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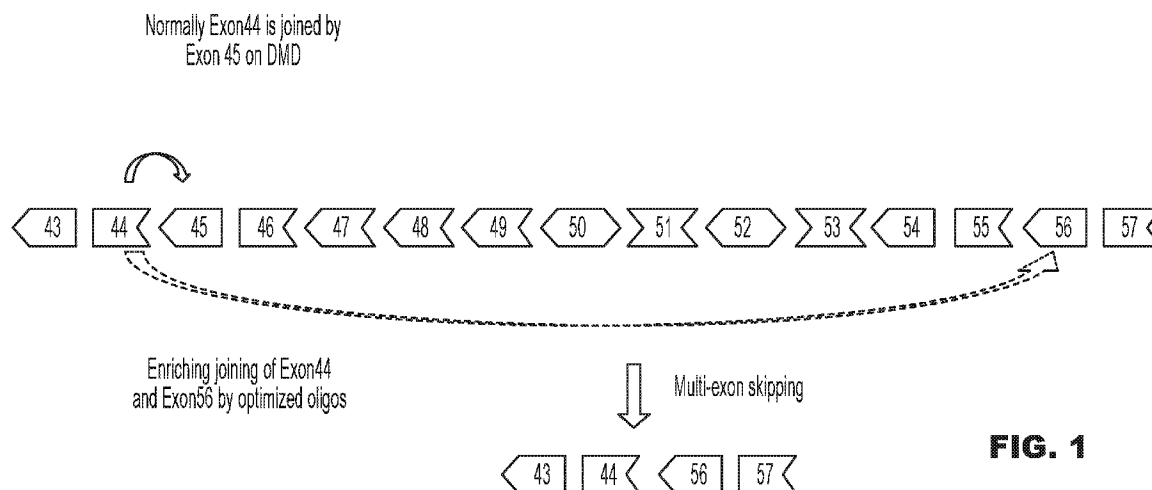
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(54) Title: OLIGONUCLEOTIDE COMPOSITIONS AND METHODS OF USE THEREOF



(57) Abrégé/Abstract:

Among other things, the present disclosure provides designed oligonucleotides, compositions, and methods of use thereof. In some embodiments, the present disclosure provides technologies useful for reducing levels of transcripts. In some embodiments, the present disclosure provides technologies useful for modulating transcript splicing. In some embodiments, provided technologies can alter splicing of a dystrophin (DMD) transcript. In some embodiments, the present disclosure provides methods for treating diseases, such as Duchenne muscular dystrophy, Becker's muscular dystrophy, etc.

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(54) Title: OLIGONUCLEOTIDE COMPOSITIONS AND METHODS OF USE THEREOF

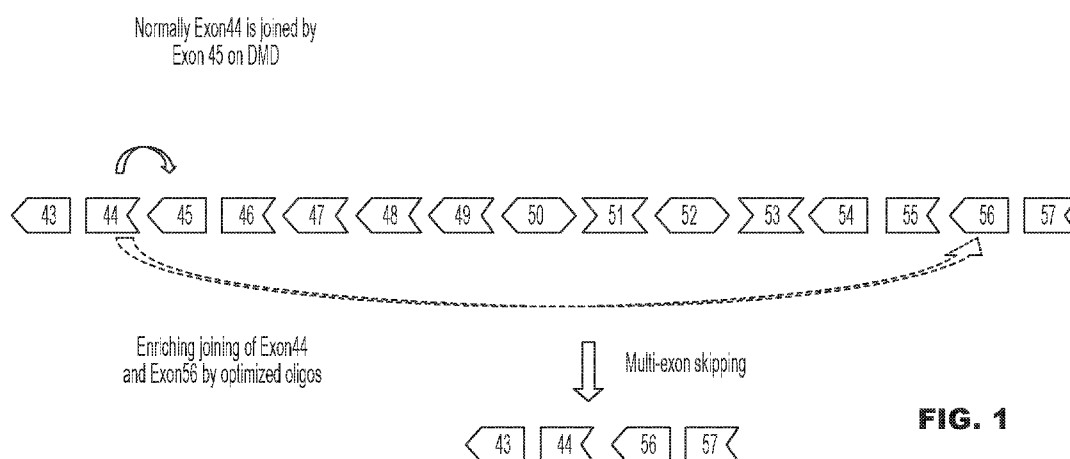


FIG. 1

(57) Abstract: Among other things, the present disclosure provides designed oligonucleotides, compositions, and methods of use thereof. In some embodiments, the present disclosure provides technologies useful for reducing levels of transcripts. In some embodiments, the present disclosure provides technologies useful for modulating transcript splicing. In some embodiments, provided technologies can alter splicing of a dystrophin (DMD) transcript. In some embodiments, the present disclosure provides methods for treating diseases, such as Duchenne muscular dystrophy, Becker's muscular dystrophy, etc.

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OLIGONUCLEOTIDE COMPOSITIONS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to United States Provisional Application Nos. 62/656,949, filed April 12, 2018, 62/670,709, filed May 11, 2018, 62/715,684, filed August 07, 2018, 62/723,375, filed August 27, 2018, and 62/776,432, filed December 06, 2018, the entirety of each of which is incorporated herein by reference.

BACKGROUND

[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. There is a need for new and improved oligonucleotides and oligonucleotide compositions, such as, *e.g.*, new oligonucleotides and oligonucleotide compositions capable of modulating exon skipping of Dystrophin for treatment of muscular dystrophy.

SUMMARY

[0003] Among other things, the present disclosure encompasses the recognition that structural elements of oligonucleotides, such as base sequence, chemical modifications (*e.g.*, modifications of sugar, base, and/or internucleotidic linkages, and patterns thereof), and/or stereochemistry (*e.g.*, stereochemistry of backbone chiral centers (chiral internucleotidic linkages), and/or patterns thereof), can have significant impact on oligonucleotide properties, *e.g.*, activities, toxicities, *e.g.*, as may be mediated by protein binding characteristics, stability, splicing-altering capabilities, *etc.* In some embodiments, the present disclosure demonstrates that oligonucleotide compositions comprising oligonucleotides with controlled structural elements, *e.g.*, controlled chemical modification and/or controlled backbone stereochemistry patterns, provide unexpected properties, including but not limited to certain activities, toxicities, *etc.* In some embodiments, the present disclosure demonstrates that oligonucleotide properties, *e.g.*, activities, toxicities, *etc.*, can be modulated by chemical modifications (*e.g.*, modifications of sugars, bases, internucleotidic linkages, *etc.*), chiral structures (*e.g.*, stereochemistry of chiral internucleotidic linkages and patterns thereof, *etc.*), and/or combinations thereof.

[0004] In some embodiments, the present disclosure provides an oligonucleotide or an oligonucleotide composition. In some embodiments, an oligonucleotide or an oligonucleotide

composition is a DMD oligonucleotide or a DMD oligonucleotide composition. In some embodiments, a DMD oligonucleotide or a DMD oligonucleotide composition is an oligonucleotide or an oligonucleotide composition capable of modulating skipping of one or more exons of the target gene Dystrophin (DMD). In some embodiments, a DMD oligonucleotide or a DMD oligonucleotide composition is useful for treatment of muscular dystrophy. In some embodiments, an oligonucleotide or oligonucleotide composition is an oligonucleotide or oligonucleotide composition which comprises a non-negatively charged internucleotidic linkage. In some embodiments, an oligonucleotide or oligonucleotide composition which comprises a non-negatively charged internucleotidic linkage is capable of modulating the expression, level and/or activity of a gene target or a gene product thereof, including but not limited to, increasing or decreasing the expression, level and/or activity of a gene target or gene product thereof via any mechanism, including but not limited to: an RNase H-dependent mechanism, steric hindrance, RNA interference, modulation of skipping of one or more exon, etc. In some embodiments, the present disclosure pertains to an oligonucleotide or oligonucleotide composition which comprises a non-negatively charged internucleotidic linkage, in combination with any other structure or chemical moiety described herein. In some embodiments, the present disclosure pertains to a DMD oligonucleotide or DMD oligonucleotide composition which comprises a non-negatively charged internucleotidic linkage.

[0005] In some embodiments, the present disclosure provides technologies related to an oligonucleotide or an oligonucleotide composition for reducing levels of a transcript and/or a protein encoded thereby. In some embodiments, as demonstrated by example data described herein, provided technologies are particularly useful for reducing levels of mRNA and/or proteins encoded thereby.

[0006] In some embodiments, the present disclosure provides technologies, e.g., oligonucleotides, compositions and methods, etc., for altering gene expression, levels and/or splicing of transcripts. In some embodiments, a transcript is Dystrophin (DMD). Splicing of a transcript, such as pre-mRNA, is an essential step for the transcript to perform its biological functions in many higher eukaryotes. In some embodiments, the present disclosure recognizes that targeting splicing, especially through compositions comprising oligonucleotides having base sequences and/or chemical modifications and/or stereochemistry patterns (and/or patterns thereof) described in this disclosure, can effectively correct disease-associated mutations and/or aberrant splicing, and/or introduce and/or enhance beneficial splicing that lead to desired products, e.g., mRNA, proteins, *etc.* which can repair, restore, or add new desired biological functions. e.g., one or more functions of Dystrophin.

[0007] In some embodiments, the present disclosure provides compositions and methods for altering splicing of DMD transcripts, wherein altered splicing deletes or compensates for an exon(s) comprising a disease-associated mutation.

[0008] For example, in some embodiments, a Dystrophin gene can comprise an exon comprising

one or more mutations associated with a disease, e.g., muscular dystrophy (including but not limited to Duchenne (Duchenne's) muscular dystrophy (DMD) and Becker (Becker's) muscular dystrophy (BMD)). In some embodiments, a disease-associated exon comprises a mutation (e.g., a missense mutation, a frameshift mutation, a nonsense mutation, a premature stop codon, etc.) in an exon. In some embodiments, the present disclosure provides compositions and methods for effectively skipping a disease-associated Dystrophin exon(s) and/or a different or an adjacent exon(s), while maintaining or restoring the reading frame so that a shorter (e.g., internally truncated) but partially functional dystrophin can be produced. A person having ordinary skill in the art appreciates that provided technologies (oligonucleotides, compositions, methods, *etc.*) can also be utilized for skipping of other exons, for example, those described in WO 2017/062862 and incorporated herein by reference, in accordance with the present disclosure to treat a disease and/or condition.

[0009] Among other things, the present disclosure demonstrates that chemical modifications and/or stereochemistry can be used to modulate transcript splicing by oligonucleotide compositions. In some embodiments, the present disclosure provides combinations of chemical modifications and stereochemistry to improve properties of oligonucleotides, *e.g.*, their capabilities to alter splicing of transcripts. In some embodiments, the present disclosure provides chirally controlled oligonucleotide compositions that, when compared to a reference condition (*e.g.*, absence of the composition, presence of a reference composition (*e.g.*, a stereorandom composition of oligonucleotides having the same constitution (as understood by those skilled in the art, unless otherwise indicated constitution generally refers to the description of the identity and connectivity (and corresponding bond multiplicities) of the atoms in a molecular entity but omitting any distinction arising from their spatial arrangement), a different chirally controlled oligonucleotide composition, etc.), combinations thereof, etc.), provide altered splicing that can deliver one or more desired biological effects, for example, increase production of desired proteins, knockdown of a gene by producing mRNA with frameshift mutations and/or premature termination codons, knockdown of a gene expressing a mRNA with a frameshift mutation and/or premature termination codon, *etc.* In some embodiments, compared to a reference condition, provided chirally controlled oligonucleotide compositions are surprisingly effective. In some embodiments, desired biological effects (*e.g.*, as measured by increased levels of desired mRNA, proteins, *etc.*, decreased levels of undesired mRNA, proteins, *etc.*) can be enhanced by more than 5, 10, 15, 20, 25, 30, 40, 50, or 100 fold.

[0010] The present disclosure recognizes challenges of providing low toxicity oligonucleotide compositions and methods of use thereof. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced toxicity. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced immune responses. In some

embodiments, the present disclosure recognizes that various toxicities induced by oligonucleotides are related to cytokine and/or complement activation. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced cytokine and/or complement activation. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced complement activation via the alternative pathway. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced complement activation via the classical pathway. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced drug-induced vascular injury. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced injection site inflammation. In some embodiments, reduced toxicity can be evaluated through one or more assays widely known to and practiced by a person having ordinary skill in the art, *e.g.*, evaluation of levels of complete activation product, protein binding, *etc.*

[0011] In some embodiments, the present disclosure provides oligonucleotides with enhanced antagonism of hTLR9 activity. In some embodiments, certain diseases, *e.g.*, DMD, are associated with inflammation in, *e.g.*, muscle tissues. In some embodiments, provided technologies (*e.g.*, oligonucleotides, compositions, methods, *etc.*) provides both enhanced activities (*e.g.*, exon-skipping activities) and hTLR9 antagonist activities which can be beneficial to one or more conditions and/or diseases associated with inflammation. In some embodiments, provided oligonucleotides and/or compositions thereof provides both exon-skipping capabilities and decreased levels of toxicity and/or inflammation. In some embodiments, the present disclosure provides an oligonucleotide which comprises one or more non-negatively charged internucleotidic linkages, wherein the oligonucleotide agonizes TLR9 activity less than another oligonucleotide which does not comprise a non-negatively charged internucleotidic linkage or which comprises fewer non-negatively charged internucleotidic linkages and which is otherwise identical. In some embodiments, the present disclosure provides an oligonucleotide which comprises one or more non-negatively charged internucleotidic linkages, wherein the oligonucleotide agonizes TLR9 activity less than an otherwise identical oligonucleotide which does not comprise a non-negatively charged internucleotidic linkage or which comprises fewer non-negatively charged internucleotidic linkages. In some embodiments, the present disclosure pertains to an oligonucleotide comprising at least one non-negatively charged internucleotidic linkage. In some embodiments, the non-negatively charged internucleotidic is selected from: n001, n002, n003, n004, n005, n006, n007, n008, n009, or n010, or a chirally controlled stereoisomer of n001, n002, n003, n004, n005, n006, n007, n008, n009, or n010. In some embodiments, the present disclosure pertains to an oligonucleotide which comprises at least two non-negatively charged internucleotidic linkages, wherein the linkages are different from each other. In some embodiments, the present disclosure pertains to an

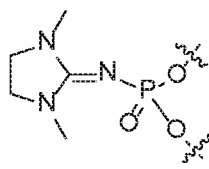
oligonucleotide comprising a CpG motif, wherein at least one internucleotidic linkage in the CpG (e.g., the p in CpG) or immediately upstream of the CpG (toward the 5' end of the oligonucleotide) or immediately downstream of the CpG (toward the 3' end of the oligonucleotide) is a non-negatively charged internucleotidic linkage. In some embodiments, TLR9 is a human TLR9. In some embodiments, TLR9 is a mouse TLR9.

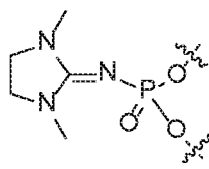
[0012] In some embodiments, the present disclosure demonstrates that oligonucleotide properties, *e.g.*, activities, toxicities, *etc.*, can be modulated through chemical modifications. In some embodiments, the present disclosure provides an oligonucleotide composition comprising a plurality of oligonucleotides which have a common base sequence, and comprise one or more modified internucleotidic linkages (or “non-natural internucleotidic linkages”, linkages that are not but can be utilized in place of a natural phosphate internucleotidic linkage ($-\text{OP}(\text{O})(\text{OH})\text{O}-$, which may exist as a salt form ($-\text{OP}(\text{O})(\text{O}^-)\text{O}-$) at a physiological pH) found in natural DNA and RNA), one or more modified sugar moieties, and/or one or more natural phosphate linkages. In some embodiments, provided oligonucleotides may comprise two or more types of modified internucleotidic linkages. In some embodiments, a provided oligonucleotide comprises a non-negatively charged internucleotidic linkage. In some embodiments, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, a neutral internucleotidic linkage comprises a triazole, alkyne, or guanidine (*e.g.*, cyclic guanidine) moiety. Such moieties are optionally substituted. In some embodiments, a provided oligonucleotide comprises a neutral internucleotidic linkage and another internucleotidic linkage which is not a neutral backbone. In some embodiments, a provided oligonucleotide comprises a neutral internucleotidic linkage and a phosphorothioate internucleotidic linkage. In some embodiments, provided oligonucleotide compositions comprising a plurality of oligonucleotides are chirally controlled and level of the plurality of oligonucleotides in the composition is controlled or pre-determined, and oligonucleotides of the plurality share a common stereochemistry configuration at one or more chiral internucleotidic linkages. For example, in some embodiments, oligonucleotides of a plurality share a common stereochemistry configuration at 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, 35, 40, 45, 50 or more chiral internucleotidic linkages, each of which is independently *Rp* or *Sp*; in some embodiments, oligonucleotides of a plurality share a common stereochemistry configuration at each chiral internucleotidic linkages. In some embodiments, a chiral internucleotidic linkage where a controlled level of oligonucleotides of a composition share a common stereochemistry configuration (independently in the *Rp* or *Sp* configuration) is referred to as a chirally controlled internucleotidic linkage.

[0013] In some embodiments, a modified internucleotidic linkage is a non-negatively charged (neutral or cationic) internucleotidic linkage in that at a pH, (*e.g.*, human physiological pH (~ 7.4), pH of

a delivery site (e.g., an organelle, cell, tissue, organ, organism, etc.), etc.), it largely (e.g., at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, etc.; in some embodiments, at least 30%; in some embodiments, at least 40%; in some embodiments, at least 50%; in some embodiments, at least 60%; in some embodiments, at least 70%; in some embodiments, at least 80%; in some embodiments, at least 90%; in some embodiments, at least 99%; etc.) exists as a neutral or cationic form (as compared to an anionic form (e.g., $-\text{O}-\text{P}(\text{O})(\text{O}^-)-\text{O}-$ (the anionic form of natural phosphate linkage), $-\text{O}-\text{P}(\text{O})(\text{S}^-)-\text{O}-$ (the anionic form of phosphorothioate linkage), etc.)), respectively. In some embodiments, a modified internucleotidic linkage is a neutral internucleotidic linkage in that at a pH, it largely exists as a neutral form. In some embodiments, a modified internucleotidic linkage is a cationic internucleotidic linkage in that at a pH, it largely exists as a cationic form. In some embodiments, a pH is human physiological pH (~ 7.4). In some embodiments, a modified internucleotidic linkage is a neutral internucleotidic linkage in that at pH 7.4 in a water solution, at least 90% of the internucleotidic linkage exists as its neutral form. In some embodiments, a modified internucleotidic linkage is a neutral internucleotidic linkage in that in a water solution of the oligonucleotide, at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the internucleotidic linkage exists in its neutral form. In some embodiments, the percentage is at least 90%. In some embodiments, the percentage is at least 95%. In some embodiments, the percentage is at least 99%. In some embodiments, a non-negatively charged internucleotidic linkage, e.g., a neutral internucleotidic linkage, when in its neutral form has no moiety with a pKa that is less than 8, 9, 10, 11, 12, 13, or 14. In some embodiments, pKa of an internucleotidic linkage in the present disclosure can be represented by pKa of CH_3 —the internucleotidic linkage— CH_3 (i.e., replacing the two nucleoside units connected by the internucleotidic linkage with two $-\text{CH}_3$ groups). Without wishing to be bound by any particular theory, in at least some cases, a neutral internucleotidic linkage in an oligonucleotide can provide improved properties and/or activities, e.g., improved delivery, improved resistance to exonucleases and endonucleases, improved cellular uptake, improved endosomal escape and/or improved nuclear uptake, etc., compared to a comparable nucleic acid which does not comprises a neutral internucleotidic linkage.

[0014] In some embodiments, a non-negatively charged internucleotidic linkage has the structure of e.g., of formula **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, etc. In some embodiments, a non-negatively charged internucleotidic linkage comprises a triazole or alkyne moiety. In some embodiments, a non-negatively charged internucleotidic linkage comprises a guanidine moiety. In some embodiments, a non-negatively charged internucleotidic linkage comprises a cyclic guanidine moiety. In some embodiments, a modified internucleotidic linkage comprising a cyclic



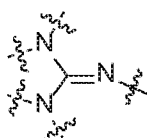
guanidine moiety has the structure of: . In some embodiments, a neutral internucleotidic linkage comprising a cyclic guanidine moiety is chirally controlled. In some embodiments, the present disclosure pertains to a composition comprising an oligonucleotide comprising at least one neutral internucleotidic linkage and at least one phosphorothioate internucleotidic linkage.

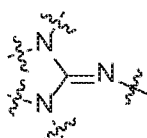
[0015] In some embodiments, a non-negatively charged internucleotidic linkage is n001, n002, n003, n004, n005, n006, n007, or n008. In some embodiments, a non-negatively charged internucleotidic linkage is chirally controlled, *e.g.*, n001R, n002R, n003R, n004R, n005R, n006R, n007R, n008R, n009R, n001S, n002S, n003S, n004S, n005S, n006S, n007S, n008S, n009S, *etc.*

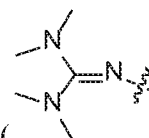
[0016] In some embodiments, the present disclosure pertains to a composition comprising an oligonucleotide comprising at least one neutral internucleotidic linkage and at least one phosphorothioate internucleotidic linkage, wherein the phosphorothioate internucleotidic linkage is a chirally controlled internucleotidic linkage in the Sp configuration.

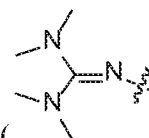
[0017] In some embodiments, the present disclosure pertains to a composition comprising an oligonucleotide comprising at least one neutral internucleotidic linkage and at least one phosphorothioate internucleotidic linkage, wherein the phosphorothioate internucleotidic linkage is a chirally controlled internucleotidic linkage in the Rp configuration.

[0018] In some embodiments, the present disclosure pertains to a composition comprising an oligonucleotide comprising at least one neutral internucleotidic linkage selected from a neutral internucleotidic linkage comprising an optionally substituted triazolyl group, a neutral internucleotidic linkage comprising an optionally substituted alkynyl group, and a neutral internucleotidic linkage



comprising a moiety , and at least one phosphorothioate internucleotidic linkage. In some embodiments, the present disclosure pertains to a composition comprising an oligonucleotide comprising at least one neutral internucleotidic linkage selected from a neutral internucleotidic linkage comprising an optionally substituted triazolyl group, a neutral internucleotidic linkage comprising an optionally



substituted alkynyl group, and a neutral internucleotidic linkage comprising a Tmg group () , and at least one phosphorothioate internucleotidic linkage. In some embodiments, an oligonucleotide comprises at least one non-negatively charged internucleotidic linkage and at least one phosphorothioate

internucleotidic linkage. In some embodiments, the non-negatively charged internucleotidic linkage is n001. In some embodiments, the non-negatively charged internucleotidic linkage and the phosphorothioate internucleotidic linkage are independently chirally controlled. In some embodiments, each of the non-negatively charged internucleotidic linkage and the phosphorothioate internucleotidic linkages are independently chirally controlled.

[0019] In some embodiments, the present disclosure pertains to a composition comprising an oligonucleotide comprising at least one neutral internucleotidic linkage selected from a neutral internucleotidic linkage comprising an optionally substituted triazolyl group, a neutral internucleotidic linkage comprising an optionally substituted alkynyl group, and a neutral internucleotidic linkage comprising a Tmg group, and at least one phosphorothioate, wherein the phosphorothioate is a chirally controlled internucleotidic linkage in the Sp configuration.

[0020] In some embodiments, the present disclosure pertains to a composition comprising an oligonucleotide comprising at least one neutral internucleotidic linkage selected from a neutral internucleotidic linkage comprising an optionally substituted triazolyl group, a neutral internucleotidic linkage comprising an optionally substituted alkynyl group, and a neutral internucleotidic linkage comprising a Tmg group, and at least one phosphorothioate, wherein the phosphorothioate is a chirally controlled internucleotidic linkage in the Rp configuration.

[0021] Various types of internucleotidic linkages differ in properties. Without wishing to be bound by any theory, the present disclosure notes that a natural phosphate linkage (phosphodiester internucleotidic linkage) is anionic and may be unstable when used by itself without other chemical modifications in vivo; a phosphorothioate internucleotidic linkage is anionic, generally more stable in vivo than a natural phosphate linkage, and generally more hydrophobic; a neutral internucleotidic linkage such as one exemplified in the present disclosure comprising a cyclic guanidine moiety is neutral at physiological pH, can be more stable in vivo than a natural phosphate linkage, and more hydrophobic.

[0022] In some embodiments, an internucleotidic linkage (e.g., a non-negatively charged internucleotidic linkage, a chirally controlled non-negatively charged internucleotidic linkage, etc.) is neutral at physiological pH, chirally controlled, stable in vivo, hydrophobic, and may increase endosomal escape.

[0023] In some embodiments, an oligonucleotide or oligonucleotide composition is: a DMD oligonucleotide or oligonucleotide composition; an oligonucleotide or oligonucleotide composition comprising a non-negatively charged internucleotidic linkage; or a DMD oligonucleotide comprising a non-negatively charged internucleotidic linkage.

[0024] In some embodiments, an oligonucleotide has, as non-limiting examples, a wing-core-wing, wing-core, core-wing, wing-wing-core-wing-wing, wing-wing-core-wing, or wing-core-wing-wing

structure (in some embodiments, a wing-wing comprises or consists of a first wing and a second wing, wherein the first wing is different than the second wing, and the first and second wings are different than the core). A wing or core can be defined by any structural elements and/or patterns and/or combinations thereof. In some embodiments, a wing and core is defined by nucleoside modifications, sugar modifications, and/or internucleotidic linkages, wherein a wing comprises a nucleoside modification, sugar modification and/or internucleotidic linkage and/or pattern and/or combination thereof, that the core region does not have, or vice versa. In some embodiments, oligonucleotides of the present disclosure comprise or consist of a 5'-end region, a middle region, and a 3'-end region. In some embodiments, a 5'-end region is a 5'-wing region. In some embodiments, a 5'-wing region is a 5'-end region. In some embodiments, a 3'-end region is a 3'-wing region. In some embodiments, a 3'-wing region is a 3'-end region. In some embodiments, a core region is a middle region.

[0025] In some embodiments, each wing region (or each of the 5'-end and 3'-end regions) independently comprises one or more modified phosphate linkages and no natural phosphate linkages, and the core region (the middle region) comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, each wing region (or each of the 5'-end and 3'-end regions) independently comprises one or more natural phosphate linkages and optionally one or more modified internucleotidic linkages, and the core (or the middle region) comprises one or more modified internucleotidic linkages and optionally one or more natural phosphate linkages. In some embodiments, a wing (or a 5'-end or 3'-end region) comprises modified sugar moieties. In some embodiments, a modified internucleotidic linkage is a phosphorothioate internucleotidic linkage.

[0026] Among other things, the present disclosure encompasses the recognition that stereorandom oligonucleotide preparations contain a plurality of distinct chemical entities that differ from one another, *e.g.*, in the stereochemical structure of individual backbone chiral centers within the oligonucleotide chain. Without control of stereochemistry of backbone chiral centers, stereorandom oligonucleotide preparations provide uncontrolled (or stereorandom) compositions comprising undetermined levels of oligonucleotide stereoisomers. Even though these stereoisomers may have the same base sequence and/or chemical modifications, they are different chemical entities at least due to their different backbone stereochemistry, and they can have, as demonstrated herein, different properties, *e.g.*, activities, toxicities, distribution *etc.* Among other things, the present disclosure provides chirally controlled compositions that are or contain particular stereoisomers of oligonucleotides of interest; in contrast to chirally uncontrolled compositions, chirally controlled compositions comprise controlled levels of particular stereoisomers of oligonucleotides. In some embodiments, a particular stereoisomer may be defined, for example, by its base sequence, its pattern of backbone linkages, its pattern of backbone chiral centers, and pattern of backbone phosphorus modifications, etc. As is understood in the

art, in some embodiments, base sequence may refer solely to the sequence of bases and/or to the identity and/or modification status of nucleoside residues (*e.g.*, of sugar and/or base components, relative to standard naturally occurring nucleotides such as adenine, cytosine, guanosine, thymine, and uracil) in an oligonucleotide and/or to the hybridization character (*i.e.*, the ability to hybridize with particular complementary residues) of such residues. In some embodiments, the present disclosure demonstrates that property improvements (*e.g.*, improved activities, lower toxicities, *etc.*) 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 chemical modifications, *e.g.*, particular backbone linkages, residue modifications, *etc.* (*e.g.*, through use of certain types of modified phosphates [*e.g.*, phosphorothioate, substituted phosphorothioate, *etc.*], sugar modifications [*e.g.*, 2'-modifications, *etc.*], and/or base modifications [*e.g.*, methylation, *etc.*]). In some embodiments, the present disclosure demonstrates that chirally controlled oligonucleotide compositions of oligonucleotides comprising certain chemical modifications (*e.g.*, 2'-F, 2'-OMe, phosphorothioate internucleotidic linkages, lipid conjugation, *etc.*) demonstrate unexpectedly high exon-skipping efficiency.

[0027] In some embodiments, provided oligonucleotides are blockmers. In some embodiments, a blockmer is an oligonucleotide comprising one or more blocks.

[0028] In some embodiments, a block is a portion of an oligonucleotide. In some embodiments, a block is a wing or a core. In some embodiments, a blockmer comprises one or more blocks. In some embodiments, a 5'-block is a 5'-end region or 5'-wing. In some embodiments, a 3'-block is a 3'-end region or 3'-wing.

[0029] In some embodiments, provided oligonucleotides are altmers. In some embodiments, provided oligonucleotides are altmers comprising alternating blocks. In some embodiments, a blockmer or an altmer can be defined by chemical modifications (including presence or absence), *e.g.*, base modifications, sugar modification, internucleotidic linkage modifications, stereochemistry, *etc.*

[0030] In some embodiments, provided oligonucleotides comprise blocks comprising different internucleotidic linkages. In some embodiments, provided oligonucleotides comprise blocks comprising modified internucleotidic linkages and/or natural phosphate linkages.

[0031] In some embodiments, provided oligonucleotides comprise blocks comprising sugar modifications. In some embodiments, provided oligonucleotides comprise one or more blocks comprising one or more 2'-F modifications (2'-F blocks). In some embodiments, provided oligonucleotides comprise blocks comprising consecutive 2'-F modifications. In some embodiments, a block comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more consecutive 2'-F modifications.

[0032] In some embodiments, provided oligonucleotides comprises one or more blocks

comprising one or more 2'-OR¹ modifications (2'-OR¹ blocks), wherein R¹ is independently as defined and described herein and below. In some embodiments, provided oligonucleotides comprise both 2'-F and 2'-OR¹ blocks. In some embodiments, provided oligonucleotides comprise alternating 2'-F and 2'-OR¹ blocks. In some embodiments, provided oligonucleotides comprise a first 2'-F block at the 5'-end, and a second 2'-F block at the 3'-end, each of which independently comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more consecutive 2'-F modifications.

[0033] In some embodiments, provided oligonucleotides comprise a 5'-block wherein each sugar moiety of the 5'-block comprises a 2'-F modification. In some embodiments, provided oligonucleotides comprise a 3'-block wherein each sugar moiety of the 3'-block comprises a 2'-F modification. In some embodiments, such provided oligonucleotides comprise one or more 2'-OR¹ blocks, and optionally one or more 2'-F blocks, between the 5' and 3' 2'-F blocks. In some embodiments, such provided oligonucleotides comprise one or more 2'-OR¹ blocks, and one or more 2'-F blocks, between the 5' and 3' 2'-F blocks (*e.g.*, WV-3047, WV-3048, *etc.*).

[0034] In some embodiments, a block is a stereochemistry block. In some embodiments, a block is an *Rp* block in that each internucleotidic linkage of the block is *Rp*. In some embodiments, a 5'-block is an *Rp* block. In some embodiments, a 3'-block is an *Rp* block. In some embodiments, a block is an *Sp* block in that each internucleotidic linkage of the block is *Sp*. In some embodiments, a 5'-block is an *Sp* block. In some embodiments, a 3'-block is an *Sp* block. In some embodiments, provided oligonucleotides comprise both *Rp* and *Sp* blocks. In some embodiments, provided oligonucleotides comprise one or more *Rp* but no *Sp* blocks. In some embodiments, provided oligonucleotides comprise one or more *Sp* but no *Rp* blocks.

[0035] In some embodiments, provided oligonucleotides comprise one or more PO blocks wherein each internucleotidic linkage in a natural phosphate linkage.

[0036] In some embodiments, a 5'-block is an *Sp* block wherein each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block is an *Sp* block wherein each internucleotidic linkage is a modified internucleotidic linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block is an *Sp* block wherein each internucleotidic linkage is a phosphorothioate linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block comprises 4 or more nucleoside units.

[0037] In some embodiments, a 3'-block is an *Sp* block wherein each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block is an *Sp* block wherein each internucleotidic linkage is a modified internucleotidic linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block is an *Sp* block wherein each internucleotidic linkage is a phosphorothioate linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block

comprises 4 or more nucleoside units.

[0038] In some embodiments, provided oligonucleotides comprise alternating blocks comprising different modified sugar moieties and/or unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise alternating blocks comprising different modified sugar moieties and unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise alternating blocks comprising different modified sugar moieties. In some embodiments, provided oligonucleotides comprise alternating blocks comprising different modified sugar moieties, wherein the modified sugar moieties comprise different 2'-modifications. For example, in some embodiments, provided oligonucleotide comprises alternating blocks comprising 2'-OMe and 2'-F, respectively.

[0039] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a plurality of oligonucleotides which:

- 1) have a common base sequence complementary to a target sequence in a transcript; and
- 2) comprise one or more modified sugar moieties and modified internucleotidic linkages.

[0040] In some embodiments, a provided oligonucleotide composition is characterized in that, when it is contacted with the transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under a reference condition selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[0041] In some embodiments, a reference condition is absence of the composition. In some embodiments, a reference condition is presence of a reference composition. Example reference compositions comprising a reference plurality of oligonucleotides are extensively described in this disclosure. In some embodiments, oligonucleotides of the reference plurality have a different structural elements (chemical modifications, stereochemistry, *etc.*) compared with oligonucleotides of the plurality in a provided composition. In some embodiments, a reference composition is a stereorandom preparation of oligonucleotides having the same chemical modifications. In some embodiments, a reference composition is a mixture of stereoisomers while a provided composition is a chirally controlled oligonucleotide composition of one stereoisomer. In some embodiments, oligonucleotides of the reference plurality have the same base sequence, same sugar modifications, same base modifications, same internucleotidic linkage modifications, and/or same stereochemistry as oligonucleotide of the plurality in a provided composition but different chemical modifications, e.g., base modification, sugar modification, internucleotidic linkage modifications, etc.

[0042] Example splicing systems are widely known in the art. In some embodiments, a splicing system is an *in vivo* or *in vitro* system including components sufficient to achieve splicing of a relevant target transcript. In some embodiments, a splicing system is or comprises a spliceosome (*e.g.*, protein and/or RNA components thereof). In some embodiments, a splicing system is or comprises an organellar

membrane (*e.g.*, a nuclear membrane) and/or an organelle (*e.g.*, a nucleus). In some embodiments, a splicing system is or comprises a cell or population thereof. In some embodiments, a splicing system is or comprises a tissue. In some embodiments, a splicing system is or comprises an organism, *e.g.*, an animal, *e.g.*, a mammal such as a mouse, rat, monkey, dog, human, *etc.*

[0043] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a plurality of oligonucleotides which:

- 1) have a common base sequence complementary to a target sequence in a transcript; and
- 2) comprise one or more modified sugar moieties and modified internucleotidic linkages,

the oligonucleotide composition being characterized in that, when it is contacted with the transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[0044] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and
- 4) pattern of backbone phosphorus modifications.

[0045] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and
- 4) pattern of backbone phosphorus modifications,

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

the oligonucleotide composition being characterized in that, when it is contacted with the transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[0046] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

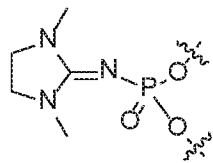
- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and
- 4) pattern of backbone phosphorus modifications,

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.

[0047] In some embodiments, each region (e.g., a block, wing, core, 5'-end, 3'-end, or middle region, etc.) of an oligonucleotide independently comprises 3, 4, 5, 6, 7, 8, 9, 10 or more bases. In some embodiments, each region independently comprises 3 or more bases. In some embodiments, each region independently comprises 4 or more bases. In some embodiments, each region independently comprises 5 or more bases. In some embodiments, each region independently comprises 6 or more bases. In some embodiments, each sugar moiety in a region is modified. In some embodiments, a modification is a 2'-modification. In some embodiments, each modification is a 2'-modification. In some embodiments, a modification is 2'-F. In some embodiments, each modification is 2'-F. In some embodiments, a modification is 2'-OR¹. In some embodiments, each modification is 2'-OR¹. In some embodiments, a modification is 2'-OR¹. In some embodiments, each modification is 2'-OMe. In some embodiments, each modification is 2'-OMe. In some embodiments, each modification is 2'-MOE. In some embodiments, each modification is 2'-MOE. In some embodiments, a modification is an LNA sugar modification. In some embodiments, each modification is an LNA sugar modification. In some embodiments, each internucleotidic linkage in a region is a chiral internucleotidic linkage. In some embodiments, each internucleotidic linkage in a wing, or 5'-end or 3'-end region, is an *Sp* chiral internucleotidic linkage. In some embodiments, a chiral internucleotidic linkage is a phosphorothioate linkage. In some embodiments, a core or middle region comprises one or more natural phosphate linkages and one or more modified internucleotidic linkages. In some embodiments, a core or middle region comprises one or more natural phosphate linkages and one or more chiral internucleotidic linkages. In some embodiments, a core region comprises one or more natural phosphate linkages and one or more *Sp* chiral internucleotidic linkages. In some embodiments, a core or middle region comprises one or more natural phosphate linkages and one or more *Sp* phosphorothioate linkages.

[0048] In some embodiments, a region (e.g., a block, wing, core, 5'-end, 3'-end, middle region, etc.) of an oligonucleotide comprises a non-negatively charged internucleotidic linkage, e.g., of formula **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, etc. In some embodiments, a region comprises a neutral internucleotidic linkage. In some embodiments, a region comprises an internucleotidic linkage which comprises a triazole or alkyne moiety. In some embodiments,

a region comprises an internucleotidic linkage which comprises a cyclic guanidine guanidine. In some embodiments, a region comprises an internucleotidic linkage which comprises a cyclic guanidine moiety. In some embodiments, a region comprises an internucleotidic linkage having the structure of



. In some embodiments, such internucleotidic linkages are chirally controlled.

[0049] In some embodiments, the base sequence of an oligonucleotide, e.g., the base sequence of a plurality of oligonucleotides of a particular oligonucleotide type, is or comprises a base sequence disclosed herein (e.g., a base sequence of an example oligonucleotide (e.g., those listed in the tables, examples, etc.), a target sequence, etc.) (or a portion thereof which is at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 bases long). In some embodiments, a provided oligonucleotide has a base sequence comprising the base sequence of any example oligonucleotides or another base sequence disclosed herein, and a length of up to 30 bases. In some embodiments, a provided oligonucleotide has a base sequence comprising the base sequence of any example oligonucleotides or another base sequence disclosed herein, and a length of up to 40 bases. In some embodiments, a provided oligonucleotide has a base sequence comprising the base sequence of any example oligonucleotides or another base sequence disclosed herein, and a length of up to 50 bases. In some embodiments, a provided oligonucleotide has a base sequence comprising at least 15 contiguous bases of the base sequence of an oligonucleotide example or another sequence disclosed herein, and a length of up to 30 bases. In some embodiments, a provided oligonucleotide has a base sequence comprising at least 15 contiguous bases of the base sequence of an oligonucleotide example or another sequence disclosed herein, and a length of up to 40 bases. In some embodiments, a provided oligonucleotide has a base sequence comprising at least 15 contiguous bases of the base sequence of an oligonucleotide example or another sequence disclosed herein, and a length of up to 50 bases. In some embodiments, a provided oligonucleotide has a base sequence comprising a sequence having no more than 5 mismatches from the base sequence of an example oligonucleotide or another sequence disclosed herein, and a length of up to 30 bases. In some embodiments, a provided oligonucleotide has a base sequence comprising a sequence having no more than 5 mismatches from the base sequence of an example oligonucleotide or another sequence disclosed herein, and a length of up to 40 bases. In some embodiments, a provided oligonucleotide has a base sequence comprising a sequence having no more than 5 mismatches from the base sequence of an example oligonucleotide or another sequence disclosed herein, and a length of up to 50 bases.

[0050] In some embodiments, the base sequence of a provided oligonucleotide is the base sequence of an example oligonucleotide or another sequence disclosed herein, and a pattern of backbone

chiral centers comprises at least one chirally controlled center which is a *Sp* linkage phosphorus of a phosphorothioate linkage. In some embodiments, the base sequence of a provided oligonucleotide is the base sequence of an example oligonucleotide or another sequence disclosed herein, the oligonucleotide has a length of up to 30 bases, and a pattern of backbone chiral centers comprises at least one chirally controlled center which is a *Sp* linkage phosphorus of a phosphorothioate linkage. In some embodiments, the base sequence of a provided oligonucleotide is the base sequence of an example oligonucleotide or another sequence disclosed herein, the oligonucleotide has a length of up to 40 bases, and a pattern of backbone chiral centers comprises at least one chirally controlled center which is a *Sp* linkage phosphorus of a phosphorothioate linkage. In some embodiments, the base sequence of a provided oligonucleotide comprises at least 15 contiguous bases of any example oligonucleotides or another sequence disclosed herein, the oligonucleotide has a length of up to 30, 40, or 50 bases, and a pattern of backbone chiral centers comprises at least one chirally controlled center which is a *Sp* linkage phosphorus of a phosphorothioate linkage.

[0051] In some embodiments, a mismatch is a difference between the base sequence or length when two sequences are maximally aligned and compared. As a non-limiting example, a mismatch is counted if a difference exists between the base at a particular location in one sequence and the base at the corresponding position in another sequence. Thus, a mismatch is counted, for example, if a position in one sequence has a particular base (*e.g.*, A), and the corresponding position on the other sequence has a different base (*e.g.*, G, C or U). A mismatch is also counted, *e.g.*, if a position in one sequence has a base (*e.g.*, A), and the corresponding position on the other sequence has no base (*e.g.*, that position is an abasic nucleotide which comprises a phosphate-sugar backbone but no base) or that position is skipped. A single-stranded nick in either sequence (or in the sense or antisense strand) may not be counted as mismatch, for example, no mismatch would be counted if one sequence comprises the sequence 5'-AG-3', but the other sequence comprises the sequence 5'-AG-3' with a single-stranded nick between the A and the G. A base modification is generally not considered a mismatch, for example, if one sequence comprises a C, and the other sequence comprises a modified C (*e.g.*, with a 2'-modification) at the same position, no mismatch may be counted.

[0052] In some embodiments, oligonucleotides of a particular type are chemically identical in that they have the same base sequence (including length), the same pattern of chemical modifications to sugar and base moieties, the same pattern of backbone linkages (*e.g.*, pattern of natural phosphate linkages, phosphorothioate linkages, phosphorothioate triester linkages, non-negatively charged linkages, and combinations thereof), the same pattern of backbone chiral centers (*e.g.*, pattern of stereochemistry (*Rp/Sp*) of chiral internucleotidic linkages), and the same pattern of backbone phosphorus modifications (*e.g.*, pattern of modifications on the internucleotidic phosphorus atom, such as $-S^-$, and $-L-R^1$ of

formula I).

[0053] In some embodiments, the present disclosure provides chirally controlled oligonucleotide compositions of oligonucleotides comprising multiple (*e.g.*, more than 5, 6, 7, 8, 9, or 10) internucleotidic linkages, and particularly for oligonucleotides comprising multiple (*e.g.*, more than 5, 6, 7, 8, 9, or 10) chiral internucleotidic linkages, wherein the oligonucleotides comprise at least one, and in some embodiments, more than 5, 6, 7, 8, 9, or 10 chirally controlled internucleotidic linkages. In some embodiments, in a chirally controlled composition of oligonucleotides each chiral internucleotidic linkage of the oligonucleotides is independently a chirally controlled internucleotidic linkage. In some embodiments, in a stereorandom or racemic composition of oligonucleotides, each chiral internucleotidic linkage is formed with less than 90:10, 95:5, 96:4, 97:3, or 98:2 diastereoselectivity. In some embodiments, in a stereoselective or chirally controlled composition of oligonucleotides, each chirally controlled internucleotidic linkage of the oligonucleotides independently has a diastereopurity of at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% at its chiral linkage phosphorus (either *R_p* or *S_p*). Among other things, the present disclosure provides technologies to prepare oligonucleotides of high diastereopurity. In some embodiments, diastereopurity of a chiral internucleotidic linkage in an oligonucleotide may be measured through a model reaction, *e.g.* formation of a dimer under essentially the same or comparable conditions wherein the dimer has the same internucleotidic linkage as the chiral internucleotidic linkage, the 5'-nucleoside of the dimer is the same as the nucleoside to the 5'-end of the chiral internucleotidic linkage, and the 3'-nucleoside of the dimer is the same as the nucleoside to the 3'-end of the chiral internucleotidic linkage.

[0054] As described herein, provided compositions and methods are capable of altering splicing of transcripts. In some embodiments, provided compositions and methods provide improved splicing patterns of transcripts compared to reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof. An improvement can be an improvement of any desired biological functions. In some embodiments, for example, in DMD, an improvement is production of an mRNA from which a dystrophin protein with improved biological activities is produced.

[0055] In some embodiments, the present disclosure provides a method for altering splicing of a target transcript, comprising administering a provided composition, wherein the splicing of the target transcript is altered relative to reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[0056] In some embodiments, the present disclosure provides a method of generating a set of spliced products from a target transcript, the method comprising steps of:

contacting a splicing system containing the target transcript with an oligonucleotide composition

comprising a plurality of oligonucleotides (e.g., a provided chirally controlled oligonucleotide composition), in an amount, for a time, and under conditions sufficient for a set of spliced products to be generated that is different from a set generated under reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[0057] In some embodiments, the present disclosure provides a method for treating or preventing a disease, comprising administering to a subject an oligonucleotide composition described herein.

[0058] In some embodiments, the present disclosure provides a method for treating or preventing a disease, comprising administering to a subject an oligonucleotide composition comprising a plurality of oligonucleotides, which:

- 1) have a common base sequence complementary to a target sequence in a transcript; and
- 2) comprise one or more modified sugar moieties and modified internucleotidic linkages,

the oligonucleotide composition being characterized in that, when it is contacted with the transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[0059] In some embodiments, the present disclosure provides a method for treating or preventing a disease, comprising administering to a subject a chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and
- 4) pattern of backbone phosphorus modifications,

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

the oligonucleotide composition being characterized in that, when it is contacted with the transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[0060] In some embodiments, a disease is one in which, after administering a provided composition, one or more spliced transcripts repair, restore or introduce a new beneficial function. For example, in DMD, after skipping one or more exons, functions of dystrophin can be restored, or partially restored, through a truncated but (at least partially) active version. In some embodiments, a disease is one in which, after administering a provided composition, one or more spliced transcripts repair, a gene is

effectively knockdown by altering splicing of the gene transcript.

[0061] In some embodiments, a disease is muscular dystrophy, including but not limited to Duchenne (Duchenne's) muscular dystrophy (DMD) and Becker (Becker's) muscular dystrophy (BMD).

[0062] In some embodiments, a transcript is of Dystrophin gene or a variant thereof.

[0063] In some embodiments, the present disclosure provides a method of treating a disease by administering a composition comprising a plurality of oligonucleotides sharing a common base sequence comprising a nucleotide sequence, which nucleotide sequence is complementary to a target sequence in the target transcript,

the improvement that comprises using as the oligonucleotide composition a chirally controlled oligonucleotide composition characterized in that, when it is contacted with the transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[0064] In some embodiments, a common sequence comprises a sequence (or at least 15 base long portion thereof) of any oligonucleotide in Table A1.

[0065] In some embodiments, the present disclosure provides a method of administering an oligonucleotide composition comprising a plurality of oligonucleotides having a common nucleotide sequence, the improvement that comprises:

administering an oligonucleotide composition comprising the plurality of oligonucleotides each of which independently comprises one or more negatively charged internucleotidic linkages and one or more non-negatively charged internucleotidic linkages, wherein the oligonucleotide composition is optionally chirally controlled.

[0066] In some embodiments, the present disclosure provides a method of administering an oligonucleotide composition comprising a plurality of oligonucleotides having a common nucleotide sequence, the improvement that comprises:

administering an oligonucleotide composition comprising the plurality of oligonucleotides that is chirally controlled and that is characterized by reduced toxicity relative to a reference oligonucleotide composition of the same common nucleotide sequence.

[0067] In some embodiments, the present disclosure provides a method of administering an oligonucleotide composition comprising a plurality of oligonucleotides having a common nucleotide sequence, the improvement that comprises:

administering an oligonucleotide composition in which each oligonucleotide in the plurality includes one or more natural phosphate linkages and one or more modified phosphate linkages;

wherein the oligonucleotide composition is characterized by reduced toxicity when tested in at

least one assay that is observed with an otherwise comparable reference composition whose oligonucleotides do not comprise natural phosphate linkages.

[0068] In some embodiments, oligonucleotides can elicit proinflammatory responses. In some embodiments, the present disclosure provides compositions and methods for reducing inflammation. In some embodiments, the present disclosure provides compositions and methods for reducing proinflammatory responses. In some embodiments, the present disclosure provides methods for reducing injection site inflammation using provided compositions. In some embodiments, the present disclosure provides methods for reducing drug-induced vascular injury using provided compositions.

[0069] In some embodiments, the present disclosure provides a method, comprising administering a composition comprising a plurality of oligonucleotides of a common base sequence, which composition displays reduced injection site inflammation as compared with a reference composition comprising a plurality of oligonucleotides, each of which also has the common base sequence, but which differs structurally from the oligonucleotides of the plurality in that:

individual oligonucleotides within the reference plurality differ from one another in stereochemical structure; and/or

at least some oligonucleotides within the reference plurality have a structure different from a structure represented by the plurality of oligonucleotides of the composition; and/or

at least some oligonucleotides within the reference plurality do not comprise a wing region and a core region.

[0070] In some embodiments, the present disclosure provides a method, comprising administering a composition comprising a plurality of oligonucleotides of a common base sequence, which composition displays altered protein binding as compared with a reference composition comprising a plurality of oligonucleotides, each of which also has the common base sequence but which differs structurally from the oligonucleotides of the plurality in that:

individual oligonucleotides within the reference plurality differ from one another in stereochemical structure; and/or

at least some oligonucleotides within the reference plurality have a structure different from a structure represented by the plurality of oligonucleotides of the composition; and/or

at least some oligonucleotides within the reference plurality do not comprise a wing region and a core region.

[0071] In some embodiments, the present disclosure provides a method of administering an oligonucleotide composition comprising a plurality of oligonucleotides having a common nucleotide sequence, the improvement that comprises:

administering an oligonucleotide composition comprising a plurality of oligonucleotides that is

characterized by altered protein binding relative to a reference oligonucleotide composition of the same common nucleotide sequence.

[0072] In some embodiments, the present disclosure provides a method comprising administering a composition comprising a plurality of oligonucleotides of a common base sequence, which composition displays improved delivery as compared with a reference composition comprising a reference plurality of oligonucleotides, each of which also has the common base sequence but which differs structurally from the oligonucleotides of the plurality in that:

individual oligonucleotides within the reference plurality differ from one another in stereochemical structure; and/or

at least some oligonucleotides within the reference plurality have a structure different from a structure represented by the plurality of oligonucleotides of the composition; and/or

at least some oligonucleotides within the reference plurality do not comprise a wing region and a core region.

[0073] In some embodiments, the present disclosure provides a method of administering an oligonucleotide composition comprising a plurality of oligonucleotides having a common nucleotide sequence, the improvement that comprises:

administering an oligonucleotide comprising a plurality of oligonucleotides that is characterized by improved delivery relative to a reference oligonucleotide composition of the same common nucleotide sequence.

[0074] In some embodiments, the present disclosure provides a composition comprising any oligonucleotide disclosed herein. In some embodiments, the present disclosure provides a composition comprising any chirally controlled oligonucleotide disclosed herein.

[0075] In some embodiments, the present disclosure provides a composition comprising an oligonucleotide disclosed herein which is capable of mediating skipping of Dystrophin exon 45. In some embodiments, the present disclosure provides a composition comprising an oligonucleotide disclosed herein which is capable of mediating skipping of Dystrophin exon 51. In some embodiments, the present disclosure provides a composition comprising an oligonucleotide disclosed herein which is capable of mediating skipping of Dystrophin exon 53. In some embodiments, the present disclosure provides a composition comprising an oligonucleotide(s) disclosed herein which is capable of mediating skipping of multiple Dystrophin exons. In some embodiments, such a composition is a chirally controlled oligonucleotide composition.

[0076] In some embodiments, the present disclosure pertains to an oligonucleotide or an oligonucleotide composition capable of mediating skipping of a DMD exon or multiple DMD exons. In

some embodiments, a DMD exon is exon 51. In some embodiments, a DMD exon is exon 53. In some embodiments, a DMD exon is exon 45. In some embodiments, the present disclosure pertains to an oligonucleotide composition capable of mediating skipping of a DMD exon 53, wherein the oligonucleotide composition comprises at least one chirally controlled internucleotidic linkage.

[0077] In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of DMD exon 45. In some embodiments, the present disclosure pertains to an oligonucleotide composition capable of mediating skipping of DMD exon 45, wherein the oligonucleotide composition comprises at least one chirally controlled internucleotidic linkage and comprises at least one non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of DMD exon 45 and comprises at least one non-negatively charged internucleotidic linkage.

[0078] In some embodiments, the present disclosure pertains to an oligonucleotide composition capable of mediating skipping of DMD exon 45, wherein the oligonucleotide composition comprises at least one non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of DMD exon 45 and comprises at least one non-negatively charged internucleotidic linkage.

[0079] In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of DMD exon 51. In some embodiments, the present disclosure pertains to an oligonucleotide composition capable of mediating skipping of DMD exon 51, wherein the oligonucleotide composition comprises at least one chirally controlled internucleotidic linkage and comprises at least one non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of DMD exon 51 and comprises at least one non-negatively charged internucleotidic linkage.

[0080] In some embodiments, the present disclosure pertains to an oligonucleotide composition capable of mediating skipping of DMD exon 51, wherein the oligonucleotide composition comprises at least one non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of DMD exon 51 and comprises at least one non-negatively charged internucleotidic linkage.

[0081] In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of DMD exon 53. In some embodiments, the present disclosure pertains to an oligonucleotide composition capable of mediating skipping of DMD exon 53, wherein the oligonucleotide composition comprises at least one chirally controlled internucleotidic linkage and comprises at least one non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of DMD exon 53 and comprises at least one non-negatively charged internucleotidic linkage.

[0082] In some embodiments, the present disclosure pertains to an oligonucleotide composition capable of mediating skipping of DMD exon 53, wherein the oligonucleotide composition comprises at least one non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of DMD exon 53 and comprises at least one non-negatively charged internucleotidic linkage.

[0083] In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of multiple DMD exons. In some embodiments, the present disclosure pertains to an oligonucleotide composition capable of mediating skipping of multiple DMD exons, wherein the oligonucleotide composition comprises at least one chirally controlled internucleotidic linkage and comprises at least one non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of multiple DMD exons and comprises at least one non-negatively charged internucleotidic linkage.

[0084] In some embodiments, the present disclosure pertains to an oligonucleotide composition capable of mediating skipping of a DMD exon, wherein the oligonucleotide composition comprises at least one non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of a DMD exon and comprises at least one non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of multiple DMD exons. In some embodiments, the present disclosure pertains to an oligonucleotide composition capable of mediating skipping of multiple DMD exons, wherein the oligonucleotide composition comprises at least one chirally controlled internucleotidic linkage and comprises at least one non-negatively charged

internucleotidic linkage. In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of multiple DMD exons and comprises at least one non-negatively charged internucleotidic linkage. In some embodiments, a DMD exon is any DMD exon disclosed herein, including but not limited to exon 45, exon 51, exon 52, exon 53, exon 55, exon 56, and exon 57.

[0085] In some embodiments, the present disclosure pertains to an oligonucleotide composition capable of mediating skipping of multiple DMD exons, wherein the oligonucleotide composition comprises at least one non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a chirally controlled oligonucleotide composition, wherein the oligonucleotide is capable of mediating skipping of multiple DMD exons and comprises at least one non-negatively charged internucleotidic linkage.

[0086] In some embodiments, the present disclosure provides a chirally controlled composition of an oligonucleotide capable of mediating skipping of Dystrophin exon 51. In some embodiments, the present disclosure provides a chirally controlled composition of an oligonucleotide capable of mediating skipping of Dystrophin exon 51 and disclosed herein.

[0087] In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which is, comprises, or comprises a 15-base portion of the base sequence of UCAAGGAAGAUGGCAUUUCU, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled. In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which is UCAAGGAAGAUGGCAUUUCU, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled. In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which comprises UCAAGGAAGAUGGCAUUUCU, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled. In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which comprises a 15-base portion of the base sequence of UCAAGGAAGAUGGCAUUUCU, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled. In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which is, comprises, or comprises a 15-base portion of any of: UCAAGGAAGAUGGCAUUUCU, UCAAGGAAGAUGGCAUUUC, UCAAGGAAGAUGGCAUUU, UCAAGGAAGAUGGCAUU, UCAAGGAAGAUGGCAU, UCAAGGAAGAUGGCA, CAAGGAAGAUGGCAUUUCU, AAGGAAGAUGGCAUUUCU, AGGAAGAUGGCAUUUCU,

GGAAGAUGGCAUUUCU, GAAGAUGGCAUUUCU, CAAGGAAGAUGGCAUUUC, CAAGGAAGAUGGCAUUU, AAGGAAGAUGGCAUUUC, AAGGAAGAUGGCAUUU, AGGAAGAUGGCAUUU, or AAGGAAGAUGGCAUU, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled.

[0088] In some embodiments, the present disclosure provides a chirally controlled composition of an oligonucleotide capable of mediating skipping of Dystrophin exon 53. In some embodiments, the present disclosure provides a chirally controlled composition of an oligonucleotide capable of mediating skipping of Dystrophin exon 53 and disclosed herein.

[0089] In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9517. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9519. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9521. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9524. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9714. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9715. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9747. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9748. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9749. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9897. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9898. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9899. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9900. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9906. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-9912. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-10670. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-10671. In some embodiments, the present disclosure provides a chirally controlled composition of oligonucleotide WV-10672.

[0090] In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which is, comprises, or comprises a 15-base portion of the base sequence of CUCCGGUUCUGAAGGUGUUC, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled. In some embodiments, the

present disclosure provides a composition of an oligonucleotide having a base sequence which is CUCCGGUUCUGAAGGUGUUC, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled. In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which comprises CUCCGGUUCUGAAGGUGUUC, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled. In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which is, comprises, or comprises a 15-base portion of CUCCGGUUCUGAAGGUGUUC, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled. In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which is or comprises CUCCGGUUCUGAAGGUGUCC, UCCGGUUCUGAAGGUGUUC, UCCGGUUCUGAAGGUGUUC, CCGGUUCUGAAGGUGUUC, CGGUUCUGAAGGUGUUC, GGUUCUGAAGGUGUUC, GUUCUGAAGGUGUUC, CUCCGGUUCUGAAGGUGUU, CUCCGGUUCUGAAGGUGU, CUCCGGUUCUGAAGGUG, CUCCGGUUCUGAAGGU, CUCCGGUUCUGAAGG, UCCGGUUCUGAAGGUGUU, CCGGUUCUGAAGGUGUU, UCCGGUUCUGAAGGUGU, CCGGUUCUGAAGGUGU, UCCGGUUCUGAAGGUG, CCGGUUCUGAAGGUG, CGGUUCUGAAGGUGUU, UCCGGUUCUGAAGGUGUUC, UCCGGUUCUGAAGGUG, UCCGGUUCUGAAGGU, CGGUUCUGAAGGUGUU, GGUUCUGAAGGUGUU, or GGUUCUGAAGGUGUU, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled. In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which is, comprises, or comprises a 15-base portion of the base sequence of UUCUGAAGGUGUUCUUGUAC, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled. In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which is UUCUGAAGGUGUUCUUGUAC, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled. In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which comprises UUCUGAAGGUGUUCUUGUAC, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled. In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which comprises a 15-base portion of the base sequence of UUCUGAAGGUGUUCUUGUAC, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally

controlled. In some embodiments, the present disclosure provides a composition of an oligonucleotide having a base sequence which is or comprises UUCUGAAGGUGUUCUUGUAC, UCUGAAGGUGUUCUUGUAC, CUGAAGGUGUUCUUGUAC, UGAAGGUGUUCUUGUAC, GAAGGUGUUCUUGUAC, AAGGUGUUCUUGUAC, UUCUGAAGGUGUUCUUGUA, UUCUGAAGGUGUUCUUGU, UUCUGAAGGUGUUCUUG, UUCUGAAGGUGUUCUU, UUCUGAAGGUGUUCU, UCUGAAGGUGUUCUUGUA, UCUGAAGGUGUUCUUGU, UCUGAAGGUGUUCUUG, UCUGAAGGUGUUCUU, CUGAAGGUGUUCUUGUA, CUGAAGGUGUUCUUGU, CUGAAGGUGUUCUUG, UGAAGGUGUUCUUGU, or UGAAGGUGUUCUUGUA, wherein each U can be optionally and independently replaced by T, and wherein the composition is optionally chirally controlled.

[0091] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide selected from any of the Tables. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide selected from any of the Tables, wherein the oligonucleotide is conjugated to a lipid or a targeting moiety.

[0092] In some embodiments, an oligonucleotide is at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 bases long, and optionally no more than 25, 30, 35, 40, 45, 50, 55, or 60 bases long. In some embodiments, an oligonucleotide is no more than 25 bases long. In some embodiments, an oligonucleotide is no more than 30 bases long. In some embodiments, an oligonucleotide is no more than 35 bases long. In some embodiments, an oligonucleotide is no more than 40 bases long. In some embodiments, an oligonucleotide is no more than 45 bases long. In some embodiments, an oligonucleotide is no more than 50 bases long. In some embodiments, an oligonucleotide is no more than 55 bases long. In some embodiments, an oligonucleotide is no more than 60 bases long. In some embodiments, each base is independently optionally substituted A, T, C, G, or U, or an optionally substituted tautomer of A, T, C, G, or U.

[0093] In some embodiments, provided oligonucleotides comprise additional chemical moieties besides their oligonucleotide chains (oligonucleotide backbones and bases), e.g., lipid moieties, targeting moieties, etc. In some embodiments, a lipid is a fatty acid. In some embodiments, an oligonucleotide is conjugated to a fatty acid. In some embodiments, a fatty acid comprises 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more carbon atoms.

[0094] In some embodiments, a lipid is stearic acid or turbinaric acid. In some embodiments, a lipid is stearic acid. In some embodiments, a lipid is turbinaric acid.

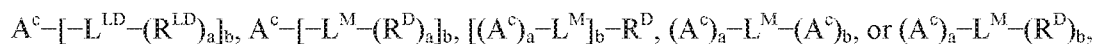
[0095] In some embodiments, a lipid comprises an optionally substituted, C₁₀-C₈₀, C₁₀-C₆₀, or C₁₀-C₄₀ saturated or partially unsaturated aliphatic group, wherein one or more methylene units are

optionally and independently replaced by C_1-C_6 alkylene, C_1-C_6 alkenylene, $-C\equiv C-$, a C_1-C_6 heteroaliphatic moiety, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, $-SC(O)-$, $-C(O)S-$, $-OC(O)-$, and $-C(O)O-$, wherein each variable is independently as defined and described herein.

[0096] In some embodiments, a lipid is selected from the group consisting of: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (DHA or cis-DHA), turbinaric acid and dilinoleyl.

[0097] In some embodiments, a lipid is conjugated to an oligonucleotide chain, optionally through one or more linker moieties. In some embodiments, a lipid is not conjugated to an oligonucleotide chain.

[0098] In some embodiments, a provided oligonucleotide is conjugated, optionally through a linker, to a chemical moiety, e.g., a lipid moiety, a peptide moiety, a targeting moiety, a carbohydrate moiety, a sulfonamide moiety, an antibody or a fragment thereof. In some embodiments, a provided compound, e.g., an oligonucleotide, has the structure of:



or a salt thereof, wherein:

A^c is an oligonucleotide chain (e.g., $H-A^c$, $[H]_a-A^c$ or $[H]_b-A^c$ is an oligonucleotide);

a is 1-1000;

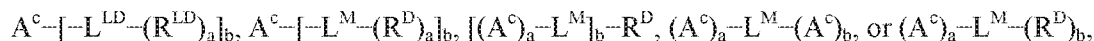
b is 1-1000;

each of L^{LD} and L^M is independently a linker moiety;

R^{LD} is a lipid moiety; and

each R^D is independently a lipid moiety or a targeting moiety.

[0099] In some embodiments, a provided compound, e.g., an oligonucleotide, has the structure of:



or a salt thereof, wherein:

A^c is an oligonucleotide chain (e.g., $H-A^c$, $[H]_a-A^c$ or $[H]_b-A^c$ is an oligonucleotide);

a is 1-1000;

b is 1-1000;

each R^D is independently R^{LD} , R^{CD} or R^{TD} ;

R^{CD} is an optionally substituted, linear or branched group selected from a C_{1-100} aliphatic group and a C_{1-100} heteroaliphatic group having 1-30 heteroatoms, wherein one or more methylene units are optionally and independently replaced with C_{1-6} alkylene, C_{1-6} alkenylene, $-C\equiv C-$, a bivalent C_1-C_6

heteroaliphatic group having 1-5 heteroatoms, $-\text{C}(\text{R}')_2-$, $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{C}(\text{O})\text{S}-$, $-\text{C}(\text{O})\text{O}-$, $-\text{P}(\text{O})(\text{OR}')-$, $-\text{P}(\text{O})(\text{SR}')-$, $-\text{P}(\text{O})(\text{R}')-$, $-\text{P}(\text{O})(\text{NR}')-$, $-\text{P}(\text{S})(\text{OR}')-$, $-\text{P}(\text{S})(\text{SR}')-$, $-\text{P}(\text{S})(\text{R}')-$, $-\text{P}(\text{S})(\text{NR}')-$, $-\text{P}(\text{R}')-$, $-\text{P}(\text{OR}')-$, $-\text{P}(\text{SR}')-$, $-\text{P}(\text{NR}')-$, $-\text{P}(\text{OR}')[\text{B}(\text{R}')_3]-$, $-\text{OP}(\text{O})(\text{OR}')\text{O}-$, $-\text{OP}(\text{O})(\text{SR}')\text{O}-$, $-\text{OP}(\text{O})(\text{R}')\text{O}-$, $-\text{OP}(\text{O})(\text{NR}')\text{O}-$, $-\text{OP}(\text{OR}')\text{O}-$, $-\text{OP}(\text{SR}')\text{O}-$, $-\text{OP}(\text{NR}')\text{O}-$, $-\text{OP}(\text{R}')\text{O}-$, or $-\text{OP}(\text{OR}')[\text{B}(\text{R}')_3]\text{O}-$; and one or more CH or carbon atoms are optionally and independently replaced with Cy^{L} ;

R^{LD} is an optionally substituted, linear or branched C_{1-100} aliphatic group wherein one or more methylene units are optionally and independently replaced with C_{1-6} alkylene, C_{1-6} alkenylene, $-\text{C}\equiv\text{C}-$, $-\text{C}(\text{R}')_2-$, $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{C}(\text{O})\text{S}-$, $-\text{C}(\text{O})\text{O}-$, $-\text{P}(\text{O})(\text{OR}')-$, $-\text{P}(\text{O})(\text{SR}')-$, $-\text{P}(\text{O})(\text{R}')-$, $-\text{P}(\text{O})(\text{NR}')-$, $-\text{P}(\text{S})(\text{OR}')-$, $-\text{P}(\text{S})(\text{SR}')-$, $-\text{P}(\text{S})(\text{R}')-$, $-\text{P}(\text{S})(\text{NR}')-$, $-\text{P}(\text{R}')-$, $-\text{P}(\text{OR}')-$, $-\text{P}(\text{SR}')-$, $-\text{P}(\text{NR}')-$, $-\text{P}(\text{OR}')[\text{B}(\text{R}')_3]-$, $-\text{OP}(\text{O})(\text{OR}')\text{O}-$, $-\text{OP}(\text{O})(\text{SR}')\text{O}-$, $-\text{OP}(\text{O})(\text{R}')\text{O}-$, $-\text{OP}(\text{O})(\text{NR}')\text{O}-$, $-\text{OP}(\text{OR}')\text{O}-$, $-\text{OP}(\text{SR}')\text{O}-$, $-\text{OP}(\text{NR}')\text{O}-$, $-\text{OP}(\text{R}')\text{O}-$, or $-\text{OP}(\text{OR}')[\text{B}(\text{R}')_3]\text{O}-$; and one or more CH or carbon atoms are optionally and independently replaced with Cy^{L} ;

R^{TD} is a targeting moiety;

each of L^{LD} and L^{M} is independently a covalent bond, or a bivalent or multivalent, optionally substituted, linear or branched group selected from a C_{1-100} aliphatic group and a C_{1-100} heteroaliphatic group having 1-30 heteroatoms, wherein one or more methylene units are optionally and independently replaced with C_{1-6} alkylene, C_{1-6} alkenylene, $-\text{C}\equiv\text{C}-$, a bivalent C_1-C_6 heteroaliphatic group having 1-5 heteroatoms, $-\text{C}(\text{R}')_2-$, $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{C}(\text{O})\text{S}-$, $-\text{C}(\text{O})\text{O}-$, $-\text{P}(\text{O})(\text{OR}')-$, $-\text{P}(\text{O})(\text{SR}')-$, $-\text{P}(\text{O})(\text{R}')-$, $-\text{P}(\text{O})(\text{NR}')-$, $-\text{P}(\text{S})(\text{OR}')-$, $-\text{P}(\text{S})(\text{SR}')-$, $-\text{P}(\text{S})(\text{R}')-$, $-\text{P}(\text{S})(\text{NR}')-$, $-\text{P}(\text{R}')-$, $-\text{P}(\text{OR}')-$, $-\text{P}(\text{SR}')-$, $-\text{P}(\text{NR}')-$, $-\text{P}(\text{OR}')[\text{B}(\text{R}')_3]-$, $-\text{OP}(\text{O})(\text{OR}')\text{O}-$, $-\text{OP}(\text{O})(\text{SR}')\text{O}-$, $-\text{OP}(\text{O})(\text{R}')\text{O}-$, $-\text{OP}(\text{O})(\text{NR}')\text{O}-$, $-\text{OP}(\text{OR}')\text{O}-$, $-\text{OP}(\text{SR}')\text{O}-$, $-\text{OP}(\text{NR}')\text{O}-$, $-\text{OP}(\text{R}')\text{O}-$, or $-\text{OP}(\text{OR}')[\text{B}(\text{R}')_3]\text{O}-$; and one or more CH or carbon atoms are optionally and independently replaced with Cy^{L} ;

each $-\text{Cy}-$ is independently an optionally substituted bivalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each Cy^{L} is independently an optionally substituted trivalent or tetravalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each R' is independently -R, -C(O)R, -C(O)OR, or -S(O)₂R; and

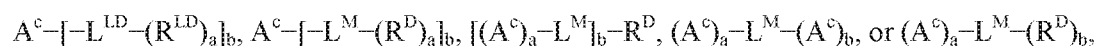
each R is independently -H, or an optionally substituted group selected from C₁₋₃₀ aliphatic, C₁₋₃₀ heteroaliphatic having 1-10 heteroatoms, C₆₋₃₀ aryl, C₆₋₃₀ arylaliphatic, C₆₋₃₀ arylheteroaliphatic having 1-10 heteroatoms, 5-30 membered heteroaryl having 1-10 heteroatoms, and 3-30 membered heterocyclyl having 1-10 heteroatoms, or

two R groups are optionally and independently taken together to form a covalent bond, or

two or more R groups on the same atom are optionally and independently taken together with the atom to form an optionally substituted, 3-30 membered monocyclic, bicyclic or polycyclic ring having, in addition to the atom, 0-10 heteroatoms, or

two or more R groups on two or more atoms are optionally and independently taken together with their intervening atoms to form an optionally substituted, 3-30 membered monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms.

[00100] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a plurality of oligonucleotides each having the structure of:



or a salt thereof.

[00101] In some embodiments, [H]_b-Ac (wherein b is 1-1000) is an oligonucleotide of any one of the Tables. In some embodiments, [H]_b-Ac is an oligonucleotide of Table A1.

[00102] In some embodiments, a is 1-100. In some embodiments, a is 1-50. In some embodiments, a is 1-40. In some embodiments, a is 1-30. In some embodiments, a is 1-20. In some embodiments, a is 1-15. In some embodiments, a is 1-10. In some embodiments, a is 1-9. In some embodiments, a is 1-8. In some embodiments, a is 1-7. In some embodiments, a is 1-6. In some embodiments, a is 1-5. In some embodiments, a is 1-4. In some embodiments, a is 1-3. In some embodiments, a is 1-2. In some embodiments, a is 1. In some embodiments, a is 2. In some embodiments, a is 3. In some embodiments, a is 4. In some embodiments, a is 5. In some embodiments, a is 6. In some embodiments, a is 7. In some embodiments, a is 8. In some embodiments, a is 9. In some embodiments, a is 10. In some embodiments, a is more than 10. In some embodiments, b is 1-100. In some embodiments, b is 1-50. In some embodiments, b is 1-40. In some embodiments, b is 1-30. In some embodiments, b is 1-20. In some embodiments, b is 1-15. In some embodiments, b is 1-10. In some embodiments, b is 1-9. In some embodiments, b is 1-8. In some embodiments, b is 1-7. In some embodiments, b is 1-6. In some embodiments, b is 1-5. In some embodiments, b is 1-4. In some embodiments, b is 1-3. In some embodiments, b is 1-2. In some embodiments, b is 1. In some embodiments, b is 2. In some embodiments, b is 3. In some embodiments, b is 4. In some embodiments, b is 5. In some embodiments, b is 6. In some embodiments, b is 7. In some embodiments, b is 8. In

some embodiments, b is 9. In some embodiments, b is 10. In some embodiments, b is more than 10. In some embodiments, an oligonucleotide has the structure of $A^c-L^{LD}-R^{LD}$. In some embodiments, A^c is conjugated through one or more of its sugar, base and/or internucleotidic linkage moieties. In some embodiments, A^c is conjugated through its 5'-OH (5'-O-). In some embodiments, A^c is conjugated through its 3'-OH (3'-O-). In some embodiments, before conjugation, $A^c-(H)_b$ (b is an integer of 1-1000 depending on valency of A^c) is an oligonucleotide as described herein, for example, one of those described in any one of the Tables. In some embodiments, L^M is $-L-$. In some embodiments, L^M comprises a phosphorothioate group. In some embodiments, L^M is $-C(O)NH-(CH_2)_6-OP(=O)(S^-)-O-$. In some embodiments, the $-C(O)NH$ end is connected to R^{LD} , and the $-O-$ end is connected to the oligonucleotide, *e.g.*, through 5'- or 3'-end. In some embodiments, R^{LD} is optionally substituted C_{10} , C_{15} , C_{16} , C_{17} , C_{18} , C_{19} , C_{20} , C_{21} , C_{22} , C_{23} , C_{24} , or C_{25} to C_{20} , C_{21} , C_{22} , C_{23} , C_{24} , C_{25} , C_{26} , C_{27} , C_{28} , C_{29} , C_{30} , C_{35} , C_{40} , C_{45} , C_{50} , C_{60} , C_{70} , or C_{80} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{10-80} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{20-80} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{10-70} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{20-70} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{10-60} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{20-60} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{10-50} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{20-50} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{10-40} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{20-40} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{10-30} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{20-30} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{10} , C_{15} , C_{16} , C_{17} , C_{18} , C_{19} , C_{20} , C_{21} , C_{22} , C_{23} , C_{24} , or C_{25} to C_{20} , C_{21} , C_{22} , C_{23} , C_{24} , C_{25} , C_{26} , C_{27} , C_{28} , C_{29} , C_{30} , C_{35} , C_{40} , C_{45} , C_{50} , C_{60} , C_{70} , or C_{80} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{10-80} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{20-80} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{10-70} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{20-70} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{10-60} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{20-60} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{10-50} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{20-50} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{10-40} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{20-40} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{10-30} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{20-30} aliphatic.

[00103] In some embodiments, incorporation of a lipid moiety into an oligonucleotide improves at least one property of the oligonucleotide compared to an otherwise identical oligonucleotide without the lipid moiety. In some embodiments, improved properties include increased activity (*e.g.*, increased ability to induce desirable skipping of a deleterious exon), decreased toxicity, and/or improved distribution to a tissue. In some embodiments, a tissue is muscle tissue. In some embodiments, a tissue is

skeletal muscle, gastrocnemius, triceps, heart or diaphragm. In some embodiments, improved properties include reduced hTLR9 agonist activity. In some embodiments, improved properties include hTLR9 antagonist activity. In some embodiments, improved properties include increased hTLR9 antagonist activity.

[00104] In some embodiments, an oligonucleotide or oligonucleotide composition is: a DMD oligonucleotide or oligonucleotide composition; an oligonucleotide or oligonucleotide composition comprising a non-negatively charged internucleotidic linkage; or a DMD oligonucleotide comprising a non-negatively charged internucleotidic linkage.

[00105] In some embodiments, the present disclosure pertains to a composition comprising an a DMD oligonucleotide comprising at least one chirally controlled phosphorothioate internucleotidic linkage in the Rp or Sp configuration, at least one natural phosphate internucleotidic linkage, and at least one non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a composition comprising an a DMD oligonucleotide comprising at least one phosphorothioate internucleotidic linkage, at least one natural phosphate internucleotidic linkage, and at least one non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a composition comprising an a DMD oligonucleotide comprising at least one phosphorothioate internucleotidic linkage, at least one natural phosphate internucleotidic linkage, and at least one chirally controlled non-negatively charged internucleotidic linkage. In some embodiments, the present disclosure pertains to a composition comprising an a DMD oligonucleotide comprising at least one chirally controlled phosphorothioate internucleotidic linkage in the Rp or Sp configuration, at least one natural phosphate internucleotidic linkage, and at least one chirally controlled non-negatively charged internucleotidic linkage.

[00106] In some embodiments, a DMD oligonucleotide (e.g., an oligonucleotide whose base sequence contains no more than 5, 4, 3, 2, or 1 mismatches when hybridizing to a portion of a DMD transcript or a DMD genetic sequence having the same length) is capable of mediating skipping of one or more exons of the Dystrophin transcript.

[00107] In some embodiments, a DMD oligonucleotide has a base sequence which consists of the base sequence of an example oligonucleotide disclosed herein (e.g., an oligonucleotide listed in a Table), or a base sequence which comprises a 15-base portion of an example oligonucleotide nucleotide described herein. In some embodiments, a DMD oligonucleotide has a length of 15 to 50 bases.

[00108] In some embodiments, an oligonucleotide comprises a nucleobase modification, a sugar modification, and/or an internucleotidic linkage. In some embodiments, a DMD oligonucleotide has a pattern of nucleobase modifications, sugar modifications, and/or internucleotidic linkages of an example oligonucleotide described herein (or any portion thereof having a length of at least 5 bases).

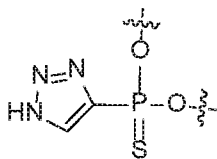
[00109] In some embodiments, an oligonucleotide comprises a nucleobase modification which is BrU.

[00110] In some embodiments, an oligonucleotide comprises a sugar modification which is 2'-OMe, 2'-F, 2'-MOE, or LNA.

[00111] In some embodiments, an oligonucleotide comprises an internucleotidic linkage which is a natural phosphate linkage or a phosphorothioate internucleotidic linkage. In some embodiments, a phosphorothioate internucleotidic linkage is not chirally controlled. In some embodiments, a phosphorothioate internucleotidic linkage is a chirally controlled internucleotidic linkage (e.g., Sp or Rp).

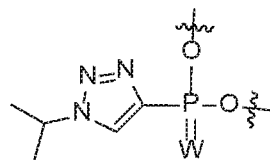
[00112] In some embodiments, an oligonucleotide comprises a non-negatively charged internucleotidic linkage. In some embodiments, a DMD oligonucleotide comprises a neutral internucleotidic linkage. In some embodiments, a neutral internucleotidic linkage is or comprises a triazole, alkyne, or cyclic guanidine moiety.

[00113] In some embodiments, an internucleotidic linkage comprising a triazole moiety (e.g., an optionally substituted triazolyl group) in a provided oligonucleotide, e.g., a DMD oligonucleotide, has the



structure of:

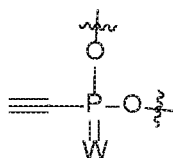
. In some embodiments, an internucleotidic linkage comprising a triazole



moiety has the formula of

, where W is O or S. In some embodiments, an

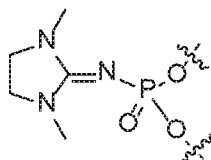
internucleotidic linkage comprising an alkyne moiety (e.g., an optionally substituted alkynyl group) has



the formula of:

, wherein W is O or S. In some embodiments, an internucleotidic linkage

comprises a guanidine moiety. In some embodiments, an internucleotidic linkage comprises a cyclic guanidine moiety. In some embodiments, an internucleotidic linkage comprising a cyclic guanidine

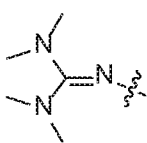


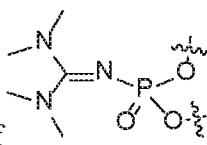
moiety has the structure of:

. In some embodiments, a neutral internucleotidic linkage

or internucleotidic linkage comprising a cyclic guanidine moiety is stereochemically controlled.

[00114] In some embodiments, a DMD oligonucleotide comprises a lipid moiety In some

embodiments, an internucleotidic linkage comprises a Tmg group (). In some embodiments,

an internucleotidic linkage comprises a Tmg group and has the structure of  (the “Tmg internucleotidic linkage”). In some embodiments, neutral internucleotidic linkages include internucleotidic linkages of PNA and PMO, and an Tmg internucleotidic linkage.

[00115] In general, properties of oligonucleotide compositions as described herein can be assessed using any appropriate assay. Relative toxicity and/or protein binding properties for different compositions (*e.g.*, stereocontrolled vs non-stereocontrolled, and/or different stereocontrolled compositions) are typically desirably determined in the same assay, in some embodiments substantially simultaneously and in some embodiments with reference to historical results.

[00116] Those of skill in the art will be aware of and/or will readily be able to develop appropriate assays for particular oligonucleotide compositions. The present disclosure provides descriptions of certain particular assays, for example that may be useful in assessing one or more features of oligonucleotide composition behavior *e.g.*, complement activation, injection site inflammation, protein binding, *etc.*

[00117] For example, certain assays that may be useful in the assessment of toxicity and/or protein binding properties of oligonucleotide compositions may include any assay described and/or exemplified herein.

[00118] Among other things, in some embodiments, the present disclosure provides an oligonucleotide composition, comprising a plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and
- 4) pattern of backbone phosphorus modifications,

wherein:

oligonucleotides of the plurality comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 chirally controlled internucleotidic linkages; and

the oligonucleotide composition being characterized in that, when it is contacted with a transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under a

reference condition selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[00119] In some embodiments, the present disclosure provides a composition comprising a plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and
- 4) pattern of backbone phosphorus modifications,

which composition is chirally controlled and it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence, pattern of backbone linkages and pattern of backbone phosphorus modifications, for oligonucleotides of the particular oligonucleotide type, wherein:

the oligonucleotide composition is characterized in that, when it is contacted with a transcript in a transcript splicing system, splicing of the transcript is altered in that level of skipping of an exon is increased relative to that observed under a reference condition selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[00120] In some embodiments, the present disclosure provides a composition comprising a plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages; and
- 3) pattern of backbone phosphorus modifications,

wherein:

oligonucleotides of the plurality comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 non-negatively charged internucleotidic linkages;

the oligonucleotide composition is characterized in that, when it is contacted with a transcript in a transcript splicing system, splicing of the transcript is altered in that level of skipping of an exon is increased relative to that observed under a reference condition selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[00121] In some embodiments, the present disclosure provides a composition comprising a plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages; and
- 3) pattern of backbone phosphorus modifications,

wherein:

oligonucleotides of the plurality comprise:

- 1) a 5'-end region comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleoside units comprising a 2'-F modified sugar moiety;
- 2) a 3'-end region comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleoside units comprising a 2'-F modified sugar moiety; and
- 3) a middle region between the 5'-end region and the 3'-region comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotidic units comprising a phosphodiester linkage.

[00122] In some embodiments, the present disclosure provides a composition comprising a plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and
- 4) pattern of backbone phosphorus modifications,

wherein:

oligonucleotides of the plurality comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 chirally controlled internucleotidic linkages; and

oligonucleotides of the plurality comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 non-negatively charged internucleotidic linkages.

[00123] In some embodiments, the present disclosure provides a composition comprising a plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and
- 4) pattern of backbone phosphorus modifications,

wherein:

the oligonucleotides of the plurality comprise cholesterol; L-carnitine (amide and carbamate bond); Folic acid; Cleavable lipid (1,2-dilaurin and ester bond); Insulin receptor ligand; Gambogic acid; CPP; Glucose (tri- and hex-antennary); or Mannose (tri- and hex-antennary, alpha and beta).

[00124] In some embodiments, the present disclosure provides a pharmaceutical composition comprising an oligonucleotide or an oligonucleotide composition of the present disclosure and a pharmaceutically acceptable carrier.

[00125] In some embodiments, the present disclosure provides a method for altering splicing of a target transcript, comprising administering an oligonucleotide composition of the present disclosure. In some embodiments, the present disclosure provides a method for reducing level of a transcript or a

product thereof, comprising administering an oligonucleotide composition of the present disclosure. In some embodiments, the present disclosure provides a method for increase level of a transcript or a product thereof, comprising administering an oligonucleotide composition of the present disclosure. A method for treating muscular dystrophy, Duchenne (Duchenne's) muscular dystrophy (DMD), or Becker (Becker's) muscular dystrophy (BMD), comprising administering to a subject susceptible thereto or suffering therefrom a composition described in the present disclosure.

[00126] In some embodiments, the present disclosure provides a method for treating muscular dystrophy, Duchenne (Duchenne's) muscular dystrophy (DMD), or Becker (Becker's) muscular dystrophy (BMD), comprising administering to a subject susceptible thereto or suffering therefrom a composition comprising any DMD oligonucleotide disclosed herein.

[00127] In some embodiments, the present disclosure provides a method for treating muscular dystrophy, Duchenne (Duchenne's) muscular dystrophy (DMD), or Becker (Becker's) muscular dystrophy (BMD), comprising (a) administering to a subject susceptible thereto or suffering therefrom a composition comprising any oligonucleotide disclosed herein, and (b) administering to the subject additional treatment which is capable of preventing, treating, ameliorating or slowing the progress of muscular dystrophy, Duchenne (Duchenne's) muscular dystrophy (DMD), or Becker (Becker's) muscular dystrophy (BMD).

BRIEF DESCRIPTION OF THE DRAWINGS

[00128] *Figure 1.* Figure 1 shows an example of multiple exon skipping.

[00129] *Figure 2.* Figure 2 shows a cartoon of a method for detecting multiple exon skipping.

[00130] *Figure 3.* Figure 3 illustrates various strategies for multiple exon skipping.

DEFINITIONS

[00131] As used herein, the following definitions shall apply unless otherwise indicated. For purposes of this disclosure, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry", 5th Ed., Ed.: Smith, M.B. and March, J., John Wiley & Sons, New York: 2001.

[00132] *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"), or combinations thereof. In some embodiments, aliphatic groups contain 1-100 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-20 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-10 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-9 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-8 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-7 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-6 aliphatic carbon atoms. In still other embodiments, aliphatic groups contain 1-5 aliphatic carbon atoms, and in yet other embodiments, aliphatic groups contain 1, 2, 3, or 4 aliphatic carbon atoms. In some embodiments, "cycloaliphatic" (or "carbocycle" or "cycloalkyl") refers to a monocyclic or bicyclic or polycyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic. In some embodiments, "cycloaliphatic" (or "carbocycle" or "cycloalkyl") refers to a monocyclic C₃-C₆ hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic. 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.

[00133] *Alkenyl:* As used herein, the term "alkenyl" refers to an aliphatic group, as defined herein, having one or more double bonds.

[00134] *Alkyl:* As used herein, the term "alkyl" is given its ordinary meaning in the art and may include saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In some embodiments, an alkyl has 1-100 carbon atoms. In certain embodiments, a straight chain or branched chain alkyl has about 1-20 carbon atoms in its backbone (e.g., C₁-C₂₀ for straight chain, C₂-C₂₀ for branched chain), and alternatively, about 1-10. In some embodiments, cycloalkyl rings have from about 3-10 carbon atoms in their ring structure where such rings are monocyclic, bicyclic, or polycyclic, and alternatively about 5, 6 or 7 carbons in the ring structure. In some embodiments, an alkyl group may be a lower alkyl group, wherein a lower alkyl group comprises 1-4 carbon atoms (e.g., C₁-C₄ for straight chain lower alkyls).

[00135] *Alkynyl:* As used herein, the term "alkynyl" refers to an aliphatic group, as defined herein, having one or more triple bonds.

[00136] *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.

[00137] *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.

[00138] *Aryl:* The term “aryl”, as used herein, used alone or as part of a larger moiety as in “aralkyl,” “aralkoxy,” or “aryloxyalkyl,” refers to monocyclic, bicyclic or polycyclic ring systems having a total of, *e.g.*, five to thirty ring members, wherein at least one ring in the system is aromatic. In some embodiments, an aryl group is a monocyclic, bicyclic or polycyclic ring system 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 3 to 7 ring members. In some embodiments, an aryl group is a biaryl group. The term “aryl” may be used interchangeably with the term “aryl ring.” In certain embodiments of the present disclosure, “aryl” refers to an aromatic ring system which includes, but not limited to, phenyl, biphenyl, naphthyl, binaphthyl, 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 an aromatic ring fused to one or more non-aromatic rings, such as indanyl, phthalimidyl, naphthimidyl, phenanthridinyl, or tetrahydronaphthyl, and the like.

[00139] *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.

[00140] *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.

[00141] *Cycloaliphatic:* The term “cycloaliphatic,” “carbocycle,” “carbocyclyl,” “carbocyclic radical,” and “carbocyclic ring,” are used interchangeably, and as used herein, refer to saturated or partially unsaturated, but non-aromatic, cyclic aliphatic monocyclic, bicyclic, or polycyclic ring systems,

as described herein, having, unless otherwise specified, from 3 to 30 ring members. Cycloaliphatic groups include, without limitation, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, cycloheptenyl, cyclooctyl, cyclooctenyl, norbornyl, adamantyl, and cyclooctadienyl. In some embodiments, a cycloaliphatic group has 3–6 carbons. In some embodiments, a cycloaliphatic group is saturated and is cycloalkyl. The term “cycloaliphatic” may also include aliphatic rings that are fused to one or more aromatic or nonaromatic rings, such as decahydronaphthyl or 1,2,3,4-tetrahydronaphth-1-yl. In some embodiments, a cycloaliphatic group is bicyclic. In some embodiments, a cycloaliphatic group is tricyclic. In some embodiments, a cycloaliphatic group is polycyclic. In some embodiments, “cycloaliphatic” refers to C₃-C₆ monocyclic hydrocarbon, or C₈-C₁₀ bicyclic or polycyclic hydrocarbon, that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, or a C₉-C₁₆ polycyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic.

[00142] *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.

[00143] *Heteroaliphatic:* The term “heteroaliphatic” refers to an aliphatic group wherein one or more units selected from C, CH, CH₂, and CH₃ are independently replaced by one or more heteroatoms. In some embodiments, a heteroaliphatic group is heteroalkyl. In some embodiments, a heteroaliphatic group is heteroalkenyl.

[00144] *Heteroaryl:* The terms “heteroaryl” and “heteroar—”, as used herein, used alone or as part of a larger moiety, e.g., “heteroaralkyl,” or “heteroaralkoxy,” refer to monocyclic, bicyclic or polycyclic ring systems having a total of, e.g., five to thirty ring members, wherein at least one ring in the system is aromatic and at least one aromatic ring atom is a heteroatom. In some embodiments, a heteroaryl group is a group having 5 to 10 ring atoms (i.e., monocyclic, bicyclic or polycyclic), in some embodiments 5, 6, 9, or 10 ring atoms. In some embodiments, a heteroaryl group has 6, 10, or 14 π electrons shared in a cyclic

array; and having, in addition to carbon atoms, from one to five heteroatoms. Heteroaryl groups include, without limitation, thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, indoliziny, purinyl, naphthyridinyl, and pteridinyl. In some embodiments, a heteroaryl is a heterobiaryl group, such as bipyridyl and the like. 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. Non-limiting examples include indolyl, isoindolyl, benzothienyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4H-quinoliziny, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolyl, tetrahydroisoquinolyl, and pyrido[2,3-b]-1,4-oxazin-3(4H)-one. A heteroaryl group may be monocyclic, bicyclic or polycyclic. 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 group, wherein the alkyl and heteroaryl portions independently are optionally substituted.

[00145] *Heteroatom:* The term “heteroatom” means an atom that is not carbon or hydrogen. In some embodiments, a heteroatom is oxygen, sulfur, nitrogen, phosphorus, boron or silicon (including any oxidized form of nitrogen, 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-2H-pyrrolyl), NH (as in pyrrolidinyl) or NR⁺ (as in N-substituted pyrrolidinyl); *etc.*). In some embodiments, a heteroatom is boron, nitrogen, oxygen, silicon, sulfur, or phosphorus. In some embodiments, a heteroatom is nitrogen, oxygen, silicon, sulfur, or phosphorus. In some embodiments, a heteroatom is nitrogen, oxygen, sulfur, or phosphorus. In some embodiments, a heteroatom is nitrogen, oxygen or sulfur.

[00146] *Heterocycle:* As used herein, the terms “heterocycle,” “heterocyclyl,” “heterocyclic radical,” and “heterocyclic ring”, as used herein, are used interchangeably and refer to a monocyclic, bicyclic or polycyclic ring moiety (e.g., 3-30 membered) that is saturated or partially unsaturated and has one or more heteroatom ring atoms. In some embodiments, a heterocyclyl group is a stable 5- to 7-membered monocyclic or 7- to 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 substituted nitrogen. As an example, in a saturated or partially unsaturated ring having 0-3 heteroatoms selected from oxygen, sulfur and nitrogen, the nitrogen may be N (as in 3,4-dihydro-2H-pyrrolyl), NH (as in pyrrolidinyl), or ⁺NR (as in N-substituted pyrrolidinyl). 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, tetrahydrothienyl, 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 heterocyclyl rings fused to one or more aryl, heteroaryl, or cycloaliphatic rings, such as indolinyl, 3H-indolyl, chromanyl, phenanthridinyl, or tetrahydroquinolinyl. A heterocyclyl group may be monocyclic, bicyclic or polycyclic. The term “heterocyclylalkyl” refers to an alkyl group substituted by a heterocyclyl, wherein the alkyl and heterocyclyl portions independently are optionally substituted.

[00147] *Intraperitoneal:* The phrases “intraperitoneal administration” and “administered intraperitoneally” as used herein have their art-understood meaning referring to administration of a compound or composition into the peritoneum of a subject.

[00148] *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).

[00149] *In vivo:* As used herein, the term “*in vivo*” refers to events that occur within an organism (*e.g.*, animal, plant, and/or microbe).

[00150] *Lower alkyl:* The term “lower alkyl” refers to a C₁₋₄ straight or branched alkyl group. Example lower alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tert-butyl.

[00151] *Lower haloalkyl:* The term “lower haloalkyl” refers to a C₁₋₄ straight or branched alkyl group that is substituted with one or more halogen atoms.

[00152] *Optionally substituted:* As described herein, compounds of the disclosure, *e.g.*, oligonucleotides, lipids, carbohydrates, *etc.*, 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 disclosure 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.

[00153] Suitable monovalent substituents are halogen; $-(CH_2)_{0-4}R^\circ$; $-(CH_2)_{0-4}OR^\circ$; $-O(CH_2)_{0-4}R^\circ$, $-O-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}CH(OR^\circ)_2$; $-(CH_2)_{0-4}Ph$, 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° ; $-NO_2$; $-CN$; $-N_3$; $-(CH_2)_{0-4}N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$; $-N(R^\circ)C(S)R^\circ$; $-(CH_2)_{0-4}N(R^\circ)C(O)N(R^\circ)_2$; $-N(R^\circ)C(S)N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $-N(R^\circ)N(R^\circ)C(O)R^\circ$; $-N(R^\circ)N(R^\circ)C(O)N(R^\circ)_2$; $-N(R^\circ)N(R^\circ)C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)R^\circ$; $-C(S)R^\circ$; $-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)SR^\circ$; $-(CH_2)_{0-4}C(O)OSi(R^\circ)_3$; $-(CH_2)_{0-4}OC(O)R^\circ$; $-OC(O)(CH_2)_{0-4}SR^\circ$; $-SC(S)SR^\circ$; $-(CH_2)_{0-4}SC(O)R^\circ$; $-(CH_2)_{0-4}C(O)N(R^\circ)_2$; $-C(S)N(R^\circ)_2$; $-C(S)SR^\circ$; $-SC(S)SR^\circ$; $-(CH_2)_{0-4}OC(O)N(R^\circ)_2$; $-C(O)N(OR^\circ)R^\circ$; $-C(O)C(O)R^\circ$; $-C(O)CH_2C(O)R^\circ$; $-C(NOR^\circ)R^\circ$; $-(CH_2)_{0-4}SSR^\circ$; $-(CH_2)_{0-4}S(O)_2R^\circ$; $-(CH_2)_{0-4}S(O)_2OR^\circ$; $-(CH_2)_{0-4}OS(O)_2R^\circ$; $-S(O)_2N(R^\circ)_2$; $-(CH_2)_{0-4}S(O)R^\circ$; $-N(R^\circ)S(O)_2N(R^\circ)_2$; $-N(R^\circ)S(O)_2R^\circ$; $-N(OR^\circ)R^\circ$; $-C(NH)N(R^\circ)_2$; $-Si(R^\circ)_3$; $-OSi(R^\circ)_3$; $-P(R^\circ)_2$; $-P(OR^\circ)_2$; $-P(R^\circ)(OR^\circ)$; $-OP(R^\circ)_2$; $-OP(OR^\circ)_2$; $-OP(R^\circ)(OR^\circ)$; $-P[N(R^\circ)_2]_2$; $-P(R^\circ)[N(R^\circ)_2]$; $-P(OR^\circ)[N(R^\circ)_2]$; $-OP[N(R^\circ)_2]_2$; $-OP(R^\circ)[N(R^\circ)_2]$; $-OP(OR^\circ)[N(R^\circ)_2]$; $-N(R^\circ)P(R^\circ)_2$; $-N(R^\circ)P(OR^\circ)_2$; $-N(R^\circ)P(R^\circ)(OR^\circ)$; $-N(R^\circ)P[N(R^\circ)_2]_2$; $-N(R^\circ)P(R^\circ)[N(R^\circ)_2]$; $-N(R^\circ)P(OR^\circ)[N(R^\circ)_2]$; $-B(R^\circ)_2$; $-B(R^\circ)(OR^\circ)$; $-B(OR^\circ)_2$; $-OB(R^\circ)_2$; $-OB(R^\circ)(OR^\circ)$; $-OB(OR^\circ)_2$; $-P(O)(R^\circ)_2$; $-P(O)(R^\circ)(OR^\circ)$; $-P(O)(R^\circ)(SR^\circ)$; $-P(O)(R^\circ)[N(R^\circ)_2]$; $-P(O)(OR^\circ)_2$; $-P(O)(SR^\circ)_2$; $-P(O)(OR^\circ)[N(R^\circ)_2]$; $-P(O)(SR^\circ)[N(R^\circ)_2]$; $-P(O)(OR^\circ)(SR^\circ)$; $-P(O)[N(R^\circ)_2]_2$; $-OP(O)(R^\circ)_2$; $-OP(O)(R^\circ)(OR^\circ)$; $-OP(O)(R^\circ)(SR^\circ)$; $-OP(O)(R^\circ)[N(R^\circ)_2]$; $-OP(O)(OR^\circ)_2$; $-OP(O)(SR^\circ)_2$; $-OP(O)(OR^\circ)[N(R^\circ)_2]$; $-OP(O)(SR^\circ)[N(R^\circ)_2]$; $-OP(O)(OR^\circ)(SR^\circ)$; $-OP(O)[N(R^\circ)_2]_2$; $-SP(O)(R^\circ)_2$; $-SP(O)(R^\circ)(OR^\circ)$; $-SP(O)(R^\circ)(SR^\circ)$; $-SP(O)(R^\circ)[N(R^\circ)_2]$; $-SP(O)(OR^\circ)_2$; $-SP(O)(SR^\circ)_2$; $-SP(O)(OR^\circ)[N(R^\circ)_2]$; $-SP(O)(SR^\circ)[N(R^\circ)_2]$; $-SP(O)(OR^\circ)(SR^\circ)$; $-SP(O)[N(R^\circ)_2]_2$; $-N(R^\circ)P(O)(R^\circ)_2$; $-N(R^\circ)P(O)(R^\circ)(OR^\circ)$; $-N(R^\circ)P(O)(R^\circ)(SR^\circ)$; $-N(R^\circ)P(O)(R^\circ)[N(R^\circ)_2]$; $-N(R^\circ)P(O)(OR^\circ)_2$; $-N(R^\circ)P(O)(SR^\circ)_2$; $-N(R^\circ)P(O)(OR^\circ)[N(R^\circ)_2]$; $-N(R^\circ)P(O)(SR^\circ)[N(R^\circ)_2]$; $-N(R^\circ)P(O)(OR^\circ)(SR^\circ)$; $-N(R^\circ)P(O)[N(R^\circ)_2]_2$; $-P(R^\circ)_2[B(R^\circ)_3]$; $-P(OR^\circ)_2[B(R^\circ)_3]$; $-P(NR^\circ)_2[B(R^\circ)_3]$; $-P(R^\circ)(OR^\circ)[B(R^\circ)_3]$; $-P(R^\circ)[N(R^\circ)_2][B(R^\circ)_3]$; $-P(OR^\circ)[N(R^\circ)_2][B(R^\circ)_3]$; $-OP(R^\circ)_2[B(R^\circ)_3]$; $-OP(OR^\circ)_2[B(R^\circ)_3]$; $-OP(NR^\circ)_2[B(R^\circ)_3]$; $-OP(R^\circ)(OR^\circ)[B(R^\circ)_3]$; $-OP(R^\circ)[N(R^\circ)_2][B(R^\circ)_3]$; $-OP(OR^\circ)[N(R^\circ)_2][B(R^\circ)_3]$; $-N(R^\circ)P(R^\circ)_2[B(R^\circ)_3]$; $-N(R^\circ)P(OR^\circ)_2[B(R^\circ)_3]$; $-N(R^\circ)P(NR^\circ)_2[B(R^\circ)_3]$; $-N(R^\circ)P(R^\circ)(OR^\circ)[B(R^\circ)_3]$; $-N(R^\circ)P(R^\circ)[N(R^\circ)_2][B(R^\circ)_3]$; $-N(R^\circ)P(OR^\circ)[N(R^\circ)_2][B(R^\circ)_3]$; $-P(OR^\circ)[B(R^\circ)_3]$; $-(C_{1-4}$ straight or branched alkylene) $O-N(R^\circ)_2$; or $-(C_{1-4}$ straight or branched alkylene) $C(O)O-N(R^\circ)_2$, wherein each R° may be substituted as defined below and is independently hydrogen, C_{1-20} aliphatic, C_{1-20} heteroaliphatic having 1-5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, $-CH_2-(C_{6-20}$ aryl), $-O(CH_2)_{0-1}(C_{6-20}$ aryl), $-CH_2-(5-20$ membered heteroaryl ring having 1-5 heteroatoms

independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus), a 5–20 membered, monocyclic, bicyclic, or polycyclic, saturated, partially unsaturated or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, or, notwithstanding the definition above, two independent occurrences of R° , taken together with their intervening atom(s), form a 3–20 membered, monocyclic, bicyclic, or polycyclic, saturated, partially unsaturated or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, which may be substituted as defined below.

[00154] Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $-(CH_2)_{0-2}R^*$, $-(haloR^*)$, $-(CH_2)_{0-2}OH$, $-(CH_2)_{0-2}OR^*$, $-(CH_2)_{0-2}CH(OR^*)_2$, $-O(haloR^*)$, $-CN$, $-N_3$, $-(CH_2)_{0-2}C(O)R^*$, $-(CH_2)_{0-2}C(O)OH$, $-(CH_2)_{0-2}C(O)OR^*$, $-(CH_2)_{0-2}SR^*$, $-(CH_2)_{0-2}SH$, $-(CH_2)_{0-2}NH_2$, $-(CH_2)_{0-2}NHR^*$, $-(CH_2)_{0-2}NR^{*2}$, $-NO_2$, $-SiR^{*3}$, $-OSiR^{*3}$, $-C(O)SR^*$, $-(C_{1-4}$ straight or branched alkylene) $C(O)OR^*$, or $-SSR^*$ 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, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, and a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, and sulfur. Suitable divalent substituents on a saturated carbon atom of R° include $=O$ and $=S$.

[00155] Suitable divalent substituents, e.g., on a suitable carbon atom, nitrogen atom, are independently the following: $=O$, $=S$, $=CR^{*2}$, $=NNR^{*2}$, $=NNHC(O)R^*$, $=NNHC(O)OR^*$, $=NNHS(O)_2R^*$, $=NR^*$, $=NOR^*$, $-O(C(R^{*2}))_{2-3}O-$, or $-S(C(R^{*2}))_{2-3}S-$, wherein each R^* may be substituted as defined below and is independently hydrogen, C_{1-20} aliphatic, C_{1-20} heteroaliphatic having 1–5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, $-CH_2-(C_{6-20}$ aryl), $-O(CH_2)_{0-1}(C_{6-20}$ aryl), $-CH_2$ -(5-20 membered heteroaryl ring having 1–5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus), a 5–20 membered, monocyclic, bicyclic, or polycyclic, saturated, partially unsaturated or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, or, notwithstanding the definition above, two independent occurrences of R^* , taken together with their intervening atom(s), form a 3–20 membered, monocyclic, bicyclic, or polycyclic, saturated, partially unsaturated or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, which may be substituted as defined below. Suitable divalent substituents that are bound to vicinal substitutable atoms of an “optionally substituted” group include: $-O(CR^{*2})_{2-3}O-$.

[00156] Suitable monovalent substituents on R^* (or the ring formed by taking two independent occurrences of R^* together with their intervening atoms), are independently halogen, $-(CH_2)_{0-2}R^*$, $-(haloR^*)$, $-(CH_2)_{0-2}OH$, $-(CH_2)_{0-2}OR^*$, $-(CH_2)_{0-2}CH(OR^*)_2$, $-O(haloR^*)$, $-CN$, $-N_3$, $-(CH_2)_{0-2}C(O)R^*$, $-$

$(\text{CH}_2)_{0-2}\text{C}(\text{O})\text{OH}$, $-(\text{CH}_2)_{0-2}\text{C}(\text{O})\text{OR}^*$, $-(\text{CH}_2)_{0-2}\text{SR}^*$, $-(\text{CH}_2)_{0-2}\text{SH}$, $-(\text{CH}_2)_{0-2}\text{NH}_2$, $-(\text{CH}_2)_{0-2}\text{NHR}^*$, $-(\text{CH}_2)_{0-2}\text{NR}^{*2}$, $-\text{NO}_2$, $-\text{SiR}^{*3}$, $-\text{OSiR}^{*3}$, $-\text{C}(\text{O})\text{SR}^*$, $-(\text{C}_{1-4} \text{ straight or branched alkylene})\text{C}(\text{O})\text{OR}^*$, or $-\text{SSR}^*$ 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, $-\text{CH}_2\text{Ph}$, $-\text{O}(\text{CH}_2)_{0-1}\text{Ph}$, and a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, and sulfur. Suitable divalent substituents on a saturated carbon atom of R^* include $=\text{O}$ and $=\text{S}$.

[00157] In some embodiments, suitable substituents on a substitutable nitrogen of an “optionally substituted” group include $-\text{R}^\dagger$, $-\text{NR}^\dagger_2$, $-\text{C}(\text{O})\text{R}^\dagger$, $-\text{C}(\text{O})\text{OR}^\dagger$, $-\text{C}(\text{O})\text{C}(\text{O})\text{R}^\dagger$, $-\text{C}(\text{O})\text{CH}_2\text{C}(\text{O})\text{R}^\dagger$, $-\text{S}(\text{O})_2\text{R}^\dagger$, $-\text{S}(\text{O})_2\text{NR}^\dagger_2$, $-\text{C}(\text{S})\text{NR}^\dagger_2$, $-\text{C}(\text{NH})\text{NR}^\dagger_2$, or $-\text{N}(\text{R}^\dagger)\text{S}(\text{O})_2\text{R}^\dagger$; wherein each $\text{R}^\dagger$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted $-\text{OPh}$, 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 $\text{R}^\dagger$, 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.

[00158] In some embodiments, suitable substituents on the aliphatic group of $\text{R}^\dagger$ are independently halogen, $-\text{R}^*$, $-(\text{haloR}^*)$, $-\text{OH}$, $-\text{OR}^*$, $-\text{O}(\text{haloR}^*)$, $-\text{CN}$, $-\text{C}(\text{O})\text{OH}$, $-\text{C}(\text{O})\text{OR}^*$, $-\text{NH}_2$, $-\text{NHR}^*$, $-\text{NR}^{*2}$, or $-\text{NO}_2$, wherein each R^* is unsubstituted or where preceded by “halo” is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-\text{CH}_2\text{Ph}$, $-\text{O}(\text{CH}_2)_{0-1}\text{Ph}$, or a 5–6 membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00159] *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.

[00160] *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, intraarticular, subcapsular, subarachnoid, intraspinal, and intrasternal injection and infusion.

[00161] *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.

[00162] *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 controlled 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.

[00163] *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.

[00164] *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.

[00165] *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 salts 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. In some embodiments, a provided compound comprises one or more acidic groups, e.g., an oligonucleotide, and a pharmaceutically acceptable salt is an alkali, alkaline earth metal, or ammonium (e.g., an ammonium salt of $N(R)_3$, wherein each R is independently as defined and described in the present disclosure) salt. Representative alkali or alkaline earth metal salts include salts of sodium, lithium, potassium, calcium, magnesium, and the like. In some embodiments, a pharmaceutically acceptable salt is a sodium salt. In some embodiments, a pharmaceutically acceptable salt is a potassium salt. In some embodiments, a pharmaceutically acceptable salt is a calcium salt. 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. In some embodiments, a provided compound comprises more than one acid groups, for example, a provided

oligonucleotide may comprise two or more acidic groups (e.g., in natural phosphate linkages and/or modified internucleotidic linkages). In some embodiments, a pharmaceutically acceptable salt, or generally a salt, of such a compound comprises two or more cations, which can be the same or different. In some embodiments, in a pharmaceutically acceptable salt (or generally, a salt), each acidic group having sufficient acidity independently exists as its salt form (e.g., in an oligonucleotide comprising natural phosphate linkages and phosphorothioate internucleotidic linkages, each of the natural phosphate linkages and phosphorothioate internucleotidic linkages independently exists as its salt form). In some embodiments, a pharmaceutically acceptable salt of an oligonucleotide is a sodium salt of a provided oligonucleotide. In some embodiments, a pharmaceutically acceptable salt of an oligonucleotide is a sodium salt of a provided oligonucleotide, wherein each acidic linkage, e.g., each natural phosphate linkage and phosphorothioate internucleotidic linkage, exists as a sodium salt form (all sodium salt).

[00166] *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, e.g., those 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 carbamate, 9-fluorenylmethyl carbamate (Fmoc), 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluorenylmethyl 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-biphenyl)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*-hydroxypiperidinyll carbamate, alkylthio carbamate, benzyl carbamate (Cbz), *p*-methoxybenzyl carbamate (Moz), *p*-nitrobenzyl 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 (Tercoc), *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*-nitrophenylacetamide, *o*-nitrophenoxycetamide, 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-pyrroline-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)phenylmethyleamine, *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 -trimethylsilyl ethanesulfonamide (SES), 9-anthracenesulfonamide, 4-(4',8'-dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzyisulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamide.

[00167] 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.

[00168] Suitable hydroxyl protecting groups include methyl, methoxymethyl (MOM), methylthiomethyl (MTM), *t*-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), *p*-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (*p*-AOM), guaiacolmethyl (GUM), *t*-butoxymethyl, 4-pentenylloxymethyl (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-picoyl, 4-picoyl, 3-methyl-2-picoyl *N*-oxido, diphenylmethyl, *p,p'*-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, α -naphthylidiphenylmethyl, *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), dimethylhexylsilyl, *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-naphthyl 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-butenate, *o*-(methoxycarbonyl)benzoate, α -naphthoate, nitrate, alkyl *N,N,N',N'*-tetramethylphosphorodiamidate, alkyl *N*-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-

dinitrophenylsulfonate, sulfate, methanesulfonate (mesylate), benzyisulfonate, 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, acetone, 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-ethoxyethylidene 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-tetraisopropylidisiloxanylidene) derivative (TIPDS), tetra-*t*-butoxydisiloxane-1,3-diylidene derivative (TBDS), cyclic carbonates, cyclic boronates, ethyl boronate, and phenyl boronate.

[00169] In some embodiments, a hydroxyl protecting group is acetyl, *t*-butyl, *t*-butoxymethyl, methoxymethyl, tetrahydropyranyl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 2-trimethylsilyl, *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, trifluoroacetyl, 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-phenylxanthine-9-yl (pixyl) or 9-(*p*-methoxyphenyl)xanthine-9-yl (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.

[00170] 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-1-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.

[00171] *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.

[00172] *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 disclosure *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.

[00173] *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.

[00174] *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.

[00175] *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.

[00176] *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.

[00177] *Tautomeric forms:* The phrase “tautomeric forms,” as used herein and generally understood in the art, 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 disclosure. 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 disclosure, chemical compositions may be provided that are or include pure preparations of a single tautomeric form of a compound. In some embodiments of the disclosure, 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 disclosure, chemical compositions may contain all tautomeric forms of a compound. In some embodiments of the disclosure, chemical compositions may contain less than all tautomeric forms of a compound. In some embodiments of the disclosure, 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 disclosure, 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 disclosure encompasses all tautomeric forms of relevant compounds, whether in pure form or in admixture with one another.

[00178] *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.

[00179] *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.

[00180] *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.

[00181] *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.

[00182] *Unsaturated:* The term "unsaturated," as used herein, means that a moiety has one or more units of unsaturation.

[00183] *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).

[00184] *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) or analogs thereof. These terms refer to the primary structure of the molecules and 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 "internucleotidic linkages"). The term encompasses

nucleic acids containing any combinations of nucleobases, modified nucleobases, sugars, modified sugars, natural natural phosphate internucleotidic linkages or non-natural internucleotidic linkages. Examples include, and are not limited to, nucleic acids containing ribose moieties, nucleic acids containing deoxy-ribose moieties, nucleic acids containing both ribose and deoxyribose moieties, nucleic acids containing ribose and modified ribose moieties. Unless otherwise specified, 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.

[00185] *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. 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. Naturally occurring sugars include the pentose (five-carbon sugar) deoxyribose (which is found in natural DNA) or ribose (which is found in natural RNA), though it should be understood that naturally and non-naturally occurring sugar analogs are also included, such as sugars with 2'-modifications, sugars in locked nucleic acid (LNA) and phosphorodiamidate morpholino oligomer (PMO). 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, natural phosphate linkage, phosphorothioate linkages, boranophosphate linkages 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, etc. In some embodiments, a nucleotide is a natural nucleotide comprising a naturally occurring nucleobase, a natural occurring sugar and the natural phosphate linkage. In some embodiments, a nucleotide is a modified nucleotide or a nucleotide analog, which is a structural analog that can be used in lieu of a natural nucleotide.

[00186] *Modified nucleotide:* The term “modified nucleotide” includes any chemical moiety which differs structurally from a natural nucleotide but is capable of performing at least one function of a natural nucleotide. In some embodiments, a modified nucleotide comprises a modification at a sugar, base and/or internucleotidic linkage. In some embodiments, a modified nucleotide comprises a modified sugar, modified nucleobase and/or modified internucleotidic linkage. In some embodiments, a modified nucleotide is capable of at least one function of a nucleotide, e.g., forming a subunit in a polymer capable of base-pairing to a nucleic acid comprising an at least complementary sequence of bases.

[00187] *Analog:* The term “analog” includes any chemical moiety which differs structurally from a reference chemical moiety or class of moieties, but which is capable of performing at least one function

of such a reference chemical moiety or class of moieties. As non-limiting examples, a nucleotide analog differs structurally from a nucleotide but performs at least one function of a nucleotide; a nucleobase analog differs structurally from a nucleobase but performs at least one function of a nucleobase; a sugar analog differs structurally from a nucleobase but performs at least one function of a sugar, etc.

[00188] *Nucleoside*: The term “nucleoside” refers to a moiety wherein a nucleobase or a modified nucleobase is covalently bound to a sugar or modified sugar.

[00189] *Modified nucleoside*: The term “modified nucleoside” refers to a chemical moiety which is chemically distinct from a natural nucleoside, but which is capable of performing at least one function of a nucleoside. In some embodiments, a modified nucleoside is derived from or chemically similar to a natural nucleoside, but which comprises a chemical modification which differentiates it from a natural nucleoside. Non-limiting examples of modified nucleosides include those which comprise a modification at the base and/or the sugar. Non-limiting examples of modified nucleosides include those with a 2'-modification at a sugar. Non-limiting examples of modified nucleosides also include abasic nucleosides (which lack a nucleobase). In some embodiments, a modified nucleoside is capable of at least one function of a nucleoside, e.g., forming a moiety in a polymer capable of base-pairing to a nucleic acid comprising an at least complementary sequence of bases.

[00190] *Nucleoside analog*: The term “nucleoside analog” refers to a chemical moiety which is chemically distinct from a natural nucleoside, but which is capable of performing at least one function of a nucleoside. In some embodiments, a nucleoside analog comprises an analog of a sugar and/or an analog of a nucleobase. In some embodiments, a modified nucleoside is capable of at least one function of a nucleoside, e.g., forming a moiety in a polymer capable of base-pairing to a nucleic acid comprising a complementary sequence of bases.

[00191] *Sugar*: The term “sugar” refers to a monosaccharide or polysaccharide in closed and/or open form. In some embodiments, sugars are monosaccharides. In some embodiments, sugars are polysaccharides. Sugars include, but are not limited to, ribose, deoxyribose, pentofuranose, pentopyranose, and hexopyranose moieties. As used herein, the term “sugar” 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”), etc. As used herein, the term “sugar” also encompasses structural analogs used in lieu of natural or naturally-occurring nucleotides, such as modified sugars and nucleotide sugars. In some embodiments, a sugar is D-2-deoxyribose. In some embodiments, a sugar is beta-D-deoxyribofuranose. In some embodiments, a sugar moiety is a beta-D-deoxyribofuranose moiety. In some embodiments, a sugar is D-ribose. In some embodiments, a sugar is beta-D-ribofuranose. In some embodiments, a sugar moiety is a beta-D-ribofuranose moiety. In some embodiments, a sugar is optionally substituted beta-D-deoxyribofuranose or beta-D-ribofuranose. In

some embodiments, a sugar moiety is an optionally substituted beta-D-deoxyribofuranose or beta-D-ribofuranose moiety. In some embodiments, a sugar moiety/unit in an oligonucleotide, nucleic acid, etc. is a sugar which comprises one or more carbon atoms each independently connected to an internucleotidic linkage, e.g., optionally substituted beta-D-deoxyribofuranose or beta-D-ribofuranose whose 5'-C and/or 3'-C are each independently connected to an internucleotidic linkage (e.g., a natural phosphate linkage, a modified internucleotidic linkage, a chirally controlled internucleotidic linkage, etc.).

[00192] *Modified sugar:* The term “modified sugar” refers to a moiety that can replace a sugar. A modified sugar mimics the spatial arrangement, electronic properties, or some other physicochemical property of a sugar. In some embodiments, a modified sugar is substituted beta-D-deoxyribofuranose or beta-D-ribofuranose. In some embodiments, a modified sugar comprises a 2'-modification. In some embodiments, a modified sugar comprises a linker (e.g., optionally substituted bivalent heteroaliphatic) connecting two sugar carbon atoms (e.g., C2 and C4), e.g., as found in LNA. In some embodiments, a linker is --O--CH(R)-- , wherein R is as described in the present disclosure. In some embodiments, a linker is --O--CH(R)-- , wherein O is connected to C2, and --CH(R)-- is connected to C4 of a sugar, and R is as described in the present disclosure. In some embodiments, R is methyl. In some embodiments, R is --H . In some embodiments, --CH(R)-- is of S configuration. In some embodiments, --CH(R)-- is of R configuration.

[00193] *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, a modified nucleobase is a substituted nucleobase which nucleobase is selected from A, T, C, G, U, and tautomers thereof. 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. As used herein, the term “nucleobase” also encompasses structural analogs used in lieu of natural or naturally-occurring nucleotides, such as modified nucleobases and

nucleobase analogs. In some embodiments, a nucleobase is an optionally substituted A, T, C, G, or U, or a substituted nucleobase which nucleobase is selected from A, T, C, G, U, and tautomers thereof.

[00194] *Modified nucleobase:* The terms "modified nucleobase", "modified base" and the like refer to a chemical moiety which is chemically distinct from a nucleobase, but which is capable of performing at least one function of a nucleobase. In some embodiments, a modified nucleobase is a nucleobase which comprises a modification. In some embodiments, a modified nucleobase is capable of at least one function of a nucleobase, e.g., forming a moiety in a polymer capable of base-pairing to a nucleic acid comprising an at least complementary sequence of bases. In some embodiments, a modified nucleobase is a substituted nucleobase which nucleobase is selected from A, T, C, G, U, and tautomers thereof.

[00195] *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. In some embodiments, the term may also refer to a compound that comprises such a moiety.

[00196] *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.

[00197] *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. In some embodiments, a moiety of a compound is a monovalent, bivalent, or polyvalent group formed from the compound by removing one or more -H and/or equivalents thereof from a compound. In some embodiments, depending on its context, "moiety" may also refer to a compound or entity from which the moiety is derived from.

[00198] *Solid support:* The term "solid support" when used in the context of preparation of nucleic acids, oligonucleotides, or other compounds refers to any support which enables synthesis of nucleic acids, oligonucleotides or other compounds. 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).

[00199] *Reading frame:* The term "reading frame" refers to one of the six possible reading frames, three in each direction, of a 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.

[00200] *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. In some embodiments, transcripts may be generated from both strands. In some embodiments, transcripts may or may not encode protein products. In some embodiments, when directed or targeted to a particular nucleic acid sequence, a "antisense" sequence may refer to a sequence that is complementary to the particular nucleic acid sequence.

[00201] *Oligonucleotide:* the term "oligonucleotide" refers to a polymer or oligomer of nucleotide monomers, containing any combination of nucleobases, modified nucleobases, sugars, modified sugars, natural phosphate linkages, or non-natural internucleotidic linkages.

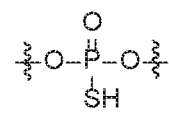
[00202] Oligonucleotides can be single-stranded or double-stranded. As used herein, the term "oligonucleotide strand" encompasses a single-stranded oligonucleotide. A single-stranded oligonucleotide can have double-stranded regions and a double-stranded oligonucleotide can have single-stranded regions. Example 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, UI adaptors, triplex-forming oligonucleotides, G-quadruplex oligonucleotides, RNA activators, immuno-stimulatory oligonucleotides, and decoy oligonucleotides.

[00203] Double-stranded and single-stranded oligonucleotides that are effective in inducing RNA interference may also be referred to as siRNA, RNAi agent, or iRNA agent. 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.

[00204] Oligonucleosides of the present disclosure can be of various lengths. In particular embodiments, oligonucleosides can range from about 2 to about 200 nucleosides in length. In various related embodiments, oligonucleosides, single-stranded, double-stranded, and triple-stranded, can range in length from about 4 to about 10 nucleosides, from about 10 to about 50 nucleosides, from about 20 to about 50 nucleosides, from about 15 to about 30 nucleosides, from about 20 to about 30 nucleosides in length. In some embodiments, the oligonucleoside is from about 9 to about 39 nucleosides in length. In some embodiments, the oligonucleoside is at least 15 nucleosides in length. In some embodiments, the

oligonucleoside is at least 20 nucleosides in length. In some embodiments, the oligonucleoside is at least 25 nucleosides in length. In some embodiments, the oligonucleoside is at least 30 nucleosides in length. In some embodiments, the oligonucleoside is a duplex of complementary strands of at least 18 nucleosides in length. In some embodiments, the oligonucleoside is a duplex of complementary strands of at least 21 nucleosides in length. In some embodiments, for the purpose of oligonucleotide lengths, each nucleoside counted independently comprises an optionally substituted nucleobase selected from A, T, C, G, U and their tautomers.

[00205] *Internucleotidic linkage:* As used herein, the phrase “internucleotidic linkage” refers generally to a linkage, typically a phosphorus-containing linkage, between nucleotide units of a nucleic acid or an oligonucleotide, and is interchangeable with “inter-sugar linkage”, “internucleosidic linkage,” and “phosphorus atom bridge,” as used above and herein. As appreciated by those skilled in the art, natural DNA and RNA contain natural phosphate linkages. In some embodiments, an internucleotidic linkage is a natural phosphate linkage ($-\text{OP}(\text{O})(\text{OH})\text{O}-$, typically existing as its anionic form $-\text{OP}(\text{O})(\text{O}^-)\text{O}-$ at pH e.g., ~ 7.4), as found in naturally occurring DNA and RNA molecules. In some embodiments, an internucleotidic linkage is a modified internucleotidic linkage (or non-natural internucleotidic linkage), which is structurally different from a natural phosphate linkage but may be utilized in place of a natural phosphate linkage, e.g., phosphorothioate internucleotidic linkage, PMO linkages, etc. In some embodiments, an internucleotidic linkage is a modified internucleotidic linkage wherein one or more oxygen atoms of a natural phosphodiester linkage are independently replaced by one or more organic or inorganic moieties. In some embodiments, such an organic or inorganic moiety is selected from but not limited to $=\text{S}$, $=\text{Se}$, $=\text{NR}'$, $-\text{SR}'$, $-\text{SeR}'$, $-\text{N}(\text{R}')_2$, $\text{B}(\text{R}')_3$, $-\text{S}-$, $-\text{Se}-$, and $-\text{N}(\text{R}')-$, wherein each R' is independently as defined and described below. In some embodiments, an internucleotidic linkage is a phosphotriester linkage. In some embodiments, an internucleotidic linkage is



a phosphorothioate diester linkage (phosphorothioate internucleotidic linkage, typically existing as its anionic form $-\text{OP}(\text{O})(\text{S}^-)\text{O}-$ at pH e.g., ~ 7.4). It is understood by a person of ordinary skill in the art that an 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. In some embodiments, an internucleotidic linkage is a non-negatively charged internucleotidic linkage at a given pH. In some embodiments, an internucleotidic linkage is a neutral internucleotidic linkage at a given pH. In some embodiments, a given pH is pH ~ 7.4 . In some embodiments, a given pH is in the range of pH about 0, 1, 2, 3, 4, 5, 6 or 7 to pH about 7, 8, 9, 10, 11, 12, 13 or 14. In some embodiments, a given pH is in the range of pH 5-9. In some embodiments, a given pH is in the range of pH 6-8. In some embodiments, an internucleotidic linkage has the structure

of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, etc., as described in the present disclosure. In some embodiments, a non-negatively charged internucleotidic linkage has the structure of formula I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, etc., as described in the present disclosure. In some embodiments, an internucleotidic linkage is one of, e.g., PNA (peptide nucleic acid) or PMO (phosphorodiamidate Morpholino oligomer) linkage. In some embodiments, an internucleotidic linkage comprises a chiral linkage phosphorus. In some embodiments, an internucleotidic linkage is a chirally controlled internucleotidic linkage. In some embodiments, an internucleotidic linkage is selected from: s (phosphorothioate), s1, s2, s3, s4, s5, s6, s7, s8, s9, s10, s11, s12, s13, s14, s15, s16, s17 or s18, wherein each of s1, s2, s3, s4, s5, s6, s7, s8, s9, s10, s11, s12, s13, s14, s15, s16, s17 and s18 is independently as described in WO 2017/062862.

[00206] Unless otherwise specified, the Rp/Sp designations preceding an oligonucleotide sequence describe the configurations of linkage phosphorus in chirally controlled internucleotidic linkages sequentially from 5' to 3' of the oligonucleotide sequence. For instance, in (Rp, Sp)-ATsCs1GA, the phosphorus in the “s” linkage between T and C has Rp configuration and the phosphorus in “s1” linkage between C and G has Sp configuration. In some embodiments, “All-(Rp)” or “All-(Sp)” is used to indicate that all chiral linkage phosphorus atoms in chirally controlled internucleotidic linkages have the same Rp or Sp configuration, respectively. For instance, All-(Rp)-GsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC indicates that all the chiral linkage phosphorus atoms in the oligonucleotide have Rp configuration; All-(Sp)-GsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC indicates that all the chiral linkage phosphorus atoms in the oligonucleotide have Sp configuration.

[00207] *Oligonucleotide type:* As used herein, the phrase “oligonucleotide type” is used to define oligonucleotides that have a particular base sequence, pattern of backbone linkages (i.e., pattern of internucleotidic linkage types, for example, natural phosphate linkages, phosphorothioate internucleotidic linkages, negatively charged internucleotidic linkages, neutral internucleotidic linkages *etc.*), pattern of backbone chiral centers (i.e. pattern of linkage phosphorus stereochemistry (Rp/Sp)), and pattern of backbone phosphorus modifications (e.g., pattern of “-X-L-R¹” groups in formula I). In some embodiments, oligonucleotides of a common designated “type” are structurally identical to one another.

[00208] One of skill in the art will appreciate that synthetic methods of the present disclosure 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 disclosure 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. In some embodiments, all such molecules are structurally identical to one another. In some embodiments, provided compositions comprise a plurality of oligonucleotides of different types, typically in pre-determined (non-random) relative amounts.

[00209] *Chiral control:* As used herein, “chiral control” refers to control of the stereochemical designation of a chiral linkage phosphorus in a chiral internucleotidic linkage within an oligonucleotide. In some embodiments, a control is achieved through a chiral element that is absent from the sugar and base moieties of an oligonucleotide, for example, in some embodiments, a control is achieved through use of one or more chiral auxiliaries during oligonucleotide preparation as exemplified in the present disclosure, which chiral auxiliaries often are part of chiral phosphoramidites used during oligonucleotide preparation. In contrast to chiral control, a person having ordinary skill in the art appreciates that conventional oligonucleotide synthesis which does not use chiral auxiliaries cannot control stereochemistry at a chiral internucleotidic linkage if such conventional oligonucleotide synthesis is used to form the chiral internucleotidic linkage. In some embodiments, the stereochemical designation of each chiral linkage phosphorus in a chiral internucleotidic linkage within an oligonucleotide is controlled.

[00210] *Chirally controlled oligonucleotide composition:* The terms “chirally controlled (stereocontrolled or stereodefined) oligonucleotide composition”, “chirally controlled (stereocontrolled or stereodefined) nucleic acid composition”, and the like, as used herein, refers to a composition that comprises a plurality of oligonucleotides (or nucleic acids, chirally controlled oligonucleotides or chirally controlled nucleic acids) which share 1) a common base sequence, 2) a common pattern of backbone linkages; 3) a common pattern of backbone chiral centers, and 4) a common pattern of backbone phosphorus modifications (oligonucleotides of a particular type), wherein the plurality of oligonucleotides (or nucleic acids) share the same stereochemistry at one or more chiral internucleotidic linkages (chirally controlled internucleotidic linkages, whose chiral linkage phosphorus is Rp or Sp, not a random Rp and Sp mixture as non-chirally controlled internucleotidic linkages). Level of the plurality of oligonucleotides (or nucleic acids) in a chirally controlled oligonucleotide composition is non-random (pre-determined, controlled). Chirally controlled oligonucleotide compositions are typically prepared through chirally

controlled oligonucleotide preparation to stereoselectively form one or more chiral internucleotidic linkages (e.g., using chiral auxiliaries as exemplified in the present disclosure, compared to non-chirally controlled (stereorandom, non-stereoselective, racemic) oligonucleotide synthesis such as traditional phosphoramidite-based oligonucleotide synthesis using no chiral auxiliaries or chiral catalysts to purposefully control stereoselectivity). A chirally controlled oligonucleotide composition is enriched, relative to a substantially racemic preparation of oligonucleotides having the common base sequence, the common pattern of backbone linkages, and the common pattern of backbone phosphorus modifications, for oligonucleotides of the plurality. In some embodiments, a chirally controlled oligonucleotide composition comprises a plurality of oligonucleotides of a particular oligonucleotide type defined by: 1) base sequence; 2) pattern of backbone linkages; 3) pattern of backbone chiral centers; and 4) pattern of backbone phosphorus modifications, wherein it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence, pattern of backbone linkages, and pattern of backbone phosphorus modifications, for oligonucleotides of the particular oligonucleotide type. As one having ordinary skill in the art readily appreciates, such enrichment can be characterized in that compared to a substantially racemic preparation, at each chirally controlled internucleotidic linkage, a higher level of the linkage phosphorus has the desired configuration. In some embodiments, each chirally controlled internucleotidic linkage independently has a diastereopurity of at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% with respect to its chiral linkage phosphorus. In some embodiments, each independently has a diastereopurity of at least 90%. In some embodiments, each independently has a diastereopurity of at least 95%. In some embodiments, each independently has a diastereopurity of at least 97%. In some embodiments, each independently has a diastereopurity of at least 98%. In some embodiments, oligonucleotides of a plurality have the same constitution. In some embodiments, oligonucleotides of a plurality have the same constitution and stereochemistry, and are structurally identical.

[00211] In some embodiments, the plurality of oligonucleotides in a chirally controlled oligonucleotide composition share the same base sequence, the same, if any, nucleobase, sugar, and internucleotidic linkage modifications, and the same stereochemistry (*Rp* or *Sp*) independently at linkage phosphorus chiral centers of one or more chirally controlled internucleotidic linkages, though stereochemistry of certain linkage phosphorus chiral centers may differ. In some embodiments, about 0.1%-100%, (e.g., about 1%-100%, 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80%-100%, 90%-100%, 95%-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all oligonucleotides in a chirally controlled oligonucleotide composition are

oligonucleotides of the plurality. In some embodiments, about 0.1%-100%, (*e.g.*, about 1%-100%, 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80%-100%, 90%-100%, 95-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all oligonucleotides in a chirally controlled oligonucleotide composition that share the common base sequence are oligonucleotides of the plurality. In some embodiments, about 0.1%-100%, (*e.g.*, about 1%-100%, 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80%-100%, 90-100%, 95-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all oligonucleotides in a chirally controlled oligonucleotide composition that share the common base sequence, the common pattern of backbone linkages, and the common pattern of backbone phosphorus modifications are oligonucleotides of the plurality. In some embodiments, about 0.1%-100%, (*e.g.*, about 1%-100%, 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80-100%, 90-100%, 95-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all oligonucleotides in a chirally controlled oligonucleotide composition, or of all oligonucleotides in a composition that share a common base sequence (*e.g.*, of a plurality of oligonucleotide or an oligonucleotide type), or of all oligonucleotides in a composition that share a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone phosphorus modifications (*e.g.*, of a plurality of oligonucleotide or an oligonucleotide type), or of all oligonucleotides in a composition that share a common base sequence, a common pattern of base modifications, a common pattern of sugar modifications, a common pattern of internucleotidic linkage types, and/or a common pattern of internucleotidic linkage modifications (*e.g.*, of a plurality of oligonucleotide or an oligonucleotide type), or of all oligonucleotides in a composition that share the same constitution, are oligonucleotides of the plurality. In some embodiments, a percentage is at least $(DP)^{NCI}$, wherein DP is a percentage selected from 85%-100%, and NCI is the number of chirally controlled internucleotidic linkage. In some embodiments, DP is at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. In some embodiments, DP is at least 85%. In some embodiments, DP is at least 90%. In some embodiments, DP is at least 95%. In some embodiments, DP is at least 96%. In some embodiments, DP is at least 97%. In some embodiments, DP is at least 98%. In some embodiments, DP is at least 99%. In some embodiments, DP reflects diastereopurity of linkage phosphorus chiral centers chirally controlled

internucleotidic linkages. In some embodiments, diastereopurity of a linkage phosphorus chiral center of an internucleotidic linkage may be typically assessed using an appropriate dimer comprising such an internucleotidic linkage and the two nucleoside units being linked by the internucleotidic linkage. In some embodiments, the plurality of oligonucleotides share the same stereochemistry at about 1-50 (*e.g.*, about 1-10, 1-20, 5-10, 5-20, 10-15, 10-20, 10-25, 10-30, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) chiral internucleotidic linkages. In some embodiments, the plurality of oligonucleotides share the same stereochemistry at about 0.1%-100% (*e.g.*, about 1%-100%, 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80%-100%, 90%-100%, 95%-100%, 50%-90%, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%, or at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%) of chiral internucleotidic linkages. In some embodiments, each chiral internucleotidic linkage is a chiral controlled internucleotidic linkage, and the composition is a completely chirally controlled oligonucleotide composition. In some embodiments, not all chiral internucleotidic linkages are chiral controlled internucleotidic linkages, and the composition is a partially chirally controlled oligonucleotide composition. In some embodiments, a chirally controlled oligonucleotide composition comprises predetermined levels of individual oligonucleotide or nucleic acids types. For instance, in some embodiments a chirally controlled oligonucleotide composition comprises one oligonucleotide type at a predetermined level (*e.g.*, as described above). In some embodiments, a chirally controlled oligonucleotide composition comprises more than one oligonucleotide type, each independently at a predetermined level. In some embodiments, a chirally controlled oligonucleotide composition comprises multiple oligonucleotide types, each independently at a predetermined level. In some embodiments, a chirally controlled oligonucleotide composition is a composition of oligonucleotides of an oligonucleotide type, which composition comprises a predetermined level of a plurality of oligonucleotides of the oligonucleotide type.

[00212] *Chirally pure*: as used herein, the phrase “chirally pure” is used to describe an oligonucleotide or compositions thereof, in which all or nearly all (the rest are impurities) of the oligonucleotide molecules exist in a single diastereomeric form with respect to the linkage phosphorus atoms. In many embodiments, as appreciated by those skilled in the art, a chirally pure oligonucleotide composition is substantially pure in that substantially all of the oligonucleotides in the composition are structurally identical (being the same stereoisomer).

[00213] *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 an internucleotidic linkage, which phosphorus atom corresponds to the phosphorus atom of a natural

phosphate linkage as occurs in naturally occurring DNA and RNA. In some embodiments, a linkage phosphorus atom is in a modified internucleotidic linkage. In some embodiments, a linkage phosphorus atom is the P of P^L of formula I. In some embodiments, a linkage phosphorus atom is chiral.

[00214] *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 W, Y, Z, or –X–L–R¹ of formula I.

[00215] *Blockmer*: the term “blockmer,” as used herein, refers to an oligonucleotide 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 nucleobase, sugar and/or internucleotidic linkage. By common structural feature is meant common chemistry and/or stereochemistry, e.g., common modifications at nucleobases, sugars, and/or internucleotidic linkages and common stereochemistry at linkage phosphorus chiral centers. In some embodiments, the at least two consecutive nucleotide units sharing a common structural feature are referred to as a “block”.

[00216] 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 least two consecutive nucleotide units form a “stereoblock.” For instance, (Sp, Sp)-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 Sp). In the same oligonucleotide (Sp, Sp)-ATsCs1GA, TsCs1 forms a block, and it is a stereoblock.

[00217] 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 least two consecutive nucleotide units form a “P-modification block.” For instance, (Rp, Sp)-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 (Rp, Sp)-ATsCsGA, TsCs forms a block, and it is a P-modification block.

[00218] 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, (Rp, Rp)-ATsCsGA is a linkage blockmer because at least two consecutive nucleotide units, the Ts and the Cs, have the same stereochemistry (both Rp) and P-modification (both phosphorothioate). In the same oligonucleotide of (Rp, Rp)-ATsCsGA, TsCs forms a block, and it is a linkage block.

[00219] In some embodiments, a blockmer is a “sugar modification blockmer,” e.g., at least two

modification, while nucleotide units flanking the gap at each end independently comprise a 2'-OR modification. In some embodiments, a provided oligonucleotide comprising a gap, wherein each nucleotide unit in the gap region contains no 2'-F modification, while nucleotide units flanking the gap at each end independently comprise a 2'-F modification.

[00224] *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 (a natural phosphate 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 (a non-natural internucleotidic linkage).

[00225] For purposes of this disclosure, 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.

[00226] Unless otherwise specified, salts, such as pharmaceutically acceptable acid or base addition salts, stereoisomeric forms, and tautomeric forms, of compounds (e.g., oligonucleotides, agents, etc.) are included. Unless otherwise specified, singular forms “a”, “an”, and “the” include the plural reference unless the context clearly indicates otherwise (and vice versa). Thus, for example, a reference to “a compound” may include a plurality of such compounds.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[00227] 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 chemical modification, e.g., base modifications, sugar modifications, backbone modifications, etc., which, among other things, render these molecules less susceptible to degradation and improve other properties of oligonucleotides. Chemical modifications may also lead to certain undesired effects, such as increased toxicities, etc. From a structural point of view, modifications to natural phosphate linkages can introduce chirality, and certain properties of oligonucleotides may be affected by the configurations of the phosphorus atoms that form the backbone of the oligonucleotides.

[00228] In some embodiments, an oligonucleotide or oligonucleotide composition is: a DMD oligonucleotide or oligonucleotide composition; an oligonucleotide or oligonucleotide composition comprising a non-negatively charged internucleotidic linkage; or a DMD oligonucleotide comprising a non-negatively charged internucleotidic linkage.

[00229] In some embodiments, the chirality of the backbone (*e.g.*, the configurations of the phosphorus atoms) or inclusion of natural phosphate linkages or non-natural internucleotidic linkages in the backbone and/or modifications of a sugar and/or nucleobase, and/or the addition of chemical moieties can affect properties and activities of oligonucleotides, *e.g.*, the ability of a DMD oligonucleotide (*e.g.*, an oligonucleotide antisense to a Dystrophin (DMD) transcript sequence) to skip one or more exons, and/or other properties of a DMD oligonucleotide, including but not limited to, increased stability, improved pharmacokinetics, and/or decreased immunogenicity, etc. Suitable assays for assessing properties and/or activities of provided compounds, *e.g.*, oligonucleotides, and compositions thereof are widely known in the art and can be utilized in accordance with the present disclosure. For example, to test immunogenicity, various DMD oligonucleotides were tested in mouse serum *in vivo* and demonstrated minimal activation of cytokines, and various DMD oligonucleotides were tested *ex vivo* in human PBMC (peripheral blood mononuclear cells) for cytokine activity (*e.g.*, IL-12p40, IL-12p70, IL-1 α , IL-1 β , IL-6, MCP-1, MIP-1 α , MIP-1 β , and TNF- α).

[00230] In some embodiments, technologies (*e.g.*, oligonucleotides, compositions, and methods of use thereof) of the present disclosure can be utilized to target various nucleic acids (*e.g.*, by hybridizing to a target sequence of a target nucleic acid, and/or providing level reduction, degradation, splicing modulation, transcription suppression, etc. of the target nucleic acid, etc.) In some embodiments, provided technologies are particularly useful for modulating splicing of transcripts, *e.g.*, to increase levels of desired splicing products and/or to reduce levels of undesired splicing products. In some embodiments, provided technologies are particularly useful for reducing levels of transcripts, *e.g.*, pre-mRNA, RNA, etc., and in many instances, reducing levels of products arising from or encoded by such transcripts such as mRNA, proteins, etc.

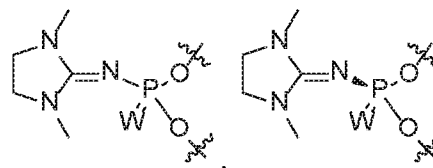
[00231] In some embodiments, a transcript is pre-mRNA. In some embodiments, a splicing product is mature RNA. In some embodiments, a splicing product is mRNA. In some embodiments, splicing modulation or alteration comprises skipping one or more exons. In some embodiments, splicing of a transcript is improved in that exon skipping increases levels of mRNA and proteins that have improved beneficial activities compared with absence of exon skipping. In some embodiments, an exon causing frameshift is skipped. In some embodiments, an exon comprising an undesired mutation is skipped. In some embodiments, an exon comprising a premature termination codon is skipped. An undesired mutation can be a mutation causing changes in protein sequences; it can also be a silent mutation. In some embodiments, a transcript is a transcript of Dystrophin (DMD).

[00232] In some embodiments, splicing of a transcript is improved in that exon skipping lowers levels of mRNA and proteins that have undesired activities compared with absence of exon skipping. In some embodiments, a target is knocked down through exon skipping which, by skipping one or more

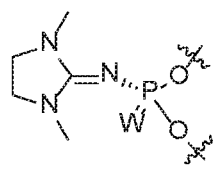
exons, causes premature stop codon and/or frameshift mutations. In some embodiments, provided oligonucleotides in provided compositions, *e.g.*, oligonucleotides of a plurality, comprise base modifications, sugar modifications, and/or internucleotidic linkage modifications. In some embodiments, provided oligonucleotides comprise base modifications and sugar modifications. In some embodiments, provided oligonucleotides comprise base modifications and internucleotidic linkage modifications. In some embodiments, provided oligonucleotides comprise sugar modifications and internucleotidic linkage modifications. In some embodiments, provided compositions comprise base modifications, sugar modifications, and internucleotidic linkage modifications. Example chemical modifications, such as base modifications, sugar modifications, internucleotidic linkage modifications, *etc.* are widely known in the art including but not limited to those described in this disclosure. In some embodiments, a modified base is substituted A, T, C, G or U. In some embodiments, a sugar modification is 2'-modification. In some embodiments, a 2'-modification is 2-F modification. In some embodiments, a 2'-modification is 2'-OR¹, wherein R¹ is not hydrogen. In some embodiments, a 2'-modification is 2'-OR¹, wherein R¹ is optionally substituted alkyl. In some embodiments, a 2'-modification is 2'-OMe. In some embodiments, a 2'-modification is 2'-MOE. In some embodiments, a modified sugar moiety is a bridged bicyclic or polycyclic ring. In some embodiments, a modified sugar moiety is a bridged bicyclic or polycyclic ring having 5-20 ring atoms wherein one or more ring atoms are optionally and independently heteroatoms. Example ring structures are widely known in the art, such as those found in BNA, LNA, *etc.* In some embodiments, provided oligonucleotides comprise both one or more modified internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, oligonucleotides comprising both modified internucleotidic linkage and natural phosphate linkage and compositions thereof provide improved properties, *e.g.*, activities and toxicities, *etc.* In some embodiments, a modified internucleotidic linkage is a chiral internucleotidic linkage. In some embodiments, a modified internucleotidic linkage is a phosphorothioate linkage. In some embodiments, a modified internucleotidic linkage is a substituted phosphorothioate linkage.

[00233] In some embodiments, provided oligonucleotides comprise one or more non-negatively charged internucleotidic linkages. In some embodiments, a non-negatively charged internucleotidic linkage is a positively charged internucleotidic linkage. In some embodiments, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, a modified internucleotidic linkage (*e.g.*, a non-negatively charged internucleotidic linkage) comprises optionally substituted triazolyl. In some embodiments, a modified internucleotidic linkage (*e.g.*, a non-negatively charged internucleotidic linkage) comprises optionally substituted alkynyl. In some embodiments, a modified internucleotidic linkage comprises a triazole or alkyne moiety. In some embodiments, a triazole moiety, *e.g.*, a triazolyl group, is optionally substituted. In some embodiments, a triazole moiety, *e.g.*, a

triazolyl group) is substituted. In some embodiments, a triazole moiety is unsubstituted. In some embodiments, a modified internucleotidic linkage comprises an optionally substituted guanidine moiety. In some embodiments, a modified internucleotidic linkage comprises an optionally substituted cyclic guanidine moiety. In some embodiments, a modified internucleotidic linkage comprises an optionally



substituted cyclic guanidine moiety and has the structure of:



, wherein W is O or S. In some embodiments, W is O. In some embodiments, W is S. In some embodiments, a non-negatively charged internucleotidic linkage is stereochemically controlled.

[00234] In some embodiments, an internucleotidic linkage comprising an optionally substituted guanidine moiety is an internucleotidic linkage of formula **I-n-2**, **I-n-3**, **I-n-4**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, or **II-d-2** as described herein. In some embodiments, an internucleotidic linkage comprising an optionally substituted cyclic guanidine moiety is an internucleotidic linkage of formula **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, or **II-d-2**.

[00235] Among other things, the present disclosure encompasses the recognition that stereorandom oligonucleotide preparations contain a plurality of distinct chemical entities that differ from one another, *e.g.*, in the stereochemical structure of individual backbone linkage phosphorus chiral centers within the oligonucleotide chain. Without control of stereochemistry of backbone chiral centers, stereorandom oligonucleotide preparations provide uncontrolled compositions comprising undetermined levels of oligonucleotide stereoisomers with respect to the uncontrolled chiral centers, *e.g.*, chiral linkage phosphorus. Even though these stereoisomers may have the same base sequence, they are different chemical entities at least due to their different backbone stereochemistry, and they can have, as demonstrated herein, different properties, *e.g.*, activities, toxicities, *etc.* Among other things, the present disclosure provides new oligonucleotide compositions wherein stereochemistry of one or more linkage phosphorus chiral centers are independently controlled (*e.g.*, in chirally controlled internucleotidic linkages). In some embodiments, the present disclosure provides chirally controlled oligonucleotide compositions which are or contain particular stereoisomers of oligonucleotides of interest.

[00236] In some embodiments, provided oligonucleotides contain increased levels of one or more isotopes. In some embodiments, provided oligonucleotides are labeled, *e.g.*, by one or more isotopes of one or more elements, *e.g.*, hydrogen, carbon, nitrogen, *etc.* In some embodiments, provided

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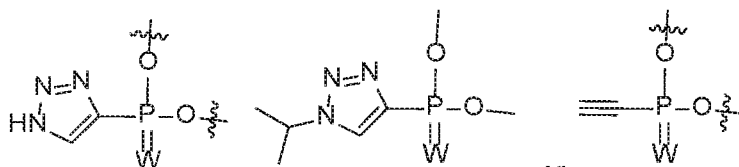
SSSRSSRRSSRS, SSSSRSSRRSSRS, SSSSRSSRRSSRS, SSSSRSSRRSSRS, SSSSRSSRR, SSSSRSSRRS, SSSSRSSRRS, SSSSRSSRRSS, SSSSRSSRRSSS, RSSSSSRSSRRSSS, RSSSSSRSSRRS, RSSSSSRSSRRSS, RSSSSSRSSRRSSS, RSSSSSRSSRRSSS, RSSSSSRSSRRSSS, $(R)_n(S)_m$, $(S)_t(R)_n$, $(O)_t(R)_n(S)_m$, $(S)_t(O)_m$, $(O)_m(S)_t$, $(S)_t(R)_n(S)_m$, $(S)_t(O)_m(S)_n$, $(S)_t(O)_m$, wherein t , m and n are independently 1 to 20, O is a non-chiral internucleotidic linkage, R is a R_p chiral internucleotidic linkage, and S is an S_p chiral internucleotidic linkage. In some embodiments, the non-chiral center is a phosphodiester linkage. In some embodiments, the chiral center in a S_p configuration is a phosphorothioate linkage.

[00239] In some embodiments, the 5'-end region of provided oligonucleotides, *e.g.*, a 5'-wing, comprises a stereochemistry pattern of S , SS , SSS , $SSSS$, $SSSSS$, $SSSSSS$, or $SSSSSSS$. In some embodiments, each S is or represents an S_p phosphorothioate internucleotidic linkage. In some embodiments, the 5'-end region of provided oligonucleotides, *e.g.*, a 5'-wing, comprises a stereochemistry pattern of S , SS , SSS , $SSSS$, $SSSSS$, $SSSSSS$, or $SSSSSSS$, wherein the first S represents the first (the 5'-end) internucleotidic linkage of a provided oligonucleotide. In some embodiments, one or more nucleotidic units comprising an S_p internucleotidic linkage in the 5'-end region independently comprise $-F$. In some embodiments, each nucleotidic unit comprising an S_p internucleotidic linkage in the 5'-end region independently comprises $-F$. In some embodiments, one or more nucleotidic units comprising an S_p internucleotidic linkage in the 5'-end region independently comprise a sugar modification. In some embodiments, each nucleotidic unit comprising an S_p internucleotidic linkage in the 5'-end region independently comprises a sugar modification. In some embodiments, each 2'-modification is the same. In some embodiments, a sugar modification is a 2'-modification. In some embodiments, a 2'-modification is 2'-OR¹. In some embodiments, a 2'-modification is 2'-F. In some embodiments, the 3'-end region of provided oligonucleotides, *e.g.*, a 3'-wing, comprises a stereochemistry pattern of S , SS , SSS , $SSSS$, $SSSSS$, $SSSSSS$, or $SSSSSSS$. In some embodiments, each S is or represents an S_p phosphorothioate internucleotidic linkage. In some embodiments, the 3'-end region of provided oligonucleotides, *e.g.*, a 3'-wing, comprises a stereochemistry pattern of S , SS , SSS , $SSSS$, $SSSSS$, $SSSSSS$, or $SSSSSSS$, wherein the last S represents the last (the 3'-end) internucleotidic linkage of a provided oligonucleotide. In some embodiments, each S represents an S_p phosphorothioate internucleotidic linkage. In some embodiments, one or more nucleotidic units comprising an S_p internucleotidic linkage in the 3'-end region independently comprise $-F$. In some embodiments, each nucleotidic unit comprising an S_p internucleotidic linkage in the 3'-end region independently comprises $-F$. In some embodiments, one or more nucleotidic units comprising an S_p internucleotidic linkage in the 3'-end region independently comprise a sugar modification. In some embodiments, each nucleotidic unit comprising an S_p internucleotidic linkage in the 3'-end region independently comprises a sugar

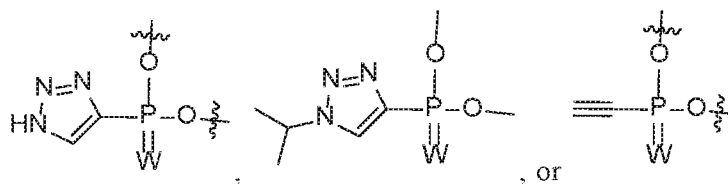
modification. In some embodiments, each 2'-modification is the same. In some embodiments, a sugar modification is a 2'-modification. In some embodiments, a 2'-modification is 2'-OR¹. In some embodiments, a 2'-modification is 2'-F. In some embodiments, provided oligonucleotides comprise both a 5'-end region, *e.g.*, a 5'-wing, and a 3'-end region, *e.g.*, a 3'-end wing, as described herein. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SS, wherein the first S represents the first internucleotidic linkage of a provided oligonucleotide, the 3'-end region comprises a stereochemistry pattern of SS, wherein one or more nucleotidic unit comprising an Sp internucleotidic linkage in the 5'- or 3'-end region comprise -F. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SS, wherein the first S represents the first internucleotidic linkage of a provided oligonucleotide, the 3'-end region comprises a stereochemistry pattern of SS, wherein one or more nucleotidic unit comprising an Sp internucleotidic linkage in the 5'- or 3'-end region comprise a 2'-F sugar modification. In some embodiments, provided oligonucleotides further comprise a middle region between the 5'-end and 3'-end regions, *e.g.*, a core region, which comprises one or more natural phosphate linkages. In some embodiments, provided oligonucleotides further comprise a middle region between the 5'-end and 3'-end regions, *e.g.*, a core region, which comprises one or more natural phosphate linkages and one or more internucleotidic linkages. In some embodiments, a middle region comprises one or more sugar moieties, wherein each sugar moiety independently comprises a 2'-OR¹ modification. In some embodiments, a middle region comprises one or more sugar moieties comprising no 2'-F modification. In some embodiments, a middle region comprises one or more Sp internucleotidic linkages. In some embodiments, a middle region comprises one or more Sp internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, a middle region comprises one or more Rp internucleotidic linkages. In some embodiments, a middle region comprises one or more Rp internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, a middle region comprises one or more Rp internucleotidic linkages and one or more Sp internucleotidic linkages.

[00240] In some embodiments, provided oligonucleotides comprise one or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise one or more chiral modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise one or more chirally controlled chiral modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise one or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise one or more modified internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, a modified internucleotidic linkage is a phosphorothioate linkage. In some embodiments, each modified internucleotidic linkage is a phosphorothioate linkage. In some embodiments, a modified internucleotidic linkage comprises a triazole, substituted triazole, alkyne or Tmg.

[00241] In some embodiments, the present disclosure pertains to a nucleic acid which comprises a modified internucleotidic linkage comprising a triazole or alkyne moiety. In some embodiments, the present disclosure pertains to a nucleic acid which comprises a modified internucleotidic linkage comprising an optionally substituted triazolyl or alkynyl. In some embodiments, such a nucleic acid is a siRNA, double-stranded siRNA, single-stranded siRNA, oligonucleotide, gapmer, skipmer, blockmer, antisense oligonucleotide, antagomir, microRNA, pre-microRNA, antimir, supermir, ribozyme, U1 adaptor, RNA activator, RNAi agent, decoy oligonucleotide, triplex forming oligonucleotide, aptamer or adjuvant. In some embodiments, the present disclosure pertains to an oligonucleotide which comprises a modified internucleotidic linkage comprising a triazole or alkyne moiety. In some embodiments, the present disclosure pertains to a DMD oligonucleotide which comprises a modified internucleotidic linkage comprising a triazole or alkyne moiety. In some embodiments, the present disclosure pertains to a nucleic acid which comprises a modified internucleotidic linkage comprising a triazole moiety. In some embodiments, the present disclosure pertains to a nucleic acid which comprises a modified internucleotidic linkage comprising optionally substituted triazolyl. In some embodiments, the present disclosure pertains to a nucleic acid which comprises a modified internucleotidic linkage comprising a substituted triazole moiety. In some embodiments, the present disclosure pertains to a nucleic acid which comprises a modified internucleotidic linkage comprising an alkyne moiety. In some embodiments, the present disclosure pertains to a nucleic acid or oligonucleotide which comprises, at a 5' end, a structure of

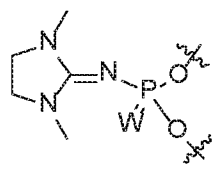


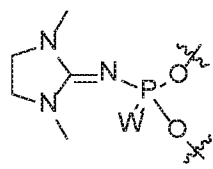
the formula: , or , wherein W is O or S. In some embodiments, an oligonucleotide is a single-stranded siRNA which comprises, at a 5' end, a

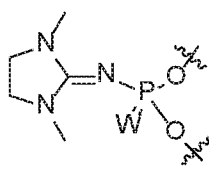


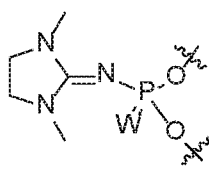
structure of the formula: , or , wherein W is O or S. In some embodiments, a modified internucleotidic linkage is any modified internucleotidic linkage described in Krishna et al. 2012 J. Am. Chem. Soc. 134: 11618–11631.

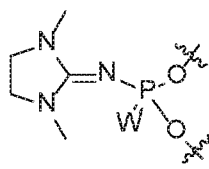
[00242] In some embodiments, the present disclosure pertains to a nucleic acid which comprises a modified internucleotidic linkage which comprises a guanidine moiety. In some embodiments, the present disclosure pertains to a nucleic acid which comprises a modified internucleotidic linkage which comprises a cyclic guanidine moiety. In some embodiments, the present disclosure pertains to a nucleic acid which comprises a modified internucleotidic linkage which comprises a cyclic guanidine moiety and

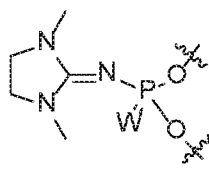


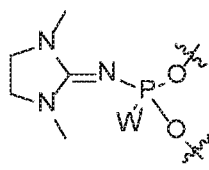
has the structure of: , wherein W is O or S. In some embodiments, a neutral internucleotidic linkage or internucleotidic linkage comprising a cyclic guanidine is chirally controlled. In some embodiments, a nucleic acid comprising a non-negatively charged internucleotidic linkage or a modified internucleotidic linkage comprising a cyclic guanidine moiety is a siRNA, double-stranded siRNA, single-stranded siRNA, oligonucleotide, gapmer, skipmer, blockmer, antisense oligonucleotide, antagomir, microRNA, pre-microRNA, antimir, supermir, ribozyme, U1 adaptor, RNA activator, RNAi agent, decoy oligonucleotide, triplex forming oligonucleotide, aptamer or adjuvant. In some embodiments, the present disclosure pertains to an oligonucleotide which comprises a modified internucleotidic linkage which comprises a cyclic guanidine moiety. In some embodiments, the present disclosure pertains to an oligonucleotide which comprises a modified internucleotidic linkage which has

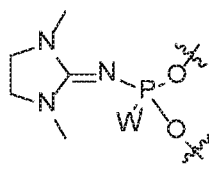


the structure of: , wherein W is O or S. In some embodiments, a neutral internucleotidic linkage or internucleotidic linkage comprising a cyclic guanidine moiety is chirally controlled. In some embodiments, the present disclosure pertains to a DMD oligonucleotide which comprises a modified internucleotidic linkage comprising a cyclic guanidine moiety. In some embodiments, the present disclosure pertains to a DMD oligonucleotide which comprises a modified

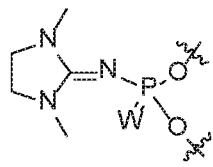


internucleotidic linkage which has the structure of: , wherein W is O or S. In some embodiments, a neutral internucleotidic linkage or internucleotidic linkage comprising a cyclic guanidine moiety is chirally controlled. In some embodiments, the present disclosure pertains to a nucleic acid which comprises a modified internucleotidic linkage comprising a cyclic guanidine moiety. In some embodiments, the present disclosure pertains to a nucleic acid which comprises a modified

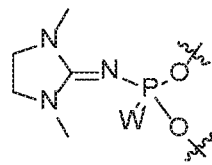


internucleotidic linkage which has the structure of: , wherein W is O or S. In some embodiments, the present disclosure pertains to a nucleic acid or oligonucleotide which comprises, at a 5' end, a structure comprising a cyclic guanidine moiety. In some embodiments, the present disclosure

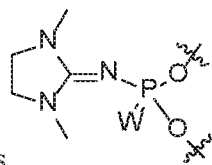
pertains to a nucleic acid or oligonucleotide which comprises, at a 5' end, a structure of the formula:



, wherein W is O or S. In some embodiments, the oligonucleotide is a single-stranded siRNA which comprises, at a 5' end, a structure comprising a cyclic guanine moiety. In some embodiments, the oligonucleotide is a single-stranded siRNA which comprises, at a 5' end, a structure of



the formula: , wherein W is O or S. In some embodiments, the internucleotidic linkage



comprises (wherein W is O or S) and is chirally controlled.

[00243] In some embodiments, provided oligonucleotides can bind to a transcript, and change the splicing pattern of the transcript. In some embodiments, provided oligonucleotides provides exon-skipping of an exon, with efficiency greater than a comparable oligonucleotide under one or more suitable conditions, *e.g.*, as described herein. In some embodiments, a provided skipping efficiency is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190% more than, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50 or more fold of, that of a comparable oligonucleotide under one or more suitable conditions, *e.g.*, as described herein. In some embodiments, a comparable oligonucleotide is an oligonucleotide which has fewer or no chirally controlled internucleotidic linkages and/or fewer or no non-negatively charged internucleotidic linkages but is otherwise identical.

[00244] In some embodiments, the present disclosure demonstrates that 2'-F modifications, among other things, can improve exon-skipping efficiency. In some embodiments, the present disclosure demonstrates that *Sp* internucleotidic linkages, among other things, at the 5'- and 3'-ends can improve oligonucleotide stability. In some embodiments, the present disclosure demonstrates that, among other things, natural phosphate linkages and/or *Rp* internucleotidic linkages can improve removal of oligonucleotides from a system. As appreciated by a person having ordinary skill in the art, various assays known in the art can be utilized to assess such properties in accordance with the present disclosure.

[00245] In some embodiments, provided oligonucleotides comprise one or more modified sugar moieties. In some embodiments, a modified sugar moiety comprises a 2'-modification. In some embodiments, a modified sugar moiety comprises a 2'-modification. In some embodiments, a 2'-

modification is 2'-OR¹. In some embodiments, a 2'-modification is a 2'-OMe. In some embodiments, a 2'-modification is a 2'-MOE. In some embodiments, a 2'-modification is an LNA sugar modification. In some embodiments, a 2'-modification is 2'-F. In some embodiments, each sugar modification is independently a 2'-modification. In some embodiments, each sugar modification is independently 2'-OR¹ or 2'-F. In some embodiments, each sugar modification is independently 2'-OR¹ or 2'-F, wherein R¹ is optionally substituted C₁₋₆ alkyl. In some embodiments, each sugar modification is independently 2'-OR¹ or 2'-F, wherein at least one is 2'-F. In some embodiments, each sugar modification is independently 2'-OR¹ or 2'-F, wherein R¹ is optionally substituted C₁₋₆ alkyl, and wherein at least one is 2'-OR¹. In some embodiments, each sugar modification is independently 2'-OR¹ or 2'-F, wherein at least one is 2'-F, and at least one is 2'-OR¹. In some embodiments, each sugar modification is independently 2'-OR¹ or 2'-F, wherein R¹ is optionally substituted C₁₋₆ alkyl, and wherein at least one is 2'-F, and at least one is 2'-OR¹.

[00246] In some embodiments, 5% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, each sugar moiety of provided oligonucleotides is modified. In some embodiments, a modified sugar moiety comprises a 2'-modification. In some embodiments, a modified sugar moiety comprises a 2'-modification. In some embodiments, a 2'-modification is 2'-OR¹. In some embodiments, a 2'-modification is a 2'-OMe. In some embodiments, a 2'-modification is a 2'-MOE. In some embodiments, a 2'-modification is an LNA sugar modification. In some embodiments, a 2'-modification is 2'-F. In some embodiments, each sugar modification is independently a 2'-modification. In some embodiments, each sugar modification is independently 2'-OR¹ or 2'-F. In some embodiments, each sugar modification is independently 2'-OR¹ or 2'-F, wherein R¹ is optionally substituted C₁₋₆ alkyl. In some embodiments, each sugar modification is independently 2'-OR¹ or 2'-F, wherein at least one is 2'-F. In some embodiments, each sugar modification is independently 2'-OR¹ or 2'-F, wherein R¹ is optionally substituted C₁₋₆ alkyl, and wherein at least one is 2'-OR¹. In some embodiments, each sugar modification is independently 2'-OR¹ or 2'-F, wherein at least one is 2'-F, and at least one is 2'-OR¹. In some embodiments, each sugar modification is independently 2'-OR¹ or 2'-F, wherein R¹ is optionally substituted C₁₋₆ alkyl, and wherein at least one is 2'-F, and at least one is 2'-OR¹.

[00247] In some embodiments, provided oligonucleotides comprise one or more 2'-F. In some embodiments, provided oligonucleotides comprise two or more 2'-F.

[00248] In some embodiments, provided oligonucleotides comprise alternating 2'-F modified sugar moieties and 2'-OR¹ modified sugar moieties. In some embodiments, provided oligonucleotides comprise alternating 2'-F modified sugar moieties and 2'-OMe modified sugar moieties, *e.g.*, [(2'-F)(2'-OMe)]_x, [(2'-OMe)(2'-F)]_x, *etc.*, wherein x is 1-50. In some embodiments, provided oligonucleotides

comprise at least two pairs of alternating 2'-F and 2'-OMe modifications. In some embodiments, provided oligonucleotides comprises alternating phosphodiester and phosphorothioate internucleotidic linkages, *e.g.*, [(PO)(PS)]_x, [(PS)(PO)]_x, *etc.*, wherein x is 1-50. In some embodiments, provided oligonucleotides comprise at least two pairs of alternating phosphodiester and phosphorothioate internucleotidic linkages.

[00249] In some embodiments, provided oligonucleotides comprise one or more natural phosphate linkages and one or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise one or more natural phosphate linkages and one or more modified internucleotidic linkages and one or more non-negatively charged internucleotidic linkages.

[00250] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a plurality of oligonucleotides, wherein:

oligonucleotides of the plurality have the same base sequence; and

oligonucleotides of the plurality comprise one or more modified sugar moieties, or comprise one or more natural phosphate linkages and one or more modified internucleotidic linkages.

[00251] In some embodiments, oligonucleotides of a plurality comprise one or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise one or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 2 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 3 or more modified sugar moieties.

[00252] In some embodiments, provided compositions alter transcript splicing so that an undesired target and/or biological function are suppressed.

[00253] In some embodiments, provided compositions alter transcript splicing so a desired target and/or biological function is enhanced.

[00254] In some embodiments, each oligonucleotide of a plurality comprises one or more modified sugar moieties and modified internucleotidic linkages.

[00255] In some embodiments, each oligonucleotide of a plurality comprises no more than about 25 consecutive unmodified sugar moieties

[00256] In some embodiments, each oligonucleotide of a plurality comprises no more than about 95% unmodified sugar moieties. In some embodiments, each oligonucleotide of a plurality comprises no more than about 90% unmodified sugar moieties. In some embodiments, each oligonucleotide of a plurality comprises no more than about 85% unmodified sugar moieties. In some embodiments, each oligonucleotide of a plurality comprises no more than about 15 consecutive unmodified sugar moieties.

[00257] In some embodiments, each oligonucleotide of a plurality comprises no more than about 95% unmodified sugar moieties.

[00258] In some embodiments, each oligonucleotide of a plurality comprises two or more

modified internucleotidic linkages.

[00259] In some embodiments, about 5% of the internucleotidic linkages in each oligonucleotide of a plurality are modified internucleotidic linkages.

[00260] In some embodiments, each oligonucleotide of a plurality comprises no more than about 25 consecutive natural phosphate linkages. In some embodiments, each oligonucleotide of a plurality comprises no more than about 20 natural phosphate linkages.

[00261] In some embodiments, oligonucleotides of a plurality comprise no natural DNA nucleotide units. In some embodiments, oligonucleotides of a plurality comprise no more than 30 natural DNA nucleotides. In some embodiments, oligonucleotides of a plurality comprise no more than 30 consecutive DNA nucleotides.

[00262] In some embodiments, compared to a reference condition, provided chirally controlled oligonucleotide compositions are surprisingly effective. In some embodiments, desired biological effects (*e.g.*, as measured by increased levels of desired mRNA, proteins, *etc.*, decreased levels of undesired mRNA, proteins, *etc.*) can be enhanced by more than 5, 10, 15, 20, 25, 30, 40, 50, or 100 fold. In some embodiments, a change is measured by increase of a desired mRNA level compared to a reference condition. In some embodiments, a change is measured by decrease of an undesired mRNA level compared to a reference condition. In some embodiments, a reference condition is absence of oligonucleotide treatment. In some embodiments, a reference condition is a stereorandom composition of oligonucleotides having the same base sequence and chemical modifications.

[00263] In some embodiments, a desired biological effect is: improved skipping of one or more exons, increased stability, increased activity, increased stability and activity, low toxicity, low immune response, improved protein binding profile, increased binding to certain proteins, and/or enhanced delivery. In some embodiments, a desired biological effect is enhanced by more than 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 11 fold, 12 fold, 13 fold, 14 fold, 15 fold, 20 fold, 25 fold, 30 fold, 35 fold, 40 fold, 45 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, 100 fold, 200 fold, or 500 fold.

[00264] In some embodiments, the structure of a DMD oligonucleotide is or comprises a wing-core-wing, wing-core, or core-wing structure. In some embodiments, a 5'-wing is a 5'-end region. In some embodiments, a 3'-wing is a 3'-end region. In some embodiments, a core is a middle region. In some embodiments, a 5'-end region is a 5'-wing region. In some embodiments, a 3'-end region is a 3'-wing region. In some embodiments, a middle region is a core region.

[00265] In some embodiments, an oligonucleotide having a wing-core-wing structure is designated a gapmer. In some embodiments, a gapmer is asymmetric, in that the chemistry of one wing is different from the chemistry of the other wing. In some embodiments, a gapmer is asymmetric, in that the

chemistry of one wing is different from the chemistry of the other wing, wherein the wings differ in sugar modifications and/or internucleotidic linkages, or patterns thereof. In some embodiments, a gapmer is asymmetric, in that the chemistry of one wing is different from the chemistry of the other wing, wherein the wings differ in sugar modifications, wherein one wing comprises a sugar modification not present in the other wing; or both wings each comprise a sugar modification not found in the other wing; or both wings comprise different patterns of the same types of sugar modifications; or one wing comprises only one type of sugar modification, while the other wing comprises two types of sugar modifications; etc.

[00266] In some embodiments, an internucleotidic linkage between a wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic linkage between a 5'-wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic linkage between a 3'-wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic linkage between a wing region and a core region is considered part of the core region. In some embodiments, an internucleotidic linkage between a 5'-wing region and a core region is considered part of the core region. In some embodiments, an internucleotidic linkage between a 3'-wing region and a core region is considered part of the core region.

[00267] In some embodiments, a region (e.g., a wing region, a core region, a 5'-end region, a middle region, a 3'-end region, etc.) 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, 25, or more nucleoside units.

[00268] In some embodiments, provided oligonucleotides comprise two wing and one core regions. In some embodiments, provided oligonucleotides comprises a 5'-wing-core-wing-3' structure. In some embodiments, provided oligonucleotides are of a 5'-wing-core-wing-3' gapmer structure. In some embodiments, the two wing regions are identical. In some embodiments, the two wing regions are different. In some embodiments, the two wing regions are identical in chemical modifications. In some embodiments, the two wing regions are identical in 2'-modifications. In some embodiments, the two wing regions are identical in internucleotidic linkage modifications. In some embodiments, the two wing regions are identical in patterns of backbone chiral centers. In some embodiments, the two wing regions are identical in pattern of backbone linkages. In some embodiments, the two wing regions are identical in pattern of backbone linkage types. In some embodiments, the two wing regions are identical in pattern of backbone phosphorus modifications.

[00269] A wing region can be differentiated from a core region in that a wing region contains a different structure feature than a core region. For example, in some embodiments, a wing region differs from a core region in that they have different sugar modifications, base modifications, internucleotidic linkages, internucleotidic linkage stereochemistry, *etc.* In some embodiments, a wing region differs from a core region in that they have different 2'-modifications of the sugars.

[00270] In some embodiments, a region (e.g., a wing region, a core region, a 5'-end region, a middle region, a 3'-end region, etc.) 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, 25, or more modified internucleotidic linkages. In some embodiments, a region comprises 2 or more modified internucleotidic linkages. In some embodiments, a region comprises 3 or more modified internucleotidic linkages. In some embodiments, a region comprises 4 or more modified internucleotidic linkages. In some embodiments, a region comprises 5 or more modified internucleotidic linkages. In some embodiments, a region comprises 6 or more modified internucleotidic linkages. In some embodiments, a region comprises 7 or more modified internucleotidic linkages. In some embodiments, a region comprises 8 or more modified internucleotidic linkages. In some embodiments, a region comprises 9 or more modified internucleotidic linkages. In some embodiments, a region comprises 10 or more modified internucleotidic linkages.

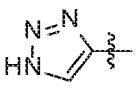
[00271] In some embodiments, provided oligonucleotides comprise consecutive nucleoside units each of which comprises no 2'-OR¹ modifications (wherein R¹ is not hydrogen). In some embodiments, provided oligonucleotides comprise consecutive nucleoside units whose 2'-positions are independently unsubstituted or substituted with 2'-F. In some embodiments, such an oligonucleotide is a DMD oligonucleotide. In some embodiments, each of the consecutive nucleoside units is independently preceded and/or followed by a modified internucleotidic linkage. In some embodiments, each of the consecutive nucleoside units is independently preceded and/or followed by a phosphorothioate linkage. In some embodiments, each of the consecutive nucleoside units is independently preceded and/or followed by a chirally controlled modified internucleotidic linkage. In some embodiments, each of the consecutive nucleoside units is independently preceded and/or followed by a chirally controlled phosphorothioate linkage.

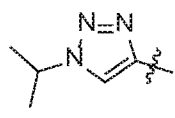
[00272] In some embodiments, a modified internucleotidic linkage has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, III, etc., or a salt form thereof. In some embodiments, a modified internucleotidic linkage has a structure of formula I or a salt form thereof. In some embodiments, a modified internucleotidic linkage has a structure of formula I-a or a salt form thereof.

[00273] In some embodiments, a modified internucleotidic linkage is a non-negatively charged internucleotidic linkage. In some embodiments, a modified internucleotidic linkage is a positively-charged internucleotidic linkage. In some embodiments, a modified internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, a non-negatively charged internucleotidic linkage has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, etc., or a salt form thereof. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 3-20 membered heterocyclyl or heteroaryl

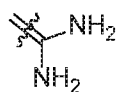
group having 1-10 heteroatoms. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 3-20 membered heterocyclyl or heteroaryl group having 1-10 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, such a heterocyclyl or heteroaryl group is of a 5-membered ring. In some embodiments, such a heterocyclyl or heteroaryl group is of a 6-membered ring.

[00274] In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heteroaryl group having 1-10 heteroatoms. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heteroaryl group having 1-10 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-6 membered heteroaryl group having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-membered heteroaryl group having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, a heteroaryl group is directly bonded to a linkage phosphorus. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted triazolyl group. In some embodiments, a non-negatively charged internucleotidic linkage comprises an unsubstituted

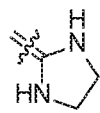
triazolyl group, e.g., . In some embodiments, a non-negatively charged internucleotidic

linkage comprises a substituted triazolyl group, e.g., .

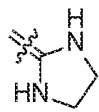
[00275] In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heterocyclyl group having 1-10 heteroatoms. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heterocyclyl group having 1-10 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-6 membered heterocyclyl group having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-membered heterocyclyl group having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, at least two heteroatoms are nitrogen. In some embodiments, a heterocyclyl group is directly bonded to a linkage phosphorus. In some embodiments, a heterocyclyl group is bonded to a linkage phosphorus through a linker, e.g., =N— when the heterocyclyl group is part of a guanidine moiety who directed bonded to a linkage phosphorus through its =N—. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted



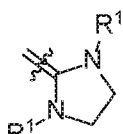
group. In some embodiments, a non-negatively charged internucleotidic linkage comprises an



optionally substituted group. In some embodiments, a non-negatively charged internucleotidic



linkage comprises an optionally substituted group. In some embodiments, a non-negatively charged



internucleotidic linkage comprises a group. In some embodiments, each R^1 is independently optionally substituted C_{1-20} alkyl. In some embodiments, each R^1 is independently optionally substituted C_{1-6} alkyl. In some embodiments, each R^1 is independently methyl. In some embodiments, the two R^1 groups are different; for example, in some embodiments, one R^1 is methyl, and the other is $-\text{CH}_2(\text{CH}_2)_{10}\text{CH}_3$.

[00276] In some embodiments, a modified internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, comprises a triazole or alkyne moiety, each of which is optionally substituted. In some embodiments, a modified internucleotidic linkage comprises a triazole moiety. In some embodiments, a modified internucleotidic linkage comprises a unsubstituted triazole moiety. In some embodiments, a modified internucleotidic linkage comprises a substituted triazole moiety. In some embodiments, a modified internucleotidic linkage comprises an alkyl moiety. In some embodiments, a modified internucleotidic linkage comprises an optionally substituted alkynyl group. In some embodiments, a modified internucleotidic linkage comprises an unsubstituted alkynyl group. In some embodiments, a modified internucleotidic linkage comprises a substituted alkynyl group. In some embodiments, an alkynyl group is directly bonded to a linkage phosphorus.

[00277] In some embodiments, an oligonucleotide comprising a non-negatively charged internucleotidic linkage can comprise any structure, format, or portion thereof described herein. In some embodiments, an oligonucleotide comprising a non-negatively charged internucleotidic linkage can comprise any structure, format, or portion thereof described herein as being a component of a DMD oligonucleotide. In some embodiments, any structure, format, or portion thereof described as being a component of any DMD oligonucleotide can be used in any oligonucleotide comprising a non-negatively charged internucleotidic linkage, whether or not that oligonucleotide targets DMD or not, or whether the oligonucleotide is capable of mediating skipping of a DMD exon or not. In some embodiments, an

oligonucleotide comprising a non-negatively charged internucleotidic is double-stranded or single-stranded.

[00278] In some embodiments, a provided oligonucleotide composition is characterized in that, when it is contacted with the transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof. In some embodiments, a desired splicing product is increased 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or 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, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 fold or more. In some embodiments, a desired splicing reference is absent (*e.g.*, cannot be reliably detected by quantitative PCR) under reference conditions. In some embodiments, as exemplified in the present disclosure, levels of the plurality of oligonucleotides, *e.g.*, a plurality of oligonucleotides, in provided compositions are pre-determined.

[00279] In some embodiments, provided oligonucleotides, *e.g.*, oligonucleotides of a plurality in a provided composition, comprise two or more regions. In some embodiments, provided comprise a 5'-end region, a 3'-end region, and a middle region in between. In some embodiments, provided oligonucleotides have two wing and one core regions. In some embodiments, provided oligonucleotides are of a wing-core-wing structure. In some embodiments, the two wing regions are identical. In some embodiments, the two wing regions are different. In some embodiments, a 5'-end region is a 5'-wing region. In some embodiments, a 5'-wing region is a 5'-end region. In some embodiments, a 3'-end region is a 3'-wing region. In some embodiments, a 3'-wing region is a 3'-end region. In some embodiments, a core region is a middle region.

[00280] In some embodiments, a region (*e.g.*, a 5'-wing region, a 3'-wing, a core region, a 5'-end region, a middle region, etc.) 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, 25, or more nucleoside units. In some embodiments, a region comprises 2 or more nucleoside units. In some embodiments, a region comprises 3 or more nucleoside units. In some embodiments, a region comprises 4 or more nucleoside units. In some embodiments, a region comprises 5 or more nucleoside units. In some embodiments, a region comprises 6 or more nucleoside units. In some embodiments, a region comprises 7 or more nucleoside units. In some embodiments, a region comprises 8 or more nucleoside units. In some embodiments, a region comprises 9 or more nucleoside units. In some embodiments, a region comprises 10 or more nucleoside units.

[00281] In some embodiments, a region (*e.g.*, a 5'-wing region, a 3'-wing, a core region, a 5'-end region, a middle region, etc.) 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, 25, or more modified internucleotidic linkages. In some embodiments, a region comprises 2 or more modified internucleotidic linkages. In some embodiments, the one or more modified

internucleotidic linkages are consecutive. In some embodiments, a region comprises 2 or more consecutive modified internucleotidic linkages. In some embodiments, each internucleotidic linkage in a region is independently a modified internucleotidic linkage, wherein each chiral internucleotidic linkage is optionally and independently chirally controlled. In some embodiments, a chiral internucleotidic linkage or a modified internucleotidic linkage has the structure of formula I or a salt form thereof. In some embodiments, a chiral internucleotidic linkage or a modified internucleotidic linkage is a phosphorothioate internucleotidic linkage. In some embodiments, each chiral internucleotidic linkage or a modified internucleotidic linkage independently has the structure of formula I or a salt form thereof. In some embodiments, each chiral internucleotidic linkage or a modified internucleotidic linkage is a phosphorothioate internucleotidic linkage. In some embodiments, a region comprises 3 or consecutive modified internucleotidic linkages.

[00282] In some embodiments, a wing region comprises one or more natural phosphate linkages. In some embodiments, a core region comprises one or more natural phosphate linkages. In some embodiments, a 5'-end region comprises one or more natural phosphate linkages. In some embodiments, a 3'-end region comprises one or more natural phosphate linkages. In some embodiments, a middle region comprises one or more natural phosphate linkages. In some embodiments, the one or more natural phosphate linkages are consecutive.

[00283] In some embodiments, a natural phosphate linkage follows (e.g., connected to a 3'-position of a sugar moiety) or precedes (e.g., connected to a 5'-position of a sugar moiety) a nucleoside unit whose sugar moiety comprises a 2'-OR¹ modification, wherein R¹ is not hydrogen. In some embodiments, R¹ is optionally substituted C₁₋₆ aliphatic. In some embodiments, a modified internucleotidic linkage follows (e.g., connected to a 3'-position of a sugar moiety) or precedes (e.g., connected to a 5'-position of a sugar moiety) all or most (e.g., more than 55%, 60%, 70%, 80%, 90%, 95%, etc.) nucleoside units whose sugar moiety comprises no 2'-OR¹ modification, wherein R¹ is not hydrogen (e.g., those having two 2'-H at the 2'-position, those having a 2'-H and a 2'-F at the 2'-position (2'-F modified), etc.).

[00284] In some embodiments, a region comprises one or more nucleoside units comprising sugar modifications, e.g., 2'-F, 2'-OR¹, LNA sugar modifications, etc. In some embodiments, each sugar in a region is independently modified. In some embodiments, each sugar moiety in a wing, a 5'-end region, and/or a 3'-end region is modified. In some embodiments, a modification is a 2'-modification. In some embodiments, a modification can increase stability, e.g., 2'-OR¹ where in R¹ is not -H (e.g., is optionally substituted C₁₋₆ aliphatic), LNA sugar modifications, etc. In some embodiments, a region, e.g., a core region or a middle region, comprise no sugar modifications (or no 2'-OR¹ sugar modifications/LNA modifications etc.). In some embodiments, such a core/middle region can form a duplex with a RNA for

recognition/binding of a protein, e.g., RNase H, for the protein to perform one or more of its functions (e.g., in the case of RNase H, its binding and cleavage of DNA/RNA duplex).

[00285] A region and/or a provided oligonucleotide may have various patterns of backbone chiral centers. In some embodiments, each internucleotidic linkage in a region is a chirally controlled internucleotidic linkage and is *Sp*. In some embodiments, the 5'-end and/or the 3'-end internucleotidic linkage is a chirally controlled internucleotidic linkage and is *Sp*. In some embodiments, the pattern of backbone chiral centers of a wing region, a 5'-end region, and/or a 3'-end region is or comprises a 5'-end and/or a 3'-end internucleotidic linkage which is a chirally controlled internucleotidic linkage and is *Sp*, with the other internucleotidic linkages in the region independently being an natural phosphate linkage, a modified internucleotidic linkage, or a chirally controlled internucleotidic linkage (*Sp* or *Rp*). In some embodiments, such patterns provide stability. Many example patterns of backbone chiral centers are described in the present disclosure.

[00286] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides defined by having:

- 1) a common base sequence;
- 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 a controlled level 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.

[00287] In some embodiments, oligonucleotides having a common base sequence may have the same pattern of nucleoside modifications, e.g., sugar modifications, base modifications, *etc.* In some embodiments, a pattern of nucleoside modifications may be represented by a combination of locations and modifications. In some embodiments, all non-chiral linkages (e.g., PO) may be omitted. In some embodiments, oligonucleotides having the same base sequence have the same constitution.

[00288] As understood by a person having ordinary skill in the art, a stereorandom or racemic preparation of oligonucleotides is prepared by non-stereoselective and/or low-stereoselective coupling of nucleotide monomers, typically without using any chiral auxiliaries, chiral modification reagents, and/or chiral catalysts. 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 example substantially racemic preparation of oligonucleotides is the preparation of phosphorothioate oligonucleotides through sulfurizing phosphite triesters from commonly used phosphoramidite oligonucleotide synthesis with either tetraethylthiuram disulfide or (TETD) or 3H-1, 2-benzodithiol-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). In some embodiments, at least one coupling of a nucleotide monomer has a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, each internucleotidic linkage independently has a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, a diastereoselectivity is lower than about 60:40. In some embodiments, a diastereoselectivity is lower than about 70:30. In some embodiments, a diastereoselectivity is lower than about 80:20. In some embodiments, a diastereoselectivity is lower than about 90:10. In some embodiments, a diastereoselectivity is lower than about 91:9. In some embodiments, at least one internucleotidic linkage has a diastereoselectivity lower than about 90:10. In some embodiments, at least two internucleotidic linkages have a diastereoselectivity lower than about 90:10. In some embodiments, at least three internucleotidic linkages have a diastereoselectivity lower than about 90:10. In some embodiments, at least four internucleotidic linkages have a diastereoselectivity lower than about 90:10. In some embodiments, at least five internucleotidic linkages have a diastereoselectivity lower than about 90:10. In some embodiments, each internucleotidic linkage independently has a diastereoselectivity lower than about 90:10. In some embodiments, a non-chirally controlled internucleotidic linkage has a diastereomeric purity no more than 90%, 85%, 80%, 75%, 70%, 65%, 60%, or 55%. In some embodiments, the purity is no more than 90%. In some embodiments, the purity is no more than 85%. In some embodiments, the purity is no more than 80%.

[00289] In contrast, in chirally controlled oligonucleotide composition, at least one and typically each chirally controlled internucleotidic linkage, such as those of oligonucleotides of chirally controlled oligonucleotide compositions, independently has a diastereomeric purity of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more with respect to the chiral linkage phosphorus. In some embodiments, a diastereomeric purity is 95% or more. In some embodiments, a diastereomeric purity is 96% or more. In some embodiments, a diastereomeric purity is 97% or more. In some embodiments, a diastereomeric purity is 98% or more. In some embodiments, a diastereomeric purity is 99% or more. Among other things, technologies of the present disclosure routinely provide chirally controlled internucleotidic linkages with high diastereomeric purity.

[00290] As appreciated by a person having ordinary skill in the art, diastereoselectivity of a coupling or diastereomeric purity (diastereopurity) of an internucleotidic linkage can be assessed through the diastereoselectivity of a dimer formation/diastereomeric purity of the internucleotidic linkage of a dimer formed under the same or comparable conditions, wherein the dimer has the same 5'- and 3'-nucleosides and internucleotidic linkage.

[00291] In some embodiments, the present disclosure provides chirally controlled (and/or stereochemically pure) oligonucleotide compositions comprising a plurality of oligonucleotides defined by having:

- 1) a common base sequence;
- 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.

[00292] In some embodiments, the present disclosure provides chirally controlled oligonucleotide composition of a plurality of oligonucleotides, wherein 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 disclosure provides chirally controlled oligonucleotide composition of a plurality of oligonucleotides wherein the composition is enriched, relative to a substantially racemic preparation of the same oligonucleotides, for oligonucleotides of a single oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and
- 4) pattern of backbone phosphorus modifications.

[00293] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and
- 4) pattern of backbone phosphorus modifications.

wherein the composition is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type.

[00294] In some embodiments, oligonucleotides having a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone chiral centers have a common pattern of backbone phosphorus modifications and a common pattern of base modifications. In some embodiments, oligonucleotides having a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone chiral centers have a common pattern of backbone phosphorus

modifications and a common pattern of nucleoside modifications. In some embodiments, oligonucleotides having a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone chiral centers have identical structures.

[00295] In some embodiments, oligonucleotides of an oligonucleotide type have a common pattern of backbone phosphorus modifications and a common pattern of sugar modifications. In some embodiments, oligonucleotides of an oligonucleotide type have a common pattern of backbone phosphorus modifications and a common pattern of base modifications. In some embodiments, oligonucleotides of an oligonucleotide type have a common pattern of backbone phosphorus modifications and a common pattern of nucleoside modifications. In some embodiments, oligonucleotides of a particular type have the same constitution. In some embodiments, oligonucleotides of an oligonucleotide type are identical.

[00296] In some embodiments, a chirally controlled oligonucleotide composition is a substantially pure preparation of an oligonucleotide type in that oligonucleotides in the composition that are not of the oligonucleotide type are impurities from the preparation process of said oligonucleotide type, in some case, after certain purification procedures.

[00297] In some embodiments, at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of the oligonucleotides in the composition have a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone chiral centers.

[00298] In some embodiments, oligonucleotides having a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone chiral centers have a common pattern of backbone phosphorus modifications. In some embodiments, oligonucleotides having a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone chiral centers have a common pattern of backbone phosphorus modifications and a common pattern of nucleoside modifications. In some embodiments, oligonucleotides having a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone chiral centers have a common pattern of backbone phosphorus modifications and a common pattern of sugar modifications. In some embodiments, oligonucleotides having a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone chiral centers have a common pattern of backbone phosphorus modifications and a common pattern of base modifications. In some embodiments, oligonucleotides having a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone chiral centers are identical.

[00299] 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 oligonucleotide type. In some embodiments, at least about 20% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the oligonucleotide type. In some embodiments, at least about 30% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the oligonucleotide type. In some embodiments, at least about 40% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the oligonucleotide type. In some embodiments, at least about 50% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the oligonucleotide type. In some embodiments, at least about 60% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the oligonucleotide type. In some embodiments, at least about 70% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the oligonucleotide type. In some embodiments, at least about 80% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the oligonucleotide type. In some embodiments, at least about 90% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the oligonucleotide type. In some embodiments, at least about 92% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the oligonucleotide type. In some embodiments, at least about 94% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the oligonucleotide type. In some embodiments, at least about 95% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the 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 oligonucleotide type. In some embodiments, at least about 98% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the oligonucleotide type. In some embodiments, at least about 99% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the oligonucleotide type.

[00300] 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, use of a nuclease which stereoselectively cleaves phosphorothioates, *etc*) has the intended stereoselectivity. In some embodiments, stereoselectivity of a chiral internucleotidic linkage in an oligonucleotide may be measured through a model reaction, *e.g.* formation of a dimer under essentially the same or comparable conditions wherein the dimer has the same internucleotidic linkage as the chiral internucleotidic linkage, the 5'-nucleoside of the dimer is the same as the nucleoside to the 5'-end of the chiral internucleotidic linkage, and the 3'-nucleoside of the dimer is the same as the nucleoside to the 3'-end of the chiral internucleotidic linkage (*e.g.*, for fU*SfU*SfC*SfU, through the dimer of fU*SfC). As appreciated by a person having ordinary skill in the art, percentage of oligonucleotides of a particular type having *n* chirally controlled internucleotidic linkages in a preparation may be calculated as $DP^1 * DP^2 * DP^3 * \dots * DP^n$, wherein each of DP^1 , DP^2 , DP^3 , ..., and DP^n is independently the diastereomeric purity of the 1st, 2nd, 3rd, ..., and *n*th chirally controlled internucleotidic linkage. In some embodiments, each of DP^1 , DP^2 , DP^3 , ..., and DP^n is independently 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 97% or 99% or more. . In some embodiments, each of DP^1 , DP^2 , DP^3 , ..., and DP^n is independently 95% or more.

[00301] In some embodiments, in provided compositions, at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 97% or 99% of oligonucleotides that have the base sequence of a particular oligonucleotide type (defined by 1) base sequence; 2) pattern of backbone linkages; 3) pattern of backbone chiral centers; and 4) pattern of backbone phosphorus modifications) are oligonucleotides of the particular oligonucleotide type. In some embodiments, at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 97% or 99% of oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of a particular oligonucleotide type are oligonucleotides of the particular oligonucleotide type.

[00302] In some embodiments, oligonucleotides of a particular type in a chirally controlled oligonucleotide composition is enriched at least 5 fold (oligonucleotides of the particular type have a fraction of $5 \cdot (1/2^n)$ of oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type, wherein n is the number of chiral internucleotidic linkages; or oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type but are not of the particular oligonucleotide type are no more than $[1 - (1/2^n)]/5$ of oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type) compared to a stereorandom preparation of the oligonucleotides (oligonucleotides of the particular type are typically considered to have a fraction of $1/2^n$ of oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type, wherein n is the number of chiral internucleotidic linkages, and oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type but are not of the particular oligonucleotide type are typically considered to have a fraction of $[1 - (1/2^n)]$ of oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type). In some embodiments, the enrichment is at least 20 fold. In some embodiments, the enrichment is at least 30 fold. In some embodiments, the enrichment is at least 40 fold. In some embodiments, the enrichment is at least 50 fold. In some embodiments, the enrichment is at least 60 fold. In some embodiments, the enrichment is at least 70 fold. In some embodiments, the enrichment is at least 80 fold. In some embodiments, the enrichment is at least 90 fold. In some embodiments, the enrichment is at least 100 fold. In some embodiments, the enrichment is at least 20,000 fold. In some embodiments, the enrichment is at least $(1.5)^n$. In some embodiments, the enrichment is at least $(1.6)^n$. In some embodiments, the enrichment is at least $(1.7)^n$. In some embodiments, the enrichment is at least $(1.1)^n$. In some embodiments, the enrichment is at least $(1.8)^n$. In some embodiments, the enrichment is at least $(1.9)^n$. In some embodiments, the enrichment is at least 2^n . In some embodiments, the enrichment is at least 3^n . In some embodiments, the enrichment is at least 4^n . In some embodiments, the enrichment is at least 5^n . In some embodiments, the enrichment is at least 6^n . In some embodiments, the enrichment is at least 7^n . In some embodiments, the enrichment is at least 8^n . In some embodiments, the enrichment is at least 9^n . In some embodiments, the enrichment is at least 10^n . In some embodiments, the enrichment is at least 15^n . In some embodiments, the enrichment is at least 20^n . In some embodiments, the enrichment is at least 25^n . In some embodiments, the enrichment is at least 30^n . In some embodiments, the enrichment is at least 40^n . In some embodiments, the enrichment is at least 50^n . In some embodiments, the enrichment is at

least 100ⁿ. In some embodiments, enrichment is measured by increase of the fraction of oligonucleotides of the particular oligonucleotide type in oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type. In some embodiments, an enrichment is measured by decrease of the fraction of oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type but are not of the particular oligonucleotide type in oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type.

[00303] In some embodiments, provided oligonucleotides are antisense oligonucleotides. In some embodiments, provided oligonucleotides are siRNA oligonucleotides. In some embodiments, a provided chirally controlled oligonucleotide composition is of oligonucleotides that can be antisense oligonucleotide, antagomir, microRNA, pre-microRNA, antimir, supermir, ribozyme, UI 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 siRNA 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 UI 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.

[00304] In some embodiments, a provided oligonucleotide comprises one or more chiral, modified phosphate linkages. 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.

[00305] 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%.

[00306] In some embodiments, at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the internucleotidic linkages of an oligonucleotide are independently chiral internucleotidic linkages. In some embodiments, all chiral, modified internucleotidic linkages are chiral phosphorothioate internucleotidic linkages. In some embodiments, all chiral, modified internucleotidic linkages except non-negatively charged internucleotidic linkages are chiral phosphorothioate internucleotidic linkages. In some embodiments, each chiral internucleotidic linkage is chirally controlled. In some embodiments, at least about 10, 20, 30, 40, 50, 60, 70, 80, or 90% chiral internucleotidic linkages of an oligonucleotide are chirally controlled and are of the *Sp* conformation. In some embodiments, at least about 10, 20, 30, 40, 50, 60, 70, 80, or 90% phosphorothioate internucleotidic linkages of an oligonucleotide are chirally controlled and are of the *Sp* conformation. In some embodiments, the percentage is at least about 10%. In some embodiments, the percentage is at least about 20%. In some embodiments, the percentage is at least about 30%. In some embodiments, the percentage is at least about 40%. In some embodiments, the percentage is at least about 50%. In some embodiments, the percentage is at least about 60%. In some embodiments, the percentage is at least about 70%. In some embodiments, the percentage is at least about 80%. In some embodiments, the percentage is at least about 90%.

[00307] In some embodiments, at least about 10, 20, 30, 40, 50, 60, 70, 80, or 90% chiral internucleotidic linkages of an oligonucleotide are chirally controlled and are of the *Rp* conformation. In some embodiments, at least about 10, 20, 30, 40, 50, 60, 70, 80, or 90% chiral phosphorothioate internucleotidic linkages of an oligonucleotide are chirally controlled and are of the *Rp* conformation. In some embodiments, the percentage is at least about 10%. In some embodiments, the percentage is at least about 20%. In some embodiments, the percentage is at least about 30%. In some embodiments, no more than 10, 20, 30, 40, 50, 60, 70, 80, or 90% chiral internucleotidic linkages of an oligonucleotide are chirally controlled and are of the *Rp* conformation. In some embodiments, no more than 10, 20, 30, 40, 50, 60, 70, 80, or 90% phosphorothioate internucleotidic linkages of an oligonucleotide are of the *Rp* conformation. In some embodiments, the percentage is no more than 10%. In some embodiments, the percentage is no more than 20%. In some embodiments, the percentage is no more than 30%.

[00308] In some embodiments, provided chirally controlled (and/or stereochemically pure) compositions are of oligonucleotides that contain one or more modified bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) compositions are of oligonucleotides that contain no modified bases. As appreciated by those skilled in the art, many types of modified bases can be utilized in accordance with the present disclosure. Example modified bases are described herein.

[00309] In some embodiments, oligonucleotides of provided compositions comprise at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least one natural phosphate linkage. In some embodiments, oligonucleotides of provided compositions comprise at least two natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least three natural phosphate linkages.

[00310] In some embodiments, oligonucleotides of provided compositions comprise 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise one natural phosphate linkage. In some embodiments, oligonucleotides of provided compositions comprise two natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise three natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise four natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise five natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise six natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise seven natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise eight natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise nine natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise ten natural phosphate linkages.

[00311] In some embodiments, oligonucleotides of provided compositions comprise at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least two consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least three consecutive natural phosphate linkages.

[00312] In some embodiments, oligonucleotides of the present disclosure have at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, or 75 nucleobases in length. In some embodiments, oligonucleotides of the present disclosure comprises at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, or 75 nucleobases in length, wherein each nucleobase is independently optionally substituted A, T, C, G, U, or a tautomer thereof.

[00313] In some embodiments, provided compositions comprise oligonucleotides containing one or more residues which are modified at the sugar moiety. In some embodiments, provided compositions 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"). Examples of such modifications are described herein and include, but are not limited to, 2'-OMe, 2'-MOE, 2'-LNA, 2'-F, FRNA, FANA, S-cEt, *etc.* In some embodiments, provided compositions 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 compositions comprise oligonucleotides which do not contain any 2'-modifications. In some embodiments, provided compositions are oligonucleotides which do not contain any 2'-MOE residues. That is, in some embodiments, provided oligonucleotides are not MOE-modified. Additional example sugar modifications are described in the present disclosure.

[00314] In some embodiments, one or more is one. In some embodiments, one or more is two. In some embodiments, one or more is three. In some embodiments, one or more is four. In some embodiments, one or more is five. In some embodiments, one or more is six. In some embodiments, one or more is seven. In some embodiments, one or more is eight. In some embodiments, one or more is nine. In some embodiments, one or more is ten. In some embodiments, one or more is at least one. In some embodiments, one or more is at least two. In some embodiments, one or more is at least three. In some embodiments, one or more is at least four. In some embodiments, one or more is at least five. In some embodiments, one or more is at least six. In some embodiments, one or more is at least seven. In some embodiments, one or more is at least eight. In some embodiments, one or more is at least nine. In some embodiments, one or more is at least ten.

[00315] In some embodiments, a base sequence, e.g., a common base sequence of a plurality of

oligonucleotide, a base sequence of a particular oligonucleotide type, etc., comprises or is a sequence complementary to a gene or transcript (e.g., of Dystrophin or DMD). In some embodiments, a common base sequence comprises or is a sequence 100% complementary to a gene. In some embodiments, a common base sequence comprises or is a sequence complementary to a characteristic sequence element of a gene, which characteristic sequences differentiate the gene from a similar sequence sharing homology with the gene. In some embodiments, a common base sequence comprises or is a sequence 100% complementary to a characteristic sequence element of a gene, which characteristic sequences differentiate the gene from another allele of the gene. In some embodiments, a common base sequence comprises or is a sequence 100% complementary to a characteristic sequence element of a gene, which characteristic sequences differentiate the gene from a similar sequence sharing homology with the gene. In some embodiments, a common base sequence comprises or is a sequence complementary to characteristic sequence element of a target gene, which characteristic sequences comprises a mutation that is not found in other copies of the gene, *e.g.*, the wild-type copy of the gene, another mutant copy the gene, *etc.* In some embodiments, a common base sequence comprises or is a sequence 100% complementary to characteristic sequence element of a target gene, which characteristic sequences comprises a mutation that is not found in other copies of the gene, *e.g.*, the wild-type copy of the gene, another mutant copy the gene, *etc.* In some embodiments, a common base sequence comprises or is a sequence 100% complementary to a characteristic sequence element of a gene, which characteristic sequences differentiate the gene from another allele of the gene. In some embodiments, a characteristic sequence element is a mutation. In some embodiments, a characteristic sequence element is a SNP.

[00316] In some embodiments, a chiral internucleotidic linkage has the structure of formula **I**, **I-a**, **I-b**, **I-c**, **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, **III**, etc., or a salt form thereof. In some embodiments, linkage phosphorus of chiral internucleotidic linkages are chirally controlled. In some embodiments, a chiral internucleotidic linkage is phosphorothioate internucleotidic linkage. In some embodiments, each chiral internucleotidic linkage in an oligonucleotide of a provided composition independently has the structure of formula **I**. In some embodiments, each chiral internucleotidic linkage in an oligonucleotide of a provided composition independently has the structure of formula **II**. In some embodiments, each chiral internucleotidic linkage in an oligonucleotide of a provided composition independently has the structure of formula **III**. In some embodiments, each chiral internucleotidic linkage in an oligonucleotide of a provided composition is a phosphorothioate internucleotidic linkage.

[00317] As appreciated by those skilled in the art, internucleotidic linkages, *e.g.*, those of formula **I**, natural phosphate linkages, phosphorothioate internucleotidic linkages, etc. may exist in their salt forms depending on pH of their environment. Unless otherwise indicated, such salt forms are included in the

present application when such internucleotidic linkages are referred to.

[00318] In some embodiments, oligonucleotides of the present disclosure comprise one or more modified sugar moieties. In some embodiments, oligonucleotides of the present disclosure comprise one or more modified base moieties. As known by a person of ordinary skill in the art and described in the disclosure, various modifications can be introduced to sugar and base moieties. For example, in some embodiments, a modification is a modification described in US9006198, WO2014/012081, WO/2015/107425, and WO/2017/062862, the sugar and base modifications of each of which are incorporated herein by reference.

[00319] In some embodiments, a sugar modification is a 2'-modification. Commonly used 2'-modifications include but are not limited to 2'-OR¹, wherein R¹ 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 disclosure 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.

[00320] In some embodiments, a 2'-modification is -O-L- or -L- which connects the 2'-carbon of a sugar moiety to another carbon of a sugar moiety. In some embodiments, a 2'-modification is -O-L- or -L- which connects the 2'-carbon of a sugar moiety to the 4'-carbon of a sugar moiety. In some embodiments, a 2'-modification is S-cEt. In some embodiments, a modified sugar moiety is an LNA sugar moiety.

[00321] In some embodiments, a 2'-modification is -F. In some embodiments, a 2'-modification is FANA. In some embodiments, a 2'-modification is FRNA.

[00322] In some embodiments, a sugar modification is a 5'-modification. In some embodiments, a modification is 5'-R¹, wherein R¹ is not hydrogen. In some embodiments, a sugar modification is 5'-R, wherein R is not hydrogen and is otherwise as described in the present disclosure. In some embodiments, a sugar modification is 5'-R, wherein R is optionally substituted C₁₋₆ aliphatic. In some embodiments, a sugar modification is 5'-R, wherein R is optionally substituted C₁₋₆ alkyl. In some embodiments, a sugar modification is 5'-R, wherein R is optionally substituted methyl. In some embodiments, a sugar modification is 5'-R, wherein R is optionally substituted methyl, wherein no substituents of the methyl group comprises a carbon atom. In some embodiments, a 5'-modification is methyl. In some

embodiments, each substituent is independently halogen. In some embodiments, a substituted 5'-carbon is diastereomerically pure. In some embodiments, a substituted 5'-carbon has the *R* configuration. In some embodiments, a substituted 5'-carbon has the *S* configuration. In some embodiments, a 5'-modification is 5'-(*R*)-Me. In some embodiments, a 5'-modification is 5'-(*S*)-Me.

[00323] In some embodiments, a sugar moiety has one and no more than one modification at a position, *e.g.*, a 2'-position, 5'-position, *etc.* In some embodiments, a 2'-modification takes the position corresponding to the position of the 2'-OH in a natural RNA sugar moiety. In some embodiments, a 2'-modification takes the position corresponding to the position of the 2'-H in a natural RNA sugar moiety.

[00324] In some embodiments, a sugar modification changes the size of the sugar ring. In some embodiments, a sugar modification changes the conformation of the sugar ring. In some embodiments, a sugar modification is the sugar moiety in FHNA.

[00325] In some embodiments, a sugar modification replaces a sugar moiety with another cyclic or acyclic moiety. Examples of such moieties are widely known in the art, including but not limited to those used in Morpholino, glycol nucleic acids, *etc.*

Certain Embodiments of Internucleotidic Linkages, Chirally Controlled Oligonucleotides and Chirally Controlled Oligonucleotide Compositions

[00326] Among other things, the present disclosure provides chirally controlled oligonucleotides and chirally controlled oligonucleotide compositions. In some embodiments, the present disclosure provides chirally controlled oligonucleotides and chirally controlled oligonucleotide compositions which are of high crude purity. In some embodiments, the present disclosure provides chirally controlled oligonucleotides, and chirally controlled oligonucleotide compositions which are of high diastereomeric purity. Chirally controlled oligonucleotides are oligonucleotides comprise one or more chirally controlled internucleotidic linkages, such as oligonucleotides of a plurality in chirally controlled oligonucleotide compositions. In some embodiments, chirally controlled oligonucleotides comprise 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 or more chirally controlled internucleotidic linkages. In some embodiments, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more chiral internucleotidic linkages of a chirally controlled oligonucleotide are independently chirally controlled internucleotidic linkages. In some embodiments, each chiral internucleotidic linkage in a chirally controlled oligonucleotide is a chirally controlled internucleotidic linkage, and a chirally controlled oligonucleotide is diastereomerically pure.

[00327] In some embodiments, a chirally controlled oligonucleotide composition is a substantially pure composition of an oligonucleotide type in that oligonucleotides in the composition that are not of the oligonucleotide type are impurities. In some embodiments, such impurities are formed during the

preparation process of oligonucleotides of said oligonucleotide type, in some case, after certain purification procedures.

[00328] In some embodiments, the present disclosure provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages with respect to the chiral linkage phosphorus (e.g., linkage phosphorus of chirally controlled internucleotidic linkages). In some embodiments, the present disclosure provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages having the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, III, etc., or a salt form thereof. In some embodiments, the present disclosure provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages with respect to the chiral linkage phosphorus, and one or more natural phosphate linkages (unless otherwise indicated, reference in the present application to internucleotidic linkages, such as natural phosphate linkages and other types of internucleotidic linkages when applicable, includes salt forms of such linkages). Thus, diastereomerically pure internucleotidic linkages here include salt forms of diastereomerically pure internucleotidic linkages; natural phosphate linkages here include salt forms of natural phosphate linkages. A person having ordinary skill in the art appreciates that many internucleotidic linkages, such as natural phosphate linkages, exist as salt forms when at physiological pH, in many buffers (e.g., PBS buffers having a pH around 7, e.g., PH 7.4), etc.). In some embodiments, the present disclosure provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages having the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, III, etc., or a salt form thereof, and one or more natural phosphate linkages. In some embodiments, the present disclosure 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 designed diastereomerically pure internucleotidic linkages with respect to the chiral linkage phosphorus.

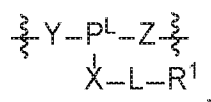
[00329] In some embodiments, an oligonucleotide of the present disclosure comprises at least one internucleotidic linkage, e.g., a modified (non-natural) internucleotidic linkage (e.g., non-negatively charged internucleotidic linkage) within or at the terminus (e.g. 5' or 3') of the oligonucleotide. In some embodiments, an oligonucleotide comprises a P-modification moiety within or at the terminus (e.g. 5' or 3') of the oligonucleotide.

[00330] In some embodiments, an oligonucleotide of the present disclosure comprises at least one chirally controlled internucleotidic linkage within the oligonucleotide. In some embodiments, an

oligonucleotide of the present disclosure comprises at least one chirally controlled internucleotidic linkage within the oligonucleotide, and at least one natural phosphate linkage. In some embodiments, an oligonucleotide of the present disclosure comprises at least one chirally controlled internucleotidic linkage within the oligonucleotide, at least one natural phosphate linkage, and at least one phosphorothioate internucleotidic linkage. In some embodiments, an oligonucleotide of the present disclosure comprises at least one chirally controlled internucleotidic linkage within the oligonucleotide, and at least one phosphorothioate triester internucleotidic linkage. In some embodiments, an oligonucleotide of the present disclosure comprises at least one chirally controlled internucleotidic linkage within the oligonucleotide, at least one natural phosphate linkage, and at least one phosphorothioate triester internucleotidic linkage.

[00331] In some embodiments, an oligonucleotide of the present disclosure comprises at least two chirally controlled internucleotidic linkages within the oligonucleotide that have different stereochemistry and/or different P-modifications relative to one another. In some embodiments, such at least two internucleotidic linkages have different stereochemistry. In some embodiments, such at least two internucleotidic linkages have different P-modifications. In some embodiments, an oligonucleotide of the present disclosure comprises at least two chirally controlled internucleotidic linkages within the oligonucleotide that have different P-modifications relative to one another, and at least one natural phosphate linkage. In some embodiments, an oligonucleotide of the present disclosure comprises at least two chirally controlled internucleotidic linkages within the oligonucleotide that have different P-modifications relative to one another, at least one natural phosphate linkage, and at least one phosphorothioate internucleotidic linkage. In some embodiments, an oligonucleotide of the present disclosure comprises at least two chirally controlled internucleotidic linkages within the oligonucleotide that have different P-modifications relative to one another, and at least one phosphorothioate triester internucleotidic linkage. In some embodiments, an oligonucleotide of the present disclosure comprises at least two chirally controlled internucleotidic linkages within the oligonucleotide that have different P-modifications relative to one another, at least one natural phosphate linkage, and at least one phosphorothioate triester internucleotidic linkage.

[00332] In certain embodiments, an internucleotidic linkage (e.g., a modified (non-natural) internucleotidic linkage when formula I is not a natural phosphate linkage) has the structure of formula I:



I

or a salt form thereof, wherein:

P^L is $P(=W)$, P , or $P \rightarrow B(R')_3$;

W is O , $N(-L-R^5)$, S or Se ;

each of R^1 and R^5 is independently $-H$, $-L-R'$, halogen, $-CN$, $-NO_2$, $-L-Si(R')_3$, $-OR'$, $-SR'$, or $-N(R')_2$;

each of X , Y and Z is independently $-O-$, $-S-$, $-N(-L-R^5)-$, or L ;

each L is independently a covalent bond, or a bivalent, optionally substituted, linear or branched group selected from a C_{1-30} aliphatic group and a C_{1-30} heteroaliphatic group having 1-10 heteroatoms, wherein one or more methylene units are optionally and independently replaced with C_{1-6} alkylene, C_{1-6} alkenylene, $-C \equiv C-$, a bivalent C_1-C_6 heteroaliphatic group having 1-5 heteroatoms, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, $-C(O)O-$, $-P(O)(OR')-$, $-P(O)(SR')-$, $-P(O)(R')-$, $-P(O)(NR')-$, $-P(S)(OR')-$, $-P(S)(SR')-$, $-P(S)(R')-$, $-P(S)(NR')-$, $-P(R')-$, $-P(OR')-$, $-P(SR')-$, $-P(NR')-$, $-P(OR')[B(R')_3]-$, $-OP(O)(OR')O-$, $-OP(O)(SR')O-$, $-OP(O)(R')O-$, $-OP(O)(NR')O-$, $-OP(OR')O-$, $-OP(SR')O-$, $-OP(NR')O-$, $-OP(R')O-$, or $-OP(OR')[B(R')_3]O-$, and one or more CH or carbon atoms are optionally and independently replaced with Cy^L ;

each $-Cy-$ is independently an optionally substituted bivalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each Cy^L is independently an optionally substituted trivalent or tetravalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each R' is independently $-R$, $-C(O)R$, $-C(O)OR$, or $-S(O)_2R$;

each R is independently $-H$, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms, 5-30 membered heteroaryl having 1-10 heteroatoms, and 3-30 membered heterocyclyl having 1-10 heteroatoms, or

two R groups are optionally and independently taken together to form a covalent bond, or

two or more R groups on the same atom are optionally and independently taken together with the atom to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the atom, 0-10 heteroatoms, or

two or more R groups on two or more atoms are optionally and independently taken together with their intervening atoms to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms.

[00333] In some embodiments, a linkage of formula **I** is chiral at the linkage phosphorus (P in P^L).

In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising one or more modified internucleotidic linkages of formula I. In some embodiments, the present disclosure 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 disclosure 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 disclosure 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 disclosure 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. In some embodiments, a chirally controlled oligonucleotide is an oligonucleotide in a provided composition that is of the particular oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide is an oligonucleotide in a provided composition that has the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

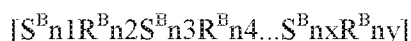
[00334] As extensively described herein, in some embodiments, $-X-L-R^1$ is a moiety useful for oligonucleotide preparation. For example, in some embodiments, $-X-L-R^1$ is $-OCH_2CH_2CN$ (*e.g.*, in non-chirally controlled internucleotidic linkages); in some embodiments, $-X-L-R^1$ is of such a structure that $H-X-L-R^1$ is a chiral auxiliary, optionally capped, as described herein (*e.g.*, DPSE, PSM, *etc.*; particularly in chirally controlled internucleotidic linkages, although may also in non-chirally controlled internucleotidic linkages (*e.g.*, precursors of natural phosphate linkages)).

[0001] In some embodiments, a chirally controlled oligonucleotide is an oligonucleotide in a chirally controlled composition that is of a particular oligonucleotide type, and the chirally controlled oligonucleotide is of the type. In some embodiments, a chirally controlled oligonucleotide is an oligonucleotide in a provided composition that comprises a controlled level of a plurality of oligonucleotides that share 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, and the chirally controlled oligonucleotide shares the common base sequence, the common pattern of backbone linkages, the common pattern of backbone chiral centers, and the common pattern of backbone phosphorus modifications.

[00335] In some embodiments, the present disclosure provides a chirally controlled

oligonucleotide, wherein at least two chirally controlled 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, and/or in that they have different $-XLR^1$ moieties.

[00336] In some embodiments, the present disclosure 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:



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 n_1 - n_y 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 n_1 - n_y 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.

[00337] 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.

[00338] 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.

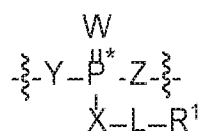
[00339] In some embodiments, at least two skip-adjacent ns are equal to one another, so that a provided oligonucleotide includes at least two blocks of linkages of a first stereochemistry that are equal in length to one another and are separated by a block of linkages of the other stereochemistry, which separating block may be of the same length or a different length from the blocks of first stereochemistry.

[00340] 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.

[00341] In some embodiments, a provided oligonucleotide of formula $[S^B_n1R^B_n2S^B_n3R^B_n4...S^B_{nx}R^B_{ny}]$ is a stereoblockmer. In some embodiments, a provided oligonucleotide of formula $[S^B_n1R^B_n2S^B_n3R^B_n4...S^B_{nx}R^B_{ny}]$ is a stereoskipmer. In some embodiments, a provided oligonucleotide of formula $[S^B_n1R^B_n2S^B_n3R^B_n4...S^B_{nx}R^B_{ny}]$ is a stereoaltmer. In some embodiments, a provided oligonucleotide of formula $[S^B_n1R^B_n2S^B_n3R^B_n4...S^B_{nx}R^B_{ny}]$ is a gapmer.

[00342] In some embodiments, a provided oligonucleotide of formula $[S^B_n1R^B_n2S^B_n3R^B_n4...S^B_{nx}R^B_{ny}]$ 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 $[S^B_n1R^B_n2S^B_n3R^B_n4...S^B_{nx}R^B_{ny}]$ and is a stereoskipmer and P-modification skipmer. In some embodiments, a provided oligonucleotide of formula $[S^B_n1R^B_n2S^B_n3R^B_n4...S^B_{nx}R^B_{ny}]$ and is a stereoblockmer and P-modification altmer. In some embodiments, a provided oligonucleotide of formula $[S^B_n1R^B_n2S^B_n3R^B_n4...S^B_{nx}R^B_{ny}]$ and is a stereoaltmer and P-modification blockmer.

[00343] In some embodiments, an internucleotidic linkage of formula **I** has the structure of:



wherein:

P* is an asymmetric phosphorus atom and is either *Rp* or *Sp*;

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 C_1-C_6 alkylene, C_1-C_6 alkenylene, $-C\equiv C-$, a C_1-C_6 heteroaliphatic moiety, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, $-SC(O)-$, $-C(O)S-$, $-OC(O)-$, and $-C(O)O-$;

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 C_1 - C_6 alkylene, C_1 - C_6 alkenylene, $—C\equiv C—$, a C_1 - C_6 heteroaliphatic moiety, $—C(R')_2—$, $—Cy—$, $—O—$, $—S—$, $—S—S—$, $—N(R')—$, $—C(O)—$, $—C(S)—$, $—C(NR')—$, $—C(O)N(R')—$, $—N(R')C(O)N(R')—$, $—N(R')C(O)—$, $—N(R')C(O)O—$, $—OC(O)N(R')—$, $—S(O)—$, $—S(O)_2—$, $—S(O)_2N(R')—$, $—N(R')S(O)_2—$, $—SC(O)—$, $—C(O)S—$, $—OC(O)—$, and $—C(O)O—$;

each R' is independently $—R$, $—C(O)R$, $—CO_2R$, or $—SO_2R$, or:

two R' 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, and heterocyclylene;

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

each $\text{---}\frac{3}{\text{---}}\text{---}$ independently represents a connection to a nucleoside.

[00344] In some embodiments, 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, $—C\equiv C—$, $—C(R')_2—$, $—Cy—$, $—O—$, $—S—$, $—S—S—$, $—N(R')—$, $—C(O)—$, $—C(S)—$, $—C(NR')—$, $—C(O)N(R')—$, $—N(R')C(O)N(R')—$, $—N(R')C(O)—$, $—N(R')C(O)O—$, $—OC(O)N(R')—$, $—S(O)—$, $—S(O)_2—$, $—S(O)_2N(R')—$, $—N(R')S(O)_2—$, $—SC(O)—$, $—C(O)S—$, $—OC(O)—$, or $—C(O)O—$;

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, $—C\equiv C—$, $—C(R')_2—$, $—Cy—$, $—O—$, $—S—$, $—S—S—$, $—N(R')—$, $—C(O)—$, $—C(S)—$, $—C(NR')—$, $—C(O)N(R')—$, $—N(R')C(O)N(R')—$, $—N(R')C(O)—$, $—N(R')C(O)O—$, $—OC(O)N(R')—$, $—S(O)—$, $—S(O)_2—$, $—S(O)_2N(R')—$, $—N(R')S(O)_2—$, $—SC(O)—$, $—C(O)S—$, $—OC(O)—$, or $—C(O)O—$;

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

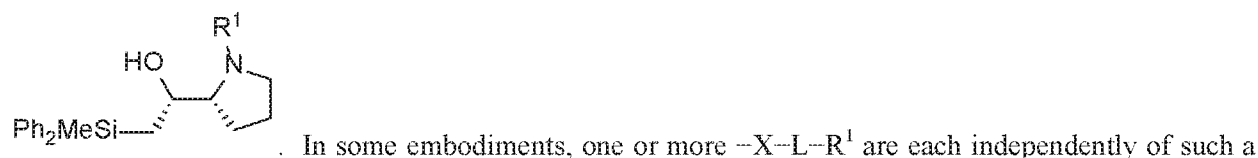
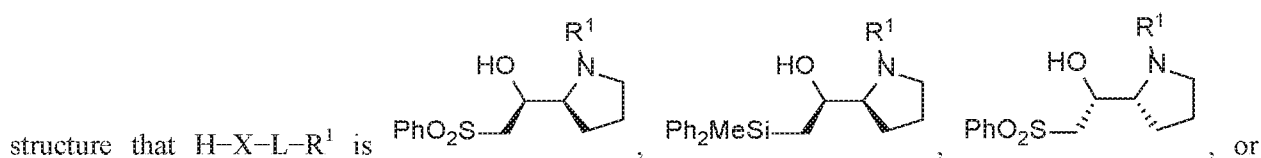
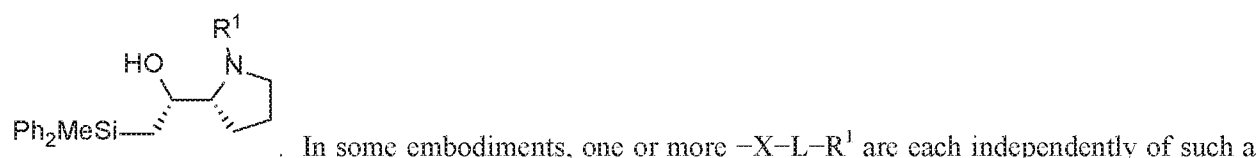
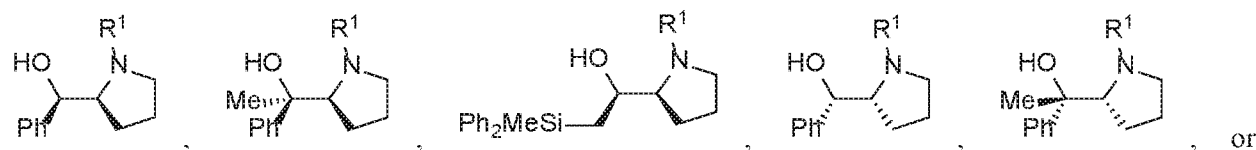
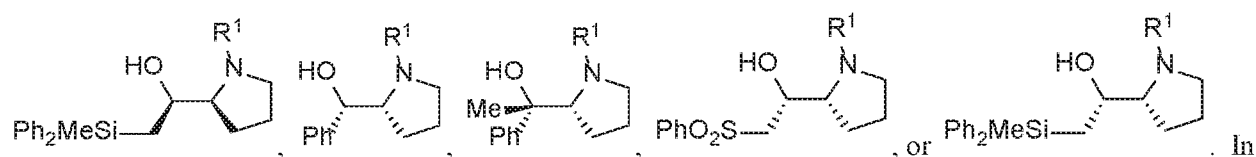
each $\text{---}\overline{\text{Z}}\text{---}$ independently represents a connection to a nucleoside.

[00345] In some embodiments, a chirally controlled oligonucleotide comprises one or more modified internucleotidic linkages. In some embodiments, a chirally controlled oligonucleotide comprises, *e.g.*, a phosphorothioate or a phosphorothioate triester internucleotidic linkage. In some embodiments, a chirally controlled oligonucleotide comprises a chirally controlled phosphorothioate triester linkage. In some embodiments, a chirally controlled oligonucleotide comprises 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, or 25 chirally controlled phosphorothioate triester internucleotidic linkages. In some embodiments, a chirally controlled oligonucleotide comprises 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, or 25 chirally controlled phosphorothioate internucleotidic linkages ($\text{---O---P(O)(SH)---O---}$ or salt forms thereof).

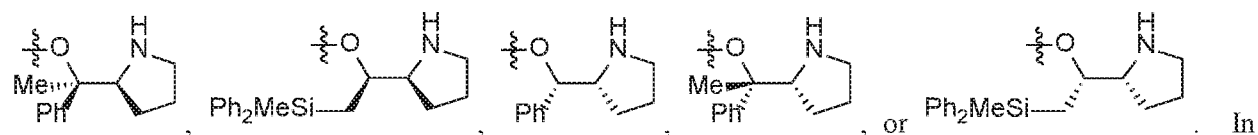
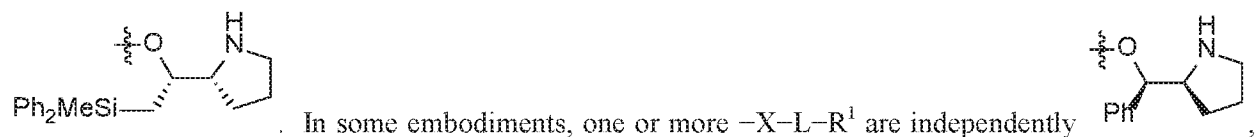
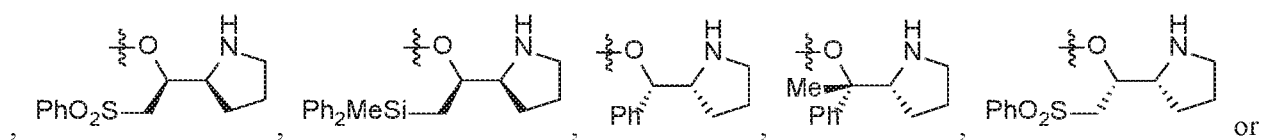
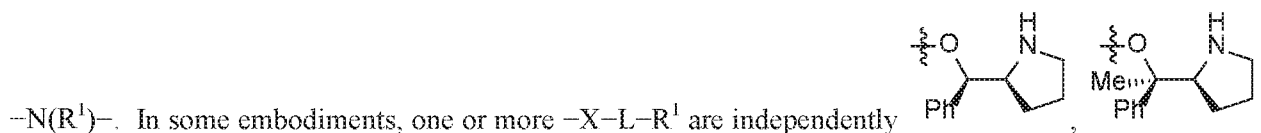
[00346] In some embodiments, an oligonucleotide comprises different types of internucleotidic phosphorus linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one natural phosphate linkage and at least one modified (non-natural) internucleotidic linkage. In some embodiments, an oligonucleotide comprises at least one natural phosphate linkage and at least one phosphorothioate. In some embodiments, an oligonucleotide comprises at least one non-negatively charged internucleotidic linkage. In some embodiments, an oligonucleotide comprises at least one natural phosphate linkage and at least one non-negatively charged internucleotidic linkage. In some embodiments, an oligonucleotide comprises at least one phosphorothioate internucleotidic linkage and at least one non-negatively charged internucleotidic linkage. In some embodiments, an oligonucleotide comprises at least one phosphorothioate internucleotidic linkage, at least one natural phosphate linkage, and at least one non-negatively charged internucleotidic linkage.

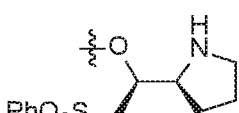
[00347] In some embodiments, an internucleotidic linkage comprises a chiral auxiliary. In some embodiments, an internucleotidic linkage of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, etc., comprises a chiral auxiliary, wherein P^{L} is $\text{P}=\text{S}$. In some embodiments, an internucleotidic linkage of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, etc., comprises a chiral auxiliary, wherein P^{L} is $\text{P}=\text{O}$. 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. Example chiral auxiliaries that can be utilized in accordance with the present disclosure include those described in US 9394333, US 9744183, US 9605019, US 20130178612, US 20150211006, US 9598458, US 20170037399, WO 2017/015555, WO 2017/062862, WO 2018/237194, WO 2019/055951, the chiral auxiliaries of each of which is incorporated

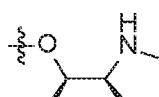
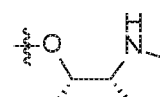
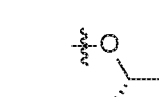
independently of such a structure that H-X-L-R¹ is



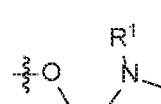
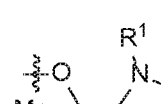
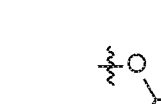
structure that $H-X-L-R^1$ is a compound selected from Tables CA-1, CA-2, CA-3, CA-4, CA-5, CA-6, CA-7, CA-8, CA-9, CA-10, CA-11, CA-12, or CA-13, or a related (having the same constitution) diastereomer or enantiomer thereof, wherein the $-NH-$ of the 5-membered pyrrolidinyl is replaced with

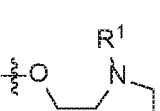
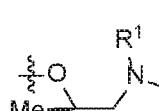
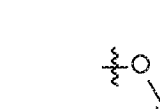
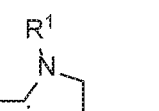


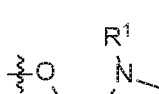
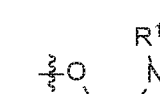
some embodiments, one or more $-X-L-R^1$ are independently ,

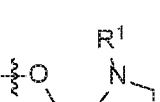
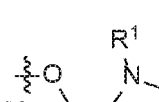
,  or . In some embodiments, one or

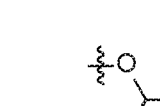
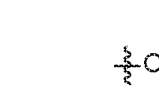
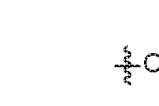
more $-X-L-R^1$ are each independently of such a structure that $H-X-L-R^1$ is a compound selected from Tables CA-1, CA-2, CA-3, CA-4, CA-5, CA-6, CA-7, CA-8, CA-9, CA-10, CA-11, CA-12, or CA-13, or a related (having the same constitution) diastereomer or enantiomer thereof, wherein the connection to the linkage phosphorus is through the alcohol hydroxyl group. In some embodiments, one or more $-X-L-R^1$

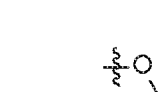
are independently , , , ,

, ,  or . In some embodiments,

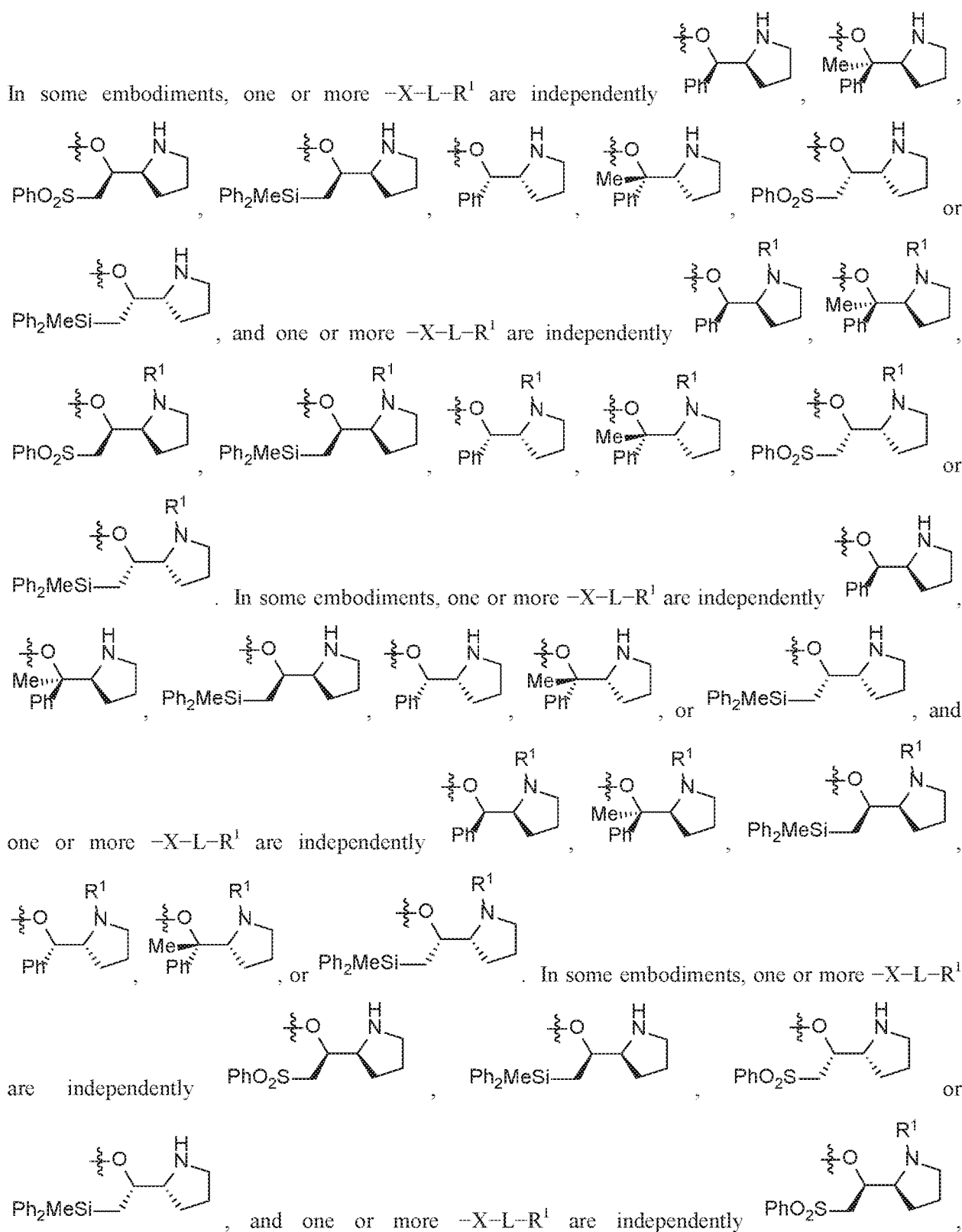
one or more $-X-L-R^1$ are independently , , ,

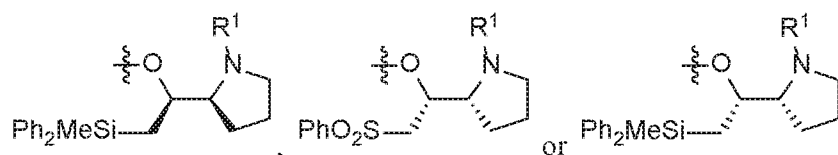
,  or . In some embodiments, one or more $-X-L-R^1$

are independently , ,  or

. In some embodiments, one or more $-X-L-R^1$ are each independently of such a

structure that $H-X-L-R^1$ is a compound selected from Tables CA-1, CA-2, CA-3, CA-4, CA-5, CA-6, CA-7, CA-8, CA-9, CA-10, CA-11, CA-12, or CA-13, or a related (having the same constitution) diastereomer or enantiomer thereof, wherein the $-NH-$ of the 5-membered pyrrolidinyl is replaced with $-N(R^1)-$, and wherein the connection to the linkage phosphorus is through the alcohol hydroxyl group.





In some embodiments, R^1 is a capping group utilized in oligonucleotide synthesis. In some embodiments, R^1 is $-C(O)-R'$. In some embodiments, R^1 is $-C(O)-R'$, wherein R' is optionally substituted C_{1-6} aliphatic. In some embodiments, R^1 is $-C(O)CH_3$.

[00348] In some embodiments, an oligonucleotide, e.g., a chirally controlled oligonucleotide, an oligonucleotide of a plurality, etc. is linked to a solid support. In some embodiments, an oligonucleotide is not linked to a solid support.

[00349] In some embodiments, an oligonucleotide comprises at least one natural phosphate linkage and at least two consecutive chirally controlled modified internucleotidic linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one natural phosphate linkage and at least two consecutive chirally controlled phosphorothioate internucleotidic linkages.

[00350] 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.

[00351] 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.

[00352] In some embodiments, a chirally controlled oligonucleotide is a unimer.

[00353] In some embodiments, in a unimer, all nucleotide units within a strand share at least one common structural feature at the internucleotidic phosphorus linkage. In some embodiments, a common structural feature is a common stereochemistry at the linkage phosphorus or a common modification at the linkage phosphorus. 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.

[00354] In some embodiments, a chirally controlled oligonucleotide is a gapmer.

[00355] In some embodiments, a chirally controlled oligonucleotide is a skipmer.

[00356] In some embodiments, the present disclosure provides oligonucleotides comprising one or more modified internucleotidic linkages independently having the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, III, or a salt form

thereof.

[00357] In some embodiments, L is a covalent bond or an optionally substituted, linear or branched C₁–C₁₀ alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C₁–C₆ alkylene, C₁–C₆ alkenylene, $\text{---C}\equiv\text{C---}$, $\text{---C(R')}_2\text{---}$, ---Cy--- , ---O--- , ---S--- , ---S---S--- , ---N(R')--- , ---C(O)--- , ---C(S)--- , ---C(NR')--- , ---C(O)N(R')--- , $\text{---N(R')C(O)N(R')---}$, ---N(R')C(O)--- , ---N(R')C(O)O--- , ---OC(O)N(R')--- , ---S(O)--- , $\text{---S(O)}_2\text{---}$, $\text{---S(O)}_2\text{N(R')---}$, $\text{---N(R')S(O)}_2\text{---}$, ---SC(O)--- , ---C(O)S--- , ---OC(O)--- , or ---C(O)O--- ;

R¹ is halogen, R, or an optionally substituted C₁–C₅₀ aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C₁–C₆ alkylene, C₁–C₆ alkenylene, $\text{---C}\equiv\text{C---}$, $\text{---C(R')}_2\text{---}$, ---Cy--- , ---O--- , ---S--- , ---S---S--- , ---N(R')--- , ---C(O)--- , ---C(S)--- , ---C(NR')--- , ---C(O)N(R')--- , $\text{---N(R')C(O)N(R')---}$, ---N(R')C(O)--- , ---N(R')C(O)O--- , ---OC(O)N(R')--- , ---S(O)--- , $\text{---S(O)}_2\text{---}$, $\text{---S(O)}_2\text{N(R')---}$, $\text{---N(R')S(O)}_2\text{---}$, ---SC(O)--- , ---C(O)S--- , ---OC(O)--- , or ---C(O)O--- ;

each R' is independently ---R , ---C(O)R , $\text{---CO}_2\text{R}$, or $\text{---SO}_2\text{R}$, 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 C₁–C₆ aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl; and

each $\text{---}\overset{\text{3}}{\text{S}}\text{---}$ independently represents a connection to a nucleoside.

[00358] 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. Example modified internucleotidic phosphorus linkages are described further herein. 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.

[00359] In some embodiments, P* is an asymmetric phosphorus atom and is either *Rp* or *Sp*. In some embodiments, P* is *Rp*. In other embodiments, P* is *Sp*. In some embodiments, an oligonucleotide comprises one or more internucleotidic linkages of formula **I** wherein each P* is independently *Rp* or *Sp*. In some embodiments, an oligonucleotide comprises one or more internucleotidic linkages of formula **I** wherein each P* is *Rp*. In some embodiments, an oligonucleotide comprises one or more internucleotidic linkages of formula **I** wherein each P* is *Sp*. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein P* is *Rp*. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein P* is *Sp*. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein P* is *Rp*, and at least one internucleotidic linkage of formula **I** wherein P* is *Sp*.

[00360] In some embodiments, 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.

[00361] 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.

[00362] 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, $-\text{C}\equiv\text{C}-$, $-\text{C}(\text{R}')_2-$, $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{N}(\text{R}')-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{S}(\text{O})_2-$, $-\text{SC}(\text{O})-$, $-\text{C}(\text{O})\text{S}-$, $-\text{OC}(\text{O})-$, or $-\text{C}(\text{O})\text{O}-$.

[00363] In some embodiments, X is $-\text{N}(-\text{L}-\text{R}^1)-$. In some embodiments, X is $-\text{N}(\text{R}^1)-$. In some embodiments, X is $-\text{N}(\text{R}')-$. In some embodiments, X is $-\text{N}(\text{R})-$. In some embodiments, X is $-\text{NH}-$.

[00364] 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, $-\text{C}\equiv\text{C}-$, $-\text{C}(\text{R}')_2-$, $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{N}(\text{R}')-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{S}(\text{O})_2-$, $-\text{SC}(\text{O})-$, $-\text{C}(\text{O})\text{S}-$, $-\text{OC}(\text{O})-$, or $-\text{C}(\text{O})\text{O}-$. 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.

[00365] In some embodiments, Y is $-\text{O}-$. In some embodiments, Y is $-\text{S}-$.

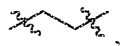
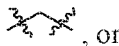
[00366] In some embodiments, Y is $-\text{N}(-\text{L}-\text{R}^1)-$. In some embodiments, Y is $-\text{N}(\text{R}^1)-$. In some embodiments, Y is $-\text{N}(\text{R}')-$. In some embodiments, Y is $-\text{N}(\text{R})-$. In some embodiments, Y is $-\text{NH}-$.

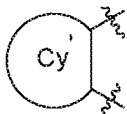
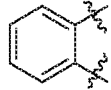
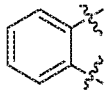
[00367] In some embodiments, Y is L. In some embodiments, Y is a covalent bond. In some embodiments, Y is or an optionally substituted, linear or branched C_1 - C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1 - C_6 alkylene, C_1 - C_6 alkenylene, $-\text{C}\equiv\text{C}-$, $-\text{C}(\text{R}')_2-$, $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{N}(\text{R}')-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{S}(\text{O})_2-$, $-\text{SC}(\text{O})-$, $-\text{C}(\text{O})\text{S}-$, $-\text{OC}(\text{O})-$, or $-\text{C}(\text{O})\text{O}-$. 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.

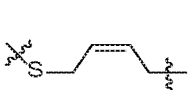
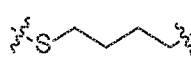
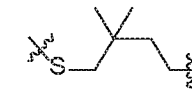
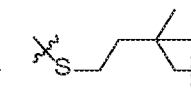
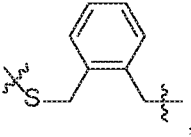
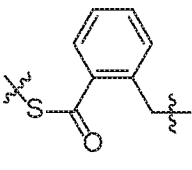
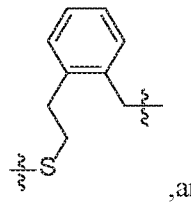
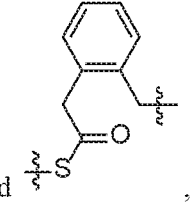
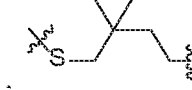
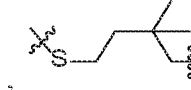
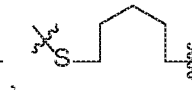
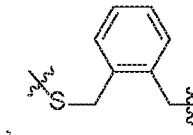
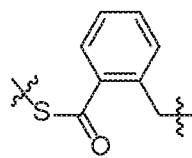
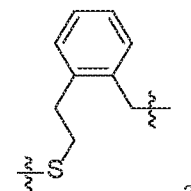
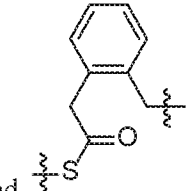
[00368] In some embodiments, Z is $-\text{O}-$. In some embodiments, Z is $-\text{S}-$.

[00369] In some embodiments, Z is $-\text{N}(-\text{L}-\text{R}^1)-$. In some embodiments, Z is $-\text{N}(\text{R}^1)-$. In some embodiments, Z is $-\text{N}(\text{R}')-$. In some embodiments, Z is $-\text{N}(\text{R})-$. In some embodiments, Z is $-\text{NH}-$.

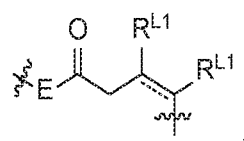
[00370] In some embodiments, Z is L. In some embodiments, Z is a covalent bond. In some embodiments, Z is or an optionally substituted, linear or branched C_1 - C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1 - C_6 alkylene, C_1 - C_6 alkenylene, $-\text{C}\equiv\text{C}-$, $-\text{C}(\text{R}')_2-$, $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{N}(\text{R}')-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{S}(\text{O})_2-$, $-\text{SC}(\text{O})-$, $-\text{C}(\text{O})\text{S}-$, $-\text{OC}(\text{O})-$, or $-\text{C}(\text{O})\text{O}-$. In some

[00374] In some embodiments, L^1 is , , , , , , or .

[00375] 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 , , or .

[00376] 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.

[00377] In some embodiments, L has the structure of:



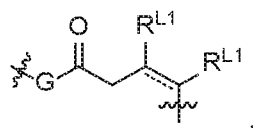
wherein:

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.

[00378] In some embodiments, L has the structure of:



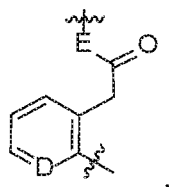
wherein:

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.

[00379] In some embodiments, L has the structure of:



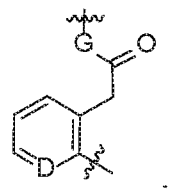
wherein:

E is $-O-$, $-S-$, $-NR'$ or $-C(R')_2-$;

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

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

[00380] In some embodiments, L has the structure of:

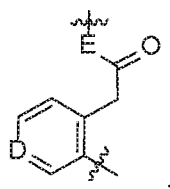


wherein:

G is $-O-$, $-S-$, or $-NR'$;

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

[00381] In some embodiments, L has the structure of:



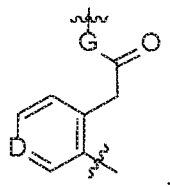
wherein:

E is $-\text{O}-$, $-\text{S}-$, $-\text{NR}'-$ or $-\text{C}(\text{R}')_2-$;

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

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

[00382] In some embodiments, L has the structure of:

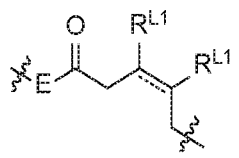


wherein:

G is $-\text{O}-$, $-\text{S}-$, or $-\text{NR}'$;

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

[00383] In some embodiments, L has the structure of:



wherein:

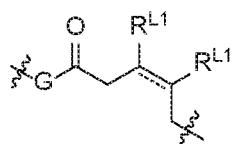
E is $-\text{O}-$, $-\text{S}-$, $-\text{NR}'-$ or $-\text{C}(\text{R}')_2-$;

==== 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.

[00384] In some embodiments, L has the structure of:



wherein:

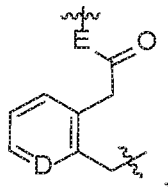
G is $-O-$, $-S-$, or $-NR'$;

— is a single or double bond;

the two R^{LI} 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.

[00385] In some embodiments, L has the structure of:



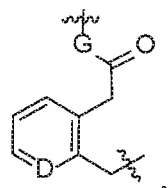
wherein:

E is $-O-$, $-S-$, $-NR'$ or $-C(R')_2-$;

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

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

[00386] In some embodiments, L has the structure of:



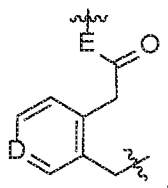
wherein:

G is $-O-$, $-S-$, or $-NR'$;

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

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

[00387] In some embodiments, L has the structure of:



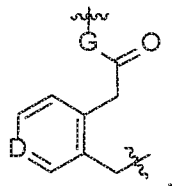
wherein:

E is $-O-$, $-S-$, $-NR'$ or $-C(R')_2-$;

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

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

[00388] In some embodiments, L has the structure of:



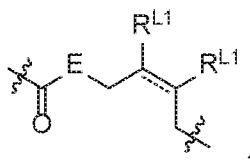
wherein:

G is -O-, -S-, or -NR';

D is =N-, =C(F)-, =C(Cl)-, =C(Br)-, =C(I)-, =C(CN)-, =C(NO₂)-, =C(CO₂-(C₁-C₆ aliphatic))-, or =C(CF₃)-; and

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

[00389] In some embodiments, L has the structure of:



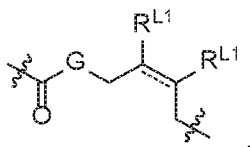
wherein:

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, C₃-C₁₀ carbocyclic, heteroaryl or heterocyclic ring; and each R' is independently as defined above and described herein.

[00390] In some embodiments, L has the structure of:



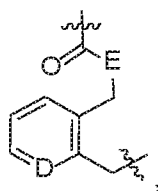
wherein:

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, C₃-C₁₀ carbocyclic, heteroaryl or heterocyclic ring; and each R' is independently as defined above and described herein.

[00391] In some embodiments, L has the structure of:



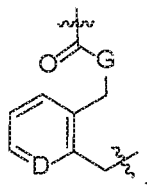
wherein:

E is $-\text{O}-$, $-\text{S}-$, $-\text{NR}'-$ or $-\text{C}(\text{R}')_2-$;

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

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

[00392] In some embodiments, L has the structure of:



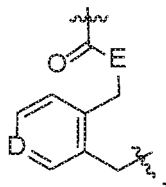
wherein:

G is $-\text{O}-$, $-\text{S}-$, or $-\text{NR}'$;

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

R' is as defined above and described herein.

[00393] In some embodiments, L has the structure of:



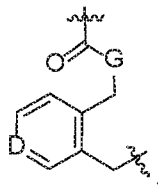
wherein:

E is $-\text{O}-$, $-\text{S}-$, $-\text{NR}'-$ or $-\text{C}(\text{R}')_2-$;

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

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

[00394] In some embodiments, L has the structure of:



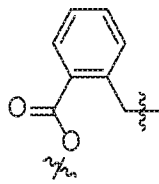
wherein:

G is $-O-$, $-S-$, or $-NR^*$;

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

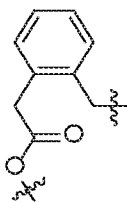
R^* is as defined above and described herein.

[00395] In some embodiments, L has the structure of:



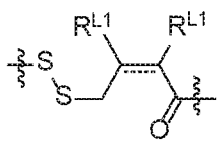
wherein the phenyl ring is optionally substituted. In some embodiments, the phenyl ring is not substituted. In some embodiments, the phenyl ring is substituted.

[00396] 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.

[00397] In some embodiments, L has the structure of:

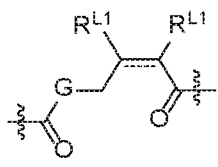


wherein:

— 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.

[00398] In some embodiments, L has the structure of:



wherein:

G is $-\text{O}-$, $-\text{S}-$, or $-\text{NR}'$;

— is a single or double bond; and

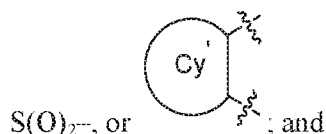
the two R^{L1} are taken together with the two carbon atoms to which they are bound to form an optionally substituted aryl, C_3 - C_{10} carbocyclic, heteroaryl or heterocyclic ring.

[00399] In some embodiments, E is $-\text{O}-$, $-\text{S}-$, $-\text{NR}'$ or $-\text{C}(\text{R}')_2-$, wherein each R' independently as defined above and described herein. In some embodiments, E is $-\text{O}-$, $-\text{S}-$, or $-\text{NR}'$. In some embodiments, E is $-\text{O}-$, $-\text{S}-$, or $-\text{NH}-$. In some embodiments, E is $-\text{O}-$. In some embodiments, E is $-\text{S}-$. In some embodiments, E is $-\text{NH}-$.

[00400] In some embodiments, G is $-\text{O}-$, $-\text{S}-$, or $-\text{NR}'$, wherein each R' independently as defined above and described herein. In some embodiments, G is $-\text{O}-$, $-\text{S}-$, or $-\text{NH}-$. In some embodiments, G is $-\text{O}-$. In some embodiments, G is $-\text{S}-$. In some embodiments, G is $-\text{NH}-$.

[00401] In some embodiments, L is $-\text{L}^3-\text{G}-$, wherein:

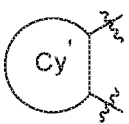
L^3 is an optionally substituted C_1 - C_5 alkylene or alkenylene, wherein one or more methylene units are optionally and independently replaced by $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}')$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$,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



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

[00402] In some embodiments, L is $-\text{L}^3-\text{S}-$, wherein L^3 is as defined above and described herein. In some embodiments, L is $-\text{L}^3-\text{O}-$, wherein L^3 is as defined above and described herein. In some embodiments, L is $-\text{L}^3-\text{N}(\text{R}')$, wherein each of L^3 and R' is independently as defined above and described herein. In some embodiments, L is $-\text{L}^3-\text{NH}-$, wherein each of L^3 and R' is independently as defined above and described herein.

[00403] 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 $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}')$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, 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, $-\text{L}^3-\text{G}-$ is .

[00404] 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 $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}')$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, or

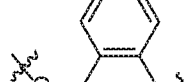
[00405] In some embodiments, $-L^3-G-$ is

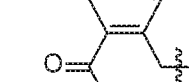


The image shows five chemical structures for the $-L^3-G-$ group, separated by commas. The first structure is a 2,2-dimethyl-1,3-propanedithiol derivative. The second is a 2-phenyl-1,3-propanedithiol derivative. The third is a 2-phenyl-1,3-propanedithiol derivative with a carbonyl group. The fourth is a 2-phenyl-1,3-propanedithiol derivative with a carbonyl group and an ester linkage. The fifth is a 2-phenyl-1,3-propanedithiol derivative with a carbonyl group and an ester linkage, and a methyl group. The word "or" is placed between the fourth and fifth structures.

[00407] In some embodiments, $-L^3-G-$ is







or

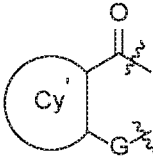






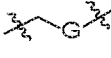
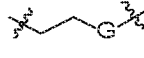
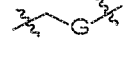
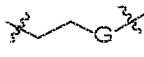
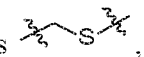
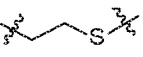
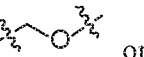
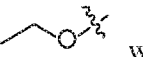
[00409] In some embodiments, L³ is an optionally substituted C₂ alkylene or alkenylene, wherein one or more methylene units are optionally and independently replaced by -O-, -S-, -N(R')-, -C(O)-, -

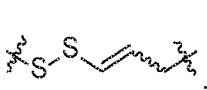
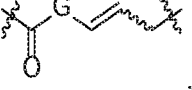
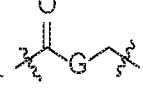
128

[00410] 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

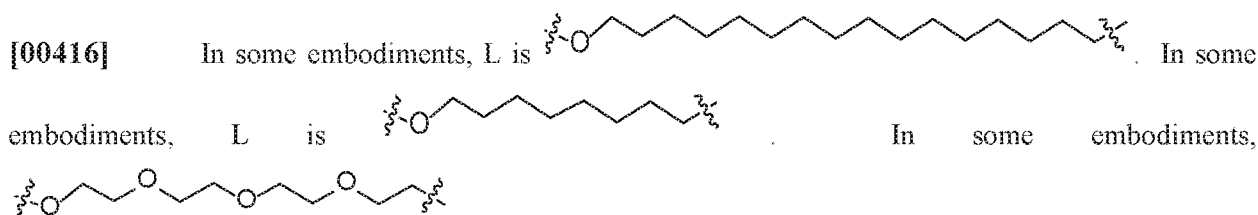
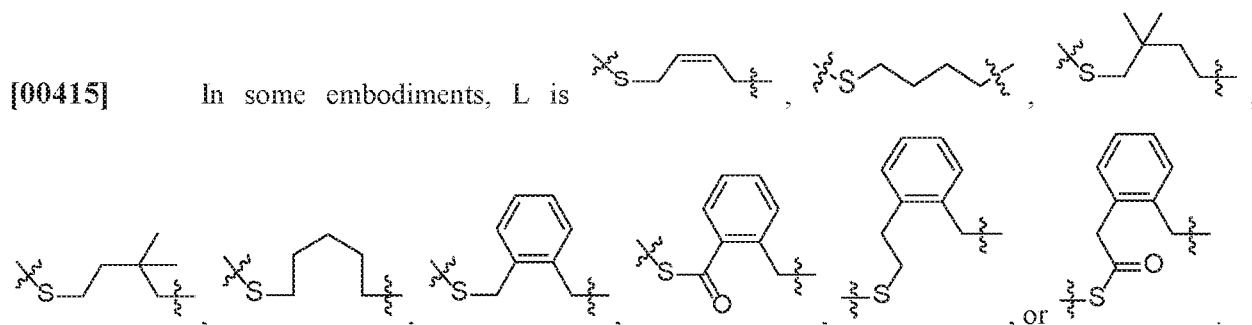
[00411] In some embodiments, L is $-L^4-G-$, wherein L^4 is an optionally substituted C_1-C_2 alkylene; and G is as defined above and described herein. In some embodiments, L is $-L^4-G-$, wherein L^4 is an optionally substituted C_1-C_2 alkylene; G is as defined above and described herein; and G is 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 .

[00412] 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 .

[00413] In some embodiments, L is , or , or , wherein G is as defined above and described herein.

[00414] 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, $-C\equiv C-$, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, $-SC(O)-$, $-C(O)S-$, $-OC(O)-$, or $-C(O)O-$, wherein each 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 $-S-(C_1-C_6 \text{ alkenylene})-$, $-S-(C_1-C_6 \text{ alkylene})-$, $-S-(C_1-C_6 \text{ alkylene})- \text{arylene}-(C_1-C_6 \text{ alkylene})-$, $-S-CO- \text{arylene}-(C_1-C_6 \text{ alkylene})-$, or $-S-CO-(C_1-C_6 \text{ alkylene})- \text{arylene}-(C_1-C_6 \text{ alkylene})-$.



[00417] 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 .

[00418] In some embodiments, 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, $-C\equiv C-$, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, $-SC(O)-$, $-C(O)S-$, $-OC(O)-$, or $-C(O)O-$, wherein each variable is independently as defined above and described herein. In some embodiments, R^1 is 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, $-C\equiv C-$, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, $-SC(O)-$, $-C(O)S-$, $-OC(O)-$, or $-C(O)O-$, wherein each variable is

independently as defined above and described herein.

[00419] 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 $-I$.

[00420] In some embodiments, R^1 is R wherein R is as defined above and described herein.

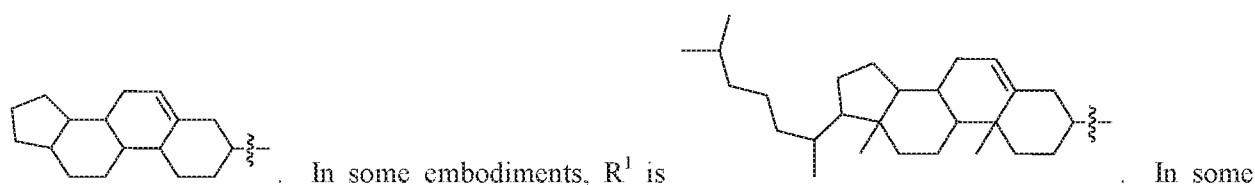
[00421] In some embodiments, R^1 is hydrogen. In some embodiments, R^1 is an optionally substituted group selected from C_1 - C_{50} aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl.

[00422] 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.

[00423] In some embodiments, R^1 is optionally substituted phenyl. In some embodiments, R^1 is substituted phenyl. In some embodiments, R^1 is phenyl.

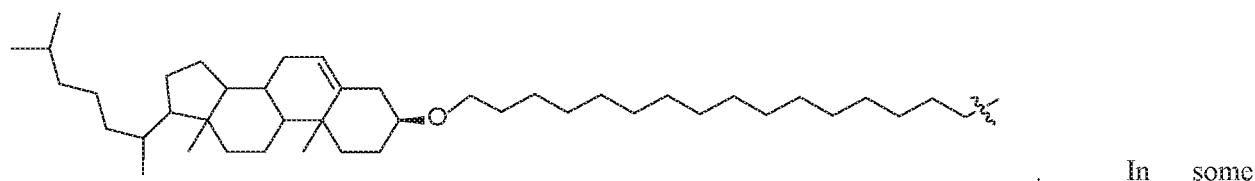
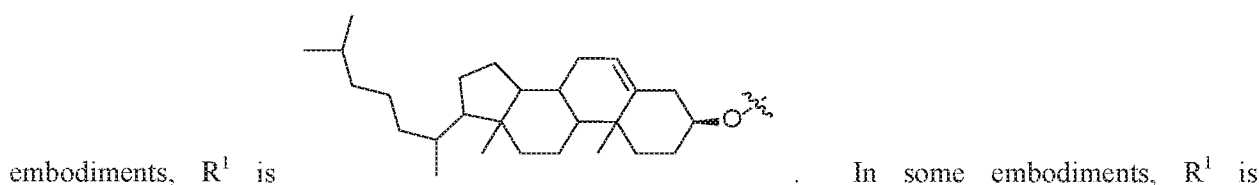
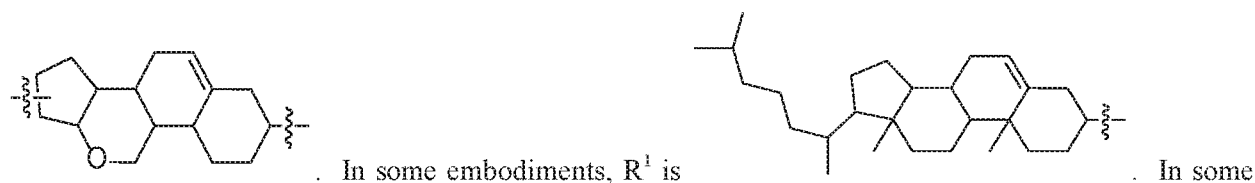
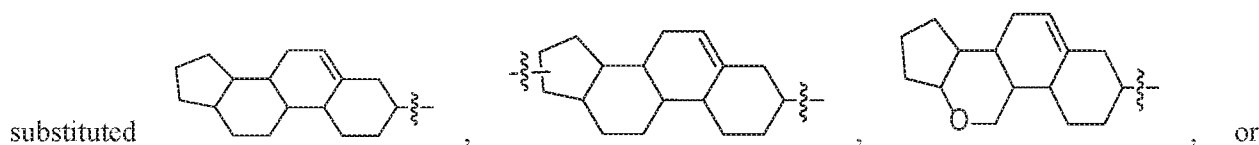
[00424] In some embodiments, R^1 is optionally substituted carbocyclyl. In some embodiments, R^1 is optionally substituted C_3 - C_{10} carbocyclyl. In some embodiments, R^1 is optionally substituted monocyclic carbocyclyl. In some embodiments, R^1 is optionally substituted cycloheptyl. In some embodiments, 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.

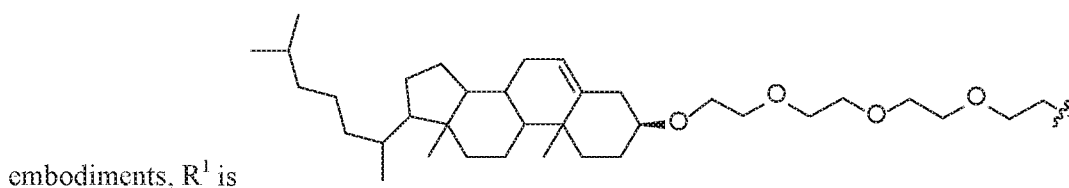
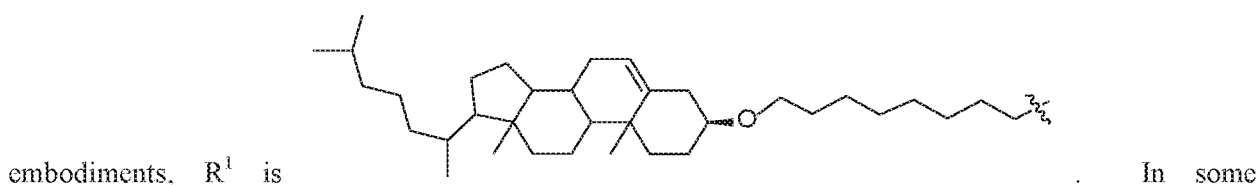
[00425] In some embodiments, R^1 is an optionally substituted C_1 - C_{50} polycyclic hydrocarbon. In some embodiments, R^1 is an optionally substituted C_1 - C_{50} polycyclic hydrocarbon wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1 - 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



embodiments, R¹ is optionally substituted

[00426] In some embodiments, R¹ is an optionally substituted C₁–C₅₀ aliphatic comprising one or more optionally substituted polycyclic hydrocarbon moieties. In some embodiments, R¹ is an optionally substituted C₁–C₅₀ 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 C₁–C₆ alkylene, C₁–C₆ alkenylene, $\text{—C}\equiv\text{C—}$, $\text{—C(R')}_2\text{—}$, —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)— , $\text{—S(O)}_2\text{—}$, $\text{—S(O)}_2\text{N(R')—}$, $\text{—N(R')S(O)}_2\text{—}$, —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, R¹ is an optionally substituted C₁–C₅₀ aliphatic comprising one or more optionally





[00427] In some embodiments, R^1 is an optionally substituted aryl. In some embodiments, R^1 is an optionally substituted bicyclic aryl ring.

[00428] 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.

[00429] 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.

[00430] 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.

[00431] 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. Example R^1 groups include optionally substituted pyrazolyl, imidazolyl, thiazolyl, isothiazolyl, oxazolyl or isoxazolyl.

[00432] 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. Example R^1 groups include optionally substituted pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, or tetrazinyl.

[00433] 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.

[00434] 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.

[00435] 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.

[00436] 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.

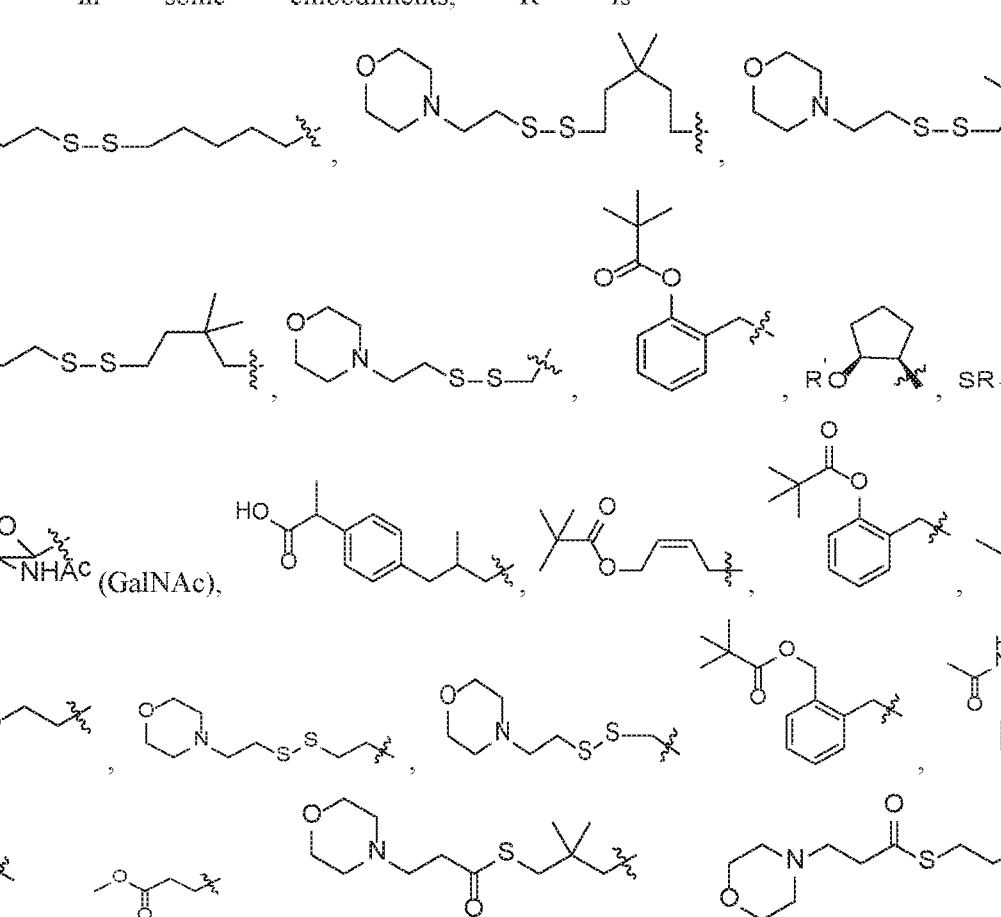
[00437] In certain embodiments, R^1 is a 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R^1 is oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, oxepanyl, aziridineyl, azetidiny, 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, pyrrolidinonyl, 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.

[00438] 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 oxazoliny group.

[00439] 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 indoliny. In some embodiments, R^1 is an optionally substituted isoindoliny. 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.

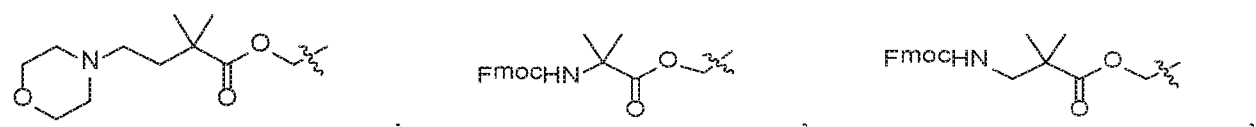
[00440] In some embodiments, R^1 is an optionally substituted C_1 - C_{10} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1 - C_6 alkylene, C_1 - C_6 alkenylene, $\text{---C}\equiv\text{C---}$, $\text{---C(R')}_2\text{---}$, ---Cy--- , ---O--- , ---S--- , ---S---S--- , ---N(R')--- , ---C(O)--- , ---C(S)--- , ---C(NR')--- , ---C(O)N(R')--- , $\text{---N(R')C(O)N(R')---}$, ---N(R')C(O)--- , ---N(R')C(O)O--- , ---OC(O)N(R')--- , ---S(O)--- , $\text{---S(O)}_2\text{---}$, $\text{---S(O)}_2\text{N(R')---}$, $\text{---N(R')S(O)}_2\text{---}$, ---SC(O)--- , ---C(O)S--- , ---OC(O)--- , or ---C(O)O--- , wherein each variable

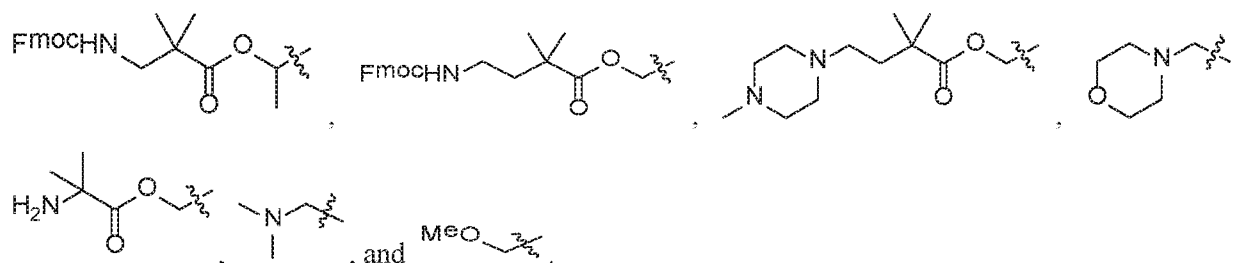
[00441] In some embodiments, R¹ is



The image displays a collection of chemical structures representing various possible R¹ groups. These include:

- Linear alkyl chains (e.g., hexyl, octyl) terminated with a wavy line.
- Alkyl chains with various branching patterns (e.g., isobutyl, 2,2-dimethylpropyl).
- Alkyl chains containing functional groups such as hydroxyl (OH), carboxylic acid (COOH), and amide (NHAc).
- Cycloalkyl groups (e.g., cyclopentyl, cyclohexyl) in both R¹-SR and SR-S¹ configurations.
- Aromatic groups (e.g., phenyl, benzyl) with various substituents.
- Unsaturated chains (e.g., allyl, 4-pentenyl).
- Complex structures including a galactose derivative (GalNAc) and a complex bicyclic system with multiple ester and ether linkages.



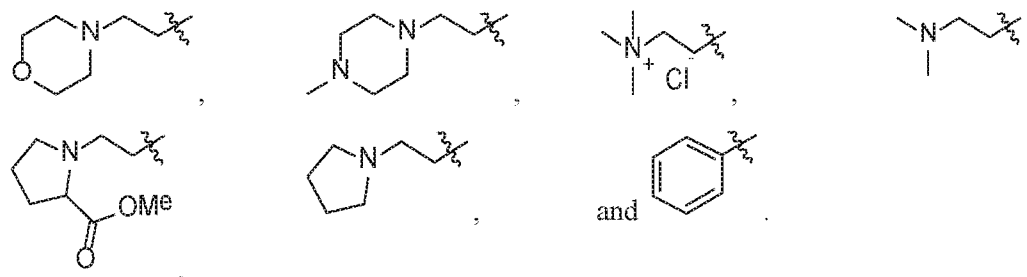


[00445] In some embodiments, R^1 is $-S-R^{L2}$, wherein R^{L2} is an optionally substituted C_1-C_9 aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1-C_6 alkylene, C_1-C_6 alkenylene, $-\text{C}\equiv\text{C}-$, $-\text{C}(\text{R}')_2-$, $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{N}(\text{R}')-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{S}(\text{O})_2-$, $-\text{SC}(\text{O})-$, $-\text{C}(\text{O})\text{S}-$, $-\text{OC}(\text{O})-$, or $-\text{C}(\text{O})\text{O}-$, and each of R' and $-\text{Cy}-$ 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.

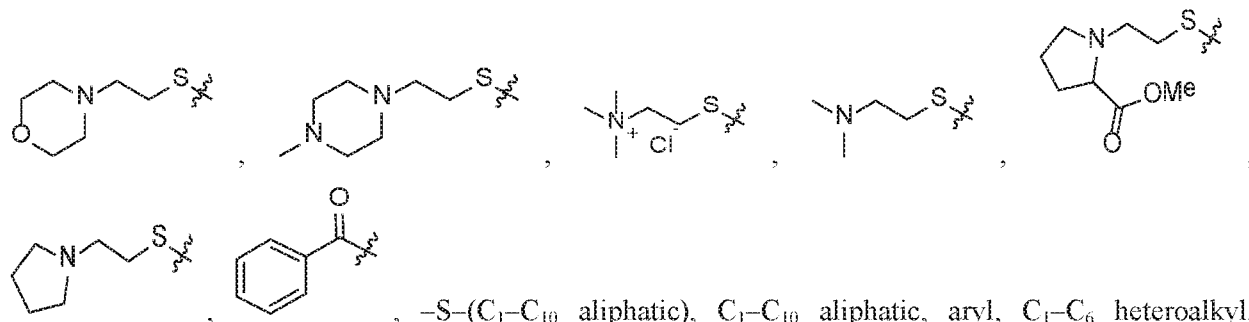
[00446] In some embodiments, R^1 is $-\text{C}(\text{O})-R^{L2}$, wherein R^{L2} is an optionally substituted C_1-C_9 aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1-C_6 alkylene, C_1-C_6 alkenylene, $-\text{C}\equiv\text{C}-$, $-\text{C}(\text{R}')_2-$, $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{N}(\text{R}')-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{S}(\text{O})_2-$, $-\text{SC}(\text{O})-$, $-\text{C}(\text{O})\text{S}-$, $-\text{OC}(\text{O})-$, or $-\text{C}(\text{O})\text{O}-$, and each of R' and $-\text{Cy}-$ is independently as defined above and described herein. In some embodiments, R^1 is $-\text{C}(\text{O})-R^{L2}$, wherein the carbonyl group is connected with G in L group. In some embodiments, R^1 is $-\text{C}(\text{O})-R^{L2}$, wherein the carbonyl group is connected with the sulfur atom in L group.

[00447] 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 $-\text{Cy}-$ or $-\text{C}(\text{O})-$. 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 $-\text{Cy}-$. 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 heterocyclylene. 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

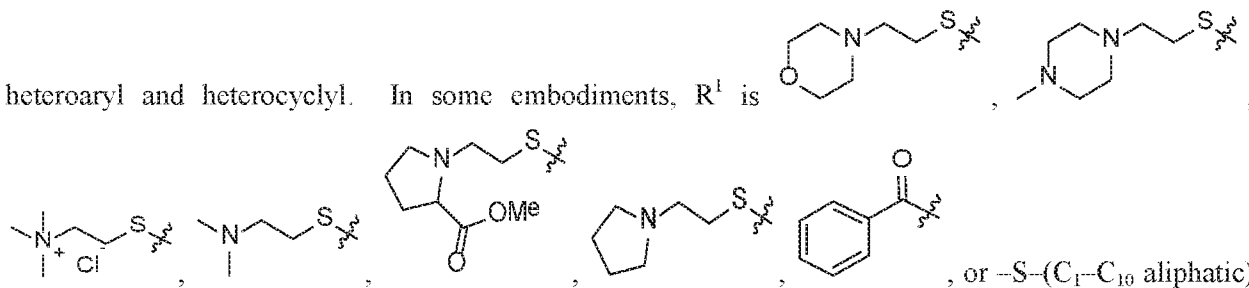
optionally and independently replaced by an optionally substituted C₃-C₁₀ carbocyclylene. In some embodiments, R^{1,2} is an optionally substituted C₁-C₉ aliphatic wherein two methylene units are optionally and independently replaced by -Cy- or -C(O)-. In some embodiments, R^{1,2} is an optionally substituted C₁-C₉ aliphatic wherein two methylene units are optionally and independently replaced by -Cy- or -C(O)-. Example R^{1,2} groups are depicted below:



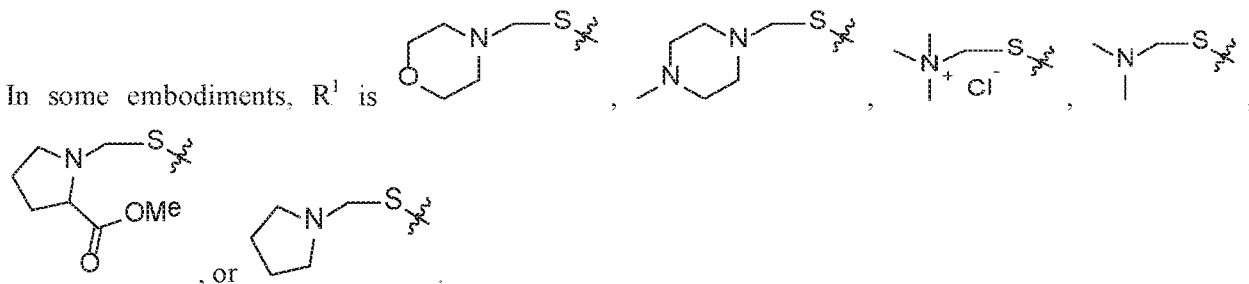
[00448] In some embodiments, R¹ is hydrogen, or an optionally substituted group selected from



heteroaryl and heterocyclyl. In some embodiments, R¹ is



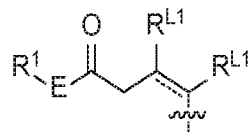
In some embodiments, R¹ is



[00449] In some embodiments, R¹ is an optionally substituted group selected from -S-(C₁-C₆ aliphatic), C₁-C₁₀ aliphatic, C₁-C₆ heteroaliphatic, aryl, heterocyclyl and heteroaryl.

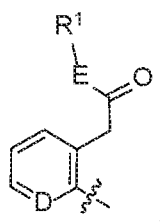
[00458] In some embodiments, $-L-R^1$ is $-R^{L3}-S-S-R^{L2}$, wherein each variable is independently as defined above and described herein. In some embodiments, $-L-R^1$ is $-R^{L3}-C(O)-S-S-R^{L2}$, wherein each variable is independently as defined above and described herein.

[00459] In some embodiments, $-L-R^1$ has the structure of:



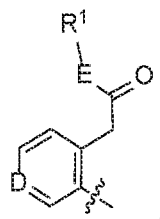
wherein each variable is independently as defined above and described herein.

[00460] In some embodiments, $-L-R^1$ has the structure of:



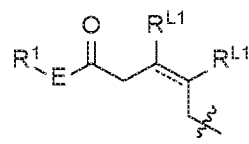
wherein each variable is independently as defined above and described herein.

[00461] In some embodiments, $-L-R^1$ has the structure of:



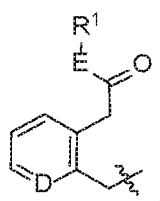
wherein each variable is independently as defined above and described herein.

[00462] In some embodiments, $-L-R^1$ has the structure of:



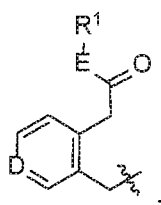
wherein each variable is independently as defined above and described herein.

[00463] In some embodiments, $-L-R^1$ has the structure of:



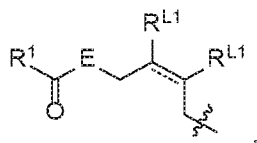
wherein each variable is independently as defined above and described herein.

[00464] In some embodiments, $-L-R^1$ has the structure of:



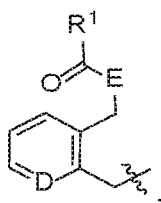
wherein each variable is independently as defined above and described herein.

[00465] In some embodiments, $-L-R^1$ has the structure of:



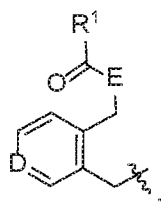
wherein each variable is independently as defined above and described herein.

[00466] In some embodiments, $-L-R^1$ has the structure of:



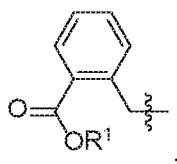
wherein each variable is independently as defined above and described herein.

[00467] In some embodiments, $-L-R^1$ has the structure of:



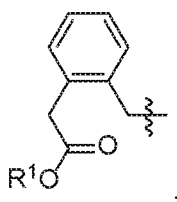
wherein each variable is independently as defined above and described herein.

[00468] In some embodiments, $-L-R^1$ has the structure of:



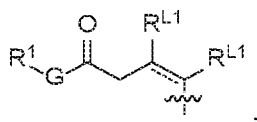
wherein each variable is independently as defined above and described herein.

[00469] In some embodiments, $-L-R^1$ has the structure of:



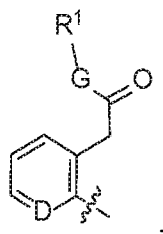
wherein each variable is independently as defined above and described herein.

[00470] In some embodiments, $-L-R^1$ has the structure of:



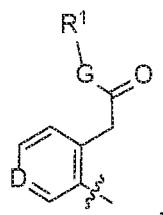
wherein each variable is independently as defined above and described herein.

[00471] In some embodiments, $-L-R^1$ has the structure of:



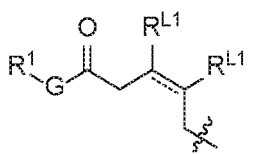
wherein each variable is independently as defined above and described herein.

[00472] In some embodiments, $-L-R^1$ has the structure of:



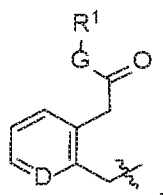
wherein each variable is independently as defined above and described herein.

[00473] In some embodiments, $-L-R^1$ has the structure of:



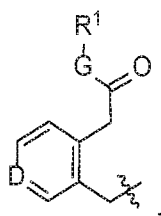
wherein each variable is independently as defined above and described herein.

[00474] In some embodiments, $-L-R^1$ has the structure of:

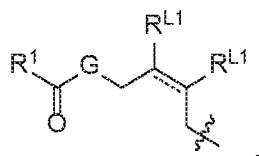


wherein each variable is independently as defined above and described herein.

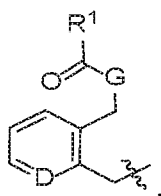
[00475] In some embodiments, $-L-R^1$ has the structure of:



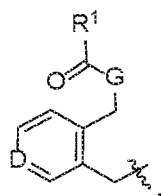
[00476] In some embodiments, $-L-R^1$ has the structure of:



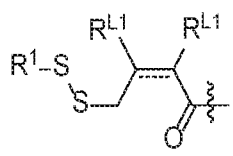
[00477] In some embodiments, $-L-R^1$ has the structure of:



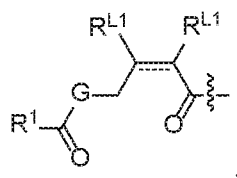
[00478] In some embodiments, $-L-R^1$ has the structure of:



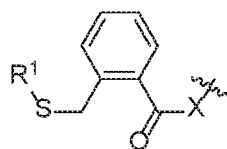
[00479] In some embodiments, $-L-R^1$ has the structure of:



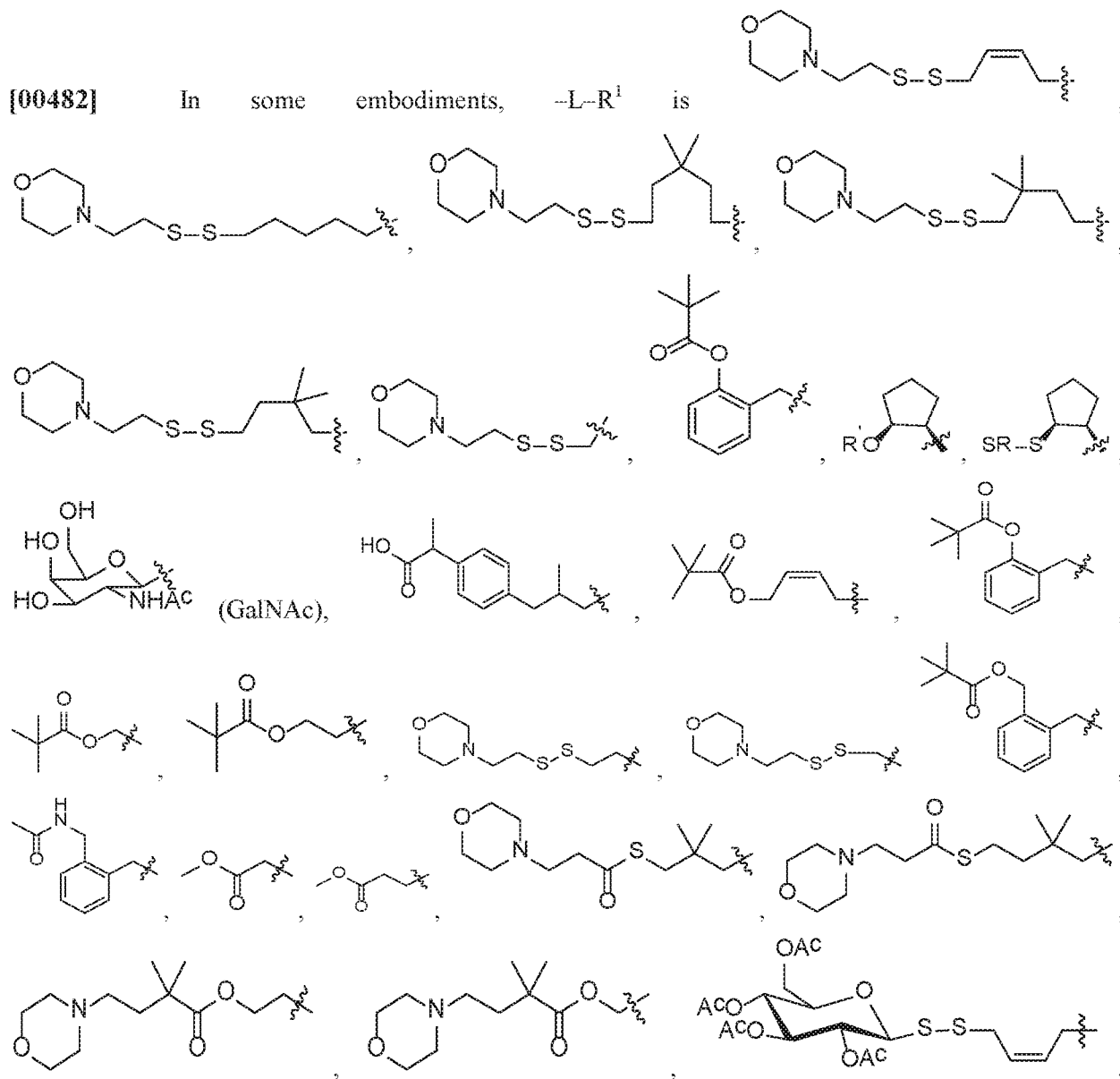
[00480] In some embodiments, L has the structure of:

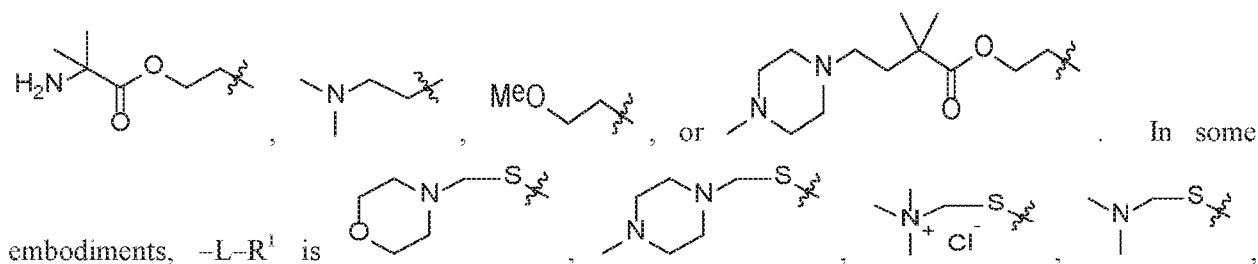
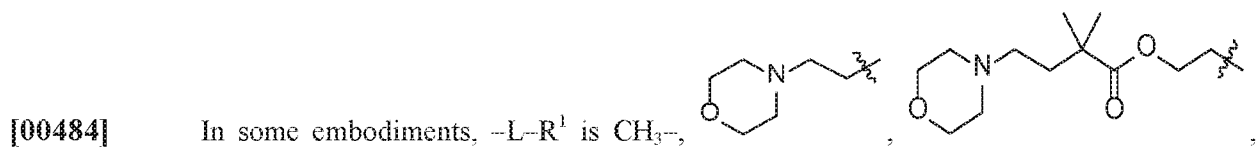
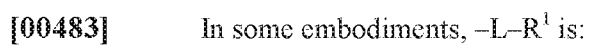


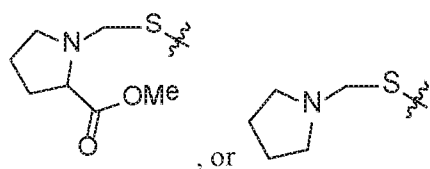
[00481] In some embodiments, $-X-L-R^1$ has the structure of:



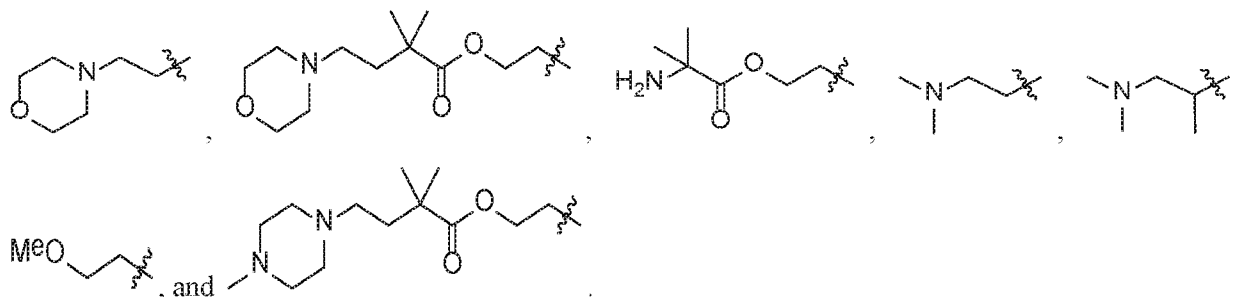
each of R^1 and X is independently as defined above and described herein.



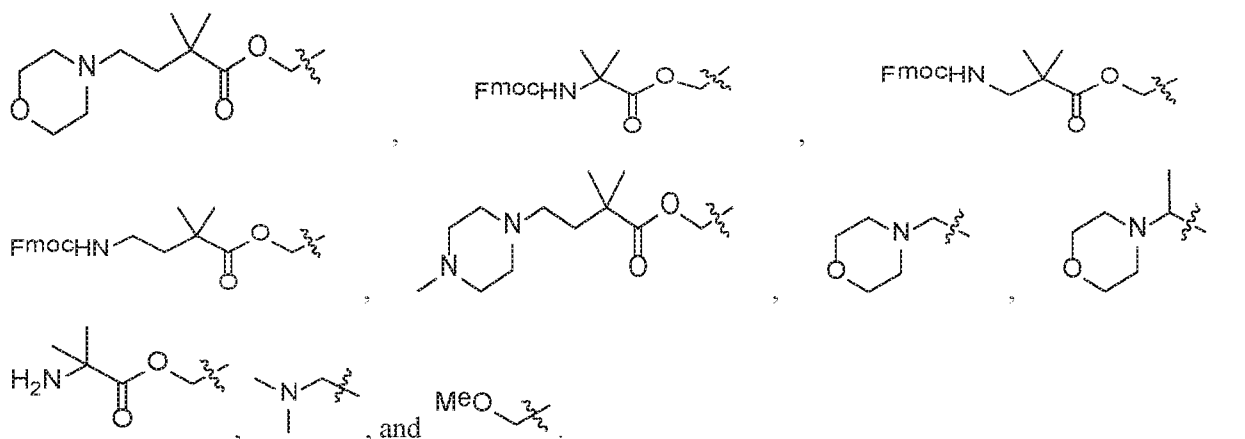




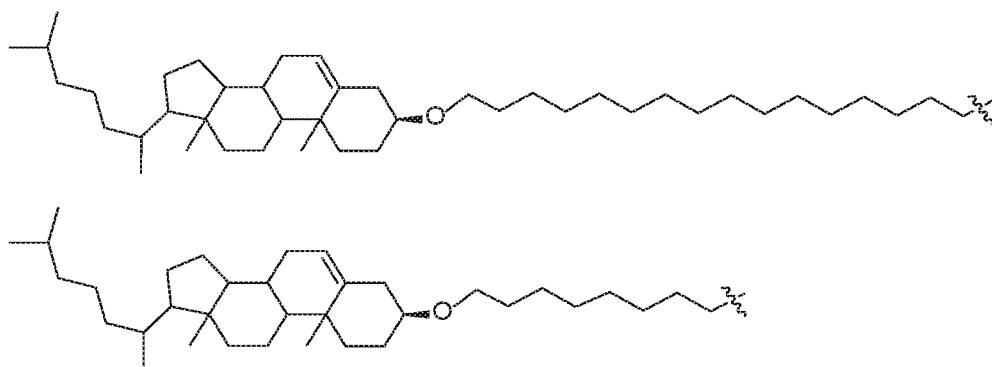
[00485] In some embodiments, $-L-R^1$ comprises a terminal optionally substituted $-(CH_2)_2-$ moiety which is connected to X. In some embodiments, $-L-R^1$ comprises a terminal $-(CH_2)_2-$ moiety which is connected to X. Examples of such $-L-R^1$ moieties are depicted below:



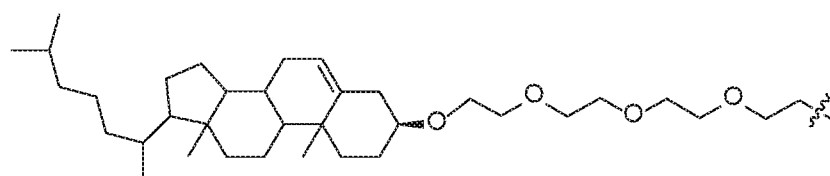
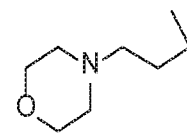
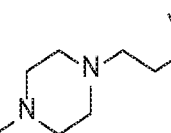
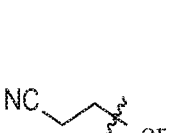
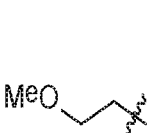
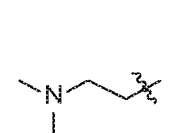
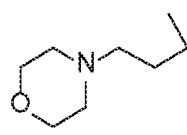
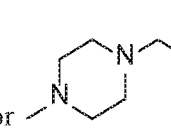
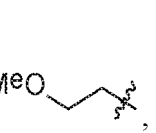
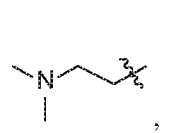
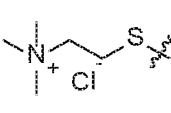
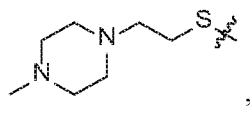
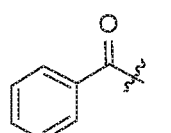
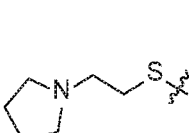
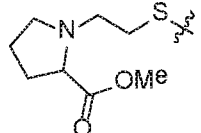
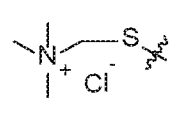
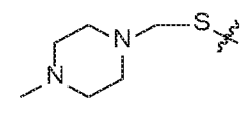
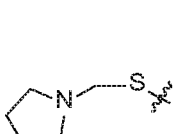
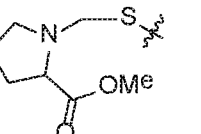
[00486] In some embodiments, $-L-R^1$ comprises a terminal optionally substituted $-(CH_2)-$ moiety which is connected to X. In some embodiments, $-L-R^1$ comprises a terminal $-(CH_2)-$ moiety which is connected to X. Examples of such $-L-R^1$ moieties are depicted below:

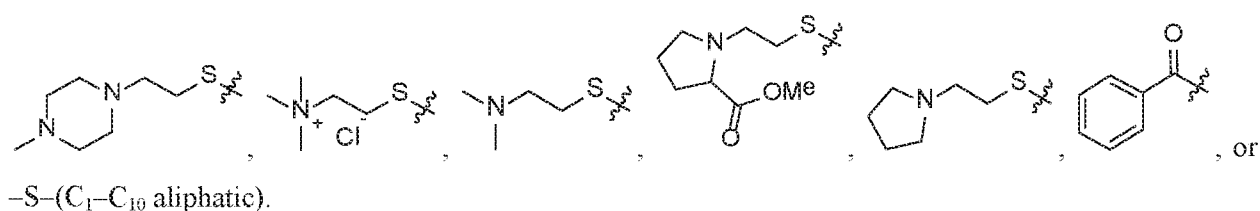


[00487] In some embodiments, $-L-R^1$ is

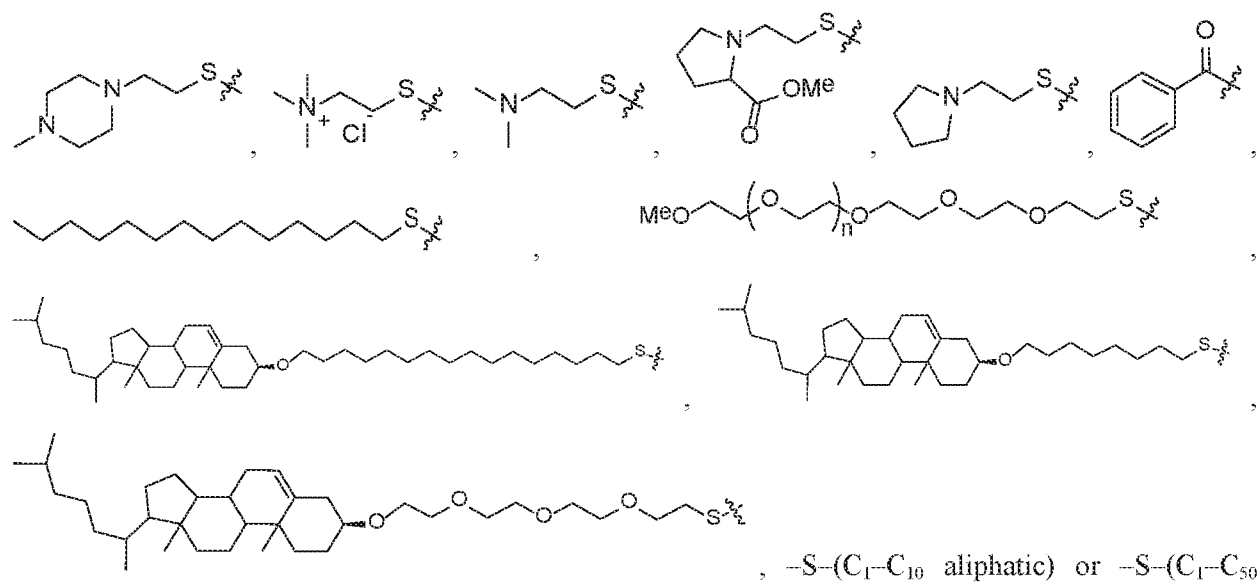


or

CCCN1CCOCC1CC(C)(N)C(=O)OCCSCCCN1CCOCC1CC(C)(N)C(=O)OCCCCSCN1CCOCC1CNCCSCN1CCOCC1SC2=CC=CC=C2CN(C)SCSN1CCOCC1



[00493] In some embodiments, X is -O- or -S-, and R¹ is

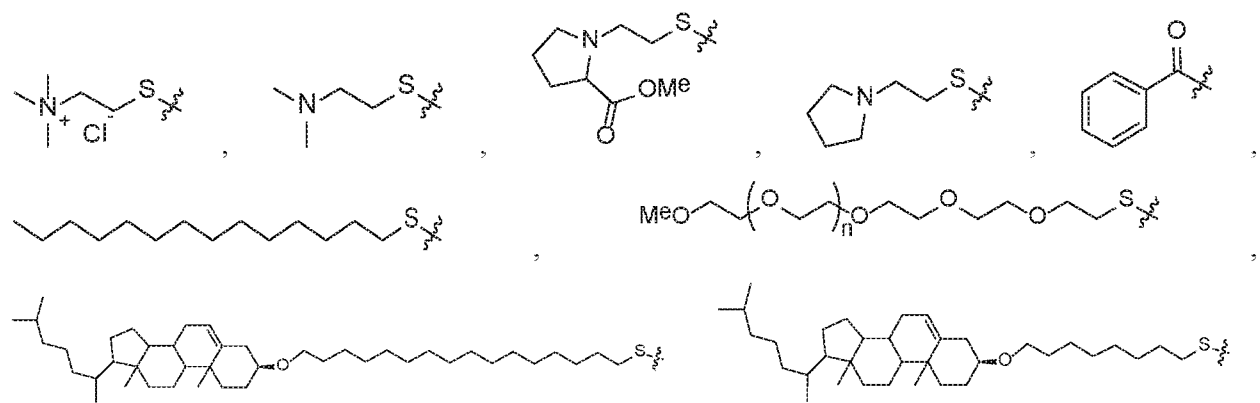


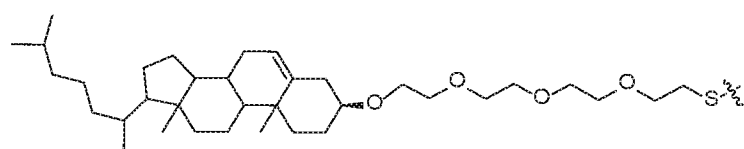
aliphatic).

[00494] In some embodiments, L is a covalent bond and -L-R¹ is R¹.

[00495] In some embodiments, -L-R¹ is not hydrogen.

[00496] In some embodiments, -X-L-R¹ is R¹ is

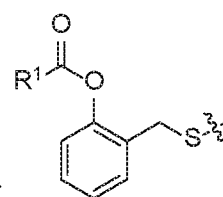




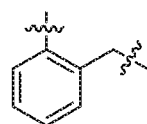
, $-\text{S}-(\text{C}_1-\text{C}_{10})$ aliphatic) or $-\text{S}-(\text{C}_1-\text{C}_{50})$

aliphatic).

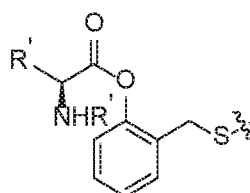
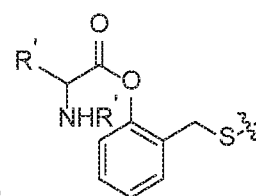
[00497] In some embodiments, $-\text{X}-\text{L}-\text{R}^1$ has the structure of



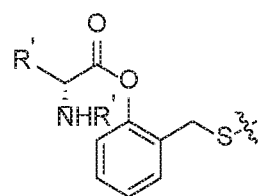
, wherein the



moiety is optionally substituted. In some embodiments, $-\text{X}-\text{L}-\text{R}^1$ is



In some embodiments, $-\text{X}-\text{L}-\text{R}^1$ is

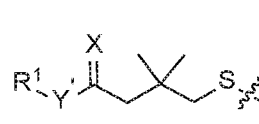


In some embodiments, $-\text{X}-\text{L}-\text{R}^1$ is

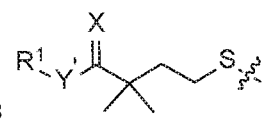
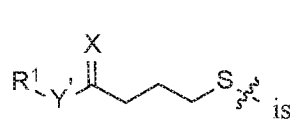
In some embodiments, $-\text{X}-\text{L}-\text{R}^1$ has the structure of

wherein X' is O or S, Y' is $-\text{O}-$, $-\text{S}-$ or $-\text{NR}'-$, and the moiety is optionally substituted. In

some embodiments, Y' is $-\text{O}-$, $-\text{S}-$ or $-\text{NH}-$. In some embodiments,

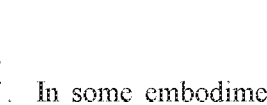
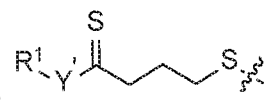
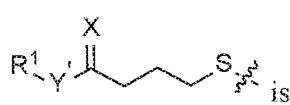


In some embodiments,

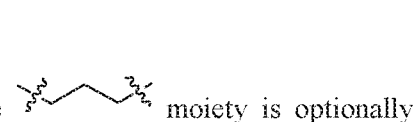
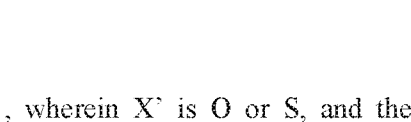
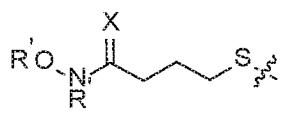


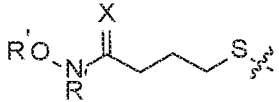
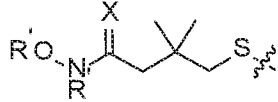
In some

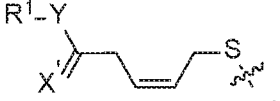
embodiments,

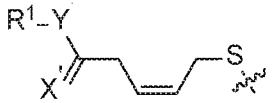


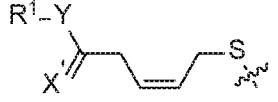
In some embodiments, $-\text{X}-\text{L}-\text{R}^1$ has the structure of

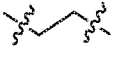
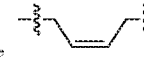
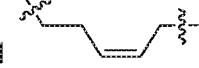


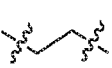
substituted. In some embodiments,  is . In some

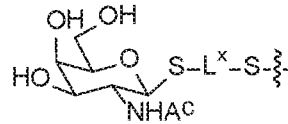
embodiments, $-X-L-R^1$ is , wherein the  is optionally substituted. In

some embodiments, $-X-L-R^1$ is , wherein the  is substituted. In

some embodiments, $-X-L-R^1$ is , wherein the  is unsubstituted.

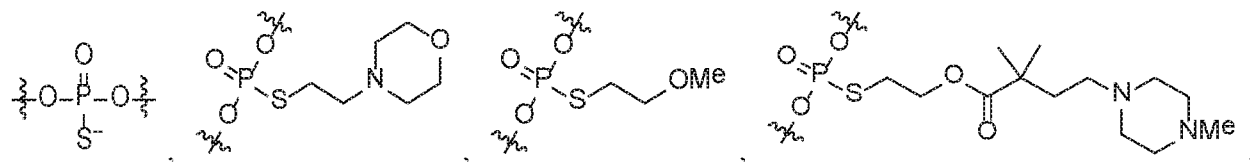
[00498] In some embodiments, $-X-L-R^1$ is $R^1-C(O)-S-L^x-S-$, wherein L^x is an optionally substituted group selected from , , , , and . In

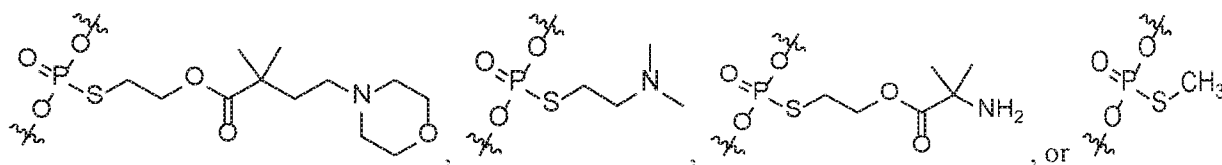
some embodiments, L^x is , , , , and . In some embodiments, $-X-L-R^1$ is $(CH_3)_3C-S-S-L^x-S-$. In some embodiments, $-X-L-R^1$ is $R^1-C(=X')-Y'-C(R)_2-S-L^x-S-$. In some embodiments, $-X-L-R^1$ is $R-C(=X')-Y'-CH_2-S-L^x-S-$. In some

embodiments, $-X-L-R^1$ is .

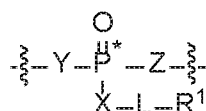
[00499] As will be appreciated by a person skilled in the art, many of the $-X-L-R^1$ groups described herein are cleavable and can be converted to $-X^-$ after administration to a subject. In some embodiments, $-X-L-R^1$ is cleavable. In some embodiments, $-X-L-R^1$ is $-S-L-R^1$, and is converted to $-S^-$ 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.

[00500] In some embodiments, the internucleotidic linkage having the structure of formula I is





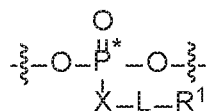
[00501] In some embodiments, the internucleotidic linkage of formula **I** has the structure of formula **I-a**:



(I-a)

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

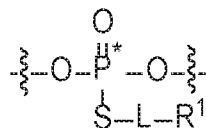
[00502] In some embodiments, the internucleotidic linkage of formula **I** has the structure of formula **I-b**:



(I-b)

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

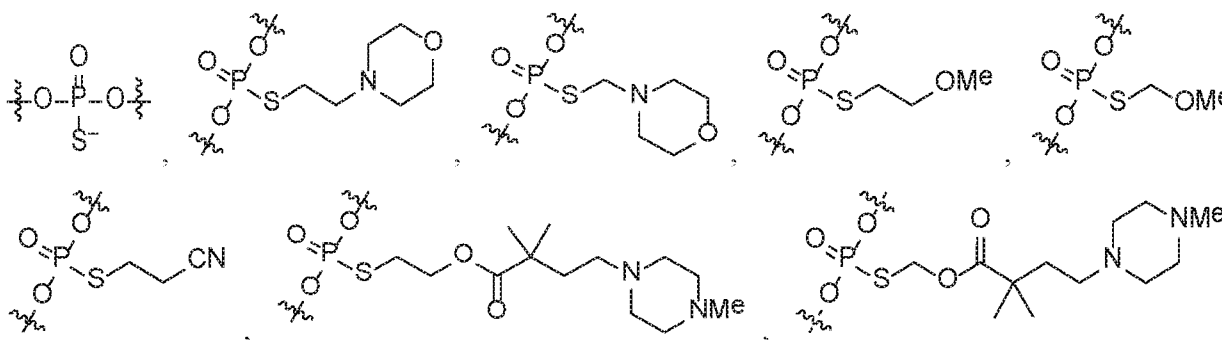
[00503] In some embodiments, the internucleotidic linkage of formula **I** is an phosphorothioate triester linkage having the structure of formula **I-c**:

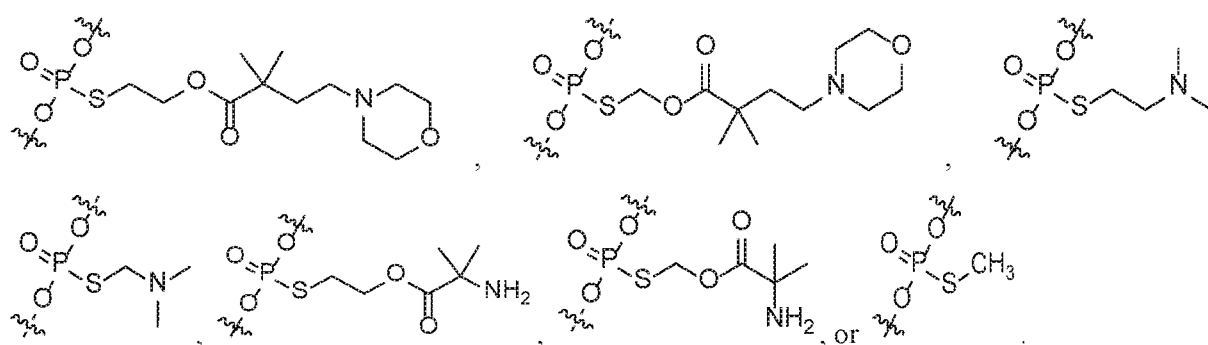


(I-c)

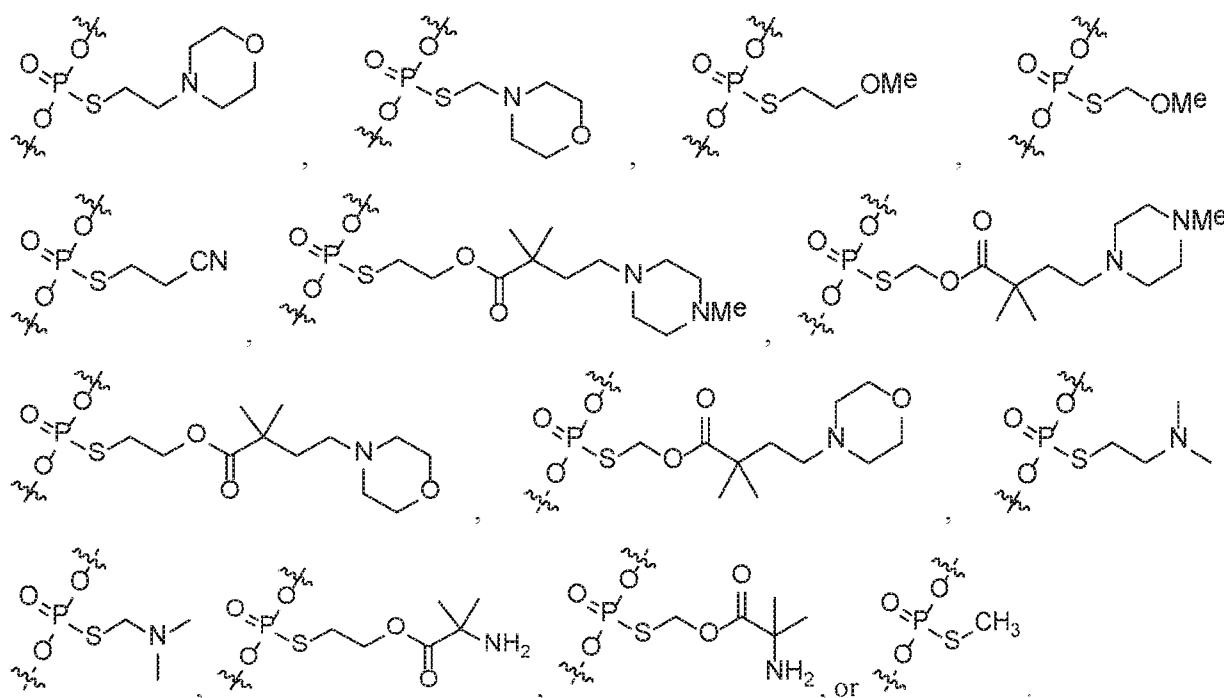
wherein R^1 is not $-H$ when L is a covalent bond.

[00504] In some embodiments, the internucleotidic linkage having the structure of formula **I** is





[00505] In some embodiments, the internucleotidic linkage having the structure of formula I-c is



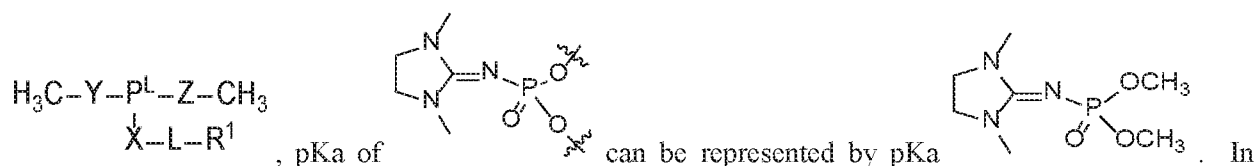
[00506] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising one or more natural phosphate linkages, and one or more modified internucleotidic linkages having the formula of I-a, I-b, or I-c.

[00507] In some embodiments, a modified internucleotidic linkage has the structure of I. In some embodiments, a modified internucleotidic linkage has the structure of I-a. In some embodiments, a modified internucleotidic linkage has the structure of I-b. In some embodiments, a modified internucleotidic linkage has the structure of I-c.

[00508] In some embodiments, a modified internucleotidic linkage is phosphorothioate internucleotidic linkage. Examples of internucleotidic linkages having the structure of formula I that can be utilized in accordance with the present disclosure include those described in US 9394333, US 9744183, US 9605019, US 20130178612, US 20150211006, US 9598458, US 20170037399, WO 2017/015555, WO 2017/062862, the internucleotidic linkages of each of which is incorporated herein by reference.

[00509] Non-limiting examples of internucleotidic linkages that can be utilized in accordance with the present disclosure also include those described in the art, including, but not limited to, those described in any of: Gryaznov, S.; Chen, J.-K. *J. Am. Chem. Soc.* 1994, 116, 3143, Jones et al. *J. Org. Chem.* 1993, 58, 2983, Koshkin et al. 1998 *Tetrahedron* 54: 3607-3630, Lauritsen et al. 2002 *Chem. Comm.* 5: 530-531, Lauritsen et al. 2003 *Bioo. Med. Chem. Lett.* 13: 253-256, Mesmaeker et al. *Angew. Chem., Int. Ed. Engl.* 1994, 33, 226, Petersen et al. 2003 *TRENDS Biotech.* 21: 74-81, Schultz et al. 1996 *Nucleic Acids Res.* 24: 2966, Ts'o et al. *Ann. N. Y. Acad. Sci.* 1988, 507, 220, and Vasseur et al. *J. Am. Chem. Soc.* 1992, 114, 4006.

[00510] In some embodiments, oligonucleotides comprise one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more non-negatively charged internucleotidic linkages. In some embodiments, a non-negatively charged internucleotidic linkage is not negatively charged in that at a given pH in an aqueous solution less than 50%, 40%, 40%, 30%, 20%, 10%, 5%, or 1% of the internucleotidic linkage exists in a negatively charged salt form. In some embodiments, a pH is about pH 7.4. In some embodiments, a pH is about 4-9. In some embodiments, the percentage is less than 10%. In some embodiments, the percentage is less than 5%. In some embodiments, the percentage is less than 1%. In some embodiments, an internucleotidic linkage is a non-negatively charged internucleotidic linkage in that the neutral form of the internucleotidic linkage has no pKa that is no more than about 1, 2, 3, 4, 5, 6, or 7 in water. In some embodiments, no pKa is 7 or less. In some embodiments, no pKa is 6 or less. In some embodiments, no pKa is 5 or less. In some embodiments, no pKa is 4 or less. In some embodiments, no pKa is 3 or less. In some embodiments, no pKa is 2 or less. In some embodiments, no pKa is 1 or less. In some embodiments, pKa of the neutral form of an internucleotidic linkage can be represented by pKa of the neutral form of a compound having the structure of $\text{CH}_3\text{---the internucleotidic linkage---CH}_3$. For example, pKa of the neutral form of an internucleotidic linkage having the structure of formula I may be represented by the pKa of the neutral form of a compound having the structure of

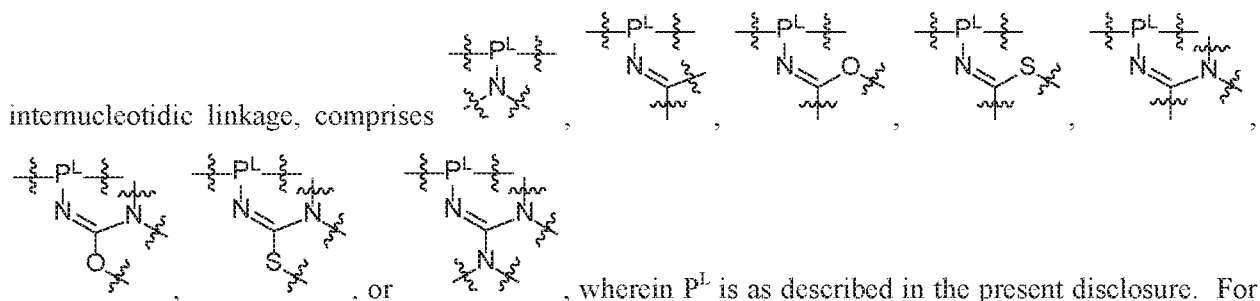


some embodiments, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, a non-negatively charged internucleotidic linkage is a positively-charged internucleotidic linkage. In some embodiments, a non-negatively charged internucleotidic linkage comprises a guanidine moiety. In some embodiments, a non-negatively charged internucleotidic linkage comprises a heteroaryl base moiety. In some embodiments, a non-negatively charged internucleotidic linkage comprises a triazole moiety. In some embodiments, a non-negatively charged internucleotidic

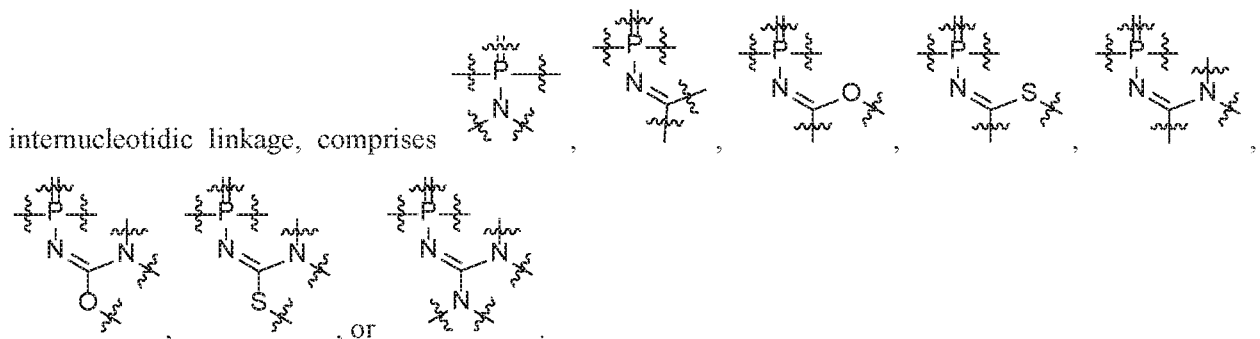
linkage comprises an alkynyl moiety.

[00511] In some embodiments, a non-negatively charged internucleotidic linkage, e.g., a neutral internucleotidic linkage, comprises $-P^L(-N=)-$, wherein P^L is as described in the present disclosure. In some embodiments, a non-negatively charged internucleotidic linkage, e.g., a neutral internucleotidic linkage, comprises $-P(-N=)-$. In some embodiments, a non-negatively charged internucleotidic linkage, e.g., a neutral internucleotidic linkage, comprises $-P(=)(-N=)-$. In some embodiments, a non-negatively charged internucleotidic linkage, e.g., a neutral internucleotidic linkage, comprises $-P(=O)(-N=)-$. In some embodiments, a non-negatively charged internucleotidic linkage, e.g., a neutral internucleotidic linkage, comprises $-P(=S)(-N=)-$.

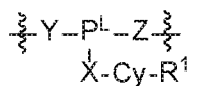
[00512] In some embodiments, a non-negatively charged internucleotidic linkage, e.g., a neutral

internucleotidic linkage, comprises , wherein P^L is as described in the present disclosure. For

example, in some embodiments, P^L is P; in some embodiments, P^L is P(O); in some embodiments, P^L is P(S); etc. In some embodiments, a non-negatively charged internucleotidic linkage, e.g., a neutral

internucleotidic linkage, comprises .

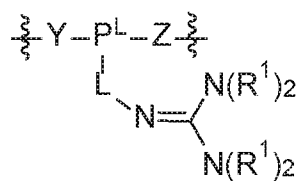
[00513] In some embodiments, a non-negatively charged internucleotidic linkage has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, or a salt form thereof (not negatively charged). In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, has the structure of formula I-n-1 or a salt form thereof:



I-n-1

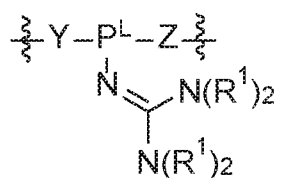
[00514] In some embodiments, X is a covalent bond and $-X-Cy-R^1$ is $-Cy-R^1$. In some embodiments, $-Cy-$ is an optionally substituted bivalent group selected from a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms. In some embodiments, $-Cy-$ is an optionally substituted bivalent 5-20 membered heteroaryl ring having 1-10 heteroatoms. In some embodiments, $-Cy-R^1$ is optionally substituted 5-20 membered heteroaryl ring having 1-10 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, $-Cy-R^1$ is optionally substituted 5-membered heteroaryl ring having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, $-Cy-R^1$ is optionally substituted 6-membered heteroaryl ring having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, $-Cy-R^1$ is optionally substituted triazolyl.

[00515] In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, has the structure of formula I-n-2 or a salt form thereof:



I-n-2

[00516] In some embodiments, R^1 is R' . In some embodiments, L is a covalent bond. In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, has the structure of formula I-n-3 or a salt form thereof:

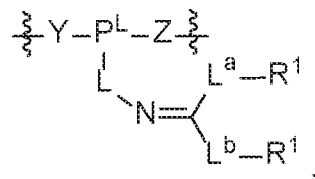


I-n-3

[00517] In some embodiments, two R' on different nitrogen atoms are taken together to form a ring as described. In some embodiments, a formed ring is 5-membered. In some embodiments, a formed ring is 6-membered. In some embodiments, a formed ring is substituted. In some embodiments, the two R' group that are not taken together to form a ring are each independently R. In some embodiments, the two R' group that are not taken together to form a ring are each independently hydrogen or an optionally substituted C_{1-6} aliphatic. In some embodiments, the two R' group that are not taken together to form a ring are each independently hydrogen or an optionally substituted C_{1-6} alkyl. In some embodiments, the two R' group that are not taken together to form a ring are the same. In some embodiments, the two R' group that are not taken together to form a ring are different. In some embodiments, both of them are

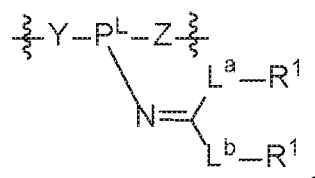
—CH₃.

[00518] In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, has the structure of formula **I-n-4** or a salt form thereof:



I-n-4

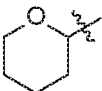
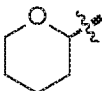
wherein each of L^a and L^b is independently L or —N(R¹)—, and each other variable is independently as described in the present disclosure. In some embodiments, L is a covalent bond, and an internucleotidic linkage of formula **I-n-4** has the structure of:

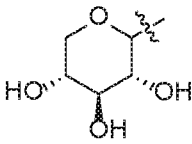


or a salt form thereof, wherein each variable is independently as described in the present disclosure.

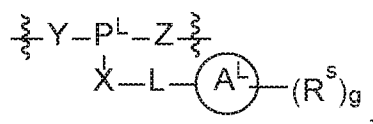
[00519] In some embodiments, L^a is —N(R¹)—. In some embodiments, L^a is L as described in the present disclosure. In some embodiments, L^a is a covalent bond. In some embodiments, L^a is —N(R¹)—. In some embodiments, L^a is —N(R)—. In some embodiments, L^a is —O—. In some embodiments, L^a is —S—. In some embodiments, L^a is —S(O)—. In some embodiments, L^a is —S(O)₂—. In some embodiments, L^a is —S(O)₂N(R¹)—. In some embodiments, L^b is —N(R¹)—. In some embodiments, L^b is L as described in the present disclosure. In some embodiments, L^b is a covalent bond. In some embodiments, L^b is —N(R¹)—. In some embodiments, L^b is —N(R)—. In some embodiments, L^b is —O—. In some embodiments, L^b is —S—. In some embodiments, L^b is —S(O)—. In some embodiments, L^b is —S(O)₂—. In some embodiments, L^b is —S(O)₂N(R¹)—. In some embodiments, L^a and L^b are the same. In some embodiments, L^a and L^b are different. In some embodiments, at least one of L^a and L^b is —N(R¹)—. In some embodiments, at least one of L^a and L^b is —O—. In some embodiments, at least one of L^a and L^b is —S—. In some embodiments, at least one of L^a and L^b is a covalent bond. In some embodiments, as described herein, R¹ is R. In some embodiments, R¹ is —H. In some embodiments, R¹ is optionally substituted C₁₋₁₀ aliphatic. In some embodiments, R¹ is optionally substituted C₁₋₁₀ alkyl. In some embodiments, a structure of formula **I-n-4** is a structure of formula **I-n-2**. In some embodiments, a structure of formula **I-n-4** is a structure of formula **I-n-3**. In some embodiments, a non-negatively charged internucleotidic linkage, e.g., a neutral internucleotidic linkage, has the structure of formula I. In some embodiments, X, e.g., in formula I, II, etc., is —N(—L—R⁵)—, wherein R⁵ is R as described herein. In

some embodiments, X is $-\text{NH}-$. In some embodiments, L, e.g., in $-\text{X}-\text{L}-$ of formula I, II, etc., comprises $-\text{SO}_2-$. In some embodiments, L is $-\text{SO}_2-$. In some embodiments, L is a covalent bond. In some embodiments, L is $-\text{C}(\text{O})\text{O}-(\text{C}_{1-4} \text{ alkylene})-$ wherein the alkylene is optionally substituted. In some embodiments, L is $-\text{C}(\text{O})\text{OCH}_2-$. In some embodiments, R^1 , e.g., in formula I, III, etc., comprise an optionally substituted ring. In some embodiments, R^1 is R as described herein. In some embodiments, R^1 is optionally substituted phenyl. In some embodiments, R^1 is 4-methylphenyl. In some embodiments, R^1 is 4-methoxyphenyl. In some embodiments, R^1 is 4-aminophenyl. In some embodiments, R^1 is an optionally substituted heteroaliphatic ring. In some embodiments, R^1 is an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, or 8) membered heteroaliphatic ring. In some embodiments, R^1 is an optionally substituted 5- or 6-membered saturated monocyclic heteroaliphatic ring having 1-3 heteroatoms. In some embodiments, the ring is 5-membered. In some embodiments, the ring is 6-membered. In some embodiments, the number of ring heteroatom(s) is 1. In some embodiments, the number of ring heteroatoms is 2. In some embodiments, a heteroatom is oxygen. In some embodiments, R^1 is optionally

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

embodiments, R^1 is . In some embodiments, R^1 is optionally substituted C_{1-30} aliphatic. In some embodiments, R^1 is optionally substituted C_{1-10} alkyl.

[00520] In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, has the structure of formula II or a salt form thereof:



II

or a salt form thereof, wherein:

P^{L} is $\text{P}(=\text{W})$, P , or $\text{P} \rightarrow \text{B}(\text{R}')_3$;

W is O, N($-\text{L}-\text{R}^{\text{S}}$), S or Se;

each of X, Y and Z is independently $-\text{O}-$, $-\text{S}-$, $-\text{N}(-\text{L}-\text{R}^{\text{S}})-$, or L;

R^{S} is $-\text{H}$, $-\text{L}-\text{R}'$, halogen, $-\text{CN}$, $-\text{NO}_2$, $-\text{L}-\text{Si}(\text{R}')_3$, $-\text{OR}'$, $-\text{SR}'$, or $-\text{N}(\text{R}')_2$;

Ring A^{L} is an optionally substituted 3-20 membered monocyclic, bicyclic or polycyclic ring having 0-10 heteroatoms;

each R^{S} is independently $-\text{H}$, halogen, $-\text{CN}$, $-\text{N}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{L}-\text{R}'$, $-\text{L}-\text{Si}(\text{R})_3$, $-\text{L}-\text{OR}'$,

$-L-SR'$, $-L-N(R')_2$, $-O-L-R'$, $-O-L-Si(R)_3$, $-O-L-OR'$, $-O-L-SR'$, or $-O-L-N(R')_2$;

g is 0-20;

each L is independently a covalent bond, or a bivalent, optionally substituted, linear or branched group selected from a C_{1-30} aliphatic group and a C_{1-30} heteroaliphatic group having 1-10 heteroatoms, wherein one or more methylene units are optionally and independently replaced with C_{1-6} alkylene, C_{1-6} alkenylene, $-C\equiv C-$, a bivalent C_1-C_6 heteroaliphatic group having 1-5 heteroatoms, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, $-C(O)O-$, $-P(O)(OR')-$, $-P(O)(SR')-$, $-P(O)(R')-$, $-P(O)(NR')-$, $-P(S)(OR')-$, $-P(S)(SR')-$, $-P(S)(R')-$, $-P(S)(NR')-$, $-P(R')-$, $-P(OR')-$, $-P(SR')-$, $-P(NR')-$, $-P(OR')[B(R')_3]-$, $-OP(O)(OR')O-$, $-OP(O)(SR')O-$, $-OP(O)(R')O-$, $-OP(O)(NR')O-$, $-OP(OR')O-$, $-OP(SR')O-$, $-OP(NR')O-$, $-OP(R')O-$, or $-OP(OR')[B(R')_3]O-$, and one or more CH or carbon atoms are optionally and independently replaced with Cy^L ;

each $-Cy-$ is independently an optionally substituted bivalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each Cy^L is independently an optionally substituted trivalent or tetravalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each R' is independently $-R$, $-C(O)R$, $-C(O)OR$, or $-S(O)_2R$;

each R is independently $-H$, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms, 5-30 membered heteroaryl having 1-10 heteroatoms, and 3-30 membered heterocyclyl having 1-10 heteroatoms, or

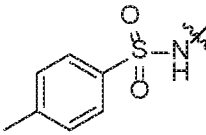
two R groups are optionally and independently taken together to form a covalent bond, or,

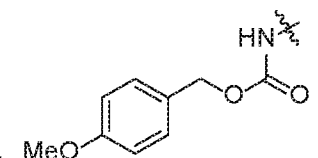
two or more R groups on the same atom are optionally and independently taken together with the atom to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the atom, 0-10 heteroatoms, or

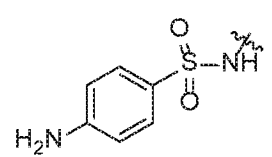
two or more R groups on two or more atoms are optionally and independently taken together with their intervening atoms to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms.

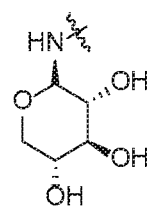
[00521] In some embodiments, Ring A^L in various structures of the present disclosure is an optionally substituted aryl ring. In some embodiments, Ring A^L is an optionally substituted phenyl ring. In some embodiments, Ring A^L is an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, or 8) membered heteroaliphatic ring. In some embodiments, Ring A^L is an optionally substituted 5- or 6-membered

saturated monocyclic heteroaliphatic ring having 1-3 heteroatoms. In some embodiments, the ring is 5-membered. In some embodiments, the ring is 6-membered. In some embodiments, the number of ring heteroatom(s) is 1. In some embodiments, the number of ring heteroatoms is 2. In some embodiments, a heteroatom is oxygen. In some embodiments, R^s is optionally substituted C_1 - C_6 alkyl group. In some embodiments, R^s is Me. In some embodiments, R^s is OR, wherein R is hydrogen or C_1 - C_6 alkyl group. In some embodiments, R^s is OH. In some embodiments, R^s is OMe. In some embodiments, R^s is $-N(R')_2$.

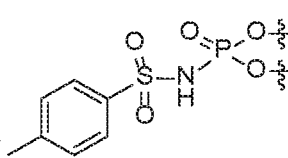
In some embodiments, R^s is $-NH_2$. In some embodiments, $\frac{1}{2}-X-L-(A^L)-(R^s)_g$ is 

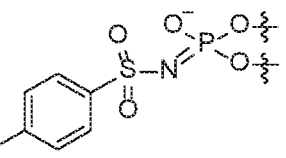
In some embodiments, $\frac{1}{2}-X-L-(A^L)-(R^s)_g$ is . In some embodiments,

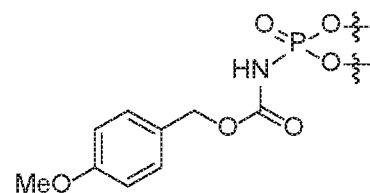
$\frac{1}{2}-X-L-(A^L)-(R^s)_g$ is . In some embodiments, $\frac{1}{2}-X-L-(A^L)-(R^s)_g$ is



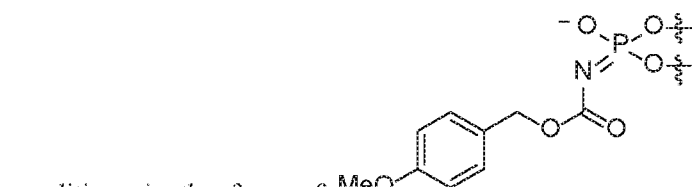
. In some embodiments, an internucleotidic linkage, e.g. a neutral internucleotidic linkage of

formula I or II, is n002 () , which, as one skilled in the art will appreciate, can

exist under certain conditions in the form of ). In some embodiments, an internucleotidic linkage, e.g. a neutral internucleotidic linkage of formula I or II, is n005 (

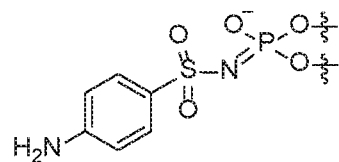


, which, as one skilled in the art will appreciate, can exist under certain

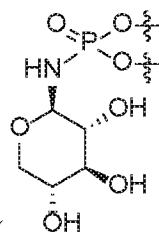


conditions in the form of $\text{MeO}-\text{C}_6\text{H}_4-\text{CH}_2-\text{O}-\text{C}(=\text{O})-\text{O}-\text{P}(=\text{O})(\text{O}^-)(\text{O}^-)-\text{O}-\text{Sugar}$ (n006). In some embodiments, an internucleotidic

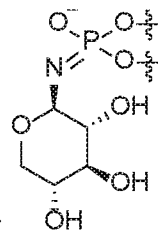
linkage, e.g. a neutral internucleotidic linkage of formula I or II, is n006 ($\text{H}_2\text{N}-\text{C}_6\text{H}_4-\text{SO}_2-\text{NH}-\text{P}(=\text{O})(\text{O}^-)(\text{O}^-)-\text{O}-\text{Sugar}$), which, as one skilled in the art will appreciate, can exist under certain conditions in the form of



(n007). In some embodiments, an internucleotidic linkage, e.g. a neutral

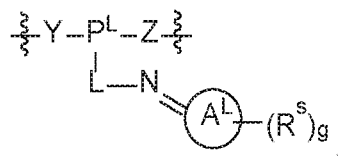


internucleotidic linkage of formula I or II, is n007 ($\text{Sugar}-\text{P}(=\text{O})(\text{O}^-)(\text{O}^-)-\text{O}-\text{Sugar}$), which, as one skilled in the art will



appreciate, can exist under certain conditions in a form of $\text{Sugar}-\text{P}(=\text{O})(\text{O}^-)(\text{O}^-)-\text{O}-\text{Sugar}$ (n008).

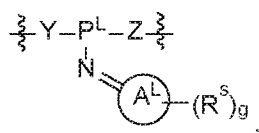
[00522] In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage of formula II, has the structure of formula II-a-1 or a salt form thereof:



II-a-1

or a salt form thereof.

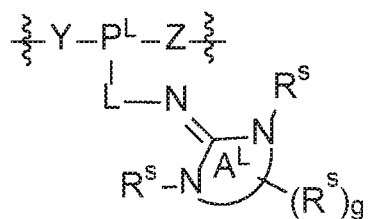
[00523] In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage of formula II, has the structure of formula II-a-2 or a salt form thereof:



II-a-2

or a salt form thereof.

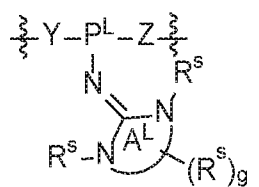
[00524] In some embodiments, A^L is bonded to $-N=$ or L through a carbon atom. In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage of formula II or II-a-1, II-a-2, has the structure of formula II-b-1 or a salt form thereof:



II-b-1

[00525] In some embodiments, a structure of formula II-a-1 or II-a-2 may be referred to a structure of formula II-a. In some embodiments, a structure of formula II-b-1 or II-b-2 may be referred to a structure of formula II-b. In some embodiments, a structure of formula II-c-1 or II-c-2 may be referred to a structure of formula II-c. In some embodiments, a structure of formula II-d-1 or II-d-2 may be referred to a structure of formula II-d.

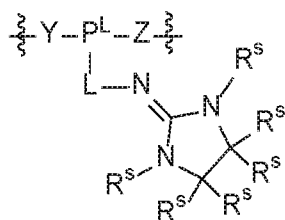
[00526] In some embodiments, A^L is bonded to $-N=$ or L through a carbon atom. In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage of formula II or II-a-1, II-a-2, has the structure of formula II-b-2 or a salt form thereof:



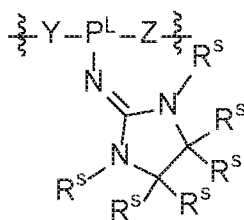
II-b-2

[00527] In some embodiments, Ring A^L is an optionally substituted 3-20 membered monocyclic ring having 0-10 heteroatoms (in addition to the two nitrogen atoms for formula II-b). In some embodiments, Ring A^L is an optionally substituted 5-membered monocyclic saturated ring.

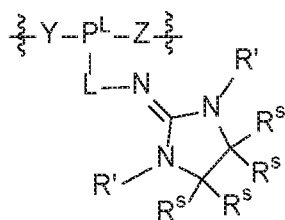
[00528] In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage of formula II, II-a, or II-b, has the structure of formula II-c-1 or a salt form thereof:

**II-c-1**

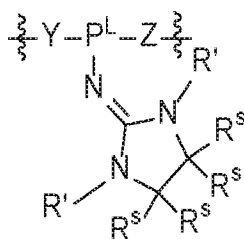
[00529] In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage of formula II, II-a, or II-b, has the structure of formula II-c-2 or a salt form thereof:

**II-c-2**

[00530] In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage of formula II, II-a, II-b, or II-c has the structure of formula II-d-1 or a salt form thereof:

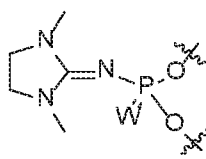
**II-d-1**

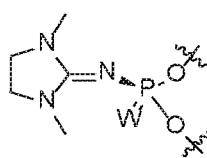
[00531] In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage of formula II, II-a, II-b, or II-c has the structure of formula II-d-2 or a salt form thereof:

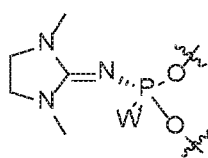
**II-d-2**

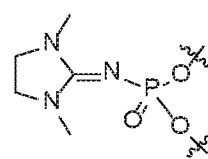
[00532] In some embodiments, each R' is independently optionally substituted C₁₋₆ aliphatic. In some embodiments, each R' is independently optionally substituted C₁₋₆ alkyl. In some embodiments, each R' is independently -CH₃. In some embodiments, each R^s is -H.

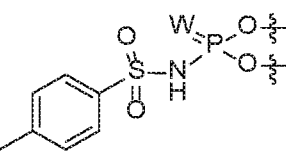
[00533] In some embodiments, a non-negatively charged internucleotidic linkage has the structure

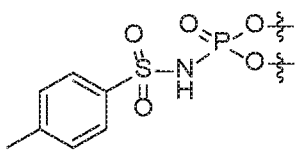
of . In some embodiments, a non-negatively charged internucleotidic linkage has the

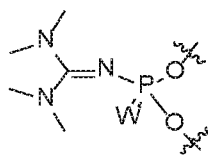
structure of . In some embodiments, a non-negatively charged internucleotidic linkage

has the structure of . In some embodiments, a non-negatively charged internucleotidic

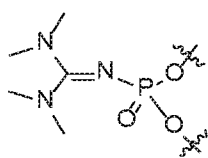
linkage has the structure of . In some embodiments, a non-negatively charged

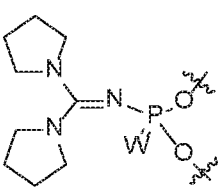
internucleotidic linkage has the structure of . In some embodiments, a non-

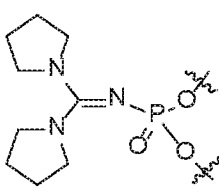
negatively charged internucleotidic linkage has the structure of . In some

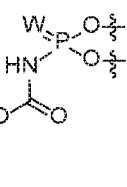
embodiments, a non-negatively charged internucleotidic linkage has the structure of .

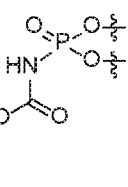
In some embodiments, a non-negatively charged internucleotidic linkage has the structure of

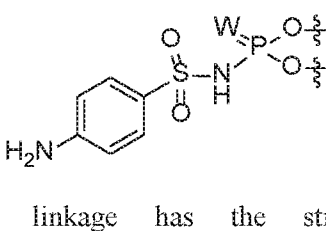
. In some embodiments, a non-negatively charged internucleotidic linkage has the

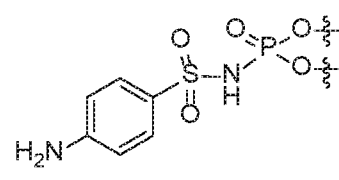
structure of . In some embodiments, a non-negatively charged internucleotidic linkage

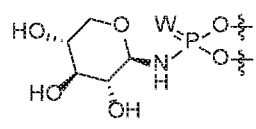
has the structure of . In some embodiments, a non-negatively charged internucleotidic

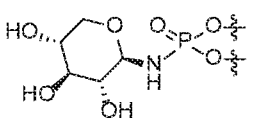
linkage has the structure of . In some embodiments, a non-negatively charged

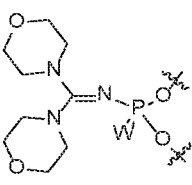
internucleotidic linkage has the structure of . In some embodiments, a non-

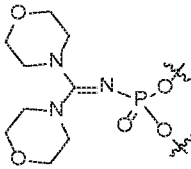
negatively charged internucleotidic linkage has the structure of . In some

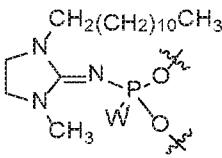
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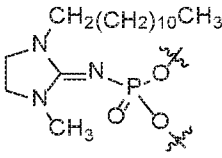
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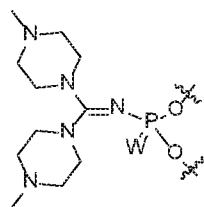
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a non-negatively charged internucleotidic linkage has the structure of . In some

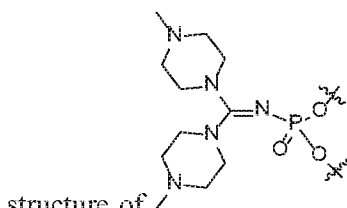
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embodiments, a non-negatively charged internucleotidic linkage has the structure of .

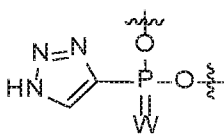
In some embodiments, a non-negatively charged internucleotidic linkage has the structure of

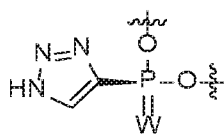


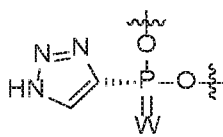
. In some embodiments, a non-negatively charged internucleotidic linkage has the

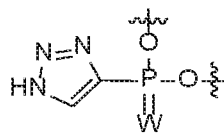


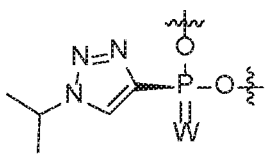
structure of . In some embodiments, a non-negatively charged internucleotidic linkage

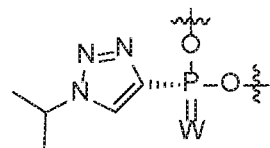
has the structure of . In some embodiments, a non-negatively charged internucleotidic

linkage has the structure of . In some embodiments, a non-negatively charged

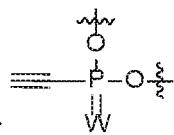
internucleotidic linkage has the structure of . In some embodiments, a non-negatively

charged internucleotidic linkage has the structure of . In some embodiments, a non-

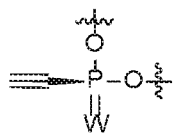
negatively charged internucleotidic linkage has the structure of . In some embodiments, a non-negatively charged internucleotidic linkage has the structure of



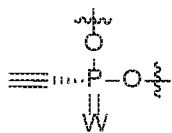
. In some embodiments, a non-negatively charged internucleotidic linkage has the



structure of . In some embodiments, a non-negatively charged internucleotidic linkage has



the structure of . In some embodiments, a non-negatively charged internucleotidic linkage

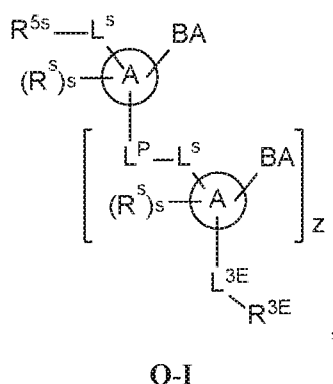


has the structure of . In some embodiments, W is O. In some embodiments, W is S. In some embodiments, a non-negatively charged internucleotidic linkage is chirally controlled. In some embodiments, the linkage phosphorus is *Rp*. In some embodiments, the linkage phosphorus is *Sp*.

[00534] In some embodiments, each non-negatively charged internucleotidic linkage or neutral internucleotidic linkage (e.g., those of formula **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, or **II-d-2**) is independently *Rp* at its linkage phosphorus. In some embodiments, each negatively charged chiral internucleotidic linkage is *Sp* at its linkage phosphorus. In some embodiments, each phosphorothioate internucleotidic linkages is *Sp* at its linkage phosphorus. In some embodiments, each natural phosphate linkage is independently bonded to a sugar comprising a 2'-OR modification, wherein R is not -H. In some embodiments, each natural phosphate linkage is independently bonded to a sugar comprising a 2'-OR modification, wherein R is not -H, at a 3'-position. In some embodiments, each sugar that contains no 2'-OR modification wherein R is not -H is independently bonded to at least one non-natural phosphate linkages, in many cases, two non-natural natural phosphate linkages. In some embodiments, each 2'-F modified sugar is independently bonded to at least one non-natural phosphate linkages, in many cases, two non-natural natural phosphate linkages. In some embodiments, each non-natural phosphate linkage is a phosphorothioate internucleotidic linkage. In some embodiments, each non-natural phosphate linkage is a *Sp* phosphorothioate internucleotidic

linkage. In some embodiments, each sugar bonded to non-negatively charged internucleotidic linkage or neutral internucleotidic linkage (e.g., those of formula I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, or II-d-2) independently contains no 2'-OR. In some embodiments, each sugar bonded to non-negatively charged internucleotidic linkage or neutral internucleotidic linkage (e.g., those of formula I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, or II-d-2) is a 2'-F modified sugar.

[00535] In some embodiments, the present disclosure provides a compound, e.g., an oligonucleotide, a chirally controlled oligonucleotide, an oligonucleotide of a provided composition (e.g., of a plurality of oligonucleotides), having the structure of formula O-I:



or a salt thereof, wherein:

R^{5s} is independently R' or $-OR'$;

each BA is independently an optionally substituted group selected from C_{3-30} cycloaliphatic, C_{6-30} aryl, C_{5-30} heteroaryl having 1-10 heteroatoms, C_{3-30} heterocyclyl having 1-10 heteroatoms, a natural nucleobase moiety, and a modified nucleobase moiety;

each R^s is independently $-H$, halogen, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L-R'$, $-L-Si(R)_3$, $-L-OR'$, $-L-SR'$, $-L-N(R')_2$, $-O-L-R'$, $-O-L-Si(R)_3$, $-O-L-OR'$, $-O-L-SR'$, or $-O-L-N(R')_2$;

each s is independently 0-20;

each L^s is independently $-C(R^{5s})_2-$, or L ;

each L is independently a covalent bond, or a bivalent, optionally substituted, linear or branched group selected from a C_{1-30} aliphatic group and a C_{1-30} heteroaliphatic group having 1-10 heteroatoms, wherein one or more methylene units are optionally and independently replaced with C_{1-6} alkylene, C_{1-6} alkenylene, $-C\equiv C-$, a bivalent C_{1-6} heteroaliphatic group having 1-5 heteroatoms, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, $-C(O)O-$, $-P(O)(OR')-$, $-P(O)(SR')-$, $-P(O)(R')-$, $-P(O)(NR')-$, $-P(S)(OR')-$, $-P(S)(SR')-$, $-P(S)(R')-$, $-P(S)(NR')-$, $-P(R')-$, $-P(OR')-$, $-P(SR')-$, $-P(NR')-$, $-P(OR')[B(R')_3]-$, $-OP(O)(OR')O-$, $-OP(O)(SR')O-$, $-OP(O)(R')O-$,

$-\text{OP}(\text{O})(\text{NR}')\text{O}-$, $-\text{OP}(\text{OR}')\text{O}-$, $-\text{OP}(\text{SR}')\text{O}-$, $-\text{OP}(\text{NR}')\text{O}-$, $-\text{OP}(\text{R}')\text{O}-$, or $-\text{OP}(\text{OR}')[\text{B}(\text{R}')_3]\text{O}-$, and one or more CH or carbon atoms are optionally and independently replaced with Cy^{L} ;

each $-\text{Cy}-$ is independently an optionally substituted bivalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each Cy^{L} is independently an optionally substituted trivalent or tetravalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each Ring A is independently an optionally substituted 3-20 membered monocyclic, bicyclic or polycyclic ring having 0-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon;

each L^{P} is independently an internucleotidic linkage;

z is 1-1000;

$\text{L}^{3\text{E}}$ is L or $-\text{L}-\text{L}-$;

$\text{R}^{3\text{E}}$ is $-\text{R}'$, $-\text{L}-\text{R}'$, $-\text{OR}'$, or a solid support;

each R' is independently $-\text{R}$, $-\text{C}(\text{O})\text{R}$, $-\text{C}(\text{O})\text{OR}$, or $-\text{S}(\text{O})_2\text{R}$;

each R is independently $-\text{H}$, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms, 5-30 membered heteroaryl having 1-10 heteroatoms, and 3-30 membered heterocyclyl having 1-10 heteroatoms, or

two R groups are optionally and independently taken together to form a covalent bond, or

two or more R groups on the same atom are optionally and independently taken together with the atom to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the atom, 0-10 heteroatoms, or

two or more R groups on two or more atoms are optionally and independently taken together with their intervening atoms to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms.

[00536] In some embodiments, each L^{P} independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, III, or a salt form thereof. In some embodiments, each L^{P} independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, or a salt form thereof. In some embodiments, each L^{P} independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3,

I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, III, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, or a salt form thereof. In some embodiments, each internucleotidic linkage independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, III, or a salt form thereof. In some embodiments, each internucleotidic linkage independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, or a salt form thereof. In some embodiments, each internucleotidic linkage independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, or a salt form thereof.

[00537] In some embodiments, each BA is independently an optionally substituted group selected from C₅₋₃₀ heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, and C₃₋₃₀ heterocyclyl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus, boron and silicon;

each Ring A is independently an optionally substituted 3-20 membered monocyclic, bicyclic or polycyclic ring having 0-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon; and

each L^P independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, III, or a salt form thereof. In some embodiments, each L^P independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, or a salt form thereof.

[00538] In some embodiments, each BA is independently an optionally substituted C₅₋₃₀ heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, wherein the heteroaryl comprises one or more heteroatoms selected from oxygen and nitrogen;

each Ring A is independently an optionally substituted 5-10 membered monocyclic or bicyclic saturated ring having 0-5 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, wherein the ring comprises at least one oxygen atom; and

each L^P independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, III, or a salt form thereof. In some embodiments, each L^P independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, or a salt form thereof.

[00539] In some embodiments, each BA is independently an optionally substituted A, T, C, G, or

U, or an optionally substituted tautomer of A, T, C, G, or U;

each Ring A is independently an optionally substituted 5-7 membered monocyclic or bicyclic saturated ring having one or more oxygen atoms; and

each L^P independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, III, or a salt form thereof. In some embodiments, each L^P independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, or a salt form thereof.

[00540] In some embodiments, each BA is independently an optionally substituted or protected nucleobase selected from adenine, cytosine, guanosine, thymine, and uracil and tautomers thereof;

each Ring A is independently an optionally substituted 5-7 membered monocyclic or bicyclic saturated ring having one or more oxygen atoms; and

each L^P independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, III, or a salt form thereof. In some embodiments, each L^P independently has the structure of formula I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1, II-d-2, or a salt form thereof.

[00541] In some embodiments, BA is an optionally substituted group selected from C₃₋₃₀ cycloaliphatic, C₆₋₃₀ aryl, C₅₋₃₀ heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, C₃₋₃₀ heterocyclyl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, a natural nucleobase moiety, and a modified nucleobase moiety. In some embodiments, BA is an optionally substituted group selected from C₅₋₃₀ heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, C₃₋₃₀ heterocyclyl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, a natural nucleobase moiety, and a modified nucleobase moiety. In some embodiments, BA is an optionally substituted group selected from C₅₋₃₀ heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, a natural nucleobase moiety, and a modified nucleobase moiety. In some embodiments, BA is optionally substituted C₅₋₃₀ heteroaryl having 1-10 heteroatoms independently selected from oxygen, nitrogen, and sulfur. In some embodiments, BA is optionally substituted natural nucleobases and tautomers thereof. In some embodiments, BA is protected natural nucleobases and tautomers thereof. Various nucleobase protecting groups for oligonucleotide synthesis are known and can be utilized in accordance with the present disclosure. In some embodiments, BA is an optionally substituted nucleobase selected from adenine, cytosine, guanosine, thymine, and uracil, and tautomers thereof. In some embodiments, BA is an optionally protected nucleobase selected from adenine, cytosine, guanosine, thymine, and uracil, and tautomers thereof.

[00542] In some embodiments, BA is optionally substituted C_{3-30} cycloaliphatic. In some embodiments, BA is optionally substituted C_{6-30} aryl. In some embodiments, BA is optionally substituted C_{3-30} heterocyclyl. In some embodiments, BA is optionally substituted C_{5-30} heteroaryl. In some embodiments, BA is an optionally substituted natural base moiety. In some embodiments, BA is an optionally substituted modified base moiety. BA is an optionally substituted group selected from C_{3-30} cycloaliphatic, C_{6-30} aryl, C_{3-30} heterocyclyl, and C_{5-30} heteroaryl. In some embodiments, BA is an optionally substituted group selected from C_{3-30} cycloaliphatic, C_{6-30} aryl, C_{3-30} heterocyclyl, C_{5-30} heteroaryl, and a natural nucleobase moiety.

[00543] In some embodiments, BA is connected through an aromatic ring. In some embodiments, BA is connected through a heteroatom. In some embodiments, BA is connected through a ring heteroatom of an aromatic ring. In some embodiments, BA is connected through a ring nitrogen atom of an aromatic ring.

[00544] In some embodiments, BA is a natural nucleobase moiety. In some embodiments, BA is an optionally substituted natural nucleobase moiety. In some embodiments, BA is a substituted natural nucleobase moiety. In some embodiments, BA is optionally substituted, or an optionally substituted tautomer of, A, T, C, U, or G. In some embodiments, BA is natural nucleobase A, T, C, U, or G. In some embodiments, BA is an optionally substituted group selected from natural nucleobases A, T, C, U, and G.

[00545] In some embodiments, BA is an optionally substituted purine base residue. In some embodiments, BA is a protected purine base residue. In some embodiments, BA is an optionally substituted adenine residue. In some embodiments, BA is a protected adenine residue. In some embodiments, BA is an optionally substituted guanine residue. In some embodiments, BA is a protected guanine residue. In some embodiments, BA is an optionally substituted cytosine residue. In some embodiments, BA is a protected cytosine residue. In some embodiments, BA is an optionally substituted thymine residue. In some embodiments, BA is a protected thymine residue. In some embodiments, BA is an optionally substituted uracil residue. In some embodiments, BA is a protected uracil residue. In some embodiments, BA is an optionally substituted 5-methylcytosine residue. In some embodiments, BA is a protected 5-methylcytosine residue.

[00546] In some embodiments, BA is a protected base residue as used in oligonucleotide preparation. In some embodiments, BA is a base residue illustrated in US 2011/0294124, US 2015/0211006, US 2015/0197540, and WO 2015/107425, each of which is incorporated herein by reference.

[00547] In some embodiments, $R^{5s}-L^s-$ is $-CH_2OH$. In some embodiments, $R^{5s}-L^s-$ is $-CH(R^{5s})-OH$, wherein R^{5s} is as described in the present disclosure. In some embodiments, L^s is $-CH_2-$. In some embodiments, L^s is $-CH(R^{5s})-$ wherein R^{5s} is not $-H$. In some embodiments, L^s is $-CH(R^{5s})-$

wherein R^{5s} is not $-H$ and is otherwise R . In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted C_{1-6} alkyl. In some embodiments, R is methyl. In some embodiments, $-CH(R^{5s})-$ wherein R^{5s} is not $-H$ has is R . In some embodiments, $-CH(R^{5s})-$ wherein R^{5s} is not $-H$ has is S .

[00548] Example embodiments for variables, e.g., variables of each of the formulae, are additionally described in the present disclosure, and may be independently and optionally combined.

[00549] In some embodiments, the present disclosure provides oligonucleotides and oligonucleotide compositions that are chirally controlled. For instance, in some embodiments, a provided composition contains controlled 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. In some embodiments, oligonucleotides of the same oligonucleotide type are identical.

[00550] 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.

[00551] 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.

[00552] In some embodiments, a provided oligonucleotide is a gapmer.

[00553] In some embodiments, a provided oligonucleotide is a skipmer.

[00554] 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 example 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 LNATM or ENATM bicyclic sugar 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 disclosure. In some embodiments, a hemimer structure provides advantageous benefits. In some embodiments, provided oligonucleotides are 5'-hemimers that comprises modified sugar moieties in a 5'-end sequence. In some embodiments, provided oligonucleotides are 5'-hemimers that comprises modified 2'-sugar moieties in a 5'-end sequence.

[00555] 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 nucleosides or sugars of LNAs.

[00556] 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.

[00557] 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

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CECI EST LE TOME 1 DE 6
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CONTAINING PAGES 1 TO 174

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CLAIMS

1. An oligonucleotide composition, comprising a plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and
- 4) pattern of backbone phosphorus modifications,

wherein:

oligonucleotides of the plurality comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 chirally controlled internucleotidic linkages; and

oligonucleotides of the plurality comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 non-negatively charged internucleotidic linkages.

2. An oligonucleotide composition, comprising a plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and
- 4) pattern of backbone phosphorus modifications,

wherein:

oligonucleotides of the plurality comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 chirally controlled internucleotidic linkages; and

the oligonucleotide composition being characterized in that, when it is contacted with a transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under a reference condition selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

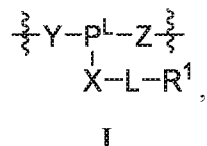
3. The oligonucleotide of claim 2, wherein the pattern of backbone linkages comprises at least one non-negatively charged internucleotidic linkage.

4. The oligonucleotide composition of claim 1, wherein when the oligonucleotide composition is contacted with a transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under a reference condition selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

5. The oligonucleotide of any one of claims 1-4, wherein one or more non-negatively charged internucleotidic linkage are independently chirally controlled.

6. The composition of claim 5, wherein a non-negatively charged internucleotidic linkage has the

structure of formula I:



or a salt form thereof, wherein:

P^L is $P(=W)$, P , or $P \rightarrow B(R')_3$;

W is O , $N(-L-R^5)$, S or Se ;

each of R^1 and R^5 is independently $-H$, $-L-R'$, halogen, $-CN$, $-NO_2$, $-L-Si(R')_3$, $-OR'$, $-SR'$, or $-N(R')_2$;

X is $-N(-L-R^5)-$;

each of Y and Z is independently $-O-$, $-S-$, $-N(-L-R^5)-$, or L ;

each L is independently a covalent bond, or a bivalent, optionally substituted, linear or branched group selected from a C_{1-30} aliphatic group and a C_{1-30} heteroaliphatic group having 1-10 heteroatoms, wherein one or more methylene units are optionally and independently replaced with C_{1-6} alkylene, C_{1-6} alkenylene, $-C \equiv C-$, a bivalent C_1-C_6 heteroaliphatic group having 1-5 heteroatoms, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, $-C(O)O-$, $-P(O)(OR')-$, $-P(O)(SR')-$, $-P(O)(R')-$, $-P(O)(NR')-$, $-P(S)(OR')-$, $-P(S)(SR')-$, $-P(S)(R')-$, $-P(S)(NR')-$, $-P(R')-$, $-P(OR')-$, $-P(SR')-$, $-P(NR')-$, $-P(OR')[B(R')_3]-$, $-OP(O)(OR')O-$, $-OP(O)(SR')O-$, $-OP(O)(R')O-$, $-OP(O)(NR')O-$, $-OP(OR')O-$, $-OP(SR')O-$, $-OP(NR')O-$, $-OP(R')O-$, or $-OP(OR')[B(R')_3]O-$, and one or more CH or carbon atoms are optionally and independently replaced with Cy^1 ;

each $-Cy-$ is independently an optionally substituted bivalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each Cy^1 is independently an optionally substituted trivalent or tetravalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each R' is independently $-R$, $-C(O)R$, $-C(O)OR$, or $-S(O)_2R$;

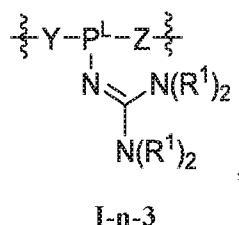
each R is independently $-H$, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms, 5-30 membered heteroaryl having 1-10 heteroatoms, and 3-30 membered heterocyclyl having 1-10 heteroatoms, or

two R groups are optionally and independently taken together to form a covalent bond, or

two or more R groups on the same atom are optionally and independently taken together with the atom to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the atom, 0-10 heteroatoms, or

two or more R groups on two or more atoms are optionally and independently taken together with their intervening atoms to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms.

7. The composition of claim 5, wherein a non-negatively charged internucleotidic linkage has the structure of formula **I-n-3**:



or a salt form thereof, wherein:

P^{L} is $\text{P}(=\text{W})$, P , or $\text{P} \rightarrow \text{B}(\text{R}')_3$;

W is O , $\text{N}(-\text{L}-\text{R}^5)$, S or Se ;

each of R^1 and R^5 is independently $-\text{H}$, $-\text{L}-\text{R}'$, halogen, $-\text{CN}$, $-\text{NO}_2$, $-\text{L}-\text{Si}(\text{R}')_3$, $-\text{OR}'$, $-\text{SR}'$, or $-\text{N}(\text{R}')_2$;

each of Y and Z is independently $-\text{O}-$, $-\text{S}-$, $-\text{N}(-\text{L}-\text{R}^5)-$, or L ;

each L is independently a covalent bond, or a bivalent, optionally substituted, linear or branched group selected from a C_{1-30} aliphatic group and a C_{1-30} heteroaliphatic group having 1-10 heteroatoms, wherein one or more methylene units are optionally and independently replaced with C_{1-6} alkylene, C_{1-6} alkenylene, $-\text{C}\equiv\text{C}-$, a bivalent C_1-C_6 heteroaliphatic group having 1-5 heteroatoms, $-\text{C}(\text{R}')_2-$, $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{C}(\text{O})\text{S}-$, $-\text{C}(\text{O})\text{O}-$, $-\text{P}(\text{O})(\text{OR}')-$, $-\text{P}(\text{O})(\text{SR}')-$, $-\text{P}(\text{O})(\text{R}')-$, $-\text{P}(\text{O})(\text{NR}')-$, $-\text{P}(\text{S})(\text{OR}')-$, $-\text{P}(\text{S})(\text{SR}')-$, $-\text{P}(\text{S})(\text{R}')-$, $-\text{P}(\text{S})(\text{NR}')-$, $-\text{P}(\text{R}')-$, $-\text{P}(\text{OR}')-$, $-\text{P}(\text{SR}')-$, $-\text{P}(\text{NR}')-$, $-\text{P}(\text{OR}')[\text{B}(\text{R}')_3]-$, $-\text{OP}(\text{O})(\text{OR}')\text{O}-$, $-\text{OP}(\text{O})(\text{SR}')\text{O}-$, $-\text{OP}(\text{O})(\text{R}')\text{O}-$, $-\text{OP}(\text{O})(\text{NR}')\text{O}-$, $-\text{OP}(\text{OR}')\text{O}-$, $-\text{OP}(\text{SR}')\text{O}-$, $-\text{OP}(\text{NR}')\text{O}-$, $-\text{OP}(\text{R}')\text{O}-$, or $-\text{OP}(\text{OR}')[\text{B}(\text{R}')_3]\text{O}-$, and one or more CH or carbon atoms are optionally and independently replaced with Cy^{L} ;

each $-\text{Cy}-$ is independently an optionally substituted bivalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each Cy^{L} is independently an optionally substituted trivalent or tetravalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and

a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each R' is independently $-R$, $-C(O)R$, $-C(O)OR$, or $-S(O)_2R$;

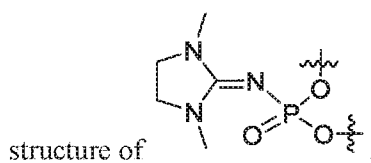
each R is independently $-H$, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms, 5-30 membered heteroaryl having 1-10 heteroatoms, and 3-30 membered heterocyclyl having 1-10 heteroatoms, or

two R groups are optionally and independently taken together to form a covalent bond, or

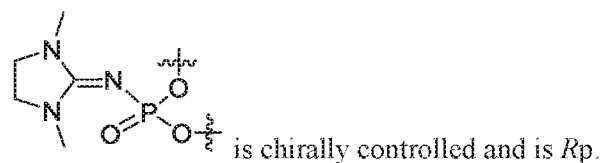
two or more R groups on the same atom are optionally and independently taken together with the atom to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the atom, 0-10 heteroatoms, or

two or more R groups on two or more atoms are optionally and independently taken together with their intervening atoms to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms.

8. The composition of claim 5, wherein a non-negatively charged internucleotidic linkage has the



9. The composition of claim 8, wherein the non-negatively charged internucleotidic linkage



10. The composition of claim 8, wherein the transcript is a Dystrophin transcript.

11. The composition of claim 10, wherein splicing of the transcript is altered such that the level of skipping of exon 45, 51, or 53, or multiple exons is increased.

12. The composition of claim 8, wherein each chiral internucleotidic linkage of the oligonucleotides of the plurality is independently a chirally controlled internucleotidic linkage.

13. The composition of claim 8, wherein the base sequence is or comprises or comprises 15 contiguous bases of the base sequence of any oligonucleotide in Table A1.

14. The composition of claim 11, wherein the oligonucleotide type comprises any of: cholesterol; L-carnitine (amide and carbamate bond); Folic acid; Gambogic acid; Cleavable lipid (1,2-dilaurin and ester bond); Insulin receptor ligand; CPP; Glucose (tri- and hex-antennary); or Mannose (tri- and hex-antennary, alpha and beta).

15. The composition of claim 11, wherein each non-negatively charged internucleotidic linkage is

independently an internucleotidic linkage at least 50% of which exists in its non-negatively charged form at pH 7.4.

16. The composition of claim 11, wherein the oligonucleotides of the plurality each comprise one or more sugar modifications.

17. The composition of claim 16, wherein one or more sugar modifications are 2'-F modifications.

18. The composition of any one of the preceding claims, wherein each heteroatom is independently boron, nitrogen, oxygen, silicon, sulfur, or phosphorus.

19. A pharmaceutical composition comprising an oligonucleotide composition of any one of the preceding claims and a pharmaceutically acceptable carrier.

20. A method for altering splicing of a target transcript, comprising administering an oligonucleotide composition of any one of the preceding claims.

21. The method of claim 20, wherein the target transcript is pre-mRNA of dystrophin.

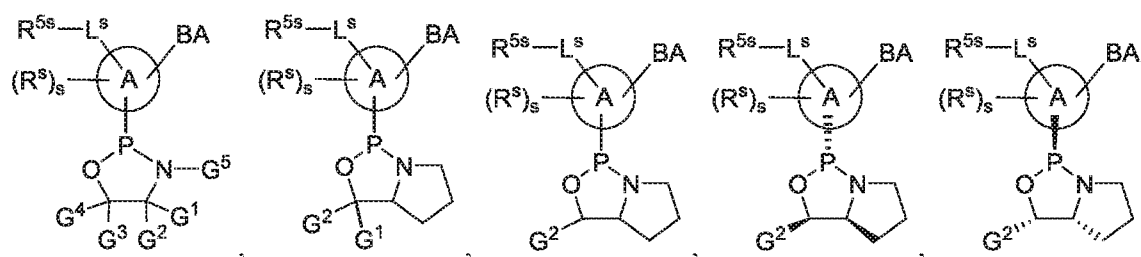
22. The method of claim 21, wherein exon 45 of dystrophin is skipped at an increased level relative to absence of the composition.

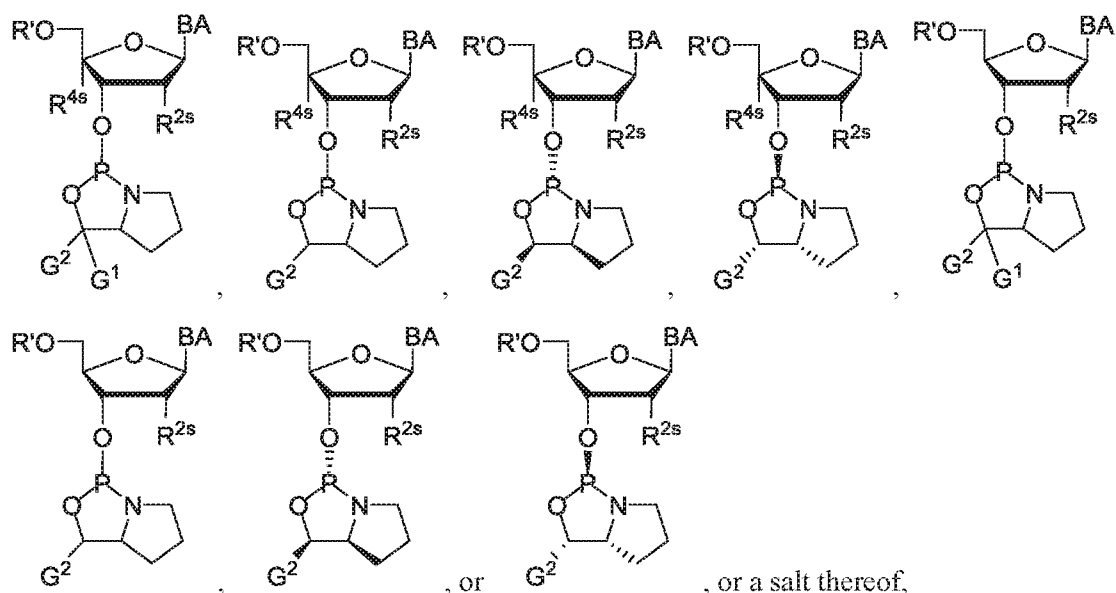
23. The method of claim 21, wherein exon 51 of dystrophin is skipped at an increased level relative to absence of the composition.

24. The method of claim 21, wherein exon 53 of dystrophin is skipped at an increased level relative to absence of the composition.

25. A method for treating muscular dystrophy, Duchenne (Duchenne's) muscular dystrophy (DMD), or Becker (Becker's) muscular dystrophy (BMD), comprising administering to a subject susceptible thereto or suffering therefrom a composition of any one of the preceding claims.

26. A method for preparing an oligonucleotide or an oligonucleotide composition thereof, wherein the oligonucleotide comprises one or more non-negatively charged internucleotidic linkages, comprising providing a phosphoramidite compound having the structure of:





wherein:

R^{5s} is independently R' or $-OR'$;

each BA is independently an optionally substituted group selected from C_{3-30} cycloaliphatic, C_{6-30} aryl, C_{5-30} heteroaryl having 1-10 heteroatoms, C_{3-30} heterocyclyl having 1-10 heteroatoms, a natural nucleobase moiety, and a modified nucleobase moiety;

each R^s is independently $-H$, halogen, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L-R'$, $-L-Si(R)_3$, $-L-OR'$, $-L-SR'$, $-L-N(R')_2$, $-O-L-R'$, $-O-L-Si(R)_3$, $-O-L-OR'$, $-O-L-SR'$, or $-O-L-N(R')_2$;

each s is independently 0-20;

each L^s is independently $-C(R^{5s})_2-$, or L ;

each L is independently a covalent bond, or a bivalent, optionally substituted, linear or branched group selected from a C_{1-30} aliphatic group and a C_{1-30} heteroaliphatic group having 1-10 heteroatoms, wherein one or more methylene units are optionally and independently replaced with C_{1-6} alkylene, C_{1-6} alkenylene, $-C\equiv C-$, a bivalent C_1-C_6 heteroaliphatic group having 1-5 heteroatoms, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, $-C(O)O-$, $-P(O)(OR')-$, $-P(O)(SR')-$, $-P(O)(R')-$, $-P(O)(NR')-$, $-P(S)(OR')-$, $-P(S)(SR')-$, $-P(S)(R')-$, $-P(S)(NR')-$, $-P(R')-$, $-P(OR')-$, $-P(SR')-$, $-P(NR')-$, $-P(OR')[B(R')_3]-$, $-OP(O)(OR')O-$, $-OP(O)(SR')O-$, $-OP(O)(R')O-$, $-OP(O)(NR')O-$, $-OP(OR')O-$, $-OP(SR')O-$, $-OP(NR')O-$, $-OP(R')O-$, or $-OP(OR')[B(R')_3]O-$, and one or more CH or carbon atoms are optionally and independently replaced with Cy^1 ;

each $-Cy-$ is independently an optionally substituted bivalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each Cy^1 is independently an optionally substituted trivalent or tetravalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each Ring A is independently an optionally substituted 3-20 membered monocyclic, bicyclic or polycyclic ring having 0-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon;

each of G^1 , G^2 , G^3 , G^4 , G^5 , and G^8 is independently R^1 ;

each R^1 is independently $-H$, $-L-R'$, halogen, $-CN$, $-NO_2$, $-L-Si(R')_3$, $-OR'$, $-SR'$, or $-N(R')_2$;

each R' is independently $-R$, $-C(O)R$, $-C(O)OR$, or $-S(O)_2R$;

each R is independently $-H$, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms, 5-30 membered heteroaryl having 1-10 heteroatoms, and 3-30 membered heterocyclyl having 1-10 heteroatoms, or

two R groups are optionally and independently taken together to form a covalent bond, or

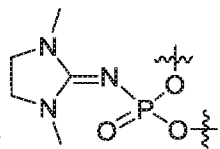
two or more R groups on the same atom are optionally and independently taken together with the atom to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the atom, 0-10 heteroatoms, or

two or more R groups on two or more atoms are optionally and independently taken together with their intervening atoms to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms; and

wherein G^2 comprises an electron-withdrawing group.

27. The method of claim 26, wherein G^5 and one of G^3 and G^4 are taken together to form an optionally substituted 3-8 membered saturated ring having 0-3 heteroatoms in addition to the nitrogen of $-NG^5-$.

28. The method of claim 26, wherein the oligonucleotide comprises an internucleotidic linkage



having the structure of

29. The method of any one of claims 26-28, wherein G^2 comprises an electron-withdrawing group.

30. The method of claim 29, wherein G^2 is $-L'-S(O)_2R'$, wherein L' is optionally substituted $-CH_2-$.

31. The method of claim 30, wherein R' is optionally substituted C_{1-6} aliphatic.

32. The method of claim 30, wherein R' is t-butyl.

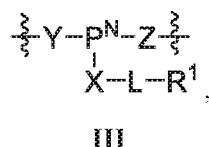
33. The method of claim 30, wherein R' is optionally substituted phenyl.

34. The method of claim 30, wherein R' is phenyl.

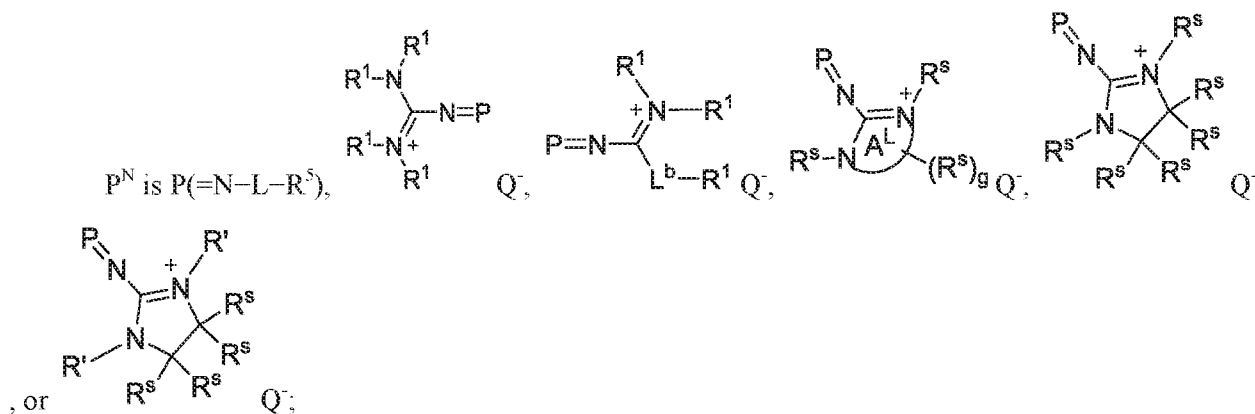
35. The method of claim 29, comprising one or more cycles, each of which independently comprises or consisting of:

- 1) deblocking;
- 2) coupling;
- 3) optionally a first capping;
- 4) modifying; and
- 5) optionally a second capping.

36. An oligonucleotide, comprising an internucleotidic linkage having the structure of formula III:



wherein:



Q^- is an anion;

each of R^1 and R^5 is independently $-\text{H}$, $-\text{L-R}'$, halogen, $-\text{CN}$, $-\text{NO}_2$, $-\text{L-Si(R}^*)_3$, $-\text{OR}'$, $-\text{SR}'$, or $-\text{N(R}^*)_2$;

each of Y and Z is independently $-\text{O-}$, $-\text{S-}$, $-\text{N(-L-R}^5\text{)-}$, or L;

each L is independently a covalent bond, or a bivalent, optionally substituted, linear or branched group selected from a C_{1-30} aliphatic group and a C_{1-30} heteroaliphatic group having 1-10 heteroatoms, wherein one or more methylene units are optionally and independently replaced with C_{1-6} alkylene, C_{1-6} alkenylene, $-\text{C}\equiv\text{C}-$, a bivalent $\text{C}_1\text{-C}_6$ heteroaliphatic group having 1-5 heteroatoms, $-\text{C(R}^*)_2-$, $-\text{Cy-}$, $-\text{O-}$, $-\text{S-}$, $-\text{S-S-}$, $-\text{N(R}^*)-$, $-\text{C(O)-}$, $-\text{C(S)-}$, $-\text{C(NR}^*)-$, $-\text{C(O)N(R}^*)-$, $-\text{N(R}^*)\text{C(O)N(R}^*)-$, $-\text{N(R}^*)\text{C(O)O-}$, $-\text{S(O)-}$, $-\text{S(O)}_2-$, $-\text{S(O)}_2\text{N(R}^*)-$, $-\text{C(O)S-}$, $-\text{C(O)O-}$, $-\text{P(O)(OR}^*)-$, $-\text{P(O)(SR}^*)-$, $-\text{P(O)(R}^*)-$, $-\text{P(O)(NR}^*)-$, $-\text{P(S)(OR}^*)-$, $-\text{P(S)(SR}^*)-$, $-\text{P(S)(R}^*)-$, $-\text{P(S)(NR}^*)-$, $-\text{P(R}^*)-$, $-\text{P(OR}^*)-$, $-\text{P(SR}^*)-$, $-\text{P(NR}^*)-$, $-\text{P(OR}^*)[\text{B(R}^*)_3]-$, $-\text{OP(O)(OR}^*)\text{O-}$, $-\text{OP(O)(SR}^*)\text{O-}$, $-\text{OP(O)(R}^*)\text{O-}$, $-\text{OP(O)(NR}^*)\text{O-}$, $-\text{OP(OR}^*)\text{O-}$, $-\text{OP(SR}^*)\text{O-}$, $-\text{OP(NR}^*)\text{O-}$, $-\text{OP(R}^*)\text{O-}$, or $-\text{OP(OR}^*)[\text{B(R}^*)_3]\text{O-}$, and

one or more CH or carbon atoms are optionally and independently replaced with Cy^L ;

each $-\text{Cy}-$ is independently an optionally substituted bivalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

each Cy^L is independently an optionally substituted trivalent or tetravalent group selected from a C_{3-20} cycloaliphatic ring, a C_{6-20} aryl ring, a 5-20 membered heteroaryl ring having 1-10 heteroatoms, and a 3-20 membered heterocyclyl ring having 1-10 heteroatoms;

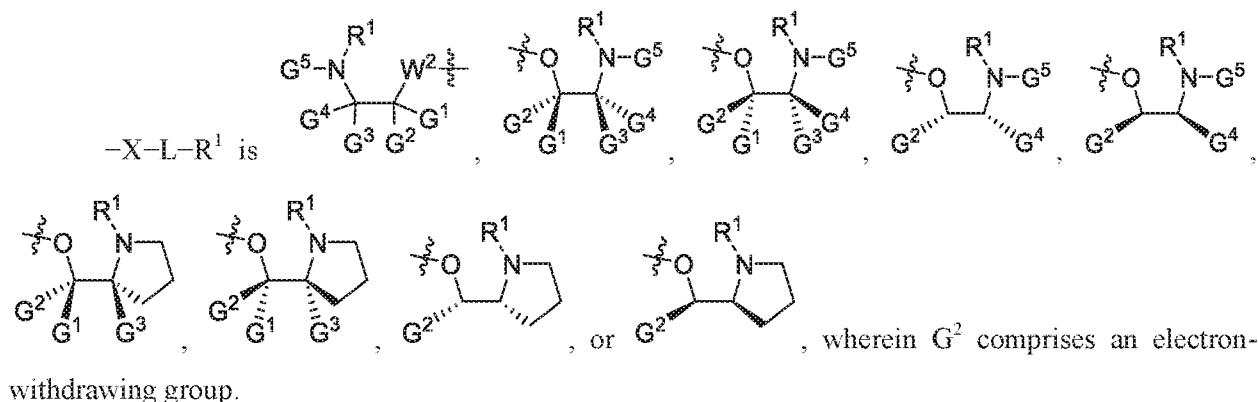
each R' is independently $-\text{R}$, $-\text{C}(\text{O})\text{R}$, $-\text{C}(\text{O})\text{OR}$, or $-\text{S}(\text{O})_2\text{R}$;

each R is independently $-\text{H}$, or an optionally substituted group selected from C_{1-30} aliphatic, C_{1-30} heteroaliphatic having 1-10 heteroatoms, C_{6-30} aryl, C_{6-30} arylaliphatic, C_{6-30} arylheteroaliphatic having 1-10 heteroatoms, 5-30 membered heteroaryl having 1-10 heteroatoms, and 3-30 membered heterocyclyl having 1-10 heteroatoms, or

two R groups are optionally and independently taken together to form a covalent bond, or

two or more R groups on the same atom are optionally and independently taken together with the atom to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the atom, 0-10 heteroatoms, or

two or more R groups on two or more atoms are optionally and independently taken together with their intervening atoms to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms; and



37. The oligonucleotide of claim 36, wherein G^2 is $-\text{L}'-\text{S}(\text{O})_2\text{R}'$, wherein L' is optionally substituted $-\text{CH}_2-$.

38. The oligonucleotide of claim 37, wherein R' is optionally substituted C_{1-6} aliphatic.

39. The oligonucleotide of claim 38, wherein R' is t-butyl.

40. The oligonucleotide of claim 37, wherein R' is optionally substituted phenyl.

41. The oligonucleotide of claim 40, wherein R' is phenyl.

42. The oligonucleotide of any one of claims 36-41, wherein R^1 is $-\text{C}(\text{O})\text{R}'$.

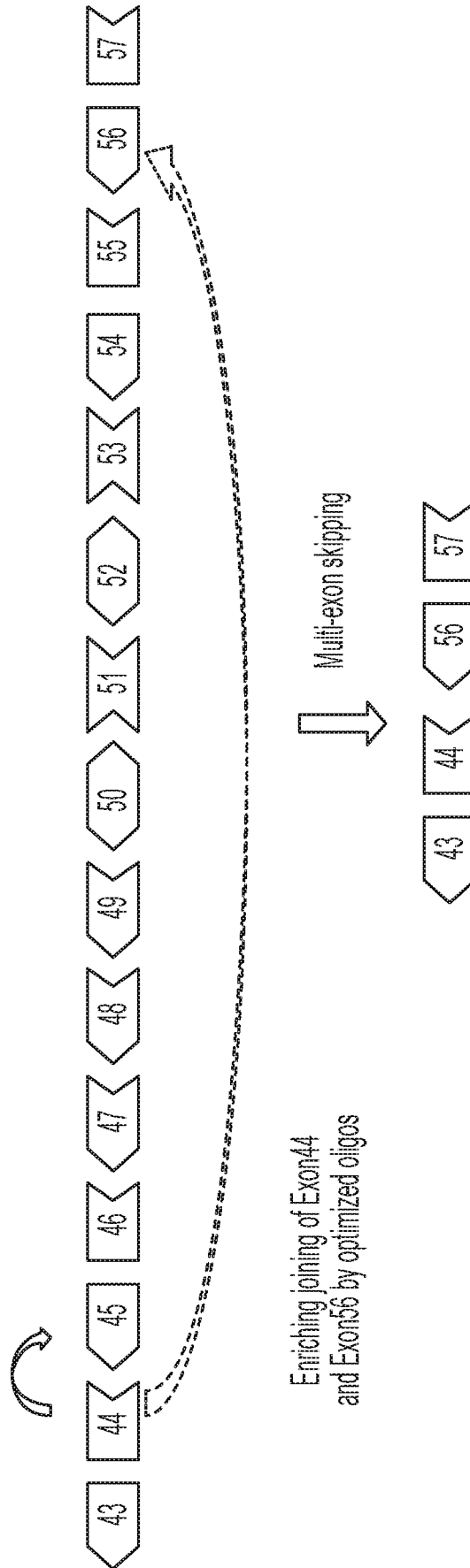
43. The oligonucleotide of claim 42, wherein R' is $-\text{CH}_3$.
44. The oligonucleotide of any one of claims 36-41, wherein Q⁻ is F⁻, Cl⁻, Br⁻, BF₄⁻, PF₆⁻, TfO⁻, Tf₂N⁻, AsF₆⁻, ClO₄⁻, or SbF₆⁻.
45. The oligonucleotide of any one of claims 36-44, wherein the oligonucleotide is attached to a solid support.
46. The oligonucleotide of claim 45, wherein the solid support is CPG.
47. A method for preparing an oligonucleotide, comprising contacting an oligonucleotide of any one of claims 36-46 with a base.
48. The method of claim 47, wherein the contact is performed substantially absent of water.
49. The method of claim 47 or 48, wherein the contact is after the oligonucleotide length is achieved before deprotection and cleavage of oligonucleotide.
50. The method of any one of claims 47-49, wherein the base is an amine base having the structure of NR₃.
51. The method of claim 50, wherein the base is *N,N*-diethylamine.
52. The oligonucleotide, compound or method of any one of Example Embodiments 1-420.
53. An oligonucleotide, wherein the oligonucleotide is, WV-20104, WV-20103, WV-20102, WV-20101, WV-20100, WV-20099, WV-20098, WV-20097, WV-20096, WV-20095, WV-20094, WV-20106, WV-20119, WV-20118, WV-13739, WV-13740, WV-9079, WV-9082, WV-9100, WV-9096, WV-9097, WV-9106, WV-9133, WV-9148, WV-9154, WV-9898, WV-9899, WV-9900, WV-9906, WV-9907, WV-9908, WV-9909, WV-9756, WV-9757, WV-9517, WV-9714, WV-9715, WV-9519, WV-9521, WV-9747, WV-9748, WV-9749, WV-9897, WV-9898, WV-9900, WV-9899, WV-9906, WV-9912, WV-9524, WV-9912, WV-9906, WV-9900, WV-9899, WV-9899, WV-9898, WV-9898, WV-9898, WV-9898, WV-9897, WV-9897, WV-9897, WV-9897, WV-9897, WV-9747, WV-9714, WV-9699, WV-9517, WV-9517, WV-13409, WV-13408, WV-12887, WV-12882, WV-12881, WV-12880, WV-12880, WV-WV12880, WV-12878, WV-12877, WV-12877, WV-12876, WV-12873, WV-12872, WV-12559, WV-12559, WV-12558, WV-12558, WV-12557, WV-12556, WV-12556, WV-12555, WV-12555, WV-12554, WV-12553, WV-12129, WV-12127, WV-12125, WV-12123, WV-11342, WV-11342, WV-11341, WV-11341, WV-11340, WV-10672, WV-10671, WV-10670, WV-10461, WV-10455, WV-9897, WV-9898, WV-13826, WV-13827, WV-13835, WV-12880, WV-14344, WV-13864, WV-13835, WV-14791, WV-14344, WV-13754, WV-13766, WV-11086, WV-11089, WV-17859, WV-17860, WV-20070, WV-20073, WV-20076, WV-20052, WV-20099, WV-20049, WV-20085, WV-20087, WV-20034, WV-20046, WV-20052, WV-20061, WV-20064, WV-20067, WV-20092, WV-20091, WV-20093, WV-20084, WV-9738, WV-9739, WV-9740, WV-9741, WV-15860, WV-15862, WV-11084, WV-11086, WV-11088, WV-11089, WV-14522, WV-14523, WV-17861, WV-

17862, WV-13815, WV-13816, WV-13817, WV-13780, WV-17862, WV-17863, WV-17864, WV-17865, WV-17866, WV-20082, WV-20081, WV-20080, WV-20079, WV-20076, WV-20075, WV-20074, WV-20073, WV-20072, WV-20071, WV-20064, WV-20059, WV-20058, WV-20057, WV-20056, WV-20053, WV-20052, WV-20051, WV-20050, WV-20049, WV-20094, WV-20095, or a salt form thereof.

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FIG. 1

Normally Exon44 is joined by
Exon 45 on DMD



Enriching joining of Exon44
and Exon56 by optimized oligos

FIG. 2

Monitor splicing variants and frequency of each variant

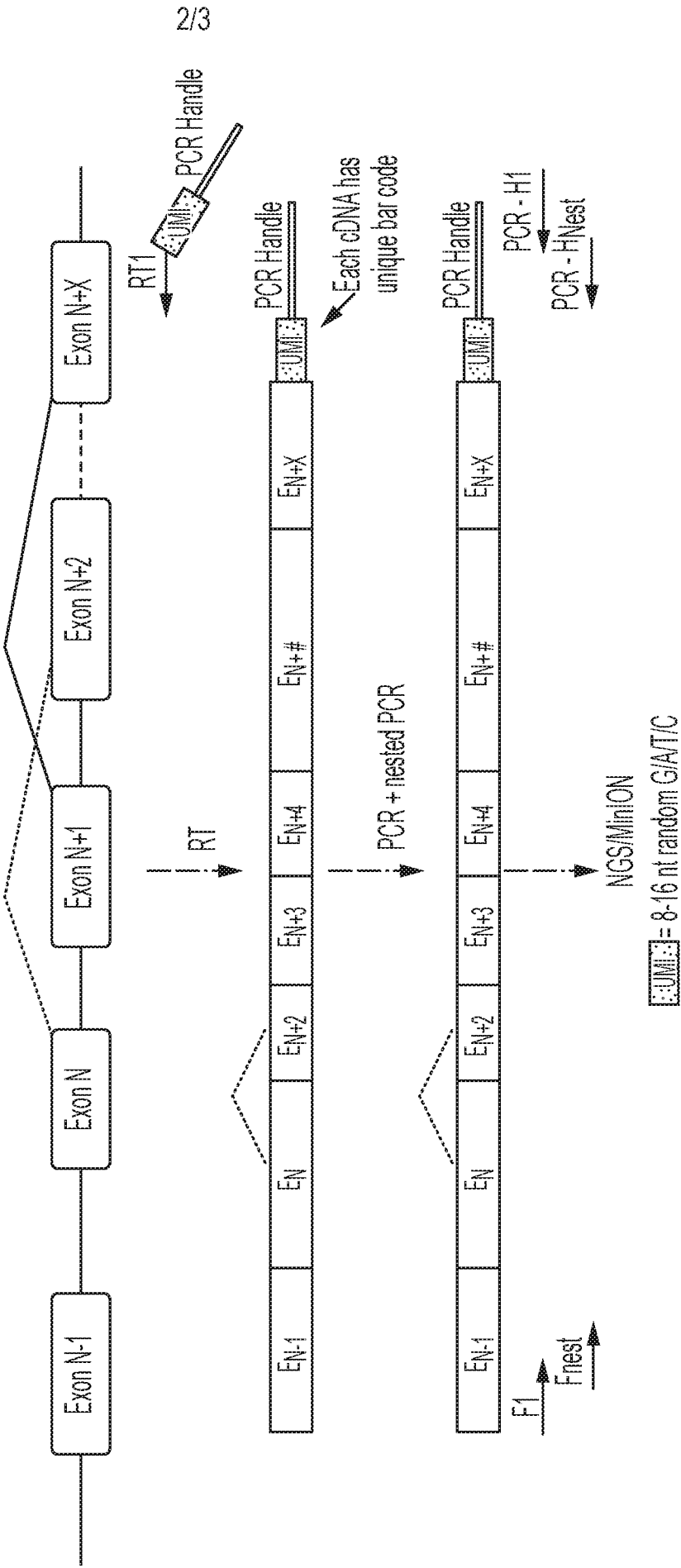
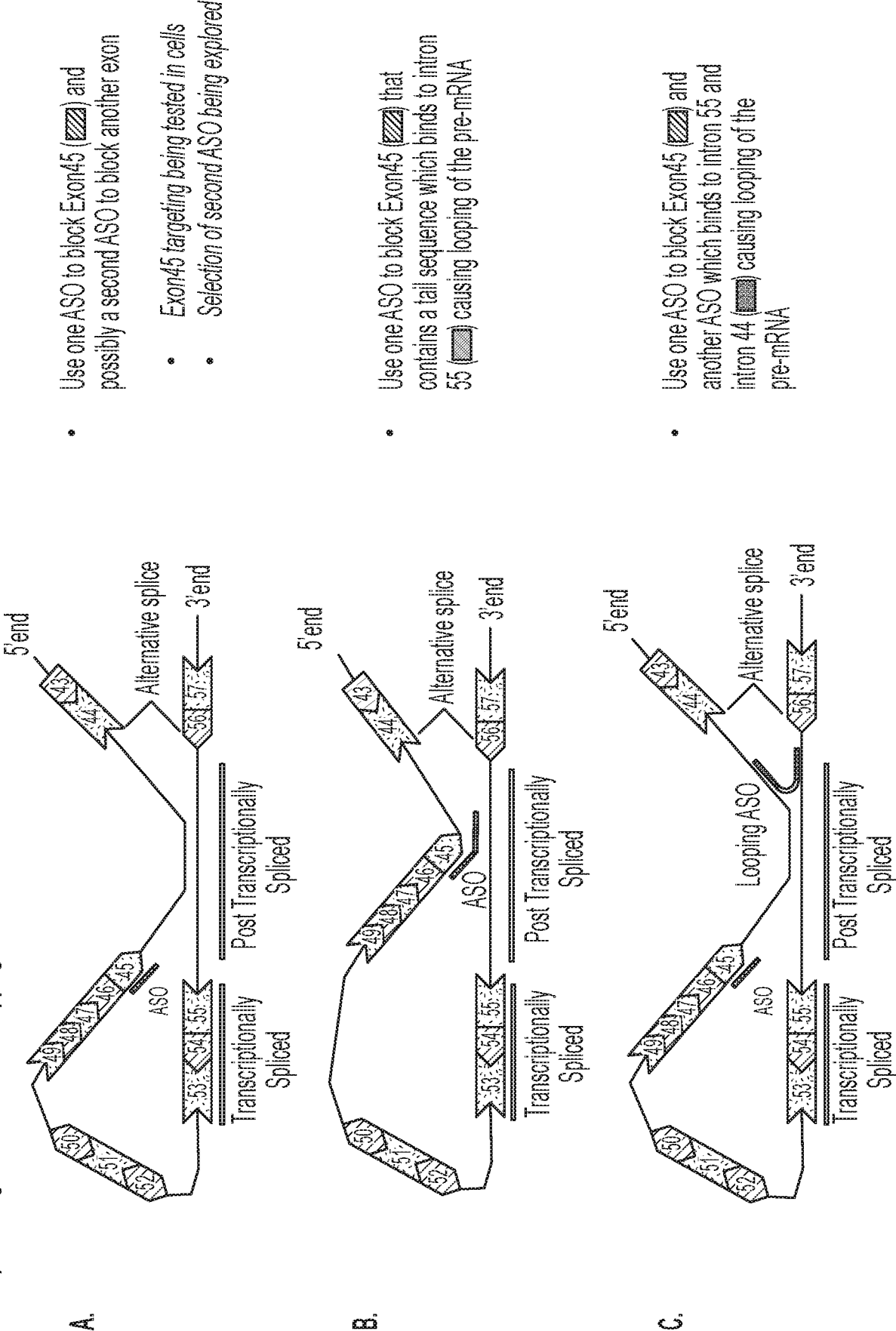
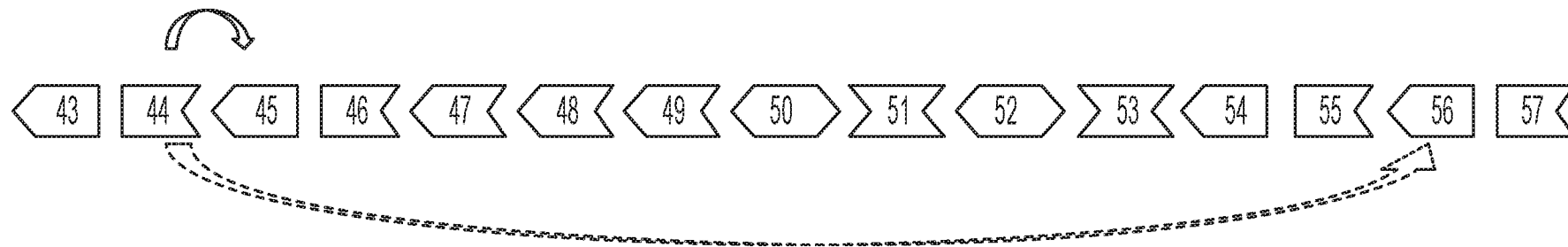


FIG. 3

Example strategies for multi-exon skipping



Normally Exon44 is joined by
Exon 45 on DMD



Enriching joining of Exon44
and Exon56 by optimized oligos



Multi-exon skipping

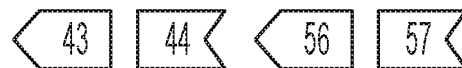


FIG. 1