2–(2'– and 4'–pyridyl)ethyl carbamate (Pyoc), 2–(*N*,*N*–dicyclohexylcarboxamido)ethyl carbamate, *t*–butyl carbamate (BOC), 1–adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1–isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4–nitrocinnamyl carbamate (Noc), 8–quinolyl carbamate, *N*–hydroxypiperidinyl carbamate, alkyldithio carbamate, benzyl carbamate (Cbz), *p*–methoxybenzyl carbamate (Moz), *p*–nitobenzyl carbamate, *p*–bromobenzyl carbamate, *p*–chlorobenzyl carbamate, 2,4–dichlorobenzyl carbamate, 4–methylsulfinylbenzyl carbamate (Msz), 9–anthrylmethyl carbamate, diphenylmethyl carbamate, 2–methylthioethyl carbamate, 2–methylsulfonylethyl carbamate, 2–(*p*–toluenesulfonyl)ethyl carbamate, [2–(1,3–dithianyl)]methyl carbamate (Dmoc), 4–methylthiophenyl carbamate (Mtpc), 2,4–dimethylthiophenyl carbamate (Bmpc), 2–phosphonioethyl carbamate (Peoc), 2–triphenylphosphonioisopropyl carbamate (Ppoc), 1,1–dimethyl–2–cyanoethyl carbamate, *m*–chloro–*p*–acyloxybenzyl carbamate, *p*–(dihydroxyboryl)benzyl carbamate, 5–benzisoxazolylmethyl carbamate, 2–(trifluoromethyl)–6–chromonylmethyl carbamate (Tcroc), *m*–nitrophenyl carbamate, 3,5–dimethoxybenzyl carbamate, *o*–nitrobenzyl carbamate, 3,4–dimethoxy–6–nitrobenzyl carbamate, phenyl(*o*–nitrophenyl)methyl carbamate, phenothiazinyl–(10)–carbonyl derivative, *N'*–*p*–toluenesulfonylaminocarbonyl derivative, *N'*–phenylaminothiocarbonyl derivative, *t*–amyl carbamate, *S*–benzyl thiocarbamate, *p*–cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, *p*–decyloxybenzyl carbamate, 2,2–dimethoxycarbonylvinyl carbamate, *o*–(*N*,*N*–dimethylcarboxamido)benzyl carbamate, 1,1–dimethyl–3–(*N*,*N*–dimethylcarboxamido)propyl carbamate, 1,1–dimethylpropynyl carbamate, di(2–pyridyl)methyl carbamate, 2–furanylmethyl carbamate, 2–iodoethyl carbamate, isoborynl carbamate, isobutyl carbamate, isonicotinyl carbamate, *p*–(*p'*–methoxyphenylazo)benzyl carbamate, 1–

methylcyclobutyl carbamate, 1–methylcyclohexyl carbamate, 1–methyl–1–cyclopropylmethyl carbamate, 1–methyl–1–(3,5–dimethoxyphenyl)ethyl carbamate, 1–methyl–1–(*p*–phenylazophenyl)ethyl carbamate, 1–methyl–1–phenylethyl carbamate, 1–methyl–1–(4–pyridyl)ethyl carbamate, phenyl carbamate, *p*–(phenylazo)benzyl carbamate, 2,4,6–tri–*t*–butylphenyl carbamate, 4–(trimethylammonium)benzyl carbamate, 2,4,6–trimethylbenzyl carbamate, formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3–phenylpropanamide, picolinamide, 3–pyridylcarboxamide, *N*–benzoylphenylalanyl derivative, benzamide, *p*–phenylbenzamide, *o*–nitophenylacetamide, *o*–nitrophenoxyacetamide, acetoacetamide, (*N'*–dithiobenzyloxycarbonylamino)acetamide, 3–(*p*–hydroxyphenyl)propanamide, 3–(*o*–nitrophenyl)propanamide, 2–methyl–2–(*o*–nitrophenoxy)propanamide, 2–methyl–2–(*o*–phenylazophenoxy)propanamide, 4–chlorobutanamide, 3–methyl–3–nitrobutanamide, *o*–nitrocinnamide, *N*–acetylmethionine derivative, *o*–nitrobenzamide, *o*–(benzoyloxymethyl)benzamide, 4,5–diphenyl–3–oxazolin–2–one, *N*–phthalimide, *N*–dithiasuccinimide (Dts), *N*–2,3–diphenylmaleimide, *N*–2,5–dimethylpyrrole, *N*–1,1,4,4–tetramethyldisilylazacyclopentane adduct (STABASE), 5–substituted 1,3–dimethyl–1,3,5–triazacyclohexan–2–one, 5–substituted 1,3–dibenzyl–1,3,5–triazacyclohexan–2–one, 1–substituted 3,5–dinitro–4–pyridone, *N*–methylamine, *N*–allylamine, *N*–[2–(trimethylsilyl)ethoxy]methylamine (SEM), *N*–3–acetoxypropylamine, *N*–(1–isopropyl–4–nitro–2–oxo–3–pyroolin–3–yl)amine, quaternary ammonium salts, *N*–benzylamine, *N*–di(4–methoxyphenyl)methylamine, *N*–5–dibenzosuberylamine, *N*–triphenylmethylamine (Tr), *N*–[(4–methoxyphenyl)diphenylmethyl]amine (MMTr), *N*–9–phenylfluorenylamine (PhF), *N*–2,7–dichloro–9–fluorenylmethyleneamine, *N*–ferrocenylmethylamino (Fcm), *N*–2–picolylamino *N'*–oxide, *N*–1,1–dimethylthiomethyleneamine, *N*–benzylideneamine, *N*–*p*–methoxybenzylideneamine, *N*–diphenylmethyleneamine, *N*–[(2–pyridyl)mesityl]methyleneamine, *N*–(*N',N'*–dimethylaminomethylene)amine, *N,N'*–isopropylidenediamine, *N*–*p*–nitrobenzylideneamine, *N*–salicylideneamine, *N*–5–chlorosalicylideneamine, *N*–(5–chloro–2–hydroxyphenyl)phenylmethyleneamine, *N*–cyclohexylideneamine, *N*–(5,5–dimethyl–3–oxo–1–cyclohexenyl)amine, *N*–borane derivative, *N*–diphenylborinic acid derivative, *N*–[phenyl(pentacarbonylchromium– or tungsten)carbonyl]amine, *N*–copper chelate, *N*–zinc chelate, *N*–nitroamine, *N*–nitrosoamine, amine *N*–oxide, diphenylphosphinamide (Dpp), dimethylthiophosphinamide (Mpt),

diphenylthiophosphinamide (Ppt), dialkyl phosphoramidates, dibenzyl phosphoramidate, diphenyl phosphoramidate, benzenesulfenamide, *o*–nitrobenzenesulfenamide (Nps), 2,4–dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2–nitro–4–methoxybenzenesulfenamide, triphenylmethylsulfenamide, 3–nitropyridinesulfenamide (Npys), *p*–toluenesulfonamide (Ts), benzenesulfonamide, 2,3,6,–trimethyl–4–methoxybenzenesulfonamide (Mtr), 2,4,6–trimethoxybenzenesulfonamide (Mtb), 2,6–dimethyl–4–methoxybenzenesulfonamide (Pme), 2,3,5,6–tetramethyl–4–methoxybenzenesulfonamide (Mte), 4–methoxybenzenesulfonamide (Mbs), 2,4,6–trimethylbenzenesulfonamide (Mts), 2,6–dimethoxy–4–methylbenzenesulfonamide (iMds), 2,2,5,7,8–pentamethylchroman–6–sulfonamide (Pmc), methanesulfonamide (Ms), β–trimethylsilylethanesulfonamide (SES), 9–anthracenesulfonamide, 4–(4',8'–dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzylsulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamide.

[0047] Suitably protected carboxylic acids further include, but are not limited to, silyl–, alkyl–, alkenyl–, aryl–, and arylalkyl–protected carboxylic acids. Examples of suitable silyl groups include trimethylsilyl, triethylsilyl, t–butyldimethylsilyl, t–butyldiphenylsilyl, triisopropylsilyl, and the like. Examples of suitable alkyl groups include methyl, benzyl, p–methoxybenzyl, 3,4–dimethoxybenzyl, trityl, t–butyl, tetrahydropyran–2–yl. Examples of suitable alkenyl groups include allyl. Examples of suitable aryl groups include optionally substituted phenyl, biphenyl, or naphthyl. Examples of suitable arylalkyl groups include optionally substituted benzyl (*e.g.,* p–methoxybenzyl (MPM), 3,4–dimethoxybenzyl, O–nitrobenzyl, p–nitrobenzyl, p–halobenzyl, 2,6–dichlorobenzyl, p–cyanobenzyl), and 2– and 4–picolyl.

[0048] Suitable hydroxyl protecting groups include methyl, methoxylmethyl (MOM), methylthiomethyl (MTM), *t*–butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), *p*–methoxybenzyloxymethyl (PMBM), (4–methoxyphenoxy)methyl (*p*–AOM), guaiacolmethyl (GUM), *t*–butoxymethyl, 4–pentenyloxymethyl (POM), siloxymethyl, 2–methoxyethoxymethyl (MEM), 2,2,2–trichloroethoxymethyl, bis(2–chloroethoxy)methyl, 2–(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3–bromotetrahydropyranyl, tetrahydrothiopyranyl, 1–methoxycyclohexyl, 4–methoxytetrahydropyranyl (MTHP), 4–methoxytetrahydrothiopyranyl, 4–methoxytetrahydrothiopyranyl S,S–dioxide, 1–[(2–chloro–4–methyl)phenyl]–4–

methoxypiperidin–4–yl (CTMP), 1,4–dioxan–2–yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a–octahydro–7,8,8–trimethyl–4,7–methanobenzofuran–2–yl, 1–ethoxyethyl, 1–(2–chloroethoxy)ethyl, 1–methyl–1–methoxyethyl, 1–methyl–1–benzyloxyethyl, 1–methyl–1–benzyloxy–2–fluoroethyl, 2,2,2–trichloroethyl, 2–trimethylsilylethyl, 2–(phenylselenyl)ethyl, *t*–butyl, allyl, *p*–chlorophenyl, *p*–methoxyphenyl, 2,4–dinitrophenyl, benzyl, *p*–methoxybenzyl, 3,4–dimethoxybenzyl, *o*–nitrobenzyl, *p*–nitrobenzyl, *p*–halobenzyl, 2,6–dichlorobenzyl, *p*–cyanobenzyl, *p*–phenylbenzyl, 2–picolyl, 4–picolyl, 3–methyl–2–picolyl *N*–oxido, diphenylmethyl, *p,p'*–dinitrobenzhydryl, 5–dibenzosuberyl, triphenylmethyl, α–naphthyldiphenylmethyl, *p*–methoxyphenyldiphenylmethyl, di(*p*–methoxyphenyl)phenylmethyl, tri(*p*–methoxyphenyl)methyl, 4–(4'–bromophenacyloxyphenyl)diphenylmethyl, 4,4',4''–tris(4,5–dichlorophthalimidophenyl)methyl, 4,4',4''–tris(levulinoyloxyphenyl)methyl, 4,4',4''–tris(benzoyloxyphenyl)methyl, 3–(imidazol–1–yl)bis(4',4''–dimethoxyphenyl)methyl, 1,1–bis(4–methoxyphenyl)–1'–pyrenylmethyl, 9–anthryl, 9–(9–phenyl)xanthenyl, 9–(9–phenyl–10–oxo)anthryl, 1,3–benzodithiolan–2–yl, benzisothiazolyl S,S–dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylthexylsilyl, *t*–butyldimethylsilyl (TBDMS), *t*–butyldiphenylsilyl (TBDPS), tribenzylsilyl, tri–*p*–xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), *t*–butylmethoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, *p*–chlorophenoxyacetate, 3–phenylpropionate, 4–oxopentanoate (levulinate), 4,4–(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantoate, crotonate, 4–methoxycrotonate, benzoate, *p*–phenylbenzoate, 2,4,6–trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9–fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2–trichloroethyl carbonate (Troc), 2–(trimethylsilyl)ethyl carbonate (TMSEC), 2–(phenylsulfonyl) ethyl carbonate (Psec), 2–(triphenylphosphonio) ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate alkyl allyl carbonate, alkyl *p*–nitrophenyl carbonate, alkyl benzyl carbonate, alkyl *p*–methoxybenzyl carbonate, alkyl 3,4–dimethoxybenzyl carbonate, alkyl *o*–nitrobenzyl carbonate, alkyl *p*–nitrobenzyl carbonate, alkyl *S*–benzyl thiocarbonate, 4–ethoxy–1–napththyl carbonate, methyl dithiocarbonate, 2–iodobenzoate, 4–azidobutyrate, 4–nitro–4–methylpentanoate, *o*–(dibromomethyl)benzoate, 2–formylbenzenesulfonate, 2–(methylthiomethoxy)ethyl, 4–(methylthiomethoxy)butyrate, 2–

(methylthiomethoxymethyl)benzoate, 2,6–dichloro–4–methylphenoxyacetate, 2,6–dichloro–4–(1,1,3,3–tetramethylbutyl)phenoxyacetate, 2,4–bis(1,1–dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (*E*)–2–methyl–2–butenoate, *o*–(methoxycarbonyl)benzoate, α–naphthoate, nitrate, alkyl *N,N,N',N'*–tetramethylphosphorodiamidate, alkyl *N*–phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4–dinitrophenylsulfenate, sulfate, methanesulfonate (mesylate), benzylsulfonate, and tosylate (Ts). For protecting 1,2– or 1,3–diols, the protecting groups include methylene acetal, ethylidene acetal, 1–*t*–butylethylidene ketal, 1–phenylethylidene ketal, (4–methoxyphenyl)ethylidene acetal, 2,2,2–trichloroethylidene acetal, acetonide, cyclopentylidene ketal, cyclohexylidene ketal, cycloheptylidene ketal, benzylidene acetal, *p*–methoxybenzylidene acetal, 2,4–dimethoxybenzylidene ketal, 3,4–dimethoxybenzylidene acetal, 2–nitrobenzylidene acetal, methoxymethylene acetal, ethoxymethylene acetal, dimethoxymethylene ortho ester, 1–methoxyethylidene ortho ester, 1–ethoxyethylidine ortho ester, 1,2–dimethoxyethylidene ortho ester, α–methoxybenzylidene ortho ester, 1–(*N,N*–dimethylamino)ethylidene derivative, α–(*N,N'*–dimethylamino)benzylidene derivative, 2–oxacyclopentylidene ortho ester, di–*t*–butylsilylene group (DTBS), 1,3–(1,1,3,3–tetraisopropyldisiloxanylidene) derivative (TIPDS), tetra–*t*–butoxydisiloxane–1,3–diylidene derivative (TBDS), cyclic carbonates, cyclic boronates, ethyl boronate, and phenyl boronate.

[0049] In some embodiments, a hydroxyl protecting group is acetyl, t-butyl, t-butoxymethyl, methoxymethyl, tetrahydropyranyl, 1 -ethoxyethyl, 1 -(2-chloroethoxy)ethyl, 2-trimethylsilylethyl, p-chlorophenyl, 2,4-dinitrophenyl, benzyl, benzoyl, p-phenylbenzoyl, 2,6-dichlorobenzyl, diphenylmethyl, p-nitrobenzyl, triphenylmethyl (trityl), 4,4'-dimethoxytrityl, trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triphenylsilyl, triisopropylsilyl, benzoylformate, chloroacetyl, trichloroacetyl, trifiuoroacetyl, pivaloyl, 9-fluorenylmethyl carbonate, mesylate, tosylate, triflate, trityl, monomethoxytrityl (MMTr), 4,4'-dimethoxytrityl, (DMTr) and 4,4',4"-trimethoxytrityl (TMTr), 2-cyanoethyl (CE or Cne), 2-(trimethylsilyl)ethyl (TSE), 2-(2-nitrophenyl)ethyl, 2-(4-cyanophenyl)ethyl 2-(4-nitrophenyl)ethyl (NPE), 2-(4-nitrophenylsulfonyl)ethyl, 3,5-dichlorophenyl, 2,4-dimethylphenyl, 2-nitrophenyl, 4-nitrophenyl, 2,4,6-trimethylphenyl, 2-(2-nitrophenyl)ethyl, butylthiocarbonyl, 4,4',4"-tris(benzoyloxy)trityl, diphenylcarbamoyl, levulinyl, 2-(dibromomethyl)benzoyl (Dbmb), 2-(isopropylthiomethoxymethyl)benzoyl (Ptmt), 9-

phenylxanthen-9-yl (pixyl) or 9-(p-methoxyphenyl)xanthine-9-y1 (MOX). In some embodiments, each of the hydroxyl protecting groups is, independently selected from acetyl, benzyl, t- butyldimethylsilyl, t-butyldiphenylsilyl and 4,4'-dimethoxytrityl. In some embodiments, the hydroxyl protecting group is selected from the group consisting of trityl, monomethoxytrityl and 4,4'-dimethoxytrityl group.

[0050] In some embodiments, a phosphorous protecting group is a group attached to the internucleotide phosphorous linkage throughout oligonucleotide synthesis. In some embodiments, the phosphorous protecting group is attached to the sulfur atom of the internucleotide phosphorothioate linkage. In some embodiments, the phosphorous protecting group is attached to the oxygen atom of the internucleotide phosphorothioate linkage. In some embodiments, the phosphorous protecting group is attached to the oxygen atom of the internucleotide phosphate linkage. In some embodiments the phosphorous protecting group is 2-cyanoethyl (CE or Cne), 2-trimethylsilylethyl, 2-nitroethyl, 2-sulfonylethyl, methyl, benzyl, *o*-nitrobenzyl, 2-(*p*-nitrophenyl)ethyl (NPE or Npe), 2-phenylethyl, 3-(*N*-*tert*-butylcarboxamido)-1-propyl, 4-oxopentyl, 4-methylthio-l-butyl, 2-cyano-1,1-dimethylethyl, 4-*N*-methylaminobutyl, 3-(2-pyridyl)-1-propyl, 2-[*N*-methyl-*N*-(2-pyridyl)]aminoethyl, 2-(*N*-formyl,*N*-methyl)aminoethyl, 4-[*N*-methyl-*N*-(2,2,2-trifluoroacetyl)amino]butyl.

[0051] *Protein:* As used herein, the term "protein" refers to a polypeptide (*i.e.*, a string of at least two amino acids linked to one another by peptide bonds). In some embodiments, proteins include only naturally-occurring amino acids. In some embodiments, proteins include one or more non-naturally-occurring amino acids (e.g., moieties that form one or more peptide bonds with adjacent amino acids). In some embodiments, one or more residues in a protein chain contain a non-amino-acid moiety (e.g., a glycan, etc). In some embodiments, a protein includes more than one polypeptide chain, for example linked by one or more disulfide bonds or associated by other means. In some embodiments, proteins contain L-amino acids, D-amino acids, or both; in some embodiments, proteins contain one or more amino acid modifications or analogs known in the art. Useful modifications include, *e.g.*, terminal acetylation, amidation, methylation, *etc.* The term "peptide" is generally used to refer to a polypeptide having a length of less than about 100 amino acids, less than about 50 amino acids, less than 20 amino acids, or less than 10 amino acids. In some embodiments, proteins are antibodies, antibody fragments, biologically active portions thereof, and/or characteristic portions thereof.

[0052] *Sample*: A "sample" as used herein is a specific organism or material obtained therefrom. In some embodiments, a sample is a biological sample obtained or derived from a source of interest, as described herein, . In some embodiments, a source of interest comprises an organism, such as an animal or human. In some embodiments, a biological sample comprises biological tissue or fluid. In some embodiments, a biological sample is or comprises bone marrow; blood; blood cells; ascites; tissue or fine needle biopsy samples; cell-containing body fluids; free floating nucleic acids; sputum; saliva; urine; cerebrospinal fluid, peritoneal fluid; pleural fluid; feces; lymph; gynecological fluids; skin swabs; vaginal swabs; oral swabs; nasal swabs; washings or lavages such as a ductal lavages or broncheoalveolar lavages; aspirates; scrapings; bone marrow specimens; tissue biopsy specimens; surgical specimens; feces, other body fluids, secretions, and/or excretions; and/or cells therefrom, *etc*. In some embodiments, a biological sample is or comprises cells obtained from an individual. In some embodiments, a sample is a "primary sample" obtained directly from a source of interest by any appropriate means. For example, in some embodiments, a primary biological sample is obtained by methods selected from the group consisting of biopsy (*e.g.*, fine needle aspiration or tissue biopsy), surgery, collection of body fluid (*e.g.*, blood, lymph, feces *etc.*), *etc.* In some embodiments, as will be clear from context, the term "sample" refers to a preparation that is obtained by processing (e.g., by removing one or more components of and/or by adding one or more agents to) a primary sample. For example, filtering using a semi-permeable membrane. Such a "processed sample" may comprise, for example nucleic acids or proteins extracted from a sample or obtained by subjecting a primary sample to techniques such as amplification or reverse transcription of mRNA, isolation and/or purification of certain components, etc. In some embodiments, a sample is an organism. In some embodiments, a sample is a plant. In some embodiments, a sample is an animal. In some embodiments, a sample is a human. In some embodiments, a sample is an organism other than a human.

[0053] *Stereochemically isomeric forms:* The phrase "stereochemically isomeric forms," as used herein, refers to different compounds made up of the same atoms bonded by the same sequence of bonds but having different three-dimensional structures which are not interchangeable. In some embodiments of the invention, provided chemical compositions may be or include pure preparations of individual stereochemically isomeric forms of a compound; in some embodiments, provided chemical compositions may be or include mixtures of two or more

stereochemically isomeric forms of the compound. In certain embodiments, such mixtures contain equal amounts of different stereochemically isomeric forms; in certain embodiments, such mixtures contain different amounts of at least two different stereochemically isomeric forms. In some embodiments, a chemical composition may contain all diastereomers and/or enantiomers of the compound. In some embodiments, a chemical composition may contain less than all diastereomers and/or enantiomers of a compound. In some embodiments, if a particular enantiomer of a compound of the present invention is desired, it may be prepared, for example, by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, diastereomeric salts are formed with an appropriate optically-active acid, and resolved, for example, by fractional crystallization.

[0054] *Subject:* As used herein, the term "subject" or "test subject" refers to any organism to which a provided compound or composition is administered in accordance with the present invention *e.g.*, for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and humans; insects; worms; *etc.*) and plants. In some embodiments, a subject may be suffering from, and/or susceptible to a disease, disorder, and/or condition.

[0055] *Substantially:* As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and/or chemical phenomena.

[0056] *Suffering from*: An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with and/or displays one or more symptoms of a disease, disorder, and/or condition.

[0057] *Susceptible to*: An individual who is "susceptible to" a disease, disorder, and/or condition is one who has a higher risk of developing the disease, disorder, and/or condition than does a member of the general public. In some embodiments, an individual who is susceptible to a disease, disorder and/or condition may not have been diagnosed with the disease, disorder,

and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may not exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[0058] *Systemic:* The phrases "systemic administration," "administered systemically," "peripheral administration," and "administered peripherally" as used herein have their art-understood meaning referring to administration of a compound or composition such that it enters the recipient's system.

[0059] *Tautomeric forms:* The phrase "tautomeric forms," as used herein, is used to describe different isomeric forms of organic compounds that are capable of facile interconversion. Tautomers may be characterized by the formal migration of a hydrogen atom or proton, accompanied by a switch of a single bond and adjacent double bond. In some embodiments, tautomers may result from prototropic tautomerism (*i.e.*, the relocation of a proton). In some embodiments, tautomers may result from valence tautomerism (*i.e.*, the rapid reorganization of bonding electrons). All such tautomeric forms are intended to be included within the scope of the present invention. In some embodiments, tautomeric forms of a compound exist in mobile equilibrium with each other, so that attempts to prepare the separate substances results in the formation of a mixture. In some embodiments, tautomeric forms of a compound are separable and isolatable compounds. In some embodiments of the invention, chemical compositions may be provided that are or include pure preparations of a single tautomeric form of a compound. In some embodiments of the invention, chemical compositions may be provided as mixtures of two or more tautomeric forms of a compound. In certain embodiments, such mixtures contain equal amounts of different tautomeric forms; in certain embodiments, such mixtures contain different amounts of at least two different tautomeric forms of a compound. In some embodiments of the invention, chemical compositions may contain all tautomeric forms of a compound. In some embodiments of the invention, chemical compositions may contain less than all tautomeric forms of a compound. In some embodiments of the invention, chemical compositions may contain one or more tautomeric forms of a compound in

amounts that vary over time as a result of interconversion. In some embodiments of the invention, the tautomerism is keto-enol tautomerism. One of skill in the chemical arts would recognize that a keto-enol tautomer can be "trapped" (i.e., chemically modified such that it remains in the "enol" form) using any suitable reagent known in the chemical arts in to provide an enol derivative that may subsequently be isolated using one or more suitable techniques known in the art. Unless otherwise indicated, the present invention encompasses all tautomeric forms of relevant compounds, whether in pure form or in admixture with one another.

[0060] *Therapeutic agent:* As used herein, the phrase "therapeutic agent" refers to any agent that, when administered to a subject, has a therapeutic effect and/or elicits a desired biological and/or pharmacological effect. In some embodiments, a therapeutic agent is any substance that can be used to alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition.

[0061] *Therapeutically effective amount:* As used herein, the term "therapeutically effective amount" means an amount of a substance (*e.g.*, a therapeutic agent, composition, and/or formulation) that elicits a desired biological response when administered as part of a therapeutic regimen. In some embodiments, a therapeutically effective amount of a substance is an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition. As will be appreciated by those of ordinary skill in this art, the effective amount of a substance may vary depending on such factors as the desired biological endpoint, the substance to be delivered, the target cell or tissue, *etc.* For example, the effective amount of compound in a formulation to treat a disease, disorder, and/or condition is the amount that alleviates, ameliorates, relieves, inhibits, prevents, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of the disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is administered in a single dose; in some embodiments, multiple unit doses are required to deliver a therapeutically effective amount.

[0062] *Treat:* As used herein, the term "treat," "treatment," or "treating" refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease,

disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition. In some embodiments, treatment may be administered to a subject who exhibits only early signs of the disease, disorder, and/or condition, for example for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[0063] *Unsaturated:* The term "unsaturated," as used herein, means that a moiety has one or more units of unsaturation.

[0064] *Unit dose*: The expression “unit dose” as used herein refers to an amount administered as a single dose and/or in a physically discrete unit of a pharmaceutical composition. In many embodiments, a unit dose contains a predetermined quantity of an active agent. In some embodiments, a unit dose contains an entire single dose of the agent. In some embodiments, more than one unit dose is administered to achieve a total single dose. In some embodiments, administration of multiple unit doses is required, or expected to be required, in order to achieve an intended effect. A unit dose may be, for example, a volume of liquid (e.g., an acceptable carrier) containing a predetermined quantity of one or more therapeutic agents, a predetermined amount of one or more therapeutic agents in solid form, a sustained release formulation or drug delivery device containing a predetermined amount of one or more therapeutic agents, etc. It will be appreciated that a unit dose may be present in a formulation that includes any of a variety of components in addition to the therapeutic agent(s). For example, acceptable carriers (e.g., pharmaceutically acceptable carriers), diluents, stabilizers, buffers, preservatives, etc., may be included as described infra. It will be appreciated by those skilled in the art, in many embodiments, a total appropriate daily dosage of a particular therapeutic agent may comprise a portion, or a plurality, of unit doses, and may be decided, for example, by the attending physician within the scope of sound medical judgment. In some embodiments, the specific effective dose level for any particular subject or organism may depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of specific active compound employed; specific composition employed; age, body weight, general health, sex and diet of the subject; time of administration, and rate of excretion of the specific active compound employed; duration of the treatment; drugs and/or additional therapies used in combination or coincidental with specific compound(s) employed, and like factors well known in the medical arts.

[0065] *Wild-type:* As used herein, the term "wild-type" has its art-understood meaning that refers to an entity having a structure and/or activity as found in nature in a "normal" (as contrasted with mutant, diseased, altered, etc) state or context. Those of ordinary skill in the art will appreciate that wild type genes and polypeptides often exist in multiple different forms (e.g., alleles).

[0066] *Nucleic acid*: The term "nucleic acid" includes any nucleotides, analogs thereof, and polymers thereof. The term "polynucleotide" as used herein refer to a polymeric form of nucleotides of any length, either ribonucleotides (RNA) or deoxyribonucleotides (DNA). These terms refer to the primary structure of the molecules and, thus, include double- and single-stranded DNA, and double- and single-stranded RNA. These terms include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs and modified polynucleotides such as, though not limited to, methylated, protected and/or capped nucleotides or polynucleotides. The terms encompass poly- or oligo-ribonucleotides (RNA) and poly- or oligo-deoxyribonucleotides (DNA); RNA or DNA derived from N-glycosides or C-glycosides of nucleobases and/or modified nucleobases; nucleic acids derived from sugars and/or modified sugars; and nucleic acids derived from phosphate bridges and/or modified phosphorus-atom bridges (also referred to herein as "internucleotide linkages"). The term encompasses nucleic acids containing any combinations of nucleobases, modified nucleobases, sugars, modified sugars, phosphate bridges or modified phosphorus atom bridges. Examples include, and are not limited to, nucleic acids containing ribose moieties, the nucleic acids containing deoxy-ribose moieties, nucleic acids containing both ribose and deoxyribose moieties, nucleic acids containing ribose and modified ribose moieties. The prefix poly- refers to a nucleic acid containing 2 to about 10,000 nucleotide monomer units and wherein the prefix oligo- refers to a nucleic acid containing 2 to about 200 nucleotide monomer units.

[0067] *Nucleotide:* The term "nucleotide" as used herein refers to a monomeric unit of a polynucleotide that consists of a heterocyclic base, a sugar, and one or more phosphate groups or phosphorus-containing internucleotidic linkages. The naturally occurring bases, (guanine, (G), adenine, (A), cytosine, (C), thymine, (T), and uracil (U)) are derivatives of purine or pyrimidine, though it should be understood that naturally and non-naturally occurring base analogs are also included. The naturally occurring sugar is the pentose (five-carbon sugar) deoxyribose (which forms DNA) or ribose (which forms RNA), though it should be understood that naturally and

non-naturally occurring sugar analogs are also included. Nucleotides are linked via internucleotidic linkages to form nucleic acids, or polynucleotides. Many internucleotidic linkages are known in the art (such as, though not limited to, phosphate, phosphorothioates, boranophosphates and the like). Artificial nucleic acids include PNAs (peptide nucleic acids), phosphotriesters, phosphorothionates, *H*-phosphonates, phosphoramidates, boranophosphates, methylphosphonates, phosphonoacetates, thiophosphonoacetates and other variants of the phosphate backbone of native nucleic acids, such as those described herein.

[0068] *Nucleoside*: The term "nucleoside" refers to a moiety wherein a nucleobase or a modified nucleobase is covalently bound to a sugar or modified sugar.

[0069] *Sugar:* The term "sugar" refers to a monosaccharide in closed and/or open form. Sugars include, but are not limited to, ribose, deoxyribose, pentofuranose, pentopyranose, and hexopyranose moieties. As used herein, the term also encompasses structural analogs used in lieu of conventional sugar molecules, such as glycol, polymer of which forms the backbone of the nucleic acid analog, glycol nucleic acid ("GNA").

[0070] *Modified sugar*: The term "modified sugar" refers to a moiety that can replace a sugar. The modified sugar mimics the spatial arrangement, electronic properties, or some other physicochemical property of a sugar.

[0071] *Nucleobase*: The term "nucleobase" refers to the parts of nucleic acids that are involved in the hydrogen-bonding that binds one nucleic acid strand to another complementary strand in a sequence specific manner. The most common naturally-occurring nucleobases are adenine (A), guanine (G), uracil (U), cytosine (C), and thymine (T). In some embodiments, the naturally-occurring nucleobases are modified adenine, guanine, uracil, cytosine, or thymine. In some embodiments, the naturally-occurring nucleobases are methylated adenine, guanine, uracil, cytosine, or thymine. In some embodiments, a nucleobase is a "modified nucleobase," e.g., a nucleobase other than adenine (A), guanine (G), uracil (U), cytosine (C), and thymine (T). In some embodiments, the modified nucleobases are methylated adenine, guanine, uracil, cytosine, or thymine. In some embodiments, the modified nucleobase mimics the spatial arrangement, electronic properties, or some other physicochemical property of the nucleobase and retains the property of hydrogen-bonding that binds one nucleic acid strand to another in a sequence specific manner. In some embodiments, a modified nucleobase can pair with all of the five naturally occurring bases (uracil, thymine, adenine, cytosine, or guanine) without substantially affecting

the melting behavior, recognition by intracellular enzymes or activity of the oligonucleotide duplex.

[0072] *Chiral ligand*: The term "chiral ligand" or "chiral auxiliary" refers to a moiety that is chiral and can be incorporated into a reaction so that the reaction can be carried out with certain stereoselectivity.

[0073] *Condensing reagent*: In a condensation reaction, the term "condensing reagent" refers to a reagent that activates a less reactive site and renders it more susceptible to attack by another reagent. In some embodiments, such another reagent is a nucleophile.

[0074] *Blocking group*: The term "blocking group" refers to a group that masks the reactivity of a functional group. The functional group can be subsequently unmasked by removal of the blocking group. In some embodiments, a blocking group is a protecting group.

[0075] *Moiety*: The term "moiety" refers to a specific segment or functional group of a molecule. Chemical moieties are often recognized chemical entities embedded in or appended to a molecule.

[0076] *Solid support*: The term "solid support" refers to any support which enables synthesis of nucleic acids. In some embodiments, the term refers to a glass or a polymer, that is insoluble in the media employed in the reaction steps performed to synthesize nucleic acids, and is derivatized to comprise reactive groups. In some embodiments, the solid support is Highly Cross-linked Polystyrene (HCP) or Controlled Pore Glass (CPG). In some embodiments, the solid support is Controlled Pore Glass (CPG). In some embodiments, the solid support is hybrid support of Controlled Pore Glass (CPG) and Highly Cross-linked Polystyrene (HCP).

[0077] *Linking moiety*: The term "linking moiety" refers to any moiety optionally positioned between the terminal nucleoside and the solid support or between the terminal nucleoside and another nucleoside, nucleotide, or nucleic acid.

[0078] *DNA molecule*: A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences can be described herein according to the normal convention of giving only

the sequence in the 5’ to 3’ direction along the non-transcribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

[0079] *Coding sequence*: A DNA "coding sequence" or “coding region” is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate expression control sequences. The boundaries of the coding sequence (the “open reading frame” or “ORF”) are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and synthetic DNA sequences. A polyadenylation signal and transcription termination sequence is, usually, be located 3' to the coding sequence. The term “non-coding sequence” or “non-coding region” refers to regions of a polynucleotide sequence that are not translated into amino acids (e.g. 5′ and 3′ un-translated regions).

[0080] *Reading frame*: The term “reading frame” refers to one of the six possible reading frames, three in each direction, of the double stranded DNA molecule. The reading frame that is used determines which codons are used to encode amino acids within the coding sequence of a DNA molecule.

[0081] *Antisense*: As used herein, an "antisense" nucleic acid molecule comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule, complementary to an mRNA sequence or complementary to the coding strand of a gene. Accordingly, an antisense nucleic acid molecule can associate via hydrogen bonds to a sense nucleic acid molecule.

[0082] *Wobble position*: As used herein, a “wobble position” refers to the third position of a codon. Mutations in a DNA molecule within the wobble position of a codon, in some embodiments, result in silent or conservative mutations at the amino acid level. For example, there are four codons that encode Glycine, i.e., GGU, GGC, GGA and GGG, thus mutation of any wobble position nucleotide, to any other nucleotide selected from A, U , C and G, does not result in a change at the amino acid level of the encoded protein and, therefore, is a silent substitution.

[0083] *Silent substitution*: a “silent substitution” or “silent mutation” is one in which a nucleotide within a codon is modified, but does not result in a change in the amino acid residue

encoded by the codon. Examples include mutations in the third position of a codon, as well in the first position of certain codons such as in the codon "CGG" which, when mutated to AGG, still encodes Arg.

[0084] *Gene*: The terms "gene," "recombinant gene" and "gene construct" as used herein, refer to a DNA molecule, or portion of a DNA molecule, that encodes a protein or a portion thereof. The DNA molecule can contain an open reading frame encoding the protein (as exon sequences) and can further include intron sequences. The term "intron" as used herein, refers to a DNA sequence present in a given gene which is not translated into protein and is found in some, but not all cases, between exons. It can be desirable for the gene to be operably linked to, (or it can comprise), one or more promoters, enhancers, repressors and/or other regulatory sequences to modulate the activity or expression of the gene, as is well known in the art.

[0085] *Complementary DNA*: As used herein, a "complementary DNA" or "cDNA" includes recombinant polynucleotides synthesized by reverse transcription of mRNA and from which intervening sequences (introns) have been removed.

[0086] *Homology*: "Homology" or "identity" or "similarity" refers to sequence similarity between two nucleic acid molecules. Homology and identity can each be determined by comparing a position in each sequence which can be aligned for purposes of comparison. When an equivalent position in the compared sequences is occupied by the same base, then the molecules are identical at that position; when the equivalent site occupied by the same or a similar nucleic acid residue (e.g., similar in steric and/or electronic nature), then the molecules can be referred to as homologous (similar) at that position. Expression as a percentage of homology/similarity or identity refers to a function of the number of identical or similar nucleic acids at positions shared by the compared sequences. A sequence which is "unrelated" or "non-homologous" shares less than 40% identity, less than 35% identity, less than 30% identity, or less than 25% identity with a sequence described herein. In comparing two sequences, the absence of residues (amino acids or nucleic acids) or presence of extra residues also decreases the identity and homology/similarity.

[0087] In some embodiments, the term "homology" describes a mathematically based comparison of sequence similarities which is used to identify genes with similar functions or motifs. The nucleic acid sequences described herein can be used as a "query sequence" to

perform a search against public databases, for example, to identify other family members, related sequences or homologs. In some embodiments, such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. In some embodiments, BLAST nucleotide searches can be performed with the NBLAST program, score=100, wordlength=12 to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. In some embodiments, to obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and BLAST) can be used (See www.ncbi.nlm.nih.gov).

[0088] *Identity*: As used herein, "identity" means the percentage of identical nucleotide residues at corresponding positions in two or more sequences when the sequences are aligned to maximize sequence matching, i.e., taking into account gaps and insertions. Identity can be readily calculated by known methods, including but not limited to those described in (Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48: 1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., Nucleic Acids Research 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Altschul, S. F. et al., J. Molec. Biol. 215: 403-410 (1990) and Altschul et al. Nuc. Acids Res. 25: 3389-3402 (1997)). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, Md. 20894; Altschul, S., et al., J. Mol. Biol. 215: 403-410 (1990). The well-known Smith Waterman algorithm can also be used to determine identity.

[0089] *Heterologous*: A "heterologous" region of a DNA sequence is an identifiable segment of DNA within a larger DNA sequence that is not found in association with the larger sequence in nature. Thus, when the heterologous region encodes a mammalian gene, the gene can usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example of a heterologous coding sequence is a sequence where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns or synthetic sequences having codons or motifs different than the unmodified gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

[0090] *Transition mutation:* The term "transition mutations" refers to base changes in a DNA sequence in which a pyrimidine (cytidine (C) or thymidine (T) is replaced by another pyrimidine, or a purine (adenosine (A) or guanosine (G) is replaced by another purine.

[0091] *Transversion mutation*: The term "transversion mutations" refers to base changes in a DNA sequence in which a pyrimidine (cytidine (C) or thymidine (T) is replaced by a purine (adenosine (A) or guanosine (G), or a purine is replaced by a pyrimidine.

[0092] *Oligonucleotide:* the term "oligonucleotide" refers to a polymer or oligomer of nucleotide monomers, containing any combination of nucleobases, modified nucleobases, sugars, modified sugars, phosphate bridges, or modified phosphorus atom bridges (also referred to herein as "internucleotidic linkage", defined further herein).

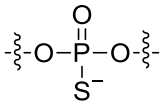
[0093] Oligonucleotides can be single-stranded or double-stranded. As used herein, the term "oligonucleotide strand" encompasses a single-stranded oligonucleotide. A single-stranded oligonucleotide can have double-stranded regions and a double-stranded oligonucleotide can have single-stranded regions. Exemplary oligonucleotides include, but are not limited to structural genes, genes including control and termination regions, self-replicating systems such as viral or plasmid DNA, single-stranded and double-stranded siRNAs and other RNA interference reagents (RNAi agents or iRNA agents), shRNA, antisense oligonucleotides, ribozymes, microRNAs, microRNA mimics, supermirs, aptamers, antimirs, antagomirs, Ul adaptors, triplex-forming oligonucleotides, G-quadruplex oligonucleotides, RNA activators, immuno-stimulatory oligonucleotides, and decoy oligonucleotides.

[0094] Double-stranded and single-stranded oligonucleotides that are effective in inducing RNA interference are also referred to as siRNA, RNAi agent, or iRNA agent, herein.

In some embodiments, these RNA interference inducing oligonucleotides associate with a cytoplasmic multi-protein complex known as RNAi-induced silencing complex (RISC). In many embodiments, single-stranded and double-stranded RNAi agents are sufficiently long that they can be cleaved by an endogenous molecule, e.g., by Dicer, to produce smaller oligonucleotides that can enter the RISC machinery and participate in RISC mediated cleavage of a target sequence, e.g. a target mRNA.

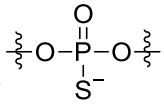
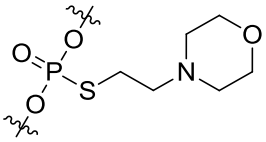
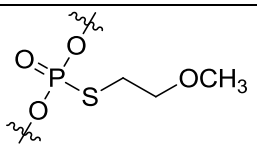
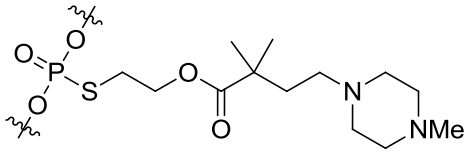
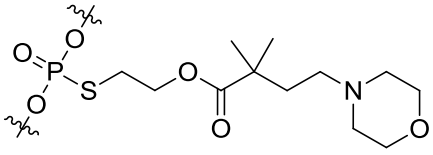
[0095] Oligonucleotides of the present invention can be of various lengths. In particular embodiments, oligonucleotides can range from about 2 to about 200 nucleotides in length. In various related embodiments, oligonucleotides, single-stranded, double-stranded, and triple-stranded, can range in length from about 4 to about 10 nucleotides, from about 10 to about 50 nucleotides, from about 20 to about 50 nucleotides, from about 15 to about 30 nucleotides, from about 20 to about 30 nucleotides in length. In some embodiments, the oligonucleotide is from about 9 to about 39 nucleotides in length. In some embodiments, the oligonucleotide is at least 4 nucleotides in length. In some embodiments, the oligonucleotide is at least 5 nucleotides in length. In some embodiments, the oligonucleotide is at least 6 nucleotides in length. In some embodiments, the oligonucleotide is at least 7 nucleotides in length. In some embodiments, the oligonucleotide is at least 8 nucleotides in length. In some embodiments, the oligonucleotide is at least 9 nucleotides in length. In some embodiments, the oligonucleotide is at least 10 nucleotides in length. In some embodiments, the oligonucleotide is at least 11 nucleotides in length. In some embodiments, the oligonucleotide is at least 12 nucleotides in length. In some embodiments, the oligonucleotide is at least 15 nucleotides in length. In some embodiments, the oligonucleotide is at least 20 nucleotides in length. In some embodiments, the oligonucleotide is at least 25 nucleotides in length. In some embodiments, the oligonucleotide is at least 30 nucleotides in length. In some embodiments, the oligonucleotide is a duplex of complementary strands of at least 18 nucleotides in length. In some embodiments, the oligonucleotide is a duplex of complementary strands of at least 21 nucleotides in length.

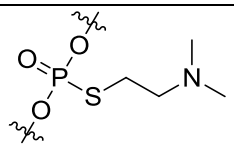
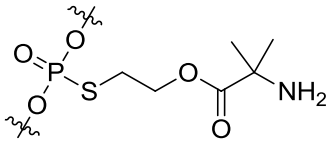
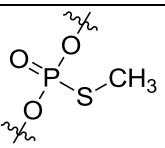
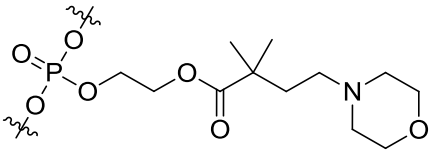
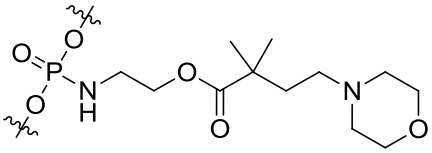
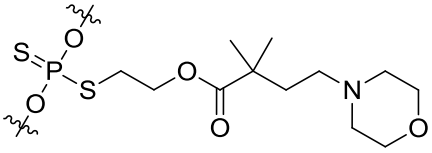
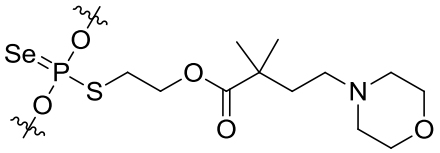
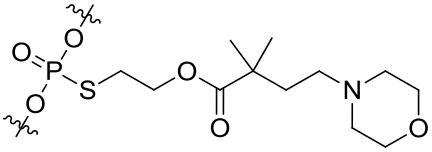
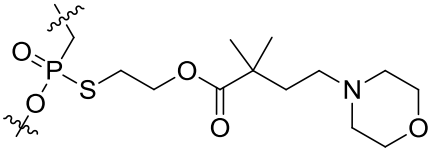
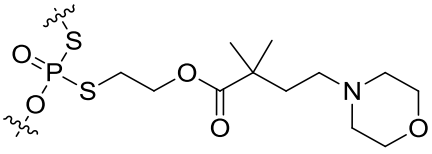
[0096] *Internucleotidic linkage:* As used herein, the phrase "internucleotidic linkage" refers generally to the phosphorus-containing linkage between nucleotide units of an oligonucleotide, and is interchangeable with "inter-sugar linkage" and "phosphorus atom bridge," as used above and herein. In some embodiments, an internucleotidic linkage is a phosphodiester linkage, as found in naturally occurring DNA and RNA molecules. In some

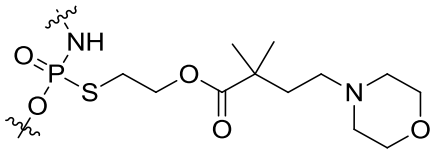
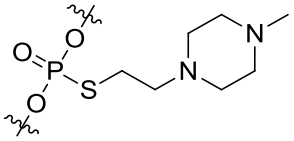
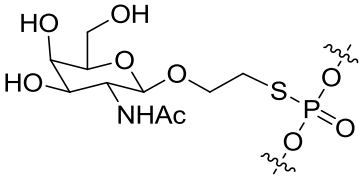
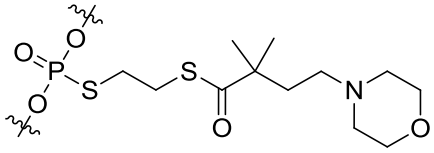
embodiments, an internucleotidic linkage is a "modified internucleotidic linkage" wherein each oxygen atom of the phosphodiester linkage is optionally and independently replaced by an organic or inorganic moiety. In some embodiments, such an organic or inorganic moiety is selected from but not limited to =S, =Se, =NR', –SR', –SeR', $–N(R')_2$, $B(R')_3$, –S–, –Se–, and –N(R')–, wherein each R' is independently as defined and described below. In some embodiments, an internucleotidic linkage is a phosphotriester linkage, phosphorothioate diester linkage (), or modified phosphorothioate triester linkage. It is understood by a person of ordinary skill in the art that the internucleotidic linkage may exist as an anion or cation at a given pH due to the existence of acid or base moieties in the linkage.

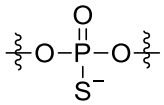
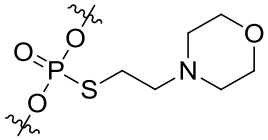
[0097] Unless otherwise specified, when used with an oligonucleotide sequence, each of s, s1, s2, s3, s4, s5, s6 and s7 independently represents the following modified internucleotidic linkage as illustrated in Table 1, below.

[0098] Table 1. Exemplary Modified Internucleotidic Linkage.

Symbol	Modified Internucleotidic Linkage
s	phosphorothioate ()
s1	
s2	
s3	
s4	

s5	
s6	
s7	
s8	
s9	
s10	
s11	
s12	
s13	
s14	

s15	
s16	
s17	
s18	

[0099] For instance, (*R*p, *S*p)–ATsCs1GA has 1) a phosphorothioate internucleotidic linkage () between T and C; and 2) a phosphorothioate triester internucleotidic linkage having the structure of  between C and G. Unless otherwise specified, the *R*p/*S*p designations preceding an oligonucleotide sequence describe the configurations of chiral linkage phosphorus atoms in the internucleotidic linkages sequentially from 5' to 3' of the oligonucleotide sequence. For instance, in (*R*p, *S*p)–ATsCs1GA, the phosphorus in the "s" linkage between T and C has *R*p configuration and the phosphorus in "s1" linkage between C and G has *S*p configuration. In some embodiments, "All-(*R*p)" or "All-(*S*p)" is used to indicate that all chiral linkage phosphorus atoms in oligonucleotide have the same *R*p or *S*p configuration, respectively. For instance, All-(*R*p)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC indicates that all the chiral linkage phosphorus atoms in the oligonucleotide have *R*p configuration; All-(*S*p)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC indicates that all the chiral linkage phosphorus atoms in the oligonucleotide have *S*p configuration.

[00100] *Oligonucleotide type:* As used herein, the phrase "oligonucleotide type" is used to define an oligonucleotide that has a particular base sequence, pattern of backbone linkages (i.e., pattern of internucleotidic linkage types, for example, phosphate, phosphorothioate, etc), pattern of backbone chiral centers (i.e. pattern of linkage phosphorus stereochemistry (*R*p/*S*p)), and pattern of backbone phosphorus modifications (e.g., pattern of "$-XLR^1$" groups in formula **I**). Oligonucleotides of a common designated "type" are structurally identical to one another.

[00101] One of skill in the art will appreciate that synthetic methods of the present invention provide for a degree of control during the synthesis of an oligonucleotide strand such that each nucleotide unit of the oligonucleotide strand can be designed and/or selected in advance to have a particular stereochemistry at the linkage phosphorus and/or a particular modification at the linkage phosphorus, and/or a particular base, and/or a particular sugar. In some embodiments, an oligonucleotide strand is designed and/or selected in advance to have a particular combination of stereocenters at the linkage phosphorus. In some embodiments, an oligonucleotide strand is designed and/or determined to have a particular combination of modifications at the linkage phosphorus. In some embodiments, an oligonucleotide strand is designed and/or selected to have a particular combination of bases. In some embodiments, an oligonucleotide strand is designed and/or selected to have a particular combination of one or more of the above structural characteristics. The present invention provides compositions comprising or consisting of a plurality of oligonucleotide molecules (e.g., chirally controlled oligonucleotide compositions). In some embodiments, all such molecules are of the same type (i.e., are structurally identical to one another). In many embodiments, however, provided compositions comprise a plurality of oligonucleotides of different types, typically in pre-determined relative amounts.

[00102] *Chiral control:* As used herein, "chiral control" refers to an ability to control the stereochemical designation of every chiral linkage phosphorus within an oligonucleotide strand. The phrase "chirally controlled oligonucleotide" refers to an oligonucleotide which exists in a single diastereomeric form with respect to the chiral linkage phosphorus.

[00103] *Chirally controlled oligonucleotide composition:* As used herein, the phrase "chirally controlled oligonucleotide composition" refers to an oligonucleotide composition that contains predetermined levels of individual oligonucleotide types. For instance, in some embodiments a chirally controlled oligonucleotide composition comprises one oligonucleotide

type. In some embodiments, a chirally controlled oligonucleotide composition comprises more than one oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide composition comprises a mixture of multiple oligonucleotide types. Exemplary chirally controlled oligonucleotide compositions are described further herein.

[00104] *Chirally pure:* as used herein, the phrase "chirally pure" is used to describe a chirally controlled oligonucleotide composition in which all of the oligonucleotides exist in a single diastereomeric form with respect to the linkage phosphorus.

[00105] *Chirally uniform:* as used herein, the phrase "chirally uniform" is used to describe an oligonucleotide molecule or type in which all nucleotide units have the same stereochemistry at the linkage phosphorus. For instance, an oligonucleotide whose nucleotide units all have *R*p stereochemistry at the linkage phosphorus is chirally uniform. Likewise, an oligonucleotide whose nucleotide units all have *S*p stereochemistry at the linkage phosphorus is chirally uniform.

[00106] *Predetermined:* By predetermined is meant deliberately selected, for example as opposed to randomly occurring or achieved. Those of ordinary skill in the art, reading the present specification, will appreciate that the present invention provides new and surprising technologies that permit selection of particular oligonucleotide types for preparation and/or inclusion in provided compositions, and further permits controlled preparation of precisely the selected particular types, optionally in selected particular relative amounts, so that provided compositions are prepared. Such provided compositions are "predetermined" as described herein. Compositions that may contain certain individual oligonucleotide types because they happen to have been generated through a process that cannot be controlled to intentionally generate the particular oligonucleotide types is not a "predetermined" composition. In some embodiments, a predetermined composition is one that can be intentionally reproduced (e.g., through repetition of a controlled process).

[00107] *Linkage phosphorus:* as defined herein, the phrase "linkage phosphorus" is used to indicate that the particular phosphorus atom being referred to is the phosphorus atom present in the internucleotidic linkage, which phosphorus atom corresponds to the phosphorus atom of a phosphodiester of an internucleotidic linkage as occurs in naturally occurring DNA and RNA. In some embodiments, a linkage phosphorus atom is in a modified internucleotidic linkage, wherein each oxygen atom of a phosphodiester linkage is optionally and independently replaced by an organic or inorganic moiety. In some embodiments, a linkage phosphorus atom is P* of formula

I. In some embodiments, a linkage phosphorus atom is chiral. In some embodiments, a chiral linkage phosphorus atom is P* of formula **I**.

[00108] *P-modification:* as used herein, the term "P-modification" refers to any modification at the linkage phosphorus other than a stereochemical modification. In some embodiments, a P-modification comprises addition, substitution, or removal of a pendant moiety covalently attached to a linkage phosphorus. In some embodiments, the "P-modification" is –X–L–R^1 wherein each of X, L and R^1 is independently as defined and described herein and below.

[00109] *Blockmer:* the term "blockmer," as used herein, refers to an oligonucleotide strand whose pattern of structural features characterizing each individual nucleotide unit is characterized by the presence of at least two consecutive nucleotide units sharing a common structural feature at the internucleotidic phosphorus linkage. By common structural feature is meant common stereochemistry at the linkage phosphorus or a common modification at the linkage phosphorus. In some embodiments, the at least two consecutive nucleotide units sharing a common structure feature at the internucleotidic phosphours linkage are referred to as a "block".

[00110] In some embodiments, a blockmer is a "stereoblockmer," e.g., at least two consecutive nucleotide units have the same stereochemistry at the linkage phosphorus. Such at lest two consecutive nucleotide units form a "stereoblock." For instance, (*S*p, *S*p)-ATsCs1GA is a stereoblockmer because at least two consecutive nucleotide units, the Ts and the Cs1, have the same stereochemistry at the linkage phosphorus (both *S*p). In the same oligonucleotide (*S*p, *S*p)-ATsCs1GA, TsCs1 forms a block, and it is a stereoblock.

[00111] In some embodiments, a blockmer is a "P-modification blockmer," e.g., at least two consecutive nucleotide units have the same modification at the linkage phosphorus. Such at lest two consecutive nucleotide units form a "P-modification block". For instance, (*R*p, *S*p)-ATsCsGA is a P-modification blockmer because at least two consecutive nucleotide units, the Ts and the Cs, have the same P-modification (i.e., both are a phosphorothioate diester). In the same oligonucleotide of (*R*p, *S*p)-ATsCsGA, TsCs forms a block, and it is a P-modification block.

[00112] In some embodiments, a blockmer is a "linkage blockmer," e.g., at least two consecutive nucleotide units have identical stereochemistry and identical modifications at the linkage phosphorus. At least two consecutive nucleotide units form a "linkage block". For instance, (*R*p, *R*p)-ATsCsGA is a linkage blockmer because at least two consecutive nucleotide

units, the Ts and the Cs, have the same stereochemistry (both *R*p) and P-modification (both phosphorothioate). In the same oligonucleotide of (*R*p, *R*p)-ATsCsGA, TsCs forms a block, and it is a linkage block.

[00113] In some embodiments, a blockmer comprises one or more blocks independently selected from a stereoblock, a P-modification block and a linkage block. In some embodiments, a blockmer is a stereoblockmer with respect to one block, and/or a P-modification blockmer with respect to another block, and/or a linkage blockmer with respect to yet another block. For instance, (*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p)–AAsTsCsGsAs1Ts1Cs1Gs1ATCG is a stereoblockmer with respect to the stereoblock AsTsCsGsAs1 (all *R*p at linkage phosphorus) or Ts1Cs1Gs1 (all *S*p at linkage phosphorus), a P-modification blockmer with respect to the P-modification block AsTsCsGs (all s linkage) or As1Ts1Cs1Gs1 (all s1 linkage), or a linkage blockmer with respect to the linkage block AsTsCsGs (all *R*p at linkage phosphorus and all s linkage) or Ts1Cs1Gs1 (all *S*p at linkage phosphorus and all s1 linkage).

[00114] *Altmer:* the term "altmer," as used herein, refers to an oligonucleotide strand whose pattern of structural features characterizing each individual nucleotide unit is characterized in that no two consecutive nucleotide units of the oligonucleotide strand share a particular structural feature at the internucleotidic phosphorus linkage. In some embodiments, an altmer is designed such that it comprises a repeating pattern. In some embodiments, an altmer is designed such that it does not comprise a repeating pattern.

[00115] In some embodiments, an altmer is a "stereoaltmer," e.g., no two consecutive nucleotide units have the same stereochemistry at the linkage phosphorus. For instance, (*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC.

[00116] In some embodiments, an altmer is a "P-modification altmer" e.g., no two consecutive nucleotide units have the same modification at the linkage phosphorus. For instance, All-(*S*p)-CAs1GsT, in which each linkage phosphorus has a different P-modification than the others.

[00117] In some embodiments, an altmer is a "linkage altmer," e.g., no two consecutive nucleotide units have identical stereochemistry or identical modifications at the linkage phosphorus. For instance, (*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-GsCs1CsTs1CsAs1GsTs1CsTs1GsCs1TsTs2CsGs3CsAs4CsC.

[00118] *Unimer:* the term "unimer," as used herein, refers to an oligonucleotide strand whose pattern of structural features characterizing each individual nucleotide unit is such that all nucleotide units within the strand share at least one common structural feature at the internucleotidic phosphorus linkage. By common structural feature is meant common stereochemistry at the linkage phosphorus or a common modification at the linkage phosphorus.

[00119] In some embodiments, a unimer is a "stereounimer," e.g., all nucleotide units have the same stereochemistry at the linkage phosphorus. For instance, All-(*S*p)-CsAs1GsT, in which all the linkages have *S*p phosphorus.

[00120] In some embodiments, a unimer is a "P-modification unimer", e.g., all nucleotide units have the same modification at the linkage phosphorus. For instance, (*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC, in which all the internucleotidic linkages are phosphorothioate diester.

[00121] In some embodiments, a unimer is a "linkage unimer," e.g., all nucleotide units have the same stereochemistry and the same modifications at the linkage phosphorus. For instance, All-(*S*p)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC, in which all the internucleotidic linkages are phosphorothioate diester having *S*p linkage phosphorus.

[00122] *Gapmer*: as used herein, the term "gapmer" refers to an oligonucleotide strand characterized in that at least one internucleotidic phosphorus linkage of the oligonucleotide strand is a phosphate diester linkage, for example such as those found in naturally occurring DNA or RNA. In some embodiments, more than one internucleotidic phosphorus linkage of the oligonucleotide strand is a phosphate diester linkage such as those found in naturally occurring DNA or RNA. For instance, All-(*S*p)-CAs1GsT, in which the internucleotidic linkage between C and A is a phosphate diester linkage.

[00123] *Skipmer*: as used herein, the term "skipmer" refers to a type of gapmer in which every other internucleotidic phosphorus linkage of the oligonucleotide strand is a phosphate diester linkage, for example such as those found in naturally occurring DNA or RNA, and every other internucleotidic phosphorus linkage of the oligonucleotide strand is a modified internucleotidic linkage. For instance, All-(*S*p)-AsTCs1GAs2TCs3G.

[00124] For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, *Handbook of Chemistry and Physics*, 67th Ed., 1986-87, inside cover.

[00125] The methods and structures described herein relating to compounds and compositions of the invention also apply to the pharmaceutically acceptable acid or base addition salts and all stereoisomeric forms of these compounds and compositions.

Brief Description of the Drawing

[00126] *Figure 1.* Reverse phase HPLCs after incubation with rat liver homogenate. Total amounts of oligonucleotides remaining when incubated with rat whole liver homogenate at 37°C at different days were measured. The *in-vitro* metabolic stability of ONT-154 was found to be similar to ONT-87 which has 2'-MOE wings while both have much better stability than 2'-MOE gapmer which is stereorandom (ONT-41, Mipomersen). The amount of full length oligomer remaining was measured by reverse phase HPLC where peak area of the peak of interest was normalized with internal standard.

[00127] *Figure 2.* Degradation of various chirally pure analogues of Mipomersen (ONT-41) in rat whole liver homogenate. Total amounts of oligonucleotide remaining when incubated with rat whole liver homogenate at 37°C at different days were measured. The *in-vitro* metabolic stability of chirally pure diastereomers of human ApoB sequence ONT-41 (Mipomersen) was found to increase with increased *S*p internucleotidic linkages. The amount of full length oligomer remaining was measured by reverse phase HPLC where peak area of the peak of interest was normalized with internal standard.

[00128] *Figure 3.* Degradation of various chirally pure analogues of mouse ApoB sequence (ISIS 147764, ONT-83) in rat whole liver homogenate. Total amounts of oligonucleotide remaining when incubated with rat whole liver homogenate at 37°C at different days were measured. The *in-vitro* metabolic stability of chirally pure diastereomers of murine ApoB sequence (ONT-83, 2'-MOE gapmer, stereorandom phosphorothioate) was found to increase with increased *S*p internucleotidic linkages. The amount of full length oligomer remaining was measured by reverse phase HPLC where peak area of the peak of interest was normalized with internal standard.

[00129] *Figure 4.* Degradation of Mipomersen analogue ONT-75 in rat whole liver homogenate over a period of 24hrs. This figure illustrates stability of ONT-75 in rate whole liver homogenate.

[00130] *Figure 5.* Degradation of Mipomersen analogue ONT-81 in rat whole liver homogenate over a period of 24hrs. This figure illustrates stability of ONT-81 in rate whole liver homogenate.

[00131] *Figure 6.* Durations of knockdown for ONT-87, ONT-88, and ONT-89. Stereoisomers can exhibit substantially different durations of knockdown. ONT-87 results in substantially more durable suppression than other stereoisomers. Increased duration of action of ONT-87 in multiple *in vivo* studies were observed. ONT-88 showed similar efficacy and recovery profile as ONT-41 (Mipomersen) in certain *in-vivo* studies. Hu ApoB transgenic mice, n=4, were dosed with 10 mpk IP bolus, 2X/week for three weeks. The mice were randomized to study groups, and dosed intraperitoneally (IP) at 10 mg/kg on Days 1, 4, 8, 11, 15, 18, and 22, based on individual mouse body weight measured prior to dosing on each dosing day. Blood was collected on days 0, 17, 24, 31, 38, 45 and 52 by submandibular (cheek) bleed and at sacrifice on Day 52 by cardiac puncture and then processed to serum. ApoB was measured by ELISA. Highlighted: 72% vs. 35% knock-down maintained at 3 weeks postdose.

[00132] *Figure 7.* HPLC profiles exhibiting the difference in metabolic stability determined in Human Serum for siRNA duplexes having several *R*p, *S*p or stereorandom phosphorothioate linkages.

[00133] *Figure 8.* Effect of stereochemistry on RNase H activity. Oligonucleotides were hybridized with RNA and then incubated with RNase H at 37°C in the presence of 1X RNase H buffer. From top to bottom at 120 min: ONT-89, ONT-77, ONT-81, ONT-80, ONT-75, ONT-41, ONT-88, ONT-154, ONT-87, with ONT-77/154 very close to each other.

[00134] *Figure 9.* Analysis of human RNase H1 cleavage of a 20-mer RNA when hybridized with different preparations of stereoisomers of phosphorothioate oligonucleotides targeting the same region of human ApoB mRNA. Specific sites of cleavage are strongly influenced by the distinct stereochemistries. Arrows represent position of cleavage (cleavage sites). Products were analyzed by UPLC/MS. The length of the arrow signifies the amount of products present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment (the larger the arrow, the more the detected

cleavage products). (A): Legend for cleavage maps. (B) and (C): cleavage maps of oligonucleotides.

[00135] *Figure 10.* Cleavage maps of different oligonucleotide compositions ((A)-(C)). These three sequences target different regions in FOXO1 mRNA. Each sequence was studied with five different chemistries. Cleavage maps are derived from reaction mixtures obtained after 30 minutes of incubation of respective duplexes with RNase H1C in the presence of 1XPBS buffer at 37°C. Arrows indicate sites of cleavage. (┬) indicates that both fragments, 5'-phosphate specie as well as 5'-OH 3'-OH specie were identified in reaction mixtures. (┌) indicates that only 5'-phosphate specie was detected and (┐) indicates that 5'-OH 3'-OH component was detected in mass spectrometry analysis. The length of the arrow signifies the amount of products present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment (the larger the arrow, the more the detectable cleavage products). Only in the cases where 5'-OH 3'-OH was not detected in the reaction mixture, 5'-phosphate specie peak was used for quantification. Cleavage rates were determined by measuring amount of full length RNA remaining in the reaction mixtures by reverse phase HPLC. Reactions were quenched at fixed time points by 30mM Na_2EDTA.

[00136] *Figure 11.* Cleavage maps of oligonucleotide compositions having different common base sequences and lengths ((A)-(B)). The maps show a comparison of stereorandom DNA compositions (top panel) with three distinct and stereochemically pure oligonucleotide compositions. Data compare results of chirally controlled oligonucleotide compositions with two stereorandom phosphorothioate oligonucleotide compositions (ONT-366 and ONT-367) targeting different regions in FOXO1 mRNA. Each panel shows a comparison of setreorandom DNA (top panel) with three distinct and stereochemically pure oligonucleotide preparaitons. Cleavage maps were derived from reaction mixtures obtained after 30 minutes of incubation of respective duplexes with RNase H1C in the presence of 1XPBS buffer at 37°C. Arrows indicate sites of cleavage. (┬) indicates that both fragments, 5'-phosphate specie as well as 5'-OH 3'-OH specie were identified in reaction mixtures. (┌) indicates that only 5'-phosphate specie was detected and (┐) indicates that 5'-OH 3'-OH component was detected in mass spectrometry analysis. The length of the arrow signifies the amount of metabolite present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment (the larger the arrow, the more the detectable cleavage products).

Only in the cases where 5'-OH 3'-OH was not detected in the reaction mixture, 5'-phosphate specie peak was used for quantification.

[00137] *Figure 12.* Effect of stereochemistry on RNase H activity. In two independent experiments, antisense oligonucleotides targeting an identical region of FOXO1 mRNA were hybridized with RNA and then incubated with RNase H at 37°C in the presence of 1X RNase H buffer. Disappearance of full length RNA was measured from its peak area at 254nm using RP-HPLC. (A): from top to bottom at 60 min: ONT-355, ONT-316, ONT-367, ONT-392, ONT-393 and ONT-394 (ONT-393 and ONT-394 about the same at 60 min; ONT-393 had higher %RNA substrate remaining at 5 min). (B): from top to bottom at 60 min: ONT-315, ONT-354, ONT-366, ONT-391, ONT-389 and ONT-390. Cleavage rates were determined by measuring amount of full length RNA remaining in the reaction mixtures by reverse phase HPLC. Reactions were quenched at fixed time points by 30mM Na_2EDTA.

[00138] *Figure 13.* Turnover of antisense oligonucleotides. The duplexes were made with each DNA strand concentration equal to 6 μM and RNA being 100 μM. These duplexes were incubated with 0.02 μM RNase H enzyme and disappearance of full length RNA was measured from its peak area at 254 nm using RP-HPLC. Cleavage rates were determined by measuring amount of full length RNA remaining in the reaction mixtures by reverse phase HPLC. Reactions were quenched at fixed time points by 30 mM Na_2EDTA. From top to bottom at 40 min: ONT-316, ONT-367 and ONT-392.

[00139] *Figure 14.* Cleavage map comparing a stereorandom phosphorothioate oligonucleotide with six distinct and stereochemically pure oligonucleotide preparations targeting the same FOXO1 mRNA region.

[00140] *Figure 15.* Effect of stereochemistry on RNase H activity. Antisense oligonucleotides were hybridized with RNA and then incubated with RNase H at 37°C in the presence of 1X RNase H buffer. Dependence of stereochemistry upon RNase H activity was observed. Also evident in comparing ONT-367 (stereorandom DNA) and ONT-316 (5-10-5 2'-MOE Gapmer) is the strong dependence of compositional chemistry upon RNase H activity. From top to bottom at 40 min: ONT-316, ONT-421, ONT-367, ONT-392, ONT-394, ONT-415, and ONT-422 (ONT-394/415/422 have similar levels at 40 min; at 5 min, ONT-422 > ONT-394 > ONT-415 in % RNA remaining in DNA/RNA duplex).

[00141] *Figure 16.* Effect of stereochemistry on RNase H activity. Antisense oligonucleotides targeting an identical region of FOXO1 mRNA were hybridized with RNA and then incubated with RNase H at 37°C in the presence of 1X RNase H buffer. Dependence of stereochemistry upon RNase H activity was observed. Form top to bottom at 40 min: ONT-396, ONT-409, ONT-414, ONT-408 (ONT-396/409/414/408 have similar levels at 40 min), ONT-404, ONT-410, ONT-402 (ONT-404/410/408 have similar levels at 40 min), ONT-403, ONT-407, ONT-405, ONT-401, ONT-406 and ONT-400 (ONT-401/405/406/400 have similar levels at 40 min).

[00142] *Figure 17.* Effect of stereochemistry on RNase H activity. Antisense oligonucleotides targeting an identical region of FOXO1 mRNA were hybridized with RNA and then incubated with RNase H at 37°C in the presence of 1X RNase H buffer. Dependence of stereochemistry upon RNase H activity was observed. ONT-406 was observed to elicit cleavage of duplexed RNA at a rate in slight excess of that of the phosphodiester oligonucleotide ONT-415. From top to bottom at 40 min: ONT-396, ONT-421, ONT-392, ONT-394, ONT-415 ONT-406, and ONT-422 (ONT-394/415/406 have similar levels at 40 min; at 5 min, ONT-394 > ONT-415 > ONT-406 in % RNA remaining in DNA/RNA duplex).

[00143] *Figure 18.* Exemplary UV chromatograms of RNA cleavage products obtained when RNA (ONT-388) was duplexed with stereorandom DNA, ONT-367 (top) and stereopure DNA with repeat triplet motif-3'-SSR-5', ONT-394 (bottom).). 2.35min: 7mer; 3.16min: 8mer and p-6mer; 4.48min: P-7mer; 5.83min: P-8mer; 6.88min: 12mer; 9.32min: 13mer; 10.13min: P-11mer; 11.0min: P-12mer and 14mer; 11.93min: P-13mer; 13.13min: P-14mer. ONT-394 (on the bottom) peak assignment: 4.55min: p-7mer; 4.97min: 10mer; 9.53min: 13mer.

[00144] *Figure 19.* Electrospray Ionization Spectrum of RNA cleavage products. RNA fragments obtained from the duplex ONT-387, RNA/ONT-354, (7-6-7, DNA-2'-OMe-DNA) on the top and ONT-387, RNA/ONT-315, (5-10-5,2'-MOE Gapmer) at the bottom when these duplexes were incubated with RNase H for 30min in the presence of 1X RNse H buffer.

[00145] *Figure 20.* UV Chromatogram and TIC of ONT-406 and ONT-388 duplex after 30 minutes of incubation with RNase H.

[00146] *Figure 21*. An exemplary proposed cleavage. Provided chirally controlled oligonucleotide compositions are capable of cleaving targets as depicted.

[00147] *Figure 22*. Exemplary allele specific cleavage targeting mutant Huntingtin mRNA. (A) and (B): exemplary oligonucleotides. (C)-(E): cleavage maps. (F)-(H): RNA cleavage. Stereorandom and chirally controlled oligonucleotide compositions were prepared to target single nucleotide polymorphisms for allele selective suppression of mutant Huntingtin. ONT-450 (stereorandom) targeting ONT-453 (muHTT) and ONT-454 (wtHTT) showed marginal differentiation in RNA cleavage and their cleavage maps. Chirally controlled ONT-451 with selective placement of 3'-SSR-5' motif in RNase H recognition site targeting ONT-453 (muHTT) and ONT-454 (wtHTT) showed large differentiation in RNA cleavage rate. From the cleavage map, it is notable that 3'-SSR-5' motif is placed to direct the cleavage between positions 8 and 9 which is after the mismatch if read from 5'-end of RNA. ONT-452 with selective placement of 3'-SSR-5' motif in RNase H recognition site targeting ONT-453 (muHTT) and ONT-454 (wtHTT) showed moderate differentiation in RNA cleavage rate. 3'-SSR-5' motif was placed to direct the cleavage at positions 7 and 8 which is before the mismatch if read from 5'-end of RNA. Exemplary data illustrate significance of position in placement of 3'-SSR-5' motif to achieve enhanced discrimination for allele specific cleavage. All cleavage maps are derived from the reaction mixtures obtained after 5 minutes of incubation of respective duplexes with RNase H1C in the presence of 1XPBS buffer at 37°C. Arrows indicate sites of cleavage. (┳) indicates that both fragments, 5'-phosphate specie as well as 5'-OH 3'-OH specie were identified in reaction mixtures. (┳) indicates that only 5'-phosphate specie was detected and (┓) indicates that 5'-OH 3'-OH component was detected in mass spectrometry analysis. The length of the arrow signifies the amount of metabolite present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment. Only in the cases where 5'-OH 3'-OH was not detected in the reaction mixture, 5'-phosphate specie peak was used for quantification.

[00148] *Figure 23*. (A)-(C): exemplary allele specific cleavage targeting FOXO1 mRNA.

[00149] *Figure 24*. *In vitro* dose response silencing of ApoB mRNA after treatment with ApoB oligonucleotides. Stereochemically pure diasetereomers with and without 2'-MOE wings show similar efficacy as ONT-41 (Mipomersen).

[00150] *Figure 25*. Comparison of RNase H cleavage maps (A) and RNA cleavage rates (B) for stereorandom composition (ONT-367) and chirally controlled oligonucleotide compositions (ONT-421, all *S*p and ONT-455, all *R*p) and DNA (ONT-415). These sequences

target the same region in FOXO1 mRNA. Cleavage maps were derived from the reaction mixtures obtained after 5 minutes of incubation of respective duplexes with RNase H1C in the presence of 1XPBS buffer at 37°C. Arrows indicate sites of cleavage. (┬) indicates that both fragments, 5'-phosphate specie as well as 5'-OH 3'-OH specie were identified in reaction mixtures. (┌) indicates that only 5'-phosphate specie was detected and (┐) indicates that 5'-OH 3'-OH component was detected in mass spectrometry analysis. The length of the arrow signifies the amount of metabolite present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment. Only in the cases where 5'-OH 3'-OH was not detected in the reaction mixture, 5'-phosphate specie peak was used for quantification. Cleavage rates were determined by measuring amount of full length RNA remaining in the reaction mixtures by reverse phase HPLC. Reactions are quenched at fixed time points by 30mM Na_2EDTA.

[00151] *Figure 26.* Comparison of cleavage maps of sequences containing one *R*p with change of position starting from 3'-end of DNA. These sequences target the same region in FOXO1 mRNA. Cleavage maps are derived from the reaction mixtures obtained after 5 minutes of incubation of respective duplexes with RNase H1C in the presence of 1XPBS buffer at 37°C. Arrows indicate sites of cleavage. (┬) indicates that both fragments, 5'-phosphate specie as well as 5'-OH 3'-OH specie were identified in reaction mixtures. (┌) indicates that only 5'-phosphate specie was detected and (┐) indicates that 5'-OH 3'-OH component was detected in mass spectrometry analysis. The length of the arrow signifies the amount of metabolite present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment. Only in the cases where 5'-OH 3'-OH was not detected in the reaction mixture, 5'-phosphate specie peak was used for quantification.

[00152] *Figure 27.* (A) Comparison of RNase H cleavage rates for stereopure oligonucleotides (ONT-406), (ONT-401), (ONT-404) and (ONT-408). All four sequences are stereopure phosphorothioates with one *R*p linkage. These sequences target the same region in FOXO1 mRNA. All duplexes were incubation with RNase H1C in the presence of 1XPBS buffer at 37°C. Reactions were quenched at fixed time points by 30mM Na_2EDTA. Cleavage rates were determined by measuring amount of full length RNA remaining in the reaction mixtures by reverse phase HPLC. ONT-406 and ONT-401 were found to have superior cleavage rates. (B) Correlation between %RNA cleaved in RNase H assay (10 μM oligonucleotide) and

%mRNA knockdown in *in vitro* assay (20 nM oligonucleotide). All sequences target the same region of mRNA in the FOXO1 target. The quantity of RNA remaining is determined by UV peak area for RNA when normalized to DNA in the same reaction mixture. All of the above maps are derived from the reaction mixture obtained after 5 minutes of incubation of respective duplexes with RNase H1C in the presence of 1XPBS buffer at 37°C. All sequences from ONT-396 to ONT-414 have one *R*p phosphorothioate and they vary in the position of *R*p. ONT-421 (All *S*p) phosphorothioate was inactive *in-vitro* assay. It relates poor cleavage rate of RNA in RNase H assay when ONT-421 is duplexed with complementary RNA.

[00153] *Figure 28.* Serum stability assay of single *R*p walk PS DNA (ONT-396-ONT-414), stereorandom PS DNA(ONT-367), all-*S*p PS DNA (ONT-421) and all-*R*p PS DNA (ONT-455) in rat serum for 2 days. Note ONT-396 and ONT-455 decomposed at tested time point.

[00154] *Figure 29.* Exemplary oligonucleotides including hemimers. (A): cleavage maps. (B): RNA cleavage assay. (C): FOXO1 mRNA knockdown. In some embodiments, introduction of 2'-modifications on 5'-end of the sequences increases stability for binding to target RNA while maintaining RNase H activity. ONT-367 (stereorandom phosphorothioate DNA) and ONT-440 (5-15, 2'-F-DNA) have similar cleavage maps and similar rate of RNA cleavage in RNase H assay (10 μM oligonucleotide). In some embodiments, ONT-440 (5-11, 2'-F-DNA) sequence can have better cell penetration properties. In some embodiments, asymmetric 2'-modifications provide Tm advantage while maintaining RNase H activity. Introduction of RSS motifs can further enhance RNase H efficiency in the hemimers. Cleavage maps are derived from the reaction mixtures obtained after 5 minutes of incubation of respective duplexes with RNase H1C in the presence of 1XPBS buffer at 37°C. Arrows indicate sites of cleavage. (┳) indicates that both fragments, 5'-phosphate specie as well as 5'-OH 3'-OH specie were identified in reaction mixtures. (┏) indicates that only 5'-phosphate specie was detected and (┓) indicates that 5'-OH 3'-OH component was detected in mass spectrometry analysis. The length of the arrow signifies the amount of metabolite present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment. Only in the cases where 5'-OH 3'-OH was not detected in the reaction mixture, 5'-phosphate specie peak was used for quantification.

[00155] *Figure 30.* Exemplary mass spectrometry data of cleavage assay. Top: data for ONT-367: 2.35 min: 7 mer; 3.16 min: 8 mer and P-6 mer; 4.58 min: P-7 mer; 5.91 min: P-8 mer;

7.19 min: 12 mer; 9.55 min: 13 mer; 10.13 min: P-11 mer; 11.14 min: P-12 mer and 14 mer; 12.11 min: P-13 mer; 13.29 min: P-14 mer; 14.80 min: full length RNA (ONT-388) and 18.33 min: stereorandom DNA (ONT-367). Bottom: data for ONT-406: 4.72 min: p-rArUrGrGrCrUrA, 5'-phosphorylated 7 mer RNA; 9.46 min: 5'-rGrUrGrArGrCrArGrCrUrGrCrA, 5'-OH 3'-OH 13 mer RNA; 16.45 min: full length RNA (ONT-388); 19.48 and 19.49 min: stereopure DNA (ONT-406).

Detailed Description of Certain Embodiments

[00156] Synthetic oligonucleotides provide useful molecular tools in a wide variety of applications. For example, oligonucleotides are useful in therapeutic, diagnostic, research, and new nanomaterials applications. The use of naturally occurring nucleic acids (e.g., unmodified DNA or RNA) is limited, for example, by their susceptibility to endo- and exo-nucleases. As such, various synthetic counterparts have been developed to circumvent these shortcomings. These include synthetic oligonucleotides that contain backbone modifications, which render these molecules less susceptible to degradation. From a structural point of view, such modifications to internucleotide phosphate linkages introduce chirality. It has become clear that certain properties of oligonucleotides may be affected by the configurations of the phosphorus atoms that form the backbone of the oligonucleotides. For example, *in vitro* studies have shown that the properties of antisense nucleotides such as binding affinity, sequence specific binding to the complementary RNA, stability to nucleases are affected by, inter alia, chirality of the backbone (e.g., the configurations of the phosphorus atoms).

[00157] Among other things, the present invention encompasses the recognition that stereorandom oligonucleotide preparations contain a plurality of distinct chemical entities that differ from one another in the stereochemical structure of individual backbone chiral centers within the oligonucleotide chain. Moreover, the present invention encompasses the insight that it is typically unlikely that a stereorandom oligonucleotide preparation will include every possible stereoisomer of the relevant oligonucleotide. Thus, among other things, the present invention provides new chemical entities that are particular stereoisomers of oligonucleotides of interest. That is, the present invention provides substantially pure preparations of single oligonucleotide compounds, where a particular oligonucleotide compound may be defined by its base sequence, its length, its pattern of backbone linkages, and its pattern of backbone chiral centers.

[00158] The present invention demonstrates, among other things, that individual stereoisomers of a particular oligonucleotide can show different stability and/or activity from each other. Moreover, the present disclosure demonstrates that stability improvements achieved through inclusion and/or location of particular chiral structures within an oligonucleotide can be comparable to, or even better than those achieved through use of modified backbone linkages, bases, and/or sugars (e.g., through use of certain types of modified phosphates, 2'-modifications, base modifications, etc.). The present disclosure, in some embodiments, also demonstrates that activity improvements achieved through inclusion and/or location of particular chiral structures within an oligonucleotide can be comparable to, or even better than those achieved through use of modified backbone linkages, bases, and/or sugars (e.g., through use of certain types of modified phosphates, 2'-modifications, base modifications, etc.). In some embodiments, inclusion and/or location of particular chiral linkages within an oligonucleotide can surprisingly change the cleavage pattern of a nucleic acid polymer when such an oligonucleotide is utilized for cleaving said nucleic acid polymer. For example, in some embodiments, a pattern of backbone chiral centers provides unexpectedly high cleavage efficiency of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers provides new cleavage sites. In some embodiments, a pattern of backbone chiral centers provides fewer cleavage sites, for example, by blocking certain existing cleavage sites. Even more unexpectedly, in some embodiments, a pattern of backbone chiral centers provides cleavage at only one site of a target nucleic acid polymer within the sequence that is complementary to a oligonucleotide utilized for cleavage. In some embodiments, higher cleavage efficiency is achieved by selecting a pattern of backbone chiral centers to minimize the number of cleavage sites.

[00159] In some embodiments, the present invention provides chirally controlled (and/or stereochemically pure) oligonucleotide compositions comprising oligonucleotides defined by having:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which composition is a substantially pure preparation of a single oligonucleotide in that at least about 10% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers. A pattern of backbone chiral

centers of an oligonucleotide can be designated by a combination of linkage phosphorus stereochemistry (*R*p/*S*p) from 5' to 3'. For example, as exemplified below ONT-154 has a pattern of 5S-$(SSR)_3$-5S, and ONT-80 has S_{19}.

[00160] In some embodiments, the present invention provides chirally controlled oligonucleotide composition of oligonucleotides in that the composition is enriched, relative to a substantially racemic preparation of the same oligonucleotides, for oligonucleotides of a single oligonucleotide type. In some embodiments, the present invention provides chirally controlled oligonucleotide composition of oligonucleotides in that the composition is enriched, relative to a substantially racemic preparation of the same oligonucleotides, for oligonucleotides of a single oligonucleotide type that share:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers.

In some embodiments, in a substantially racemic (or chirally uncontrolled) preparation of oligonucleotides, all or most coupling steps are not chirally controlled in that the coupling steps are not specifically conducted to provide enhanced stereoselectivity. An exemplary substantially racemic preparation of oligonucleotides is the preparation of phosphorothioate oligonucleotides through sulfurizing phosphite triesters with either tteraethylthiuram disulfide or (TETD) or 3H-1, 2-bensodithiol-3-one 1, 1-dioxide (BDTD), a well-known process in the art. In some embodiments, substantially racemic preparation of oligonucleotides provides substantially racemic oligonucleotide compositions (or chirally uncontrolled oligonucleotide compositions).

[00161] In some embodiments, the present invention provides a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide composition is a substantially pure preparation of a oligonucleotide type in that

oligonucleotides in the composition that are not of the oligonucleotide type are impurities form the preparation process of said oligonucleotide type, in some case, after certain purification procedures.

[00162] In some embodiments, at least about 20% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 25% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 30% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 35% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 40% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 45% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 50% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 55% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 60% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 65% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 70% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 75% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of

backbone chiral centers. In some embodiments, at least about 80% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 85% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 90% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 92% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 94% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 95% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, greater than about 99% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, purity of a chirally controlled oligonucleotide composition of an oligonucleotide can be expressed as the percentage of oligonucleotides in the composition that have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers.

[00163] In some embodiments, purity of a chirally controlled oligonucleotide composition of an oligonucleotide type is expressed as the percentage of oligonucleotides in the composition that are of the oligonucleotide type. In some embodiments, at least about 10% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 20% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 30% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 40% of

the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 50% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 60% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 70% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 80% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 90% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 92% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 94% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 95% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 96% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 97% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 98% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 99% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type.

[00164] In some embodiments, purity of a chirally controlled oligonucleotide composition can be controlled by stereoselectivity of each coupling step in its preparation process. In some embodiments, a coupling step has a stereoselectivity (*e.g.*, diastereoselectivity) of 60% (60% of the new internucleotidic linkage formed from the coupling step has the intended stereochemistry). After such a coupling step, the new internucleotidic linkage formed may be referred to have a 60% purity. In some embodiments, each coupling step has a stereoselectivity of at least 60%. In some embodiments, each coupling step has a stereoselectivity of at least 70%. In some embodiments, each coupling step has a stereoselectivity of at least 80%. In some embodiments, each coupling step has a stereoselectivity of at least 85%. In some embodiments,

each coupling step has a stereoselectivity of at least 90%. In some embodiments, each coupling step has a stereoselectivity of at least 91%. In some embodiments, each coupling step has a stereoselectivity of at least 92%. In some embodiments, each coupling step has a stereoselectivity of at least 93%. In some embodiments, each coupling step has a stereoselectivity of at least 94%. In some embodiments, each coupling step has a stereoselectivity of at least 95%. In some embodiments, each coupling step has a stereoselectivity of at least 96%. In some embodiments, each coupling step has a stereoselectivity of at least 97%. In some embodiments, each coupling step has a stereoselectivity of at least 98%. In some embodiments, each coupling step has a stereoselectivity of at least 99%. In some embodiments, each coupling step has a stereoselectivity of at least 99.5%. In some embodiments, each coupling step has a stereoselectivity of virtually 100%. In some embodiments, a coupling step has a stereoselectivity of virtually 100% in that all detectable product from the coupling step by an analytical method (*e.g.*, NMR, HPLC, *etc*) has the intended stereoselectivity.

[00165] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are antisense oligonucleotides (e.g., chiromersen). In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are siRNA oligonucleotides. In some embodiments, a provided chirally controlled oligonucleotide composition is of oligonucleotides that can be antisense oligonucleotide, antagomir, microRNA, pre-microRNs, antimir, supermir, ribozyme, Ul adaptor, RNA activator, RNAi agent, decoy oligonucleotide, triplex forming oligonucleotide, aptamer or adjuvant. In some embodiments, a chirally controlled oligonucleotide composition is of antisense oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of antagomir oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of microRNA oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of pre-microRNA oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of antimir oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of supermir oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of ribozyme oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of Ul adaptor oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of

RNA activator oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of RNAi agent oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of decoy oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of triplex forming oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of aptamer oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of adjuvant oligonucleotides.

[00166] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides that include one or more modified backbone linkages, bases, and/or sugars.

[00167] In some embodiments, a provided oligonucleotide comprises one or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide comprises two or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide comprises three or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide comprises four or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide comprises five or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide comprises 1, 2, 3, 4, 5, 6, 7, 8,9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 5 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 6 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 7 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 8 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 9 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 10 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 11 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 12 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 13 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 14 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises

15 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 16 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 17 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 18 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 19 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 20 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 21 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 22 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 23 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 24 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 25 or more chiral, modified phosphate linkages.

[00168] In some embodiments, a provided oligonucleotide comprises at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% chiral, modified phosphate linkages. Exemplary such chiral, modified phosphate linkages are described above and herein. In some embodiments, a provided oligonucleotide comprises at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% chiral, modified phosphate linkages in the *S*p configuration.

[00169] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 80%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 85%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 90%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 91%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 92%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater

than about 93%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 94%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 95%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 96%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 97%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 98%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 99%.

[00170] In some embodiments, a chiral, modified phosphate linkage is a chiral phosphorothioate linkage, i.e., phosphorothioate internucleotidic linkage. In some embodiments, a provided oligonucleotide comprises at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% chiral phosphorothioate internucleotidic linkages. In some embodiments, all chiral, modified phosphate linkages are chiral phosphorothioate internucleotidic linkages. In some embodiments, at least about 10, 20, 30, 40, 50, 60, 70, 80, or 90% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 10% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 20% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 30% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 40% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 50% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 60% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 70% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 80% chiral phosphorothioate

internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 90% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 95% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 10, 20, 30, 40, 50, 60, 70, 80, or 90% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 10% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 20% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 30% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 40% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 50% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 60% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 70% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 80% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 90% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 95% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, less than about 10, 20, 30, 40, 50, 60, 70, 80, or 90% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, less than about 10% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, less than about 20% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, less than about 30% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, less than about 40% chiral phosphorothioate internucleotidic linkages of a

provided oligonucleotide are of the *R*p conformation. In some embodiments, less than about 50% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, less than about 60% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, less than about 70% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, less than about 80% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, less than about 90% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, less than about 95% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, a provided oligonucleotide has only one *R*p chiral phosphorothioate internucleotidic linkages. In some embodiments, a provided oligonucleotide has only one *R*p chiral phosphorothioate internucleotidic linkages, wherein all internucleotide linkages are chiral phosphorothioate internucleotidic linkages. In some embodiments, a chiral phosphorothioate internucleotidic linkage is a chiral phosphorothioate diester linkage. In some embodiments, each chiral phosphorothioate internucleotidic linkage is independently a chiral phosphorothioate diester linkage. In some embodiments, each internucleotidic linkage is independently a chiral phosphorothioate diester linkage. In some embodiments, each internucleotidic linkage is independently a chiral phosphorothioate diester linkage, and only one is *R*p.

[00171] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides that contain one or more modified bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides that contain no modified bases. Exemplary such modified bases are described above and herein.

[00172] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 8 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 9 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 10 bases. In some embodiments, provided chirally

controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 11 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 12 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 13 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 14 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 15 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 16 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 17 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 18 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 19 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 20 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 21 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 22 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 23 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 24 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 25 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 30, 35, 40, 45, 50, 55, 60, 65, 70, or 75 bases.

[00173] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations comprise oligonucleotides containing one or more residues which are modified at the sugar moiety. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations comprise oligonucleotides containing one or more residues which are modified at the 2' position of the sugar moiety (referred to herein as a "2'-modification"). Exemplary such modifications are described above and herein and include, but are not limited to, 2'-OMe, 2'-MOE, 2'-LNA, 2'-F, etc. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations comprise oligonucleotides containing one or more residues which are 2'-modified. For example, in some embodiments, provided oligonucleotides contain one or more residues which are 2'-O-methoxyethyl (2'-MOE)-modified residues. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations comprise oligonucleotides which do not contain any 2'-modifications. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are oligonucleotides which do not contain any 2'-MOE residues. That is, in some embodiments, provided oligonucleotides are not MOE-modified.

[00174] In some embodiments, provided chirally controlled (and/or stereochemically pure) oligonucleotides are of a general motif of wing-core-wing (also represented herein generally as X-Y-X). In some embodiments, each wing contains one or more residues having a particular modification, which modification is absent from the core "Y" portion. In some embodiment, each wing contains one or more residues having a 2' modification that is not present in the core portion. For instance, in some embodiments, provided chirally controlled (and/or stereochemically pure) oligonucleotides have a wing-core-wing motif represented as X-Y-X, wherein the residues at each "X" portion are 2'-modified residues of a particular type and the residues in the core "Y" portion are not 2'-modified residues of the same particular type. For instance, in some embodiments, provided chirally controlled (and/or stereochemically pure) oligonucleotides have a wing-core-wing motif represented as X-Y-X, wherein the residues at each "X" portion are 2'-MOE-modified residues and the residues in the core "Y" portion are not 2'-MOE-modified residues. In some embodiments, provided chirally controlled (and/or stereochemically pure) oligonucleotides have a wing-core-wing motif represented as X-Y-X, wherein the residues at each "X" portion are 2'-MOE-modified residues and the residues in the core "Y" portion are 2'-deoxyribonucleotide. One of skill in the relevant arts will recognize that

all such 2'-modifications described above and herein are contemplated in the context of such X-Y-X motifs.

[00175] In some embodiments, each wing region independently has a length of one or more bases. In some embodiments, each wing region independently has a length of two or more bases. In some embodiments, each wing region independently has a length of three or more bases. In some embodiments, each wing region independently has a length of four or more bases. In some embodiments, each wing region independently has a length of five or more bases. In some embodiments, each wing region independently has a length of six or more bases. In some embodiments, each wing region independently has a length of seven or more bases. In some embodiments, each wing region independently has a length of eight or more bases. In some embodiments, each wing region independently has a length of nine or more bases. In some embodiments, each wing region independently has a length of ten or more bases. In certain embodiments, each wing region has a length of one base. In certain embodiments, each wing region has a length of two bases. In certain embodiments, each wing region has a length of three bases. In certain embodiments, each wing region has a length of four bases. In certain embodiments, each wing region has a length of five bases.

[00176] In some embodiments, a core region has a length of one or more bases. In some embodiments, a core region has a length of one or more bases. In some embodiments, a core region has a length of two or more bases. In some embodiments, a core region has a length of three or more bases. In some embodiments, a core region has a length of four or more bases. In some embodiments, a core region has a length of five or more bases. In some embodiments, a core region has a length of six or more bases. In some embodiments, a core region has a length of seven or more bases. In some embodiments, a core region has a length of eight or more bases. In some embodiments, a core region has a length of nine or more bases. In some embodiments, a core region has a length of ten or more bases. In some embodiments, a core region has a length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or more bases. In certain embodiments, a core region has a length of ten bases. In certain embodiments, a core region has a length of 3 bases. In certain embodiments, a core region has a length of 4 bases. In certain embodiments, a core region has a length of 5 bases. In certain embodiments, a core region has a length of 6 bases. In certain embodiments, a core region has a length of 7 bases. In certain embodiments, a core region has a length of 8 bases. In certain embodiments, a core region has a length of 9 bases. In

certain embodiments, a core region has a length of 10 bases. In certain embodiments, a core region has a length of 11 bases. In certain embodiments, a core region has a length of 12 bases. In certain embodiments, a core region has a length of 13 bases. In certain embodiments, a core region has a length of 14 bases. In certain embodiments, a core region has a length of 15 bases. In certain embodiments, a core region has a length of 16 bases. In certain embodiments, a core region has a length of 17 bases. In certain embodiments, a core region has a length of 18 bases. In certain embodiments, a core region has a length of 19 bases. In certain embodiments, a core region has a length of 11 or more bases. In certain embodiments, a core region has a length of 12 or more bases. In certain embodiments, a core region has a length of 13 or more bases. In certain embodiments, a core region has a length of 14 or more bases. In certain embodiments, a core region has a length of 15 or more bases. In certain embodiments, a core region has a length of 16 or more bases. In certain embodiments, a core region has a length of 17 or more bases. In certain embodiments, a core region has a length of 18 or more bases. In certain embodiments, a core region has a length of 19 or more bases. In certain embodiments, a core region has a length of 20 or more bases. In certain embodiments, a core region has a length of more than 20 bases.

[00177] In some embodiments, a wing-core-wing (i.e., X-Y-X) motif of a provided oligonucleotide is represented numerically as, e.g., 5-10-5, meaning each wing region of the oligonucleotide is 5 bases in length and the core region of the oligonucleotide is 10 bases in length. In some embodiments, a wing-core-wing motif is any of, e.g. 2-16-2, 3-14-3, 4-12-4, 5-10-5, etc. In certain embodiments, a wing-core-wing motif is 5-10-5.

[00178] In some embodiments, the internucleosidic linkages of provided oligonucleotides of such wing-core-wing (i.e., X-Y-X) motifs are all chiral, modified phosphate linkages. In some embodiments, the internucleosidic linkages of provided oligonucleotides of such wing-core-wing (i.e., X-Y-X) motifs are all chiral phosphorothioate internucleotidic linkages. In some embodiments, chiral internucleotidic linkages of provided oligonucleotides of such wing-core-wing motifs are at least about 10, 20, 30, 40, 50, 50, 70, 80, or 90% chiral, modified phosphate internucleotidic linkages. In some embodiments, chiral internucleotidic linkages of provided oligonucleotides of such wing-core-wing motifs are at least about 10, 20, 30, 40, 50, 60, 70, 80, or 90% chiral phosphorothioate internucleotidic linkages. In some embodiments, chiral internucleotidic linkages of provided oligonucleotides of such wing-core-wing motifs are at least

about 10, 20, 30, 40, 50, 50, 70, 80, or 90% chiral phosphorothioate internucleotidic linkages of the *S*p conformation.

[00179] In some embodiments, each wing region of a wing-core-wing motif optionally contains chiral, modified phosphate internucleotidic linkages. In some embodiments, each wing region of a wing-core-wing motif optionally contains chiral phosphorothioate internucleotidic linkages. In some embodiments, each wing region of a wing-core-wing motif contains chiral phosphorothioate internucleotidic linkages. In some embodiments, the two wing regions of a wing-core-wing motif have the same internucleotidic linkage stereochemistry. In some embodiments, the two wing regions have different internucleotidic linkage stereochemistry. In some embodiments, each internucleotidic linkage in the wings is independently a chiral internucleotidic linkage.

[00180] In some embodiments, the core region of a wing-core-wing motif optionally contains chiral, modified phosphate internucleotidic linkages. In some embodiments, the core region of a wing-core-wing motif optionally contains chiral phosphorothioate internucleotidic linkages. In some embodiments, the core region of a wing-core-wing motif comprises a repeating pattern of internucleotidic linkage stereochemistry. In some embodiments, the core region of a wing-core-wing motif has a repeating pattern of internucleotidic linkage stereochemistry. In some embodiments, the core region of a wing-core-wing motif comprises repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is (*S*p)m*R*p or *R*p(*S*p)m, wherein m is 2, 3, 4, 5, 6, 7 or 8. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is (*S*p)m*R*p or *R*p(*S*p)m, wherein m is 2, 3, 4, 5, 6, 7 or 8. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is (*S*p)m*R*p, wherein m is 2, 3, 4, 5, 6, 7 or 8. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is *R*p(*S*p)m, wherein m is 2, 3, 4, 5, 6, 7 or 8. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is (*S*p)m*R*p or *R*p(*S*p)m, wherein m is 2, 3, 4, 5, 6, 7 or 8. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a motif comprising at least 33% of internucleotidic linkage in the

S conformation. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a motif comprising at least 50% of internucleotidic linkage in the S conformation. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a motif comprising at least 66% of internucleotidic linkage in the S conformation. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a repeating triplet motif selected from *R*p*R*p*S*p and *S*p*S*p*R*p. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a repeating *R*p*R*p*S*p. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a repeating *S*p*S*p*R*p.

[00181] In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*S*p)m*R*p or *R*p(*S*p)m. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises *R*p(*S*p)m. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*S*p)m*R*p. In some embodiments, m is 2. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises *R*p(*S*p)$_2$. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*S*p)$_2$*R*p(*S*p)$_2$. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*R*p)$_2$*R*p(*S*p)$_2$. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises *R*p*S*p*R*p(*S*p)$_2$. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises

*S*p*R*p*R*p(*S*p)$_2$. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*S*p)$_2$*R*p.

[00182] In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*S*p)m*R*p or *R*p(*S*p)m. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises *R*p(*S*p)m. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*S*p)m*R*p. In some embodiments, m is 2. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises *R*p(*S*p)$_2$. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*S*p)$_2$*R*p(*S*p)$_2$. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*R*p)$_2$*R*p(*S*p)$_2$. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises *R*p*S*p*R*p(*S*p)$_2$. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises *S*p*R*p*R*p(*S*p)$_2$. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*S*p)$_2$*R*p.

[00183] As defined herein, m is 2, 3, 4, 5, 6, 7 or 8. In some embodiments, m is 3, 4, 5, 6, 7 or 8. In some embodiments, m is 4, 5, 6, 7 or 8. In some embodiments, m is 5, 6, 7 or 8. In some embodiments, m is 6, 7 or 8. In some embodiments, m is 7 or 8. In some embodiments, m is 2. In some embodiments, m is 3. In some embodiments, m is 4. In some embodiments, m is 5. In some embodiments, m is 6. In some embodiments, m is 7. In some embodiments, m is 8.

[00184] In some embodiments, a repeating pattern is (*S*p)m(*R*p)n, wherein n is independently 1, 2, 3, 4, 5, 6, 7 or 8, and m is independently as defined above and described herein. In some embodiments, the present invention provides a chirally controlled

oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*S*p)m(*R*p)n. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*S*p)m(*R*p)n. In some embodiments, a repeating pattern is (*R*p)n(*S*p)m, wherein n is independently 1, 2, 3, 4, 5, 6, 7 or 8, and m is independently as defined above and described herein. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*R*p)n(*S*p)m. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*R*p)n(*S*p)m. In some embodiments, (*R*p)n(*S*p)m is (*R*p)(*S*p)$_2$. In some embodiments, (*S*p)n(*R*p)m is (*S*p)$_2$(*R*p).

[00185] In some embodiments, a repeating pattern is (*S*p)m(*R*p)n(*S*p)t, wherein each of n and t is independently 1, 2, 3, 4, 5, 6, 7 or 8, and m is as defined above and described herein. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*S*p)m(*R*p)n(*S*p)t. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*S*p)m(*R*p)n(*S*p)t. In some embodiments, a repeating pattern is (*S*p)t(*R*p)n(*S*p)m, wherein each of n and t is independently 1, 2, 3, 4, 5, 6, 7 or 8, and m is as defined above and described herein. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*S*p)t(*R*p)n(*S*p)m. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*S*p)t(*R*p)n(*S*p)m.

[00186] In some embodiments, a repeating pattern is (*N*p)t(*R*p)n(*S*p)m, wherein each of n and t is independently 1, 2, 3, 4, 5, 6, 7 or 8, *N*p is independently *R*p or *S*p, and m is as defined above and described herein. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*N*p)t(*R*p)n(*S*p)m. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*N*p)t(*R*p)n(*S*p)m. In some embodiments, a

repeating pattern is (*N*p)m(*R*p)n(*S*p)t, wherein each of n and t is independently 1, 2, 3, 4, 5, 6, 7 or 8, *N*p is independently *R*p or *S*p, and m is as defined above and described herein. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*N*p)m(*R*p)n(*S*p)t. In some embodiments, the present invention provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*N*p)m(*R*p)n(*S*p)t. In some embodiments, *N*p is *R*p. In some embodiments, *N*p is *S*p. In some embodiments, all *N*p are the same. In some embodiments, all *N*p are *S*p. In some embodiments, at least one *N*p is different from the other *N*p. In some embodiments, t is 2.

[00187] As defined herein, n is 1, 2, 3, 4, 5, 6, 7 or 8. In some embodiments, n is 2, 3, 4, 5, 6, 7 or 8. In some embodiments, n is 3, 4, 5, 6, 7 or 8. In some embodiments, n is 4, 5, 6, 7 or 8. In some embodiments, n is 5, 6, 7 or 8. In some embodiments, n is 6, 7 or 8. In some embodiments, n is 7 or 8. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5. In some embodiments, n is 6. In some embodiments, n is 7. In some embodiments, n is 8.

[00188] As defined herein, t is 1, 2, 3, 4, 5, 6, 7 or 8. In some embodiments, t is 2, 3, 4, 5, 6, 7 or 8. In some embodiments, t is 3, 4, 5, 6, 7 or 8. In some embodiments, t is 4, 5, 6, 7 or 8. In some embodiments, t is 5, 6, 7 or 8. In some embodiments, t is 6, 7 or 8. In some embodiments, t is 7 or 8. In some embodiments, t is 1. In some embodiments, t is 2. In some embodiments, t is 3. In some embodiments, t is 4. In some embodiments, t is 5. In some embodiments, t is 6. In some embodiments, t is 7. In some embodiments, t is 8.

[00189] In some embodiments, at least one of m and t is greater than 2. In some embodiments, at least one of m and t is greater than 3. In some embodiments, at least one of m and t is greater than 4. In some embodiments, at least one of m and t is greater than 5. In some embodiments, at least one of m and t is greater than 6. In some embodiments, at least one of m and t is greater than 7. In some embodiments, each one of m and t is greater than 2. In some embodiments, each one of m and t is greater than 3. In some embodiments, each one of m and t is greater than 4. In some embodiments, each one of m and t is greater than 5. In some embodiments, each one of m and t is greater than 6. In some embodiments, each one of m and t is greater than 7.

[00190] In some embodiments, n is 1, and at least one of m and t is greater than 1. In some embodiments, n is 1 and each of m and t is independent greater than 1. In some embodiments, m>n and t>n. In some embodiments, (*S*p)m(*R*p)n(*S*p)t is $(Sp)_2Rp(Sp)_2$. In some embodiments, (*S*p)t(*R*p)n(*S*p)m is $(Sp)_2Rp(Sp)_2$. In some embodiments, (*S*p)t(*R*p)n(*S*p)m is $SpRp(Sp)_2$. In some embodiments, (*N*p)t(*R*p)n(*S*p)m is (*N*p)t*R*p(*S*p)m. In some embodiments, (*N*p)t(*R*p)n(*S*p)m is $(Np)_2Rp(Sp)m$. In some embodiments, (*N*p)t(*R*p)n(*S*p)m is $(Rp)_2Rp(Sp)m$. In some embodiments, (*N*p)t(*R*p)n(*S*p)m is $(Sp)_2Rp(Sp)m$. In some embodiments, (*N*p)t(*R*p)n(*S*p)m is *R*p*S*p*R*p(*S*p)m. In some embodiments, (*N*p)t(*R*p)n(*S*p)m is *S*p*R*p*R*p(*S*p)m.

[00191] In some embodiments, (*S*p)t(*R*p)n(*S*p)m is *S*p*R*p*S*p*S*p. In some embodiments, (*S*p)t(*R*p)n(*S*p)m is $(Sp)_2Rp(Sp)_2$. In some embodiments, (*S*p)t(*R*p)n(*S*p)m is $(Sp)_3Rp(Sp)_3$. In some embodiments, (*S*p)t(*R*p)n(*S*p)m is $(Sp)_4Rp(Sp)_4$. In some embodiments, (*S*p)t(*R*p)n(*S*p)m is (*S*p)t*R*p$(Sp)_5$. In some embodiments, (*S*p)t(*R*p)n(*S*p)m is $SpRp(Sp)_5$. In some embodiments, (*S*p)t(*R*p)n(*S*p)m is $(Sp)_2Rp(Sp)_5$. In some embodiments, (*S*p)t(*R*p)n(*S*p)m is $(Sp)_3Rp(Sp)_5$. In some embodiments, (*S*p)t(*R*p)n(*S*p)m is $(Sp)_4Rp(Sp)_5$. In some embodiments, (*S*p)t(*R*p)n(*S*p)m is $(Sp)_5Rp(Sp)_5$.

[00192] In some embodiments, (*S*p)m(*R*p)n(*S*p)t is $(Sp)_2Rp(Sp)_2$. In some embodiments, (*S*p)m(*R*p)n(*S*p)t is $(Sp)_3Rp(Sp)_3$. In some embodiments, (*S*p)m(*R*p)n(*S*p)t is $(Sp)_4Rp(Sp)_4$. In some embodiments, (*S*p)m(*R*p)n(*S*p)t is (*S*p)m*R*p$(Sp)_5$. In some embodiments, (*S*p)m(*R*p)n(*S*p)t is $(Sp)_2Rp(Sp)_5$. In some embodiments, (*S*p)m(*R*p)n(*S*p)t is $(Sp)_3Rp(Sp)_5$. In some embodiments, (*S*p)m(*R*p)n(*S*p)t is $(Sp)_4Rp(Sp)_5$. In some embodiments, (*S*p)m(*R*p)n(*S*p)t is $(Sp)_5Rp(Sp)_5$.

[00193] In some embodiments, the core region of a wing-core-wing motif comprises at least one *R*p internucleotidic linkage. In some embodiments, the core region of a wing-core-wing motif comprises at least one *R*p phosphorothioate internucleotidic linkage. In some embodiments, the core region of a wing-core-wing motif comprises at least two *R*p internucleotidic linkages. In some embodiments, the core region of a wing-core-wing motif comprises at least two *R*p phosphorothioate internucleotidic linkages. In some embodiments, the core region of a wing-core-wing motif comprises at least three *R*p internucleotidic linkages. In some embodiments, the core region of a wing-core-wing motif comprises at least three *R*p phosphorothioate internucleotidic linkages. In some embodiments, the core region of a wing-core-wing motif comprises at least 4, 5, 6, 7, 8, 9, or 10 *R*p internucleotidic linkages. In some

embodiments, the core region of a wing-core-wing motif comprises at least 4, 5, 6, 7, 8, 9, or 10 *R*p phosphorothioate internucleotidic linkages.

[00194] In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each "X" wing region are 2'-MOE-modified residues. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues in the core "Y" region are 2'-deoxyribonucleotide residues. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif, wherein all internucleosidic linkages are phosphorothioate internucleosidic linkages. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif, wherein all internucleosidic linkages are chiral phosphorothioate internucleosidic linkages. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each "X" wing region are 2'-MOE-modified residues, the residues in the core "Y" region are 2'-deoxyribonucleotide, and all internucleosidic linkages are chiral phosphorothioate internucleosidic linkages.

[00195] In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each "X" wing region are not 2'-MOE-modified residues. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues in the core "Y" region are 2'-deoxyribonucleotide residues. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif, wherein all internucleosidic linkages are phosphorothioate internucleosidic linkages. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif, wherein all internucleosidic linkages are chiral phosphorothioate internucleosidic linkages. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each "X" wing region are not 2'-MOE-modified residues, the residues in the core "Y" region are 2'-deoxyribonucleotide, and all internucleosidic linkages are chiral phosphorothioate internucleosidic linkages.

[00196] In certain embodiments, provided chirally controlled (and/or stereochemically pure) preparations comprise oligonucleotides of base sequence GCCTCAGTCTGCTTCGCACC.

[00197] In some embodiments, the present invention provides stereochemical design parameters for oligonucleotides. That is, among other things, the present disclosure demonstrates impact of stereochemical structure at different positions along an oligonucleotide chain, for example on stability and/or activity of the oligonucleotide, including on interaction of the oligonucleotide with a cognate ligand and/or with a processing enzyme. The present invention specifically provides oligonucleotides whose structure incorporates or reflects the

design parameters. Such oligonucleotides are new chemical entities relative to stereorandom preparations having the same base sequence and length.

[00198] In some embodiments, the present invention provides stereochemical design parameters for antisense oligonucleotides. In some embodiments, the present invention specifically provides design parameter for oligonucleotides that may be bound and/or cleaved by RNaseH. In ome embodiments, the present invention provides stereochemical design parameters for siRNA oligonucleotides. In some embodiments, the present invention specifically provides design parameters for oligonucleotides that may be bound and/or cleaved by, e.g., DICER, Argonaute proteins (e.g., Argonaute-1 and Argonaute-2), etc.

[00199] In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral. In some embodiments, at least two of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least three of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least four of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least five of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least six of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least seven of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least eight of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least nine of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, one of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral. In some embodiments, two of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, three of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, four of the

first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, five of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, six of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, seven of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, eight of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, nine of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, ten of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral.

[00200] In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral. In some embodiments, at least two of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least three of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least four of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least five of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least six of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least seven of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, one of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral. In some embodiments, two of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, three of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, four of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, five of the first, second, third, fifth, seventh, eighteenth, nineteenth and

twentieth internucleotidic linkages are chiral. In some embodiments, six of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, seven of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, eight of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral.

[00201] In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral, and at least one internucleotidic linkage is achiral. In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral, and at least one internucleotidic linkage is achiral. In some embodiments, at least two internucleotidic linkages are achiral. In some embodiments, at least three internucleotidic linkages are achiral. In some embodiments, at least four internucleotidic linkages are achiral. In some embodiments, at least five internucleotidic linkages are achiral. In some embodiments, at least six internucleotidic linkages are achiral. In some embodiments, at least seven internucleotidic linkages are achiral. In some embodiments, at least eight internucleotidic linkages are achiral. In some embodiments, at least nine internucleotidic linkages are achiral. In some embodiments, at least 10 internucleotidic linkages are achiral. In some embodiments, at least 11 internucleotidic linkages are achiral. In some embodiments, at least 12 internucleotidic linkages are achiral. In some embodiments, at least 13 internucleotidic linkages are achiral. In some embodiments, at least 14 internucleotidic linkages are achiral. In some embodiments, at least 15 internucleotidic linkages are achiral. In some embodiments, at least 16 internucleotidic linkages are achiral. In some embodiments, at least 17 internucleotidic linkages are achiral. In some embodiments, at least 18 internucleotidic linkages are achiral. In some embodiments, at least 19 internucleotidic linkages are achiral. In some embodiments, at least 20 internucleotidic linkages are achiral. In some embodiments, one internucleotidic linkage is achiral. In some embodiments, two internucleotidic linkages are achiral. In some embodiments, three internucleotidic linkages are achiral. In some embodiments, four internucleotidic linkages are achiral. In some embodiments, five internucleotidic linkages are achiral. In some embodiments, six internucleotidic linkages are achiral. In some embodiments, seven internucleotidic linkages are achiral. In some

embodiments, eight internucleotidic linkages are achiral. In some embodiments, nine internucleotidic linkages are achiral. In some embodiments, 10 internucleotidic linkages are achiral. In some embodiments, 11 internucleotidic linkages are achiral. In some embodiments, 12 internucleotidic linkages are achiral. In some embodiments, 13 internucleotidic linkages are achiral. In some embodiments, 14 internucleotidic linkages are achiral. In some embodiments, 15 internucleotidic linkages are achiral. In some embodiments, 16 internucleotidic linkages are achiral. In some embodiments, 17 internucleotidic linkages are achiral. In some embodiments, 18 internucleotidic linkages are achiral. In some embodiments, 19 internucleotidic linkages are achiral. In some embodiments, 20 internucleotidic linkages are achiral. In some embodiments, a single oligonucleotide of a provided composition comprises a region in which all internucleotidic linkages, except the at least one of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages which is chiral, are achiral.

[00202] In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral, and at least one internucleotidic linkage is phosphate. In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral, and at least one internucleotidic linkage is phosphate. In some embodiments, at least two internucleotidic linkages are phosphate. In some embodiments, at least three internucleotidic linkages are phosphate. In some embodiments, at least four internucleotidic linkages are phosphate. In some embodiments, at least five internucleotidic linkages are phosphate. In some embodiments, at least six internucleotidic linkages are phosphate. In some embodiments, at least seven internucleotidic linkages are phosphate. In some embodiments, at least eight internucleotidic linkages are phosphate. In some embodiments, at least nine internucleotidic linkages are phosphate. In some embodiments, at least 10 internucleotidic linkages are phosphate. In some embodiments, at least 11 internucleotidic linkages are phosphate. In some embodiments, at least 12 internucleotidic linkages are phosphate. In some embodiments, at least 13 internucleotidic linkages are phosphate. In some embodiments, at least 14 internucleotidic linkages are phosphate. In some embodiments, at least 15 internucleotidic linkages are phosphate. In some embodiments, at least 16 internucleotidic linkages are phosphate. In some embodiments, at least

17 internucleotidic linkages are phosphate. In some embodiments, at least 18 internucleotidic linkages are phosphate. In some embodiments, at least 19 internucleotidic linkages are phosphate. In some embodiments, at least 20 internucleotidic linkages are phosphate. In some embodiments, one internucleotidic linkage is phosphate. In some embodiments, two internucleotidic linkages are phosphate. In some embodiments, three internucleotidic linkages are phosphate. In some embodiments, four internucleotidic linkages are phosphate. In some embodiments, five internucleotidic linkages are phosphate. In some embodiments, six internucleotidic linkages are phosphate. In some embodiments, seven internucleotidic linkages are phosphate. In some embodiments, eight internucleotidic linkages are phosphate. In some embodiments, nine internucleotidic linkages are phosphate. In some embodiments, 10 internucleotidic linkages are phosphate. In some embodiments, 11 internucleotidic linkages are phosphate. In some embodiments, 12 internucleotidic linkages are phosphate. In some embodiments, 13 internucleotidic linkages are phosphate. In some embodiments, 14 internucleotidic linkages are phosphate. In some embodiments, 15 internucleotidic linkages are phosphate. In some embodiments, 16 internucleotidic linkages are phosphate. In some embodiments, 17 internucleotidic linkages are phosphate. In some embodiments, 18 internucleotidic linkages are phosphate. In some embodiments, 19 internucleotidic linkages are phosphate. In some embodiments, 20 internucleotidic linkages are phosphate. In some embodiments, a single oligonucleotide of a provided composition comprises a region in which all internucleotidic linkages, except the at least one of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages which is chiral, are phosphate.

[00203] In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral, and at least 10% of all the internucleotidic linkages in the region is achiral. In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral, and at least 10% of all the internucleotidic linkages in the region are achiral. In some embodiments, at least 20% of all the internucleotidic linkages in the region are achiral. In some embodiments, at least 30% of all the internucleotidic linkages in the region are achiral. In some

embodiments, at least 40% of all the internucleotidic linkages in the region are achiral. In some embodiments, at least 50% of all the internucleotidic linkages in the region are achiral. In some embodiments, at least 60% of all the internucleotidic linkages in the region are achiral. In some embodiments, at least 70% of all the internucleotidic linkages in the region are achiral. In some embodiments, at least 80% of all the internucleotidic linkages in the region are achiral. In some embodiments, at least 90% of all the internucleotidic linkages in the region are achiral. In some embodiments, at least 50% of all the internucleotidic linkages in the region are achiral. In some embodiments, an achiral internucleotidic linkage is a phosphate linkage. In some embodiments, each achiral internucleotidic linkage in a phosphate linkage.

[00204] In some embodiments, the first internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the first internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the second internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the second internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the third internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the third internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the fifth internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the fifth internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the seventh internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the seventh internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the eighth internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the eighth internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the ninth internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the ninth internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the eighteenth internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the eighteenth internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the nineteenth internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the nineteenth

internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the twentieth internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the twentieth internucleotidic linkage of the region is an *R*p modified internucleotidic linkage.

[00205] In some embodiments, the region has a length of at least 21 bases. In some embodiments, the region has a length of 21 bases. In some embodiments, a single oligonucleotide in a provided composition has a length of at least 21 bases. In some embodiments, a single oligonucleotide in a provided composition has a length of 21 bases.

[00206] In some embodiments, a chiral internucleotidic linkage has the structure of formula **I**. In some embodiments, a chiral internucleotidic linkage is phosphorothioate. In some embodiments, each chiral internucleotidic linkage in a single oligonucleotide of a provided composition independently has the structure of formula **I**. In some embodiments, each chiral internucleotidic linkage in a single oligonucleotide of a provided composition is a phosphorothioate.

[00207] As known by a person of ordinary skill in the art and described in the disclosure, various modifications can be introduced to the 2'-position of the sugar moiety. Commonly used 2'-modifications include but are not limited to 2'–OR^1, wherein R^1 is not hydrogen. In some embodiments, a modification is 2'−OR, wherein R is optionally substituted aliphatic. In some embodiments, a modification is 2'−OMe. In some embodiments, a modification is 2'-*O*-MOE. In some embodiments, the present invention demonstrates that inclusion and/or location of particular chirally pure internucleotidic linkages can provide stability improvements comparable to or better than those achieved through use of modified backbone linkages, bases, and/or sugars. In some embodiments, a provided single oligonucleotide of a provided composition has no modifications on the sugars. In some embodiments, a provided single oligonucleotide of a provided composition has no modifications on 2'-positions of the sugars (i.e., the two groups at the 2'-position are either −H/−H or −H/−OH). In some embodiments, a provided single oligonucleotide of a provided composition does not have any 2'-MOE modifications.

[00208] In some embodiments, a single oligonucleotide in a provided composition is a better substrate for Argonaute proteins (*e.g.*, hAgo-1 and hAgo-2) compared to stereorandom oligonucleotide compositions. Selection and/or location of chirally pure linkages as described in

the present closure are useful design parameters for oligonucleotides that interacting with such proteins, such as siRNA.

[00209] In some embodiments, a single oligonucleotide in a provided composition has at least about 25% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 30% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 35% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 40% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 45% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 50% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 55% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 60% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 65% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 70% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 75% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 80% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 85% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 90% of its internucleotidic linkages in *S*p configuration.

[00210] In some embodiments, a single oligonucleotide in a provided composition is not an oligonucleotide selected from:

143	(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	$(SSR)_3$-SS
ONT-87	(*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p)-Gs5mCs5mCsTs5mCsAs GsTs5mCsTsGs5mCsTsTs5mCsGs5mCsAs 5mCs5mC	(5R-$(SSR)_3$-5R)

underlined nucleotides are 2′-modified.

[00211] In some embodiments, a single oligonucleotide in a provided composition is not an oligonucleotide selected from:

143	(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	$(SSR)_3$-SS
ONT-87	(*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p)-Gs5mCs5mCsTs5mCsAs GsTs5mCsTsGs5mCsTsTs5mCsGs5mCsAs 5mCs5mC	$(5R-(SSR)_3-5R)$

underlined nucleotides are 2′-*O*-MOE modified.

[00212] In some embodiments, a single oligonucleotide in a provided composition is not an oligonucleotide selected from:

ONT-106	(*R*p)- uucuAGAccuGuuuuGcuudT***s***dT	PCSK9 sense
ONT-107	(*S*p)- uucuAGAccuGuuuuGcuudT***s***dT	PCSK9 sense
ONT-108	(*R*p)- AAGcAAAAcAGGUCuAGAAdT**s**dT	PCSK9 antisense
ONT-109	(*S*p)- AAGcAAAAcAGGUCuAGAAdT***s***dT	PCSK9 antisense
ONT-110	(*R*p, *R*p)- a***s***AGcAAAAcAGGUCuAGAAdT***s***dT	PCSK9 antisense
ONT-111	(*S*p, *R*p)- a***s***GcAAAAcAGGUCuAGAAdT***s***dT	PCSK9 antisense
ONT-112	(*S*p, *S*p)- a***s***GcAAAAcAGGUCuAGAAdT***s***dT	PCSK9 antisense
ONT-113	(*R*p, *S*p)- a***s***GcAAAAcAGGUCuAGAAdT***s***dT	PCSK9 antisense

wherein lower case letters represent 2'-OMe RNA residues; capital letters represent 2'-OH RNA residues; and bolded and "***s***" indicates a phosphorothioate moiety; and

PCSK9 (1)	(All (*S*p))- ususcsusAsGsAscscsusGsususususGscsususdTsdT
PCSK9 (2)	(All (*R*p))- ususcsusAsGsAscscsusGsususususGscsususdTsdT
PCSK9 (3)	(All (*S*p))- usucuAsGsAsccuGsuuuuGscuusdTsdT
PCSK9 (4)	(All (*R*p))- usucuAsGsAsccuGsuuuuGscuusdTsdT
PCSK9 (5)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)-ususcsusAsGsAscscsusGsususususGscsususdTsdT
PCSK9 (6)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-ususcsusAsGsAscscsusGsususususGscsususdTsdT

wherein lower case letters represent 2'-OMe RNA residues; capital letters represent RNA residues; d = 2'-deoxy residues; and "s" indicates a phosphorothioate moiety; and

PCSK9 (7)	(All (*R*p))- AsAsGscsAsAsAsAscsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (8)	(All (*S*p))- AsAsGscsAsAsAsAscsAsGsGsUsCsusAsGsAsAsdTsdT

PCSK9 (9)	(All (*R*p))- AsAGcAAAAcsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (10)	(All (*S*p))- AsAGcAAAAcsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (11)	(All (*R*p))- AAsGscsAsAsAsAscAGGUCuAGAAdTsdT
PCSK9 (12)	(All (*S*p))- AAsGscsAsAsAsAscAGGUCuAGAAdTsdT
PCSK9 (13)	(All (*R*p))- AsAsGscAsAsAsAscAsGsGsUsCsuAsGsAsAsdTsdT
PCSK9 (14)	(All (*S*p))- AsAsGscAsAsAsAscAsGsGsUsCsuAsGsAsAsdTsdT
PCSK9 (15)	(All (*R*p))- AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (16)	(All (*S*p))- AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (17)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)-AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (18)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT

wherein lower case letters represent 2'-OMe RNA residues; capital letters represent RNA residues; d = 2'-deoxy residues; "s" indicates a phosphorothioate moiety; and

PCSK9 (19)	(All (*R*p))- UfsusCfsusAfsgsAfscsCfsusGfsusUfsusUfsgsCfsusUfsdTsdT
PCSK9 (20)	(All (*S*p))- UfsusCfsusAfsgsAfscsCfsusGfsusUfsusUfsgsCfsusUfsdTsdT
PCSK9 (21)	(All (*R*p))- UfsuCfsuAfsgAfscCfsuGfsuUfsuUfsgCfsuUfsdTsdT
PCSK9 (22)	(All (*S*p))- UfsuCfsuAfsgAfscCfsuGfsuUfsuUfsgCfsuUfsdTsdT
PCSK9 (23)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)- UfsusCfsusAfsgsAfscsCfsusGfsusUfsusUfsgsCfsusUfsdTsdT
PCSK9 (24)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)- UfsusCfsusAfsgsAfscsCfsusGfsusUfsusUfsgsCfsusUfsdTsdT

wherein lower case letters represent 2'-OMe RNA residues; capital letters represent 2'-F RNA residues; d = 2'-deoxy residues; and "s" indicates a phosphorothioate moiety; and

PCSK9 (25)	(All (*R*p))- asAfsgsCfsasAfsasAfscsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (26)	(All (*S*p))- asAfsgsCfsasAfsasAfscsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (27)	(All (*R*p))- asAfgCfaAfaAfcsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (28)	(All (*S*p))- asAfgCfaAfaAfcsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (29)	(All (*R*p))- asAfsgCfsaAfsaAfscAfsgGfsuCfsuAfsgAfsadTsdT

PCSK9 (30)	(All (*S*p))- asAfsgCfsaAfsaAfscAfsgGfsuCfsuAfsgAfsadTsdT
PCSK9 (31)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)-asAfgCfaAfasAfscAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (32)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-asAfgCfaAfasAfscAfsgsGfsusCfsusAfsgsAfsasdTsdT

wherein lower case letters represent 2'-OMe RNA residues; capital letters represent 2'-F RNA residues; d = 2'-deoxy residues; and "s" indicates a phosphorothioate moiety.

[00213] In some embodiments, a single oligonucleotide in a provided composition is not an oligonucleotide selected from: $d[A_RC_SA_RC_SA_RC_SA_RC_SA_RC]$, $d[C_SC_SC_SC_RC_RC_SC_SC_SC_SC]$, $d[C_SC_SC_SC_SC_SC_SC_RC_RC_SC]$ and $d[C_SC_SC_SC_SC_SC_SC_RC_RC_SC_SC]$, wherein R is *R*p phosphorothioate linkage, and S is *S*p phosphorothioate linkage.

[00214] In some embodiments, a single oligonucleotide in a provided composition is not an oligonucleotide selected from: $GGA_RT_SG_RT_ST_R{}^mC_STCGA$, $GGA_RT_RG_ST_ST_R{}^mC_RTCGA$, $GGA_ST_SG_RT_RT_S{}^mC_STCGA$, wherein R is *R*p phosphorothioate linkage, S is *S*p phosphorothioate linkage, all other linkages are PO, and each ^mC is a 5-methyl cytosine modified nucleoside.

[00215] In some embodiments, a single oligonucleotide in a provided composition is not an oligonucleotide selected from : $T_kT_k{}^mC_kAGT^mCATGA^mCT_kT^mC_k{}^mC_k$, wherein each nucleoside followed by a subscript 'k' indicates a (S)-cEt modification, R is *R*p phosphorothioate linkage, S is *S*p phosphorothioate linkage, each ^mC is a 5-methyl cytosine modified nucleoside, and all internucleoside linkages are phosphorothioates (PS) with stereochemistry patterns selected from RSSSRSRRRS, RSSSSSSSSS, SRRSRSSSSR, SRSRSSRSSR, RRRSSSRSSS, RRRSRSSRSR, RRSSSRSRSR, SRSSSRSSSS, SSRRSSRSRS, SSSSSSRRSS, RRRSSRRRSR, RRRRSSSSRS, SRRSRRRRRR, RSSRSSRRRR, RSRRSRRSRR, RRSRSSRSRS, SSRRRRRSRR, RSRRSRSSSR, RRSSRSRRRR, RRSRSRRSSS, RRSRSSSRRR, RSRRRRSRSR, SSRSSSRRRS, RSSRSRSRSR, RSRSRSSRSS, RRRSSRRSRS, SRRSSRRSRS, RRRRSRSRRR, SSSSRRRRSR, RRRRRRRRRR and SSSSSSSSSS.

In some embodiments, a single oligonucleotide in a provided composition is not an oligonucleotide selected from : $T_kT_k{}^mC_kAGT^mCATGA^mCTT_k{}^mC_k{}^mC_k$, wherein each nucleoside followed by a subscript 'k' indicates a (S)-cEt modification, R is *R*p phosphorothioate linkage, S is *S*p phosphorothioate linkage, each ^mC is a 5-methyl cytosine modified nucleoside and all

internucleoside linkages are phosphorothioates (PS) with stereochemistry patterns selected from: RSSSRSRRRS, RSSSSSSSSS, SRRSRSSSSR, SRSRSSRSSR, RRRSSSRSSS, RRRSRSSRSR, RRSSSRSRSR, SRSSSRSSSS, SSRRSSRSRS, SSSSSSRRSS, RRRSSRRRSR, RRRRSSSSRS, SRRSRRRRRR, RSSRSSRRRR, RSRRSRRSRR, RRSRSSRSRS, SSRRRRRSRR, RSRRSRSSSR, RRSSRSRRRR, RRSRSRRSSS, RRSRSSSRRR, RSRRRRSRSR, SSRSSSRRRS, RSSRSRSRSR, RSRSRSSRSS, RRRSSRRSRS, SRRSSRRSRS, RRRRSRSRRR, SSSSRRRRSR, RRRRRRRRRR and SSSSSSSSSS.

Modified Oligonucleotide Structures

[00216] As noted above, in light of the usefulness of oligonucleotide compositions in various applications and indications, those skilled in the art have endeavoured to develop modifications of oligonucleotide structures that may have preferred or desirable characteristics or attributes as compared with naturally-occurring oligonucleotide molecules, for example as used in particular applications and indications. Exemplary such modifications are described below.

[00217] WO2010/141471 (herein "Traversa I") teaches the modification of different types of nucleic acid constructs modified to have a reduced net polyanionic charge. WO2010/039543 (herein "Travera II") teaches compositions and methods for making neutral polynucleotides (NNs) with reduced polyanionic charge. WO2008/008476 (herein, "Traversa III") describes the synthesis of SATE (Imbach-type) phosphate prodrugs. Traversa I, II, and III do not teach chirally controlled oligonucleotides, compositions thereof, and methods of making and using the same, as described by the present invention.

[00218] WO2010/072831 (herein "Girindus et al.") also teaches the modification of oligonucleotides. In particular, Girindus et al. teaches the use of sulfurization reagents to generate phosphorothioate triesters as prodrugs. Girindus et al. does not teach chirally controlled oligonucleotides, compositions thereof, and methods of making and using the same, as described by the present invention.

[00219] Similarly, WO2004/085454 (herein "Avecia I") teaches the preparation of phosphorothioate oligonucleotides through, e.g., transient silylation of poly-*H*-phosphonate diesters. WO2001/027126 (herein "Avecia II") teaches processes for the solid phase synthesis of phosphotriester oligonucleotides by coupling *H*-phosphonate monomers to a solid supported 5'-hydroxyl oligonucleotide and further sulfurization of the resulting *H*-phosphonte diester into a

phosphorothioate triester. The disclosure of WO2001/064702 (herein "Avecia III") is similar to Avecia II and further describes solid-phase synthesis on different solid supports. Avecia I, II, and III do not teach chirally controlled oligonucleotides, compositions thereof, and methods of making and using the same, as described by the present invention.

[00220] WO1997/006183 (herein "Chiron") teaches oligonucleotides with cationic internucleotide linkages comprising asymmetric phosphorus, such as stereopure amidates. Chiron teaches stereopure oligonucleotides obtained via crystallization of a mixture of diastereomers or via resolution using, e.g., column chromatography. Chiron does not teach chirally controlled oligonucleotides, compositions thereof, and methods of making and using the same, as described by the present invention.

[00221] WO2009/146123 (herein "Spring Bank I") teaches compositions and methods for treating viral infections using substituted phosphate oligonucleotides and phosphorothioate triesters. WO2007/070598 (herein "Spring Bank II") teaches phosphotriester prodrugs as antiviral nucleic acids and teaches the synthesis of phosphorothioate prodrugs. Spring Bank I and II do not teach chirally controlled oligonucleotides, compositions thereof, and methods of making and using the same, as described by the present invention.

[00222] EP0779893 (herein "Hybridon") teaches lipophilic prodrugs for the increased cellular uptake of antisense oligonucleotides and observes that *R*p and *S*p phosphorothioates and phosphorothioate triester dimers can have different enzymatic stability properties. Hybridon does not teach chirally controlled oligonucleotides, compositions thereof, and methods of making and using the same, as described by the present invention.

[00223] WO1997/047637 (herein "Imbach I") teaches generally the Imbach "SATE" (S-acyl thioethyl) prodrug oligonucleotide compositions and methods. Imbach I describes, for example, bioreversible phosphotriester prodrugs and the preparation of certain prodrug oligonucleotides using post-synthestic alkylation or prodrug-group-containing phosphoramidites. US 6,124,445 (herein "Imbach II") teaches modified antisense and chimeric prodrug oligonucleotides. Imbach I and II do not teach chirally controlled oligonucleotides, compositions thereof, and methods of making and using the same, as described by the present invention.

[00224] WO2006/065751 (herein "Beaucage") teaches CpG oligonucleotide phosphorothioate prodrugs that comprise thermolabile substituents (which substituents are introduced via a phosphoramidite monomer), and applications thereof. Beaucage does not teach

chirally controlled oligonucleotides, compositions thereof, and methods of making and using the same, as described by the present invention.

[00225] Takeshi Wada et al. developed novel methods for the stereo-controlled synthesis of P-chiral nucleic acids using amidite chiral auxiliaries (JP4348077, WO2005/014609, WO2005/092909, and WO2010/064146, cumulatively referred to herein as "Wada I"). In particular, WO2010/064146 (referred to herein as "Wada II") discloses methods for synthesizing phosphorus atom-modified nucleic acids wherein the stereochemical configuration at phosphorus is controlled. However, the methods of Wada II are limited in that they do not provide for individual P-modification of each chiral linkage phosphorus in a controlled and designed manner. That is, the methods for P-modified linkages of Wada II provide for the generation of a condensed intermediate poly *H*-phosphonate oligonucleotide strand that, once built to a desired length, is mass modified at the linkage phosphorus to provide, e.g., a desired phosphorothioate diester, phosphoramidate or boranophosphate or other such phosphorus atom-modified nucleic acids (referred to as Route B in the document – Scheme 6, page 36). Furthermore, the *H*-phosphonate oligonucleotide strands of Wada II are of shorter lengths (e.g., dimer trimer, or tetramer). Combined with the fact that there is no capping step in route B, which generally presents low crude purity as a result of the accumulation of "n-1"-type byproducts, the Wada II route contains limitations in regards of the synthesis of longer oligonucleotides. While Wada II contemplates generally that a particular oligonucleotide could be envisaged to contain different modifications at each linkage phosphorus, Wada II does not describe or suggest methods for controlled iterative installation of such modifications, as are described herein. To the extent that Wada II depicts a synthetic cycle that does not require an *H*-phosphonate intermediate oligonucleotide to be completely assembled prior to modification at the linkage phosphorus (therein referred to as Route A, page 35, Scheme 5, "Synthesis of a nucleic acid comprising a chiral X-phosphonate moiety of Formula 1 via Route A"), this general disclosure does not teach certain key steps that are required to install certain P-modifications, as provided by the present invention, and especially not with any degree of efficiency and versatility such that this cycle would be useful in the synthesis of chirally controlled P-modified oligonucleotides, and especially oligonucleotides of longer lengths.

[00226] At least one such inefficiency of Wada II is noted by Wada et al. in WO2012/039448 (herein "Wada III"). Wada III teaches novel chiral auxiliaries for use in Wada

II methods to produce *H*-phosphonate oligonucleotides that, once built, can be subsequently modified to provide, *inter alia*, phosphorothioates and the like. Wada et al. observe in Wada III that the four types of chiral auxiliaries disclosed in Wada II formed strong bonds with phosphorus at the linkage phosphorus and thus did not allow for efficient removal. Wada III notes that removal of the Wada II chiral auxiliaries required harsh conditions, which conditions were prone to compromising the integrity of the product oligonucleotide. Wada III observes that this is especially problematic when synthesizing long chain oligonucleotides for at least the reason that as the degradation reaction(s) proceed, additional byproducts are generated that can further react with and degrade the product oligonucleotide. Wada III therefore provides chiral auxiliaries that can be more efficiently cleaved from the oligonucleotide under mild acidic conditions by way of an S_N1 mechanism releasing the *H*-phosphonate internucleotide linkage (route B), or under relatively mild basic conditions, by a β-elimation pathway.

[00227] One of skill in the chemical and synthetic arts will immediately appreciate the complexities associated with generating chirally controlled oligonucleotides such as those provided by the present invention. For instance, in order to synthesize and isolate a chirally controlled oligonucleotide, conditions for each monomer addition must be designed such that (1) the chemistry is compatible with every portion of the growing oligonucleotide; (2) the byproducts generated during each monomer addition do not compromise the structural and stereochemical integrity of the growing oligonucleotide; and (3) the crude final product composition is a composition which allows for isolation of the desired chirally controlled oligonucleotide product.

[00228] Oligonucleotide phosphorothioates have shown therapeutic potential (Stein *et al.*, Science (1993), 261:1004-12; Agrawal *et al.*, Antisence Res. and Dev. (1992), 2:261-66; Bayever *et al.*, Antisense Res. and Dev. (1993), 3:383-390). Oligonucleotide phosphorothioates prepared without regard to the stereochemistry of the phosphorothioate exist as a mixture of 2^n diastereomers, where n is the number of internucleotide phosphorothioates linkages. The chemical and biological properties of these diastereomeric phosphorothioates can be distinct. For example, Wada *et al* (Nucleic Acids Symposium Series No. 51 p. 119-120; doi:10.1093/nass/nrm060) found that stereodefined-(*R*p)-(Ups)9U/(Ap)9A duplex showed a higher Tm value than that of natural-(Up)9U/(Ap)9A and stereodefined-(*S*p)-(Ups)9U did not form a duplex. In another example, in a study by Tang *et al.*, (Nucleosides Nucleotides (1995),

14:985-990) stereopure *R*p-oligodeoxyribonucleoside phosphorothioates were found to possess lower stability to nucleases endogenous to human serum that the parent oligodeoxyribonucleoside phosphorothioates with undefined phosphorus chirality.

Chirally Controlled Oligonucleotides and Chirally Controlled Oligonucleotide Compositions

[00229] The present invention provides chirally controlled oligonucleotides, and chirally controlled oligonucleotide compositions which are of high crude purity and of high diastereomeric purity. In some embodiments, the present invention provides chirally controlled oligonucleotides, and chirally controlled oligonucleotide compositions which are of high crude purity. In some embodiments, the present invention provides chirally controlled oligonucleotides, and chirally controlled oligonucleotide compositions which are of high diastereomeric purity.

[00230] In some embodiments, the present invention provides a chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which composition is a substantially pure preparation of a single oligonucleotide in that at least about 10% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

[00231] In some embodiments, the present invention provides chirally controlled oligonucleotide composition of oligonucleotides in that the composition is enriched, relative to a substantially racemic preparation of the same oligonucleotides, for oligonucleotides of a single oligonucleotide type. In some embodiments, the present invention provides chirally controlled oligonucleotide composition of oligonucleotides in that the composition is enriched, relative to a substantially racemic preparation of the same oligonucleotides, for oligonucleotides of a single oligonucleotide type that share:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers.

[00232] In some embodiments, the present invention provides a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type.

[00233] In some embodiments, as understood by a person having ordinary skill in the art, in a substantially racemic (or chirally uncontrolled) preparation of oligonucleotides, all or most coupling steps are not chirally controlled in that the coupling steps are not specifically conducted to provide enhanced stereoselectivity. An exemplary substantially racemic preparation of oligonucleotides is the preparation of phosphorothioate oligonucleotides through sulfurizing phosphite triesters with either tteraethylthiuram disulfide or (TETD) or 3H-1, 2-bensodithiol-3-one 1, 1-dioxide (BDTD), a well-known process in the art. In some embodiments, substantially racemic preparation of oligonucleotides provides substantially racemic oligonucleotide compositions (or chirally uncontrolled oligonucleotide compositions).

[00234] In some embodiments, a chirally controlled oligonucleotide composition is a substantially pure preparation of a oligonucleotide type in that oligonucleotides in the composition that are not of the oligonucleotide type are impurities form the preparation process of said oligonucleotide type, in some case, after certain purification procedures.

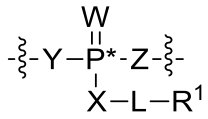
[00235] In some embodiments, the present invention provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages with respect to the chiral linkage phosphorus. In some embodiments, the present invention provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages having the structure of formula **I**. In some embodiments, the present invention provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages with respect to the chiral linkage phosphorus, and one or more phosphate diester linkages. In some embodiments, the present invention provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages having the structure of formula **I**, and one or

more phosphate diester linkages. In some embodiments, the present invention provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages having the structure of formula **I-c**, and one or more phosphate diester linkages. In some embodiments, such oligonucleotides are prepared by using stereoselective oligonucleotide synthesis, as described in this application, to form pre-designed diastereomerically pure internucleotidic linkages with respect to the chiral linkage phosphorus. For instance, in one exemplary oligonucleotide of (*R*p/*S*p, *R*p/*S*p, *R*p/*S*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGs1Cs1As1CsC], the first three internucleotidic linkages are constructed using traditional oligonucleotide synthesis method, and the diastereomerically pure internucleotidic linkages are constructed with stereochemical control as described in this application. Exemplary internucleotidic linkages, including those having structures of formula **I**, are further described below.

[00236] In some embodiments, the present invention provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different stereochemistry and/or different P-modifications relative to one another. In certain embodiments, the present invention provides a chirally controlled oligonucleotide, wherein at least two individual internucleotidic linkages within the oligonucleotide have different P-modifications relative to one another. In certain embodiments, the present invention provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different P-modifications relative to one another, and wherein the chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage. In certain embodiments, the present invention provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different P-modifications relative to one another, and wherein the chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least one phosphorothioate diester internucleotidic linkage. In certain embodiments, the present invention provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different P-modifications relative to one another, and wherein the chirally controlled oligonucleotide comprises at least one phosphorothioate triester internucleotidic linkage. In certain embodiments, the present invention provides a chirally

controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different P-modifications relative to one another, and wherein the chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least one phosphorothioate triester internucleotidic linkage.

[00237] In certain embodiments, the present invention provides a chirally controlled oligonucleotide comprising one or more modified internucleotidic linkages independently having the structure of formula **I**:



(**I**)

wherein each variable is as defined and described below. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising one or more modified internucleotidic linkages of formula **I**, and wherein individual internucleotidic linkages of formula **I** within the oligonucleotide have different P-modifications relative to one another. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising one or more modified internucleotidic linkages of formula **I**, and wherein individual internucleotidic linkages of formula **I** within the oligonucleotide have different –X–L–R^1 relative to one another. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising one or more modified internucleotidic linkages of formula **I**, and wherein individual internucleotidic linkages of formula **I** within the oligonucleotide have different X relative to one another. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising one or more modified internucleotidic linkages of formula **I**, and wherein individual internucleotidic linkages of formula **I** within the oligonucleotide have different –L–R^1 relative to one another.

[00238] In some embodiments, the present invention provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different stereochemistry and/or different P-modifications relative to one another. In some embodiments, the present invention provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different stereochemistry relative to one another, and wherein at least a

portion of the structure of the chirally controlled oligonucleotide is characterized by a repeating pattern of alternating stereochemisty.

[00239] In some embodiments, the present invention provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different P-modifications relative to one another, in that they have different X atoms in their $–XLR^1$ moieties, and/or in that they have different L groups in their $–XLR^1$ moieties, and/or that they have different R^1 atoms in their $–XLR^1$ moieties.

[00240] In some embodiments, the present invention provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different stereochemistry and/or different P-modifications relative to one another and the oligonucleotide has a structure represented by the following formula:

$$[S^Bn1R^Bn2S^Bn3R^Bn4...S^BnxR^Bny]$$

wherein:

each R^B independently represents a block of nucleotide units having the R configuration at the linkage phosphorus;

each S^B independently represents a block of nucleotide units having the S configuration at the linkage phosphorus;

each of n1-ny is zero or an integer, with the requirement that at least one odd n and at least one even n must be non-zero so that the oligonucleotide includes at least two individual internucleotidic linkages with different stereochemistry relative to one another; and

wherein the sum of n1-ny is between 2 and 200, and in some embodiments is between a lower limit selected from the group consisting of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more and an upper limit selected from the group consisting of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, and 200, the upper limit being larger than the lower limit.

[00241] In some such embodiments, each n has the same value; in some embodiments, each even n has the same value as each other even n; in some embodiments, each odd n has the same value each other odd n; in some embodiments, at least two even ns have different values from one another; in some embodiments, at least two odd ns have different values from one another.

[00242] In some embodiments, at least two adjacent ns are equal to one another, so that a provided oligonucleotide includes adjacent blocks of S stereochemistry linkages and R stereochemistry linkages of equal lengths. In some embodiments, provided oligonucleotides include repeating blocks of S and R stereochemistry linkages of equal lengths. In some embodiments, provided oligonucleotides include repeating blocks of S and R stereochemistry linkages, where at least two such blocks are of different lengths from one another; in some such embodiments each S stereochemistry block is of the same length, and is of a different length from each R stereochemistry length, which may optionally be of the same length as one another.

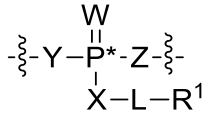
[00243] In some embodiments, at least two skip-adjacent ns are equal to one another, so that a provided oligonucleotide includes at least two blocks of linkages of a first steroechemistry that are equal in length to one another and are separated by a block of linkages of the other stereochemistry, which separating block may be of the same length or a different length from the blocks of first steroechemistry.

[00244] In some embodiments, ns associated with linkage blocks at the ends of a provided oligonucleotide are of the same length. In some embodiments, provided oligonucleotides have terminal blocks of the same linkage stereochemistry. In some such embodiments, the terminal blocks are separated from one another by a middle block of the other linkage stereochemistry.

[00245] In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ is a stereoblockmer. In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ is a stereoskipmer. In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ is a stereoaltmer. In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ is a gapmer.

[00246] In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ is of any of the above described patterns and further comprises patterns of P-modifications. For instance, in some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ and is a stereoskipmer and P-modification skipmer. In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ and is a stereoblockmer and P-modification altmer. In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ and is a stereoaltmer and P-modification blockmer.

[00247] In some embodiments, a provided oligonucleotide of formula [S^Bn1R^Bn2S^Bn3R^Bn4...S^BnxR^Bny] is a chirally controlled oligonucleotide comprising one or more modified internuceotidic linkages independently having the structure of formula I:



(**I**)

wherein:

P* is an asymmetric phosphorus atom and is either *R*p or *S*p;

W is O, S or Se;

each of X, Y and Z is independently –O–, –S–, –N(–L–R^1)–, or L;

L is a covalent bond or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

R^1 is halogen, R, or an optionally substituted C_1–C_{50} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

each R′ is independently –R, -C(O)R, -CO_2R, or –SO_2R, or:

two R′ on the same nitrogen are taken together with their intervening atoms to form an optionally substituted heterocyclic or heteroaryl ring, or

two R′ on the same carbon are taken together with their intervening atoms to form an optionally substituted aryl, carbocyclic, heterocyclic, or heteroaryl ring;

–Cy– is an optionally substituted bivalent ring selected from phenylene, carbocyclylene, arylene, heteroarylene, or heterocyclylene;

each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl; and

each $-\!\!\!\!\!\!\sim$ independently represents a connection to a nucleoside.

[00248] In some embodiments, a chirally controlled oligonucleotide comprises one or more modified internucleotidic phosphorus linkages. In some embodiments, a chirally controlled oligonucleotide comprises, e.g., a phosphorothioate or a phosphorothioate triester linkage. In some embodiments, a chirally controlled oligonucleotide comprises a phosphorothioate triester linkage. In some embodiments, a chirally controlled oligonucleotide comprises at least two phosphorothioate triester linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least three phosphorothioate triester linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least four phosphorothioate triester linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least five phosphorothioate triester linkages. Exemplary such modified internucleotidic phosphorus linkages are described further herein.

[00249] In some embodiments, a chirally controlled oligonucleotide comprises different internucleotidic phosphorus linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least one modified internucleotidic linkage. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least one phosphorothioate triester linkage. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least two phosphorothioate triester linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least three phosphorothioate triester linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least four phosphorothioate triester linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least five phosphorothioate triester linkages. Exemplary such modified internucleotidic phosphorus linkages are described further herein.

[00250] In some embodiments, a phosphorothioate triester linkage comprises a chiral auxiliary, which, for example, is used to control the stereoselectivity of a reaction. In some embodiments, a phosphorothioate triester linkage does not comprise a chiral auxiliary. In some

embodiments, a phosphorothioate triester linkage is intentionally maintained until and/or during the administration to a subject.

[00251] In some embodiments, a chirally controlled oligonucleotide is linked to a solid support. In some embodiments, a chirally controlled oligonucleotide is cleaved from a solid support.

[00252] In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least two consecutive modified internucleotidic linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least two consecutive phosphorothioate triester internucleotidic linkages.

[00253] In some embodiments, a chirally controlled oligonucleotide is a blockmer. In some embodiments, a chirally controlled oligonucleotide is a stereoblockmer. In some embodiments, a chirally controlled oligonucleotide is a P-modification blockmer. In some embodiments, a chirally controlled oligonucleotide is a linkage blockmer.

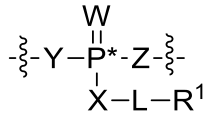
[00254] In some embodiments, a chirally controlled oligonucleotide is an altmer. In some embodiments, a chirally controlled oligonucleotide is a stereoaltmer. In some embodiments, a chirally controlled oligonucleotide is a P-modification altmer. In some embodiments, a chirally controlled oligonucleotide is a linkage altmer.

[00255] In some embodiments, a chirally controlled oligonucleotide is a unimer. In some embodiments, a chirally controlled oligonucleotide is a stereounimer. In some embodiments, a chirally controlled oligonucleotide is a P-modification unimer. In some embodiments, a chirally controlled oligonucleotide is a linkage unimer.

[00256] In some embodiments, a chirally controlled oligonucleotide is a gapmer.

[00257] In some embodiments, a chirally controlled oligonucleotide is a skipmer.

[00258] In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising one or more modified internucleotidic linkages independently having the structure of formula **I**:



(**I**)

wherein:

P* is an asymmetric phosphorus atom and is either *R*p or *S*p;

W is O, S or Se;

each of X, Y and Z is independently –O–, –S–, –N(–L–R^1)–, or L;

L is a covalent bond or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

R^1 is halogen, R, or an optionally substituted C_1–C_{50} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

each R′ is independently –R, -C(O)R, -CO_2R, or –SO_2R, or:

two R′ on the same nitrogen are taken together with their intervening atoms to form an optionally substituted heterocyclic or heteroaryl ring, or

two R′ on the same carbon are taken together with their intervening atoms to form an optionally substituted aryl, carbocyclic, heterocyclic, or heteroaryl ring;

–Cy– is an optionally substituted bivalent ring selected from phenylene, carbocyclylene, arylene, heteroarylene, or heterocyclylene;

each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl; and

each $-\!\!\wwave\!\!-$ independently represents a connection to a nucleoside.

[00259] As defined generally above and herein, P* is an asymmetric phosphorus atom and is either *R*p or *S*p. In some embodiments, P* is *R*p. In other embodiments, P* is *S*p. In some embodiments, an oligonucleotide comprises one or more internucleotidic linkages of formula **I** wherein each P* is independently *R*p or *S*p. In some embodiments, an oligonucleotide comprises one or more internucleotidic linkages of formula **I** wherein each P* is *R*p. In some embodiments, an oligonucleotide comprises one or more internucleotidic linkages of formula **I**

wherein each P* is *S*p. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein P* is *R*p. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein P* is *S*p. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein P* is *R*p, and at least one internucleotidic linkage of formula **I** wherein P* is *S*p.

[00260] As defined generally above and herein, W is O, S, or Se. In some embodiments, W is O. In some embodiments, W is S. In some embodiments, W is Se. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein W is O. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein W is S. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein W is Se.

[00261] As defined generally above and herein, each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl.

[00262] In some embodiments, R is hydrogen. In some embodiments, R is an optionally substituted group selected from C_1–C_6 aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl.

[00263] In some embodiments, R is an optionally substituted C_1–C_6 aliphatic. In some embodiments, R is an optionally substituted C_1–C_6 alkyl. In some embodiments, R is optionally substituted, linear or branched hexyl. In some embodiments, R is optionally substituted, linear or branched pentyl. In some embodiments, R is optionally substituted, linear or branched butyl. In some embodiments, R is optionally substituted, linear or branched propyl. In some embodiments, R is optionally substituted ethyl. In some embodiments, R is optionally substituted methyl.

[00264] In some embodiments, R is optionally substituted phenyl. In some embodiments, R is substituted phenyl. In some embodiments, R is phenyl.

[00265] In some embodiments, R is optionally substituted carbocyclyl. In some embodiments, R is optionally substituted C_3-C_{10} carbocyclyl. In some embodiments, R is optionally substituted monocyclic carbocyclyl. In some embodiments, R is optionally substituted cycloheptyl. In some embodiments, R is optionally substituted cyclohexyl. In some embodiments, R is optionally substituted cyclopentyl. In some embodiments, R is optionally

substituted cyclobutyl. In some embodiments, R is an optionally substituted cyclopropyl. In some embodiments, R is optionally substituted bicyclic carbocyclyl.

[00266] In some embodiments, R is an optionally substituted aryl. In some embodiments, R is an optionally substituted bicyclic aryl ring.

[00267] In some embodiments, R is an optionally substituted heteroaryl. In some embodiments, R is an optionally substituted 5-6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, sulfur, or oxygen. In some embodiments, R is a substituted 5-6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an unsubstituted 5-6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, sulfur, or oxygen.

[00268] In some embodiments, R is an optionally substituted 5 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen or sulfur. In some embodiments, R is an optionally substituted 6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00269] In some embodiments, R is an optionally substituted 5-membered monocyclic heteroaryl ring having 1 heteroatom selected from nitrogen, oxygen, or sulfur. In some embodiments, R is selected from pyrrolyl, furanyl, or thienyl.

[00270] In some embodiments, R is an optionally substituted 5-membered heteroaryl ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R is an optionally substituted 5-membered heteroaryl ring having 1 nitrogen atom, and an additional heteroatom selected from sulfur or oxygen. Exemplary R groups include optionally substituted pyrazolyl, imidazolyl, thiazolyl, isothiazolyl, oxazolyl or isoxazolyl.

[00271] In some embodiments, R is a 6-membered heteroaryl ring having 1–3 nitrogen atoms. In other embodiments, R is an optionally substituted 6-membered heteroaryl ring having 1–2 nitrogen atoms. In some embodiments, R is an optionally substituted 6-membered heteroaryl ring having 2 nitrogen atoms. In certain embodiments, R is an optionally substituted 6-membered heteroaryl ring having 1 nitrogen. Exemplary R groups include optionally substituted pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, or tetrazinyl.

[00272] In certain embodiments, R is an optionally substituted 8–10 membered bicyclic heteroaryl ring having 1–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

In some embodiments, R is an optionally substituted 5,6–fused heteroaryl ring having 1–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, R is an optionally substituted 5,6–fused heteroaryl ring having 1–2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R is an optionally substituted 5,6–fused heteroaryl ring having 1 heteroatom independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted indolyl. In some embodiments, R is an optionally substituted azabicyclo[3.2.1]octanyl. In certain embodiments, R is an optionally substituted 5,6–fused heteroaryl ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted azaindolyl. In some embodiments, R is an optionally substituted benzimidazolyl. In some embodiments, R is an optionally substituted benzothiazolyl. In some embodiments, R is an optionally substituted benzoxazolyl. In some embodiments, R is an optionally substituted indazolyl. In certain embodiments, R is an optionally substituted 5,6–fused heteroaryl ring having 3 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00273] In certain embodiments, R is an optionally substituted 6,6–fused heteroaryl ring having 1–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted 6,6–fused heteroaryl ring having 1–2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, R is an optionally substituted 6,6–fused heteroaryl ring having 1 heteroatom independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted quinolinyl. In some embodiments, R is an optionally substituted isoquinolinyl. According to one aspect, R is an optionally substituted 6,6–fused heteroaryl ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is a quinazoline or a quinoxaline.

[00274] In some embodiments, R is an optionally substituted heterocyclyl. In some embodiments, R is an optionally substituted 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is a substituted 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an unsubstituted 3-7 membered saturated or partially

unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00275] In some embodiments, R is an optionally substituted heterocyclyl. In some embodiments, R is an optionally substituted 6 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted 6 membered partially unsaturated heterocyclic ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted 6 membered partially unsaturated heterocyclic ring having 2 oxygen atom.

[00276] In certain embodiments, R is a 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R is oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, oxepaneyl, aziridineyl, azetidineyl, pyrrolidinyl, piperidinyl, azepanyl, thiiranyl, thietanyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, thiepanyl, dioxolanyl, oxathiolanyl, oxazolidinyl, imidazolidinyl, thiazolidinyl, dithiolanyl, dioxanyl, morpholinyl, oxathianyl, piperazinyl, thiomorpholinyl, dithianyl, dioxepanyl, oxazepanyl, oxathiepanyl, dithiepanyl, diazepanyl, dihydrofuranonyl, tetrahydropyranonyl, oxepanonyl, pyrolidinonyl, piperidinonyl, azepanonyl, dihydrothiophenonyl, tetrahydrothiopyranonyl, thiepanonyl, oxazolidinonyl, oxazinanonyl, oxazepanonyl, dioxolanonyl, dioxanonyl, dioxepanonyl, oxathiolinonyl, oxathianonyl, oxathiepanonyl, thiazolidinonyl, thiazinanonyl, thiazepanonyl, imidazolidinonyl, tetrahydropyrimidinonyl, diazepanonyl, imidazolidinedionyl, oxazolidinedionyl, thiazolidinedionyl, dioxolanedionyl, oxathiolanedionyl, piperazinedionyl, morpholinedionyl, thiomorpholinedionyl, tetrahydropyranyl, tetrahydrofuranyl, morpholinyl, thiomorpholinyl, piperidinyl, piperazinyl, pyrrolidinyl, tetrahydrothiophenyl, or tetrahydrothiopyranyl. In some embodiments, R is an optionally substituted 5 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00277] In certain embodiments, R is an optionally substituted 5–6 membered partially unsaturated monocyclic ring having 1–2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R is an optionally substituted tetrahydropyridinyl, dihydrothiazolyl, dihydrooxazolyl, or oxazolinyl group.

[00278] In some embodiments, R is an optionally substituted 8-10 membered bicyclic saturated or partially unsaturated heterocyclic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted indolinyl. In some embodiments, R is an optionally substituted isoindolinyl. In some embodiments, R is an optionally substituted 1, 2, 3, 4-tetrahydroquinoline. In some embodiments, R is an optionally substituted 1, 2, 3, 4-tetrahydroisoquinoline.

[00279] As defined generally above and herein, each R′ is independently –R, -C(O)R, -CO_2R, or –SO_2R, or:

two R′ on the same nitrogen are taken together with their intervening atoms to form an optionally substituted heterocyclic or heteroaryl ring, or

two R′ on the same carbon are taken together with their intervening atoms to form an optionally substituted aryl, carbocyclic, heterocyclic, or heteroaryl ring.

[00280] In some embodiments, R' is –R, -C(O)R, -CO_2R, or –SO_2R, wherein R is as defined above and described herein.

[00281] In some embodiments, R' is –R, wherein R is as defined and described above and herein. In some embodiments, R' is hydrogen.

[00282] In some embodiments, R' is –C(O)R, wherein R is as defined above and described herein. In some embodiments, R' is –CO_2R, wherein R is as defined above and described herein. In some embodiments, R' is –SO_2R, wherein R is as defined above and described herein.

[00283] In some embodiments, two R′ on the same nitrogen are taken together with their intervening atoms to form an optionally substituted heterocyclic or heteroaryl ring. In some embodiments, two R′ on the same carbon are taken together with their intervening atoms to form an optionally substituted aryl, carbocyclic, heterocyclic, or heteroaryl ring.

[00284] As defined generally above and herein, –Cy– is an optionally substituted bivalent ring selected from phenylene, carbocyclylene, arylene, heteroarylene, or heterocyclylene.

[00285] In some embodiments, –Cy– is optionally substituted phenylene. In some embodiments, –Cy– is optionally substituted carbocyclylene. In some embodiments, –Cy– is optionally substituted arylene. In some embodiments, –Cy– is optionally substituted heteroarylene. In some embodiments, –Cy– is optionally substituted heterocyclylene.

[00286] As defined generally above and herein, each of X, Y and Z is independently –O–, –S–, –N(–L–R^1)–, or L, wherein each of L and R^1 is independently as defined above and described below.

[00287] In some embodiments, X is –O–. In some embodiments, X is –S–. In some embodiments, X is –O– or –S–. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein X is –O–. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein X is –S–. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein X is –O–, and at least one internucleotidic linkage of formula **I** wherein X is –S–. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein X is –O–, and at least one internucleotidic linkage of formula **I** wherein X is –S–, and at least one internucleotidic linkage of formula **I** wherein L is an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–.

[00288] In some embodiments, X is –N(–L–R^1)–. In some embodiments, X is –N(R^1)–. In some embodiments, X is –N(R’)–. In some embodiments, X is –N(R)–. In some embodiments, X is –NH–.

[00289] In some embodiments, X is L. In some embodiments, X is a covalent bond. In some embodiments, X is or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–. In some embodiments, X is an optionally substituted C_1–C_{10} alkylene or C_1–C_{10} alkenylene. In some embodiments, X is methylene.

[00290] In some embodiments, Y is –O–. In some embodiments, Y is –S–.

[00291] In some embodiments, Y is –N(–L–R^1)–. In some embodiments, Y is –N(R^1)–. In some embodiments, Y is –N(R')–. In some embodiments, Y is –N(R)–. In some embodiments, Y is –NH–.

[00292] In some embodiments, Y is L. In some embodiments, Y is a covalent bond. In some embodiments, Y is or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–. In some embodiments, Y is an optionally substituted C_1–C_{10} alkylene or C_1–C_{10} alkenylene. In some embodiments, Y is methylene.

[00293] In some embodiments, Z is –O–. In some embodiments, Z is –S–.

[00294] In some embodiments, Z is –N(–L–R^1)–. In some embodiments, Z is –N(R^1)–. In some embodiments, Z is –N(R')–. In some embodiments, Z is –N(R)–. In some embodiments, Z is –NH–.

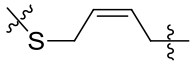
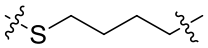
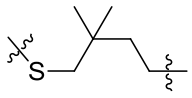
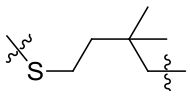
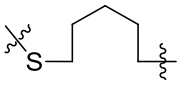
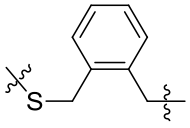
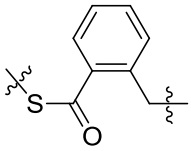
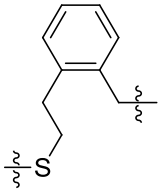
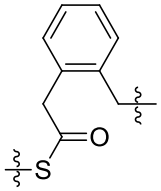
[00295] In some embodiments, Z is L. In some embodiments, Z is a covalent bond. In some embodiments, Z is or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–. In some embodiments, Z is an optionally substituted C_1–C_{10} alkylene or C_1–C_{10} alkenylene. In some embodiments, Z is methylene.

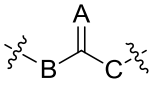
[00296] As defined generally above and herein, L is a covalent bond or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–.

[00297] In some embodiments, L is a covalent bond. In some embodiments, L is an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units

of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–.

[00298] In some embodiments, L has the structure of –L^1–V–, wherein:

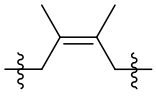
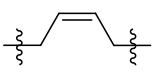
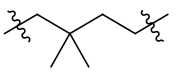
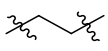
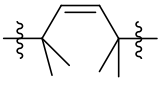
L^1 is an optionally substituted group selected from , , , , , , , , , C_1–C_6 alkylene, C_1-C_6 alkenylene, carbocyclylene, arylene, C_1–C_6 heteroalkylene, heterocyclylene, and heteroarylene;

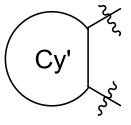
V is selected from –O–, –S–, –NR’–, C(R’)$_2$, –S–S–, –B–S–S–C–, , or an optionally substituted group selected from C_1–C_6 alkylene, arylene, C_1–C_6 heteroalkylene, heterocyclylene, and heteroarylene;

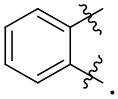
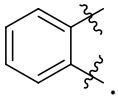
A is =O, =S, =NR’, or =C(R’)$_2$;

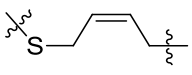
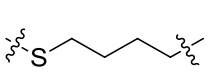
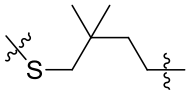
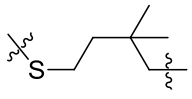
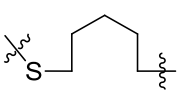
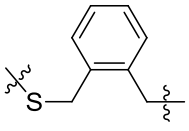
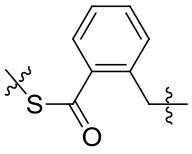
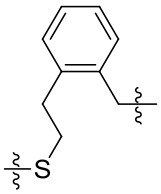
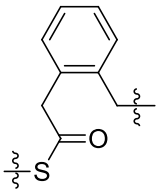
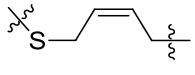
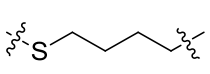
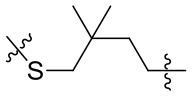
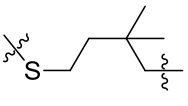
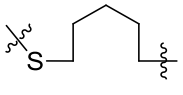
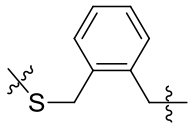
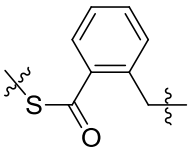
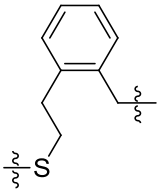
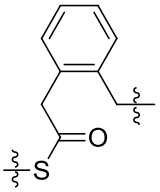
each of B and C is independently –O–, –S–, –NR’–, –C(R’)$_2$–, or an optionally substituted group selected from C_1–C_6 alkylene, carbocyclylene, arylene, heterocyclylene, or heteroarylene; and

each R’ is independently as defined above and described herein.

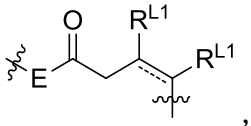
[00299] In some embodiments, L^1 is , , , , , , or .

[00300] In some embodiments, L^1 is 

, wherein Ring Cy' is an optionally substituted arylene, carbocyclylene, heteroarylene, or heterocyclylene. In some embodiments, L^1 is optionally substituted . In some embodiments, L^1 is .

[00301] In some embodiments, L^1 is connected to X. In some embodiments, L^1 is an optionally substituted group selected from , , , , , , , ,and , and the sulfur atom is connect to V. In some embodiments, L^1 is an optionally substituted group selected from , , , , , , , ,and , and the carbon atom is connect to X.

[00302] In some embodiments, L has the structure of:



,

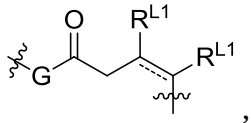
wherein:

E is –O–, –S–, –NR'– or $–C(R')_2–$;

----- is a single or double bond;

the two R^{L1} are taken together with the two carbon atoms to which they are bound to form an optionally substituted aryl, carbocyclic, heteroaryl or heterocyclic ring; and each R' is independently as defined above and described herein.

[00303] In some embodiments, L has the structure of:



,

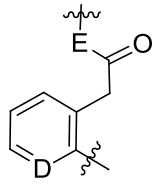
wherein:

G is –O–, –S–, or –NR’;

----- is a single or double bond; and

the two R^{L1} are taken together with the two carbon atoms to which they are bound to form an optionally substituted aryl, C_3-C_{10} carbocyclic, heteroaryl or heterocyclic ring.

[00304] In some embodiments, L has the structure of:



,

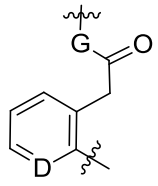
wherein:

E is –O–, –S–, –NR’– or –C(R’)$_2$–;

D is =N–, =C(F)–, =C(Cl)–, =C(Br)–, =C(I)–, =C(CN)–, =C(NO$_2$)–, =C(CO$_2$–(C_1-C_6 aliphatic))–, or =C(CF$_3$)–; and

each R’ is independently as defined above and described herein.

[00305] In some embodiments, L has the structure of:



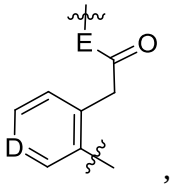
,

wherein:

G is –O–, –S–, or –NR’;

D is =N–, =C(F)–, =C(Cl)–, =C(Br)–, =C(I)–, =C(CN)–, =C(NO$_2$)–, =C(CO$_2$–(C_1-C_6 aliphatic))–, or =C(CF$_3$)–.

[00306] In some embodiments, L has the structure of:



,

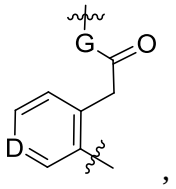
wherein:

E is –O–, –S–, –NR'– or –C(R')$_2$–;

D is =N–, =C(F)–, =C(Cl)–, =C(Br)–, =C(I)–, =C(CN)–, =C(NO$_2$)–, =C(CO$_2$–(C$_1$-C$_6$ aliphatic))–, or =C(CF$_3$)–; and

each R' is independently as defined above and described herein.

[00307] In some embodiments, L has the structure of:



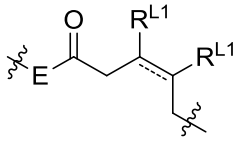
,

wherein:

G is –O–, –S–, or –NR';

D is =N–, =C(F)–, =C(Cl)–, =C(Br)–, =C(I)–, =C(CN)–, =C(NO$_2$)–, =C(CO$_2$–(C$_1$-C$_6$ aliphatic))–, or =C(CF$_3$)–.

[00308] In some embodiments, L has the structure of:



,

wherein:

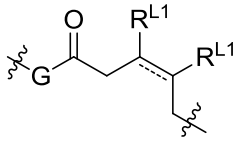
E is –O–, –S–, –NR'– or –C(R')$_2$–;

----- is a single or double bond;

the two R^{L1} are taken together with the two carbon atoms to which they are bound to form an optionally substituted aryl, C$_3$-C$_{10}$ carbocyclic, heteroaryl or heterocyclic ring;

and each R' is independently as defined above and described herein.

[00309] In some embodiments, L has the structure of:



,

wherein:

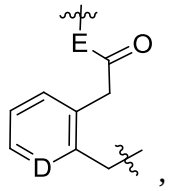
G is –O–, –S–, or –NR’;

----- is a single or double bond;

the two R^{L1} are taken together with the two carbon atoms to which they are bound to form an optionally substituted aryl, C_3-C_{10} carbocyclic, heteroaryl or heterocyclic ring;

and each R’ is independently as defined above and described herein.

[00310] In some embodiments, L has the structure of:



,

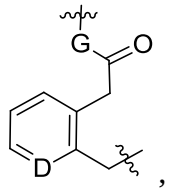
wherein:

E is –O–, –S–, –NR’– or –C(R’)$_2$–;

D is =N–, =C(F)–, =C(Cl)–, =C(Br)–, =C(I)–, =C(CN)–, =C(NO_2)–, =C(CO_2–(C_1-C_6 aliphatic))–, or =C(CF_3)–; and

each R’ is independently as defined above and described herein.

[00311] In some embodiments, L has the structure of:



,

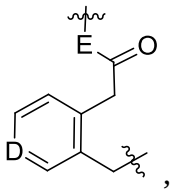
wherein:

G is –O–, –S–, or –NR’;

D is =N–, =C(F)–, =C(Cl)–, =C(Br)–, =C(I)–, =C(CN)–, =C(NO_2)–, =C(CO_2–(C_1-C_6 aliphatic))–, or =C(CF_3)–; and

each R’ is independently as defined above and described herein.

[00312] In some embodiments, L has the structure of:



,

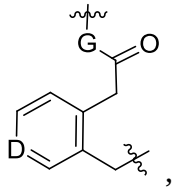
wherein:

E is –O–, –S–, –NR'– or $–C(R')_2–$;

D is =N–, =C(F)–, =C(Cl)–, =C(Br)–, =C(I)–, =C(CN)–, $=C(NO_2)–$, $=C(CO_2–(C_1\text{-}C_6$ aliphatic))–, or $=C(CF_3)–$; and

each R' is independently as defined above and described herein.

[00313] In some embodiments, L has the structure of:



,

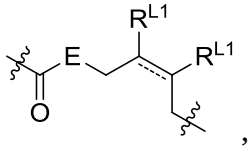
wherein:

G is –O–, –S–, or –NR';

D is =N–, =C(F)–, =C(Cl)–, =C(Br)–, =C(I)–, =C(CN)–, $=C(NO_2)–$, $=C(CO_2–(C_1\text{-}C_6$ aliphatic))–, or $=C(CF_3)–$; and

each R' is independently as defined above and described herein.

[00314] In some embodiments, L has the structure of:



,

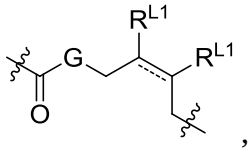
wherein:

E is –O–, –S–, –NR'– or $–C(R')_2–$;

⁻⁻⁻⁻ is a single or double bond;

the two R^{L1} are taken together with the two carbon atoms to which they are bound to form an optionally substituted aryl, C_3-C_{10} carbocyclic, heteroaryl or heterocyclic ring; and each R' is independently as defined above and described herein.

[00315] In some embodiments, L has the structure of:



,

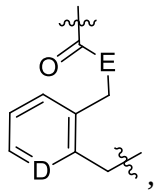
wherein:

G is –O–, –S–, or –NR';

⁻⁻⁻⁻ is a single or double bond;

the two R^{L1} are taken together with the two carbon atoms to which they are bound to form an optionally substituted aryl, C_3-C_{10} carbocyclic, heteroaryl or heterocyclic ring; and each R' is independently as defined above and described herein.

[00316] In some embodiments, L has the structure of:



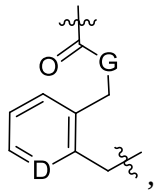
wherein:

E is –O–, –S–, –NR'– or $–C(R')_2–$;

D is =N–, =C(F)–, =C(Cl)–, =C(Br)–, =C(I)–, =C(CN)–, $=C(NO_2)–$, $=C(CO_2–(C_1\text{-}C_6$ aliphatic))–, or $=C(CF_3)–$; and

each R' is independently as defined above and described herein.

[00317] In some embodiments, L has the structure of:



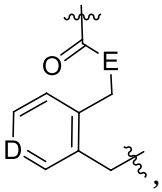
wherein:

G is –O–, –S–, or –NR';

D is =N–, =C(F)–, =C(Cl)–, =C(Br)–, =C(I)–, =C(CN)–, $=C(NO_2)–$, $=C(CO_2–(C_1\text{-}C_6$ aliphatic))–, or $=C(CF_3)–$; and

R' is as defined above and described herein.

[00318] In some embodiments, L has the structure of:



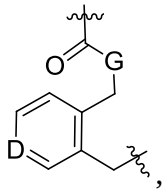
wherein:

E is –O–, –S–, –NR'– or $–C(R')_2–$;

D is =N–, =C(F)–, =C(Cl)–, =C(Br)–, =C(I)–, =C(CN)–, $=C(NO_2)–$, $=C(CO_2–(C_1\text{-}C_6$ aliphatic))–, or $=C(CF_3)–$; and

each R’ is independently as defined above and described herein.

[00319] In some embodiments, L has the structure of:



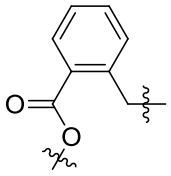
wherein:

G is –O–, –S–, or –NR’;

D is =N–, =C(F)–, =C(Cl)–, =C(Br)–, =C(I)–, =C(CN)–, =C(NO_2)–, =C(CO_2–(C_1-C_6 aliphatic))–, or =C(CF_3)–; and

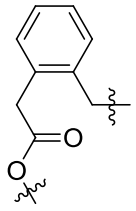
R’ is as defined above and described herein.

[00320] In some embodiments, L has the structure of:



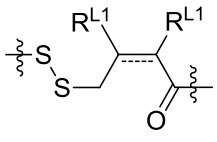
wherein the phenyl ring is optionally substituted. In some embodiments, the phenyl ring is not substituted. In some embodiments, the phenyl ring is substituted.

[00321] In some embodiments, L has the structure of:



wherein the phenyl ring is optionally substituted. In some embodiments, the phenyl ring is not substituted. In some embodiments, the phenyl ring is substituted.

[00322] In some embodiments, L has the structure of:

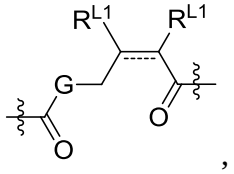


wherein:

----- is a single or double bond; and

the two R^{L1} are taken together with the two carbon atoms to which they are bound to form an optionally substituted aryl, C_3-C_{10} carbocyclic, heteroaryl or heterocyclic ring.

[00323] In some embodiments, L has the structure of:



,

wherein:

G is –O–, –S–, or –NR'–;

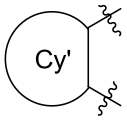
⋯⋯ is a single or double bond; and

the two R^{L1} are taken together with the two carbon atoms to which they are bound to form an optionally substituted aryl, C_3-C_{10} carbocyclic, heteroaryl or heterocyclic ring.

[00324] As defined generally above and herein, E is –O–, –S–, –NR'– or –$C(R')_2$–, wherein each R' independently as defined above and described herein. In some embodiments, E is –O–, –S–, or –NR'–. In some embodiments, E is –O–, –S–, or –NH–. In some embodiments, E is –O–. In some embodiments, E is –S–. In some embodiments, E is –NH–.

[00325] As defined generally above and herein, G is –O–, –S–, or –NR', wherein each R' independently as defined above and described herein. In some embodiments, G is –O–, –S–, or –NH–. In some embodiments, G is –O–. In some embodiments, G is –S–. In some embodiments, G is –NH–.

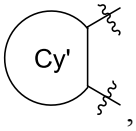
[00326] In some embodiments, L is –L^3–G–, wherein:

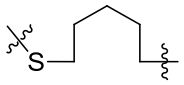
L^3 is an optionally substituted C_1–C_5 alkylene or alkenylene, wherein one or more methylene units are optionally and independently replaced by –O–, –S–,–N(R')–, –C(O)–, –C(S)–, –C(NR')–, –S(O)–, –$S(O)_2$–, or 

; and

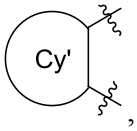
wherein each of G, R' and Ring Cy' is independently as defined above and described herein.

[00327] In some embodiments, L is –L^3–S–, wherein L^3 is as defined above and described herein. In some embodiments, L is –L^3–O–, wherein L^3 is as defined above and described herein. In some embodiments, L is –L^3–N(R')–, wherein each of L^3 and R' is independently as defined above and described herein. In some embodiments, L is –L^3–NH–, wherein each of L^3 and R' is independently as defined above and described herein.

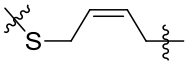
[00328] In some embodiments, L^3 is an optionally substituted C_5 alkylene or alkenylene, wherein one or more methylene units are optionally and independently replaced by –O–, –S–,–N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –S(O)–, –$S(O)_2$–, or 

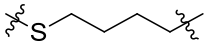
, and each of R' and Ring Cy' is independently as defined above and described herein. In some embodiments, L^3 is an optionally substituted C_5 alkylene. In some embodiments, –L^3–G– is 

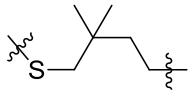
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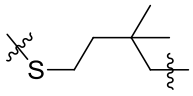
[00329] In some embodiments, L^3 is an optionally substituted C_4 alkylene or alkenylene, wherein one or more methylene units are optionally and independently replaced by –O–, –S–,–N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –S(O)–, –$S(O)_2$–, or 

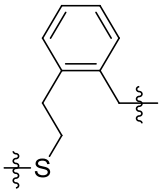
, and each of R' and Cy' is independently as defined above and described herein.

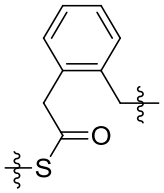
[00330] In some embodiments, –L^3–G– is 

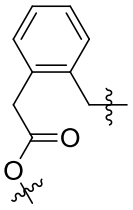
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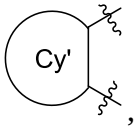
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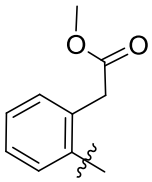
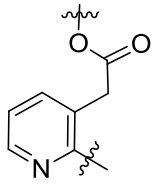
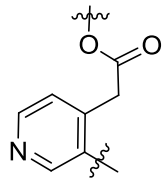
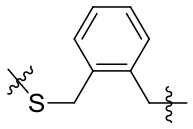
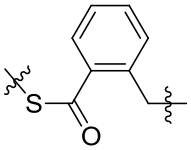
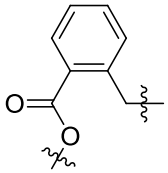
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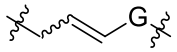
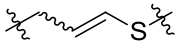
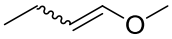
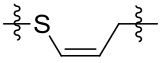
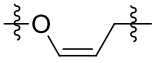
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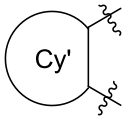
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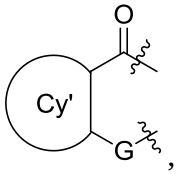
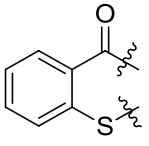
[00331] In some embodiments, L^3 is an optionally substituted C_3 alkylene or alkenylene, wherein one or more methylene units are optionally and independently replaced by –O–, –S–,–N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –S(O)–, –$S(O)_2$–, or 

, and each of R' and Cy' is independently as defined above and described herein.

[00332] In some embodiments, $–L^3–G–$ is , , , , , or .

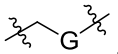
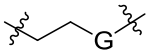
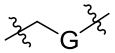
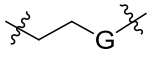
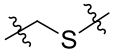
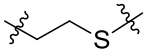
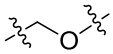
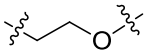
[00333] In some embodiments, L is . In some embodiments, L is  or . In some embodiments, L is  or .

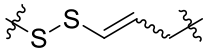
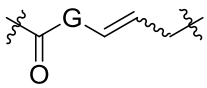
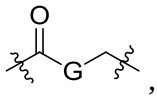
[00334] In some embodiments, L^3 is an optionally substituted C_2 alkylene or alkenylene, wherein one or more methylene units are optionally and independently replaced by –O–, –S–,–N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –S(O)–, –$S(O)_2$–, or , and each of R' and Cy' is independently as defined above and described herein.

[00335] In some embodiments, $–L^3–G–$ is , wherein each of G and Cy' is independently as defined above and described herein. In some embodiments, L is .

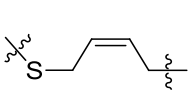
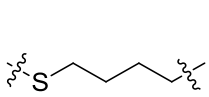
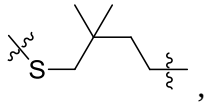
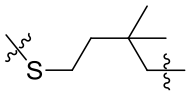
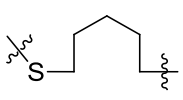
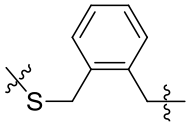
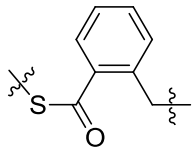
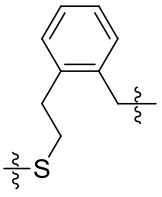
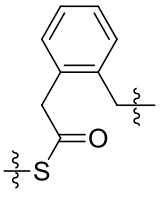
[00336] In some embodiments, L is $–L^4–G–$, wherein L^4 is an optionally substituted C_1–C_2 alkylene; and G is as defined above and described herein. In some embodiments, L is $–L^4–G–$, wherein L^4 is an optionally substituted C_1–C_2 alkylene; G is as defined above and described herein; and G is connected to R^1. In some embodiments, L is $–L^4–G–$, wherein L^4 is an optionally substituted methylene; G is as defined above and described herein; and G is connected to R^1. In some embodiments, L is $–L^4–G–$, wherein L^4 is methylene; G is as defined above and described herein; and G is connected to R^1. In some embodiments, L is $–L^4–G–$, wherein L^4 is an optionally substituted $–(CH_2)_2–$; G is as defined above and described herein; and G is

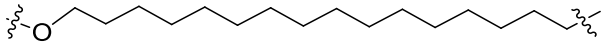
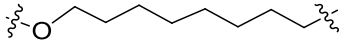
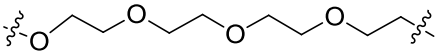
connected to R^1. In some embodiments, L is $–L^4–G–$, wherein L^4 is $–(CH_2)_2–$; G is as defined above and described herein; and G is connected to R^1.

[00337] In some embodiments, L is , or , wherein G is as defined above and described herein, and G is connected to R^1. In some embodiments, L is , wherein G is as defined above and described herein, and G is connected to R^1. In some embodiments, L is , wherein G is as defined above and described herein, and G is connected to R^1. In some embodiments, L is , or , wherein the sulfur atom is connected to R^1. In some embodiments, L is , or , wherein the oxygen atom is connected to R^1.

[00338] In some embodiments, L is , , or , wherein G is as defined above and described herein.

[00339] In some embodiments, L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$, wherein R^{L3} is an optionally substituted, linear or branched, $C_1–C_9$ alkylene, wherein one or more methylene units are optionally and independently replaced by an optionally substituted $C_1–C_6$ alkylene, $C_1–C_6$ alkenylene, —C≡C—, $–C(R')_2–$, –Cy–, –O–, –S–, –S–S–, $–N(R')–$, $–C(O)–$, $–C(S)–$, $–C(NR')–$, $–C(O)N(R')–$, $–N(R')C(O)N(R')-$, $–N(R')C(O)–$, $–N(R')C(O)O–$, $–OC(O)N(R')-$, $–S(O)–$, $–S(O)_2–$, $–S(O)_2N(R')–$, $–N(R')S(O)_2–$, $–SC(O)–$, $–C(O)S–$, $–OC(O)–$, or $–C(O)O–$, wherein each of R' and –Cy– is independently as defined above and described herein. In some embodiments, L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$, wherein R^{L3} is an optionally substituted $C_1–C_6$ alkylene. In some embodiments, L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$, wherein R^{L3} is an optionally substituted $C_1–C_6$ alkenylene. In some embodiments, L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$, wherein R^{L3} is an optionally substituted C_1.C_6 alkylene wherein one or more methylene units are optionally and independently replaced by an optionally substituted $C_1–C_6$ alkenylene, arylene, or heteroarylene. In some embodiments, In some embodiments, R^{L3} is an optionally substituted $–S–(C_1–C_6$ alkenylene$)–$, $–S–(C_1–C_6$ alkylene$)–$, $–S–(C_1–C_6$ alkylene$)–$arylene$–(C_1–C_6$ alkylene$)–$, $–S–CO–$arylene$–(C_1–C_6$ alkylene$)–$, or $–S–CO–(C_1–C_6$ alkylene$)–$arylene$–(C_1–C_6$ alkylene$)–$.

[00340] In some embodiments, L is , , , , , , , , or .

[00341] In some embodiments, L is . In some embodiments, L is . In some embodiments, .

[00342] In some embodiments, the sulfur atom in the L embodiments described above and herein is connected to X. In some embodiments, the sulfur atom in the L embodiments described above and herein is connected to R^1.

[00343] As defined generally above and herein, R^1 is halogen, R, or an optionally substituted C_1–C_{50} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, wherein each variable is independently as defined above and described herein. In some embodiments, R^1 is halogen, R, or an optionally substituted C_1–C_{10} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C— , –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, wherein each variable is independently as defined above and described herein.

[00344] In some embodiments, R^1 is hydrogen. In some embodiments, R^1 is halogen. In some embodiments, R^1 is –F. In some embodiments, R^1 is –Cl. In some embodiments, R^1 is –Br. In some embodiments, R^1 is –I.

[00345] In some embodiments, R^1 is R wherein R is as defined above and described herein.

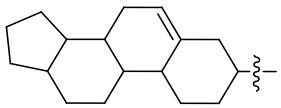
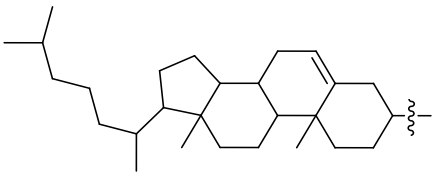
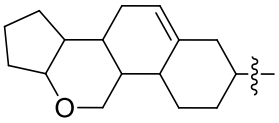
[00346] In some embodiments, R^1 is hydrogen. In some embodiments, R^1 is an optionally substituted group selected from C_1–C_{50} aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl.

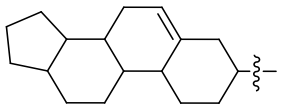
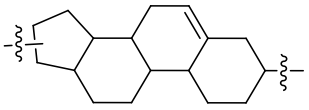
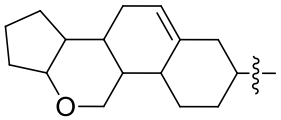
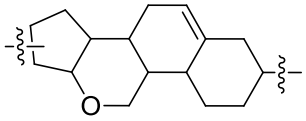
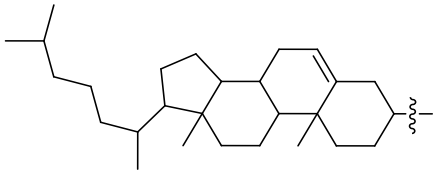
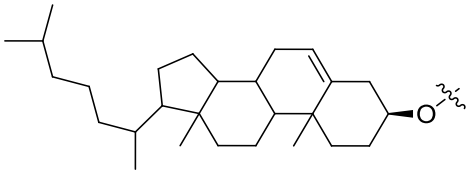
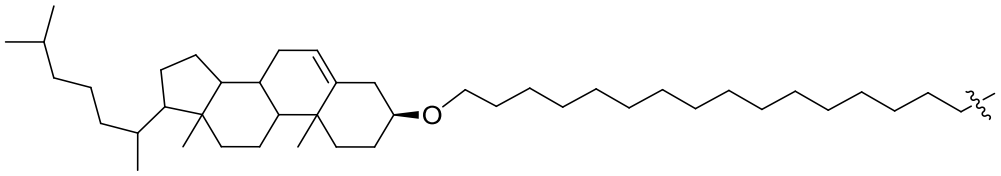
[00347] In some embodiments, R^1 is an optionally substituted C_1–C_{50} aliphatic. In some embodiments, R^1 is an optionally substituted C_1–C_{10} aliphatic. In some embodiments, R^1 is an optionally substituted C_1–C_6 aliphatic. In some embodiments, R^1 is an optionally substituted C_1–C_6 alkyl. In some embodiments, R^1 is optionally substituted, linear or branched hexyl. In some embodiments, R^1 is optionally substituted, linear or branched pentyl. In some embodiments, R^1 is optionally substituted, linear or branched butyl. In some embodiments, R^1 is optionally substituted, linear or branched propyl. In some embodiments, R^1 is optionally substituted ethyl. In some embodiments, R^1 is optionally substituted methyl.

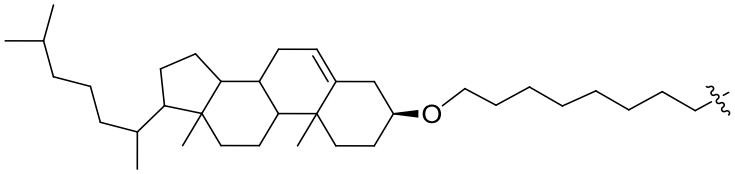
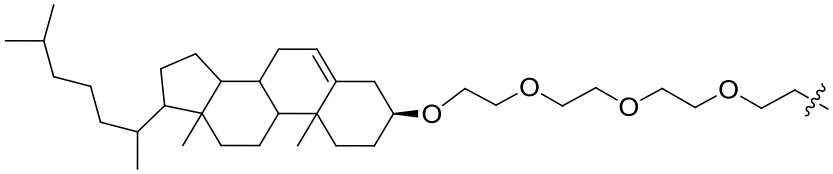
[00348] In some embodiments, R^1 is optionally substituted phenyl. In some embodiments, R^1 is substituted phenyl. In some embodiments, R^1 is phenyl.

[00349] In some embodiments, R^1 is optionally substituted carbocyclyl. In some embodiments, R^1 is optionally substituted C_3-C_{10} carbocyclyl. In some embodiments, R^1 is optionally substituted monocyclic carbocyclyl. In some embodiments, R^1 is optionally substituted cycloheptyl. In some embodiments, R^1 is optionally substituted cyclohexyl. In some embodiments, R^1 is optionally substituted cyclopentyl. In some embodiments, R^1 is optionally substituted cyclobutyl. In some embodiments, R^1 is an optionally substituted cyclopropyl. In some embodiments, R^1 is optionally substituted bicyclic carbocyclyl.

[00350] In some embodiments, R^1 is an optionally substituted C_1–C_{50} polycyclic hydrocarbon. In some embodiments, R^1 is an optionally substituted C_1–C_{50} polycyclic hydrocarbon wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, $-C\equiv C-$, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, wherein each variable is independently as defined above and described herein. In some embodiments, R^1 is optionally substituted

. In some embodiments, R^1 is . In some embodiments, R^1 is optionally substituted .

[00351] In some embodiments, R^1 is an optionally substituted C_1–C_{50} aliphatic comprising one or more optionally substituted polycyclic hydrocarbon moieties. In some embodiments, R^1 is an optionally substituted C_1–C_{50} aliphatic comprising one or more optionally substituted polycyclic hydrocarbon moieties, wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, wherein each variable is independently as defined above and described herein. In some embodiments, R^1 is an optionally substituted C_1–C_{50} aliphatic comprising one or more optionally substituted , , , or . In some embodiments, R^1 is . In some embodiments, R^1 is . In some embodiments, R^1 is . In some

embodiments, R^1 is . In some embodiments, R^1 is .

[00352] In some embodiments, R^1 is an optionally substituted aryl. In some embodiments, R^1 is an optionally substituted bicyclic aryl ring.

[00353] In some embodiments, R^1 is an optionally substituted heteroaryl. In some embodiments, R^1 is an optionally substituted 5-6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, sulfur, or oxygen. In some embodiments, R^1 is a substituted 5-6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an unsubstituted 5-6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, sulfur, or oxygen.

[00354] In some embodiments, R^1 is an optionally substituted 5 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen or sulfur. In some embodiments, R^1 is an optionally substituted 6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00355] In some embodiments, R^1 is an optionally substituted 5-membered monocyclic heteroaryl ring having 1 heteroatom selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is selected from pyrrolyl, furanyl, or thienyl.

[00356] In some embodiments, R^1 is an optionally substituted 5-membered heteroaryl ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R^1 is an optionally substituted 5-membered heteroaryl ring having 1 nitrogen atom, and an additional heteroatom selected from sulfur or oxygen. Exemplary R^1 groups include optionally substituted pyrazolyl, imidazolyl, thiazolyl, isothiazolyl, oxazolyl or isoxazolyl.

[00357] In some embodiments, R^1 is a 6-membered heteroaryl ring having 1–3 nitrogen atoms. In other embodiments, R^1 is an optionally substituted 6-membered heteroaryl ring having

1–2 nitrogen atoms. In some embodiments, R^1 is an optionally substituted 6-membered heteroaryl ring having 2 nitrogen atoms. In certain embodiments, R^1 is an optionally substituted 6-membered heteroaryl ring having 1 nitrogen. Exemplary R^1 groups include optionally substituted pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, or tetrazinyl.

[00358] In certain embodiments, R^1 is an optionally substituted 8–10 membered bicyclic heteroaryl ring having 1–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted 5,6–fused heteroaryl ring having 1–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, R^1 is an optionally substituted 5,6–fused heteroaryl ring having 1–2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R^1 is an optionally substituted 5,6–fused heteroaryl ring having 1 heteroatom independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted indolyl. In some embodiments, R^1 is an optionally substituted azabicyclo[3.2.1]octanyl. In certain embodiments, R^1 is an optionally substituted 5,6–fused heteroaryl ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted azaindolyl. In some embodiments, R^1 is an optionally substituted benzimidazolyl. In some embodiments, R^1 is an optionally substituted benzothiazolyl. In some embodiments, R^1 is an optionally substituted benzoxazolyl. In some embodiments, R^1 is an optionally substituted indazolyl. In certain embodiments, R^1 is an optionally substituted 5,6–fused heteroaryl ring having 3 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00359] In certain embodiments, R^1 is an optionally substituted 6,6–fused heteroaryl ring having 1–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted 6,6–fused heteroaryl ring having 1–2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, R^1 is an optionally substituted 6,6–fused heteroaryl ring having 1 heteroatom independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted quinolinyl. In some embodiments, R^1 is an optionally substituted isoquinolinyl. According to one aspect, R^1 is an optionally substituted 6,6–fused heteroaryl ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is a quinazoline or a quinoxaline.

[00360] In some embodiments, R^1 is an optionally substituted heterocyclyl. In some embodiments, R^1 is an optionally substituted 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is a substituted 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an unsubstituted 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00361] In some embodiments, R^1 is an optionally substituted heterocyclyl. In some embodiments, R^1 is an optionally substituted 6 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted 6 membered partially unsaturated heterocyclic ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted 6 membered partially unsaturated heterocyclic ring having 2 oxygen atoms.

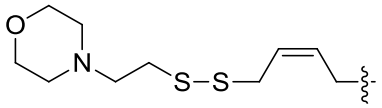
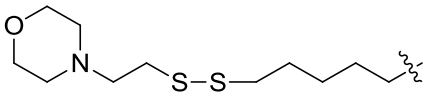
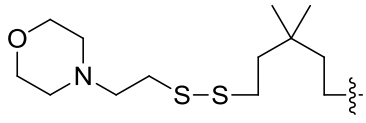
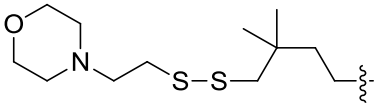
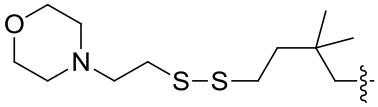
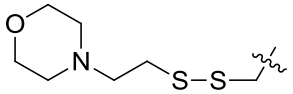
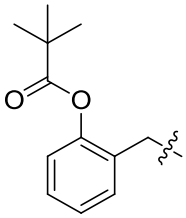
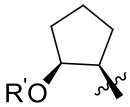
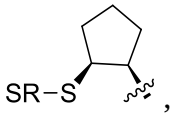
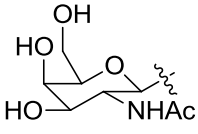
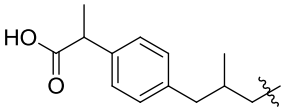
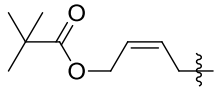
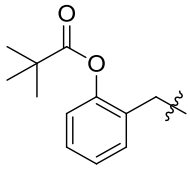
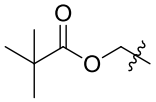
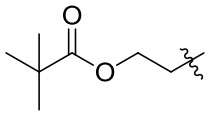
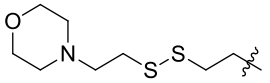
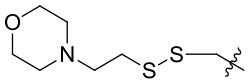
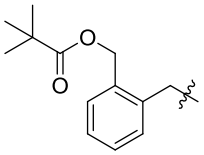
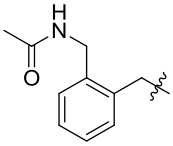
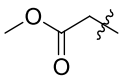
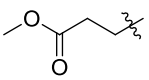
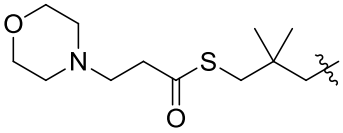
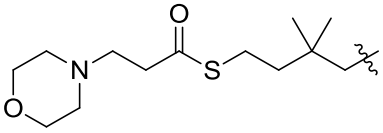
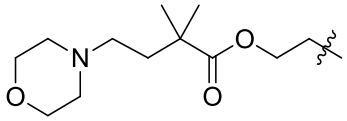
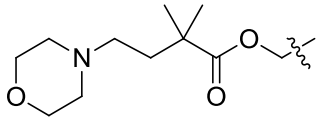
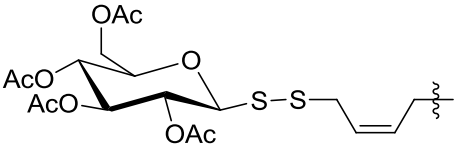
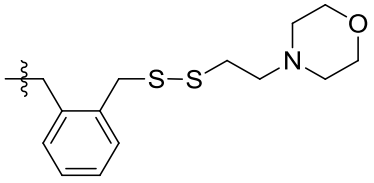
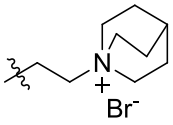
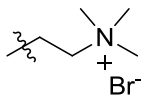
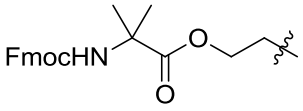
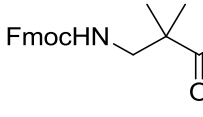
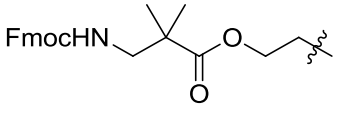
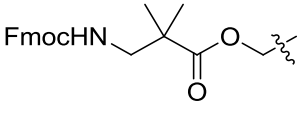
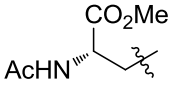
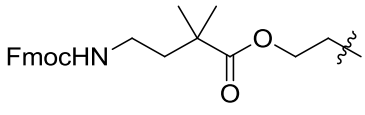
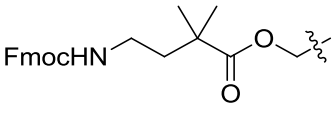
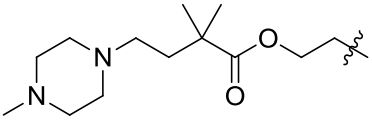
[00362] In certain embodiments, R^1 is a 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R^1 is oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, oxepaneyl, aziridineyl, azetidineyl, pyrrolidinyl, piperidinyl, azepanyl, thiiranyl, thietanyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, thiepanyl, dioxolanyl, oxathiolanyl, oxazolidinyl, imidazolidinyl, thiazolidinyl, dithiolanyl, dioxanyl, morpholinyl, oxathianyl, piperazinyl, thiomorpholinyl, dithianyl, dioxepanyl, oxazepanyl, oxathiepanyl, dithiepanyl, diazepanyl, dihydrofuranonyl, tetrahydropyranonyl, oxepanonyl, pyrolidinonyl, piperidinonyl, azepanonyl, dihydrothiophenonyl, tetrahydrothiopyranonyl, thiepanonyl, oxazolidinonyl, oxazinanonyl, oxazepanonyl, dioxolanonyl, dioxanonyl, dioxepanonyl, oxathiolinonyl, oxathianonyl, oxathiepanonyl, thiazolidinonyl, thiazinanonyl, thiazepanonyl, imidazolidinonyl, tetrahydropyrimidinonyl, diazepanonyl, imidazolidinedionyl, oxazolidinedionyl, thiazolidinedionyl, dioxolanedionyl, oxathiolanedionyl, piperazinedionyl, morpholinedionyl, thiomorpholinedionyl, tetrahydropyranyl, tetrahydrofuranyl, morpholinyl, thiomorpholinyl, piperidinyl, piperazinyl, pyrrolidinyl, tetrahydrothiophenyl, or tetrahydrothiopyranyl. In some embodiments, R^1 is an optionally substituted 5 membered saturated or partially unsaturated

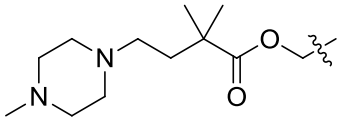
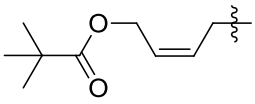
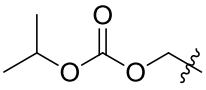
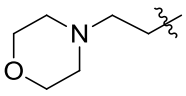
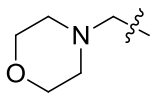
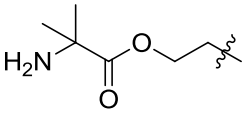
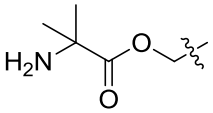
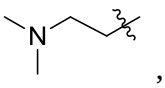
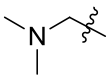
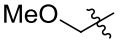
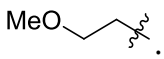
heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

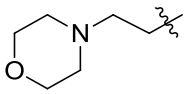
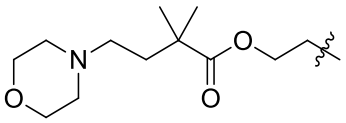
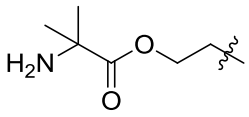
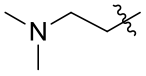
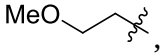
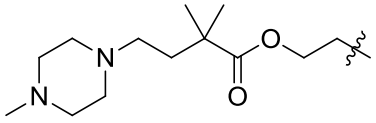
[00363] In certain embodiments, R^1 is an optionally substituted 5–6 membered partially unsaturated monocyclic ring having 1–2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R^1 is an optionally substituted tetrahydropyridinyl, dihydrothiazolyl, dihydrooxazolyl, or oxazolinyl group.

[00364] In some embodiments, R^1 is an optionally substituted 8-10 membered bicyclic saturated or partially unsaturated heterocyclic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted indolinyl. In some embodiments, R^1 is an optionally substituted isoindolinyl. In some embodiments, R^1 is an optionally substituted 1, 2, 3, 4-tetrahydroquinoline. In some embodiments, R^1 is an optionally substituted 1, 2, 3, 4-tetrahydroisoquinoline.

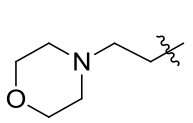
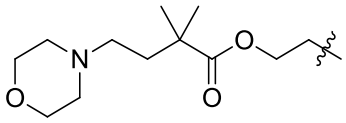
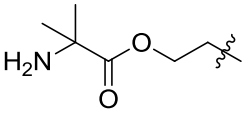
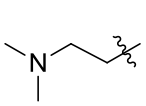
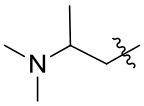
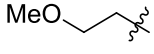
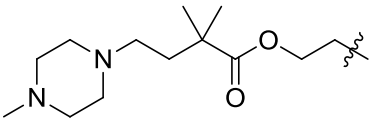
[00365] In some embodiments, R^1 is an optionally substituted C_1–C_{10} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, wherein each variable is independently as defined above and described herein. In some embodiments, R^1 is an optionally substituted C_1–C_{10} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally–Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –OC(O)–, or –C(O)O–, wherein each R’ is independently as defined above and described herein. In some embodiments, R^1 is an optionally substituted C_1–C_{10} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally–Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –OC(O)–, or –C(O)O–, wherein each R’ is independently as defined above and described herein.

[00366] In some embodiments, R^1 is , , , , , , , , , (GalNAc), , , , , , , , , , , , , , , , , , , , , , , , , , , 

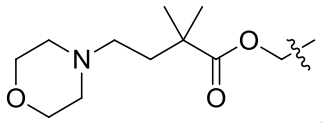
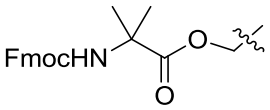
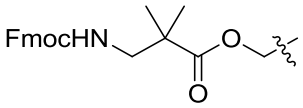
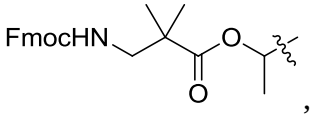
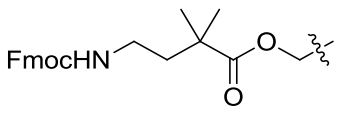
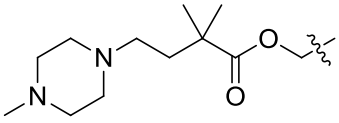
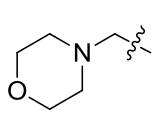
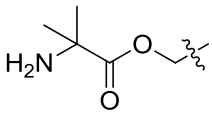
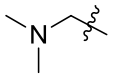
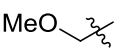
, , , CH_3–, , , , , , , , or .

[00367] In some embodiments, R^1 is CH_3–, , , , , , or .

[00368] In some embodiments, R^1 comprises a terminal optionally substituted –$(CH_2)_2$– moiety which is connected to L. Exemplary such R^1 groups are depicted below:

, , , , , , and .

[00369] In some embodiments, R^1 comprises a terminal optionally substituted –(CH_2)– moiety which is connected to L. Ecemplary such R^1 groups are depicted below:

, , , , , , , , , and .

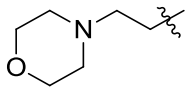
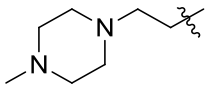
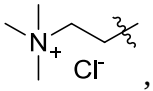
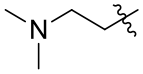
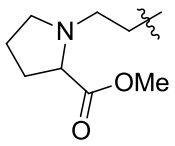
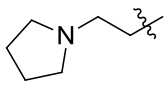
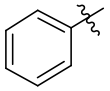
[00370] In some embodiments, R^1 is –S–R^{L2}, wherein R^{L2} is an optionally substituted C_1–C_9 aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R')$_2$–, –Cy–, –O–, –

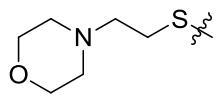
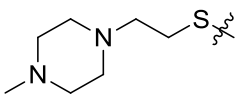
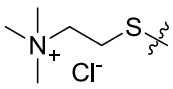
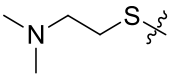
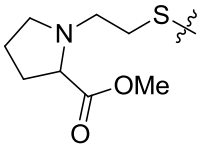
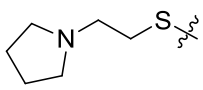
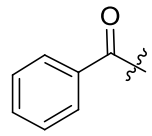
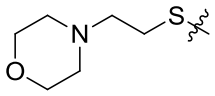
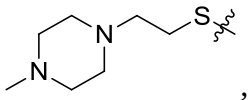
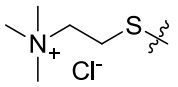
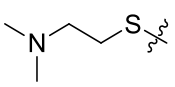
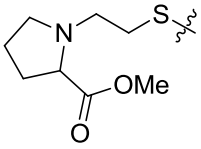
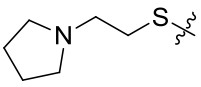
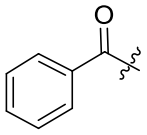
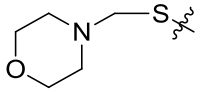
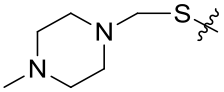
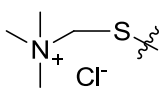
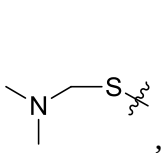
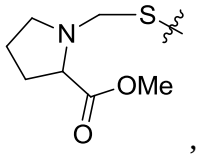
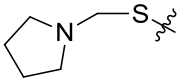
S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, $–S(O)_2–$, $–S(O)_2N(R′)–$, $–N(R′)S(O)_2–$, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, and each of R' and –Cy– is independently as defined above and described herein. In some embodiments, R^1 is $–S–R^{L2}$, wherein the sulfur atom is connected with the sulfur atom in L group.

[00371] In some embodiments, R^1 is $–C(O)–R^{L2}$, wherein R^{L2} is an optionally substituted $C_1–C_9$ aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted $C_1–C_6$ alkylene, $C_1–C_6$ alkenylene, —C≡C—, $–C(R')_2–$, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, $–S(O)_2–$, $–S(O)_2N(R′)–$, $–N(R′)S(O)_2–$, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, and each of R' and –Cy– is independently as defined above and described herein. In some embodiments, R^1 is $–C(O)–R^{L2}$, wherein the carbonyl group is connected with G in L group. In some embodiments, R^1 is $–C(O)–R^{L2}$, wherein the carbonyl group is connected with the sulfur atom in L group.

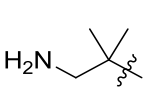
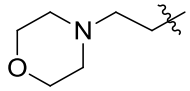
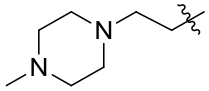
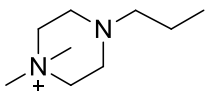
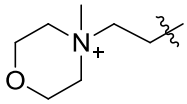
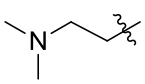
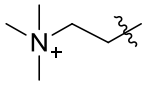
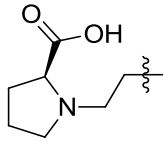
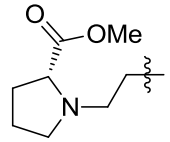
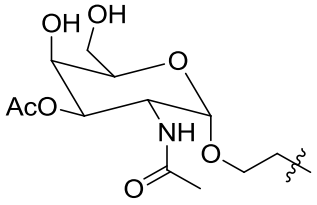
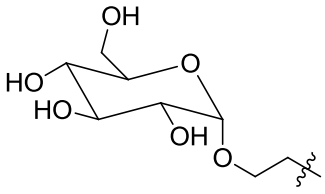
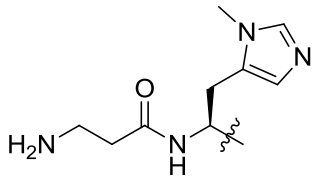
[00372] In some embodiments, R^{L2} is optionally substituted $C_1–C_9$ aliphatic. In some embodiments, R^{L2} is optionally substituted $C_1–C_9$ alkyl. In some embodiments, R^{L2} is optionally substituted $C_1–C_9$ alkenyl. In some embodiments, R^{L2} is optionally substituted $C_1–C_9$ alkynyl. In some embodiments, R^{L2} is an optionally substituted $C_1–C_9$ aliphatic wherein one or more methylene units are optionally and independently replaced by –Cy– or –C(O)–. In some embodiments, R^{L2} is an optionally substituted $C_1–C_9$ aliphatic wherein one or more methylene units are optionally and independently replaced by –Cy–. In some embodiments, R^{L2} is an optionally substituted $C_1–C_9$ aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted heterocycylene. In some embodiments, R^{L2} is an optionally substituted $C_1–C_9$ aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted arylene. In some embodiments, R^{L2} is an optionally substituted $C_1–C_9$ aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted heteroarylene. In some embodiments, R^{L2} is an optionally substituted $C_1–C_9$ aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_3-C_{10} carbocyclylene. In some embodiments, R^{L2} is an optionally substituted $C_1–C_9$ aliphatic wherein two methylene units are optionally and independently replaced by –Cy– or –C(O)–. In some embodiments, R^{L2} is an

optionally substituted C_1–C_9 aliphatic wherein two methylene units are optionally and independently replaced by –Cy– or –C(O)–. Exemplary R^{L2} groups are depicted below:


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[00373] In some embodiments, R^1 is hydrogen, or an optionally substituted group selected from 
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, –S–(C_1–C_{10} aliphatic), C_1–C_{10} aliphatic, aryl, C_1–C_6 heteroalkyl, heteroaryl and heterocyclyl. In some embodiments, R^1 is 
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, or –S–(C_1–C_{10} aliphatic). In some embodiments, R^1 is 
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, or 
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[00374] In some embodiments, R^1 is an optionally substituted group selected from –S–(C_1-C_6 aliphatic), C_1-C_{10} aliphatic, C_1-C_6 heteroaliphatic, aryl, heterocyclyl and heteroaryl.

[00375] In some embodiments, R^1 is , , , , , , , , , , , or .

[00376] In some embodiments, the sulfur atom in the R^1 embodiments described above and herein is connected with the sulfur atom, G, E, or –C(O)– moiety in the L embodiments described above and herein. In some embodiments, the –C(O)– moiety in the R^1 embodiments described above and herein is connected with the sulfur atom, G, E, or –C(O)– moiety in the L embodiments described above and herein.

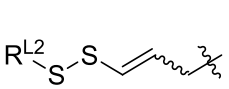
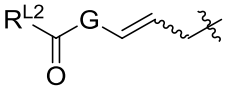
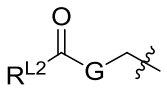
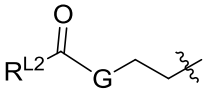
[00377] In some embodiments, $–L–R^1$ is any combination of the L embodiments and R^1 embodiments described above and herein.

[00378] In some embodiments, $–L–R^1$ is $–L^3–G–R^1$ wherein each variable is independently as defined above and described herein.

[00379] In some embodiments, $–L–R^1$ is $–L^4–G–R^1$ wherein each variable is independently as defined above and described herein.

[00380] In some embodiments, $–L–R^1$ is $–L^3–G–S–R^{L2}$, wherein each variable is independently as defined above and described herein.

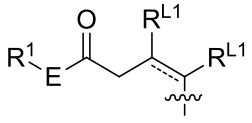
[00381] In some embodiments, $–L–R^1$ is $–L^3–G–C(O)–R^{L2}$, wherein each variable is independently as defined above and described herein.

[00382] In some embodiments, $–L–R^1$ is , , , or , wherein R^{L2} is an optionally substituted $C_1–C_9$ aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted $C_1–C_6$ alkylene, $C_1–C_6$ alkenylene, $—C\equiv C—$, $–C(R')_2–$, –Cy–, –O–, –S–, –S–S–, –

N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, $–S(O)_2–$, $–S(O)_2N(R′)–$, $–N(R′)S(O)_2–$, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, and each G is independently as defined above and described herein.

[00383] In some embodiments, $–L–R^1$ is $–R^{L3}–S–S–R^{L2}$, wherein each variable is independently as defined above and described herein. In some embodiments, $–L–R^1$ is $–R^{L3}–C(O)–S–S–R^{L2}$, wherein each variable is independently as defined above and described herein.

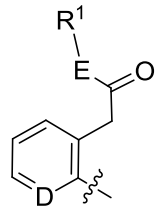
[00384] In some embodiments, $–L–R^1$ has the structure of:



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wherein each variable is independently as defined above and described herein.

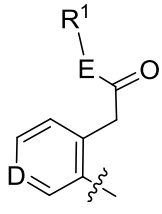
[00385] In some embodiments, $–L–R^1$ has the structure of:



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wherein each variable is independently as defined above and described herein.

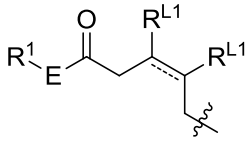
[00386] In some embodiments, $–L–R^1$ has the structure of:



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wherein each variable is independently as defined above and described herein.

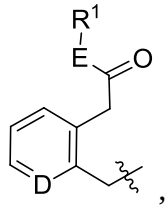
[00387] In some embodiments, $–L–R^1$ has the structure of:



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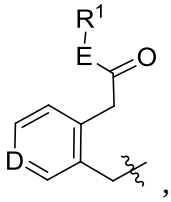
wherein each variable is independently as defined above and described herein.

[00388] In some embodiments, $–L–R^1$ has the structure of:



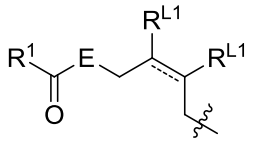
wherein each variable is independently as defined above and described herein.

[00389] In some embodiments, –L–R^1 has the structure of:



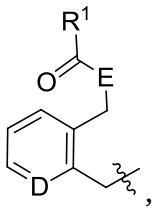
wherein each variable is independently as defined above and described herein.

[00390] In some embodiments, –L–R^1 has the structure of:



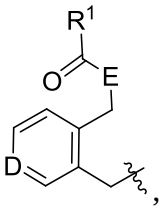
wherein each variable is independently as defined above and described herein.

[00391] In some embodiments, –L–R^1 has the structure of:



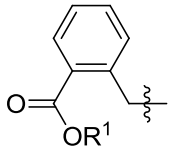
wherein each variable is independently as defined above and described herein.

[00392] In some embodiments, –L–R^1 has the structure of:



wherein each variable is independently as defined above and described herein.

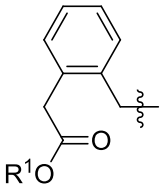
[00393] In some embodiments, –L–R^1 has the structure of:



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wherein each variable is independently as defined above and described herein.

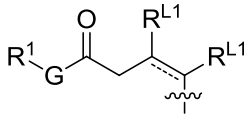
[00394] In some embodiments, –L–R^1 has the structure of:



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wherein each variable is independently as defined above and described herein.

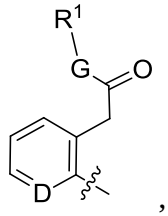
[00395] In some embodiments, –L–R^1 has the structure of:



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wherein each variable is independently as defined above and described herein.

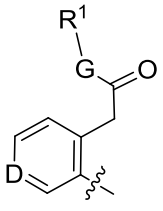
[00396] In some embodiments, –L–R^1 has the structure of:



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wherein each variable is independently as defined above and described herein.

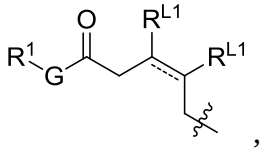
[00397] In some embodiments, –L–R^1 has the structure of:



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wherein each variable is independently as defined above and described herein.

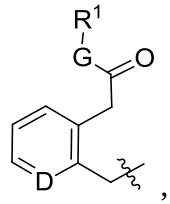
[00398] In some embodiments, –L–R^1 has the structure of:



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wherein each variable is independently as defined above and described herein.

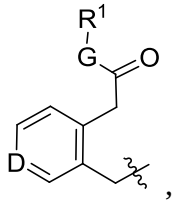
[00399] In some embodiments, –L–R^1 has the structure of:



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wherein each variable is independently as defined above and described herein.

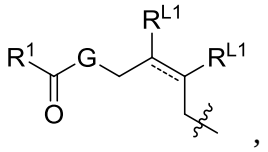
[00400] In some embodiments, –L–R^1 has the structure of:



,

wherein each variable is independently as defined above and described herein.

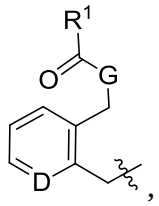
[00401] In some embodiments, –L–R^1 has the structure of:



,

wherein each variable is independently as defined above and described herein.

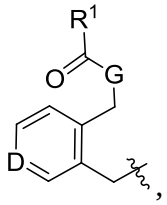
[00402] In some embodiments, –L–R^1 has the structure of:



,

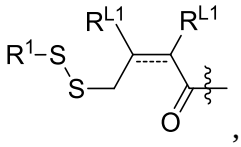
wherein each variable is independently as defined above and described herein.

[00403] In some embodiments, –L–R^1 has the structure of:



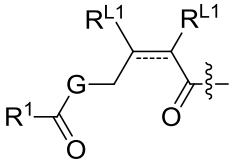
wherein each variable is independently as defined above and described herein.

[00404] In some embodiments, –L–R^1 has the structure of:



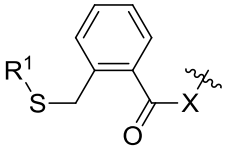
wherein each variable is independently as defined above and described herein.

[00405] In some embodiments, L has the structure of:



wherein each variable is independently as defined above and described herein.

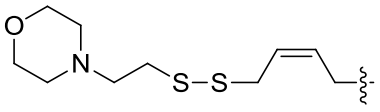
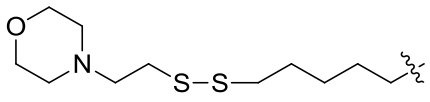
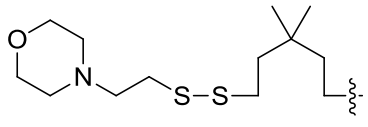
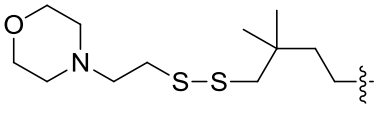
[00406] In some embodiments, –X–L–R^1 has the structure of:

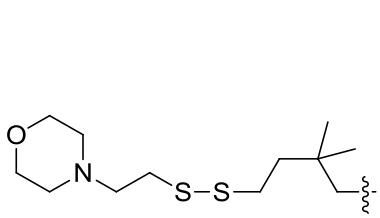
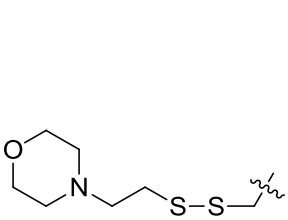
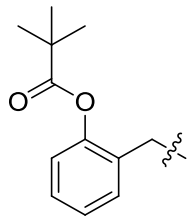
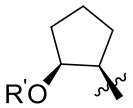
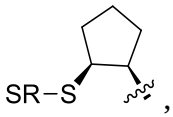
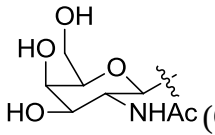
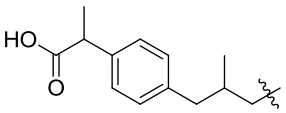
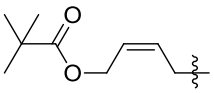
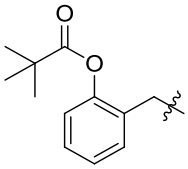
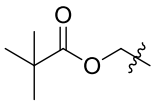
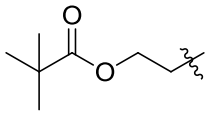
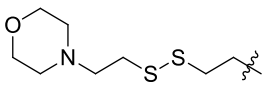
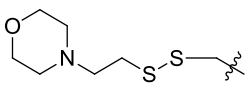
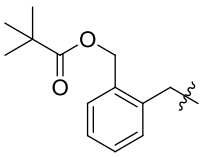
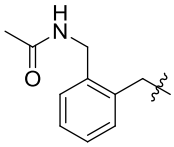
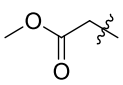
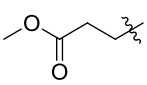
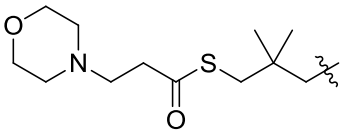
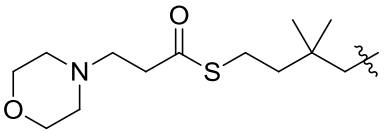
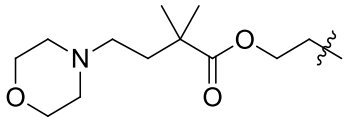
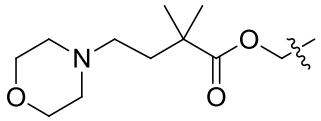
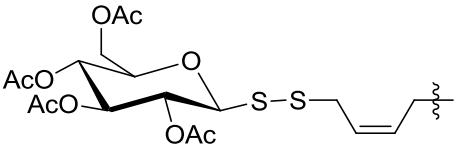
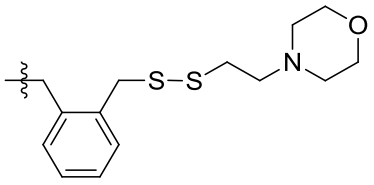
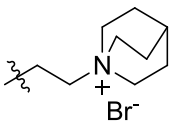
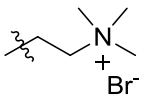
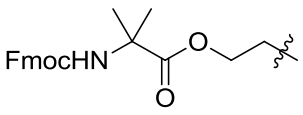
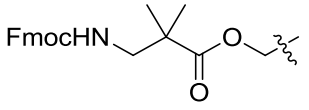
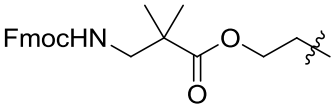
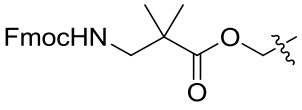
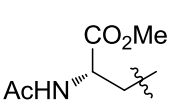
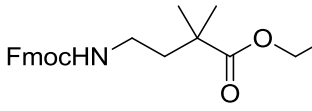
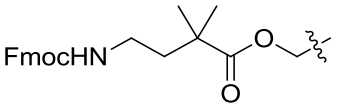
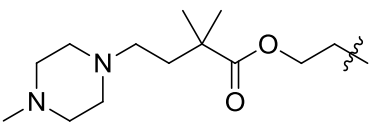
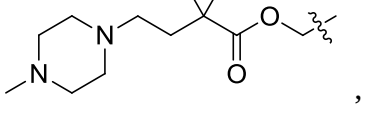
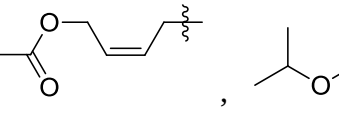
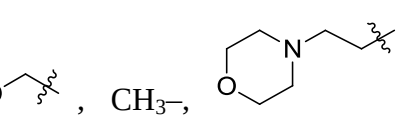


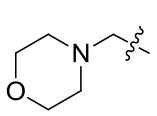
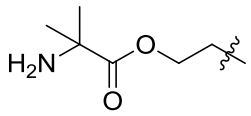
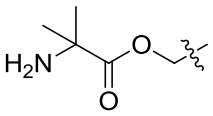
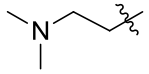
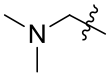
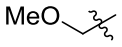
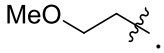
wherein:

the phenyl ring is optionally substituted, and

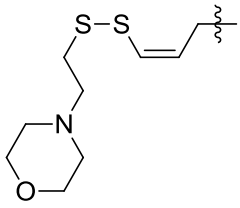
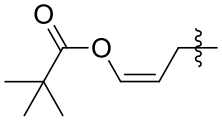
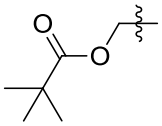
each of R^1 and X is independently as defined above and described herein.

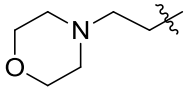
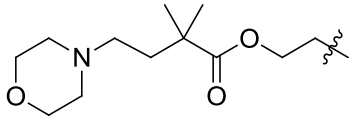
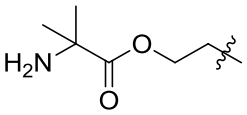
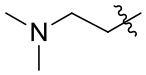
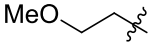
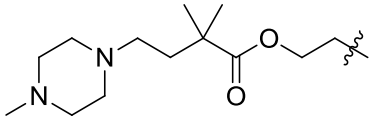
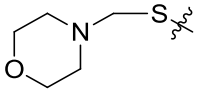
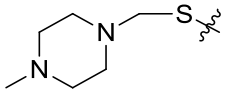
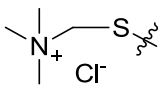
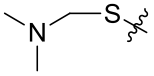
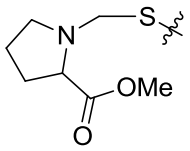
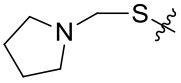
[00407] In some embodiments, –L–R^1 is , , , ,

, , , , ,  (GalNAc), , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , CH₃–, ,

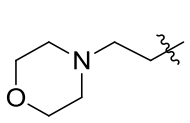
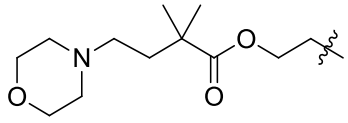
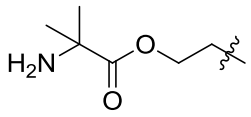
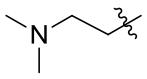
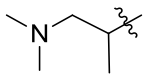
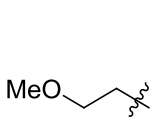
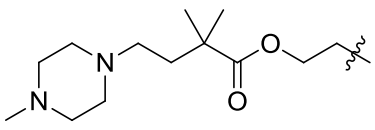

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[00408] In some embodiments, –L–R^1 is:

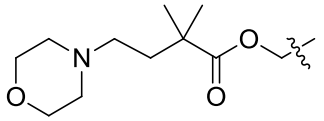
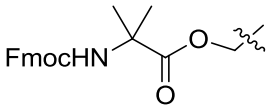
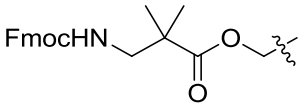
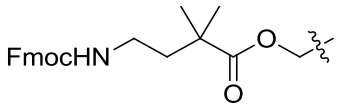
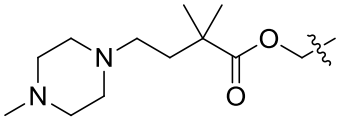
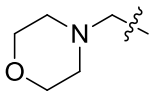
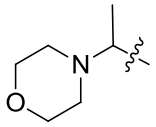
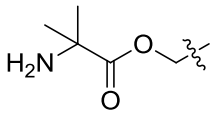
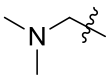
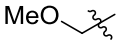

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[00409] In some embodiments, –L–R^1 is CH_3–, 
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. In some embodiments, –L–R^1 is 
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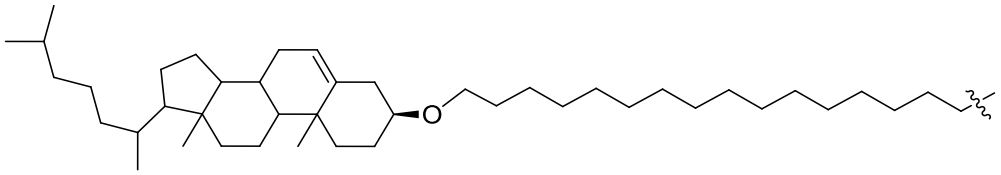
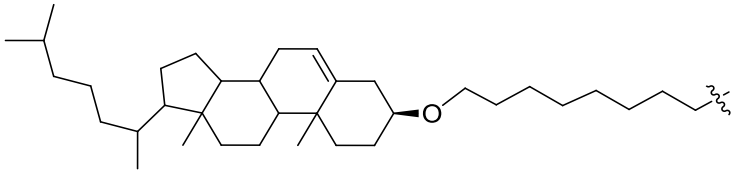
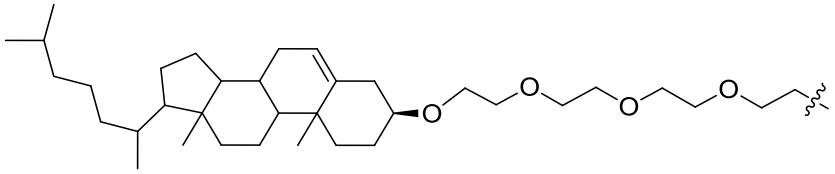
[00410] In some embodiments, –L–R^1 comprises a terminal optionally substituted –$(CH_2)_2$– moiety which is connected to X. In some embodiments, –L–R^1 comprises a terminal –$(CH_2)_2$– moiety which is connected to X. Exemplary such –L–R^1 moieties are depicted below:

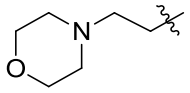
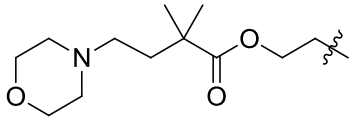
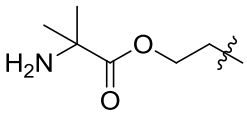
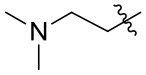
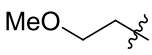
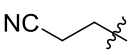
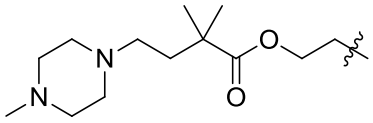

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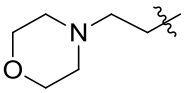
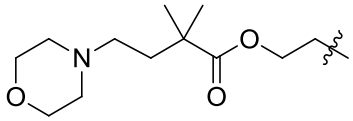
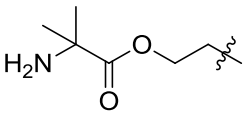
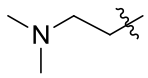
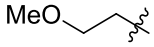
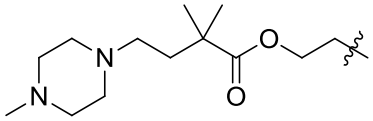
[00411] In some embodiments, –L–R^1 comprises a terminal optionally substituted –(CH_2)– moiety which is connected to X. In some embodiments, –L–R^1 comprises a terminal –(CH_2)– moiety which is connected to X. Exemplary such –L–R^1 moieties are depicted below:

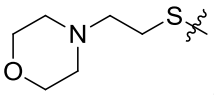
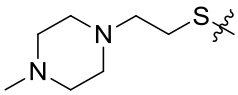
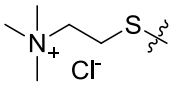
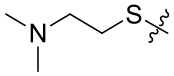
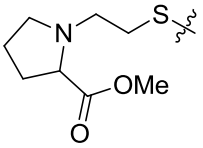
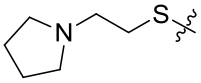
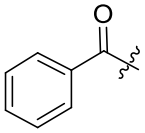











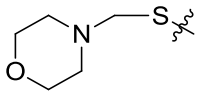
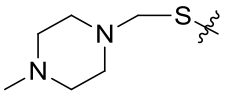
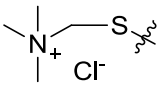
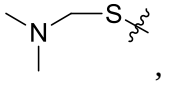
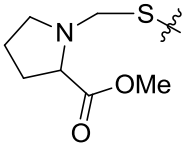
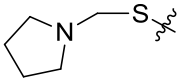
[00412] In some embodiments, –L–R^1 is

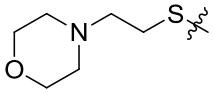
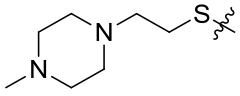
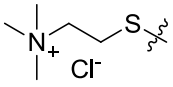
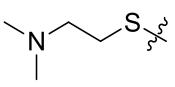
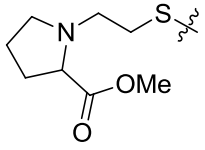
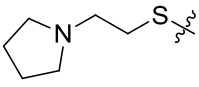
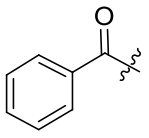

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, or

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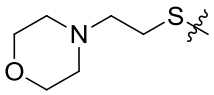
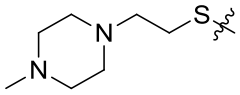
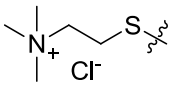
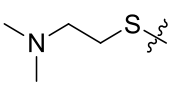
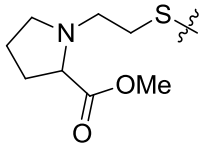
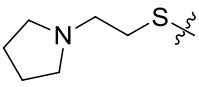
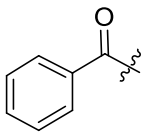
[00413] In some embodiments, –L–R^1 is CH_3–,

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,or

; and X is –S–.

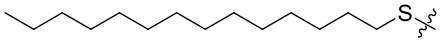
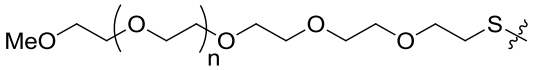
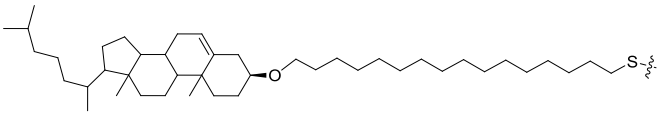
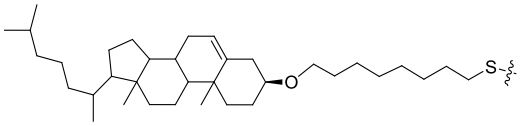
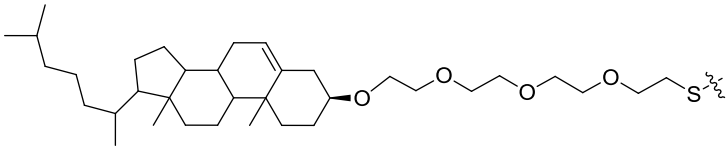
[00414] In some embodiments, –L–R^1 is CH_3–, , , , , , or , X is –S–, W is O, Y is –O–, and Z is –O–.

[00415] In some embodiments, R^1 is , , , , , , , or –S–(C_1–C_{10} aliphatic).

[00416] In some embodiments, R^1 is , , , , , or .

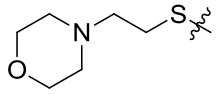
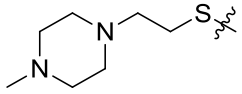
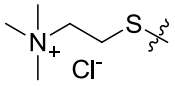
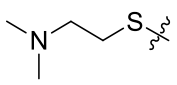
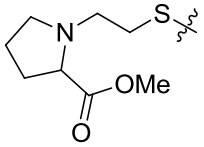
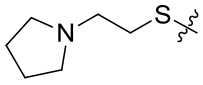
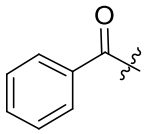
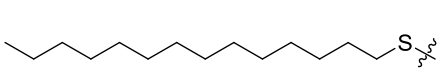
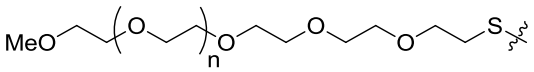
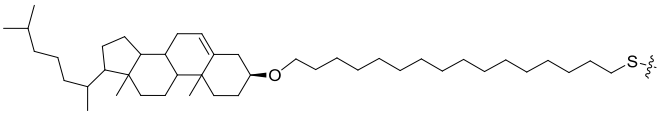
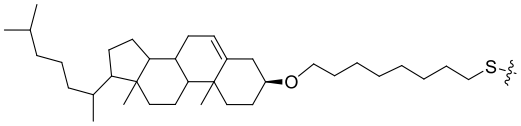
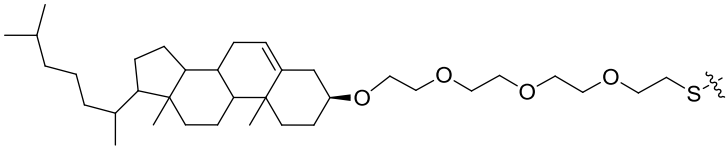
[00417] In some embodiments, X is –O– or –S–, and R^1 is , , , , , , , or –S–(C_1–C_{10} aliphatic).

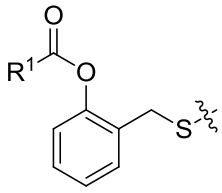
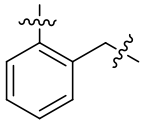
[00418] In some embodiments, X is –O– or –S–, and R^1 is , , , , , , ,

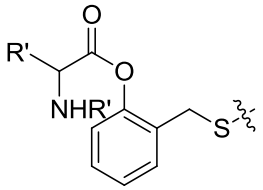
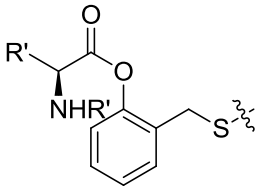
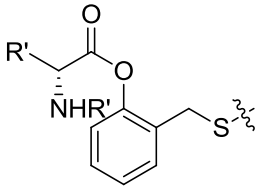
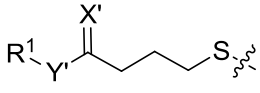
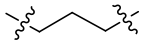
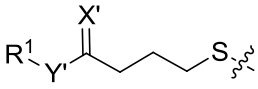
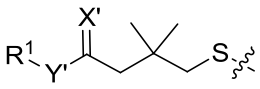
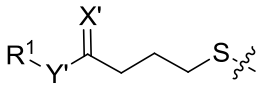
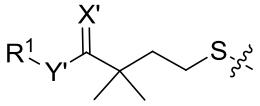
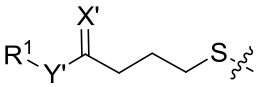
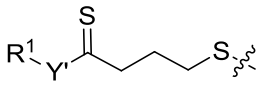
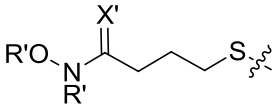
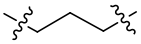
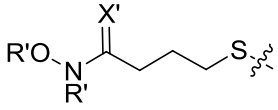
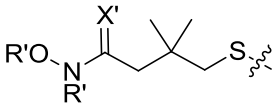
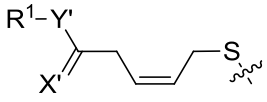
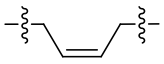
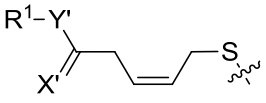
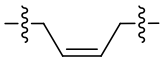
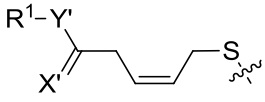
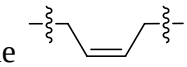

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, –S–(C_1–C_{10} aliphatic) or –S–(C_1–C_{50} aliphatic).

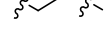
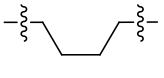
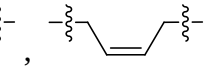
[00419] In some embodiments, L is a covalent bond and –L–R^1 is R^1.

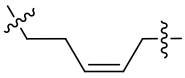
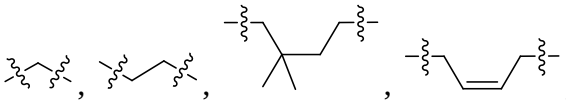
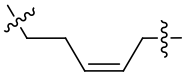
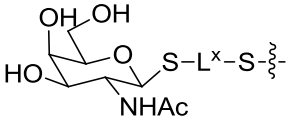
[00420] In some embodiments, –L–R^1 is not hydrogen.

[00421] In some embodiments, –X–L–R^1 is R^1 is

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, –S–(C_1–C_{10} aliphatic) or –S–(C_1–C_{50} aliphatic).

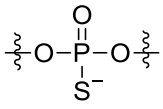
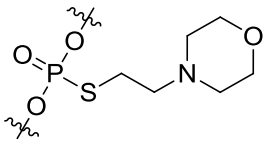
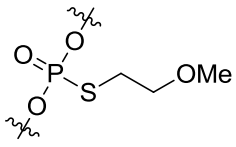
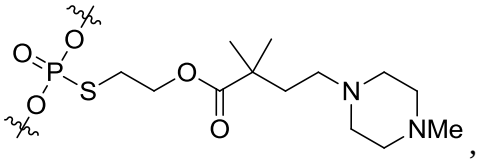
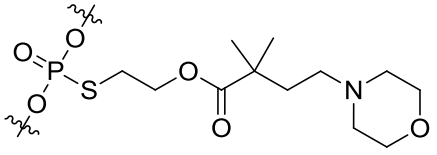
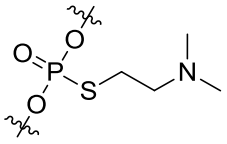
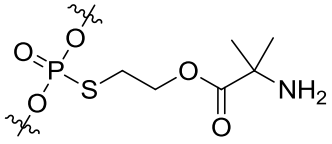
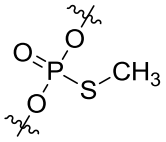
[00422] In some embodiments, –X–L–R^1 has the structure of

, wherein the

moiety is optionally substituted. In some embodiments, –X–L–R^1 is


. In some embodiments, –X–L–R^1 is 
. In some embodiments, –X–L–R^1 is 
. In some embodiments, –X–L–R^1 has the structure of 
, wherein X' is O or S, Y' is –O–, –S– or –NR'–, and the 
moiety is optionally substituted. In some embodiments, Y' is –O–, –S– or –NH–. In some embodiments, 
is 
. In some embodiments, 
is 
. In some embodiments, 
is 
. In some embodiments, –X–L–R^1 has the structure of 
, wherein X' is O or S, and the 
moiety is optionally substituted. In some embodiments, 
is 
. In some embodiments, –X–L–R^1 is 
, wherein the 
is optionally substituted. In some embodiments, –X–L–R^1 is 
, wherein the 
is substituted. In some embodiments, –X–L–R^1 is 
, wherein the 
is unsubstituted.

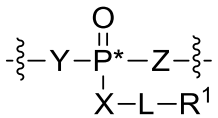
[00423] In some embodiments, –X–L–R^1 is R^1–C(O)–S–L^x–S–, wherein L^x is an optionally substituted group selected from 
, 
, 
, 
, and

. In some embodiments, L^x is , and . In some embodiments, –X–L–R^1 is $(CH_3)_3C$–S–S–L^x–S–. In some embodiments, –X–L–R^1 is R^1–C(=X')–Y'–$C(R)_2$–S–L^x–S–. In some embodiments, –X–L–R^1 is R–C(=X')–Y'–CH_2–S–L^x–S–. In some embodiments, –X–L–R^1 is .

[00424] As will be appreciated by a person skilled in the art, many of the –X–L–R^1 groups described herein are cleavable and can be converted to $–X^-$ after administration to a subject. In some embodiments, –X–L–R^1 is cleavable. In some embodiments, –X–L–R^1 is –S–L–R^1, and is converted to $–S^-$ after administration to a subject. In some embodiments, the conversion is promoted by an enzyme of a subject. As appreciated by a person skilled in the art, methods of determining whether the –S–L–R^1 group is converted to $–S^-$ after administration is widely known and practiced in the art, including those used for studying drug metabolism and pharmacokinetics.

[00425] In some embodiments, the internucleotidic linkage having the structure of formula **I** is , , , , , , , or .

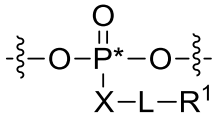
[00426] In some embodiments, the internucleotidic linkage of formula **I** has the structure of formula **I-a**:



(**I-a**)

wherein each variable is independently as defined above and described herein.

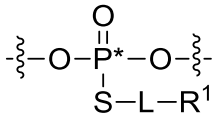
[00427] In some embodiments, the internucleotidic linkage of formula **I** has the structure of formula **I-b**:



(**I-b**)

wherein each variable is independently as defined above and described herein.

[00428] In some embodiments, the internucleotidic linkage of formula **I** is an phosphorothioate triester linkage having the structure of formula **I-c**:



(**I-c**)

wherein:

P* is an asymmetric phosphorus atom and is either *R*p or *S*p;

L is a covalent bond or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

R^1 is halogen, R, or an optionally substituted C_1–C_{50} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

each R′ is independently –R, -C(O)R, -CO_2R, or –SO_2R, or:

two R′ on the same nitrogen are taken together with their intervening atoms to form an optionally substituted heterocyclic or heteroaryl ring, or

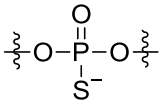
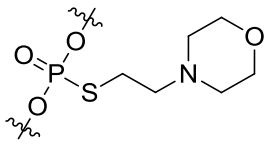
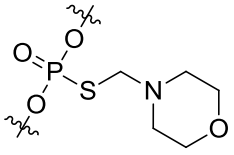
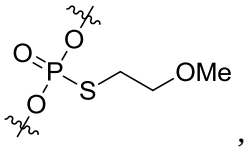
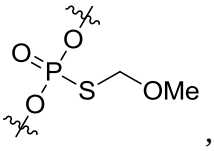
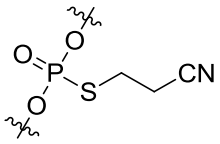
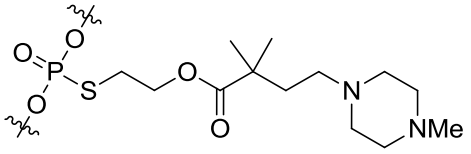
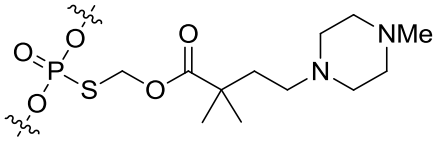
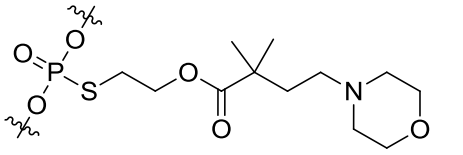
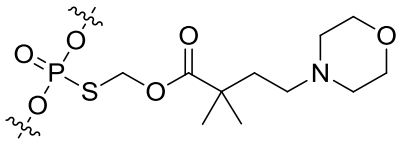
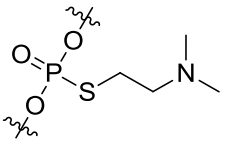
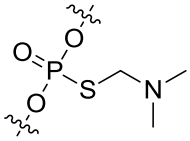
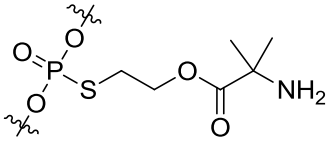
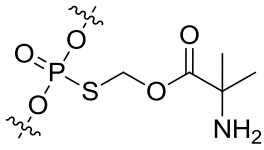
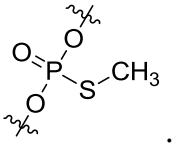
two R′ on the same carbon are taken together with their intervening atoms to form an optionally substituted aryl, carbocyclic, heterocyclic, or heteroaryl ring;

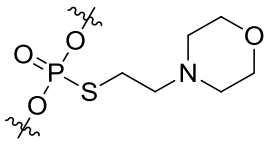
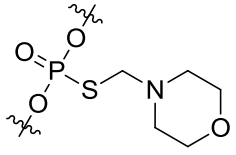
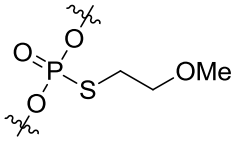
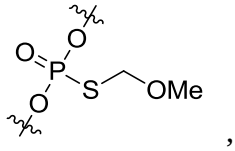
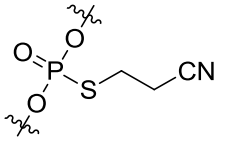
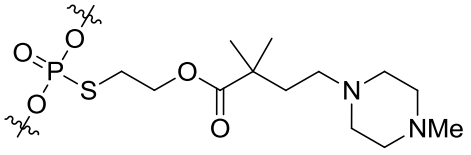
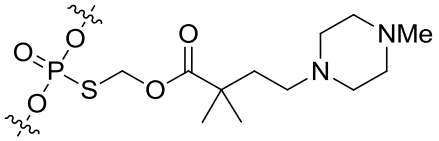
–Cy– is an optionally substituted bivalent ring selected from phenylene, carbocyclylene, arylene, heteroarylene, or heterocyclylene;

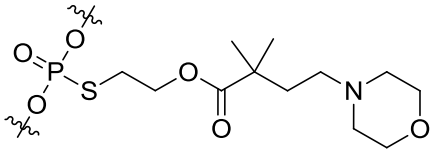
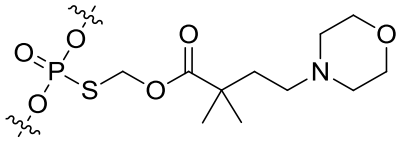
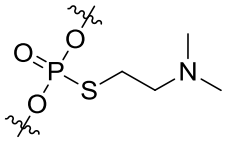
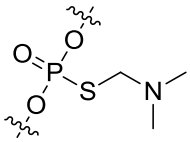
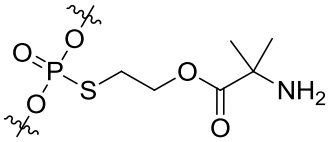
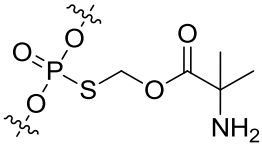
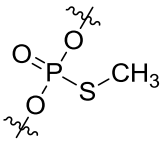
each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl;

each $^{-\!\!\!\sim\!\!\!-}$ independently represents a connection to a nucleoside; and

R^1 is not –H when L is a covalent bond.

[00429] In some embodiments, the internucleotidic linkage having the structure of formula **I** is , , , , , , , , , , , , , , and .

[00430] In some embodiments, the internucleotidic linkage having the structure of formula **I-c** is , , , , , , ,

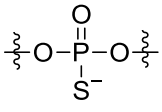

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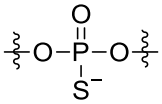
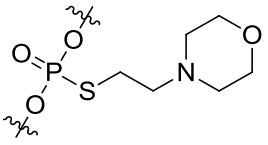
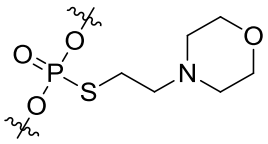
[00431] In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising one or more phosphate diester linkages, and one or more modified internucleotide linkages having the formula of **I-a**, **I-b**, or **I-c**.

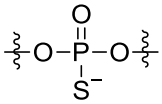
[00432] In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising at least one phosphate diester internucleotidic linkage and at least one phosphorothioate triester linkage having the structure of formula **I-c**. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising at least one phosphate diester internucleotidic linkage and at least two phosphorothioate triester linkages having the structure of formula **I-c**. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising at least one phosphate diester internucleotidic linkage and at least three phosphorothioate triester linkages having the structure of formula **I-c**. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising at least one phosphate diester internucleotidic linkage and at least four phosphorothioate triester linkages having the structure of formula **I-c**. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising at least one phosphate diester internucleotidic linkage and at least five phosphorothioate triester linkages having the structure of formula **I-c**.

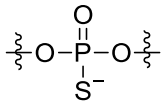
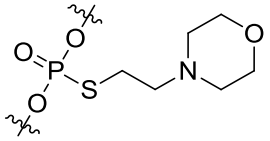
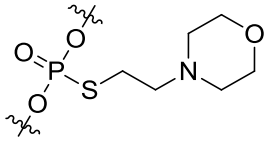
[00433] In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein the said sequence has over 50% identity with GCCTCAGTCTGCTTCGCACC. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein the said sequence has over 60% identity with GCCTCAGTCTGCTTCGCACC. In some embodiments, the present invention provides a

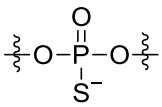
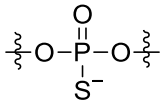
chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein the said sequence has over 70% identity with GCCTCAGTCTGCTTCGCACC. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein the said sequence has over 80% identity with GCCTCAGTCTGCTTCGCACC. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein the said sequence has over 90% identity with GCCTCAGTCTGCTTCGCACC. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein the said sequence has over 95% identity with GCCTCAGTCTGCTTCGCACC. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising the sequence of GCCTCAGTCTGCTTCGCACC. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC.

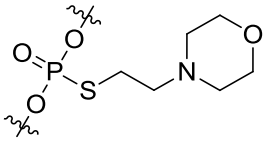
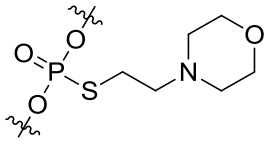
[00434] In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage has a chiral linkage phosphorus. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage has the structure of formula **I**. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein each internucleotidic linkage has the structure of formula **I**. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein each internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage is .

In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein each internucleotidic linkage is . In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage is . In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising a sequence found in GCCTCAGTCTGCTTCGCACC, wherein each internucleotidic linkage is .

[00435] In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage has a chiral linkage phosphorus. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage has the structure of formula **I**. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising the sequence of GCCTCAGTCTGCTTCGCACC, wherein each internucleotidic linkage has the structure of formula **I**. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising the sequence of GCCTCAGTCTGCTTCGCACC, wherein each internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage is . In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising the sequence of GCCTCAGTCTGCTTCGCACC, wherein each internucleotidic

linkage is  . In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage is  . In some embodiments, the present invention provides a chirally controlled oligonucleotide comprising the sequence of GCCTCAGTCTGCTTCGCACC, wherein each internucleotidic linkage is  .

[00436] In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage has a chiral linkage phosphorus. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage has the structure of formula **I**. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein each internucleotidic linkage has the structure of formula **I**. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein each internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage is  . In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein each internucleotidic linkage is  . In some embodiments, the present invention provides a chirally controlled

oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one internucleotidic linkage is . In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein each internucleotidic linkage is .

[00437] In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one linkage phosphorus is *R*p. It is understood by a person of ordinary skill in the art that in certain embodiments wherein the chirally controlled oligonucleotide comprises an RNA sequence, each T is independently and optionally replaced with U. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein each linkage phosphorus is *R*p. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one linkage phosphorus is *S*p. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein each linkage phosphorus is *S*p. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a blockmer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a stereoblockmer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a P-modification blockmer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a linkage blockmer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is an altmer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the

sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a stereoaltmer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a P-modification altmer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a linkage altmer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a unimer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a stereounimer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a P-modification unimer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a linkage unimer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a gapmer. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein the oligonucleotide is a skipmer.

[00438] In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein each cytosine is optionally and independently replaced by 5-methylcytosine. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein at least one cytosine is optionally and independently replaced by 5-methylcytosine. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of GCCTCAGTCTGCTTCGCACC, wherein each cytosine is optionally and independently replaced by 5-methylcytosine.

[00439] In some embodiments, a chirally controlled oligonucleotide is designed such that one or more nucleotides comprise a phosphorus modification prone to “autorelease” under certain conditions. That is, under certain conditions, a particular phosphorus modification is designed such that it self-cleaves from the oligonucleotide to provide, e.g., a phosphate diester

such as those found in naturally occurring DNA and RNA. In some embodiments, such a phosphorus modification has a structure of –O–L–R^1, wherein each of L and R^1 is independently as defined above and described herein. In some embodiments, an autorelease group comprises a morpholino group. In some embodiments, an autorelease group is characterized by the ability to deliver an agent to the internucleotidic phosphorus linker, which agent facilitates further modification of the phosphorus atom such as, e.g., desulfurization. In some embodiments, the agent is water and the further modification is hydrolysis to form a phosphate diester as is found in naturally occurring DNA and RNA.

[00440] In some embodiments, a chirally controlled oligonucleotide is designed such that the resulting pharmaceutical properties are improved through one or more particular modifications at phosphorus. It is well documented in the art that certain oligonucleotides are rapidly degraded by nucleases and exhibit poor cellular uptake through the cytoplasmic cell membrane (Poijarvi-Virta *et al*., Curr. Med. Chem. (2006), 13(28);3441-65; Wagner *et al*., Med. Res. Rev. (2000), 20(6):417-51; Peyrottes *et al.*, Mini Rev. Med. Chem. (2004), 4(4):395-408; Gosselin *et al.,* (1996), 43(1):196-208; Bologna *et al*., (2002), Antisense & Nucleic Acid Drug Development 12:33-41). For instance, Vives *et al.,* (Nucleic Acids Research (1999), 27(20):4071-76) found that tert-butyl SATE pro-oligonucleotides displayed markedly increased cellular penetration compared to the parent oligonucleotide.

[00441] In some embodiments, a modification at a linkage phosphorus is characterized by its ability to be transformed to a phosphate diester, such as those present in naturally occurring DNA and RNA, by one or more esterases, nucleases, and/or cytochrome P450 enzymes, including but not limited to, those listed in Table 1, below.

Table 1. Exemplary enzymes.

Family	Gene
CYP1	CYP1A1, CYP1A2, CYP1B1
CYP2	CYP2A6, CYP2A7, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2R1, CYP2S1, CYP2U1, CYP2W1

CYP3	CYP3A4, CYP3A5, CYP3A7, CYP3A43
CYP4	CYP4A11, CYP4A22, CYP4B1, CYP4F2, CYP4F3, CYP4F8, CYP4F11, CYP4F12, CYP4F22, CYP4V2, CYP4X1, CYP4Z1
CYP5	CYP5A1
CYP7	CYP7A1, CYP7B1
CYP8	CYP8A1 (prostacyclin synthase), CYP8B1 (bile acid biosynthesis)
CYP11	CYP11A1, CYP11B1, CYP11B2
CYP17	CYP17A1
CYP19	CYP19A1
CYP20	CYP20A1
CYP21	CYP21A2
CYP24	CYP24A1
CYP26	CYP26A1, CYP26B1, CYP26C1
CYP27	CYP27A1 (bile acid biosynthesis), CYP27B1 (vitamin D3 1-alpha hydroxylase, activates vitamin D3), CYP27C1 (unknown function)
CYP39	CYP39A1
CYP46	CYP46A1
CYP51	CYP51A1 (lanosterol 14-alpha demethylase)

[00442] In some embodiments, a modification at phosphorus results in a P-modification moiety characterized in that it acts as a pro-drug, e.g., the P-modification moiety facilitates delivery of an oligonucleotide to a desired location prior to removal. For instance, in some embodiments, a P-modification moiety results from PEGylation at the linkage phosphorus. One of skill in the relevant arts will appreciate that various PEG chain lengths are useful and that the selection of chain length will be determined in part by the result that is sought to be achieved by PEGylation. For instance, in some embodiments, PEGylation is effected in order to reduce RES uptake and extend *in vivo* circulation lifetime of an oligonucleotide.

[00443] In some embodiments, a PEGylation reagent for use in accordance with the present invention is of a molecular weight of about 300 g/mol to about 100,000 g/mol. In some embodiments, a PEGylation reagent is of a molecular weight of about 300 g/mol to about 10,000 g/mol. In some embodiments, a PEGylation reagent is of a molecular weight of about 300 g/mol to about 5,000 g/mol. In some embodiments, a PEGylation reagent is of a molecular weight of about 500 g/mol. In some embodiments, a PEGylation reagent of a molecular weight of about 1000 g/mol. In some embodiments, a PEGylation reagent is of a molecular weight of about 3000 g/mol. In some embodiments, a PEGylation reagent is of a molecular weight of about 5000 g/mol.

[00444] In certain embodiments, a PEGylation reagent is PEG500. In certain embodiments, a PEGylation reagent is PEG1000. In certain embodiments, a PEGylation reagent is PEG3000. In certain embodiments, a PEGylation reagent is PEG5000.

[00445] In some embodiments, a P-modification moiety is characterized in that it acts as a PK enhancer, e.g., lipids, PEGylated lipids, etc.

[00446] In some embodiments, a P-modification moiety is characterized in that it acts as an agent which promotes cell entry and/or endosomal escape, such as a membrane-disruptive lipid or peptide.

[00447] In some embodiments, a P-modification moiety is characterized in that it acts as a targeting agent. In some embodiments, a P-modification moiety is or comprises a targeting agent. The phrase "targeting agent," as used herein, is an entity that is associates with a payload of interest (e.g., with an oligonucleotide or oligonucleotide composition) and also interacts with a target site of interest so that the payload of interest is targeted to the target site of interest when associated with the targeting agent to a materially greater extent than is observed under otherwise

comparable conditions when the payload of interest is not associated with the targeting agent. A targeting agent may be, or comprise, any of a variety of chemical moieties, including, for example, small molecule moieties, nucleic acids, polypeptides, carbohydrates, etc. Targeting agents are described further by Adarsh et al., "Organelle Specific Targeted Drug Delivery – A Review," International Journal of Research in Pharmaceutical and Biomedical Sciences, 2011, p. 895.

[00448] Exemplary such targeting agents include, but are not limited to, proteins (e.g. Transferrin), oligopeptides (e.g., cyclic and acylic RGD-containing oligopedptides), antibodies (monoclonal and polyclonal antibodies, e.g. IgG, IgA, IgM, IgD, IgE antibodies), sugars / carbohydrates (e.g., monosaccharides and/or oligosaccharides (mannose, mannose-6-phosphate, galactose, and the like)), vitamins (e.g., folate), or other small biomolecules. In some embodiments, a targeting moiety is a steroid molecule (e.g., bile acids including cholic acid, deoxycholic acid, dehydrocholic acid; cortisone; digoxigenin; testosterone; cholesterol; cationic steroids such as cortisone having a trimethylaminomethyl hydrazide group attached via a double bond at the 3-position of the cortisone ring, etc.). In some embodiments, a targeting moiety is a lipophilic molecule (e.g., alicyclic hydrocarbons, saturated and unsaturated fatty acids, waxes, terpenes, and polyalicyclic hydrocarbons such as adamantine and buckminsterfullerenes). In some embodiments, a lipophilic molecule is a terpenoid such as vitamin A, retinoic acid, retinal, or dehydroretinal. In some embodiments, a targeting moiety is a peptide.

[00449] In some embodiments, a P-modification moiety is a targeting agent of formula –-X-L-R^1 wherein each of X, L, and R^1 are as defined in Formula **I** above.

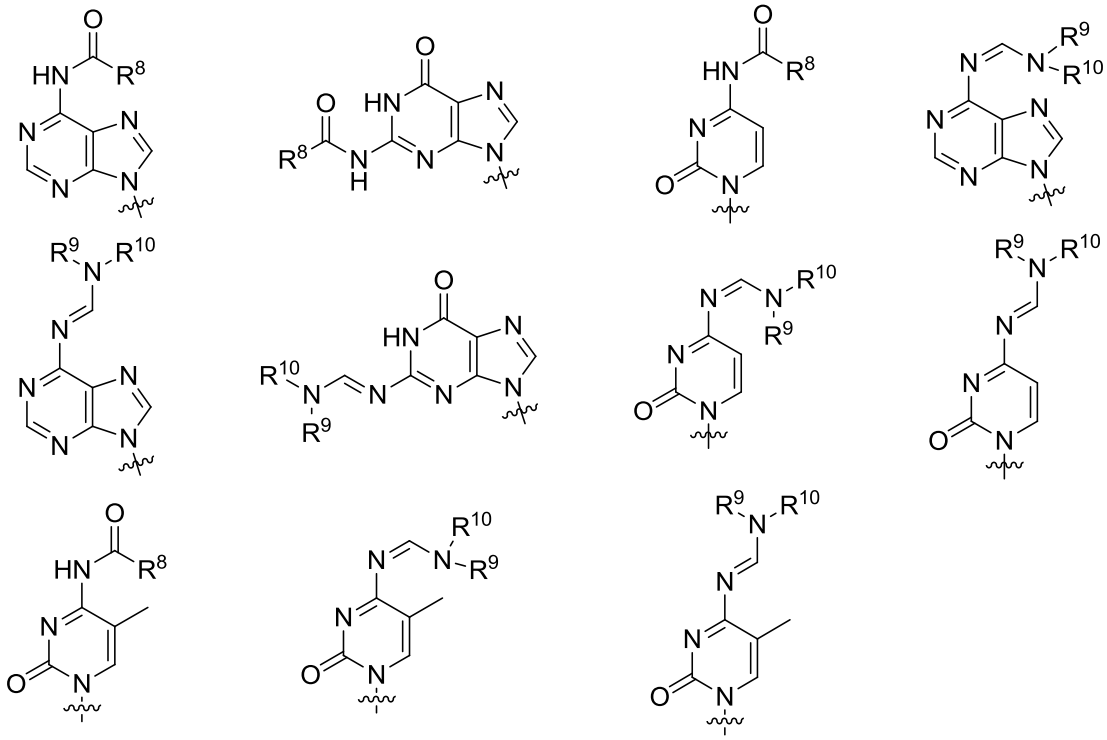
[00450] In some embodiments, a P-modification moiety is characterized in that it facilitates cell specific delivery.

[00451] In some embodiments, a P-modification moiety is characterized in that it falls into one or more of the above-described categories. For instance, in some embodiments, a P-modification moiety acts as a PK enhancer and a targeting ligand. In some embodiments, a P-modification moiety acts as a pro-drug and an endosomal escape agent. One of skill in the relevant arts would recognize that numerous other such combinations are possible and are contemplated by the present invention.

Nucleobases

[00452] In some embodiments, a nucleobase present in a provided oligonucleotide is a natural nucleobase or a modified nucleobase derived from a natural nucleobase. Examples include, but are not limited to, uracil, thymine, adenine, cytosine, and guanine having their respective amino groups protected by acyl protecting groups, 2-fluorouracil, 2-fluorocytosine, 5-bromouracil, 5-iodouracil, 2,6-diaminopurine, azacytosine, pyrimidine analogs such as pseudoisocytosine and pseudouracil and other modified nucleobases such as 8-substituted purines, xanthine, or hypoxanthine (the latter two being the natural degradation products). Exemplary modified nucleobases are disclosed in Chiu and Rana, *RNA*, **2003**, *9*, 1034-1048, Limbach *et al. Nucleic Acids Research*, **1994**, *22*, 2183-2196 and Revankar and Rao, *Comprehensive Natural Products Chemistry*, vol. 7, 313.

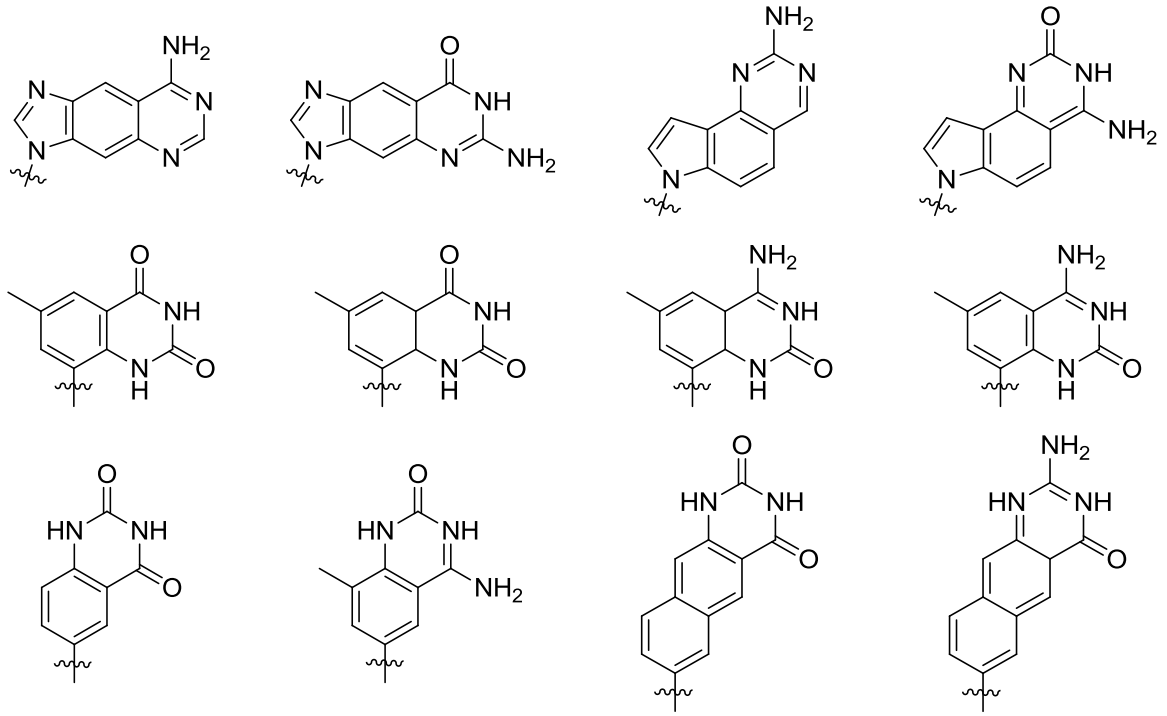
[00453] Compounds represented by the following general formulae are also contemplated as modified nucleobases:



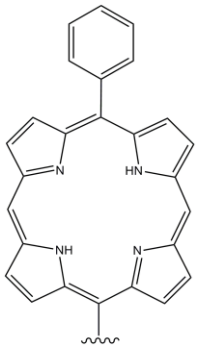
wherein R^8 is an optionally substituted, linear or branched group selected from aliphatic, aryl, aralkyl, aryloxylalkyl, carbocyclyl, heterocyclyl or heteroaryl group having 1 to 15 carbon atoms, including, by way of example only, a methyl, isopropyl, phenyl, benzyl, or

phenoxymethyl group; and each of R^9 and R^{10} is independently an optionally substituted group selected from linear or branched aliphatic, carbocyclyl, aryl, heterocyclyl and heteroaryl.

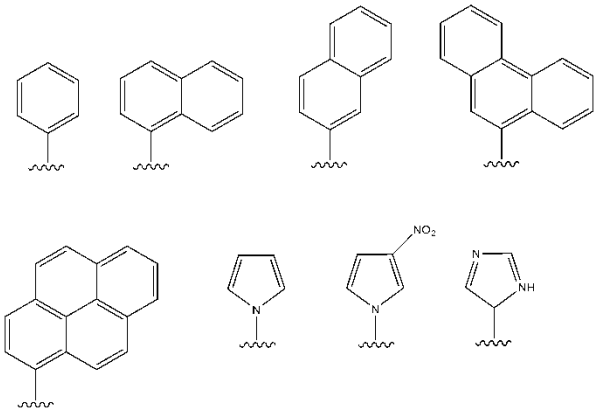
[00454] Modified nucleobases also include expanded-size nucleobases in which one or more aryl rings, such as phenyl rings, have been added. Nucleic base replacements described in the Glen Research catalog (www.glenresearch.com); Krueger AT *et al*, *Acc. Chem. Res.*, **2007**, *40*, 141-150; Kool, ET, *Acc. Chem. Res.*, **2002**, *35*, 936-943; Benner S.A., *et al*., *Nat. Rev. Genet.*, **2005**, *6*, 553-543; Romesberg, F.E., *et al.*, *Curr. Opin. Chem. Biol.*, **2003**, *7*, 723-733; Hirao, I., *Curr. Opin. Chem. Biol.*, **2006**, *10*, 622-627, are contemplated as useful for the synthesis of the nucleic acids described herein. Some examples of these expanded-size nucleobases are shown below:



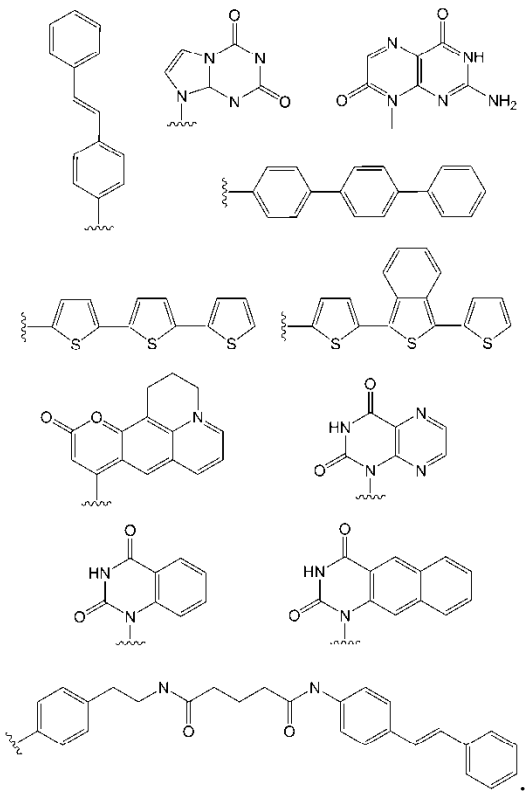
[00455] Herein, modified nucleobases also encompass structures that are not considered nucleobases but are other moieties such as, but not limited to, corrin- or porphyrin-derived rings. Porphyrin-derived base replacements have been described in Morales-Rojas, H and Kool, ET, *Org. Lett.*, 2002, *4*, 4377-4380. Shown below is an example of a porphyrin-derived ring which can be used as a base replacement:



[00456] In some embodiments, modified nucleobases are of any one of the following structures, optionally substituted:



[00457] In some embodiments, a modified nucleobase is fluorescent. Exemplary such fluorescent modified nucleobases include phenanthrene, pyrene, stillbene, isoxanthine, isozanthopterin, terphenyl, terthiophene, benzoterthiophene, coumarin, lumazine, tethered stillbene, benzo-uracil, and naphtho-uracil, as shown below:



[00458] In some embodiments, a modified nucleobase is unsubstituted. In some embodiments, a modified nucleobase is substituted. In some embodiments, a modified nucleobase is substituted such that it contains, e.g., heteroatoms, alkyl groups, or linking moieties connected to fluorescent moieties, biotin or avidin moieties, or other protein or peptides. In some embodiments, a modified nucleobase is a "universal base" that is not a nucleobase in the most classical sense, but that functions similarly to a nucleobase. One representative example of such a universal base is 3-nitropyrrole.

[00459] In some embodiments, other nucleosides can also be used in the process disclosed herein and include nucleosides that incorporate modified nucleobases, or nucleobases covalently bound to modified sugars. Some examples of nucleosides that incorporate modified nucleobases include 4-acetylcytidine; 5-(carboxyhydroxylmethyl)uridine; 2′-*O*-methylcytidine; 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyluridine; dihydrouridine; 2′-*O*-methylpseudouridine; beta,D-galactosylqueosine; 2′-*O*-methylguanosine; N^6-isopentenyladenosine; 1-methyladenosine; 1-methylpseudouridine; 1-methylguanosine; l-methylinosine; 2,2-dimethylguanosine; 2-methyladenosine; 2-methylguanosine; N^7-methylguanosine; 3-methyl-cytidine; 5-methylcytidine; 5-hydroxymethylcytidine; 5-

formylcytosine; 5-carboxylcytosine; N^6-methyladenosine; 7-methylguanosine; 5-methylaminoethyluridine; 5-methoxyaminomethyl-2-thiouridine; beta,D-mannosylqueosine; 5-methoxycarbonylmethyluridine; 5-methoxyuridine; 2-methylthio-N^6-isopentenyladenosine; *N*-((9-beta,D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine; *N*-((9-beta,D-ribofuranosylpurine-6-yl)-*N*-methylcarbamoyl)threonine; uridine-5-oxyacetic acid methylester; uridine-5-oxyacetic acid (v); pseudouridine; queosine; 2-thiocytidine; 5-methyl-2-thiouridine; 2-thiouridine; 4-thiouridine; 5-methyluridine; 2′-*O*-methyl-5-methyluridine; and 2′-*O*-methyluridine.

[00460] In some embodiments, nucleosides include 6′-modified bicyclic nucleoside analogs that have either (*R*) or (*S*)-chirality at the 6′-position and include the analogs described in US Patent No. 7,399,845. In other embodiments, nucleosides include 5′-modified bicyclic nucleoside analogs that have either (*R*) or (*S*)-chirality at the 5′-position and include the analogs described in US Patent Application Publication No. 20070287831.

[00461] In some embodiments, a nucleobase or modified nucleobase comprises one or more biomolecule binding moieties such as e.g., antibodies, antibody fragments, biotin, avidin, streptavidin, receptor ligands, or chelating moieties. In other embodiments, a nucleobase or modified nucleobase is 5-bromouracil, 5-iodouracil, or 2,6-diaminopurine. In some embodiments, a nucleobase or modified nucleobase is modified by substitution with a fluorescent or biomolecule binding moiety. In some embodiments, the substituent on a nucleobase or modified nucleobase is a fluorescent moiety. In some embodiments, the substituent on a nucleobase or modified nucleobase is biotin or avidin.

[00462] Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,457,191; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, each of which is herein incorporated by reference in its entirety.

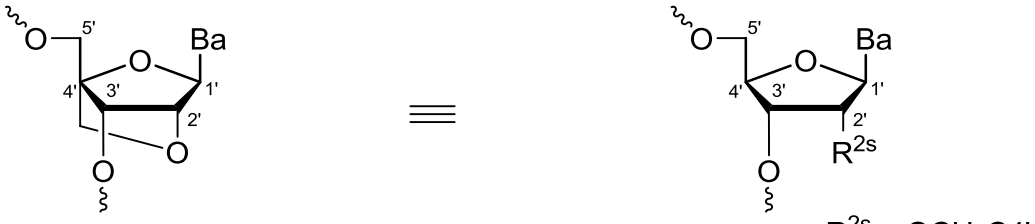
Sugars

[00463] The most common naturally occurring nucleotides are comprised of ribose sugars linked to the nucleobases adenosine (A), cytosine (C), guanine (G), and thymine (T) or uracil (U). Also contemplated are modified nucleotides wherein a phosphate group or linkage phosphorus in the nucleotides can be linked to various positions of a sugar or modified sugar. As non-limiting examples, the phosphate group or linkage phosphorus can be linked to the 2′, 3′, 4′ or 5′ hydroxyl moiety of a sugar or modified sugar. Nucleotides that incorporate modified nucleobases as described herein are also contemplated in this context. In some embodiments, nucleotides or modified nucleotides comprising an unprotected –OH moiety are used in accordance with methods of the present invention.

[00464] Other modified sugars can also be incorporated within a provided oligonucleotide. In some embodiments, a modified sugar contains one or more substituents at the 2′ position including one of the following: –F; $–CF_3$, –CN, $–N_3$, –NO, $–NO_2$, –OR', –SR', or $–N(R')_2$, wherein each R' is independently as defined above and described herein; $–O–(C_1–C_{10}$ alkyl), $–S–(C_1–C_{10}$ alkyl), $–NH–(C_1–C_{10}$ alkyl), or $–N(C_1–C_{10}$ alkyl$)_2$; $–O–(C_2–C_{10}$ alkenyl), $–S–(C_2–C_{10}$ alkenyl), $–NH–(C_2–C_{10}$ alkenyl), or $–N(C_2–C_{10}$ alkenyl$)_2$; $–O–(C_2–C_{10}$ alkynyl), $–S–(C_2–C_{10}$ alkynyl), $–NH–(C_2–C_{10}$ alkynyl), or $–N(C_2–C_{10}$ alkynyl$)_2$; or $–O—(C_1–C_{10}$ alkylene$)–O—(C_1–C_{10}$ alkyl), $–O–(C_1–C_{10}$ alkylene$)–NH–(C_1–C_{10}$ alkyl) or $–O–(C_1–C_{10}$ alkylene$)–NH(C_1–C_{10}$ alkyl$)_2$, $–NH–(C_1–C_{10}$ alkylene$)–O–(C_1–C_{10}$ alkyl), or $–N(C_1–C_{10}$ alkyl$)–(C_1–C_{10}$ alkylene$)–O–(C_1–C_{10}$ alkyl), wherein the alkyl, alkylene, alkenyl and alkynyl may be substituted or unsubstituted. Examples of substituents include, and are not limited to, $–O(CH_2)_nOCH_3$, and $–O(CH_2)_nNH_2$, wherein n is from 1 to about 10, MOE, DMAOE, DMAEOE. Also contemplated herein are modified sugars described in WO 2001/088198; and Martin *et al.*, *Helv. Chim. Acta*, **1995**, *78*, 486-504. In some embodiments, a modified sugar comprises one or more groups selected from a substituted silyl group, an RNA cleaving group, a reporter group, a fluorescent label, an intercalator, a group for improving the pharmacokinetic properties of a nucleic acid, a group for improving the pharmacodynamic properties of a nucleic acid, or other substituents having similar properties. In some embodiments, modifications are made at one or more of the the 2′, 3′, 4′, 5′, or 6′ positions of the sugar or modified sugar, including the 3′ position of the sugar on the 3′-terminal nucleotide or in the 5′ position of the 5′-terminal nucleotide.

[00465] In some embodiments, the 2'-OH of a ribose is replaced with a substituent including one of the following: –H, –F; $–CF_3$, –CN, $–N_3$, –NO, $–NO_2$, –OR', –SR', or $–N(R')_2$, wherein each R' is independently as defined above and described herein; –O–(C_1–C_{10} alkyl), –S–(C_1–C_{10} alkyl), –NH–(C_1–C_{10} alkyl), or –N(C_1–C_{10} alkyl)$_2$; –O–(C_2–C_{10} alkenyl), –S–(C_2–C_{10} alkenyl), –NH–(C_2–C_{10} alkenyl), or –N(C_2–C_{10} alkenyl)$_2$; –O–(C_2–C_{10} alkynyl), –S–(C_2–C_{10} alkynyl), –NH–(C_2–C_{10} alkynyl), or –N(C_2–C_{10} alkynyl)$_2$; or –O—(C_1–C_{10} alkylene)–O—(C_1–C_{10} alkyl), –O–(C_1–C_{10} alkylene)–NH–(C_1–C_{10} alkyl) or –O–(C_1–C_{10} alkylene)–NH(C_1–C_{10} alkyl)$_2$, –NH–(C_1–C_{10} alkylene)–O–(C_1–C_{10} alkyl), or –N(C_1–C_{10} alkyl)–(C_1–C_{10} alkylene)–O–(C_1–C_{10} alkyl), wherein the alkyl, alkylene, alkenyl and alkynyl may be substituted or unsubstituted. In some embodiments, the 2'–OH is replaced with –H (deoxyribose). In some embodiments, the 2'–OH is replaced with –F. In some embodiments, the 2'–OH is replaced with –OR'. In some embodiments, the 2'–OH is replaced with –OMe. In some embodiments, the 2'–OH is replaced with $–OCH_2CH_2OMe$.

[00466] Modified sugars also include locked nucleic acids (LNAs). In some embodiments, two substituents on sugar carbon atoms are taken together to form a bivalent moiety. In some embodiments, two substituents are on two different sugar carbon atoms. In some embodiments, a formed bivalent moiety has the structure of −L− as defined herein. In some embodiments, −L− is $−O−CH_2−$, wherein $−CH_2−$ is optionally substituted. In some embodiments, −L− is $−O−CH_2−$. In some embodiments, −L− is −O−CH(Et)−. In some embodiments, −L− is between C2 and C4 of a sugar moiety. In some embodiments, a locked nucleic acid has the structure indicated below. A locked nucleic acid of the structure below is indicated, wherein Ba represents a nucleobase or modified nucleobase as described herein, and wherein R^{2s} is $–OCH_2C4'–$.



C2'OCH$_2$C4' = LNA (Locked Nucleic Acid)

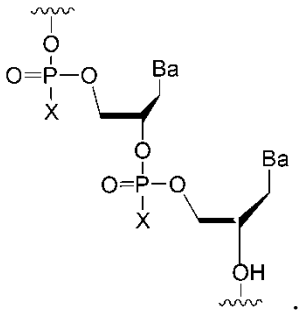
R^{2s} = OCH_2C4'

[00467] In some embodiments, a modified sugar is an ENA such as those described in, e.g., Seth et al., J Am Chem Soc. 2010 October 27; 132(42): 14942–14950. In some

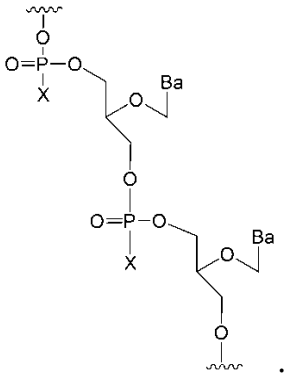
embodiments, a modified sugar is any of those found in an XNA (xenonucleic acid), for instance, arabinose, anhydrohexitol, threose, 2'fluoroarabinose, or cyclohexene.

[00468] Modified sugars include sugar mimetics such as cyclobutyl or cyclopentyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, US Patent Nos.: 4,981,957; 5,118,800; 5,319,080 ; and 5,359,044. Some modified sugars that are contemplated include sugars in which the oxygen atom within the ribose ring is replaced by nitrogen, sulfur, selenium, or carbon. In some embodiments, a modified sugar is a modified ribose wherein the oxygen atom within the ribose ring is replaced with nitrogen, and wherein the nitrogen is optionally substituted with an alkyl group (e.g., methyl, ethyl, isopropyl, etc).

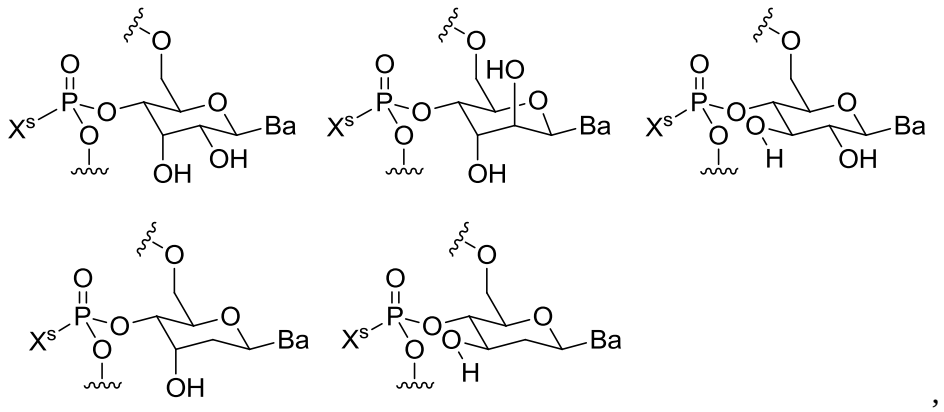
[00469] Non-limiting examples of modified sugars include glycerol, which form glycerol nucleic acid (GNA) analogues. One example of a GNA analogue is shown below and is described in Zhang, R *et al.*, *J. Am. Chem. Soc.*, 2008, *130*, 5846-5847; Zhang L, *et al.*, *J. Am. Chem. Soc.*, 2005, *127*, 4174-4175 and Tsai CH *et al.*, *PNAS*, 2007, 14598-14603 (X = O^-):



[00470] Another example of a GNA derived analogue, flexible nucleic acid (FNA) based on the mixed acetal aminal of formyl glycerol, is described in Joyce GF *et al.*, *PNAS*, 1987, *84*, 4398-4402 and Heuberger BD and Switzer C, *J. Am. Chem. Soc.*, 2008, *130*, 412-413, and is shown below:

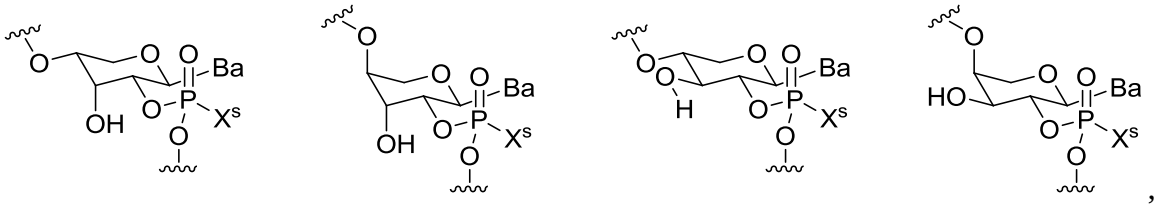


[00471] Additional non-limiting examples of modified sugars include hexopyranosyl (6' to 4'), pentopyranosyl (4' to 2'), pentopyranosyl (4' to 3'), or tetrofuranosyl (3' to 2') sugars. In some embodiments, a hexopyranosyl (6' to 4') sugar is of any one in the following formulae:



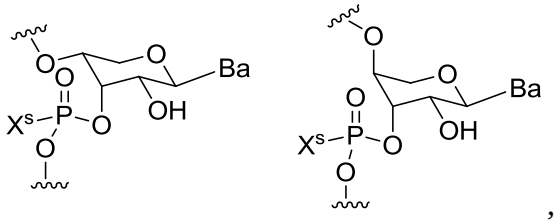
wherein X^s corresponds to the P-modification group "-XLR^1" described herein and Ba is as defined herein.

[00472] In some embodiments, a pentopyranosyl (4' to 2') sugar is of any one in the following formulae:



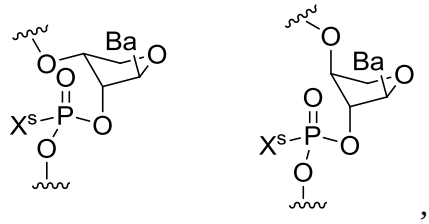
wherein X^s corresponds to the P-modification group "-XLR^1" described herein and Ba is as defined herein.

[00473] In some embodiments, a pentopyranosyl (4' to 3') sugar is of any one in the following formulae:



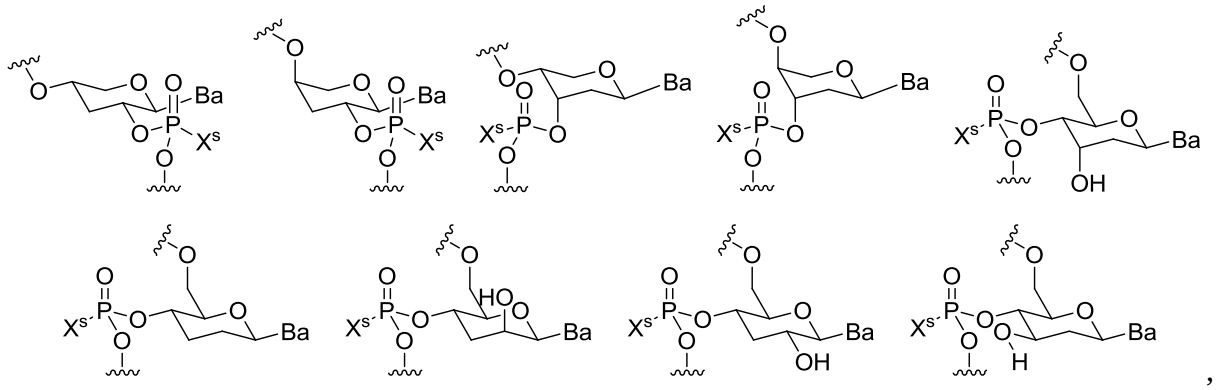
wherein X^s corresponds to the P-modification group "-XLR^1" described herein and Ba is as defined herein.

[00474] In some embodiments, a tetrofuranosyl (3' to 2') sugar is of either in the following formulae:



wherein X^s corresponds to the P-modification group "-XLR^1" described herein and Ba is as defined herein.

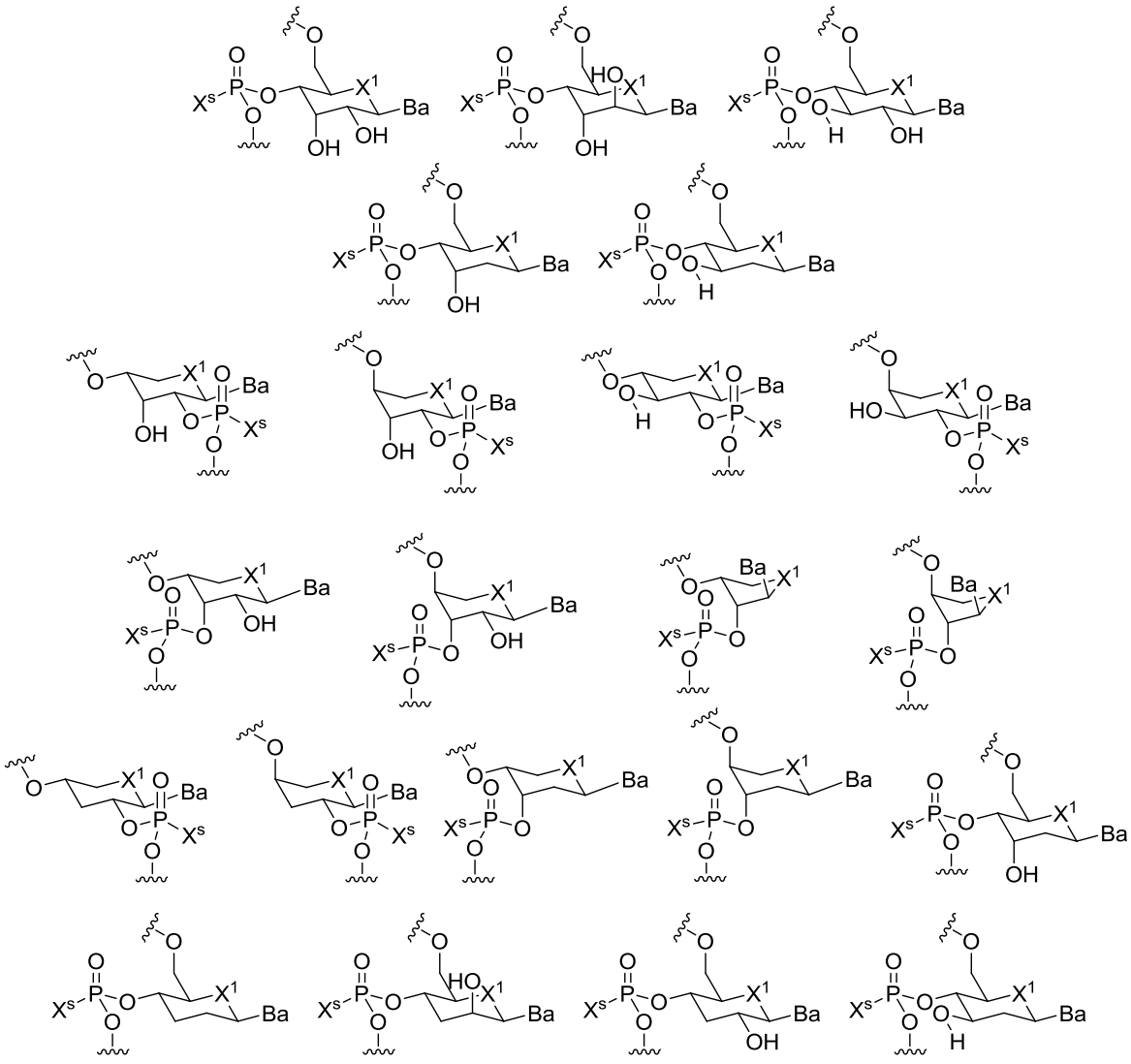
[00475] In some embodiments, a modified sugar is of any one in the following formulae:



wherein X^s corresponds to the P-modification group "-XLR^1" described herein and Ba is as defined herein.

[00476] In some embodiments, one or more hydroxyl group in a sugar moiety is optionally and independently replaced with halogen, R' $-N(R')_2$, $-OR'$, or $-SR'$, wherein each R' is independently as defined above and described herein.

[00477] In some embodiments, a sugar mimetic is as illustrated below, wherein X^s corresponds to the P-modification group "-XLR^1" described herein, Ba is as defined herein, and X^1 is selected from –S–, –Se–, $-CH_2-$, –NMe–, –NEt– or –N*i*Pr–.



[00478] In some embodiments, at least 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%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50% or more (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more), inclusive, of the sugars in a chirally controlled oligonucleotide composition are modified. In some embodiments, only purine residues are modified (e.g., about 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%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50% or more [e.g., 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more] of the purine residues

are modified). In some embodiments, only pyrimidine residues are modified (e.g., about 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%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50% or more [e.g., 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more] of the pyridimine residues are modified). In some embodiments, both purine and pyrimidine residues are modified.

[00479] Modified sugars and sugar mimetics can be prepared by methods known in the art, including, but not limited to: A. Eschenmoser, Science (1999), 284:2118; M. Bohringer *et al*, Helv. Chim. Acta (1992), 75:1416-1477; M. Egli *et al*, J. Am. Chem. Soc. (2006), 128(33):10847-56; A. Eschenmoser in *Chemical Synthesis: Gnosis to Prognosis*, C. Chatgilialoglu and V. Sniekus, Ed., (Kluwer Academic, Netherlands, 1996), p.293; K.-U. Schoning *et al*, Science (2000), 290:1347-1351; A. Eschenmoser *et al*, Helv. Chim. Acta (1992), 75:218; J. Hunziker *et al*, Helv. Chim. Acta (1993), 76:259; G. Otting *et al*, Helv. Chim. Acta (1993), 76:2701; K. Groebke *et al*, Helv. Chim. Acta (1998), 81:375; and A. Eschenmoser, Science (1999), 284:2118. Modifications to the 2' modifications can be found in Verma, S. *et al*. *Annu. Rev. Biochem.* **1998,** 67, 99-134 and all references therein. Specific modifications to the ribose can be found in the following references: 2'-fluoro (Kawasaki et. al., *J. Med. Chem.*, **1993,** 36, 831- 841), 2'-MOE (Martin, P. *Helv. Chim. Acta* **1996,** 79, 1930-1938), "LNA" (Wengel, J. *Acc. Chem. Res.* **1999,** *32*, 301-310). In some embodiments, a modified sugar is any of those described in PCT Publication No. WO2012/030683, incorporated herein by reference, and depicted in the Figures 26-30 of the present application.

Oligonucleotides

[00480] In some embodiments, the present invention provides oligonucleotides and oligonucleotide compositions that are chirally controlled. For instance, in some embodiments, a provided composition contains predetermined levels of one or more individual oligonucleotide types, wherein an oligonucleotide type is defined by: 1) base sequence; 2) pattern of backbone linkages; 3) pattern of backbone chiral centers; and 4) pattern of backbone P-modifications.

[00481] In some embodiments, a provided oligonucleotide is a unimer. In some embodiments, a provided oligonucleotide is a P-modification unimer. In some embodiments, a provided oligonucleotide is a stereounimer. In some embodiments, a provided oligonucleotide is

a stereounimer of configuration *R*p. In some embodiments, a provided oligonucleotide is a stereounimer of configuration *S*p.

[00482] In some embodiments, a provided oligonucleotide is an altmer. In some embodiments, a provided oligonucleotide is a P-modification altmer. In some embodiments, a provided oligonucleotide is a stereoaltmer.

[00483] In some embodiments, a provided oligonucleotide is a blockmer. In some embodiments, a provided oligonucleotide is a P-modification blockmer. In some embodiments, a provided oligonucleotide is a stereoblockmer.

[00484] In some embodiments, a provided oligonucleotide is a gapmer.

[00485] In some embodiments, a provided oligonucleotide is a skipmer.

[00486] In some embodiments, a provided oligonucleotide is a hemimer. In some embodiments, a hemimer is an oligonucleotide wherein the 5'-end or the 3'-end has a sequence that possesses a structure feature that the rest of the oligonucleotide does not have. In some embodiments, the 5'-end or the 3'-end has or comprises 2 to 20 nucleotides. In some embodiments, a structural feature is a base modification. In some embodiments, a structural feature is a sugar modification. In some embodiments, a structural feature is a P-modification. In some embodiments, a structural feature is stereochemistry of the chiral internucleotidic linkage. In some embodiments, a structural feature is or comprises a base modification, a sugar modification, a P-modification, or stereochemistry of the chiral internucleotidic linkage, or combinations thereof. In some embodiments, a hemimer is an oligonucleotide in which each sugar moiety of the 5'-end sequence shares a common modification. In some embodiments, a hemimer is an oligonucleotide in which each sugar moiety of the 3'-end sequence shares a common modification. In some embodiments, a common sugar modification of the 5' or 3' end sequence is not shared by any other sugar moieties in the oligonucleotide. In some embodiments, an exemplary hemimer is an oligonucleotide comprising a sequence of substituted or unsubstituted 2'-O-alkyl sugar modified nucleosides, bicyclic sugar modified nucleosides, β-D-ribonucleosides or β-D- deoxyribonucleosides (for example 2'-MOE modified nucleosides, and LNA™ or ENA™ bicyclic syugar modified nucleosides) at one terminus and a sequence of nucleosides with a different sugar moiety (such as a substituted or unsubstituted 2'-O-alkyl sugar modified nucleosides, bicyclic sugar modified nucleosides or natural ones) at the other terminus. In some embodiments, a provided oligonucleotide is a combination of one or more of unimer,

altmer, blockmer, gapmer, hemimer and skipmer. In some embodiments, a provided oligonucleotide is a combination of one or more of unimer, altmer, blockmer, gapmer, and skipmer. For instance, in some embodiments, a provided oligonucleotide is both an altmer and a gapmer. In some embodiments, a provided nucleotide is both a gapmer and a skipmer. One of skill in the chemical and synthetic arts will recognize that numerous other combinations of patterns are available and are limited only by the commercial availability and / or synthetic accessibility of constituent parts required to synthesize a provided oligonucleotide in accordance with methods of the present invention. In some embodiments, a hemimer structure provides advantageous benefits, as exemplified by Figure 29. In some embodiments, provided oligonucleotides are 5'-hemmimers that comprises modified sugar moieties in a 5'-end sequence. In some embodiments, provided oligonucleotides are 5'-hemmimers that comprises modified 2'-sugar moieties in a 5'-end sequence.

[00487] In some embodiments, a provided oligonucleotide comprises one or more optionally substituted nucleotides. In some embodiments, a provided oligonucleotide comprises one or more modified nucleotides. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted nucleosides. In some embodiments, a provided oligonucleotide comprises one or more modified nucleosides. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted LNAs.

[00488] In some embodiments, a provided oligonucleotide comprises one or more optionally substituted nucleobases. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted natural nucleobases. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted modified nucleobases. In some embodiments, a provided oligonucleotide comprises one or more 5-methylcytidine; 5-hydroxymethylcytidine, 5-formylcytosine, or 5-carboxylcytosine. In some embodiments, a provided oligonucleotide comprises one or more 5-methylcytidine.

[00489] In some embodiments, a provided oligonucleotide comprises one or more optionally substituted sugars. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted sugars found in naturally occurring DNA and RNA. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted ribose or deoxyribose. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted ribose or deoxyribose, wherein one or more hydroxyl groups of the ribose

or deoxyribose moiety is optionally and independently replaced by halogen, R', $–N(R')_2$, –OR', or –SR', wherein each R' is independently as defined above and described herein. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with halogen, R', $–N(R')_2$, –OR', or –SR', wherein each R' is independently as defined above and described herein. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with halogen. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with one or more –F. halogen. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with –OR', wherein each R' is independently as defined above and described herein. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with –OR', wherein each R' is independently an optionally substituted $C_1–C_6$ aliphatic. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with –OR', wherein each R' is independently an optionally substituted $C_1–C_6$ alkyl. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with –OMe. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with –O–methoxyethyl.

[00490] In some embodiments, a provided oligonucleotide is single-stranded oligonucleotide.

[00491] In some embodiments, a provided oligonucleotide is a hybridized oligonucleotide strand. In certain embodiments, a provided oligonucleotide is a partially hydridized oligonucleotide strand. In certain embodiments, a provided oligonucleotide is a completely hydridized oligonucleotide strand. In certain embodiments, a provided oligonucleotide is a

double-stranded oligonucleotide. In certain embodiments, a provided oligonucleotide is a triple-stranded oligonucleotide (e.g., a triplex).

[00492] In some embodiments, a provided oligonucleotide is chimeric. For example, in some embodiments, a provided oligonucleotide is DNA-RNA chimera, DNA-LNA chimera, etc.

[00493] In some embodiments, any one of the structures comprising an oligonucleotide depicted in WO2012/030683 can be modified in accordance with methods of the present invention to provide chirally controlled variants thereof. For example, in some embodiments the chirally controlled variants comprise a stereochemical modification at any one or more of the linkage phosphorus and/or a P-modification at any one or more of the linkage phosphorus. For example, in some embodiments, a particular nucleotide unit of a oligonucleotide of WO2012/030683 is preselected to be stereochemically modified at the linkage phosphorus of that nucleotide unit and/or P-modified at the linkage phosphorus of that nucleotide unit. In some embodiments, a chirally controlled oligonucleotide is of any one of the structures depicted in Figures 26-30. In some embodiments, a chirally controlled oligonucleotide is a variant (e.g., modified version) of any one of the structures depicted in Figures 26-30. The disclosure of WO2012/030683 is herein incorporated by reference in its entirety.

[00494] In some embodiments, a provided oligonucleotide is a therapeutic agent.

[00495] In some embodiments, a provided oligonucleotide is an antisense oligonucleotide.

[00496] In some embodiments, a provided oligonucleotide is an antigene oligonucleotide.

[00497] In some embodiments, a provided oligonucleotide is a decoy oligonucleotide.

[00498] In some embodiments, a provided oligonucleotide is part of a DNA vaccine.

[00499] In some embodiments, a provided oligonucleotide is an immunomodulatory oligonucleotide, e.g., immunostimulatory oligonucleotide and immunoinhibitory oligonucleotide.

[00500] In some embodiments, a provided oligonucleotide is an adjuvant.

[00501] In some embodiments, a provided oligonucleotide is an aptamer.

[00502] In some embodiments, a provided oligonucleotide is a ribozyme.

[00503] In some embodiments, a provided oligonucleotide is a deoxyribozyme (DNAzymes or DNA enzymes).

[00504] In some embodiments, a provided oligonucleotide is an siRNA.

[00505] In some embodiments, a provided oligonucleotide is a microRNA, or miRNA.

[00506] In some embodiments, a provided oligonucleotide is a ncRNA (non-coding RNAs), including a long non-coding RNA (lncRNA) and a small non-coding RNA, such as piwi-interacting RNA (piRNA).

[00507] In some embodiments, a provided oligonucleotide is complementary to a structural RNA, e.g., tRNA.

[00508] In some embodiments, a provided oligonucleotide is a nucleic acid analog, e.g., GNA, LNA, PNA, TNA and Morpholino.

[00509] In some embodiments, a provided oligonucleotide is a P-modified prodrug.

[00510] In some embodiments, a provided oligonucleotide is a primer. In some embodiments, a primers is for use in polymerase-based chain reactions (i.e., PCR) to amplify nucleic acids. In some embodiments, a primer is for use in any known variations of PCR, such as reverse transcription PCR (RT-PCR) and real-time PCR.

[00511] In some embodiments, a provided oligonucleotide is characterized as having the ability to modulate RNase H activation. For example, in some embodiments, RNase H activation is modulated by the presence of stereocontrolled phosphorothioate nucleic acid analogs, with natural DNA/RNA being more or equally susceptible than the *R*p stereoisomer, which in turn is more susceptible than the corresponding *S*p stereoisomer.

[00512] In some embodiments, a provided oligonucleotide is characterized as having the ability to indirectly or directly increase or decrease activity of a protein or inhibition or promotion of the expression of a protein. In some embodiments, a provided oligonucleotide is characterized in that it is useful in the control of cell proliferation, viral replication, and/or any other cell signaling process.

[00513] In some embodiments, a provided oligonucleotide is from about 2 to about 200 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 180 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 160 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 140 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 120 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 100 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 90 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 80 nucleotide

units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 70 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 60 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 50 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 40 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 30 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 29 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 28 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 27 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 26 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 25 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 24 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 23 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 22 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 21 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 20 nucleotide units in length.

[00514] In some embodiments, a provided oligonucleotide is from about 4 to about 200 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 180 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 160 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 140 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 120 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 100 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 90 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 80 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 70 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 60 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 50 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 40 nucleotide units in length. In some embodiments, a provided

oligonucleotide is from about 4 to about 30 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 29 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 28 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 27 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 26 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 25 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 24 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 23 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 22 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 21 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 20 nucleotide units in length.

[00515] In some embodiments, a provided oligonucleotide is from about 5 to about 10 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 10 to about 30 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 15 to about 25 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotide units in length.

[00516] In some embodiments, the oligonucleotide is at least 2 nucleotide units in length. In some embodiments, the oligonucleotide is at least 3 nucleotide units in length. In some embodiments, the oligonucleotide is at least 4 nucleotide units in length. In some embodiments, the oligonucleotide is at least 5 nucleotide units in length. In some embodiments, the oligonucleotide is at least 6 nucleotide units in length. In some embodiments, the oligonucleotide is at least 7 nucleotide units in length. In some embodiments, the oligonucleotide is at least 8 nucleotide units in length. In some embodiments, the oligonucleotide is at least 9 nucleotide units in length. In some embodiments, the oligonucleotide is at least 10 nucleotide units in length. In some embodiments, the oligonucleotide is at least 11 nucleotide units in length. In some embodiments, the oligonucleotide is at least 12 nucleotide units in length. In some embodiments, the oligonucleotide is at least 13 nucleotide units in length. In some embodiments, the oligonucleotide is at least 14 nucleotide units in length. In some embodiments, the

oligonucleotide is at least 15 nucleotide units in length. In some embodiments, the oligonucleotide is at least 16 nucleotide units in length. In some embodiments, the oligonucleotide is at least 17 nucleotide units in length. In some embodiments, the oligonucleotide is at least 18 nucleotide units in length. In some embodiments, the oligonucleotide is at least 19 nucleotide units in length. In some embodiments, the oligonucleotide is at least 20 nucleotide units in length. In some embodiments, the oligonucleotide is at least 21 nucleotide units in length. In some embodiments, the oligonucleotide is at least 22 nucleotide units in length. In some embodiments, the oligonucleotide is at least 23 nucleotide units in length. In some embodiments, the oligonucleotide is at least 24 nucleotide units in length. In some embodiments, the oligonucleotide is at least 25 nucleotide units in length. In some other embodiments, the oligonucleotide is at least 30 nucleotide units in length. In some other embodiments, the oligonucleotide is a duplex of complementary strands of at least 18 nucleotide units in length. In some other embodiments, the oligonucleotide is a duplex of complementary strands of at least 21 nucleotide units in length.

[00517] In some embodiments, the 5'-end and/or the 3'-end of a provided oligonucleotide is modified. In some embodiments, the 5'-end and/or the 3'-end of a provided oligonucleotide is modified with a terminal cap moiety. Exemplary such modifications, including terminal cap moieties are extensively described herein and in the art, for example but not limited to those described in US Patent Application Publication US 2009/0023675A1.

[00518] In some embodiments, oligonucleotides of an oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

have the same chemical structure. For example, they have the same base sequence, the same pattern of backbone linkages (i.e., pattern of internucleotidic linkage types, for example, phosphate, phosphorothioate, etc), the same pattern of backbone chiral centers (i.e. pattern of linkage phosphorus stereochemistry (*R*p/*S*p)), and the same pattern of backbone phosphorus modifications (e.g., pattern of "$-XLR^1$" groups in formula **I**).

Species of oligonucleotides

[00519] In some embodiments, a provided chirally controlled oligonucleotide comprises the sequence of, or part of the sequence of mipomersen. Mipomersen is based on the following base sequence GCCT/UCAGT/UCT/UGCT/UT/UCGCACC. In some embodiments, one or more of any of the nucleotide or linkages may be modified in accordance of the present invention. In some embodiments, the present invention provides a chirally controlled oligonucleotide having the sequence of G*-C*-C*-U*-C*-dA-dG-dT-dC-dT-dG-dmC-dT-dT-dmC-G*-C*-A*-C*-C* [d = 2'-deoxy, * = 2'-O-(2-methoxyethyl)] with 3'→5' phosphorothioate linkages. Exemplary modified mipomersen sequences are described throughout the application, including but not limited to those in Table 2.

[00520] In certain embodiments, a provided oligonucleotide is a mipomersen unimer. In certain embodiments, a provided oligonucleotide is a mipomersen unimer of configuration *R*p. In certain embodiments, a provided oligonucleotide is a mipomersen unimer of configuration *S*p.

[00521] Exempary chirally controlled oligonucleotides comprising the sequence of, or part of the sequence of mipomersen is depicted in Table 2, below.

[00522] Table 2. Exemplary Mipomersen related sequences.

Oligo	Stereochemistry/Sequence	Description
101	All-(*R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	All-R
102	All-(*S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	All-S
103	(*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	5R-9S-5R
104	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	5S-9R-5S
105	(*S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	1S-17R-1S
106	(*R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	1R-17S-1R
107	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	$(R/S)_9R$
108	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	$(S/R)_9S$
109	(*S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p)d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	3S-13R-3S
110	(*R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	3R-13S-3R
111	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	$18S/R^{19}$
112	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p,	$18S/R^{9}$

	*S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	
113	(*S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	18S/R[2]
114	(*R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	$(RRS)_6$-R
115	(*S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	S-$(RRS)_6$
116	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	RS-$(RRS)_5$-RR
122	All-(*R*p)-d[Gs1Cs1Cs1Ts1Cs1As1Gs1Ts1Cs1Ts1Gs1Cs1Ts1Ts1Cs1Gs1Cs1As1Cs1C]	All-R
123	(*S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *S*p)-d[Gs1Cs1Cs1Ts1Cs1As1Gs1Ts1Cs1 Ts1Gs1Cs1Ts1Ts1Cs1Gs1Cs1As1Cs1C]	1S-17R-1S
124	All-(*S*p)-d[Gs1Cs1Cs1Ts1Cs1As1Gs1Ts1Cs1Ts1 Gs1Cs1Ts1Ts1Cs1Gs1Cs1As1Cs1C]	All-S
126	All-(*R*p)-d[Cs2As2Gs2T]	All-R
127	All-(*R*p)-d[Cs3As3Gs3T]	All-R
128	All-(*S*p)-d[Cs4As4Gs4T]	All-S
129	All-(*S*p)-d[Cs5As5Gs5T]	All-S
130	All-(*S*p)-d[Cs6As6Gs6T]	All-S
131	All-(*R*p)-d[Gs7Cs7Cs7Ts7Cs7As7Gs7Ts7Cs7Ts7Gs7 Cs7Ts7Ts7Cs7Gs7Cs7As7Cs7C]	All-R
132	All-(*S*p)-d[Gs7Cs7Cs7Ts7Cs7As7Gs7Ts7Cs7Ts7Gs7 Cs7Ts7Ts7Cs7Gs7Cs7As7Cs7C]	All-S
133	(*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p)-d[Gs15mCs15mCs1Ts15mCs1As1Gs1Ts15mCs1Ts1 Gs15mCs1Ts1Ts15mCs1Gs15mCs1As15mCs15mC]	5R-9S-5R
134	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[Gs15mCs15mCs1Ts15mCs1As1Gs1Ts15mCs1Ts1 Gs15mCs1Ts1Ts15mCs1Gs15mCs1As15mCs15mC]	5S-9R-5S
135	All-(*R*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	All-R
136	All-(*S*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	All-S
137	(*S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *S*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	1S-9R-1S
138	(*S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	2S-7R-2S
139	(*R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	1R-9S-1R
140	(*R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	2R-7S-2R
141	(*S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	3S-5R-3S
142	(*R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	3R-5S-3R

143	(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	$(SSR)_3$-SS
144	(*R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	$(RRS)_3$-RR
145	All-(*R*p)-d[5mCs1Ts15mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1 Gs15mC]	All-R
146	All-(*R*p)-d[Gs15mCs1Ts1G]	All-R
147	All-(*R*p)-d[5mCs1As1Gs1T]	All-R
148	All-(*R*p)-d[5mCs2As2Gs2Ts25mCs2Ts2Gs25mCs2Ts2Ts25mCs2G]	All-R
149	All-(*R*p)-d[5mCs4As4Gs4Ts45mCs4Ts4Gs45mCs4Ts4Ts45mCs4G]	All-R
151	All-(*S*p)-d[Cs1AsGs1T]	All-S
152	All-(*S*p)-d[Cs1AGs1T]	All-S
153	All-(*S*p)-d[CAs1GsT]	All-S
157	All-(*S*p)-d[5mCs1As1Gs1T]	All-S
158	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCs1GsCsACsC]	5S-9R-4S
159	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[Gs1Cs1Cs1Ts1CsAsGsTsCsTsGsCsTsTsCs1GsCs2As2Cs2C]	5S-9R-5S
160	All-(*R*p)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	All-R
161	All-(*S*p)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	All-S
162	(*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	5R-9S-5R
163	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	5S-9R-5S
164	(*S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *S*p)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	1S-17R-1S
165	(*R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	1R-17S-1R
166	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	$(R/S)_9R$
167	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	$(S/R)_9S$
168	(*S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p)$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]	3S-13R-3S

	(Gs5mCsAs5mCs5mC)$_{MOE}$	
169	(*R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	3R-13S-3R
170	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	18S/R[19]
171	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	18S/R[9]
172	(*S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	18S/R[2]
173	(*R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	(RRS)$_6$-R
174	(*S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	S-(RRS)$_6$
175	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	RS-(RRS)$_5$-RR
176	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)(Gs15mCs15mCs1Ts15mCs1)$_{MOE}$d[As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1] (Gs15mCs1As15mCs15mC)$_{MOE}$	RS-(RRS)$_5$-RR
177	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)(Gs15mCs15mCs1Ts15mCs1)$_{MOE}$d[AGT5mCTG5mCTT5mC] (Gs25mCs2As25mCs25mC)$_{MOE}$	RS-(RRS)$_5$-RR
178	(*S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_F$ (F: 2-fluorodeoxyribose)	S-(RRS)$_6$
179	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)d[Gs8Cs8Cs8Ts8Cs8As8Gs8Ts8Cs8Ts8Gs8Cs8Ts8Ts8Cs8Gs8Cs8As8Cs8C]	RS-(RRS)$_5$-RR
180	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)d[Gs9Cs9Cs9Ts9Cs9As9Gs9Ts9Cs9Ts9Gs9Cs9Ts9Ts9Cs9Gs9Cs9As9Cs9C]	RS-(RRS)$_5$-RR
181	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)d[Gs10Cs10Cs10Ts10Cs10As10Gs10Ts10Cs10Ts10Gs10Cs10Ts10Ts10Cs10Gs10Cs10As10Cs10C]	RS-(RRS)$_5$-RR
182	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)d[Gs11Cs11Cs11Ts11Cs11As11Gs11Ts11Cs11Ts11Gs11Cs11Ts11Ts11Cs11Gs11Cs11As11Cs11C]	RS-(RRS)$_5$-RR
183	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)d[Gs12Cs12Cs12Ts12Cs12As12Gs12Ts12Cs12Ts12Gs12Cs12Ts12Ts12Cs12Gs12Cs12As12Cs12C]	RS-(RRS)$_5$-RR

184	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)d[Gs13Cs13Cs13Ts13Cs13As13Gs13Ts13Cs13Ts13Gs13Cs13Ts13Ts13Cs13Gs13Cs13As13Cs13C]	RS-(RRS)$_5$-RR
185	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)d[Gs14Cs14Cs14Ts14Cs14As14Gs14Ts14Cs14Ts14Gs14Cs14Ts14Ts14Cs14Gs14Cs14As14Cs14C]	RS-(RRS)$_5$-RR
186	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)d[Gs15Cs15Cs15Ts15Cs15As15Gs15Ts15Cs15Ts15Gs15Cs15Ts15Ts15Cs15Gs15Cs15As15Cs15C]	RS-(RRS)$_5$-RR
187	(*R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p *R*p)d[GsCsCs1TsCsAs]GsUs2CsUsGsd[CsTs3TsCsGs]CsAs4CsC	RS-(RRS)$_5$-RR
188	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsACsC]	5S-9R-4S
189	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[Gs1Cs1Cs1Ts1Cs1As1Gs1Ts1Cs1Ts1Gs1Cs1Ts1Ts1Cs1Gs1CsACs1C]	5S-9R-4S
190	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[Gs8Cs8Cs8Ts8Cs8As8Gs8Ts8Cs8Ts8Gs8Cs8Ts8Ts8Cs8Gs8Cs1ACs8C]	5S-9R-4S
191	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[Gs9Cs9Cs9Ts9Cs9As9Gs9Ts9Cs9Ts9Gs9Cs9Ts9Ts9Cs9Gs9Cs1ACs9C]	5S-9R-4S
192	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[Gs10Cs10Cs10Ts10Cs10As10Gs10Ts10Cs10Ts10Gs10Cs10Ts10Ts10Cs10Gs10Cs1ACs10C]	5S-9R-4S
193	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[Gs11Cs11Cs11Ts11Cs11As11Gs11Ts11Cs11Ts11Gs11Cs11Ts11Ts11Cs11Gs11Cs1ACs11C]	5S-9R-4S
194	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[Gs12Cs12Cs12Ts12Cs12As12Gs12Ts12Cs12Ts12Gs12Cs12Ts12Ts12Cs12Gs12Cs1ACs12C]	5S-9R-4S
195	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[Gs13Cs13Cs13Ts13Cs13As13Gs13Ts13Cs13Ts13Gs13Cs13Ts13Ts13Cs13Gs13Cs1ACs13C]	5S-9R-4S
196	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[Gs14Cs14Cs14Ts14Cs14As14Gs14Ts14Cs14Ts14Gs14Cs14Ts14Ts14Cs14Gs14Cs1ACs14C]	5S-9R-4S
197	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[Gs15Cs15Cs15Ts15Cs15As15Gs15Ts15Cs15Ts15Gs15Cs15Ts15Ts15Cs15Gs15Cs1ACs15C]	5S-9R-4S
198	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-GsCsCsUsCsAsGsUsCsUsGsCsUsUsCsGsCsACsC	5S-9R-4S
199	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-Gs1Cs1Cs1Us1Cs1As1Gs1Us1Cs1Us1Gs1Cs1Us1Us1Cs1Gs1CsACs1C	5S-9R-4S
200	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-	5S-9R-4S

	Gs8Cs8Cs8Us8Cs8As8Gs8Us8Cs8Us8Gs8Cs8Us8Us8Cs8Gs8Cs1ACs8C	
201	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-Gs9Cs9Cs9Us9Cs9As9Gs9Us9Cs9Us9Gs9Cs9Us9Us9Cs9Gs9Cs1ACs9C	5S-9R-4S
202	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-Gs10Cs10Cs10Us10Cs10As10Gs10Us10Cs10Us10Gs10Cs10Us10Us10Cs10Gs10Cs1ACs10C	5S-9R-4S
203	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-Gs11Cs11Cs11Us11Cs11As11Gs11Us11Cs11Us11Gs11Cs11Us11Us11Cs11Gs11Cs1ACs11C	5S-9R-4S
204	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-Gs12Cs12Cs12Us12Cs12As12Gs12Us12Cs12Us12Gs12Cs12Us12Us12Cs12Gs12Cs1ACs12C	5S-9R-4S
205	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-Gs13Cs13Cs13Us13Cs13As13Gs13Us13Cs13Us13Gs13Cs13Us13Us13Cs13Gs13Cs1ACs13C	5S-9R-4S
206	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-Gs14Cs14Cs14Us14Cs14As14Gs14Us14Cs14Us14Gs14Cs14Us14Us14Cs14Gs14Cs1ACs14C	5S-9R-4S
207	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-Gs15Cs15Cs15Us15Cs15As15Gs15Us15Cs15Us15Gs15Cs15Us15Us15Cs15Gs15Cs1ACs15C	5S-9R-4S

Oligonucleotide compositions

[00523] The present invention provides compositions comprising or consisting of a plurality of provided oligonucleotides (e.g., chirally controlled oligonucleotide compositions). In some embodiments, all such provided oligonucleotides are of the same type, i.e., all have the same base sequence, pattern of backbone linkages (i.e., pattern of internucleotidic linkage types, for example, phosphate, phosphorothioate, etc), pattern of backbone chiral centers (i.e. pattern of linkage phosphorus stereochemistry (*R*p/*S*p)), and pattern of backbone phosphorus modifications (e.g., pattern of "-XLR^1" groups in formula **I**). In many embodiments, however, provided compositions comprise a plurality of oligonucleotides types, typically in pre-determined relative amounts.

[00524] In some embodiments, a provided chirally controlled oligonucleotide composition is a chirally pure mipomersen composition. That is to say, in some embodiments, a provided chirally controlled oligonucleotide composition provides mipomersen as a single diastereomer with respect to the configuration of the linkage phosphorus.

[00525] In some embodiments, a provided chirally controlled oligonucleotide composition is a chirally uniform mipomersen composition. That is to say, in some embodiments, every linkage phosphorus of mipomersen is in the *R*p configuration or every linkage phosphorus of mipomersen is in the *S*p configuration.

[00526] In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of one or more provided oligonucleotide types. One of skill in the chemical and medicinal arts will recognize that the selection and amount of each of the one or more types of provided oligonucleotides in a provided composition will depend on the intended use of that composition. That is to say, one of skill in the relevant arts would design a provided chirally controlled oligonucleotide composition such that the amounts and types of provided oligonucleotides contained therein cause the composition as a whole to have certain desirable characteristics (e.g., biologically desirable, therapeutically desirable, etc.).

[00527] In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of two or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of three or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of four or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of five or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of six or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of seven or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of eight or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of nine or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of ten or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of fifteen or more provided oligonucleotide types.

[00528] In some embodiments, a provided chirally controlled oligonucleotide composition is a combination of an amount of chirally uniform mipomersen of the *R*p configuration and an amount of chirally uniform mipomersen of the *S*p configuration.

[00529] In some embodiments, a provided chirally controlled oligonucleotide composition is a combination of an amount of chirally uniform mipomersen of the *R*p configuration, an amount of chirally uniform mipomersen of the *S*p configuration, and an amount of one or more chirally pure mipomersen of a desired diastereomeric form.

[00530] In some embodiments, a provided oligonucleotide type is selected from those described in PCT/US2013/050407, which is incorporated herein by reference. In some embodiments, a provided chirally controlled oligonucleotide composition comprises oligonucleotides of a oligonucleotide type selected from those described in PCT/US2013/050407.

Methods for Making Chirally Controlled Oligonucleotides and Compositions Thereof

[00531] The present invention provides methods for making chirally controlled oligonucleotides and chirally controlled compositions comprising one or more specific nucleotide types. As noted above, the phrase "oligonucleotide type," as used herein, defines an oligonucleotide that has a particular base sequence, pattern of backbone linkages, pattern of backbone chiral centers, and pattern of backbone phosphorus modifications (e.g., "-XLR^1" groups). Oligonucleotides of a common designated "type" are structurally identical to one another with respect to base sequence, pattern of backbone linkages, pattern of backbone chiral centers, and pattern of backbone phosphorus modifications.

[00532] In some embodiments, a provided chirally controlled oligonucleotide in the invention has properties different from those of the corresponding stereorandom oligonucleotide mixture. In some embodiments, a chirally controlled oligonucleotide has lipophilicity different from that of the stereorandom oligonucleotide mixture. In some embodiments, a chirally controlled oligonucleotide has different retention time on HPLC. In some embodiments, a chirally controlled oligonucleotide may have a peak retention time significantly different from that of the corresponding stereorandom oligonucleotide mixture. During oligonucleotide purification using HPLC as generally practiced in the art, certain chirally controlled oligonucleotides will be largely if not totally lost. During oligonucleotide purification using

HPLC as generally practiced in the art, certain chirally controlled oligonucleotides will be largely if not totally lost. One of the consequences is that certain diastereomers of a stereorandom oligonucleotide mixture (certain chirally controlled oligonucleotides) are not tested in assays. Another consequence is that from batches to batches, due to the inevitable instrumental and human errors, the supposedly "pure" stereorandom oligonucleotide will have inconsistent compositions in that diastereomers in the composition, and their relative and absolute amounts, are different from batches to batches. The chirally controlled oligonucleotide and chirally controlled oligonucleotide composition provided in this invention overcome such problems, as a chirally controlled oligonucleotide is synthesized in a chirally controlled fashion as a single diastereomer, and a chirally controlled oligonucleotide composition comprise predetermined levels of one or more individual oligonucleotide types.

[00533] One of skill in the chemical and synthetic arts will appreciate that synthetic methods of the present invention provide for a degree of control during each step of the synthesis of a provided oligonucleotide such that each nucleotide unit of the oligonucleotide can be designed and/or selected in advance to have a particular stereochemistry at the linkage phosphorus and/or a particular modification at the linkage phosphorus, and/or a particular base, and/or a particular sugar. In some embodiments, a provided oligonucleotide is designed and/or selected in advance to have a particular combination of stereocenters at the linkage phosphorus of the internucleotidic linkage.

[00534] In some embodiments, a provided oligonucleotide made using methods of the present invention is designed and/or determined to have a particular combination of linkage phosphorus modifications. In some embodiments, a provided oligonucleotide made using methods of the present invention is designed and/or determined to have a particular combination of bases. In some embodiments, a provided oligonucleotide made using methods of the present invention is designed and/or determined to have a particular combination of sugars. In some embodiments, a provided oligonucleotide made using methods of the present invention is designed and/or determined to have a particular combination of one or more of the above structural characteristics.

[00535] Methods of the present invention exhibit a high degree of chiral control. For instance, methods of the present invention facilitate control of the stereochemical configuration of every single linkage phosphorus within a provided oligonucleotide. In some embodiments,

methods of the present invention provide an oligonucleotide comprising one or more modified internucleotidic linkages independently having the structure of formula **I**.

[00536] In some embodiments, methods of the present invention provide an oligonucleotide which is a mipomersen unimer. In some embodiments, methods of the present invention provide an oligonucleotide which is a mipomersen unimer of configuration *R*p. In some embodiments, methods of the present invention provide an oligonucleotide which is a mipomersen unimer of configuration *S*p.

[00537] In some embodiments, methods of the present invention provide a chirally controlled oligonucleotide composition, i.e., an oligonucleotide composition that contains predetermined levels of individual oligonucleotide types. In some embodiments a chirally controlled oligonucleotide composition comprises one oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide composition comprises more than one oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide composition comprises a plurality of oligonucleotide types. Exemplary chirally controlled oligonucleotide compositions made in accordance with the present invention are described herein.

[00538] In some embodiments, methods of the present invention provide chirally pure mipomersen compositions with respect to the configuration of the linkage phosphorus. That is to say, in some embodiments, methods of the present invention provide compositions of mipomersen wherein mipomersen exists in the composition in the form of a single diastereomer with respect to the configuration of the linkage phosphorus.

[00539] In some embodiments, methods of the present invention provide chirally uniform mipomersen compositions with respect to the configuration of the linkage phosphorus. That is to say, in some embodiments, methods of the present invention provide compositions of mipomersen in which all nucleotide units therein have the same stereochemistry with respect to the configuration of the linkage phosphorus, e.g., all nucleotide units are of the *R*p configuration at the linkage phosphorus or all nucleotide units are of the *S*p configuration at the linkage phosphorus.

[00540] In some embodiments, a provided chirally controlled oligonucleotide is over 50% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 55% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 60% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 65%

pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 70% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 75% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 80% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 85% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 90% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 91% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 92% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 93% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 94% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 95% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 96% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 97% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 98% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 99% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 99.5% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 99.6% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 99.7% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 99.8% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 99.9% pure. In some embodiments, a provided chirally controlled oligonucleotide is over at least about 99% pure.

[00541] In some embodiments, a chirally controlled oligonucleotide composition is a composition designed to comprise a single oligonucleotide type. In certain embodiments, such compositions are about 50% diastereomerically pure. In some embodiments, such compositions are about 50% diastereomerically pure. In some embodiments, such compositions are about 50% diastereomerically pure. In some embodiments, such compositions are about 55% diastereomerically pure. In some embodiments, such compositions are about 60% diastereomerically pure. In some embodiments, such compositions are about 65% diastereomerically pure. In some embodiments, such compositions are about 70% diastereomerically pure. In some embodiments, such compositions are about 75% diastereomerically pure. In some embodiments, such compositions are about 80%

diastereomerically pure. In some embodiments, such compositions are about 85% diastereomerically pure. In some embodiments, such compositions are about 90% diastereomerically pure. In some embodiments, such compositions are about 91% diastereomerically pure. In some embodiments, such compositions are about 92% diastereomerically pure. In some embodiments, such compositions are about 93% diastereomerically pure. In some embodiments, such compositions are about 94% diastereomerically pure. In some embodiments, such compositions are about 95% diastereomerically pure. In some embodiments, such compositions are about 96% diastereomerically pure. In some embodiments, such compositions are about 97% diastereomerically pure. In some embodiments, such compositions are about 98% diastereomerically pure. In some embodiments, such compositions are about 99% diastereomerically pure. In some embodiments, such compositions are about 99.5% diastereomerically pure. In some embodiments, such compositions are about 99.6% diastereomerically pure. In some embodiments, such compositions are about 99.7% diastereomerically pure. In some embodiments, such compositions are about 99.8% diastereomerically pure. In some embodiments, such compositions are about 99.9% diastereomerically pure. In some embodiments, such compositions are at least about 99% diastereomerically pure.

[00542] In some embodiments, a chirally controlled oligonucleotide composition is a composition designed to comprise multiple oligonucleotide types. In some embodiments, methods of the present invention allow for the generation of a library of chirally controlled oligonucleotides such that a pre-selected amount of any one or more chirally controlled oligonucleotide types can be mixed with any one or more other chirally controlled oligonucleotide types to create a chirally controlled oligonucleotide composition. In some embodiments, the pre-selected amount of an oligonucleotide type is a composition having any one of the above-described diastereomeric purities.

[00543] In some embodiments, the present invention provides methods for making a chirally controlled oligonucleotide comprising steps of:

(1) coupling;

(2) capping;

(3) modifying;

(4) deblocking; and

(5) repeating steps (1) – (4) until a desired length is achieved.

[00544] When describing the provided methods, the word "cycle" has its ordinary meaning as understood by a person of ordinary skill in the art. In some embodiments, one round of steps (1)-(4) is referred to as a cycle.

[00545] In some embodiments, the present invention provides methods for making chirally controlled oligonucleotide compositions, comprising steps of:

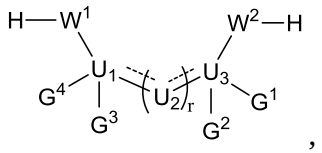
(a) providing an amount of a first chirally controlled oligonucleotide; and

(b) optionally providing an amount of one or more additional chirally controlled oligonucleotides.

[00546] In some embodiments, a first chirally controlled oligonucleotide is an oligonucleotide type, as described herein. In some embodiments, a one or more additional chirally controlled oligonucleotide is a one or more oligonucleotide type, as described herein.

[00547] One of skill in the relevant chemical and synthetic arts will recognize the degree of versatility and control over structural variation and stereochemical configuration of a provided oligonucleotide when synthesized using methods of the present invention. For instance, after a first cycle is complete, a subsequent cycle can be performed using a nucleotide unit individually selected for that subsequent cycle which, in some embodiments, comprises a nucleobase and/or a sugar that is different from the first cycle nucleobase and/or sugar. Likewise, the chiral auxiliary used in the coupling step of the subsequent cycle can be different from the chiral auxiliary used in the first cycle, such that the second cycle generates a phosphorus linkage of a different stereochemical configuration. In some embodiments, the stereochemistry of the linkage phosphorus in the newly formed internucleotidic linkage is controlled by using stereochemically pure phosphoramidites. Additionally, the modification reagent used in the modifying step of a subsequent cycle can be different from the modification reagent used in the first or former cycle. The cumulative effect of this iterative assembly approach is such that each component of a provided oligonucleotide can be structurally and configurationally tailored to a high degree. An additional advantage to this approach is that the step of capping minimizes the formation of "n-1" impurities that would otherwise make isolation of a provided oligonucleotide extremely challenging, and especially oligonucleotides of longer lengths.

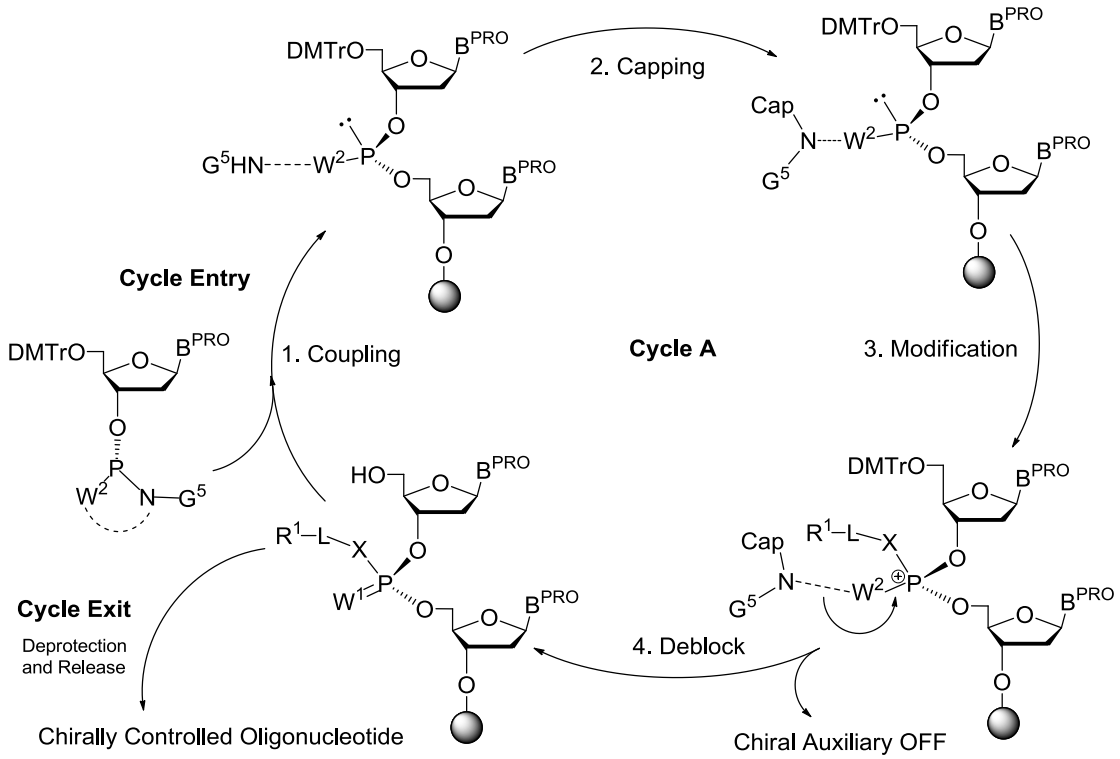
[00548] In some embodiments, an exemplary cycle of the method for making chirally controlled oligonucleotides is illustrated in Scheme I. In Scheme I, ● represents the solid support, and optionally a portion of the growing chirally controlled oligonucleotide attached to the solid support. The chiral auxiliary exemplified has the structure of formula 3-I:



Formula 3-I

which is further described below. "Cap" is any chemical moiety introduced to the nitrogen atom by the capping step, and in some embodiments, is an amino protecting group. One of ordinary skill in the art understands that in the first cycle, there may be only one nucleoside attached to the solid support when started, and cycle exit can be performed optionally before deblocking. As understood by a person of skill in the art, B^{PRO} is a protected base used in oligonucleotide synthesis. Each step of the above-depicted cycle of Scheme I is described further below.

[00549] **Scheme I.** Synthesis of chirally controlled oligonucleotide.



Synthesis on solid support

[00550] In some embodiments, the synthesis of a provided oligonucleotide is performed on solid phase. In some embodiments, reactive groups present on a solid support are protected. In some embodiments, reactive groups present on a solid support are unprotected. During oligonucleotide synthesis a solid support is treated with various reagents in several synthesis cycles to achieve the stepwise elongation of a growing oligonucleotide chain with individual nucleotide units. The nucleoside unit at the end of the chain which is directly linked to the solid support is termed "the first nucleoside" as used herein. A first nucleoside is bound to a solid support via a linker moiety, *i.e.* a diradical with covalent bonds between either of a CPG, a polymer or other solid support and a nucleoside. The linker stays intact during the synthesis cycles performed to assemble the oligonucleotide chain and is cleaved after the chain assembly to liberate the oligonucleotide from the support.

[00551] Solid supports for solid-phase nucleic acid synthesis include the supports described in, *e.g.*, US patents 4,659,774 , 5,141,813, 4,458,066; Caruthers U.S. Pat. Nos. 4,415,732, 4,458,066, 4,500,707, 4,668,777, 4,973,679, and 5,132,418; Andrus *et al*. U.S. Pat. Nos. 5,047,524, 5,262,530; and Koster U.S. Pat. Nos. 4,725,677 (reissued as Re34,069). In some embodiments, a solid phase is an organic polymer support. In some embodiments, a solid phase is an inorganic polymer support. In some embodiments, an organic polymer support is polystyrene, aminomethyl polystyrene, a polyethylene glycol-polystyrene graft copolymer, polyacrylamide, polymethacrylate, polyvinylalcohol, highly cross-linked polymer (HCP), or other synthetic polymers, carbohydrates such as cellulose and starch or other polymeric carbohydrates, or other organic polymers and any copolymers, composite materials or combination of the above inorganic or organic materials. In some embodiments, an inorganic polymer support is silica, alumina, controlled polyglass (CPG), which is a silica-gel support, or aminopropyl CPG. Other useful solid supports include fluorous solid supports (see *e.g.*, WO/2005/070859), long chain alkylamine (LCAA) controlled pore glass (CPG) solid supports (see *e.g.*, S. P. Adams, K. S. Kavka, E. J. Wykes, S. B. Holder and G. R. Galluppi, *J. Am. Chem. Soc.*, **1983**, *105*, 661-663; G. R. Gough, M. J. Bruden and P. T. Gilham, *Tetrahedron Lett.*, **1981**, *22*, 4177-4180). Membrane supports and polymeric membranes (see *e.g.* Innovation and Perspectives in Solid Phase Synthesis, Peptides, Proteins and Nucleic Acids, ch 21 pp 157-162,

1994, Ed. Roger Epton and U.S. Pat. No. 4,923,901) are also useful for the synthesis of nucleic acids. Once formed, a membrane can be chemically functionalized for use in nucleic acid synthesis. In addition to the attachment of a functional group to the membrane, the use of a linker or spacer group attached to the membrane is also used in some embodiments to minimize steric hindrance between the membrane and the synthesized chain.

[00552] Other suitable solid supports include those generally known in the art to be suitable for use in solid phase methodologies, including, for example, glass sold as PrimerTM 200 support, controlled pore glass (CPG), oxalyl-controlled pore glass (see, *e.g.*, Alul, *et al.*, *Nucleic Acids Research*, **1991**, *19*, 1527), TentaGel Support-an aminopolyethyleneglycol derivatized support (see, *e.g.*, Wright, *et al.*, *Tetrahedron Lett.*, **1993**, *34*, 3373), and Poros-a copolymer of polystyrene/divinylbenzene.

[00553] Surface activated polymers have been demonstrated for use in synthesis of natural and modified nucleic acids and proteins on several solid supports mediums. A solid support material can be any polymer suitably uniform in porosity, having sufficient amine content, and sufficient flexibility to undergo any attendant manipulations without losing integrity. Examples of suitable selected materials include nylon, polypropylene, polyester, polytetrafluoroethylene, polystyrene, polycarbonate, and nitrocellulose. Other materials can serve as a solid support, depending on the design of the investigator. In consideration of some designs, for example, a coated metal, in particular gold or platinum can be selected (see *e.g.*, US publication No. 20010055761). In one embodiment of oligonucleotide synthesis, for example, a nucleoside is anchored to a solid support which is functionalized with hydroxyl or amino residues. Alternatively, a solid support is derivatized to provide an acid labile trialkoxytrityl group, such as a trimethoxytrityl group (TMT). Without being bound by theory, it is expected that the presence of a trialkoxytrityl protecting group will permit initial detritylation under conditions commonly used on DNA synthesizers. For a faster release of oligonucleotide material in solution with aqueous ammonia, a diglycoate linker is optionally introduced onto the support.

[00554] In some embodiments, a provided oligonucleotide alternatively is synthesized from the 5′ to 3′ direction. In some embodiments, a nucleic acid is attached to a solid support through its 5′ end of the growing nucleic acid, thereby presenting its 3′ group for reaction, i.e. using 5′-nucleoside phosphoramidites or in enzymatic reaction (*e.g.* ligation and polymerization

using nucleoside 5′-triphosphates). When considering the 5’ to 3’ synthesis the iterative steps of the present invention remain unchanged (i.e. capping and modification on the chiral phosphorus).

Linking moiety

[00555] A linking moiety or linker is optionally used to connect a solid support to a compound comprising a free nucleophilic moiety. Suitable linkers are known such as short molecules which serve to connect a solid support to functional groups (*e.g.*, hydroxyl groups) of initial nucleosides molecules in solid phase synthetic techniques. In some embodiments, the linking moiety is a succinamic acid linker, or a succinate linker (-CO-CH_2-CH_2-CO-), or an oxalyl linker (-CO-CO-). In some embodiments, the linking moiety and the nucleoside are bonded together through an ester bond. In some embodiments, a linking moiety and a nucleoside are bonded together through an amide bond. In some embodiments, a linking moiety connects a nucleoside to another nucleotide or nucleic acid. Suitable linkers are disclosed in, for example, *Oligonucleotides And Analogues A Practical Approach*, Ekstein, F. Ed., IRL Press, N.Y., **1991**, Chapter 1 and Solid-Phase Supports for Oligonucleotide Synthesis, Pon, R. T., *Curr. Prot. Nucleic Acid Chem.*, **2000**, 3.1.1-3.1.28.

[00556] A linker moiety is used to connect a compound comprising a free nucleophilic moiety to another nucleoside, nucleotide, or nucleic acid. In some embodiments, a linking moiety is a phosphodiester linkage. In some embodiments, a linking moiety is an *H*-phosphonate moiety. In some embodiments, a linking moiety is a modified phosphorus linkage as described herein. In some embodiments, a universal linker (UnyLinker) is used to attached the oligonucleotide to the solid support (Ravikumar et al., *Org. Process Res. Dev.*, **2008**, *12* (3), 399–410). In some embodiments, other universal linkers are used (Pon, R. T., *Curr. Prot. Nucleic Acid Chem.*, **2000**, 3.1.1-3.1.28). In some embodiments, various orthogonal linkers (such as disulfide linkers) are used (Pon, R. T., *Curr. Prot. Nucleic Acid Chem.*, **2000**, 3.1.1-3.1.28).

General conditions - solvents for synthesis

[00557] Syntheses of provided oligonucleotides are generally performed in aprotic organic solvents. In some embodiments, a solvent is a nitrile solvent such as, e.g., acetonitrile. In some embodiments, a solvent is a basic amine solvent such as, e.g., pyridine. In some embodiments, a solvent is an ethereal solvent such as, e.g., tetrahydrofuran. In some embodiments, a solvent is a

halogenated hydrocarbon such as, e.g., dichloromethane. In some embodiments, a mixture of solvents is used. In certain embodiments a solvent is a mixture of any one or more of the above-described classes of solvents.

[00558] In some embodiments, when an aprotic organic solvent is not basic, a base is present in the reacting step. In some embodiments where a base is present, the base is an amine base such as, e.g., pyridine, quinoline, or *N*,*N*-dimethylaniline. Exemplary other amine bases include pyrrolidine, piperidine, *N*-methyl pyrrolidine, pyridine, quinoline, *N*,*N*-dimethylaminopyridine (DMAP), or *N*,*N*-dimethylaniline.

[00559] In some embodiments, a base is other than an amine base.

[00560] In some embodiments, an aprotic organic solvent is anhydrous. In some embodiments, an anhydrous aprotic organic solvent is freshly distilled. In some embodiments, a freshly distilled anhydrous aprotic organic solvent is a basic amine solvent such as, e.g., pyridine. In some embodiments, a freshly distilled anhydrous aprotic organic solvent is an ethereal solvent such as, e.g., tetrahydrofuran. In some embodiments, a freshly distilled anhydrous aprotic organic solvent is a nitrile solvent such as, e.g., acetonitrile.

Activation

[00561] An achiral *H*-phosphonate moiety is treated with the first activating reagent to form the first intermediate. In one embodiment, the first activating reagent is added to the reaction mixture during the condensation step. Use of the first activating reagent is dependent on reaction conditions such as solvents that are used for the reaction. Examples of the first activating reagent are phosgene, trichloromethyl chloroformate, bis(trichloromethyl)carbonate (BTC), oxalyl chloride, Ph_3PCl_2, $(PhO)_3PCl_2$, *N*,*N'*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BopCl), 1,3-dimethyl-2-(3-nitro-1,2,4-triazol-1-yl)-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium hexafluorophosphate (MNTP), or 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyNTP).

[00562] The example of achiral *H*-phosphonate moiety is a compound shown in the above Scheme. DBU represents 1,8-diazabicyclo[5.4.0]undec-7-ene. H^+DBU may be, for example, ammonium ion, alkylammonium ion, heteroaromatic iminium ion, or heterocyclic iminium ion, any of which is primary, secondary, tertiary or quaternary, or a monovalent metal ion.

Reacting with Chiral Reagent

[00563] After the first activation step, the activated achiral *H*-phosphonate moiety reacts with a chiral reagent, which is represented by formula (Z-I) or (Z-I'), to form a chiral intermediate of formula (Z-Va), (Z-Vb), (Z-Va'), or (Z-Vb').

Stereospecific Condensation Step

[00564] A chiral intermediate of Formula Z-Va ((Z-Vb), (Z-Va'), or (Z-Vb')) is treated with the second activating reagent and a nucleoside to form a condensed intermediate. The nucleoside may be on solid support. Examples of the second activating reagent are 4,5-dicyanoimidazole (DCI), 4,5-dichloroimidazole, 1-phenylimidazolium triflate (PhIMT), benzimidazolium triflate (BIT), benztriazole, 3-nitro-l,2,4-triazole (NT), tetrazole, 5-ethylthiotetrazole (ETT), 5-benzylthiotetrazole (BTT), 5-(4-nitrophenyl)tetrazole, *N*-cyanomethylpyrrolidinium triflate (CMPT), *N*-cyanomethylpiperidinium triflate, *N*-cyanomethyldimethylammonium triflate. A chiral intermediate of Formula Z-Va ((Z-Vb), (Z-Va'), or (Z-Vb')) may be isolated as a monomer. Usually, the chiral intermediate of Z-Va ((Z-Vb), (Z-Va'), or (Z-Vb')) is not isolated and undergoes a reaction in the same pot with a nucleoside or modified nucleoside to provide a chiral phosphite compound, a condensed intermediate. In other embodiments, when the method is performed via solid phase synthesis, the solid support comprising the compound is filtered away from side products, impurities, and/or reagents.

Capping Step

[00565] If the final nucleic acid is larger than a dimer, the unreacted -OH moiety is capped with a blocking group and the chiral auxiliary in the compound may also be capped with a blocking group to form a capped condensed intermediate. If the final nucleic acid is a dimer, then the capping step is not necessary.

Modifying Step

[00566] The compound is modified by reaction with an electrophile. The capped condensed intermediate may be executed modifying step. In some embodiments, the modifying

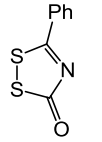
step is performed using a sulfur electrophile, a selenium electrophile or a boronating agent. Examples of modifying steps are step of oxidation and sulfurization.

[00567] In some embodiments of the method, the sulfur electrophile is a compound having one of the following formulas:

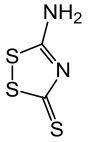
S_8 (Formula Z-B), Z^{z1}-S-S-Z^{z2}, or Z^{z1}-S-V^z-Z^{z2};

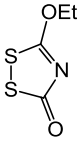
wherein Z^{z1} and Z^{z2} are independently alkyl, aminoalkyl, cycloalkyl, heterocyclic, cycloalkylalkyl, heterocycloalkyl, aryl, heteroaryl, alkyloxy, aryloxy, heteroaryloxy, acyl, amide, imide, or thiocarbonyl, or Z^{z1} and Z^{z2} are taken together to form a 3 to 8 membered alicyclic or heterocyclic ring, which may be substituted or unsubstituted; V^z is SO_2, O, or NR^f; and R^f is hydrogen, alkyl, alkenyl, alkynyl, or aryl.

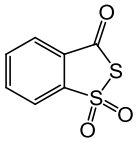
[00568] In some embodiments of the method, the sulfur electrophile is a compound of following Formulae Z-A, Z-B, Z-C, Z-D, Z-E, or Z-F:

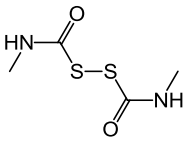

Formula Z-A


Formula Z-B


Formula Z-C


Formula Z-D


Formula Z-E


Formula Z-F

[00569] In some embodiments, the selenium electrophile is a compound having one of the following formulae:

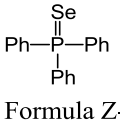
Se (Formula Z-G), Z^{z3}-Se-Se-Z^{z4}, or Z^{z3}-Se-V^z-Z^{z4};

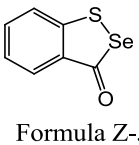
wherein Z^{z3} and Z^{z4} are independently alkyl, aminoalkyl, cycloalkyl, heterocyclic, cycloalkylalkyl, heterocycloalkyl, aryl, heteroaryl, alkyloxy, aryloxy, heteroaryloxy, acyl, amide, imide, or thiocarbonyl, or Z^{z3} and Z^{z4} are taken together to form a 3 to 8 membered alicyclic or heterocyclic ring, which may be substituted or unsubstituted; V^z is SO_2, S, O, or NR^f; and R^f is hydrogen, alkyl, alkenyl, alkynyl, or aryl.

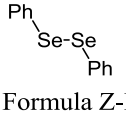
[00570] In some embodiments, the selenium electrophile is a compound of Formula Z-G, Z-H, Z-I, Z-J, Z-K, or Z-L.

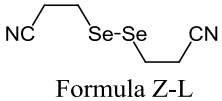

Formula Z-G


Formula Z-H


Formula Z-I


Formula Z-J


Formula Z-K


Formula Z-L

[00571] In some embodiments, the boronating agent is borane-*N*,*N*-diisopropylethylamine (BH_3 DIPEA), borane-pyridine (BH_3 Py), borane-2-chloropyridine (BH_3 CPy), borane-aniline (BH_3 An), borane-tetrahydrofiirane (BH_3 THF), or borane-dimethylsulfide (BH_3 Me_2S).

[00572] In some embodiments of the method, the modifying step is an oxidation step. In some embodiments of the method, the modifying step is an oxidation step using similar conditions as described above in this application. In some embodiments, an oxidation step is as disclosed in, *e.g.*, JP 2010-265304 A and WO2010/064146.

Chain Elongation Cycle and De-protection Step

[00573] The capped condensed intermediate is deblocked to remove the blocking group at the 5'-end of the growing nucleic acid chain to provide a compound. The compound is optionally allowed to re-enter the chain elongation cycle to form a condensed intermediate, a capped condensed intermediate, a modified capped condensed intermediate, and a 5'-deprotected modified capped intermediate. Following at least one round of chain elongation cycle, the 5'-deprotected modified capped intermediate is further deblocked by removal of the chiral auxiliary ligand and other protecting groups for, *e.g.*, nucleobase, modified nucleobase, sugar and modified sugar protecting groups, to provide a nucleic acid. In other embodiments, the nucleoside comprising a 5'-OH moiety is an intermediate from a previous chain elongation cycle as described herein. In yet other embodiments, the nucleoside comprising a 5'-OH moiety is an intermediate obtained from another known nucleic acid synthetic method. In embodiments where a solid support is used, the phosphorus-atom modified nucleic acid is then cleaved from the solid support. In certain embodiments, the nucleic acids is left attached on the solid support for purification purposes and then cleaved from the solid support following purification.

[00574] In yet other embodiments, the nucleoside comprising a 5'-OH moiety is an intermediate obtained from another known nucleic acid synthetic method. In yet other embodiments, the nucleoside comprising a 5'-OH moiety is an intermediate obtained from another known nucleic acid synthetic method as described in this application. In yet other embodiments, the nucleoside comprising a 5'-OH moiety is an intermediate obtained from another known nucleic acid synthetic method comprising one or more cycles illustrated in Scheme I. In yet other embodiments, the nucleoside comprising a 5'-OH moiety is an

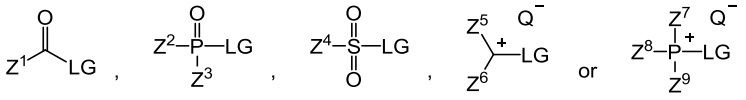
intermediate obtained from another known nucleic acid synthetic method comprising one or more cycles illustrated in Scheme I-b, I-c or I-d.

[00575] In some embodiments, the present invention provides oligonucleotide synthesis methods that use stable and commercially available materials as starting materials. In some embodiments, the present invention provides oligonucleotide synthesis methods to produce stereocontrolled phosphorus atom-modified oligonucleotide derivatives using an achiral starting material.

[00576] In some embodiments, the method of the present invention does not cause degradations under the de-protection steps. Further the method does not require special capping agents to produce phosphorus atom-modified oligonucleotide derivatives.

Condensing reagent

[00577] Condensing reagents (C_R) useful in accordance with methods of the present invention are of any one of the following general formulae:



C_R1 C_R2 C_R3 C_R4 C_R5

wherein Z^1, Z^2, Z^3, Z^4, Z^5, Z^6, Z^7, Z^8, and Z^9 are independently optionally substituted group selected from alkyl, aminoalkyl, cycloalkyl, heterocyclic, cycloalkylalkyl, heterocycloalkyl, aryl, heteroaryl, alkyloxy, aryloxy, or heteroaryloxy, or wherein any of Z^2 and Z^3, Z^5 and Z^6, Z^7 and Z^8, Z^8 and Z^9, Z^9 and Z^7, or Z^7 and Z^8 and Z^9 are taken together to form a 3 to 20 membered alicyclic or heterocyclic ring; Q^- is a counter anion; and LG is a leaving group.

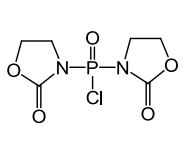
[00578] In some embodiments, a counter ion of a condensing reagent C_R is Cl^-, Br^-, BF_4^-, PF_6^-, TfO^-, Tf_2N^-, AsF_6^-, ClO_4^-, or SbF_6^-, wherein Tf is CF_3SO_2. In some embodiments, a leaving group of a condensing reagent C_R is F, Cl, Br, I, 3-nitro-1,2,4-triazole, imidazole, alkyltriazole, tetrazole, pentafluorobenzene, or 1-hydroxybenzotriazole.

[00579] Examples of condensing reagents used in accordance with methods of the present invention include, but are not limited to, pentafluorobenzoyl chloride, carbonyldiimidazole (CDI), 1-mesitylenesulfonyl-3-nitrotriazole (MSNT), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDCI-HCl), benzotriazole-1-

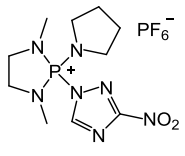
yloxytris(dimethylamino)phosphonium hexafluorophosphate (PyBOP), *N,N'*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BopCl), 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), and *O*-benzotriazole-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), DIPCDI; *N,N'*-bis(2-oxo-3-oxazolidinyl)phosphinic bromide (BopBr), 1,3-dimethyl-2-(3-nitro-1,2,4-triazol-1-yl)-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium hexafluorophosphate (MNTP), 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyNTP), bromotripyrrolidinophosphonium hexafluorophosphate (PyBrOP); *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU); and tetramethylfluoroformamidinium hexafluorophosphate (TFFH). In certain embodiments, a counter ion of the condensing reagent C_R is Cl^-, Br^-, BF_4^-, PF_6^-, TfO^-, Tf_2N^-, AsF_6^-, ClO_4^-, or SbF_6^-, wherein Tf is CF_3SO_2.

[00580] In some embodiments, a condensing reagent is 1-(2,4,6-triisopropylbenzenesulfonyl)-5-(pyridin-2-yl) tetrazolide, pivaloyl chloride, bromotrispyrrolidinophosphonium hexafluorophosphate, *N,N'*-bis(2-oxo-3-oxazolidinyl) phosphinic chloride (BopCl), or 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane. In some embodiment, a condensing reagent is *N,N'*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BopCl). In some embodiments, a condensing reagent is selected from those described in WO/2006/066260).

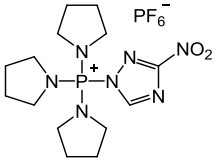
[00581] In some embodiments, a condensing reagent is 1,3-dimethyl-2-(3-nitro-1,2,4-triazol-1-yl)-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium hexafluorophosphate (MNTP), or 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyNTP):



BopCl



MNTP



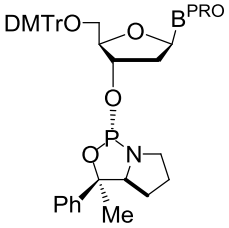
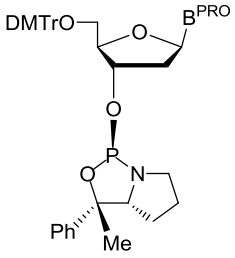
PyNTP .

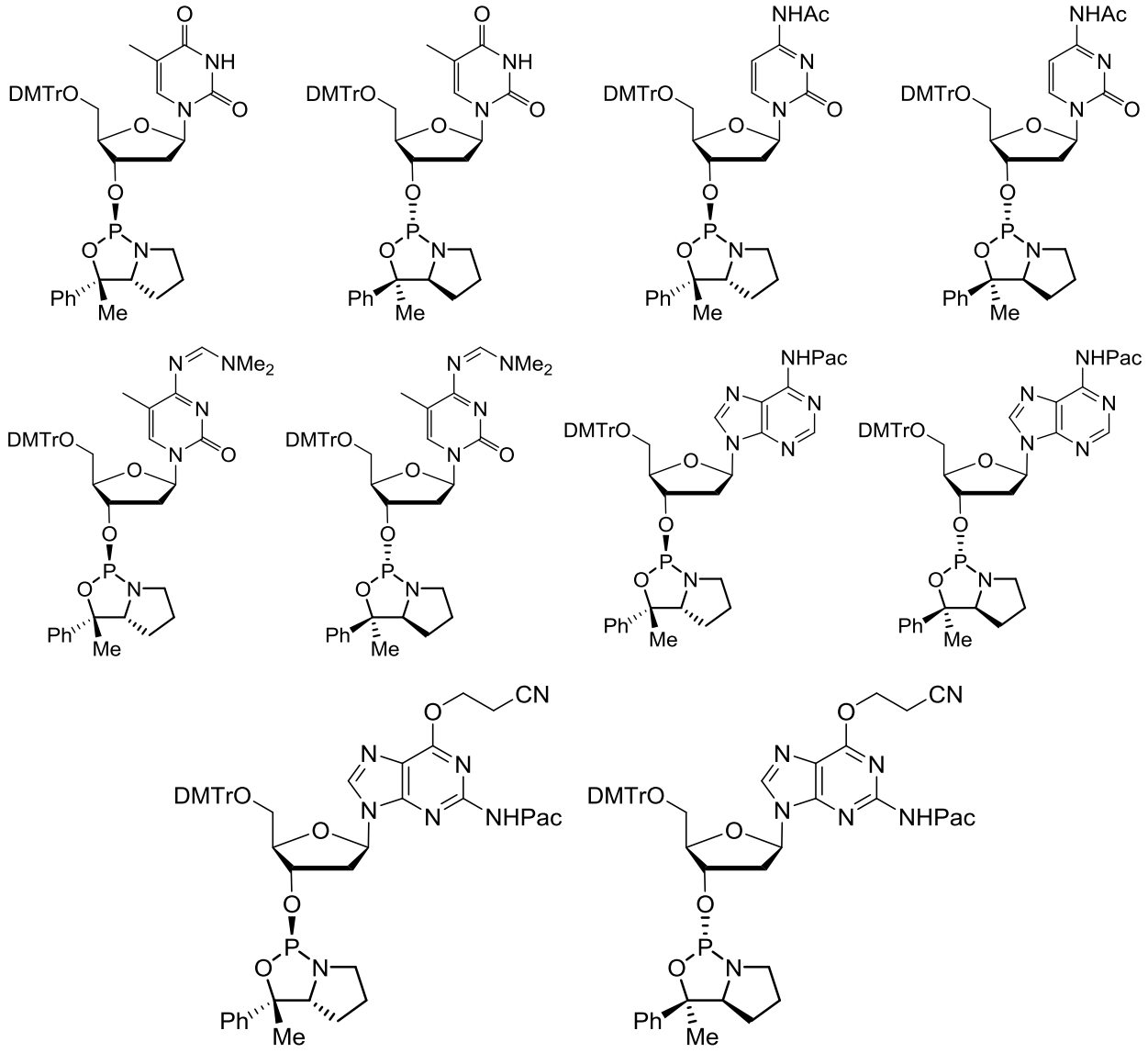
Selection of base and sugar of nucleoside coupling partner

[00582] As described herein, nucleoside coupling partners for use in accordance with methods of the present invention can be the same as one another or can be different from one another. In some embodiments, nucleoside coupling partners for use in the synthesis of a provided oligonucleotide are of the same structure and/or stereochemical configuration as one

another. In some embodiments, each nucleoside coupling partner for use in the synthesis of a provided oligonucleotide is not of the same structure and/or stereochemical configuration as certain other nucleoside coupling partners of the oligonucleotide. Exemplary nucleobases and sugars for use in accordance with methods of the present invention are described herein. One of skill in the relevant chemical and synthetic arts will recognize that any combination of nucleobases and sugars described herein are contemplated for use in accordance with methods of the present invention.

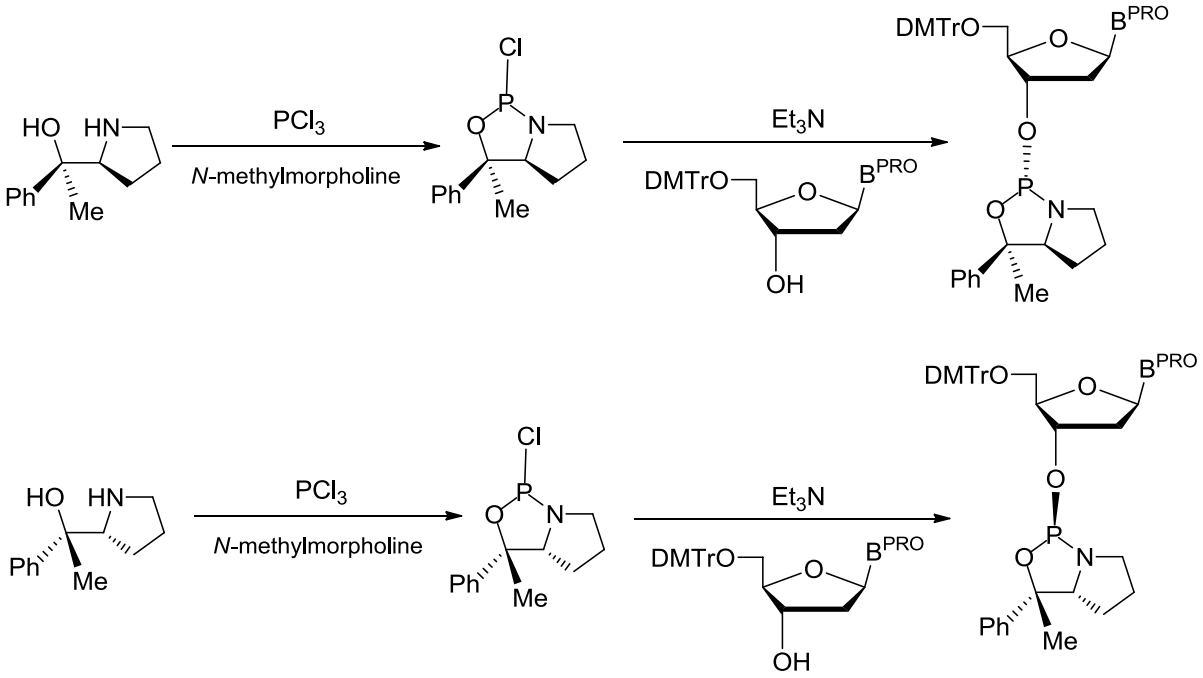
Coupling step:

[00583] Exemplary coupling procedures and chiral reagents and condensing reagents for use in accordance with the present invention are outlined in, *inter alia*, Wada I (JP4348077; WO2005/014609; WO2005/092909), Wada II (WO2010/064146), and Wada III (WO2012/039448). Chiral nucleoside coupling partners for use in accordance with the present invention are also referred to herein as "Wada amidites." In some embodiments, a coupling partner has the structure of , wherein B^{PRO} is a protected nucleobase. In some embodiments, a coupling partner has the structure of , wherein B^{PRO} is a protected nucleobase. Exemplary chiral phosphoramidites as coupling partner are depicted below:



[00584] One of the methods used for synthesizing the coupling partner is depicted in Scheme II, below.

Scheme II. Exemplary synthesis of coupling partner.



[00585] In some embodiments, the step of coupling comprises reacting a free hydroxyl group of a nucleotide unit of an oligonucleotide with a nucleoside coupling partner under suitable conditions to effect the coupling. In some embodiments, the step of coupling is preceded by a step of deblocking. For instance, in some embodiments, the 5' hydroxyl group of the growing oligonucleotide is blocked (i.e., protected) and must be deblocked in order to subsequently react with a nucleoside coupling partner.

[00586] Once the appropriate hydroxyl group of the growing oligonucleotide has been deblocked, the support is washed and dried in preparation for delivery of a solution comprising a chiral reagent and a solution comprising an activator. In some embodiments, a chiral reagent and an activator are delivered simultaneously. In some embodiments, co-delivery comprises delivering an amount of a chiral reagent in solution (e.g., a phosphoramidite solution) and an amount of activator in a solution (e.g., a CMPT solution) in a polar aprotic solvent such as a nitrile solvent (e.g., acetonitrile).

[00587] In some embodiments, the step of coupling provides a crude product composition in which the chiral phosphite product is present in a diastereomeric excess of > 95%. In some embodiments, the chiral phosphite product is present in a diastereomeric excess of > 96%. In some embodiments, the chiral phosphite product is present in a diastereomeric excess of > 97%. In some embodiments, the chiral phosphite product is present in a diastereomeric excess of >

98%. In some embodiments, the chiral phosphite product is present in a diastereomeric excess of > 99%.

Capping step:

[00588] Provided methods for making chirally controlled oligonucleotides comprise a step of capping. In some embodiments, a step of capping is a single step. In some embodiments, a step of capping is two steps. In some embodiments, a step of capping is more than two steps.

[00589] In some embodiments, a step of capping comprises steps of capping the free amine of the chiral auxiliary and capping any residual unreacted 5' hydroxyl groups. In some embodiments, the free amine of the chiral auxiliary and the unreacted 5' hydroxyl groups are capped with the same capping group. In some embodiments, the free amine of the chiral auxiliary and the unreacted 5' hydroxyl groups are capped with different capping groups. In certain embodiments, capping with different capping groups allows for selective removal of one capping group over the other during synthesis of the oligonucleotide. In some embodiments, the capping of both groups occurs simultaneously. In some embodiments, the capping of both groups occurs iteratively.

[00590] In certain embodiments, capping occurs iteratively and comprises a first step of capping the free amine followed by a second step of capping the free 5' hydroxyl group, wherein both the free amine and the 5' hydroxyl group are capped with the same capping group. For instance, in some embodiments, the free amine of the chiral auxiliary is capped using an anhydride (e.g., phenoxyacetic anhydride, i.e., Pac_2O) prior to capping of the 5' hydroxyl group with the same anhydride. In certain embodiments, the capping of the 5' hydroxyl group with the same anhydride occurs under different conditions (e.g., in the presence of one or more additional reagents). In some embodiments, capping of the 5' hydroxyl group occurs in the presence of an amine base in an etherial solvent (e.g., NMI (N-methylimidazole) in THF). The phrase "capping group" is used interchangeably herein with the phrases "protecting group" and "blocking group".

[00591] In some embodiments, an amine capping group is characterized in that it effectively caps the amine such that it prevents rearrangement and/or decomposition of the intermediate phosphite species. In some embodiments, a capping group is selected for its ability to protect the amine of the chiral auxiliary in order to prevent intramolecular cleavage of the internucleotide linkage phosphorus.

[00592] In some embodiments, a 5' hydroxyl group capping group is characterized in that it effectively caps the hydroxyl group such that it prevents the occurrence of "shortmers," e.g., "n-m" (m and n are integers and m<n; n is the number of bases in the targeted oligonucleotide) impurities that occur from the reaction of an oligonucleotide chain that fails to react in a first cycle but then reacts in one or more subsequent cycles. The presence of such shortmers, especially "n-1", has a deleterious effect upon the purity of the crude oligonucleotide and makes final purification of the oligonucleotide tedious and generally low-yielding.

[00593] In some embodiments, a particular cap is selected based on its tendency to facilitate a particular type of reaction under particular conditions. For instance, in some embodiments, a capping group is selected for its ability to facilitate an E1 elimination reaction, which reaction cleaves the cap and/or auxiliary from the growing oligonucleotide. In some embodiments, a capping group is selected for its ability to facilitate an E2 elimination reaction, which reaction cleaves the cap and/or auxiliary from the growing oligonucleotide. In some embodiments, a capping group is selected for its ability to facilitate a β-elimination reaction, which reaction cleaves the cap and/or auxiliary from the growing oligonucleotide.

Modifying step:

[00594] As used herein, the phrase "modifying step", "modification step" and "P-modification step" are used interchangeably and refer generally to any one or more steps used to install a modified internucleotidic linkage. In some embodiments, the modified internucleotidic linkage having the structure of formula I. A P-modification step of the present invention occurs during assembly of a provided oligonucleotide rather than after assembly of a provided oligonucleotide is complete. Thus, each nucleotide unit of a provided oligonucleotide can be individually modified at the linkage phosphorus during the cycle within which the nucleotide unit is installed.

[00595] In some embodiments, a suitable P-modification reagent is a sulfur electrophile, selenium electrophile, oxygen electrophile, boronating reagent, or an azide reagent.

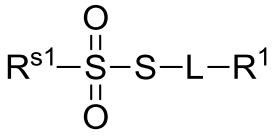
[00596] For instance, in some embodiments, a selemium reagent is elemental selenium, a selenium salt, or a substituted diselenide. In some embodiments, an oxygen electrophile is elemental oxygen, peroxide, or a substituted peroxide. In some embodiments, a boronating reagent is a borane-amine (e.g., *N*,*N*-diisopropylethylamine (BH_3·DIPEA), borane-pyridine

(BH_3·Py), borane-2-chloropyridine (BH_3·CPy), borane-aniline (BH_3·An)), a borane-ether reagent (e.g., borane-tetrahydrofuran (BH_3·THF)), a borane-dialkylsulfide reagent (e.g., BH_3·Me_2S), aniline-cyanoborane, or a triphenylphosphine-carboalkoxyborane. In some embodiments, an azide reagent is comprises an azide group capable of undergoing subsequent reduction to provide an amine group.

[00597] In some embodiments, a P-modification reagent is a sulfurization reagent as described herein. In some embodiments, a step of modifying comprises sulfurization of phosphorus to provide a phosphorothioate linkage or phosphorothioate triester linkage. In some embodiments, a step of modifying provides an oligonucleotide having an internucleotidic linkage of formula I.

[00598] In some embodiments, the present invention provides sulfurizing reagents, and methods of making, and use of the same.

[00599] In some embodiments, such sulfurizing reagents are thiosulfonate reagents. In some embodiments, a thiosulfonate reagent has a structure of formula **S-I**:



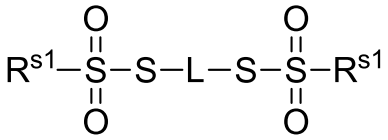
S-I

wherein:

R^{s1} is R; and

each of R, L and R^1 is independently as defined and described above and herein.

[00600] In some embodiments, the sulfurizing reagent is a bis(thiosulfonate) reagent. In some embodiments, the bis(thiosulfonate) reagent has the structure of formula **S-II**:

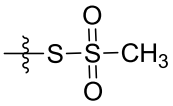
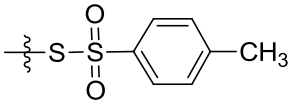
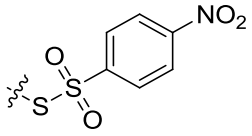
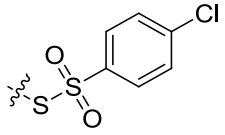
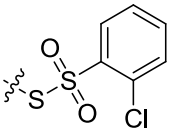
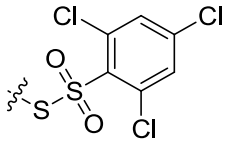
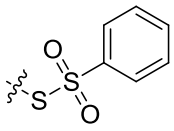
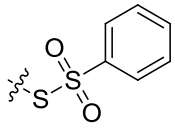
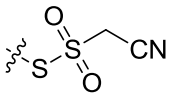
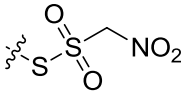


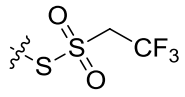
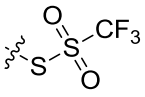
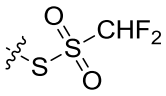
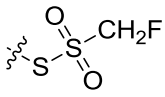
S-II

wherein each of R^{s1} and L is independently as defined and described above and herein.

[00601] As defined generally above, R^{s1} is R, wherein R is as defined and described above and herein. In some embodiments, R^{s1} is optionally substituted aliphatic, aryl, heterocyclyl or heteroaryl. In some embodiments, R^{s1} is optionally substituted alkyl. In some embodiments, R^{s1}

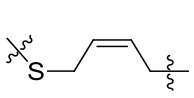
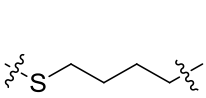
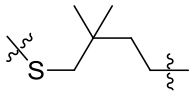
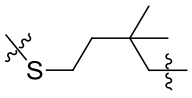
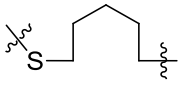
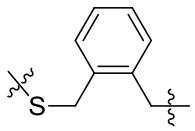
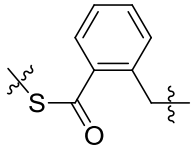
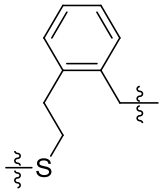
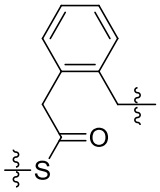
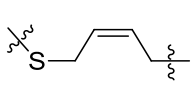
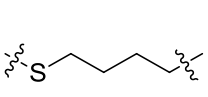
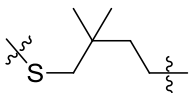
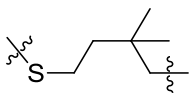
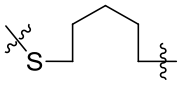
is optionally substituted alkyl. In some embodiments, R^{s1} is methyl. In some embodiments, R^{s1} is cyanomethyl. In some embodiments, R^{s1} is nitromethyl. In some embodiments, R^{s1} is optionally substituted aryl. In some embodiments, R^{s1} is optionally substituted phenyl. In some embodiments, R^{s1} is phenyl. In some embodiments, R^{s1} is *p*-nitrophenyl. In some embodiments, R^{s1} is *p*-methylphenyl. In some embodiments, R^{s1} is *p*-chlorophenyl. In some embodiments, R^{s1} is *o*-chlorophenyl. In some embodiments, R^{s1} is 2,4,6-trichlorophenyl. In some embodiments, R^{s1} is pentafluorophenyl. In some embodiments, R^{s1} is optionally substituted heterocyclyl. In some embodiments, R^{s1} is optionally substituted heteroaryl.

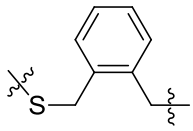
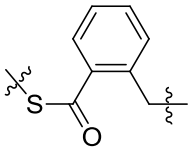
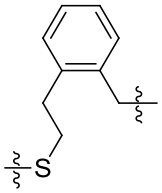
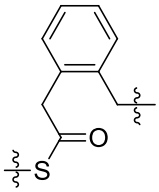
[00602] In some embodiments, R^{s1}–$S(O)_2S$– is  (MTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (TTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (NO_2PheTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (p-ClPheTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (o-ClPheTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (2,4,6-TriClPheTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (PheTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (PFPheTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (a-CNMTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (a-NO_2MTS). In some embodiments, R^{s1}–

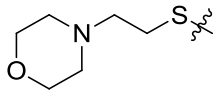
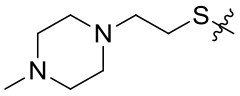
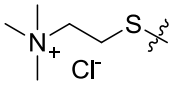
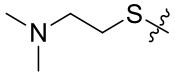
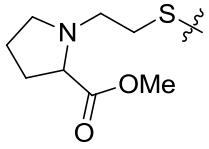
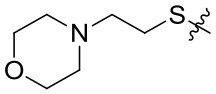
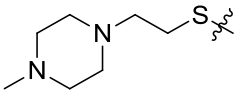
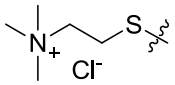
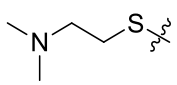
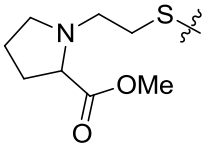
$S(O)_2S–$ is  (a-CF_3MTS). In some embodiments, $R^{s1}–S(O)_2S–$ is  (a-CF_3TS). In some embodiments, $R^{s1}–S(O)_2S–$ is  (a-CHF_2TS). In some embodiments, $R^{s1}–S(O)_2S–$ is  (a-CH_2FTS).

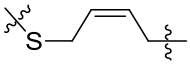
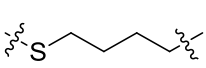
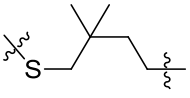
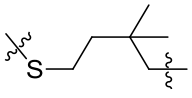
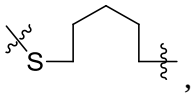
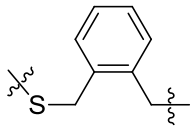
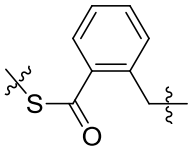
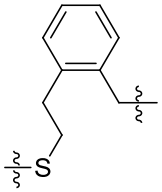
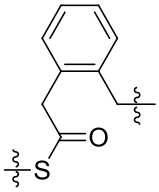
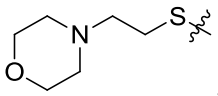
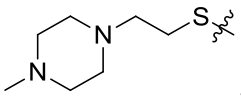
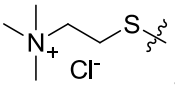
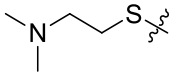
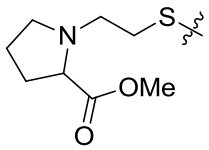
[00603] In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$. In some embodiments, L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$, wherein R^{L3} is an optionally substituted $C_1–C_6$ alkylene. In some embodiments, L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$, wherein R^{L3} is an optionally substituted $C_1–C_6$ alkenylene. In some embodiments, L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$, wherein R^{L3} is an optionally substituted C_1-C_6 alkylene wherein one or more methylene units are optionally and independently replaced by an optionally substituted $C_1–C_6$ alkenylene, arylene, or heteroarylene. In some embodiments, In some embodiments, R^{L3} is an optionally substituted $–S–(C_1–C_6$ alkenylene$)–$, $–S–(C_1–C_6$ alkylene$)–$, $–S–(C_1–C_6$ alkylene$)$–arylene–$(C_1–C_6$ alkylene$)–$, –S–CO–arylene–$(C_1–C_6$ alkylene$)–$, or $–S–CO–(C_1–C_6$ alkylene$)$–arylene–$(C_1–C_6$ alkylene$)–$. In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$, and the sulfur atom is connected to R^1.

[00604] In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein L is alkylene, alkenylene, arylene or heteroarylene.

[00605] In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein L is , , , , , , , , or . In some embodiments, L is , , , , ,





, wherein the sulfur atom is connected to R^1.

[00606] In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein R^1 is 



, or 
. In some embodiments, R^1 is 




, wherein the sulfur atom is connected to L.

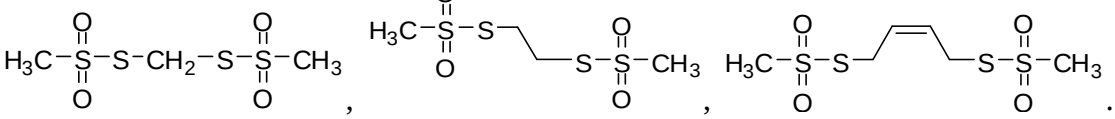
[00607] In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein L is 








, wherein the sulfur atom is connected to R^1; and R^1 is 



, or 
, wherein the sulfur atom is connected to L.

[00608] In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein R^1 is $–S–R^{L2}$, wherein R^{L2} is as defined and described above and herein. In some embodiments, R^{L2} is an optionally substituted group selected from $–S–(C_1–C_6$ alkylene)–

heterocyclyl, –S–(C_1–C_6 alkenylene)–heterocyclyl, –S–(C_1–C_6 alkylene)–N(R')$_2$, –S–(C_1–C_6 alkylene)–N(R')$_3$, wherein each R' is as defined above and described herein.

[00609] In some embodiments, –L–R^1 is –R^{L3}–S–S–R^{L2}, wherein each variable is independently as defined above and described herein. In some embodiments, –L–R^1 is –R^{L3}–C(O)–S–S–R^{L2}, wherein each variable is independently as defined above and described herein.

[00610] Exemplary bis(thiosulfonate) reagents of formula **S-II** are depicted below:



[00611] In some embodiments, the sulfurization reagent is a compound having one of the following formulae:

S_8, R^{s2}–S–S–R^{s3}, or R^{s2}–S–X^s–R^{s3},

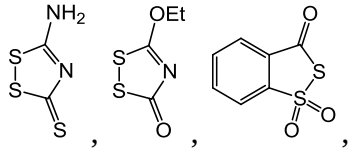
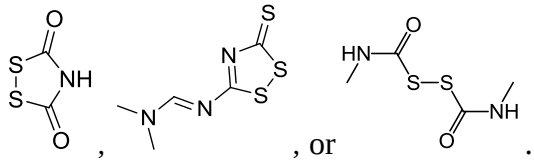
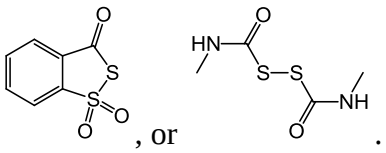
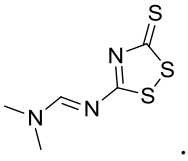
wherein:

each of R^{s2} and R^{s3} is independently an optionally substituted group selected from aliphatic, aminoalkyl, carbocyclyl, heterocyclyl, heterocycloalkyl, aryl, heteroaryl, alkyloxy, aryloxy, heteroaryloxy, acyl, amide, imide, or thiocarbonyl; or

R^{s2} and R^{s3} are taken together with the atoms to which they are bound to form an optionally substituted heterocyclic or heteroaryl ring;

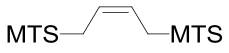
X^s is –$S(O)_2$–, –O–, or –N(R')–; and

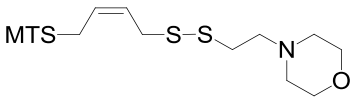
R' is as defined and described above and herein.

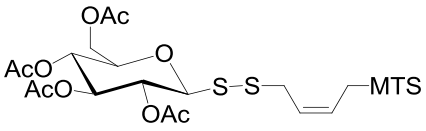
[00612] In some embodiments, the sulfurization reagent is S_8, , , or . In some embodiments, the sulfurization reagent is S_8, , or . In some embodiments, the sulfurization reagent is .

[00613] Exemplary sulfuring reagents are depicted in Table 5 below.

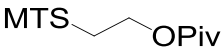
Table 5. Exemplary sulfurization reagents.

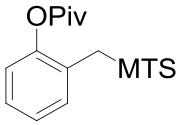

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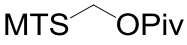

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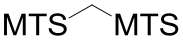

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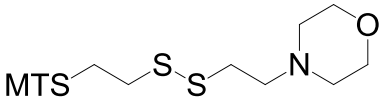

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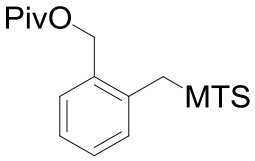

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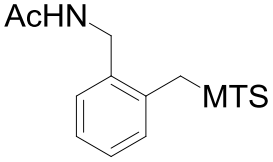

OPiv
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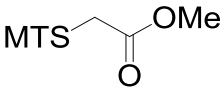

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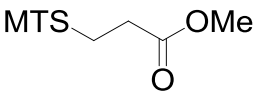

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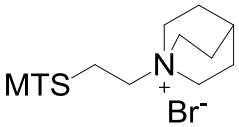

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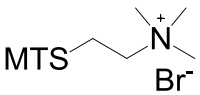

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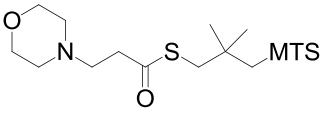

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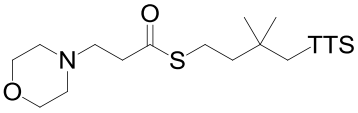

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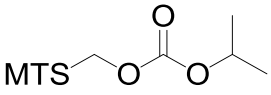

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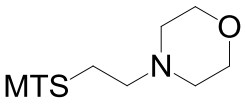

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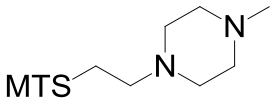

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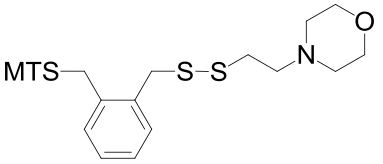

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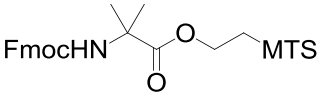

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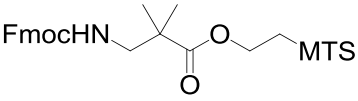

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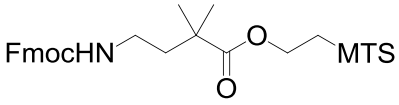

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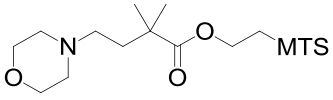

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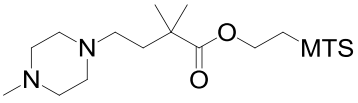

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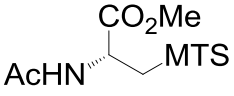

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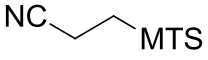

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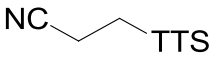

FmocHN
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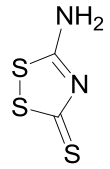

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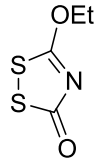

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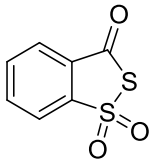

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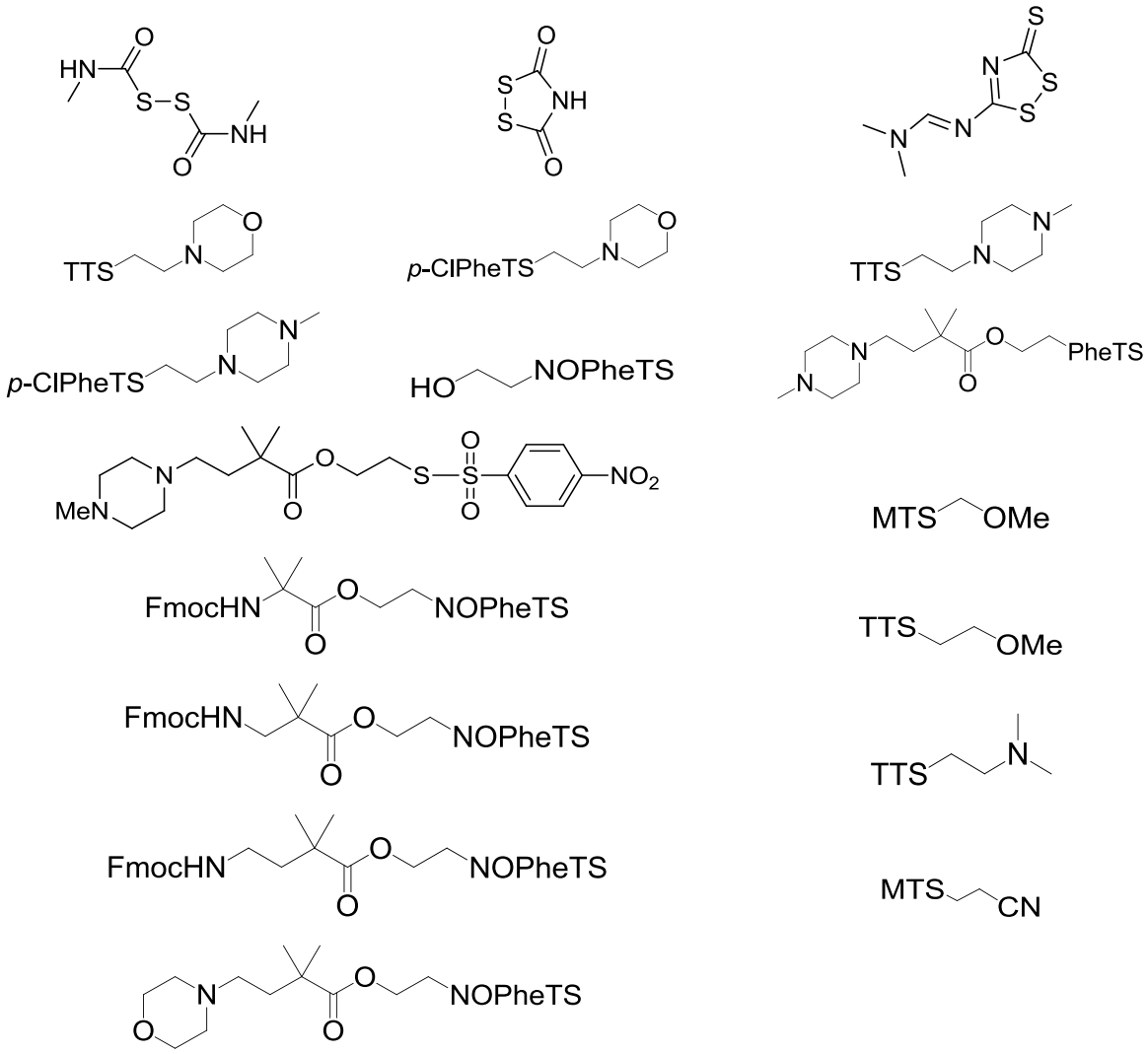

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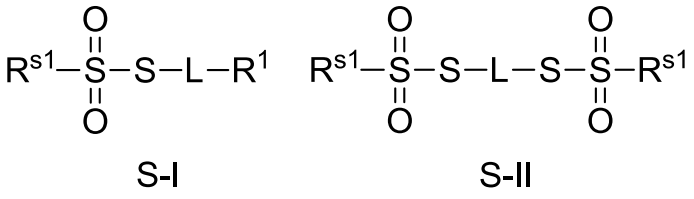

NH2
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OEt
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[00614] In some embodiments, a provided sulfurization reagent is used to modify an H-phosphonate. For instance, in some embodiments, an H-phosphonate oligonucleotide is synthesized using, e.g., a method of Wada I or Wada II, and is modified using a sulfurization reagent of formula **S-I** or **S-II**:

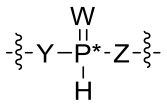


,

wherein each of R^{S1}, L, and R^1 are as described and defined above and herein.

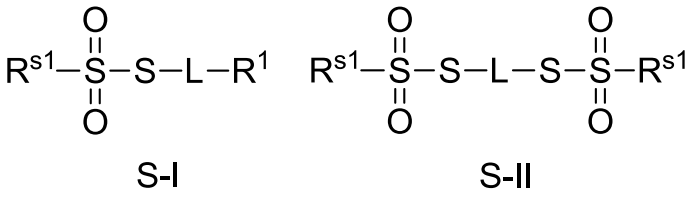
[00615] In some embodiments, the present invention provides a process for synthesizing a phosphorothioate triester, comprising steps of:

i) reacting an H-phosphonate of structure:



wherein each of W, Y, and Z are as described and defined above and herein, with a silylating reagent to provide a silyloxyphosphonate; and

ii) reacting the silyloxyphosphonate with a sulfurization reagent of structure **S-I** or **S-II**:



S-I S-II ,

to provide a phosphorothiotriester.

[00616] In some embodiments, a selenium electrophile is used instead of a sulfurizing reagent to introduce modification to the internucleotidic linkage. In some embodiments, a selenium electrophile is a compound having one of the following formulae:

Se, R^{s2}–Se–Se– R^{s3}, or R^{s2}–Se–X^s–R^{s3},

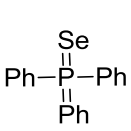
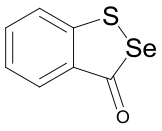
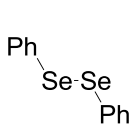
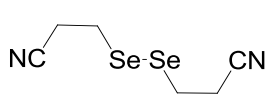
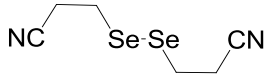
wherein:

each of R^{s2} and R^{s3} is independently an optionally substituted group selected from aliphatic, aminoalkyl, carbocyclyl, heterocyclyl, heterocycloalkyl, aryl, heteroaryl, alkyloxy, aryloxy, heteroaryloxy, acyl, amide, imide, or thiocarbonyl; or

R^{s2} and R^{s3} are taken together with the atoms to which they are bound to form an optionally substituted heterocyclic or heteroaryl ring;

X^s is $–S(O)_2–$, –O–, or –N(R')–; and

R' is as defined and described above and herein.

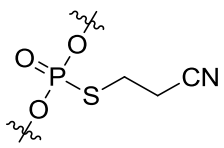
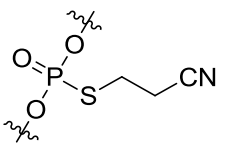
[00617] In other embodiments, the selenium electrophile is a compound of Se, KSeCN, , , , or . In some embodiments, the selenium electrophile is Se or .

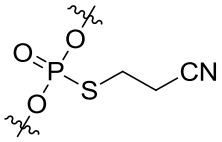
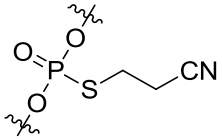
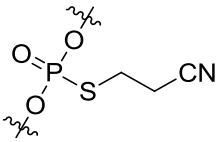
[00618] In some embodiments, a sulfurization reagent for use in accordance with the present invention is characterized in that the moiety transferred to phosphorus during sulfurization is a substituted sulfur (e.g., -SR) as opposed to a single sulfur atom (e.g., $-S^-$ or =S).

[00619] In some embodiments, a sulfurization reagent for use in accordance with the present invention is characterized in that the activity of the reagent is tunable by modifying the reagent with a certain electron withdrawing or donating group.

[00620] In some embodiments, a sulfurization reagent for use in accordance with the present invention is characterized in that it is crystalline. In some embodiments, a sulfurization reagent for use in accordance with the present invention is characterized in that it has a high degree of crystallinity. In certain embodiments, a sulfurization reagent for use in accordance with the present invention is characterized by ease of purification of the reagent via, e.g., recrystallization. In certain embodiments, a sulfurization reagent for use in accordance with the present invention is characterized in that it is substantially free from sulfur-containing impurities. In some embodiments, sulfurization reagents which are substantially free from sulfur-containing impurities show increased efficiency.

[00621] In some embodiments, the provided chirally controlled oligonucleotide comprises one or more phosphate diester linkages. To synthesize such chirally controlled oligonucleotides, one or more modifying steps are optionally replaced with an oxidation step to install the corresponding phosphate diester linkages. In some embodiments, the oxidation step is performed in a fashion similar to ordinary oligonucleotide synthesis. In some embodiments, an oxidation step comprises the use of I_2. In some embodiments, an oxidation step comprises the use of I_2 and pyridine. In some embodiments, an oxidation step comprises the use of 0.02 M I_2 in a THF/pyridine/water (70:20:10 – v/v/v) co-solvent system. An exemplary cycle is depicted in Scheme I-c.

[00622] In some embodiments, a phosphorothioate precursor is used to synthesize chirally controlled oligonucleotides comprising phosphorothioate linkages. In some embodiments, such a phosphorothioate precursor is . In some embodiments,  is converted into phosphorothioate diester linkages during standard deprotection/release procedure after cycle exit. Examples are further depicted below.

[00623] In some embodiments, the provided chirally controlled oligonucleotide comprises one or more phosphate diester linkages and one or more phosphorothioate diester linkages. In some embodiments, the provided chirally controlled oligonucleotide comprises one or more phosphate diester linkages and one or more phosphorothioate diester linkages, wherein at least one phosphate diester linkage is installed after all the phosphorothioate diester linkages when synthesized from 3' to 5'. To synthesize such chirally controlled oligonucleotides, in some embodiments, one or more modifying steps are optionally replaced with an oxidation step to install the corresponding phosphate diester linkages, and a phosphorothioate precursor is installed for each of the phosphorothioate diester linkages. In some embodiments, a phosphorothioate precursor is converted to a phosphorothioate diester linkage after the desired oligonucleotide length is achieved. In some embodiments, the deprotection/release step during or after cycle exit converts the phosphorothioate precursors into phosphorothioate diester linkages. In some embodiments, a phosphorothioate precursor is characterized in that it has the ability to be removed by a beta-elimination pathway. In some embodiments, a phosphorothioate precursor is . As understood by one of ordinary skill in the art, one of the benefits of using a phosphorothioate precursor, for instance, , during synthesis is that  is more stable than phosphorothioate in certain conditions.

[00624] In some embodiments, a phosphorothioate precursor is a phosphorus protecting group as described herein, e.g., 2-cyanoethyl (CE or Cne), 2-trimethylsilylethyl, 2-nitroethyl, 2-sulfonylethyl, methyl, benzyl, *o*-nitrobenzyl, 2-(*p*-nitrophenyl)ethyl (NPE or Npe), 2-phenylethyl, 3-(*N*-*tert*-butylcarboxamido)-1-propyl, 4-oxopentyl, 4-methylthio-l-butyl, 2-cyano-1,1-dimethylethyl, 4-*N*-methylaminobutyl, 3-(2-pyridyl)-1-propyl, 2-[*N*-methyl-*N*-(2-pyridyl)]aminoethyl, 2-(*N*-formyl,*N*-methyl)aminoethyl, 4-[*N*-methyl-*N*-(2,2,2-trifluoroacetyl)amino]butyl. Examples are further depicted below.

[00625] Methods for synthesizing a desired sulfurization reagent are described herein and in the examples section.

[00626] As noted above, in some embodiments, sulfurization occurs under conditions which cleave the chiral reagent from the growing oligonucleotide. In some embodiments, sulfurization occurs under conditions which do not cleave the chiral reagent from the growing oligonucleotide.

[00627] In some embodiments, a sulfurization reagent is dissolved in a suitable solvent and delivered to the column. In certain embodiments, the solvent is a polar aprotic solvent such as a nitrile solvent. In some embodiments, the solvent is acetonitrile. In some embodiments, a solution of sulfurization reagent is prepared by mixing a sulfurization reagent (e.g., a thiosulfonate derivative as described herein) with BSTFA (N,O-bis-trimethylsilyl-trifluoroacetamide) in a nitrile solvent (e.g., acetonitrile). In some embodiments, BSTFA is not included. For example, the present inventors have found that relatively more reactive sulfurization reagents of general formula $R^{s2}–S–S(O)_2–R^{s3}$ can often successfully participate in sulfurization reactions in the absence of BSTFA. To give but one example, the inventors have demonstrated that where R^{s2} is *p*-nitrophenyl and R^{s3} is methyl then no BSTFA is required. In light of this disclosure, those skilled in the art will readily be able to determine other situations and/or sulfurization reagents that do not require BSTFA.

[00628] In some embodiments, the sulfurization step is performed at room temperature. In some embodiments, the sulfurization step is performed at lower temperatures such as about 0 ^{o}C, about 5 ^{o}C, about 10 ^{o}C, or about 15 ^{o}C. In some embodiments, the sulfurization step is performed at elevated temperatures of greater than about 20 ^{o}C.

[00629] In some embodiments, a sulfurization reaction is run for about 1 minute to about 120 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 90 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 60 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 30 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 25 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 20 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 15 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 10

minutes. In some embodiments, a sulfurization reaction is run for about 5 minute to about 60 minutes.

[00630] In some embodiments, a sulfurization reaction is run for about 5 minutes. In some embodiments, a sulfurization reaction is run for about 10 minutes. In some embodiments, a sulfurization reaction is run for about 15 minutes. In some embodiments, a sulfurization reaction is run for about 20 minutes. In some embodiments, a sulfurization reaction is run for about 25 minutes. In some embodiments, a sulfurization reaction is run for about 30 minutes. In some embodiments, a sulfurization reaction is run for about 35 minutes. In some embodiments, a sulfurization reaction is run for about 40 minutes. In some embodiments, a sulfurization reaction is run for about 45 minutes. In some embodiments, a sulfurization reaction is run for about 50 minutes. In some embodiments, a sulfurization reaction is run for about 55 minutes. In some embodiments, a sulfurization reaction is run for about 60 minutes.

[00631] It was unexpectedly found that certain of the sulfurization modification products made in accordance with methods of the present invention are unexpectedly stable. In some embodiments, it the unexpectedly stable products are phosphorothioate triesters. In some embodiments, the unexpectedly stable products are chirally controlled oligonucleotides comprising one or more internucleotidic linkages having the structure of formula I-c.

[00632] One of skill in the relevant arts will recognize that sulfurization methods described herein and sulfurization reagents described herein are also useful in the context of modifying H-phosphonate oligonucleotides such as those described in Wada II (WO2010/064146).

[00633] In some embodiments, the sulfurization reaction has a stepwise sulfurization efficiency that is at least about 80%, 85%, 90%, 95%, 96%, 97%, or 98%. In some embodiments, the sulfurization reaction provides a crude dinucleotide product compositon that is at least 98% pure. In some embodiments, the sulfurization reaction provides a crude tetranucleotide product compositon that is at least 90% pure. In some embodiments, the sulfurization reaction provides a crude dodecanucleotide product compositon that is at least 70% pure. In some embodiments, the sulfurization reaction provides a crude icosanucleotide product compositon that is at least 50% pure.

[00634] Once the step of modifying the linkage phosphorus is complete, the oligonucleotide undergoes another deblock step in preparation for re-entering the cycle. In some

embodiments, a chiral auxiliary remains intact after sulfurization and is deblocked during the subsequent deblock step, which necessarily occurs prior to re-entering the cycle. The process of deblocking, coupling, capping, and modifying, are repeated until the growing oligonucleotide reaches a desired length, at which point the oligonucleotide can either be immediately cleaved from the solid support or left attached to the support for purification purposes and later cleaved. In some embodiments, one or more protecting groups are present on one or more of the nucleotide bases, and cleaveage of the oligonucleotide from the support and deprotection of the bases occurs in a single step. In some embodiments, one or more protecting groups are present on one or more of the nucleotide bases, and cleaveage of the oligonucleotide from the support and deprotection of the bases occurs in more than one steps. In some embodiments, deprotection and cleavage from the support occurs under basic conditions using, e.g., one or more amine bases. In certain embodiments, the one or more amine bases comprise propyl amine. In certain embodiments, the one or more amine bases comprise pyridine.

[00635] In some embodiments, cleavage from the support and/or deprotection occurs at elevated temperatures of about 30 ºC to about 90 ºC. In some embodiments, cleavage from the support and/or deprotection occurs at elevated temperatures of about 40 ºC to about 80 ºC. In some embodiments, cleavage from the support and/or deprotection occurs at elevated temperatures of about 50 ºC to about 70 ºC. In some embodiments, cleavage from the support and/or deprotection occurs at elevated temperatures of about 60 ºC. In some embodiments, cleavage from the support and/or deprotection occurs at ambient temperatures.

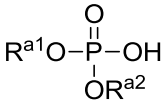
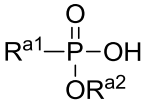
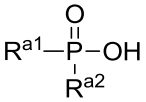
[00636] Exemplary purification procedures are described herein and/or are known generally in the relevant arts.

[00637] Noteworthy is that the removal of the chiral auxiliary from the growing oligonucleotide during each cycle is beneficial for at least the reasons that (1) the auxiliary will not have to be removed in a separate step at the end of the oligonucleotide synthesis when potentially sensitive functional groups are installed on phosphorus; and (2) unstable phosphorus-auxiliary intermediates prone to undergoing side reactions and/or interfering with subsequent chemistry are avoided. Thus, removal of the chiral auxiliary during each cycle makes the overall synthesis more efficient.

[00638] While the step of deblocking in the context of the cycle is described above, additional general methods are included below.

Deblocking Step

[00639] In some embodiments, the step of coupling is preceded by a step of deblocking. For instance, in some embodiments, the 5' hydroxyl group of the growing oligonucleotide is blocked (i.e., protected) and must be deblocked in order to subsequently react with a nucleoside coupling partner.

[00640] In some embodiments, acidification is used to remove a blocking group. In some embodiments, the acid is a Brønsted acid or Lewis acid. Useful Brønsted acids are carboxylic acids, alkylsulfonic acids, arylsulfonic acids, phosphoric acid and its derivatives, phosphonic acid and its derivatives, alkylphosphonic acids and their derivatives, arylphosphonic acids and their derivatives, phosphinic acid, dialkylphosphinic acids, and diarylphosphinic acids which have a pKa (25 °C in water) value of -0.6 (trifluoroacetic acid) to 4.76 (acetic acid) in an organic solvent or water (in the case of 80% acetic acid). The concentration of the acid (1 to 80%) used in the acidification step depends on the acidity of the acid. Consideration to the acid strength must be taken into account as strong acid conditions will result in depurination/depyrimidination, wherein purinyl or pyrimidinyl bases are cleaved from ribose ring and or other sugar ring. In some embodiments, an acid is selected from $R^{a1}COOH$, $R^{a1}SO_3H$, $R^{a3}SO_3H$, , , or , wherein each of R^{a1} and R^{a2} is independently hydrogen or an optionally substituted alkyl or aryl, and R^{a3} is an optionally substituted alkyl or aryl.

[00641] In some embodiments, acidification is accomplished by a Lewis acid in an organic solvent. Exemplary such useful Lewis acids are $Zn(X^a)_2$ wherein X^a is Cl, Br, I, or CF_3SO_3.

[00642] In some embodiments, the step of acidifying comprises adding an amount of a Brønsted or Lewis acid effective to remove a blocking group without removing purine moieties from the condensed intermediate.

[00643] Acids that are useful in the acidifying step also include, but are not limited to 10% phosphoric acid in an organic solvent, 10% hydrochloric acid in an organic solvent, 1% trifluoroacetic acid in an organic solvent, 3% dichloroacetic acid or trichloroacetic acid in an

organic solvent or 80% acetic acid in water. The concentration of any Brønsted or Lewis acid used in this step is selected such that the concentration of the acid does not exceed a concentration that causes cleavage of a nucleobase from a sugar moiety.

[00644] In some embodiments, acidification comprises adding 1% trifluoroacetic acid in an organic solvent. In some embodiments, acidification comprises adding about 0.1% to about 8% trifluoroacetic acid in an organic solvent. In some embodiments, acidification comprises adding 3% dichloroacetic acid or trichloroacetic acid in an organic solvent. In some embodiments, acidification comprises adding about 0.1% to about 10% dichloroacetic acid or trichloroacetic acid in an organic solvent. In some embodiments, acidification comprises adding 3% trichloroacetic acid in an organic solvent. In some embodiments, acidification comprises adding about 0.1% to about 10% trichloroacetic acid in an organic solvent. In some embodiments, acidification comprises adding 80% acetic acid in water. In some embodiments, acidification comprises adding about 50% to about 90%, or about 50% to about 80%, about 50% to about 70%, about 50% to about 60%, about 70% to about 90% acetic acid in water. In some embodiments, the acidification comprises the further addition of cation scavengers to an acidic solvent. In certain embodiments, the cation scavengers can be triethylsilane or triisopropylsilane. In some embodiments, a blocking group is deblocked by acidification, which comprises adding 1% trifluoroacetic acid in an organic solvent. In some embodiments, a blocking group is deblocked by acidification, which comprises adding 3% dichloroacetic acid in an organic solvent. In some embodiments, a blocking group is deblocked by acidification, which comprises adding 3% trichloroacetic acid in an organic solvent. In some embodiments, a blocking group is deblocked by acidification, which comprises adding 3% trichloroacetic acid in dichloromethane.

[00645] In certain embodiments, methods of the present invention are completed on a synthesizer and the step of deblocking the hydroxyl group of the growing oligonucleotide comprises delivering an amount solvent to the synthesizer column, which column contains a solid support to which the oligonucleotide is attached. In some embodiments, the solvent is a halogenated solvent (e.g., dichloromethane). In certain embodiments, the solvent comprises an amount of an acid. In some embodiments, the solvent comprises an amount of an organic acid such as, for instance, trichloroacetic acid. In certain embodiments, the acid is present in an amount of about 1% to about 20% w/v. In certain embodiments, the acid is present in an amount

of about 1% to about 10% w/v. In certain embodiments, the acid is present in an amount of about 1% to about 5% w/v. In certain embodiments, the acid is present in an amount of about 1 to about 3% w/v. In certain embodiments, the acid is present in an amount of about 3% w/v. Methods for deblocking a hydroxyl group are described further herein. In some embodiments, the acid is present in 3% w/v is dichloromethane.

[00646] In some embodiments, the chiral auxiliary is removed before the deblocking step. In some embodiments, the chiral auxiliary is removed during the deblocking step.

[00647] In some embodiments, cycle exit is performed before the deblocking step. In some embodiments, cycle exit is preformed after the deblocking step.

General conditions for blocking group/protecting group removal

[00648] Functional groups such as hydroxyl or amino moieties which are located on nucleobases or sugar moieties are routinely blocked with blocking (protecting) groups (moieties) during synthesis and subsequently deblocked. In general, a blocking group renders a chemical functionality of a molecule inert to specific reaction conditions and can later be removed from such functionality in a molecule without substantially damaging the remainder of the molecule (see *e.g.*, Green and Wuts, Protective Groups in Organic Synthesis, 2nd Ed., John Wiley & Sons, New York, 1991). For example, amino groups can be blocked with nitrogen blocking groups such as phthalimido, 9-fludrenylmethoxycarbonyl (FMOC), triphenylmethylsulfenyl, *t*-BOC, 4,4'-dimethoxytrityl (DMTr), 4-methoxytrityl (MMTr), 9-phenylxanthin-9-yl (Pixyl), trityl (Tr), or 9-(*p*-methoxyphenyl)xanthin-9-yl (MOX). Carboxyl groups can be protected as acetyl groups. Hydroxy groups can be protected such as tetrahydropyranyl (THP), *t*-butyldimethylsilyl (TBDMS), 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (Ctmp), 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp), 1-(2-chloroethoxy)ethyl, 3-methoxy-1,5-dicarbomethoxypentan-3-yl (MDP), bis(2-acetoxyethoxy)methyl (ACE), triisopropylsilyloxymethyl (TOM), 1-(2-cyanoethoxy)ethyl (CEE), 2-cyanoethoxymethyl (CEM), [4-(*N*-dichloroacetyl-*N*-methylamino)benzyloxy]methyl, 2-cyanoethyl (CN), pivaloyloxymethyl (PivOM), levunyloxymethyl (ALE). Other representative hydroxyl blocking groups have been described (see *e.g.*, Beaucage *et al.*, *Tetrahedron*, **1992**, *46*, 2223). In some embodiments, hydroxyl blocking groups are acid-labile groups, such as the trityl, monomethoxytrityl, dimethoxytrityl, trimethoxytrityl, 9-phenylxanthin-9-yl (Pixyl) and 9-(p-

methoxyphenyl)xanthin-9-yl (MOX). Chemical functional groups can also be blocked by including them in a precursor form. Thus an azido group can be considered a blocked form of an amine as the azido group is easily converted to the amine. Further representative protecting groups utilized in nucleic acid synthesis are known (see e.g. Agrawal et al., Protocols for Oligonucleotide Conjugates, Eds., Humana Press, New Jersey, 1994, Vol. 26, pp. 1-72).

[00649] Various methods are known and used for removal of blocking groups from nucleic acids. In some embodiments, all blocking groups are removed. In some embodiments, a portion of blocking groups are removed. In some embodiments, reaction conditions can be adjusted to selectively remove certain blocking groups.

[00650] In some embodiments, nucleobase blocking groups, if present, are cleavable with an acidic reagent after the assembly of a provided oligonucleotide. In some embodiment, nucleobase blocking groups, if present, are cleavable under neither acidic nor basic conditions, *e.g.* cleavable with fluoride salts or hydrofluoric acid complexes. In some embodiments, nucleobase blocking groups, if present, are cleavable in the presence of base or a basic solvent after the assembly of a provided oligonucleotide. In certain embodiments, one or more of the nucleobase blocking groups are characterized in that they are cleavable in the presence of base or a basic solvent after the assembly of a provided oligonucleotide but are stable to the particular conditions of one or more earlier deprotection steps occurring during the assembly of the provided oligonucleotide.

[00651] In some embodiments, blocking groups for nucleobases are not required. In some embodiments, blocking groups for nucleobases are required. In some embodiments, certain nucleobases require one or more blocking groups while other nucleobases do not require one or more blocking groups.

[00652] In some embodiments, the oligonucleotide is cleaved from the solid support after synthesis. In some embodiments, cleavage from the solid support comprises the use of propylamine. In some embodiments, cleavage from the solid support comprises the use of propylamine in pyridine. In some embodiments, cleavage from the solid support comprises the use of 20% propylamine in pyridine. In some embodiments, cleavage from the solid support comprises the use of propylamine in anhydrous pyridine. In some embodiments, cleavage from the solid support comprises the use of 20% propylamine in anhydrous pyridine. In some embodiments, cleavage from the solid support comprises use of a polar aprotic solvent such as

acetonitrile, NMP, DMSO, sulfone, and/or lutidine. In some embodiments, cleavage from the solid support comprises use of solvent, e.g., a polar aprotic solvent, and one or more primary amines (e.g., a C_{1-10} amine), and/or one or more of methoxylamine, hydrazine, and pure anhydrous ammonia.

[00653] In some embodiments, deprotection of oligonucleotide comprises the use of propylamine. In some embodiments, deprotection of oligonucleotide comprises the use of propylamine in pyridine. In some embodiments, deprotection of oligonucleotide comprises the use of 20% propylamine in pyridine. In some embodiments deprotection of oligonucleotide comprises the use of propylamine in anhydrous pyridine. In some embodiments, deprotection of oligonucleotide comprises the use of 20% propylamine in anhydrous pyridine.

[00654] In some embodiments, the oligonucleotide is deprotected during cleavage.

[00655] In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about room temperature. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at elevated temperature. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at above about 30 °C, 40 °C, 50 °C, 60 °C, 70°C, 80 °C 90 °C or 100 °C. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about 30 °C, 40 °C, 50 °C, 60 °C, 70°C, 80 °C 90 °C or 100 °C. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about 40-80 °C. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about 50-70 °C. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about 60 °C.

[00656] In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for more than 0.1 hr, 1 hr, 2 hrs, 5 hrs, 10 hrs, 15 hrs, 20 hrs, 24 hrs, 30 hrs, or 40 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 0.1-5 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 3-10 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 5-15 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide,

is performed for about 10-20 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 15-25 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 20-40 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 2 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 5 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 10 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 15 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 18 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 24 hrs.

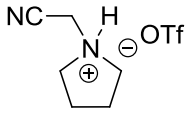
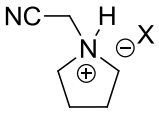
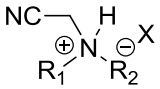
[00657] In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at room temperature for more than 0.1 hr, 1 hr, 2 hrs, 5 hrs, 10 hrs, 15 hrs, 20 hrs, 24 hrs, 30 hrs, or 40 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at room temperature for about 5-48 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at room temperature for about 10-24 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at room temperature for about 18 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at elevated temperature for more than 0.1 hr, 1 hr, 2 hrs, 5 hrs, 10 hrs, 15 hrs, 20 hrs, 24 hrs, 30 hrs, or 40 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at elevated temperature for about 0.5-5 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about 60 °C for about 0.5-5 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about 60 °C for about 2 hrs.

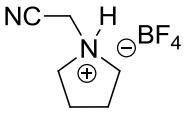
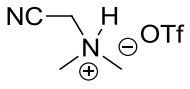
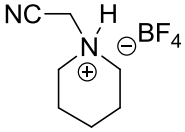
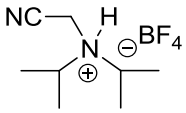
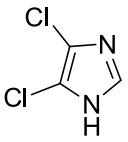
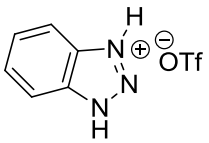
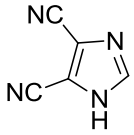
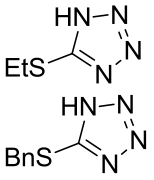
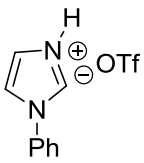
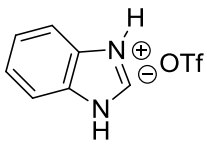
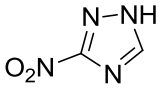
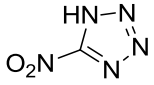
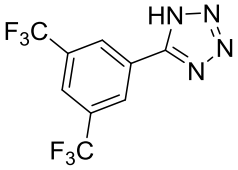
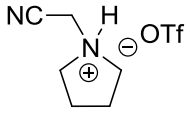
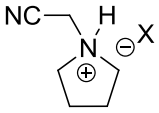
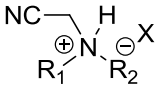
[00658] In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide comprises the use of propylamine and is performed at room

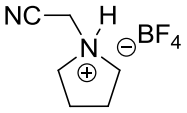
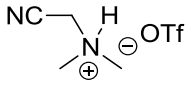
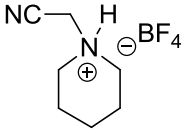
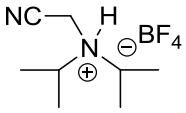
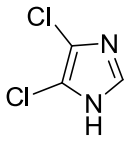
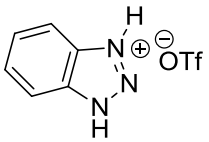
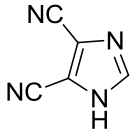
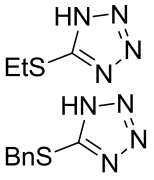
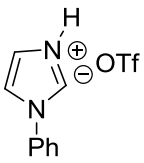
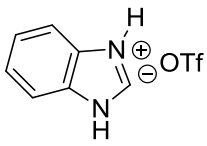
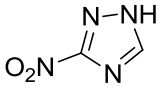
temperature or elevated temperature for more than 0.1 hr, 1 hr, 2 hrs, 5 hrs, 10 hrs, 15 hrs, 20 hrs, 24 hrs, 30 hrs, or 40 hrs. Exemplary conditions are 20% propylamine in pyridine at room temperature for about 18 hrs, and 20% propylamine in pyridine at 60 °C for about 18 hrs,

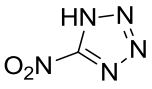
[00659] In some embodiments, an activator is a "Wada" activator, i.e., the activator is from any one of Wada I, II, or III documents cited above.

[00660] Exemplary activating groups are depicted below:



X = non nucleophilic anion

X = $^{-}$OTf, BF_4^{-}, $PF6^{-}$, TfN^{-}

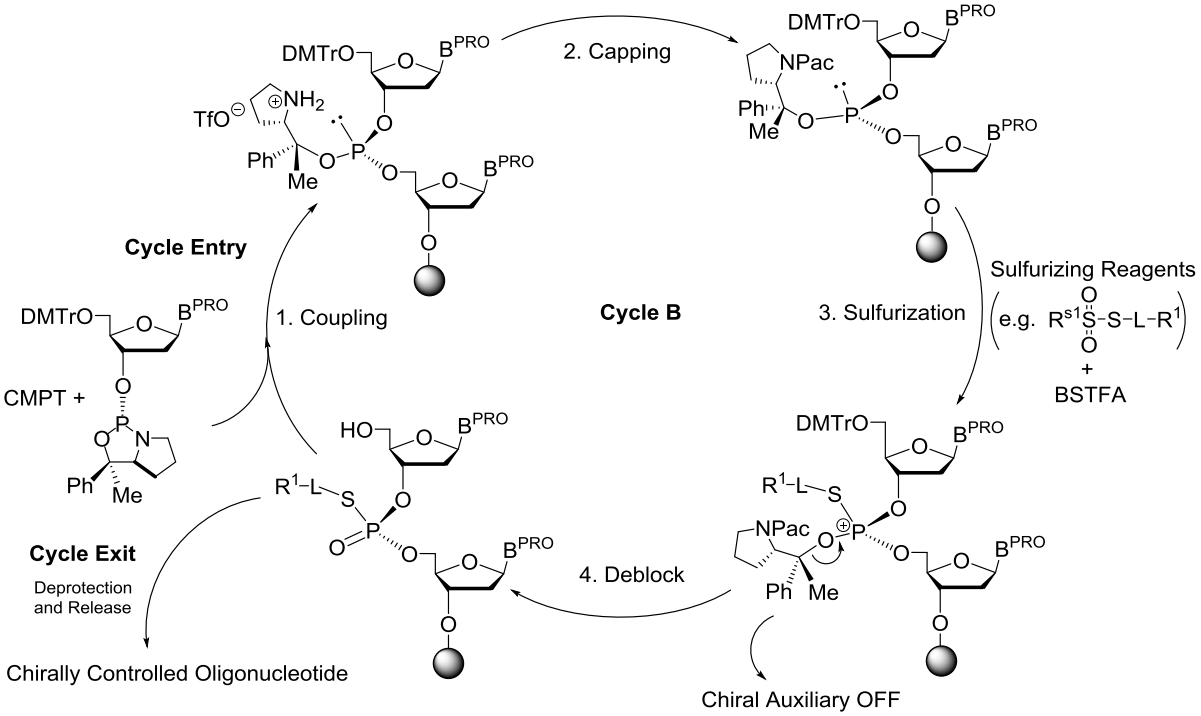













In some embodiments, an activating reagent is selected from


X = non nucleophilic anion

X = $^{-}$OTf, BF_4^{-}, $PF6^{-}$, TfN^{-}



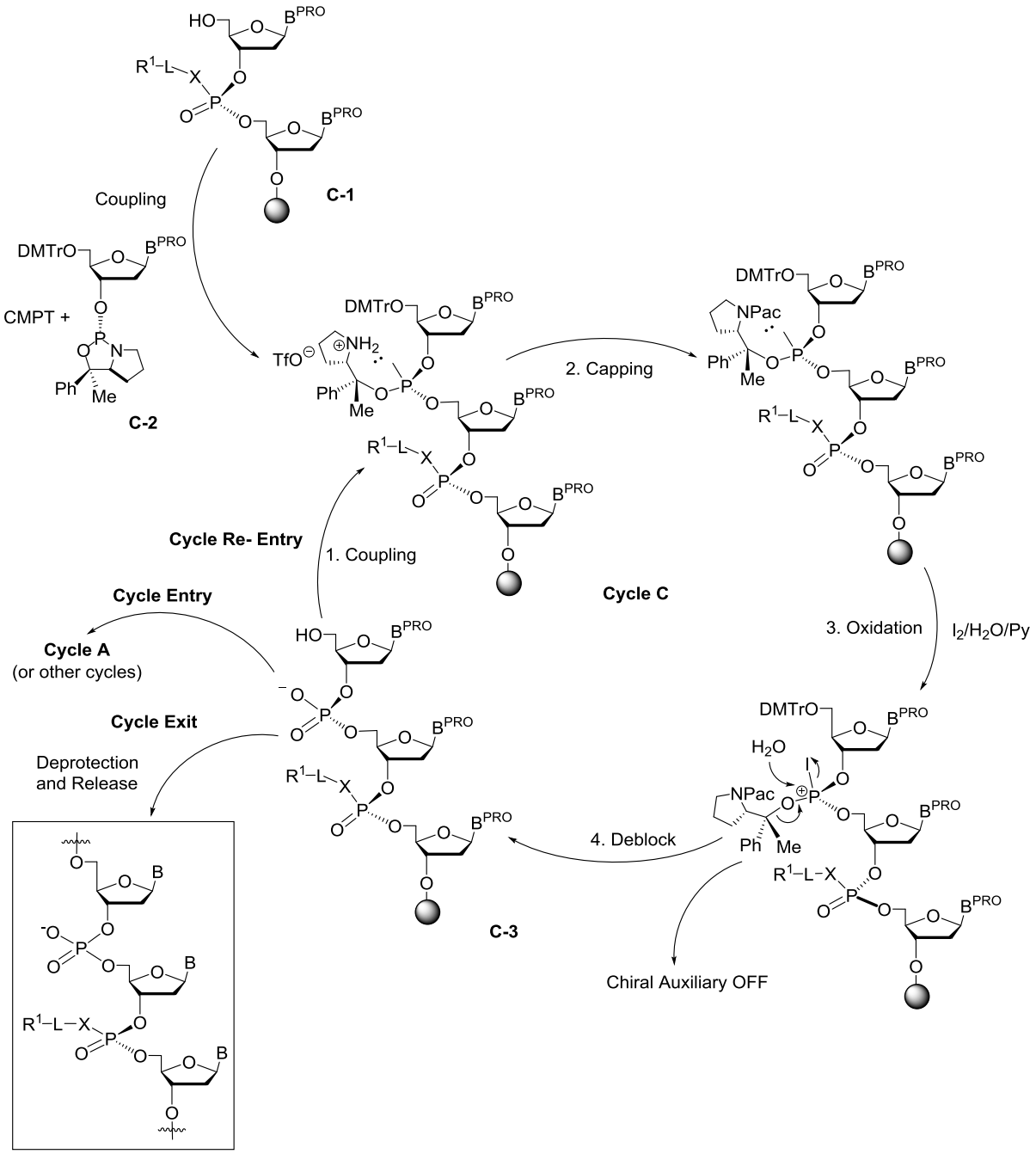
[00661] An exemplary cycle is depicted in Scheme I-b.

Scheme I-b. Installation of phosphorothioate linkages.



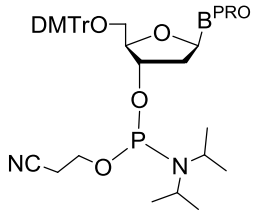
[00662] An exemplary cycle is illustrated in Scheme I-c.

Scheme I-c. Installation of both phosphate diester and modified internucleotidic linkages in a chirally controlled oligonucleotide.



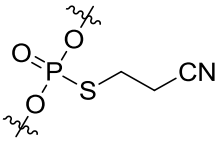
[00663] In Scheme I-c, oligonucleotide (or nucleotide, or oligonucleotide with modified internucleotidic linkage) on solid support (C-1) is coupled with phosphoramidite C-2. After coupling and capping, an oxidation step is performed. After deblocking, a phosphate diester linkage is formed. The cycle product C-3 can either re-enter cycle C to install more phosphate diester linkage, or enter other cycles to install other types of internucleotidic linkages, or go to cycle exit.

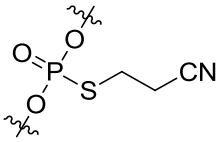
[00664] In some embodiments, non-chirally pure phosphoramidite can be used instead of C-2 in Scheme I-c. In some embodiments, β-cyanoethylphosphoramidites protected with DMTr is used. In some embodiments, the phosphoramidite being used has the structure of



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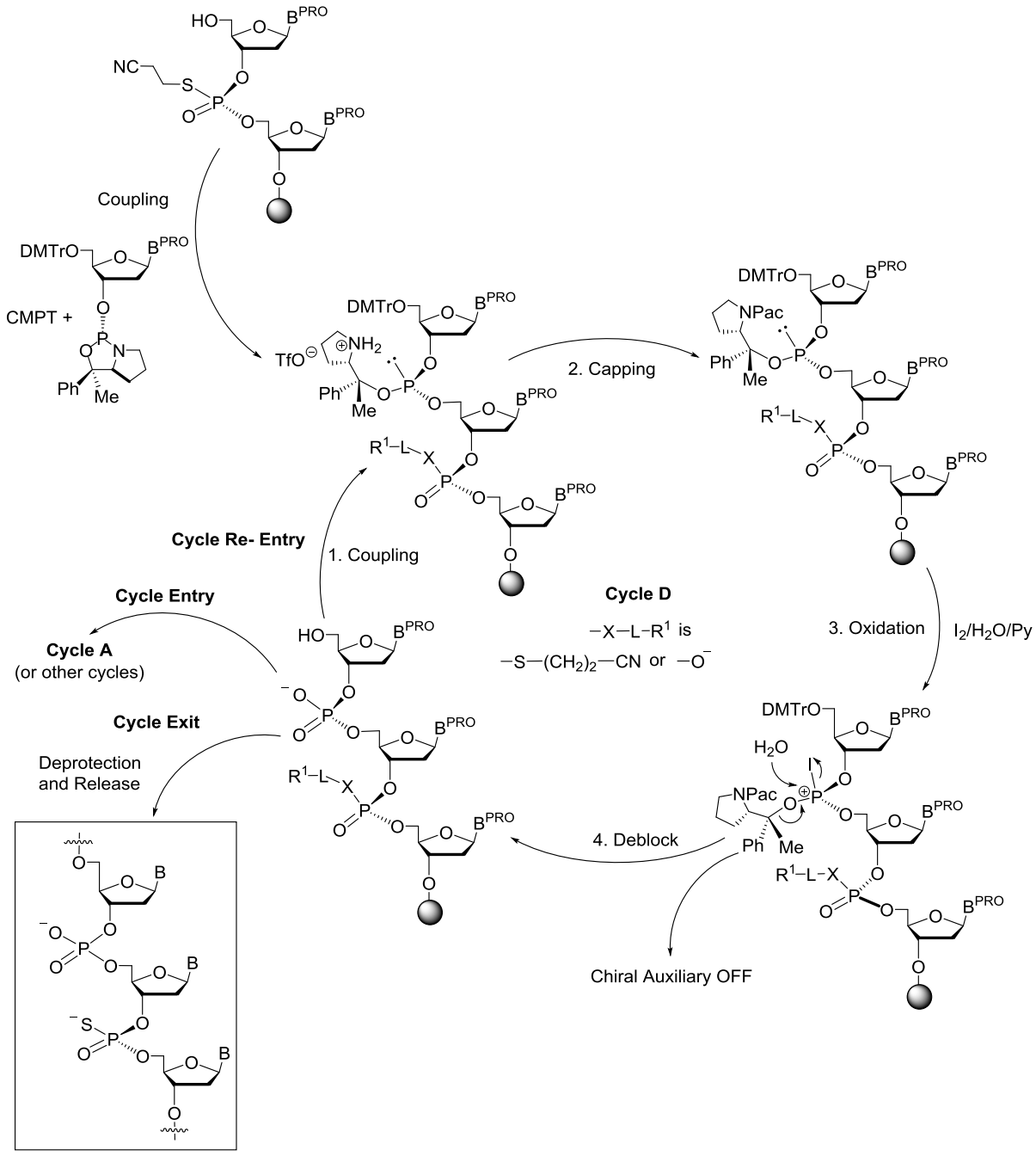
[00665] In some embodiments, the use of a phosphorothioate diester precursor increases the stability of oligonucleotide during synthesis. In some embodiments, the use of a phosphorothioate diester precursor improves the efficiency of chirally controlled oligonucleotide synthesis. In some embodiments, the use of a phosphorothioate diester precursor improves the yield of chirally controlled oligonucleotide synthesis. In some embodiments, the use of a phosphorothioate diester precursor improves the product purity of chirally controlled oligonucleotide synthesis.

[00666] In some embodiments, the phosphorothioate diester precursor in the above-mentioned methods is 

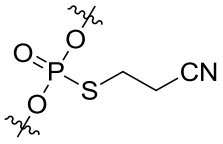
. In some embodiments, 

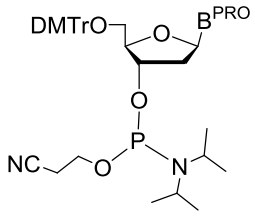
is converted to a phosphorothioate diester linkage during deprotection/release. An exemplary cycle is depicted in Scheme I-d. More examples are depicted below.

Scheme I-d. Phosphorothioate diester precursor in chirally controlled oligonucleotide synthesis.



[00667] As illustrated in Scheme I-d, both phosphorothioate and phosphate diester linkages can be incorporated into the same chirally controlled oligonucleotide. As understood by a person of ordinary skill in the art, the provided methods do not require that the phosphorothioate diester and the phosphate diester to be consecutive – other internucleotidic linkages can form between them using a cycle as described above. In Scheme I-d,

phosphorothioate diester precursors, , are installed in place of the phosphorothioate diester linkages. In some embodiments, such replacement provided increased synthesis efficiency during certain steps, for instance, the oxidation step. In some embodiments, the use of phosphorothioate diester precursors generally improve the stability of chirally controlled oligonucleotides during synthesis and/or storage. After cycle exit, during deprotection/release, the phosphorothioate diester precursor is converted to phosphorothioate diester linkage. In some embodiments, it is benefical to use phosphorothioate diester precursor even when no phosphate diester linkage is present in the chirally controlled oligonucleotide, or no oxidation step is required during synthesis.

[00668] As in Scheme I-c, in some embodiments, non-chirally pure phosphoramidite can be used for cycles comprising oxidation steps. In some embodiments, β-cyanoethylphosphoramidites protected with DMTr is used. In some embodiments, the phosphoramidite being used has the structure of 

[00669] In some embodiments, methods of the present invention provide chirally controlled oligonucleotide compositions that are enriched in a particular oligonucleotide type.

[00670] In some embodiments, at least about 10% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 20% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 30% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 40% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 50% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 60% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 70% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 80% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 90% of a provided crude composition is of a particular

oligonucleotide type. In some embodiments, at least about 95% of a provided crude composition is of a particular oligonucleotide type.

[00671] In some embodiments, at least about 1% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 2% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 3% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 4% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 5% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 10% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 20% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 30% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 40% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 50% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 60% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 70% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 80% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 90% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 95% of a provided composition is of a particular oligonucleotide type.

Biological Applications and Exemplary Use

[00672] Among other things, the present invention recognizes that properties and activities of an oligonucleotide can be adjusted by optimizing its pattern of backbone chiral centers through the use of provided chirally controlled oligonucleotide compositions. In some embodiments, the present invention provides chirally controlled oligonucleotide compositions, wherein the oligonucleotides have a common pattern of backbone chiral centers which enhances their stability and/or biological activity. In some embodiments, a pattern of backbone chiral centers provides unexpectedly increased stability. In some embodiments, a pattern of backbone chiral centers, surprisingly, provides greatly increased activity. In some embodiments, a pattern of backbone chiral centers provides both increased stability and activity. In some embodiments,

when an oligonucleotide is utilized to cleave a nucleic acid polymer, a pattern of backbone chiral centers of the oligonucleotide, surprisingly by itself, changes the cleavage pattern of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers effectively prevents cleavage at secondary sites. In some embodiments, a pattern of backbone chiral centers creates new cleavage sites. In some embodiments, a pattern of backbone chiral centers minimizes the number of cleavage sites. In some embodiments, a pattern of backbone chiral centers minimizes the number of cleavage sites so that a target nucleic acid polymer is cleaved at only one site within the sequence of the target nucleic acid polymer that is complementary to the oligonucleotide. In some embodiments, a pattern of backbone chiral centers enhances cleavage efficiency at a cleavage site. In some embodiments, a pattern of backbone chiral centers of the oligonucleotide improves cleavage of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers increases selectivity. In some embodiments, a pattern of backbone chiral centers minimizes off-target effect. In some embodiments, a pattern of backbone chiral centers increase selectivity, *e.g.*, cleavage selectivity among target sequences differing by point mutations or single nucleotide polymorphisms (SNPs). In some embodiments, a pattern of backbone chiral centers increase selectivity, *e.g.*, cleavage selectivity among target sequences differing by only one point mutation or single nucleotide polymorphism (SNP).

[00673] In some embodiments, the present invention provides a method for controlled cleavage of a nucleic acid polymer, comprising providing a chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers, which composition is a substantially pure preparation of a single oligonucleotide in that at least about 10% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers; and

wherein the nucleic acid polymer is cleaved in a cleavage pattern that is different than the cleavage pattern when chirally uncontrolled oligonucleotide composition is provided.

[00674] As used herein, a cleavage pattern of a nucleic acid polymer is defined by the number of cleavage sites, the locations of the cleavage sites, and the percentage of cleavage at each sites. In some embodiments, a cleavage pattern has multiple cleavage sites, and the percentage of cleavage at each site is different. In some embodiments, a cleavage pattern has multiple cleavage sites, and the percentage of cleavage at each site is the same. In some embodiments, a cleavage pattern has only one cleavage site. In some embodiments, cleavage patterns differ from each other in that they have different numbers of cleavage sites. In some embodiments, cleavage patterns differ from each other in that at least one cleavage location is different. In some embodiments, cleavage patterns differ from each other in that the percentage of cleavage at at least one common cleavage site is different. In some embodiments, cleavage patterns differ from each other in that they have different numbers of cleavage sites, and/or at least one cleavage location is different, and/or the percentage of cleavage at at least one common cleavage site is different.

[00675] In some embodiments, the present invention provides a method for controlled cleavage of a nucleic acid polymer, the method comprising steps of:

contacting a nucleic acid polymer whose nucleotide sequence comprises a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a target sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type.

[00676] In some embodiments, the present invention provides a method for controlled cleavage of a nucleic acid polymer, the method comprising steps of:

contacting a nucleic acid polymer whose nucleotide sequence comprises a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a target sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type, the contacting being performed under conditionsso that cleavage of the nucleic acid polymer occurs.

[00677] In some embodiments, the present invention provides a method for changing a first cleavage pattern of a nucleic acid polymer resulted from using a first oligonucleotide composition, comprising providing a second chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers, which composition is a substantially pure preparation of a single oligonucleotide in that at least about 10% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers; and

wherein the nucleic acid polymer is cleaved in a cleavage pattern that is different than the first cleavage pattern.

[00678] In some embodiments, the present invention provides a method for altering a cleavage pattern observed when a nucleic acid polymer whose nucleotide sequence includes a target sequence is contacted with a reference oligonucleotide composition that comprises oligonucleotides having a particular base sequence and length, which particular base sequence is or comprises a sequence that is complementary to the target sequence, the method comprising:

contacting the nucleic acid polymer with a chirally controlled oligonucleotide composition of oligonucleotides having the particular base sequence and length, which

composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of a single oligonucleotide type characterized by:

1) the particular base sequence and length;

2) a particular pattern of backbone linkages; and

3) a particular pattern of backbone chiral centers,

the contacting being performed under conditions so that cleavage of the nucleic acid polymer occurs.

[00679] In some embodiments, a provided chirally controlled oligonucleotide composition reduces the number of cleavage sites within the target sequence. In some embodiments, a provided chirally controlled oligonucleotide composition provides single-site cleavage within the target sequence. In some embodiments, a chirally controlled oligonucleotide composition provides enhanced cleavage rate at a cleavage site within the target sequence. In some embodiments, a chirally controlled oligonucleotide composition provides enhanced efficiency at a cleavage site within the target sequence. In some embodiments, a chirally controlled oligonucleotide composition provides increased turn-over in cleaving a target nucleic acid polymer.

[00680] In some embodiments, cleavage occurs with a cleavage pattern differs from a reference cleavage pattern. In some embodiments, a reference cleavage pattern is one observed when a nucleic acid polymer is contacted under comparable conditions with a reference oligonucleotide composition. In some embodiments, a reference oligonucleotide composition is a chirally uncontrolled (*e.g.*, stereorandom) oligonucleotide composition of oligonucleotides that share the common base sequence and length of a chirally controlled oligonucleotide composition. In some embodiments, a reference oligonucleotide composition is a substantially racemic preparation of oligonucleotides that share the common sequence and length.

[00681] In some embodiments, a nucleic acid polymer is RNA. In some embodiments, a nucleic acid polymer is an oligonucleotide. In some embodiments, a nucleic acid polymer is an RNA oligonucleotide. In some embodiments, a nucleic acid polymer is a transcript. In some embodiments, oligonucleotides of a provided chirally controlled oligonucleotide composition form duplexes with a nucleic acid polymer to be cleaved.

[00682] In some embodiments, a nucleic acid polymer is cleaved by an enzyme. In some embodiments, an enzyme cleaves a duplex formed by a nucleic acid polymer. In some embodiments, an enzyme is RNase H. In some embodiments, an enzyme is Dicer. In some embodiments, an enzyme is an Argonaute protein. In some embodiments, an enzyme is Ago2. In some embodiments, an enzyme is within a protein complex. An exemplary protein complex is RNA-induced silencing complex (RISC).

[00683] In some embodiments, a provided chirally controlled oligonucleotide composition comprising oligonucleotides with a common pattern of backbone chiral centers provides unexpectedly high selectivity so that nucleic acid polymers that have only small sequence variations within a target region can be selectively targeted. In some embodiments, a nucleic acid polymer is a transcript from an allele. In some embodiments, transcripts from different alleles can be selectively targeted by provided chirally controlled oligonucleotide compositions.

[00684] In some embodiments, provided chirally controlled oligonucleotide compositions and methods thereof enables precise control of cleavage sites within a target sequence. In some embodiments, a cleavage site is around a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is upstream of and near a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 5 base pairs upstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 4 base pairs upstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 3 base pairs upstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 2 base pairs upstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 1 base pair upstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is downstream of and near a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 5 base pairs downstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 4 base pairs downstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 3 base pairs downstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 2 base pairs downstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 1 base pair downstream of a sequence of *R*p*S*p*S*p backbone chiral centers. Among other things, the present invention therefore provides control of

cleavage sites with in a target sequence. In some embodiments, an exemplary cleavage is depicted in Figure 21. In some embodiments, cleavage depicted in Figure 21 is designated as cleavage at a site two base pairs downstream a sequence of *R*p*S*p*S*p backbone chiral centers. As extensively described in the present disclosure, a sequence of *R*p*S*p*S*p backbone chiral centers can be found in a single or repeating units of (*N*p)m(*R*p)n(*S*p)t, (*N*p)t(*R*p)n(*S*p)m, (*S*p)m(*R*p)n(*S*p)t, (*S*p)t(*R*p)n(*S*p)m, (*R*p)n(*S*p)m, (*R*p)m(*S*p)n, (*S*p)m*R*p and/or *R*p(*S*p)m, each of which is independently as defined above and described herein. In some embodiments, a provided chirally controlled oligonucleotide composition creates a new cleavage site 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule (*e.g.*, see Figure 21), wherein said new cleavage site does not exist if a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used (cannot be detected). In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a cleavage site 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule (*e.g.*, see Figure 21), wherein cleavage at such a site occurs at a higher percentage than when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, cleavage at such a site by a provided chirally controlled oligonucleotide composition is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500 or 1000 fold of that by a reference oligonucleotide composition (for example, when measured by percentage of cleavage at a site). In some embodiments, a provided chirally controlled oligonucleotide composition provides accelerated cleavage at a cleavage site 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule (*e.g.*, see Figure 21), compared to when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, cleavage by a provided chirally controlled oligonucleotide composition is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500 or 1000 fold faster than that by a reference oligonucleotide composition. In some embodiments, a cleavage site of a provided chirally controlled oligonucleotide composition 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule (*e.g.*, see Figure 21) is a cleavage site when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, a cleavage site of a provided chirally controlled oligonucleotide composition 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule (*e.g.*, see Figure 21) is within one base pair of a cleavage site when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used.

In some embodiments, a cleavage site of a provided chirally controlled oligonucleotide composition 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule (*e.g.*, see Figure 21) is within 2 base pairs of a cleavage site when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, it is within 3 base pairs. In some embodiments, it is within 4 base pairs. In some embodiments, it is within 5 base pairs. In some embodiments, a cleavage site of a provided chirally controlled oligonucleotide composition 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule is one of the major cleavage sites when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, such a site is the cleavage site with the highest cleavage percentage when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, a cleavage site of a provided chirally controlled oligonucleotide composition 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule is one of the cleavage sites with higher cleavage rate when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, such a site is the cleavage site with the highest cleavage rate when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used.

[00685] In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at one or more sites, *e.g.*, relative to a reference (*e.g.*, chirally uncontrolled/stereorandom) oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition selectively enhances cleavage at a single site relative to a reference (*e.g.*, chirally uncontrolled/stereorandom) composition. In some embodiments, a chirally controlled oligonucleotide composition enhances cleavage at a site by providing a higher cleavage rate. In some embodiments, a chirally controlled oligonucleotide composition enhances cleavage at a site by providing a higher percentage of cleavage at said site. Percentage of cleavage at a site can be determined by various methods widely known and practiced in the art. In some embodiments, percentage of cleavage at a site is determined by analysis of cleavage products, for example, as by HPLC-MS as illustrated in Figure 18, Figure 19 and Figure 30; see also exemplary cleavage maps such as Figure 9, Figure 10, Figure 11, Figure 14, Figure 22, Figure 25 and Figure 26. In some embodiments, enhancement is relative to a reference oligonucleotide composition. In some embodiments, enhancement is relative to another cleavage site. In some embodiments, a provided chirally controlled oligonucleotide

composition enhances cleavage at a site that is a preferred cleavage site of a reference oligonucleotide composition. In some embodiments, a preferred cleavage site, or a group of preferred cleavage sites, is a site or sites that have relatively higher percentage of cleavage compared to one or more other cleavage sites. In some embodiments, preferred cleavage sites can indicate preference of an enzyme. For example, for RNase H, when a DNA oligonucleotide is used, resulting cleavage sites may indicate preference of RNase H. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site that is a preferred cleavage site of an enzyme. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site that is not a preferred cleavage site of a reference oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site that is not a cleavage site of a reference oligonucleotide composition, effectively creating a new cleavage site which does not exist when a reference oligonucleotide composition is used. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site within 5 base pairs from a targeted mutation or SNP, thereby increasing selective cleavage of the undesired target oligonucleotide. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site within 4 base pairs from a targeted mutation or SNP, thereby increasing selective cleavage of the undesired target oligonucleotide. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site within 3 base pairs from a targeted mutation or SNP, thereby increasing selective cleavage of the undesired target oligonucleotide. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site within 2 base pairs from a targeted mutation or SNP, thereby increasing selective cleavage of the undesired target oligonucleotide. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site immediately upstream or downstream targeted mutation or SNP, thereby increasing selective cleavage of the undesired target oligonucleotide (*e.g.*, Figure 22, Panel D, muRNA).

[00686] In some embodiments, a provided chirally controlled oligonucleotide composition suppresses cleavage at one or more sites, *e.g.*, relative to a reference (*e.g.*, chirally uncontrolled/stereorandom) oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition selectively suppresses cleavage at a single site

relative to a reference (*e.g.*, chirally uncontrolled/stereorandom) composition. In some embodiments, a chirally controlled oligonucleotide composition suppresses cleavage at a site by providing a lower cleavage rate. In some embodiments, a chirally controlled oligonucleotide composition suppresses cleavage at a site by providing a lower percentage of cleavage at said site. In some embodiments, suppression is relative to a reference oligonucleotide composition. In some embodiments, suppression is relative to another cleavage site. In some embodiments, a provided chirally controlled oligonucleotide composition suppresses cleavage at a site that is a preferred cleavage site of a reference oligonucleotide composition. In some embodiments, a preferred cleavage site, or a group of preferred cleavage sites, is a site or sites that have relatively higher percentage of cleavage compared to one or more other cleavage sites. In some embodiments, preferred cleavage sites can indicate preference of an enzyme. For example, for RNase H, when a DNA oligonucleotide is used, resulting cleavage sites may indicate preference of RNase H. In some embodiments, a provided chirally controlled oligonucleotide composition suppresses cleavage at a site that is a preferred cleavage site of an enzyme. In some embodiments, a provided chirally controlled oligonucleotide composition suppresses cleavage at a site that is not a preferred cleavage site of a reference oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition suppresses all cleavage sites of a reference oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition generally enhances cleavage of target oligonucleotides. In some embodiments, a provided chirally controlled oligonucleotide composition generally suppresses cleavage of non-target oligonucleotides. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage of target oligonucleotides and suppresses cleavage of non-target oligonucleotides. Using Figure 22, Panel D, as an example, a target oligonucleotide for cleavage is muRNA, while a non-target oligonucleotide is wtRNA. In a subject comprising a diseased tissue comprising a mutation or SNP, a target oligonucleotide for cleavage can be transcripts with a mutation or SNP, while a non-target oligonucleotide can be normal transcripts without a mutation or SNP, such as those expressed in healthy tissues.

[00687] In some embodiments, besides patterns of backbone chiral centers described herein, provided oligonucleotides optionally comprises modified bases, modified sugars, modified backbone linkages and any combinations thereof. In some embodiments, a provided oligonucleotide is a unimer, altmer, blockmer, gapmer, hemimer and skipmer. In some

embodiments, a provided oligonucleotide comprises one or more unimer, altmer, blockmer, gapmer, hemimer or skipmer moieties, or any combinations thereof. In some embodiments, besides patterns of backbone chiral centers herein, a provided oligonucleotide is a hemimer. In some embodiments, besides patterns of backbone chiral centers herein, a provided oligonucleotide is a 5'-hemimer with modified sugar moieties. In some embodiments, a provided oligonucleotide is 5'-hemimer with 2'-modified sugar moieties. Suitable modifications are widely known in the art, *e.g.*, those described in the present application. In some embodiments, a modification is 2'−F. In some embodiments, a modification is 2'−MOE. In some embodiments, a modification is s-cEt.

[00688] In some embodiments, the present invention provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

[00689] In some embodiments, the present invention provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles

exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same nucleic acid seqeunce, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence,

the contacting being performed under conditions determined to permit the composition to suppress transcripts of the particular allele.

[00690] In some embodiments, the present invention provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

[00691] In some embodiments, the present invention provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with

a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particule allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence,

the contacting being performed under conditions determined to permit the composition to suppress transcripts of the particular allele.

[00692] In some embodiments, a transcript is suppressed by cleavage of said transcript. In some embodiments, a specific nucleotide characteristic sequence element is in an intron. In some embodiments, a specific nucleotide characteristic sequence element is in an exon. In some embodiments, a specific nucleotide characteristic sequence element is partially in an exon and partially in an intron. In some embodiments, a specific nucleotide characteristic sequence element comprises a mutation that differentiates an allele from other alleles. In some embodiments, a mutation is a deletion. In some embodiments, a mutation is an insertion. In some embodiments, a mutation is a point mutation. In some embodiments, a specific nucleotide characteristic sequence element comprises at least one single nucleotide polymorphism (SNP) that differentiates an allele from other alleles.

[00693] In some embodiments, a target nucleic acid sequence is a target gene.

[00694] In some embodiments, the present invention provides a method for allele-specific suppression of a gene whose sequence comprises at least one single nucleotide polymorphism (SNP), comprising providing a chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is completely complementary to a sequence found in a transcript from the first allele but not to the corresponding sequence found in a transcript from the second allele, wherein the sequence found in the transcripts comprises a SNP site;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers, which composition is a substantially pure preparation of a single oligonucleotide in that at least about 10% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers;

wherein the transcript from the first allele is suppressed at least five folds more than that from the second allele.

[00695] In some embodiments, the present invention provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[00696] In some embodiments, the present invention provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene,

the contacting being performed under conditions determined to permit the composition to suppress transcripts of the particular allele.

[00697] In some embodiments, the present invention provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene,

the contacting being performed under conditions determined to permit the composition to suppress expression of the particular allele.

[00698] In some embodiments, the present invention provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in thatwhen it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[00699] In some embodiments, the present invention provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene,

the contacting being performed under conditions determined to permit the composition to suppress transcripts of the particular allele.

[00700] In some embodiments, the present invention provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene,

the contacting being performed under conditions determined to permit the composition to suppress expression of the particular allele.

[00701] In some embodiments, suppression of transcripts of a particular allele is at a level that is greater than when the composition is absent. In some embodiments, suppression of transcripts of a particular allele is at a level that is at least 1.1 fold relative to when the composition is absent, in that transcripts from the particular allele are detected in amounts that

are at least 1.1 fold lower when the composition is present relative to when it is absent. In some embodiments, a level is at least 1.2 fold. In some embodiments, a level is at least 1.3 fold. In some embodiments, a level is at least 1.4 fold. In some embodiments, a level is at least 1.5 fold. In some embodiments, a level is at least 1.6 fold. In some embodiments, a level is at least 1.7 fold. In some embodiments, a level is at least 1.8 fold. In some embodiments, a level is at least 1.9 fold. In some embodiments, a level is at least 2 fold. In some embodiments, a level is at least 3 fold. In some embodiments, a level is at least 4 fold. In some embodiments, a level is at least 5 fold. In some embodiments, a level is at least 6 fold. In some embodiments, a level is at least 7 fold. In some embodiments, a level is at least 8 fold. In some embodiments, a level is at least 9 fold. In some embodiments, a level is at least 10 fold. In some embodiments, a level is at least 11 fold. In some embodiments, a level is at least 12 fold. In some embodiments, a level is at least 13 fold. In some embodiments, a level is at least 14 fold. In some embodiments, a level is at least 15 fold. In some embodiments, a level is at least 20 fold. In some embodiments, a level is at least 30 fold. In some embodiments, a level is at least 40 fold. In some embodiments, a level is at least 50 fold. In some embodiments, a level is at least 75 fold. In some embodiments, a level is at least 100 fold. In some embodiments, a level is at least 150 fold. In some embodiments, a level is at least 200 fold. In some embodiments, a level is at least 300 fold. In some embodiments, a level is at least 400 fold. In some embodiments, a level is at least 500 fold. In some embodiments, a level is at least 750 fold. In some embodiments, a level is at least 1000 fold. In some embodiments, a level is at least 5000 fold.

[00702] In some embodiments, suppression of transcripts of a particular allele is at a level that is greater than a level of suppression observed for another allele of the same nucleic acid sequence. In some embodiments, suppression of transcripts of a particular allele is at a level that is at least 1.1 fold greater than a level of suppression observed for another allele of the same nucleic acid sequence. In some embodiments, a level is at least 1.2 fold. In some embodiments, a level is at least 1.3 fold. In some embodiments, a level is at least 1.4 fold. In some embodiments, a level is at least 1.5 fold. In some embodiments, a level is at least 1.6 fold. In some embodiments, a level is at least 1.7 fold. In some embodiments, a level is at least 1.8 fold. In some embodiments, a level is at least 1.9 fold. In some embodiments, a level is at least 2 fold. In some embodiments, a level is at least 3 fold. In some embodiments, a level is at least 4 fold. In some embodiments, a level is at least 5 fold. In some embodiments, a level is at least 6 fold.

In some embodiments, a level is at least 7 fold. In some embodiments, a level is at least 8 fold. In some embodiments, a level is at least 9 fold. In some embodiments, a level is at least 10 fold. In some embodiments, a level is at least 11 fold. In some embodiments, a level is at least 12 fold. In some embodiments, a level is at least 13 fold. In some embodiments, a level is at least 14 fold. In some embodiments, a level is at least 15 fold. In some embodiments, a level is at least 20 fold. In some embodiments, a level is at least 30 fold. In some embodiments, a level is at least 40 fold. In some embodiments, a level is at least 50 fold. In some embodiments, a level is at least 75 fold. In some embodiments, a level is at least 100 fold. In some embodiments, a level is at least 150 fold. In some embodiments, a level is at least 200 fold. In some embodiments, a level is at least 300 fold. In some embodiments, a level is at least 400 fold. In some embodiments, a level is at least 500 fold. In some embodiments, a level is at least 750 fold. In some embodiments, a level is at least 1000 fold. In some embodiments, a level is at least 5000 fold.

[00703] In some embodiments, suppression of transcripts of a particular allele is at a level that is greater than when the composition is absent, and at a level that is greater than a level of suppression observed for another allele of the same nucleic acid sequence. In some embodiments, suppression of transcripts of a particular allele is at a level that is at least 1.1 fold relative to when the composition is absent, and at least 1.1 fold greater than a level of suppression observed for another allele of the same nucleic acid sequence. In some embodiments, each fold is independently as described above.

[00704] In some embodiments, a system is a composition comprising a transcript. In some embodiments, a system is a composition comprising transcripts from different alleles. In some embodiments, a system can be *in vivo* or *in vitro*, and in either way can comprise one or more cells, tissues, organs or organisms. In some embodiments, a system comprises one or more cells. In some embodiments, a system comprises one or more tissues. In some embodiments, a system comprises one or more organs. In some embodiments, a system comprises one or more organisms. In some embodiments, a system is a subject.

[00705] In some embodiments, suppression of a transcript, or suppression of expression of an allele from which a transcript is transcribed, can be measured in *in vitro* assay. In some embodiments, a sequence from a transcript and comprising a specific nucleotide characteristic sequence element is usned in assays instead of the full-length transcript. In some embodiments, an assay is a biochemical assay. In some embodiments, an assay is a biochemical assay wherein

a nucleic acid polymer, for example, a transcript or a sequence from a transcript and comprising a specific nucleotide characteristic sequence element, is tested for cleavage by an enzyme in the presence of a chirally controlled oligonucleotide composition.

[00706] In some embodiments, a provided chirally controlled oligonucleotide composition is administered to a subject. In some embodiments, a subject is an animal. In some embodiments, a subject is a plant. In some embodiments, a subject is a human.

[00707] In some embodiments, for allele-specific suppression of transcripts from a particular allele, transcripts are cleaved at a site near a sequence difference, for example a mutation, within a specific nucleotide characteristic sequence element, which sequence difference differentiates transcripts from a particular allele from transcripts from the other alleles. In some embodiments, transcripts are selectively cleaved at a site near such a sequence difference. In some embodiments, transcripts are cleaved at a higher percentage at a site near such a sequence difference that when a chirally uncontrolled oligonucleotide composition is used. In some embodiments, transcripts are cleaved at the site of a sequence difference. In some embodiments, transcripts are cleaved only at the site of a sequence difference within a specific nucleotide characteristic sequence element. In some embodiments, transcripts are cleaved at a site within 5 base pairs downstream or upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 4 base pairs downstream or upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 3 base pairs downstream or upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 2 base pairs downstream or upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 1 base pair downstream or upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 5 base pairs downstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 4 base pairs downstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 3 base pairs downstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 2 base pairs downstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 1 base pair downstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 5 base pairs upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 4 base pairs upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 3 base pairs upstream a

sequence difference. In some embodiments, transcripts are cleaved at a site within 2 base pairs upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 1 base pair upstream a sequence difference. Such precise control of cleavage patterns, and the resulting highly selective suppression of transcripts from a particular allele, would not be possible without chirally controlled oligonucleotide compositions and methods thereof provided by Applicant in this disclosure.

[00708] In some embodiments, the present invention provides methods for treating a subject, or preventing a disease in a subject, by specifically suppress transcripts from a particular allele, for example, an allele that causes or may cause a disease. In some embodiments, the present invention provides methods for treating a subject suffering from a disease, comprising administering to the subject a pharmaceutical composition comprising a chirally controlled oligonucleotide composition, wherein transcripts from an allele that causes or contributes to the disease is selectively suppressed. In some embodiments, the present invention provides methods for treating a subject suffering from a disease, comprising administering to the subject a pharmaceutical composition comprising a chirally controlled oligonucleotide composition, wherein transcripts from an allele that causes the disease is selectively suppressed. In some embodiments, the present invention provides methods for treating a subject suffering from a disease, comprising administering to the subject a pharmaceutical composition comprising a chirally controlled oligonucleotide composition, wherein transcripts from an allele that contributes to the disease is selectively suppressed. In some embodiments, the present invention provides methods for treating a subject suffering from a disease, comprising administering to the subject a pharmaceutical composition comprising a chirally controlled oligonucleotide composition, wherein transcripts from an allele that is related to the disease is selectively suppressed. In some embodiments, the present invention provides methods for preventing a disease in a subject, by specifically suppress transcripts from a particular allele that may cause a disease. In some embodiments, the present invention provides methods for preventing a disease in a subject, by specifically suppress transcripts from a particular allele that increases risk of a disease in the subject. In some embodiments, a provided method comprises administering to the subject a pharmaceutical composition comprising a chirally controlled oligonucleotide composition. In some embodiments, a pharmaceutical composition further comprises a pharmaceutical carrier.

[00709] Diseases that involves disease-causing alleles are widely known in the art, including but not limited to those described in Hohjoh, *Pharmaceuticals* **2013**, *6*, 522-535; US patent application publication US 2013/0197061; Østergaard *et al*., *Nucleic Acids Research* **2013**, *41*(21), 9634–9650; and Jiang *et al.*, *Science* **2013**, *342*, 111-114. In some embodiments, a disease is Huntington's disease. In some embodiments, a disease is human hypertrophic cardiomyopathy (HCM). In some embodiments, a disease is dilated cardiomyopathy. In some embodiments, a disease-causing allele is an allele of myosin heavy chains (MHC). In some embodiments, an exemplary disease is selected from:

Disease	Target gene	Target variation
Familial Alzheimer's disease	Amyloid precursor protein (APP)	K670N-M671L (Swedish mutant)
	Amyloid precursor protein (APP)	K670N-M671L (Swedish mutant)
	Amyloid precursor protein (APP)	V717F (London mutant)
	Amyloid precursor protein (APP)	V717I (London mutant)
	Preseniline 1 (PSEN1)	L392V
Amyotrophic lateral sclerosis (ALS)	Superoxide dismutase (SOD1)	G93A
	Superoxide dismutase (SOD1)	G85R
Slow channel congenital myasthenic syndrome (SCCMS)	Acetylcholine receptor (AChR)	aS226F
Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17)	Microtubule-associated protein TAU (MAPT)	V337M
Ehlers-Danlos syndrome (vEDS)	Procollagen type III (COL3A1)	G252V
Sickle cell anemia	Hemoglobin-beta locus (HBB)	E6V
Familial amyloidotic polyneuropathy (FAP)	Transthyretin (TTR)	V30M
Fibrodysplasia ossificans progressiva (FOP)	Activin A receptor type I (ACVR1)	R206H, G356D
	Activin A receptor type I (ACVR1)	R206H
Tumors	Phosphoinositide-3-kinase, catalytic, alpha polypeptide (PIK3CA)	1633G -> A 3140A -> G
Spinocerebellar ataxia type 1 (SCA1)	Ataxin-1 (ATXN1)	flanking region of expanded CAG repeat

Disease	Target gene	Target variation
Machado-Joseph disease/spinocerebellar ataxia type 3 (MJD/SCA3)	ATAXIN3/MJD1	SNPs linked to expanded CAG repeat
Spinocerebellar ataxia type 7 (SCA7)	Ataxin-7 (ATXN7)	SNP linked to expanded CAG repeat
Parkinson's disease	Leucine-rich repeat kinase 2 (LRRK2)	R1441G, R1441C
	Leucine-rich repeat kinase 2 (LRRK2)	G20195S
	alpha-synuclein	A30P
Huntington disease	Huntingtin (HTT)	SNPs linked to expanded CAG repeat
Hypertrophic cardiomyopathy	MYH7	R403Q

In some embodiments, exemplary targets of, and diseases that can be treated by, provided chirally controlled oligonucleotide compositions and methods, comprises:

Disease	Target gene	Target variation
Familial Alzheimer's disease	Amyloid precursor protein (APP)	K670N-M671L (Swedish mutant)
	Amyloid precursor protein (APP)	K670N-M671L (Swedish mutant)
	Amyloid precursor protein (APP)	V717F (London mutant)
	Amyloid precursor protein (APP)	V717I (London mutant)
	Preseniline 1 (PSEN1)	L392V
Amyotrophic lateral sclerosis (ALS)	Superoxide dismutase (SOD1)	G93A
	Superoxide dismutase (SOD1)	G85R

Disease	Target gene	Target variation
Slow channel congenital myasthenic syndrome (SCCMS)	Acetylcholine receptor (AChR)	aS226F, aT254I, aS269I
Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17)	Microtubule-associated protein TAU (MAPT)	V337M
Ehlers-Danlos syndrome (vEDS)	Procollagen type III (COL3A1)	G252V
Sickle cell anemia	Hemoglobin-beta locus (HBB)	E6V
Familial amyloidotic polyneuropathy (FAP)	Transthyretin (TTR)	V30M
Fibrodysplasia ossificans progressiva (FOP)	Activin A receptor type I (ACVR1)	R206H, G356D
	Activin A receptor type I (ACVR1)	R206H
Tumors	KRAS	G12V, G12D, G13D
Tumors	Phosphoinositide-3-kinase, catalytic, alpha polypeptide (PIK3CA)	G1633A, A3140G
Spinocerebellar ataxia type 1 (SCA1)	Ataxin-1 (ATXN1)	SNPs linked to expanded CAG repeat
Spinocerebellar ataxia type 7 (SCA7)	Ataxin-7 (ATXN7)	SNPs linked to expanded CAG repeat
Spinocerebellar Ataxia Type 3 (SCA3) /Machado-Joseph Disease	Ataxin-3 (ATXN3)	SNPs linked to expanded CAG repeat
Parkinson's disease	Leucine-rich repeat kinase 2 (LRRK2)	R1441G, R1441C
	Leucine-rich repeat kinase 2 (LRRK2)	G20195S
	Alpha-synuclein (SNCA)	A30P, A53T, E46K
Huntington's disease	Huntingtin (HTT)	SNPs linked to expanded CAG repeat
Huntington's disease-like 2	JPH3	SNPs linked to expanded CTG repeat
Friedreich's ataxia	FXN	SNPs linked to expanded GAA repeat

Disease	Target gene	Target variation
Fragile X mental retardation syndrome/fragile X tremor ataxia syndrome	FMR1	SNPs linked to expanded CGG repeat
Myotonic Dystophy (DM1)	DMPK	SNPs linked to expanded CTG repeat
Myotonic Dystophy (DM2)	ZNF9	SNPs linked to expanded CTG repeat
Spinal-Bulbar Muscular Atrophy	AR	SNPs linked to expanded CAG repeat
Hypertrophic cardiomyopathy	MHY7	R403Q

[00710] In some embodiments, a target Huntingtin site is selected from rs9993542_C, rs362310_C, rs362303_C, rs10488840_G, rs363125_C, rs363072_A, rs7694687_C, rs363064_C, rs363099_C, rs363088_A, rs34315806_C, rs2298967_T, rs362272_G, rs362275_C, rs362306_G, rs3775061_A, rs1006798_A, rs16843804_C, rs3121419_C, rs362271_G, rs362273_A, rs7659144_C, rs3129322_T, rs3121417_G, rs3095074_G, rs362296_C, rs108850_C, rs2024115_A, rs916171_C, rs7685686_A, rs6844859_T, rs4690073_G, rs2285086_A, rs362331_T, rs363092_C, rs3856973_G, rs4690072_T, rs7691627_G, rs2298969_A, rs2857936_C, rs6446723_T, rs762855_A, rs1263309_T, rs2798296_G, rs363096_T, rs10015979_G, rs11731237_T, rs363080_C, rs2798235_G and rs362307_T. In some embodiments, a target Huntingtin site is selected from rs34315806_C, rs362273_A, rs362331_T, rs363099_C, rs7685686_A, rs362306_G, rs363064_C, rs363075_G, rs2276881_G, rs362271_G, rs362303_C, rs362322_A, rs363088_A, rs6844859_T, rs3025838_C, rs363081_G, rs3025849_A, rs3121419_C, rs2298967_T, rs2298969_A, rs16843804_C, rs4690072_T, rs362310_C, rs3856973_G, and rs2285086_A. In some embodiments, a target Huntingtin site is selected from rs362331_T, rs7685686_A, rs6844859_T, rs2298969_A, rs4690072_T, rs2024115_A, rs3856973_G, rs2285086_A, rs363092_C, rs7691627_G, rs10015979_G, rs916171_C, rs6446723_T, rs11731237_T, rs362272_G, rs4690073_G, and rs363096_T. In some embodiments, a target Huntingtin site is selected from rs362267, rs6844859, rs1065746, rs7685686, rs362331, rs362336, rs2024115, rs362275, rs362273, rs362272, rs3025805, rs3025806, rs35892913, rs363125, rs17781557, rs4690072,

rs4690074, rs1557210, rs363088, rs362268, rs362308, rs362307, rs362306, rs362305, rs362304, rs362303, rs362302, rs363075 and rs2298969. In some embodiments, a target Huntingtin site is selected from:

Frequency of Heterozygosity for 24 SNP Sites in the Huntingtin mRNA			
Location in mRNA (Position, nt)	Reference Number	Percent Heterozygosity	
		Controls	HD Patients
ORF, exon 20 (2822)	rs363075	G/A, 10.3% (G/G, 89.7%)	G/A, 12.8% (G/G, 86.2%; A/A, 0.9%)
ORF, exon 25 (3335)	rs35892913	G/A, 10.3% (G/G, 89.7%)	G/A, 13.0% (G/G, 86.1%; A/A, 0.9%)
ORF, exon 25 (3389)	rs1065746	G/C, 0% (G/G, 100%)	G/C, 0.9% (G/G, 99.1%)
ORF, exon 25 (3418)	rs17781557	T/G, 12.9% (T/T, 87.1%)	T/G, 1.9% (T/T, 98.1%)
ORF, exon 29 (3946)	rs4690074	C/T, 37.9% (C/C, 50.9%; T/T, 11.2)	C/T, 35.8% (C/C, 59.6%; T/T, 4.6%)
ORF, exon 39 (5304)	rs363125	C/A, 17.5% (C/C, 79.0%; A/A, 3.5%)	C/A, 11.0% (C/C, 87.2%; A/A, 1.8%)
ORF, exon 44 (6150)	exon 44	G/A, 0% (G/G, 100%)	G/A, 2.8% (G/G, 97.2%)
ORF, exon 48 (6736)	rs362336	G/A, 38.7% (G/G, 49.6%; A/A, 11.7%)	G/A, 37.4% (G/G, 57.9%; A/A, 4.7%)
ORF, exon 50 (7070)	rs362331	T/C, 45.7% (T/T, 31.0%; C/C, 23.3%)	T/C, 39.4% (T/T, 49.5%; C/C, 11.0%)
ORF, exon 57 (7942)	rs362273	A/G, 40.3% (A/A, 48.2%; G/G, 11.4%)	A/G, 35.2% (A/A, 60.2%; G/G, 4.6%)
ORF, exon 61 (8501)	rs362272	G/A, 37.1% (G/G, 51.7%; A/A, 11.2%)	G/A, 36.1% (G/G, 59.3%; A/A, 4.6%)
ORF, exon 65 (9053)	rs3025806	A/T, 0% (C/C, 100%)	A/T, 0% (C/C, 100%)
ORF, exon 65 (9175)	exon 65	G/A, 2.3% (G/G, 97.7%)	G/A, 0% (G/G, 100%)
ORF, exon 67 (9523)	rs362308	T/C, 0% (T/T, 100%)	T/C, 0% (T/T, 100%)
3′UTR, exon 67 (9633)	rs362307	C/T, 13.0% (C/C, 87.0%)	C/T, 48.6% (C/C, 49.5%; T/T, 1.9%)
3′UTR, exon 67 (9888)	rs362306	G/A, 36.0% (G/G, 52.6%; A/A, 11.4%)	G/A, 35.8% (G/G, 59.6%; A/A, 4.6%)
3′UTR, exon 67 (9936)	rs362268	C/G, 36.8% (C/C, 50.0%; G/G 13.2%)	C/G, 35.8% (C/C, 59.6%; G/G, 4.6%)
3′UTR, exon 67 (9948)	rs362305	C/G, 20.2% (C/C, 78.1%; G/G 1.8%)	C/G, 11.9% (C/C, 85.3%; G/G, 2.8%)
3′UTR, exon 67	rs362304	C/A, 22.8% (C/C, 73.7%;	C/A, 11.9% (C/C, 85.3%;

(10060)		A/A, 3.5%)	AA, 2.8%)
3′UTR, exon 67 (10095)	rs362303	C/T, 18.4% (C/C, 79.8%; T/T, 1.8%)	C/A, 11.9% (C/C, 85.3%; T/T, 2.8%)
3′UTR, exon 67 (10704)	rs1557210	C/T, 0% (C/C, 100%)	C/T, 0% (C/C, 100%)
3′UTR, exon 67 (10708)	rs362302	C/T, 4.3% (C/C, 95.7%)	C/T, 0% (C/C, 100%)
3′UTR, exon 67 (10796)	rs3025805	G/T, 0% (G/G, 100%)	G/T, 0% (G/G, 100%)
3′UTR, exon 67 (11006)	rs362267	C/T, 36.2% (C/C, 52.6%; T/T, 11.2%)	C/T, 35.5% (C/C, 59.8%; T/T, 4.7%)

In some embodiments, a chirally controlled oligonucleotide composition targets two or more sites. In some embodiments, targeted two or more sites are selected from sited listed herein.

[00711] It is understood by a person having ordinary skill in the art that provided methods apply to any similar targets containing a mismatch. In some embodiments, a mismatch is between a maternal and paternal gene. Additional exemplary targets for suppression and/or knockdown, including allele-specific suppression and/or knockdown, can be any genetic abnormalties, *e.g.*, mutations, related to any diseases. In some embodiments, a target, or a set of targets, is selected from genetic determinants of diseases, *e.g.*, as disclosed in Xiong, *et al.*, The human splicing code reveals new insights into the genetic determinants of disease. *Science* Vol. 347 no. 6218 DOI: 10.1126/science.1254806. In some embodiments, a mismatch is between a mutant and a wild type.

[00712] In some embodiments, provided chirally controlled oligonucleotide compositions and methods are used to selectively suppress oligonucleotides with a mutation in a disease. In some embodiments, a disease is cancer. In some embodiments, provided chirally controlled oligonucleotide compositions and methods are used to selectively suppress transcripts with mutations in cancer. In some embodiments, provided chirally controlled oligonucleotide compositions and methods are used to suppress transcripts of KRAS. Exemplary target KRAS sites comprises G12V = GGU -> GUU Position 227 G->U, G12D = GGU->GAU Position 227 G->A and G13D = GGC -> GAC Position 230 G->A.

[00713] In some embodiments, provided chirally controlled oligonucleotide compositions and methods provide allele-specific suppression of a transcript in an organism. In some embodiments, an organism comprises a target gene for which two or more alleles exist. For example, a subject has a wild type gene in its normal tissues, while the same gene is mutated in

diseased tissues such as in a tumor. In some embodiments, the present invention provides chirally controlled oligonucleotide compositions and methods that selectively suppress one allele, for example, one with a mutation or SNP. In some embodiments, the present invention provides treatment with higher efficacy and/or low toxicity, and/or other benefits as described in the application.

[00714] In some embodiments, provided chirally controlled oligonucleotide compositions comprises oligonucleotides of one oligonucleotide type. In some embodiments, provided chirally controlled oligonucleotide compositions comprises oligonucleotides of only one oligonucleotide type. In some embodiments, provided chirally controlled oligonucleotide compositions has oligonucleotides of only one oligonucleotide type. In some embodiments, provided chirally controlled oligonucleotide compositions comprises oligonucleotides of two or more oligonucleotide types. In some embodiments, using such compositions, provided methods can target more than one target. In some embodiments, a chirally controlled oligonucleotide composition comprising two or more oligonucleotide types targets two or more targets. In some embodiments, a chirally controlled oligonucleotide composition comprising two or more oligonucleotide types targets two or more mismatches. In some embodiments, a single oligonucleotide type targets two or more targets, *e.g.*, mutations. In some embodiments, a target region of oligonucleotides of one oligonucleotide type comprises two or more "target sites" such as two mutations or SNPs.

[00715] In some embodiments, oligonucleotides in a provided chirally controlled oligonucleotide composition optionally comprise modified bases or sugars. In some embodiments, a provided chirally controlled oligonucleotide composition does not have any modified bases or sugars. In some embodiments, a provided chirally controlled oligonucleotide composition does not have any modified bases. In some embodiments, oligonucleotides in a provided chirally controlled oligonucleotide composition comprise modified bases and sugars. In some embodiments, oligonucleotides in a provided chirally controlled oligonucleotide composition comprise a modified base. In some embodiments, oligonucleotides in a provided chirally controlled oligonucleotide composition comprise a modified sugar. Modified bases and sugars for oligonucleotides are widely known in the art, including but not limited in those described in the present disclosure. In some embodiments, a modified base is 5-mC. In some embodiments, a modified sugar is a 2'-modified sugar. Suitable 2'-modification of

oligonucleotide sugars are widely known by a person having ordinary skill in the art. In some embodiments, 2'-modifications include but are not limited to 2'–OR^1, wherein R^1 is not hydrogen. In some embodiments, a 2'-modification is 2'–OR^1, wherein R^1 is optionally substituted C_{1-6} aliphatic. In some embodiments, a 2'-modification is 2'-MOE.In some embodiments, a modification is 2'-halogen. In some embodiments, a modification is 2'−F. In some embodiments, modified bases or sugars may further enhance activity, stability and/or selectivity of a chirally controlled oligonucleotide composition, whose common pattern of backbone chiral centers provides unexpected activity, stability and/or selectivity.

[00716] In some embodiments, a provided chirally controlled oligonucleotide composition does not have any modified sugars. In some embodiments, a provided chirally controlled oligonucleotide composition does not have any 2'- modified sugars. In some embodiments, the present invention surprising found that by using chirally controlled oligonucleotide compositions, modified sugars are not needed for stability, activity, and/or control of cleavage patterns. Furthermore, in some embodiments, the present invention surprisingly found that chirally controlled oligonucleotide compositions of oligonucleotides without modified sugars deliver better properties in terms of stability, activity, turn-over and/or control of cleavage patterns. For example, in some embodiments, it is surprising found that chirally controlled oligonucleotide compositions of oligonucleotides having no modified sugars dissociates much faster from cleavage products and provide significantly increased turn-over than compositions of oligonucleotides with modified sugars.

[00717] In some embodiments, oligonucleotides of provided chirally controlled oligonucleotide compositions useful for provided methods have structures as extensively described in the present disclosure. In some embodiments, an oligonucleotide has a wing-core-wing structure as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)m*R*p as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_2Rp$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)m(*R*p)n as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*R*p)n(*S*p)m as described. In some embodiments, the common pattern of backbone chiral centers

of a provided chirally controlled oligonucleotide composition comprises *R*p(*S*p)m as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises *R*p(*S*p)$_2$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)m(*R*p)n(*S*p)t as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)m*R*p(*S*p)t as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)t(*R*p)n(*S*p)m as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)t*R*p(*S*p)m as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises *S*p*R*p*S*p*S*p. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)$_2$*R*p(*S*p)$_2$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)$_3$*R*p(*S*p)$_3$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)$_4$*R*p(*S*p)$_4$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)t*R*p(*S*p)$_5$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises *S*p*R*p(*S*p)$_5$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)$_2$*R*p(*S*p)$_5$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)$_3$*R*p(*S*p)$_5$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)$_4$*R*p(*S*p)$_5$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises (*S*p)$_5$*R*p(*S*p)$_5$. In some embodiments, a common pattern of backbone chiral centers has only one *R*p, and each of the other internucleotidic linkages is *S*p. In some embodiments, a common base length is greater than 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 35, 40, 45 or 50 as described in the present disclosure. In some embodiments, a common base length is

greater than 10. In some embodiments, a common base length is greater than 11. In some embodiments, a common base length is greater than 12. In some embodiments, a common base length is greater than 13. In some embodiments, a common base length is greater than 14. In some embodiments, a common base length is greater than 15. In some embodiments, a common base length is greater than 16. In some embodiments, a common base length is greater than 17. In some embodiments, a common base length is greater than 18. In some embodiments, a common base length is greater than 19. In some embodiments, a common base length is greater than 20. In some embodiments, a common base length is greater than 21. In some embodiments, a common base length is greater than 22. In some embodiments, a common base length is greater than 23. In some embodiments, a common base length is greater than 24. In some embodiments, a common base length is greater than 25. In some embodiments, a common base length is greater than 26. In some embodiments, a common base length is greater than 27. In some embodiments, a common base length is greater than 28. In some embodiments, a common base length is greater than 29. In some embodiments, a common base length is greater than 30. In some embodiments, a common base length is greater than 31. In some embodiments, a common base length is greater than 32. In some embodiments, a common base length is greater than 33. In some embodiments, a common base length is greater than 34. In some embodiments, a common base length is greater than 35.

[00718] In some embodiments, a provided chirally controlled oligonucleotide composition provides higher turn-over. In some embodiments, cleavage products from a nucleic acid polymer dissociate from oligonucleotides of a provided chirally controlled oligonucleotide composition at a faster rate than from oligonucleotides of a reference oligonucleotide composition, for example, a chirally uncontrolled oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition can be administered in lower unit dosage, and/or total dosage, and/or fewer doses than chirally uncontrolled oligonucleotide composition.

[00719] In some embodiments, a chirally controlled oligonucleotide composition provides fewer cleavage sites in the sequence of a nucleic acid polymer that is complementary to its common base sequence or a sequence within its common base sequence when compared to a reference oligonucleotide composition. In some embodiments, a chirally controlled oligonucleotide composition provides fewer cleavage sites in the sequence of a nucleic acid

polymer that is complementary to its common base sequence. In some embodiments, a nucleic acid polymer is selectively cleaved at a single site within the sequence that is complimentary to the common base sequence, or a sequence within the common base sequence, of a chirally controlled oligonucleotide composition. In some embodiments, a chirally controlled oligonucleotide composition provides higher cleavage percentage at a cleavage site within the sequence that is complimentary to the common base sequence, or a sequence within the common base sequence, of the chirally controlled oligonucleotide composition. In some embodiments, a chirally controlled oligonucleotide composition provides higher cleavage percentage at a cleavage site within the sequence that is complimentary to the common base sequence of the chirally controlled oligonucleotide composition. In some embodiments, a site having a higher cleavage percentage is a cleavage site when a reference oligonucleotide composition is used. In some embodiments, a site having a higher cleavage percentage is a cleavage site that is not present when a reference oligonucleotide composition is used.

[00720] It is surprisingly found that with reduced number of cleavage sites in the complimentary sequence, cleavage rate can be unexpectedly increased and/or higher cleavage percentage can be achieved. As demonstrated in the examples of this disclosure, provided chirally controlled oligonucleotide compositions that produce fewer cleavage sites, especially those that provide single-site cleavage, within the complementary sequences of target nucleic acid polymers provide much higher cleavage rates and much lower levels of remaining un-cleaved nucleic acid polymers. Such results are in sharp contrast to general teachings in the art in which more cleavage sites have been pursued in order to increase the cleavage rate.

[00721] In some embodiments, a chirally controlled oligonucleotide composition increases cleavage rate by 1.5 fold compared to a reference oligonucleotide composition. In some embodiments, cleavage rate is increased by at least 2 fold. In some embodiments, cleavage rate is increased by at least 3 fold. In some embodiments, cleavage rate is increased by at least 4 fold. In some embodiments, cleavage rate is increased by at least 5 fold. In some embodiments, cleavage rate is increased by at least 6 fold. In some embodiments, cleavage rate is increased by at least 7 fold. In some embodiments, cleavage rate is increased by at least 8 fold. In some embodiments, cleavage rate is increased by at least 9 fold. In some embodiments, cleavage rate is increased by at least 10 fold. In some embodiments, cleavage rate is increased by at least 11 fold. In some embodiments, cleavage rate is increased by at least 12 fold. In some

embodiments, cleavage rate is increased by at least 13 fold. In some embodiments, cleavage rate is increased by at least 14 fold. In some embodiments, cleavage rate is increased by at least 15 fold. In some embodiments, cleavage rate is increased by at least 20 fold. In some embodiments, cleavage rate is increased by at least 30 fold. In some embodiments, cleavage rate is increased by at least 40 fold. In some embodiments, cleavage rate is increased by at least 50 fold. In some embodiments, cleavage rate is increased by at least 60 fold. In some embodiments, cleavage rate is increased by at least 70 fold. In some embodiments, cleavage rate is increased by at least 80 fold. In some embodiments, cleavage rate is increased by at least 90 fold. In some embodiments, cleavage rate is increased by at least 100 fold. In some embodiments, cleavage rate is increased by at least 200 fold. In some embodiments, cleavage rate is increased by at least 300 fold. In some embodiments, cleavage rate is increased by at least 400 fold. In some embodiments, cleavage rate is increased by at least 500 fold. In some embodiments, cleavage rate is increased by at least more than 500 fold.

[00722] In some embodiments, a chirally controlled oligonucleotide composition provides a lower level of remaining, un-cleaved target nucleic acid polymer compared to a reference oligonucleotide composition. In some embodiments, it is 1.5 fold lower. In some embodiments, it is at least 2 fold lower. In some embodiments, it is at least 3 fold lower. In some embodiments, it is at least 4 fold lower. In some embodiments, it is at least 5 fold lower. In some embodiments, it is at least 6 fold lower. In some embodiments, it is at least 7 fold lower. In some embodiments, it is at least 8 fold lower. In some embodiments, it is at least 9 fold lower. In some embodiments, it is at least 10 fold lower. In some embodiments, it is at least 11 fold lower. In some embodiments, it is at least 12 fold lower. In some embodiments, it is at least 13 fold lower. In some embodiments, it is at least 14 fold lower. In some embodiments, it is at least 15 fold lower. In some embodiments, it is at least 20 fold lower. In some embodiments, it is at least 30 fold lower. In some embodiments, it is at least 40 fold lower. In some embodiments, it is at least 50 fold lower. In some embodiments, it is at least 60 fold lower. In some embodiments, it is at least 70 fold lower. In some embodiments, it is at least 80 fold lower. In some embodiments, it is at least 90 fold lower. In some embodiments, it is at least 100 fold lower. In some embodiments, it is at least 200 fold lower. In some embodiments, it is at least 300 fold lower. In some embodiments, it is at least 400 fold lower. In some embodiments, it is at least 500 fold lower. In some embodiments, it is at least 1000 fold lower.

[00723] As discussed in detail herein, the present invention provides, among other things, a chirally controlled oligonucleotide composition, meaning that the composition contains a plurality of oligonucleotides of at least one type. Each oligonucleotide molecule of a particular "type" is comprised of preselected (e.g., predetermined) structural elements with respect to: (1) base sequence; (2) pattern of backbone linkages; (3) pattern of backbone chiral centers; and (4) pattern of backbone P-modification moieties. In some embodiments, provided oligonucloetide compositions contain oligonucleotides that are prepared in a single synthesis process. In some embodiments, provided compositions contain oligonucloetides having more than one chiral configuration within a single oligonucleotide molecule (e.g., where different residues along the oligonucleotide have different stereochemistry); in some such embodiments, such oligonucleotides may be obtained in a single synthesis process, without the need for secondary conjugation steps to generate individual oligonucleotide molecules with more than one chiral configuration.

[00724] Oligonucleotide compositions as provided herein can be used as agents for modulating a number of cellular processes and machineries, including but not limited to, transcription, translation, immune responses, epigenetics, etc. In addition, oligonucleotide compositions as provided herein can be used as reagents for research and/or diagnostic purposes. One of ordinary skill in the art will readily recognize that the present invention disclosure herein is not limited to particular use but is applicable to any situations where the use of synthetic oligonucleitides is desirable. Among other things, provided compositions are useful in a variety of therapeutic, diagnostic, agricultural, and/or research applications.

[00725] In some embodiments, provided oligonucloetide compositions comprise oligonucleotides and/or residues thereof that include one or more structural modifications as described in detail herein. In some embodiments, provided oligonucleotide compositions comprise oligonucleoties that contain one or more nucleic acid analogs. In some embodiments, provided oligonucleotide compositions comprise oligonucleotides that contain one or more artificial nucleic acids or residues, including but not limited to: peptide nucleic acids (PNA), Morpholino and locked nucleic acids (LNA), glycon nucleic acids (GNA), threose nucleic acids (TNA), Xeno nucleic acids (ZNA), and any combination thereof.

[00726] In any of the embodiments, the invention is useful for oligonucleotide-based modulation of gene expression, immune response, etc. Accordingly, stereo-

defined,oligonucleotide compositions of the invention, which contain oligonucleotides of predetermined type (i.e., which are chirally controlled, and optionally chirally pure), can be used in lieu of conventional stereo-random or chirally impure counterparts. In some embodiments, provided compositions show enhanced intended effects and/or reduced unwanted side effects. Certain embodimetns of biological and clinical/therapeutic applications of the invention are discussed explicitly below.

[00727] Various dosing regimens can be utilized to administer .provided chirally controlled oligonucleotide compositions. In some embodiments, multiple unit doses are administered, separated by periods of time. In some embodiments, a given composition has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second (or subsequent) dose amount that is same as or different from the first dose (or another prior dose) amount. In some embodiments, a dosing regimen comprises administering at least one unit dose for at least one day. In some embodiments, a dosing regimen comprises administering more than one dose over a time period of at least one day, and sometimes more than one day. In some embodiments, a dosing regimen comprises administering multiple doses over a time period of at least week. In some embodiments, the time period is at least 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, 39, 40 or more (e.g., about 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more) weeks. In some embodiments, a dosing regimen comprises administering one dose per week for more than one week. In some embodiments, a dosing regimen comprises administering one dose per week for 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, 39, 40 or more (e.g., about 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more) weeks. In some embodiments, a dosing regimen

comprises administering one dose every two weeks for more than two week period. In some embodiments, a dosing regimen comprises administering one dose every two weeks over a time period of 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, 39, 40 or more (e.g., about 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more) weeks. In some embodiments, a dosing regimen comprises administering one dose per month for one month. In some embodiments, a dosing regimen comprises administering one dose per month for more than one month. In some embodiments, a dosing regimen comprises administering one dose per month for 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more months. In some embodiments, a dosing regimen comprises administering one dose per week for about 10 weeks. In some embodiments, a dosing regimen comprises administering one dose per week for about 20 weeks. In some embodiments, a dosing regimen comprises administering one dose per week for about 30 weeks. In some embodiments, a dosing regimen comprises administering one dose per week for 26 weeks. In some embodiments, a chirally controlled oligonucleotide composition is administered according to a dosing regimen that differs from that utilized for a chirally uncontrolled (e.g., stereorandom) oligonucleotide composition of the same sequence, and/or of a different chirally controlled oligonucleotide composition of the same sequence. In some embodiments, a chirally controlled oligonucleotide composition is administered according to a dosing regimen that is reduced as compared with that of a chirally uncontrolled (e.g., sterorandom) oligonucleotide composition of the same sequence in that it achieves a lower level of total exposure over a given unit of time, involves one or more lower unit doses, and/or includes a smaller number of doses over a given unit of time. In some embodiments, a chirally controlled oligonucleotide composition is administered according to a dosing regimen that extends for a longer period of time than does that of a chirally uncontrolled (e.g., stereorandom) oligonucleotide composition of the same sequence Without wishing to be limited by theory, Applicant notes that in some embodiments, the shorter dosing regimen, and/or longer time periods between doses, may be due to the improved stability, bioavailability, and/or efficacy of a chirally controlled oligonucleotide composition. In some embodiments, a chirally controlled oligonucleotide composition has a longer dosing regimen compared to the corresponding chirally uncontrolled oligonucleotide composition. In some embodiments, a chirally controlled oligonucleotide composition has a shorter time period between at least two doses compared to the corresponding chirally uncontrolled oligonucleotide composition.

Without wishing to be limited by theory, Applicant notes that in some embodiments longer dosing regimen, and/or shorter time periods between doses, may be due to the improved safety of a chirally controlled oligonucleotide composition.

[00728] A single dose can contain various amounts of a type of chirally controlled oligonucleotide, as desired suitable by the application. In some embodiments, a single dose contains about 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300 or more (e.g., about 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more) mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 1 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 5 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 10 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 15 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 20 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 50 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 100 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 150 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 200 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 250 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 300 mg of a type of chirally controlled oligonucleotide. In some embodiments, a chirally controlled oligonucleotide is administered at a lower amount in a single dose, and/or in total dose, than a chirally uncontrolled oligonucleotide. In some embodiments, a chirally controlled oligonucleotide is administered at a lower amount in a single dose, and/or in total dose, than a chirally uncontrolled oligonucleotide due to improved efficacy. In some embodiments, a chirally controlled oligonucleotide is administered at a higher amount in a single dose, and/or in total dose, than a chirally uncontrolled oligonucleotide. In some embodiments, a chirally controlled oligonucleotide is administered at a higher amount in a single dose, and/or in total dose, than a chirally uncontrolled oligonucleotide due to improved safety.

Biologically Active Oligonucleotides

[00729] A provided oligonucleotide composition as used herein may comprise single

stranded and/or multiply stranded oligonucleotides. In some embodiments, single-stranded oligonucleotides contain self-complementary portions that may hybridize under relevant conditions so that, as used, even single-stranded oligonucleotides may have at least partially double-stranded character. In some embodiments, an oligonucleotide included in a provided composition is single-stranded, double-stranded, or triple-stranded. In some embodiments, an oligonucleotide included in a provided composition comprises a single-stranded portion and a multiple-stranded portion within the oligonucleotide. In some embodiments, as noted above, individual single-stranded oligonucleotides can have double-stranded regions and single-stranded regions.

[00730] In some embodiments, provided compositions include one or more oligonucleotides fully or partially complementary to strand of: structural genes, genes control and/or termination regions, and/or self-replicating systems such as viral or plasmid DNA. In some embodiments, provided compositions include one or more oligonucleotides that are or act as siRNAs or other RNA interference reagents (RNAi agents or iRNA agents), shRNA, antisense oligonucleotides, self-cleaving RNAs, ribozymes, fragment thereof and/or variants thereof (such as Peptidyl transferase 23S rRNA, RNase P, Group I and Group II introns, GIR1 branching ribozymes, Leadzyme, Hairpin ribozymes, Hammerhead ribozymes, HDV ribozymes, Mammalian CPEB3 ribozyme, VS ribozymes, glmS ribozymes, CoTC ribozyme, etc.), microRNAs, microRNA mimics, supermirs, aptamers, antimirs, antagomirs, Ul adaptors, triplex-forming oligonucleotides, RNA activators, long non-coding RNAs, short non-coding RNAs (e.g., piRNAs), immunomodulatory oligonucleotides (such as immunostimulatory oligonucleotides, immunoinhibitory oligonucleotides), GNA, LNA, ENA, PNA, TNA, morpholinos, G-quadruplex (RNA and DNA), antiviral oligonucleotides, and decoy oligonucleotides.

[00731] In some embodiments, provided compositions include one or more hybrid (e.g., chimeric) oligonucleotides. In the context of the present disclosure, the term “hybrid” broadly refers to mixed structural components of oligonucloetides. Hybrid oliogonucleotides may refer to, for example, (1) an oligonucleotide molecule having mixed classes of nucleotides, e.g., part DNA and part RNA within the single molecule (e.g., DNA-RNA); (2) complementary pairs of nucleic acids of different classes, such that DNA:RNA base pairing occurs either intramolecularly or intermolecularly; or both; (3) an oligonucleotide with two or more kinds of the backbone or internucleotide linkages.

[00732] In some embodiments, provided compositions include one or more oligonucleotide that comprises more than one classes of nucleic acid residues within a single molecule. For example, in any of the embodiments described herein, an oligonucleotide may comprise a DNA portion and an RNA portion. In some embodiments, an oligonucleotide may comprise a unmodified portion and modified portion.

[00733] Provided oligonucleotide compositions can include oligonucleotides containing any of a variety of modifications, for example as described herein. In some embodiments, particular modifications are selected, for example, in light of intended use. In some embodiments, it is desirable to modify one or both strands of a double-stranded oligonucleotide (or a double-stranded portion of a single-stranded oligonucleotie). In some embodiments, the two strands (or portions) include different modifications. In some embodiments, the two strands include the same modificatinons. One of skill in the art will appreciate that the degree and type of modifications enabled by methods of the present invention allow for numerous permutations of modifications to be made. Exemplary such modifications are described herein and are not meant to be limiting.

[00734] The phrase "antisense strand" as used herein, refers to an oligonucleotide that is substantially or 100% complementary to a target sequence of interest. The phrase "antisense strand" includes the antisense region of both oligonucleotides that are formed from two separate strands, as well as unimolecular oligonucleotides that are capable of forming hairpin or dumbbell type structures. The terms "antisense strand" and "guide strand" are used interchangeably herein.

[00735] The phrase "sense strand" refers to an oligonucleotide that has the same nucleoside sequence, in whole or in part, as a target sequence such as a messenger RNA or a sequence of DNA. The terms "sense strand" and "passenger strand" are used interchangeably herein.

[00736] By "target sequence" is meant any nucleic acid sequence whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA, such as endogenous DNA or RNA, viral DNA or viral RNA, or other RNA encoded by a gene, virus, bacteria, fungus, mammal, or plant. In some embodiments, a target sequence is associated with a disease or disorder.

[00737] By "specifically hybridizable" and "complementary" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-

Crick or other non- traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner et al, 1987, *CSH Symp. Quant. Biol.* LIT pp.123-133; Frier et al., 1986, *Proc. Nat. Acad. Sci.* USA83:9373-9377; Turner et al., 1987, /. *Ain. Chem. Soc.* 109:3783-3785)

[00738] A percent complcmentarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9,10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" or 100% complementarity means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. Less than perfect complementarity refers to the situation in which some, but not all, nucleoside units of two strands can hydrogen bond with each other. "Substantial complementarity" refers to polynucleotide strands exhibiting 90% or greater complementarity, excluding regions of the polynucleotide strands, such as overhangs, that are selected so as to be noncomplementary. Specific binding requires a sufficient degree of complementarity to avoid non-specific binding of the oligomeric compound to non-target sequences under conditions in which specific binding is desired, e.g., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, or in the case of *in vitro* assays, under conditions in which the assays are performed. In some embodiments, non-target sequences differ from corresponding target sequences by at least 5 nucleotides.

[00739] When used as therapeutics, a provided oligonucleotide is administered as a pharmaceutical composition. In some embodiments, the pharmaceutical composition comprises a therapeutically effective amount of a provided oligonucleotide comprising, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable inactive ingredient selected from pharmaceutically acceptable diluents, pharmaceutically acceptable excipients, and pharmaceutically acceptable carriers. In another embodiment, the pharmaceutical composition is formulated for intravenous injection, oral administration, buccal administration, inhalation, nasal administration, topical administration, ophthalmic administration or otic administration. In further embodiments, the pharmaceutical composition

is a tablet, a pill, a capsule, a liquid, an inhalant, a nasal spray solution, a suppository, a suspension, a gel, a colloid, a dispersion, a suspension, a solution, an emulsion, an ointment, a lotion, an eye drop or an ear drop.

Pharmaceutical compositions

[00740] When used as therapeutics, a provided oligonucleotide or oligonucleotide composition described herein is administered as a pharmaceutical composition. In some embodiments, the pharmaceutical composition comprises a therapeutically effective amount of a provided oligonucleotides, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable inactive ingredient selected from pharmaceutically acceptable diluents, pharmaceutically acceptable excipients, and pharmaceutically acceptable carriers. In some embodiments, the pharmaceutical composition is formulated for intravenous injection, oral administration, buccal administration, inhalation, nasal administration, topical administration, ophthalmic administration or otic administration. In some embodiments, the pharmaceutical composition is a tablet, a pill, a capsule, a liquid, an inhalant, a nasal spray solution, a suppository, a suspension, a gel, a colloid, a dispersion, a suspension, a solution, an emulsion, an ointment, a lotion, an eye drop or an ear drop.

[00741] In some embodiments, the present invention provides a pharmaceutical composition comprising chirally controlled oligonucleotide, or composition thereof, in admixture with a pharmaceutically acceptable excipient. One of skill in the art will recognize that the pharmaceutical compositions include the pharmaceutically acceptable salts of the chirally controlled oligonucleotide, or composition thereof, described above.

[00742] A variety of supramolecular nanocarriers can be used to deliver nucleic acids. Exemplary nanocarriers include, but are not limited to liposomes, cationic polymer complexes and various polymeric. Complexation of nucleic acids with various polycations is another approach for intracellular delivery; this includes use of PEGlyated polycations, polyethyleneamine (PEI) complexes, cationic block co-polymers, and dendrimers. Several cationic nanocarriers, including PEI and polyamidoamine dendrimers help to release contents from endosomes. Other approaches include use of polymeric nanoparticles, polymer micelles, quantum dots and lipoplexes.

[00743] Additional nucleic acid delivery strategies are known in addition to the exemplary delivery strategies described herein.

[00744] In therapeutic and/or diagnostic applications, the compounds of the invention can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remington, The Science and Practice of Pharmacy, (20th ed. 2000).

[00745] Provided oligonucleotides, and compositions thereof, are effective over a wide dosage range. For example, in the treatment of adult humans, dosages from about 0.01 to about 1000 mg, from about 0.5 to about 100 mg, from about 1 to about 50 mg per day, and from about 5 to about 100 mg per day are examples of dosages that may be used. The exact dosage will depend upon the route of administration, the form in which the compound is administered, the subject to be treated, the body weight of the subject to be treated, and the preference and experience of the attending physician.

[00746] Pharmaceutically acceptable salts are generally well known to those of ordinary skill in the art, and may include, by way of example but not limitation, acetate, benzenesulfonate, besylate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, carnsylate, carbonate, citrate, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, mucate, napsylate, nitrate, pamoate (embonate), pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, or teoclate. Other pharmaceutically acceptable salts may be found in, for example, Remington, The Science and Practice of Pharmacy (20th ed. 2000). Preferred pharmaceutically acceptable salts include, for example, acetate, benzoate, bromide, carbonate, citrate, gluconate, hydrobromide, hydrochloride, maleate, mesylate, napsylate, pamoate (embonate), phosphate, salicylate, succinate, sulfate, or tartrate.

[00747] Depending on the specific conditions being treated, such agents may be formulated into liquid or solid dosage forms and administered systemically or locally. The agents may be delivered, for example, in a timed- or sustained- low release form as is known to those skilled in the art. Techniques for formulation and administration may be found in Remington, The Science and Practice of Pharmacy (20th ed. 2000). Suitable routes may include

oral, buccal, by inhalation spray, sublingual, rectal, transdermal, vaginal, transmucosal, nasal or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intra-articullar, intra-sternal, intra-synovial, intra-hepatic, intralesional, intracranial, intraperitoneal, intranasal, or intraocular injections or other modes of delivery.

[00748] For injection, the agents of the invention may be formulated and diluted in aqueous solutions, such as in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[00749] Use of pharmaceutically acceptable inert carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection.

[00750] The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject (e.g., patient) to be treated.

[00751] For nasal or inhalation delivery, the agents of the invention may also be formulated by methods known to those of skill in the art, and may include, for example, but not limited to, examples of solubilizing, diluting, or dispersing substances such as, saline, preservatives, such as benzyl alcohol, absorption promoters, and fluorocarbons.

[00752] Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[00753] In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used

pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions.

[00754] Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethyl-cellulose (CMC), and/or polyvinylpyrrolidone (PVP: povidone). If desired, disintegrating agents may be added, such as the cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[00755] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol (PEG), and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dye-stuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[00756] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin, and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols (PEGs). In addition, stabilizers may be added.

[00757] Depending upon the particular condition, or disease state, to be treated or prevented, additional therapeutic agents, which are normally administered to treat or prevent that condition, may be administered together with oligonucleotides of this invention. For example, chemotherapeutic agents or other anti-proliferative agents may be combined with the oligonucleotides of this invention to treat proliferative diseases and cancer. Examples of known chemotherapeutic agents include, but are not limited to, adriamycin, dexamethasone, vincristine, cyclophosphamide, fluorouracil, topotecan, taxol, interferons, and platinum derivatives.

[00758] The function and advantage of these and other embodiments of the present invention will be more fully understood from the examples described below. The following examples are intended to illustrate the benefits of the present invention, but do not exemplify the full scope of the invention.

[00759] In some embodiments, the present invention provides the following exemplary embodiments:

E1. A chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which composition is a substantially pure preparation of a single oligonucleotide in that at least about 10% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

E2. The composition of example E1, wherein one or more bases are modified.

E3. The composition of example E1, wherein none of the bases are modified.

E4. The composition of any one of the preceding examples, wherein the common base sequence has at least 8 bases.

E5. The composition of any one of the preceding examples, wherein the common base sequence has at least 10 bases.

E6. The composition of any one of the preceding examples, wherein the common base sequence has at least 15 bases.

E7. The composition of any one of the preceding examples, wherein at least about 20% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

E8. The composition of any one of the preceding examples, wherein at least about 50% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

E9. The composition of any one of the preceding examples, wherein at least about 80% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

E10. The composition of any one of the preceding examples, wherein at least about 85% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

E11. The composition of any one of the preceding examples, wherein at least about 90% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

E12. The composition of any one of the preceding examples, wherein at least about 95% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

E13. The composition of any one of the preceding examples, wherein at least about 97% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

E14. The composition of any one of the preceding examples, wherein at least about 98% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

E15. The composition of any one of the preceding examples, wherein at least about 99% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

E16. The composition of any one of the preceding examples, wherein the single oligonucleotide comprises one or more chiral, modified phosphate linkages.

E17. The composition of any one of the preceding examples, wherein the single oligonucleotide has a wing-core-wing structure.

E18. The composition of any one of the preceding examples, wherein each wing optionally contains chiral internucleotidic linkages.

E19. The composition of any one of the preceding examples, wherein the chiral internucleotidic linkages within each wing independently have the same stereochemistry.

E20. The composition of any one of the preceding examples, wherein the chiral internucleotidic linkages of both wings are of the same stereochemistry.

E21. The composition of any one of the preceding examples, wherein the chiral internucleotidic linkages within each wing independently have the same stereochemistry, and the stereochemistry of the first wing is different from that of the second wing.

E22. The composition of any one of the preceding examples, wherein the first wing region independently has a length of one or more bases.

E23. The composition of any one of the preceding examples, wherein the first wing region independently has a length of two or more bases.

E24. The composition of any one of the preceding examples, wherein the first wing region independently has a length of three or more bases.

E25. The composition of any one of the preceding examples, wherein the first wing region independently has a length of four or more bases.

E26. The composition of any one of the preceding examples, wherein the first wing region independently has a length of five or more bases.

E27. The composition of any one of the preceding examples, wherein the first wing region independently has a length of less than eight bases.

E28. The composition of any one of the preceding examples, wherein the second wing region independently has a length of one or more bases.

E29. The composition of any one of the preceding examples, wherein the second wing region independently has a length of two or more bases.

E30. The composition of any one of the preceding examples, wherein the second wing region independently has a length of three or more bases.

E31. The composition of any one of the preceding examples, wherein the second wing region independently has a length of four or more bases.

E32. The composition of any one of the preceding examples, wherein the second wing region independently has a length of five or more bases.

E33. The composition of any one of the preceding examples, wherein the second wing region independently has a length of less than eight bases.

E34. The composition of any one of the preceding examples, wherein the core region has a length of five or more bases.

E36. The composition of any one of the preceding examples, wherein the core region has a length of six or more bases.

E37. The composition of any one of the preceding examples, wherein the core region has a length of seven or more bases.

E38. The composition of any one of the preceding examples, wherein the core region has a

length of eight or more bases.

E39. The composition of any one of the preceding examples, wherein the core region has a length of nine or more bases.

E40. The composition of any one of the preceding examples, wherein the core region has a length of 10 or more bases.

E41. The composition of any one of the preceding examples, wherein the core region has a length of 15 or more bases.

E42. The composition of any one of the preceding examples, wherein the core region has repeating pattern of internucleotidic linkage stereochemistry.

E43. The composition of any one of the preceding examples, wherein the repeating pattern of internucleotidic linkage stereochemistry is (*S*p)m(*R*p)n or (*R*p)n(*S*p)m, wherein each of m and n is independently 1, 2, 3, 4, 5, 6, 7 or 8.

E44. The composition of example E43, wherein m>n.

E45. The composition of example E43 or E44, wherein n is 1.

E46. The composition of any one of the preceding examples, wherein the core region comprises a internucleotidic linkage stereochemistry pattern of (*S*p)m(*R*p)n or (*R*p)n(*S*p)m, wherein each of m and n is independently 2, 3, 4, 5, 6, 7 or 8.

E47. The composition of exampleE 46, wherein m>n.

E48. The composition of example E47, wherein n is 1.

E49. The composition of any one of the preceding examples, wherein 50% percent or more of the chiral internucleotidic linkages of the core region have *S*p configuration.

E50. The composition of any one of the preceding examples, wherein 60% percent or more of the chiral internucleotidic linkages of the core region have *S*p configuration.

E51. The composition of any one of the preceding examples, wherein the core region comprises at least 2 *R*p internucleotidic linkages.

E52. The composition of any one of the preceding examples, wherein the core region comprises at least 3 *R*p internucleotidic linkages.

E53. The composition of any one of the preceding examples, wherein the core region comprises at least 4 *R*p internucleotidic linkages.

E54. The composition of any one of the preceding examples, wherein the core region comprises at least 5 *R*p internucleotidic linkages.

E55. The composition of any one of the preceding examples, wherein at least 5 backbone internucleotidic linkages are chiral.

E56. The composition of any one of the preceding examples, wherein each backbone internucleotidic linkage is chiral.

E57. The composition of any one of examples E1-E55, wherein at least one backbone internucleotidic linkage is a phosphate linkage.

E58. The composition of any one of examples E1-E16, wherein the single oligonucleotide comprises a region in which at least one of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral.

E59. The composition of example E58, wherein at least two of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral.

E60. The composition of any one of examples E58-E59, wherein at least three of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral.

E61. The composition of any one of examples E58-E60, wherein at least four of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral.

E62. The composition of any one of examples E58-E61, wherein at least one internucleotidic linkage in the region is achiral.

E63. The composition of any one of examples E58-E62, wherein at least one internucleotidic linkage in the region is a phosphate linkage.

E64. The composition of any one of examples E58-E63, wherein at least 10% of the internucleotidic linkages in the region are phosphate linkages.

E65. The composition of any one of examples E58-E64, wherein the first internucleotidic linkage is an *S*p modified internucleotidic linkage.

E66. The composition of any one of examples E58-E64, wherein the first internucleotidic linkage is an *R*p modified internucleotidic linkage.

E67. The composition of any one of examples E58-E66, wherein the second internucleotidic linkage is an *S*p modified internucleotidic linkage.

E68. The composition of any one of examples E58-E66, wherein the second internucleotidic linkage is an *R*p modified internucleotidic linkage.

E69. The composition of any one of examples E58-E68, wherein the thrid internucleotidic linkage is an *S*p modified internucleotidic linkage.

E70. The composition of any one of examples E58-E68, wherein the third internucleotidic linkage is an *R*p modified internucleotidic linkage.

E71. The composition of any one of examples E58-E70, wherein the fifth internucleotidic linkage is an *S*p modified internucleotidic linkage.

E72. The composition of any one of examples E58-E70, wherein the fifth internucleotidic linkage is an *R*p modified internucleotidic linkage.

E73. The composition of any one of examples E58-E72, wherein the seventh internucleotidic linkage is an *S*p modified internucleotidic linkage.

E74. The composition of any one of examples E58-E72, wherein the seventh internucleotidic linkage is an *R*p modified internucleotidic linkage.

E75. The composition of any one of examples E58-E74, wherein the eighteenth internucleotidic linkage is an *S*p modified internucleotidic linkage.

E76. The composition of any one of examples E58-E74, wherein the eighteenth internucleotidic linkage is an *R*p modified internucleotidic linkage.

E77. The composition of any one of examples E58-E76, wherein the nineteenth internucleotidic linkage is an *S*p modified internucleotidic linkage.

E78. The composition of any one of examples E58-E76, wherein the nineteenth internucleotidic linkage is an *R*p modified internucleotidic linkage.

E79. The composition of any one of examples E58-E78, wherein the twentieth internucleotidic linkage is an *S*p modified internucleotidic linkage.

E80. The composition of any one of examples E58-E78, wherein the twentieth internucleotidic linkage is an *R*p modified internucleotidic linkage.

E81. The composition of any one of examples E58-E80, wherein the region has a length of 21 bases.

E82. The composition of any one of examples E58-E81, wherein the single oligonucleotide has a length of 21 bases.

E83. The composition of any one of examples E58-E82, wherein the chiral internucleotidic linkage is phosphorothioate.

E84. The composition of any one of the preceding examples, wherein the chiral

internucleotidic linkage has the structure of formula **I**.

E85. The composition of any one of the preceding examples, wherein the single oligonucleotide does not have 2'−OR^1 on a sugar moiety.

E86. The composition of any one of the preceding examples, wherein each sugar moiety does not have 2'-MOE.

E87. The composition of any one of the preceding examples, wherein the single oligonucleotide is not (*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G] or (*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p)-Gs5mCs5mCsTs5mCsAsGsTs5mCsTsGs5mCsTsTs5mCsGs5mCsAs5mCs5mC (5R-(SSR)3-5R), wherein in the underlined nucleotide are 2'-*O*-MOE modified.

E88. The composition of any one of the preceding examples, wherein the single oligonucleotide is not an oligonucleotide selected from:

ONT-106	(*R*p)- uucuAGAccuGuuuuGcuudT**s**dT	PCSK9 sense
ONT-107	(*S*p)- uucuAGAccuGuuuuGcuudT**s**dT	PCSK9 sense
ONT-108	(*R*p)- AAGcAAAAcAGGUCuAGAAdT**s**dT	PCSK9 antisense
ONT-109	(*S*p)- AAGcAAAAcAGGUCuAGAAdT**s**dT	PCSK9 antisense
ONT-110	(*R*p, *R*p)- a**s**AGcAAAAcAGGUCuAGAAdT**s**dT	PCSK9 antisense
ONT-111	(*S*p, *R*p)- a**s**GcAAAAcAGGUCuAGAAdT**s**dT	PCSK9 antisense
ONT-112	(*S*p, *S*p)- a**s**GcAAAAcAGGUCuAGAAdT**s**dT	PCSK9 antisense
ONT-113	(*R*p, *S*p)- a**s**GcAAAAcAGGUCuAGAAdT**s**dT	PCSK9 antisense

wherein lower case letters represent 2'OMe RNA residues; capital letters represent 2'OH RNA residues; and bolded and "**s**" indicates a phosphorothioate moiety; and

PCSK9 (1)	(All (*S*p))- ususcsusAsGsAscscsusGsususususGscsususdTsdT
PCSK9 (2)	(All (*R*p))- ususcsusAsGsAscscsusGsususususGscsususdTsdT
PCSK9 (3)	(All (*S*p))- usucuAsGsAsccuGsuuuuGscuusdTsdT
PCSK9 (4)	(All (*R*p))- usucuAsGsAsccuGsuuuuGscuusdTsdT
PCSK9 (5)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)-ususcsusAsGsAscscsusGsususususGscsususdTsdT

PCSK9 (6)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-ususcsusAsGsAscscsusGsususususGscsususdTsdT

wherein lower case letters represent 2'-OMe RNA residues; capital letters represent RNA residues; d = 2'-deoxy residues; and "s" indicates a phosphorothioate moiety; and

PCSK9 (7)	(All (*R*p))- AsAsGscsAsAsAsAscsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (8)	(All (*S*p))- AsAsGscsAsAsAsAscsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (9)	(All (*R*p))- AsAGcAAAAcsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (10)	(All (*S*p))- AsAGcAAAAcsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (11)	(All (*R*p))- AAsGscsAsAsAsAscAGGUCuAGAAdTsdT
PCSK9 (12)	(All (*S*p))- AAsGscsAsAsAsAscAGGUCuAGAAdTsdT
PCSK9 (13)	(All (*R*p))- AsAsGscAsAsAsAscAsGsGsUsCsuAsGsAsAsdTsdT
PCSK9 (14)	(All (*S*p))- AsAsGscAsAsAsAscAsGsGsUsCsuAsGsAsAsdTsdT
PCSK9 (15)	(All (*R*p))- AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (16)	(All (*S*p))- AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (17)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)-AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (18)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT

wherein lower case letters represent 2'-OMe RNA residues; capital letters represent RNA residues; d = 2'-deoxy residues; "s" indicates a phosphorothioate moiety; and

PCSK9 (19)	(All (*R*p))- UfsusCfsusAfsgsAfscsCfsusGfsusUfsusUfsgsCfsusUfsdTsdT
PCSK9 (20)	(All (*S*p))- UfsusCfsusAfsgsAfscsCfsusGfsusUfsusUfsgsCfsusUfsdTsdT
PCSK9 (21)	(All (*R*p))- UfsuCfsuAfsgAfscCfsuGfsuUfsuUfsgCfsuUfsdTsdT
PCSK9 (22)	(All (*S*p))- UfsuCfsuAfsgAfscCfsuGfsuUfsuUfsgCfsuUfsdTsdT
PCSK9 (23)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)- UfsusCfsusAfsgsAfscsCfsusGfsusUfsusUfsgsCfsusUfsdTsdT
PCSK9 (24)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)- UfsusCfsusAfsgsAfscsCfsusGfsusUfsusUfsgsCfsusUfsdTsdT

wherein lower case letters represent 2'-OMe RNA residues; capital letters represent 2'-F RNA residues; d = 2'-deoxy residues; and "s" indicates a phosphorothioate moiety; and

PCSK9 (25)	(All (*R*p))- asAfsgsCfsasAfsasAfscsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (26)	(All (*S*p))- asAfsgsCfsasAfsasAfscsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (27)	(All (*R*p))- asAfgCfaAfaAfcsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (28)	(All (*S*p))- asAfgCfaAfaAfcsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (29)	(All (*R*p))- asAfsgCfsaAfsaAfscAfsgGfsuCfsuAfsgAfsadTsdT
PCSK9 (30)	(All (*S*p))- asAfsgCfsaAfsaAfscAfsgGfsuCfsuAfsgAfsadTsdT
PCSK9 (31)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)-asAfgCfaAfasAfscAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (32)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-asAfgCfaAfasAfscAfsgsGfsusCfsusAfsgsAfsasdTsdT

wherein lower case letters represent 2'-OMe RNA residues; capital letters represent 2'-F RNA residues; d = 2'-deoxy residues; and "s" indicates a phosphorothioate moiety.

E89. The composition of any one of the preceding examples, wherein at least about 50% of the internucleotidic linkages are in the *S*p configuration.

E90. The composition of any one of the preceding examples, wherein the core portion comprises at least about 5 nucleotides.

E91. The composition of any one of the preceding examples, wherein the core portion comprises at least about 10 nucleotides.

E92. The composition of any one of the preceding examples, wherein the core portion comprises at least about 15 nucleotides.

E93. The composition of any one of the preceding examples, wherein the core portion comprises at least about 20 nucleotides.

E94. The composition of any one of the preceding examples, wherein the core portion comprises at least about 25 nucleotides.

E95. The composition of any one of the preceding examples, wherein (*S*p)m(*R*p)n or (*R*p)n(*S*p)m is SSR.

E96. The composition of any one of examples E1-E95, wherein (*S*p)m(*R*p)n or (*R*p)n(*S*p)m is RRS.

E97. The composition of any one of the preceding examples, wherein a repeating pattern is a motif comprising at least about 20% of backbone chiral centers in the Sp conformation.

E98. The composition of any one of the preceding examples, wherein a repeating pattern is a motif comprising at least about 50% of backbone chiral centers in the Sp conformation.

E99. The composition of any one of the preceding examples, wherein a repeating pattern is a motif comprising at least about 66% of backbone chiral centers in the Sp conformation.

E100. The composition of any one of the preceding examples, wherein a repeating pattern is a motif comprising at least about 75% of backbone chiral centers in the Sp conformation.

E101. The composition of any one of the preceding examples, wherein a repeating pattern is a motif comprising at least about 80% of backbone chiral centers in the Sp conformation.

E102. A chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type.

E103. The composition of example E102, wherein the common base sequence is or comprises a sequence that is complementary to a target sequence, wherein when contacted with a nucleic acid polymer comprising the target sequence, the chirally controlled oligonucleotide composition provides an altered cleavage pattern than a reference cleavage pattern from a reference oligonucleotide composition.

E104. The composition of example E103, wherein the nucleic acid polymer is RNA, and a reference oligonucleotide composition is a substantially racemic preparation of oligonucleotides that share the common sequence and length.

E105. The composition of example E103, wherein the nucleic acid polymer is RNA, and a reference oligonucleotide composition is a chirally uncontrolled oligonucleotide composition of oligonucleotides that share the common sequence and length.

E106. The composition of any one of examples E103-E105, wherein the altered cleavage pattern has fewer cleavage sites than the reference cleavage pattern.

E107. The composition of any one of examples E103-E106, wherein the altered cleavage pattern has only one cleavage site within the target sequence, and the reference cleavage pattern has two or more cleavage sites within the target sequence.

E108. The composition of example E102, wherein the common base sequence for the oligonucleotides of the single oligonucleotide type is or comprises a sequence that is complementary to a characteristic sequence element that defines a particular allele of a target gene relative to other alleles of the same target gene that exist in a population, the composition being characterized in that, when it is contacted with a system expressing transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

E109. The composition of example E102, wherein the common base sequence for the oligonucleotides of the single oligonucleotide type is or comprises a sequence that is complementary to a characteristic sequence element that defines a particular allele of a target gene relative to other alleles of the same target gene that exist in a population, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

E110. The composition of any one of examples E102-E109, wherein oligonucleotides of the particular oligonucleotide type comprise a modified base.

E111. The composition of any one of examples E102-E110, wherein oligonucleotides of the particular oligonucleotide type comprise a modified sugar.

E112. The composition of example E111, wherein the modified sugar comprises a 2'-modification.

E113. The composition of example E112, wherein the 2'-modification is 2'-OR^1.

E114. The composition of example E113, wherein the 2’-modification is 2’-MOE.

E115. The composition of any one of examples E102-E109, wherein oligonucleotides of the particular oligonucleotide type have no modified base or modified sugar.

E116. The composition of any one of examples E102-E115, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises (*S*p)m(*R*p)n, wherein m is 2, 3, 4, 5, 6, 7 or 8 and n is 1, 2, 3, 4, 5, 6, 7 or 8.

E117. The composition of any one of examples E102-E116, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises (*S*p)m*R*p, wherein m is 2, 3, 4, 5, 6, 7 or 8.

E118. The composition of any one of examples E102-E117, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises $(Sp)_2Rp$.

E119. The composition of any one of examples E102-E118, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises repeating (*S*p)m(*R*p)n, wherein m is 2, 3, 4, 5, 6, 7 or 8 and n is 1, 2, 3, 4, 5, 6, 7 or 8.

E120. The composition of any one of examples E102-E119, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises repeating (*S*p)m*R*p, wherein m is 2, 3, 4, 5, 6, 7 or 8.

E121. The composition of any one of examples E102-E120, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises repeating $(Sp)_2Rp$.

E122. The composition of any one of examples E102-E115, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises (*R*p)n(*S*p)m, wherein m is 2, 3, 4, 5, 6, 7 or 8 and n is 1, 2, 3, 4, 5, 6, 7 or 8.

E123. The composition of any one of examples E102-E115, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises *R*p(*S*p)m, wherein m is 2, 3, 4, 5, 6, 7 or 8.

E124. The composition of any one of examples E102-E115, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises $Rp(Sp)_2$.

E125. The composition of any one of examples E102-E115, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises repeating (*R*p)n(*S*p)m, wherein m is 2, 3, 4, 5, 6, 7 or 8 and n is 1, 2, 3, 4, 5, 6, 7 or 8.

E126. The composition of any one of examples E102-E115, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises repeating *R*p(*S*p)m, wherein m is 2, 3, 4, 5, 6, 7 or 8.

E127. The composition of any one of examples E102-E115, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises repeating *R*p(*S*p)$_2$.

E128. The composition of any one of examples E102-E115, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises (*N*p)t(*R*p)n(*S*p)m, wherein each n and t is independently 1, 2, 3, 4, 5, 6, 7 or 8, m is 2, 3, 4, 5, 6, 7 or 8, and each *N*p is independent *R*p or *S*p.

E129. The composition of any one of examples E102-E115, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises (*S*p)t(*R*p)n(*S*p)m, wherein each n and t is independently 1, 2, 3, 4, 5, 6, 7 or 8 and m is 2, 3, 4, 5, 6, 7 or 8.

E130. The composition of example E128 or E129, wherein n is 1.

E131. The composition of any one of examples E128-E130, wherein t is 2, 3, 4, 5, 6, 7 or 8.

E132. The composition of any one of examples E128-E131, wherein m is 2, 3, 4, 5, 6, 7 or 8.

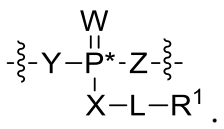
E133. The composition of any one of examples E128-E131, wherein at least one of t and m is greater than 5.

E134. The composition of any one of examples E102-E115, wherein the pattern of backbone chiral centers of the particular oligonucleotide type comprises *S*p*S*p*R*p*S*p*S*p.

E135. The composition of any one of examples E102-E134, wherein oligonucleotides of the particularly oligonucleotide type has only one *R*p, and each of the other internucleotidic linkages is *S*p.

E136. The composition of any one of examples E102-E135, wherein the common base length of the particular oligonucleotide type is greater than 10.

E137. The composition of any one of examples E102-E136, wherein each chiral internucleotidic linkage in oligonucleotides of the particular oligonucleotide type has a structure of formula **I**:



(I)

E138. The composition of example E137, wherein X is S, and Y and Z are O.

E139. The composition of example E137 or E138, wherein −L−R^1 is not −H.

E140. The composition of example E137 or E138, wherein a structure of formula **I** is a phosphorothioate diester linkage.

E141. The composition of any one of examples E102-E140, wherein oligonucleotides of the particular oligonucleotide type are antisense oligonucleotide, antagomir, microRNA, pre-microRNs, antimir, supermir, ribozyme, Ul adaptor, RNA activator, RNAi agent, decoy oligonucleotide, triplex forming oligonucleotide, aptamer or adjuvant.

E142. A method for controlled cleavage of a nucleic acid polymer, the method comprising steps of:

contacting a nucleic acid polymer whose nucleotide sequence comprises a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a target sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type.

E143. The method of example E142, wherein the contacting being performed under conditions so that cleavage of the nucleic acid polymer occurs.

E144. The method of any one of examples E142-E143, wherein the cleavage occurs with a cleavage pattern that differs from a reference cleavage pattern observed when the nucleic acid polymer is contacted under comparable conditions with a reference oligonucleotide composition.

E145. A method for altering a cleavage pattern observed when a nucleic acid polymer whose nucleotide sequence includes a target sequence is contacted with a reference oligonucleotide composition that comprises oligonucleotides having a particular base sequence and length, which particular base sequence is or comprises a sequence that is complementary to the target sequence, the method comprising:

contacting the nucleic acid polymer with a chirally controlled oligonucleotide composition of oligonucleotides having the particular base sequence and length, which

composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of a single oligonucleotide type characterized by:

1) the particular base sequence and length;

2) a particular pattern of backbone linkages; and

3) a particular pattern of backbone chiral centers.

E146. The method of example E145, wherein the contacting being performed under conditions so that cleavage of the nucleic acid polymer occurs.

E147. The method of any one of examples E144-E146, wherein the reference oligonucleotide composition is a substantially racemic preparation of oligonucleotides that share the common sequence and length.

E148. The method of any one of examples E144-E146, wherein the reference oligonucleotide composition is a chirally uncontrolled oligonucleotide composition of oligonucleotides that share the common sequence and length.

E149. The method of any one of examples E144-E148, wherein the cleavage pattern provided by the chirally controlled oligonucleotide composition differs from a reference cleavage pattern in that it has fewer cleavage sites within the target sequence found in the nucleic acid polymer than the reference cleavage pattern.

E150. The method of example E149, wherein the cleavage pattern provided by the chirally controlled oligonucleotide composition has a single cleavage site within the target sequence found in the nucleic acid polymer than the reference cleavage pattern.

E151. The method of example E150, wherein the single cleavage site is a cleavage site in the reference cleavage pattern.

E152. The method of example E150, wherein the single cleavage site is a cleavage site not in the reference cleavage pattern.

E153. The method of any one of examples E144-E148, wherein the cleavage pattern provided by the chirally controlled oligonucleotide composition differs from a reference cleavage pattern in that it increases cleavage percentage at a cleavage site.

E154. The method of example E153, wherein the cleavage site with increased cleavage percentage is a cleavage site in the reference cleavage pattern.

E155. The method of example E153, wherein the cleavage site with increased cleavage percentage is a cleavage site not in the reference cleavage pattern.

E156. The method of any one of examples E142-E155, wherein the chirally controlled oligonucleotide composition provides a higher cleavage rate of the target nucleic acid polymer than a reference oligonucleotide composition.

E157. The method of any one of examples E142-E156, where the cleavage rate is at least 5 fold higher.

E158. The method of any one of examples E142-E157, wherein the chirally controlled oligonucleotide composition provides a lower level of remaining un-cleaved target nucleic acid polymer than a reference oligonucleotide composition.

E159. The method of any one of examples E142-E158, wherein the remaining un-cleaved target nucleic acid polymer is at least 5 fold lower.

E160. The methods of any one of examples E142-E159, wherein the cleavage products from the nucleic acid polymer dissociate from oligonucleotides of the particular oligonucleotide type in the chirally controlled oligonucleotide composition at a faster rate than from oligonucleotides of the reference oligonucleotide composition.

E161. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that

defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

E162. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

E163. The method of example E161 or E162, the contacting being performed under conditions determined to permit the composition to suppress transcripts of the particular allele.

E164. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

E165. The method of example E164, wherein the contacting being performed under conditions determined to permit the composition to suppress expression of the particular allele.

E166. The method of any one of examples E161-E165, wherein transcripts of the particular allele are suppressed at a level at least 5, 10, 20, 50, 100, 200 or 500 fold greater than a level of suppression observed for another allele of the same gene.

E167. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

E168. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

E169. The method of example E167 or E168, the contacting being performed under conditions determined to permit the composition to suppress transcripts of the particular allele.

E170. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

E171. The method of example E170, wherein the contacting being performed under conditions determined to permit the composition to suppress expression of the particular allele.

E172. The method of any one of examples E167-E171, wherein transcripts of the particular allele are suppressed at a level that is at least 5, 10, 20, 50, 100, 200 or 500 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent.

E173. The method of any one of examples E167-E172, wherein transcripts of the particular allele are suppressed at a level that is at least 5, 10, 20, 50, 100, 200 or 500 fold greater than a level of suppression observed for another allele of the same gene.

E174. The method of any one of example E161-E173, wherein the system is an *in vitro* or *in vivo* system.

E175. The method of any one of example E161-E174, wherein the system comprises one or more cells, tissues or organs.

E176. The method of any one of example E161-E174, wherein the system comprises one or more organisms.

E177. The method of any one of example E161-E174, wherein the system comprises one or more subjects.

E178. The method of any one of examples E161-E177, wherein transcripts of the particular allele are cleaved.

E179. The method of any one of examples E161-E178, wherein the specific nucleotide characteristic sequence element is present within an intron of the target nucleic acid sequence or gene.

E180. The method of any one of examples E161-E178, wherein the specific nucleotide characteristic sequence element is present within an exon of the target nucleic acid sequence or gene.

E181. The method of any one of examples E161-E178, wherein the specific nucleotide characteristic sequence element spans an exon and an intron of the target nucleic acid sequence or gene.

E182. The method of any one of examples E161-E181, wherein the specific nucleotide characteristic sequence element comprises a mutation.

E183. The method of any one of examples E161-E181, wherein the specific nucleotide characteristic sequence element comprises a SNP.

E184. The method of any one of example E142-E183, wherein the chirally controlled oligonucleotide composition is administered to a subject.

E185. The method of any one of example E142-E184, wherein the target nucleic acid polymer or transcripts are oligonucleotides.

E186. The method of any one of example E142-E185, wherein the target nucleic acid polymer or transcripts are RNA.

E187. The method of any one of example E142-E186, wherein oligonucleotides of the particular oligonucleotide type in the chirally controlled oligonucleotide composition form duplexes with the nucleic acid polymer or transcripts.

E188. The method of any one of example E142-E187, wherein the nucleic acid polymer or transcripts are cleaved by an enzyme.

E189. The method of any one of example E142-E188, wherein the enzyme is RNase H.

E190. The method of any one of example E142-E188, wherein the enzyme is an Argonaute protein or within the RNA-induced silencing complex (RISC).

E191. The composition of any one of examples E102-E141, wherein the oligonucleotide type is not (*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G] or (*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p)-Gs5mCs5mCsTs5mCsAsGsTs5mCsTsGs5mCsTsTs5mCsGs5mCsAs5mCs5mC (5R-(SSR)3-5R), wherein in the underlined nucleotide are 2'-*O*-MOE modified.

E192. The composition of any one of examples E102-E141, wherein the oligonucleotide has a wing-core-wing motif, wherein the pattern of backbone chiral centers of the core region comprises (*N*p)t(*R*p)n(*S*p)m, wherein each n and t is independently 1, 2, 3, 4, 5, 6, 7 or 8, m is 2, 3, 4, 5, 6, 7 or 8, and each *N*p is independent *R*p or *S*p.

E193. The composition of any one of examples E102-E141, wherein the oligonucleotide has a wing-core-wing motif, wherein the pattern of backbone chiral centers of the core region

comprises (*S*p)t(*R*p)n(*S*p)m, wherein each n and t is independently 1, 2, 3, 4, 5, 6, 7 or 8, and m is 2, 3, 4, 5, 6, 7 or 8.

E194. The composition of any one of examples E192 or E193, wherein n is 1.

E195. The composition of any one of examples E192-E194, wherein m is 2.

E196. The composition of any one of examples E192-E194, wherein m is 3, 4, 5, 6, 7 or 8.

E197. The composition of any one of examples E192-E194, wherein m is 4, 5, 6, 7 or 8.

E198. The composition of any one of examples E192-E194, wherein m is 5, 6, 7, or 8.

E199. The composition of any one of examples E192-E194, wherein m is 6, 7 or 8.

E200. The composition of any one of examples E192-E194, wherein m is 7 or 8.

E201. The composition of any one of examples E192-E194, wherein m is 8.

E202. The composition of any one of examples E192-E201, wherein t is 1.

E203. The composition of any one of examples E192-E201, wherein t is 2, 3, 4, 5, 6, 7 or 8.

E204. The composition of any one of examples E192-E201, wherein t is 3, 4, 5, 6, 7 or 8.

E205. The composition of any one of examples E192-E201, wherein t is 4, 5, 6, 7 or 8.

E206. The composition of any one of examples E192-E201, wherein t is 5, 6, 7 or 8.

E207. The composition of any one of examples E192-E201, wherein t is 6, 7 or 8.

E208. The composition of any one of examples E192-E201, wherein t is 7 or 8.

E209. The composition of any one of examples E192-E201, wherein t is 8.

E210. The composition of any one of examples E192-E209, wherein each wing region independently has a length of two or more bases.

E211. The composition of any one of examples E192-E210, wherein all internucleotidic linkages are chiral, modified phosphate linkages.

E212. The composition of any one of examples E192-E211, wherein the core region has a length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or more bases.

E213. The composition of any one of examples E102-E141 and E192-E212, wherein the common base sequence has at least 18 bases.

E214. The composition of any one of examples E102-E141 and E192-E212, wherein the common base sequence has at least 19 bases.

E215. The composition of any one of examples E102-E141 and E192-E212, wherein the common base sequence has at least 19 bases.

E216. The composition of any one of examples E102-E141 and E192-E212, wherein the common base sequence has at least 19 bases.

E217. The composition of any one of examples E102-E141 and E192-E212, wherein the common base sequence has at least 19 bases.

E218. The composition of any one of examples E102-E141 and E192-E212, wherein the common base sequence has at least 19 bases.

E219. The composition of any one of examples E102-E141 and E192-E212, wherein the common base sequence has at least 19 bases.

E220. The composition of any one of examples E102-E141 and E192-E212, wherein the common base sequence has at least 19 bases.

E221. The composition of any one of examples E102-E141 and E192-E220, wherein the oligonucleotide is not an oligonucleotide selected from: (*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G] or (*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p)-Gs5mCs5mCsTs5mCsAsGsTs5mCsTsGs5mCsTsTs5mCsGs5mCsAs5mCs5mC (5R-(SSR)3-5R), wherein in the underlined nucleotide are 2'-*O*-MOE modified.

E222. The composition of any one of examples E102-E141 and E192-E221, wherein the oligonucleotide is not an oligonucleotide selected from:

ONT-106	(*R*p)- uucuAGAccuGuuuuGcuudT***s***dT	PCSK9 sense
ONT-107	(*S*p)- uucuAGAccuGuuuuGcuudT***s***dT	PCSK9 sense
ONT-108	(*R*p)- AAGcAAAAcAGGUCuAGAAdT**s**dT	PCSK9 antisense
ONT-109	(*S*p)- AAGcAAAAcAGGUCuAGAAdT***s***dT	PCSK9 antisense
ONT-110	(*R*p, *R*p)- a***s***AGcAAAAcAGGUCuAGAAdT***s***dT	PCSK9 antisense
ONT-111	(*S*p, *R*p)- a***s***GcAAAAcAGGUCuAGAAdT***s***dT	PCSK9 antisense
ONT-112	(*S*p, *S*p)- a***s***GcAAAAcAGGUCuAGAAdT***s***dT	PCSK9 antisense
ONT-113	(*R*p, *S*p)- a***s***GcAAAAcAGGUCuAGAAdT***s***dT	PCSK9 antisense

wherein lower case letters represent 2'OMe RNA residues; capital letters represent 2'OH RNA residues; and bolded and "***s***" indicates a phosphorothioate moiety; and

PCSK9 (1)	(All (*S*p))- ususcsusAsGsAscscsusGsususususGscsususdTsdT
PCSK9 (2)	(All (*R*p))- ususcsusAsGsAscscsusGsususususGscsususdTsdT

PCSK9 (3)	(All (*S*p))- usucuAsGsAsccuGsuuuuGscuusdTsdT
PCSK9 (4)	(All (*R*p))- usucuAsGsAsccuGsuuuuGscuusdTsdT
PCSK9 (5)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)-ususcsusAsGsAscscsusGsusususususGscsususdTsdT
PCSK9 (6)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-ususcsusAsGsAscscsusGsusususususGscsususdTsdT

wherein lower case letters represent 2'-OMe RNA residues; capital letters represent RNA residues; d = 2'-deoxy residues; and "s" indicates a phosphorothioate moiety; and

PCSK9 (7)	(All (*R*p))- AsAsGscsAsAsAsAscsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (8)	(All (*S*p))- AsAsGscsAsAsAsAscsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (9)	(All (*R*p))- AsAGcAAAAcsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (10)	(All (*S*p))- AsAGcAAAAcsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (11)	(All (*R*p))- AAsGscsAsAsAsAscAGGUCuAGAAdTsdT
PCSK9 (12)	(All (*S*p))- AAsGscsAsAsAsAscAGGUCuAGAAdTsdT
PCSK9 (13)	(All (*R*p))- AsAsGscAsAsAsAscAsGsGsUsCsuAsGsAsAsdTsdT
PCSK9 (14)	(All (*S*p))- AsAsGscAsAsAsAscAsGsGsUsCsuAsGsAsAsdTsdT
PCSK9 (15)	(All (*R*p))- AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (16)	(All (*S*p))- AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (17)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)-AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (18)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT

wherein lower case letters represent 2'-OMe RNA residues; capital letters represent RNA residues; d = 2'-deoxy residues; "s" indicates a phosphorothioate moiety; and

PCSK9 (19)	(All (*R*p))- UfsusCfsusAfsgsAfscsCfsusGfsusUfsusUfsgsCfsusUfsdTsdT
PCSK9 (20)	(All (*S*p))- UfsusCfsusAfsgsAfscsCfsusGfsusUfsusUfsgsCfsusUfsdTsdT
PCSK9 (21)	(All (*R*p))- UfsuCfsuAfsgAfscCfsuGfsuUfsuUfsgCfsuUfsdTsdT
PCSK9 (22)	(All (*S*p))- UfsuCfsuAfsgAfscCfsuGfsuUfsuUfsgCfsuUfsdTsdT
PCSK9 (23)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p,

	*S*p)- UfsusCfsusAfsgsAfscsCfsusGfsusUfsusUfsgsCfsusUfsdTsdT
PCSK9 (24)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)- UfsusCfsusAfsgsAfscsCfsusGfsusUfsusUfsgsCfsusUfsdTsdT

wherein lower case letters represent 2'-OMe RNA residues; capital letters represent 2'-F RNA residues; d = 2'-deoxy residues; and "s" indicates a phosphorothioate moiety; and

PCSK9 (25)	(All (*R*p))- asAfsgsCfsasAfsasAfscsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (26)	(All (*S*p))- asAfsgsCfsasAfsasAfscsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (27)	(All (*R*p))- asAfgCfaAfaAfcsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (28)	(All (*S*p))- asAfgCfaAfaAfcsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (29)	(All (*R*p))- asAfsgCfsaAfsaAfscAfsgGfsuCfsuAfsgAfsadTsdT
PCSK9 (30)	(All (*S*p))- asAfsgCfsaAfsaAfscAfsgGfsuCfsuAfsgAfsadTsdT
PCSK9 (31)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)- asAfgCfaAfasAfscAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (32)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)- asAfgCfaAfasAfscAfsgsGfsusCfsusAfsgsAfsasdTsdT

E223. The composition of any one of examples E1-E141 and E192-E222, wherein the oligonucleotide is not an oligonucleotide selected from:

$d[A_RC_SA_RC_SA_RC_SA_RC_SA_RC]$, $d[C_SC_SC_SC_RC_RC_SC_SC_SC_SC]$, $d[C_SC_SC_SC_SC_SC_SC_RC_RC_SC]$ and $d[C_SC_SC_SC_SC_SC_RC_RC_SC_SC]$, wherein R is *R*p phosphorothioate linkage, and S is *S*p phosphorothioate linkage.

E224. The composition of any one of examples E1-E141 and E192-E223, wherein the oligonucleotide is not an oligonucleotide selected from: $GGA_RT_SG_RT_ST_R{}^mC_STCGA$, $GGA_RT_RG_ST_ST_R{}^mC_RTCGA$, $GGA_ST_SG_RT_RT_S{}^mC_STCGA$, wherein R is *R*p phosphorothioate linkage, S is *S*p phosphorothioate linkage, all other linkages are PO, and each ^mC is a 5-methyl cytosine modified nucleoside.

E225. The composition of any one of examples E1-E141 and E192-E224, wherein the oligonucleotide is not an oligonucleotide selected from: $T_kT_k{}^mC_kAGT^mCATGA^mCT_kT^mC_k{}^mC_k$, wherein each nucleoside followed by a subscript 'k' indicates a (S)-cEt modification, R is *R*p phosphorothioate linkage, S is *S*p phosphorothioate linkage, each ^mC is a 5-methyl cytosine modified nucleoside, and all internucleoside linkages are phosphorothioates (PS) with stereochemistry patterns selected from RSSSRSRRRS, RSSSSSSSSS, SRRSRSSSSR,

SRSRSSRSSR, RRRSSSRSSS, RRRSRSSRSR, RRSSSRSRSR, SRSSSRSSSS, SSRRSSRSRS, SSSSSSRRSS, RRRSSRRRSR, RRRRSSSSRS, SRRSRRRRRR, RSSRSSRRRR, RSRRSRRSRR, RRSRSSRSRS, SSRRRRRSRR, RSRRSRSSSR, RRSSRSRRRR, RRSRSRRSSS, RRSRSSSRRR, RSRRRRSRSR, SSRSSSRRRS, RSSRSRSRSR, RSRSRSSRSS, RRRSSRRSRS, SRRSSRRSRS, RRRRSRSRRR, SSSSRRRRSR, RRRRRRRRRR and SSSSSSSSSS.

E226. The composition of any one of examples E1-E141 and E192-E225, wherein the oligonucleotide is not an oligonucleotide selected from: $T_kT_k{}^mC_kAGT^mCATGA^mCTT_k{}^mC_k{}^mC_k$, wherein each nucleoside followed by a subscript 'k' indicates a (S)-cEt modification, R is *R*p phosphorothioate linkage, S is *S*p phosphorothioate linkage, each ^mC is a 5-methyl cytosine modified nucleoside and all internucleoside linkages are phosphorothioates (PS) with stereochemistry patterns selected from: RSSSRSRRRS, RSSSSSSSSS, SRRSRSSSSR, SRSRSSRSSR, RRRSSSRSSS, RRRSRSSRSR, RRSSSRSRSR, SRSSSRSSSS, SSRRSSRSRS, SSSSSSRRSS, RRRSSRRRSR, RRRRSSSSRS, SRRSRRRRRR, RSSRSSRRRR, RSRRSRRSRR, RRSRSSRSRS, SSRRRRRSRR, RSRRSRSSSR, RRSSRSRRRR, RRSRSRRSSS, RRSRSSSRRR, RSRRRRSRSR, SSRSSSRRRS, RSSRSRSRSR, RSRSRSSRSS, RRRSSRRSRS, SRRSSRRSRS, RRRRSRSRRR, SSSSRRRRSR, RRRRRRRRRR and SSSSSSSSSS.

E227. The composition of any one of examples E1-E141 and E192-E226, wherein the composition comprises a predetermined level of oligonucleotides of one oligonucleotide type characterized by a common base sequence, a common pattern of backbone linkages, a common pattern of backbone chiral centers, and a common pattern of backbone phosphorus modifications.

E228. The composition of any one of claims E1-E141 and E192-E226, wherein the common pattern of backbone chiral centers comprises at least about 20% of backbone chiral centers in the Sp conformation.

E229. The composition of any one of claims E1-E141 and E192-E226, wherein the common pattern of backbone chiral centers comprises at least about 50% of backbone chiral centers in the Sp conformation.

E230. The composition of any one of claims E1-E141 and E192-E226, wherein the common pattern of backbone chiral centers comprises at least about 66% of backbone chiral centers in the Sp conformation.

E231. The composition of any one of claims E1-E141 and E192-E226, wherein the common pattern of backbone chiral centers comprises at least about 75% of backbone chiral centers in the Sp conformation.

E232. The composition of any one of claims E1-E141 and E192-E226, wherein the common pattern of backbone chiral centers comprises at least about 80% of backbone chiral centers in the Sp conformation.

E233. A pharmaceutical composition, comprising a composition of any one of claims E1-E141 and E191-E232.

E234. The method of any one of examples E142-E190, wherein the chirally controlled oligonucleotide composition is a composition of any one of examples E1-E101.

E235. The method of any one of examples E142-E190, wherein the chirally controlled oligonucleotide composition is a composition of any one of examples E102-E141 and E191.

E236. The method of any one of examples E142-E190, wherein the chirally controlled oligonucleotide composition is a composition of any one of examples E102-E141 and E191-E233.

Exemplification

[00760] The foregoing has been a description of certain non–limiting embodiments of the invention. Accordingly, it is to be understood that the embodiments of the invention herein described are merely illustrative of the application of the principles of the invention. Reference herein to details of the illustrated embodiments is not intended to limit the scope of the claims.

Example 1. In Vitro Metabolic Stabilities of human Chiromersens in preincubated rat whole liver homogenates

[00761] The present Example describes comparisons of *in vitro* whole rat liver homogenate stability of Mipomersen (stereochemical mixture) with chirally controlled oligonucleotide compositions of Mipomersen (“chiromersens”). The method, among other things, is useful in screening compounds to predict *in vivo* half lives.

[00762] As is known in the art, Mipomersen (previously ISIS 301012, sold under the trade name Kynamro) is a 20mer oligonucleotide whose base sequence is antisense to a portion of the

apolipoprotein B gene. Mipomersen inhibits apolipoprotein B gene expression, presumably by targetging mRNA. Mipomersen has the following structure:

G*-C*-C*-U*-C*-dA-dG-dT-dC-dT-dG-dmC-dT-dT-dmC-G*-C*-A*-C*-C*

[d = 2'-deoxy, * = 2'-*O*-(2-methoxyethyl)]

with 3'→5' phosphorothioate linkages. Thus, Mipomersen has 2'-O-methoxyethyl-modified ribose residues at both ends, and deoxyribose residues in the middle.

[00763] Tested chirally pure Mipomersen analogs described in this Example included 3'→5' phosphorothioate linkages. In some embodiments, tested analogs include one or more 2'-O-(2-methoxyethyl)-modified residues; in some embodiments, tested analogs include only 2'-deoxy residues. Particular tested analogs had the structures set forth below in Tables 3 and 4.

[00764] **Protocol:** We used the protocol reported by Geary et al. (Oligonucleotides, Volume 20, Number 6, 2010) with some modifications.

[00765] **Test system:** Six male Sprague-Dawley rats (Rattus norvegicus) were supplied by Charles River Laboratories, Inc., (Hollister, CA), and were received at SNBL USA.

[00766] **Tissue Collection:** Animals were acclimated to the study room for two days prior to tissue collection. At the time of tissue collection, animals were anesthetized with an intraperitoneal (IP) injection of sodium pentobarbital solution. Liver perfusion was performed using 500 mL of chilled saline/animal, administered via the hepatic portal vein. After perfusion, the livers were dissected and maintained on ice. Livers were minced into small pieces then weighed.

[00767] **Liver Homogenate Preparation:** The minced pieces of liver tissues were transferred to tared 50 mL centrifuge tubes and weighed. Chilled homogenization buffer (100 mM Tris pH 8.0, 1 mM magnesium acetate, with antibiotic-antimycotic agents) was added to each tube, such that the tube(s) contained 5 mL of buffer per gram of tissue. Using a QIAGEN TissueRuptor tissue homogenizer, the liver/buffer mixture was homogenized while maintaining the tube on ice. The protein concentration of the liver homogenate pool was determined using a Pierce BCA protein assay. Liver homogenates were divided into 5 mL aliquots, transferred to appropriately sized labeled cryovials and stored at -60 °C.

[00768] **Incubation Conditions:** 5ml aliquots of frozen liver homogenate (protein concentration = 22.48mg/ml) were thawed and incubated at 37 ºC for 24 hrs. Six eppendorf tubes

(2ml) were taken for each oligomer in table 1and 450ul of homogenate was added in each tube. 50ul ASO (200uM) was added to each tube. Immediately after mixing, 125ul of (5X) stop buffer (2.5% IGEPAL, 0.5M NaCl, 5mM EDTA, 50mM Tris, pH=8.0) and 12.5 ul of 20 mg/ml Proteinase K (Ambion, # AM2546) was added to one tube for 0 hour time point. The remaining reaction mixtures were incubated at 37 ºC with shaking at 400 rpm on VWR Incubating Microplate shaker. After incubation for a designated period (1, 2, 3, 4, and 5 days), each mixture was treated with 125ul of (5X) stop buffer (2.5% IGEPAL, 0.5M NaCl, 5mM EDTA, 50mM Tris, pH=8.0) and 12.5ul of 20mg/ml Proteinase K (Ambion, # AM2546).

[00769] Work up and Bioanalysis: ISIS 355868 (5'-GCGTTTGCTCTTCTTCTTGCGTTTTTT-3'), a 27-mer oligonucleotide (underlined bases are MOE modified) was used as the internal standard for quantitation of chiromersens. 50 ul of internal standard (200uM) was added to each tube followed by addition of 250 ul of 30% ammonium hydroxide, 800 ul of Phenol: Chloroform: isoamyl alcohol (25:24:1). After mixing and centrifugation at 600 rpg, the aqueous layer was evaporated on speed vac to 100 ul and loaded on Sep Pak column (C18, 1g, WAT 036905). All the aqueous washings (2x20ml) of Sep pak column were tested with quick Ion Exchange method to ensure that no product was found there. 50% ACN (3.5ml) was used to elute the oligonucleotide and metabolites and the column was further washed with 70% CAN (3.5) ensure that there was nothing left on the column. Five fractions were collected for each sequence. Water wash1, 2, 3, ACN1 and 2 using Visiprep system (Sigma, part number: 57031-U).

Ion Exchange method

	Time	Flow (ml/min)	%A	%B	Curve
	Time	1.0	95	5	
1	2	1.0	95	5	1
2	3	1.0	75	25	6
3	10	1.0	35	65	6
4	10.1	1.0	95	5	6
5	12.5	1.0	95	5	1

Buffer A = 10mM Tris HCl, 50%ACN, pH=8.0

Buffer B = A+800mM NaClO4

Column = DNA pac 100

Column Temperature 60°C

Wash method was used after each run (Described in M9-Exp21) using the same buffers as above and 50:50 (methanol:water) in buffer line C.

	Time	Flow (ml/min)	%A	%B	%C	Curve
	Time	1.0	0	0	100	
1	5.5	1.0	0	0	100	1
2	5.6	1.0	100	0	0	6
3	7.5	1.0	100	0	0	6
4	7.6	1.0	95	5	0	6
5	12.5	1.0	95	5	0	1

Acetonitrile eluate was concentrated to dryness and dissolved in 100ul water to be analyzed using RPHPIPC.

Eluant A = 10mM Tributylammonium acetate, pH=7.0

Eluant B= ACN (HPLC grade, B& J)

Coulmn : XTerra MS C18, 3.5um, 4.6x50mm, Part number: 186000432

Guard column from Phenomenex, part number: KJ0-4282

Column Temperature = 60°C

HPLC Gradient:

	Time	Flow (ml/min)	%A	%B	Curve
1		1.0	65	35	
2	5.0	1.0	65	35	1
3	30.0	1.0	40	60	6
4	35.0	1.0	5	90	6
5	36.0	1.0	65	35	6
6	40.0	1.0	65	35	1

For Analytical RP HPLC, 10 ul of this stock solution was added to 40 ul water and 40 ul was injected.

Table 3.

S.NO.	Sequence	Description
ONT-41	Gs5mCs5mCs Ts5mCsAs GsTs5mCs TsGs5mCs TsTs5mCs Gs5mCsAs 5mCs5mC	Mipomersen
ONT-87	Gs5mCs5mCsTs5mCsAsGsTs5mCsTsGs5mCsTsTs5mCsGs5mCsAs5mCs5mC	MOE-wing-core-wing design – (human) RNAse H substrate 1 5R-(SSR)$_3$-5R
ONT-154	Gs5mCs5mCsTs5mCsAsGsTs5mCsTsGs5mCsTsTs5mCsGs5mCsAs5mCs5mC	All deoxy, (5S-(SSR)$_3$-5S)
ONT-70	Gs5mCsGsTsTsTsGs5mCsTs5mCsTsTs5mCsTsTs5mCsTsTsGs5mCGsTsTsTsTsTsT	ISIS 355868 internal standard for quantitation of Mipomersen

[00770] **Discussion:** 2′ modifications in antisense and siRNAs are predicted to stabilize these molecules and increase their the persistence in plasma and tissues compared with wild-type DNAs and siRNAs.

[00771] 2'-MOE wing-core-wing design in Mipomersen. The first generation antisense oligonucleotides employed in the first antisense clinical trials had 2'-deoxy ribonucleotide residues and phosphorothioate internucleoside linkages. Subsequently, second generation antisense oligonucleotides were developed, which were typically of what is referred to herein as "5-10-5 2'-MOE wing-core-wing design", in that five (5) residues at each end were 2'-O-methoxyethyl (2'-MOE)-modified residues and ten (10) residues in the middle were 2'-deoxy ribonucleotides; the internucleotide linkages of such oligonucleotides were phosphorothioates. Such "5-10-5 2'-MOE wing-core-wing" oligonucleotides exhibited marked improvement in potency over first generation (PCT/US2005/033837). Similar wing-core-wing motifs like 2-16-2, 3-14-3, 4-12-4, or 5-10-5 were designed to improve the stability of oligonucleotides to nucleases, while at the same time maintaining enough DNA structure for RNase activity.

[00772] Chirally pure oligonucleotides. The present invention provides chirally pure oligonucleotides and demonstrates, among other things, that selection of stereochemistry in and of itself can improve oligonucleotide stability (i.e., independent of residue modification such as 2'MOE modification). Indeed, the present invention demonstrates that chirally pure phosphorothioate oligonucleotides can provide same or better stability than corresponding 2'-modified stereorandom phosphorothioate compounds.

[00773] In some embodiments, tested chirally pure oligonucleotides are of the general structure X-Y-X with respect to stereochemistry in that they contain wing "X" regions (typically about 1-10 residues long) where all residues have the same stereochemistry flanking a core "Y" region in which stereochemistry varies. In many embodiments, about 20-50% of the nucleotide analogs in tested such oligonucleotides are not substrates for RNase H. The ability to control the stereochemistry of phosphorothioates in DNA enables us to protect the oligomers from degradation by nucleases while maintaining the RNase active sites. One of these designs is ONT-154 where wings of the oligonucleotide have been stabilized by *S*p phosphorothioate chemistry with retention of few Rp phosphorothioates which are better substrates for RNase H (Molecular Cell, 2007). The crystal structure of human RNase H complexed with DNA/RNA duplex shows that the Phosphate-binding pocket of the enzyme makes contacts with four contiguous phosphates of DNA. The first three contacts seem stronger than fourth one and they prefer Pro-R/Pro-R/Pro-S oxygen atoms of each of these three phosphates. Combining the stability advantage coming from Sp stereochemistry with RNase H active sites, several sequences can be designed to compete with/or improve upon 2'-modifications. From rat whole liver homogenate stability experiment comparing Mipomersen (ONT-41) with our rational (chiral control) design with and without 2'-modifications (ONT-87 and ONT-154) (Table 1 and Figure 1), it is evident that through removal of the 2'-modifications and careful chiral control with *R*p and *S*p phosphorothioates, we can improve the stability of these oligonucleotides which later affect the efficacy *in vivo.*

Table 4. Hu chiromersens studied for rat whole live homogenate stability

Sequence	Description	Target	Tm (°C)
ONT-41	Gs5mCs5mCs Ts5mCsAs GsTs5mCs TsGs5mCs TsTs5mCs Gs5mCsAs 5mCs5mC	Hu ApoB	80.7

ONT-75	**Gs5mCs5mCsTs5mC**sAsGsTs5mCsTsGs5mCsTsTs 5mCs**Gs5mCsAs5mCs5mC**	Hu ApoB	85.0
ONT-77	**Gs5mCs5mCsTs5mC**sAsGsTs5mCsTsGs5mCsTsTs 5mCs**Gs5mCsAs5mCs5mC**	Hu ApoB	79.9
ONT-80	**Gs5mCs5mCsTs5mCs**AsGsTs5mCsTsGs5mCsTsTs5 mCs**Gs5mCsAs5mCs5mC**	Hu ApoB	75.8
ONT-81	**Gs5mCs5mCsTs5mCs**AsGsTs5mCsTsGs5mCsTsTs5 mCs**Gs5mCsAs5mCs5mC**	Hu ApoB	80.7
ONT-87	**Gs5mCs5mCsTs5mCs**AsGsTs5mCsTsGs5mCsTsTs5 mCs**Gs5mCsAs5mCs5mC**	Hu ApoB	82.4
ONT-88	**Gs5mCs5mCsTs5mCs**AsGsTs5mCsTsGs5mCsTsTs5 mCs**Gs5mCsAs5mCs5mC**	Hu ApoB	78.9
ONT-89	**Gs5mCs5mCsTs5mCs**AsGsTs5mCsTsGs5mCsTsTs5 mCs**Gs5mCsAs5mCs5mC**	Hu ApoB	80.9
ONT-70	Gs5mCsGsTsTsTsGs5mCsTs5mCsTsTs5mCsTsTs5m CsTsTsGs5mCGsTsTsTsTsTsT	ISIS 355868 internal standard	

Table 5. Mouse chiromersens studied for rat whole live homogenate stability

Sequence	Description	Target
ONT-83	**GsTs5mCs5mCs5mCs**TsGsAsAsGsAsTsGsTs5mCs**AsAsTsGs5mC**	Mouse ApoB
ONT-82	**GsTs5mCs5mCs5mCs**TsGsAsAsGsAsTsGsTs5mCs**AsAsTsGs5mC**	Mouse ApoB
ONT-84	**GsTs5mCs5mCs5mCs**TsGsAsAsGsAsTsGsTs5mCs**AsAsTsGs5mC**	Mouse ApoB
ONT-85	**GsTs5mCs5mCs5mCs**TsGsAsAsGsAsTsGsTs5mCs**AsAsTsGs5mC**	Mouse ApoB
ONT-86	**GsTs5mCs5mCs5mCs**TsGsAsAsGsAsTsGsTs5mCs**AsAsTsGs5mC**	Mouse ApoB

Example 2. Exemplary Chirally Controlled siRNA Molecules

Table 1. Summary of Phosphodiester Polar interactions with h-Ago-2 and h-Ago-1

Science 2012 hAgo-2

Phosphate*	Residue	Length/A	Config
2	Asn551	2.7	Pro(S)
	Gln548	2.9	Pro(S)
3	Lys566	3.1	Pro(R)
	Arg792	3.4	Pro(R)
4	Tyr790	2.6	Pro(R)
	Arg792	3.0	Pro(R)
		2.8	Pro(R)
		3.4	Pro(S)
5	Ser798	2.7	Pro(R)
		2.9	Pro(R)
	Tyr804	2.8	Pro(S)
6	Lys709	3.0	Pro(S)
	Arg761	2.9	Pro(R)
	His753	2.8	Pro(R)
7	Arg714	2.9	Pro(R)
		3.0	Pro(R)
	Arg761	3.0	Pro(S)

*Phosphate No. from 5'-end

Cell 2012 hAgo-2

Phosphate	Residue	Length/ Å	Config
2	Asn551	2.7	Pro(S)
	Gln548	3.1	Pro(S)
	Gln548	2.9	Pro(R)
3	Lys566	2.9	Pro(R)
	Arg792	3.3	Pro(R)
4	Tyr790	2.8	Pro(R)
	Arg792	2.8	Pro(R)
5	Ser798	2.6	Pro(R)
		2.9	Pro(R)
	Tyr804	2.5	Pro(S)
6	Lys709	3.2	Pro(S)
	Arg761	2.8	Pro(R)
	His753	3.0	Pro(R)
7	Arg714	2.8	Pro(R)
		3.1	Pro(R)
	Arg761	2.8	Pro(S)
8	Arg761	2.4	Pro(S)
	Ala221	3.5	Pro(R)
9	Arg351	2.2	Pro(R)
10	Arg710	2.5	Pro(R)
18	No contacts		
19	Tyr311	3.1	Pro(R)
	Arg315	2.8	Pro(R)
20	His271	3.1	Pro(R)
	His319	3.4	Pro(S)
	Tyr311	2.2	Pro(S)

Cell Rep 2013, h-Ago-1†

Phosphate	Residue	Length/ Å	Config
2	Asn549	2.7	Pro(S)
	Gln546	2.9	Pro(S)
		2.8	Pro(R)
2	Lys564	2.9	Pro(R)
	Arg790	3.4	Pro(R)
		3.3	Pro(R)
4	Try788	2.7	Pro(R)
	Arg790	3.3	Pro(R)
5	Ser796	2.5	Pro(R)
		2.8	Pro(R)
	Tyr802	2.6	Pro(S)
6	Lys707	2.8	Pro(S)
	Arg759	2.7	Pro(R)
	His751	3.0	Pro(R)
7	Arg712	3.1	Pro(S)
		3.3	Pro(S)
	Arg373	3.4	Pro(R)
	Thr757	2.9	Pro(R)
	Arg759	2.2	Pro(S)
8	His710	3.4	Pro(R)
	Ser218	2.7	Pro(R)
9	Arg349	3.5	Pro(R)
	Arg708	2.9	Pro(S)
10	Arg708	3.2	Pro(R)
		2.9	Pro(R)
21	Tyr309	2.6	Pro(S)
	Tyr314	2.6	Pro(S)
	His269	3.0	Pro(R)

†Complexed with h-let-7 22mer

[00774] The present invention, despite teachings in the art to the contrary, recognizes that stereochemistry of internucleotidic linkages can be utilized to increase stability and activity of oligonucleotides through chirally controlled oligonucleotide compositions. Such chirally controlled oligonucleotide compositions can provide much better results than chirally uncontrolled oligonucleotide compositions as demonstrated in this disclosure.

[00775] There are two reported crystal structures of RNA complexed with human Argonaute-2 protein (hAgo2): The Crystal Structure of Human Argonaute-2, Science, 2012 (PDB-4ei3); and The Structure of Human Argonaute-2 in Complex with miR-20a Cell, 2012 PDB-4f3t). In addition, there is one reported crystal structure of Let-7 RNA complexed with human Argonaute-1 protein (hAgo-1): The Making of a Slicer: Activation of Human Argonaute-1, Cell Rep. 2013 (PDB-4krf).

[00776] Based upon the information contained in these publications, it was anticipated that some judgments could be made about advantageous preferences for stereochemistry at the internucleotidic phosphate linkage if the phosphodiester bonds were to be replaced by

phosphorothioate diester bonds. These advantages could relate to significantly improved potency, stability and other pharmacological properties. With this in mind, the computer program Pymol was used to locate all polar interactions between the protein and the internucleotidic phosphodiester linkage of the crystallized RNA for all three structures. Polar interactions at a distance of more than 3.5 Å were ignored.

[00777] The results of this analysis are summarized in Table 1. A particular phosphorus atom from the phosphodiester backbone on the RNA was assigned a Pro(R) or a Pro(S) configuration based upon the assumption that in the phosphorothioate diester analog the quite similar bond would be made between the polar group on an amino acid residue and the respectful phosphate oxygen atom.. The sulfur substitution, instead of non-bridging oxygen would therefore confer a unique stereochemistry (either (Sp) or (Rp) absolute configuration) on the phosphorus atom within that motif.

[00778] Of note is the extraordinarily good agreement between the two structures of hAgo-2 in complex with RNA. Also, there is an excellent agreement between the structures of hAgo-1 and hAgo-2 in complex with RNA, indicating that the conformation that the RNA molecule adopts is highly conserved between these two proteins. Any conclusions or rules which are formed based upon the results of this analysis are likely, therefore, to be valid for both protein molecules.

[00779] As can be seen, there is usually more than one polar interaction at any one phospodiester group, with the exception of those between the phosphodiesters at phosphate positions 9 and 10 and hAgo-2 (Cell 2012) which adopt exclusively Pro(Rp) preference through bonding with Arg351 and Arg710 respectively.

[00780] However, shorter distances (corresponding to stronger interactions) as well as the number of bonds per oxygen can suggest a predominant interaction for the Pro(Rp) or the Pro(Sp) oxygens: henceresulting in several interactions which are predominantly of one stereochemical type or the other. Within this group are the interactions between the phosphodiesters at phosphate positions 2 (Sp), 3 (Rp), 4 (Rp), 6 (Rp), 8 (Sp), 19 (Rp), 20 (Sp) and 21 (Sp).

[00781] Of the remaining interactions, there does not appear to be a preference for one particular stereochemistry to be adopted over the other, so the preferred stereochemistry could be either (Sp) or (Rp).

[00782] Within this category are the interactions formed between the phosphodiesters at phosphate positions 5 (Rp or Sp) and 7 (Rp or Sp).

[00783] For interactions at the other phosphate backbone, there is no crystal structure information, so stereochemistry at these positions can similarly be either (Rp) or (Sp) until empirical data shows otherwise.

[00784] To this end, Table 6 contains several non-limiting exemplary siRNA general constructs which can be conceived to take advantage of this preference for stereochemistry at individual phosphorothioate diester motifs.

Table 6. Exemplary general siRNA constructs

PS*	Chirally Controlled Antisense Strand Construct					
2	(Sp)	(Rp)	(Sp)	(Rp)	(Sp)	(Rp)
3	(Rp)	(Sp)	(Rp)	(Sp)	(Rp)	(Sp)
4	(Rp)	(Sp)	PO	PO	(Rp)	(Sp)
5	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
6	(Rp)	(Sp)	PO	PO	(Rp)	(Sp)
7	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
8	(Sp)	(Rp)	PO	PO	(Sp)	(Rp)
9	(Rp)	(Sp)	PO	PO	(Rp)	(Sp)
10	(Rp)	(Sp)	PO	PO	(Rp)	(Sp)
11	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
12	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
13	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
14	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
15	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
16	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
17	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
18	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
19	(Rp)	(Sp)	PO	PO	(Rp)	(Sp)
20	(Sp)	(Rp)	(Sp)	(Rp)	(Sp)	(Rp)
21	(Sp)	(Rp)	(Sp)	(Rp)	(Sp)	(Rp)

*The number indicates the phosphate position from the 5′ end of the antisense strand of the siRNA, (e.g. #2 is located between nucleotides 1 and 2 and #21 is located between nucleotides 20 and 21). (Sp) and (Rp) designates stereochemistry of phosphorus atom on phosphorothioate

(PS) diester internucleotidic linkage at the indicated position. PO designates a phosphodiester internucleotidic linkage at the indicated position.

[00785] Exemplary siRNAs include but are not limited to siRNAs having a Sp configuration for a chiral phosphorothioate at the 3'end and at the 5'end of the antisense strand of the siRNA duplex, which confers unprecedentedly increased stability in human serum or biological fluids. That same Sp configuration for the chiral phosphorothioate at the 3'end and at the 5'end of the antisense strand of the siRNA duplex confers unprecedentedly increased biological potency caused by increased affitnity to the Ago2 protein leading to increased activity within the RISC RNAi silencing complex.

[00786] In one embodiment, a single chiral phosphorothioate motif is introduced independently at each position along the antisense or sense strand of the siRNA molecule. For a 21mer, this provides 80 unique sequences, with either an (Sp) or an (Rp) chirally controlled phosphorothioate group. When duplexed independently, 1600 unique combinations of siRNAs are prepared.

siRNA Transfection of Chiral siRNA Molecules

[00787] Hep3B, or HeLa cells are reverse transfected at a density of 2.0×10^4 cells/well in 96-well plates. Transfection of siRNA is carried out with lipofectamine RNAiMax (Life Technologies, cat. No. 13778-150) using the manufacturer's protocol, except with a decreased amount of Lipofectamine RNAiMax of 0.2ul per well. Twelve, 1:3 siRNA duplex dilutions are created starting at 1uM. 10ul of 10x siRNA duplex is then lipoplexed with a prepared mixture of 9.8ul of serum-free medium and 0.2ul of Lipofectamine RNAiMax per well. After a 10-15 minute incubation, 2.0×10^4 cells in 80ul of EMEM cell growing media (ATCC, 30-2003) is added to bring the final volume to 100ul per well. Two separate transfection events are performed for each dose.

[00788] 24 hours after transfection Hep3B or HeLa cells are lysed and mRNA against which the siRNA is targeted is purified using MagMAX™-96 Total RNA Isolation Kit (Life Technologies, AM1830); 15ul of cDNA is synthesized with High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Life Technologies, 4374967). Gene expression is evaluated by Real-Time PCR on a Lightcycler 480(Roche) using a Probes Master Mix(Roche, 04 707 494 001) according to manufacturer's protocol.

IC50s and Data Analysis

[00789] Delta delta Ct method is used to calculate values. Samples are normalized to hGAPDH and calibrated to mock transfected and untreated samples. A stereo-random molecule is used as a control. The data is represented as a mean of 2 biological replicates using Graphpad Prism. A four-parameter linear regression curve is fitted to the data and the bottom and top are constrained to a 0 and 100 constants respectively in order to calculate a relative IC50.

[00790] The present Example demonstrates successful inhibition of target gene expression using siRNA agents comprised of chirally controlled oligonucleotides as described herein. Specifically, this Example describes hybridization of individual oligonucleotide strands prepared through chirally controlled synthesis as described herein, so that double-stranded chirally controlled siRNA oligonucleotide compositions are provided. This Example further demonstrates successful transfection of cells with such agents and, moreover, successful inhibition of target gene expression.

In Vitro Metabolic Stabilities of Human PCSK9 siRNA Duplexes Having Stereocontrolled Phosphorothioate Diester Linkages in Human Serum.

[00791] 10 μM siRNA duplexes were incubated in 90% human serum (50 μL, Sigma, H4522) at 37 ^{o}C for 24 hours. A 0 min time point (50 μL) was prepared as well as a PBS control incubation time point (50 μL), where the 10 μM siRNA duplex was incubated in 90% 1×PBS (50 μL at 37 ^{o}C for 24 hours. After completion of the incubation, to each time point, were added 10 μL of Stop-Solution (0.5 M NaCl, 50 mM TRIS, 5 mM EDTA, 2.5% IGEPAL), followed by 3.2 μL of Proteinase K (20 mg/mL, Ambion). The samples were incubated at 60 ^{o}C for 20 min, and then centrifuged at 2000 rpm for 15 min. The final reaction mixtures were directly analyzed in denaturing IEX HPLC (injection volume 50 μL). The ratio of integrated area at 24 h and 0 min was used to determine the % of degradation for each siRNA.

[00792] It was observed that the stereochemistry configuration of the single phosphorothioate at position 21 (3'end) of both the antisense strand and the sense strand of the siRNA had a crucial impact on the stability of the duplex upon incubation in Human Serum (Figure 1). As illustrated in the Figure 1 and as determined following the integration ratio of the degradation pattern, an (Rp, Rp) siRNA duplex exhibited a significant 55.0% degradation after 24 h. The stereorandom mixture of phosphorothioates in the stereorandom siRNA showed 25.2% degradation after 24 h. The (Sp/Sp) siRNA showed only minor 7.3% degradation after 24 h. This

illustrates the drastic impact that phosphorothioate stereochemistry confers to therapeutic siRNAs. Additional exemplary data were presented in Figure 2, Figure 3, Figure 4 and Figure 5.

[00793] It is observed that each of the stereopure constructs show different potency (IC_{50} values) dependent on the position of the phosphorothioate motif along the backbone. It is also observered that different IC_{50} values are obtained dependent upon whether the phosphorothioate motif at any single position is (Sp) or (Rp). The impact of stereochemistry upon stability is likewise clear and differentiating, using either Human Serum described above, or Human Hepatic Cytosol extract or Snake Venom Phosphodiesterase, or isolated endonuclease or isolated exonuclease.

[00794] Certain design rules may be formulated based upon data obtained in the above example. These design information can be applied for the introduction of multiple chiral phosphorothioate linkages within the antisense and/or sense strand of the siRNA as exemplified below. The present invention recognizes that an increased amount of chiral phosphorothioate within the antisense and/or sense strand of the siRNA, introduced at the right positions and having the right stereochemistry configuration leads to greatly improved siRNA constructs in terms of potency and metabolic stability in vitro – translating into greatly pharmacologically enhanced therapeutic siRNAs.

Exemplary Chirally Controlled siRNA Oligonucleotides Targeting PCSK9

[00795] Proprotein convertase subtilisin/kexin type 9 (PCSK9), is an enzyme involved in cholesterol metabolism. PCSK9 binds to the receptor for low density lipoprotein (LDL), triggering its destruction. Although LDL associated with the receptor is also eliminated when the receptor is destroyed, the net effect of PCSK9 binding in fact increases LDL levels, as the receptor would otherwise cycle back to the cell surface and remove more cholesterol.

[00796] Several companies are developing therapeutic agents that target PCSK9. Of particular relevance to the present disclosure, each of Isis Pharmaceuticals, Santaris Pharma, and Alnylam Pharmaceuticals is developing a nucleic acid agent that inhibits PCSK9. The Isis Pharmaceuticals product, an antisense oligonucleotide, has been shown to increase expression of the LDLR and decrease circulating total cholesterol levels in mice (Graham et al "Antisense inhibition of proprotein convertase subtilisin/kexin type 9 reduces serum LDL in hyperlipidemic mice". *J. Lipid Res.* **48** (4): 763–7, April 2007). Initial clinical trials with the Alnylam

Pharmaceuticals product, ALN-PCS, reveal that RNA interference offers an effective mechanism for inhibiting PCSK9 (Frank-Kamenetsky et al "Therapeutic RNAi targeting PCSK9 acutely lowers plasma cholesterol in rodents and LDL cholesterol in nonhuman primates". *Proc. Natl. Acad. Sci. U.S.A.* **105** (33): 11915–20, August 2008).

[00797] In some embodiments, despite known results to the contrary, the present invention recognizes that phosphorothioate motifs of one stereochemical conformation or another can be rationally designed to take advantage of increased potency, stability and other pharmacological qualities through chirally controlled oligonucleotide compositions. To reinforce this concept, table 3 contains exemplary stereochemically pure constructs based on an siRNA sequence which targets PCSK9 messenger RNA.

[00798] In this exemplary embodiment, a single chiral phosphorothioate motif is introduced independently at each position along the antisense or sense strand of the siRNA molecule. For a 21mer, this provides 80 unique sequences, with either an (Sp) or an (Rp) chirally controlled phosphorothioate group. When duplexed independently, 1600 unique combinations of siRNAs are prepared.

[00799] In other exemplary embodiments, a single chiral phosphorothioate motif is introduced independently at each position along the antisense or sense strand of the siRNA molecule, while a 3'-(Sp) phosphorothioate linkage is conserved. For a 21mer, this provides another additional 80 unique sequences, with either an (Sp) or an (Rp) chirally controlled phosphorothioate group. When duplexed independently, 1600 unique combinations of siRNAs are prepared.

[00800] In other exemplary embodiments, multiple chiral phosphorothioate motifs are introduced independently at several positions along the antisense or sense strand of the siRNA molecule, following the codes described in Table 7, while a 3'-(Sp) phosphorothioate linkage is conserved.

Table 7. Example of PCSK-9 Sense and Antisense RNAs

	PCSK9 siRNA Sense Strands
PCSK9 (1)	(*R*p)- uucuAGAccuGuuuuGcuudTsdT
PCSK9 (2)	(*S*p)- uucuAGAccuGuuuuGcuudTsdT
PCSK9 (3)	(*R*p)- uucuAGAccuGuuuuGcuusdTdT

PCSK9 (4)	(*S*p)- uucuAGAccuGuuuuGcuusdTdT
PCSK9 (5)	(*R*p)- uucuAGAccuGuuuuGcusudTdT
PCSK9 (6)	(*S*p)- uucuAGAccuGuuuuGcusudTdT
PCSK9 (7)	(*R*p)- uucuAGAccuGuuuuGcsuudTdT
PCSK9 (8)	(*S*p)- uucuAGAccuGuuuuGcsuudTdT
PCSK9 (9)	(*R*p)- uucuAGAccuGuuuuGscuudTdT
PCSK9 (10)	(*S*p)- uucuAGAccuGuuuuGscuudTdT
PCSK9 (11)	(*R*p)- uucuAGAccuGuuuusGcuudTdT
PCSK9 (12)	(*S*p)- uucuAGAccuGuuuusGcuudTdT
PCSK9 (13)	(*R*p)- uucuAGAccuGuuusuGcuudTdT
PCSK9 (14)	(*S*p)- uucuAGAccuGuuusuGcuudTdT
PCSK9 (15)	(*R*p)- uucuAGAccuGuusuuGcuudTdT
PCSK9 (16)	(*S*p)- uucuAGAccuGuusuuGcuudTdT
PCSK9 (17)	(*R*p)- uucuAGAccuGusuuuGcuudTdT
PCSK9 (18)	(*S*p)- uucuAGAccuGusuuuGcuudTdT
PCSK9 (19)	(*R*p)- uucuAGAccuGsuuuuGcuudTdT
PCSK9 (20)	(*S*p)- uucuAGAccuGsuuuuGcuudTdT
PCSK9 (21)	(*R*p)- uucuAGAccusGuuuuGcuudTdT
PCSK9 (22)	(*S*p)- uucuAGAccusGuuuuGcuudTdT
PCSK9 (23)	(*R*p)- uucuAGAccsuGuuuuGcuudTdT
PCSK9 (24)	(*S*p)- uucuAGAccsuGuuuuGcuudTdT
PCSK9 (25)	(*R*p)- uucuAGAcscuGuuuuGcuudTdT
PCSK9 (26)	(*S*p)- uucuAGAcscuGuuuuGcuudTdT
PCSK9 (27)	(*R*p)- uucuAGAsccuGuuuuGcuudTdT
PCSK9 (28)	(*S*p)- uucuAGAsccuGuuuuGcuudTdT
PCSK9 (29)	(*R*p)- uucuAGsAccuGuuuuGcuudTdT
PCSK9 (30)	(*S*p)- uucuAGsAccuGuuuuGcuudTdT
PCSK9 (31)	(*R*p)- uucuAsGAccuGuuuuGcuudTdT
PCSK9 (32)	(*S*p)- uucuAsGAccuGuuuuGcuudTdT
PCSK9 (33)	(*R*p)- uucusAGAccuGuuuuGcuudTdT

PCSK9 (34)	(*S*p)- uucusAGAccuGuuuuGcuudTdT
PCSK9 (35)	(*R*p)- uucsuAGAccuGuuuuGcuudTdT
PCSK9 (36)	(*S*p)- uucsuAGAccuGuuuuGcuudTdT
PCSK9 (37)	(*R*p)- uuscuAGAccuGuuuuGcuudTdT
PCSK9 (38)	(*S*p)- uuscuAGAccuGuuuuGcuudTdT
PCSK9 (38)	(*R*p)- usucuAGAccuGuuuuGcuudTdT
PCSK9 (40)	(*S*p)- usucuAGAccuGuuuuGcuudTdT

NOTE: lower case letters represent 2'-OMe RNA residues; capital letters represent RNA residues; d = 2'-deoxy residues; and "s" indicates a phosphorothioate moiety.

[00801] Synthesis examples for Human PCSK9 siRNA Antisense Strands having several chiral phosphorothioate internucleotide linkages and full chiral phosphorothioate internucleotide linkages.

	Human PCSK9 siRNA Antisense Strands
PCSK9 (41)	(*R*p)- AAGcAAAAcAGGUCuAGAAdTsdT
PCSK9 (42)	(*S*p)- AAGcAAAAcAGGUCuAGAAdTsdT
PCSK9 (43)	(*R*p)- AAGcAAAAcAGGUCuAGAAsdTdT
PCSK9 (44)	(*S*p)- AAGcAAAAcAGGUCuAGAAsdTdT
PCSK9 (45)	(*R*p)- AAGcAAAAcAGGUCuAGAsAdTdT
PCSK9 (46)	(*S*p)- AAGcAAAAcAGGUCuAGAsAdTdT
PCSK9 (47)	(*R*p)- AAGcAAAAcAGGUCuAGsAAdTdT
PCSK9 (48)	(*S*p)- AAGcAAAAcAGGUCuAGsAAdTdT
PCSK9 (49)	(*R*p)- AAGcAAAAcAGGUCuAsGAAdTdT
PCSK9 (50)	(*S*p)- AAGcAAAAcAGGUCuAsGAAdTdT
PCSK9 (51)	(*R*p)- AAGcAAAAcAGGUCusAGAAdTdT
PCSK9 (52)	(*S*p)- AAGcAAAAcAGGUCusAGAAdTdT
PCSK9 (53)	(*R*p)- AAGcAAAAcAGGUCsuAGAAdTdT
PCSK9 (54)	(*S*p)- AAGcAAAAcAGGUCsuAGAAdTdT
PCSK9 (55)	(*R*p)- AAGcAAAAcAGGUsCuAGAAdTdT

PCSK9 (56)	(*S*p)- AAGcAAAAcAGGUsCuAGAAdTdT
PCSK9 (57)	(*R*p)- AAGcAAAAcAGGsUCuAGAAdTdT
PCSK9 (58)	(*S*p)- AAGcAAAAcAGGsUCuAGAAdTdT
PCSK9 (59)	(*R*p)- AAGcAAAAcAGsGUCuAGAAdTdT
PCSK9 (60)	(*S*p)- AAGcAAAAcAGsGUCuAGAAdTdT
PCSK9 (61)	(*R*p)- AAGcAAAAcAsGGUCuAGAAdTdT
PCSK9 (62)	(*S*p)- AAGcAAAAcAsGGUCuAGAAdTdT
PCSK9 (63)	(*R*p)- AAGcAAAAcsAGGUCuAGAAdTdT
PCSK9 (64)	(*S*p)- AAGcAAAAcsAGGUCuAGAAdTdT
PCSK9 (65)	(*R*p)- AAGcAAAAscAGGUCuAGAAdTdT
PCSK9 (66)	(*S*p)- AAGcAAAAscAGGUCuAGAAdTdT
PCSK9 (67)	(*R*p)- AAGcAAAsAcAGGUCuAGAAdTdT
PCSK9 (68)	(*S*p)- AAGcAAAsAcAGGUCuAGAAdTdT
PCSK9 (69)	(*R*p)- AAGcAAsAAcAGGUCuAGAAdTdT
PCSK9 (70)	(*S*p)- AAGcAAsAAcAGGUCuAGAAdTdT
PCSK9 (71)	(*R*p)- AAGcAsAAAcAGGUCuAGAAdTdT
PCSK9 (72)	(*S*p)- AAGcAsAAAcAGGUCuAGAAdTdT
PCSK9 (73)	(*R*p)- AAGcsAAAAcAGGUCuAGAAdTdT
PCSK9 (74)	(*S*p)- AAGcsAAAAcAGGUCuAGAAdTdT
PCSK9 (75)	(*R*p)- AAGscAAAAcAGGUCuAGAAdTdT
PCSK9 (76)	(*S*p)- AAGscAAAAcAGGUCuAGAAdTdT
PCSK9 (77)	(*R*p)- AAsGcAAAAcAGGUCuAGAAdTdT
PCSK9 (78)	(*S*p)- AAsGcAAAAcAGGUCuAGAAdTdT
PCSK9 (77)	(*R*p)- AsAGcAAAAcAGGUCuAGAAdTdT
PCSK9 (78)	(*S*p)- AsAGcAAAAcAGGUCuAGAAdTdT
PCSK9 (79)	(*R*p, *S*p)- AAGcAAAAcAGGUCuAGAAsdTsdT
PCSK9 (80)	(*S*p, *S*p)- AAGcAAAAcAGGUCuAGAAsdTsdT
PCSK9 (81)	(*R*p, *S*p)- AAGcAAAAcAGGUCuAGAsAdTsdT
PCSK9 (82)	(*S*p, *S*p)- AAGcAAAAcAGGUCuAGAsAdTsdT
PCSK9 (83)	(*R*p, *S*p)- AAGcAAAAcAGGUCuAGsAAdTsdT

PCSK9 (84)	(*S*p, *S*p)- AAGcAAAAcAGGUCuAGsAAdTsdT
PCSK9 (85)	(*R*p, *S*p)- AAGcAAAAcAGGUCuAsGAAdTsdT
PCSK9 (86)	(*S*p, *S*p)- AAGcAAAAcAGGUCuAsGAAdTsdT
PCSK9 (87)	(*R*p, *S*p)- AAGcAAAAcAGGUCusAGAAdTsdT
PCSK9 (88)	(*S*p, *S*p)- AAGcAAAAcAGGUCusAGAAdTsdT
PCSK9 (89)	(*R*p, *S*p)- AAGcAAAAcAGGUCsuAGAAdTsdT
PCSK9 (90)	(*S*p, *S*p)- AAGcAAAAcAGGUCsuAGAAdTsdT
PCSK9 (91)	(*R*p, *S*p)- AAGcAAAAcAGGUsCuAGAAdTsdT
PCSK9 (92)	(*S*p, *S*p)- AAGcAAAAcAGGUsCuAGAAdTsdT
PCSK9 (93)	(*R*p, *S*p)- AAGcAAAAcAGGsUCuAGAAdTsdT
PCSK9 (94)	(*S*p, *S*p)- AAGcAAAAcAGGsUCuAGAAdTsdT
PCSK9 (95)	(*R*p, *S*p)- AAGcAAAAcAGsGUCuAGAAdTsdT
PCSK9 (96)	(*S*p, *S*p)- AAGcAAAAcAGsGUCuAGAAdTsdT
PCSK9 (97)	(*R*p, *S*p)- AAGcAAAAcAsGGUCuAGAAdTsdT
PCSK9 (98)	(*S*p, *S*p)- AAGcAAAAcAsGGUCuAGAAdTsdT
PCSK9 (99)	(*R*p, *S*p)- AAGcAAAAcsAGGUCuAGAAdTsdT
PCSK9 (100)	(*S*p, *S*p)- AAGcAAAAcsAGGUCuAGAAdTsdT
PCSK9 (101)	(*R*p, *S*p)- AAGcAAAAscAGGUCuAGAAdTsdT
PCSK9 (102)	(*S*p, *S*p)- AAGcAAAAscAGGUCuAGAAdTsdT
PCSK9 (103)	(*R*p, *S*p)- AAGcAAAsAcAGGUCuAGAAdTsdT
PCSK9 (104)	(*S*p, *S*p)- AAGcAAAsAcAGGUCuAGAAdTsdT
PCSK9 (105)	(*R*p, *S*p)- AAGcAAsAAcAGGUCuAGAAdTsdT
PCSK9 (106)	(*S*p, *S*p)- AAGcAAsAAcAGGUCuAGAAdTsdT
PCSK9 (107)	(*R*p, *S*p)- AAGcAsAAAcAGGUCuAGAAdTsdT
PCSK9 (108)	(*S*p, *S*p)- AAGcAsAAAcAGGUCuAGAAdTsdT
PCSK9 (109)	(*R*p, *S*p)- AAGcsAAAAcAGGUCuAGAAdTsdT
PCSK9 (110)	(*S*p, *S*p)- AAGcsAAAAcAGGUCuAGAAdTsdT
PCSK9 (111)	(*R*p, *S*p)- AAGscAAAAcAGGUCuAGAAdTsdT
PCSK9 (112)	(*S*p, *S*p)- AAGscAAAAcAGGUCuAGAAdTsdT
PCSK9 (113)	(*R*p, *S*p)- AAsGcAAAAcAGGUCuAGAAdTsdT

PCSK9 (114)	(*S*p, *S*p)- AAsGcAAAAcAGGUCuAGAAdTsdT
PCSK9 (115)	(*R*p, *S*p)- AsAGcAAAAcAGGUCuAGAAdTsdT
PCSK9 (116)	(*S*p, *S*p)- AsAGcAAAAcAGGUCuAGAAdTsdT
PCSK9 (117)	(*R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *R*p)- AsAsGscAsAAsAscsAGGUCuAGAsAsdTsdT
PCSK9 (118)	(*S*p, *R*p, *R*p, *R*p, *S*p, *R*p, *R*p, *R*p, *S*p, *S*p)- AsAsGscAsAAsAscsAGGUCuAGAsAsdTsdT

NOTE: lower case letters represent 2'-OMe RNA residues; capital letters represent RNA residues; d = 2'-deoxy residues; and "s" indicates a phosphorothioate moiety.

Example 3. Stereopure FOXO-1 antisense analogs.

Rational Design − Chirally Controlled Antisense Oligonucleotide Compositions

[00802] The unprecedented nuclease stability determined *in vivo* and in a whole rat liver homogenate model of the Sp-chiral phosphorothioate internucleotide linkage is applied in the novel design of new types of RNaseH substrate gapmers, whereby the external flanks are composed of unmodified DNA and the internal gap core is modified with 2' chemical modifications (2'OMe, 2'MOE, 2'LNA, 2'F, etc). Eventually this design is extended to fully unmodified DNA therapeutic oligonucleotides wherein careful chiral control of the phosphorothioate backbone confers the desired pharmacological properties of the RNaseH therapeutic oligonucleotide.

[00803] The application of the triplet-phosphate repeating motif designed after studying the crystal structure of human RNaseH has been employed as well. The crystal structure of RNaseH has been previously published (Structure of Human RNase H1 Complexed with an RNA/DNA Hybrid: Insight into HIV Reverse Transcription, Nowotny et al., Molecular Cell, Volume 28, Issue 2, 264-276, 2007, pdb file: 2qkb). Among other things, the present invention recognizes the importance of internucleotidic linkage stereochemistry of oligonucleotides, for example, in settings herein. Upon performing *in silico* analysis upon this structure using the program Pymol, Applicant found that the phosphate-binding pocket of Human RNase H1 makes polar contacts with three contiguous phosphates of the complexed DNA, and interacts preferentially with the Pro-R/Pro-R/Pro-S (or with the Pro-S/Pro-S/Pro-R) respective oxygen atoms of each of these three phosphates. Based on this observation we designed two chiral

architectures with repeating (RRS) and (SSR) triplet phosphorothioates motifs as designed RNase H substrates. Applicant also designed other internucleotidic linkage stereochemical patterns. As demonstrated by exemplary results provide herein, provided chirally controlled oligonucleotide compositions of oligonucleotide types that comprises certain backbone internucleotidic linkage patterns (patterns backbone chiral centers) provides significantly increased activity and/or kinetics. Among others, a sequence of 5'-RSS-3' backbone chiral centers is particularly useful and delivers unexpected results as described in the present disclosure.

[00804] The combination of increased Sp chiral backbone (for enzymatic stability and other pharmacologically advantageous properties) and (RRS) or (SSR) repeating triplet chiral backbone motifs (for enhancing the property as RNase H substrate) are also utilized in the novel designs.; "S" represents Sp-phosphorothioate linkage and "R" represents Rp-phosphorothioate linkage.

[00805] Another alternative design is based on the increased amount of *S*p chiral phosphorothioate backbone in extended repeating motifs such as:

(SSSR)n, SR(SSSR)n, SSR(SSSR)n, SSR(SSSR)n;

(SSSSR)n, SR(SSSSR)n, SSR(SSSSR)n, SSR(SSSSR)n, SSSR(SSSSR)n;

(SSSSSR)n; SR(SSSSSR)n, SSR(SSSSSR)n, SSR(SSSSSR)n, SSSR(SSSSSR)n, SSSSR(SSSSSR)n; etc., where n = 0-50, depending on the number of respective internucleotide linkages.; "S" represents *S*p-phosphorothioate linkage and "R" represents *R*p-phosphorothioate linkage. In some embodiments, n is 0. In some embodiments, R is 1-50. In some embodiments, R is 1. In some embodiments, a common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises a motif described herein. In some embodiments, a motif is in the core region. In some embodiments, n is 0. In some embodiments, R is 1-50. In some embodiments, R is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5.

[00806] Another alternative design is based on the "invert" architecture design of the stereo backbone ("stereo invert-mers"). These result from positioning the stereochemistry of the chiral phosphorothioate in a inverting manner, exposing some *S*p-rich motifs at the 5' and 3' end extremities of the oligonucleotide as well as the middle portion of the oligonucleotide and having the repeating stereochemistry motifs positioned in a invert image manner on both sides, such as:

SS(SSR)n(SSS)(RSS)nSS;
SS(SSR)n(SRS)(RSS)nSS;
SS(SSR)n(SSR)(RSS)nSS;
SS(SSR)n(RSS)(RSS)nSS;
SS(RSS)n(SSS)(SSR)nSS;
SS(RSS)n(SRS)(SSR)nSS;
SS(RSS)n(SSR)(SSR)nSS;
SS(RSS)n(RSS)(SSR)nSS; etc.,

where n = 0-50, depending on the number of respective internucleotide linkages. ; "S" represents Sp-phosphorothioate linkage and "R" represents Rp-phosphorothioate linkage. In some embodiments, a common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises a motif described herein. In some embodiments, a motif is in the core region. In some embodiments, n is 0. In some embodiments, n is 1. In some embodiments, n is 1-50. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5.

Initial screen

Synthesis: Summary for Oligonucleotide Synthesis on a DNA/RNA Synthesizer MerMade-12 (2'-deoxy and 2'-OMe cycle)

step	reaction	reagent	delivery volume (mL)	wait time (sec)
1	detritylation	3% TCA in DCM	4 x 1	N. A.
2	coupling	0.15 M phosphoramidite in ACN + 0.45 M ETT in ACN	2 x 0.5 mL	60 + 60 (DNA), 300 + 300 (2'-OMe RNA)
3	capping	5% Pac_2O in THF/2,6-lutidine + 16% NMI in THF	1	60

4	oxidation	0.02 Iodine in water/pyridine	1	240

Stereorandom PS oligonucleotides having DNA-2'-OMe-DNA (7-6-7) design:

ONT-141 d(CsCsCsTsCsTsGs)gsaststsgsasd(GsCsAsTsCsCsA)
ONT-142 d(AsAsGsCsTsTsTs)gsgststsgsgsd(GsCsAsAsCsAsC)
ONT-143 d(AsGsTsCsAsCsTs)tsgsgsgsasgsd(CsTsTsCsTsCsC)
ONT-144 d(CsAsCsTsTsGsGs)gsasgscststsd(CsTsCsCsTsGsG)
ONT-145 d(AsTsAsGsCsCsAs)tstsgscsasgsd(CsTsGsCsTsCsA)
ONT-146 d(TsGsGsAsTsTsGs)asgscsastscsd(CsAsCsCsAsAsG)
ONT-147 d(CsCsAsTsAsGsCs)csaststsgscsd(AsGsCsTsGsCsT)
ONT-148 d(GsTsCsAsCsTsTs)gsgsgsasgscsd(TsTsCsTsCsCsT)
ONT-149 d(CsCsAsGsGsGsCs)ascstscsastsd(CsTsGsCsAsTsG)
ONT-150 d(GsCsCsAsTsCsCs)asasgstscsasd(CsTsTsGsGsGsA)
ONT-151 d(GsAsAsGsCsTsTs)tsgsgststsgsd(GsGsCsAsAsCsA)
ONT-152 d(CsTsGsGsAsTsTs)gsasgscsastsd(CsCsAsCsCsAsA)
ONT-183 d(CsAsAsGsTsCsAs)cststsgsgsgsd(AsGsCsTsTsCsT)
ONT-184 d(AsTsGsCsCsAsTs)cscsasasgstsd(CsAsCsTsTsGsG)
ONT-185 d(AsTsGsAsGsAsTs)gscscstsgsgsd(CsTsGsCsCsAsT)
ONT-186 d(TsTsGsGsGsAsGs)cststscstscsd(CsTsGsGsTsGsG)
ONT-187 d(TsGsGsGsAsGsCs)tstscstscscsd(TsGsGsTsGsGsA)
ONT-188 d(TsTsAsTsGsAsGs)astsgscscstsd(GsGsCsTsGsCsC)
ONT-189 d(GsTsTsAsTsGsAs)gsastsgscscsd(TsGsGsCsTsGsC)
ONT-190 d(CsCsAsAsGsTsCs)ascststsgsgsd(GsAsGsCsTsTsC)
ONT-191 d(AsGsCsTsTsTsGs)gststsgsgsgsd(CsAsAsCsAsCsA)
ONT-192 d(TsAsTsGsAsGsAs)tsgscscstsgsd(GsCsTsGsCsCsA)
ONT-193 d(TsGsTsTsAsTsGs)asgsastsgscsd(CsTsGsGsCsTsG)
ONT-194 d(AsTsCsCsAsAsGs)tscsascststsd(GsGsGsAsGsCsT)
ONT-195 d(GsGsGsAsAsGsCs)tststsgsgstsd(TsGsGsGsCsAsA)
ONT-196 d(CsTsCsCsAsTsCs)csastsgsasgsd(GsTsCsAsTsTsC)
ONT-197 d(AsAsGsTsCsAsCs)tstsgsgsgsasd(GsCsTsTsCsTsC)
ONT-198 d(CsCsAsTsCsCsAs)asgstscsascsd(TsTsGsGsGsAsG)
ONT-199 d(TsCsCsAsAsGsTs)csascststsgsd(GsGsAsGsCsTsT)
ONT-200 d(CsCsTsCsTsGsGs)aststsgsasgsd(CsAsTsCsCsAsC)
ONT-201 d(AsCsTsTsGsGsGs)asgscststscsd(TsCsCsTsGsGsT)
ONT-202 d(CsTsTsGsGsGsAs)gscststscstsd(CsCsTsGsGsTsG)
ONT-203 d(CsAsTsGsCsCsAs)tscscsasasgsd(TsCsAsCsTsTsG)
ONT-204 d(TsGsCsCsAsTsCs)csasasgstscsd(AsCsTsTsGsGsG)
ONT-205 d(TsCsCsAsTsCsCs)astsgsasgsgsd(TsCsAsTsTsCsC)
ONT-206 d(AsGsGsGsCsAsCs)tscsastscstsd(GsCsAsTsGsGsG)
ONT-207 d(CsCsAsGsTsTsCs)cststscsastsd(TsCsTsGsCsAsC)
ONT-208 d(CsAsTsAsGsCsCs)aststsgscsasd(GsCsTsGsCsTsC)
ONT-209 d(TsCsTsGsGsAsTs)tsgsasgscsasd(TsCsCsAsCsCsA)
ONT-210 d(GsGsAsTsTsGsAs)gscsastscscsd(AsCsCsAsAsGsA)

Biology *in vitro* data in HepG2 cells for the initial DNA-2'-OMe-DNA (7-6-7) design: (d Upper case) = DNA; lower case = 2'-OMe; s = phosphorothioate.

FOXO1

Levels at 20 nM	(%)	SD
ONT-141	89	6
ONT-142	45	1
ONT-143	98	2
ONT-144	89	1
ONT-145	46	5
ONT-146	99	1
ONT-147	66	6
ONT-148	101	2
ONT-149	95	6
ONT-150	58	4
ONT-151	41	5
ONT-152	84	5
ONT-183	95	2
ONT-184	58	4
ONT-185	42	2
ONT-186	96	4
ONT-187	92	3
ONT-188	47	5
ONT-189	63	5
ONT-190	83	2
ONT-191	58	4
ONT-192	46	2
ONT-193	58	2
ONT-194	76	1
ONT-195	66	0
ONT-196	77	2
ONT-197	90	6
ONT-198	42	4
ONT-199	68	1
ONT-200	89	6
ONT-201	91	2
ONT-202	94	2
ONT-203	86	1
ONT-204	58	2
ONT-205	75	3
ONT-206	94	5
ONT-207	96	0
ONT-208	54	0
ONT-209	87	4
ONT-210	92	4

FOXO1

Levels at 200 nM	(%)	SD
ONT-141	37	4
ONT-142	45	4
ONT-143	46	2
ONT-144	42	5
ONT-145	53	4
ONT-146	31	2
ONT-147	28	8
ONT-148	45	4
ONT-149	29	5
ONT-150	32	6
ONT-151	38	4
ONT-152	30	5
ONT-183	60	5
ONT-184	34	2
ONT-185	50	2
ONT-186	86	3
ONT-187	76	6
ONT-188	50	5
ONT-189	38	2
ONT-190	51	1
ONT-191	43	5
ONT-192	54	7
ONT-193	41	6
ONT-194	50	1
ONT-195	43	6
ONT-196	33	7
ONT-197	57	4
ONT-198	40	5
ONT-199	50	5
ONT-200	28	9
ONT-201	46	6
ONT-202	57	9
ONT-203	27	7
ONT-204	36	6
ONT-205	29	5
ONT-206	81	0
ONT-207	37	4
ONT-208	43	3
ONT-209	35	4
ONT-210	40	4

Stereorandom PS oligonucleotides having 2'-OMe-DNA-2'OMe (3-14-3) design: (d Upper case) = DNA; lower case = 2'-OMe; s = phosphorothioate.

ONT-129	cscscsd(TsCsTsGsGsAsTsTsGsAsGsCsAsTs)cscsa
ONT-130	asasgsd(CsTsTsTsGsGsTsTsGsGsGsCsAsAs)csasc
ONT-131	asgstsd(CsAsCsTsTsGsGsGsAsGsCsTsTsCs)tscsc
ONT-132	csascsd(TsTsGsGsGsAsGsCsTsTsCsTsCsCs)tsgsg
ONT-133	astsasd(GsCsCsAsTsTsGsCsAsGsCsTsGsCs)tscsa
ONT-134	tsgsgsd(AsTsTsGsAsGsCsAsTsCsCsAsCsCs)asasg
ONT-135	cscsasd(TsAsGsCsCsAsTsTsGsCsAsGsCsTs)gscst
ONT-136	gstscsd(AsCsTsTsGsGsGsAsGsCsTsTsCsTs)cscst
ONT-137	cscsasd(GsGsGsCsAsCsTsCsAsTsCsTsGsCs)astsg
ONT-138	gscscsd(AsTsCsCsAsAsGsTsCsAsCsTsTsGs)gsgsa
ONT-139	gsasasd(GsCsTsTsTsGsGsTsTsGsGsGsCsAs)ascsa
ONT-140	cstsgsd(GsAsTsTsGsAsGsCsAsTsCsCsAsCs)csasa
ONT-155	csasasd(GsTsCsAsCsTsTsGsGsGsAsGsCsTs)tscst
ONT-156	astsgsd(CsCsAsTsCsCsAsAsGsTsCsAsCsTs)tsgsg
ONT-157	astsgsd(AsGsAsTsGsCsCsTsGsGsCsTsGsCs)csast
ONT-158	tstsgsd(GsGsAsGsCsTsTsCsTsCsCsTsGsGs)tsgsg
ONT-159	tsgsgsd(GsAsGsCsTsTsCsTsCsCsTsGsGsTs)gsgsa
ONT-160	tstsasd(TsGsAsGsAsTsGsCsCsTsGsGsCsTs)gscsc
ONT-161	gststsd(AsTsGsAsGsAsTsGsCsCsTsGsGsCs)tsgsc
ONT-162	cscsasd(AsGsTsCsAsCsTsTsGsGsGsAsGsCs)tstsc
ONT-163	asgscsd(TsTsTsGsGsTsTsGsGsGsCsAsAsCs)ascsa
ONT-164	tsastsd(GsAsGsAsTsGsCsCsTsGsGsCsTsGs)cscsa
ONT-165	tsgstsd(TsAsTsGsAsGsAsTsGsCsCsTsGsGs)cstsg
ONT-166	astscsd(CsAsAsGsTsCsAsCsTsTsGsGsGsAs)gscst
ONT-167	gsgsgsd(AsAsGsCsTsTsTsGsGsTsTsGsGsGs)csasa
ONT-168	cstscsd(CsAsTsCsCsAsTsGsAsGsGsTsCsAs)tstsc
ONT-169	asasgsd(TsCsAsCsTsTsGsGsGsAsGsCsTsTs)cstsc
ONT-170	cscsasd(TsCsCsAsAsGsTsCsAsCsTsTsGsGs)gsasg
ONT-171	tscscsd(AsAsGsTsCsAsCsTsTsGsGsGsAsGs)cstst
ONT-172	cscstsd(CsTsGsGsAsTsTsGsAsGsCsAsTsCs)csasc
ONT-173	ascstsd(TsGsGsGsAsGsCsTsTsCsTsCsCsTs)gsgst
ONT-174	cststsd(GsGsGsAsGsCsTsTsCsTsCsCsTsGs)gstsg
ONT-175	csastsd(GsCsCsAsTsCsCsAsAsGsTsCsAsCs)tstsg
ONT-176	tsgscsd(CsAsTsCsCsAsAsGsTsCsAsCsTsTs)gsgsg
ONT-177	tscscsd(AsTsCsCsAsTsGsAsGsGsTsCsAsTs)tscsc
ONT-178	asgsgsd(GsCsAsCsTsCsAsTsCsTsGsCsAsTs)gsgsg
ONT-179	cscsasd(GsTsTsCsCsTsTsCsAsTsTsCsTsGs)csasc
ONT-180	csastsd(AsGsCsCsAsTsTsGsCsAsGsCsTsGs)cstsc
ONT-181	tscstsd(GsGsAsTsTsGsAsGsCsAsTsCsCsAs)cscsa
ONT-182	gsgsasd(TsTsGsAsGsCsAsTsCsCsAsCsCsAs)asgsa

Biology *in vitro* data in HepG2 cells for the 2'-OMe-DNA-2'-OMe (3-14-3) design:

FOXO1

Levels at 20 nM	(%)	SD
ONT-129	82	5

ONT-130	49	4
ONT-131	92	3
ONT-132	91	2
ONT-133	58	3
ONT-134	73	2
ONT-135	65	5
ONT-136	92	2
ONT-137	94	2
ONT-138	78	1
ONT-139	61	1
ONT-140	82	4
ONT-155	95	2
ONT-156	74	1
ONT-157	56	2
ONT-158	93	1
ONT-159	94	1
ONT-160	71	1
ONT-161	67	1
ONT-162	89	1
ONT-163	55	7
ONT-164	68	4
ONT-165	70	1
ONT-166	89	4
ONT-167	81	0
ONT-168	81	0
ONT-169	94	0
ONT-170	88	1
ONT-171	92	4
ONT-172	86	2
ONT-173	90	1
ONT-174	93	2
ONT-175	84	1
ONT-176	80	2
ONT-177	83	2
ONT-178	95	2
ONT-179	93	8
ONT-180	68	7
ONT-181	85	5
ONT-182	80	5

FOXO1

Levels at 200 nM	(%)	SD
ONT-129	27	1
ONT-130	46	4
ONT-131	53	9

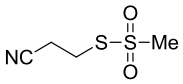
ONT-132	53	2
ONT-133	48	6
ONT-134	35	9
ONT-135	45	15
ONT-136	40	7
ONT-137	50	4
ONT-138	80	3
ONT-139	40	3
ONT-140	33	13
ONT-155	52	2
ONT-156	35	4
ONT-157	39	2
ONT-158	87	6
ONT-159	89	5
ONT-160	33	10
ONT-161	40	11
ONT-162	60	7
ONT-163	42	8
ONT-164	34	10
ONT-165	38	1
ONT-166	62	9
ONT-167	64	1
ONT-168	38	2
ONT-169	67	3
ONT-170	74	8
ONT-171	65	5
ONT-172	33	18
ONT-173	72	15
ONT-174	65	15
ONT-175	38	21
ONT-176	48	8
ONT-177	28	5
ONT-178	97	11
ONT-179	47	6
ONT-180	56	12
ONT-181	45	26
ONT-182	33	17

Hit selection:

ONT-151	d(GsAsAsGsCsTsTs)tsgsgststsgsd(GsGsCsAsAsCsA)
ONT-198	d(CsCsAsTsCsCsAs)asgstscsascsd(TsTsGsGsGsAsG)
ONT-185	d(AsTsGsAsGsAsTs)gscscstsgsgsd(CsTsGsCsCsAsT)
ONT-142	d(AsAsGsCsTsTsTs)gsgststsgsgsd(GsCsAsAsCsAsC)
ONT-145	d(AsTsAsGsCsCsAs)tstsgscsasgsd(CsTsGsCsTsCsA)
ONT-192	d(TsAsTsGsAsGsAs)tsgscscstsgsd(GsCsTsGsCsCsA)
ONT-188	d(TsTsAsTsGsAsGs)astsgscscstsd(GsGsCsTsGsCsC)

Secondary screen. Chemistry and Stereochemistry Screen

Summary for Oligonucleotide Synthesis on a DNA/RNA Synthesizer MerMade-12 (stereodefined phosphorothioate 2'-deoxy and 2'-OMe cycle)

step	reaction	reagent	delivery vol (mL)	wait time (sec)
1	detritylation	3% TCA in DCM	4 x 1	N. A.
2	coupling	0.15 M chiral phosphoramidite in ACN + 2 M CMPT in ACN	2 x 0.5	2 x 450 (2'-OMe RNA) 2 x 300 (DNA)
3	capping 1	5% Pac_2O in THF/2,6-lutidine	1	60
4	capping 2	5% Pac_2O in THF/2,6-lutidine + 16% NMI in THF	1	60
5	sulfurization	0.3 M *S*-(2-cyanoethyl) methylthiosulfonate  in ACN/BSTFA	1	600

Examples applied on the FOXO1 Hit sequences:

Examples include but are not limited to:

(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p)d[GsAsAsGsCsTsTsTsGsGsTsTsGsGsGsCsAsAsCsA]

(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)d[GsAsAsGsCsTsTsTsGsGsTsTsGsGsGsCsAsAsCsA]

(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)d[GsAsAsGsCsTsTsTsGsGsTsTsGsGsGsCsAsAsCsA]

(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)d[CsCsAsTsCsCsAs]<u>(AsGsTsCsAsCs)</u>$_{OMe}$d[TsTsGsGsGsAsG]

(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)d[AsTsGsAsGsAsTs]<u>(GsCsCsTsGsGs)</u>$_{OMe}$d[CsTsGsCsCsAsT]

(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)d[CsCsAsTsCsCsAs]<u>(AsGsTsCsAsCs)</u>$_{LNA}$d[TsTsGsGsGsAsG]

(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)d[AsTsGsAsGsAsTs]<u>(GsCsCsTsGsGs)</u>$_{LNA}$d[CsTsGsCsCsAsT]

(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)d[CsCsAsTsCsCsAs]<u>(AsGsTsCsAsCs)</u>$_{MOE}$d[TsTsGsGsGsAsG]

(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)d[AsTsGsAsGsAsTs]<u>(GsCsCsTsGsGs)</u>$_{MOE}$d[CsTsGsCsCsAsT]

(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *R*p, *S*p, *S*p)<u>(CsCsAs)</u>$_{OMe}$d[TsCsCsAsAsGsTsCsAsCsTsTsGsGs]<u>(GsAsG)</u>$_{OMe}$

(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *R*p, *S*p, *S*p)(AsTsGs)$_{MOE}$d[AsGsAsTsGsCsCsTsGsGsCsTsGsCs](CsAsT)$_{MOE}$

(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)(CsCsAs)$_{LNA}$d[TsCsCsAsAsGsTsCsAsCsTsTsGsGs](GsAsG)$_{LNA}$

(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)(AsTsGs)$_{OMe}$d[AsGsAsTsGsCsCsTsGsGsCsTsGsCs](CsAsT)$_{OMe}$

(*S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)d[GsAsAsGsCsTsTsTsGsGsTsTsGsGsGsCsAsAsCsA]

(*S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p)d[GsAsAsGsCsTsTsTsGsGsTsTsGsGsGsCsAsAsCsA]

(*S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p,
*S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]
(*S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p,
*S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]
(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p,
*S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]
(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p,
*S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]
(*S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p,
*S*p)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]
(*S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p,
*S*p)(CsCs)$_{OMe}$d[AsTsCsCsAsAs](GsTsCs)$_{OMe}$d[AsCsTsTsGsGsGs](AsG)$_{OMe}$
(*S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)
(CsCs)$_{LNA}$d[AsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGs](AsG)$_{LNA}$
(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)
(CsCs)$_{MOE}$d[AsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGs](AsG)$_{MOE}$
(*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)
(CsCs)$_{OMe}$d[AsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGs](AsG)$_{OMe}$

Example 4. Suppression of nucleic acid polymer

[00807] Among other things, the present invention provides chirally controlled oligonucleotide compositions and methods thereof that deliver unexpected results when, *e.g.*, used for suppressing nucleic acid polymers through, in some cases, cleavage of such nucleic acid polymers. Examples include but are not limited to those presented herein.

[00808] RNase H Assay

[00809] Cleavage rate of nucleic acid polymers by nucleases, for example, RNA by RNase H, is important with respect to the use of oligonucleotides in therapeutic technologies such as antisense technology. Using our assay, we investigated the cleavage rates and analyzed the metabolites for chirally controlled oligonucleotide compositions of particular oligonucleotide types (*P*-diastereomers) when oligonucleotides of the particular oligonucleotide types are bound

to complementary RNA. Results below also illustrate the importance of cleavage patterns recognized by the present invention.

[00810] RNase H used herein is a ubiquitously expressed endonuclease that hydrolyses the RNA strand of a RNA/DNA hybrid. It plays an important role in the mode of action of antisense oligonucleotides. In some embodiments, RNase H cleavage rate is significantly reduced when the RNA substrate is structured (Lima, W. F., Venkatraman, M., Crooke , S.T. The Influence of Antisense Oligonucleotide-induced RNA Structure on Escherichia coli RNase H1 Activity *The Journal Of Biological Chemistry* **272**, No. 29, 18191–18199, (1997)). Furthermore, the 2'-MOE gapmer designs (5-10-5) offer higher affinities for RNA targets leading to minimal turnover of the antisense strand. Presence of 2'-MOE modifications in the wings also reduce the number of RNase H cleavage sites.

[00811] To study the RNA cleavage rate, the present invention provides a simple assay to quantify the length of RNA remaining after incubation with RNase H. The provided method, among other things, provides the relative rates of RNase H cleavage for stereorandom 2'-modified gapmers, stereorandom DNA oligonucleotide compositions and chirally pure *P*-diastereomers (chirally controlled oligonucleotide compositions of a corresponding oligonucleotide type) for various oligomers for different targets. Changing the stereochemistry at 2'-modified regions and the DNA core provides information with respect to how stereochemistry in these regions affects the interaction of RNase H to its substrates. RNase H reaction mixtures at different time points were analyzed by LCMS to determine the cleavage pattern. The present invention, among other things, provides nucleic acid polymer, for example RNA, cleavage rates and cleavage patterns (maps) that are critical to design stereochemical nucleic acid architectures for optimal activity, *e.g.*, antisense activity.

[00812] **Equipment :**

[00813] Alliance HPLC, 2489 – TUV, 2695E - Equipped with autosampler

[00814] Cary100 (Agilent Technologies)

[00815] **Methods:**

[00816] **DNA/RNA Duplex Preparation**: Oligonucleotide concentrations were determined by measuring the absorbance in water at 260nm. DNA/RNA duplexes were prepared by mixing equimolar solutions oligonucleotides with each strand concentration of 10uM. The

mixtures were heated at 90°C for 2 minutes in water bath and were cooled down slowly over several hours.

[00817] **Human RNase H Protein Expression and Purification**: Human RNase HC clone was obtained from Prof. Wei Yang's laboratory at NIH Bethesda. The protocol for obtaining this human RNase HC (residues 136-286) has been described (Nowotny, M. et al. Structure of Human RNase H1 Complexed with an RNA/DNA Hybrid: Insight into HIV Reverse Transcription. *Molecular Cell* 28, 264–276, (2007). The protein expression was carried out by following reported protocol with the exception that the resulting protein had an N-terminal His6 tag. BL21(DE3) *E.coli* cells in LB medium were used for protein expression. Cells were grown at 37°C till OD600 reached around 0.7. The cultures were then cooled and 0.4 mM IPTG was added to induce protein expression overnight at 16°C. *E.coli* extract was prepared by sonication in buffer A (40 mM NaH_2PO_4 (pH 7.0), 1 M NaCl, 5% glycerol, 2.8 mM β-mercaptoethanol and 10 mM imidazole) with the addition of protease inhibitors (Sigma-Aldrich). The extract was purified by Ni affinity column using buffer A plus 60 mM imidazole. The protein was eluted with a linear gradient of 60 to 300 mM imidazole. The protein peak was collected and was further purified on a Mono S column (GE Healthcare) with a 100 mM - 500mM gradient of NaCl in buffer B. Fractions containing RNase HC were concentrated to 0.3 mg/ml in the storage buffer (20 mM HEPES (pH 7.0), 100mM NaCl, 5% glycerol, 0.5 mM EDTA, 2 mM DTT) and stored at -20°C. 0.3mg/ml enzyme concentration corresponds to 17.4uM based on its reported extinction coefficient (32095 $cm^{-1}M^{-1}$) and MW (18963.3 Da units).

[00818] **RNase H assay**: In a 96-well plate, to 25 μL DNA/RNA duplex (10 μM) was added 5 μL of 10X RNase H buffer followed by 15 μL water. The mixture was incubated at 37°C for a few minutes and then 5 μL of 0.1 μM stock solution of enzyme was added to give total volume of 50 μL with final substrate/enzyme concentration 5 μM/0.01 μM (500:1) and was further incubated at 37°C. Various ratios of the DNA/RNA duplex: RNase H protein were studied using these conditions to find an optimal ratio to study the kinetics. The reactions were quenched at different time points using 10 μL of 500 mM EDTA disodium solution in water. For zero min time point, EDTA was added to the reaction mixture before the addition of enzyme. Controls were run to ensure that EDTA was able to successfully inhibit the enzyme activity completely. After all the reactions were quenched 10 μL of each reaction mixture was injected on to analytical HPLC column (XBridge C18, 3.5um, 4.6x150mm, Waters Part# 186003034).

Kcat/Km can be measured by a number of methods, such as FRET (Fluorescence Resonance Energy Transfer) dependent RNase H assay using dual labeled RNA and monitored by SpectraMax.

[00819] **Solid Phase Extraction Protocol for sample preparation for LCMS:** 96 well plate (Waters part# 186002321) was used to clean the RNase H reaction mixture before running LCMS. 500 µL of acetonitrile followed by water was used to equilibrate the plate under mild vacuum with the help of manifold (Millipore part# MSV MHTS00). Precaution was taken not to let the plate dry. About 50-100 µL of RNase H reaction mixture was loaded in each well followed by water washings (2 mL) under mild vacuum. 2x500 µL of 70% ACN/Water was used to recover the sample. The recovered samples were transferred to 2 mL centrifuge tubes and were concentrated to dryness in speed vac. Each dry sample was reconstituted in 100 µL water and 10 µL was injected on Acquity UPLC@OST C18 1.7um, 2.1x50mm (part# 186003949) for LCMS analysis.

[00820] For mass spectrometry analysis, the reaction mixtures after quenching were cleaned using C_{18} 96 well plate (Waters). The oligomers were eluted in 70% Acetonitrile/Water. The Acetonitrile was evaporated using speedvac and the resulting residue was reconstituted in water for injection.

Eluent A = 50mM Triethyl ammonium acetate

Eluent B= Acetonitrile

Column Temperature = 60°C

UV was recorded at 254nm and 280nm

RP-HPLC Gradient Method

	Time (min)	**Flow (ml/min)**	**%A**	**%B**	**Curve**
1	0.0	1.00	95.0	5.0	
2	2.00	1.00	95.0	5.0	1
3	22.00	1.00	80.0	20.0	6
4	25.00	1.00	5.0	95.0	6
5	25.5	1.00	95.0	5.0	1
6	30	1.00	95.0	5.0	1

[00821] On HPLC chromatograms, peak areas corresponding to full length RNA oligomer (ONT-28) were integrated, normalized using the DNA peak and were plotted against time (Figure 8). ONT-87 demonstrated superior cleavage for complementary RNA when in duplex form, in comparison to the other product candidates and Mipomersen. Since all the diastereomers in this panel have 2'-MOE modified wings that do not activate RNase H enzyme, without the intention to be limited by theory, Applicant notes that activity is likely dictated by the stereochemistry in the DNA core. Heteroduplexes with ONT-77 to ONT-81 including Mipomersen in the antisense strand show very similar RNA cleavage rates. ONT-89 with alternating Sp/Rp stereochemistry showed the least activity in the tested time frame under the tested conditions. Among the tested oligonucleotides with MOE modifications, ONT-87 and ONT-88 units in the antisense strand exhibited increased in activity in comparison to the rest of the heteroduplexes. Particularly, ONT-87 provided surprisingly high cleavage rate and unexpected low level of remaining target RNA. Additional exemplary data were illustrated in Figure 6 and Figure 24.

[00822] ***In Vitro*** **Oligonucleotide Transfection Assay:** Transfection assays are widely known and practiced by persons having ordinary skill in the art. An exemplary protocol is described herein. Hep3B cells are reverse transfected with Lipofectamine 2000 (Life Technologies, Cat. No. 11668-019) at 18×10^3 cells/well density in 96-well plates using the manufacturer's protocol. For dose response curves eight 1/3 serial dilutions are used starting from 60-100 nM. 25 μL of 6x oligonucleotide concentration is mixed with a prepared mixture of 0.4 μL Lipofectamine 2000 with 25 μL of serum-free medium Opti-MEM medium (Gibco, Cat. No. 31985-062) per well. After a 20min minute incubation, 100 μL of 180×10^3 cells/ml suspended in 10% FBS in DMEM cell culture media (Gibco, Cat. No. 11965-092) is added to bring the final volume to 150 μL per well. 24-48 hours post transfection Hep3B cells are lysed by adding 75 μL of Lysis Mixture with 0.5mg/ml Proteinase K using QuantiGene Sample Processing Kit for Cultured Cells (Affymetrix, Cat. No. QS0103). The Target mRNA and GAPDH mRNA expression levels in cell lysates are measured using Affymetrix QuantiGene 2.0 Assay Kit (Cat. No. QS0011) according to the manufacturer's protocol. The Target mRNA expression is normalized to GAPDH mRNA expression from the same sample; and relative Target/GAPDH levels are compared to transfections using Lipofectamine 2000 only (no oligonucleotide) control. Dose response curves are generated by GraphPad Prism 6 using

nonlinear regression log (inhibitor) vs. response curve fit with variable slope (4 parameters). For exemplary results, see Figure 24, Figure 27 and Figure 29.

Example 5. Provided compositions and methods provide control of cleavage patterns

[00823] The present invention surprisingly found that internucleotidic linkage stereochemistry pattern has unexpected impact on cleavage patterns of nucleic acid polymers. By changing common patterns of backbone chiral centers of chirally controlled oligonucleotide compositions, numbers of cleavage sites, cleavage percentage at a cleavage site, and/or locations of cleavage sits can be surprisingly altered, both independently and in combination. As described in the example herein, provided compositions and methods can provide control over cleavage patterns of nucleic acid polymers.

[00824] Using similar assay conditions, various chirally controlled oligonucleotide compositions of different oligonucleotide types were tested. Exemplary cleavage patterns of the target RNA sequence is presented in Figure 9. Certain pattern of backbone chiral centers, such as that in ONT-87 and ONT-154, surprisingly produces only one cleavage site in the target sequence. Moreover, it is surprisingly found that oligonucleotides providing single cleavage site, such as ONT-87 and ONT-154, provide unexpectedly high cleavage rate and low level of remaining target nucleic acid polymer. See also Figure 8, Figure 10 and Figure 11.

Example 6. Exemplary cleavage of FOXO1 mRNA

[00825] Oligonucleotide compositions targeting different regions of FOXO1 mRNA were tested in cleavage assays as described above. In each case, chirally controlled oligonucleotide compositions were shown to be capable of providing altered cleavage patterns relative to reference cleavage patterns from chirally uncontrolled oligonucleotide compositions sharing the same common base sequence and length. For exemplary results, see Figure 10 and Figure 11. As shown in Figure 12, exemplary chirally controlled oligonucleotide compositions provide both significantly faster cleavage rates and unexpectedly low levels of remaining substrates when compared to reference chirally uncontrolled oligonucleotide compositions. In some embodiments, as shown in Figure 11, the cleavage sites are associated with *R*p*S*p*S*p backbone chiral center sequence. In some embodiments, cleavage sites are two base pairs upstream of *R*p*S*p*S*p.

[00826] Exemplary oligonucleotide compositions are listed below.

Oligo	Sequence	Description	Tm (°C)
ONT-366	dTsdGsdAsdGsdAsdTsdGsdCsdCsdTsdGsdGsdCsdTsdGsdCsdCsdAsdTsdA	All DNA	66.5
ONT-389	dTsdGsdAsdGsdAsdTsdGsdCsdCsdTsdGsdGsdCsdTsdGsdCsdCsdAsdTsdA	S_7RSSRSSRS$_5$	64.3
ONT-390	dTsdGsdAsdGsdAsdTsdGsdCsdCsdTsdGsdGsdCsdTsdGsdCsdCsdAsdTsdA	S_6RSSRSSRS$_6$	64.6
ONT-391	dTsdGsdAsdGsdAsdTsdGsdCsdCsdTsdGsdGsdCsdTsdGsdCsdCsdAsdTsdA	S_5RSSRSSRS$_7$	64.3
ONT-387	rUrArUrGrGrCrArGrCrCrArGrGrCrArUrCrUrCrA	complementary RNA	
ONT-367	dTsdAsdGsdCsdCsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGsdCsdTsdCsdAsdC	All DNA	62.9
ONT-392	dTsdAsdGsdCsdCsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGsdCsdTsdCsdAsdC	S_7RSSRSSRS$_5$	59.5
ONT-393	dTsdAsdGsdCsdCsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGsdCsdTsdCsdAsdC	S_6RSSRSSRS$_6$	60
ONT-394	dTsdAsdGsdCsdCsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGsdCsdTsdCsdAsdC	S_5RSSRSSRS$_7$	59.5
ONT-388	rGrUrGrArGrCrArGrCrUrGrCrArArUrGrGrCrUrA	complementary RNA	

Example 7. Exemplary chirally controlled oligonucleotide compositions provide higher turn-over

[00827] In cases where the Tm of cleaved nucleic acid polymer fragments, for example RNA fragments, to oligonucleotides is greater than a physiological temperature, product dissociation may be inhibited and oligonucleotides may not be able to dissociate and find other target strands to form duplexes and cause the target strands to be cleaved. The Tm of ONT-316 (5-10-5 2'-MOE Gapmer) to complementary RNA is 76°C. After a cut or a few cuts in the RNA sequence complementary to the oligonucleotides, the 2'-MOE fragments may remain bound to RNA and thus cannot cause the other target molecules to be cleaved. Thermal melting temperatures of DNA strands generally are much lower when duplexed to RNA, for example, ONT-367 (63°C) and ONT-392 60°C). Additionally, thermal stability in DNA sequences is

often relatively uniformly distributed compared to 2'-MOE modified oligonucleotides. In some embodiments, oligonucleotides in provided chirally controlled oligonucleotide compositions do not contain 2'-modifications such as 2'-MOE. In some embodiments, oligonucleotides in provided chirally controlled oligonucleotide compositions, which do not contain 2'-modifications such as 2'-MOE, more easily dissociate from nucleic acid polymer cleavage fragments, and have higher turn-over than oligonucleotides having 2'-modifications such as 2'-MOE. In some embodiments, the present invention provides an all DNA designs, in which oligonucleotides do not have 2'-modifications. In some embodiments, chirally controlled oligonucleotide compositions wherein oligonucleotides having no 2'-modification provides higher turn-over of a nuclease such as RNase H. In some embodiments, after cleavage RNase H dissociates more easily from duplex formed by RNA and oligonucleotides of provided chirally controlled oligonucleotide compositions. Using similar protocols as described above, turn-over of two exemplary chirally controlled oligonucleotide compositions of oligonucleotide type ONT-367 and ONT-392 indeed showed higher turn-over rate than reference chirally uncontrolled oligonucleotide compositions (see Figure 13).

Example 8. Exemplary cleavage of FOXO1 mRNA

[00828] As exemplified in Figure 14, chirally controlled oligonucleotide compositions and methods thereof in the present disclosure can provide controlled cleavage of nucleic acid polymers. In some embodiments, chirally controlled oligonucleotide compositions of the present invention produces altered cleavage pattern in terms of number of cleavage sites, location of cleavage sites, and/or relative cleavage percentage of cleavage sites. In some embodiments, as exemplified by ONT-401 and ONT-406, chirally controlled oligonucleotide compositions provide single site cleavage.

[00829] In some embodiments, only one component from RNA cleavage was detected. Without the intention to be limited by theory, Applicant notes that such observation could be due to the processive nature of RNase H enzyme which could make multiple cuts on the same duplex resulting in much shorter 5'-OH 3'-OH fragments.

[00830] Additional chirally controlled oligonucleotide compositions were further tested. As described above, provided chirally controlled oligonucleotide compositions provides unexpected results, for example, in terms of cleavage rate and %RNA remaining in DNA/RNA

duplex. See Figures 15-17. Exemple analytical data were presented in Figures 18-20. Without the intention to be limited by theory, Applicant notes that in some embodiments, cleavage may happen as depicted in Figure 21. In Figure 17, it is noted ONT-406 was observed to elicit cleavage of duplexed RNA at a rate in slight excess of that of the natural DNA oligonucleotide ONT-415 having the same base sequence and length. Applicant notes that chirally controlled oligonucleotide compositions of ONT-406, and other chirally controlled oligonucleotide compositions provided in this disclosure, have other preferred properties that an ONT-415 composition does not have, for example, better stability profiles *in vitro* and/or *in vivo*. Additional exemplary data were presented in Figure 25. Also, as will be appreciated by those skilled in the art, exemplary data illustrated in Figure 26 and Figure 27 confirm that provided exemplary chirally controlled oligonucleotide compositions, especially when so designed to control the cleavage patterns through patterns of backbone chiral centers, produced much better results than reference oligonucleotide compositions, *e.g.*, a stereorandom oligonucleotide composition. As exemplified in Figure 26, controlled patterns of backbone chiral centers, among other things, can selectively increase and/or decrease cleavage at existing cleavage site when a DNA oligonucleotide is used, or creates entirely new cleavage sites that do not exist when a DNA oligonucleotide is used (see Figure 25, ONT-415). In some embodiments, cleavage sites from a DNA oligonucleotide indicate endogenous cleavage preference of RNase H. As confirmed by Figure 27, provided chirally controlled oligonucleotide compositions are capable of modulating target cleavage rate. In some embodiments, approximately 75% of the variance in cellular activity is accounted for by differences in cleavage rate which can be controlled through patterns of backbone chiral centers. As provided in this Application, further structural features such as base modifications and their patterns, sugar modification and their patterns, internucleotidic linkage modifications and their patterns, and/or any combinations thereof, can be combined with patterns of backbone chiral centers to provide desired oligonucleotide properties.

Example 9. Exemplary allele-specific suppression of mHTT

[00831] In some embodiments, the present invention provides chirally controlled oligonucleotide compositions and methods thereof for allele-specific suppression of transcripts from one particular allele with selectivity over the others. In some embodiments, the present invention provides allele-specific suppression of mHTT.

[00832] Figure 22 illustrates exemplary chirally controlled oligonucleotide compositions that specifically suppress transcripts from one allele but not the others. Oligonucleotides 451 and 452 were tested with transcripts from both exemplified alleles using biochemical assays described above. Allele-specific suppression is also tested in cells and animal models using similar procedures as described in Hohjoh, *Pharmaceuticals* **2013**, *6*, 522-535; US patent application publication US 2013/0197061; and Østergaard *et al*., *Nucleic Acids Research*, **2013**, *41*(21), 9634–9650. In all cases, transcripts from the target allele are selectively suppressed over those from the other alleles. As will be appreciated by those skilled in the art, exemplary data illustrated in Figure 22 confirm that provided exemplary chirally controlled oligonucleotide compositions, especially when so designed to control the cleavage patterns through stereochemistry, produced much better results than reference oligonucleotide compositions, in this case, a stereorandom oligonucleotide composition. As confirmed by Figure 22, patterns of backbone chiral centers can dramatically change cleavage patterns (Figure 22 C-E), and stereochemistry patterns can be employed to position cleavage site at the mismatch site(Figure 22 C-E), and/or can dramatically improve selectivity between the mutant and wild type (Figure 22 G-H). In some embodiments, chirally controlled oligonucleotide compositions are incubated with wtRNA and muRNA of a target and both the duplexes are incubated with RNase H.

[00833] Huntingtin Allele Tm

Mutant Huntingtin Allele ONT-453/ONT-451	38.8 °C
Wild Type Huntingtin Allele ONT-454/ONT-451	37.3 °C
Mutant Huntingtin Allele ONT-453/ONT-452	38.8 °C
Wild Type Huntingtin Allele ONT-454/ONT-452	36.5 °C
Mutant Huntingtin Allele ONT-453/ONT-450	40.3 °C
Wild Type Huntingtin Allele ONT-454/ONT-450	38.8 °C

Example 10. Exemplary allele-specific suppression of FOXO1

[00834] In some embodiments, the present invention provides allele-specific suppression of FOXO1.

[00835] Figure 23 illustrates exemplary chirally controlled oligonucleotide compositions that specifically suppress transcripts from one allele but not the others. Oligonucleotides ONT-400, ONT-402 and ONT-406 were tested with transcripts from both exemplified alleles using

biochemical assays described above. Allele-specific suppression is also tested in cells and animal models using similar procedures as described in Hohjoh, *Pharmaceuticals* **2013**, *6*, 522-535; US patent application publication US 2013/0197061; Østergaard *et al*., *Nucleic Acids Research* **2013**, *41*(21), 9634–9650; and Jiang *et al.*, *Science* **2013**, *342*, 111-114. Transcripts from the target allele are selectively suppressed over those from the other alleles. In some cases, two RNAs with mismatch ONT-442 (A/G, position 7th) and ONT-443 (A/G, position 13th) from ONT-388 are synthesized and are duplexed with ONT-396 to ONT-414. RNase H assay are performed to obtain cleavage rates and cleavage maps.

Example 11. Certain exemplary oligonucleotides and oligonucleotide compositions

[00836] Stereorandom oligonucleotides with different 2' substitution chemistries targeting three distinct regions of FOXO1 mRNA with the thermal melting temperatures when duplexed with complementary RNA. The concentration of each strand was 1uM in 1X PBS buffer.

Oligo	Sequence	Description	Tm(°C)
ONT-316	TeosAeosGeos5mCeos5mCeosdAsdTsdTsdGs5mdCsdAsdGs5mdCsdTsdGs5mCeosTeos5mCeosAeos5mCeo	5-10-5 (2'-MOE Gapmer)	76.7
ONT-355	dTsdAsdGsdCsdCsdAsdTstsgscsasgscsdTsdGsdCsdTsdCsdAsdC	7-6-7 (DNA-2'-OMe-DNA) Gapmer	71.2
ONT-361	tsasgsdCsdCsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGsdCsdTscsascs	3-14-3 (2'-OMe-DNA-2'-OMe) Gapmer	65.8
ONT-367	dTsdAsdGsdCsdCsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGsdCsdTsdCsdAsdC	All DNA	62.9
ONT-373	tsasgscscsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGscstscsasc	5-10-5 (2'-OMe Gapmer)	71.8
ONT-388	rGrUrGrArGrCrArGrCrUrGrCrArArUrGrGrCrUrA	Complementary RNA	
ONT-302	Teos5mCeos5mCeosAeosGeosdTsdTs5mdCs5mdCsdTsdTs5mdCsdAsdTsdTs5mCeosTeosGeos5mCeosAeo	5-10-5 (2'-MOE Gapmer)	72.5
ONT-352	dTsdCsdCsdAsdGsdTsdTscscststscsasdTsdTsdCsdTsdGsdCsdA	7-6-7 (DNA-2'-OMe-DNA) Gapmer	65.4
ONT-358	tscscsdAsdGsdTsdTsdCsdCsdTsdTsdCsdAsdTsdTsdCsdTsgscsas	3-14-3 (2'-OMe-DNA-2'-OMe) Gapmer	62.6
ONT-364	dTsdCsdCsdAsdGsdTsdTsdCsdCsdTsdTsdCsdAsdTsdTsdCsdTsdGsdCsdA	All DNA	58.4

Oligo	Sequence	Description	Tm(°C)
ONT-370	tscscsasgsdTsdTsdCsdCsdTsdTsdCsdAsdTsdTscstsgscsa	5-10-5 (2'-OMe Gapmer)	68
ONT-386	rUrGrCrArGrArArUrGrArArGrGrArArCrUrGrGrA	Complementary RNA	
ONT-315	TeosGeosAeosGeosAeosdTsdGs5mdCs5mdCsdTsdGsdGs5mdCsdTsdGs5mCeos5mCeosAeosTeosAeo	5-10-5 (2'-MOE Gapmer)	77.5
ONT-354	dTsdGsdAsdGsdAsdTsdGscscstsgsgscsdTsdGsdCsdCsdAsdTsdA	7-6-7 (DNA-2'-OMe-DNA) Gapmer	75.5
ONT-360	tsgsasdGsdAsdTsdGsdCsdCsdTsdGsdGsdCsdTsdGsdCsdCsastsas	3-14-3 (2'-OMe-DNA-2'-OMe) Gapmer	69
ONT-366	dTsdGsdAsdGsdAsdTsdGsdCsdCsdTsdGsdGsdCsdTsdGsdCsdCsdAsdTsdA	All DNA	66.5
ONT-372	tsgsasgsasdTsdGsdCsdCsdTsdGsdGsdCsdTsdGscscsastsa	5-10-5 (2'-OMe Gapmer)	74.4
ONT-387	rUrArUrGrGrCrArGrCrCrArGrGrCrArUrCrUrCrA	Complementary RNA	

[00837] Additional exemplary stereorandom oligonucleotide compositions are listed below.

Oligo	Sequence (5′ to 3′)
ONT-41	$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$
ONT-70	$(Gs5mCs)_{MOE}$d[GsTsTsTsGs5mCsTs5mCsTsTs5mCsTsTs](5mCsTsTsGs5mCGs)$_{MOE}$d[TsTsTsTs](TsT)$_{MOE}$
ONT-83	$(GsTs5mCs5mCs5mCs)_{MOE}$d(TsGsAsAsGsAsTsGsTs5mCs](AsAsTsGs5mC)$_{MOE}$
ONT-302	$(Ts5mCs5mCsAsGs)_{MOE}$d[TsTs5mCs5mCsTsTs5mCsAsTsTs](5mCsTsGs5mCsA)$_{MOE}$
ONT-315	$(TsGsAsGsAs)_{MOE}$d[TsGs5mCs5mCsTsGsGs5mCsTsGs](5mCs5mCsAsTsA)$_{MOE}$
ONT-316	$(TsAsGs5mCs5mCs)_{MOE}$d[AsTsTsGs5mCsAsGs5mCsTsGs5m](CsTs5mCsAs5mC)$_{MOE}$
ONT-352	[TsCsCsAsGsTsTs](cscststscsas)$_{OMe}$d[TsTsCsTsGsCsA]
ONT-354	[TsGsAsGsAsTsGs](CsCsTsGsGsCs)$_{OMe}$d[TsGsCsCsAsTsA]
ONT-355	[TsAsGsCsCsAsTs](TsGsCsAsGsCs)$_{OMe}$d[TsGsCsTsCsAsC]
ONT-358	$(TsCsCs)_{OMe}$d[AsGsTsTsCsCsTsTsCsAsTsTsCsTs](GsCsA)$_{OMe}$
ONT-360	$(TsGsAs)_{OMe}$d[GsAsTsGsCsCsTsGsGsCsTsGsCsCs](AsTsA)$_{OMe}$
ONT-361	$(TsAsGs)_{OMe}$d[CsCsAsTsTsGsCsAsGsCsTsGsCsTs](CsAsC)$_{OMe}$

Oligo	Sequence (5′ to 3′)
ONT-364	[TsCsCsAsGsTsTsCsCsTsTsCsAsTsTsCsTsGsCsA]
ONT-366	[TsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsTsA]
ONT-367	[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]
ONT-370	$(TsCsCsAsGs)_{OMe}$d[TsTsCsCsTsTsCsAsTsTs]$(CsTsGsCsA)_{OMe}$
ONT-372	$(TsGsAsGsAs)_{OMe}$d[TsGsCsCsTsGsGsCsTsGs]$(CsCsAsTsA)_{OMe}$
ONT-373	$(TsAsGsCsCs)_{OMe}$d[AsTsTsGsCsAsGsCsTsGs]$(CsTsCsAsC)_{OMe}$
ONT-440	$(UsAsGsCsCs)_{F}$d[AsTsTsGsCsAsGsCsTsGsCsTsCsAsC]
ONT-441	$(UsAsGsCsCs)_{F}$d[AsTsTsGsCsAsGsCsTsGsC]
ONT-460	$(TsAsGsCsCs)_{OMe}$d[AsTsTsGsCsAsGsCsTsGsCsTsCsAsC]
ONT-450	[AsTsTsAsAsTsAsAsAsTsTsGsTsCsAsTsCsAsCsC]

[00838] Exemplary RNA and DNA oligonucleotides are listed below.

Oligo	Sequence (5′ to 3′)
ONT-28	rGrGrUrGrCrGrArArGrCrArGrArCrUrGrArGrGrC
ONT-386	rUrGrCrArGrArArUrGrArArGrGrArArCrUrGrGrA
ONT-387	rUrArUrGrGrCrArGrCrCrArGrGrCrArUrCrUrCrA
ONT-388	rGrUrGrArGrCrArGrCrUrGrCrArArUrGrGrCrUrA
ONT-415	d[TAGCCATTGCAGCTGCTCAC]
ONT-442	rGrUrGrArGrCrGrGrCrUrGrCrArArUrGrGrCrUrA
ONT-443	rGrUrGrArGrCrArGrCrUrGrCrGrArUrGrGrCrUrA
ONT-453	rGrGrUrGrArUrGrArCrArArUrUrUrArUrUrArArU
ONT-454	rGrGrUrGrArUrGrGrCrArArUrUrUrArUrUrArArU

[00839] Exemplary chirally pure oligonucleotides are presented below. In some embodiments, the present invention provides corresponding chirally controlled oligonucleotide compositions of each of the following exemplary oligonucleotides.

Oligo	Stereochemistry/Sequence (5′ to 3′)	Description
ONT-389	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsTsA]	7S-$(RSS)_3$-3S
ONT-390	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsTsA]	6S-$(RSS)_3$-4S
ONT-391	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsTsA]	5S-$(RSS)_3$-5S
ONT-392	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	7S-$(RSS)_3$-3S
ONT-393	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	6S-$(RSS)_3$-4S

Oligo	Stereochemistry/Sequence (5′ to 3′)	Description
ONT-394	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	5S-(RSS)$_3$-5S
ONT-396	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	18S-1R
ONT-397	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	17S-RS
ONT-398	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	16S-(RSS)
ONT-399	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	15S-(RSS)-1S
ONT-400	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	14S-(RSS)-2S
ONT-401	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	13S-(RSS)-3S
ONT-402	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	12S-(RSS)-4S
ONT-403	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	11S-(RSS)-5S
ONT-404	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	10S-(RSS)-6S
ONT-405	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	9S-(RSS)-7S
ONT-406	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	8S-(RSS)-8S
ONT-407	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	7S-(RSS)-9S
ONT-408	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	6S-(RSS)-10S
ONT-409	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	5S-(RSS)-11S
ONT-410	(*S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	4S-(RSS)-12S
ONT-411	(*S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	3S-(RSS)-13S
ONT-412	(*S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	2S-(RSS)-14S
ONT-413	(*S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	S-(RSS)-15S
ONT-414	(*R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	(RSS)-16S
ONT-421	All-(*S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	All S
ONT-422	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)-C6-amino-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	8S-(RSS)-3S-(RSS)-2S
ONT-455	All-(*R*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	All R

Oligo	Stereochemistry/Sequence (5′ to 3′)	Description
ONT-451	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[AsTsTsAsAsTsAsAsAsTsTsGsTsCsAsTsCsAsCsC]	13S-(RSS)-3S
ONT-452	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p)-d[AsTsTsAsAsTsAsAsAsTsTsGsTsCsAsTsCsAsCsC]	14S-(RSS)-2S
ONT-75	All-(*R*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs 5mCsTsTs5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	All R
ONT-76	(*S*p, *R*p, *R*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *R*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCs TsTs5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	SRRSR-11S-RSR
ONT-77	(*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTs Ts5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	5R-10S-4R
ONT-80	All-(*S*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTs Ts5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	All S
ONT-81	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCs TsTs5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	5S-10R-4S
ONT-82	All-(*R*p)-(GsTs5mCs5mCs5mCs)$_{MOE}$d[TsGsAsAsGsAsTsGsTs 5mCs](AsAsTsGs5mC)$_{MOE}$	All R
ONT-84	All-(*S*p)-(GsTs5mCs5mCs5mCs)$_{MOE}$d[TsGsAsAsGsAsTsGsTs 5mCs](AsAsTsGs5mC)$_{MOE}$	All S
ONT-85	(*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p)-(GsTs5mCs5mCs5mCs)$_{MOE}$d[TsGsAsAsGsAsTsGsTs 5mCs](AsAsTsGs5mC)$_{MOE}$	5R-10S-4R
ONT-86	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p)-(GsTs5mCs5mCs5mCs)$_{MOE}$d[TsGsAsAsGsAsTsGsTs 5mCs](AsAsTsGs5mC)$_{MOE}$	5S-10R-4S
ONT-87	(*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCs TsTs5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	5R-2S-(RSS)$_2$-6R
ONT-88	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTs Ts5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	5S-(RRS)$_3$-5S
ONT-89	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCs TsTs5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	(SR)$_9$S
ONT-154	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[Gs5mCs5mCsTs5mCsAsGsTs5mCsTsGs5mCsTsTs 5mCsGs5mCsAs5mCs5mC]	7S-(RSS)$_3$-3S
ONT-75	All-(*R*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCs TsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	All-R
ONT-80	All-(*S*p)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCs TsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	All-S

[00840] Additonal exemplary oligonucleotides targeting FOXO1 with Tm are presented below. In some embodiments, the present invention provides corresponding chirally controlled oligonucleotide compositions of each of the following exemplary oligonucleotides.

Oligo	Sequence (5' to 3')	Tm (°C)
ONT-439	$[UsAsGs]_F$d[CsCsAsTsTsGsCsAsGsCsTsGsCsTs][$CsAsC]_F$	68.3
ONT-440	$[UsAsGsCsCs]_F$d[AsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	70.0
ONT-441	$[UsAsGsCsCs]_F$d[AsTsTsGsCsAsGsCsTsGsC]	65.5
ONT-455	All-(*R*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	66.8
ONT-316	$[TsAsGs5mCs5mCs]_{MOE}$d[AsTsTsGs5mCsAsGs5mCsTsGs][5mCsTs5mCsAs5mC]$_{MOE}$	76.9
ONT-367	d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	62.8
ONT-415	d[TAGCCATTGCAGCTGCTCAC]	72.6
ONT-416	$[TsAsGsCsCsAsTsTsGsCsAsGsCs]_{OMe}$d[TsGsCsTsCsAsC]	78.4
ONT-421	All-(*S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	59.2
ONT-394	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	60.0
ONT-406	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	58.5

Example 12. Exemplary additional controlled cleavage by provided chirally controlled oligonucleotide compositions

[00841] As will be appreciated by those skilled in the art, exemplary data illustrated in Figure 26 confirm that provided chirally controlled oligonucleotide compositions and methods thereof provided unexpected results compared to reference compositions, such as stereorandom oligonucleotide compositions. Among other things, chirally controlled oligonucleotide compositions can produce controlled cleavage patterns, including but not limited to controlling of positions of cleavage sites, numbers of cleavage sites, and relative cleavage percentage of cleavage sites. See also exemplary data presented in Figure 27.

Example 13. Stability of chirally controlled oligonucleotide compositions

[00842] As will be appreciated by those skilled in the art, exemplary data illustrated in Figure 26 confirm that stability of provided chirally controlled oligonucleotide compositions can be adjusted by varying patterns of backbone chiral centers. For exemplary data, see Figure 7 and Figure 28. An exemplary protocol for performing serum stability experiment is described below.

[00843] **Protocol:** P-stereochemically pure PS DNA (ONT-396-ONT-414 (single Rp walk from 3'end to 5'end)), stereorandom PS DNA (ONT-367), all-Sp PS DNA (ONT-421) and all-Rp PS DNA (ONT-455) were incubated in Rat serum (Sigma, R9759) (0 h and 48 h) and analyzed by IEX-HPLC.

[00844] **Incubation Method:** 5 µL of 250 µM of each DNA solutions and 45 µL of Rat serum were mixed and incubated at 37 °C for each time points (0 h and 48 h). At each time points, reaction was stopped by adding 25 µL of 150 mM EDTA solution, 30 µL of Lysis buffer (erpicentre, MTC096H) and 3 µL of Proteinase K solution (20 mg/mL). The mixture was incubated at 60 °C for 20 min then 20 µL of the mixture was injected to IEX-HPLC and analyzed.

[00845] **Incubation Control Sample:** Mixture of 5 µL of 250 µM of each DNA solutions and 103 µL of 1x PBS buffer were prepared and 20 µL of the mixture was analyzed by IEX-HPLC as controls in order to check the absolute quantification.

[00846] **Exemplary Analytical Method:**

IEx-HPLC

A: 10 mM TrisHCl, 50%ACN (pH 8.0)

B: 10 mM TrisHCl, 800 mM NaCl, 50%ACN (pH 8.0)

C: Water-ACN (1:1, v/v)

Temp : 60 °C

Column: DIONEX DNAPac PA-100, 250x4 mm

Gradient:

	Time	Flow	%A	%B	%C	%D	Curve
1	0.00	1.00	95.0	5.0	0.0	0.0	6
2	1.00	1.00	95.0	5.0	0.0	0.0	1
3	2.00	1.00	75.0	25.0	0.0	0.0	6
4	10.00	1.00	5.0	95.0	0.0	0.0	6
5	10.10	1.00	95.0	5.0	0.0	0.0	6
6	12.50	1.00	95.0	5.0	0.0	0.0	1

Washing:

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	1.00	0.0	0.0	100.0	0.0	6
2	5.50	1.00	0.0	0.0	100.0	0.0	1
3	5.60	1.00	0.0	100.0	0.0	0.0	6
4	7.50	1.00	0.0	100.0	0.0	0.0	1
5	7.60	1.00	95.0	5.0	0.0	0.0	6
6	12.50	1.00	95.0	5.0	0.0	0.0	1

Column Temperature: 60 ^{o}C.

Washing was performed every after the sample run.

Percentage of remained PS DNA was calculated by the analysis of the ratio from the 0 h to 48 h using the area of integration of HPLC chromatogram.

Example 14. Exemplary analytical results (Figure 19)

[00847] Peak assignments for Fig. 19 (Top panel, M12-Exp11 B10, ONT-354, 30min)

Retention time (minutes)	$(M-2)^{2-}$	$(M-3)^{3-}$	$(M-4)^{4-}$	$(M-5)^{5-}$	$(M-6)^{6-}$
2.34	1100.6	733.7			
11.91		1390.6	1042.6		
13.07		1500.08	1125.5		750.73
		1805.29	1354.19		
13.58		1603.39	1202.2	961.35	801.15
14.80			1589.9	1271.4	1059.5
18.59			1653.3	1323.3	1101.6

Retention time (minutes)	Observed MW	Assignment based on mass match		
		5'-p-RNA fragment	3'-OH and 5'-OH, RNA	DNA
2.34	2203.2		7mer	
11.91	4176	13mer		
13.07	4505.7	14mer		
	5418.87		17mer	
13.58	4812.8	15mer		
14.80	6362.5		20mer, ONT-387	
18.59	6615.4			ONT-354

[00848] Peak assignments for Fig. 19 (Bottom panel, M12-Exp11 A10, ONT-315, 30min)

Retention time	$(M-2)^{2-}$	$(M-3)^{3-}$	$(M-4)^{4-}$	$(M-5)^{5-}$	$(M-6)^{6-}$

(minutes)					
4.01	1425.33	950.15			
4.4	1100.83	733.69			
4.94	1578.34	1051.54			
6.21	1741.91	1161.89	870.37		
	1445.42	963.31	722.97		
8.48	1610	1073.3			
9.15		1391.2	1043.1		
9.93	1763.4	1174.7			
11.8		1602.3	1201.7		
14.82					
20.73			1809.94	1447.82	1205.9

Retention time (minutes)	Observed MW	Assignment based on mass match		
		5'-p-RNA fragment	3'-OH and 5'-OH, RNA	DNA
4.01	2853.45		9mer	
4.4	2203.66	7mer		
4.94	3158.47		10mer	
6.21	3487.52		11mer	
	2892.84	9mer		
8.48	3220.94	10mer		
9.15	4177		13mer	
9.93	3528.88	11mer		
11.8	4810		15mer	
14.82			20mer, ONT-387	
20.73	7244.3			ONT-315

Example 15. Exemplary analytical results (Figure 30)

[00849] Peak assignments for Fig. 30 (Top panel, M12-Exp11 D2, ONT-367, 30min)

Retention time (minutes)	$(M-2)^{2-}$	$(M-3)^{3-}$	$(M-4)^{4-}$	$(M-5)^{5-}$	$(M-6)^{6-}$
2.36	1120.28	746.25			
3.15	1292.41	861.32			
4.04	975.92				
4.49	1140.6	759.78			
5.83	1305.21	869.65	652.31		
6.88	1923.23	1281.69	961.28		
9.32		1390.76	1043.29	833.72	
9.96	1783.85	1187.98	891.6	712.94	
11.01	1936.14	1289.93			
		1501.52	1125.4	899.89	

11.93		1405.25	1053.78	842.84	
13.15		1514.72	1135.72		
14.81			1609.95	1287.53	1072.58
18.33			1587.9	1270.2	1058.3

Retention time (minutes)	Observed MW	Assignment based on mass match		
		5'-p-RNA fragment	3'-OH and 5'-OH, RNA	DNA
2.36	2242.56		7mer	
3.15	2586.82		8mer	
4.04	1953.84	6mer		
4.49	2283.2	7mer		
5.83	2612.42	8mer		
6.88	3849.14		12mer	
9.32	4175.28		13mer	
9.96	3569.7	11mer		
11.01	3874.28	12mer		
	4507.56		14mer	
11.93	4218.75	13mer		
13.15	4547.16	14mer		
14.81	6441.8		20mer, ONT-388	
18.33	6355.6			ONT-367

[00850] Peak assignments for Fig. 30 (Bottom panel, M12-Exp21 NM Plate1 (pool) F11 ONT-406 30min

Retention time (minutes)	$(M-2)^{2-}$	$(M-3)^{3-}$	$(M-4)^{4-}$	$(M-5)^{5-}$	$(M-6)^{6-}$
4.72	1140.6	759.78			
9.46		1390.76	1043.29	833.72	
16.45			1609.95	1287.53	1072.58
19.48			1588.1	1270.4	1058.4

Retention time (minutes)	Observed MW	Assignment based on mass match		
		5'-p-RNA fragment	3'-OH and 5'-OH, RNA	DNA
4.72	2203.2	2283.2	7mer	
9.46	4176	4175.28		13mer
16.45	6362.5	6441.8		20mer, ONT-388
19.48	6615.4	6355.9		

Equivalents

[00851] Having described some illustrative embodiments of the invention, it should be apparent to those skilled in the art that the foregoing is merely illustrative and not limiting, having been presented by way of example only. Numerous modifications and other illustrative embodiments are within the scope of one of ordinary skill in the art and are contemplated as falling within the scope of the invention. In particular, although many of the examples presented herein involve specific combinations of method acts or system elements, it should be understood that those acts and those elements may be combined in other ways to accomplish the same objectives. Acts, elements, and features discussed only in connection with one embodiment are not intended to be excluded from a similar role in other embodiments. Further, for the one or more means-plus-function limitations recited in the following claims, the means are not intended to be limited to the means disclosed herein for performing the recited function, but are intended to cover in scope any means, known now or later developed, for performing the recited function.

[00852] Use of ordinal terms such as "first", "second", "third", *etc*., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements. Similarly, use of a), b), *etc*., or i), ii), *etc*. does not by itself connote any priority, precedence, or order of steps in the claims. Similarly, the use of these terms in the specification does not by itself connote any required priority, precedence, or order.

[00853] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by examples provided, since the examples are intended as a single illustration of one aspect of the invention and other functionally equivalent embodiments are within the scope of the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The advantages and objects of the invention are not necessarily encompassed by each embodiment of the invention.

Claims

1. A chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

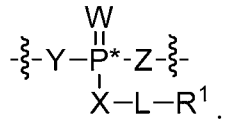
3) a common pattern of backbone chiral centers,

which composition is a substantially pure preparation of a single oligonucleotide in that at least about 10% of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers; wherein:

the common base sequence has at least 17 bases; or

the single oligonucleotide comprises 11 or more chiral, modified phosphate linkages.

2. The composition of claim 1, wherein each chiral, modified phosphate linkage independent has the structure of formula **I**:



(I)

wherein:

P* is an asymmetric phosphorus atom and is either *R*p or *S*p;

W is O, S or Se;

each of X, Y and Z is independently –O–, –S–, –N(–L–R^1)–, or L;

L is a covalent bond or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

R^1 is halogen, R, or an optionally substituted C_1–C_{50} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene,

C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–;

each R′ is independently –R, -C(O)R, -CO_2R, or –SO_2R, or:

two R′ on the same nitrogen are taken together with their intervening atoms to form an optionally substituted heterocyclic or heteroaryl ring, or

two R′ on the same carbon are taken together with their intervening atoms to form an optionally substituted aryl, carbocyclic, heterocyclic, or heteroaryl ring;

–Cy– is an optionally substituted bivalent ring selected from phenylene, carbocyclylene, arylene, heteroarylene, or heterocyclylene;

each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl; and

each —§— independently represents a connection to a nucleoside.

3. The composition of claim 2, wherein each chiral, modified phosphate linkage is a phosphorothioate linkage.

4. The composition of claim 2, wherein X is −S− and −L−R^1 is not hydrogen.

5. The composition of any one of claims 1-4, wherein the common pattern of backbone chiral centers comprises from 5’ to 3’ (*R*p)n(*S*p)m, wherein m is 2, 3, 4, 5, 6, 7 or 8 and n is 1, 2, 3, 4, 5, 6, 7 or 8.

6. The composition of any one of claims 1-4, wherein the common pattern of backbone chiral centers comprises from 5’ to 3’ *R*p(*S*p)m, wherein m is 2, 3, 4, 5, 6, 7 or 8.

7. The composition of any one of claims 1-4, wherein the common pattern of backbone chiral centers comprises from 5’ to 3’ *R*p(*S*p)$_2$.

8. The composition of any one of claims 1-4, wherein the pattern of backbone chiral centers comprises from 5' to 3' (*N*p)t(*R*p)n(*S*p)m, wherein each n and t is independently 1, 2, 3, 4, 5, 6, 7 or 8, m is 2, 3, 4, 5, 6, 7 or 8, and each *N*p is independent *R*p or *S*p.

9. The composition of claim 8, wherein n is 1.

10. The composition of claim 9, wherein t is 2, 3, 4, 5, 6, 7 or 8.

11. The composition of claim 10, wherein m is 2, 3, 4, 5, 6, 7 or 8.

12. The composition of claim 8, wherein at least one of t and m is greater than 5.

13. The composition of any one of claims 1-12, wherein oligonucleotides of the particular oligonucleotide type are antisense oligonucleotide, antagomir, microRNA, pre-microRNs, antimir, supermir, ribozyme, Ul adaptor, RNA activator, RNAi agent, decoy oligonucleotide, triplex forming oligonucleotide, aptamer or adjuvant.

14. A method for controlled cleavage of a nucleic acid polymer, the method comprising steps of:

contacting a nucleic acid polymer whose nucleotide sequence comprises a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a target sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type.

15. The method of claim 14, wherein the cleavage occurs with a cleavage pattern that differs from a reference cleavage pattern observed when the nucleic acid polymer is contacted under comparable conditions with a reference oligonucleotide composition.

16. A method for altering a cleavage pattern observed when a nucleic acid polymer whose nucleotide sequence includes a target sequence is contacted with a reference oligonucleotide composition that comprises oligonucleotides having a particular base sequence and length, which particular base sequence is or comprises a sequence that is complementary to the target sequence, the method comprising:

contacting the nucleic acid polymer with a chirally controlled oligonucleotide composition of oligonucleotides having the particular base sequence and length, which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of a single oligonucleotide type characterized by:

1) the particular base sequence and length;

2) a particular pattern of backbone linkages; and

3) a particular pattern of backbone chiral centers.

17. The method of claim 15 or 16, wherein the reference oligonucleotide composition is a substantially racemic preparation of oligonucleotides that share the common sequence and length.

18. The method of any one of claims 15-17, wherein the cleavage pattern provided by the chirally controlled oligonucleotide composition differs from a reference cleavage pattern in that it has fewer cleavage sites within the target sequence found in the nucleic acid polymer than the reference cleavage pattern.

19. The method of claim 18, wherein the cleavage pattern provided by the chirally controlled oligonucleotide composition has a single cleavage site within the target sequence found in the nucleic acid polymer.

20. The method of any one of claims 14-19, wherein the cleavage pattern provided by the chirally controlled oligonucleotide composition differs from a reference cleavage pattern in that it increases cleavage percentage at a cleavage site.

21. The method of any one of claims 14-20, wherein the chirally controlled oligonucleotide composition provides a higher cleavage rate of the target nucleic acid polymer than a reference oligonucleotide composition.

22. The method of any one of claims 14-21, wherein the chirally controlled oligonucleotide composition provides a lower level of remaining un-cleaved target nucleic acid polymer than a reference oligonucleotide composition.

23. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

24. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

25. The method of claim 23 or 24, the contacting being performed under conditions determined to permit the composition to suppress transcripts of the particular allele.

26. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

27. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

28. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

29. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type;

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

30. The method of any one of claims 23-29, wherein the specific nucleotide characteristic sequence element is present within an intron of the target nucleic acid sequence or gene.

31. The method of any one of claims 23-29, wherein the specific nucleotide characteristic sequence element is present within an exon of the target nucleic acid sequence or gene.

32. The method of any one of claims 23-29, wherein the specific nucleotide characteristic sequence element spans an exon and an intron of the target nucleic acid sequence or gene.

33. The method of any one of claims 23-29, wherein the specific nucleotide characteristic sequence element comprises a mutation.

34. The method of any one of claims 23-29, wherein the specific nucleotide characteristic sequence element comprises a SNP.

35. The method of any one of claims 14-34, wherein the chirally controlled oligonucleotide composition is a composition of claim 8.