OLIGONUCLEOTIDE COMPOSITIONS AND METHODS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to United States Provisional Application Serial No. 63/586,377, filed September 28, 2023, the entirety of which is incorporated herein by reference.

BACKGROUND

[0002] Oligonucleotides are useful in various applications, e.g., therapeutic, diagnostic, and/or research applications. For example, oligonucleotides targeting various genes can be useful for treatment of conditions, disorders or diseases related to such target genes.

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 a significant impact on oligonucleotide properties, e.g., exon skipping (e.g., of exon 52 of DMD), toxicities, stability, protein binding characteristics, etc. In some embodiments, the present disclosure provides oligonucleotides that, among other things, have certain numbers of PN linkages at certain locations. Such PN linkages, in combination with other structure features (e.g., 2'-F modified sugars, 2'-OMe modified sugars, phosphorothioate internucleotidic linkages, natural phosphate linkages, linkage phosphorus stereochemistry, etc.) of such oligonucleotides, can provide useful therapeutic profiles, such as pharmacokinetic and pharmacodynamic profiles including delivery, distribution, stability, exon skipping efficiency, toxicity, etc., for such oligonucleotides to be utilized for treating conditions, disorders or diseases including DMD.

[0004] In some embodiments, the present disclosure provides an oligonucleotide or an oligonucleotide composition capable of mediating skipping of an exon, e.g., exon 52, of the DMD gene and useful for treating muscular dystrophy. In some embodiments, an oligonucleotide or an oligonucleotide composition is useful for treatment of muscular dystrophy. In some embodiments, an oligonucleotide is an oligonucleotide disclosed herein (e.g., in Table 1, e.g., in Table 1A, Table 1B, Table 1C). In some embodiments, an oligonucleotide composition is a composition comprising an oligonucleotide disclosed herein (e.g., in Table 1, e.g., in Table 1A, Table 1B, Table 1C). In some embodiments, an oligonucleotide composition is a chirally controlled oligonucleotide composition comprising an oligonucleotide disclosed herein (e.g., in Table 1, e.g., in Table 1A, Table 1B, Table 1C).

[0005] Among other things, the present disclosure provides oligonucleotides and compositions thereof that have demonstrated various advantages and improvements. For example, as demonstrated herein, certain oligonucleotides and compositions have demonstrated not only high exon skipping levels, but also high levels

of stability and low toxicity when assessed in a variety of assays.

[0006] In some embodiments, the present disclosure provides an oligonucleotide, wherein the base sequence of the oligonucleotide comprises 15 or more contiguous nucleobases, e.g., 20 contiguous nucleobases, of a complement of a DMD transcript, wherein the oligonucleotide comprises a PN (e.g., a phosphoryl guanidine, e.g., n001) internucleotidic linkage between the 5' terminal (+1) nucleoside and the immediately downstream (+2) nucleoside in the *R*p configuration, a PN internucleotidic linkage between the +3 nucleoside and the +4 nucleoside in the *R*p configuration, a PN internucleotidic linkage between the +6 nucleoside and the +7 nucleoside in the *R*p configuration, and a PN internucleotidic linkage between the +17 nucleoside and the +18 nucleoside in the *R*p configuration, and wherein the sugar moiety of the 5' terminal (+1) nucleoside comprises a 2'-OMe modification. In some embodiments, the present disclosure provides an oligonucleotide, wherein the base sequence of the oligonucleotide comprises 15 or more contiguous nucleosides, e.g., 20 contiguous nucleosides, of a complement of a DMD transcript, wherein the oligonucleotide comprises a PN internucleotidic linkage between the 5' terminal (+1) nucleoside and the immediately downstream (+2) nucleoside in the *R*p configuration, a PN internucleotidic linkage between the +3 nucleoside and the +4 nucleoside in the *R*p configuration, a PN internucleotidic linkage between the +6 nucleoside and the +7 nucleoside in the *R*p configuration, and a PN internucleotidic linkage between the +17 nucleoside and the +18 nucleoside in the *R*p configuration, and wherein the sugar moiety of the 5' terminal (+1) nucleoside comprises a 2'-F modification. In some embodiments, the oligonucleotide comprises a PS (e.g., a phosphorothioate) linkage between the +2 nucleoside and the +3 nucleoside in the *S*p configuration, a PS linkage between the +4 nucleoside and the +5 nucleoside in the *S*p configuration, a PS linkage between the +5 nucleoside and the +6 nucleoside in the *S*p configuration, a PS linkage between the +8 nucleoside and the +9 nucleoside in the *S*p configuration, a PS linkage between the +9 nucleoside and the +10 nucleoside in the *S*p configuration, a PS linkage between the +10 nucleoside and the +11 nucleoside in the *S*p configuration, a PS linkage between the +11 nucleoside and the +12 nucleoside in the *S*p configuration, a PS linkage between the +13 nucleoside and the +14 nucleoside in the *S*p configuration, a PS linkage between the +14 nucleoside and the +15 nucleoside in the *S*p configuration, a PS linkage between the +15 nucleoside and the +16 nucleoside in the *S*p configuration, a PS linkage between the +16 nucleoside and the +17 nucleoside in the *S*p configuration, and a PS linkage between the +18 nucleoside and the +19 nucleoside in the *S*p configuration. In some embodiments, the oligonucleotide comprises a PS linkage between the 3' terminal (N) nucleoside and the penultimate (N-1) nucleoside in the *S*p configuration. In some embodiments, the oligonucleotide comprises a PN linkage between the 3' terminal (N) nucleoside and the penultimate (N-1) nucleoside in the *R*p configuration. In some embodiments, the sugar moiety of the +7 nucleoside comprises a 2'-OMe modification, the sugar moiety of the +10 nucleoside comprises a 2'-OMe modification, the sugar moiety of the +12 nucleoside, comprises a 2'-OMe modification, and the sugar moiety of the +13 nucleoside comprises a 2'-OMe modification. In some embodiments, the sugar moiety of the 3' terminal (N) nucleoside, comprises a 2'-OMe modification. In some embodiments, a PN internucleotidic linkage is a phosphoryl guanidine internucleotidic linkage. In some

embodiments, a PN internucleotidic linkage is n001. In some embodiments, each PN internucleotidic linkage is n001. In some embodiments, each PN internucleotidic linkage is *R*p n001. In some embodiments, a PS linkage is a phosphorothioate internucleotidic linkage. In some embodiments, each PS linkage is a phosphorothioate internucleotidic linkage. In some embodiments, each PS linkage is a *S*p phosphorothioate internucleotidic linkage.

[0007] In some embodiments, the present disclosure provides an oligonucleotide, wherein the base sequence of the oligonucleotide comprises 15 or more contiguous nucleobases, e.g., 20 contiguous nucleobases, of a complement of a DMD transcript, wherein the oligonucleotide comprises a PN (e.g., a phosphoryl guanidine, e.g., n001) internucleotidic linkage between the 5' terminal (+1) nucleoside and the immediately downstream (+2) nucleoside in the *R*p configuration, a PN internucleotidic linkage between the +3 nucleoside and the +4 nucleoside in the *R*p configuration, a PN internucleotidic linkage between the +6 nucleoside and the +7 nucleoside in the *R*p configuration, and a PN internucleotidic linkage between the +17 nucleoside and the +18 nucleoside in the *R*p configuration, and wherein the sugar moiety of the 5' terminal (+1) nucleoside comprises a 2'-OMe modification. In some embodiments, the present disclosure provides an oligonucleotide, wherein the base sequence of the oligonucleotide comprises 15 or more contiguous nucleosides, e.g., 20 contiguous nucleosides, of a complement of a DMD transcript, wherein the oligonucleotide comprises a PN internucleotidic linkage between the 5' terminal (+1) nucleoside and the immediately downstream (+2) nucleoside in the *R*p configuration, a PN internucleotidic linkage between the +3 nucleoside and the +4 nucleoside in the *R*p configuration, a PN internucleotidic linkage between the +6 nucleoside and the +7 nucleoside in the *R*p configuration, and a PN internucleotidic linkage between the +17 nucleoside and the +18 nucleoside in the *R*p configuration, and wherein the sugar moiety of the 5' terminal (+1) nucleoside comprises a 2'-F modification. In some embodiments, the oligonucleotide comprises a phosphorothioate (PS) linkage between the +2 nucleoside and the +3 nucleoside in the *S*p configuration, a PS linkage between the +4 nucleoside and the +5 nucleoside in the *S*p configuration, a PS linkage between the +5 nucleoside and the +6 nucleoside in the *S*p configuration, a PS linkage between the +8 nucleoside and the +9 nucleoside in the *S*p configuration, a PS linkage between the +9 nucleoside and the +10 nucleoside in the *S*p configuration, a PS linkage between the +10 nucleoside and the +11 nucleoside in the *S*p configuration, a PS linkage between the +11 nucleoside and the +12 nucleoside in the *S*p configuration, a PS linkage between the +13 nucleoside and the +14 nucleoside in the *S*p configuration, a PS linkage between the +14 nucleoside and the +15 nucleoside in the *S*p configuration, a PS linkage between the +15 nucleoside and the +16 nucleoside in the *S*p configuration, a PS linkage between the +16 nucleoside and the +17 nucleoside in the *S*p configuration, and a PS linkage between the +18 nucleoside and the +19 nucleoside in the *S*p configuration. In some embodiments, the oligonucleotide comprises a PS linkage between the 3' terminal (N) nucleoside and the penultimate (N-1) nucleoside in the *S*p configuration. In some embodiments, the oligonucleotide comprises a PN linkage between the 3' terminal (N) nucleoside and the penultimate (N-1) nucleoside in the *R*p configuration. In some embodiments, the sugar moiety of the +7 nucleoside comprises a 2'-OMe modification, the sugar moiety of

the +10 nucleoside comprises a 2'-OMe modification, the sugar moiety of the +12 nucleoside, comprises a 2'-OMe modification, and the sugar moiety of the +13 nucleoside comprises a 2'-OMe modification. In some embodiments, the sugar moiety of the 3' terminal (N) nucleoside comprises a 2'-OMe modification.

[0008] In some embodiments, an oligonucleotide is selected from

[fl2r](U)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A),

[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](C)[Ssp].m(A)[Ssp].[fl2r](A)[Ssp].m(A)p.m(U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](U),

[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G),

[fl2r](U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A),

[fl2r](C)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A),

[fl2r](C)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G),

[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[n001R].[fl2r](G),

[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](

C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C),

[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C),

[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C),

m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

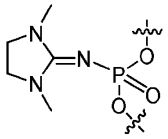
m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].m(G),

m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A),

m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].m(A),

[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C), and

m(C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C), or a salt thereof,

wherein: [fl2r] represents a 2'-F modified nucleoside; m represents a 2'-OMe modified nucleoside; p represents a phosphodiester; [Ssp] represents a phosphorothioate in the *S*p configuration; and [n001R] represents  wherein the phosphorus is in the *R*p configuration.

[0009] In some embodiments, as demonstrated herein, provided technologies (e.g., oligonucleotides, compositions, methods, etc.) are particularly useful for reducing levels of a mutant mRNA (e.g., a DMD transcript comprising a deleterious mutation) and/or proteins encoded thereby, and increasing levels of repaired mRNA (e.g., a DMD transcript in which exon 52 is skipped to delete, correct or compensate for a deleterious mutation) and/or proteins encoded thereby.

[0010] In some embodiments, provided technologies are particularly useful for modulating splicing of DMD 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 DMD transcripts (e.g., mutant DMD transcripts), e.g., pre-mRNA, RNA, etc., and in many instances, reducing levels of products arising from or encoded by such DMD transcripts such as mRNA, polypeptides, proteins, etc. In some embodiments, a pre-mRNA or mRNA or RNA is transported from one cellular compartment (e.g., nucleus, cytoplasm, etc.) to another, and/or has been modified by one or more enzyme.

[0011] For example, in some embodiments, a dystrophin gene can comprise an exon comprising one or more mutations associated with 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, while maintaining or restoring the reading frame so that a shorter (e.g., truncated, e.g., internally truncated) but partially functional dystrophin (e.g., a variant) can be produced.

[0012] In some embodiments, the present disclosure provides an oligonucleotide, wherein the base sequence of the oligonucleotide comprises 10 or more contiguous nucleobases of a complement of a DMD transcript and the oligonucleotide comprises 3 or more PN internucleotidic linkages. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases of a complement of a DMD transcript. In some embodiments, a base sequence of an oligonucleotide comprises 20 or more contiguous nucleobases of a complement of a DMD transcript. In some embodiments, a base sequence of an oligonucleotide is the same as an equal length portion in a complement of a DMD transcript. In some embodiments, the present disclosure provides an oligonucleotide, wherein the base sequence of the oligonucleotide comprises 10 or more contiguous nucleobases that are complementary to an equal length portion in a DMD transcript and the oligonucleotide comprises 3 or more PN internucleotidic linkages. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases that are complementary to an equal length portion in a DMD transcript. In some embodiments, a base sequence of an oligonucleotide comprises 20 or more contiguous nucleobases that are complementary to an equal length portion in a DMD transcript. In some embodiments, a base sequence of the oligonucleotide is complementary to the base sequence of an equal length portion in a DMD transcript. In some embodiments, the present disclosure provides an oligonucleotide, wherein the oligonucleotide can hybridize to an equal length portion in a DMD transcript and the oligonucleotide comprises 3 or more PN internucleotidic linkages. In some embodiments, a DMD transcript is a DMD mRNA. In some embodiments, a DMD transcript or a wild-type version thereof encodes dystrophin. In some embodiments, a DMD transcript comprises a mutation that is amenable to exon 52 skipping.

[0013] In some embodiments, each nucleobase of an oligonucleotide is independently an optionally

substituted nucleobase selected from A, T, C, G and U, or an optionally substituted tautomer of a nucleobase selected from A, T, C, G and U. In some embodiments, each nucleobase of an oligonucleotide is independently A, T, C, 5mC, G or U. In some embodiments, length of an oligonucleotide is 20 or more nucleobases. In some embodiments, length of an oligonucleotide is 20 nucleobases.

[0014] In some embodiments, an oligonucleotide comprises 3, 4, 5, 6 or more PN internucleotidic linkages. In some embodiments, an oligonucleotide comprises 4, 5, 6 or more PN internucleotidic linkages. In some embodiments, an oligonucleotide comprises 3 or more PN internucleotidic linkages. In some embodiments, an oligonucleotide comprises 4 or more PN internucleotidic linkages. In some embodiments, an oligonucleotide comprises 5 or more PN internucleotidic linkages. In some embodiments, an oligonucleotide comprises 6 or more PN internucleotidic linkages. In some embodiments, an oligonucleotide comprises 3, 4, 5, 6 or more *R*p PN internucleotidic linkages. In some embodiments, an oligonucleotide comprises 4, 5, 6 or more *R*p PN internucleotidic linkages. In some embodiments, an oligonucleotide comprises 3 or more *R*p PN internucleotidic linkages. In some embodiments, an oligonucleotide comprises 4 or more *R*p PN internucleotidic linkages. In some embodiments, an oligonucleotide comprises 5 or more *R*p PN internucleotidic linkages. In some embodiments, an oligonucleotide comprises 6 or more *R*p PN internucleotidic linkages. In some embodiments, each PN internucleotidic linkage is a *R*p PN internucleotidic linkage. In some embodiments, an oligonucleotide comprises 3, 4, 5, 6 or more phosphoryl guanidine internucleotidic linkages. In some embodiments, an oligonucleotide comprises 4, 5, 6 or more phosphoryl guanidine internucleotidic linkages. In some embodiments, an oligonucleotide comprises 3 or more phosphoryl guanidine internucleotidic linkages. In some embodiments, an oligonucleotide comprises 4 or more phosphoryl guanidine internucleotidic linkages. In some embodiments, an oligonucleotide comprises 5 or more phosphoryl guanidine internucleotidic linkages. In some embodiments, an oligonucleotide comprises 6 or more phosphoryl guanidine internucleotidic linkages. In some embodiments, each PN internucleotidic linkage is a phosphoryl guanidine internucleotidic linkage. In some embodiments, an oligonucleotide comprises 3, 4, 5, 6 or more *R*p phosphoryl guanidine internucleotidic linkages. In some embodiments, an oligonucleotide comprises 4, 5, 6 or more *R*p phosphoryl guanidine internucleotidic linkages. In some embodiments, an oligonucleotide comprises 3 or more *R*p phosphoryl guanidine internucleotidic linkages. In some embodiments, an oligonucleotide comprises 4 or more *R*p phosphoryl guanidine internucleotidic linkages. In some embodiments, an oligonucleotide comprises 5 or more *R*p phosphoryl guanidine internucleotidic linkages. In some embodiments, an oligonucleotide comprises 6 or more *R*p phosphoryl guanidine internucleotidic linkages. In some embodiments, each phosphoryl guanidine internucleotidic linkage is a *R*p phosphoryl guanidine internucleotidic linkage. In some embodiments, an oligonucleotide comprises 3, 4, 5, 6 or more n001 internucleotidic linkages. In some embodiments, an oligonucleotide comprises 4, 5, 6 or more n001 internucleotidic linkages. In some embodiments, an oligonucleotide comprises 3 or more n001 internucleotidic linkages. In some embodiments, an oligonucleotide comprises 4 or more n001 internucleotidic linkages. In some embodiments, an oligonucleotide comprises 5 or more n001 internucleotidic

linkages. In some embodiments, an oligonucleotide comprises 6 or more n001 internucleotidic linkages. In some embodiments, each phosphoryl guanidine internucleotidic linkage is a n001 internucleotidic linkage. In some embodiments, an oligonucleotide comprises 3, 4, 5, 6 or more *R*p n001 internucleotidic linkages. In some embodiments, an oligonucleotide comprises 4, 5, 6 or more *R*p n001 internucleotidic linkages. In some embodiments, an oligonucleotide comprises 3 or more *R*p n001 internucleotidic linkages. In some embodiments, an oligonucleotide comprises 4 or more *R*p n001 internucleotidic linkages. In some embodiments, an oligonucleotide comprises 5 or more *R*p n001 internucleotidic linkages. In some embodiments, an oligonucleotide comprises 6 or more *R*p n001 internucleotidic linkages. In some embodiments, each n001 internucleotidic linkage is a *R*p n001 internucleotidic linkage.

[0015] In some embodiments, an internucleotidic linkage between the first and the second nucleosides, the third and the fourth nucleosides, the sixth and the seventh nucleosides, the 17th and the 18th nucleosides, and/or the last two nucleosides (unless otherwise noted, 5' to 3' direction) of an oligonucleotide is a PN linkage. In some embodiments, an internucleotidic linkage between the first and the second nucleosides, the third and the fourth nucleosides, the sixth and the seventh nucleosides, and the 17th and the 18th nucleosides of an oligonucleotide is a PN linkage. In some embodiments, an internucleotidic linkage between the third and the fourth nucleosides, the sixth and the seventh nucleosides, the 17th and the 18th nucleosides, and the last two nucleosides of an oligonucleotide is a PN linkage. In some embodiments, an internucleotidic linkage between the first and the second nucleosides of an oligonucleotide is a PN linkage. In some embodiments, an internucleotidic linkage between the third and the fourth nucleosides of an oligonucleotide is a PN linkage. In some embodiments, an internucleotidic linkage between the sixth and the seventh nucleosides of an oligonucleotide is a PN linkage. In some embodiments, an internucleotidic linkage between the 17th and the 18th nucleosides of an oligonucleotide is a PN linkage. In some embodiments, an internucleotidic linkage between the last two nucleosides of an oligonucleotide is a PN linkage.

[0016] In some embodiments, an oligonucleotide comprises a PS internucleotidic linkage. In some embodiments, an oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more PS internucleotidic linkages. In some embodiments, an oligonucleotide comprises a PS internucleotidic linkage. In some embodiments, an oligonucleotide comprises a *S*p PS internucleotidic linkage. In some embodiments, an oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more *S*p PS internucleotidic linkages. In some embodiments, each PS internucleotidic linkage is *S*p. In some embodiments, each PS internucleotidic linkage is independently a phosphorothioate internucleotidic linkage. In some embodiments, an oligonucleotide comprises a phosphorothioate internucleotidic linkage. In some embodiments, an oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more phosphorothioate internucleotidic linkages. In some embodiments, an oligonucleotide comprises a *S*p phosphorothioate internucleotidic linkage. In some embodiments, an oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more *S*p phosphorothioate internucleotidic linkages. In some embodiments, each phosphorothioate internucleotidic linkage is independently *S*p.

[0017] In some embodiments, an internucleotidic linkage between the first and the second nucleosides, the second and the third nucleosides, the fourth and the fifth nucleosides, the fifth and the sixth nucleosides, the eighth and the ninth nucleosides, the ninth and the tenth nucleosides, the 10th and the 11th nucleosides, the 11th and the 12th nucleosides, the 13th and the 14th nucleosides, the 14th and the 15th nucleosides, the 15th and the 16th nucleosides, the 16th and the 17th nucleosides, the 18th and the 19th nucleosides, and/or the last two nucleosides (unless otherwise noted, 5' to 3' direction) of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the second and the third nucleosides, the fourth and the fifth nucleosides, the fifth and the sixth nucleosides, the eighth and the ninth nucleosides, the ninth and the tenth nucleosides, the 10th and the 11th nucleosides, the 11th and the 12th nucleosides, the 13th and the 14th nucleosides, the 14th and the 15th nucleosides, the 15th and the 16th nucleosides, the 16th and the 17th nucleosides, the 18th and the 19th nucleosides, and the last two nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the first and the second nucleosides, the second and the third nucleosides, the fourth and the fifth nucleosides, the fifth and the sixth nucleosides, the eighth and the ninth nucleosides, the ninth and the tenth nucleosides, the 10th and the 11th nucleosides, the 11th and the 12th nucleosides, the 13th and the 14th nucleosides, the 14th and the 15th nucleosides, the 15th and the 16th nucleosides, the 16th and the 17th nucleosides, and the 18th and the 19th nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the first and the second nucleosides of an oligonucleotide, if it is not a PN linkage, is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the second and the third nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the fourth and the fifth nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the fifth and the sixth nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the eighth and the ninth nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the ninth and the tenth nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the 10th and the 11th nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the 11th and the 12th nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the 13th and the 14th nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the 14th and the 15th nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the 15th and the 16th nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the 16th and the 17th nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the 18th and the 19th nucleosides of an oligonucleotide is a PS internucleotidic linkage. In some embodiments, an internucleotidic linkage between the last two nucleosides of an oligonucleotide, if it is

not a PN linkage, is a PS internucleotidic linkage.

[0018] In some embodiments, an oligonucleotide comprises a PO internucleotidic linkage. In some embodiments, an oligonucleotide comprises 2, 3, 4, 5 or more PO internucleotidic linkages. In some embodiments, each PO internucleotidic linkage is independently a natural phosphate linkage. In some embodiments, an oligonucleotide comprises a natural phosphate internucleotidic linkage. In some embodiments, an oligonucleotide comprises 2, 3, 4, 5 or more natural phosphate internucleotidic linkages. In some embodiments, an internucleotidic linkage between the seventh and the eighth nucleoside of an oligonucleotide is a PO internucleotidic linkage. In some embodiments, an internucleotidic linkage between the 12th and the 13th nucleoside of an oligonucleotide is a PO internucleotidic linkage.

[0019] In some embodiments, an oligonucleotide comprises a sugar modification. In some embodiments, an oligonucleotide comprises a 2'-modified sugar. In some embodiments, an oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more 2'-modified sugars. In some embodiments, an oligonucleotide comprises a 2'-F modified sugar. In some embodiments, an oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more 2'-F modified sugars. In some embodiments, sugar of nucleosides 1, 2, 3, 4, 5, 6, 8, 9, 11, 14, 15, 16, 17, 18, 19 and 20 of an oligonucleotide are 2'-F modified sugar. In some embodiments, an oligonucleotide comprises a 2'-OR modified sugar, wherein R is optionally substituted C_1-C_6 aliphatic. In some embodiments, an oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 or more 2'-OR modified sugars, wherein R is optionally substituted C_1-C_6 aliphatic. In some embodiments, sugar of nucleosides 7, 10, 12 and 13 of an oligonucleotide are independently 2'-OR modified sugars, wherein R is optionally substituted C_1-C_6 aliphatic. In some embodiments, an oligonucleotide comprises a 2'-OMe modified sugar. In some embodiments, an oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 or more 2'-OMe modified sugars. In some embodiments, an oligonucleotide comprises 2'-OR modified sugar is a 2'-OMe modified sugar.

[0020] In some embodiments, an oligonucleotide can hybridize to a region of a DMD transcript. In some embodiments, a region of a DMD transcript comprises GGCUUCAACUAUCUGAGUGA, wherein each U is optionally and independently replaced with T. In some embodiments, a region of a DMD transcript comprises CCAGUUGGAAGAACUCAUUA, wherein each U is optionally and independently replaced with T. In some embodiments, a region of a DMD transcript comprises CCCCAGUUGGAAGAACUCAU, wherein each U is optionally and independently replaced with T. In some embodiments, a region of a DMD transcript comprises UGCAGGAUUUGGAACAGAGG, wherein each U is optionally and independently replaced with T. In some embodiments, a region of a DMD transcript comprises AUGCAGGAUUUGGAACAGAG, wherein each U is optionally and independently replaced with T. In some embodiments, a region of a DMD transcript comprises CAGGAUUUGGAACAGAGGCG, wherein each U is optionally and independently replaced with T. In some embodiments, a region of a DMD transcript comprises GGAAGAACUCAUUACCGCUG, wherein each U is optionally and independently replaced with T.

[0021] In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of a DMD transcript. In some

embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of a DMD transcript. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of a DMD transcript. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of GGCUUCAACUAUCUGAGUGA, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of GGCUUCAACUAUCUGAGUGA, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of GGCUUCAACUAUCUGAGUGA, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of CCAGUUGGAAGAACUCAUUA, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of CCAGUUGGAAGAACUCAUUA, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 20 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of CCAGUUGGAAGAACUCAUUA, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of CCCCAGUUGGAAGAACUCAU, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of CCCCAGUUGGAAGAACUCAU, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of CCCCAGUUGGAAGAACUCAU, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of UGCAGGAUUUGGAACAGAGG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of UGCAGGAUUUGGAACAGAGG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of UGCAGGAUUUGGAACAGAGG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of AUGCAGGAUUUGGAACAGAG, wherein each U is optionally and independently replaced with T. In some

embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of AUGCAGGAUUUGGAACAGAG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of AUGCAGGAUUUGGAACAGAG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of CAGGAUUUGGAACAGAGGCG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of CAGGAUUUGGAACAGAGGCG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of CAGGAUUUGGAACAGAGGCG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of GGAAGAACUCAUUACCGCUG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of GGAAGAACUCAUUACCGCUG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of GGAAGAACUCAUUACCGCUG, wherein each U is optionally and independently replaced with T.

[0022] In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases of UCACUCAGAUAGUUGAAGCC, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases of UCACUCAGAUAGUUGAAGCC, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises UCACUCAGAUAGUUGAAGCC, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide is UCACUCAGAUAGUUGAAGCC. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases of UAAUGAGUUCUUCCAACUGG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases of UAAUGAGUUCUUCCAACUGG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises UAAUGAGUUCUUCCAACUGG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide is UAAUGAGUUCUUCCAACUGG. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases of AUGAGUUCUUCCAACUGGGG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an

oligonucleotide comprises 15 or more contiguous nucleobases of AUGAGUUCUUCCAACUGGGG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises AUGAGUUCUUCCAACUGGGG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide is AUGAGUUCUUCCAACUGGGG. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases of CCUCUGUUCCAAAUCCUGCA, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases of CCUCUGUUCCAAAUCCUGCA, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises CCUCUGUUCCAAAUCCUGCA, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide is CCUCUGUUCCAAAUCCUGCA. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases of CUCUGUUCCAAAUCCUGCAU, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases of CUCUGUUCCAAAUCCUGCAU, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises CUCUGUUCCAAAUCCUGCAU, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide is CUCUGUUCCAAAUCCUGCAU. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases of CGCCUCUGUUCCAAAUCCUG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases of CGCCUCUGUUCCAAAUCCUG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises CGCCUCUGUUCCAAAUCCUG, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide is CGCCUCUGUUCCAAAUCCUG. In some embodiments, a base sequence of an oligonucleotide comprises 10 or more contiguous nucleobases of CAGCGGUAAUGAGUUCUUCC, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises 15 or more contiguous nucleobases of CAGCGGUAAUGAGUUCUUCC, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide comprises CAGCGGUAAUGAGUUCUUCC, wherein each U is optionally and independently replaced with T. In some embodiments, a base sequence of an oligonucleotide is CAGCGGUAAUGAGUUCUUCC.

[0023] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising an oligonucleotide described herein, wherein the composition is enriched, relative to a substantially racemic preparation of the oligonucleotide, for the oligonucleotide. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides each of which is an oligonucleotide described herein, wherein oligonucleotides of the plurality

share the same constitution and at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of all oligonucleotides in the composition that share the same constitution are oligonucleotides of the plurality. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising an oligonucleotide described herein, wherein at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of all oligonucleotides in the composition that share the same constitution as the oligonucleotide are the oligonucleotide. In some embodiments, the present disclosure provides an oligonucleotide composition comprising a plurality of oligonucleotides, wherein oligonucleotides of the plurality share 1) a common base sequence and 2) the same linkage phosphorus stereochemistry independently at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more chiral internucleotidic linkages, wherein oligonucleotides of the plurality are an oligonucleotide described herein and wherein at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the oligonucleotides in the composition that share the common base sequence are oligonucleotides of the plurality. In some embodiments, oligonucleotides of plurality share the same linkage phosphorus stereochemistry independently at each chiral internucleotidic linkage. In some embodiments, the present disclosure provides an oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of an oligonucleotide described herein (e.g., in Table 1, e.g., in Table 1A, Table 1B, Table 1C). In some embodiments, a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of all oligonucleotides that share the same base sequence as an oligonucleotide having the structure or a pharmaceutically acceptable salt thereof. In some embodiments, a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of all oligonucleotides that share the same constitution as an oligonucleotide having the structure or a pharmaceutically acceptable salt thereof. In some embodiments, a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% based on UV peak area at 260 nm.

[0024] In some embodiments, the present disclosure provides a pharmaceutical composition comprising an oligonucleotide or oligonucleotide composition described herein. In some embodiments, a pharmaceutical composition comprises one or more pharmaceutically acceptable salts of an oligonucleotide. In some embodiments, a pharmaceutical composition is a solution.

[0025] In some embodiments, the present disclosure provides methods using oligonucleotides and compositions described herein. In some embodiments, the present disclosure provides a method for altering splicing of a DMD transcript in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition described herein. In some embodiments, the present disclosure provides a method for providing DMD exon skipping in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition described herein. In some embodiments, the present disclosure provides a method for increasing level of DMD polypeptide in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition described

herein. In some embodiments, the present disclosure provides a method for increasing level of DMD activity in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition described herein. In some embodiments, the present disclosure provides a method In some embodiments, the present disclosure provides a method for treating muscular dystrophy, comprising administering or delivering to a subject suffering therefrom an effective amount of an oligonucleotide or composition described herein.

[0026] In some embodiments, the present disclosure provides a method for manufacturing or preparing an oligonucleotide or composition described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] Figure 1. Provided oligonucleotides can provide dose-dependent exon skipping. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed. H2K mdx cells were plated at 40,000 cells/well in 24 well plates coated with matrigel and differentiation media and allowed to differentiate for 4 days. Following 4 days, differentiated cells were gymnotically treated with 3 uM, 1 uM, or 0.3 uM of indicated oligonucleotides targeting DMD by refreshing the differentiation medium. After 3 hours of incubation, oligonucleotide-containing medium was aspirated and replaced with fresh differentiation medium. Cells were allowed to differentiate for a further 4 days and subsequently harvested. RNA was collected and transcribed into cDNA. Exon skipping was quantified by qPCR. From top to bottom for each oligonucleotide, horizontal bars represent data from treatment with 3 uM, 1 uM, or 0.3 uM of indicated oligonucleotide. Error bars represent SEM.

[0028] Figure 2. Provided oligonucleotides can provide exon skipping. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed. Patient-derived immortalized fibroblasts containing a deletion in DMD exon 51 were engineered to express MyoD from a Tet-inducible promoter to drive transdifferentiation to muscle fate. To transdifferentiate, cells were seeded at a pre-determined density on matrigel-coated plates in appropriate medium on day -1. At day 2, cells were dosed gymnotically with 10 uM, 5 uM, or 2.5 uM of indicated oligonucleotides. After 16 hours, a complete media change was performed to remove oligonucleotides and cells were allowed to continue to grow. At day 9, cells were harvested and RNA was collected and transcribed into cDNA. Exon skipping was quantified using qPCR. From left to right for each oligonucleotide, vertical bars represent data from treatment with 10 uM, 5 uM, or 2 uM of indicated oligonucleotide. % skipping was calculated by dividing skipped DMD transcript by total DMD transcript.

[0029] Figure 3. Provided oligonucleotides can provide increased levels of protein encoded by target transcripts. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and

stereochemistry and patterns thereof were designed and assessed. Patient-derived immortalized fibroblasts containing a deletion in DMD exon 51 were engineered to express MyoD from a Tet-inducible promoter to drive transdifferentiation to muscle fate. To transdifferentiate, cells were seeded at a pre-determined density on matrigel-coated plates in appropriate medium on day -1. At day 2, cells were dosed gymnotically with 10 uM, 5 uM, or 2.5 uM of indicated oligonucleotides. After 16 hours, a complete media change was performed to remove oligonucleotides and cells were allowed to continue to grow. At day 9, cells were harvested and lysates collected using RIPA buffer for protein analysis. Dystrophin protein levels were quantified using Jess capillary electrophoresis. % dystrophin relative to wild-type (WT) was calculated by normalizing to vinculin levels and mapping to a dystrophin protein standard curve generated from wild-type transdifferentiated fibroblast lysate from a healthy donor.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0030] Technologies of the present disclosure may be understood more readily by reference to the following detailed description of certain embodiments.

Definitions

[0031] 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.

[0032] As used herein in the present disclosure, unless otherwise clear from context, (i) the term "a" or "an" may be understood to mean "at least one"; (ii) the term "or" may be understood to mean "and/or"; (iii) the terms "comprising", "comprise", "including" (whether used with "not limited to" or not), and "include" (whether used with "not limited to" or not) may be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; (iv) the term "another" may be understood to mean at least an additional/second one or more; (v) the terms "about" and "approximately" may be understood to permit standard variation as would be understood by those of ordinary skill in the art; and (vi) where ranges are provided, endpoints are included.

[0033] Unless otherwise specified, description of oligonucleotides and elements thereof (e.g., base sequence, sugar modifications, internucleotidic linkages, linkage phosphorus stereochemistry, patterns thereof, etc.) is from 5' to 3'. As those skilled in the art will appreciate, in some embodiments, oligonucleotides may be provided and/or utilized as salt forms, particularly pharmaceutically acceptable salt forms, e.g., sodium salts. As those skilled in the art will also appreciate, in some embodiments, individual oligonucleotides within a composition may be considered to be of the same constitution and/or structure even though, within such

composition (e.g., a liquid composition), particular such oligonucleotides might be in different salt form(s) (and may be dissolved and the oligonucleotide chain may exist as an anion form when, e.g., in a liquid composition) at a particular moment in time. For example, those skilled in the art will appreciate that, at a given pH, individual internucleotidic linkages along an oligonucleotide chain may be in an acid (H) form, or in one of a plurality of possible salt forms (e.g., a sodium salt, or a salt of a different cation, depending on which ions might be present in the preparation or composition), and will understand that, so long as their acid forms (e.g., replacing all cations, if any, with H^+) are of the same constitution and/or structure, such individual oligonucleotides may properly be considered to be of the same constitution and/or structure.

[0034] Aliphatic: As used herein, "aliphatic" 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 (but not aromatic), or a substituted or unsubstituted monocyclic, bicyclic, or polycyclic hydrocarbon ring that is completely saturated or that contains one or more units of unsaturation (but not aromatic), or combinations thereof. In some embodiments, aliphatic groups contain 1-50 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. 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.

[0035] Alkenyl: As used herein, the term "alkenyl" refers to an aliphatic group, as defined herein, having one or more double bonds.

[0036] 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, 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., C_1-C_{20} for straight chain, C_2-C_{20} 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., C_1-C_4 for straight chain lower alkyls).

[0037] Alkynyl: As used herein, the term "alkynyl" refers to an aliphatic group, as defined herein, having one or more triple bonds.

[0038] 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; etc.

[0039] 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.

[0040] 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 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, each monocyclic ring unit is aromatic. 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 is 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 a group in which an aromatic ring is fused to one or more non–aromatic rings, such as indanyl, phthalimidyl, naphthimidyl, phenanthridinyl, or tetrahydronaphthyl, and the like.

[0041] Characteristic portion: As used herein, the term "characteristic portion", in the broadest sense, refers to a portion of a substance whose presence (or absence) correlates with presence (or absence) of a particular feature, attribute, or activity of the substance. In some embodiments, a characteristic portion of a substance is a portion that is found in the substance and in related substances that share the particular feature, attribute or activity, but not in those that do not share the particular feature, attribute or activity. In certain embodiments, a characteristic portion shares at least one functional characteristic with the intact substance. For example, in some embodiments, a "characteristic portion" of a protein or polypeptide is one that contains a continuous stretch of amino acids, or a collection of continuous stretches of amino acids, that together are characteristic of a protein or polypeptide. In some embodiments, each such continuous stretch generally contains at least 2, 5, 10, 15, 20, 50, or more amino acids. In general, a characteristic portion of a substance (*e.g.*, of a protein, antibody, *etc.*) is one that, in addition to the sequence and/or structural identity specified above, shares at least one functional characteristic with the relevant intact substance. In some embodiments, a characteristic portion may be biologically active.

[0042] Chiral control: As used herein, "chiral control" refers to control of the stereochemical designation of

the chiral linkage phosphorus in a chiral internucleotidic linkage within an oligonucleotide. As used herein, a chiral internucleotidic linkage is an internucleotidic linkage whose linkage phosphorus is chiral. 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, 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 will appreciate 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 each chiral internucleotidic linkage within an oligonucleotide is controlled.

[0043] Chirally controlled oligonucleotide composition: The terms "chirally controlled oligonucleotide composition", "chirally controlled nucleic acid composition", and the like, as used herein, refers to a composition that comprises a plurality of oligonucleotides (or nucleic acids) which share a common base sequence, wherein the plurality of oligonucleotides (or nucleic acids) share the same linkage phosphorus stereochemistry at one or more chiral internucleotidic linkages (chirally controlled or stereodefined internucleotidic linkages, whose chiral linkage phosphorus is Rp or Sp in the composition ("stereodefined"), not a random Rp and Sp mixture as non-chirally controlled internucleotidic linkages). In some embodiments, a chirally controlled oligonucleotide composition comprises a plurality of oligonucleotides (or nucleic acids) that share: 1) a common base sequence, 2) a common pattern of backbone linkages, and 3) a common pattern of backbone phosphorus modifications, wherein the plurality of oligonucleotides (or nucleic acids) share the same linkage phosphorus stereochemistry at one or more chiral internucleotidic linkages (chirally controlled or stereodefined internucleotidic linkages, whose chiral linkage phosphorus is Rp or Sp in the composition ("stereodefined"), 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 pre-determined/controlled or enriched (e.g., through chirally controlled oligonucleotide preparation to stereoselectively form one or more chiral internucleotidic linkages) compared to a random level in a non-chirally controlled oligonucleotide composition. In some embodiments, about 1%-100%, (e.g., about 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%, 99%, or 100%, 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 1%-100%, (e.g., about 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%, 99%, or 100%, 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, a level is about 1%-100%, (e.g., about 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%, 99%, or 100%, 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 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, or of all oligonucleotides in a composition that share a common base sequence, a common patter 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. 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 1%-100% (e.g., about 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, oligonucleotides (or nucleic acids) of a plurality share the same pattern of sugar and/or nucleobase modifications, in any. In some embodiments, oligonucleotides (or nucleic acids) of a plurality are various forms of the same oligonucleotide (e.g., acid and/or various salts of the same oligonucleotide). In some embodiments, oligonucleotides (or nucleic acids) of a plurality are of the same constitution. In some embodiments, level of the oligonucleotides (or nucleic acids) of the plurality is about 1%-100%, (e.g., about 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%, 99%, or 100%, 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 (or nucleic acids) in a composition that share the same constitution as the oligonucleotides (or nucleic acids) of the plurality. 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, oligonucleotides (or nucleic acids) of a plurality are structurally identical. In some embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 80%, 85%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%, typically at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%. In some embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 95%. In some embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 96%. In some embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 97%. In some embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 98%. In some embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 99%. In some embodiments, a percentage of a level is or is at least $(DS)^{nc}$, wherein DS is a diastereopurity as described in the present disclosure (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% or more) and nc is the number of chiral linkage phosphorus as described in the present disclosure (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 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). In some embodiments, a percentage of a level is or is at least $(DS)^{nc}$, wherein DS is a diastereopurity as described in the present disclosure (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% or more) and nc is the number of chirally controlled internucleotidic linkages as described in the present disclosure (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 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). In some embodiments, a percentage of a level is or is at least $(DS)^{nc}$, wherein DS is 95%-100%. For example, when DS is 99% and nc is 10, the percentage is or is at least 90% ($(99\%)^{10} \approx 0.90 = 90\%$). In some embodiments, level of a plurality of oligonucleotides in a composition is represented as the product of the diastereopurity of each chiral linkage phosphorus. In some embodiments, level of a plurality of oligonucleotides in a composition is represented as the product of the diastereopurity of each chirally controlled internucleotidic linkage in the oligonucleotides. In some embodiments, diastereopurity of an internucleotidic linkage connecting two nucleosides in an oligonucleotide (or nucleic acid) is represented by the diastereopurity of an internucleotidic linkage of a dimer connecting the same two nucleosides, wherein the dimer is prepared using comparable conditions, in some instances, identical synthetic cycle conditions (e.g., for the linkage between Nx and Ny in an oligonucleotide ….NxNy….., the dimer is NxNy). 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 non-chirally controlled internucleotidic linkage has a diastereopurity of less than about 80%, 75%, 70%, 65%, 60%, 55%, or of about 50%, as typically observed in stereorandom oligonucleotide compositions (e.g., as appreciated by those skilled in the art, from traditional oligonucleotide synthesis, e.g., the phosphoramidite method). In some embodiments, oligonucleotides (or nucleic acids) of a plurality are of the same type. In some embodiments, a chirally controlled oligonucleotide composition comprises non-random or controlled levels of individual oligonucleotide or nucleic acids types. For instance, in some embodiments a chirally controlled oligonucleotide composition comprises one and no more than one oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide composition comprises more than one oligonucleotide type. In some

embodiments, a chirally controlled oligonucleotide composition comprises multiple oligonucleotide types. In some embodiments, a chirally controlled oligonucleotide composition is a composition of oligonucleotides of an oligonucleotide type, which composition comprises a non-random or controlled level of a plurality of oligonucleotides of the oligonucleotide type.

[0044] 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.

[0045] 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 tetrahydronaphthyl. 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 C_3-C_6 monocyclic hydrocarbon, or C_8-C_{10} bicyclic or polycyclic hydrocarbon, that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule, or a C_9-C_{16} polycyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule.

[0046] Heteroaliphatic: The term "heteroaliphatic", as used herein, is given its ordinary meaning in the art and refers to aliphatic groups as described herein in which one or more carbon atoms are independently replaced with one or more heteroatoms (e.g., oxygen, nitrogen, sulfur, silicon, phosphorus, and the like). In some embodiments, one or more units selected from C, CH, CH_2, and CH_3 are independently replaced by one or more heteroatoms (including oxidized and/or substituted forms thereof). In some embodiments, a heteroaliphatic group is heteroalkyl. In some embodiments, a heteroaliphatic group is heteroalkenyl.

[0047] Heteroalkyl: The term "heteroalkyl", as used herein, is given its ordinary meaning in the art and refers to alkyl groups as described herein in which one or more carbon atoms are independently replaced with one or more heteroatoms (e.g., oxygen, nitrogen, sulfur, silicon, phosphorus, and the like). Examples of heteroalkyl

groups include, but are not limited to, alkoxy, poly(ethylene glycol)-, alkyl-substituted amino, tetrahydrofuranyl, piperidinyl, morpholinyl, etc.

[0048] 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 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, each monocyclic ring unit is aromatic. 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, indolizinyl, 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, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4H–quinolizinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, 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.

[0049] Heteroatom: The term "heteroatom", as used herein, means an atom that is not carbon or hydrogen. In some embodiments, a heteroatom is boron, oxygen, sulfur, nitrogen, phosphorus, or silicon (including oxidized forms of nitrogen, sulfur, phosphorus, or silicon; charged forms of nitrogen (e.g., quaternized forms, forms as in iminium groups, etc.), phosphorus, sulfur, oxygen; etc.). In some embodiments, a heteroatom is silicon, phosphorus, oxygen, sulfur or nitrogen. In some embodiments, a heteroatom is silicon, oxygen, sulfur or nitrogen. In some embodiments, a heteroatom is oxygen, sulfur or nitrogen.

[0050] 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 groups in which a heterocyclyl ring is 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.

[0051] Identity: As used herein, the term "identity" refers to the overall relatedness between polymeric molecules, e.g., between nucleic acid molecules (e.g., oligonucleotides, DNA, RNA, etc.) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be "substantially identical" to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical. Calculation of the percent identity of two nucleic acid or polypeptide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or substantially 100% of the length of a reference sequence. The nucleotides at corresponding positions are then compared. When a position in the first sequence is occupied by the same residue (e.g., nucleotide or amino acid) as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4: 11-17), which has been incorporated into the ALIGN program (version 2.0). In some exemplary embodiments, nucleic acid sequence comparisons made with the ALIGN program use a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix.

[0052] Internucleotidic linkage: As used herein, the phrase "internucleotidic linkage" refers generally to a

linkage linking nucleoside units of an oligonucleotide or a nucleic acid. In some embodiments, an internucleotidic linkage is a phosphodiester linkage, as extensively found in naturally occurring DNA and RNA molecules (natural phosphate linkage (–OP(=O)(OH)O–), which as appreciated by those skilled in the art may exist as a salt form). In some embodiments, an internucleotidic linkage is a modified internucleotidic linkage (not a natural phosphate linkage). In some embodiments, an internucleotidic linkage is a "modified internucleotidic linkage" wherein at least one oxygen atom or –OH of a phosphodiester linkage is replaced by a different organic or inorganic moiety. In some embodiments, such an organic or inorganic moiety is selected from =S, =Se, =NR', –SR', –SeR', $–N(R')_2$, $B(R')_3$, –S–, –Se–, and –N(R')–, wherein each R' is independently as defined and described in the present disclosure. In some embodiments, an internucleotidic linkage is a phosphotriester linkage, phosphorothioate linkage (or phosphorothioate diester linkage, –OP(=O)(SH)O–, which as appreciated by those skilled in the art may exist as a salt form), or phosphorothioate triester linkage. In some embodiments, a modified internucleotidic linkage is a phosphorothioate linkage. In some embodiments, an internucleotidic linkage is one of, e.g., PNA (peptide nucleic acid) or PMO (phosphorodiamidate Morpholino oligomer) linkage. In some embodiments, a modified internucleotidic linkage is a non-negatively charged internucleotidic linkage. In some embodiments, a modified internucleotidic linkage is a neutral internucleotidic linkage (e.g., n001 in certain provided oligonucleotides). 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, a modified internucleotidic linkages is a modified internucleotidic linkages designated as s, s1, s2, s3, s4, s5, s6, s7, s8, s9, s10, s11, s12, s13, s14, s15, s16, s17 and s18 as described in WO 2017/210647.

[0053] 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).

[0054] In vivo: As used herein, the term "in vivo" refers to events that occur within an organism (e.g., animal, plant and/or microbe).

[0055] Linkage phosphorus: as defined herein, the phrase "linkage phosphorus" is used to indicate that the particular phosphorus atom being referred to is the phosphorus atom present in the internucleotidic linkage, which phosphorus atom corresponds to the phosphorus atom of a phosphodiester internucleotidic linkage as occurs in naturally occurring DNA and RNA. In some embodiments, a linkage phosphorus atom is in a modified internucleotidic linkage, wherein each oxygen atom of a phosphodiester linkage is optionally and independently replaced by an organic or inorganic moiety. In some embodiments, a linkage phosphorus atom is chiral (e.g., as in phosphorothioate internucleotidic linkages). In some embodiments, a linkage phosphorus atom is achiral (e.g., as in natural phosphate linkages).

[0056] 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 substituted A, T, C, G, or U, or a substituted tautomer of A, T, C, G, or U. In some embodiments, a modified nucleobase in the context of oligonucleotides refer to a nucleobase that is not A, T, C, G or U.

[0057] Modified nucleoside: The term "modified nucleoside" refers to a moiety 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.

[0058] 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.

[0059] *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, as described in the present disclosure, a modified sugar is substituted ribose or deoxyribose. In some embodiments, a modified sugar comprises a 2'-modification. Examples of useful 2'-modification are widely utilized in the art and described herein. In some embodiments, a 2'-modification is 2'-F. In some embodiments, a 2'-modification is 2'-OR, wherein R is optionally substituted C_{1-10} aliphatic. In some embodiments, a 2'-modification is 2'-OMe. In some embodiments, a 2'-modification is 2'-MOE. In some embodiments, a modified sugar is a bicyclic sugar (e.g., a sugar used in LNA, BNA, etc.). In some embodiments, in the context of oligonucleotides, a modified sugar is a sugar that is not ribose or deoxyribose as typically found in natural RNA or DNA.

[0060] Nucleic acid: The term "nucleic acid", as used herein, includes any nucleotides and polymers thereof. The term "polynucleotide", as used herein, refers to a polymeric form of nucleotides of any length, either ribonucleotides (RNA) or deoxyribonucleotides (DNA) or a combination thereof. These terms refer to the primary structure of the molecules and, thus, include double- and single-stranded DNA, and double- and single-stranded RNA. These terms include, as equivalents, analogs of either RNA or DNA comprising modified nucleotides and/or 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 internucleotidic linkages. The term encompasses nucleic acids containing any combinations of nucleobases, modified nucleobases, sugars, modified sugars, phosphate bridges or modified 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.

[0061] 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 naturally-occurring nucleobases are modified adenine, guanine, uracil, cytosine, or thymine. In some embodiments, a naturally-occurring nucleobases are methylated adenine, guanine, uracil, cytosine, or thymine. In some embodiments, a nucleobase comprises a heteroaryl ring wherein a ring atom is nitrogen, and when in a nucleoside, the nitrogen is bonded to a sugar moiety. In some embodiments, a nucleobase comprises a heterocyclic ring wherein a ring atom is nitrogen, and when in a nucleoside, the nitrogen is bonded to a sugar moiety. In some embodiments, a nucleobase is a "modified nucleobase," a nucleobase other than adenine (A), guanine (G), uracil (U), cytosine (C), and thymine (T). In some embodiments, a modified nucleobase is substituted A, T, C, G or U. In some embodiments, a modified nucleobase is a substituted tautomer of A, T, C, G, or U. In some embodiments, a modified nucleobases is methylated adenine, guanine, uracil, cytosine, or thymine. In some embodiments, a 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 optionally substituted A, T, C, G, or U, or an optionally substituted tautomer of A, T, C, G, or U. In some embodiments, a "nucleobase" refers to a nucleobase unit in an oligonucleotide or a nucleic acid (e.g., A, T, C, G or U as in an oligonucleotide or a nucleic acid).

[0062] Nucleoside: The term "nucleoside" refers to a moiety wherein a nucleobase or a modified nucleobase is covalently bound to a sugar or a modified sugar. In some embodiments, a nucleoside is a natural nucleoside,

e.g., adenosine, deoxyadenosine, guanosine, deoxyguanosine, thymidine, uridine, cytidine, or deoxycytidine. In some embodiments, a nucleoside is a modified nucleoside, e.g., a substituted natural nucleoside selected from adenosine, deoxyadenosine, guanosine, deoxyguanosine, thymidine, uridine, cytidine, and deoxycytidine. In some embodiments, a nucleoside is a modified nucleoside, e.g., a substituted tautomer of a natural nucleoside selected from adenosine, deoxyadenosine, guanosine, deoxyguanosine, thymidine, uridine, cytidine, and deoxycytidine. In some embodiments, a "nucleoside" refers to a nucleoside unit in an oligonucleotide or a nucleic acid.

[0063] Nucleotide: The term "nucleotide" as used herein refers to a monomeric unit of a polynucleotide that consists of a nucleobase, a sugar, and one or more internucleotidic linkages (e.g., phosphate linkages in natural DNA and RNA). The naturally occurring bases [guanine, (G), adenine, (A), cytosine, (C), thymine, (T), and uracil (U)] are derivatives of purine or pyrimidine, though it should be understood that naturally and non-naturally occurring base analogs are also included. The naturally occurring sugar is the pentose (five-carbon sugar) deoxyribose (which forms DNA) or ribose (which forms RNA), though it should be understood that naturally and non-naturally occurring sugar analogs are also included. Nucleotides are linked via internucleotidic linkages to form nucleic acids, or polynucleotides. Many internucleotidic linkages are known in the art (such as, though not limited to, phosphate, phosphorothioates, boranophosphates and the like). Artificial nucleic acids include PNAs (peptide nucleic acids), phosphotriesters, phosphorothionates, H-phosphonates, phosphoramidates, boranophosphates, methylphosphonates, phosphonoacetates, thiophosphonoacetates and other variants of the phosphate backbone of native nucleic acids, such as those described herein. In some embodiments, a natural nucleotide comprises a naturally occurring base, sugar and internucleotidic linkage. As used herein, the term "nucleotide" also encompasses structural analogs used in lieu of natural or naturally-occurring nucleotides, such as modified nucleotides and nucleotide analogs. In some embodiments, a "nucleotide" refers to a nucleotide unit in an oligonucleotide or a nucleic acid.

[0064] Oligonucleotide: The term "oligonucleotide" refers to a polymer or oligomer of nucleotides, and may contain any combination of natural and non-natural nucleobases, sugars, and internucleotidic linkages.

[0065] Oligonucleotides can be single-stranded or double-stranded. A single-stranded oligonucleotide can have double-stranded regions (formed by two portions of the single-stranded oligonucleotide) and a double-stranded oligonucleotide, which comprises two oligonucleotide chains, can have single-stranded regions for example, at regions where the two oligonucleotide chains are not complementary to each other. 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 RNAi agents and other RNA interference reagents (RNAi agents or iRNA agents), shRNA, antisense oligonucleotides, ribozymes, microRNAs, microRNA mimics, supermirs, aptamers, antimirs, antagomirs, Ul adaptors, triplex-forming oligonucleotides, G-quadruplex oligonucleotides, RNA activators, immuno-stimulatory oligonucleotides, and decoy oligonucleotides.

[0066] Oligonucleotides of the present disclosure can be of various lengths. In particular embodiments,

oligonucleotides can range from about 2 to about 200 nucleosides in length. In various related embodiments, oligonucleotides, single-stranded, double-stranded, or 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, an oligonucleotide is from about 9 to about 39 nucleosides in length. In some embodiments, an oligonucleotide is from about 25 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 26 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 27 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 28 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 29 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 30 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 31 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 32 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 25 to about 60 nucleosides in length. In some embodiments, an oligonucleotide is from about 25 to about 50 nucleosides in length. In some embodiments, an oligonucleotide is from about 25 to about 40 nucleosides in length. In some embodiments, an oligonucleotide is from about 30 to about 40 nucleosides in length. In some embodiments, the oligonucleotide is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleosides in length. In some embodiments, an oligonucleotide is at least 4 nucleosides in length. In some embodiments, an oligonucleotide is at least 5 nucleosides in length. In some embodiments, an oligonucleotide is at least 6 nucleosides in length. In some embodiments, an oligonucleotide is at least 7 nucleosides in length. In some embodiments, an oligonucleotide is at least 8 nucleosides in length. In some embodiments, an oligonucleotide is at least 9 nucleosides in length. In some embodiments, an oligonucleotide is at least 10 nucleosides in length. In some embodiments, an oligonucleotide is at least 11 nucleosides in length. In some embodiments, an oligonucleotide is at least 12 nucleosides in length. In some embodiments, an oligonucleotide is at least 15 nucleosides in length. In some embodiments, an oligonucleotide is at least 15 nucleosides in length. In some embodiments, an oligonucleotide is at least 16 nucleosides in length. In some embodiments, an oligonucleotide is at least 17 nucleosides in length. In some embodiments, an oligonucleotide is at least 18 nucleosides in length. In some embodiments, an oligonucleotide is at least 19 nucleosides in length. In some embodiments, an oligonucleotide is at least 20 nucleosides in length. In some embodiments, an oligonucleotide is at least 25 nucleosides in length. In some embodiments, an oligonucleotide is at least 26 nucleosides in length. In some embodiments, an oligonucleotide is at least 27 nucleosides in length. In some embodiments, an oligonucleotide is at least 28 nucleosides in length. In some embodiments, an oligonucleotide is at least 29 nucleosides in length. In some embodiments, an oligonucleotide is at least 30 nucleosides in length. In some embodiments, an oligonucleotide is at least 31 nucleosides in length. In some embodiments, an oligonucleotide is at least 32 nucleosides in length. In some embodiments, an oligonucleotide is at least 33 nucleosides in length. In some embodiments, an oligonucleotide is at least 34 nucleosides in length. In some embodiments, an oligonucleotide is at least 35 nucleosides in length. In some embodiments,

an oligonucleotide is at least 36 nucleosides in length. In some embodiments, an oligonucleotide is at least 37 nucleosides in length. In some embodiments, an oligonucleotide is at least 38 nucleosides in length. In some embodiments, an oligonucleotide is at least 39 nucleosides in length. In some embodiments, an oligonucleotide is at least 40 nucleosides in length. In some embodiments, an oligonucleotide is 25 nucleosides in length. In some embodiments, an oligonucleotide is 26 nucleosides in length. In some embodiments, an oligonucleotide is 27 nucleosides in length. In some embodiments, an oligonucleotide is 28 nucleosides in length. In some embodiments, an oligonucleotide is 29 nucleosides in length. In some embodiments, an oligonucleotide is 30 nucleosides in length. In some embodiments, an oligonucleotide is 31 nucleosides in length. In some embodiments, an oligonucleotide is 32 nucleosides in length. In some embodiments, an oligonucleotide is 33 nucleosides in length. In some embodiments, an oligonucleotide is 34 nucleosides in length. In some embodiments, an oligonucleotide is 35 nucleosides in length. In some embodiments, an oligonucleotide is 36 nucleosides in length. In some embodiments, an oligonucleotide is 37 nucleosides in length. In some embodiments, an oligonucleotide is 38 nucleosides in length. In some embodiments, an oligonucleotide is 39 nucleosides in length. In some embodiments, an oligonucleotide is 40 nucleosides in length. In some embodiments, each nucleoside counted in an oligonucleotide length independently comprises a nucleobase comprising a ring having at least one nitrogen ring atom. In some embodiments, each nucleoside counted in an oligonucleotide length independently comprises A, T, C, G, or U, or optionally substituted A, T, C, G, or U, or an optionally substituted tautomer of A, T, C, G or U.

[0067] Oligonucleotide type: As used herein, the phrase "oligonucleotide type" is used to define an oligonucleotide that has a particular base sequence, pattern of backbone linkages (i.e., pattern of internucleotidic linkage types, for example, phosphate, phosphorothioate, phosphorothioate triester, etc.), pattern of backbone chiral centers [i.e., pattern of linkage phosphorus stereochemistry (Rp/Sp)], and pattern of backbone phosphorus modifications. In some embodiments, oligonucleotides of a common designated "type" are structurally identical to one another.

[0068] 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. In some embodiments, 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 (i.e., are structurally identical to one another). In some embodiments, however, provided compositions comprise a plurality of oligonucleotides of different types, typically in pre-determined relative amounts.

[0069] Optionally Substituted: As described herein, compounds of the disclosure may contain optionally substituted, substituted and/or unsubstituted moieties. In general, the term "substituted," means that one or more hydrogens of the designated moiety are independently replaced with a substituent. Unless otherwise indicated, an "optionally substituted" group may independently have a substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with two or more substituents, the substituents may be either the same or different at every position. In some embodiments, an optionally substituted group is unsubstituted. In some embodiments, an optionally substituted group is substituted. Various substituents are described below.

[0070] Monovalent substituents are independently halogen; $-(CH_2)_{0-4}R°$; $-(CH_2)_{0-4}OR°$; $-O(CH_2)_{0-4}R°$, $-O-(CH_2)_{0-4}C(O)OR°$; $-(CH_2)_{0-4}CH(OR°)_2$; $-(CH_2)_{0-4}Ph$, 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; $-N_3$; $-(CH_2)_{0-4}N(R°)_2$; $-(CH_2)_{0-4}N(R°)C(O)R°$; $-N(R°)C(S)R°$; $-(CH_2)_{0-4}N(R°)C(O)N(R°)_2$; $-N(R°)C(S)N(R°)_2$; $-(CH_2)_{0-4}N(R°)C(O)OR°$; $-N(R°)N(R°)C(O)R°$; $-N(R°)N(R°)C(O)N(R°)_2$; $-N(R°)N(R°)C(O)OR°$; $-(CH_2)_{0-4}C(O)R°$; $-C(S)R°$; $-(CH_2)_{0-4}C(O)OR°$; $-(CH_2)_{0-4}C(O)SR°$; $-(CH_2)_{0-4}C(O)OSi(R°)_3$; $-(CH_2)_{0-4}OC(O)R°$; $-OC(O)(CH_2)_{0-4}SR°$, $-SC(S)SR°$; $-(CH_2)_{0-4}SC(O)R°$; $-(CH_2)_{0-4}C(O)N(R°)_2$; $-C(S)N(R°)_2$; $-C(S)SR°$; $-SC(S)SR°$, $-(CH_2)_{0-4}OC(O)N(R°)_2$; -C(O)N(OR°)R°; –C(O)C(O)R°; $-C(O)CH_2C(O)R°$; $-C(NOR°)R°$; $-(CH_2)_{0-4}SSR°$; $-(CH_2)_{0-4}S(O)_2R°$; $-(CH_2)_{0-4}S(O)_2OR°$; $-(CH_2)_{0-4}OS(O)_2R°$; $-S(O)_2N(R°)_2$; $-(CH_2)_{0-4}S(O)R°$; $-N(R°)S(O)_2N(R°)_2$; $-N(R°)S(O)_2R°$; –N(OR°)R°; –C(NH)N(R°)$_2$; $-Si(R°)_3$; $-OSi(R°)_3$; $-P(R°)_2$; $-P(OR°)_2$; $-OP(R°)_2$; $-OP(OR°)_2$; $-N(R°)P(R°)_2$; $-B(R°)_2$; $-OB(R°)_2$; $-P(O)(R°)_2$; $-OP(O)(R°)_2$; $-N(R°)P(O)(R°)_2$; $-(C_{1-4}$ straight or branched alkylene)O–N(R°)$_2$; or $-(C_{1-4}$ straight or branched alkylene)C(O)O–N(R°)$_2$; wherein each R° may be independently substituted as defined below and is independently hydrogen, C_{1-10} (e.g., C_{1-6}, C_{1-4}, etc.) aliphatic, C_{1-10} (e.g., C_{1-6}, C_{1-4}, etc.) heteroaliphatic having 1-5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, C_{6-10} (e.g., C_6, C_{10}, etc.) aryl, 5-10 (e.g., 5-9, 5-6, 5, 6, 9, 10, etc.) membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen and sulfur, $-CH_2-(C_{6-10}$ (e.g., C_6, C_{10}, etc.) aryl), $-O(CH_2)_{0-1}(C_{6-10}$ (e.g., C_6, C_{10}, etc.) aryl), $-CH_2-$(5-10 (e.g., 5-9, 5-6, 5, 6, 9, 10, etc.) membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen and sulfur), $-O(CH_2)_{0-1}$(5-10 (e.g., 5-9, 5-6, 5, 6, 9, 10, etc.) membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen and sulfur), a 3-10 (e.g., 3-6, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, etc.) membered, monocyclic, bicyclic, or polycyclic, saturated, or partially unsaturated 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-

10 (e.g., 3-6, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, etc.) membered, monocyclic, bicyclic, or polycyclic, saturated, partially unsaturated or aromatic ring (for aromatic ring, 5-10 (e.g., 5-9, 5-6, 5, 6, 9, 10, etc.) membered) having, in addition to the intervening atom(s), 0-5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, which may be substituted as defined below.

[0071] Monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $-(CH_2)_{0-2}R^{\bullet}$, $-(haloR^{\bullet})$, $-(CH_2)_{0-2}OH$, $-(CH_2)_{0-2}OR^{\bullet}$, $-(CH_2)_{0-2}CH(OR^{\bullet})_2$; $-O(haloR^{\bullet})$, $-CN$, $-N_3$, $-(CH_2)_{0-2}C(O)R^{\bullet}$, $-(CH_2)_{0-2}C(O)OH$, $-(CH_2)_{0-2}C(O)OR^{\bullet}$, $-(CH_2)_{0-2}SR^{\bullet}$, $-(CH_2)_{0-2}SH$, $-(CH_2)_{0-2}NH_2$, $-(CH_2)_{0-2}NHR^{\bullet}$, $-(CH_2)_{0-2}NR^{\bullet}_2$, $-NO_2$, $-SiR^{\bullet}_3$, $-OSiR^{\bullet}_3$, -C(O)SR•, –(C_{1-4} straight or branched alkylene)C(O)OR•, 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, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 3-6 (e.g., 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur. Divalent substituents on a saturated carbon atom of R° are independently =O or =S.

[0072] Divalent substituents are independently the following: =O, =S, $=NNR^*_2$, =NNHC(O)R*, =NNHC(O)OR*, $=NNHS(O)_2R^*$, $=NR^*$, $=NOR^*$, $-O(C(R^*_2))_{2-3}O-$, or $-S(C(R^*_2))_{2-3}S-$, wherein each independent occurrence of R* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 3-6 (e.g., 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur. Divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group are independently $-O(CR^*_2)_{2-3}O-$, wherein each independent occurrence of R* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 3-6 (e.g., 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0073] Substituents on the aliphatic group of R* are independently halogen, $-R^{\bullet}$, $-(haloR^{\bullet})$, $-OH$, $-OR^{\bullet}$, $-O(haloR^{\bullet})$, $-CN$, $-C(O)OH$, $-C(O)OR^{\bullet}$, $-NH_2$, $-NHR^{\bullet}$, $-NR^{\bullet}_2$, or $-NO_2$, 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, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 3-6 (e.g., 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0074] Substituents on a substitutable nitrogen are independently $-R^{\dagger}$, $-NR^{\dagger}_2$, $-C(O)R^{\dagger}$, $-C(O)OR^{\dagger}$, $-C(O)C(O)R^{\dagger}$, $-C(O)CH_2C(O)R^{\dagger}$, $-S(O)_2R^{\dagger}$, $-S(O)_2NR^{\dagger}_2$, $-C(S)NR^{\dagger}_2$, $-C(NH)NR^{\dagger}_2$, or $-N(R^{\dagger})S(O)_2R^{\dagger}$; wherein each $R^{\dagger}$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted –OPh, or an unsubstituted 3-6 (e.g., 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 heteroatoms independently selected from

nitrogen, oxygen, and sulfur, or, notwithstanding the definition above, two independent occurrences of $R^{\dagger}$, taken together with their intervening atom(s) form an unsubstituted 3–12 (e.g., 3-10, 3-6, 5-10, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, etc.) membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0075] Substituents on the aliphatic group of $R^{\dagger}$ are independently halogen, $-R^{\bullet}$, -(halo$R^{\bullet}$), –OH, $-OR^{\bullet}$, –O(halo$R^{\bullet}$), –CN, –C(O)OH, $-C(O)OR^{\bullet}$, $-NH_2$, $-NHR^{\bullet}$, $-NR^{\bullet}_2$, or $-NO_2$, wherein each $R^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 3-6 (e.g., 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0076] 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.

[0077] 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.

[0078] 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, an active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

[0079] 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.

[0080] 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.

[0081] Pharmaceutically acceptable salt: The term "pharmaceutically acceptable salt", as used herein, refers to salts of such compounds that are appropriate for use in pharmaceutical contexts, i.e., salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, et al. describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977). In some embodiments, pharmaceutically acceptable salt include, but are not limited to, nontoxic acid addition salts, which are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. In some embodiments, pharmaceutically acceptable salts include, but are not limited to, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. 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 defined and described in the present disclosure) salt. Representative alkali or alkaline earth metal salts include 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, an 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), all ionizable hydrogen (e.g., in an aqueous solution with a pKa no more than about 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2; in some embodiments, no more than about 7; in some embodiments, no more than about 6; in some embodiments, no more than about 5; in some embodiments, no more than about 4; in some embodiments, no more than about 3) in the acidic groups are replaced with cations. In some embodiments, each phosphorothioate and phosphate group independently exists in its salt form (e.g., if sodium salt, –O–P(O)(SNa)–O– and –O–P(O)(ONa)–O–, respectively). In some embodiments, each phosphorothioate and phosphate internucleotidic linkage independently exists in its salt form (e.g., if sodium salt, –O–P(O)(SNa)–O– and –O–P(O)(ONa)–O–, respectively). In some embodiments, a pharmaceutically acceptable salt is a sodium salt of an oligonucleotide. In some embodiments, a pharmaceutically acceptable salt is a sodium salt of an oligonucleotide, wherein each acidic phosphate and modified phosphate group (e.g., phosphorothioate, phosphate, etc.), if any, exists as a salt form (all sodium salt).

[0082] Predetermined: By predetermined (or pre-determined) is meant deliberately selected or non-random or controlled, for example as opposed to randomly occurring, random, or achieved without control. Those of ordinary skill in the art, reading the present specification, will appreciate that the present disclosure provides technologies that permit selection of particular chemistry and/or stereochemistry features to be incorporated into oligonucleotide compositions, and further permits controlled preparation of oligonucleotide compositions having such chemistry and/or stereochemistry features. Such provided compositions are "predetermined" as described herein. Compositions that may contain certain oligonucleotides because they happen to have been generated through a process that are not controlled to intentionally generate the particular chemistry and/or stereochemistry features are not "predetermined" compositions. In some embodiments, a predetermined composition is one that can be intentionally reproduced (e.g., through repetition of a controlled process). In some embodiments, a predetermined level of a plurality of oligonucleotides in a composition means that the absolute amount, and/or the relative amount (ratio, percentage, etc.) of the plurality of oligonucleotides in the composition is controlled. In some embodiments, a predetermined level of a plurality of oligonucleotides in a composition is achieved through chirally controlled oligonucleotide preparation.

[0083] Protecting group: The term "protecting group," as used herein, is well known in the art and includes those described in detail in Protecting Groups in Organic Synthesis, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of which is incorporated herein by reference. Also included

are those protecting groups specially adapted for nucleoside and nucleotide chemistry described in Current Protocols in Nucleic Acid Chemistry, edited by Serge L. Beaucage et al. 06/2012, the entirety of Chapter 2 is incorporated herein by reference. Suitable amino–protecting groups include methyl carbamate, ethyl carbamante, 9–fluorenylmethyl carbamate (Fmoc), 9–(2–sulfo)fluorenylmethyl carbamate, 9–(2,7–dibromo)fluoroenylmethyl carbamate, 2,7–di–t–butyl–[9–(10,10–dioxo–10,10,10,10–tetrahydrothioxanthyl)]methyl carbamate (DBD–Tmoc), 4–methoxyphenacyl carbamate (Phenoc), 2,2,2–trichloroethyl carbamate (Troc), 2–trimethylsilylethyl carbamate (Teoc), 2–phenylethyl carbamate (hZ), 1–(1–adamantyl)–1–methylethyl carbamate (Adpoc), 1,1–dimethyl–2–haloethyl carbamate, 1,1–dimethyl–2,2–dibromoethyl carbamate (DB–t–BOC), 1,1–dimethyl–2,2,2–trichloroethyl carbamate (TCBOC), 1–methyl–1–(4–biphenylyl)ethyl carbamate (Bpoc), 1–(3,5–di–t–butylphenyl)–1–methylethyl carbamate (t–Bumeoc), 2–(2'– and 4'–pyridyl)ethyl carbamate (Pyoc), 2–(N,N–dicyclohexylcarboxamido)ethyl carbamate, t–butyl carbamate (BOC), 1–adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1–isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4–nitrocinnamyl carbamate (Noc), 8–quinolyl carbamate, N–hydroxypiperidinyl carbamate, alkyldithio carbamate, benzyl carbamate (Cbz), p–methoxybenzyl carbamate (Moz), p–nitobenzyl carbamate, p–bromobenzyl carbamate, p–chlorobenzyl carbamate, 2,4–dichlorobenzyl carbamate, 4–methylsulfinylbenzyl carbamate (Msz), 9–anthrylmethyl carbamate, diphenylmethyl carbamate, 2–methylthioethyl carbamate, 2–methylsulfonylethyl carbamate, 2–(p–toluenesulfonyl)ethyl carbamate, [2–(1,3–dithianyl)]methyl carbamate (Dmoc), 4–methylthiophenyl carbamate (Mtpc), 2,4–dimethylthiophenyl carbamate (Bmpc), 2–phosphonioethyl carbamate (Peoc), 2–triphenylphosphonioisopropyl carbamate (Ppoc), 1,1–dimethyl–2–cyanoethyl carbamate, m–chloro–p–acyloxybenzyl carbamate, p–(dihydroxyboryl)benzyl carbamate, 5–benzisoxazolylmethyl carbamate, 2–(trifluoromethyl)–6–chromonylmethyl carbamate (Tcroc), m–nitrophenyl carbamate, 3,5–dimethoxybenzyl carbamate, o–nitrobenzyl carbamate, 3,4–dimethoxy–6–nitrobenzyl carbamate, phenyl(o–nitrophenyl)methyl carbamate, phenothiazinyl–(10)–carbonyl derivative, N'–p–toluenesulfonylaminocarbonyl derivative, N'–phenylaminothiocarbonyl derivative, t–amyl carbamate, S–benzyl thiocarbamate, p–cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, p–decyloxybenzyl carbamate, 2,2–dimethoxycarbonylvinyl carbamate, o–(N,N–dimethylcarboxamido)benzyl carbamate, 1,1–dimethyl–3–(N,N–dimethylcarboxamido)propyl carbamate, 1,1–dimethylpropynyl carbamate, di(2–pyridyl)methyl carbamate, 2–furanylmethyl carbamate, 2–iodoethyl carbamate, isoborynl carbamate, isobutyl carbamate, isonicotinyl carbamate, p–(p'–methoxyphenylazo)benzyl carbamate, 1–methylcyclobutyl carbamate, 1–methylcyclohexyl carbamate, 1–methyl–1–cyclopropylmethyl carbamate, 1–methyl–1–(3,5–dimethoxyphenyl)ethyl carbamate, 1–methyl–1–(p–phenylazophenyl)ethyl carbamate, 1–methyl–1–phenylethyl carbamate, 1–methyl–1–(4–pyridyl)ethyl carbamate, phenyl carbamate, p–(phenylazo)benzyl carbamate, 2,4,6–tri–t–butylphenyl carbamate, 4–(trimethylammonium)benzyl carbamate, 2,4,6–trimethylbenzyl carbamate, formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3–phenylpropanamide, picolinamide, 3–pyridylcarboxamide, N–benzoylphenylalanyl

derivative, benzamide, p–phenylbenzamide, o–nitophenylacetamide, o–nitrophenoxyacetamide, acetoacetamide, (N'–dithiobenzyloxycarbonylamino)acetamide, 3–(p–hydroxyphenyl)propanamide, 3–(o–nitrophenyl)propanamide, 2–methyl–2–(o–nitrophenoxy)propanamide, 2–methyl–2–(o–phenylazophenoxy)propanamide, 4–chlorobutanamide, 3–methyl–3–nitrobutanamide, o–nitrocinnamide, N–acetylmethionine derivative, o–nitrobenzamide, o–(benzoyloxymethyl)benzamide, 4,5–diphenyl–3–oxazolin–2–one, N–phthalimide, N–dithiasuccinimide (Dts), N–2,3–diphenylmaleimide, N–2,5–dimethylpyrrole, N–1,1,4,4–tetramethyldisilylazacyclopentane adduct (STABASE), 5–substituted 1,3–dimethyl–1,3,5–triazacyclohexan–2–one, 5–substituted 1,3–dibenzyl–1,3,5–triazacyclohexan–2–one, 1–substituted 3,5–dinitro–4–pyridone, N–methylamine, N–allylamine, N–[2–(trimethylsilyl)ethoxy]methylamine (SEM), N–3–acetoxypropylamine, N–(1–isopropyl–4–nitro–2–oxo–3–pyroolin–3–yl)amine, quaternary ammonium salts, N–benzylamine, N–di(4–methoxyphenyl)methylamine, N–5–dibenzosuberylamine, N–triphenylmethylamine (Tr), N–[(4–methoxyphenyl)diphenylmethyl]amine (MMTr), N–9–phenylfluorenylamine (PhF), N–2,7–dichloro–9–fluorenylmethyleneamine, N–ferrocenylmethylamino (Fcm), N–2–picolylamino N'–oxide, N–1,1–dimethylthiomethyleneamine, N–benzylideneamine, N–p–methoxybenzylideneamine, N–diphenylmethyleneamine, N–[(2–pyridyl)mesityl]methyleneamine, N–(N',N'–dimethylaminomethylene)amine, N,N'–isopropylidenediamine, N–p–nitrobenzylideneamine, N–salicylideneamine, N–5–chlorosalicylideneamine, N–(5–chloro–2–hydroxyphenyl)phenylmethyleneamine, N–cyclohexylideneamine, N–(5,5–dimethyl–3–oxo–1–cyclohexenyl)amine, N–borane derivative, N–diphenylborinic acid derivative, N–[phenyl(pentacarbonylchromium– or tungsten)carbonyl]amine, N–copper chelate, N–zinc chelate, N–nitroamine, N–nitrosoamine, amine N–oxide, diphenylphosphinamide (Dpp), dimethylthiophosphinamide (Mpt), diphenylthiophosphinamide (Ppt), dialkyl phosphoramidates, dibenzyl phosphoramidate, diphenyl phosphoramidate, benzenesulfenamide, o–nitrobenzenesulfenamide (Nps), 2,4–dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2–nitro–4–methoxybenzenesulfenamide, triphenylmethylsulfenamide, 3–nitropyridinesulfenamide (Npys), p–toluenesulfonamide (Ts), benzenesulfonamide, 2,3,6,–trimethyl–4–methoxybenzenesulfonamide (Mtr), 2,4,6–trimethoxybenzenesulfonamide (Mtb), 2,6–dimethyl–4–methoxybenzenesulfonamide (Pme), 2,3,5,6–tetramethyl–4–methoxybenzenesulfonamide (Mte), 4–methoxybenzenesulfonamide (Mbs), 2,4,6–trimethylbenzenesulfonamide (Mts), 2,6–dimethoxy–4–methylbenzenesulfonamide (iMds), 2,2,5,7,8–pentamethylchroman–6–sulfonamide (Pmc), methanesulfonamide (Ms), β–trimethylsilylethanesulfonamide (SES), 9–anthracenesulfonamide, 4–(4',8'–dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzylsulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamide.

[0084] 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.

[0085] Suitable hydroxyl protecting groups include methyl, methoxylmethyl (MOM), methylthiomethyl (MTM), t–butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), p–methoxybenzyloxymethyl (PMBM), (4–methoxyphenoxy)methyl (p–AOM), guaiacolmethyl (GUM), t–butoxymethyl, 4–pentenyloxymethyl (POM), siloxymethyl, 2–methoxyethoxymethyl (MEM), 2,2,2–trichloroethoxymethyl, bis(2–chloroethoxy)methyl, 2–(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3–bromotetrahydropyranyl, tetrahydrothiopyranyl, 1–methoxycyclohexyl, 4–methoxytetrahydropyranyl (MTHP), 4–methoxytetrahydrothiopyranyl, 4–methoxytetrahydrothiopyranyl S,S–dioxide, 1–[(2–chloro–4–methyl)phenyl]–4–methoxypiperidin–4–yl (CTMP), 1,4–dioxan–2–yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a–octahydro–7,8,8–trimethyl–4,7–methanobenzofuran–2–yl, 1–ethoxyethyl, 1–(2–chloroethoxy)ethyl, 1–methyl–1–methoxyethyl, 1–methyl–1–benzyloxyethyl, 1–methyl–1–benzyloxy–2–fluoroethyl, 2,2,2–trichloroethyl, 2–trimethylsilylethyl, 2–(phenylselenyl)ethyl, t–butyl, allyl, p–chlorophenyl, p–methoxyphenyl, 2,4–dinitrophenyl, benzyl, p–methoxybenzyl, 3,4–dimethoxybenzyl, o–nitrobenzyl, p–nitrobenzyl, p–halobenzyl, 2,6–dichlorobenzyl, p–cyanobenzyl, p–phenylbenzyl, 2–picolyl, 4–picolyl, 3–methyl–2–picolyl N–oxido, diphenylmethyl, p,p'–dinitrobenzhydryl, 5–dibenzosuberyl, triphenylmethyl, α–naphthyldiphenylmethyl, p–methoxyphenyldiphenylmethyl, di(p–methoxyphenyl)phenylmethyl, tri(p–methoxyphenyl)methyl, 4–(4''–bromophenacyloxyphenyl)diphenylmethyl, 4,4',4''–tris(4,5–dichlorophthalimidophenyl)methyl, 4,4',4''–tris(levulinoyloxyphenyl)methyl, 4,4',4''–tris(benzoyloxyphenyl)methyl, 3–(imidazol–1–yl)bis(4',4''–dimethoxyphenyl)methyl, 1,1–bis(4–methoxyphenyl)–1'–pyrenylmethyl, 9–anthryl, 9–(9–phenyl)xanthenyl, 9–(9–phenyl–10–oxo)anthryl, 1,3–benzodithiolan–2–yl, benzisothiazolyl S,S–dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylthexylsilyl, t–butyldimethylsilyl (TBDMS), t–butyldiphenylsilyl (TBDPS), tribenzylsilyl, tri–p–xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), t–butylmethoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, p–chlorophenoxyacetate, 3–phenylpropionate, 4–oxopentanoate (levulinate), 4,4–(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantoate, crotonate, 4–methoxycrotonate, benzoate, p–phenylbenzoate, 2,4,6–trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9–fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2–trichloroethyl carbonate (Troc), 2–(trimethylsilyl)ethyl carbonate (TMSEC), 2–(phenylsulfonyl) ethyl carbonate (Psec), 2–(triphenylphosphonio) ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate alkyl allyl carbonate, alkyl p–nitrophenyl carbonate, alkyl benzyl carbonate, alkyl p–methoxybenzyl carbonate, alkyl 3,4–dimethoxybenzyl carbonate, alkyl o–nitrobenzyl carbonate, alkyl p–nitrobenzyl carbonate, alkyl S–benzyl thiocarbonate, 4–ethoxy–1–napthhyl carbonate, methyl dithiocarbonate, 2–iodobenzoate, 4–azidobutyrate, 4–nitro–4–

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

[0086] In some embodiments, a hydroxyl protecting group is acetyl, t-butyl, tbutoxymethyl, methoxymethyl, tetrahydropyranyl, 1 -ethoxyethyl, 1 -(2-chloroethoxy)ethyl, 2- trimethylsilylethyl, p-chlorophenyl, 2,4-dinitrophenyl, benzyl, benzoyl, p-phenylbenzoyl, 2,6- dichlorobenzyl, diphenylmethyl, p-nitrobenzyl, triphenylmethyl (trityl), 4,4'-dimethoxytrityl, trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triphenylsilyl, triisopropylsilyl, benzoylformate, chloroacetyl, trichloroacetyl, trifiuoroacetyl, pivaloyl, 9- fluorenylmethyl carbonate, mesylate, tosylate, triflate, trityl, monomethoxytrityl (MMTr), 4,4'-dimethoxytrityl, (DMTr) and 4,4',4"-trimethoxytrityl (TMTr), 2-cyanoethyl (CE or Cnc), 2-(trimethylsilyl)ethyl (TSE), 2-(2-nitrophenyl)ethyl, 2-(4-cyanophenyl)ethyl 2-(4-nitrophenyl)ethyl (NPE), 2-(4-nitrophenylsulfonyl)ethyl, 3,5-dichlorophenyl, 2,4-dimethylphenyl, 2-nitrophenyl, 4-nitrophenyl, 2,4,6-trimethylphenyl, 2-(2-nitrophenyl)ethyl, butylthiocarbonyl, 4,4',4"-tris(benzoyloxy)trityl, diphenylcarbamoyl, levulinyl, 2-(dibromomethyl)benzoyl (Dbmb), 2-(isopropylthiomethoxymethyl)benzoyl (Ptmt), 9-phenylxanthen-9-yl (pixyl) or 9-(p-methoxyphenyl)xanthine-9-y1 (MOX). In some embodiments, each of the hydroxyl protecting groups is, independently selected from acetyl, benzyl, t- butyldimethylsilyl, t-butyldiphenylsilyl and 4,4'-dimethoxytrityl. In some embodiments, the hydroxyl protecting group is selected from the group consisting of trityl, monomethoxytrityl and 4,4'-dimethoxytrityl group. In some embodiments, a phosphorous linkage protecting group is a group attached to the phosphorous linkage (e.g., an internucleotidic linkage) throughout oligonucleotide synthesis. In some embodiments, a protecting group is attached to a sulfur atom of an phosphorothioate group. In some embodiments, a protecting group is attached to an oxygen atom

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

[0087] Subject: As used herein, the term "subject" or "test subject" refers to any organism to which a compound (e.g., an oligonucleotide) 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 is a human. In some embodiments, a subject may be suffering from and/or susceptible to a disease, disorder and/or condition.

[0088] 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. A base sequence which is substantially identical or complementary to a second sequence is not fully identical or complementary to the second sequence, but is mostly or nearly identical or complementary to the second sequence. In some embodiments, an oligonucleotide with a substantially complementary sequence to another oligonucleotide or nucleic acid forms duplex with the oligonucleotide or nucleic acid in a similar fashion as an oligonucleotide with a fully complementary sequence. In addition, one of ordinary skill in the biological and/or chemical 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.

[0089] 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 a RNA or DNA sugar (ribose or deoxyribose). In some embodiments, a sugar is a modified ribose or deoxyribose sugar, e.g., 2'-modified, 5'-modified, etc. As described herein, in some embodiments, when used in oligonucleotides and/or nucleic acids, modified sugars may provide one or more desired properties, activities, etc. In some embodiments, a sugar is optionally substituted ribose or deoxyribose. In some embodiments, a "sugar" refers to a sugar unit in an oligonucleotide or a nucleic acid.

[0090] 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 is predisposed to have that disease, disorder and/or condition. 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.

[0091] Therapeutic agent: As used herein, the term "therapeutic agent" in general refers to any agent that elicits a desired effect (e.g., a desired biological, clinical, or pharmacological effect) when administered to a subject. In some embodiments, an agent is considered to be a therapeutic agent if it demonstrates a statistically significant effect across an appropriate population. In some embodiments, an appropriate population is a population of subjects suffering from and/or susceptible to a disease, disorder or condition. In some embodiments, an appropriate population is a population of model organisms. In some embodiments, an appropriate population may be defined by one or more criterion such as age group, gender, genetic background, preexisting clinical conditions, prior exposure to therapy. In some embodiments, a therapeutic agent is a substance that alleviates, ameliorates, relieves, inhibits, prevents, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms or features of a disease, disorder, and/or condition in a subject when administered to the subject in an effective amount. In some embodiments, a "therapeutic agent" is an agent that has been or is required to be approved by a government agency before it can be marketed for administration to humans. In some embodiments, a "therapeutic agent" is an agent for which a medical prescription is required for administration to humans. In some embodiments, a therapeutic agent is a provided compound, e.g., a provided oligonucleotide.

[0092] 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.

[0093] 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.

[0094] Unsaturated: The term "unsaturated," as used herein, means that a moiety has one or more units of unsaturation.

[0095] 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).

[0096] As those skilled in the art will appreciate, methods and compositions described herein relating to provided compounds (e.g., oligonucleotides) generally also apply to pharmaceutically acceptable salts of such compounds.

Description of Certain Embodiments

[0097] Oligonucleotides are useful in various therapeutic, diagnostic, and research applications. Use of naturally occurring nucleic acids is limited, for example, by their susceptibility to endo- and exo-nucleases. As such, various synthetic counterparts have been developed to circumvent these shortcomings and/or to further improve various properties and activities. These include synthetic oligonucleotides that contain chemical modifications, e.g., base modifications, sugar modifications, backbone modifications, etc., which, among other things, render these molecules less susceptible to degradation and improve other properties and/or activities.

[0098] From a structural point of view, modifications to internucleotidic linkages can introduce chirality, and certain properties and activities may be affected by configurations of linkage phosphorus atoms of oligonucleotides. For example, binding affinity, sequence specific binding to complementary RNA, stability to nucleases, activities, delivery, pharmacokinetics, etc. can be affected by, inter alia, chirality of backbone linkage phosphorus atoms.

[0099]

Dystrophin

[0100] In some embodiments, the present disclosure provides technologies, e.g., oligonucleotides,

compositions, methods, etc., related to the dystrophin (DMD) gene or a gene product thereof (e.g., a nucleic acid (e.g., DNA, RNA), a transcript (e.g., a DMD transcript), a protein encoded thereby (e.g., a DMD polypeptide), etc.).

[0101] In some embodiments, the present disclosure provides technologies, including oligonucleotides and compositions and methods of use thereof, for treatment of muscular dystrophy, including but not limited to, Duchenne Muscular Dystrophy (also abbreviated as DMD) and Becker Muscular Dystrophy (BMD). In some embodiments, DMD comprises one or more mutations. In some embodiments, such mutations are associated with reduced biological functions of dystrophin protein in a subject suffering from or susceptible to muscular dystrophy.

[0102] In some embodiments, the DMD gene or a gene product thereof, or a variant or portion thereof, may be referred to as DMD, BMD, CMD3B, DXS142, DXS164, DXS206, DXS230, DXS239, DXS268, DXS269, DXS270, DXS272, MRX85, dystrophin, etc. Various DMD sequences including variants thereof are readily available to those of skill in the art, including those sequences associated with the following identification numbers: (i) for human: OMIM: 300377; GeneCards: DMD; Entrez ID: 1756; Ensembl: ENSG00000198947; UniProt: P11532; RefSeq (mRNA): NM_000109, NM_004006, NM_004007, NM_004009, and NM_004010; RefSeq (protein): NP_000100, NP_003997, NP_004000, NP_004001, and NP_004002; Location (UCSC): Chr X: 31.1 – 33.34 Mb; (ii) for mouse: MGI: 94909; Entrez ID: 13405; Ensembl: ENSMUSG00000045103; UniProt: P11531; RefSeq (mRNA): NM_007868, NM_001314034, NM_001314035, NM_001314036, and NM_001314037; RefSeq (protein): NP_001300963, NP_001300964, NP_001300965, NP_001300966, and NP_001300967; Location (UCSC): Chr X: 82.95 – 85.21 Mb; and (iii) for multiple species: HomoloGene: 20856.

[0103] The DMD gene reportedly contains 79 exons distributed over 2.3 million bp of genetic real estate on the X chromosome; however, only approximately 14,000 bp (<1%) is reported to be used for translation into protein (coding sequence). It is reported that about 99.5% of the genetic sequence, the intronic sequences, is spliced out of the 2.3 million bp initial heteronuclear RNA DMD transcript to provide a mature 14,000 bp mRNA which includes all key information for dystrophin protein production. In some embodiments, patients with DMD have mutation(s) in the DMD gene that prevent the appropriate construction of the wild-type DMD mRNA and/or the production of the wild-type dystrophin protein, and patients with DMD often show marked dystrophin deficiency in their muscle.

[0104] In some embodiments, a dystrophin DMD transcript, e.g., mRNA, or protein encompasses those related to or produced from alternative splicing. For example, sixteen alternative DMD transcripts of the dystrophin gene were reported following an analysis of splicing patterns of the DMD gene in skeletal muscle, brain and heart tissues (Sironi et al. 2002 FEBS Letters 517: 163-166).

[0105] It is reported that dystrophin has several isoforms. In some embodiments, dystrophin refers to a specific isoform. At least three full-length dystrophin isoforms have been reported, each controlled by a tissue-specific promoter (Klamut et al. 1990 Mol. Cell. Biol. 10: 193-205; Nudel et al. 1989 Nature 337: 76-78;

Gorecki et al. 1992 Hum. Mol. Genet. 1: 505-510). The muscle isoform is reportedly mainly expressed in skeletal muscle but also in smooth and cardiac muscles (Bies, R.D., Phelps, S.F., Cortez, M.D., Roberts, R., Caskey, C.T. and Chamberlain, J.S. 1992 Nucleic Acids Res. 20: 1725-1731), the brain dystrophin is reportedly specific for cortical neurons but can also be detected in heart and cerebellar neurons, while the Purkinje-cell type reportedly accounts for nearly all cerebellar dystrophin (Gorecki et al. 1992 Hum. Mol. Genet. 1: 505-510). Alternative splicing reportedly provides a means for dystrophin diversification: the 3' region of the gene reportedly undergoes alternative splicing resulting in tissue-specific DMD transcripts in brain neurons, cardiac Purkinje fibers, and smooth muscle cells (Bies et al. 1992 Nucleic Acids Res. 20: 1725-1731; and Feener et al. 1989 Nature 338: 509-511) while 12 patterns of alternative splicing have been reported in the 5' region of the gene in skeletal muscle (Surono et al. 1997 Biochem. Biophys. Res. Commun. 239: 895-899).

[0106] In some embodiments, a dystrophin gene, transcript (e.g., mRNA), or protein is a revertant version. Among others, revertant dystrophins were reported in, for example: Hoffman et al. 1990 J. Neurol. Sci. 99:9-25; Klein et al. 1992 Am. J. Hum. Genet. 50: 950-959; and Chelly et al. 1990 Cell 63: 1239-1348; Arahata et al. 1998 Nature 333: 861-863; Bonilla et al. 1988 Cell 54: 447-452; Fanin et al. 1992 Neur. Disord. 2: 41-45; Nicholson et al. 1989 J. Neurol. Sci. 94: 137-146; Shimizu et al. 1988 Proc. Jpn. Acad. Sci. 64: 205-208; Sicinzki et al. 1989 Science 244: 1578-1580; and Sherratt et al. Am. J. Hum. Genet. 53: 1007-1015.

[0107] Various mutations in the DMD gene can and/or were reported to cause muscular dystrophy. The variety of mutations in the DMD gene are reviewed in, e.g., Flanigan et al. 2009 Hum Mutat. 30(12): 1657-1666. Mutations in the form of large deletions (1 or more exons) are reported to account for approximately two-thirds of all DMD gene mutations; the remaining mutations are reported to be due to duplications and small deletions, insertions, point mutations, or splicing mutations. According to certain reports, deletions are typically clustered in a hotspot region between exons 45 and 55 and prevent translation of dystrophin. Absent or defective dystrophin protein, resulting from DMD gene mutations, is reported to disrupt the dystrophin-glycoprotein complex, leading to increased muscle membrane fragility, chronic muscle damage, inflammation, replacement of muscle fibers with fat and fibrotic tissue, and then loss of muscle function. Various databases of mutations in the DMD gene have been constructed, including those described in, e.g., Aartsma-Rus et al. 2006 Muscle Nerve. 34(2):135-44; Bladen et al. 2015 Hum Mutat. 36(4):395-402. In some embodiments, a mutation in a DMD gene or gene product thereof (e.g., a DMD transcript) comprises a deletion. In some embodiments, a mutation in a DMD gene or gene product thereof (e.g., a DMD transcript) comprises a point mutation. In some embodiments, a mutation in a DMD gene or gene product thereof (e.g., a DMD transcript) comprises an insertion. In some embodiments, a mutation in a DMD gene or gene product thereof (e.g., a DMD transcript) comprises a duplication. In some embodiments, a mutation in a DMD gene or gene product thereof (e.g., a DMD transcript) is a mutation amenable to exon skipping. In some embodiments, a mutation in a DMD gene or gene product thereof (e.g., a DMD transcript) is a mutation amenable to exon 52 skipping.

Oligonucleotides

[0108] Among other things, the present disclosure provides oligonucleotides of various designs, which may comprise various nucleobases and patterns thereof, sugars and patterns thereof, internucleotidic linkages and patterns thereof, and/or additional chemical moieties and patterns thereof as described in the present disclosure.
[0109] In some embodiments, the present disclosure provides oligonucleotides and/or oligonucleotide compositions that are useful for various purposes, e.g., modulating skipping, reducing levels of DMD transcripts, improving levels of beneficial proteins, treating conditions, diseases and disorders, etc. In some embodiments, the present disclosure provides oligonucleotide compositions with improved properties, *e.g.*, increased skipping of exon 52, reduced toxicities, *etc*. Among other things, oligonucleotides of the present disclosure comprise chemical modifications, stereochemistry, and/or combinations thereof which can improve various properties and activities of oligonucleotides. Non-limiting examples of oligonucleotides are listed in Table 1 (e.g., Table 1A, Table 1B, Table 1C).

Base Sequences

[0110] As appreciated by those skilled in the art, structural features of the present disclosure, such as nucleobase modification, sugar modifications, internucleotidic linkage modifications, linkage phosphorus stereochemistry, etc., and combinations thereof may be utilized with various suitable base sequences to provide oligonucleotides and compositions with desired properties and/or activities.
[0111] In some embodiments, an oligonucleotide has a base sequence described herein (e.g., in a Table) or a portion thereof (e.g., a span of 10-50, 10-40, 10-30, 10-20, or 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or at least 10, at least 15, at least 20 contiguous nucleobases) with 0-5 (e.g., 0, 1, 2, 3, 4 or 5) mismatches, wherein each T can be independently substituted with U and vice versa. In some embodiments, an oligonucleotide comprises a base sequence described herein, or a portion thereof, wherein a portion is a span of at least 10 contiguous nucleobases, or a span of at least 15 contiguous nucleobases with 0-5 mismatches. In some embodiments, provided oligonucleotides have a base sequence described herein, or a portion thereof, wherein a portion is a span of at least 10 contiguous nucleobases, or a span of at least 10 contiguous nucleobases with 1-5 mismatches, wherein each T can be independently substituted with U and vice versa.
[0112] In some embodiments, base sequences of oligonucleotides comprise or consist of 10-60 (e.g., about or at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60; in some embodiments, at least 15; in some embodiments, at least 16; in some embodiments, at least 17; in some embodiments, at least 18; in some embodiments, at least 19; in some embodiments, at least 20; in some embodiments, at least 21; in some embodiments, at least 22; in some embodiments, at least 23; in some embodiments, at least 24; in some embodiments, at least 25; in some embodiments, at least 26; in some embodiments, at least 27; in some embodiments, at least 28; in some embodiments, at least 29; in some embodiments, at least 30; in some embodiments, at least 31; in some embodiments, at least 32; in some embodiments, at least 33; in some embodiments, at least 34; in some embodiments, at least 35) bases, optionally contiguous, of a base sequence that is identical or complementary

to a base sequence of nucleic acid, e.g., a DMD gene or a transcript thereof. In some embodiments, the base sequence of an oligonucleotide is or comprises a sequence that is complementary to a target sequence in a gene or a transcript thereof.

[0113] In some embodiments, a target sequence is or comprises a characteristic sequence of a nucleic acid sequence (e.g., of an gene or a transcript thereof) in that it defines the nucleic acid sequence over others in a relevant organism; for example, a characteristic sequence is not in or has at least various mismatches from other genomic nucleic acid sequences (e.g., genes) or transcripts thereof in a relevant organism. In some embodiments, a characteristic sequence of a transcript defines that transcript over other transcripts in a relevant organism; for example, in some embodiments, a characteristic sequence is not in transcripts that are transcribed from a different nucleic acid sequence (e.g., a different gene). In some embodiments, transcript variants from a nucleic acid sequence (e.g., mRNA variants of a gene) may share a common characteristic sequence that defines them from, e.g., transcripts of other genes.

[0114] Base sequences of provided oligonucleotides, as appreciated by those skilled in the art, typically have sufficient lengths and complementarity to their target nucleic acids, e.g., RNA transcripts (e.g., pre-mRNA, mature mRNA, etc.) for, e.g., exon skipping. As appreciated by those skilled in the art, in many instances target nucleic acids are longer than oligonucleotides of the present disclosure, and complementarity may be properly assessed based on the shorter of the two, oligonucleotides. In some embodiments, the base sequence of an oligonucleotide has 90% or more identity with the base sequence of an oligonucleotide disclosed in a Table, wherein each T can be independently substituted with U and vice versa. In some embodiments, the base sequence of an oligonucleotide has 95% or more identity with the base sequence of an oligonucleotide disclosed in a Table, wherein each T can be independently substituted with U and vice versa. In some embodiments, the base sequence of an oligonucleotide comprises a continuous span of 15, 16, 17, 18, 19, 20 or more bases of an oligonucleotide disclosed in a Table, wherein each T can be independently substituted with U and vice versa, except that one or more bases within the span are abasic (e.g., a nucleobase is absent from a nucleotide).

[0115] In some embodiments, the present disclosure pertains to an oligonucleotide having a base sequence which comprises the base sequence of any oligonucleotide disclosed herein, wherein each T may be independently replaced with U and vice versa.

[0116] In some embodiments, the present disclosure pertains to an oligonucleotide having a base sequence which is the base sequence of any oligonucleotide disclosed herein, wherein each T may be independently replaced with U and vice versa.

[0117] In some embodiments, the present disclosure pertains to an oligonucleotide having a base sequence which comprises at least 15 contiguous bases of the base sequence of any oligonucleotide disclosed herein, wherein each T may be independently replaced with U and vice versa.

[0118] In some embodiments, the present disclosure pertains to an oligonucleotide having a base sequence which is at least 90% identical to the base sequence of any oligonucleotide disclosed herein, wherein each T

may be independently replaced with U and vice versa.

[0119] In some embodiments, the present disclosure pertains to an oligonucleotide having a base sequence which is at least 95% identical to the base sequence of any oligonucleotide disclosed herein, wherein each T may be independently replaced with U and vice versa.

[0120] In some embodiments, a base sequence of an oligonucleotide is, comprises, or comprises 10-40, e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 contiguous bases of the base sequence of any oligonucleotide described herein, wherein each T may be independently replaced with U and vice versa.

[0121] In some embodiments, an oligonucleotide is an oligonucleotide presented in a Table herein.

[0122] In some embodiments, the base sequence of an oligonucleotide is complementary to that of a target nucleic acid, e.g., a dystrophin gene or transcript thereof. In some embodiments, a base sequence of an oligonucleotide is at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, or 100% complementary to a target sequence in a DMD transcript. In some embodiments, a base sequence of an oligonucleotide is fully complementary to a target sequence in a DMD transcript.

[0123] In some embodiments, an oligonucleotide has a base sequence which comprises at least 15 contiguous bases (e.g., 15, 16, 17, 18, 19, or 20) of an oligonucleotide in a Table, wherein each T can be independently substituted with U and vice versa.

[0124] In some embodiments, an oligonucleotide comprises a base sequence or portion thereof (*e.g.*, a portion comprising 10-40, e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 nucleobases) described in any of the Tables, wherein each T may be independently replaced with U and vice versa, and/or a sugar, nucleobase, and/or internucleotidic linkage modification and/or stereochemistry, and/or a pattern thereof described in any of the Tables, and/or an additional chemical moiety (in addition to an oligonucleotide chain, e.g., a target moiety, a lipid moiety, a carbohydrate moiety, etc.) described in any of the Tables.

[0125] In some embodiments, the terms "complementary," "fully complementary" and "substantially complementary" may be used with respect to the base matching between an oligonucleotide and a target sequence, as will be understood by those skilled in the art from the context of their uses. It is noted that substitution of T for U, or vice versa, generally does not alter the amount of complementarity. As used herein, an oligonucleotide that is "substantially complementary" to a target sequence is largely or mostly complementary but not necessarily 100% complementary. In some embodiments, a sequence (e.g., an oligonucleotide) which is substantially complementary has one or more, e.g., 1, 2, 3, 4 or 5 mismatches when maximally aligned to its target sequence. In some embodiments, an oligonucleotide has a base sequence which is substantially complementary to a target sequence of a target nucleic acid. In some embodiments, an oligonucleotide has a base sequence which is substantially complementary to the complement of the sequence of an oligonucleotide disclosed herein. As appreciated by those skilled in the art, in some embodiments, sequences of oligonucleotides need not be 100% complementary to their targets for oligonucleotides to perform

their functions. In some embodiments, base sequences of provided oligonucleotides are fully complementary to their target sequences (A-T/U and C-G base pairing).

[0126] In some embodiments, an oligonucleotide comprises an oligonucleotide comprises a sequence that is complementary to a characteristic portion of a nucleic acid. In some embodiments, an oligonucleotide comprises a sequence that is complementary to a characteristic portion of a DMD transcript. In some embodiments, the base sequence of an oligonucleotide is complementary to a characteristic portion of a nucleic acid. In some embodiments, the base sequence of an oligonucleotide is complementary to a characteristic portion of a DMD transcript. In some embodiments, a characteristic portion is a characteristic sequence. In some embodiments, a characteristic sequence of a DMD transcript is or comprises a complementary sequence of the sequence of an oligonucleotide in Table 1 (e.g., Table 1A, Table 1B, Table 1C). In some embodiments a characteristic sequence is or comprises GGCUUCAACUAUCUGAGUGA, wherein each U may be independently replaced with T. In some embodiments a characteristic sequence is or comprises CCAGUUGGAAGAACUCAUUA, wherein each U may be independently replaced with T. In some embodiments a characteristic sequence is or comprises CCCCAGUUGGAAGAACUCAU, wherein each U may be independently replaced with T. In some embodiments a characteristic sequence is or comprises UGCAGGAUUUGGAACAGAGG, wherein each U may be independently replaced with T. In some embodiments a characteristic sequence is or comprises AUGCAGGAUUUGGAACAGAG, wherein each U may be independently replaced with T. In some embodiments a characteristic sequence is or comprises CAGGAUUUGGAACAGAGGCG, wherein each U may be independently replaced with T. In some embodiments a characteristic sequence is or comprises GGAAGAACUCAUUACCGCUG, wherein each U may be independently replaced with T.

[0127] In some embodiments, an oligonucleotide can hybridize to a region of a nucleic acid. In some embodiments, a region has a length of about 20-200 (e.g., about 20-150, 20-100, 30-200, 30-150, 40-200, 40-150, 50-100, or about 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or 200) nucleobases. In some embodiments, a region has a length of about 30 nucleobases. In some embodiments, a region has a length of about 40 nucleobases. In some embodiments, a region has a length of about 50 nucleobases. In some embodiments, a region has a length of about 60 nucleobases. In some embodiments, a region has a length of about 70 nucleobases. In some embodiments, a region has a length of about 80 nucleobases. In some embodiments, a region has a length of about 90 nucleobases. In some embodiments, a region has a length of about 100 nucleobases. In some embodiments, a region has a length of about 120 nucleobases. In some embodiments, a region has a length of about 150 nucleobases. In some embodiments, a region has a length of about 200 nucleobases. In some embodiments, a region comprises a complementary sequence of a base sequence of an oligonucleotide in Table 1 (e.g., Table 1A, Table 1B, Table 1C), which in some embodiments, is in the middle of a region. For example, in some embodiments, a region is or comprises GGCUUCAACUAUCUGAGUGA. In some embodiments, a region is or comprises CCAGUUGGAAGAACUCAUUA. In some embodiments, a region is or comprises

CCCCAGUUGGAAGAACUCAU. In some embodiments, a region is or comprises UGCAGGAUUUGGAACAGAGG. In some embodiments, a region is or comprises AUGCAGGAUUUGGAACAGAG. In some embodiments, a region is or comprises CAGGAUUUGGAACAGAGGCG. In some embodiments, a region is or comprises GGAAGAACUCAUUACCGCUG.

[0128] In some embodiments, the present disclosure provides an oligonucleotide comprising a sequence found in an oligonucleotide described in a Table, wherein one or more U is independently and optionally replaced with T or vice versa. In some embodiments, an oligonucleotide can comprise at least one T and/or at least one U. In some embodiments, the present disclosure provides an oligonucleotide comprising a sequence found in an oligonucleotide described in a Table herein, wherein the said sequence has over 50% identity with the sequence of the oligonucleotide described in a Table. In some embodiments, the present disclosure provides an oligonucleotide whose base sequence is the sequence of an oligonucleotide disclosed in a Table, wherein each T may be independently replaced with U and vice versa. In some embodiments, the present disclosure provides an oligonucleotide comprising a sequence found in an oligonucleotide in a Table, wherein the oligonucleotides have a pattern of backbone linkages, pattern of backbone chiral centers, and/or pattern of backbone phosphorus modifications of the same oligonucleotide or another oligonucleotide in a Table herein.

[0129] In some embodiments, the disclosure provides an oligonucleotide having a base sequence which is, comprises, or comprises a portion of the base sequence of an oligonucleotide disclosed herein, e.g., in a Table, wherein each T may be independently replaced with U and vice versa, wherein the oligonucleotide optionally further comprises a chemical modification, stereochemistry, format, an additional chemical moiety described herein (e.g., a targeting moiety, lipid moiety, carbohydrate moiety, etc.), and/or another structural feature.

[0130] In some embodiments, a "portion" (e.g., of a base sequence or a pattern of modifications or other structural element) is at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 monomeric units long.

[0131] As examples, certain oligonucleotides comprising certain example base sequences, nucleobase modifications and patterns thereof, sugar modifications and patterns thereof, internucleotidic linkages and patterns thereof, linkage phosphorus stereochemistry and patterns thereof, linkers, and/or additional chemical moieties, etc., are presented in Table 1, below. Among other things, these oligonucleotides may be utilized for exon skipping. In some embodiments, listed in Tables are stereorandom oligonucleotide compositions. In some embodiments, the present disclosure provides chirally controlled oligonucleotide compositions.

[0132] In some embodiments, a base sequence is or comprises a particular sequence. In some embodiments, a base sequence is complementary to a base sequence that is or comprises a base sequence that is complementary to a particular sequence. In some embodiments, a base sequence is or comprise a sequence that differs from a particular sequence at no more than 1, 2, 3, 4, or 5 positions. In some embodiments, a base sequence is or comprise a sequence that differs from about 15-30 (e.g., 15-25, 15-20, 20-30, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) consecutive nucleobases of a particular sequence at no more than 1, 2, 3, 4, or 5 positions. In some embodiments, a base sequence is or comprise a sequence that differs from a

particular sequence at no more than 1 position. In some embodiments, a base sequence is or comprise a sequence that differs from a particular sequence at no more than 2 positions. In some embodiments, a base sequence is or comprise a sequence that differs from a particular sequence at no more than 3 positions. In some embodiments, a base sequence is or comprise a sequence that differs from a particular sequence at no more than 4 positions. In some embodiments, a base sequence is or comprise a sequence that differs from a particular sequence at no more than 5 positions. In some embodiments, a particular sequence is or comprises a base sequence selected from Table 1. In some embodiments, a particular sequence is or comprises 5-20, 10-20, or 15-20 (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) consecutive bases in a base sequence selected from Table 1. In some embodiments, a particular sequence is or comprises 10 consecutive bases in a base sequence selected from Table 1. In some embodiments, a particular sequence is or comprises 11 consecutive bases in a base sequence selected from Table 1. In some embodiments, a particular sequence is or comprises 12 consecutive bases in a base sequence selected from Table 1. In some embodiments, a particular sequence is or comprises 13 consecutive bases in a base sequence selected from Table 1. In some embodiments, a particular sequence is or comprises 14 consecutive bases in a base sequence selected from Table 1. In some embodiments, a particular sequence is or comprises 15 consecutive bases in a base sequence selected from Table 1. In some embodiments, a particular sequence is or comprises 16 consecutive bases in a base sequence selected from Table 1. In some embodiments, a particular sequence is or comprises 17 consecutive bases in a base sequence selected from Table 1. In some embodiments, a particular sequence is or comprises 18 consecutive bases in a base sequence selected from Table 1. In some embodiments, a particular sequence is or comprises 19 consecutive bases in a base sequence selected from Table 1. In some embodiments, a particular sequence is or comprises 20 consecutive bases in a base sequence selected from Table 1. In some embodiments, a base sequence selected from Table 1 is a base sequence selected from Table 1A. In some embodiments, a base sequence selected from Table 1 is a base sequence selected from Table 1B. In some embodiments, a base sequence selected from Table 1 is a base sequence selected from Table 1C. In some embodiments, a particular sequence is or comprises UCACUCAGAUAGUUGAAGCC, wherein each U can be independently replaced with T and vice versa. In some embodiments, a particular sequence is or comprises UCACUCAGAUAGUUGAAGCC. In some embodiments, a particular sequence is or comprises UAAUGAGUUCUUCCAACUGG, wherein each U can be independently replaced with T and vice versa. In some embodiments, a particular sequence is or comprises UAAUGAGUUCUUCCAACUGG. In some embodiments, a particular sequence is or comprises AUGAGUUCUUCCAACUGGGG, wherein each U can be independently replaced with T and vice versa. In some embodiments, a particular sequence is or comprises AUGAGUUCUUCCAACUGGGG. In some embodiments, a particular sequence is or comprises CCUCUGUUCCAAAUCCUGCA, wherein each U can be independently replaced with T and vice versa. In some embodiments, a particular sequence is or comprises CCUCUGUUCCAAAUCCUGCA. In some embodiments, a particular sequence is or comprises CUCUGUUCCAAAUCCUGCAU, wherein each U can be independently replaced with T and vice versa. In some embodiments, a particular sequence is or comprises

CUCUGUUCCAAAUCCUGCAU. In some embodiments, a particular sequence is or comprises CGCCUCUGUUCCAAAUCCUG, wherein each U can be independently replaced with T and vice versa. In some embodiments, a particular sequence is or comprises CGCCUCUGUUCCAAAUCCUG. In some embodiments, a particular sequence is or comprises CAGCGGUAAUGAGUUCUUCC, wherein each U can be independently replaced with T and vice versa. In some embodiments, a particular sequence is or comprises CAGCGGUAAUGAGUUCUUCC.

Table 1. Example oligonucleotides and/or compositions.

Table 1A. Example oligonucleotides and/or compositions.

ID	HELM Description	Base Sequence
ASO-0119462	RNA1{m(G)|sp|.m(C)|sp|.m(A)|sp|.m(C)|sp|.m(U)|sp|.d(T)|sp|.d(T)|sp|.d(G)|sp|.d(A)|sp|.d(G)|sp|.d(|m5C])[sp].d(G)[sp].d(A)[sp].d(G)[sp].d(G)[sp].[moe](G)p.[moe]([m5C])p.[moe](G)p.[moe](G)[sp].[moe]([m5C])}$$$$V2.0	GCACUTTGAGC GAGGGCGGC
ASO-0116492	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC
ASO-0116487	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC
ASO-0120569	RNA1{[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	CACUCAGAUAG UUGAAGCC
ASO-0126328	RNA1{[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$SV2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126155	RNA1{[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$SV2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126054	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$SV2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126046	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$SV2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126044	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$SV2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126042	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$SV2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126040	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[n001R].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[	UCACUCAGAUA GUUGAAGCC

	Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	
ASO-0126038	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[n001R].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126037	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[n001R].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126035	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[n001R].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126034	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126032	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126030	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126028	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126026	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126024	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126022	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC
ASO-0126332	RNA1{[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUA GUUGAAGCC

ASO-0132574	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)[n001R].[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUAGUUGAAGCC
ASO-0132575	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(A)p.[fl2r](G)|Ssp|.|fl2r|(A)|Ssp|.m(U)|Ssp|.|fl2r|(A)|Ssp|.m(G)|n001R|.m(U)|Ssp|.|fl2r|(U)|Ssp|.|fl2r|(G)|Ssp|.|fl2r\](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUAGUUGAAGCC
ASO-0132638	RNA1{[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)[n001R].m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)}$$$$V2.0	UCACUCAGAUAGUUGAAGCC
ASO-0139739	RNA1{[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)[n001R].m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)}$$$$V2.0	UCACUCAGAUAGUUGAAGCC
ASO-0139740	RNA1{[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)}$$$$V2.0	UCACUCAGAUAGUUGAAGCC
ASO-0139741	RNA1{[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)[n001R].m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)}$$$$V2.0	UCACUCAGAUAGUUGAAGCC
ASO-0139771	RNA1{[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[n001R].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	UCACUCAGAUAGUUGAAGCC

Table 1B. Example oligonucleotides and/or compositions.

ID	HELM Description	Base Sequence
ASO-0100157	RNA1{m(U)[sp].m(C)[sp].m(A)[sp].m(A)[sp].m(G)[sp].m(G)[sp].m(A)[sp].m(A)[sp].m(G)[sp].m(A)[sp].m(U)[sp].m(G)[sp].m(G)[sp].m(C)[sp].m(A)[sp].m(U)[sp].m(U)[sp].m(U)[sp].m(C)[sp].m(U)}$$$$V2.0	UCAAGGAAGAUGGCAUUUCU
ASO-0101369	RNA1{[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[Ssp].m(A)p.[fl2r](A)[Ssp].m(G)p.m(A)[Ssp].[fl2r](U)[Ssp].m(G)p.m(G)p.[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)}$$$$V2.0	UCAAGGAAGAUGGCAUUUCU
ASO-0104323	RNA1{[moe](T)[sp].[moe]([m5C])[sp].[moe](A)[sp].[moe]([m5C])[sp].[moe](T)[sp].[moe](T)[sp].[moe](T)[sp].[moe]([m5C])[sp].[moe](A)[sp].[moe](T)[sp].[moe](A)[sp].[moe](A)[sp].[moe](T)[sp].[moe](G)[sp].[moe]([m5C])[sp].[moe](T)[sp].[moe](G)[sp].[moe](G)}$$$$V2.0	TCACTTTCATAATGCTGG
ASO-0109259	RNA1{m(C)[Ssp].[moe]([m5C])p.[moe](T)p.[moe]([m5C])p.m(A)[Ssp].d(C)[Ssp].d(T)[Ssp].d(C)[Rsp].d(A)[Ssp].d(C)[Ssp].d(C)[Rsp].d(C)[Ssp].d(A)[Ssp].d(C)[Ssp].d(T)[Ssp].m(C)[Ssp].m(G)[Ssp].m(C)[Ssp].m(C)[Ssp].m(A)}$$$$V2.0	CCTCACTCACCCACTCGCCA

ASO-0136356	RNA1{d(T)[sp].d(C)[sp].d(G)[sp].d(T)[sp].d(C)[sp].d(G)[sp].d(T)[sp].d(T)[sp].d(T)[sp].d(T)[sp].d(G)[sp].d(T)[sp].d(C)[sp].d(G)[sp].d(T)[sp].d(T)[sp].d(T)[sp].d(T)[sp].d(G)[sp].d(T)[sp].d(C)[sp].d(G)[sp].d(T)[sp].d(T)}$$$$V2.0	TCGTCGTTTTGTCGTTTTGTCGTT
ASO-0140873	RNA1{[fl2r](U)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G)}$$$$V2.0	UAAUGAGUUCUUCCAACUGG
ASO-0140874	RNA1{[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G)}$$$$V2.0	AUGAGUUCUUCCAACUGGGG
ASO-0140875	RNA1{[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A)}$$$$V2.0	CCUCUGUUCCAAAUCCUGCA
ASO-0140876	RNA1{[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](C)[Ssp].m(A)[Ssp].[fl2r](A)[Ssp].m(A)p.m(U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](U)}$$$$V2.0	CUCUGUUCCAAAUCCUGCAU
ASO-0140877	RNA1{[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)}$$$$V2.0	CGCCUCUGUUCCAAAUCCUG
ASO-0140878	RNA1{[fl2r](U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G)}$$$$V2.0	UAAUGAGUUCUUCCAACUGG
ASO-0140879	RNA1{[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G)}$$$$V2.0	AUGAGUUCUUCCAACUGGGG
ASO-0140880	RNA1{[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A)}$$$$V2.0	CCUCUGUUCCAAAUCCUGCA
ASO-0140881	RNA1{[fl2r](C)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)}$$$$V2.0	CGCCUCUGUUCCAAAUCCUG
ASO-0140882	RNA1{[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[n001R].[fl2r](G)}$$$$V2.0	AUGAGUUCUUCCAACUGGGG
ASO-0140920	RNA1{[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}S$$$V2.0	CAGCGGUAAUGAGUUCUUCC

ASO-0140921	RNA1{[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	CAGCGGUAAUGAGUUCUUCC
ASO-0140922	RNA1{[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)}$$$$V2.0	CAGCGGUAAUGAGUUCUUCC
ASO-0116227	RNA1{[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001].[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](C)[Ssp].m(A)[Ssp].[fl2r](A)[Ssp].m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A)}$$$$V2.0	CCUCUGUUCCAAAUCCUGCA
ASO-0116109	RNA1{[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](C)[Ssp].m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G)}$$$$V2.0	AUGAGUUCUUCCAACUGGGG
ASO-0116456	RNA1{[fl2r](U)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001].[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G)}$$$$V2.0	UAAUGAGUUCUUCCAACUGG
ASO-0116267	RNA1{[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](U)}$$$$V2.0	CUCUGUUCCAAAUCCUGCAU
ASO-0116234	RNA1{[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001].[fl2r](U)[Ssp].[fl2r](G)[Ssp].m(U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](C)[Ssp].m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)}$$$$V2.0	CGCCUCUGUUCCAAAUCCUG

Table 1C. Example oligonucleotides and/or compositions.

ID	HELM Description	Base Sequence
ASO-0141048	RNA1{m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G)}$$$$V2.0	UAAUGAGUUCUUCCAACUGG
ASO-0141054	RNA1{m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].m(G)}$$$$V2.0	UAAUGAGUUCUUCCAACUGG
ASO-0141049	RNA1{m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A)}$$$$V2.0	CCUCUGUUCCAAAUCCUGCA
ASO-0141055	RNA1{m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)	CCUCUGUUCCAAAUCCUGCA

	[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].m(A)}$$$$V2.0	
ASO-0141053	RNA1{[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)}$$$$V2.0	CAGCGGUAAUGAGUUCUUCC
ASO-0141056	RNA1{m(C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)}$$$$V2.0	CAGCGGUAAUGAGUUCUUCC
ASO-0141480	RNA1{[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G)}$$$$V2.0	AUGAGUUCUUCCAACUGG
ASO-0141481	RNA1{[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)}$$$$V2.0	GCGGUAAUGAGUUCUUCC

Notes:

HELM Description and Base Sequence, due to their length, may be divided into multiple lines in Table 1 (e.g., Table 1A, Table 1B, Table 1C). Unless otherwise specified, all oligonucleotides in Table 1 are single-stranded. As appreciated by those skilled in the art, nucleoside units are unmodified and contain unmodified nucleobases and 2'-deoxy sugars unless otherwise indicated (e.g., with m, [fl2r], etc.); linkages, unless otherwise indicated, are natural phosphate linkages; and acidic/basic groups may independently exist in their salt forms. If a sugar is not specified, the sugar is a natural DNA sugar; and if an internucleotidic linkage is not specified, the internucleotidic linkage is a natural phosphate linkage. A natural DNA sugar may also be indicated with "d" as in d(G), d(A), d(C), d(T), etc., and a natural phosphate linkage may be indicated with "p" in Table 1. Oligonucleotides in Table 1 (e.g., Table 1A, Table 1B, Table 1C) are described using the Hierarchical Editing Language for Macromolecules (HELM), which is described in, e.g., Zhang, T. et al. J Chem Inf Model. 2012 Oct 22;52(10):2796-806 and Milton, J. et al. J Chem Inf Model. 2017 Jun 26;57(6):1233-1239.

Moieties and modifications:

m: 2'-OMe (e.g., m(G), m(A), m(C), m(U));

[m5C]: methyl at 5-position of C (nucleobase is 5-methylcytosine);

[fl2r]: 2'-F;

r: 2'-OH (e.g., r(G), r(A), r(C), r(U));

[moe]: 2'-MOE (2'–$OCH_2CH_2OCH_3$);

[moe]([m5C]): 5-methyl 2'-O-methoxyethyl C;

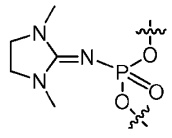
p: phosphodiester (phosphate). It can a linkage or be an end group (or a component thereof), e.g., a linkage between a linker and an oligonucleotide chain, an internucleotidic linkage (a natural phosphate linkage), etc.;

*: Phosphorothioate. It can be an end group (or a component thereof), or a linkage, e.g., a linkage between a linker and an oligonucleotide chain, an internucleotidic linkage (a phosphorothioate internucleotidic linkage), etc.;

[Rsp]: Phosphorothioate in the *R*p configuration;

[Ssp]: Phosphorothioate in the *S*p configuration;

[sp]: stereorandom phosphorothioate;

n001:  ;

[n001]: stereorandom n001;

[n001R]: n001 in Rp configuration;

[n001S]: n001 in Sp configuration.

In some embodiments, a sugar is bonded to an internucleotidic linkage through an oxygen atom, e.g., an oxygen

atom in a natural phosphate linkage such as in typical natural DNA molecules. In some embodiments, a sugar is boned to an internucleotidic linkage through an atom that is not oxygen. In some embodiments, a sugar is boned to an internucleotidic linkage through a nitrogen atom of a sugar. In some embodiments, a sugar is boned to an internucleotidic linkage through a ring nitrogen atom of a sugar; in such cases, a ring nitrogen atom of a sugar may directly form a bond with a linkage phosphorus atom, and those skilled in the art will appreciate an oxygen atom may be removed from a linkage. Certain reagents (e.g., phosphoramidites, nucleosides, etc.) and methods for utilizing various modifications, e.g., those exemplified in the Tables herein, such as modified sugars, modified nucleobases, etc., are described in the Examples, WO 2021/071858 or WO 2022/099159, each of which is incorporated herein by reference. As those skilled in the art appreciate, an oligonucleotide may be referred to by its HELM description, e.g., in the Tables above, with or without "RNA1{}$$$$V2.0".

Nucleobases

[0133] Various nucleobases may be utilized in provided oligonucleotides in accordance with the present disclosure. In some embodiments, a nucleobase is a natural nucleobase, the most commonly occurring ones being A, T, C, G and U. In some embodiments, a nucleobase is a modified nucleobase in that it is not A, T, C, G or U. In some embodiments, a nucleobase is optionally substituted A, T, C, G or U, or a substituted tautomer of A T, C, G or U. In some embodiments, a nucleobase is optionally substituted A, T, C, G or U, e.g., 5mC, 5-hydroxymethyl C, etc. In some embodiments, a nucleobase is alkyl-substituted A, T, C, G or U. In some embodiments, a nucleobase is A. In some embodiments, a nucleobase is T. In some embodiments, a nucleobase is C. In some embodiments, a nucleobase is G. In some embodiments, a nucleobase is U. In some embodiments, a nucleobase is 5mC. In some embodiments, a nucleobase is substituted A, T, C, G or U. In some embodiments, a nucleobase is a substituted tautomer of A, T, C, G or U. In some embodiments, substitution protects certain functional groups in nucleobases to minimize undesired reactions during oligonucleotide synthesis. Suitable technologies for nucleobase protection in oligonucleotide synthesis are widely known in the art and may be utilized in accordance with the present disclosure. In some embodiments, modified nucleobases improves properties and/or activities of oligonucleotides. For example, in many cases, 5mC may be utilized in place of C to modulate certain undesired biological effects, e.g., immune responses. In some embodiments, when determining sequence identity, a substituted nucleobase having the same hydrogen-bonding pattern is treated as the same as the unsubstituted nucleobase, e.g., 5mC may be treated the same as C [e.g., an oligonucleotide having 5mC in place of C (e.g., AT5mCG) is considered to have the same base sequence as an oligonucleotide having C at the corresponding location(s) (e.g., ATCG)]. In some embodiments, a nucleobase is or comprise an optionally substituted ring having at least one nitrogen atom. In some embodiments, a nucleobase comprise Ring BA as described herein, wherein at least one monocyclic ring of Ring BA comprise a nitrogen ring atom.

[0134] In some embodiments, an oligonucleotide comprises one or more A, T, C, G or U. In some

embodiments, an oligonucleotide comprises one or more optionally substituted A, T, C, G or U. In some embodiments, an oligonucleotide comprises one or more 5-methylcytidine, 5-hydroxymethylcytidine, 5-formylcytosine, or 5-carboxylcytosine. In some embodiments, an oligonucleotide comprises one or more 5-methylcytidine. In some embodiments, each nucleobase in an oligonucleotide is selected from the group consisting of optionally substituted A, T, C, G and U, and optionally substituted tautomers of A, T, C, G and U. In some embodiments, each nucleobase in an oligonucleotide is optionally protected A, T, C, G and U. In some embodiments, each nucleobase in an oligonucleotide is optionally substituted A, T, C, G or U. In some embodiments, each nucleobase in an oligonucleotide is selected from the group consisting of A, T, C, G, U, and 5mC.

[0135] As appreciated by those skilled in the art, various nucleobases are known in the art and can be utilized in accordance with the present disclosure, e.g., those described in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the sugar, base, and internucleotidic linkage modifications of each of which are independently incorporated herein by reference. In some embodiments, nucleobases are protected and useful for oligonucleotide synthesis.

[0136] In some embodiments, a nucleobase is a natural nucleobase or a modified nucleobase derived from a natural nucleobase. Examples include uracil, thymine, adenine, cytosine, and guanine optionally having their respective amino groups protected by acyl protecting groups, 2-fluorouracil, 2-fluorocytosine, 5-bromouracil, 5-iodouracil, 2,6-diaminopurine, azacytosine, pyrimidine analogs such as pseudoisocytosine and pseudouracil and other modified nucleobases such as 8-substituted purines, xanthine, or hypoxanthine (the latter two being the natural degradation products). Certain examples of modified nucleobases are disclosed in Chiu and Rana, RNA, 2003, 9, 1034-1048, Limbach et al. Nucleic Acids Research, 1994, 22, 2183-2196 and Revankar and Rao, Comprehensive Natural Products Chemistry, vol. 7, 313. In some embodiments, a modified nucleobase is substituted uracil, thymine, adenine, cytosine, or guanine. In some embodiments, a modified nucleobase is a functional replacement, e.g., in terms of hydrogen bonding and/or base pairing, of uracil, thymine, adenine, cytosine, or guanine. In some embodiments, a nucleobase is optionally substituted uracil, thymine, adenine, cytosine, 5-methylcytosine, or guanine. In some embodiments, a nucleobase is uracil, thymine, adenine, cytosine, 5-methylcytosine, or guanine.

[0137] In some embodiments, a provided oligonucleotide comprises one or more 5-methylcytosine. In some embodiments, the present disclosure provides an oligonucleotide whose base sequence is disclosed herein, e.g., in Table 1, wherein each T may be independently replaced with U and vice versa, and each cytosine is optionally and independently replaced with 5-methylcytosine or vice versa. As appreciated by those skilled in the art, in some embodiments, 5mC may be treated as C with respect to base sequence of an oligonucleotide -

such oligonucleotide comprises a nucleobase modification at the C position (e.g., see various oligonucleotides in Table 1). In description of oligonucleotides, typically unless otherwise noted, nucleobases, sugars and internucleotidic linkages are non-modified.

[0138] In some embodiments, a modified base is optionally substituted adenine, cytosine, guanine, thymine, or uracil, or a tautomer thereof. In some embodiments, a modified nucleobase is a modified adenine, cytosine, guanine, thymine or uracil, modified by one or more modifications by which:

a nucleobase is modified by one or more optionally substituted groups independently selected from acyl, halogen, amino, azide, alkyl, alkenyl, alkynyl, aryl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocyclyl, heteroaryl, carboxyl, hydroxyl, biotin, avidin, streptavidin, substituted silyl, and combinations thereof;

one or more atoms of a nucleobase are independently replaced with a different atom selected from carbon, nitrogen and sulfur;

one or more double bonds in a nucleobase are independently hydrogenated; or

one or more aryl or heteroaryl rings are independently inserted into a nucleobase.

[0139] In some embodiments, a base is optionally substituted A, T, C, G or U. In some embodiments, a modified base is substituted A, T, C, G or U, wherein the modified base is different than the natural A, T, C, G and U. In some embodiments, a modified base is a substituted tautomer of A, T, C, G or U.

[0140] In some embodiments, a modified nucleobase is a modified nucleobase known in the art, e.g., WO2017/210647. In some embodiments, modified nucleobases are expanded-size nucleobases in which one or more aryl and/or heteroaryl rings, such as phenyl rings, have been added. Certain examples of modified nucleobases, including nucleobase replacements, are described in the Glen Research catalog (Glen Research, Sterling, Virginia); Krueger AT et al., Acc. Chem. Res., 2007, 40, 141-150; Kool, ET, Acc. Chem. Res., 2002, 35, 936-943; Benner S.A., et al., Nat. Rev. Genet., 2005, 6, 553-543; Romesberg, F.E., et al., Curr. Opin. Chem. Biol., 2003, 7, 723-733; or Hirao, I., Curr. Opin. Chem. Biol., 2006, 10, 622-627. In some embodiments, an expanded-size nucleobase is an expanded-size nucleobase described in, e.g., WO2017/210647. In some embodiments, modified nucleobases are moieties such as corrin- or porphyrin-derived rings. Certain porphyrin-derived base replacements have been described in, e.g., Morales-Rojas, H and Kool, ET, Org. Lett., 2002, 4, 4377-4380. In some embodiments, a porphyrin-derived ring is a porphyrin-derived ring described in, e.g., WO2017/219647. In some embodiments, a modified nucleobase is a modified nucleobase described in, e.g., WO2017/219647. In some embodiments, a modified nucleobase is fluorescent. Examples of such fluorescent modified nucleobases include phenanthrene, pyrene, stillbene, isoxanthine, isozanthopterin, terphenyl, terthiophene, benzoterthiophene, coumarin, lumazine, tethered stillbene, benzo-uracil, naphtho-uracil, etc., and those described in e.g., WO2017/210647. In some embodiments, a nucleobase or modified nucleobase is selected from: C5-propyne T, C5-propyne C, C5-Thiazole, phenoxazine, 2-thio-thymine, 5-triazolylphenyl-thymine, diaminopurine, and N2-aminopropylguanine.

[0141] In some embodiments, a modified nucleobase is selected from 5-substituted pyrimidines, 6-

azapyrimidines, alkyl or alkynyl substituted pyrimidines, alkyl substituted purines, and N-2, N-6 and O-6 substituted purines. In certain embodiments, modified nucleobases are selected from 2-aminopropyladenine, 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-N-methylguanine, 6-N-methyladenine, 2-propyladenine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl ($-C\equiv C-CH_3$) uracil, 5-propynylcytosine, 6-azouracil, 6-azocytosine, 6-azothymine, 5-ribosyluracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl, 8-aza and other 8-substituted purines, 5-halo, particularly 5-bromo, 5-trifluoromethyl, 5-halouracil, and 5-halocytosine, 7-methylguanine, 7-methyladenine, 2-F-adenine, 2-aminoadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, 3-deazaadenine, 6-N-benzoyladenine, 2-N-isobutyrylguanine, 4-N-benzoylcytosine, 4-N-benzoyluracil, 5-methyl 4-N-benzoylcytosine, 5-methyl 4-N-benzoyluracil, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. In some embodiments, modified nucleobases are tricyclic pyrimidines, such as 1,3-diazaphenoxazine-2-one, 1,3-diazaphenothiazine-2-one or 9-(2-aminoethoxy)-1,3-diazaphenoxazine-2- one (G-clamp). In some embodiments, modified nucleobases are those in which the purine or pyrimidine base is replaced with other heterocycles, for example, 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine or 2- pyridone. In some embodiments, modified nucleobases are those disclosed in US 3687808, The Concise Encyclopedia Of Polymer Science And Engineering, Kroschwitz, J.I., Ed., John Wiley & Sons, 1990, 858-859; Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613; Sanghvi, Y.S., Chapter 15, Antisense Research and Applications, Crooke, S.T. and Lebleu, B., Eds., CRC Press, 1993, 273-288; or in Chapters 6 and 15, Antisense Drug Technology, Crooke S.T., Ed., CRC Press, 2008, 163-166 and 442-443.

[0142] In some embodiments, modified nucleobases and methods thereof are those described in US 20030158403, US 3687808, US 4845205, US 5130302, US 5134066, US 5 175273, US 5367066, US 5432272, US 5434257, US 5457187, US 5459255, US 5484908, US 5502177, US 5525711, US 5552540, US 5587469, US 5594 121, US 5596091, US 5614617, US 5645985, US 5681941, US 5750692, US 5763588, US 5830653, or US 6005096.

[0143] In some embodiments, a modified nucleobase is substituted. In some embodiments, a modified nucleobase is substituted such that it contains, e.g., heteroatoms, alkyl groups, or linking moieties connected to fluorescent moieties, biotin or avidin moieties, or other protein or peptides. In some embodiments, a modified nucleobase is a "universal base" that is not a nucleobase in the most classical sense, but that functions similarly to a nucleobase. One example of a universal base is 3-nitropyrrole.

[0144] In some embodiments, nucleosides that can be utilized in provided technologies comprise modified nucleobases and/or modified sugars, e.g., 4-acetylcytidine; 5-(carboxyhydroxylmethyl)uridine; 2'-O-methylcytidine; 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyluridine; dihydrouridine; 2'-O-methylpseudouridine; beta,D-galactosylqueosine; 2'-O-methylguanosine; N^6-isopentenyladenosine; 1-methyladenosine; 1-methylpseudouridine; 1-methylguanosine; 1-methylinosine; 2,2-

dimethylguanosine; 2-methyladenosine; 2-methylguanosine; N^7-methylguanosine; 3-methyl-cytidine; 5-methylcytidine; 5-hydroxymethylcytidine; 5-formylcytosine; 5-carboxylcytosine; N^6-methyladenosine; 7-methylguanosine; 5-methylaminoethyluridine; 5-methoxyaminomethyl-2-thiouridine; beta,D-mannosylqueosine; 5-methoxycarbonylmethyluridine; 5-methoxyuridine; 2-methylthio-N^6-isopentenyladenosine; N-((9-beta,D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine; N-((9-beta,D-ribofuranosylpurine-6-yl)-N-methylcarbamoyl)threonine; uridine-5-oxyacetic acid methylester; uridine-5-oxyacetic acid (v); pseudouridine; queosine; 2-thiocytidine; 5-methyl-2-thiouridine; 2-thiouridine; 4-thiouridine; 5-methyluridine; 2'-O-methyl-5-methyluridine; and 2'-O-methyluridine.

[0145] In some embodiments, a nucleobase, e.g., a modified nucleobase comprises one or more biomolecule binding moieties such as e.g., antibodies, antibody fragments, biotin, avidin, streptavidin, receptor ligands, or chelating moieties. In other embodiments, a nucleobase is 5-bromouracil, 5-iodouracil, or 2,6-diaminopurine. In some embodiments, a nucleobase comprises substitution with a fluorescent or biomolecule binding moiety. In some embodiments, a substituent is a fluorescent moiety. In some embodiments, a substituent is biotin or avidin.

[0146] Certain examples of nucleobases and related methods are described in US 3687808, 4845205, US 513030, US 5134066, US 5175273, US 5367066, US 5432272, US 5457187, US 5457191, US 5459255, US 5484908, US 5502177, US 5525711, US 5552540, US 5587469, US 5594121, US 5596091, US 5614617, US 5681941, US 5750692, US 6015886, US 6147200, US 6166197, US 6222025, US 6235887, US 6380368, US 6528640, US 6639062, US 6617438, US 7045610, US 7427672, US or US 7495088.

[0147] In some embodiments, an oligonucleotide comprises a nucleobase, sugar, nucleoside, and/or internucleotidic linkage which is described in any of: Gryaznov, S; Chen, J.-K. J. Am. Chem. Soc. 1994, 116, 3143; Hendrix et al. 1997 Chem. Eur. J. 3: 110; Hyrup et al. 1996 Bioorg. Med. Chem. 4: 5; Jepsen et al. 2004 Oligo. 14: 130-146; Jones et al. J. Org. Chem. 1993, 58, 2983; Koizumi et al. 2003 Nuc. Acids Res. 12: 3267-3273; Koshkin et al. 1998 Tetrahedron 54: 3607-3630; Kumar et al. 1998 Bioo. Med. Chem. Let. 8: 2219-2222; 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; Morita et al. 2001 Nucl. Acids Res. Supp. 1: 241-242; Morita et al. 2002 Bioo. Med. Chem. Lett. 12: 73-76; Morita et al. 2003 Bioo. Med. Chem. Lett. 2211-2226; Nielsen et al. 1997 Chem. Soc. Rev. 73; Nielsen et al. 1997 J. Chem. Soc. Perkins Transl. 1: 3423-3433; Obika et al. 1997 Tetrahedron Lett. 38 (50): 8735–8; Obika et al. 1998 Tetrahedron Lett. 39: 5401-5404; Pallan et al. 2012 Chem. Comm. 48: 8195-8197; Petersen et al. 2003 TRENDS Biotech. 21: 74-81; Rajwanshi et al. 1999 Chem. Commun. 1395-1396; Schultz et al. 1996 Nucleic Acids Res. 24: 2966; Seth et al. 2009 J. Med. Chem. 52: 10-13; Seth et al. 2010 J. Med. Chem. 53: 8309-8318; Seth et al. 2010 J. Org. Chem. 75: 1569-1581; Seth et al. 2012 Bioo. Med. Chem. Lett. 22: 296-299; Seth et al. 2012 Mol. Ther-Nuc. Acids. 1, e47; Seth, Punit P; Siwkowski, Andrew; Allerson, Charles R; Vasquez, Guillermo; Lee, Sam; Prakash, Thazha P; Kinberger, Garth; Migawa, Michael T; Gaus, Hans; Bhat, Balkrishen; et al. From Nucleic Acids Symposium Series (2008), 52(1), 553-554; Singh et al. 1998 Chem. Comm. 1247-1248; Singh et al. 1998 J. Org. Chem.

63: 10035-39; Singh et al. 1998 J. Org. Chem. 63: 6078-6079; Sorensen 2003 Chem. Comm. 2130-2131; Ts'o et al. Ann. N. Y. Acad. Sci. 1988, 507, 220; Van Aerschot et al. 1995 Angew. Chem. Int. Ed. Engl. 34: 1338; Vasseur et al. J. Am. Chem. Soc. 1992, 114, 4006; WO 2007090071; or WO 2016/079181.

[0148] In some embodiments, an oligonucleotide comprises a modified nucleobase, nucleoside or nucleotide which is described in any of: Feldman et al. 2017 J. Am. Chem. Soc. 139: 11427-11433, Feldman et al. 2017 Proc. Natl. Acad. Sci. USA 114: E6478-E6479, Hwang et al. 2009 Nucl. Acids Res. 37: 4757-4763, Hwang et al. 2008 J. Am. Chem. Soc. 130: 14872-14882, Lavergne et al. 2012 Chem. Eur. J. 18: 1231-1239, Lavergne et al. 2013 J. Am. Chem. Soc. 135: 5408-5419, Ledbetter et al. 2018 J. Am. Chem. Soc. 140: 758-765, Malyshev et al. 2009 J. Am. Chem. Soc. 131: 14620-14621, Seo et al. 2009 Chem. Bio. Chem. 10: 2394-2400, e.g., d3FB, d2Py analogs, d2Py, d3MPy, d4MPy, d5MPy, d34DMPy, d35DMPy, d45DMPy, d5FM, d5PrM, d5SICS, dFEMO, dMMO2, dNaM, dNM01, dTPT3, nucleotides with 2'-azido, 2'-chloro, 2'-amino or arabinose sugars, isocarbostiryl-, napthyl- and azaindole-nucleotides, and modifications and derivatives and functionalized versions thereof, e.g., those in which the sugar comprises a 2'-modification and/or other modification, and dMMO2 derivatives with meta-chlorine, -bromine, -iodine, -methyl, or -propinyl substituents.

[0149] In some embodiments, a nucleobase comprises at least one optionally substituted ring which comprises a heteroatom ring atom. In some embodiments, a nucleobase comprises at least one optionally substituted ring which comprises a nitrogen ring atom. In some embodiments, such a ring is aromatic. In some embodiments, a nucleobase is bonded to a sugar through a heteroatom. In some embodiments, a nucleobase is bonded to a sugar through a nitrogen atom. In some embodiments, a nucleobase is bonded to a sugar through a ring nitrogen atom.

[0150] In some embodiments, an oligonucleotide comprises a nucleobase or modified nucleobase as described in: WO 2018/022473, WO 2018/098264, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the bases and modified nucleobases of each of which are independently incorporated herein by reference.

[0151] In some embodiments, a nucleobase is an optionally substituted purine base residue. In some embodiments, a nucleobase is a protected purine base residue. In some embodiments, a nucleobase is an optionally substituted adenine residue. In some embodiments, a nucleobase is a protected adenine residue. In some embodiments, a nucleobase is an optionally substituted guanine residue. In some embodiments, a nucleobase is a protected guanine residue. In some embodiments, a nucleobase is an optionally substituted cytosine residue. In some embodiments, a nucleobase is a protected cytosine residue. In some embodiments, a nucleobase is an optionally substituted thymine residue. In some embodiments, a nucleobase is a protected thymine residue. In some embodiments, a nucleobase is an optionally substituted uracil residue. In some embodiments, a nucleobase is a protected uracil residue. In some embodiments, a nucleobase is an optionally substituted 5-methylcytosine residue. In some embodiments, a nucleobase is a protected 5-methylcytosine

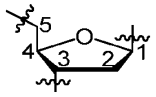
residue.

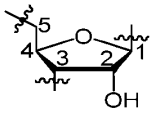
[0152] In some embodiments, a provided oligonucleotide comprises a modified nucleobase described in, e.g., US 5552540, US 6222025, US 6528640, US 4845205, US 5681941, US 5750692, US 6015886, US 5614617, US 6147200, US 5457187, US 6639062, US 7427672, US 5459255, US 5484908, US 7045610, US 3687808, US 5502177, US 5525711 6235887, US 5175273, US 6617438, US 5594121, US 6380368, US 5367066, US 5587469, US 6166197, US 5432272, US 7495088, US 5134066, or US 5596091. In some embodiments, a nucleobase is described in WO 2020/154344, WO 2020/154343, WO 2020/154342, WO 2020/165077, WO 2020/201406, WO 2020/216637, or WO 2020/252376, and can be utilized in accordance with the present disclosure.

[0153] In some embodiments, a nucleobase is a protected base residue as used in oligonucleotide preparation. In some embodiments, a nucleobase is a base residue illustrated in US 2011/0294124, US 2015/0211006, US 2015/0197540, WO 2015/107425, WO 2017/192679, WO 2018/022473, WO 2018/098264, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the base residues of each of which are independently incorporated herein by reference.

Sugars

[0154] Various sugars, including modified sugars, can be utilized in accordance with the present disclosure. In some embodiments, the present disclosure provides sugar modifications and patterns thereof optionally in combination with other structural elements (e.g., internucleotidic linkage modifications and patterns thereof, pattern of backbone chiral centers thereof, etc.) that when incorporated into oligonucleotides can provide improved properties and/or activities.

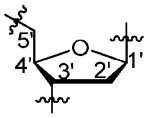
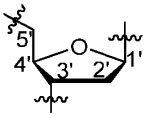
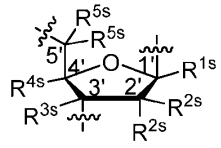
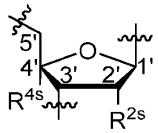
[0155] The most common naturally occurring nucleosides comprise ribose sugars (e.g., in RNA) or deoxyribose sugars (e.g., in DNA) linked to the nucleobases adenosine (A), cytosine (C), guanine (G), thymine (T) or uracil (U). In some embodiments, a sugar, e.g., various sugars in many oligonucleotides in Table 1 (unless otherwise notes), is a natural DNA sugar (in DNA nucleic acids or oligonucleotides, having the structure of 

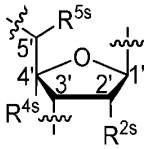
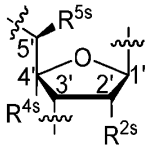
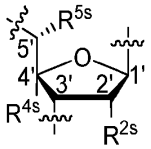
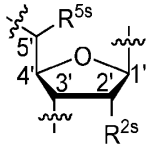
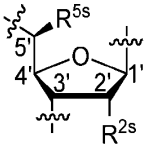
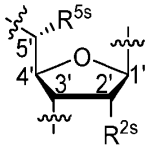
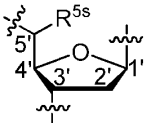
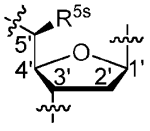
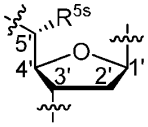
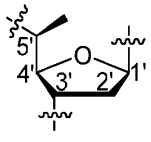
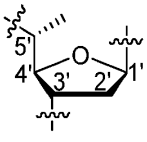
, wherein a nucleobase is attached to the 1' position, and the 3' and 5' positions are connected to internucleotidic linkages (as appreciated by those skilled in the art, if at the 5'-end of oligonucleotide, the 5' position may be connected to a 5'-end group (e.g., –OH), and if at the 3'-end of an oligonucleotide, the 3' position may be connected to a 3'-end group (e.g., –OH). In some embodiments, a sugar is a natural RNA sugar (in RNA nucleic acids or oligonucleotides, having the structure of 

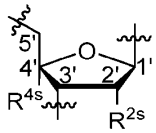
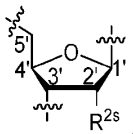
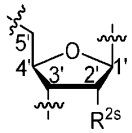
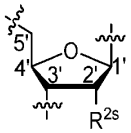
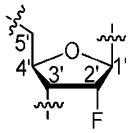
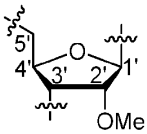
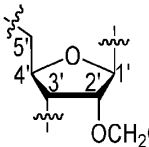
, wherein a nucleobase is attached to the 1' position, and the 3' and 5' positions are connected to internucleotidic linkages (as appreciated by those skilled in the art, if at the 5'-end of an oligonucleotide, the 5' position may be connected to a 5'-end group (e.g., –OH), and if at the 3'-end of an oligonucleotide, the 3' position may be connected to a 3'-end group (e.g., –OH). In some embodiments, a sugar is a modified sugar in that it is not a natural DNA sugar or a natural RNA sugar. Among other things, modified sugars may provide improved stability. In some embodiments, modified sugars can be utilized to alter and/or optimize one or more hybridization characteristics. In some embodiments, modified sugars can be utilized to alter and/or optimize target nucleic acid recognition. In some embodiments, modified sugars can be utilized to optimize Tm. In some embodiments, modified sugars can be utilized to improve oligonucleotide activities.

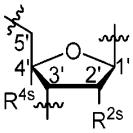
[0156] Among other things, the present disclosure demonstrates that various non-natural RNA sugars, such as natural DNA sugar, various modified sugars (e.g., 2'-F modified sugars, 2'-OMe modified sugars), etc., may be utilized in accordance with the present disclosure.

[0157] Sugars can be bonded to internucleotidic linkages at various positions. As non-limiting examples, internucleotidic linkages can be bonded to the 2', 3', 4' or 5' positions of sugars. In some embodiments, as most commonly in natural nucleic acids, an internucleotidic linkage connects with one sugar at the 5' position and another sugar at the 3' position unless otherwise indicated.

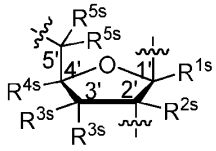
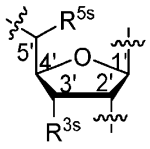
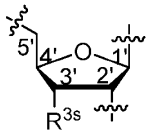
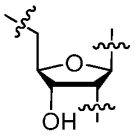
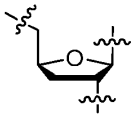
[0158] In some embodiments, a sugar is an optionally substituted natural DNA or RNA sugar. In some embodiments, a sugar is optionally substituted . In some embodiments, the 2' position is optionally substituted. In some embodiments, a sugar is . In some embodiments, a sugar has the structure of  or , wherein each of R^{1s}, R^{2s}, R^{3s}, R^{4s}, and R^{5s} is independently –H, a suitable substituent or suitable sugar modification (e.g., those described in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the substituents, sugar modifications, descriptions of R^{1s}, R^{2s}, R^{3s}, R^{4s}, and R^{5s}, and modified sugars of each of which are independently incorporated herein by reference). In some embodiments, each of R^{1s}, R^{2s}, R^{3s}, R^{4s},

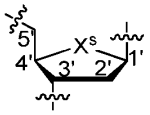
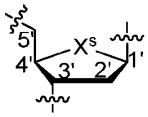
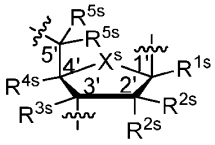
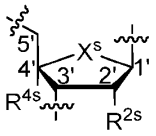
and R^{5s} is independently R^s, wherein each R^s is independently –F, –Cl, –Br, –I, –CN, $–N_3$, –NO, $–NO_2$, $–L^s–R'$, $–L^s–OR'$, $–L^s–SR'$, $–L^s–N(R')_2$, $–O–L^s–OR'$, $–O–L^s–SR'$, or $–O–L^s–N(R')_2$, wherein each R' is independently as described herein, and each L^s is independently a covalent bond or optionally substituted bivalent C_{1-6} aliphatic or heteroaliphatic having 1-4 heteroatoms; or two R^s are taken together to form a bridge $–L^s–$. In some embodiments, R' is optionally substituted C_{1-10} aliphatic. In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, R^{5s} is optionally substituted C_{1-6} aliphatic. In some embodiments, R^{5s} is optionally substituted C_{1-6} alkyl. In some embodiments, R^{5s} is optionally substituted methyl. In some embodiments, R^{5s} is methyl. In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a

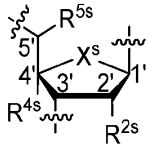
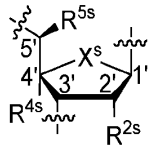
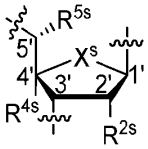
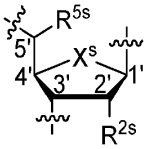
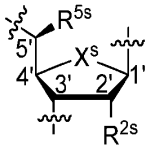
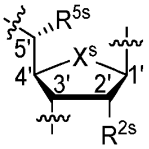
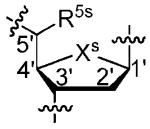
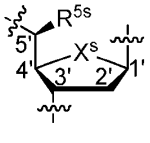
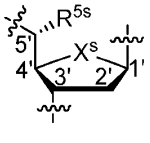
sugar has the structure of . Various such sugars are utilized in Table 1. In some embodiments, a sugar has the structure of . In some embodiments, a 2'-modified sugar has the structure of , wherein R^{2s} is a 2'-modification. In some embodiments, a sugar has the structure of , wherein R^{2s} is –H, halogen, or –OR, wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, R^{2s} is –H. In some embodiments, R^{2s} is –F. In some embodiments, R^{2s} is –OMe. In some embodiments, a modified nucleoside is mA, mT, mC, m5mC, mG, mU, etc., in which R^{2s} is –OMe. In some embodiments, R^{2s} is $-OCH_2CH_2OMe$. In some embodiments, a modified nucleoside is Aeo, Teo, Ceo, m5Ceo, Geo, Ueo, etc., in which R^{2s} is $-OCH_2CH_2OMe$. In some embodiments, R^{2s} is $-OCH_2CH_2OH$. In some embodiments, an oligonucleotide comprises a 2'-F modified sugar having the structure of  (e.g., as in fA, fT, fC, f5mC, fG, fU, [fl2r](A), [fl2r](T), [fl2r](C), [fl2r](5mC), [fl2r](G), [fl2r](U),etc.). In some embodiments, an oligonucleotide comprises a 2'-OMe modified sugar having the structure of  (e.g., as in mA, mT, mC, m5mC, mG, mU, m(A), m(T), m(C), m(5mC), m(G), m(U), etc.). In some embodiments, an oligonucleotide comprises a 2'-MOE modified sugar having the structure of  (e.g., as in Aeo, Teo, Ceo, m5Ceo, Geo, Ueo, [moe](A), [moe](T), [moe](C), [moe](5mC), [moe](G), [moe](U), etc.).

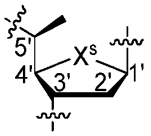
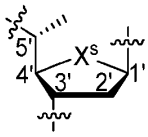
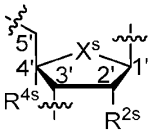
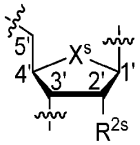
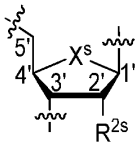
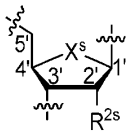
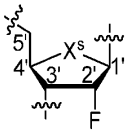
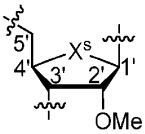
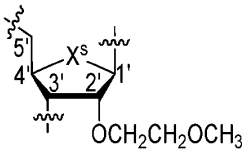
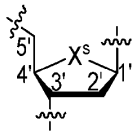
[0159] In some embodiments, a sugar has the structure of , wherein R^{2s} and R^{4s} are taken together to form $-L^s-$, wherein L^s is a covalent bond or optionally substituted bivalent C_{1-6} aliphatic or heteroaliphatic having 1-4 heteroatoms. In some embodiments, each heteroatom is independently selected from nitrogen,

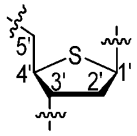
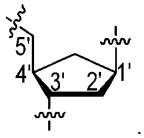
oxygen or sulfur). In some embodiments, L^s is optionally substituted C2–O–CH_2–C4. In some embodiments, L^s is C2–O–CH_2–C4. In some embodiments, L^s is C2–O–(*R*)-CH(CH_2CH_3)–C4. In some embodiments, L^s is C2–O–(*S*)-CH(CH_2CH_3)–C4.

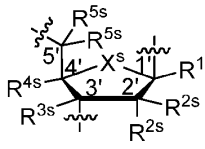
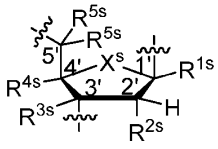
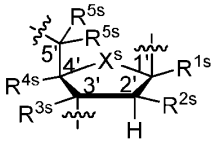
[0160] In some embodiments, a sugar has the structure of , wherein each variable is independently as described herein. In some embodiments, a sugar has the structure of , wherein each variable is independently as described herein. In some embodiments, R^{5s} is –H. In some embodiments, a sugar has the structure of , wherein each variable is independently as described herein. In some embodiments, R^{3s} is –OH. In some embodiments, R^{3s} is –H. In some embodiments, a sugar is . In some embodiments, a sugar is .

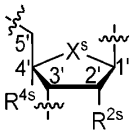
[0161] In some embodiments, a sugar is optionally substituted , wherein X^s is –S–, –Se–, or optionally substituted –CH_2–. In some embodiments, the 2' position is optionally substituted. In some embodiments, a sugar is . In some embodiments, a sugar has the structure of  or , wherein each of R^{1s}, R^{2s}, R^{3s}, R^{4s}, and R^{5s} is independently –H, a suitable substituent or suitable sugar modification (e.g., those described in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO

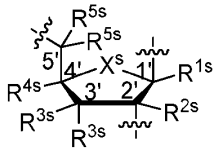
2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the substituents, descriptions of R^{1s}, R^{2s}, R^{3s}, R^{4s}, and R^{5s}, and modified sugars of each of which are independently incorporated herein by reference). In some embodiments, each of R^{1s}, R^{2s}, R^{3s}, R^{4s}, and R^{5s} is independently R^s, wherein each R^s is independently –F, –Cl, –Br, –I, –CN, $–N_3$, –NO, $–NO_2$, $–L^s–R'$, $–L^s–OR'$, $–L^s–SR'$, $–L^s–N(R')_2$, $–O–L^s–OR'$, $–O–L^s–SR'$, or $–O–L^s–N(R')_2$, wherein each R' is independently as described herein, and each L^s is independently a covalent bond or optionally substituted bivalent C_{1-6} aliphatic or heteroaliphatic having 1-4 heteroatoms; or two R^s are taken together to form a bridge $–L^s–$. In some embodiments, R' is optionally substituted C_{1-10} aliphatic. In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, R^{5s} is optionally substituted C_{1-6} aliphatic. In some embodiments, R^{5s} is optionally substituted C_{1-6} alkyl. In some embodiments, R^{5s} is optionally substituted methyl. In some embodiments, R^{5s} is methyl.

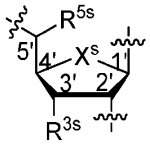
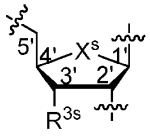
In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure of . Various such sugars are utilized in Table 1. In some embodiments, a sugar has the structure of . In some embodiments, a 2'-modified sugar has the structure of , wherein R^{2s} is a 2'-modification. In some embodiments, a sugar has the structure of , wherein R^{2s} is –H, halogen, or –OR, wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, R^{2s} is –H. In some embodiments, R^{2s} is –F. In some embodiments, R^{2s} is –OMe. In some embodiments, R^{2s} is $-OCH_2CH_2OMe$. In some embodiments, R^{2s} is $-OCH_2CH_2OH$. In some embodiments, a modified sugar has the structure of . In some embodiments, a modified sugar has the structure of . In some embodiments, a modified sugar having the structure of . In some embodiments, a modified sugar having the structure of . In some embodiments, X^s is –S–. In some embodiments, X^s is optionally substituted $-CH_2-$.

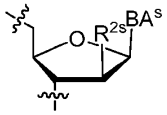
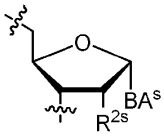
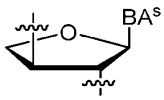
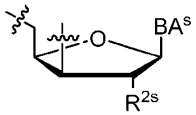
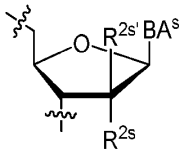
In some embodiments, X^s is $-CH_2-$. In some embodiments, a modified sugar having the structure of . In some embodiments, a modified sugar having the structure of .

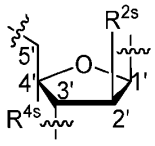
[0162] In some embodiments, a sugar has the structure of , , or , wherein each R^{2s} is independently –H, –F, –OH or $-OR^{ak}$, wherein R^{ak} is optionally substituted C_{1-6} aliphatic, and each of the other variables is independently as described herein. In some embodiments, each of R^{1s}, R^{3s}, R^{4s}, and R^{5s} is independently –H. In some embodiments, each of R^{1s}, R^{3s} and R^{4s}, and one of R^{5s}, are independently –H, and the other R^{5s} is independently C_{1-6} aliphatic. In some embodiments, an occurrence of R^{5s} is C_{1-6} aliphatic, e.g., methyl. In some embodiments, R^{2s} is –H. In some embodiments, R^{2s} is –F. In some embodiments, R^{2s} is $-OR^{ak}$. In some embodiments, R^{2s} is –OMe. In some embodiments, R^{2s} is $-OCH_2CH_2CH_3$. In some embodiments, at least one occurrence of R^{2s} is –H. In some embodiments, at least one occurrence of R^{2s} is not –H. In some embodiments, X^s is –O–. In some embodiments, X^s is –S–. In some embodiments, X^s is optionally substituted $-CH_2-$. In some embodiments, X^s is $-CH_2-$.

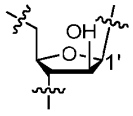
[0163] In some embodiments, a sugar has the structure of , wherein R^{2s} and R^{4s} are taken together to form $-L^s-$, wherein L^s is a covalent bond or optionally substituted bivalent C_{1-6} aliphatic or heteroaliphatic having 1-4 heteroatoms. In some embodiments, each heteroatom is independently selected from nitrogen, oxygen or sulfur). In some embodiments, L^s is optionally substituted C2–O–CH_2–C4. In some embodiments, L^s is C2–O–CH_2–C4. In some embodiments, L^s is C2–O–(*R*)-$CH(CH_2CH_3)$–C4. In some embodiments, L^s is C2–O–(*S*)-$CH(CH_2CH_3)$–C4. In some embodiments, X^s is –S–. In some embodiments, X^s is optionally substituted $-CH_2-$. In some embodiments, X^s is $-CH_2-$. In some embodiments, X^s is –Se–.

[0164] In some embodiments, a sugar has the structure of , wherein each variable is

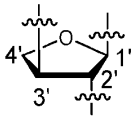
independently as described herein. In some embodiments, a sugar has the structure of , wherein each variable is independently as described herein. In some embodiments, R^{5s} is –H. In some embodiments, a sugar has the structure of , wherein each variable is independently as described herein. In some embodiments, R^{3s} is –OH. In some embodiments, R^{3s} is –H. In some embodiments, X^s is –S–. In some embodiments, X^s is optionally substituted $–CH_2–$. In some embodiments, X^s is $–CH_2–$.

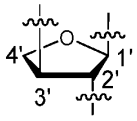
[0165] In some embodiments, a nucleoside comprising a modified sugar has the structure of  or a salt form thereof, wherein BA^s is –H or an optionally substituted or protected nucleobase (e.g., BA), and R^{2s} is as described herein. In some embodiments, R^{2s} is –OH, halogen, or optionally substituted C_1-C_6 alkoxy. In some embodiments, BA^s is –H. In some embodiments, BA^s is an optionally substituted or protected nucleobase. In some embodiments, BA^s is BA. In some embodiments, R^{2s} is –F. In some embodiments, a nucleoside comprising a modified sugar has the structure of  or a salt form thereof, wherein each variable is independently as described herein. In some embodiments, R^{2s} is –H, –OH, halogen, or optionally substituted C_1-C_6 alkoxy. In some embodiments, R^{2s} is –H. In some embodiments, R^{2s} is –F. In some embodiments, a nucleoside comprising a modified sugar has the structure of , wherein each variable is as described herein. In some embodiments, a nucleoside comprising a modified sugar has the structure of  or a salt form thereof, wherein each variable is independently as described herein. In some embodiments, R^{2s} is –H, –OH, halogen, or optionally substituted C_1-C_6 alkoxy. In some embodiments, R^{2s} is –H. In some embodiments, R^{2s} is –F. In some embodiments, a nucleoside comprising a modified sugar has the structure of  or a salt form thereof, wherein $R^{2s'}$ is R^s, and each of R^s,

R^{2s} and BA^s is independently as described herein. In some embodiments, each of R^{2s} and $R^{2s'}$ is independently –H, –OH, halogen, or optionally substituted C_1-C_6 alkoxy. In some embodiments, R^{2s} is –H. In some embodiments, R^{2s} is –OH. In some embodiments, R^{2s} is halogen. In some embodiments, R^{2s} is –F. In some embodiments, R^{2s} is optionally substituted C_1-C_6 alkoxy. In some embodiments, $R^{2s'}$ is –H. In some embodiments, $R^{2s'}$ is –OH. In some embodiments, $R^{2s'}$ is halogen. In some embodiments, $R^{2s'}$ is –F. In some embodiments, $R^{2s'}$ is optionally substituted C_1-C_6 alkoxy. In some embodiments, BA^s is –H. In some embodiments, BA^s is an optionally substituted or protected nucleobase. In some embodiments, BA^s is BA. In some embodiments, nucleobases such as BA are optionally substituted or protected for oligonucleotide synthesis. Certain such nucleosides including sugars and nucleobases and uses thereof are described in WO 2020/154342. In some embodiments, an oligonucleotide comprises arabinoside, 2'-deoxy-2'-fluoro-arabinoside, 2'-OR arabinoside, adeoxycytidine, DNA-abasic, RNA-abasic, or 2'-OR abasic, wherein R is not hydrogen (e.g., optionally substituted C_{1-6} aliphatic). In some embodiments, 2'-OR is 2'-OMe. In some embodiments, 2'-OR is 2'-MOE. In some embodiments, an oligonucleotide comprises 2'-O-methyl-arabinocytidine (amC). In some embodiments, oligonucleotides comprise such nucleosides. In some embodiments, monomers comprise such nucleosides. In some embodiments, phosphoramidites comprise such nucleosides (in some embodiments, one connecting site (e.g., a $-CH_2-$ connecting site) is bonded to an optionally substituted –OH, e.g., (–ODMTr), and one connecting site (e.g., a ring connecting site) is bonded to O which is also bonded to P of a phosphoramidite). In some embodiments, a sugar has the structure of 

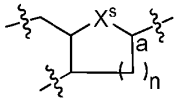
, wherein each variable is as described herein and C1' is bonded to a nucleobase. In some embodiments, a sugar is an arabinose. In some embodiments, a sugar has the structure of 

, wherein C1' is bonded to a nucleobase.

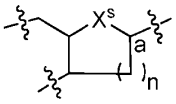
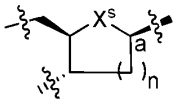
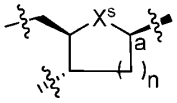
[0166] In some embodiments, a sugar is optionally substituted 

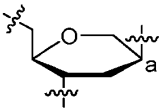
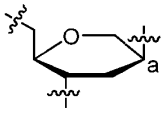
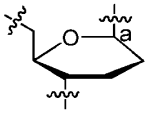
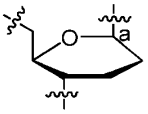
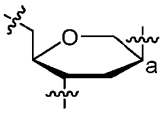
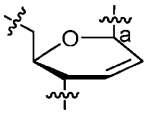
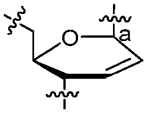
, wherein a nucleobase is bonded at position 1'. In some embodiments, a sugar is 

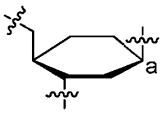
, wherein a nucleobase is bonded at position 1'.

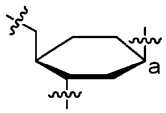
[0167] In some embodiments, a sugar is optionally substituted 

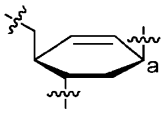
, wherein position a is bonded

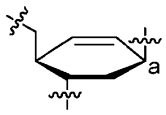
to a nucleobase, X^s is –O–, –S–, –Se– or optionally substituted $–CH_2–$. In some embodiments, a sugar is  . In some embodiments, a sugar is optionally substituted  , wherein position a is bonded to a nucleobase, X^s is –O–, –S–, –Se– or optionally substituted $–CH_2–$. In some embodiments, a sugar is  . In some embodiments, X^s is –O–. In some embodiments, X^s is –S–. In some embodiments, X^s is –Se–. In some embodiments, X^s is optionally substituted $–CH_2–$. In some embodiments, X^s is $–CH_2–$. In some embodiments, n is 0. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3.

[0168] In some embodiments, a modified sugar comprises an optionally substituted 6-membered ring having 0-1 oxygen atom. In some embodiments, a modified sugar comprises an optionally substituted 6-membered ring having an oxygen atom. For example, in some embodiments, a modified sugar has the structure of optionally substituted  , wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure of  , wherein position a is bonded to a nucleobase. in some embodiments, a modified sugar has the structure of optionally substituted  , wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure of  , wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure of  , wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure of optionally substituted  , wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure of  , wherein position a is bonded to a

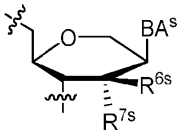
nucleobase. In some embodiments, a modified sugar has the structure of optionally substituted 

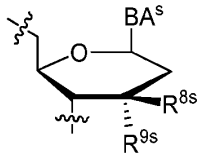
, wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure of 

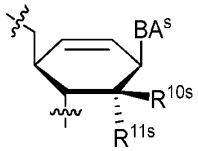
, wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure of optionally substituted 

, wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure of 

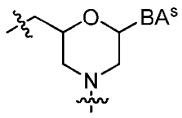
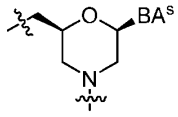
, wherein position a is bonded to a nucleobase.

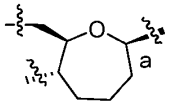
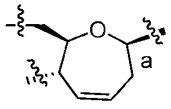
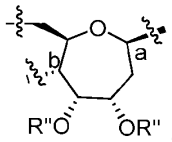
[0169] In some embodiments, a nucleoside comprising a modified sugar has the structure of 

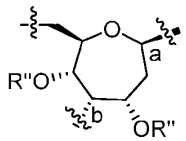
or a salt form thereof, wherein each of R^{6s} and R^{7s} is independently R^s, BA^s is –H or an optionally substituted or protected nucleobase (e.g., BA), and R^s is independently as described herein. In some embodiments, R^{6s} is –H, –OH or halogen, and R^{7s} is –H, –OH, halogen or optionally substituted C_1-C_6 alkoxy. In some embodiments, BA^s is –H. In some embodiments, BA^s is an optionally substituted or protected nucleobase. In some embodiments, BA^s is BA. In some embodiments, a nucleoside comprising a modified sugar has the structure of 

or a salt form thereof, wherein each of R^{8s} and R^{9s} is independently R^s, and each of R^s and BA^s is independently as described herein. In some embodiments, R^{8s} is –H or halogen, and R^{9s} is –H, –OH, halogen, or optionally substituted C_1-C_6 alkoxy. In some embodiments, a nucleoside comprising a modified sugar has the structure of 

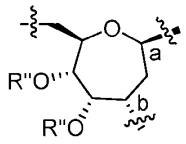
or a salt form thereof, wherein each of R^{10s} and R^{11s} is independently R^s, and each of R^s and BA^s is independently as described herein. In some embodiments, R^{10s} is –H or halogen, and R^{11s} is –H, –OH, halogen, or optionally substituted C_1-C_6 alkoxy. In some embodiments,

a nucleoside comprising a modified sugar has the structure of  or a salt form thereof, wherein BA^s is as described herein. In some embodiments, a nucleoside comprising a modified sugar has the structure of  or a salt form thereof, wherein BA^s is as described herein. Those skilled in the art appreciate that in some embodiments, the nitrogen may be directly bonded to linkage phosphorus. In some embodiments, a halogen is –F. In some embodiments, BA^s is –H. In some embodiments, BA^s is an optionally substituted or protected nucleobase. In some embodiments, BA^s is BA. In some embodiments, nucleobases such as BA are optionally substituted or protected for oligonucleotide synthesis. In some embodiments, an oligonucleotide comprises alpha-homo-DNA, beta-homo-DNA moieties. In some embodiments, an oligonucleotide comprises an alpha- or beta-homo-DNA sugar. In some embodiments, an oligonucleotide comprises an alpha-homo-DNA sugar. In some embodiments, an oligonucleotide comprises a beta-homo-DNA sugar. Certain such nucleosides including sugars and nucleobases and uses thereof are described in WO 2020/154343. In some embodiments, oligonucleotides comprise such nucleosides. In some embodiments, monomers comprise such nucleosides. In some embodiments, phosphoramidites comprise such nucleosides (in some embodiments, one connecting site (e.g., a $-CH_2-$ connecting site) is bonded to an optionally substituted –OH, e.g., –ODMTr, and one connecting site (e.g., a ring connecting site) is bonded to P of a phosphoramidite (e.g., when the connecting ring atom is N) or to O which is also bonded to P of a phosphoramidite(e.g., when the connecting ring atom is C)).

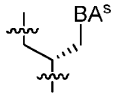
[0170] In some embodiments, a modified sugar has the structure of , wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure of , wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure of , wherein position a is bonded to a nucleobase, position b is bonded to an internucleoside linkage and R'' is –H or optionally substituted C_{1-6} aliphatic. In some embodiments, a modified sugar has the

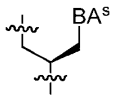


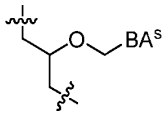
structure of , wherein position a is bonded to a nucleobase, position b is bonded to an internucleoside linkage and R'' is –H or C_{1-6} aliphatic. In some embodiments, a modified sugar has the

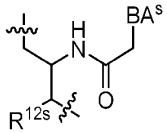


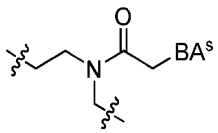
structure of , wherein position a is bonded to a nucleobase, position b is bonded to an internucleoside linkage and R'' is –H or C_{1-6} aliphatic. In some embodiments, R" is methyl.

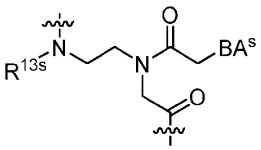
[0171] In some embodiments, a nucleoside comprising a modified sugar has the structure of 

or a salt form thereof, wherein each variable is as described herein. In some embodiments, a nucleoside comprising a modified sugar has the structure of 

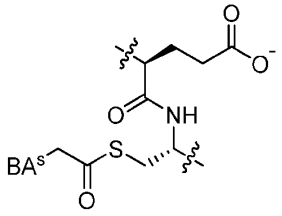
or a salt form thereof, wherein each variable is as described herein. In some embodiments, a nucleoside comprising a modified sugar has the structure of 

or a salt form thereof, wherein each variable is as described herein. In some embodiments, a nucleoside comprising a modified sugar has the structure of 

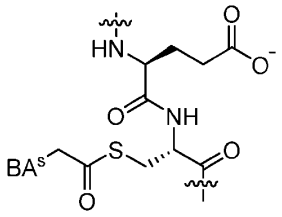
or a salt form thereof, wherein R^{12s} is R^s, and each of R^s and BA^s is independently as described herein. In some embodiments, R^{12s} is –H, –OH, halogen, optionally substituted C_{1-6} alkyl, optionally substituted C_{1-6} heteroalkyl, or optionally substituted C_{1-6} alkoxy. In some embodiments, a halogen is –F. In some embodiments, a nucleoside comprising a modified sugar has the structure of 

or a salt form thereof, wherein each variable is as described herein. In some embodiments, a nucleotide comprising a modified sugar has the structure of 

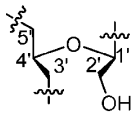
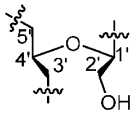
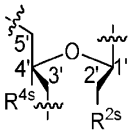
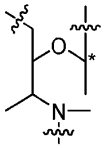
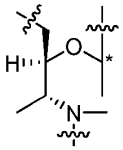
or a salt form thereof, wherein R^{13s} is R^s, and each of R^s and BA^s is independently as described herein. In some embodiments, R^{13s} is –H or optionally substituted C_1-C_6 alkyl. In some embodiments, a nucleoside comprising



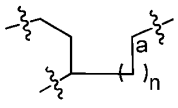
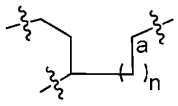
a modified sugar has the structure of or a salt form thereof, wherein each variable is as described herein. In some embodiments, a nucleotide comprising a modified sugar has the structure of



or a salt form thereof, wherein each variable is as described herein. In some embodiments, a linkage is an amide linkage. In some embodiments, BA^s is –H. In some embodiments, BA^s is an optionally substituted or protected nucleobase. In some embodiments, BA^s is BA. In some embodiments, nucleobases such as BA are optionally substituted or protected for oligonucleotide synthesis. Certain such nucleosides and nucleotides including sugars and nucleobases and uses thereof are described in WO 2020/154344. In some embodiments, oligonucleotides comprise such nucleosides. In some embodiments, oligonucleotides comprise such nucleosides (in some embodiments, one connecting site (e.g., a $–CH_2–$ connecting site) is bonded to an optionally substituted –OH, e.g., (–ODMTr), and one connecting site (e.g., a ring connecting site) is bonded to O which is also bonded to P of a phosphoramidite.

[0172] In some embodiments, a sugar is an acyclic sugar, e.g. a UNA sugar. In some embodiments, a sugar is optionally substituted . In some embodiments, the 2' position is optionally substituted. In some embodiments, a sugar is . In some embodiments, a sugar has the structure of . In some embodiments, R^{2s} is –OH. In some embodiments, a sugar is , wherein "*" indicates the carbon atom bonded to a nucleobase. In some embodiments, a sugar is , wherein "*" indicates the

carbon atom bonded to a nucleobase. In some embodiments, the carbon atom bonded to a nitrogen atom of a nucleobase and is of *R* configuration (e.g., sm18). In some embodiments, an oligonucleotide comprises a sugar described herein.

[0173] In some embodiments, a sugar is optionally substituted , wherein position a is bonded to a nucleobase, X^s is –O–, –S–, –Se– or optionally substituted $–CH_2–$. In some embodiments, a sugar is . In some embodiments, X^s is –O–. In some embodiments, X^s is –S–. In some embodiments, X^s is –Se–. In some embodiments, X^s is optionally substituted $–CH_2–$. In some embodiments, X^s is $–CH_2–$. In some embodiments, n is 0. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3.

[0174] In some embodiments, a sugar is connected not through 5' and 3' positions. Those skilled in the art appreciate that for such sugars, 5' can refer to the side/direction toward 5'-end of an oligonucleotide, and 3' can refer to the side/direction toward to 3'-end of an oligonucleotide.

[0175] In some embodiments, each of R^{1s}, R^{2s}, R^{3s}, R^{4s}, and R^{5s} is independently R^s, wherein R^s is independently –H, halogen, –CN, $–N_3$, –NO, $–NO_2$, $–L^s–R'$, $–L^s–Si(R')_3$, $–L^s–OR'$, $–L^s–SR'$, $–L^s–N(R')_2$, $–O–L^s–R'$, $–O–L^s–Si(R)_3$, $–O–L^s–OR'$, $–O–L^s–SR'$, or $–O–L^s–N(R')_2$; wherein L^s is L^B as described herein, and each other variable is independently as described herein. In some embodiments, each of R^{1s} and R^{2s} is independently R^s. In some embodiments, R^s is –H. In some embodiments, R^s is not –H. In some embodiments, L^s is a covalent bond. In some embodiments, each of R^{2s} and R^{4s} are independently –H, –F, –OR, $–N(R)_2$. In some embodiments, R^{2s} is –H, –F, –OR, $–N(R)_2$. In some embodiments, R^{4s} is –H. In some embodiments, R^{2s} and R^{4s} form $2'–O–L^s–$, wherein L^s is optionally substituted C_{1-6} alkylene. In some embodiments, L^s is optionally substituted $–CH_2–$. In some embodiments, L^s is optionally substituted $–CH_2–$.

[0176] In some embodiments, R is hydrogen. In some embodiments, R is not hydrogen. In some embodiments, R is an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, C_{6-20} aryl, a 5-20 membered heteroaryl ring having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon, and a 3-20 membered heterocyclic ring having 1-10 heteroatoms independently selected from oxygen, nitrogen, sulfur, phosphorus and silicon.

[0177] In some embodiments, R is optionally substituted C_{1-30} aliphatic. In some embodiments, R is optionally substituted C_{1-20} aliphatic. In some embodiments, R is optionally substituted C_{1-15} aliphatic. In some embodiments, R is optionally substituted C_{1-10} aliphatic. 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 optionally substituted hexyl, pentyl, butyl, propyl, ethyl or methyl. In some embodiments, R is optionally

substituted hexyl. In some embodiments, R is optionally substituted pentyl. In some embodiments, R is optionally substituted butyl. In some embodiments, R is optionally substituted propyl. In some embodiments, R is optionally substituted ethyl. In some embodiments, R is optionally substituted methyl. In some embodiments, R is hexyl. In some embodiments, R is pentyl. In some embodiments, R is butyl. In some embodiments, R is propyl. In some embodiments, R is ethyl. In some embodiments, R is methyl. In some embodiments, R is isopropyl. In some embodiments, R is n-propyl. In some embodiments, R is tert-butyl. In some embodiments, R is sec-butyl. In some embodiments, R is n-butyl. In some embodiments, R is $-(CH_2)_2OCH_3$.

[0178] In some embodiments, R is optionally substituted phenyl. In some embodiments, R is phenyl.

[0179] In some embodiments, R^{2s} is a 2'-modification as described in the present disclosure, and R^{4s} is –H. In some embodiments, R^{2s} is –OR, wherein R is not hydrogen. In some embodiments, R^{2s} is –F. In some embodiments, R^{2s} is –OMe. In some embodiments, R^{2s} is $-OCH_2CH_2CH_3$, e.g., in various X_{eo} utilized in Table 1 (X being m5C, T, G, A, etc.). In some embodiments, R^{2s} is selected from –H, –F, and –OR, wherein R is optionally substituted C_{1-6} alkyl. In some embodiments, R^{2s} is selected from –H, –F, and –OMe.

[0180] In some embodiments, a sugar is a bicyclic sugar, e.g., sugars wherein R^{2s} and R^{4s} are taken to form an optionally substituted ring as described in the present disclosure. In some embodiments, a sugar is selected from LNA sugars, BNA sugars, cEt sugars, etc. In some embodiments, a bridge is between the 2' and 4'-carbon atoms (corresponding to R^{2s} and R^{4s} taken together with their intervening atoms to form an optionally substituted ring as described herein). In some embodiments, a bridge is 2'–L^a–L^b–4', wherein L^a is –O–, –S– or N(R), and L^b is an optionally substituted C_{1-4} bivalent aliphatic chain, e.g., methylene.

[0181] In some embodiments, a sugar is a 2'-OMe, 2'-MOE, 2'-F, a LNA (locked nucleic acid) sugar, an ENA (ethylene bridged nucleic acid) sugar, a BNA(NMe) (Methylamino bridged nucleic acid) sugar, 2'-F ANA (2'-F arabinose), alpha-DNA (alpha-D-ribose), 2'/5' ODN (e.g., 2'/5' linked oligonucleotide), Inv (inverted sugar, e.g., inverted desoxyribose), AmR (Amino-Ribose), ThioR (Thio-ribose), HNA (hexose nucleic acid), CcNA (cyclohexene nucleic acid), or MOR (Morpholino) sugar.

[0182] Those skilled in the art after reading the present disclosure will appreciate that various types of sugar modifications are known and can be utilized in accordance with the present disclosure. In some embodiments, a sugar modification is a 2'-modification (e.g., R^{2s}). In some embodiments, a 2'-modification is 2'-F. In some embodiments, a 2'-modification is 2'-OR, wherein R is not hydrogen. In some embodiments, a 2'-modification is 2'-OR, wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, a 2'-modification is 2'-OR, wherein R is optionally substituted C_{1-6} alkyl. In some embodiments, a 2'-modification is 2'-OMe. In some embodiments, a 2'-modification is 2'-MOE. In some embodiments, a 2'-modification is –O–L^b– or –L^b–L^b– which connects the 2'-carbon of a sugar moiety to another carbon of a sugar moiety. In some embodiments, a 2'-modification is 2'–O–L^b–4' or 2'–L^b–L^b–4' 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 is an LNA sugar. In some embodiments, –L^b– is $-C(R)_2-$. In some embodiments, a 2'-modification is

(C2–O–C(R)$_2$–C4), wherein each R is independently as described in the present disclosure. In some embodiments, a 2'-modification is a LNA sugar modification (C2–O–CH$_2$–C4). In some embodiments, a 2'-modification is (C2–O–CHR–C4), wherein R is as described in the present disclosure. In some embodiments, a 2'-modification is (C2–O–(*R*)-CHR–C4), wherein R is as described in the present disclosure and is not hydrogen. In some embodiments, a 2'-modification is (C2–O–(*S*)-CHR–C4), wherein R is as described in the present disclosure and is not hydrogen. 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 unsubstituted C_{1-6} alkyl. In some embodiments, R is methyl. In some embodiments, R is ethyl. In some embodiments, a 2'-modification is (C2–O–CHR–C4), wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, a 2'-modification is (C2–O–CHR–C4), wherein R is optionally substituted C_{1-6} alkyl. In some embodiments, a 2'-modification is (C2–O–CHR–C4), wherein R is methyl. In some embodiments, a 2'-modification is (C2–O–CHR–C4), wherein R is ethyl. In some embodiments, a 2'-modification is (C2–O–(*R*)-CHR–C4), wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, a 2'-modification is (C2–O–(*R*)-CHR–C4), wherein R is optionally substituted C_{1-6} alkyl. In some embodiments, a 2'-modification is (C2–O–(*R*)-CHR–C4), wherein R is methyl. In some embodiments, a 2'-modification is (C2–O–(*R*)-CHR–C4), wherein R is ethyl. In some embodiments, a 2'-modification is (C2–O–(*S*)-CHR–C4), wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, a 2'-modification is (C2–O–(*S*)-CHR–C4), wherein R is optionally substituted C_{1-6} alkyl. In some embodiments, a 2'-modification is (C2–O–(*S*)-CHR–C4), wherein R is methyl. In some embodiments, a 2'-modification is (C2–O–(*S*)-CHR–C4), wherein R is ethyl. In some embodiments, a 2'-modification is C2–O–(*R*)-CH(CH$_2$CH$_3$)–C4. In some embodiments, a 2'-modification is C2–O–(*S*)-CH(CH$_2$CH$_3$)–C4. In some embodiments, a sugar is a natural DNA sugar. In some embodiments, a sugar is a natural RNA sugar. In some embodiments, a sugar is an optionally substituted natural DNA sugar. In some embodiments, a sugar is a natural DNA sugar optionally substituted at 2'. In some embodiments, a sugar is a natural DNA sugar substituted at 2' (2'-modification). In some embodiments, a sugar is a natural DNA sugar modified at 2' (2'-modification).

[0183] In some embodiments, a sugar is an optionally substituted ribose or deoxyribose. In some embodiments, a sugar is an optionally modified ribose or deoxyribose, wherein one or more hydroxyl groups of the ribose or deoxyribose moiety is optionally and independently replaced by halogen, R', –N(R')$_2$, –OR', or –SR', wherein each R' is as described herein. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with halogen, R', –N(R')$_2$, –OR', or –SR', wherein each R' is independently described in the present disclosure. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with halogen. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with one or more –F. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position

of the deoxyribose is optionally substituted with –OR', wherein each R' is independently described in the present disclosure. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with –OR', wherein each R' is independently optionally substituted C_1–C_6 aliphatic. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with –OR', wherein each R' is independently an optionally substituted C_1–C_6 alkyl. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with –OMe. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with –O–methoxyethyl.

[0184] In some embodiments, provided oligonucleotides comprise one or more modified sugars. In some embodiments, provided oligonucleotides comprise one or more modified sugars and one or more natural sugars.

[0185] Examples of bicyclic sugars include sugars of alpha-L-methyleneoxy (4'-CH_2-O-2') LNA, beta-D-methyleneoxy (4'-CH_2-O-2') LNA, ethyleneoxy (4' -$(CH_2)_2$-O-2') LNA, aminooxy (4' -CH_2-O-N(R)-2') LNA, and oxyamino (4'-CH_2-N(R)-O-2') LNA. In some embodiments, a bicyclic sugar, e.g., a LNA or BNA sugar, is sugar having at least one bridge between two sugar carbons. In some embodiments, a bicyclic sugar in a nucleoside may have the stereochemical configurations of alpha-L-ribofuranose or beta-D-ribofuranose.

[0186] In some embodiments, a bicyclic sugar may be further defined by isomeric configuration. For example, a sugar comprising a 4'-(CH_2)-O-2' bridge may be in the alpha-L configuration or in the beta-D configuration. In some embodiments, a 4' to 2' bridge is a -L-4'-(CH_2)-O-2', b-D-4'-CH_2-O-2', 4'-$(CH_2)_2$-O-2', 4'-CH_2-O-N(R')-2', 4'-CH_2-N(R')-O-2', 4'-CH(R')-O-2', 4'-CH(CH_3)-O-2', 4'-CH_2-S-2', 4'-CH_2-N(R')-2', 4'-CH_2-CH(R')-2', 4'-CH_2-CH(CH_3)-2', and 4'-$(CH_2)_3$-2', wherein each R' is as described in the present disclosure. In some embodiments, R' is –H, a protecting group or optionally substituted C_1-C_{12} alkyl. In some embodiments, R' is –H or optionally substituted C_1-C_{12} alkyl.

[0187] In some embodiments, a bicyclic sugar is a sugar of alpha-L-methyleneoxy (4'-CH_2-O-2') BNA, beta-D-methyleneoxy (4'-CH_2-O-2') BNA, ethyleneoxy (4'-$(CH_2)_2$-O-2') BNA, aminooxy (4'-CH_2-O-N(R)-2') BNA, oxyamino (4'-CH_2-N(R)-O-2') BNA, methyl(methyleneoxy) (4'-CH(CH_3)-O-2') BNA (also referred to as constrained ethyl or cEt), methylene-thio (4'-CH_2-S-2') BNA, methylene-amino (4'-CH_2-N(R)-2') BNA, methyl carbocyclic (4'-CH_2-CH(CH_3)-2') BNA, propylene carbocyclic (4'-$(CH_2)_3$-2') BNA, or vinyl BNA.

[0188] In some embodiments, a sugar modification is a modification described in US 9006198. In some embodiments, a modified sugar is described in US 9006198. In some embodiments, a sugar modification is a modification described in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858,

and/or WO 2022/099159, the sugar modifications and modified sugars of each of which are independently incorporated herein by reference.

[0189] In some embodiments a modified sugar is one described in US 5658873, US 5118800, US 5393878, US 5514785, US 5627053, US 7034133;7084125, US 7399845, US 5319080, US 5591722, US 5597909, US 5466786, US 6268490, US 6525191, US 5519134, US 5576427, US 6794499, US 6998484, US 7053207, US 4981957, US 5359044, US 6770748, US 7427672, US 5446137, US 6670461, US 7569686, US 7741457, US 8022193, US 8030467, US 8278425, US 5610300, US 5646265, US 8278426, US 5567811, US 5700920, US 8278283, US 5639873, US 5670633, US 8314227, US 2008/0039618, US 2009/0012281, WO 2021/030778, WO 2020/154344, WO 2020/154343, WO 2020/154342, WO 2020/165077, WO 2020/201406, WO 2020/216637, or WO 2020/252376.

[0190] In some embodiments, a sugar modification is 2'-OMe, 2'-MOE, 2'-LNA, 2'-F, 5'-vinyl, or S-cEt. In some embodiments, a modified sugar is a sugar of FRNA, FANA, or morpholino. In some embodiments, an oligonucleotide comprises a nucleic acid analog, e.g., GNA, LNA, PNA, TNA, F-HNA (F-THP or 3'-fluoro tetrahydropyran), MNA (mannitol nucleic acid, e.g., Leumann 2002 Bioorg. Med. Chem. 10: 841-854), ANA (anitol nucleic acid), or morpholino, or a portion thereof. In some embodiments, a sugar is as in flexible nucleic acids or serinol nucleic acids. In some embodiments, a sugar modification replaces a natural sugar with another cyclic or acyclic moiety. Examples of such moieties are widely known in the art, e.g., those used in morpholino, glycol nucleic acids, etc. and may be utilized in accordance with the present disclosure. As appreciated by those skilled in the art, when utilized with modified sugars, in some embodiments internucleotidic linkages may be modified, e.g., as in morpholino, PNA, etc. In some embodiments, a sugar is a (R)-GNA sugar. In some embodiments, a sugar is a (S)-GNA sugar. In some embodiments, a sugar is bicyclic sugar. In some embodiments, a sugar is a LNA sugar. In some embodiments, a sugar is an acyclic sugar. In some embodiments, a sugar is a UNA sugar. In some embodiments, a nucleoside is abasic.

[0191] In some embodiments, a sugar is a 6'-modified bicyclic sugar that have either (R) or (S)-chirality at the 6-position, e.g., those described in US 7399845. In some embodiments, a sugar is a 5'-modified bicyclic sugar that has either (R) or (S)-chirality at the 5-position, e.g., those described in US 20070287831.

[0192] In some embodiments, a modified sugar contains one or more substituents at the 2' position (typically one substituent, and often at the axial position) independently selected from –F; $–CF_3$, –CN, $–N_3$, –NO, $–NO_2$, –OR', –SR', or $–N(R')_2$, wherein each R' is independently described in the present disclosure; $–O–(C_1–C_{10}$ alkyl), $–S–(C_1–C_{10}$ alkyl), $–NH–(C_1–C_{10}$ alkyl), or $–N(C_1–C_{10}$ alkyl)$_2$; $–O–(C_2–C_{10}$ alkenyl), $–S–(C_2–C_{10}$ alkenyl), $–NH–(C_2–C_{10}$ alkenyl), or $–N(C_2–C_{10}$ alkenyl)$_2$; $–O–(C_2–C_{10}$ alkynyl), $–S–(C_2–C_{10}$ alkynyl), $–NH–(C_2–C_{10}$ alkynyl), or $–N(C_2–C_{10}$ alkynyl)$_2$; or $–O—(C_1–C_{10}$ alkylene)$–O—(C_1–C_{10}$ alkyl), $–O–(C_1–C_{10}$ alkylene)$–NH–(C_1–C_{10}$ alkyl) or $–O–(C_1–C_{10}$ alkylene)$–NH(C_1–C_{10}$ alkyl)$_2$, $–NH–(C_1–C_{10}$ alkylene)$–O–(C_1–C_{10}$ alkyl), or $–N(C_1–C_{10}$ alkyl)$–(C_1–C_{10}$ alkylene)$–O–(C_1–C_{10}$ alkyl), wherein each of the alkyl, alkylene, alkenyl and alkynyl is independently and optionally substituted. In some embodiments, a substituent is $–O(CH_2)_nOCH_3$, $–O(CH_2)_nNH_2$, MOE, DMAOE, or DMAEOE, wherein n is from 1 to about 10. In some

embodiments, a modified sugar is one described in WO 2001/088198; and Martin et al., Helv. Chim. Acta, 1995, 78, 486-504. In some embodiments, a modified sugar comprises one or more groups selected from a substituted silyl group, an RNA cleaving group, a reporter group, a fluorescent label, an intercalator, a group for improving the pharmacokinetic properties of a nucleic acid, a group for improving the pharmacodynamic properties of a nucleic acid, or other substituents having similar properties. In some embodiments, modifications are made at one or more of the 2', 3', 4', or 5' positions, including the 3' position of the sugar on the 3'-terminal nucleoside or in the 5' position of the 5'-terminal nucleoside.

[0193] In some embodiments, the 2'-OH of a ribose is replaced with a group selected from –H, –F; $–CF_3$, –CN, $–N_3$, –NO, $–NO_2$, –OR', –SR', or $–N(R')_2$, wherein each R' is independently described in the present disclosure; $–O–(C_1–C_{10}$ alkyl), $–S–(C_1–C_{10}$ alkyl), $–NH–(C_1–C_{10}$ alkyl), or $–N(C_1–C_{10}$ alkyl)$_2$; $–O–(C_2–C_{10}$ alkenyl), $–S–(C_2–C_{10}$ alkenyl), $–NH–(C_2–C_{10}$ alkenyl), or $–N(C_2–C_{10}$ alkenyl)$_2$; $–O–(C_2–C_{10}$ alkynyl), $–S–(C_2–C_{10}$ alkynyl), $–NH–(C_2–C_{10}$ alkynyl), or $–N(C_2–C_{10}$ alkynyl)$_2$; or $–O–(C_1–C_{10}$ alkylene)–O—($C_1–C_{10}$ alkyl), $–O–(C_1–C_{10}$ alkylene)–NH–($C_1–C_{10}$ alkyl) or $–O–(C_1–C_{10}$ alkylene)–NH($C_1–C_{10}$ alkyl)$_2$, $–NH–(C_1–C_{10}$ alkylene)–O–($C_1–C_{10}$ alkyl), or $–N(C_1–C_{10}$ alkyl)–($C_1–C_{10}$ alkylene)–O–($C_1–C_{10}$ alkyl), wherein each of the alkyl, alkylene, alkenyl and alkynyl is independently and optionally substituted. In some embodiments, the 2'–OH is replaced with –H (deoxyribose). In some embodiments, the 2'–OH is replaced with –F. In some embodiments, the 2'–OH is replaced with –OR'. In some embodiments, the 2'–OH is replaced with –OMe. In some embodiments, the 2'–OH is replaced with $–OCH_2CH_2OMe$.

[0194] In some embodiments, a sugar modification is a 2'-modification. Commonly used 2'-modifications include but are not limited to 2'–OR, wherein R is not hydrogen and is as described in the present disclosure. In some embodiments, a modification is 2'–OR, wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, a modification is 2'–OR, wherein R is optionally substituted C_{1-6} alkyl. In some embodiments, a modification is 2'–OMe. In some embodiments, a modification is 2'-MOE. In some embodiments, a 2'-modification is S-cEt. In some embodiments, a modified sugar is an LNA sugar. In some embodiments, a 2'-modification is –F. In some embodiments, a 2'-modification is FANA. In some embodiments, a 2'-modification is FRNA. In some embodiments, a sugar modification is a 5'-modification, e.g., 5'-Me. In some embodiments, a sugar modification changes the size of the sugar ring. In some embodiments, a sugar modification is the sugar moiety in FHNA.

[0195] 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 (optionally with its phosphorodiamidate linkage), glycol nucleic acids, etc.

[0196] In some embodiments, one or more of the sugars of an oligonucleotide are modified. In some embodiments, a modified sugar comprises a 2'-modification. In some embodiments, each modified sugar independently comprises a 2'-modification. In some embodiments, a 2'-modification is 2'-OR. 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'-OR or 2'-F. In some embodiments, each sugar modification is independently 2'-OR or 2'-F, wherein R is optionally substituted C_{1-6} alkyl. In some embodiments, each sugar modification is independently 2'-OR or 2'-F, wherein at least one is 2'-F. In some embodiments, each sugar modification is independently 2'-OR or 2'-F, wherein R is optionally substituted C_{1-6} alkyl, and wherein at least one is 2'-OR. In some embodiments, each sugar modification is independently 2'-OR or 2'-F, wherein at least one is 2'-F, and at least one is 2'-OR. In some embodiments, each sugar modification is independently 2'-OR or 2'-F, wherein R is optionally substituted C_{1-6} alkyl, and wherein at least one is 2'-F, and at least one is 2'-OR. In some embodiments, each sugar modification is independently 2'-OR. In some embodiments, each sugar modification is independently 2'-OR, wherein R is optionally substituted C_{1-6} alkyl. In some embodiments, each sugar modification is 2'-OMe. In some embodiments, each sugar modification is 2'-MOE. In some embodiments, each sugar modification is independently 2'-OMe or 2'-MOE. In some embodiments, each sugar modification is independently 2'-OMe, 2'-MOE, or a LNA sugar.

[0197] Modified sugars include cyclobutyl or cyclopentyl moieties in place of a pentofuranosyl sugar. Representative examples of such modified sugars include those described in US 4,981,957, US 5,118,800, US 5,319,080, or US 5,359,044. In some embodiments, the oxygen atom within the ribose ring is replaced by nitrogen, sulfur, selenium, or carbon. In some embodiments, –O– is replaced with –N(R')–, –S–, –Se– or $–C(R')_2–$. In some embodiments, a modified sugar is a modified ribose wherein the oxygen atom within the ribose ring is replaced with nitrogen, and wherein the nitrogen is optionally substituted with an alkyl group (e.g., methyl, ethyl, isopropyl, etc.).

[0198] A non-limiting example of modified sugars is glycerol, which is part of glycerol nucleic acids (GNAs), e.g., as described in Zhang, R et al., J. Am. Chem. Soc., 2008, 130, 5846-5847; Zhang L, et al., J. Am. Chem. Soc., 2005, 127, 4174-4175 and Tsai CH et al., PNAS, 2007, 14598-14603.

[0199] A flexible nucleic acid (FNA) is based on a mixed acetal aminal of formyl glycerol, e.g., as described in Joyce GF et al., PNAS, 1987, 84, 4398-4402 and Heuberger BD and Switzer C, J. Am. Chem. Soc., 2008, 130, 412-413.

[0200] In some embodiments, an oligonucleotide, and/or a modified nucleoside thereof, comprises a sugar or modified sugar described in: WO 2018/022473, WO 2018/098264, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the sugars and modified sugars of each of which are independently incorporated herein by reference.

[0201] In some embodiments, one or more hydroxyl group in a sugar is optionally and independently replaced with halogen, R' $–N(R')_2$, –OR', or –SR', wherein each R' is independently described in the present disclosure.

[0202] In some embodiments, a modified nucleoside is any modified nucleoside described in: WO 2018/022473, WO 2018/098264, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO

2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the modified nucleosides of each of which are independently incorporated herein by reference.

[0203] In some embodiments, a sugar modification is 5'-vinyl (R or S), 5'-methyl (R or S), 2'-SH, 2'-F, 2'-OCH_3, 2'-OCH_2CH_3, 2'-OCH_2CH_2F or 2'-$O(CH_2)_{20}CH_3$. In some embodiments, a substituent at the 2' position, e.g., a 2'-modification, is allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, OCF_3, OCH_2F, $O(CH_2)_2SCH_3$, $O(CH_2)_2$-O-$N(R_m)(R_n)$, O-CH_2-C(=O)-$N(R_m)(R_n)$, and O-CH_2-C(=O)-$N(R_1)$-$(CH_2)_2$-$N(R_m)(R_n)$, wherein each allyl, amino and alkyl is optionally substituted, and each of R_l, R_m and R_n is independently R' as described in the present disclosure. In some embodiments, each of R_l, R_m and R_n is independently –H or optionally substituted C_1-C_{10} alkyl.

[0204] In some embodiments, bicyclic sugars comprise a bridge, e.g., $–L^b–L^b–$, –L–, etc. between two sugar carbons, e.g., between the 4' and the 2' ribosyl ring carbon atoms. In some embodiments, a bridge is 4'–(CH_2)–O–2' (e.g., LNA sugars), 4'–(CH_2)–S–2', 4'–$(CH_2)_2$–O–2' (e.g., ENA sugars), 4'–CH(R')–O–2' (e.g., 4'–$CH(CH_3)$–O–2', 4'–$CH(CH_2OCH_3)$–O–2', and examples in US 7399845, etc.), 4'–$CH(R')_2$–O–2' (e.g., 4'–$C(CH_3)(CH_3)$–O–2' and examples in WO 2009006478, etc.), 4'-CH_2-N(OR')-2' (e.g., 4'-CH_2-$N(OCH_3)$-2', examples in WO 2008150729, etc.), 4'–CH_2–O–N(R')–2' (e.g., 4'–CH_2–O–$N(CH_3)$–2', examples in US 20040171570, etc.), 4'–CH_2–N(R')–O–2' [e.g., wherein R is –H, C_1-C_{12} alkyl, or a protecting group (e.g., see US 7427672)], 4'–$C(R')_2$–C(H)(R')–2' (e.g., 4'–CH_2–$C(H)(CH_3)$–2', examples in Chattopadhyaya et al., J. Org. Chem., 2009, 74, 118-134, etc.), or 4'–$C(R')_2$–$C(=C(R')_2)$–2' (e.g., 4'–CH_2–$C(=CH_2)$–2', examples in WO 2008154401, etc.).

[0205] In some embodiments, a sugar is a tetrahydropyran or THP sugar. In some embodiments, a modified nucleoside is tetrahydropyran nucleoside or THP nucleoside which is a nucleoside having a six-membered tetrahydropyran sugar substituted for a pentofuranosyl residue in typical natural nucleosides. THP sugars and/or nucleosides include those used in hexitol nucleic acid (HNA), anitol nucleic acid (ANA), mannitol nucleic acid (MNA) (e.g., Leumann, Bioorg. Med. Chem., 2002, 10, 841-854) or fluoro HNA (F-HNA).

[0206] In some embodiments, sugars comprise rings having more than 5 atoms and/or more than one heteroatom, e.g., morpholino sugars which are described in e.g., Braasch et al., Biochemistry, 2002, 41, 4503-4510; US 5698685; US 5166315; US 5185444; US 5034506; etc.).

[0207] As those skilled in the art will appreciate, modifications of sugars, nucleobases, internucleotidic linkages, etc. can and are often utilized in combination in oligonucleotides, e.g., see various oligonucleotides in Table 1.

[0208] In some embodiments, a nucleoside has a six-membered cyclohexenyl in place of the pentofuranosyl residue in naturally occurring nucleosides. Example cyclohexenyl nucleosides and preparation and uses thereof are described in, e.g., WO 2010036696; Robeyns et al., J. Am. Chem. Soc., 2008, 130(6), 1979-1984; Horvath et al., Tetrahedron Letters, 2007, 48, 3621-3623; Nauwelaerts et al., J. Am. Chem. Soc., 2007, 129(30), 9340-9348; Gu et al., Nucleosides, Nucleotides & Nucleic Acids, 2005, 24(5-7), 993-998; Nauwelaerts et al., Nucleic Acids Research, 2005, 33(8), 2452-2463; Robeyns et al., Acta Crystallographica,

Section F: Structural Biology and Crystallization Communications, 2005, F61(6), 585-586; Gu et al., Tetrahedron, 2004, 60(9), 2111-2123; Gu et al., Oligonucleotides, 2003, 13(6), 479-489; Wang et al., J. Org. Chem., 2003, 68, 4499-4505; Verbeure et al., Nucleic Acids Research, 2001, 29(24), 4941-4947; Wang et al., J. Org. Chem., 2001, 66, 8478-82; Wang et al., Nucleosides, Nucleotides & Nucleic Acids, 2001, 20(4-7), 785-788; Wang et al., J. Am. Chem., 2000, 122, 8595-8602; WO 2006047842; WO 2001049687; etc.

[0209] Many monocyclic, bicyclic and tricyclic ring systems are suitable as sugar surrogates (modified sugars) and may be utilized in accordance with the present disclosure. See, e.g., Leumann, Christian J. Bioorg. & Med. Chem., 2002, 10, 841-854. Such ring systems can undergo various additional substitutions to further enhance their properties and/or activities.

[0210] In some embodiments, a 2'-modified sugar is a furanosyl sugar modified at the 2' position. In some embodiments, a 2'-modification is halogen, –R' (wherein R' is not –H), –OR' (wherein R' is not –H), –SR', $-N(R')_2$, optionally substituted $-CH_2-CH=CH_2$, optionally substituted alkenyl, or optionally substituted alkynyl. In some embodiments, a 2'-modifications is selected from $-O[(CH_2)_nO]_mCH_3$, $-O(CH_2)_nNH_2$, $-O(CH_2)_nCH_3$, $-O(CH_2)_nF$, $-O(CH_2)_nONH_2$, $-OCH_2C(=O)N(H)CH_3$, and $-O(CH_2)_nON[(CH_2)_nCH_3]_2$, wherein each n and m is independently from 1 to about 10. In some embodiments, a 2'-modification is optionally substituted C_1-C_{12} alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkaryl, optionally substituted aralkyl, optionally substituted –O–alkaryl, optionally substituted –O–aralkyl, –SH, $-SCH_3$, –OCN, –Cl, –Br, –CN, –F, $-CF_3$, $-OCF_3$, $-SOCH_3$, $-SO_2CH_3$, $-ONO_2$, $-NO_2$, $-N_3$, $-NH_2$, optionally substituted heterocycloalkyl, optionally substituted heterocycloalkaryl, optionally substituted aminoalkylamino, optionally substituted polyalkylamino, substituted silyl, a reporter group, an intercalator, a group for improving pharmacokinetic properties, a group for improving the pharmacodynamic properties, and other substituents. In some embodiments, a 2'-modification is a 2'-MOE modification (e.g., see Baker et al., J. Biol. Chem., 1997, 272, 11944-12000). In some cases, a 2'-MOE modification has been reported as having improved binding affinity compared to unmodified sugars and to some other modified nucleosides, such as 2'- O-methyl, 2'-O-propyl, and 2'-O-aminopropyl. Oligonucleotides having the 2'-MOE modification have also been reported to be capable of inhibiting gene expression with promising features for in vivo use (see, e.g., Martin, Helv. Chim. Acta, 1995, 78, 486-504; Altmann et al., Chimia, 1996, 50, 168-176; Altmann et al., Biochem. Soc. Trans., 1996, 24, 630-637; and Altmann et al., Nucleosides Nucleotides, 1997, 16, 917-926; etc.).

[0211] In some embodiments, a 2'-modified or 2'-substituted sugar or nucleoside is a sugar or nucleoside comprising a substituent at the 2' position of the sugar which is other than –H (typically not considered a substituent) or –OH. In some embodiments, a 2'-modified sugar is a bicyclic sugar comprising a bridge connecting two carbon atoms of the sugar ring one of which is the 2' carbon. In some embodiments, a 2'-modification is non-bridging, e.g., allyl, amino, azido, thio, optionally substituted –O–allyl, optionally substituted –O–C_1-C_{10} alkyl, $-OCF_3$, $-O(CH_2)_2OCH_3$, 2'-$O(CH_2)_2SCH_3$, $-O(CH_2)_2ON(R_m)(R_n)$, or $-OCH_2C(=O)N(R_m)(R_n)$, where each R_m and R_n is independently –H or optionally substituted C_1-C_{10} alkyl.

[0212] Certain modified sugars, their preparation and uses are described in US 4981957, US 5118800, US 5319080, US 5359044, US 5393878, US 5446137, US 5466786, US 5514785, US 5519134, US 5567811, US 5576427, US 5591722, US 5597909, US 5610300, US 5627053, US 5639873, US 5646265, US 5670633, US 5700920, US 5792847, US 6600032 and WO 2005121371.

[0213] In some embodiments, a sugar is the sugar of N-methanocarba, LNA, cMOE BNA, cEt BNA, α-L-LNA or related analogs, HNA, Me-ANA, MOE-ANA, Ara-FHNA, FHNA, R-6'-Me-FHNA, S-6'-Me-FHNA, ENA, or c-ANA. In some embodiments, a modified internucleotidic linkage is C3-amide (e.g., sugar that has the amide modification attached to the C3', Mutisya et al. 2014 Nucleic Acids Res. 2014 Jun 1; 42(10): 6542–6551), formacetal, thioformacetal, MMI [e.g., methylene(methylimino), Peoc'h et al. 2006 Nucleosides and Nucleotides 16 (7-9)], a PMO (phosphorodiamidate linked morpholino) linkage (which connects two sugars), or a PNA (peptide nucleic acid) linkage. In some embodiments, examples of internucleotidic linkages and/or sugars are described in Allerson et al. 2005 J. Med. Chem. 48: 901-4; BMCL 2011 21: 1122; BMCL 2011 21: 588; BMCL 2012 22: 296; Chattopadhyaya et al. 2007 J. Am. Chem. Soc. 129: 8362; Chem. Bio. Chem. 2013 14: 58; Curr. Prot. Nucl. Acids Chem. 2011 1.24.1; Egli et al. 2011 J. Am. Chem. Soc. 133: 16642; Hendrix et al. 1997 Chem. Eur. J. 3: 110; Hyrup et al. 1996 Bioorg. Med. Chem. 4: 5; Imanishi 1997 Tet. Lett. 38: 8735; J. Am. Chem. Soc. 1994, 116, 3143; J. Med. Chem. 2009 52: 10; J. Org. Chem. 2010 75: 1589; Jepsen et al. 2004 Oligo. 14: 130-146; Jones et al. J. Org. Chem. 1993, 58, 2983; Jung et al. 2014 ACIEE 53: 9893; Kodama et al. 2014 AGDS; Koizumi 2003 BMC 11: 2211; Koizumi et al. 2003 Nuc. Acids Res. 12: 3267-3273; Koshkin et al. 1998 Tetrahedron 54: 3607-3630; Kumar et al. 1998 Bioo. Med. Chem. Let. 8: 2219-2222; Lauritsen et al. 2002 Chem. Comm. 5: 530-531; Lauritsen et al. 2003 Bioo. Med. Chem. Lett. 13: 253-256; Lima et al. 2012 Cell 150: 883-894; Mesmaeker et al. Angew. Chem., Int. Ed. Engl. 1994, 33, 226; Migawa et al. 2013 Org. Lett. 15: 4316; Mol. Ther. Nucl. Acids 2012 1: e47; Morita et al. 2001 Nucl. Acids Res. Supp. 1: 241-242; Morita et al. 2002 Bioo. Med. Chem. Lett. 12: 73-76; Morita et al. 2003 Bioo. Med. Chem. Lett. 2211-2226; Murray et al. 2012 Nucl. Acids Res. 40: 6135; Nielsen et al. 1997 Chem. Soc. Rev. 73; Nielsen et al. 1997 J. Chem. Soc. Perkins Transl. 1: 3423-3433; Obika et al. 1997 Tetrahedron Lett. 38 (50): 8735–8; Obika et al. 1998 Tetrahedron Lett. 39: 5401-5404; Obika et al. 2008 J. Am. Chem. Soc. 130: 4886; Obika et al. 2011 Org. Lett. 13: 6050; Oestergaard et al. 2014 JOC 79: 8877; Pallan et al. 2012 Biochem. 51: 7; Pallan et al. 2012 Chem. Comm. 48: 8195-8197; Petersen et al. 2003 TRENDS Biotech. 21: 74-81; Prakash et al. 2010 J. Med. Chem. 53: 1636; Prakash et al. 2015 Nucl. Acids Res. 43: 2993-3011; Prakash et al. 2016 Bioorg. Med. Chem. Lett. 26: 2817-2820; Rajwanshi et al. 1999 Chem. Commun. 1395-1396; Schultz et al. 1996 Nucleic Acids Res. 24: 2966; Seth et al. 2008 Nucl. Acid Sym. Ser. 52: 553; Seth et al. 2009 J. Med. Chem. 52: 10-13; Seth et al. 2010 J. Am. Chem. Soc. 132: 14942; Seth et al. 2010 J. Med. Chem. 53: 8309-8318; Seth et al. 2010 J. Org. Chem. 75: 1569-1581; Seth et al. 2011 BMCL 21: 4690; Seth et al. 2012 Bioo. Med. Chem. Lett. 22: 296-299; Seth et al. 2012 Mol. Ther-Nuc. Acids. 1, e47; Seth et al., Nucleic Acids Symposium Series (2008), 52(1), 553-554; Singh et al. 1998 Chem. Comm. 1247-1248; Singh et al. 1998 J.

Org. Chem. 63: 10035-39; Singh et al. 1998 J. Org. Chem. 63: 6078-6079; Sorensen 2003 Chem. Comm. 2130-2131; Starrup et al. 2010 Nucl. Acids Res. 38: 7100; Swayze et al. 2007 Nucl. Acids Res. 35: 687; Ts'o et al. Ann. N. Y. Acad. Sci. 1988, 507, 220; Van Aerschot et al. 1995 Angew. Chem. Int. Ed. Engl. 34: 1338; Vasseur et al. J. Am. Chem. Soc. 1992, 114, 4006; WO 2007090071; WO 2016079181; US 6326199; US 6066500; or US 6440739.

[0214] In some embodiments, an oligonucleotide or a portion thereof (e.g., a domain, a subdomain, etc.) comprises a high level of 2'-F modified sugars, e.g., about 10%-100% (e.g., about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more, or about 100%) of sugars in an oligonucleotide or a portion thereof (e.g., a domain, a subdomain, etc.) comprises 2'-F. In some embodiments, about 50% or more of sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, about 60% or more of sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, about 70% or more of sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, about 80% or more of sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, about 90% or more of sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, about 1-20 or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, or more) sugars in an oligonucleotide or a portion thereof (e.g., a domain, a subdomain, etc.) comprises 2'-F. In some embodiments, 1 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 2 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 3 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 4 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 5 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 6 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 7 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 8 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 9 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 10 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 11 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 12 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 13 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 14 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 15 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 16 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 17 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 18 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 19 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some embodiments, 20 or more sugars in an oligonucleotide or a portion thereof comprises 2'-F. In some

embodiments, an oligonucleotide or a portion thereof also comprises one or more sugars comprising no 2'-F (e.g., sugars comprising no modifications and/or sugars comprising other modifications).

[0215] In some embodiments, no more than about 1%-95% (e.g., no more than about 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, etc.) of sugars in an oligonucleotide or a portion thereof (e.g., a domain, a subdomain, etc.) comprises 2'-OMe. In some embodiments, no more than about 50% of sugars in an oligonucleotide or a portion thereof comprises 2'-OMe. In some embodiments, no sugars in an oligonucleotide or a portion thereof comprises 2'-OMe. In some embodiments, no more than 1, 2, 3, 4, or 5 sugars in an oligonucleotide or a portion thereof comprises 2'-OMe. In some embodiments, no more than 1 sugar in an oligonucleotide or a portion thereof comprises 2'-OMe. In some embodiments, no more than 2 sugars in an oligonucleotide or a portion thereof comprises 2'-OMe. In some embodiments, no more than 3 sugars in an oligonucleotide or a portion thereof comprises 2'-OMe. In some embodiments, no more than 4 sugars in an oligonucleotide or a portion thereof comprises 2'-OMe. In some embodiments, no more than 5 sugars in an oligonucleotide or a portion thereof comprises 2'-OMe.

[0216] In some embodiments, about 1-20 or more (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, or more) sugars in an oligonucleotide or a portion thereof (e.g., a domain, a subdomain, etc.) comprises 2'-F and no more than 1, 2, 3, 4, or 5 sugars in an oligonucleotide or a portion thereof comprises 2'-OMe.

[0217] Various additional sugars useful for preparing oligonucleotides or analogs thereof are known in the art and may be utilized in accordance with the present disclosure.

Internucleotidic linkages

[0218] Among other things, the present disclosure provides various internucleotidic linkages, including various modified internucleotidic linkages, that may be utilized together with other structural elements, e.g., various sugars as described herein, to provide oligonucleotides and compositions thereof.

[0219] In some embodiments, oligonucleotides comprise base modifications, sugar modifications, and/or internucleotidic linkage modifications. Various internucleotidic linkages can be utilized in accordance with the present disclosure to link units comprising nucleobases, e.g., nucleosides. In some embodiments, provided oligonucleotides comprise both one or more modified internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, an oligonucleotide comprises one or more types of internucleotidic linkage. In some embodiments, an oligonucleotide comprises two or more types of internucleotidic linkage. In some embodiments, an oligonucleotide comprises at least three types of internucleotidic linkages. In some embodiments, a linkage contains a linkage phosphorus atom bonded to an oxygen atom which oxygen atom is not bonded to or is not part of a backbone sugar ("a PO linkage", e.g., a natural phosphate linkage). In some embodiments, a linkage contains a linkage phosphorus atom bonded to a sulfur atom which sulfur atom is not bonded to or is not part of a backbone sugar ("a PS linkage", e.g., a phosphorothioate internucleotidic linkage). In some embodiments, a linkage contains a linkage phosphorus atom bonded to a nitrogen atom which nitrogen

atom is not bonded to or is not part of a backbone sugar ("a PN linkage", e.g., n001). In some embodiments, an oligonucleotide comprises one or more PS linkages. In some embodiments, an oligonucleotide comprises one or more PO linkages. In some embodiments, an oligonucleotide comprises one or more PN linkages. In some embodiments, an oligonucleotide comprises one or more PS and one or more PO linkages. In some embodiments, an oligonucleotide comprises one or more PS and one or more PN linkages. In some embodiments, an oligonucleotide comprises one or more PS, one or more PN and one or more PO linkages. In some embodiments, a PS linkage is a phosphorothioate linkage. In some embodiments, each PS linkage is independently a phosphorothioate linkage. In some embodiments, a PO linkage is a natural phosphate linkage. In some embodiments, each PO linkage is independently a natural phosphate linkage. In some embodiments, a PN linkage is a phosphoryl guanidine linkage. In some embodiments, each PN linkage is independently a phosphoryl guanidine linkage.

[0220] As widely known by those skilled in the art, natural phosphate linkages are widely found in natural DNA and RNA molecules; they have the structure of –OP(O)(OH)O–, connect sugars in the nucleosides in DNA and RNA, and may be in various salt forms, for example, at physiological pH (about 7.4), natural phosphate linkages are predominantly exist in salt forms with the anion being $–OP(O)(O^-)O–$. A modified internucleotidic linkage, or a non-natural phosphate linkage, is an internucleotidic linkage that is not natural phosphate linkage or a salt form thereof. Modified internucleotidic linkages, depending on their structures, may also be in their salt forms. For example, as appreciated by those skilled in the art, phosphorothioate internucleotidic linkages which have the structure of –OP(O)(SH)O– may be in various salt forms, e.g., at physiological pH (about 7.4) with the anion being $–OP(O)(S^-)O–$.

[0221] In some embodiments, an oligonucleotide comprises an internucleotidic linkage which is a modified internucleotidic linkage, e.g., phosphorothioate, phosphorodithioate, methylphosphonate, phosphoroamidate, thiophosphate, 3'-thiophosphate, or 5'-thiophosphate. In some embodiments, a modified internucleotidic linkage is a PN linkage. In some embodiments, a modified internucleotidic linkage is a PS linkage. In some embodiments, a modified internucleotidic linkage is a PO linkage (e.g., other than a natural phosphate linkage). In some embodiments, each modified internucleotidic linkage is independently a PN internucleotidic linkage or a PS internucleotidic linkage. In some embodiments, an oligonucleotide comprises one or more PN internucleotidic linkages, one or more PS internucleotidic linkages, and one or more PO internucleotidic linkages. In some embodiments, one or more PN internucleotidic linkages are independently phosphoryl guanidine internucleotidic linkages. In some embodiments, one or more PN internucleotidic linkages are independently n001. In some embodiments, one or more PS internucleotidic linkages are independently phosphorothioate internucleotidic linkages. In some embodiments, each PS internucleotidic linkage is independently a phosphorothioate internucleotidic linkage. In some embodiments, one or more PO internucleotidic linkages are independently natural phosphate linkages. In some embodiments, each PO internucleotidic linkage is independently a natural phosphate linkage.

[0222] In some embodiments, a modified internucleotidic linkage is a chiral internucleotidic linkage which

comprises a chiral linkage phosphorus. In some embodiments, a chiral internucleotidic linkage is a phosphorothioate linkage. In some embodiments, a chiral internucleotidic linkage is a non-negatively charged internucleotidic linkage. In some embodiments, a chiral internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, a chiral internucleotidic linkage is chirally controlled with respect to its chiral linkage phosphorus. In some embodiments, a chiral internucleotidic linkage is stereochemically pure with respect to its chiral linkage phosphorus. In some embodiments, a chiral internucleotidic linkage is not chirally controlled. In some embodiments, a pattern of backbone chiral centers comprises or consists of positions and linkage phosphorus configurations of chirally controlled internucleotidic linkages (*Rp* or *Sp*) and positions of achiral internucleotidic linkages (e.g., natural phosphate linkages).

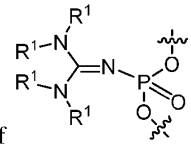
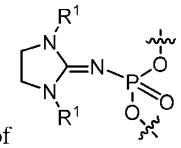
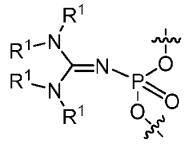
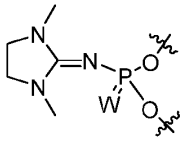
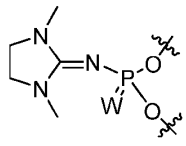
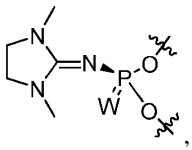
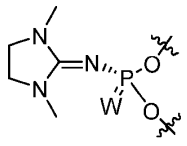
[0223] In some embodiments, an internucleotidic linkage comprises a P-modification, wherein a P-modification is a modification at a linkage phosphorus. In some embodiments, a modified internucleotidic linkage is a moiety which does not comprise a phosphorus but serves to link two sugars or two moieties that each independently comprises a nucleobase, e.g., as in peptide nucleic acid (PNA).

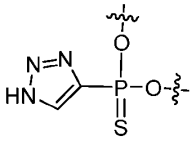
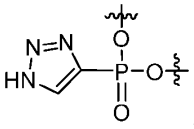
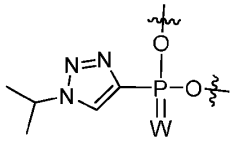
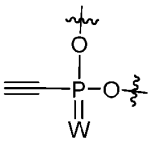
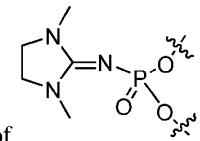
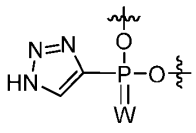
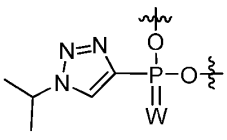
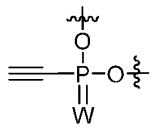
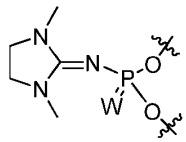
[0224] In some embodiments, an oligonucleotide comprises a modified internucleotidic linkage, e.g., those having the structure of Formula I, I-a, I-b, or I-c and described herein and/or in: WO 2018/022473, WO 2018/098264, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the internucleotidic linkages (e.g., those of Formula I, I-a, I-b, I-c, etc.) of each of which are independently incorporated herein by reference. In some embodiments, a modified internucleotidic linkage is a chiral internucleotidic linkage. In some embodiments, a modified internucleotidic linkage is a phosphorothioate internucleotidic linkage.

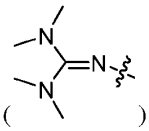
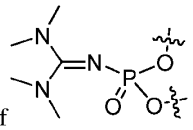
[0225] In some embodiments, a modified internucleotidic linkage is a non-negatively charged internucleotidic linkage. In some embodiments, provided oligonucleotides comprise one or more non-negatively charged internucleotidic linkages. In some embodiments, a non-negatively charged internucleotidic linkage is a positively charged internucleotidic linkage. In some embodiments, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, the present disclosure provides oligonucleotides comprising one or more neutral internucleotidic linkages. 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., or a salt form thereof, as described herein and/or in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the non-negatively charged internucleotidic linkages (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, II-d-2, etc., or a suitable salt form thereof) of each of which are

independently incorporated herein by reference.

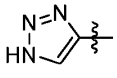
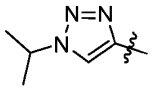
[0226] In some embodiments, a non-negatively charged internucleotidic linkage can improve the delivery and/or activities (e.g., exon skipping activity).

[0227] 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 cyclic guanidine moiety. In some embodiments, a modified internucleotidic linkage has the structure of  and is optionally chirally controlled, wherein R^1 is –L–R', wherein L is L^B as described herein, and R' is as described herein. In some embodiments, each R^1 is independently R'. In some embodiments, each R' is independently R. In some embodiments, two R^1 are R and are taken together to form a ring as described herein. In some embodiments, two R^1 on two different nitrogen atoms are R and are taken together to form a ring as described herein. In some embodiments, R^1 is independently optionally substituted C_{1-6} aliphatic as described herein. In some embodiments, R^1 is methyl. In some embodiments, two R' on the same nitrogen atom are R and are taken together to form a ring as described herein. In some embodiments, a modified internucleotidic linkage has the structure of  and is optionally chirally controlled. In some embodiments,  is . In some embodiments, a modified internucleotidic linkage comprises an optionally substituted cyclic guanidine moiety and has the structure of: , , or , 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.

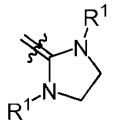
[0228] In some embodiments, a non-negatively charged internucleotidic linkage or a neutral internucleotidic linkage is an internucleotidic linkage comprising a triazole moiety. In some embodiments, a non-negatively charged internucleotidic linkage or a non-negatively charged internucleotidic linkage comprises an optionally substituted triazolyl group. In some embodiments, an internucleotidic linkage comprising a triazole moiety (e.g., an optionally substituted triazolyl group) has the structure of . In some embodiments, an internucleotidic linkage comprising a triazole moiety 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, e.g., a non-negatively charged internucleotidic linkage, a neutral 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 non-negatively charged internucleotidic linkage, or a neutral internucleotidic linkage, is or comprising a structure selected from , , , or , wherein W is O or S.

[0229] 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.

[0230] 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.

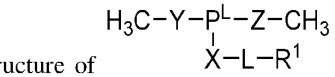
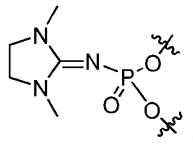
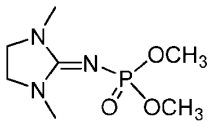
[0231] In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heteroaryl group having 1-10 heteroatoms. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heteroaryl 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.,  .

[0232] 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 substituted  group. In some embodiments, a non-negatively charged internucleotidic linkage comprises a  group, wherein each R^1 is independently –L–R. In some embodiments, each R^1 is independently optionally substituted C_{1-6} alkyl. In some embodiments, each R^1 is independently methyl.

[0233] 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.

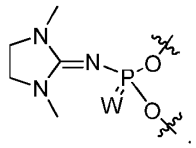
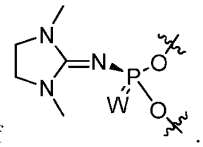
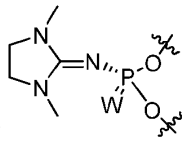
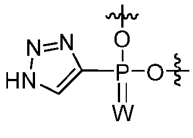
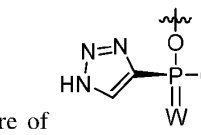
[0234] In some embodiments, an oligonucleotide comprises different types of internucleotidic phosphorus linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one natural phosphate linkage and at least one modified (non-natural) internucleotidic linkage. In some embodiments, 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-

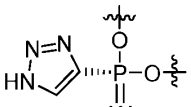
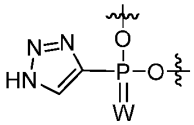
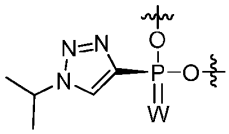
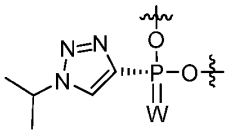
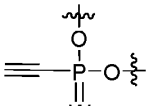
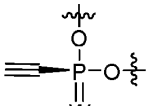
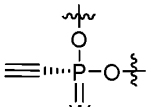
negatively charged internucleotidic linkage. In some embodiments, an oligonucleotide comprises at least one phosphorothioate internucleotidic linkage and at least one non-negatively charged internucleotidic linkage. In some embodiments, an oligonucleotide comprises at least one phosphorothioate internucleotidic linkage, at least one natural phosphate linkage, and at least one non-negatively charged internucleotidic linkage. In some embodiments, oligonucleotides comprise one or more, e.g., 1-50, 1-40, 1-30, 1-20, 1-15, 1-10, 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, oligonucleotides comprise no more than a certain number of non-negatively charged internucleotidic linkages, e.g., no more than 1, no more than 2, no more than 3, no more than 4, no more than 5, no more than 6, no more than 7, no more than 8, no more than 9, no more than 10, no more than 11, no more than 12, no more than 13, no more than 14, no more than 15, no more than 16, no more than 17, no more than 18, no more than 19, no more than 20, no more than 21, no more than 22, no more than 23, no more than 24, no more than 25, no more than 26, no more than 27, no more than 28, no more than 29, or no more than 30 non-negatively charged internucleotidic linkages. In some embodiments, oligonucleotides comprise no 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 CH_3–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  (wherein each of X, Y, Z is independently –O–, –S–, –N(R')–; L is L^B, and R^1 is –L–R'), pKa of  can be represented by pKa . In 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.

[0235] In some embodiments, a neutral or non-negatively charged internucleotidic linkage has the structure of any neutral or non-negatively charged internucleotidic linkage described in any of: US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, each neutral or non-negatively charged internucleotidic linkage of each of which is hereby incorporated by reference.

[0236] In some embodiments, each R' is independently optionally substituted C_{1-6} aliphatic. In some embodiments, each R' is independently optionally substituted C_{1-6} alkyl. In some embodiments, each R' is independently $-CH_3$. In some embodiments, each R^s is –H.

[0237] 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, W is O. In some embodiments, W is S. In some embodiments, a neutral internucleotidic linkage is a non-negatively charged internucleotidic linkage described above.

[0238] In some embodiments, provided oligonucleotides comprise 1 or more internucleotidic linkages 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, or II-d-2, which are described in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the 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, or II-d-2, or salt forms thereof, each of which are independently

incorporated herein by reference.

[0239] In some embodiments, an oligonucleotide comprises a neutral internucleotidic linkage and a chirally controlled internucleotidic linkage. In some embodiments, an oligonucleotide comprises a neutral internucleotidic linkage and a chirally controlled internucleotidic linkage which is not the neutral internucleotidic linkage. In some embodiments, an oligonucleotide comprises a neutral internucleotidic linkage and a chirally controlled phosphorothioate internucleotidic linkage. In some embodiments, the present disclosure provides an oligonucleotide comprising one or more non-negatively charged internucleotidic linkages and one or more phosphorothioate internucleotidic linkages, wherein each phosphorothioate internucleotidic linkage in the oligonucleotide is independently a chirally controlled internucleotidic linkage. In some embodiments, the present disclosure provides an oligonucleotide comprising one or more neutral internucleotidic linkages and one or more phosphorothioate internucleotidic linkage, wherein each phosphorothioate internucleotidic linkage in the oligonucleotide is independently a chirally controlled internucleotidic linkage. In some embodiments, an oligonucleotide comprises at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more chirally controlled phosphorothioate internucleotidic linkages. In some embodiments, non-negatively charged internucleotidic linkage is chirally controlled. In some embodiments, non-negatively charged internucleotidic linkage is not chirally controlled. In some embodiments, a neutral internucleotidic linkage is chirally controlled. In some embodiments, a neutral internucleotidic linkage is not chirally controlled. In some embodiments, an oligonucleotide comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) chirally controlled and one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) non-chirally controlled chiral internucleotidic linkages. In some embodiments, an oligonucleotide comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) chirally controlled and one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) non-chirally controlled non-negatively charged internucleotidic linkages (in some embodiments, each of which is independently n001). In some embodiments, a neutral internucleotidic linkage is chirally controlled. In some embodiments, a neutral internucleotidic linkage is not chirally controlled. In some embodiments, an oligonucleotide comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) chirally controlled and one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) non-chirally controlled neutral internucleotidic linkages (in some embodiments, each of which is independently n001).

[0240] Without wishing to be bound by any particular theory, the present disclosure notes that a neutral internucleotidic linkage can be more hydrophobic than a phosphorothioate internucleotidic linkage (PS), which can be more hydrophobic than a natural phosphate linkage (PO). Typically, unlike a PS or PO, a neutral internucleotidic linkage bears less charge. Without wishing to be bound by any particular theory, the present disclosure notes that incorporation of one or more neutral internucleotidic linkages into an oligonucleotide may increase oligonucleotides' ability to be taken up by a cell and/or to escape from endosomes. Without wishing to be bound by any particular theory, the present disclosure notes that incorporation of one or more neutral internucleotidic linkages can be utilized to modulate melting temperature of duplexes formed between an oligonucleotide and its target nucleic acid.

[0241] Without wishing to be bound by any particular theory, the present disclosure notes that incorporation of one or more non-negatively charged internucleotidic linkages, e.g., neutral internucleotidic linkages, into an oligonucleotide may be able to increase the oligonucleotide's ability to mediate a function such as exon skipping.

[0242] As appreciated by those skilled in the art, internucleotidic linkages such as natural phosphate linkages and those 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 salt forms thereof typically connect two nucleosides (which can either be natural or modified) as described in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the 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 salt forms thereof, each of which are independently incorporated herein by reference. A typical connection, as in natural DNA and RNA, is that an internucleotidic linkage forms bonds with two sugars (which can be either unmodified or modified as described herein). In many embodiments, as exemplified herein an internucleotidic linkage forms bonds through its oxygen atoms or heteroatoms (e.g., Y and Z in various formulae) with one optionally modified ribose or deoxyribose at its 5' carbon, and the other optionally modified ribose or deoxyribose at its 3' carbon. In some embodiments, each nucleoside units connected by an internucleotidic linkage independently comprises a nucleobase which is independently an optionally substituted A, T, C, G, or U, or a substituted tautomer of A, T, C, G or U, or a nucleobase comprising an optionally substituted heterocyclyl and/or a heteroaryl ring having at least one nitrogen atom.

[0243] In some embodiments, a linkage has the structure of or comprises $-Y-P^L(-X-R^L)-Z-$, or a salt form thereof, wherein:

P^L is P, P(=W), P–>B($-L^L-R^L)_3$, or P^N;

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

P^N is $P=N-C(-L^L-R')(=L^N-R')$ or $P=N-L^L-R^L$;

L^N is $=N-L^{LI}-$, $=CH-L^{LI}-$ wherein CH is optionally substituted, or $=N^+(R')(Q^-)-L^{LI}-$;

Q^- is an anion;

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

each R^L is independently $-L^L-N(R')_2$, $-L^L-R'$, $-N=C(-L^L-R')_2$, $-L^L-N(R')C(NR')N(R')_2$, $-L^L-N(R')C(O)N(R')_2$, a carbohydrate, or one or more additional chemical moieties optionally connected through a linker;

each of L^{LI} and L^L is independently L;

$-Cy^{IL}-$ is –Cy–;

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 by an optionally substituted group selected from C_{1-6} alkylene, C_{1-6} alkenylene, —C≡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(NR')N(R')–, –N(R')C(NR')N(R')–, –C(O)N(R')–, –N(R')C(O)N(R')–, –N(R')C(O)O–, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(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–, –OP(OR')[B(R')$_3$]O–, and –[C(R')$_2$C(R')$_2$O]n–, wherein n is 1-50, and one or more nitrogen or carbon atoms are optionally and independently replaced with Cy^L;

each –Cy– is independently an optionally substituted bivalent 3-30 membered, monocyclic, bicyclic or polycyclic ring having 0-10 heteroatoms;

each Cy^L is independently an optionally substituted trivalent or tetravalent, 3-30 membered, monocyclic, bicyclic or polycyclic ring having 0-10 heteroatoms;

each R' is independently –R, –C(O)R, –C(O)N(R)$_2$, –C(O)OR, or –S(O)$_2$R;

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.

[0244] In some embodiments, an internucleotidic linkage has the structure of –O–P^L(–X–R^L)–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –O–P(=W)(–X–R^L)–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –O–P(=W)[–N(–L^L–R^L)–R^L]–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –O–P(=W)(–NH–L^L–R^L)–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –O–P(=W)[–N(R')$_2$]–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the

structure of –O–P(=W)(–NHR')–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –O–P(=W)(–$NHSO_2R$)–O–, wherein each variable is independently as described herein. In some embodiments, R is methyl. In some embodiments, an internucleotidic linkage is –O–P(=O)(–$NHSO_2CH_3$)–O–. In some embodiments, an internucleotidic linkage has the structure of –O–P(=W)[–N=C(–L^L–R')$_2$]–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –O–P(=W)[–N=C[N(R')$_2$]$_2$]–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –OP(=W)(–N=C(R")$_2$)–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –OP(=W)(–N(R")$_2$)–O–, wherein each variable is independently as described herein. In some embodiments, W is O. In some embodiments, W is S. In some embodiments, such an internucleotidic linkage is a non-negatively charged internucleotidic linkage. In some embodiments, such an internucleotidic linkage is a neutral internucleotidic linkage.

[0245] In some embodiments, an internucleotidic linkage has the structure of –P^L(–X–R^L)–Z–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –P^L(–X–R^L)–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=W)(–X–R^L)–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=W)[–N(–L^L–R^L)–R^L]–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=W)(–NH–L^L–R^L)–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=W)[–N(R')$_2$]–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=W)(–NHR')–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=W)(–$NHSO_2R$)–O–, wherein each variable is independently as described herein. In some embodiments, R is methyl. In some embodiments, an internucleotidic linkage is –P(=O)(–$NHSO_2CH_3$)–O–. In some embodiments, an internucleotidic linkage has the structure of –P(=W)[–N=C(–L^L–R')$_2$]–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=W)[–N=C[N(R')$_2$]$_2$]–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=W)(–N=C(R")$_2$)–O–, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=W)(–N(R")$_2$)–O–, wherein each variable is independently as described herein. In some embodiments, W is O. In some embodiments, W is S. In some embodiments, such an internucleotidic linkage is a non-negatively charged internucleotidic linkage. In some embodiments, such an internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, P of such an internucleotidic linkage is bonded to N of a sugar.

[0246] In some embodiments, a linkage is a phosphoryl guanidine internucleotidic linkage. In some embodiments, a linkage is a thio-phosphoryl guanidine internucleotidic linkage.

[0247] In some embodiments, one or more methylene units are optionally and independently replaced with a moiety as described herein. In some embodiments, L or L^L is or comprises $-SO_2-$. In some embodiments, L or L^L is or comprises $-SO_2N(R')-$. In some embodiments, L or L^L is or comprises $-C(O)-$. In some embodiments, L or L^L is or comprises $-C(O)O-$. In some embodiments, L or L^L is or comprises $-C(O)N(R')-$. In some embodiments, L or L^L is or comprises $-P(=W)(R')-$. In some embodiments, L or L^L is or comprises $-P(=O)(R')-$. In some embodiments, L or L^L is or comprises $-P(=S)(R')-$. In some embodiments, L or L^L is or comprises $-P(R')-$. In some embodiments, L or L^L is or comprises $-P(=W)(OR')-$. In some embodiments, L or L^L is or comprises $-P(=O)(OR')-$. In some embodiments, L or L^L is or comprises $-P(=S)(OR')-$. In some embodiments, L or L^L is or comprises $-P(OR')-$.

[0248] In some embodiments, $-X-R^L$ is $-N(R')SO_2R^L$. In some embodiments, $-X-R^L$ is $-N(R')C(O)R^L$. In some embodiments, $-X-R^L$ is $-N(R')P(=O)(R')R^L$.

[0249] In some embodiments, a linkage, e.g., a non-negatively charged internucleotidic linkage or neutral internucleotidic linkage, has the structure of or comprises $-P(=W)(-N=C(R'')_2)-$, $-P(=W)(-N(R')SO_2R'')-$, $-P(=W)(-N(R')C(O)R'')-$, $-P(=W)(-N(R'')_2)-$, $-P(=W)(-N(R')P(O)(R'')_2)-$, $-OP(=W)(-N=C(R'')_2)O-$, $-OP(=W)(-N(R')SO_2R'')O-$, $-OP(=W)(-N(R')C(O)R'')O-$, $-OP(=W)(-N(R'')_2)O-$, $-OP(=W)(-N(R')P(O)(R'')_2)O-$, $-P(=W)(-N=C(R'')_2)O-$, $-P(=W)(-N(R')SO_2R'')O-$, $-P(=W)(-N(R')C(O)R'')O-$, $-P(=W)(-N(R'')_2)O-$, or $-P(=W)(-N(R')P(O)(R'')_2)O-$, or a salt form thereof, wherein:

W is O or S;

each R'' is independently R', $-OR'$, $-P(=W)(R')_2$, or $-N(R')_2$;

each R' is independently $-R$, $-C(O)R$, $-C(O)N(R)_2$, $-C(O)OR$, 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.

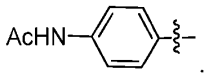
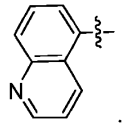
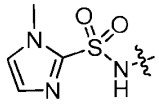
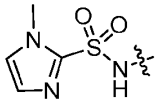
[0250] In some embodiments, W is O. In some embodiments, an internucleotidic linkage has the structure of $-P(=O)(-N=C(R'')_2)-$, $-P(=O)(-N(R')SO_2R'')-$, $-P(=O)(-N(R')C(O)R'')-$, $-P(=O)(-N(R'')_2)-$,

–P(=O)(–N(R')P(O)(R")$_2$)–, –OP(=O)(–N=C(R")$_2$)O–, –OP(=O)(–N(R')SO$_2$R")O–, –OP(=O)(–N(R')C(O)R")O–, –OP(=O)(–N(R")$_2$)O–, –OP(=O)(–N(R')P(O)(R")$_2$)O–, –P(=O)(–N=C(R")$_2$)O–, –P(=O)(–N(R')SO$_2$R")O–, –P(=O)(–N(R')C(O)R")O–, –P(=O)(–N(R")$_2$)O–, or –P(=O)(–N(R')P(O)(R")$_2$)O–, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –P(=O)(–N=C(R")$_2$)– –P(=O)(–N(R")$_2$)–, –OP(=O)(–N=C(R")$_2$)–O–, –OP(=O)(–N(R")$_2$)–O–, –P(=O)(–N=C(R")$_2$)–O– or –P(=O)(–N(R")$_2$)–O– or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –OP(=O)(–N=C(R")$_2$)–O– or –OP(=O)(–N(R")$_2$)–O–, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –OP(=O)(–N=C(R")$_2$)–O–, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –OP(=O)(–N(R")$_2$)–O–, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –OP(=O)(–N(R')SO$_2$R")O–, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –OP(=O)(–N(R')C(O)R")O–, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –OP(=O)(–N(R')P(O)(R")$_2$)O–, or a salt form thereof. In some embodiments, a internucleotidic linkage is n001.

[0251] In some embodiments, W is S. In some embodiments, an internucleotidic linkage has the structure of –P(=S)(–N=C(R")$_2$)–, –P(=S)(–N(R')SO$_2$R")–, –P(=S)(–N(R')C(O)R")–, –P(=S)(–N(R")$_2$)–, –P(=S)(–N(R')P(O)(R")$_2$)–, –OP(=S)(–N=C(R")$_2$)O–, –OP(=S)(–N(R')SO$_2$R")O–, –OP(=S)(–N(R')C(O)R")O–, –OP(=S)(–N(R")$_2$)O–, –OP(=S)(–N(R')P(O)(R")$_2$)O–, –P(=S)(–N=C(R")$_2$)O–, –P(=S)(–N(R')SO$_2$R")O–, –P(=S)(–N(R')C(O)R")O–, –P(=S)(–N(R")$_2$)O–, or –P(=S)(–N(R')P(O)(R")$_2$)O–, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –P(=S)(–N=C(R")$_2$)– –P(=S)(–N(R")$_2$)–, –OP(=S)(–N=C(R")$_2$)–O–, –OP(=S)(–N(R")$_2$)–O–, –P(=S)(–N=C(R")$_2$)–O– or –P(=S)(–N(R")$_2$)–O– or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –OP(=S)(–N=C(R")$_2$)–O– or –OP(=S)(–N(R")$_2$)–O–, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –OP(=S)(–N=C(R")$_2$)–O–, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –OP(=S)(–N(R")$_2$)–O–, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –OP(=S)(–N(R')SO$_2$R")O–, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –OP(=S)(–N(R')C(O)R")O–, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of –OP(=S)(–N(R')P(O)(R")$_2$)O–, or a salt form thereof. In some embodiments, a internucleotidic linkage is *n001.

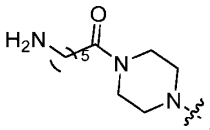
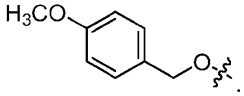
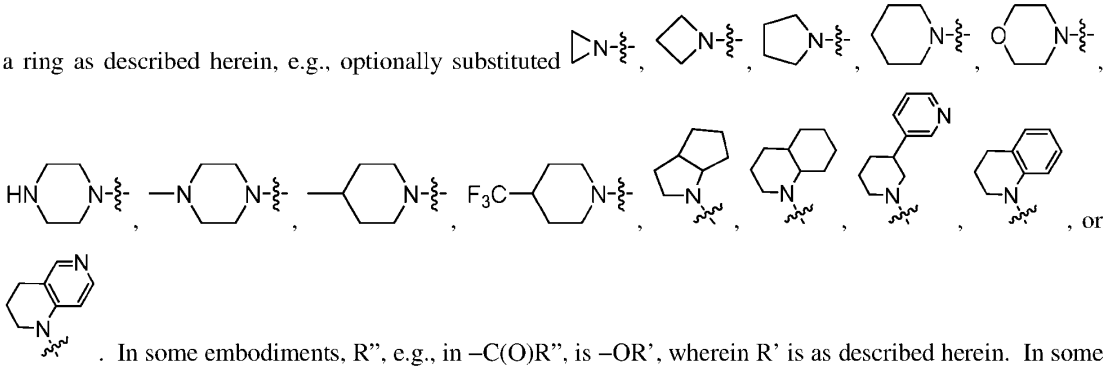
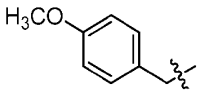
[0252] In some embodiments, an internucleotidic linkage has the structure of –P(=O)(–N(R')SO$_2$R")–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=S)(–N(R')SO$_2$R")–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=O)(–N(R')SO$_2$R")O–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=S)(–N(R')SO$_2$R")O–, wherein R" is as described herein. In

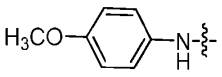
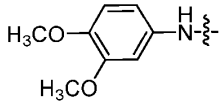
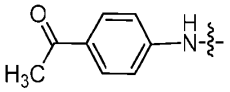
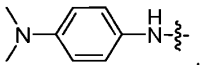
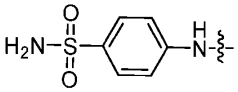
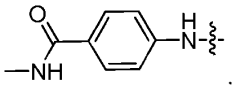
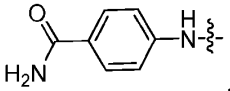
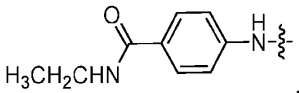
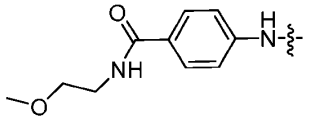
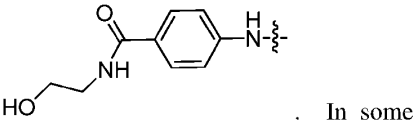
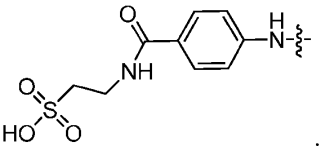
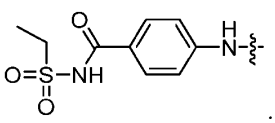
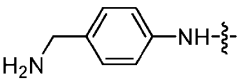
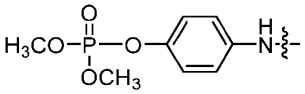
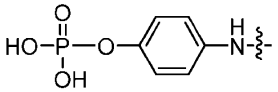
some embodiments, an internucleotidic linkage has the structure of –OP(=O)(–N(R')SO_2R")O–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –OP(=S)(–N(R')SO_2R")O–, wherein R" is as described herein. In some embodiments, R', e.g., of –N(R')–, is hydrogen or optionally substituted C_{1-6} aliphatic. In some embodiments, R' is C_{1-6} alkyl. In some embodiments, R' is hydrogen. In some embodiments, R", e.g., in –SO_2R", is R' as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=O)(–NHSO_2R")–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=S)(–NHSO_2R")–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=O)(–NHSO_2R")O–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=S)(–NHSO_2R")O–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –OP(=O)(–NHSO_2R")O–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –OP(=S)(–NHSO_2R")O–, wherein R" is as described herein. In some embodiments, –X–R^L is –N(R')SO_2R^L, wherein each of R' and R^L is independently as described herein. In some embodiments, R^L is R". In some embodiments, R^L is R'. In some embodiments, –X–R^L is –N(R')SO_2R", wherein R' is as described herein. In some embodiments, –X–R^L is –N(R')SO_2R', wherein R' is as described herein. In some embodiments, –X–R^L is –NHSO_2R', wherein R' is as described herein. In some embodiments, R' is R as described herein. 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 optionally substituted phenyl. In some embodiments, R' is optionally substituted heteroaryl. In some embodiments, R", e.g., in –SO_2R", is R. In some embodiments, R is an optionally substituted group selected from C_{1-6} aliphatic, aryl, heterocyclyl, and heteroaryl. 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 optionally substituted C_{1-6} alkenyl. In some embodiments, R is optionally substituted C_{1-6} alkynyl. In some embodiments, R is optionally substituted methyl. In some embodiments, –X–R^L is –NHSO_2CH_3. In some embodiments, R is –CF_3. In some embodiments, R is methyl. In some embodiments, R is optionally substituted ethyl. In some embodiments, R is ethyl. In some embodiments, R is –CH_2CHF_2. In some embodiments, R is –$CH_2CH_2OCH_3$. In some embodiments, R is optionally substituted propyl. In some embodiments, R is optionally substituted butyl. In some embodiments, R is n-butyl. In some embodiments, R is –$(CH_2)_6NH_2$. In some embodiments, R is an optionally substituted linear C_{2-20} aliphatic. In some embodiments, R is optionally substituted linear C_{2-20} alkyl. In some embodiments, R is linear C_{2-20} alkyl. In some embodiments, R is optionally substituted C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} aliphatic. In some embodiments, R is optionally substituted C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is optionally substituted linear C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is linear C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is phenyl. In some embodiments, R is p-methylphenyl.

In some embodiments, R is 4-dimethylaminophenyl. In some embodiments, R is 3-pyridinyl. In some embodiments, R is . In some embodiments, R is . In some embodiments, R is benzyl. In some embodiments, R is optionally substituted heteroaryl. In some embodiments, R is optionally substituted 1,3-diazolyl. In some embodiments, R is optionally substituted 2-(1,3)-diazolyl. In some embodiments, R is optionally substituted 1-methyl-2-(1,3)-diazolyl. In some embodiments, R is isopropyl. In some embodiments, R" is $-N(R')_2$. In some embodiments, R" is $-N(CH_3)_2$. In some embodiments, R", e.g., in $-SO_2R$", is –OR', wherein R' is as described herein. In some embodiments, R' is R as described herein. In some embodiments, R" is $-OCH_3$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2R)O-$, wherein R is as described herein. In some embodiments, R is optionally substituted linear alkyl as described herein. In some embodiments, R is linear alkyl as described herein. In some embodiments, a linkage is $-OP(=O)(-NHSO_2CH_3)O-$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2CH_2CH_3)O-$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2CH_2CH_2OCH_3)O-$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2CH_2Ph)O-$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2CH_2CHF_2)O-$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2(4\text{-methylphenyl}))O-$. In some embodiments, $-X-R^L$ is . In some embodiments, a linkage is $-OP(=O)(-X-R^L)O-$, wherein $-X-R^L$ is . In some embodiments, a linkage is $-OP(=O)(-NHSO_2CH(CH_3)_2)O-$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2N(CH_3)_2)O-$. In some embodiments, a linkage is n002. In some embodiments, a linkage is n006. In some embodiments, a linkage is n020. In some embodiments, such internucleotidic linkages may be utilized in place of linkages like n001.

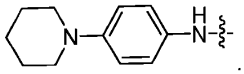
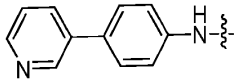
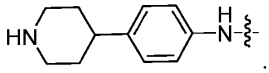
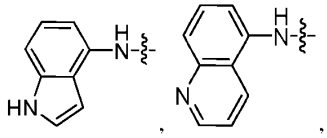
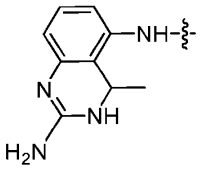
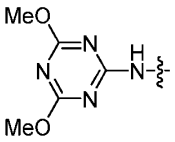
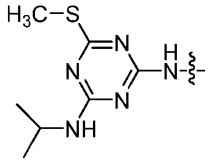
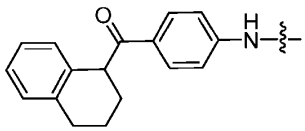
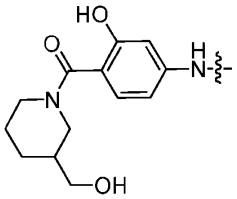
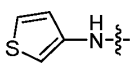
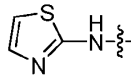
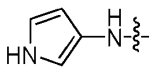
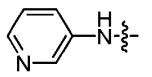
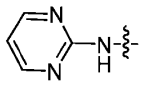
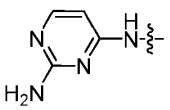
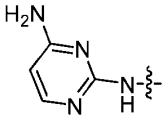
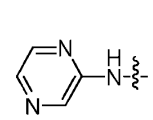
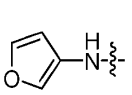
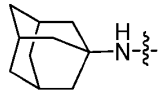
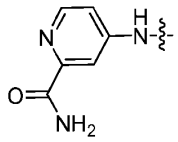
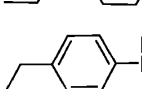
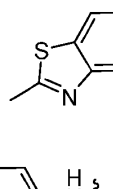
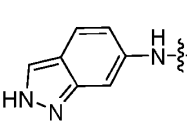
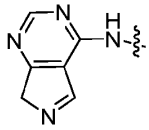
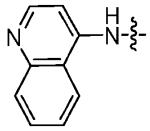
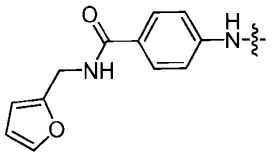
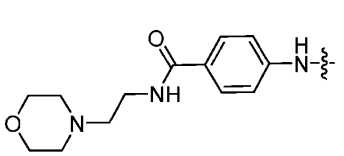
[0253] In some embodiments, an internucleotidic linkage has the structure of $-P(=O)(-N(R')C(O)R")-$, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=S)(-N(R')C(O)R")-$, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=O)(-N(R')C(O)R")O-$, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=S)(-N(R')C(O)R")O-$, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of $-OP(=O)(-N(R')C(O)R")O-$, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of $-OP(=S)(-N(R')C(O)R")O-$, wherein R" is as described herein. In some embodiments, R', e.g., of $-N(R')-$, is hydrogen or optionally substituted C_{1-6} aliphatic. In some embodiments, R' is C_{1-6} alkyl. In some embodiments, R' is hydrogen. In some embodiments, R", e.g., in –C(O)R", is R' as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=O)(-NHC(O)R")-$, wherein R" is as described herein. In some embodiments, an internucleotidic linkage

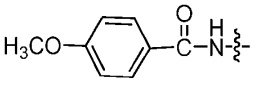
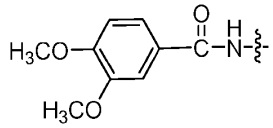
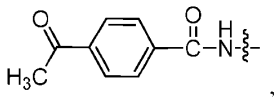
has the structure of –P(=S)(–NHC(O)R")–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=O)(–NHC(O)R")O–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –P(=S)(–NHC(O)R")O–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –OP(=O)(–NHC(O)R")O–, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of –OP(=S)(–NHC(O)R")O–, wherein R" is as described herein. In some embodiments, –X–R^L is –N(R')COR^L, wherein R^L is as described herein. In some embodiments, –X–R^L is –N(R')COR", wherein R" is as described herein. In some embodiments, –X–R^L is –N(R')COR', wherein R' is as described herein. In some embodiments, –X–R^L is –NHCOR', wherein R' is as described herein. In some embodiments, R' is R as described herein. 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 optionally substituted phenyl. In some embodiments, R' is optionally substituted heteroaryl. In some embodiments, R", e.g., in –C(O)R", is R. In some embodiments, R is an optionally substituted group selected from C_{1-6} aliphatic, aryl, heterocyclyl, and heteroaryl. 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 optionally substituted C_{1-6} alkenyl. In some embodiments, R is optionally substituted C_{1-6} alkynyl. In some embodiments, R is methyl. In some embodiments, –X–R^L is –NHC(O)CH_3. In some embodiments, R is optionally substituted methyl. In some embodiments, R is –CF_3. In some embodiments, R is optionally substituted ethyl. In some embodiments, R is ethyl. In some embodiments, R is –CH_2CHF_2. In some embodiments, R is –$CH_2CH_2OCH_3$. In some embodiments, R is optionally substituted C_{1-20} (e.g., C_{1-6}, C_{2-6}, C_{3-6}, C_{1-10}, C_{2-10}, C_{3-10}, C_{2-20}, C_{3-20}, C_{10-20}, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, etc.) aliphatic. In some embodiments, R is optionally substituted C_{1-20} (e.g., C_{1-6}, C_{2-6}, C_{3-6}, C_{1-10}, C_{2-10}, C_{3-10}, C_{2-20}, C_{3-20}, C_{10-20}, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, etc.) alkyl. In some embodiments, R is an optionally substituted linear C_{2-20} aliphatic. In some embodiments, R is optionally substituted linear C_{2-20} alkyl. In some embodiments, R is linear C_{2-20} alkyl. In some embodiments, R is optionally substituted C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} aliphatic. In some embodiments, R is optionally substituted C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is optionally substituted linear C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is linear C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is optionally substituted aryl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is p-methylphenyl. In some embodiments, R is benzyl. In some embodiments, R is optionally substituted heteroaryl. In some embodiments, R is optionally substituted 1,3-diazolyl. In some embodiments, R is optionally substituted 2-(1,3)-diazolyl. In some embodiments, R is optionally substituted 1-methyl-2-(1,3)-diazolyl. In some

embodiments, R^L is $-(CH_2)_5NH_2$. In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R" is $-N(R')_2$. In some embodiments, R" is $-N(CH_3)_2$. In some embodiments, $-X-R^L$ is $-N(R')CON(R^L)_2$, wherein each of R' and R^L is independently as described herein. In some embodiments, $-X-R^L$ is $-NHCON(R^L)_2$, wherein R^L is as described herein. In some embodiments, two R' or two R^L are taken together with the nitrogen atom to form a ring as described herein, e.g., optionally substituted . In some embodiments, R", e.g., in $-C(O)R"$, is $-OR'$, wherein R' is as described herein. In some embodiments, R' is R as described herein. In some embodiments, is optionally substituted C_{1-6} aliphatic. In some embodiments, is optionally substituted C_{1-6} alkyl. In some embodiments, R" is $-OCH_3$. In some embodiments, $-X-R^L$ is $-N(R')C(O)OR^L$, wherein each of R' and R^L is independently as described herein. In some embodiments, R is . In some embodiments, $-X-R^L$ is $-NHC(O)OCH_3$. In some embodiments, $-X-R^L$ is $-NHC(O)N(CH_3)_2$. In some embodiments, a linkage is $-OP(O)(NHC(O)CH_3)O-$. In some embodiments, a linkage is $-OP(O)(NHC(O)OCH_3)O-$. In some embodiments, a linkage is $-OP(O)(NHC(O)(p\text{-methylphenyl}))O-$. In some embodiments, a linkage is $-OP(O)(NHC(O)N(CH_3)_2)O-$. In some embodiments, $-X-R^L$ is $-N(R')R^L$, wherein each of R' and R^L is independently as described herein. In some embodiments, $-X-R^L$ is $-N(R')R^L$, wherein each of R' and R^L is independently not hydrogen. In some embodiments, $-X-R^L$ is $-NHR^L$, wherein R^L is as described herein. In some embodiments, R^L is not hydrogen. In some embodiments, R^L is optionally substituted aryl or heteroaryl. In some embodiments, R^L is optionally substituted aryl. In some embodiments, R^L is optionally substituted phenyl. In some embodiments, $-X-R^L$ is $-N(R')_2$, wherein each R' is independently as described herein. In some embodiments, $-X-R^L$ is $-NHR'$, wherein R' is as described herein. In some embodiments, $-X-R^L$ is $-NHR$, wherein R is as described herein. In some embodiments, $-X-R^L$ is R^L, wherein R^L is as described herein. In some embodiments, R^L is $-N(R')_2$, wherein each R' is independently as described herein. In some embodiments, R^L is $-NHR'$, wherein R' is as

described herein. In some embodiments, R^L is –NHR, wherein R is as described herein. In some embodiments, R^L is $-N(R')_2$, wherein each R' is independently as described herein. In some embodiments, none of R' in $-N(R')_2$ is hydrogen. In some embodiments, R^L is $-N(R')_2$, wherein each R' is independently C_{1-6} aliphatic. In some embodiments, R^L is –L–R', wherein each of L and R' is independently as described herein. In some embodiments, R^L is –L–R, wherein each of L and R is independently as described herein. In some embodiments, R^L is –N(R')–Cy–N(R')–R'. In some embodiments, R^L is –N(R')–Cy–C(O)–R'. In some embodiments, R^L is –N(R')–Cy–O–R'. In some embodiments, R^L is $-N(R')–Cy–SO_2–R'$. In some embodiments, R^L is $-N(R')–Cy–SO_2–N(R')_2$. In some embodiments, R^L is $-N(R')–Cy–C(O)–N(R')_2$. In some embodiments, R^L is $-N(R')–Cy–OP(O)(R'')_2$. In some embodiments, –Cy– is an optionally substituted bivalent aryl group. In some embodiments, –Cy– is optionally substituted phenylene. In some embodiments, –Cy– is optionally substituted 1,4-phenylene. In some embodiments, –Cy– is 1,4-phenylene. In some embodiments, R^L is $-N(CH_3)_2$. In some embodiments, R^L is $-N(i\text{-}Pr)_2$. In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is .

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

embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , or . In some embodiments, $-X-R^L$ is $-N(R')-C(O)-Cy-R^L$. In some embodiments, $-X-R^L$ is R^L. In some embodiments, R^L is $-N(R')-C(O)-Cy-O-R'$. In some

embodiments, R^L is –N(R')–C(O)–Cy–R'. In some embodiments, R^L is –N(R')–C(O)–Cy–C(O)–R'. In some embodiments, R^L is –N(R')–C(O)–Cy–N(R')$_2$. In some embodiments, R^L is –N(R')–C(O)–Cy–SO_2–N(R')$_2$. In some embodiments, R^L is –N(R')–C(O)–Cy–C(O)–N(R')$_2$. In some embodiments, R^L is –N(R')–C(O)–Cy–C(O)–N(R')–SO_2–R'. In some embodiments, R' is R as described herein. In some embodiments, R^L is , , ,

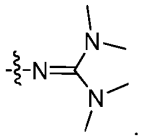
membered heteroaryl group having 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) heteroatoms. In some embodiments, –Cy– is monocyclic. In some embodiments, –Cy– is bicyclic. In some embodiments, –Cy– is polycyclic. In some embodiments, each monocyclic unit in –Cy– is independently 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) membered, and is independently saturated, partially saturated, or aromatic. In some embodiments, –Cy– is an optionally substituted 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) membered monocyclic, bicyclic or polycyclic aliphatic group. In some embodiments, –Cy– is an optionally substituted 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) membered monocyclic, bicyclic or polycyclic heteroaliphatic group having 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) heteroatoms.

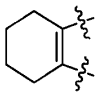
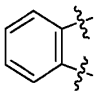
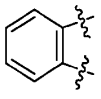
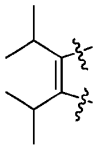
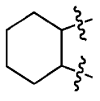
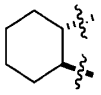
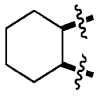
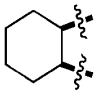
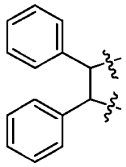
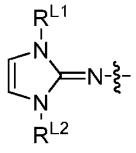
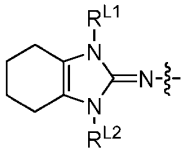
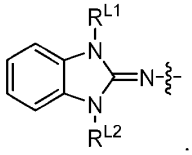
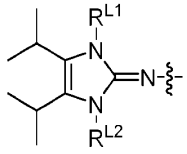
[0255] In some embodiments, an internucleotidic linkage has the structure of $-P(=O)(-N(R')P(O)(R'')_2)-$, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=S)(-N(R')P(O)(R'')_2)-$, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=O)(-N(R')P(O)(R'')_2)O-$, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=S)(-N(R')P(O)(R'')_2)O-$, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-OP(=O)(-N(R')P(O)(R'')_2)O-$, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-OP(=S)(-N(R')P(O)(R'')_2)O-$, wherein each R" is independently as described herein. In some embodiments, R', e.g., of –N(R')–, is hydrogen or optionally substituted C_{1-6} aliphatic. In some embodiments, R' is C_{1-6} alkyl. In some embodiments, R' is hydrogen. In some embodiments, R", e.g., in $-P(O)(R'')_2$, is R' as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=O)(-NHP(O)(R'')_2)-$, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=S)(-NHP(O)(R'')_2)-$, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=O)(-NHP(O)(R'')_2)O-$, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=S)(-NHP(O)(R'')_2)O-$, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-OP(=O)(-NHP(O)(R'')_2)O-$, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-OP(=S)(-NHP(O)(R'')_2)O-$, wherein each R" is independently as described herein. In some embodiments, an occurrence of R", e.g., in $-P(O)(R'')_2$, is R. In some embodiments, R is an optionally substituted group selected from C_{1-6} aliphatic, aryl, heterocyclyl, and heteroaryl. 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 optionally substituted C_{1-6} alkenyl. In some embodiments, R is optionally substituted C_{1-6} alkynyl. In some embodiments, R is methyl. In some embodiments, R is optionally substituted methyl. In some embodiments, R is $-CF_3$. In some embodiments, R is optionally substituted ethyl. In some embodiments, R is ethyl. In some embodiments, R is $-CH_2CHF_2$. In some embodiments, R is $-CH_2CH_2OCH_3$. In some embodiments, R is optionally substituted C_{1-20} (e.g., C_{1-6}, C_{2-6}, C_{3-6}, C_{1-10}, C_{2-10}, C_{3-10}, C_{2-20}, C_{3-20}, C_{10-20}, 1, 2, 3,

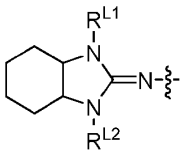
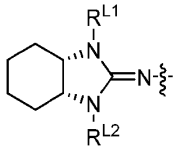
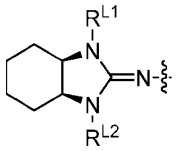
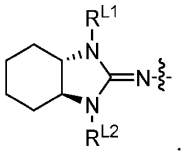
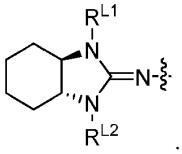
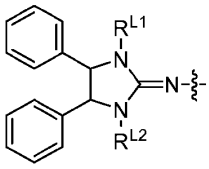
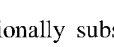
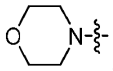
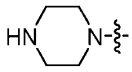
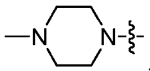
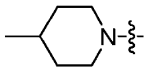
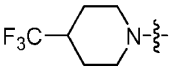
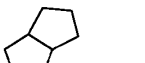
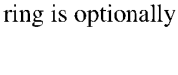
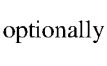
4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, etc.) aliphatic. In some embodiments, R is optionally substituted C_{1-20} (e.g., C_{1-6}, C_{2-6}, C_{3-6}, C_{1-10}, C_{2-10}, C_{3-10}, C_{2-20}, C_{3-20}, C_{10-20}, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, etc.) alkyl. In some embodiments, R is an optionally substituted linear C_{2-20} aliphatic. In some embodiments, R is optionally substituted linear C_{2-20} alkyl. In some embodiments, R is linear C_{2-20} alkyl. In some embodiments, R is isopropyl. In some embodiments, R is optionally substituted C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} aliphatic. In some embodiments, R is optionally substituted C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is optionally substituted linear C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is linear C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, each R" is independently R as described herein, for example, in some embodiments, each R" is methyl. In some embodiments, R" is optionally substituted aryl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is p-methylphenyl. In some embodiments, R is benzyl. In some embodiments, R is optionally substituted heteroaryl. In some embodiments, R is optionally substituted 1,3-diazolyl. In some embodiments, R is optionally substituted 2-(1,3)-diazolyl. In some embodiments, R is optionally substituted 1-methyl-2-(1,3)-diazolyl. In some embodiments, an occurrence of R" is $-N(R')_2$. In some embodiments, R" is $-N(CH_3)_2$. In some embodiments, an occurrence of R", e.g., in $-P(O)(R")_2$, is –OR', wherein R' is as described herein. In some embodiments, R' is R as described herein. In some embodiments, is optionally substituted C_{1-6} aliphatic. In some embodiments, is optionally substituted C_{1-6} alkyl. In some embodiments, R" is $-OCH_3$. In some embodiments, each R" is –OR' as described herein. In some embodiments, each R" is $-OCH_3$. In some embodiments, each R" is –OH. In some embodiments, a linkage is $-OP(O)(NHP(O)(OH)_2)O-$. In some embodiments, a linkage is $-OP(O)(NHP(O)(OCH_3)_2)O-$. In some embodiments, a linkage is $-OP(O)(NHP(O)(CH_3)_2)O-$.

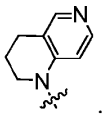
[0256] In some embodiments, $-N(R")_2$ is $-N(R')_2$. In some embodiments, $-N(R")_2$ is –NHR. In some embodiments, $-N(R")_2$ is $-NHC(O)R$. In some embodiments, $-N(R")_2$ is $-NHC(O)OR$. In some embodiments, $-N(R")_2$ is $-NHS(O)_2R$.

[0257] In some embodiments, an internucleotidic linkage is a phosphoryl guanidine internucleotidic linkage. In some embodiments, an internucleotidic linkage comprises $-X-R^L$ as described herein. In some embodiments, $-X-R^L$ is $-N=C(-L^L-R^L)_2$. In some embodiments, $-X-R^L$ is $-N=C[N(R^L)_2]_2$. In some embodiments, $-X-R^L$ is $-N=C[NR'R^L]_2$. In some embodiments, $-X-R^L$ is $-N=C[N(R')_2]_2$. In some embodiments, $-X-R^L$ is $-N=C[N(R^L)_2](CHR^{L1}R^{L2})$, wherein each of R^{L1} and R^{L2} is independently as described herein. In some embodiments, $-X-R^L$ is $-N=C(NR'R^L)(CHR^{L1}R^{L2})$, wherein each of R^{L1} and R^{L2} is independently as described herein. In some embodiments, $-X-R^L$ is $-N=C(NR'R^L)(CR'R^{L1}R^{L2})$, wherein each of R^{L1} and R^{L2} is independently as described herein. In some embodiments, $-X-R^L$ is $-N=C[N(R')_2](CHR'R^{L2})$. In some embodiments, $-X-R^L$ is $-N=C[N(R^L)_2](R^L)$. In some embodiments, $-X-R^L$ is $-N=C(NR'R^L)(R^L)$. In some embodiments, $-X-R^L$ is $-N=C(NR'R^L)(R')$. In some embodiments,

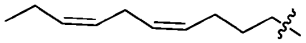
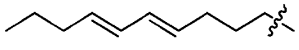
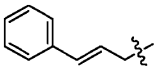
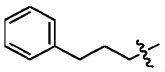
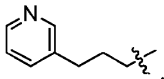
$-X-R^L$ is $-N=C[N(R')_2](R')$. In some embodiments, $-X-R^L$ is $-N=C(NR'R^{L1})(NR'R^{L2})$, wherein each R^{L1} and R^{L2} is independently R^L, and each R' and R^L is independently as described herein. In some embodiments, $-X-R^L$ is $-N=C(NR'R^{L1})(NR'R^{L2})$, wherein variable is independently as described herein. In some embodiments, $-X-R^L$ is $-N=C(NR'R^{L1})(CHR'R^{L2})$, wherein variable is independently as described herein. In some embodiments, $-X-R^L$ is $-N=C(NR'R^{L1})(R')$, wherein variable is independently as described herein. In some embodiments, each R' is independently R. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is methyl. In some embodiments, $-X-R^L$ is  . In some embodiments, two groups selected from R', R^L, R^{L1}, R^{L2}, etc. (in some embodiments, on the same atom (e.g., $-N(R')_2$, or $-NR'R^L$, or $-N(R^L)^2$, wherein R' and R^L can independently be R as described herein), etc.), or on different atoms (e.g., the two R' in $-N=C(NR'R^L)(CR'R^{L1}R^{L2})$ or $-N=C(NR'R^{L1})(NR'R^{L2})$; can also be two other variables that can be R, e.g., R^L, R^{L1}, R^{L2}, etc.)) are independently R and are taken together with their intervening atoms to form a ring as described herein. In some embodiments, two of R, R', R^L, R^{L1}, or R^{L2} on the same atom, e.g., of $-N(R')_2$, $-N(R^L)_2$, $-NR'R^L$, $-NR'R^{L1}$, $-NR'R^{L2}$, $-CR'R^{L1}R^{L2}$, etc., are taken together to form a ring as described herein. In some embodiments, two R', R^L, R^{L1}, or R^{L2} on two different atoms, e.g., the two R' in $-N=C(NR'R^L)(CR'R^{L1}R^{L2})$, $-N=C(NR'R^{L1})(NR'R^{L2})$, etc. are taken together to form a ring as described herein. In some embodiments, a formed ring is an optionally substituted 3-20 (e.g., 3-15, 3-12, 3-10, 3-9, 3-8, 3-7, 3-6, 4-15, 4-12, 4-10, 4-9, 4-8, 4-7, 4-6, 5-15, 5-12, 5-10, 5-9, 5-8, 5-7, 5-6, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc.) monocyclic, bicyclic or tricyclic ring having 0-5 additional heteroatoms. In some embodiments, a formed ring is monocyclic as described herein. In some embodiments, a formed ring is an optionally substituted 5-10 membered monocyclic ring. In some embodiments, a formed ring is bicyclic. In some embodiments, a formed ring is polycyclic. In some embodiments, two groups that are or can be R (e.g., the two R' in $-N=C(NR'R^L)(CR'R^{L1}R^{L2})$ or $-N=C(NR'R^{L1})(NR'R^{L2})$, the two R' in $-N=C(NR'R^L)(CR'R^{L1}R^{L2})$, $-N=C(NR'R^{L1})(NR'R^{L2})$, etc.) are taken together to form an optionally substituted bivalent hydrocarbon chain, e.g., an optionally substituted C_{1-20} aliphatic chain, optionally substituted $-(CH_2)n-$ wherein n is 1-20 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20). In some embodiments, a hydrocarbon chain is saturated. In some embodiments, a hydrocarbon chain is partially unsaturated. In some embodiments, a hydrocarbon chain is unsaturated. In some embodiments, two groups that are or can be R (e.g., the two R' in $-N=C(NR'R^L)(CR'R^{L1}R^{L2})$ or $-N=C(NR'R^{L1})(NR'R^{L2})$, the two R' in $-N=C(NR'R^L)(CR'R^{L1}R^{L2})$, $-N=C(NR'R^{L1})(NR'R^{L2})$, etc.) are taken together to form an optionally substituted bivalent heteroaliphatic chain, e.g., an optionally substituted C_{1-20} heteroaliphatic chain having 1-10 heteroatoms. In some embodiments, a heteroaliphatic chain is saturated. In some embodiments, a heteroaliphatic chain is partially unsaturated. In some embodiments, a heteroaliphatic chain is unsaturated. In some embodiments, a chain is optionally substituted $-(CH_2)-$. In some embodiments, a chain is optionally

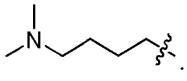
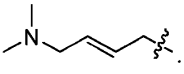
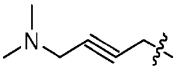
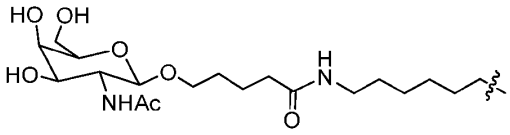
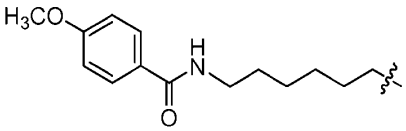
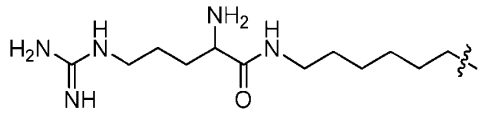
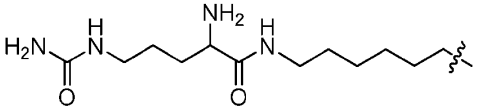
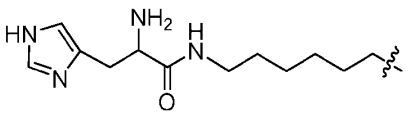
substituted $-(CH_2)_2-$. In some embodiments, a chain is optionally substituted $-(CH_2)-$. In some embodiments, a chain is optionally substituted $-(CH_2)_2-$. In some embodiments, a chain is optionally substituted $-(CH_2)_3-$. In some embodiments, a chain is optionally substituted $-(CH_2)_4-$. In some embodiments, a chain is optionally substituted $-(CH_2)_5-$. In some embodiments, a chain is optionally substituted $-(CH_2)_6-$. In some embodiments, a chain is optionally substituted $-CH=CH-$. In some embodiments, a chain is optionally substituted . In some embodiments, a chain is optionally substituted . In some embodiments, a chain is optionally substituted . In some embodiments, a chain is optionally substituted . In some embodiments, a chain is optionally substituted . In some embodiments, a chain is optionally substituted . In some embodiments, a chain is optionally substituted . In some embodiments, a chain is optionally substituted . In some embodiments, a chain is optionally substituted . In some embodiments, two of R, R', R^L, R^{L1}, R^{L2}, etc. on different atoms are taken together to form a ring as described herein. For examples, in some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some

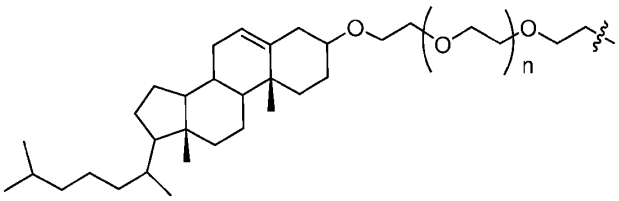
embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –N(R')$_2$, –N(R)$_2$, –N(R^L)$_2$, –NR'R^L, –NR'R^{L1}, –NR'R^{L2}, –N$R^{L1}R^{L2}$, etc. is a formed ring. In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally

substituted  .

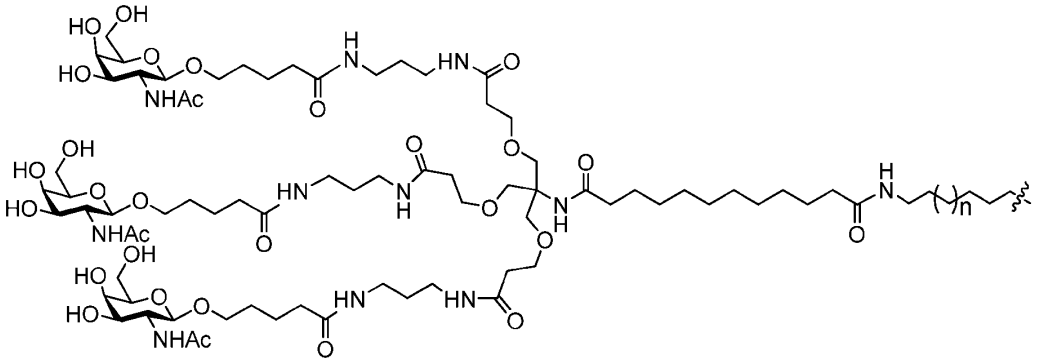
[0258] In some embodiments, R^{L1} and R^{L2} are the same. In some embodiments, R^{L1} and R^{L2} are different. In some embodiments, each of R^{L1} and R^{L2} is independently R^L as described herein, e.g., below.

[0259] In some embodiments, R^L is optionally substituted C_{1-30} aliphatic. In some embodiments, R^L is optionally substituted C_{1-30} alkyl. In some embodiments, R^L is linear. In some embodiments, R^L is optionally substituted linear C_{1-30} alkyl. In some embodiments, R^L is optionally substituted C_{1-6} alkyl. In some embodiments, R^L is methyl. In some embodiments, R^L is ethyl. In some embodiments, R^L is n-propyl. In some embodiments, R^L is isopropyl. In some embodiments, R^L is n-butyl. In some embodiments, R^L is tert-butyl. In some embodiments, R^L is (E)–CH_2–CH=CH–CH_2–CH_3. In some embodiments, R^L is (Z)–CH_2–CH=CH–CH_2–CH_3. In some embodiments, R^L is  . In some embodiments, R^L is  . In some embodiments, R^L is $CH_3(CH_2)_2C{\equiv}CC{\equiv}C(CH_2)_3$–. In some embodiments, R^L is $CH_3(CH_2)_5C{\equiv}C$–. In some embodiments, R^L optionally substituted aryl. In some embodiments, R^L is optionally substituted phenyl. In some embodiments, R^L is phenyl substituted with one or more halogen. In some embodiments, R^L is phenyl optionally substituted with halogen, –N(R'), or –N(R')C(O)R'. In some embodiments, R^L is phenyl optionally substituted with –Cl, –Br, –F, $–N(Me)_2$, or $–NHCOCH_3$. In some embodiments, R^L is $–L^L–R'$, wherein L^L is an optionally substituted C_{1-20} saturated, partially unsaturated or unsaturated hydrocarbon chain. In some embodiments, such a hydrocarbon chain is linear. In some embodiments, such a hydrocarbon chain is unsubstituted. In some embodiments, L^L is (E)–CH_2–CH=CH–. In some embodiments, L^L is $–CH_2–C{\equiv}C–CH_2–$. In some embodiments, L^L is $–(CH_2)_3–$. In some embodiments, L^L is $–(CH_2)_4–$. In some embodiments, L^L is $–(CH_2)_n–$, wherein n is 1-30 (e.g., 1-20, 5-30, 6-30, 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 or 30, etc.). In some embodiments, R' is optionally substituted aryl as described herein. In some embodiments, R' is optionally substituted phenyl. In some embodiments, R' is phenyl. In some embodiments, R' is optionally substituted heteroaryl as described herein. In some embodiments, R' is 2'-pyridinyl. In some embodiments, R' is 3'-pyridinyl. In some embodiments, R^L is  . In some embodiments, R^L is  . In some embodiments, R^L is  . In some embodiments, R^L is $–L^L–N(R')_2$, wherein each variable is independently as described herein. In some embodiments, each R' is independently C_{1-6} aliphatic as described herein. In some embodiments, $–N(R')_2$ is $–N(CH_3)_2$. In some embodiments, $–N(R')_2$ is $–NH_2$. In some embodiments, R^L is $–(CH_2)_n–N(R')_2$, wherein n is 1-30 (e.g., 1-20, 5-30, 6-30, 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 or 30, etc.). In

some embodiments, R^L is $-(CH_2CH_2O)_n-CH_2CH_2-N(R')_2$, wherein n is 1-30 (e.g., 1-20, 5-30, 6-30, 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 or 30, etc.). In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is $-(CH_2)_n-NH_2$. In some embodiments, R^L is $-(CH_2CH_2O)_n-CH_2CH_2-NH_2$. In some embodiments, R^L is $-(CH_2CH_2O)_n-CH_2CH_2-R'$, wherein n is 1-30 (e.g., 1-20, 5-30, 6-30, 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 or 30, etc.). In some embodiments, R^L is $-(CH_2CH_2O)_n-CH_2CH_2CH_3$, wherein n is 1-30 (e.g., 1-20, 5-30, 6-30, 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 or 30, etc.). In some embodiments, R^L is $-(CH_2CH_2O)_n-CH_2CH_2OH$, wherein n is 1-30 (e.g., 1-20, 5-30, 6-30, 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 or 30, etc.). In some embodiments, R^L is or comprises a carbohydrate moiety, e.g., GalNAc. In some embodiments, R^L is $-L^L-$GalNAc. In some embodiments, R^L is . In some embodiments, one or more methylene units of L^L are independently replaced with –Cy– (e.g., optionally substituted 1,4-phenylene, a 3-30 membered bivalent optionally substituted monocyclic, bicyclic, or polycyclic cycloaliphatic ring, etc.), –O–, –N(R')– (e.g., –NH), –C(O)–, –C(O)N(R')– (e.g., –C(O)NH–), –C(NR')– (e.g., –C(NH)–), –N(R')C(O)(N(R')– (e.g., –NHC(O)NH–), –N(R')C(NR')(N(R')– (e.g., –NHC(NH)NH–), $-(CH_2CH_2O)_n-$, etc. For example, in some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is



wherein n is 0-20. In some embodiments, R^L is or comprises one or more additional chemical moieties (e.g., carbohydrate moieties, GalNAc moieties, etc.) optionally substituted connected through a linker (which can be bivalent or polyvalent). For example, in some embodiments, R^L is



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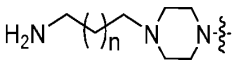
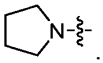
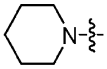
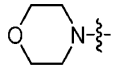
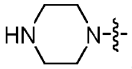
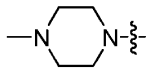
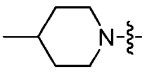
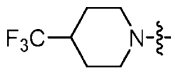
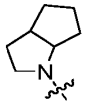
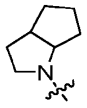
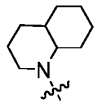
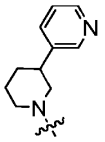
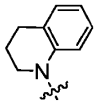
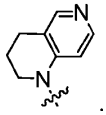
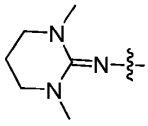
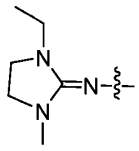
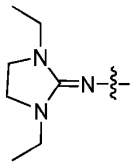
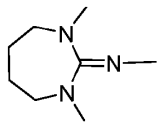
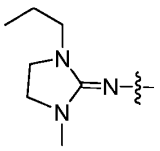
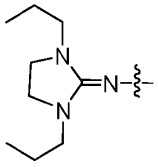
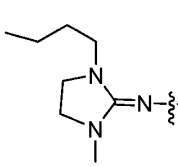
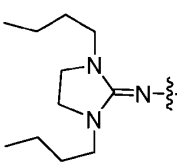
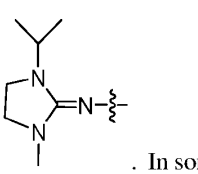
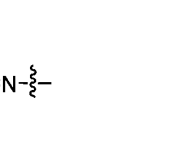
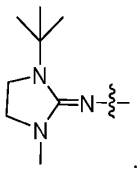
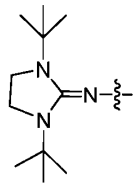
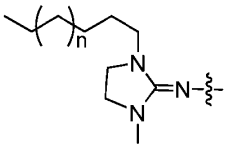
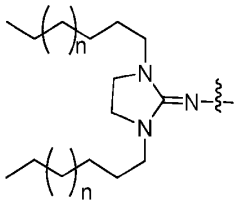
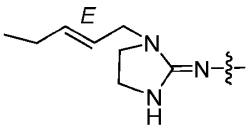
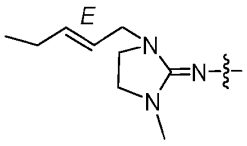
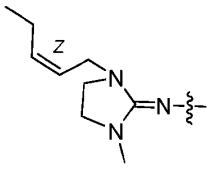
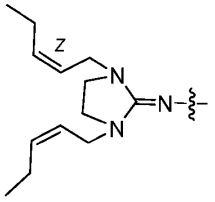
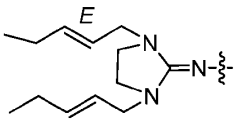
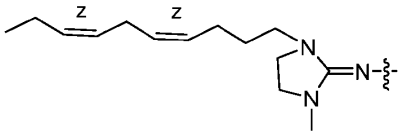
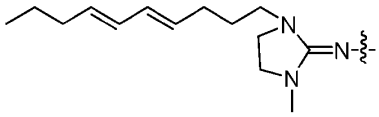
wherein n is 0-20. In some embodiments, R^L is , wherein n is 0-20. In some embodiments, R^L is R' as described herein. As described herein, many variable can independently be R'. In some embodiments, R' is R as described herein. As described herein, various variables can independently be R. In some 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, R is optionally substituted cycloaliphatic. In some embodiments, R is optionally substituted cycloalkyl. In some embodiments, R is optionally substituted aryl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is optionally substituted heteroaryl. In some embodiments, R is optionally substituted heterocyclyl. In some embodiments, R is optionally substituted C_{1-20} heterocyclyl having 1-5 heteroatoms, e.g., one of which is nitrogen. In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted

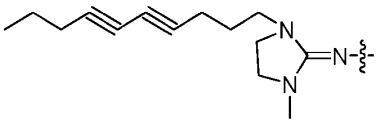
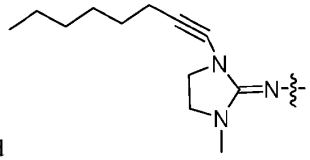
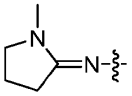
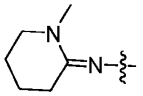
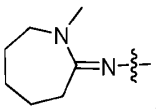
. In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted  In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted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

[0260] In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments,

$-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is , wherein n is 1-20. In some embodiments, $-X-R^L$ is , wherein n is 1-20. In some embodiments, $-X-R^L$ is selected from:

, , , ,

, , ,

 and . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is .

[0261] In some embodiments, R^L is R" as described herein. In some embodiments, R^L is R as described herein.

[0262] In some embodiments, R" or R^L is or comprises an additional chemical moiety. In some embodiments, R" or R^L is or comprises an additional chemical moiety, wherein the additional chemical moiety is or comprises a carbohydrate moiety. In some embodiments, R" or R^L is or comprises a GalNAc. In some embodiments, R^L or R" is replaced with, or is utilized to connect to, an additional chemical moiety.

[0263] In some embodiments, X is –O–. In some embodiments, X is –S–. In some embodiments, X is $-L^L-N(-L^L-R^L)-L^L-$. In some embodiments, X is $-N(-L^L-R^L)-L^L-$. In some embodiments, X is $-L^L-N(-L^L-R^L)-$. In some embodiments, X is $-N(-L^L-R^L)-$. In some embodiments, X is $-L^L-N=C(-L^L-R^L)-L^L-$. In some embodiments, X is $-N=C(-L^L-R^L)-L^L-$. In some embodiments, X is $-L^L-N=C(-L^L-R^L)-$. In some embodiments, X is $-N=C(-L^L-R^L)-$. In some embodiments, X is L^L. In some embodiments, X is a covalent bond.

[0264] In some embodiments, Y is a covalent bond. In some embodiments, Y is –O–. In some embodiments, Y is –N(R')–. In some embodiments, Z is a covalent bond. In some embodiments, Z is –O–. In some embodiments, Z is –N(R')–. In some embodiments, R' is R. In some embodiments, R is –H. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is methyl. In some embodiments, R is ethyl. In some embodiments, R is propyl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is phenyl.

[0265] As described herein, various variables in structures in the present disclosure can be or comprise R. Suitable embodiments for R are described extensively in the present disclosure. As appreciated by those skilled in the art, R embodiments described for a variable that can be R may also be applicable to another variable that can be R. Similarly, embodiments described for a component/moiety (e.g., L) for a variable may also be applicable to other variables that can be or comprise the component/moiety.

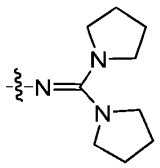
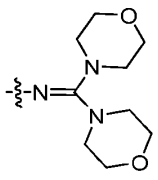
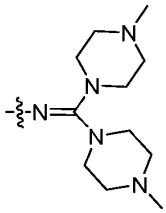
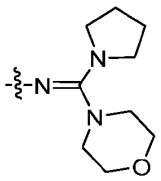
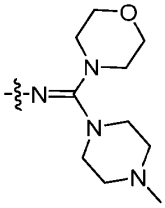
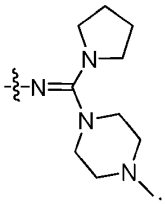
[0266] In some embodiments, R" is R'. In some embodiments, R" is $-N(R')_2$.

[0267] In some embodiments, $-X-R^L$ is –SH. In some embodiments, $-X-R^L$ is –OH.

[0268] In some embodiments, $-X-R^L$ is $-N(R')_2$. In some embodiments, each R' is independently optionally substituted C_{1-6} aliphatic. In some embodiments, each R' is independently methyl.

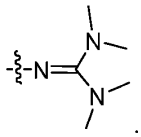
[0269] In some embodiments, a non-negatively charged internucleotidic linkage has the structure of $-OP(=O)(-N=C((N(R')_2)_2-O-$. In some embodiments, a R' group of one $N(R')_2$ is R, a R' group of the other $N(R')_2$ is R, and the two R groups are taken together with their intervening atoms to form an optionally substituted ring, e.g., a 5-membered ring as in n001. In some embodiments, each R' is independently R, wherein each R is independently optionally substituted C_{1-6} aliphatic.

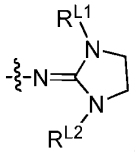
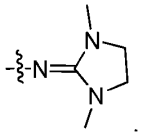
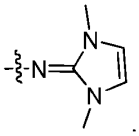
[0270] In some embodiments, $-X-R^L$ is $-N=C(-L^L-R')_2$. In some embodiments, $-X-R^L$ is $-N=C(-L^{L1}-L^{L2}-L^{L3}-R')_2$, wherein each L^{L1}, L^{L2} and L^{L3} is independently L", wherein each L" is independently a covalent bond, or a bivalent, optionally substituted, linear or branched group selected from a C_{1-10} aliphatic group and a C_{1-10} heteroaliphatic group having 1-5 heteroatoms, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_{1-6} alkylene, C_{1-6} alkenylene, —C≡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')–,

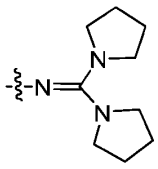
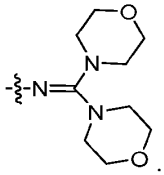
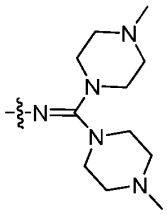
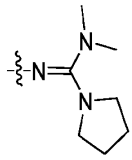
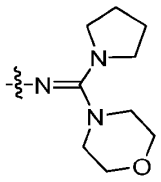
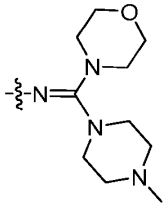
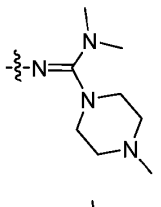
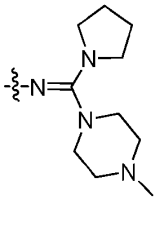
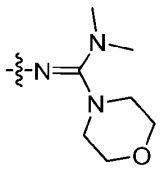
–P(NR')–, –P(OR')[B(R')$_3$]–, –OP(O)(OR')O–, –OP(O)(SR')O–, –OP(O)(R')O–, –OP(O)(NR')O–, –OP(OR')O–, –OP(SR')O–, –OP(NR')O–, –OP(R')O–, or –OP(OR')[B(R')$_3$]O–, and one or more nitrogen or carbon atoms are optionally and independently replaced with Cy^L. In some embodiments, L^{L2} is –Cy–. In some embodiments, L^{L1} is a covalent bond. In some embodiments, L^{L3} is a covalent bond. In some embodiments, –X–R^L is –N=C(–L^{L1}–Cy–L^{L3}–R')$_2$. In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments, –X–R^L is .

[0271] In some embodiments, as utilized in the present disclosure, L is covalent bond. In some embodiments, L is 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 by an optionally substituted group selected from C_{1-6} alkylene, C_{1-6} alkenylene, —C≡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)$_2$N(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 nitrogen or carbon atoms are optionally and independently replaced with Cy^L. In some embodiments, L is 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 by an optionally substituted group selected from —C≡C—, –C(R')$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R')–, –C(O)–, –C(S)–, –C(NR')–, –C(O)N(R')–, –N(R')C(O)N(R')–, –N(R')C(O)O–, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(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 nitrogen or carbon atoms are optionally and independently replaced with Cy^L. In some embodiments, L is a bivalent, optionally substituted, linear or branched group selected from a C$_{1-10}$ aliphatic group and a C$_{1-10}$ heteroaliphatic group having 1-10 heteroatoms, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from —C≡C—, –C(R')$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R')–, –C(O)–, –C(S)–, –C(NR')–, –C(O)N(R')–, –N(R')C(O)N(R')–, –N(R')C(O)O–, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(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 nitrogen or carbon atoms are optionally and independently replaced with Cy^L. In some embodiments, one or more methylene units are optionally and independently replaced by an optionally substituted group selected from —C≡C—, –C(R')$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R')–, –C(O)–, –C(S)–, –C(NR')–, –C(O)N(R')–, –N(R')C(O)N(R')–, –N(R')C(O)O–, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R')–, –C(O)S–, or –C(O)O–.

[0272] In some embodiments, an internucleotidic linkage is a phosphoryl guanidine internucleotidic linkage. In some embodiments, –X–R^L is –N=C[N(R')$_2$]$_2$. In some embodiments, each R' is independently R. In some embodiments, R is optionally substituted C$_{1-6}$ aliphatic. In some embodiments, R is methyl. In some embodiments, –X–R^L is . In some embodiments, one R' on a nitrogen atom is taken with a R' on the other nitrogen to form a ring as described herein.

[0273] In some embodiments, –X–R^L is , wherein R^1 and R^2 are independently R'. In some embodiments, –X–R^L is . In some embodiments, –X–R^L is . In some embodiments,

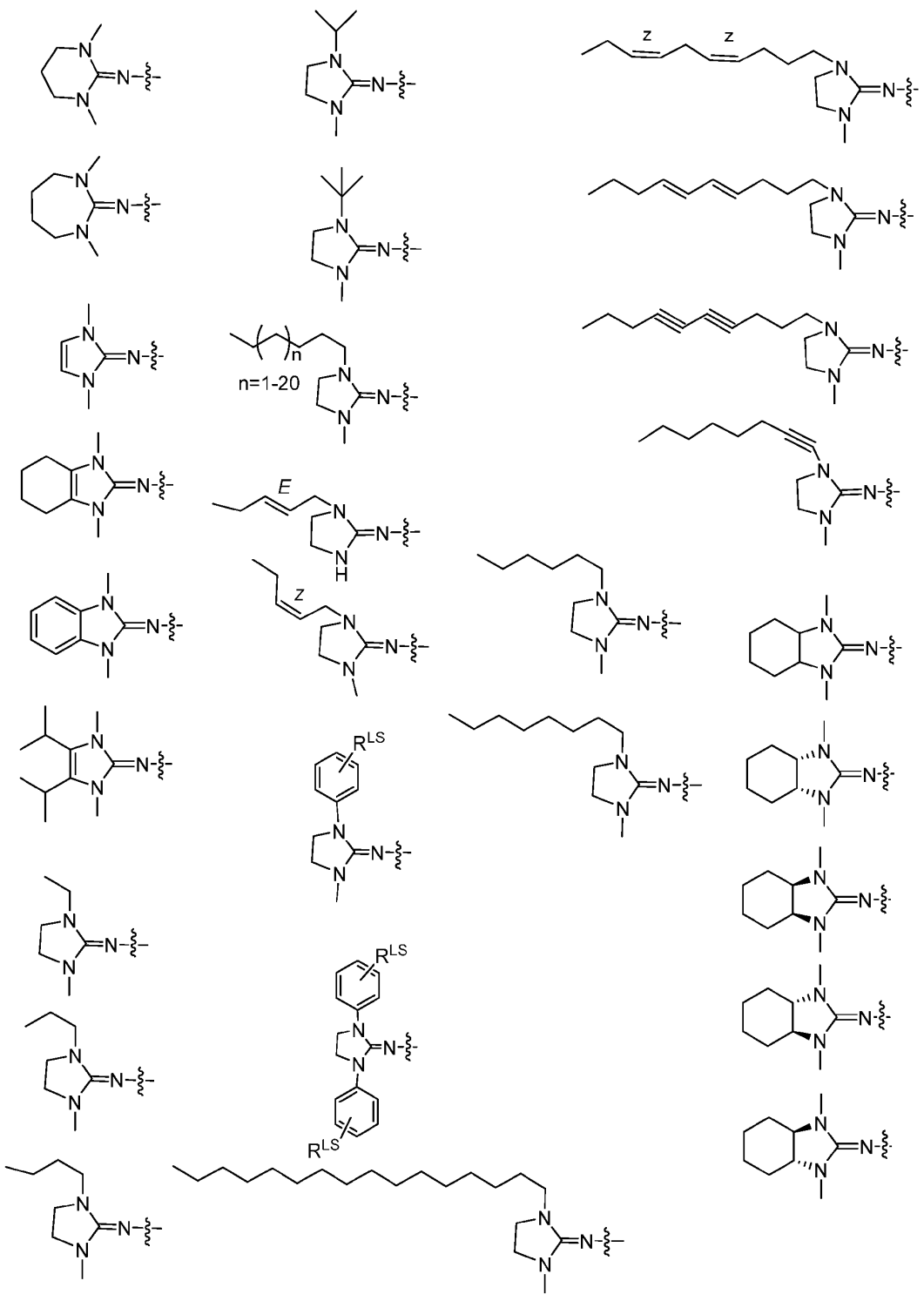
two R' on the same nitrogen are taken together to form a ring as described herein. In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is .

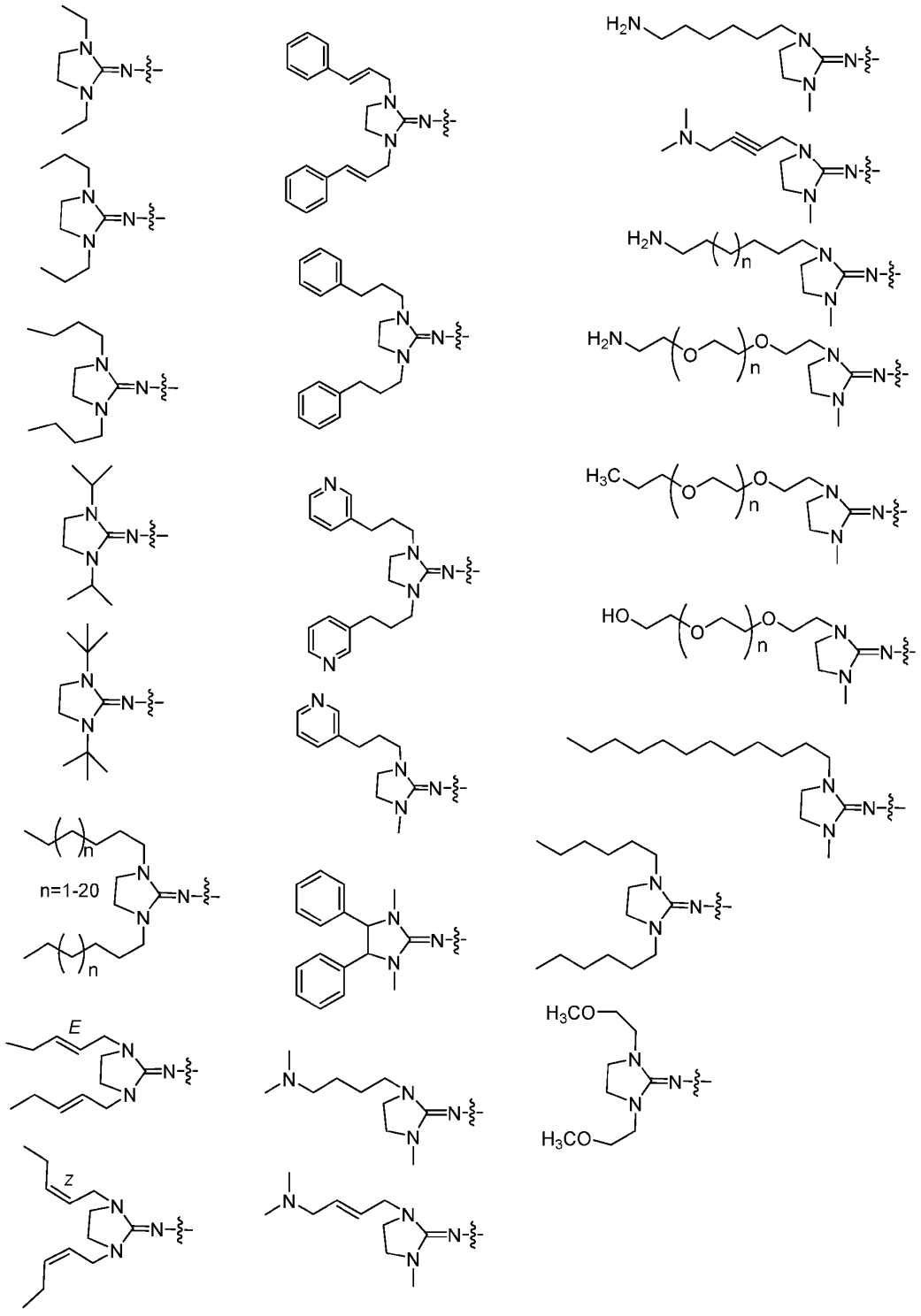
[0274] In some embodiments, $-X-R^L$ is R as described herein. In some embodiments, R is not hydrogen. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted C_{1-6} alkyl. In some embodiments, R is methyl.

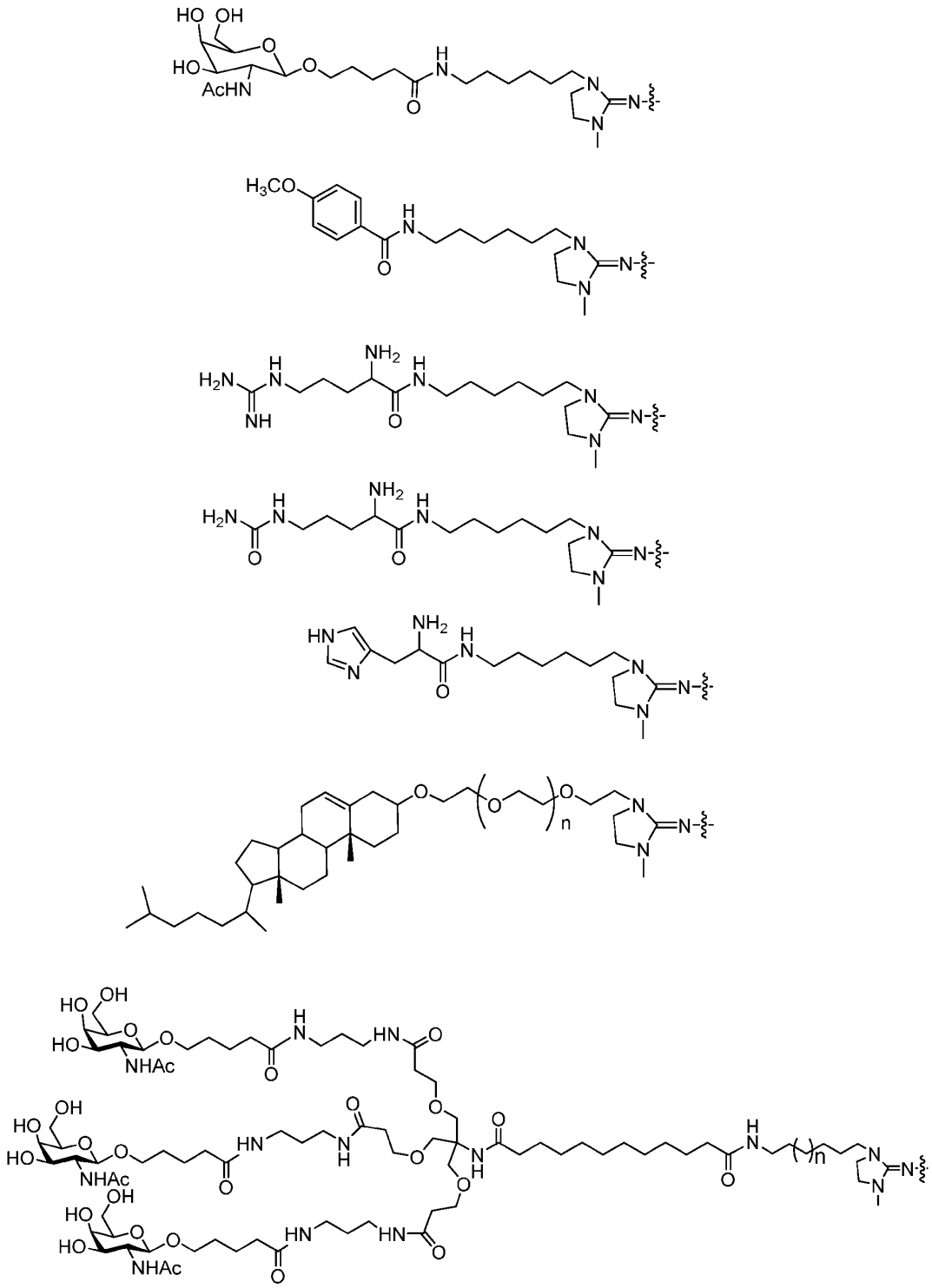
[0275] In some embodiments, $-X-R^L$ is selected from Tables below. In some embodiments, X is as described herein. In some embodiments, R^L is as described herein. In some embodiments, a linkage has the structure of $-Y-P^L(-X-R^L)-Z-$, wherein $-X-R^L$ is selected from Tables below, and each other variable is independently as described herein. In some embodiments, a linkage has the structure of or comprises $-P(O)(-X-R^L)-$, wherein $-X-R^L$ is selected from Tables below. In some embodiments, a linkage has the structure of or comprises $-P(S)(-X-R^L)-$, wherein $-X-R^L$ is selected from Tables below. In some embodiments, a linkage has the structure of or comprises $-P(-X-R^L)-$, wherein $-X-R^L$ is selected from Tables below. In some

embodiments, a linkage has the structure of or comprises –P(O)(–X–R^L)–O–, wherein –X–R^L is selected from Tables below. In some embodiments, a linkage has the structure of or comprises –P(S)(–X–R^L)–O–, wherein –X–R^L is selected from Tables below. In some embodiments, a linkage has the structure of or comprises –P(–X–R^L)–O–, wherein –X–R^L is selected from Tables below. In some embodiments, a linkage has the structure of –P(O)(–X–R^L)–O–, wherein –X–R^L is selected from Tables below. In some embodiments, a linkage has the structure of –P(S)(–X–R^L)–O–, wherein –X–R^L is selected from Tables below. In some embodiments, a linkage has the structure of –P(–X–R^L)–O–, wherein –X–R^L is selected from Tables below. In some embodiments, P is bonded to a nitrogen atom (e.g., a nitrogen atom in sm01, sm18, etc.). In some embodiments, a linkage has the structure of or comprises –O–P(O)(–X–R^L)–O–, wherein –X–R^L is selected from Tables below. In some embodiments, a linkage has the structure of or comprises –O–P(S)(–X–R^L)–O–, wherein –X–R^L is selected from Tables below. In some embodiments, a linkage has the structure of or comprises –O–P(–X–R^L)–O–, wherein –X–R^L is selected from Tables below. In some embodiments, a linkage has the structure of –O–P(O)(–X–R^L)–O–, wherein –X–R^L is selected from Tables below. In some embodiments, a linkage has the structure of –O–P(S)(–X–R^L)–O–, wherein –X–R^L is selected from Tables below. In some embodiments, a linkage has the structure of –O–P(–X–R^L)–O–, wherein –X–R^L is selected from Tables below. In some embodiments, the Tables below, n is 0-20 or as described herein. As those skilled in the art appreciate, a linkage may exist in a salt form.

Table L-1. Certain useful moieties bonded to linkage phosphorus (e.g., –X–R^L).


n=1-20
E
Z
Z
Z
RLS
RLS
RLS


n=1-20
E
Z
H2N
H3C
HO
H3CO



wherein each R^{LS} is independently R^{s}. In some embodiments, each R^{LS} is independently –Cl, –Br, –F,

–N(Me)$_2$, or –NHCOCH$_3$.

Table L-2. Certain useful moieties bonded to linkage phosphorus (e.g., –X–R^L).

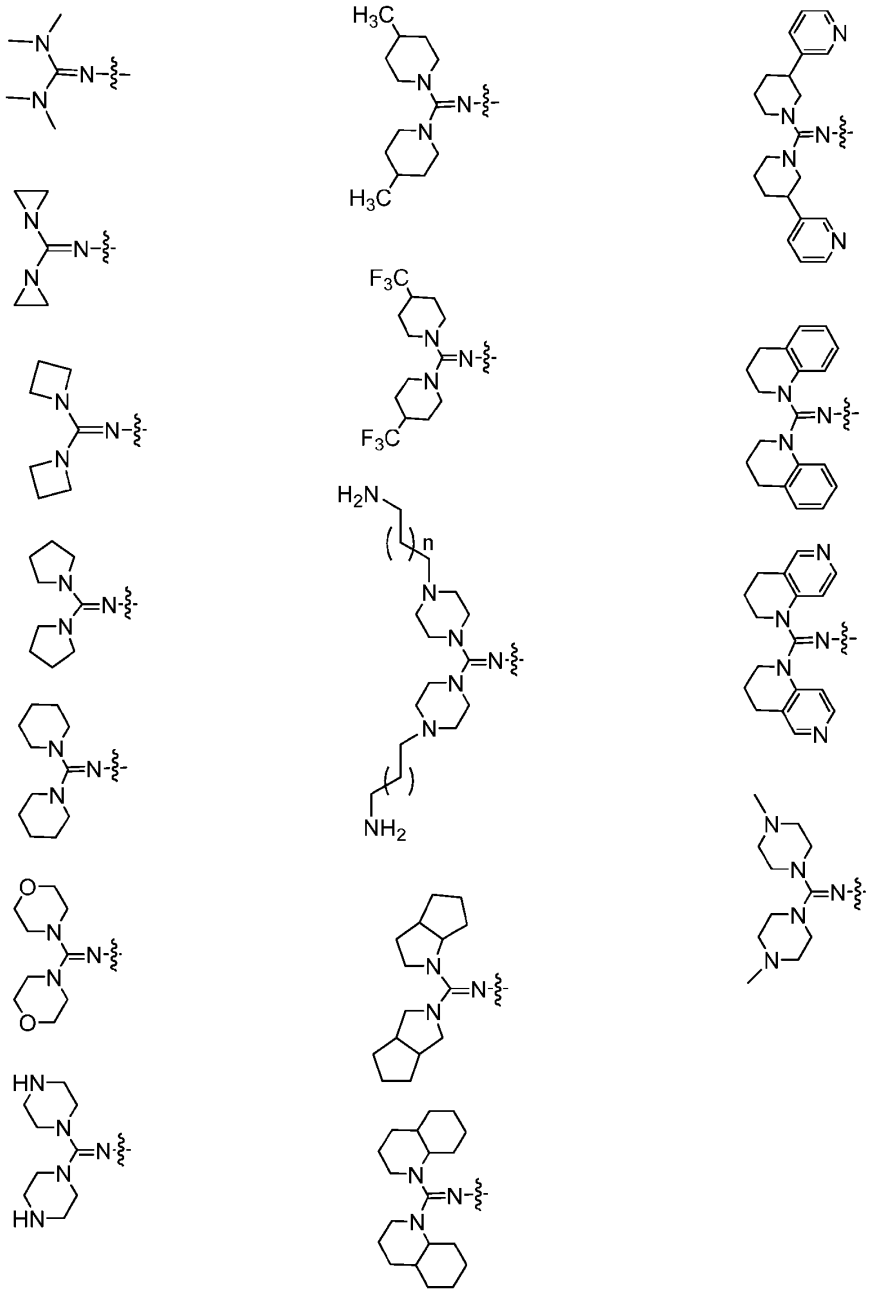


Table L-3. Certain useful moieties bonded to linkage phosphorus (e.g., –X–R^L).

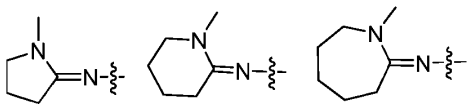
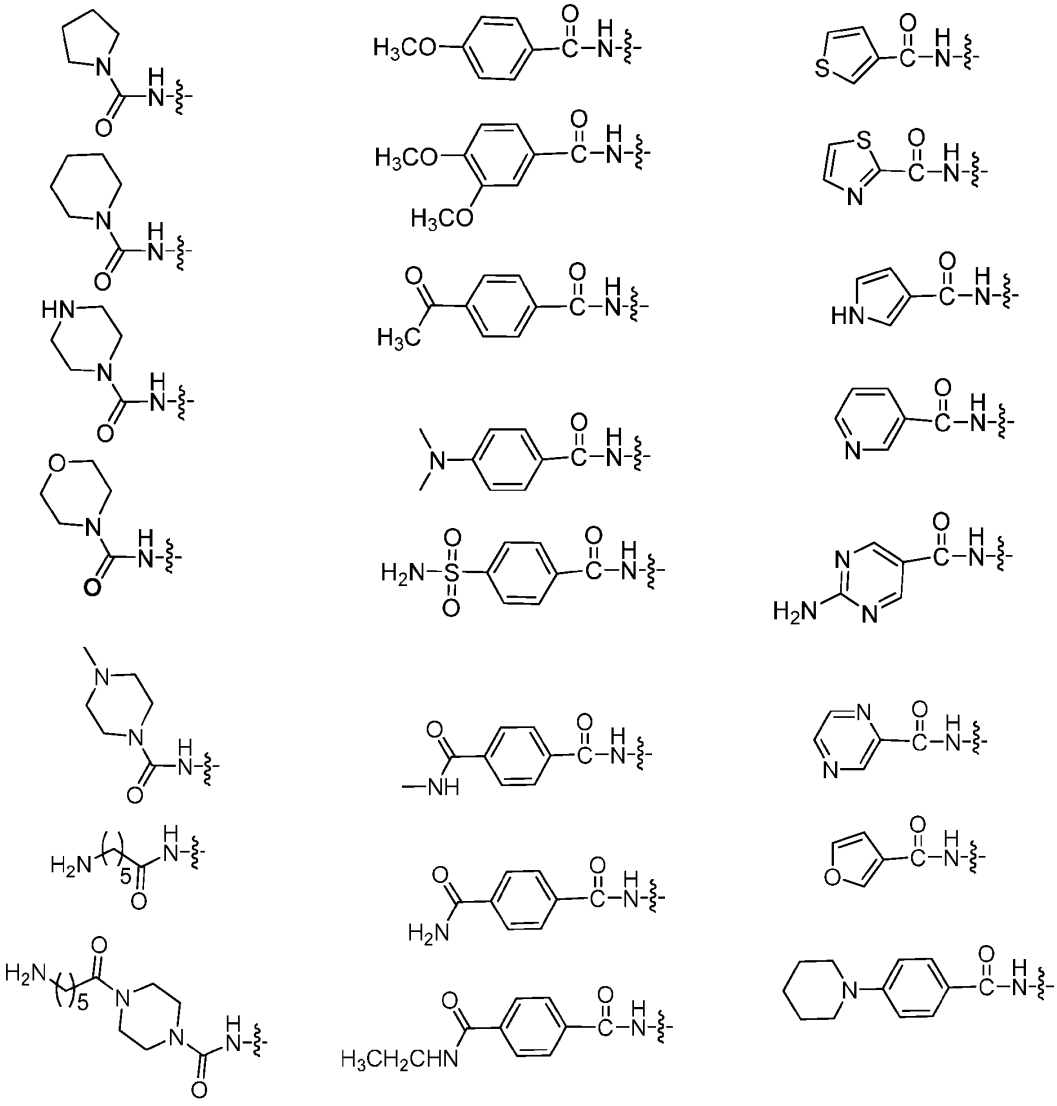


Table L-4. Certain useful moieties bonded to linkage phosphorus (e.g., –X–R^L).



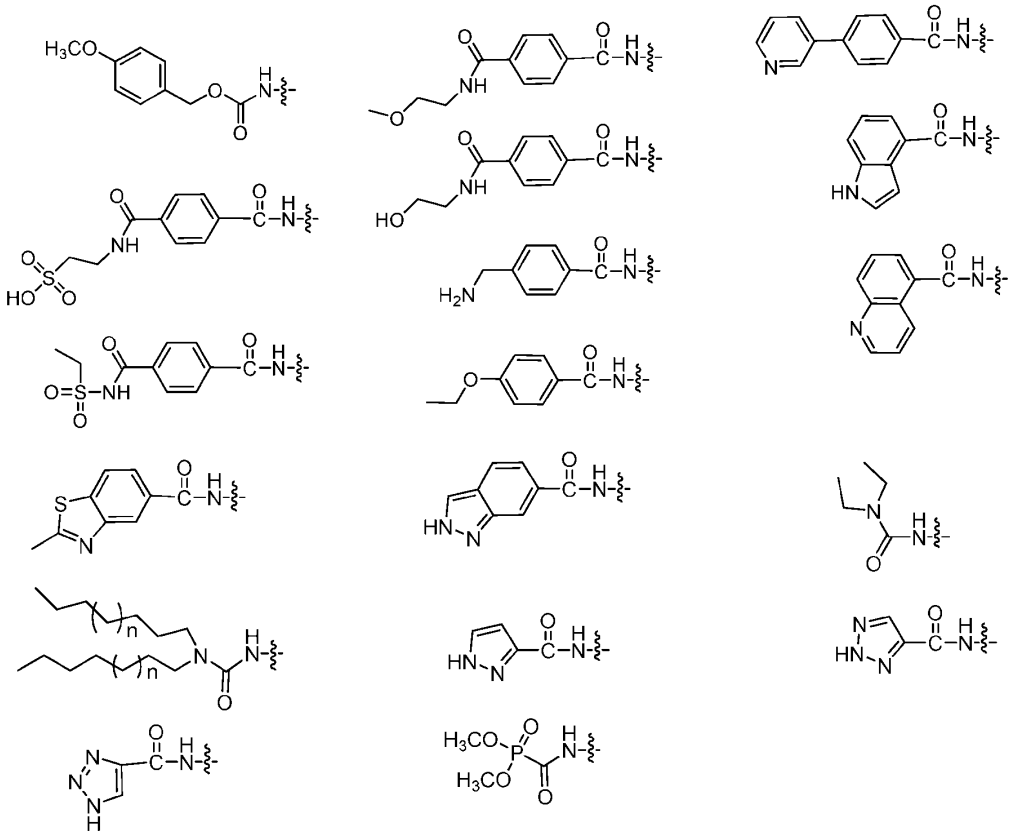
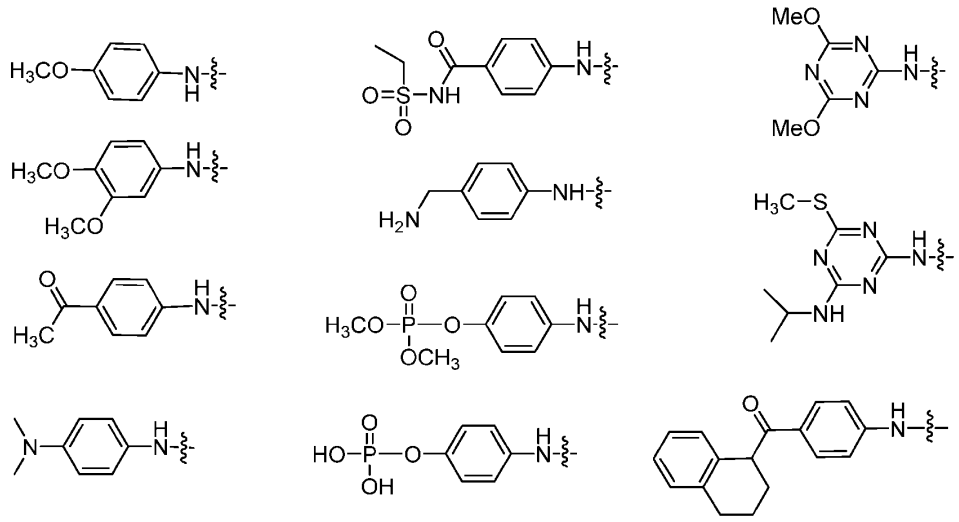
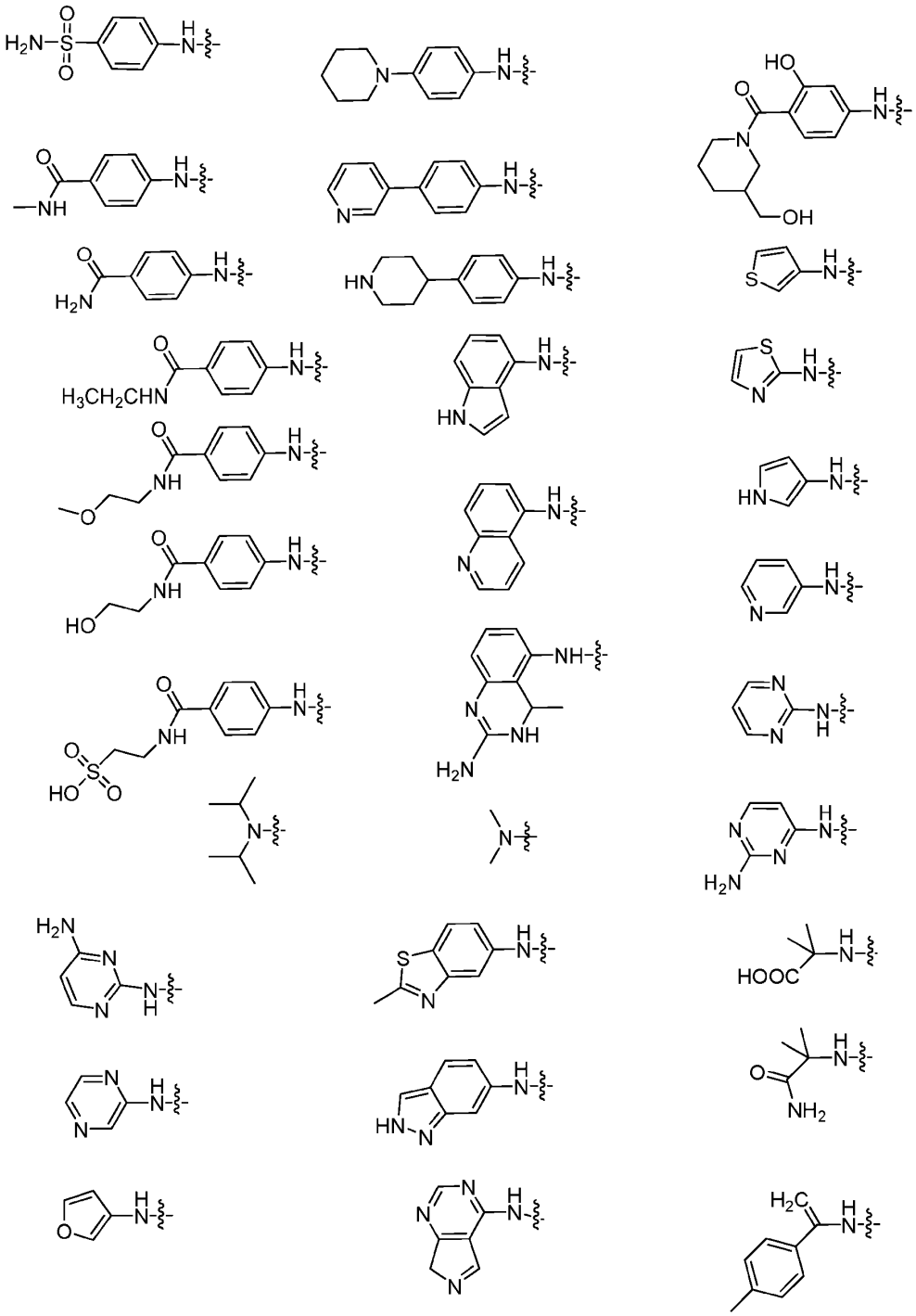


Table L-5. Certain useful moieties bonded to linkage phosphorus (e.g., $-X-R^L$).





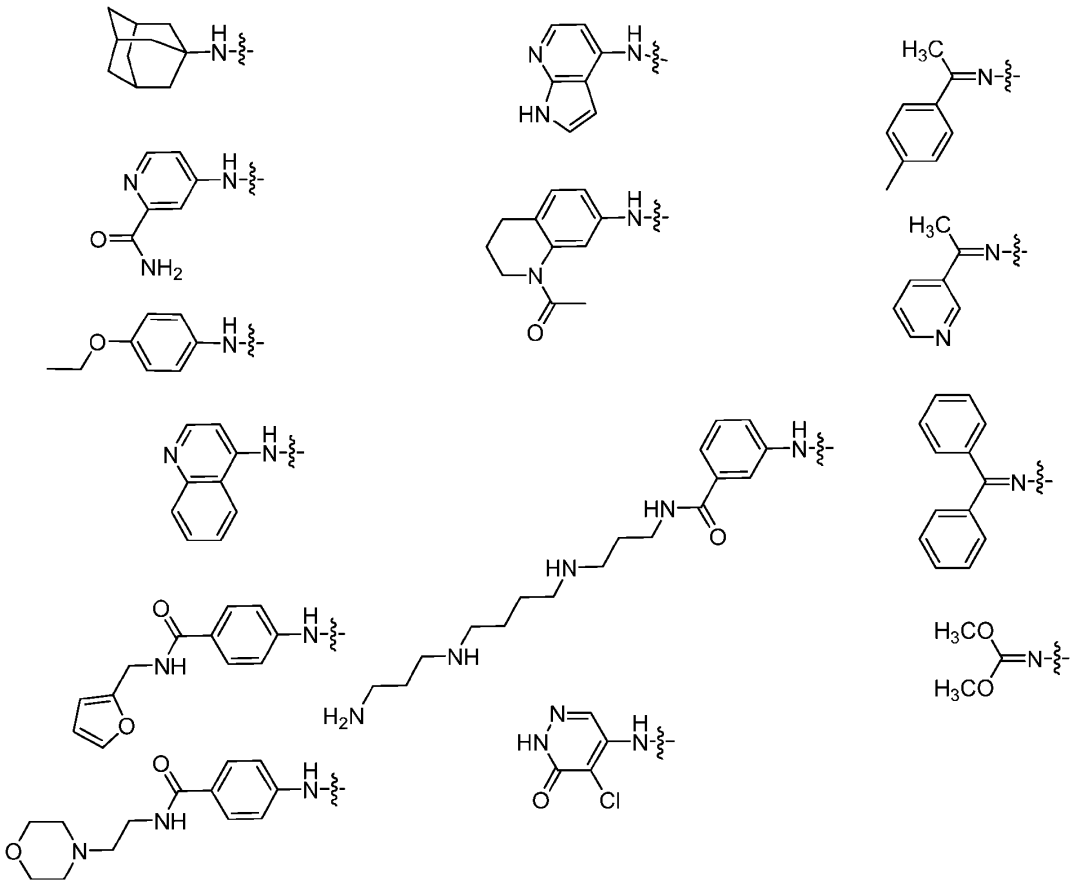
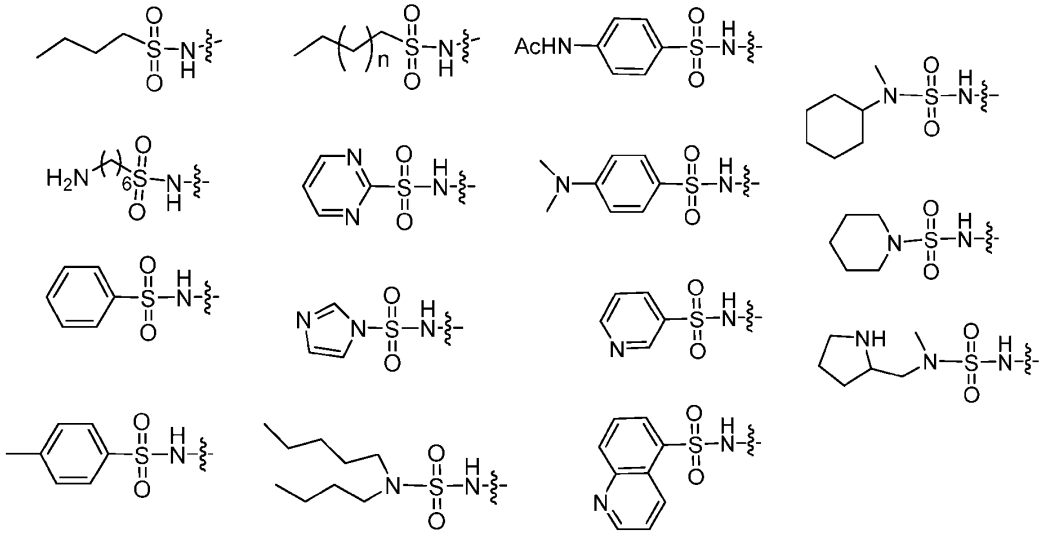
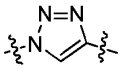
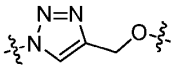


Table L-6. Certain useful moieties bonded to linkage phosphorus (e.g., –X–R^L).



[0276] In some embodiments, an internucleotidic linkage, e.g., an non-negatively charged internucleotidic

linkage or a neutral internucleotidic linkage, has the structure of $-L^{L1}-Cy^{IL}-L^{L2}-$. In some embodiments, L^{L1} is bonded to a 3'-carbon of a sugar. In some embodiments, L^{L2} is bonded to a 5'-carbon of a sugar. In some embodiments, L^{L1} is $-O-CH_2-$. In some embodiments, L^{L2} is a covalent bond. In some embodiments, L^{L2} is a $-N(R')-$. In some embodiments, L^{L2} is a $-NH-$. In some embodiments, L^{L2} is bonded to a 5'-carbon of a sugar, which 5'-carbon is substituted with =O. In some embodiments, Cy^{IL} is optionally substituted 3-10 membered saturated, partially unsaturated, or aromatic ring having 0-5 heteroatoms. In some embodiments, Cy^{IL} is an optionally substituted triazole ring. In some embodiments, Cy^{IL} is . In some embodiments, a linkage is .

[0277] In some embodiments, a non-negatively charged internucleotidic linkage has the structure of $-OP(=W)(-N(R')_2)-O-$.

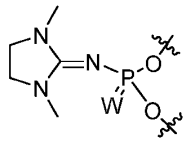
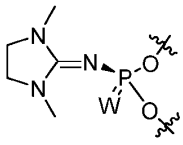
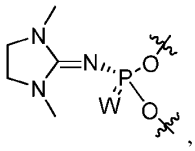
[0278] In some embodiments, R' is R. In some embodiments, R' is –H. In some embodiments, R' is –C(O)R. In some embodiments, R' is –C(O)OR. In some embodiments, R' is $-S(O)_2R$.

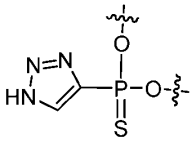
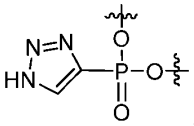
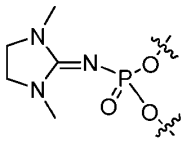
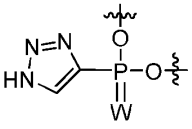
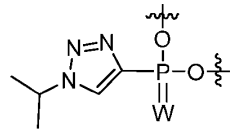
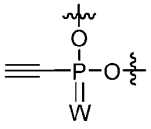
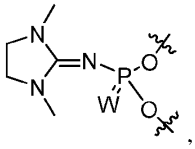
[0279] In some embodiments, R" is –NHR'. In some embodiments, $-N(R')_2$ is –NHR'.

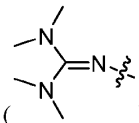
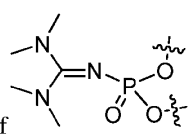
[0280] As described herein, some embodiments, R is –H. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted C_{1-6} alkyl. In some embodiments, R is methyl. In some embodiments, R is substituted methyl. In some embodiments, R is ethyl. In some embodiments, R is substituted ethyl.

[0281] In some embodiments, as described herein, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage.

[0282] In some embodiments, a modified internucleotidic linkage (e.g., a non-negatively charged internucleotidic linkage) comprises optionally substituted triazolyl. In some embodiments, R' is or 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, R' is optionally substituted alkynyl. In some embodiments, R' comprises an optionally substituted triple bond. In some embodiments, a modified internucleotidic linkage comprises a triazole or alkyne moiety. In some embodiments, R' is or comprises an optionally substituted 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, R', R^L, or $-X-R^L$, is or comprises an optionally substituted guanidine moiety. In some embodiments, R', R^L, or $-X-R^L$, is or comprises an optionally substituted cyclic guanidine moiety. In some embodiments, R', R^L, or $-X-R^L$ comprises an optionally substituted cyclic guanidine moiety and an

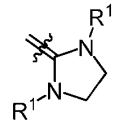
internucleotidic linkage has the structure of: , , or , wherein W is O or S. In some embodiments, W is O. In some embodiments, W is S. In some embodiments, a non-negatively charged internucleotidic linkage is stereochemically controlled.

[0283] In some embodiments, a non-negatively charged internucleotidic linkage or a neutral internucleotidic linkage is an internucleotidic linkage comprising a triazole moiety. In some embodiments, a non-negatively charged internucleotidic linkage or a non-negatively charged internucleotidic linkage comprises an optionally substituted triazolyl group. In some embodiments, an internucleotidic linkage comprising a triazole moiety (e.g., an optionally substituted triazolyl group) has the structure of . In some embodiments, an internucleotidic linkage comprising a triazole moiety has the structure of . In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, a neutral 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 non-negatively charged internucleotidic linkage, or a neutral internucleotidic linkage, is or comprising a structure selected from , , , or , wherein W is O or S.

[0284] 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.

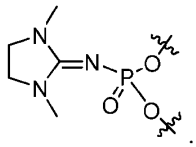
[0285] In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 3-20 membered heterocyclyl or heteroaryl group having 1-10 heteroatoms. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 3-20 membered 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.

[0286] 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 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 substituted  group. In some embodiments, a non-negatively charged internucleotidic linkage comprises a  group. In some embodiments, each R^1 is

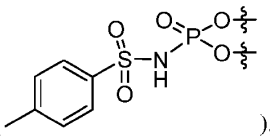
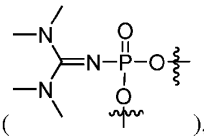
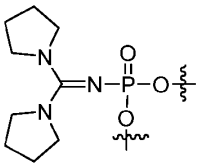
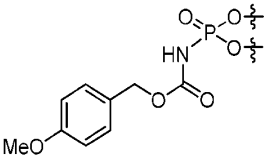
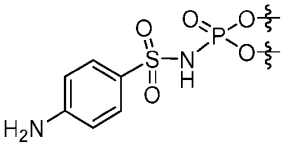
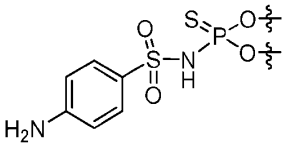
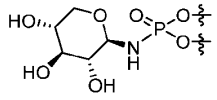
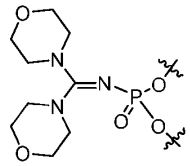
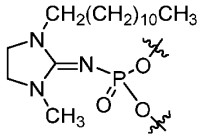
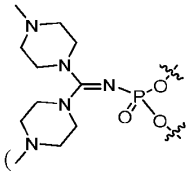
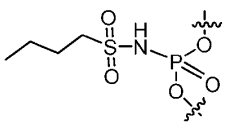
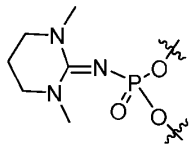
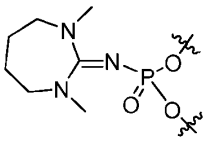
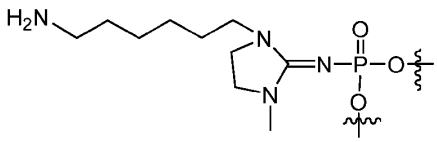
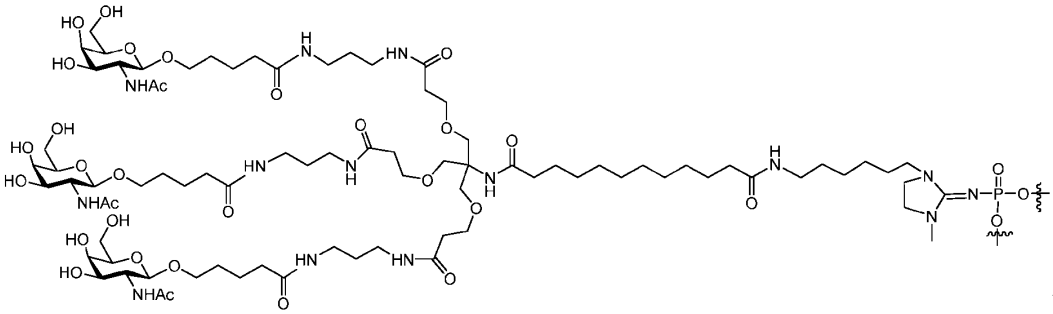
independently optionally substituted C_{1-6} alkyl. In some embodiments, each R^1 is independently methyl.

[0287] In some embodiments, a non-negatively charged internucleotidic linkage, e.g., a neutral internucleotidic linkage is not chirally controlled. In some embodiments, a non-negatively charged internucleotidic linkage is chirally controlled. In some embodiments, a non-negatively charged internucleotidic linkage is chirally controlled and its linkage phosphorus is Rp. In some embodiments, a non-negatively charged internucleotidic linkage is chirally controlled and its linkage phosphorus is Sp.

[0288] In some embodiments, an internucleotidic linkage comprises no linkage phosphorus. In some embodiments, an internucleotidic linkage has the structure of –C(O)–(O)– or –C(O)–N(R')–, wherein R' is as described herein. In some embodiments, an internucleotidic linkage has the structure of –C(O)–(O)–. In some embodiments, an internucleotidic linkage has the structure of –C(O)–N(R')–, wherein R' is as described herein. In various embodiments, –C(O)– is bonded to nitrogen. In some embodiments, an internucleotidic linkage is or comprises –C(O)–O– which is part of a carbamate moiety. In some embodiments, an internucleotidic linkage is or comprises –C(O)–O– which is part of a urea moiety.

[0289] In some embodiments, an oligonucleotide comprises 1-20, 1-15, 1-10, 1-5, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more non-negatively charged internucleotidic linkages. In some embodiments, an oligonucleotide comprises 1-20, 1-15, 1-10, 1-5, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more neutral internucleotidic linkages. In some embodiments, each of non-negatively charged internucleotidic linkage and/or neutral internucleotidic linkages is optionally and independently chirally controlled. In some embodiments, each non-negatively charged internucleotidic linkage in an oligonucleotide is independently a chirally controlled internucleotidic linkage. In some embodiments, each neutral internucleotidic linkage in an oligonucleotide is independently a chirally controlled internucleotidic linkage. In some embodiments, at least one non-negatively charged internucleotidic linkage/neutral internucleotidic linkage has the structure of . In some embodiments, an oligonucleotide comprises at least one non-negatively charged internucleotidic linkage wherein its linkage phosphorus is in Rp configuration, and at least one non-negatively charged internucleotidic linkage wherein its linkage phosphorus is in Sp configuration.

[0290] In many embodiments, as demonstrated extensively, oligonucleotides of the present disclosure comprise two or more different internucleotidic linkages. In some embodiments, an oligonucleotide comprises a phosphorothioate internucleotidic linkage and a non-negatively charged internucleotidic linkage. In some embodiments, an oligonucleotide comprises a phosphorothioate internucleotidic linkage, a non-negatively charged internucleotidic linkage, and a natural phosphate linkage. In some embodiments, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, a non-negatively

charged internucleotidic linkage is n001, n002 (), n003 (), n004 (), n005 (), n006 (), n006sp (), n007 (), n008 (), n009 (), n010 (), or n013. In some embodiments, a non-negatively charged internucleotidic linkage is n020 (), n025 (), n026 (), n051 () or n057 (). In some embodiments, a non-negatively charged internucleotidic linkage is n001. In some embodiments, each phosphorothioate internucleotidic linkage is independently chirally controlled. In some embodiments, each chiral modified internucleotidic linkage is independently chirally controlled. In some embodiments, one or more non-negatively charged internucleotidic linkage are not chirally controlled.

[0291] A typical connection, as in natural DNA and RNA, is that an internucleotidic linkage forms bonds with two sugars (which can be either unmodified or modified as described herein). In many embodiments, as exemplified herein an internucleotidic linkage forms bonds through its oxygen atoms or heteroatoms with one optionally modified ribose or deoxyribose at its 5' carbon, and the other optionally modified ribose or deoxyribose at its 3' carbon. In some embodiments, internucleotidic linkages connect sugars that are not ribose sugars, e.g., sugars comprising N ring atoms and acyclic sugars as described herein.

[0292] In some embodiments, each nucleoside units connected by an internucleotidic linkage independently comprises a nucleobase which is independently an optionally substituted A, T, C, G, or U, or an optionally substituted tautomer of A, T, C, G or U.

[0293] In some embodiments, an oligonucleotide comprises a modified internucleotidic linkage (e.g., a modified internucleotidic linkage having the structure of Formula I, I-a, I-b, or 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) as described in US 9394333, US 9744183, US 9605019, US 9598458, US 9982257, US 10160969, US 10479995, US 2020/0056173, US 2018/0216107, US 2019/0127733, US 10450568, US 2019/0077817, US 2019/0249173, US 2019/0375774, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612 the internucleotidic linkages (e.g., those of Formula I, I-a, I-b, or 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.,) of each of which are independently incorporated herein by reference. In some embodiments, a modified internucleotidic linkage is a non-negatively charged internucleotidic linkage. 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, the present disclosure provides oligonucleotides comprising one or more neutral internucleotidic linkages. In some embodiments, a non-negatively charged internucleotidic linkage or a neutral internucleotidic linkage (e.g., one 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.) is as described in US 9394333, US 9744183, US 9605019, US 9598458, US 9982257, US 10160969, US 10479995, US 2020/0056173, US 2018/0216107, US 2019/0127733, US 10450568, US 2019/0077817, US 2019/0249173, US 2019/0375774, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612. In some embodiments, a non-negatively charged internucleotidic linkage or neutral internucleotidic linkage is one 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 WO 2018/223056, WO 2019/032607, WO 2019/075357, WO 2019/032607, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, such internucleotidic linkages of each of which are independently incorporated herein by reference.

[0294] As described herein, various variables can be R, e.g., R', R^L, etc. Various embodiments for R are

described in the present disclosure (e.g., when describing variables that can be R). Such embodiments are generally useful for all variables that can be R. In some embodiments, R is hydrogen. In some embodiments, R is optionally substituted C_{1-30} (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) aliphatic. In some embodiments, R is optionally substituted C_{1-20} aliphatic. In some embodiments, R is optionally substituted C_{1-10} aliphatic. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted alkyl. In some embodiments, R is optionally substituted C_{1-6} alkyl. In some embodiments, R is optionally substituted methyl. In some embodiments, R is methyl. In some embodiments, R is optionally substituted ethyl. In some embodiments, R is optionally substituted propyl. In some embodiments, R is isopropyl. In some embodiments, R is optionally substituted butyl. In some embodiments, R is optionally substituted pentyl. In some embodiments, R is optionally substituted hexyl.

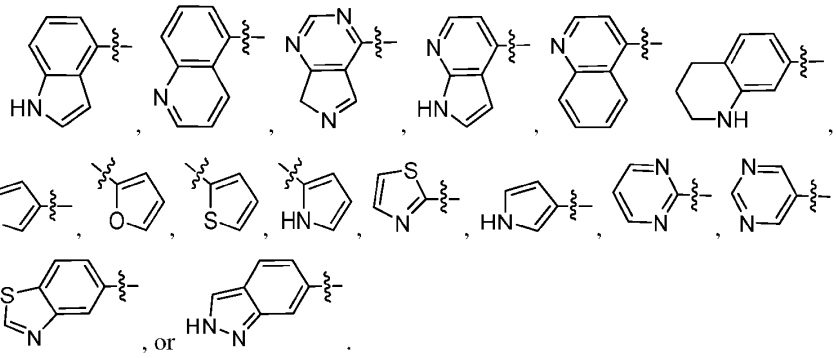
[0295] In some embodiments, R is optionally substituted 3-30 membered (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) cycloaliphatic. In some embodiments, R is optionally substituted cycloalkyl. In some embodiments, cycloaliphatic is monocyclic, bicyclic, or polycyclic, wherein each monocyclic unit is independently saturated or partially saturated. In some embodiments, R is optionally substituted cyclopropyl. In some embodiments, R is optionally substituted cyclobutyl. In some embodiments, R is optionally substituted cyclopentyl. In some embodiments, R is optionally substituted cyclohexyl. In some embodiments, R is optionally substituted adamantyl.

[0296] In some embodiments, R is optionally substituted C_{1-30} (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) heteroaliphatic having 1-10 heteroatoms. In some embodiments, R is optionally substituted C_{1-20} aliphatic having 1-10 heteroatoms. In some embodiments, R is optionally substituted C_{1-10} aliphatic having 1-10 heteroatoms. In some embodiments, R is optionally substituted C_{1-6} aliphatic having 1-3 heteroatoms. In some embodiments, R is optionally substituted heteroalkyl. In some embodiments, R is optionally substituted C_{1-6} heteroalkyl. In some embodiments, R is optionally substituted 3-30 membered (e.g., 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 or 30) heterocycloaliphatic having 1-10 heteroatoms. In some embodiments, R is optionally substituted heteroclycloalkyl. In some embodiments, heterocycloaliphatic is monocyclic, bicyclic, or polycyclic, wherein each monocyclic unit is independently saturated or partially saturated.

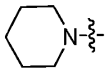
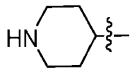
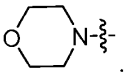
[0297] In some embodiments, R is optionally substituted C_{6-30} aryl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is C_{6-14} aryl. In some embodiments, R is optionally substituted bicyclic aryl. In some embodiments, R is optionally substituted polycyclic aryl. In some embodiments, R is optionally substituted C_{6-30} arylaliphatic. In some embodiments, R is C_{6-30} arylheteroaliphatic having 1-10 heteroatoms.

[0298] In some embodiments, R is optionally substituted 5-30 (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 or 30) membered heteroaryl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-20 membered heteroaryl having 1-10 heteroatoms. In some

embodiments, R is optionally substituted 5-10 membered heteroaryl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-5 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-4 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-3 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having one heteroatom. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-5 heteroatoms. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-4 heteroatoms. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-3 heteroatoms. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 6-membered heteroaryl having one heteroatom. In some embodiments, R is optionally substituted monocyclic heteroaryl. In some embodiments, R is optionally substituted bicyclic heteroaryl. In some embodiments, R is optionally substituted polycyclic heteroaryl. In some embodiments, a heteroatom is nitrogen.

[0299] In some embodiments, R is optionally substituted 2-pyridinyl. In some embodiments, R is optionally substituted 3-pyridinyl. In some embodiments, R is optionally substituted 4-pyridinyl. In some embodiments, R is optionally substituted 

[0300] In some embodiments, R is optionally substituted 3-30 (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 or 30) membered heterocyclyl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 3-membered heterocyclyl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 4-membered heterocyclyl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 5-20 membered heterocyclyl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-10 membered heterocyclyl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-5 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-4 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-3 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having one heteroatom. In some

embodiments, R is optionally substituted 6-membered heterocyclyl having 1-5 heteroatoms. In some embodiments, R is optionally substituted 6-membered heterocyclyl having 1-4 heteroatoms. In some embodiments, R is optionally substituted 6-membered heterocyclyl having 1-3 heteroatoms. In some embodiments, R is optionally substituted 6-membered heterocyclyl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 6-membered heterocyclyl having one heteroatom. In some embodiments, R is optionally substituted monocyclic heterocyclyl. In some embodiments, R is optionally substituted bicyclic heterocyclyl. In some embodiments, R is optionally substituted polycyclic heterocyclyl. In some embodiments, R is optionally substituted saturated heterocyclyl. In some embodiments, R is optionally substituted partially unsaturated heterocyclyl. In some embodiments, a heteroatom is nitrogen. In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted .

[0301] In some embodiments, two R groups are optionally and independently taken together to form a covalent bond. In some embodiments, 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. In some embodiments, 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.

[0302] Various variables may comprises an optionally substituted ring, or can be taken together with their intervening atom(s) to form a ring. In some embodiments, a ring is 3-30 (e.g., 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 or 30) membered. In some embodiments, a ring is 3-20 membered. In some embodiments, a ring is 3-15 membered. In some embodiments, a ring is 3-10 membered. In some embodiments, a ring is 3-8 membered. In some embodiments, a ring is 3-7 membered. In some embodiments, a ring is 3-6 membered. In some embodiments, a ring is 4-20 membered. In some embodiments, a ring is 5-20 membered. In some embodiments, a ring is monocyclic. In some embodiments, a ring is bicyclic. In some embodiments, a ring is polycyclic. In some embodiments, each monocyclic ring or each monocyclic ring unit in bicyclic or polycyclic rings is independently saturated, partially saturated or aromatic. In some embodiments, each monocyclic ring or each monocyclic ring unit in bicyclic or polycyclic rings is independently 3-10 membered and has 0-5 heteroatoms.

[0303] In some embodiments, each heteroatom is independently selected oxygen, nitrogen, sulfur, silicon, and phosphorus. In some embodiments, each heteroatom is independently selected oxygen, nitrogen, sulfur, and phosphorus. In some embodiments, each heteroatom is independently selected oxygen, nitrogen, and sulfur. In some embodiments, a heteroatom is in an oxidized form.

[0304] As described herein, various groups may be optionally substituted. Substituents are routinely utilized in chemistry including in development of various therapeutics. Many substituents can be utilized in accordance with the present disclosure. In some embodiments, an optionally substituted group is unsubstituted. In some embodiments, an optionally substituted group is substituted. Substituents are preferably those that result in the formation of compounds for a desired property, activity, use, etc., as described herein. In some embodiments, compounds are stable for therapeutic use as described herein. The term "stable," as used herein, refers to oligonucleotides, agents, etc. 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. In some embodiments, a substituent is a hydrocarbon group. In some embodiments, a substituent comprises a heteroatom. In some embodiments, a substituent comprises multiple heteroatoms. In some embodiments, each atom in a substituent is independently selected from hydrogen, carbon, halogen, nitrogen, oxygen, sulfur, phosphorus and silicon. In some embodiments, each atom in a substituent is independently selected from hydrogen, carbon, halogen, nitrogen, oxygen, and sulfur. In some embodiments, each atom in a substituent is independently selected from hydrogen, carbon, fluorine, chlorine, bromine, iodine, nitrogen, oxygen, and sulfur. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in a substituent is about or no more than about 1; in some embodiments, it is no more than about 2; in some embodiments, it is no more than about 3; in some embodiments, it is no more than about 4; in some embodiments, it is no more than about 5; in some embodiments, it is no more than about 6; in some embodiments, it is no more than about 7; in some embodiments, it is no more than about 8; in some embodiments, it is no more than about 9; in some embodiments, it is no more than about 10; in some embodiments, it is no more than about 11; in some embodiments, it is no more than about 12; in some embodiments, it is no more than about 13; in some embodiments, it is no more than about 14; in some embodiments, it is no more than about 15; in some embodiments, it is no more than about 20. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 20. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 15. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 10. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 6.

[0305] As appreciated by those skilled in the art, many other types of internucleotidic linkages may be utilized in accordance with the present disclosure, for example, those described in U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,177,195; 5,023,243; 5,034,506; 5,166,315; 5,185,444; 5,188,897; 5,214,134; 5,216,141; 5,235,033; 5,264,423; 5,264,564; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,938; 5,405,939; 5,434,257; 5,453,496; 5,455,233; 5,466,677; 5,466,677; 5,470,967; 5,476,925; 5,489,677; 5,519,126; 5,536,821; 5,541,307; 5,541,316; 5,550,111; 5,561,225; 5,563,253; 5,571,799; 5,587,361; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,625,050; 5,633,360; 5,64,562;

5,663,312; 5,677,437; 5,677,439; 6,160,109; 6,239,265; 6,028,188; 6,124,445; 6,169,170; 6,172,209; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; or RE39464. In some embodiments, a modified internucleotidic linkage is one described in US 9394333, US 9744183, US 9605019, US 9598458, US 9982257, US 10160969, US 10479995, US 2020/0056173, US 2018/0216107, US 2019/0127733, US 10450568, US 2019/0077817, US 2019/0249173, US 2019/0375774, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the nucleobases, sugars, internucleotidic linkages, chiral auxiliaries/reagents, and technologies for oligonucleotide synthesis (reagents, conditions, cycles, etc.) of each of which is independently incorporated herein by reference.

[0306] In some embodiments, each internucleotidic linkage in an oligonucleotide is independently selected from a natural phosphate linkage, a phosphorothioate linkage, and a non-negatively charged internucleotidic linkage (e.g., n001, n002, n003, n004, n005, n006, n007, n008, n009, n010, n013, etc.). In some embodiments, each internucleotidic linkage in an oligonucleotide is independently selected from a natural phosphate linkage, a phosphorothioate linkage, and a neutral internucleotidic linkage (e.g., n001, n002, n003, n004, n005, n006, n007, n008, n009, n010, n013, etc.).

[0307] Oligonucleotides can comprise various numbers of natural phosphate linkages, e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 1-10, 1-5, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more. In some embodiments, one or more (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 1-10, 1-5, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) of the natural phosphate linkages in an oligonucleotide are consecutive. In some embodiments, provided oligonucleotides comprise no natural phosphate linkages. In some embodiments, provided oligonucleotides comprise one natural phosphate linkage. In some embodiments, provided oligonucleotides comprise 1 to 30 or more natural phosphate linkages.

[0308] In some embodiments, a modified internucleotidic linkage is a chiral internucleotidic linkage which comprises a chiral linkage phosphorus. In some embodiments, a chiral internucleotidic linkage is a phosphorothioate linkage. In some embodiments, a chiral internucleotidic linkage is a non-negatively charged internucleotidic linkage. In some embodiments, a chiral internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, a chiral internucleotidic linkage is chirally controlled with respect to its chiral linkage phosphorus. In some embodiments, a chiral internucleotidic linkage is stereochemically pure with respect to its chiral linkage phosphorus. In some embodiments, a chiral internucleotidic linkage is not chirally controlled. In some embodiments, a pattern of backbone chiral centers comprises or consists of positions and linkage phosphorus configurations of chirally controlled internucleotidic linkages (*R*p or *S*p) and positions of achiral internucleotidic linkages (e.g., natural phosphate linkages).

[0309] In some embodiments, provided oligonucleotides comprise one or more non-negatively charged internucleotidic linkages. In some embodiments, provided oligonucleotides comprise one or more neutral internucleotidic linkages. In some embodiments, provided oligonucleotides comprise one or more phosphoryl

guanidine internucleotidic linkages. In some embodiments, a neutral internucleotidic linkage or non-negatively charged internucleotidic linkage is a phosphoryl guanidine internucleotidic linkage. In some embodiments, each neutral internucleotidic linkage or non-negatively charged internucleotidic linkage is independently a phosphoryl guanidine internucleotidic linkage. In some embodiments, each neutral internucleotidic linkage and non-negatively charged internucleotidic linkage is independently n001.

[0310] In some embodiments, each internucleotidic linkage in a provided oligonucleotide is independently selected from a phosphorothioate internucleotidic linkage, a phosphoryl guanidine internucleotidic linkage, and a natural phosphate linkage. In some embodiments, each internucleotidic linkage in a provided oligonucleotide is independently selected from a phosphorothioate internucleotidic linkage, n001, and a natural phosphate linkage.

[0311] Various types of internucleotidic linkages may be utilized in combination of other structural elements, e.g., sugars, to achieve desired oligonucleotide properties and/or activities. For example, the present disclosure routinely utilizes modified internucleotidic linkages and modified sugars, optionally with natural phosphate linkages and natural sugars, in designed oligonucleotides. In some embodiments, the present disclosure provides an oligonucleotide comprising one or more modified sugars. In some embodiments, the present disclosure provides an oligonucleotide comprising one or more modified sugars and one or more modified internucleotidic linkages, one or more of which are natural phosphate linkages.

[0312] In some embodiments, an internucleotidic linkage is a phosphoryl guanidine, phosphoryl amidine, phosphoryl isourea, phosphoryl isothiourea, phosphoryl imidate, or phosphoryl imidothioate internucleotidic linkage, e.g., those as described in US 20170362270.

[0313] As appreciated by those skilled in the art, many other types of internucleotidic linkages may be utilized in accordance with the present disclosure, for example, those described in U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,177,195; 5,023,243; 5,034,506; 5,166,315; 5,185,444; 5,188,897; 5,214,134; 5,216,141; 5,235,033; 5,264,423; 5,264,564; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,938; 5,405,939; 5,434,257; 5,453,496; 5,455,233; 5,466,677; 5,466,677; 5,470,967; 5,476,925; 5,489,677; 5,519,126; 5,536,821; 5,541,307; 5,541,316; 5,550,111; 5,561,225; 5,563,253; 5,571,799; 5,587,361; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,625,050; 5,633,360; 5,64,562; 5,663,312; 5,677,437; 5,677,439; 6,160,109; 6,239,265; 6,028,188; 6,124,445; 6,169,170; 6,172,209; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; or RE39464. In some embodiments, a modified internucleotidic linkage is one described in US 9982257, US 20170037399, US 20180216108, WO 2017192664, WO 2017015575, WO 2017062862, WO 2018067973, WO 2017160741, WO 2017192679, WO 2017210647, WO 2018098264, WO 2018223056, WO 2018237194, or WO 2019055951, the nucleobases, sugars, internucleotidic linkages, chiral auxiliaries/reagents, and technologies for oligonucleotide synthesis (reagents, conditions, cycles, etc.) of each of which is independently incorporated herein by reference. In some embodiments, an internucleotidic linkage is described in WO 2012/030683, WO 2021/030778, WO

2019112485, US 20170362270, WO 2018156056, WO 2018056871, WO 2020/154344, WO 2020/154343, WO 2020/154342, WO 2020/165077, WO 2020/201406, WO 2020/216637, or WO 2020/252376, and can be utilized in accordance with the present disclosure.

[0314] In some embodiments, each internucleotidic linkage in an oligonucleotide is independently selected from a natural phosphate linkage, a phosphorothioate linkage, and a non-negatively charged internucleotidic linkage (e.g., n001). In some embodiments, each internucleotidic linkage in an oligonucleotide is independently selected from a natural phosphate linkage, a phosphorothioate linkage, and a neutral internucleotidic linkage (e.g., n001).

[0315] In some embodiments, an oligonucleotide comprises one or more nucleotides that independently comprise a phosphorus modification prone to "autorelease" under certain conditions. That is, under certain conditions, a particular phosphorus modification is designed such that it self-cleaves from the oligonucleotide to provide, e.g., a natural phosphate linkage. In some embodiments, such a phosphorus modification has a structure of –O–L–R^1, wherein L is L^B as described herein, and R^1 is R' as described herein. In some embodiments, a phosphorus modification has a structure of –S–L–R^1, wherein each L and R^1 is independently as described in the present disclosure. Certain examples of such phosphorus modification groups can be found in US 9982257. In some embodiments, an autorelease group comprises a morpholino group. In some embodiments, an autorelease group is characterized by the ability to deliver an agent to the internucleotidic phosphorus linker, which agent facilitates further modification of the phosphorus atom such as, e.g., desulfurization. In some embodiments, the agent is water and the further modification is hydrolysis to form a natural phosphate linkage.

[0316] In some embodiments, an oligonucleotide comprises one or more internucleotidic linkages that improve one or more pharmaceutical properties and/or activities of the oligonucleotide. It is well documented in the art that certain oligonucleotides are rapidly degraded by nucleases and exhibit poor cellular uptake through the cytoplasmic cell membrane (Poijarvi-Virta et al., Curr. Med. Chem. (2006), 13(28);3441-65; Wagner et al., Med. Res. Rev. (2000), 20(6):417-51; Peyrottes et al., Mini Rev. Med. Chem. (2004), 4(4):395-408; Gosselin et al., (1996), 43(1):196-208; Bologna et al., (2002), Antisense & Nucleic Acid Drug Development 12:33-41). Vives et al. (Nucleic Acids Research (1999), 27(20):4071-76) reported that tert-butyl SATE pro-oligonucleotides displayed markedly increased cellular penetration compared to the parent oligonucleotide under certain conditions.

[0317] Oligonucleotides can comprise various number of natural phosphate linkages. In some embodiments, 5% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 10% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 15% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 20% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 25% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some

embodiments, 30% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 35% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 40% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, provided oligonucleotides comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprises 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages. In some embodiments, the number of natural phosphate linkages is 2. In some embodiments, the number of natural phosphate linkages is 3. In some embodiments, the number of natural phosphate linkages is 4. In some embodiments, the number of natural phosphate linkages is 5. In some embodiments, the number of natural phosphate linkages is 6. In some embodiments, the number of natural phosphate linkages is 7. In some embodiments, the number of natural phosphate linkages is 8. In some embodiments, some or all of the natural phosphate linkages are consecutive. In some embodiments, no more than a certain number of internucleotidic linkages of the provided oligonucleotides are natural phosphate linkages, e.g., no more than 1, no more than 2, no more than 3, no more than 4, no more than 5, no more than 6, no more than 7, no more than 8, no more than 9, no more than 10, no more than 11, no more than 12, no more than 13, no more than 14, no more than 15, no more than 16, no more than 17, no more than 18, no more than 19, no more than 20, no more than 21, no more than 22, no more than 23, no more than 24, no more than 25, no more than 26, no more than 27, no more than 28, no more than 29, or no more than 30 natural phosphate linkages. In some embodiments, provided oligonucleotides comprise no natural phosphate linkages.

[0318] In some embodiments, the present disclosure demonstrates that, in at least some cases, *Sp* internucleotidic linkages, among other things, at the 5'- and/or 3'-end can improve oligonucleotide stability. In some embodiments, the present disclosure demonstrates that, among other things, natural phosphate linkages and/or *Rp* internucleotidic linkages may 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.

[0319] In some embodiments, each phosphorothioate internucleotidic linkage in an oligonucleotide or a portion thereof (e.g., a domain, a subdomain, etc.) is independently chirally controlled. In some embodiments, each is independently *Sp* or *Rp*. In some embodiments, a high level is *Sp* as described herein. In some embodiments, each phosphorothioate internucleotidic linkage in an oligonucleotide or a portion thereof is chirally controlled and is *Sp*. In some embodiments, one or more, e.g., about 1-5 (e.g., about 1, 2, 3, 4, or 5) is *Rp*.

[0320] In some embodiments, as illustrated in certain examples, an oligonucleotide or a portion thereof comprises one or more non-negatively charged internucleotidic linkages, each of which is optionally and independently chirally controlled. In some embodiments, each non-negatively charged internucleotidic linkage is independently n001. In some embodiments, a chiral non-negatively charged internucleotidic linkage is not chirally controlled. In some embodiments, each chiral non-negatively charged internucleotidic linkage

is not chirally controlled. In some embodiments, a chiral non-negatively charged internucleotidic linkage is chirally controlled. In some embodiments, a chiral non-negatively charged internucleotidic linkage is chirally controlled and is *R*p. In some embodiments, a chiral non-negatively charged internucleotidic linkage is chirally controlled and is *S*p. In some embodiments, each chiral non-negatively charged internucleotidic linkage is chirally controlled. In some embodiments, the number of non-negatively charged internucleotidic linkages in an oligonucleotide or a portion thereof is about 1-10, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, it is about 1. In some embodiments, it is about 2. In some embodiments, it is about 3. In some embodiments, it is about 4. In some embodiments, it is about 5. In some embodiments, it is about 6. In some embodiments, it is about 7. In some embodiments, it is about 8. In some embodiments, it is about 9. In some embodiments, it is about 10. In some embodiments, two or more non-negatively charged internucleotidic linkages are consecutive. In some embodiments, no two non-negatively charged internucleotidic linkages are consecutive. In some embodiments, all non-negatively charged internucleotidic linkages in an oligonucleotide or a portion thereof are consecutive (e.g., 3 consecutive non-negatively charged internucleotidic linkages). In some embodiments, a non-negatively charged internucleotidic linkage, or two or more (e.g., about 2, about 3, about 4 etc.) consecutive non-negatively charged internucleotidic linkages, are at the 3'-end of an oligonucleotide or a portion thereof. In some embodiments, the last two or three or four internucleotidic linkages of an oligonucleotide or a portion thereof comprise at least one internucleotidic linkage that is not a non-negatively charged internucleotidic linkage. In some embodiments, the last two or three or four internucleotidic linkages of an oligonucleotide or a portion thereof comprise at least one internucleotidic linkage that is not n001. In some embodiments, the internucleotidic linkage linking the first two nucleosides of an oligonucleotide or a portion thereof is a non-negatively charged internucleotidic linkage. In some embodiments, the internucleotidic linkage linking the last two nucleosides of an oligonucleotide or a portion thereof is a non-negatively charged internucleotidic linkage. In some embodiments, the internucleotidic linkage linking the first two nucleosides of an oligonucleotide or a portion thereof is a phosphorothioate internucleotidic linkage. In some embodiments, it is *S*p. In some embodiments, the internucleotidic linkage linking the last two nucleosides of an oligonucleotide or a portion thereof is a phosphorothioate internucleotidic linkage. In some embodiments, it is *S*p.

[0321] In some embodiments, one or more chiral internucleotidic linkages are chirally controlled and one or more chiral internucleotidic linkages are not chirally controlled. In some embodiments, each phosphorothioate internucleotidic linkage is independently chirally controlled, and one or more non-negatively charged internucleotidic linkage are not chirally controlled. In some embodiments, each phosphorothioate internucleotidic linkage is independently chirally controlled, and each non-negatively charged internucleotidic linkage is not chirally controlled. In some embodiments, the internucleotidic linkage between the first two nucleosides of an oligonucleotide is a non-negatively charged internucleotidic linkage. In some embodiments, the internucleotidic linkage between the last two nucleosides are each independently a non-negatively charged internucleotidic linkage. In some embodiments, both are independently non-negatively charged

internucleotidic linkages. In some embodiments, each non-negatively charged internucleotidic linkage is independently neutral internucleotidic linkage. In some embodiments, each non-negatively charged internucleotidic linkage is independently n001.

[0322] As demonstrated herein, in some embodiments, non-negatively charged internucleotidic linkages such as n001 may provide improved properties and/or activities. In some embodiments, in an oligonucleotide a 5'-end internucleotidic linkage and/or a 3'-end internucleotidic linkage, each of which is independently bonded to two nucleosides comprising a nucleobase as described herein, is a non-negatively charged internucleotidic linkage as described herein. In some embodiments, the first one or more (e.g., the first 1, 2, and/or 3), and/or the last one or more (e.g., the last 1, 2, 3, 4, 5, 6 or 7) internucleotidic linkages, each of which is independently bonded to two nucleosides in a first domain, is independently a non-negatively charged internucleotidic linkage. In some embodiments, the first internucleotidic linkage of a first domain is a non-negatively charged internucleotidic linkage. In some embodiments, the last internucleotidic linkage that bonds to two nucleosides of a first domain is a non-negatively charged internucleotidic linkage. In some embodiments, the last internucleotidic linkage of a second domain is a non-negatively charged internucleotidic linkage. In some embodiments, one or more of internucleotidic linkages in the middle of a second domain, e.g., one or more of the 4th, 5th and 6th internucleotidic linkages, each of which independently bonds to two nucleosides of a second domain, is independently a non-negatively charged internucleotidic linkage. In some embodiments, the 11th internucleotidic linkage that bonds to two nucleosides of a second domain is a non-negatively charged internucleotidic linkage. In some embodiments, an internucleotidic linkage that is not bonded to a nucleoside opposite to a target nucleoside but is bonded to its 3' immediate nucleoside is a non-negatively charged internucleotidic linkage. In some embodiments, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, a non-negatively charged internucleotidic linkage is n001. In some embodiments, each non-negatively charged internucleotidic linkage is n001. In some embodiments, a non-negatively charged internucleotidic linkage is stereorandom. In some embodiments, a non-negatively charged internucleotidic linkage is chirally controlled and is *R*p. In some embodiments, a non-negatively charged internucleotidic linkage is chirally controlled and is *S*p. In some embodiments, each non-negatively charged internucleotidic linkage is independently chirally controlled. In some embodiments, one or more internucleotidic linkages of a first domain, e.g., one or more of the 4th, 5th, 6th, 7th and 8th internucleotidic linkages each of which is independently bonded to two nucleosides of a first domain, is independently not a non-negatively charged internucleotidic linkage. In some embodiments, one or more internucleotidic linkages of a second domain, e.g., one or more of the 1st, 2nd, 3rd, 7th, 8th, 9th, 12th and 13th internucleotidic linkages each of which is independently bonded to two nucleosides of a first domain, is independently not a non-negatively charged internucleotidic linkage. In some embodiments, one or both of the 2nd and the 3rd internucleotidic linkages of a second domain is not a non-negatively charged internucleotidic linkage. In some embodiments, an internucleotidic linkage that is not a non-negatively charged internucleotidic linkage is a phosphorothioate internucleotidic linkage. In some embodiments, it is a stereorandom phosphorothioate internucleotidic linkage.

In some embodiments, it is a *R*p chirally controlled phosphorothioate internucleotidic linkage. In some embodiments, it is a *S*p chirally controlled phosphorothioate internucleotidic linkage.

[0323] In some embodiments, oligonucleotides comprise one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more, PN linkages. For example, in some embodiments, an oligonucleotide comprises one and no more than one PN linkage. In some embodiments, an oligonucleotide comprises two and no more than two PN linkages. In some embodiments, an oligonucleotide comprises three and no more than three PN linkages. In some embodiments, an oligonucleotide comprises four and no more than four PN linkages. In some embodiments, an oligonucleotide comprises five and no more than five PN linkages. In some embodiments, an oligonucleotide comprises six and no more than six PN linkages. In some embodiments, an oligonucleotide comprises one or more PN linkages. In some embodiments, an oligonucleotide comprises two or more PN linkages. In some embodiments, an oligonucleotide comprises three or more PN linkages. In some embodiments, an oligonucleotide comprises four or more PN linkages. In some embodiments, an oligonucleotide comprises five or more PN linkages. In some embodiments, an oligonucleotide comprises six or more PN linkages. In some embodiments, PN linkages at certain positions, e.g., positions 1, 3, 6, 17, and/or 19 (the internucleotidic linkage bonded to the first and second nucleosides from the 5'-end is at position 1; numbering increases toward the 3'-end), provide higher levels of exon skipping than others. In some embodiments, position 19 is the last internucleotidic linkage position. In some embodiments, each such PN internucleotidic linkages is independently a phosphoryl guanidine internucleotidic linkage. In some embodiments, each is independently n001.

[0324] In some embodiments, an oligonucleotide comprises one or more (e.g., 1-10, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, etc.) natural phosphate linkages. In some embodiments, both nucleosides bonded to a natural phosphate linkage are independently a 2'-modified sugar. In some embodiments, both nucleosides bonded to a majority (e.g., at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or more) of natural phosphate linkages are independently a 2'-modified sugar. In some embodiments, both nucleosides bonded to each natural phosphate linkage are independently a 2'-modified sugar. In some embodiments, a 2'-modified sugar is a bicyclic sugar or 2'-OR modified sugar wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, each 2'-modified sugar is independently a bicyclic sugar or 2'-OR modified sugar wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, each 2'-modified sugar is independently a 2'-OR modified sugar wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, each 2'-modified sugar is independently a 2'-OMe modified sugar or a 2'-MOE modified sugar. In some embodiments, each 2'-modified sugar is independently a 2'-OMe modified sugar. In some embodiments, each 2'-modified sugar is independently a 2'-MOE modified sugar. In some embodiments, a natural phosphate linkage is utilized with a non-negatively charged internucleotidic linkage (e.g., a phosphoryl guanidine internucleotidic linkage such as n001). In some embodiments, an oligonucleotide comprises alternating natural phosphate linkages and non-negatively charged internucleotidic linkages (e.g., a phosphoryl guanidine internucleotidic linkage such as n001).

[0325] In some embodiments, one or more internucleotidic linkages at positions -1 and -2 are independently *R*p phosphorothioate internucleotidic linkages. In some embodiments, one or more internucleotidic linkages at positions -3, -2, -1, +1, +3, +4, +5, +7, +8, +9, +10, +11, +12, +13,+16, +17 and +18 are independently *R*p phosphorothioate internucleotidic linkages. In some embodiments, an internucleotidic linkage at position -3 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position -2 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position -1 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +1 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +3 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +4 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +5 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +7 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +8 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +9 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +10 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +11 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +12 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +13 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +16 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +17 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an internucleotidic linkage at position +18 is a *R*p phosphorothioate internucleotidic linkage. In some embodiments, an oligonucleotide contains one and only one *R*p phosphorothioate internucleotidic linkage. In some embodiments, it contains two and no more than two. In some embodiments, it contains three and no more than three. In some embodiments, it contains four and no more than four. In some embodiments, it contains five and no more than five.

[0326] In some embodiments, a non-negatively charged internucleotidic linkage bonded to 3'-carbon of dI is *S*p. In some embodiments, a non-negatively charged internucleotidic linkage bonded to 3'-carbon of dI is *S*p. In some embodiments, a phosphoryl guanidine internucleotidic linkage bonded to 3'-carbon of dI is *S*p. In some embodiments, a n001 internucleotidic linkage bonded to 3'-carbon of dI is *S*p. In some embodiments, each non-negatively charged internucleotidic linkage bonded to 3'-carbon of dI is independently *S*p. In some embodiments, each neutral internucleotidic linkage bonded to 3'-carbon of dI is independently *S*p. In some embodiments, each phosphoryl guanidine internucleotidic linkage bonded to 3'-carbon of dI is independently *S*p. In some embodiments, each n001 bonded to 3'-carbon of dI is independently *S*p.

[0327] In some embodiments, a controlled level of oligonucleotides in a composition are desired oligonucleotides. In some embodiments, of all oligonucleotides in a composition that share a common base

sequence (e.g., a desired sequence for a purpose), or of all oligonucleotides in a composition, level of desired oligonucleotides (which may exist in various forms (e.g., salt forms) and typically differ only at non-chirally controlled internucleotidic linkages (various forms of the same stereoisomer can be considered the same for this purpose)) is about 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%. In some embodiments, a level is at least about 50%. In some embodiments, a level is at least about 60%. In some embodiments, a level is at least about 70%. In some embodiments, a level is at least about 75%. In some embodiments, a level is at least about 80%. In some embodiments, a level is at least about 85%. In some embodiments, a level is at least about 90%. In some embodiments, a level is or is at least $(DS)^{nc}$, wherein DS is about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% and nc is the number of chiral linkage phosphorus as described in the present disclosure (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 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). In some embodiments, a level is or is at least $(DS)^{nc}$, wherein DS is about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% and nc is the number of chirally controlled internucleotidic linkages as described in the present disclosure (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 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). In some embodiments, a level is or is at least $(DS)^{nc}$, wherein DS is 95%-100%.

[0328] Various types of internucleotidic linkages may be utilized in combination of other structural elements, e.g., sugars, to achieve desired oligonucleotide properties and/or activities. For example, the present disclosure routinely utilizes modified internucleotidic linkages and modified sugars, optionally with natural phosphate linkages and natural sugars, in designing oligonucleotides. In some embodiments, the present disclosure provides an oligonucleotide comprising one or more modified sugars. In some embodiments, the present disclosure provides an oligonucleotide comprising one or more modified sugars and one or more modified internucleotidic linkages, one or more of which are natural phosphate linkages.

[0329] In some embodiments, provided oligonucleotides comprise a number of natural RNA sugars (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 or more, two or more or all of them are optionally consecutive). In some embodiments, such oligonucleotides comprise modified sugars, e.g., 2' modified sugars (e.g., 2'-F, etc.) and/or 2'-OR modified sugars wherein R is not –H (e.g., 2-OMe, 2-MOE, etc.) at one or both ends, and/or various modified internucleotidic linkages (e.g., phosphorothioate internucleotidic linkages, non-negatively charged internucleotidic linkages, etc.). In some embodiments, at the 5'-end there are one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 more such 2'-OR modified sugars, wherein R is not –H. In some embodiments, at the 3'-end there are one or more, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 more such 2'-OR modified sugars, wherein R is not –H. In some embodiments, each 2'-modified sugar is independently a 2'-OR modified sugar wherein R is not –H. In some embodiments, as described

herein, 2'-OR is 2'-OMe. In some embodiments, 2'-OR is 2'-MOE. In some embodiments, each of 2'-OR is independently 2'-OMe or 2'-MOE. In some embodiments, each 2'-OR is 2'-OMe.

[0330] In some embodiments, stability of various internucleotidic linkages is assessed. In some embodiments, internucleotidic linkages are exposed to various conditions utilized for oligonucleotide manufacturing, e.g., solid phase oligonucleotide synthesis, including reagents, solvents, temperatures (in some cases, temperatures higher than room temperature), cleavage conditions, deprotection conditions, purification conditions, etc., and stability is assessed. In some embodiments, stable internucleotidic linkages (e.g., those having no more than 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% degradation when exposed to one or more conditions and/or processes, or after a complete oligonucleotide manufacturing process) are selected for utilization in various oligonucleotide compositions and applications.

Production of Oligonucleotides and Compositions

[0331] Various methods can be utilized for production of oligonucleotides and compositions and can be utilized in accordance with the present disclosure. For example, traditional phosphoramidite chemistry (e.g., phosphoramidites comprising $-CH_2CH_2CN$ and $-N(i\text{-}Pr)_2$) can be utilized to prepare stereorandom oligonucleotides and compositions, and certain reagents and chirally controlled technologies can be utilized to prepare chirally controlled oligonucleotide compositions, e.g., as described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/223056, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the reagents and methods of each of which is incorporated herein by reference.

[0332] In some embodiments, chirally controlled/stereoselective preparation of oligonucleotides and compositions thereof comprise utilization of a chiral auxiliary, e.g., as part of monomers, dimers (e.g., chirally pure dimers from separation), monomeric phosphoramidites, dimeric phosphoramidites (e.g., chirally pure dimers from separation), etc. Examples of such chiral auxiliary reagents, monomers, dimers, and phosphoramidites are described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/223056, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the chiral auxiliary reagents, monomers, dimers, and phosphoramidites of each of which are independently incorporated herein by reference. In some embodiments, a chiral auxiliary is a chiral auxiliary described in any of: WO 2018/022473, WO 2018/098264, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the chiral auxiliaries of each of which are independently incorporated herein by reference.

[0333] In some embodiments, chirally controlled preparation technologies, including oligonucleotide synthesis cycles, reagents and conditions are described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, and/WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the oligonucleotide synthesis methods, cycles, reagents and conditions of each of which are independently incorporated herein by reference.

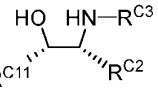
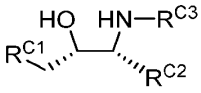
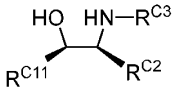
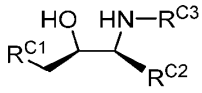
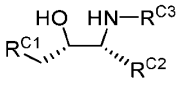
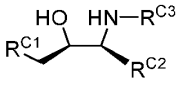
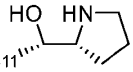
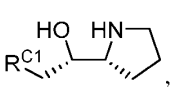
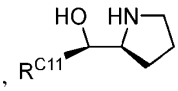
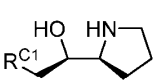
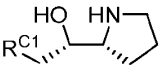
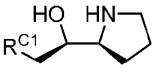
[0334] Once synthesized, provided oligonucleotides and compositions are typically further purified. Suitable purification technologies are widely known and practiced by those skilled in the art, including but not limited to those described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/223056, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the purification technologies of each of which are independently incorporated herein by reference.

[0335] In some embodiments, a cycle comprises or consists of coupling, capping, modification and deblocking. In some embodiments, a cycle comprises or consists of coupling, capping, modification, capping and deblocking. These steps are typically performed in the order they are listed, but in some embodiments, as appreciated by those skilled in the art, the order of certain steps, e.g., capping and modification, may be altered. If desired, one or more steps may be repeated to improve conversion, yield and/or purity as those skilled in the art often perform in syntheses. For example, in some embodiments, coupling may be repeated; in some embodiments, modification (e.g., oxidation to install =O, sulfurization to install =S, etc.) may be repeated; in some embodiments, coupling is repeated after modification which can convert a P(III) linkage to a P(V) linkage which can be more stable under certain circumstances, and coupling is routinely followed by modification to convert newly formed P(III) linkages to P(V) linkages. In some embodiments, when steps are repeated, different conditions may be employed (e.g., concentration, temperature, reagent, time, etc.).

[0336] Technologies for formulating provided oligonucleotides and/or preparing pharmaceutical compositions, e.g., for administration to subjects via various routes, are readily available in the art and can be utilized in accordance with the present disclosure, e.g., those described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/223056, or WO 2018/237194 and references cited therein.

[0337] Technologies for formulating provided oligonucleotides and/or preparing pharmaceutical

compositions, e.g., for administration to subjects via various routes, are readily available in the art and can be utilized in accordance with the present disclosure, e.g., those described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/223056, or WO 2018/237194 and references cited therein.

[0338] In some embodiments, a useful chiral auxiliary has the structure of , , , or , or a salt thereof, wherein R^{C11} is $-L^{C1}-R^{C1}$, L^{C1} is optionally substituted $-CH_2-$, R^{C1} is R, $-Si(R)_3$, $-SO_2R$ or an electron-withdrawing group, and R^{C2} and R^{C3} are taken together with their intervening atoms to form an optionally substituted 3-10 membered saturated ring having, in addition to the nitrogen atom, 0-2 heteroatoms. In some embodiments, a useful chiral auxiliary has the structure of  or , wherein R^{C1} is R, $-Si(R)_3$ or $-SO_2R$, and R^{C2} and R^{C3} are taken together with their intervening atoms to form an optionally substituted 3-7 membered saturated ring having, in addition to the nitrogen atom, 0-2 heteroatoms. is a formed ring is an optionally substituted 5-membered ring. In some embodiments, a useful chiral auxiliary has the structure of , ,  or , or a salt thereof. In some embodiments, a useful chiral auxiliary has the structure of  or . In some embodiments, a useful chiral auxiliary is a DPSE chiral auxiliary. In some embodiments, purity or stereochemical purity of a chiral auxiliary is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%. In some embodiments, it is at least 85%. In some embodiments, it is at least 90%. In some embodiments, it is at least 95%. In some embodiments, it is at least 96%. In some embodiments, it is at least 97%. In some embodiments, it is at least 98%. In some embodiments, it is at least 99%.

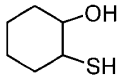
[0339] In some embodiments, L^{C1} is $-CH_2-$. In some embodiments, L^{C1} is substituted $-CH_2-$. In some embodiments, L^{C1} is mono-substituted $-CH_2-$.

[0340] In some embodiments, R^{C1} is R. In some embodiments, R^{C1} is optionally substituted phenyl. In some embodiments, R^{C1} is $-SiR_3$. In some embodiments, R^{C1} is $-SiPh_2Me$. In some embodiments, R^{C1} is $-SO_2R$. In some embodiments, R is not hydrogen. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is phenyl. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some

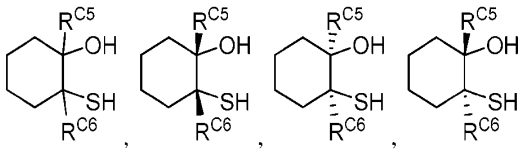
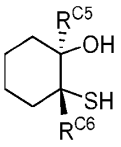
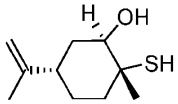
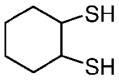
embodiments, R is C_{1-6} alkyl. In some embodiments, R is methyl. In some embodiments, R is t-butyl.

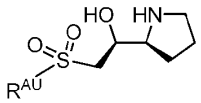
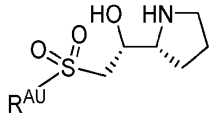
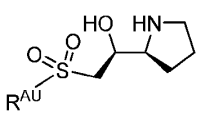
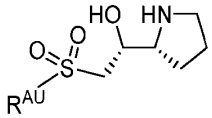
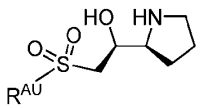
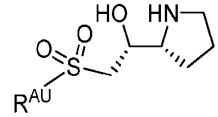
[0341] In some embodiments, R^{C1} is an electron-withdrawing group, such as –C(O)R, $-OP(O)(OR)_2$, $-OP(O)(R)_2$, $-P(O)(R)_2$, $-S(O)R$, $-S(O)_2R$, etc. In some embodiments, chiral auxiliaries comprising electron-withdrawing group R^{C1} groups are particularly useful for preparing chirally controlled non-negatively charged internucleotidic linkages and/or chirally controlled internucleotidic linkages bonded to natural RNA sugar.

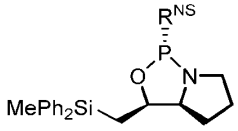
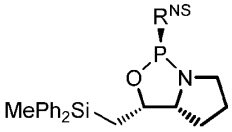
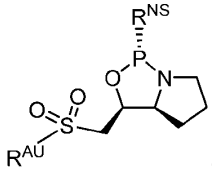
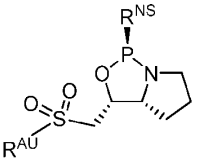
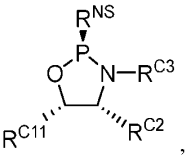
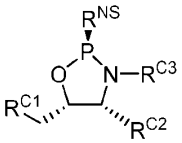
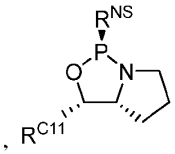
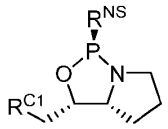
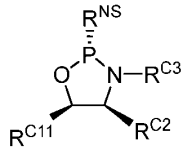
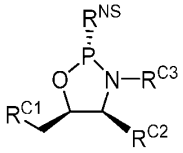
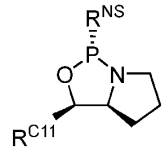
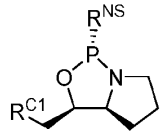
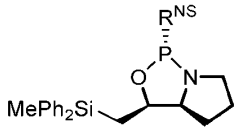
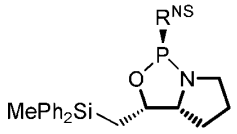
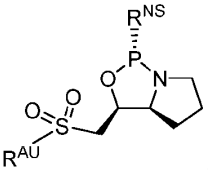
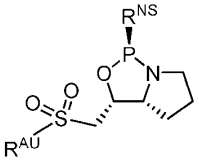
[0342] In some embodiments, R^{C2} and R^{C3} are taken together with their intervening atoms to form an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) membered saturated ring having no heteroatoms in addition to the nitrogen atom. In some embodiments, R^{C2} and R^{C3} are taken together with their intervening atoms to form an optionally substituted 5-membered saturated ring having no heteroatoms in addition to the nitrogen atom.

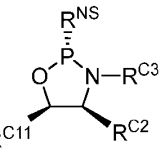
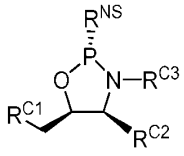
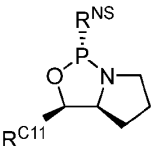
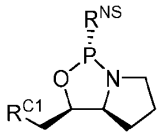
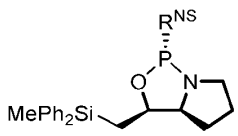
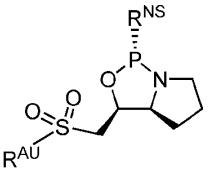
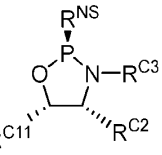
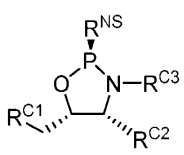
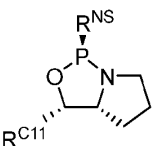
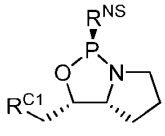
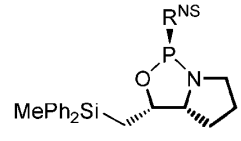
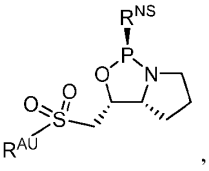
[0343] In some embodiments, a compound has the structure of $H–X^C–C(R^{C5})_2–C(R^{C6})_2–SH$ or a salt thereof, wherein X^C is O or S, and each of R^{C5} and R^{C6} is independently R as described herein. In some embodiments, such a compound is useful for preparing a monomer. In some embodiments, such a compound is useful as a chiral auxiliary. In some embodiments, such a compound is particularly useful for preparing monomer which when utilized in oligonucleotide synthesis form bonds between their nitrogen atoms with linkage phosphorus (e.g., monomers comprising sm01, sm18, etc.). In some embodiments, X^C is O. In some embodiments, X^C is S. In some embodiments, one R^{C5} is –H. In some embodiments, one R^{C6} is –H. In some embodiments, a compound has the structure of $H–X^C–CHR^{C5}–CHR^{C6}–SH$ or a salt thereof. In some embodiments, R^{C5} is optionally substituted C_{1-6} aliphatic. In some embodiments, R^{C5} is optionally substituted C_{1-6} alkyl. In some embodiments, R^{C5} is methyl. In some embodiments, R^{C6} is optionally substituted C_{1-6} aliphatic. In some embodiments, R^{C6} is optionally substituted C_{1-6} alkyl. In some embodiments, R^{C6} is methyl. In some embodiments, a compound is $HOCH(CH_3)CH(CH_3)SH$. In some embodiments, a compound is $HSCH(CH_3)CH(CH_3)SH$. In some embodiments, one R^{C5} is not hydrogen. In some embodiments, one R^{C6} is not hydrogen. In some embodiments, one R^{C5} and one R^{C6} are taken together with their intervening atoms to form an optionally substituted 3-20 (e.g., 3-15, 3-10, 5-10, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20) membered monocyclic, bicyclic or polycyclic ring having 0-5 heteroatoms. In some embodiments, a formed ring is monocyclic. In some embodiments, one R^{C5} and one R^{C6} are taken together with their intervening atoms to form an optionally substituted 4-8, 4-7, 5-8, 5-7, 4, 5, 6, 7, or 8-membered monocyclic ring. In some embodiments, a formed ring is a saturated cycloalkyl ring. In some embodiments, a formed ring is a cyclohexyl ring. In some embodiments, a formed ring is bicyclic. In some embodiments, a formed ring contain no heteroatom ring atoms. In some embodiments, each monocyclic ring unit is independently 3-10 membered, and/or is independently saturated, partially unsaturated or aromatic and has 0-5 heteroatoms. In some embodiments, a compound is 

or a salt thereof, wherein the cyclohexyl ring is optionally

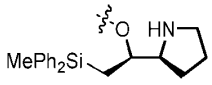
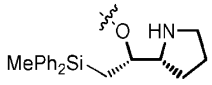
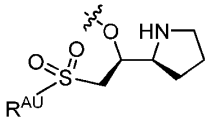
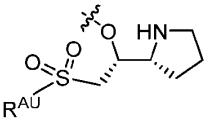
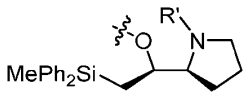
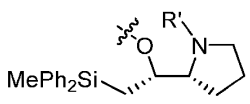
substituted. In some embodiments, a compound is , or , or a salt thereof, wherein the cyclohexyl ring is optionally substituted. In some embodiments, a substituent is C_{1-6} aliphatic, e.g., $-C(CH_3)=CH_2$. For example, in some embodiments, a compound is . In some embodiments, a compound is  or a salt thereof, wherein the cyclohexyl ring is optionally substituted.

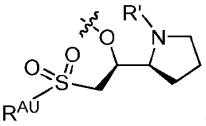
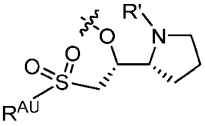
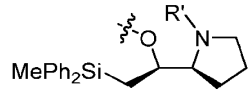
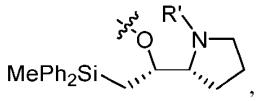
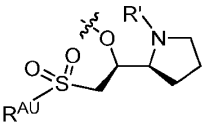
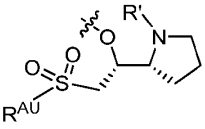
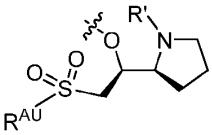
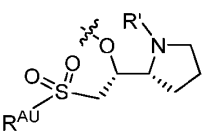
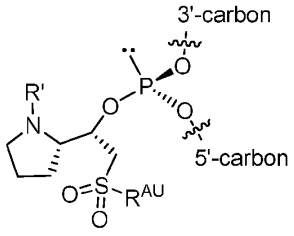
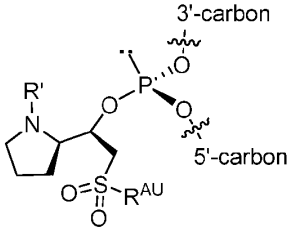
[0344] In some embodiments, methods for preparing oligonucleotides and/or compositions comprise using a chiral auxiliary described herein, e.g., for constructing one or more chirally controlled internucleotidic linkages. In some embodiments, one or more chirally controlled internucleotidic linkages are independently constructed using a DPSE chiral auxiliary. In some embodiments, each chirally controlled phosphorothioate internucleotidic linkage is independently constructed using a DPSE chiral auxiliary. In some embodiments, one or more chirally controlled internucleotidic linkages are independently constructed using  or , or a salt thereof, wherein R^{AU} is as described herein. In some embodiments, each chirally controlled non-negatively charged internucleotidic linkage (e.g., n001) is independently constructed using  or , or a salt thereof. In some embodiments, each chirally controlled internucleotidic linkage is independently constructed using  or , or a salt thereof. In some embodiments, R^{AU} is optionally substituted C_{1-20}, C_{1-10}, C_{1-6}, C_{1-5}, or C_{1-4} aliphatic. In some embodiments, R^{AU} is optionally substituted C_{1-20}, C_{1-10}, C_{1-6}, C_{1-5}, or C_{1-4} alkyl. In some embodiments, R^{AU} is optionally substituted aryl. In some embodiments, R^{AU} is phenyl. In some embodiments, one or more chirally controlled internucleotidic linkages are constructed using a PSM chiral auxiliary. In some embodiments, each chirally controlled non-negatively charged internucleotidic linkage (e.g., n001) is independently constructed using a PSM chiral auxiliary. In some

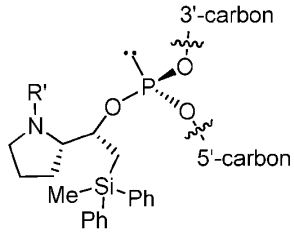
embodiments, each chirally controlled internucleotidic linkages is independently constructed using a PSM chiral auxiliary. As appreciated by those skilled in the art, a chiral auxiliary is often utilized in a phosphoramidite (e.g.,  or  (DPSE phosphoramidites),  or  (wherein R^{AU} is independently as described herein; when R^{AU} is –Ph, PSM phosphoramidites), wherein R^{NS} is an optionally substituted/protected nucleoside (e.g., optionally protected for oligonucleotide synthesis), or a salt thereof, etc.) for oligonucleotide preparation. In some embodiments, a phosphoramidite is a compound having the structure of , , , , , , , , , , , , or salt thereof, wherein each variable is independently as described herein. In some embodiments, R^{AU} is optionally substituted phenyl. In some embodiments, R^{AU} is phenyl. In some embodiments, R^{NS} is an optionally substituted or protected nucleoside comprising hypoxanthine. In some embodiments, R^{NS} comprises optionally substituted or protected hypoxanthine. In some embodiments, R^{NS} is optionally substituted or protected inosine. In some embodiments, R^{NS} is optionally substituted or protected deoxyinosine. In some embodiments, R^{NS} is optionally substituted or protected 2'-F inosine (2'-OH replaced with 2'-F). In some embodiments, R^{NS} is optionally substituted or protected 2'-OR modified inosine (2'-OH replaced with a 2'-OR modification as described herein (e.g., 2'-OMe, 2'-MOE, etc.)). In some embodiments, hypoxanthine is O^6 protected. In some embodiments, hypoxanthine is O^6 protected with $-L-Si(R)_3$, wherein L is optionally substituted $-CH_2-CH_2-$, and each R is independently as described herein and not –H. In some embodiments, each R is independently an optionally substituted group selected from C_{1-6} aliphatic and phenyl. In some embodiments, each R is

independently optionally substituted C_{1-6} alkyl. In some embodiments, $-L-Si(R)_3$ is $-CH_2CH_2Si(Me)_3$. In some embodiments, compounds comprising O^6 protected hypoxanthine (e.g., with $-CH_2CH_2Si(Me)_3$) have higher solubility than corresponding O^6 unprotected compounds and may provide various benefits and advantages when utilized for oligonucleotide synthesis in accordance with the present disclosure. In some embodiments, in a compound having the structure of , , , , , or , or salt thereof, R^{NS} comprises an O^6 protected hypoxanthine (e.g., with $-CH_2CH_2Si(Me)_3$). In some embodiments, R^{NS} is O^6-protected inosine. In some embodiments, R^{NS} is O^6-protected deoxyinosine. In some embodiments, R^{NS} is O^6-protected 2'-F inosine. In some embodiments, R^{NS} is O^6-protected 2'-OR modified inosine whose 2'-OR modification is as described herein (e.g., 2'-OMe, 2'-MOE, etc.). Among other things, the present disclosure encompasses the recognition that such a compound has sufficient solubility for oligonucleotide synthesis and can be utilized in oligonucleotide synthesis while a corresponding compound without O^6 protection may not have sufficient solubility for efficient oligonucleotide synthesis. In some embodiments, a phosphoramidite is (1S,3S,3aS)-1-(((2R,3S,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(6-(2-(trimethylsilyl)ethoxy)-9H-purin-9-yl)tetrahydrofuran-3-yl)oxy)-3-((methyldiphenylsilyl)methyl)tetrahydro-1H,3H-pyrrolo[1,2-c][1,3,2]oxazaphosphole. In some embodiments, a phosphoramidite is (1S,3S,3aS)-1-(((2R,3S,5R)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(6-(2-(trimethylsilyl)ethoxy)-9H-purin-9-yl)tetrahydrofuran-3-yl)oxy)-3-((phenylsulfonyl)methyl)tetrahydro-1H,3H-pyrrolo[1,2-c][1,3,2]oxazaphosphole. In some embodiments, in a compound having the structure of , , , , , or , or salt thereof, R^{NS} comprises an O^6 unprotected hypoxanthine. In some embodiments, R^{NS} is optionally substituted or protected inosine wherein the hypoxanthine is unprotected. In some embodiments, R^{NS} is optionally substituted or protected deoxyinosine wherein the hypoxanthine is unprotected. In some embodiments, R^{NS} is optionally substituted

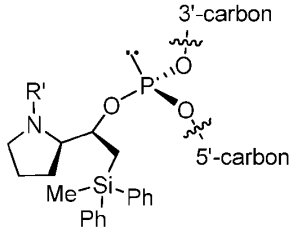
or protected 2'-F inosine wherein the hypoxanthine is unprotected. In some embodiments, R^{NS} is optionally substituted or protected 2'-OR modified inosine wherein the hypoxanthine is unprotected and whose 2'-OR modification is as described herein (e.g., 2'-OMe, 2'-MOE, etc.). Among other things, the present disclosure encompasses the recognition that such a compound has sufficient solubility for oligonucleotide synthesis and can be utilized in oligonucleotide synthesis without O^6 protection.

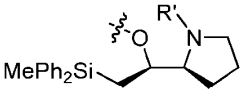
[0345] In some embodiments, a method comprises providing a DPSE and/or a PSM phosphoramidite or a salt thereof. In some embodiments, a provided method comprises contacting a DPSE and/or a PSM phosphoramidite or a salt thereof with –OH (e.g., 5'–OH of a nucleoside or an oligonucleotide chain). As those skilled in the art appreciate, contacting can be performed under various suitable conditions so that a phosphorus linkage is formed. In some embodiments, preparation of each chirally controlled internucleotidic linkage independently comprises contacting a DPSE or PSM phosphoramidite or a salt thereof with –OH (e.g., 5'–OH of a nucleoside or an oligonucleotide chain). In some embodiments, preparation of each chirally controlled phosphorothioate internucleotidic linkage independently comprises contacting a DPSE phosphoramidite or a salt thereof with –OH (e.g., 5'–OH of a nucleoside or an oligonucleotide chain). In some embodiments, preparation of each chirally controlled non-negatively charged internucleotidic linkage (e.g., n001) independently comprises contacting a PSM phosphoramidite or a salt thereof with –OH (e.g., 5'–OH of a nucleoside or an oligonucleotide chain). In some embodiments, preparation of each chirally controlled internucleotidic linkage independently comprises contacting a PSM phosphoramidite or a salt thereof with –OH (e.g., 5'–OH of a nucleoside or an oligonucleotide chain). In some embodiments, contacting forms a P(III) linkage comprising a phosphorus atom bonded to two sugars and a chiral auxiliary moiety (e.g.,  or , or a salt form thereof (e.g., from DPSE phosphoramidites or salts thereof),  or , or a salt form thereof (wherein R^{AU} is independently as described herein; when R^{AU} is –Ph, e.g., from PSM phosphoramidites or salts thereof), etc.). In some embodiments, an oligonucleotide comprises a P(III) linkage comprising a chiral auxiliary moiety, e.g., from a DPSE or PSM phosphoramidite. In some embodiments, a P(III) linkage comprising a chiral auxiliary moiety is chirally controlled. In some embodiments, a chiral auxiliary moiety may be protected, e.g., before converting a P(III) linkage to a P(V) linkage (e.g., before sulfurization, reacting with azide, etc.). In some embodiments, a protected chiral auxiliary has the structure of  or , or a salt form thereof (e.g., wherein R' is independently as described herein; e.g.,

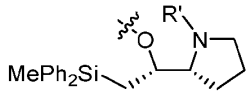
from DPSE phosphoramidites or salts thereof), or  or , or a salt form thereof (wherein each R' and R^{AU} is independently as described herein; when R^{AU} is –Ph, e.g., from PSM phosphoramidites or salts thereof), wherein each R' is independently as described herein. In some embodiments, R' is –C(O)R, wherein R is as described herein. In some embodiments, R is $–CH_3$. In some embodiments, an oligonucleotide comprises a protected chiral auxiliary. In some embodiments, each chirally controlled internucleotidic linkage in an oligonucleotide independently comprises  or , or a salt form thereof, or  or , or a salt form thereof. In some embodiments, each chirally controlled internucleotidic linkage in an oligonucleotide independently comprises  or , or a salt form thereof. In some embodiments, R' is –C(O)R. In some embodiments, R' is $–C(O)CH_3$. In some embodiments, R^{AU} is Ph. In some embodiments, an oligonucleotide comprises one or more  or a salt form thereof (PIII-1), wherein each variable independently as described herein. In some embodiments, an oligonucleotide comprises one or more  or a salt form thereof (PIII-2) , wherein each variable independently as described herein. In some embodiments, an oligonucleotide comprises one or

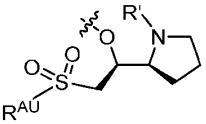


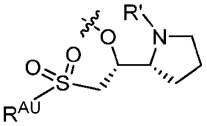
more or a salt form thereof (PIII-5) , wherein each variable independently as described herein. In some embodiments, an oligonucleotide comprises one or more

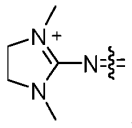


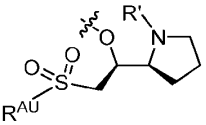
or a salt form thereof (PIII-6) , wherein each variable independently as described herein. In some embodiments, a 5'-end internucleotidic linkage is PIII-1, PIII-2, PIII-5, or PIII-6. In some embodiments, a 5'-end internucleotidic linkage is PIII-1 or PIII-2. In some embodiments, R' is –H. In some embodiments, R' is –C(O)R. In some embodiments, R' is –C(O)CH_3. In some embodiments, R^{AU} is –Ph. In some embodiments, a P(III) linkage is converted into a P(V) linkage. In some embodiments, a P(V) linkage comprises a phosphorus atom bonded to two sugars, a chiral auxiliary moiety (e.g., 

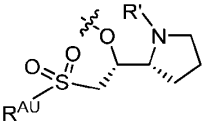
or 

, or a salt form thereof (wherein R' is as described herein; e.g., from DPSE phosphoramidites or salts thereof), 

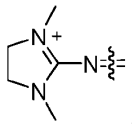
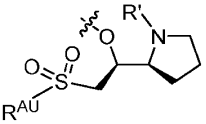
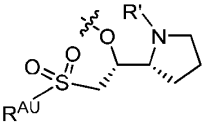
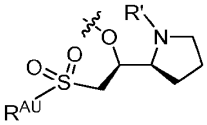
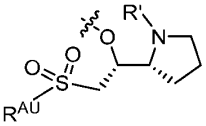
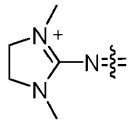
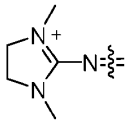
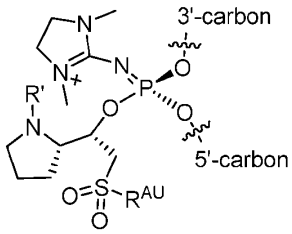
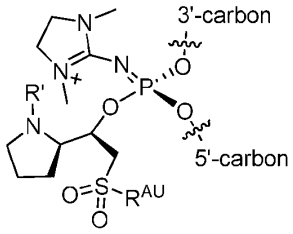
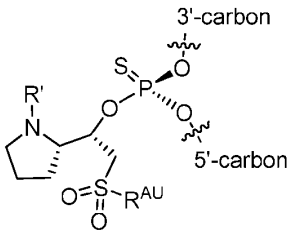
or 

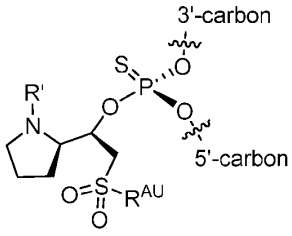
, or a salt form thereof (wherein each of R' and R^{AU} is independently as described herein; when R^{AU} is –Ph, e.g., from PSM phosphoramidites or salts thereof), etc.), and S or 

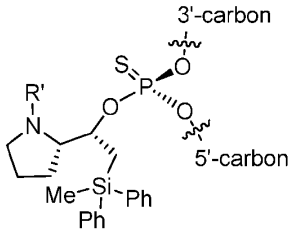
. In some embodiments, a P(V) linkage comprises a phosphorus atom bonded to two sugars, 

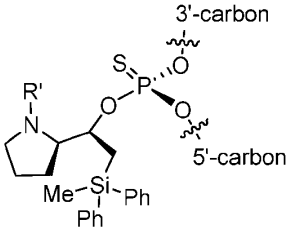
or 

, or a salt form thereof (wherein each R' and R^{AU} is independently as described herein; when R^{AU} is –Ph, e.g., from PSM

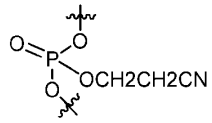
phosphoramidites or salts thereof), etc.), and S or . In some embodiments, a P(V) linkage comprises a phosphorus atom bonded to two sugars,  or , or a salt form thereof (wherein each R' and R^{AU} is independently as described herein; when R^{AU} is –Ph, e.g., from PSM phosphoramidites or salts thereof), etc.), and S. In some embodiments, a P(V) linkage comprises a phosphorus atom bonded to two sugars,  or , or a salt form thereof (wherein each R' and R^{AU} is independently as described herein; when R^{AU} is –Ph, e.g., from PSM phosphoramidites or salts thereof), etc.), and . Those skilled in the art will appreciate that  can exist with a counterion, e.g., in some embodiments, PF_6^-. In some embodiments, an oligonucleotide comprises one or more  or a salt form thereof (PV-1) , wherein each variable independently as described herein. In some embodiments, an oligonucleotide comprises one or more  or a salt form thereof (PV-2) , wherein each variable independently as described herein. In some embodiments, an oligonucleotide comprises one or more  or a

salt form thereof (PV-3) , wherein each variable independently as described herein. In some embodiments, an oligonucleotide comprises one or more 

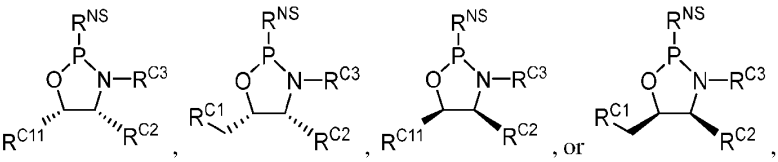
or a salt form thereof (PV-4) , wherein each variable independently as described herein. In some embodiments, an oligonucleotide comprises one or more 

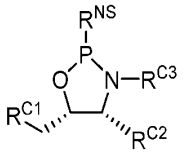
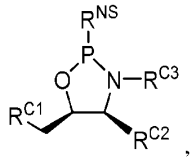
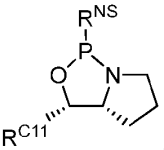
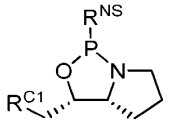
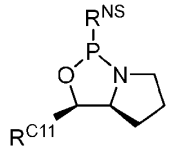
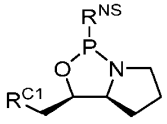
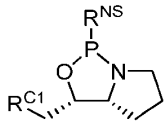
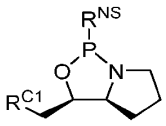
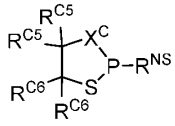
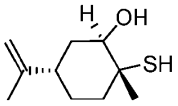
or a salt form thereof (PV-5) , wherein each variable independently as described herein. In some embodiments, an oligonucleotide comprises one or more 

or a salt form thereof (PV-6) , wherein each variable independently as described herein. In some embodiments, each chiral internucleotidic linkage, or each chirally controlled internucleotidic linkage, of an oligonucleotide is independently selected from PIII-1, PIII-2, PIII-5, PIII-6, PV-1, PV-2, PV-3, PV-4, PV-5, and PV-6. In some embodiments, each chiral internucleotidic linkage, or each chirally controlled internucleotidic linkage, of an oligonucleotide is independently selected from PIII-1, PIII-2, PV-1, PV-2, PV-3, and PV-4. In some embodiments, a linkage of PIII-1, PIII-2, PIII-5, or PIII-6 is typically the 5'-end internucleotidic linkage. In some embodiments, each chiral internucleotidic linkage, or each chirally controlled internucleotidic linkage, of an oligonucleotide is independently selected from PV-1, PV-2, PV-3, PV-4, PV-5, and PV-6. In some embodiments, each chiral internucleotidic linkage, or each chirally controlled internucleotidic linkage, of an oligonucleotide is independently selected from PV-1, PV-2, PV-3, or PV-4. In some embodiments, a provided oligonucleotide is an oligonucleotide as described herein, e.g., of Table 1, wherein each *S is independently replaced with PV-3 or PV-5, each *R is independently replaced with PV-4 or PV-6, each n001R is independently replaced with PV-1, and each n001S is independently replaced with PV-2. In some embodiments, a provided oligonucleotide is an oligonucleotide as described herein, e.g., of Table 1, wherein each *S is independently replaced with PV-3, each *R is independently replaced with PV-4, each n001R is independently replaced with PV-1, and each n001S is independently replaced with PV-2. In some

embodiments, each natural phosphate linkage is independently replaced with a precursor, e.g., . In some embodiments, R' is –H. In some embodiments, R' is –C(O)R. In some embodiments, R' is $-C(O)CH_3$. In some embodiments, R^{AU} is –Ph. In some embodiments, a method comprises removal of one or more chiral auxiliary moieties so that phosphorothioate and/or non-negatively charged internucleotidic linkages (e.g., n001) are formed (e.g., from V-1, PV-2, PV-3, PV-4, PV-5, PV-6, etc.). In some embodiments, removal of a chiral auxiliary (e.g., PSM) comprises contacting an oligonucleotide with a base (e.g., $N(R)_3$ such as DEA) under anhydrous conditions.

[0346] In some embodiments, as appreciated by those skilled in the art, for preparation of a chirally controlled internucleotidic linkage, a monomer or a phosphoramidite (e.g., a DPSE or PSM phosphoramidite) is typically utilized in a chirally enriched or pure form (e.g., of a purity as described herein (e.g., about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, or about 100%)).

[0347] In some embodiments, the present disclosure provides useful reagents for preparation of oligonucleotides and compositions thereof. In some embodiments, monomers and phosphoramidites comprise nucleosides, nucleobases and sugars as described herein. In some embodiments, nucleobases and sugars are properly protected for oligonucleotide synthesis as those skilled in the art will appreciate. In some embodiments, a phosphoramidite has the structure of R^{NS}–$P(OR)N(R)_2$, wherein R^{NS} is a optionally protected nucleoside moiety. In some embodiments, a phosphoramidite has the structure of R^{NS}–$P(OCH_2CH_2CN)N(i\text{-}Pr)_2$. In some embodiments, a monomer comprises a nucleobase which is or comprises Ring BA, wherein Ring BA has the structure of BA-I, BA-I-a, BA-I-b, BA-I-c, BA-I-d, BA-II, BA-II-a, BA-II-b, BA-II-c, BA-II-d, BA-III, BA-III-a, BA-III-b, BA-III-c, BA-III-d, BA-III-e, BA-IV, BA-IV-a, BA-IV-b, BA-V, BA-V-a, BA-V-b, or BA-VI, or a tautomer of Ring BA, wherein the nucleobase is optionally substituted or protected. In some embodiments, a phosphoramidite comprises a nucleobase which is or comprises Ring BA, wherein Ring BA has the structure of BA-I, BA-I-a, BA-I-b, BA-I-c, BA-I-d, BA-II, BA-II-a, BA-II-b, BA-II-c, BA-II-d, BA-III, BA-III-a, BA-III-b, BA-III-c, BA-III-d, BA-III-e, BA-IV, BA-IV-a, BA-IV-b, BA-V, BA-V-a, BA-V-b, or BA-VI, or a tautomer of Ring BA, wherein the nucleobase is optionally substituted or protected. In some embodiments, a phosphoramidite comprises a chiral auxiliary moiety, wherein the phosphorus is bonded to an oxygen and a nitrogen atom of the chiral auxiliary moiety. In some embodiments, a phosphoramidite has the structure of , or a salt thereof, wherein R^{NS} is a protected nucleoside moiety (e.g., 5'-OH and/or nucleobases suitably protected for oligonucleotide synthesis), and each other variable is independently as described herein. In some

embodiments, a phosphoramidite has the structure of  or , wherein R^{NS} is a protected nucleoside moiety (e.g., 5'-OH and/or nucleobases suitably protected for oligonucleotide synthesis), R^{C1} is R, $-Si(R)_3$ or $-SO_2R$, and R^{C2} and R^{C3} are taken together with their intervening atoms to form an optionally substituted 3-7 membered saturated ring having, in addition to the nitrogen atom, 0-2 heteroatoms, wherein the coupling forms an internucleotidic linkage. In some embodiments, 5'-OH of R^{NS} is protected. In some embodiments, 5'-OH of R^{NS} is protected as –ODMTr. In some embodiments, R^{NS} is bonded to phosphorus through its 3'-O-. In some embodiments, a formed ring by R^{C2} and R^{C3} is an optionally substituted 5-membered ring. In some embodiments, a phosphoramidite has the structure of , ,  or , or a salt thereof. In some embodiments, a phosphoramidite has the structure of  or . In some embodiments, as described herein R^{NS} comprises a modified nucleobase (e.g., b001A, b002A, b003A, b008U, b001C, etc.) which is optionally protected for oligonucleotide synthesis. In some embodiments, a monomer has the structure of  or a salt thereof, wherein R^{NS} is an optionally substituted/protected nucleoside (e.g., optionally protected for oligonucleotide synthesis) as described herein, and each other variable is independently as described herein. In some embodiments, $-X^C-C(R^{C5})_2-C(R^{C6})_2-S-$ is of such a structure that $H-X^C-C(R^{C5})_2-C(R^{C6})_2-SH$ is a compound as described herein, e.g., $HOCH(CH_3)CH(CH_3)SH$, $HSCH(CH_3)CH(CH_3)SH$, , etc. In some embodiments, 5'-OH of R^{NS} is protected. In some embodiments, 5'-OH of R^{NS} is protected as –ODMTr.

[0348] In some embodiments, purity or stereochemical purity of a monomer or a phosphoramidite is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%. In some embodiments, it is at least 85%. In some embodiments,

it is at least 90%. In some embodiments, it is at least 95%.

[0349] In some embodiments, the present disclosure provides a method for preparing an oligonucleotide or composition, comprising coupling a free –OH, e.g., a free 5'-OH, of an oligonucleotide or a nucleoside with a monomer as described herein. In some embodiments, the present disclosure provides a method for preparing an oligonucleotide or composition, comprising coupling a free –OH, e.g., a free 5'-OH, of an oligonucleotide or a nucleoside with a phosphoramidite as described herein.

[0350] In some embodiments, the present disclosure provides an oligonucleotide, wherein the oligonucleotide comprises one or more modified internucleotidic linkages each independently having the structure of $-O^5-P^L(W)(R^{CA})-O^3-$, wherein:

P^L is P, or P(=W);

W is O, S, or W^N;

W^N is $=N-C(-N(R^1)_2=N^+(R^1)_2Q^-$;

Q^- is an anion;

R^{CA} is or comprises an optionally capped chiral auxiliary moiety,

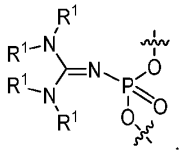
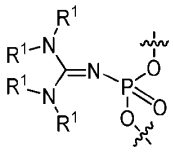
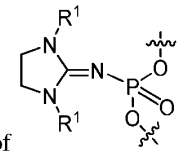
O^5 is an oxygen bonded to a 5'-carbon of a sugar, and

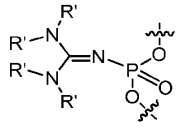
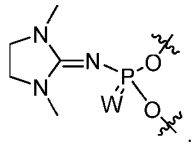
O^3 is an oxygen bonded to a 3'-carbon of a sugar.

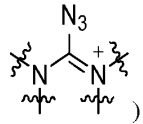
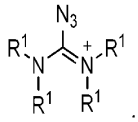
[0351] In some embodiments, a modified internucleotidic linkage is optionally chirally controlled. In some embodiments, a modified internucleotidic linkage is optionally chirally controlled.

[0352] In some embodiments, a provided methods comprising removing R^{CA} from such a modified internucleotidic linkages. In some embodiments, after removal, bonding to R^{CA} is replaced with –OH. In some embodiments, after removal, bonding to R^{CA} is replaced with =O, and bonding to W^N is replaced with $-N=C(N(R^1)_2)_2$.

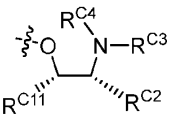
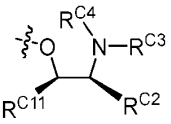
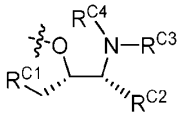
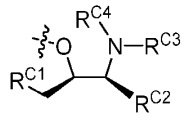
[0353] In some embodiments, P^L is P=S, and when R^{CA} is removed, such an internucleotidic linkage is converted into a phosphorothioate internucleotidic linkage.

[0354] In some embodiments, P^L is $P=W^N$, and when R^{CA} is removed, such an internucleotidic linkage is converted into an internucleotidic linkage having the structure of . In some embodiments, an internucleotidic linkage having the structure of  has the structure of . In some

embodiments, an internucleotidic linkage having the structure of  has the structure of .

[0355] In some embodiments, P^L is P (e.g., in newly formed internucleotidic linkage from coupling of a phosphoramidite with a 5'-OH). In some embodiments, W is O or S. In some embodiments, W is S (e.g., after sulfurization). In some embodiments, W is O (e.g., after oxidation). In some embodiments, certain non-negatively charged internucleotidic linkages or neutral internucleotidic linkages may be prepared by reacting a P(III) phosphite triester internucleotidic linkage with azido imidazolinium salts (*e.g.*, compounds comprising ) under suitable conditions. In some embodiments, an azido imidazolinium salt is a salt of PF_6^-. In some embodiments, an azido imidazolinium salt is a salt of . In some embodiments, an azido imidazolinium salt is 2-azido-1,3-dimethylimidazolinium hexafluorophosphate.

[0356] As appreciated by those skilled in the art, Q^- can be various suitable anion present in a system (e.g., in oligonucleotide synthesis), and may vary during oligonucleotide preparation processes depending on cycles, process stages, reagents, solvents, etc. In some embodiments, Q^- is PF_6^-.

[0357] In some embodiments, R^{CA} is  or , wherein R^{C4} is –H or –C(O)R', and each other variable is independently as described herein. In some embodiments, R^{CA} is  or , wherein R^{C1} is R, $-Si(R)_3$ or $-SO_2R$, R^{C2} and R^{C3} are taken together with their intervening atoms to form an optionally substituted 3-7 membered saturated ring having, in addition to the nitrogen atom, 0-2 heteroatoms, R^{C4} is –H or –C(O)R'. In some embodiments, R^{C4} is –H. In some embodiments, R^{C4} is $-C(O)CH_3$. In some embodiments, R^{C2} and R^{C3} are taken together to form an optionally substituted 5-

membered ring.

[0358] In some embodiments, R^{C4} is –H (e.g., in n newly formed internucleotidic linkage from coupling of a phosphoramidite with a 5'-OH). In some embodiments, R^{C4} is –C(O)R (e.g., after capping of the amine). In some embodiments, R is methyl.

[0359] In some embodiments, each chirally controlled phosphorothioate internucleotidic linkage is independently converted from $-O^5-P^L(W)(R^{CA})-O^3-$.

[0360] Among other things, the present disclosure provides oligonucleotides with high purity. In some embodiments, diastereomeric purity of each chiral linkage phosphorus is independently about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. In some embodiments, it is independently at least about 95%. In some embodiments, it is independently at least about 97%. In some embodiments, diastereomeric purity of an oligonucleotide is about or at least about least $(DS)^{nc}$, wherein DS is about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% or more, and nc is the number of chirally controlled internucleotidic linkages in the oligonucleotide (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 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).

[0361] In some embodiments, DS is about or at least about 95%. In some embodiments, DS is about or at least about 97%. In some embodiments, DS is about or at least about 98%. In some embodiments, DS is about or at least about 99%. In some embodiments, nc is about 5-20. In some embodiments, nc is about or at least about 10. In some embodiments, nc is about 10-20. In some embodiments, nc is about or at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20.

[0362] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising an oligonucleotide, wherein the composition is enriched, relative to a substantially racemic preparation of the oligonucleotide, for the oligonucleotide.

[0363] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides, wherein oligonucleotides of the plurality share the same constitution, and about or at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, of all oligonucleotides in the composition that share the same constitution are oligonucleotides of the plurality. In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of all oligonucleotides in the composition that share the same base sequence are oligonucleotides of the plurality. In some embodiments, a percentage is about or at least about $(DS)^{nc}$, wherein DS is about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%, and nc is the number of chirally controlled internucleotidic linkages an oligonucleotide of the plurality (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 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).

[0364] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising an oligonucleotide, wherein at least about or at least about 5%, 10%, 15%, 20%, 25%,

30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, of all oligonucleotides in the composition that share the same constitution are the oligonucleotide. In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of all oligonucleotides in the composition that share the same base sequence as the oligonucleotide are the oligonucleotide. In some embodiments, a percentage is about or at least about $(DS)^{nc}$, wherein DS is about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%, and nc is the number of chirally controlled internucleotidic linkages in an oligonucleotide of the plurality (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 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). In some embodiments, a percentage is about or at least about $(DS)^{nc}$, wherein DS is about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%, and nc is the number of chiral linkage phosphorus in an oligonucleotide of the plurality (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 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).

[0365] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a plurality of oligonucleotides, wherein oligonucleotides of the plurality share:

1) a common base sequence; and

2) the same linkage phosphorus stereochemistry independently at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more chiral internucleotidic linkages;

wherein at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the oligonucleotides in the composition that share the common base sequence are oligonucleotides of the plurality.

[0366] In some embodiments, oligonucleotides of plurality share the same linkage phosphorus stereochemistry independently at each chiral internucleotidic linkage. In some embodiments, a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of all oligonucleotides that share the same base sequence as an oligonucleotide having the structure or a pharmaceutically acceptable salt thereof. In some embodiments, a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of all oligonucleotides that share the same constitution as an oligonucleotide having the structure or a pharmaceutically acceptable salt thereof.

[0367] In some embodiments, a level, purity, percentage, etc. is based on UV peak area at 260 nm.

[0368] In some embodiments, a pharmaceutical composition is a chirally controlled oligonucleotide composition.

Pharmaceutical Compositions

[0369] In some embodiments, the present disclosure provides pharmaceutical compositions comprising a provided compound, e.g., an oligonucleotide, or a pharmaceutically acceptable salt thereof, and a pharmaceutical carrier. In some embodiments, for example, for therapeutic and clinical purposes, oligonucleotides of the present disclosure are provided as pharmaceutical compositions. In some

embodiments, a provided oligonucleotide is administered or delivered as a pharmaceutical composition.

[0370] As appreciated by those skilled in the art, oligonucleotides can be provided in various forms. In some embodiments, oligonucleotides can be in acid forms, e.g., for natural phosphate linkages, in the form of −OP(O)(OH)O−; for phosphorothioate internucleotidic linkages, in the form of −OP(O)(SH)O−; etc. In some embodiments, provided oligonucleotides can be in salt forms, e.g., for natural phosphate linkages, in the form of −OP(O)(ONa)O− in sodium salts; for phosphorothioate internucleotidic linkages, in the form of −OP(O)(SNa)O− in sodium salts; etc. Unless otherwise noted, oligonucleotides of the present disclosure can exist in acid, base and/or salt forms. In some embodiments, a composition comprises one or more forms of an oligonucleotide. In some embodiments, a composition comprises one or more salt forms of an oligonucleotide. In some embodiments, a composition comprises one or more pharmaceutically acceptable salt forms of an oligonucleotide.

[0371] When used as therapeutics, a provided oligonucleotide or composition described herein is typically administered as a pharmaceutical composition. In some embodiments, a pharmaceutical composition comprises a therapeutically effective amount of a provided oligonucleotide, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable inactive ingredient selected from pharmaceutically acceptable diluents, pharmaceutically acceptable excipients, and pharmaceutically acceptable carriers. In some embodiments, in provided compositions, provided oligonucleotides may exist as salts, preferably pharmaceutically acceptable salts, e.g., sodium salts, ammonium salts, etc. In some embodiments, a salt of a provided oligonucleotide comprises two or more cations; for example, in some embodiments, up to the number of negatively charged acidic groups (e.g., phosphate, phosphorothioate, etc.) in an oligonucleotide. As appreciated by those skilled in the art, oligonucleotides described herein may be provided and/or utilized in a salt form, particularly a pharmaceutically acceptable salt form.

[0372] As those skilled in the art will appreciate, oligonucleotides may exist in various salt forms. In some embodiments, a salt is a pharmaceutically acceptable salt. In some embodiments, a pharmaceutical composition comprises an oligonucleotide, optionally in its salt form, and a sodium salt. In some embodiments, a pharmaceutical composition comprises an oligonucleotide, optionally in its salt form, and sodium chloride. In some embodiments, each hydrogen ion of an oligonucleotide that may be donated to a base (e.g., under conditions of an aqueous solution, a pharmaceutical composition, etc.) is replaced by a non-H^+ cation. For example, in some embodiments, a pharmaceutically acceptable salt of an oligonucleotide is an all-metal ion salt, wherein each hydrogen ion (for example, of –OH, –SH, etc.) of each internucleotidic linkage (e.g., a natural phosphate linkage, a phosphorothioate internucleotidic linkage, etc.) is replaced by a metal ion. Various suitable metal salts for pharmaceutical compositions are widely known in the art and can be utilized in accordance with the present disclosure. In some embodiments, a pharmaceutically acceptable salt is a sodium salt. In some embodiments, a pharmaceutically acceptable salt is magnesium salt. In some embodiments, a pharmaceutically acceptable salt is a calcium salt. In some embodiments, a pharmaceutically acceptable salt is a potassium salt. In some embodiments, a pharmaceutically acceptable salt is an ammonium salt (cation

$N(R')_4^+$). In some embodiments, a pharmaceutically acceptable salt comprises one and no more than one types of cation. In some embodiments, a pharmaceutically acceptable salt comprises two or more types of cation. In some embodiments, a cation is Li^+, Na^+, K^+, Mg^{2+} or Ca^{2+}. In some embodiments, a pharmaceutically acceptable salt is an all-sodium salt. In some embodiments, a pharmaceutically acceptable salt is an all-sodium salt, wherein each internucleotidic linkage which is a natural phosphate linkage (acid form –O–P(O)(OH)–O–), if any, exists as its sodium salt form (–O–P(O)(ONa)–O–), and each internucleotidic linkage which is a phosphorothioate internucleotidic linkage (acid form –O–P(O)(SH)–O–), if any, exists as its sodium salt form (–O–P(O)(SNa)–O–).

[0373] In some embodiments, the present disclosure provides a pharmaceutical composition comprising a chirally controlled oligonucleotide, or composition thereof, in admixture with a pharmaceutically acceptable excipient. One of skill in the art will recognize that such pharmaceutical compositions include the pharmaceutically acceptable salts of the chirally controlled oligonucleotide, or composition thereof, described above.

[0374] Provided oligonucleotides, and compositions thereof, are effective over a wide dosage range. For example, in the treatment of adult humans, dosages from about 0.01 to about 1000 mg, from about 0.5 to about 100 mg, from about 1 to about 50 mg per day, and from about 5 to about 100 mg per day are examples of dosages that may be used. Exact dosage will depend upon the route of administration, the form in which the compound is administered, subject characteristics (e.g., body weight, age, body surface area, etc.), conditions, disorders or diseases, and/or preferences and experiences of physicians.

[0375] Various technologies for delivering nucleic acids and/or oligonucleotides are known in the art and can be utilized in accordance with the present disclosure. For example, a variety of supramolecular nanocarriers can be used to deliver nucleic acids. Example nanocarriers include liposomes, cationic polymer complexes and various polymeric compounds. Complexation of nucleic acids with various polycations is another approach for intracellular delivery; this includes use of PEGylated polycations, polyethyleneamine (PEI) complexes, cationic block co-polymers, and dendrimers. Several cationic nanocarriers, including PEI and polyamidoamine dendrimers may help to release contents from endosomes. Other approaches may include use of polymeric nanoparticles, microspheres, liposomes, dendrimers, biodegradable polymers, conjugates, prodrugs, inorganic colloids such as sulfur or iron, antibodies, implants, biodegradable implants, biodegradable microspheres, osmotically controlled implants, lipid nanoparticles, emulsions, oily solutions, aqueous solutions, biodegradable polymers, poly(lactide-coglycolic acid), poly(lactic acid), liquid depot, polymer micelles, quantum dots and lipoplexes. In some embodiments, an oligonucleotide is conjugated to another molecule.

[0376] In some embodiments, oligonucleotides are administered or delivered via gymnotic uptake.

[0377] In some embodiments, the pharmaceutical composition is formulated for intravenous injection, oral administration, buccal administration, inhalation, nasal administration, topical administration, ophthalmic administration, or otic administration. In some embodiments, the pharmaceutical composition is a tablet, a

pill, a capsule, a liquid, an inhalant, a nasal spray solution, a suppository, a suspension, a gel, a colloid, a dispersion, a suspension, a solution, an emulsion, an ointment, a lotion, an eye drop or an ear drop.

[0378] Depending on the specific conditions being treated, such agents may be formulated into liquid or solid dosage forms and administered systemically or locally. The agents may be delivered, for example, in a timed- or sustained- low release form as is known to those skilled in the art. Techniques for formulation and administration may be found in Remington, The Science and Practice of Pharmacy (20th ed. 2000). Suitable routes may include oral, buccal, by inhalation spray, sublingual, rectal, transdermal, vaginal, transmucosal, nasal or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intra-articullar, intra-sternal, intra-synovial, intra-hepatic, intralesional, intracranial, intraperitoneal, intranasal, or intraocular injections or other modes of delivery.

[0379] Compounds, e.g., oligonucleotides, can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the disclosure to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject (*e.g.*, patient) to be treated.

[0380] For nasal or inhalation delivery, the agents of the disclosure may also be formulated by methods known to those of skill in the art, and may include, for example, but not limited to, examples of solubilizing, diluting, or dispersing substances such as, saline, preservatives, such as benzyl alcohol, absorption promoters, and fluorocarbons.

[0381] For injection, the agents of the disclosure may be formulated and diluted in aqueous solutions, such as in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0382] In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of an active compound into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions.

[0383] Pharmaceutical preparations for oral use can be obtained by combining an active compound with solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethyl-cellulose (CMC), and/or polyvinylpyrrolidone (PVP: povidone). If desired, disintegrating agents may be added, such as the cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0384] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions

may be used, which may optionally contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol (PEG), and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dye-stuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[0385] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin, and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, an active compound may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols (PEGs). In addition, stabilizers may be added.

[0386] In some embodiments, any oligonucleotide, or combination thereof, described herein, or any composition comprising an oligonucleotide described herein, can be combined with any pharmaceutical preparation described herein or known in the art.

Characterization and Assessment

[0387] As appreciated by those skilled in the art, various technologies may be utilized to assess/characterize provided technologies in accordance with the present disclosure. Certain useful technologies are described in the Examples; as demonstrated, among other things, the present disclosure describes various *in vivo* and *in vitro* technologies suitable for assessing and characterizing provided technologies. In some embodiments, provided technologies are assessed/characterized, e.g., in cells; additionally or alternatively, in some embodiments, provided technologies are assessed/characterized, e.g., in animals, e.g., mice. Various technologies for assessing properties and/or activities of oligonucleotides can be utilized in accordance with the present disclosure, e.g., US 20170037399, WO 2017/015555, WO 2017/015575, WO 2017/192664, WO 2017/062862, WO 2017/192679, WO 2017/210647, WO 2018/067973, WO 2019/200185, WO 2019/217784, or WO 2020/118246, etc.

[0388] Among other things, the present disclosure provides oligonucleotide designs comprising sugar modifications, base modifications, internucleotidic linkage modifications, linkage phosphorus stereochemistry, and/or patterns thereof, that can greatly improve one or more properties and/or activities of oligonucleotides compared to comparable oligonucleotides of similar or identical base sequences but of reference designs. In some embodiments, a reference design is a design in WO 2017/062862, WO 2018/067973, WO 2019/200185, WO 2019/217784, or WO 2020/118246.

[0389] For example, oligonucleotides can be evaluated for their ability to mediate exon skipping in various assays, including in vitro and in vivo assays, in accordance with the present disclosure. In vitro assays can be performed in various test cells described herein or known in the art, including but not limited to, Δ48-50 patient-derived myoblast cells, Δ51 patient-derived fibroblasts. In vivo tests can be performed in test animals described herein or known in the art, including but not limited to, a mouse, rat, cat, pig, dog, monkey,

or non-human primate.

[0390] As non-limiting examples, a number of assays are described below for assessing properties/activities of oligonucleotides. Various other suitable assays are available and may be utilized to assess oligonucleotide properties/activities, including those of oligonucleotides not designed for exon skipping (e.g., for oligonucleotides that may involve RNase H for reducing levels of target DMD transcripts, assays described in US 20170037399, WO 2017/015555, WO 2017/015575, WO 2017/192664, WO 2017/192679, WO 2017/210647, WO 2018/067973, WO 2019/200185, WO 2019/217784, or WO 2020/118246, etc.).

[0391] An oligonucleotide can be evaluated for its ability to mediate skipping of exon 52 in the Dystrophin RNA, which can be tested, as non-limiting examples, using nested PCR, qRT-PCR, and/or sequencing.

[0392] An oligonucleotide can be evaluated for its ability to mediate protein restoration (e.g., production of an internally truncated Dystrophin protein variant lacking the amino acids corresponding to the codons encoded in the skipped exon, which has improved functions compared to proteins (if any) produced prior to exon skipping), which can be evaluated by a number of methods for protein detection and/or quantification, such as western blot, immunostaining, etc. Antibodies to dystrophin are commercially available or if desired, can be developed for desired purposes.

[0393] An oligonucleotide can be evaluated for its ability to mediate production of a stable restored protein. Stability of restored protein can be tested, in non-limiting examples, in assays for serum and tissue stability. An oligonucleotide can be evaluated for its ability to bind protein, such as albumin. Example related technologies include those described, e.g., in WO 2017/015555, WO 2017/015575, etc.

[0394] An oligonucleotide can be evaluated for immuno activity, e.g., through assays for cytokine activation, complement activation, TLR9 activity, etc. Example related technologies include those described, e.g., in WO 2017/015555, WO 2017/015575, WO 2017/192679, WO 2017/210647, etc.

[0395] In some embodiments, efficacy of a provided oligonucleotide can be tested, e.g., in in silico analysis and prediction, a cell-free extract, a cell transfected with artificial constructs, an animal such as a mouse with a human Dystrophin transgene or portion thereof, normal and dystrophic human myogenic cell lines, and/or clinical trials. It may be desirable to utilize more than one assay, as normal and dystrophic human myogenic cell lines may sometimes produce different efficacy results under certain conditions (Mitrpant et al. 2009 Mol. Ther. 17: 1418).

[0396] In some embodiments, provided oligonucleotides can be tested in vitro in cells. In some embodiments, testing in vitro in cells involves gymnotic delivery of the provided oligonucleotide(s), or delivery using a delivery agent or transfectant, many of which are known in the art and may be utilized in accordance with the present disclosure. In some embodiments, provided oligonucleotides can be tested in vitro in normal human skeletal muscle cells (hSkMCs). See, for example, Arechavala et al. 2007 Hum. Gene Ther. 18: 798-810.

[0397] In some embodiments, provided oligonucleotides can be tested in a muscle explant from a DMD patient. Muscle explants from DMD patients are reported in, for example, Fletcher et al. 2006 J. Gene Med.

8: 207-216; McClorey et al. 2006 Neur. Dis. 16: 583-590; and Arechavala et al. 2007 Hum. Gene Ther. 18: 798-810.

[0398] In some embodiments, cells are or comprise cultured muscle cells from DMD patients. See, for example: Aartsma-Rus et al. 2003 Hum. Mol. Genet. 8: 907-914.

[0399] In some embodiments, an individual oligonucleotide may demonstrate experiment-to-experiment variability in its ability to skip exon 52 under certain circumstances. In some embodiments, an individual oligonucleotide can demonstrate variability in its ability to skip exon 52 depending on which cells are used, the growth conditions, and other experimental factors. To control variations, typically oligonucleotides to be tested and control oligonucleotides are assayed under the same or substantially the same conditions.

[0400] In vitro experiments also include those conducted with patient-derived myoblasts. Certain results from such experiments were described herein. In certain such experiments, cells were cultured in skeletal growth media to keep them in a dividing / immature myoblast state. The media was then changed to 'differentiation' media (containing insulin and 2% horse serum) concurrent with spiking oligonucleotides in the media for dosing. The cells differentiated into myotubes as they were getting dosed for a suitable period of time, e.g., a total of 24 hrs or 4 days for RNA experiments and 6 days for protein experiments.

[0401] In some embodiments, an oligonucleotide is tested in vitro in Δ48-50 patient-derived myoblasts with, e.g., 0, 1, 2, 3, 4 or more days of pre-differentiation. In some embodiments, an oligonucleotide is tested in vitro in Δ51 patient-derived fibroblasts with, e.g., 0, 1, 2, 3, 4 or more days of pre-transdifferentiation.

[0402] In some embodiments, provided oligonucleotides can be tested in any one or more of various animal models, including non-mammalian and mammalian models; including, as non-limiting examples, Caenorhabditis, Drosophila, zebrafish, mouse, rat, cat, dog and pig. See, for example, a review in McGreevey et al. 2015 Dis. Mod. Mech. 8: 195-213.

[0403] Example use of mdx mice is reported in, for example: Lu et al. 2003 Nat. Med. 9: 1009; Jearawiriyapaisarn et al. 2008 Mol. Ther., 16, 1624–1629; Yin et al. 2008 Hum. Mol. Genet., 17, 3909–3918; Wu et al. 2009 Mol. Ther., 17, 864–871; Wu et al. 2008 Proc. Natl Acad. Sci. USA, 105, 14814–14819; Mann et al. 2001 Proc. Nat. Acad. Sci. USA 98: 42-47; and Gebski et al. 2003 Hum. Mol. Gen. 12: 1801–1811.

[0404] Efficacy of provided oligonucleotides can be tested in dogs, such as the Golden Retriever Muscular Dystrophy (GRMD) animal model. Lu et al. 2005 Proc. Natl. Acad. Sci. U S A 102:198-203; Alter et al. 2006 Nat. Med. 12:175-7; McClorey et al. 2006 Gene Ther. 13:1373-81; and Yokota et al. 2012 Nucl. Acid Ther. 22: 306.

[0405] A provided oligonucleotide can be evaluated in vivo in a test animal for efficient delivery to various tissues (e.g., skeletal, heart and/or diaphragm muscle); this can be tested, in non-limiting examples, by hybridization ELISA and tests for distribution in animal tissue.

[0406] A provided oligonucleotide can be evaluated in vivo in a test animal for plasma PK; this can be tested, as non-limiting examples, by assaying for AUC (area under the curve) and half-life.

[0407] In some embodiments, provided oligonucleotides can be tested in vivo, e.g., via intramuscular, intravenous, or subcutaneous administration in an animal. See, e.g., certain examples described herein and Bremmer-Bout et al. 2004 Mol. Ther. 10, 232–240.

[0408] Additional tests which can be performed to evaluate the efficacy of provided oligonucleotides include centrally nucleated fiber counts and dystrophin-positive fiber counts, and functional grip strength analysis. See, as non-limiting examples, experimental protocols reported in: Yin et al. 2009 Hum. Mol. Genet. 18: 4405-4414.

[0409] Additional methods of testing provided oligonucleotides include, as non-limiting example, methods reported in: Kinali et al. 2009 Lancet 8: 918; Bertoni et al. 2003 Hum. Mol. Gen. 12: 1087–1099.

[0410] Those skilled in the art reading the present disclosure will readily appreciate that other technologies, e.g., *in vitro* models (e.g., cell lines) for various conditions, disorders or diseases, animal models for various conditions, disorders or diseases, clinical trials, etc. may be designed and/or utilized to assess provided technologies (e.g., oligonucleotides, compositions, methods, etc.) in accordance with the present disclosure.

Uses and Applications

[0411] As appreciated by those skilled in the art, oligonucleotides are useful for multiple purposes. In some embodiments, provided technologies (e.g., oligonucleotides, compositions, methods, etc.) can be useful for exon skipping in target transcripts and/or modulating levels of products encoded thereby (e.g., proteins). In some embodiments, provided technologies (e.g., oligonucleotides, compositions, methods, etc.) can be useful for various purposes, e.g, those described in US 9695211, US 9605019, US 9598458, US 2013/0178612, US 20150211006, US 20170037399, WO 2017/015555, WO 2017/062862, WO 2017/160741, WO 2017/192664, WO 2017/192679, WO 2017/210647, WO 2018/067973, WO 2019/200185, WO 2019/217784, and/or WO 2020/118246.

[0412] Among other things, the present disclosure discloses and demonstrates various oligonucleotides comprising various modifications, such as linkage modifications, sugar modifications, base modifications, and stereochemistry and patterns thereof and having various base sequences and/or targeting various nucleic acids (e.g., DMD transcripts) can have various useful properties, activities, and/or advantages. In some embodiments, provided technologies can skip an exon in a target transcript. In some embodiments, provided technologies can skip exon 52 in a DMD transcript.

[0413] In some embodiments, the present disclosure provides methods for modulating level of a DMD transcript or a product encoded thereby in a system, comprising administering or delivering to the system an effective amount of a provided oligonucleotide or composition thereof. In some embodiments, the present disclosure provides methods for modulating level of a DMD transcript or a product encoded thereby in a system, comprising contacting the DMD transcript with a provided oligonucleotide or composition thereof. In some embodiments, modulating level of a DMD transcript decreases level of the DMD transcript. In some embodiments, modulating level of a DMD transcript increases level of the DMD transcript. In some

embodiments, modulating level of a product encoded by a DMD transcript decreases level of the product. In some embodiments, modulating level of a product encoded by a DMD transcript increases level the product. In some embodiments, the present disclosure provides methods for increasing level of DMD polypeptide in a system, comprising administering or delivering or delivering to the system an effective amount of a provided oligonucleotide or composition thereof.

[0414] In some embodiments, the present disclosure provides methods for altering splicing of a DMD transcript in a system, comprising administering or delivering or delivering to the system an effective amount of a provided oligonucleotide or composition thereof. In some embodiments, the present disclosure provides methods for providing DMD exon skipping in a system, comprising administering or delivering or delivering to the system an effective amount of a provided oligonucleotide or composition thereof.

[0415] In some embodiments, a system comprises an *in vitro* system. In some embodiments, a system is an *in vitro* system. In some embodiments, a system comprises an *in vivo* system. In some embodiments, a system is an *in vivo* system.

[0416] In some embodiments, a system comprises a cell. In some embodiments, a system is a cell. In some embodiments, a system comprises a population of cells. In some embodiments, a system is a population of cells. In some embodiments, a cell is a stem cell. In some embodiments, a cell is a muscle cell. In some embodiments, a cell is skeletal muscle cell. In some embodiments, a cell is a cardiac muscle cell. In some embodiments, a cell is a cell in the muscular system. In some embodiments, a cell is a neuron. In some embodiments, a cell is a cell in the neuronal system. In some embodiments, a cell is a cell in the central nervous system. In some embodiments, a cell possesses one or more characteristics, properties and/or activities of a muscle cell. In some embodiments, a cell possesses one or more characteristics, properties and/or activities of a neuronal cell.

[0417] In some embodiments, a system comprises a tissue. In some embodiments, a system is a tissue. In some embodiments, a system comprises a muscle tissue. In some embodiments, a system is a muscle tissue. In some embodiments, a system comprises an organ. In some embodiments, a system is an organ. In some embodiments, a system is a muscle or a portion thereof. In some embodiments, a system comprises an organism. In some embodiments, a system is an organism. In some embodiments, a system is a subject. In some embodiments, a system is an animal. In some embodiments, a system is a mammal, e.g., a mouse, a rat, a non-human primate, e.g., a monkey. In some embodiments, a system is a human. In some embodiments, a system is a child, e.g., a human child. In some embodiments, a system is an adult, e.g., a human adult.

[0418] In some embodiments, a system expresses DMD transcripts. In some embodiments, a system comprises a mutation of the DMD gene. In some embodiments, a system comprises a mutation of the DMD gene that is amenable to exon 52 skipping.

[0419] In some embodiments, provided oligonucleotides and compositions are useful for treating various conditions, disorders or diseases, by reducing levels and/or activities of DMD transcripts and/or products encoded thereby that are associated with the conditions, disorders or diseases. In some embodiments, provided

oligonucleotides and compositions are useful for treating various conditions, disorders or diseases, by increasing levels and/or activities of certain DMD transcripts and/or products encoded thereby, e.g. dystrophin proteins.

[0420] Exon skipping can induce cellular machinery to skip over one or more targeted exons and restore the reading frame resulting in the production of internally truncated but functional dystrophin polypeptides. In various exon-skipping approaches, oligonucleotides can bind to target complementary sequences in mRNAs, which engage with the splicing machineries to exclude exons from final transcripts. In subjects with DMD, this approach may slow disease progression by converting severe DMD symptoms to the milder symptoms such as those seen in patients with Becker muscular dystrophy (BMD), in which patients are ambulatory longer and have a longer life expectancy.

[0421] Various DMD genotypes are amenable to exon skipping including exon 52 skipping. Exon skipping can restore DMD mRNA reading frame, resulting in the production of a shorter but functional dystrophin protein. Exon 52 skipping is applicable, but not limited to, patients with deletions in the DMD gene of exons 2-51, 8-51, 20-51, 22-51, 51, 53, 53-55, 53-57, 53-59, 53-60, 53-67, 53-69, 53-75, and 53-78. According to some reports, approximately 4% of patients with DMD may be treated by exon 52 skipping (Aartsma-Rus et al. Hum Mutat. 2009 Mar;30(3):293-9). In some embodiments, a mutation of the DMD gene is amenable to exon 52 skipping. In some embodiments, a mutation of the DMD gene is Δ2-51, Δ8-51, Δ20-51, Δ22-51, Δ51, Δ53, Δ53-55, Δ53-57, Δ53-59, Δ53-60, Δ53-67, Δ53-69, Δ53-75, or Δ53-78. In some embodiments, a mutation comprises Δ51. In some embodiments, a mutation comprises Δ53.

[0422] In some embodiments, exon skipping restores or maintains a proper reading frame, and/or creating an artificially internally truncated DMD protein which provides at least partially improved or fully restored biological activity. In some embodiments, an internally truncated DMD protein produced from a dystrophin transcript with a skipped exon(s) is more functional than a terminally truncated DMD protein, e.g., produced from a dystrophin transcript with an out-of-frame deletion.

[0423] In some embodiments, an internally truncated DMD protein produced from a dystrophin transcript with a skipped exon(s) is more resistant to nonsense-mediated decay, which can degrade a terminally truncated DMD protein, e.g., produced from a dystrophin transcript with an out-of-frame deletion.

[0424] In some embodiments, a mutation of the DMD gene that is amenable to exon 52 skipping is or comprises one or more nucleobase changes (e.g., replaced by a different nucleobase), insertions, and/or deletions, etc., wherein exon 52 skipping can produce a DMD polypeptide that can provided improved functions compared to without exon 52 skipping.

[0425] Among other things, the present disclosure provides technologies, e.g., oligonucleotides and compositions thereof, for treating DMD, inducing DMD exon 52 skipping, increasing levels of exon 52-skipped DMD transcripts, producing or increasing levels of truncated DMD polypeptides from exon 52 skipping, increasing levels of and/or restoring one or more DMD functions. In some embodiments, provided technologies, e.g., oligonucleotides and compositions thereof, provide various advantages over, e.g.,

stereorandom oligonucleotide or compositions thereof.

[0426] In some embodiments, the present disclosure provides methods for preventing or treating a condition, disease, or disorder, comprising administering or delivering to a subject suffering therefrom or susceptible thereto an effective amount of a provided oligonucleotide or composition thereof. In some embodiments, the present disclosure provides methods for preventing or treating a condition, disease, or disorder associated with a DMD transcript, e.g., a mutant DMD transcript, comprising administering or delivering to a subject suffering therefrom or susceptible thereto an effective amount of a provided oligonucleotide or composition thereof, wherein the oligonucleotide or composition thereof modulate level of the DMD transcript. In some embodiments, a nucleic acid sequence is a gene. In some embodiments, modulating level of a DMD transcript decreases level of the DMD transcript. In some embodiments, modulating level of a DMD transcript increases level of the DMD transcript.

[0427] In some embodiments, a condition, disorder, or disease is muscular dystrophy. In some embodiments, muscular dystrophy (MD) is any of a group of muscle conditions, diseases, or disorders that results in (increasing) weakening and breakdown of skeletal muscles over time. The conditions, diseases, or disorders differ in which muscles are primarily affected, the degree of weakness, when symptoms begin, and how quickly symptoms worsen. Many MD patients will eventually become unable to walk. In many cases muscular dystrophy is fatal. Some types are also associated with problems in other organs, including the central nervous system. In some embodiments, the muscular dystrophy is Duchenne (Duchenne's) Muscular Dystrophy (DMD) or Becker (Becker's) Muscular Dystrophy (BMD).

[0428] In some embodiments, a condition, disorder, or disease is a symptom of a muscular dystrophy, e.g., a symptom of Duchenne Muscular Dystrophy. In some embodiments, a symptom of Duchenne Muscular Dystrophy is reportedly muscle weakness associated with muscle wasting, with the voluntary muscles being first affected, especially those of the hips, pelvic area, thighs, shoulders, and calves. Muscle weakness can reportedly also occur later, in the arms, neck, and other areas. Calves are reportedly often enlarged. Symptoms reportedly usually appear before age six and may appear in early infancy. Other physical symptoms reportedly are: awkward manner of walking, stepping, or running (in some cases, patients tend to walk on their forefeet, because of an increased calf muscle tone), frequent falls, fatigue, difficulty with motor skills (e.g., running, hopping, jumping), lumbar hyperlordosis, possibly leading to shortening of the hip-flexor muscles, unusual overall posture and/or manner of walking, stepping, or running, muscle contractures of Achilles tendon and hamstrings impair functionality, progressive difficulty walking, muscle fiber deformities, pseudohypertrophy (enlarging) of tongue and calf muscles, higher risk of neurobehavioral disorders (e.g., ADHD), learning disorders (e.g., dyslexia), and non-progressive weaknesses in specific cognitive skills (e.g., short-term verbal memory), which are believed to be the result of absent or dysfunctional dystrophin in the brain, eventual loss of ability to walk (usually by the age of 12), skeletal deformities (including scoliosis in some cases), and trouble getting up from lying or sitting position.

[0429] In some embodiments, Becker muscular dystrophy (BMD) is reportedly caused by mutations that give

rise to shortened but in-frame DMD transcripts resulting in the production of truncated but partially functional protein(s). Such partially functional protein(s) were reported to retain the critical amino terminal, cysteine rich and C-terminal domains but usually lack elements of the central rod domains which were reported to be of less functional significance (England et al. 1990 Nature, 343, 180–182). In some embodiments, BMD phenotypes range from mild DMD to virtually asymptomatic, depending on the precise mutation and the level of dystrophin produced (Yin et al. 2008 Hum. Mol. Genet. 17: 3909-3918).

[0430] In some embodiments, dystrophy patients with out-of-frame mutations are generally diagnosed with the more severe Duchenne Muscular Dystrophy, and dystrophy patients with in-frame mutations are generally diagnosed with the less severe Becker Muscular Dystrophy. However, a minority of patients with in-frame deletions are diagnosed with Duchenne Muscular Dystrophy, including those with deletion mutations starting or ending in exons 50 or 51, which encode part of the hinge region, such as deletions of exons 47 to 51, 48 to 51, and 49 to 53. Without wishing to be bound by any particular theory, the present disclosure notes that the patient-to-patient variability in disease severity despite the presence of the same exon deletion reportedly may be related to the effect of the specific deletion breakpoints on mRNA splicing efficiency and/or patterns; translation or DMD transcription efficiency after genome rearrangement; and stability or function of the truncated protein structure (Yokota et al. 2009 Arch. Neurol. 66: 32).

[0431] In some embodiments, the present disclosure provides a method for treating muscular dystrophy, comprising administering or delivering to a subject suffering therefrom an effective amount of a provided oligonucleotide or composition. In some embodiments, a subject is suffering from Duchenne's muscular dystrophy. In some embodiments, a subject is suffering from Becker's muscular dystrophy. In some embodiments, a subject has a mutation of the DMD gene that is amenable to exon 52 skipping. In some embodiments, a subject has a mutation described herein.

[0432] In some embodiments, a condition, disorder, or disease that can be treated in accordance with the present disclosure is described in WO 2017/062862, WO 2018/067973, WO 2019/200185, WO 2019/217784, or WO 2020/118246.

[0433] In some embodiments, exon 52 is skipped in DMD mRNA. In some embodiments, level of exon 52-skipped DMD mRNA is increased as compared to absence of the oligonucleotide or composition. In some embodiments, level of exon 52-skipped DMD mRNA is increased as compared to administering or delivering a reference oligonucleotide or composition. In some embodiments, a truncated DMD polypeptide is produced as compared to a wild-type DMD protein. In some embodiments, level of a truncated DMD polypeptide is increased as compared to absence of the oligonucleotide or composition. In some embodiments, level of a truncated DMD polypeptide is increased as compared to administering or delivering a reference oligonucleotide or composition. In some embodiments, a truncated DMD polypeptide performs one or more functions of a wild-type DMD protein.

[0434] In some embodiments, a subject is administered or delivered an additional treatment (including, but not limited to, a therapeutic agent or method) in addition to a provided oligonucleotide or oligonucleotide

composition. In some embodiments, a composition comprising an oligonucleotide(s) (or two or more compositions, each comprising an oligonucleotide) is administered to a patient along with an additional treatment.

[0435] In some embodiments, the present disclosure pertains to a method for treating muscular dystrophy, Duchenne (Duchenne's) muscular dystrophy (DMD), or Becker (Becker's) muscular dystrophy (BMD), comprising (a) administering or delivering to a subject susceptible thereto or suffering therefrom a composition comprising a provided oligonucleotide, and (b) administering or delivering to the subject an additional treatment which is capable of preventing, treating, ameliorating or slowing the progress of muscular dystrophy. In some embodiments, an additional treatment is a composition comprising a second oligonucleotide.

[0436] In some embodiments, an additional treatment is capable of preventing, treating, ameliorating or slowing the progress of muscular dystrophy by itself. In some embodiments, an additional treatment is capable of preventing, treating, ameliorating or slowing the progress of muscular dystrophy when administered with a provided oligonucleotide.

[0437] In some embodiments, an additional treatment is administered to the subject prior to, after, or simultaneously with a composition comprising a provided oligonucleotide. In some embodiments, a composition comprises both an oligonucleotide and an additional treatment. In some embodiments, an oligonucleotide and an additional treatment(s) are in separate compositions. In some embodiments, the present disclosure provides technologies (e.g., compositions, methods, etc.) for combination therapy, for example, with other therapeutic agents and/or medical procedures. In some embodiments, provided oligonucleotides and/or compositions may be used together with one or more other therapeutic agents. In some embodiments, provided compositions comprise provided oligonucleotides, and one or more other therapeutic agents. In some embodiments, the one or more other therapeutic agents may have one or more different targets, and/or one or more different mechanisms toward targets, when compared to provided oligonucleotides in the composition. In some embodiments, a therapeutic agent is an oligonucleotide. In some embodiments, a therapeutic agent is a small molecule drug. In some embodiments, a therapeutic agent is a protein. In some embodiments, a therapeutic agent is an antibody. A number of therapeutic agents may be utilized in accordance with the present disclosure. For example, oligonucleotides targeting DMD transcripts may be used together with one or more therapeutic agents that modulate utrophin production (utrophin modulators). In some embodiments, a utrophin modulator promotes production of utrophin. In some embodiments, a utrophin modulator is ezutromid. In some embodiments, a utrophin modulator is ezutromid, or a pharmaceutically acceptable salt thereof.

[0438] In some embodiments, provided oligonucleotides or compositions thereof are administered prior to, concurrently with, or subsequent to one or more other therapeutic agents and/or medical procedures. In some embodiments, provided oligonucleotides or compositions thereof are administered concurrently with one or more other therapeutic agents and/or medical procedures. In some embodiments, provided oligonucleotides or compositions thereof are administered prior to one or more other therapeutic agents and/or medical procedures. In some embodiments, provided oligonucleotides or compositions thereof are administered

subsequent to one or more other therapeutic agents and/or medical procedures. In some embodiments, provided compositions comprise one or more other therapeutic agents.

[0439] In some embodiments, a composition comprising an oligonucleotide is co-administered with an additional agent in order to improve skipping of a DMD exon of interest. In some embodiments, an additional agent is an antibody, an oligonucleotide, protein, or small molecule. In some embodiments, an additional agent interferes with a protein involved in splicing. In some embodiments, an additional agent interferes with a protein involved in splicing, wherein the protein is a SR protein.

[0440] In some embodiments, an additional agent interferes with a protein involved in splicing, wherein the protein is a SR protein, which contains a protein domain with one or more long repeats of serine (S) and arginine (R) amino acid residues. SR proteins are reportedly heavily phosphorylated in cells and are involved in constitutive and alternative splicing. Long et al. 2009 Biochem. J. 417: 15-27; Shepard et al. 2009 Genome Biol. 10: 242. In some embodiments, an additional agent is a chemical compound that inhibits or decreases a SR protein kinase. In some embodiments, a chemical compound that inhibits or decreases a SR protein kinase is SRPIN340. SRPIN340 is reported in, for example, Fukuhura et al. 2006 Proc. Natl. Acad. Sci. USA 103: 11329-11333. In some embodiments, a chemical compound is a kinase inhibitor specific for Cdc-like kinases (Clks) that are also able to phosphorylate SR proteins. In some embodiments, a kinase inhibitor specific for Cdc-like kinases (Clks) that are also able to phosphorylate SR proteins is TG003. TG003 reportedly affected splicing both in vitro and in vivo. Nowak et al. 2010 J. Biol. Chem. 285: 5532-5540; Muraki et al. 2004 J. Biol. Chem. 279: 24246-24254; Yomoda et al. 2008 Genes Cells 13: 233-244; and Nishida et al. 2011 Nat Commun. 2:308.

[0441] In some embodiments, in a patient afflicted with muscular dystrophy, muscle tissue is replaced by fat and connective tissue, and affected muscles may look larger due to increased fat content, a condition known as pseudohypertrophy. In some embodiments, a composition comprising an oligonucleotide is administered along with a treatment which reduces or prevents development of fat or fibrous or connective tissue, or replacement of muscle tissue by fat or fibrous or connective tissue.

[0442] In some embodiments, a composition comprising an oligonucleotide(s) is administered along with a treatment which reduces or prevents development of fat or fibrous or connective tissue, or replacement of muscle tissue by fat or fibrous or connective tissue, wherein the treatment is an antibody to connective tissue growth factor (CTGF), a central mediator of fibrosis (e.g., FG-3019). In some embodiments, a composition comprising an oligonucleotide(s) is administered along with an agent which reduces the fat content of the human body.

[0443] Additional treatments include: slowing the progression of the disease by immune modulators (e.g., steroids and transforming growth factor-beta inhibitors), inducing or introducing proteins that may compensate for dystrophin deficiency in the myofiber (e.g., utrophin, biglycan, and laminin), or bolstering the muscle's regenerative response (e.g., myostatin and activin 2B).

[0444] In some embodiments, an additional treatment is a small molecule capable of restoring normal

balance of calcium within muscle cells. In some embodiments, an additional treatment is a small molecule capable of restoring normal balance of calcium within muscle cells by correcting the activity of a type of channel called the ryanodine receptor calcium channel complex (RyR). In some embodiments, such a small molecule is Rycal ARM210 (ARMGO Pharma, Tarry Town, NY).

[0445] In some embodiments, an additional treatment is a flavonoid. In some embodiments, an additional treatment is a flavonoid such as Epicatechin. Epicatechin is a flavonoid found in dark chocolate harvested from the cacao tree which has been reported in animals and humans to increase the production of new mitochondria in heart and muscle (e.g., mitochondrial biogenesis) while concurrently stimulating the regeneration of muscle tissue.

[0446] In some embodiments, an additional treatment is follistatin gene therapy. In some embodiments, an additional treatment is adeno-associated virus delivery of follistatin 344 to increase muscle strength and prevent muscle wasting and fibrosis. In some embodiments, an additional treatment is glucocorticoid. In some embodiments, an additional treatment is prednisone. In some embodiments, an additional treatment is deflazacort. In some embodiments, an additional treatment is vamorolone (VBP15).

[0447] In some embodiments, an additional treatment is delivery of an exogenous Dystrophin gene or synthetic version or portion thereof, such as a microdystrophin gene. In some embodiments, an additional treatment is delivery of an exogenous Dystrophin gene or portion thereof, such as a microdystrophin gene, such as SGT-001, an adeno-associated viral (AAV) vector-mediated gene transfer system for delivery of a synthetic dystrophin gene or microdystrophin (Solid BioSciences, Cambridge, Mass.).

[0448] In some embodiments, an additional treatment is stem cell treatment. In some embodiments, an additional treatment is a steroid. In some embodiments, an additional treatment is a corticosteroid. In some embodiments, an additional treatment is prednisone. In some embodiments, an additional treatment is a beta-2 agonist.

[0449] In some embodiments, an additional treatment is an ion channel inhibitor. In some embodiments, an additional treatment is a calcium channel inhibitor. In some embodiments, an additional treatment is a calcium channel inhibitor which is a xanthin. In some embodiments, an additional treatment is a calcium channel inhibitor which is methylxanthine. In some embodiments, an additional treatment is a calcium channel inhibitor which is pentoxifylline. In some embodiments, an additional treatment is a calcium channel inhibitor which is a methylxanthine derivative selected from: pentoxifylline, furafylline, lisofylline, propentofylline, pentifylline, theophylline, torbafylline, albifylline, enprofylline and derivatives thereof. In some embodiments, an additional treatment is a treatment for heart disease or cardiovascular disease. In some embodiments, an additional treatment is a blood pressure medicine.

[0450] In some embodiments, an additional treatment is surgery. In some embodiments, an additional treatment is surgery to fix shortened muscles, straighten the spine, or treat a heart or lung problem. In some embodiments, an additional treatment is a brace, walker, standing walker, or other mechanical aid for walking. In some embodiments, an additional treatment is exercise and/or physical therapy. In some embodiments, an

additional treatment is assisted ventilation.

[0451] In some embodiments, an additional treatment is anticonvulsant, immunosuppressant or treatment for constipation. In some embodiments, an additional treatment is an inhibitor of NF-κB. In some embodiments, an additional treatment comprises salicylic acid and/or docosahexaenoic acid (DHA). In some embodiments, an additional treatment is edasalonexent (CAT-1004, Catabasis), a conjugate of salicylic acid and docosahexaenoic acid (DHA). In some embodiments, an additional treatment is a cell-based therapeutic. In some embodiments, an additional treatment is comprises allogeneic cardiosphere-derived cells. In some embodiments, an additional treatment is CAP-1002 (Capricor).

[0452] In some embodiments, any oligonucleotide, or combination thereof, described herein, or any composition comprising an oligonucleotide described herein, can be combined with any therapeutic agent known in the art.

[0453] Among other things, the present disclosure provides the following Example Embodiments:

1. An oligonucleotide, wherein:

the base sequence of the oligonucleotide comprises 10 or more contiguous nucleobases of a complement of a DMD transcript; and

the oligonucleotide comprises 3 or more PN internucleotidic linkages.

2. The oligonucleotide of Embodiment 1, wherein the base sequence of the oligonucleotide comprises 15 or more contiguous nucleobases of a complement of a DMD transcript.

3. The oligonucleotide of any one of the preceding Embodiments, wherein the base sequence of the oligonucleotide comprises 20 or more contiguous nucleobases of a complement of a DMD transcript.

4. The oligonucleotide of any one of the preceding Embodiments, wherein the base sequence of the oligonucleotide is the same as an equal length portion in a complement of a DMD transcript.

5. An oligonucleotide, wherein:

the base sequence of the oligonucleotide comprises 10 or more contiguous nucleobases that are complementary to an equal length portion in a DMD transcript; and

the oligonucleotide comprises 3 or more PN internucleotidic linkages.

6. The oligonucleotide of Embodiment 5, wherein the base sequence of the oligonucleotide comprises 15 or more contiguous nucleobases that are complementary to an equal length portion in a DMD transcript.

7. The oligonucleotide of Embodiment 5, wherein the base sequence of the oligonucleotide comprises 20 or more contiguous nucleobases that are complementary to an equal length portion in a DMD transcript.

8. The oligonucleotide of any one of the preceding Embodiments, wherein the base sequence of the oligonucleotide is complementary to the base sequence of an equal length portion in a DMD transcript.

9. An oligonucleotide, wherein:

the oligonucleotide can hybridize to an equal length portion in a DMD transcript; and

the oligonucleotide comprises 3 or more PN internucleotidic linkages.

10. An oligonucleotide, wherein the base sequence of the oligonucleotide comprises 15 or more contiguous nucleobases of a complement of a DMD transcript, wherein the oligonucleotide comprises 4 or more PN internucleotidic linkages, and wherein the oligonucleotide comprises a PN internucleotidic linkage between the 5' terminal (+1) nucleoside and the immediately downstream (+2) nucleoside and/or a PN internucleotidic linkage between the 3' terminal (N) nucleoside and the penultimate (N-1) nucleoside.

11. The oligonucleotide of Embodiment 10, wherein the oligonucleotide comprises a PN internucleotidic linkage between the 5' terminal (+1) nucleoside and the immediately downstream (+2) nucleoside, and wherein the oligonucleotide does not comprise a PN internucleotidic linkage between the 3' terminal (N) nucleoside and the penultimate (N-1) nucleoside.

12. The oligonucleotide of Embodiment 10 or 11, wherein the PN internucleotidic linkage between the 5' terminal (+1) nucleoside and the immediately downstream (+2) nucleoside is in the *R*p configuration.

13. The oligonucleotide of any one of Embodiments 10-12, wherein all of the PN internucleotidic linkages are in the *R*p configuration.

14. The oligonucleotide of any one of Embodiments 10-13, wherein the oligonucleotide comprises only 4 PN internucleotidic linkages.

15. The oligonucleotide of any one of Embodiments 10-14, wherein the 5' terminal (+1) nucleoside, and/or the 3' terminal (N) nucleoside, of the oligonucleotide comprises a modified sugar moiety.

16. The oligonucleotide of Embodiment 15, wherein the modified sugar moiety comprises a 2'-OMe modification.

17. The oligonucleotide of any one of Embodiments 10-16, wherein the oligonucleotide comprises a PN internucleotidic linkage between the +3 nucleoside and the +4 nucleoside, a PN internucleotidic linkage between the +6 nucleoside and the +7 nucleoside, and/or a PN internucleotidic linkage between the +17 nucleoside and the +18 nucleoside.

18. The oligonucleotide of Embodiment 17, wherein the PN internucleotidic linkage between the +3 nucleoside and the +4 nucleoside, the PN internucleotidic linkage between the +6 nucleoside and the +7 nucleoside, and/or the PN internucleotidic linkage between the +17 nucleoside and the +18 nucleoside, are in the *R*p configuration.

19. An oligonucleotide, wherein the base sequence of the oligonucleotide comprises 15 or more contiguous nucleobases of a complement of a DMD transcript, wherein the oligonucleotide comprises a PN internucleotidic linkage between the 5' terminal (+1) nucleoside and the immediately downstream (+2) nucleoside in the *R*p configuration, a PN internucleotidic linkage between the +3 nucleoside and the +4 nucleoside in the *R*p configuration, a PN internucleotidic linkage between the +6 nucleoside and the +7 nucleoside in the *R*p configuration, and/or a PN internucleotidic linkage between the +17 nucleoside and the +18 nucleoside in the *R*p configuration.

20. The oligonucleotide of Embodiment 19, wherein the sugar moiety of the 5' terminal (+1) nucleoside comprises a 2'-OMe modification.

21. The oligonucleotide of Embodiment 19, wherein the sugar moiety of the 5' terminal (+1) nucleoside comprises a 2'-F modification.

22. The oligonucleotide of any one of Embodiments 19-21, wherein the oligonucleotide comprises a PS linkage between the +2 nucleoside and the +3 nucleoside, a PS linkage between the +4 nucleoside and the +5 nucleoside, a PS linkage between the +5 nucleoside and the +6 nucleoside, a PS linkage between the +8 nucleoside and the +9 nucleoside, a PS linkage between the +9 nucleoside and the +10 nucleoside, a PS linkage between the +10 nucleoside and the +11 nucleoside, a PS linkage between the +11 nucleoside and the +12 nucleoside, a PS linkage between the +13 nucleoside and the +14 nucleoside, a PS linkage between the +14 nucleoside and the +15 nucleoside, a PS linkage between the +15 nucleoside and the +16 nucleoside, a PS linkage between the +16 nucleoside and the +17 nucleoside, and/or a PS linkage between the +18 nucleoside and the +19 nucleoside.

23. The oligonucleotide of Embodiment 23, wherein the oligonucleotide comprises a PS linkage between the 3' terminal (N) nucleoside and the penultimate (N-1) nucleoside.

24. The oligonucleotide of Embodiment 22 or 23, wherein all of the PS linkages are in the *S*p configuration.

25. The oligonucleotide of any one of Embodiments 19-24, wherein the sugar moiety of the +7 nucleoside comprises a 2'-OMe modification, the sugar moiety of the +10 nucleoside comprises a 2'-OMe modification, the sugar moiety of the +12 nucleoside comprises a 2'-OMe modification, and/or the sugar moiety of the +13 nucleoside comprises a 2'-OMe modification.

26. The oligonucleotide of Embodiment 25, wherein the sugar moiety of the 3' terminal (N) nucleoside, comprises a 2'-OMe modification.

27. The oligonucleotide of any one of the preceding Embodiments, wherein the DMD transcript is a DMD mRNA.

28. The oligonucleotide of any one of the preceding Embodiments, wherein the DMD transcript or a wild-type version thereof encodes dystrophin.

29. The oligonucleotide of any one of the preceding Embodiments, wherein the DMD transcript comprises a mutation that is amenable to exon 52 skipping.

30. The oligonucleotide of any one of the preceding Embodiments, wherein each nucleobase is independently an optionally substituted nucleobase selected from A, T, C, G and U, or an optionally substituted tautomer of a nucleobase selected from A, T, C, G and U.

31. The oligonucleotide of any one of the preceding Embodiments, wherein each nucleobase is independently A, T, C, 5mC, G or U.

32. The oligonucleotide of any one of the preceding Embodiments, wherein the length of the oligonucleotide is 20 or more nucleobases.

33. The oligonucleotide of any one of the preceding Embodiments, wherein the length of the oligonucleotide is 20 nucleobases.

34. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 4, 5, 6 or more PN internucleotidic linkages.

35. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a *R*p PN internucleotidic linkage.

36. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 4, 5, 6 or more *R*p PN internucleotidic linkages.

37. The oligonucleotide of any one of the preceding Embodiments, wherein each PN internucleotidic linkage is independently a phosphoryl guanidine internucleotidic linkage.

38. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a phosphoryl guanidine internucleotidic linkage.

39. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 4, 5, 6 or more phosphoryl guanidine internucleotidic linkages.

40. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a *R*p phosphoryl guanidine internucleotidic linkage.

41. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 4, 5, 6 or more *R*p phosphoryl guanidine internucleotidic linkages.

42. The oligonucleotide of any one of the preceding Embodiments, wherein each phosphoryl guanidine internucleotidic linkage is independently a n001 internucleotidic linkage.

43. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a n001 internucleotidic linkage.

44. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 4, 5, 6 or more n001 internucleotidic linkages.

45. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a *R*p n001 internucleotidic linkage.

46. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 4, 5, 6 or more *R*p n001 internucleotidic linkages.

47. The oligonucleotide of any one of the preceding Embodiments, wherein each n001 is *R*p.

48. The oligonucleotide of any one of the preceding Embodiments, wherein each phosphoryl guanidine internucleotidic linkage is *R*p.

49. The oligonucleotide of any one of the preceding Embodiments, wherein each PN internucleotidic linkage is *R*p.

50. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the third and the fourth nucleosides is a PN linkage (unless otherwise noted, 5' to 3' direction).

51. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the sixth and the seventh nucleosides is a PN linkage.

52. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the 17th and the 18th nucleosides is a PN linkage.

53. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the first and the second nucleosides is a PN linkage.

54. The oligonucleotide of any one of Embodiments 1-52, wherein the internucleotidic linkage between the last two nucleosides is a PN linkage.

55. The oligonucleotide of any one of the preceding Embodiments, wherein the number of *R*p n001 internucleotidic linkages in the oligonucleotide is 4.

56. The oligonucleotide of any one of the preceding Embodiments, wherein the number of n001 internucleotidic linkages in the oligonucleotide is 4.

57. The oligonucleotide of any one of the preceding Embodiments, wherein the number of *R*p phosphoryl guanidine internucleotidic linkages in the oligonucleotide is 4.

58. The oligonucleotide of any one of the preceding Embodiments, wherein the number of phosphoryl guanidine internucleotidic linkages in the oligonucleotide is 4.

59. The oligonucleotide of any one of the preceding Embodiments, wherein the number of *R*p PN internucleotidic linkages in the oligonucleotide is 4.

60. The oligonucleotide of any one of the preceding Embodiments, wherein the number of PN internucleotidic linkages in the oligonucleotide is 4.

61. The oligonucleotide of Embodiment 53, wherein the internucleotidic linkage between the last two nucleosides is a PN linkage.

62. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a PS internucleotidic linkage.

63. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more PS internucleotidic linkages.

64. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a *S*p PS internucleotidic linkage.

65. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more *S*p PS internucleotidic linkages.

66. The oligonucleotide of any one of the preceding Embodiments, wherein each PS internucleotidic linkage is *S*p.

67. The oligonucleotide of any one of the preceding Embodiments, wherein each PS internucleotidic linkage is independently a phosphorothioate internucleotidic linkage.

68. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a phosphorothioate internucleotidic linkage.

69. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more phosphorothioate internucleotidic linkages.

70. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a *Sp* phosphorothioate internucleotidic linkage.

71. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more *Sp* phosphorothioate internucleotidic linkages.

72. The oligonucleotide of any one of the preceding Embodiments, wherein each phosphorothioate internucleotidic linkage is independently *Sp*.

73. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the first and the second nucleosides, if it is not a PN linkage, is a PS internucleotidic linkage.

74. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the second and the third nucleosides is a PS internucleotidic linkage.

75. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the fourth and the fifth nucleosides is a PS internucleotidic linkage.

76. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the fifth and the sixth nucleosides is a PS internucleotidic linkage.

77. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the eighth and the ninth nucleosides is a PS internucleotidic linkage.

78. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the ninth and the tenth nucleosides is a PS internucleotidic linkage.

79. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the 10th and the 11th nucleosides is a PS internucleotidic linkage.

80. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the 11th and the 12th nucleosides is a PS internucleotidic linkage.

81. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the 13th and the 14th nucleosides is a PS internucleotidic linkage.

82. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the 14th and the 15th nucleosides is a PS internucleotidic linkage.

83. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the 15th and the 16th nucleosides is a PS internucleotidic linkage.

84. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the 16th and the 17th nucleosides is a PS internucleotidic linkage.

85. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the 18th and the 19th nucleosides is a PS internucleotidic linkage.

86. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the last two nucleosides, if it is not a PN linkage, is a PS internucleotidic linkage.

87. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a PO internucleotidic linkage.

88. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 2, 3, 4, 5 or more PO internucleotidic linkages.

89. The oligonucleotide of any one of the preceding Embodiments, wherein each PO internucleotidic linkage is independently a natural phosphate linkage.

90. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a natural phosphate internucleotidic linkage.

91. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 2, 3, 4, 5 or more natural phosphate internucleotidic linkages.

92. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the seventh and the eighth nucleoside is a PO internucleotidic linkage.

93. The oligonucleotide of any one of the preceding Embodiments, wherein the internucleotidic linkage between the 12th and the 13th nucleoside is a PO internucleotidic linkage.

94. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a sugar modification.

95. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a 2'-modified sugar.

96. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more 2'-modified sugars.

97. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a 2'-F modified sugar.

98. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more 2'-F modified sugars.

99. The oligonucleotide of any one of the preceding Embodiments, wherein the sugar of nucleosides 2, 3, 4, 5, 6, 8, 9, 11, 14, 15, 16, 17, 18, and 19 are 2'-F modified sugars.

100. The oligonucleotide of any one of Embodiments 1-99, wherein the sugar of nucleosides 2, 3, 4, 5, 6, 8, 9, 11, 14, 15, 16, 17, 18, 19 and 20 are 2'-F modified sugars.

101. The oligonucleotide of any one of Embodiments 1-99, wherein the sugar of nucleosides 1, 2, 3, 4, 5, 6, 8, 9, 11, 14, 15, 16, 17, 18, and 19 are 2'-F modified sugars.

102. The oligonucleotide of any one of the preceding Embodiments, wherein the sugar of nucleosides 1, 2, 3, 4, 5, 6, 8, 9, 11, 14, 15, 16, 17, 18, 19 and 20 are 2'-F modified sugars.

103. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a 2'-OR modified sugar, wherein R is optionally substituted C_1-C_6 aliphatic.

104. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 or more 2'-OR modified sugars, wherein R is optionally substituted C_1-C_6 aliphatic.

105. The oligonucleotide of any one of the preceding Embodiments, wherein the sugar of nucleosides 7, 10, 12 and 13 are independently 2'-OR modified sugars, wherein R is optionally substituted C_1-C_6 aliphatic.

106. The oligonucleotide of any one of the preceding Embodiments, wherein the sugar of nucleosides 1, 7, 10, 12 and 13 are independently 2'-OR modified sugars, wherein R is optionally substituted C_1-C_6 aliphatic.

107. The oligonucleotide of any one of the preceding Embodiments, wherein the sugar of nucleosides 1, 7, 10, 12, 13 and 20 are independently 2'-OR modified sugars, wherein R is optionally substituted C_1-C_6 aliphatic.

108. The oligonucleotide of any one of the preceding Embodiments, wherein the sugar of nucleosides 7, 10, 12, 13 and 20 are independently 2'-OR modified sugars, wherein R is optionally substituted C_1-C_6 aliphatic.

109. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises a 2'-OMe modified sugar.

110. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 or more 2'-OMe modified sugars.

111. The oligonucleotide of any one of the preceding Embodiments, wherein each 2'-OR modified sugar is a 2'-OMe modified sugar.

112. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide can hybridize to a region of a DMD transcript.

113. The oligonucleotide of Embodiment 112, wherein the region of the DMD transcript comprises CCAGUUGGAAGAACUCAUUA, wherein each U is optionally and independently replaced with T.

114. The oligonucleotide of Embodiment 112, wherein the region of the DMD transcript comprises CCCCAGUUGGAAGAACUCAU, wherein each U is optionally and independently replaced with T.

115. The oligonucleotide of Embodiment 112, wherein the region of the DMD transcript comprises UGCAGGAUUUGGAACAGAGG, wherein each U is optionally and independently replaced with T.

116. The oligonucleotide of Embodiment 112, wherein the region of the DMD transcript comprises AUGCAGGAUUUGGAACAGAG, wherein each U is optionally and independently replaced with T.

117. The oligonucleotide of Embodiment 112, wherein the region of the DMD transcript comprises CAGGAUUUGGAACAGAGGCG, wherein each U is optionally and independently replaced with T.

118. The oligonucleotide of Embodiment 112, wherein the region of the DMD transcript comprises GGAAGAACUCAUUACCGCUG, wherein each U is optionally and independently replaced with T.

119. The oligonucleotide of any one of the preceding Embodiments, wherein the base sequence comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of a DMD transcript.

120. The oligonucleotide of any one of the preceding Embodiments, wherein the base sequence comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of a DMD transcript.

121. The oligonucleotide of any one of the preceding Embodiments, wherein the base sequence comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of a DMD transcript.

122. The oligonucleotide of any one of Embodiments 1-121, wherein the base sequence comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of CCAGUUGGAAGAACUCAUUA, wherein each U is optionally and independently replaced with T.

123. The oligonucleotide of Embodiment 122, wherein the base sequence comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of CCAGUUGGAAGAACUCAUUA, wherein each U is optionally and independently replaced with T.

124. The oligonucleotide of Embodiment 123, wherein the base sequence comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of CCAGUUGGAAGAACUCAUUA, wherein each U is optionally and independently replaced with T.

125. The oligonucleotide of any one of Embodiments 1-121, wherein the base sequence comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of CCCCAGUUGGAAGAACUCAU, wherein each U is optionally and independently replaced with T.

126. The oligonucleotide of Embodiment 125, wherein the base sequence comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of CCCCAGUUGGAAGAACUCAU, wherein each U is optionally and independently replaced with T.

127. The oligonucleotide of Embodiment 126, wherein the base sequence comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of CCCCAGUUGGAAGAACUCAU, wherein each U is optionally and independently replaced with T.

128. The oligonucleotide of any one of Embodiments 1-121, wherein the base sequence comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of UGCAGGAUUUGGAACAGAGG, wherein each U is optionally and independently replaced with T.

129. The oligonucleotide of Embodiment 128, wherein the base sequence comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of UGCAGGAUUUGGAACAGAGG, wherein each U is optionally and independently replaced with T.

130. The oligonucleotide of Embodiment 129, wherein the base sequence comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of UGCAGGAUUUGGAACAGAGG, wherein each U is optionally and independently replaced with T.

131. The oligonucleotide of any one of Embodiments 1-121, wherein the base sequence comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of AUGCAGGAUUUGGAACAGAG, wherein each U is optionally and independently replaced with T.

132. The oligonucleotide of Embodiment 131, wherein the base sequence comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of AUGCAGGAUUUGGAACAGAG, wherein each U is optionally and independently replaced with T.

133. The oligonucleotide of Embodiment 132, wherein the base sequence comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of AUGCAGGAUUUGGAACAGAG, wherein each U is optionally and independently replaced with T.

134. The oligonucleotide of any one of Embodiments 1-121, wherein the base sequence comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of CAGGAUUUGGAACAGAGGCG wherein each U is optionally and independently replaced with T.

135. The oligonucleotide of Embodiment 134, wherein the base sequence comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of CAGGAUUUGGAACAGAGGCG wherein each U is optionally and independently replaced with T.

136. The oligonucleotide of Embodiment 135, wherein the base sequence comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of CAGGAUUUGGAACAGAGGCG wherein each U is optionally and independently replaced with T.

137. The oligonucleotide of any one of Embodiments 1-121, wherein the base sequence comprises 10 or more contiguous nucleobases complementary to 10 or more contiguous nucleobases of GGAAGAACUCAUUACCGCUG wherein each U is optionally and independently replaced with T.

138. The oligonucleotide of Embodiment 137, wherein the base sequence comprises 15 or more contiguous nucleobases complementary to 15 or more contiguous nucleobases of GGAAGAACUCAUUACCGCUG wherein each U is optionally and independently replaced with T.

139. The oligonucleotide of Embodiment 138, wherein the base sequence comprises 20 or more contiguous nucleobases complementary to 20 or more contiguous nucleobases of GGAAGAACUCAUUACCGCUG wherein each U is optionally and independently replaced with T.

140. The oligonucleotide of any one of the preceding Embodiments, wherein the base sequence comprises 10 or more contiguous nucleobases of UAAUGAGUUCUUCCAACUGG, wherein each U is optionally and independently replaced with T.

141. The oligonucleotide of Embodiment 140, wherein the base sequence comprises 15 or more contiguous nucleobases of UAAUGAGUUCUUCCAACUGG, wherein each U is optionally and independently replaced with T.

142. The oligonucleotide of Embodiment 141, wherein the base sequence comprises UAAUGAGUUCUUCCAACUGG, wherein each T is optionally and independently replaced with U.

143. The oligonucleotide of Embodiment 142, wherein the base sequence is UAAUGAGUUCUUCCAACUGG.

144. The oligonucleotide of any one of the preceding Embodiments, wherein the base sequence comprises 10 or more contiguous nucleobases of AUGAGUUCUUCCAACUGGGG, wherein each U is optionally and independently replaced with T.

145. The oligonucleotide of Embodiment 144, wherein the base sequence comprises 15 or more contiguous nucleobases of AUGAGUUCUUCCAACUGGGG, wherein each U is optionally and independently replaced with T.

146. The oligonucleotide of Embodiment 145, wherein the base sequence comprises AUGAGUUCUUCCAACUGGGG, wherein each T is optionally and independently replaced with U.

147. The oligonucleotide of Embodiment 146, wherein the base sequence is AUGAGUUCUUCCAACUGGGG.

148. The oligonucleotide of any one of the preceding Embodiments, wherein the base sequence comprises 10 or more contiguous nucleobases of CCUCUGUUCCAAAUCCUGCA, wherein each U is optionally and independently replaced with T.

149. The oligonucleotide of Embodiment 148, wherein the base sequence comprises 15 or more contiguous nucleobases of CCUCUGUUCCAAAUCCUGCA, wherein each U is optionally and independently replaced with T.

150. The oligonucleotide of Embodiment 149, wherein the base sequence comprises CCUCUGUUCCAAAUCCUGCA, wherein each T is optionally and independently replaced with U.

151. The oligonucleotide of Embodiment 150, wherein the base sequence is CCUCUGUUCCAAAUCCUGCA.

152. The oligonucleotide of any one of the preceding Embodiments, wherein the base sequence comprises 10 or more contiguous nucleobases of CUCUGUUCCAAAUCCUGCAU, wherein each U is optionally and independently replaced with T.

153. The oligonucleotide of Embodiment 152, wherein the base sequence comprises 15 or more contiguous nucleobases of CUCUGUUCCAAAUCCUGCAU, wherein each U is optionally and independently replaced with T.

154. The oligonucleotide of Embodiment 153, wherein the base sequence comprises CUCUGUUCCAAAUCCUGCAU, wherein each T is optionally and independently replaced with U.

155. The oligonucleotide of Embodiment 154, wherein the base sequence is CUCUGUUCCAAAUCCUGCAU.

156. The oligonucleotide of any one of the preceding Embodiments, wherein the base sequence comprises 10 or more contiguous nucleobases of CGCCUCUGUUCCAAAUCCUG, wherein each U is optionally and independently replaced with T.

157. The oligonucleotide of Embodiment 156, wherein the base sequence comprises 15 or more contiguous nucleobases of CGCCUCUGUUCCAAAUCCUG, wherein each U is optionally and independently replaced with T.

158. The oligonucleotide of Embodiment 157, wherein the base sequence comprises CGCCUCUGUUCCAAAUCCUG, wherein each T is optionally and independently replaced with U.

159. The oligonucleotide of Embodiment 158, wherein the base sequence is CGCCUCUGUUCCAAAUCCUG.

160. The oligonucleotide of any one of the preceding Embodiments, wherein the base sequence comprises 10 or more contiguous nucleobases of CAGCGGUAAUGAGUUCUUCC, wherein each U is optionally and independently replaced with T.

161. The oligonucleotide of Embodiment 160, wherein the base sequence comprises 15 or more contiguous nucleobases of CAGCGGUAAUGAGUUCUUCC, wherein each U is optionally and independently replaced with T.

162. The oligonucleotide of Embodiment 161, wherein the base sequence comprises CAGCGGUAAUGAGUUCUUCC, wherein each T is optionally and independently replaced with U.

163. The oligonucleotide of Embodiment 162, wherein the base sequence is CAGCGGUAAUGAGUUCUUCC.

164. An oligonucleotide, wherein the oligonucleotide is selected from

[fl2r](U)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A),

[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](C)[Ssp].m(A)[Ssp].[fl2r](A)[Ssp].m(A)p.m(U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](U),

[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G),

[fl2r](U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A),

[fl2r](C)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G),

[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[n001R].[fl2r](G),

[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C),

[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C),

[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C),

m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].m(G),

m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A),

m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].m(A),

[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C), and

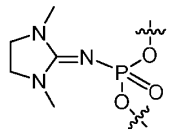
m(C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C),or a salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

165. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](U)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a salt thereof.

166. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a salt thereof.

167. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A), or a salt thereof.

168. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](C)[Ssp].m(A)[Ssp].[fl2r](A)[Ssp].m(A)p.m(U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](U), or a salt thereof.

169. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G), or a salt thereof.

170. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a salt thereof.

171. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a salt thereof.

172. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A), or a salt thereof.

173. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](C)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G), or a salt thereof.

174. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[n001R].[fl2r](G), or a salt thereof.

175. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a salt thereof.

176. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a salt thereof.

177. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C), or a salt thereof.

178. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a salt thereof.

179. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].m(G), or a salt thereof.

180. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A), or a salt thereof.

181. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].m(A), or a salt thereof.

182. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is [fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C), or a salt thereof.

183. The oligonucleotide of Embodiment 164, wherein the oligonucleotide is m(C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C), or a salt thereof.

184. The oligonucleotide of any one of the preceding Embodiments, wherein each chiral internucleotidic linkage of the oligonucleotide independently has a diastereopurity of at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%.

185. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide is a pharmaceutically acceptable salt.

186. The oligonucleotide of any one of the preceding Embodiments, wherein the oligonucleotide is a sodium salt.

187. The oligonucleotide of Embodiment 186, wherein the number of sodium ions in the sodium salt equals the total number of phosphorothioate internucleotidic linkages and natural phosphate internucleotidic linkages in the oligonucleotide.

188. A chirally controlled oligonucleotide composition comprising an oligonucleotide of any one of the preceding Embodiments, wherein the composition is enriched, relative to a substantially racemic preparation of the oligonucleotide, for the oligonucleotide.

189. A chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides each of which is an oligonucleotide of any one of Embodiments 1-187, wherein oligonucleotides of the plurality share the same constitution and at least 5% of all oligonucleotides in the composition that share the same constitution are oligonucleotides of the plurality.

190. A chirally controlled oligonucleotide composition comprising an oligonucleotide of any one of Embodiments 1-187, wherein at least 5% of all oligonucleotides in the composition that share the same constitution as the oligonucleotide are the oligonucleotide.

191. The composition of any one of Embodiments 189-190, wherein at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of all oligonucleotides in the composition that share the same constitution are oligonucleotides of the plurality.

192. The composition of any one of Embodiments 189-190, wherein at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of all oligonucleotides in the composition that share the same base sequence are oligonucleotides of the plurality.

193. An oligonucleotide composition comprising a plurality of oligonucleotides, wherein oligonucleotides of the plurality share:

1) a common base sequence; and

2) the same linkage phosphorus stereochemistry independently at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more chiral internucleotidic linkages;

wherein oligonucleotides of the plurality are an oligonucleotide of any one of Embodiments 1-187; and

wherein at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the oligonucleotides in the composition that share the common base sequence are oligonucleotides of the plurality.

194. The oligonucleotide composition of Embodiment 193, wherein oligonucleotides of plurality share the same linkage phosphorus stereochemistry independently at each chiral internucleotidic linkage.

195. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

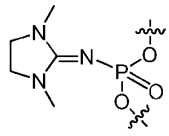
[fl2r](U)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

196. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

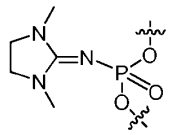
[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

197. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

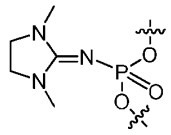
[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

198. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

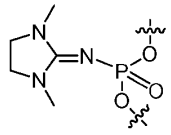
[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](C)[Ssp].m(A)[Ssp].[fl2r](A)[Ssp].m(A)p.m(U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](U), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

199. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

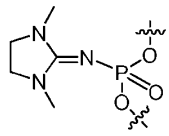
[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

200. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

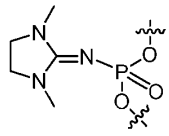
[fl2r](U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

201. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

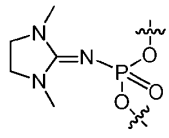
[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

202. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

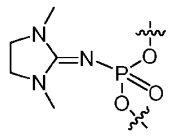
[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

203. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

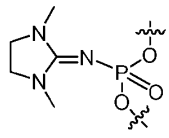
[fl2r](C)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

204. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](

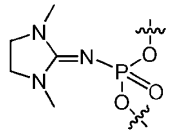
U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[n001R].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

205. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

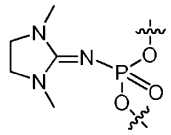
[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

206. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

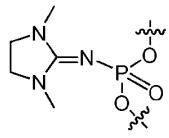
[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

207. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

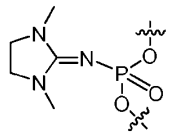
[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

208. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

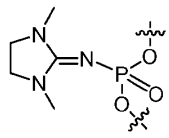
m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

209. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](

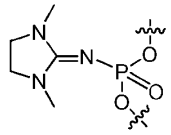
A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].m(G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

210. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

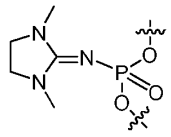
m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

211. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

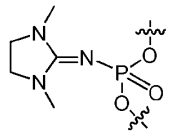
m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].m(A), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

212. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

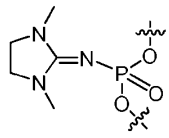
[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

213. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

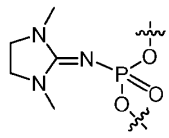
m(C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

214. The composition of any one of Embodiments 195-213, wherein a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of all oligonucleotides that share the same base sequence as an oligonucleotide having the structure or a pharmaceutically acceptable salt thereof.

215. The composition of any one of Embodiments 195-213, wherein a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of all oligonucleotides that share the same constitution as an oligonucleotide having the structure or a pharmaceutically acceptable salt thereof.

216. The composition of any one of Embodiments 195-213, wherein a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% based on UV peak area at 260 nm.

217. The composition of any one of Embodiments 188-213, wherein a level or percentage is about or at least about $(DS)^{nc}$, wherein DS is about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%, and nc is the number of chirally controlled internucleotidic linkages (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 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).

218. The composition of any one of Embodiments 188-213, wherein a level or percentage is about or at least about $(DS)^{nc}$, wherein DS is about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%, and nc is the number of chiral linkage phosphorus in an oligonucleotide of the plurality (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 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)

219. A pharmaceutical composition, comprising an oligonucleotide or oligonucleotide composition of any one of the preceding Embodiments, and a pharmaceutically acceptable carrier.

220. The pharmaceutical composition of Embodiment 219, wherein the composition comprises one or more pharmaceutically acceptable salts of an oligonucleotide.

221. The pharmaceutical composition of any one of Embodiments 219-220, wherein the pharmaceutical composition is a solution.

222. A method for altering splicing of a DMD transcript in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition of any one of the preceding Embodiments.

223. A method for providing DMD exon skipping in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition of any one of the preceding Embodiments.

224. A method for increasing level of a DMD polypeptide in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition of any one of the preceding Embodiments.

225. The method of Embodiment 224, wherein the DMD polypeptide is a truncated dystrophin.

226. A method for increasing level of dystrophin in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition of any one of the preceding Embodiments.

227. A method for increasing level of a dystrophin polypeptide encoded by exon 52-skipped DMD transcript in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition of any one of the preceding Embodiments.

228. A method for increasing level of DMD activity in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition of any one of the preceding Embodiments.

229. The method of any one of Embodiments 222-228, wherein the system expresses DMD transcripts.

230. The method of any one of Embodiments 222-229, wherein the system comprises a mutation of the DMD gene.

231. The method of any one of Embodiments 222-230, wherein the system comprises a mutation of the DMD gene that is amenable to exon 52 skipping.

232. The method of any one of Embodiments 222-231, wherein the system comprises or is a cell.

233. The method of Embodiment 232, wherein the system comprises or is a skeletal muscle cell.

234. The method of Embodiment 232, wherein the system comprises or is a cardiac muscle cell.

235. The method of Embodiment 232, wherein the system comprises or is a neuron.

236. The method of any one of Embodiments 222-235, wherein the system comprises or is a tissue.

237. The method of Embodiment 236, wherein the system comprises or is a muscle tissue.

238. The method of any one of Embodiments 222-235, wherein the system comprises or is an organ.

239. The method of any one of Embodiments 222-235, wherein the system comprises or is an animal.

240. The method of Embodiment 239, wherein the system comprises or is a mouse.

241. The method of Embodiment 239, wherein the system comprises or is a non-human primate.

242. The method of any one of Embodiments 222-235, wherein the system comprises or is a human.

243. The method of any one of Embodiments 222-235, wherein the system comprises or is a subject.

244. A method for treating muscular dystrophy, comprising administering or delivering to a subject suffering therefrom an effective amount of an oligonucleotide or composition of any one of the preceding Embodiments.

245. The method of any one of Embodiments 243-244, wherein the subject is suffering from Duchenne's muscular dystrophy.

246. The method of any one of Embodiments 243-244, wherein the subject is suffering from Becker's muscular dystrophy.

247. The method of any one of Embodiments 243-246, wherein the subject has a mutation of the DMD gene that is amenable to exon 52 skipping.

248. The oligonucleotide, composition or method of any one of the preceding Embodiments, wherein the mutation of the DMD gene is Δ2-51, Δ8-51, Δ20-51, Δ22-51, Δ51, Δ53, Δ53-55, Δ53-57, Δ53-59, Δ53-60, Δ53-67, Δ53-69, Δ53-75, or Δ53-78.

249. The oligonucleotide, composition or method of any one of Embodiments 1-248, wherein the mutation comprises Δ51.

250. The oligonucleotide, composition or method of any one of Embodiments 1-248, wherein the mutation comprises Δ53.

251. The method of any one of the preceding Embodiments, wherein exon 52 is skipped in DMD mRNA.

252. The method of any one of the preceding Embodiments, wherein level of exon 52-skipped DMD mRNA is increased as compared to absence of the oligonucleotide or composition.

253. The method of any one of the preceding Embodiments, wherein level of exon 52-skipped DMD mRNA is increased as compared to administering or delivering a reference oligonucleotide or composition.

254. The method of any one of the preceding Embodiments, wherein a truncated DMD polypeptide is produced as compared to a wild-type DMD protein.

255. The method of any one of the preceding Embodiments, wherein level of a truncated DMD polypeptide is increased as compared to absence of the oligonucleotide or composition.

256. The method of any one of the preceding Embodiments, wherein level of a truncated DMD polypeptide is increased as compared to administering or delivering a reference oligonucleotide or composition.

257. The method of any one of the preceding Embodiments, wherein the truncated DMD polypeptide performs one or more functions of wild-type dystrophin.

258. The method of Embodiment 253 or 256, wherein the reference oligonucleotide targets DMD or the reference composition comprises oligonucleotides targeting DMD.

259. An oligonucleotide or composition of any one of the preceding Embodiments, for use in a method of any one of the preceding Embodiments.

260. An oligonucleotide or composition of any one of the preceding Embodiments, for use in manufacturing a medicament for a method of any one of the preceding Embodiments.

261. Use of an oligonucleotide or composition of any one of the preceding Embodiments in a method of any one of the preceding Embodiments.

262. Use of an oligonucleotide or composition of any one of the preceding Embodiments in manufacturing a medicament for a method of any one of the preceding Embodiments.

263. A method for manufacturing an oligonucleotide or composition of any one of the preceding Embodiments according to a method described in the specification.

264. An oligonucleotide, wherein the oligonucleotide is selected from

[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[n001R].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C),

[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)[n001R].m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C),

[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C),

[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)[n001R].m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C),

[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)[n001R].m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C),

[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C), and

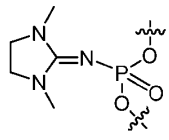
[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

265. The oligonucleotide of Embodiment 264, wherein the oligonucleotide is [fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[n001R].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a salt thereof.

266. The oligonucleotide of Embodiment 264, wherein the oligonucleotide is [fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)[n001R].m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C), or a salt thereof.

267. The oligonucleotide of Embodiment 264, wherein the oligonucleotide is [fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r]

(G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C), or a salt thereof.

268. The oligonucleotide of Embodiment 264, wherein the oligonucleotide is [fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)[n001R].m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C), or a salt thereof.

269. The oligonucleotide of Embodiment 264, wherein the oligonucleotide is [fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)[n001R].m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C), or a salt thereof.

270. The oligonucleotide of Embodiment 264, wherein the oligonucleotide is [fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a salt thereof.

271. The oligonucleotide of Embodiment 264, wherein the oligonucleotide is [fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a salt thereof.

272. The oligonucleotide of any one of Embodiments 264-271, wherein each chiral internucleotidic linkage of the oligonucleotide independently has a diastereopurity of at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%.

273. The oligonucleotide of any one of Embodiments 264-272, wherein the oligonucleotide is a pharmaceutically acceptable salt.

274. The oligonucleotide of any one of Embodiments 264-273, wherein the oligonucleotide is a sodium salt.

275. The oligonucleotide of Embodiment 274, wherein the number of sodium ions in the sodium salt equals the total number of phosphorothioate internucleotidic linkages and natural phosphate internucleotidic linkages in the oligonucleotide.

276. A chirally controlled oligonucleotide composition comprising an oligonucleotide of any one of Embodiments 264-275, wherein the composition is enriched, relative to a substantially racemic preparation of the oligonucleotide, for the oligonucleotide.

277. A chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides each of which is an oligonucleotide of any one of Embodiments 264-275, wherein oligonucleotides of the plurality share the same constitution and at least 5% of all oligonucleotides in the composition that share the same constitution are oligonucleotides of the plurality.

278. A chirally controlled oligonucleotide composition comprising an oligonucleotide of any one of Embodiments 264-275, wherein at least 5% of all oligonucleotides in the composition that share the same constitution as the oligonucleotide are the oligonucleotide.

279. The composition of any one of Embodiments 277-278, wherein at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of all oligonucleotides in the composition that share the same constitution are oligonucleotides of the plurality.

280. The composition of any one of Embodiments 277-278, wherein at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of all oligonucleotides in the composition that share the same base sequence are oligonucleotides of the plurality.

281. An oligonucleotide composition comprising a plurality of oligonucleotides, wherein oligonucleotides of the plurality share:

1) a common base sequence; and

2) the same linkage phosphorus stereochemistry independently at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more chiral internucleotidic linkages;

wherein oligonucleotides of the plurality are an oligonucleotide of any one of Embodiments 264-275; and

wherein at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the oligonucleotides in the composition that share the common base sequence are oligonucleotides of the plurality.

282. The oligonucleotide composition of Embodiment 281, wherein oligonucleotides of plurality share the same linkage phosphorus stereochemistry independently at each chiral internucleotidic linkage.

283. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

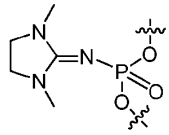
[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[n001R].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

284. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

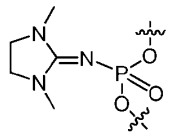
[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)[n001R].m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

285. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

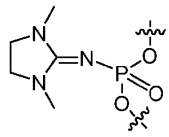
[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

286. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

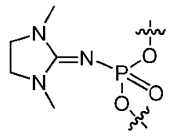
[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)[n001R].m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

287. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

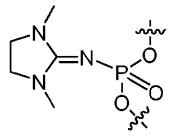
[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)[n001R].m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

288. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

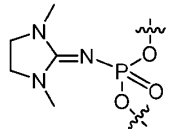
[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

289. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

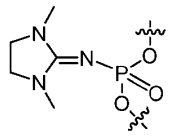
[fl2r](C)[Ssp].[fl2r](A)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(A)p.[fl2r](G)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](A)[Ssp].m(G)p.m(U)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

290. The composition of any one of Embodiments 283-289, wherein a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of all oligonucleotides that share the same base sequence as an oligonucleotide having the structure or a pharmaceutically acceptable salt thereof.

291. The composition of any one of Embodiments 283-289, wherein a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of all oligonucleotides that share the same constitution as an oligonucleotide having the structure or a pharmaceutically acceptable salt thereof.

292. The composition of any one of Embodiments 283-289, wherein a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% based on UV peak area at 260 nm.

EXEMPLIFICATION

[0454] Certain examples of provided technologies (compounds (oligonucleotides, reagents, etc.), compositions, methods (methods of preparation, use, assessment, etc.), etc.) were presented herein. Those skilled in the art appreciate that many technologies, e.g., those described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/223056, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, WO 2022/099159, etc., can be utilized to prepare and/or assess properties and/or activities of provided technologies in accordance with the present disclosure.

Example 1. Provided oligonucleotides can provide exon skipping.

[0455] As demonstrated herein, provided technologies, among other things, can provide skipping of exons in target transcripts, e.g., DMD transcripts. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed. Prior to usage in experiments, H2K mdx cells were maintained on matrigel (Scientific Laboratory Supplies) coated flasks in growth media (DMEM, 20% FBS, 2% Chick embryo extract (Sera Laboratories), 1% penicillin/streptomycin,

mouse IFN-γ (1:10,000; Millipore)). On day 0, H2K mdx cells were plated at 40,000 cells/well in 24 well plates coated with matrigel and differentiation media (DMEM, 1% penicillin/streptomycin, 5% horse serum inactivated (Thermo Fisher)) and allowed to differentiate for 4 days. On day 4, differentiated cells were gymnotically treated with 3 uM, 1 uM, or 0.3 uM of oligonucleotides targeting DMD for skipping of exon 23. Oligonucleotides were diluted to the desired concentration in 500 ul of differentiation medium. Medium on cells was aspirated and replaced with the oligonucleotide-containing medium for 3 hours. After 3 hours of incubation, oligonucleotide-containing medium was aspirated and replaced with 500 ul of fresh differentiation medium. Cells were allowed to differentiate for a further 4 days and subsequently harvested on day 8. RNA was collected using trizol-based extraction and transcribed into cDNA.

[0456] Exon skipping was quantified by qPCR. In brief, 3 ul of produced cDNA was used for pre-amplification using Taqman PreAmp Master Mix (Thermo Fisher). The pre-amplification was then diluted 1:5 with water, and 3 uL of the dilution was used for qPCR. TaqMan analysis using total and skipped TaqMan probes (see table below) and BioRad iQ Multiplex Powermix (BioRad) was performed.

Primer / Probe	Sequence
Total (mDMD20-21) Primer 1	AGATGACAACTACTGCCGAA
Total (mDMD20-21) Primer 2	GAAGAGCTGACAATCTGTTGAC
Total (mDMD20-21) Probe (FAM/ZEN/IABkFQ)	AGTCTACCACCCTATCAGAGCCAACA
Skipped (mDMD22-24) Primer 1	CTGAATATGAAATAATGGAGGAGAGACTCG
Skipped (mDMD22-24) Primer 2	CTTCAGCCATCCATTTCTGTAAGGT
Skipped (mDMD22-24) Probe (HEX/ZEN/IABkFQ)	ATGTGATTCTGTAATTTCC

Absolute quantification of exon skipping was determined using a standard curve (calculated with Bio-Rad CFX Maestro software) generated from GBlocks, which are diluted to 125,000 copies/uL and sequentially diluted 1:1 for 10 point-curve. Gblocks and cDNA were used at 3 uL per well (375,000 copies). Experiments were performed as biological triplicates (separate wells); qPCR reactions were performed as technical duplicates for each sample. Certain data are presented in the table below.

	% Skipping					
	3 uM		1 uM		0.3 uM	
	Average	Std Dev	Average	Std Dev	Average	Std Dev
Mock	0	0	0	0	0	0
ASO-0116492	21.75	1.77	13.00	0.85	5.60	1.41
ASO-0116487	7.95	2.05	4.45	0.78	1.15	0.21
ASO-0132575	10.35	0.49	5.80	2.40	2.60	1.27
ASO-0132574	8.65	0.21	5.25	2.62	2.05	0.35
ASO-0126022	5.95	0.07	3.60	0.00	1.40	0.28
ASO-0126024	10.50	2.97	5.85	2.90	2.40	1.13
ASO-0126026	9.75	2.90	3.90	0.14	1.00	0.28
ASO-0126028	8.10	0.85	3.40	0.28	2.00	0.99
ASO-0126030	8.60	0.71	6.70	3.54	2.10	0.28

ASO-0126032	10.50	0.57	4.70	0.14	1.60	0.14
ASO-0126034	10.55	0.92	5.35	0.78	2.10	0.99
ASO-0126035	5.75	0.78	2.60	0.14	0.90	0.42
ASO-0126037	5.20	0.85	3.45	3.04	1.50	1.41
ASO-0126038	7.85	0.35	4.65	1.20	1.40	0.14
ASO-0126040	4.00	0.85	1.40	0.42	0.30	0.00
ASO-0126042	12.55	0.49	5.25	0.92	1.40	0.14
ASO-0126044	2.90	0.28	1.60	0.00	0.20	0.14
ASO-0126046	10.10	0.00	3.55	0.92	0.95	0.35
ASO-0126054	11.45	0.07	5.15	1.06	3.30	1.84
ASO-0126155	5.70	0.99	2.75	0.64	0.90	0.57
ASO-0126328	17.10	0.99	9.15	0.35	4.70	0.42

[0457] As displayed in the table above, various oligonucleotides provided exon skipping in target transcripts, e.g., DMD transcripts. In some embodiments, oligonucleotides can provide skipping of exon 23 in DMD transcripts. In some embodiments, oligonucleotides can provide dose-dependent exon skipping in target transcripts. In some embodiments, oligonucleotides can provide dose-dependent skipping of exon 23 in DMD transcripts. In some embodiments, oligonucleotides comprising certain structural elements, e.g., PN internucleotidic linkages (e.g., phosphoryl guanidine linkages such as n001), controlled stereochemistry, and patterns thereof can provide higher levels of exon skipping as compared to others. In some embodiments, oligonucleotides comprising a PN internucleotidic linkage (e.g., a phosphoryl guanidine linkage such as n001) bonded to first and second nucleosides at the 5' end of the oligonucleotide can provide higher levels of exon skipping as compared to others, e.g., oligonucleotides comprising no PN internucleotidic linkages or oligonucleotides comprising a PN internucleotidic linkage at a position that is not bonded to first and second nucleosides at the 5' end of the oligonucleotide.

Example 2. Provided oligonucleotides can provide dose-dependent exon skipping.

[0458] As demonstrated herein, provided technologies, among other things, can provide skipping of exons in target transcripts, e.g., DMD transcripts. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed. Oligonucleotides were evaluated similarly as described in Example 1. In brief, on day 0, H2K mdx cells were plated at 40,000 cells/well in 24 well plates coated with matrigel and differentiation media (DMEM, 1% penicillin/streptomycin, 5% horse serum inactivated (Thermo Fisher)) and allowed to differentiate for 4 days. On day 4, differentiated cells were gymnotically treated with 3 uM, 1 uM, or 0.3 uM of oligonucleotides targeting DMD for skipping of exon 23. Oligonucleotides were diluted to the desired concentration in 500 ul of differentiation medium. Medium on cells was aspirated and replaced with the oligonucleotide-containing medium for 3 hours. After 3 hours of incubation, oligonucleotide-containing medium was aspirated and replaced with 500 ul of fresh differentiation medium. Cells were allowed to differentiate for a further 4 days and subsequently harvested on day 8. RNA was collected using trizol-based extraction and transcribed into

cDNA. Exon skipping was quantified using qPCR as described in Example 1. Certain data are presented in Figure 1 and the table below.

	% Skipping					
	3 uM		1 uM		0.3 uM	
	Average	Std Dev	Average	Std Dev	Average	Std Dev
Mock	0	0	0.09	0	0	0
ASO-0120569	7.59	0.24	3.15	0.48	0.81	0.14
ASO-0126332	38.38	1.74	23.35	3.03	9.99	0.37
ASO-0132638	30.19	3.14	16.25	1.53	6.74	0.01
ASO-0116492	18.02	1.56	8.79	1.96	3.15	0.46
ASO-0139741	24.43	1.02	14.21	0.17	6.54	0.47
ASO-0139740	25.52	0.01	12.25	0.72	4.22	0.01
ASO-0139739	29.22	1.36	14.74	0.79	6.32	0.16
ASO-0139771	36.64	3.55	19.17	0.48	8.94	0.61

[0459] As depicted in Figure 1, various oligonucleotides provided exon skipping in target transcripts, e.g., DMD transcripts. In some embodiments, oligonucleotides provided herein can provide skipping of exon 23 in DMD transcripts. In some embodiments, oligonucleotides provided herein can provide dose-dependent exon skipping in target transcripts. In some embodiments, oligonucleotides provided herein can provide dose-dependent skipping of exon 23 in DMD transcripts. In some embodiments, oligonucleotides comprising certain structural elements, e.g., PN internucleotidic linkages (e.g., phosphoryl guanidine linkages such as n001), controlled stereochemistry, and patterns thereof can provide higher levels of exon skipping in target transcripts, e.g., DMD transcripts as compared to others. In some embodiments, oligonucleotides comprising 4 or at least 4 PN internucleotidic linkages can provide comparable or higher levels of exon skipping in target transcripts, e.g., DMD transcripts as compared to oligonucleotides comprising 3 or no more than 3 PN internucleotidic linkages. In some embodiments, oligonucleotides comprising 4 or at least 4 PN internucleotidic linkages provide comparable or higher levels of exon skipping in target transcripts, e.g., DMD transcripts, e.g., DMD transcripts as compared to oligonucleotides comprising 6 or at least 6 PN internucleotidic linkages. In some embodiments, oligonucleotides comprising PN internucleotidic linkages at positions 1, 3, 6, and 17 (wherein the internucleotidic linkage bonded to the first and second nucleosides from the 5'-end is at position 1; numbering increasing towards the 3'-end) can provide comparable or higher levels of exon skipping in target transcripts, e.g., DMD transcripts as compared to oligonucleotides not comprising PN internucleotidic linkages at one or more of positions 1, 3, 6, and 17 and comprising PN internucleotidic linkages at zero or more additional positions.

Example 3. Provided oligonucleotides can provide exon skipping and increased protein levels *in vivo*.

[0460] As demonstrated herein, provided technologies, among other things, can provide skipping of exons in target transcripts, e.g., DMD transcripts *in vivo*. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed.

MDX mice were dosed intravenously weekly for 6 weeks with oligonucleotides targeting DMD for skipping of exon 23 at 75 mpk or PBS (negative control). Animals were sacrificed at 2 days after the last dose and tissues collected. Samples of frozen tissue were homogenized in trizol and RNA was collected. Collected RNA was transcribed to cDNA and diluted 1:4 with water, and 3 ul of the dilution was used to quantify exon skipping as described in Example 1. Transcribed cDNA was also used in qPCR, with appropriate probes and a 16-point standard curve of diluted oligonucleotide, to quantify oligonucleotide concentrations in tissues. Additionally, samples of frozen tissue were homogenized in tissue homogenization buffer (125 mM Tris pH 8.0, 12% SDS, 4% glycerol, 50 mM DTT, 5 mM EDTA, 1X protease inhibitor (Roche)) and spun down for 10 min at 14,000 rpm. Resulting supernatants were transferred to new tubes and total protein concentration was quantified using a Qubit protein assay kit (Thermo Fisher) according to manufacturer's protocol. Quantification of dystrophin protein in resulting supernatants was performed on a capillary immunoassay system (Wes; ProteinSimple) according to manufacturer's instructions and using the appropriate detection modules. In brief, supernatant samples were diluted to 0.5 ug/ul in 0.1x sample buffer, mixed with fluorescent master mix, denatured at 95°C for 5 min, and loaded together with blocking reagent, primary antibodies (1:50 anti-dystrophin antibody (Abcam; ab15277); 1:1000 anti-vinculin antibody (Thermo Fisher; MA5-11690)), and appropriate HRP-conjugated secondary antibodies. The instrument's default settings were used and a 7-point calibration curve was generated using pooled lysate from CB57BL10 mouse (healthy) tissue / mdx mouse (DMD) tissue. Chemiluminescence was quantified as area under the curve (AUC) of detected peaks using Compass software. Standard curves were generated by plotting percentage standard versus the ratio of AUC dystrophin / AUC vinculin. Dystrophin in treated samples was calculated using the standards.

[0461] Certain data are presented in the table below.

		% Skipping		% Protein Restoration		PK (ug oligo/g tissue)	
		Mean	SEM	Mean	SEM	Mean	SEM
Gastroc-nemius	PBS	0.19	0.06	0.08	0.20	0	0
	ASO-0116487	10.65	1.15	2.35	0.35	4.54	0.58
	ASO-0116492	24.13	2.14	8.86	2.15	41.00	8.16
Diaphragm	PBS	0.08	0.00	0.37	0.20	0	0
	ASO-0116487	6.13	1.22	1.72	0.25	3.86	0.95
	ASO-0116492	32.17	4.79	18.35	2.64	66.37	13.74
Heart	PBS	0.40	0.11	0.53	0.21	0	0
	ASO-0116487	0.89	0.22	1.21	0.05	2.28	0.32
	ASO-0116492	8.63	1.05	14.41	2.89	44.48	8.14

[0462] As depicted in the table above, various oligonucleotides provided exon skipping in target transcripts, e.g., DMD transcripts *in vivo*. In some embodiments, oligonucleotides provided herein can provide skipping of exon 23 in DMD transcripts *in vivo*. In some embodiments, oligonucleotides provided herein can provide increased levels of proteins, e.g., dystrophin encoded by target transcripts, e.g., DMD transcripts *in vivo*. In some embodiments, oligonucleotides comprising certain structural elements, e.g., PN internucleotidic linkages (e.g., phosphoryl guanidine linkages such as n001), controlled stereochemistry, and patterns thereof can

provide higher levels of exon skipping as compared to others, e.g., when utilized at certain positions. In some embodiments, oligonucleotides comprising 3 or at least 3 PN internucleotidic linkages (e.g., phosphoryl guanidine linkages such as n001) can provide higher levels of exon skipping in target transcripts *in vivo* as compared to oligonucleotides comprising no PN internucleotidic linkages. In some embodiments, oligonucleotides comprising 3 or at least 3 PN internucleotidic linkages (e.g., phosphoryl guanidine linkages such as n001) can provide higher levels of proteins encoded by target transcripts *in vivo* as compared to oligonucleotides comprising no PN internucleotidic linkages. In some embodiments, oligonucleotides comprising 3 or at least 3 PN internucleotidic linkages (e.g., phosphoryl guanidine linkages such as n001) can achieve higher levels of oligonucleotides in target tissues *in vivo* as compared to oligonucleotides comprising no PN internucleotidic linkages.

Example 4. Provided oligonucleotides can provide exon skipping and increased protein levels *in vivo*.

[0463] As demonstrated herein, provided technologies, among other things, can provide skipping of exons in target transcripts, e.g., DMD transcripts *in vivo*. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed. MDX mice were dosed intravenously at day 0 with oligonucleotides targeting DMD for skipping of exon 23 at 75 mpk or PBS (negative control). Animals were sacrificed at day 7 or day 28 and tissues collected. Samples of frozen tissue were homogenized in trizol and RNA was collected. Collected RNA was transcribed to cDNA and diluted 1:4 with water, and 3 ul of the dilution was used to quantify exon skipping as described in Example 1. Transcribed cDNA was also used in qPCR, with appropriate probes and a 16-point standard curve of diluted oligonucleotide, to quantify oligonucleotide concentrations in tissues. Additionally, samples of frozen tissue were homogenized and protein collected and quantified as described in Example 3. Certain data are presented in the table below.

			% Skipping		% Protein Restoration		Tissue Conc. (ug oligo/g tissue)	
			Mean	SEM	Mean	SEM	Mean	SEM
Gastroc-nemius	Day 7	PBS	0	0	-	-	0	0
		ASO-0116492	8.30	2.19	-	-	27.05	6.99
		ASO-0126332	20.03	5.23	-	-	21.57	4.00
		ASO-0132638	18.32	6.48	-	-	26.61	2.15
	Day 28	PBS	0.03	0.02	0.96	0.13	0	0
		ASO-0116492	4.70	0.91	2.39	0.58	3.47	0.64
		ASO-0126332	29.15	5.72	13.49	1.06	3.13	0.49
		ASO-0132638	16.62	0.54	2.20	0.64	3.09	0.35
Heart	Day 7	PBS	0	0	-	-	0	0
		ASO-0116492	2.98	0.20	-	-	8.13	1.81
		ASO-0126332	12.15	1.41	-	-	6.78	0.54
		ASO-0132638	4.49	1.21	-	-	10.27	2.00
	Day	PBS	0	0	0.90	0.31	0	0

	28	ASO-0116492	0.49	0.19	1.12	0.40	2.78	0.17
		ASO-0126332	5.47	0.54	2.40	0.49	4.08	0.66
		ASO-0132638	2.61	0.89	1.25	0.08	4.62	0.89
Diaphragm	Day 7	PBS	0	0	-	-	0	0
		ASO-0116492	7.53	0.97	-	-	17.56	0.84
		ASO-0126332	36.06	2.87	-	-	13.42	0.32
		ASO-0132638	12.84	0.75	-	-	18.69	0.88
	Day 28	PBS	0	0	0.22	0.04	0	0
		ASO-0116492	9.51	7.03	0.70	0.38	4.52	0.61
		ASO-0126332	37.96	5.69	15.57	2.80	4.35	0.37
		ASO-0132638	16.16	1.50	3.25	0.69	6.72	0.24

In the table above, "-" indicates no data reported.

[0464] As depicted in the table above, various oligonucleotides provided exon skipping in target transcripts, e.g., DMD transcripts *in vivo*. In some embodiments, oligonucleotides comprising certain structural elements, e.g., PN internucleotidic linkages (e.g., phosphoryl guanidine linkages such as n001), controlled stereochemistry, and patterns thereof can provide higher levels of exon skipping as compared to others. In some embodiments, oligonucleotides comprising certain structural elements, e.g., PN internucleotidic linkages (e.g., phosphoryl guanidine linkages such as n001), controlled stereochemistry, and patterns thereof can provide higher levels of protein restoration as compared to others.

Example 5. Provided oligonucleotides can provide exon skipping and increased levels of protein *in vivo*.

[0465] As demonstrated herein, provided technologies, among other things, can provide skipping of exons in target transcripts, e.g., DMD transcripts *in vivo*. An oligonucleotide comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof was designed and assessed. MDX mice were dosed intravenously at days 0, 14, and 28 with an oligonucleotide targeting DMD for skipping of exon 23 at 75 mpk or 150 mpk or PBS (negative control). Animals were sacrificed at day 42 or day 84 and tissues collected. Samples of frozen tissue were homogenized in trizol and RNA was collected. Collected RNA was transcribed to cDNA and diluted 1:4 with water, and 3 ul of the dilution was used to quantify exon skipping as described in Example 1. Transcribed cDNA was also used in qPCR, with appropriate probes and a 16-point standard curve of diluted oligonucleotide, to quantify oligonucleotide concentrations in tissues. Additionally, samples of frozen tissue were homogenized and protein collected and quantified as described in Example 3. Certain data are presented in the table below.

				% Skipping		% Protein Restoration		Tissue Conc. (ug oligo/g tissue)	
				Mean	SEM	Mean	SEM	Mean	SEM
Gastroc-nemius	Day 42	PBS	0 mpk	0	0	1.64	0.15	0	0
		ASO-0126332	75 mpk	63.12	5.15	15.28	2.37	31.08	8.84
			150 mpk	58.20	1.68	24.72	3.01	42.73	11.48
	Day	PBS	0 mpk	0.27	0.18	0.70	0.15	0	0

	84	ASO-0126332	75 mpk	55.53	3.20	36.30	1.64	13.41	3.98
			150 mpk	76.82	3.54	35.68	4.83	29.75	9.20
Diaphragm	Day 42	PBS	0 mpk	0	0	1.01	0.17	0	0
		ASO-0126332	75 mpk	69.08	2.25	31.12	2.19	31.16	4.20
			150 mpk	91.54	3.57	35.41	3.96	70.47	16.44
	Day 84	PBS	0 mpk	0	0	0.15	0.04	0	0
		ASO-0126332	75 mpk	49.45	2.30	30.10	1.19	12.46	2.56
			150 mpk	70.87	4.15	40.90	2.35	20.35	2.53
Heart	Day 42	PBS	0 mpk	0	0	1.10	0.08	0	0
		ASO-0126332	75 mpk	23.44	4.33	6.02	0.85	20.83	3.19
			150 mpk	32.62	1.71	7.20	0.47	46.63	5.15
	Day 84	PBS	0 mpk	0.08	0.00	1.53	0.31	0	0
		ASO-0126332	75 mpk	11.70	2.25	4.40	0.52	10.71	1.85
			150 mpk	20.79	1.99	7.88	1.06	30.53	3.88
Cortex	Day 42	PBS	0 mpk	0	0	-	-	-	-
		ASO-0126332	75 mpk	0.818	0.094	-	-	-	-
			150 mpk	2.437	0.363	-	-	-	-
	Day 84	PBS	0 mpk	0	0	-	-	0	0
		ASO-0126332	75 mpk	0.780	0.058	-	-	0.288	0.024
			150 mpk	4.560	1.573	-	-	0.611	0.051
Striatum	Day 42	PBS	0 mpk	0.015	0	-	-	-	-
		ASO-0126332	75 mpk	2.201	0.381	-	-	-	-
			150 mpk	6.074	1.153	-	-	-	-
	Day 84	PBS	0 mpk	0	0	-	-	0	0
		ASO-0126332	75 mpk	2.260	0.468	-	-	0.057	0.026
			150 mpk	10.140	2.703	-	-	0.150	0.044
Hippo-campus	Day 42	PBS	0 mpk	0	0	-	-	-	-
		ASO-0126332	75 mpk	1.457	0.061	-	-	-	-
			150 mpk	4.580	0.248	-	-	-	-
	Day 84	PBS	0 mpk	0	0	-	-	0	0
		ASO-0126332	75 mpk	1.480	0.186	-	-	0.200	0.065
			150 mpk	4.975	1.871	-	-	0.141	0.022
Cerebellum	Day 42	PBS	0 mpk	0	0	-	-	-	-
		ASO-0126332	75 mpk	1.993	0.204	-	-	-	-
			150 mpk	5.295	0.342	-	-	-	-
	Day 84	PBS	0 mpk	0	0	-	-	0	0
		ASO-0126332	75 mpk	2.640	0.169	-	-	0.398	0.050
			150 mpk	6.320	0.462	-	-	0.779	0.129
Brain Stem	Day 42	PBS	0 mpk	0	0	-	-	-	-
		ASO-0126332	75 mpk	2.921	0.102	-	-	-	-
			150 mpk	4.773	0.501	-	-	-	-
	Day 84	PBS	0 mpk	0	0	-	-	0	0
		ASO-0126332	75 mpk	2.920	0.306	-	-	0.067	0.008
			150 mpk	4.500	0.917	-	-	0.271	0.039
Spinal Cord	Day	PBS	0 mpk	-	-	-	-	-	-

	42	ASO-0126332	75 mpk	-	-	-	-	-	-
			150 mpk	-	-	-	-	-	-
	Day 84	PBS	0 mpk	0	0	-	-	0	0
		ASO-0126332	75 mpk	1.680	0.080	-	-	0.011	0.002
			150 mpk	4.040	0.269	-	-	0.012	0.001

In the table above, "-" indicates no data reported.

[0466] As depicted in the table above, various oligonucleotides provided exon skipping in target transcripts, e.g., DMD transcripts *in vivo*. In some embodiments, oligonucleotides provided herein can provide skipping of exon 23 in DMD transcripts *in vivo*. In some embodiments, oligonucleotides provided herein can provide increased levels of proteins, e.g., dystrophin encoded by target transcripts, e.g., DMD transcripts *in vivo*. In some embodiments, oligonucleotides provided herein can provide exon skipping in target transcripts (e.g., skipping of exon 23 in DMD transcripts) in various target tissues, e.g., skeletal muscle (e.g., gastrocnemius, diaphragm), cardiac muscle (e.g., heart), central nervous system (e.g., cortex, striatum, cerebellum, brain stem, spinal cord) *in vivo*. In some embodiments, oligonucleotides provided herein can provide increased levels of proteins, e.g., dystrophin encoded by target transcripts, e.g., DMD transcripts in various target tissues, e.g., skeletal muscle (e.g., gastrocnemius, diaphragm), cardiac muscle (e.g., heart) *in vivo*.

Example 6. Provided oligonucleotides can provide exon skipping.

[0467] As demonstrated herein, provided technologies, among other things, can provide skipping of exons in target transcripts, e.g., DMD transcripts. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed. Prior to usage in experiments, patient-derived immortalized fibroblasts containing a deletion in DMD exon 51 (R2811; Institut de Myologie) were cultured in a growth medium comprising DMEM (Thermo Fisher), 10% heat-inactivated FBS (Thermo Fisher), 50 ug/ml gentamycin (Thermo Fisher), and 1% penicillin-streptomycin (Thermo Fisher). Cells were engineered to express MyoD from a Tet-inducible promoter to drive transdifferentiation to muscle fate. To transdifferentiate, cells were seeded at a pre-determined density on matrigel-coated plates in medium comprising DMEM, 10% FBS, 50 ug/ml gentamycin, 1% penicillin-streptomycin, and 2 ug/ml doxycycline on day -1. At day 0, a complete media change was performed and medium comprising DMEM, 10 ug/ml insulin, 2 ug/ml doxycycline, and 1% penicillin-streptomycin was added to the cells. At day 2, cells were dosed gymnotically with 10 uM, 5 uM, or 2.5 uM, or alternatively with 0.12 uM, 0.37 uM, 1.11 uM, 3.33 uM or 10 uM of oligonucleotides targeting DMD transcripts for skipping of exon 52. After 16 hours of treatment, a complete media change was performed to remove oligonucleotides and cells were allowed to continue to grow. At day 7, a complete media was again performed and medium comprising DMEM, 10 ug/ml insulin, 2 ug/ml doxycycline, and 2% horse serum (Thermo Fisher) was added to the cells. At day 9, cells were harvested.

[0468] For quantification of exon skipping, RNA was collected using trizol-based extraction and transcribed into cDNA. Exon skipping was quantified using qPCR, which utilized the primers and probes in the table

below and hDMD53-54 FAM primers and probe (Thermo Fisher, Cat. # Hs01049450_m1) for total DMD transcript. Percent skipping was calculated by dividing skipped transcript by total transcript.

Primer / Probe	Sequence
Skipped Exon 52 Forward Primer	ACCACTATTGGAGCCTTTGA
Skipped Exon 52 Reverse Primer	CAGCCATTGTGTTGAATCCTTT
Skipped Exon 52 Probe	AAGAACACCTTCAGAACCGGAGGC

[0469] For quantification of dystrophin protein levels, lysates collected in RIPA buffer with protease inhibitor. Dystrophin protein levels were quantified using Jess capillary electrophoresis and antibodies for dystrophin (ab15277; Abcam) and vinculin (MA5-11690; Thermo Fisher). A standard curve of dystrophin was generated by diluting (from 100% to 0%) wild-type (WT) transdifferentiated fibroblast lysate from a healthy donor with lysates from the patient-derived transdifferentiated fibroblasts containing a deletion in DMD exon 51. Dystrophin signal was normalized to vinculin and % dystrophin relative to WT was calculated. Certain data are presented in Figure 5 and the table below.

[0470] Certain data are presented in Figure 2 and Figure 3 and in the tables below.

	% Skipping								
	10 uM			5 uM			2.5 uM		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
ASO-0140873	84.483	0.596	2	71.384	5.615	2	48.463	3.036	2
ASO-0140878	91.419	2.783	2	76.435	9.871	2	52.299	12.532	2
ASO-0140874	22.335	3.577	2	19.544	0.595	2	14.937	3.003	2
ASO-0140879	27.018	0.756	2	20.918	1.122	2	18.647	1.324	2
ASO-0140882	16.007	2.057	2	13.256	0.055	2	9.724	1.584	2
ASO-0140875	75.310	3.682	2	59.184	1.184	2	45.628	3.008	2
ASO-0140880	76.410	3.147	2	64.660	0.895	2	48.189	0.796	2
ASO-0140876	51.688	2.248	2	41.213	3.639	2	27.485	1.100	2
ASO-0140877	39.664	1.585	2	29.937	2.643	2	19.869	0.546	2
ASO-0140881	50.675	6.353	2	42.024	1.461	2	28.136	3.693	2
ASO-0140920	72.898	3.082	2	50.037	3.663	2	39.948	0.067	2
ASO-0140921	78.145	2.453	2	77.944	3.706	2	62.056	10.141	2
ASO-0140922	62.233	4.713	2	48.507	0.176	2	39.847	3.646	2
Mock	2.244	0.402	2	-	-	-	-	-	-

In the table above, "-" indicates no data reported.

	% Protein Restoration								
	10 uM			5 uM			2.5 uM		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
ASO-0140873	59.229	2.701	2	55.007	5.780	2	40.359	11.687	2
ASO-0140878	70.609	17.170	2	54.906	22.936	2	21.118	3.668	2
ASO-0140874	19.919	0.999	2	19.548	5.023	2	18.577	3.920	2
ASO-0140879	25.435	6.928	2	22.844	9.448	2	13.579	9.600	2

ASO-0140882	16.865	-	1	13.527	7.598	2	11.333	6.587	2
ASO-0140875	51.046	20.316	2	48.576	24.455	2	29.107	9.212	2
ASO-0140880	42.444	19.238	2	37.473	21.828	2	25.221	13.334	2
ASO-0140876	46.756	25.280	2	44.126	24.057	2	30.641	12.614	2
ASO-0140877	35.854	15.146	2	36.731	11.571	2	16.209	5.923	2
ASO-0140881	40.019	14.795	2	37.148	18.682	2	23.843	10.535	2
ASO-0140920	44.758	13.784	2	39.584	2.310	2	27.852	9.169	2
ASO-0140921	63.592	10.865	2	46.083	11.525	2	35.585	9.662	2
ASO-0140922	35.541	4.899	2	34.892	1.937	2	23.313	9.079	2
Mock	4.497	2.223	2	-	-	-	-	-	-

In the table above, "-" indicates no data reported.

		% Skipping				
	Dose (uM)	10	3.33	1.11	0.37	0.012
ASO-0140873	Mean	75.947	58.182	35.627	23.247	13.397
	SD	6.359	7.532	2.835	3.352	0.335
	n	2	2	2	2	2
ASO-0140878	Mean	86.573	65.160	39.951	24.559	11.229
	SD	3.166	2.142	1.595	1.398	1.569
	n	2	2	2	2	2
ASO-0141048	Mean	77.906	57.164	36.696	18.759	11.099
	SD	8.207	0.161	0.912	1.142	0.130
	n	2	2	2	2	2
ASO-0141054	Mean	82.266	63.520	31.926	15.474	8.693
	SD	3.010	0.633	2.209	4.254	1.923
	n	2	2	2	2	2
ASO-0140875	Mean	75.375	60.297	34.871	19.539	10.070
	SD	4.456	6.587	6.987	3.268	1.280
	n	2	2	2	2	2
ASO-0140880	Mean	82.091	66.964	47.412	22.018	10.652
	SD	5.372	3.629	7.176	0.677	0.288
	n	2	2	2	2	2
ASO-0141049	Mean	82.093	62.823	47.581	19.679	10.435
	SD	0.092	3.014	8.365	0.280	0.489
	n	2	2	2	2	2
ASO-0141055	Mean	85.015	64.760	36.963	20.210	9.049
	SD	1.237	6.508	1.736	0.257	1.072
	n	2	2	2	2	2
ASO-0140920	Mean	49.840	39.941	22.242	13.121	7.258
	SD	1.973	1.166	5.473	1.169	0.920
	n	2	2	2	2	2
ASO-0140921	Mean	85.456	75.932	47.825	32.849	17.149
	SD	10.990	2.775	6.409	3.078	3.805

	n	2	2	2	2	2
ASO-0141053	Mean	83.279	64.595	38.174	23.510	10.307
	SD	3.284	5.676	2.520	1.543	0.522
	n	2	2	2	2	2
ASO-0141056	Mean	75.925	63.491	41.864	21.826	13.438
	SD	2.023	2.407	0.256	1.239	1.846
	n	2	2	2	2	2
Mock	Mean	1.0665				
	SD	0.178				
	n	5				

		% Protein Restoration				
	Dose (uM)	10	3.33	1.11	0.37	0.012
ASO-0140873	Mean	56.002	34.430	16.062	6.003	0.476
	SD	13.832	4.814	4.359	0.836	2.850
	n	2	2	2	2	2
ASO-0140878	Mean	64.161	37.392	19.510	7.707	1.416
	SD	10.294	2.071	10.354	10.124	10.632
	n	2	2	2	2	2
ASO-0141048	Mean	58.035	39.705	20.698	5.909	-3.862
	SD	8.629	11.405	10.886	7.552	5.529
	n	2	2	2	2	2
ASO-0141054	Mean	53.510	32.867	14.141	5.077	-2.431
	SD	11.527	2.078	8.962	1.196	6.319
	n	2	2	2	2	2
ASO-0140875	Mean	41.934	14.924	15.231	5.770	-3.775
	SD	-	-	-	-	-
	n	1	1	1	1	1
ASO-0140880	Mean	53.994	43.166	19.191	8.579	-0.297
	SD	0.000	0.000	0.000	0.000	0.000
	n	1	1	1	1	1
ASO-0141049	Mean	31.300	29.379	18.775	-0.705	-4.426
	SD	-	-	-	-	-
	n	1	1	1	1	1
ASO-0141055	Mean	40.601	30.715	8.919	-0.315	-0.402
	SD	-	-	-	-	-
	n	1	1	1	1	1
ASO-0140920	Mean	34.409	19.670	7.313	5.227	-2.799
	SD	14.349	0.628	3.709	1.024	2.560
	n	2	2	2	2	2
ASO-0140921	Mean	71.790	56.156	38.794	18.799	4.621
	SD	10.196	8.623	5.627	5.345	1.055
	n	2	2	2	2	2

ASO-0141053	Mean	53.405	32.477	15.223	4.775	1.681
	SD	2.647	9.097	1.748	2.691	0.187
	n	2	2	2	2	2
ASO-0141056	Mean	62.675	52.957	22.585	12.581	2.945
	SD	10.313	0.584	2.154	5.576	3.665
	n	2	2	2	2	2
Mock	Mean	-2.324				
	SD	1.998				
	n	2				

In the table above, "-" indicates no data reported.

		% Skipping				
	Dose (uM)	10 uM	3.33 uM	1.11 uM	0.37 uM	0.12 uM
ASO-0140878	Mean	68.908	55.766	39.657	26.998	15.941
	SD	14.118	4.513	5.383	3.036	1.617
	n	2	2	2	2	2
ASO-0140873	Mean	86.807	68.772	47.898	29.165	18.945
	SD	0.376	3.459	2.395	0.373	0.129
	n	2	2	2	2	2
ASO-0141480	Mean	70.515	50.766	31.839	20.682	12.414
	SD	9.607	2.500	2.522	0.770	1.901
	n	2	2	2	2	2
ASO-0140921	Mean	89.750	73.333	59.672	35.319	21.440
	SD	2.736	1.368	4.047	0.429	1.464
	n	2	2	2	2	2
ASO-0140920	Mean	64.412	45.006	30.118	20.523	9.893
	SD	7.586	6.264	0.240	1.577	3.515
	n	2	2	2	2	2
ASO-0141481	Mean	60.671	45.375	31.670	19.803	10.503
	SD	4.346	4.355	0.651	2.075	0.672
	n	2	2	2	2	2
Mock	Mean	1.669				
	SD	0.269				
	n	4				

[0471] As depicted in Figure 2 and Figure 3 and the tables above, various oligonucleotides provided exon skipping in target transcripts, e.g., DMD transcripts and increased levels of proteins encoded by target transcripts, e.g., dystrophin proteins. In some embodiments, oligonucleotides provided herein can provide skipping of exon 52 in DMD transcripts. In some embodiments, oligonucleotides provided herein can provide dose-dependent exon skipping in target transcripts. In some embodiments, oligonucleotides provided herein can provide dose-dependent skipping of exon 52 in DMD transcripts. In some embodiments, oligonucleotides provided herein can provide increased levels of dystrophin proteins. In some embodiments, oligonucleotides

provided herein can provide does-dependent increases of dystrophin protein levels. In some embodiments, oligonucleotides comprising certain structural elements, e.g., PN internucleotidic linkages (e.g., phosphoryl guanidine linkages such as n001), controlled stereochemistry, and patterns thereof can provide higher levels of exon skipping of target transcripts, e.g., DMD transcripts as compared to others. In some embodiments, oligonucleotides comprising certain structural elements, e.g., PN internucleotidic linkages (e.g., phosphoryl guanidine linkages such as n001), controlled stereochemistry, and patterns thereof can provide higher levels of proteins, e.g., dystrophin proteins as compared to others. In some embodiments, oligonucleotides comprising one or more (e.g., 3 or 4) PN internucleotidic linkages can provide higher levels of exon skipping in target transcripts, e.g., DMD transcripts as compared to oligonucleotides comprising no PN internucleotidic linkages. In some embodiments, oligonucleotides comprising one or more (e.g., 3 or 4) PN internucleotidic linkages can provide higher levels of proteins, e.g., dystrophin proteins as compared to oligonucleotides comprising no PN internucleotidic linkages. In some embodiments, oligonucleotides comprising 4 or at least 4 PN internucleotidic linkages can provide comparable or higher levels of exon skipping in target transcripts, e.g., DMD transcripts as compared to oligonucleotides comprising 3 or no more than 3 PN internucleotidic linkages. In some embodiments, oligonucleotides comprising oligonucleotides comprising 4 or at least 4 PN internucleotidic linkages can provide comparable or higher levels of proteins, e.g., dystrophin proteins as compared to oligonucleotides comprising 3 or no more than 3 PN internucleotidic linkages. In some embodiments, oligonucleotides comprising a PN internucleotidic linkage bonded to first and second nucleosides at the 5' end of the oligonucleotide can provide comparable or higher levels of exon skipping in target transcripts, e.g., DMD transcripts as compared to others, e.g., oligonucleotides not comprising a PN internucleotidic linkage at a position that is bonded to first and second nucleosides at the 5' end of the oligonucleotide. In some embodiments, oligonucleotides comprising a PN internucleotidic linkage bonded to first and second nucleosides at the 5' end of the oligonucleotide can provide comparable or higher levels of proteins, e.g., dystrophin proteins as compared to others, e.g., oligonucleotides not comprising a PN internucleotidic linkage at a position that is bonded to first and second nucleosides at the 5' end of the oligonucleotide.

Example 7. Assessment of cytokine response induction.

[0472] Provided oligonucleotides were examined using a cell-based assay to determine potential associated induction of pro-inflammatory responses, e.g., cytokine responses. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed. As demonstrated herein, various oligonucleotides that can provide effective exon skipping showed low levels of cytokine responses. In some embodiments, oligonucleotides or compositions thereof provide low toxicity compared to a reference oligonucleotide or composition. Cells from a human Burkitt lymphoma B cell line, BJAB cells were incubated at a density of 4 x 10^5 cells/well for 24 hours with 30 uM, 10 uM, 3 uM, or 1 uM of oligonucleotides or PBS (negative control). After 24 hours, supernatant was collected

and assessed for the presence of cytokines using a commercial cytokine magnetic bead kit (EMD Millipore; HCYTOMAG-60K), according to the manufacturer's instructions and using the FLEXMAP 3D system (Luminex) for sample acquisition. Median fluorescent intensity was analyzed using a 5-parameter logistic curve-fitting method to calculate analyte concentrations in the test samples based on standard curves. For each cytokine, average fold change in concentration as compared to PBS was calculated by dividing average cytokine concentration for the tested condition by average cytokine concentration for PBS-treated wells. Cytokine fold changes were Z-standardized by subtracting the mean cytokine fold change and diving the centered values by the standard deviation of the folds changes. For each compound, the maximum responding concentration was identified as the concentration at which sum of the Z-standardized cytokine fold changes was highest. Euclidean distance versus PBS was calculated for each oligonucleotide's maximal responding concentration across Z-standardized cytokine fold changes. Certain data are presented in the table below. Determination of cytokine response induction potential by oligonucleotides can be used to assist in selection of oligonucleotides for usage in, e.g., treatment of a condition, disorder, or disease.

Oligonucleotide	Euclidean Distance vs. PBS
ASO-0140878	0.0272
ASO-0140879	0.0452
ASO-0140881	0.0500
ASO-0140873	0.0511
ASO-0140874	0.0531
ASO-0140882	0.0722
ASO-0140880	0.0951
ASO-0140921	0.2322
ASO-0104323	0.3154
ASO-0140876	0.3170
ASO-0140877	0.4010
ASO-0140875	0.4303
ASO-0140922	0.8020
ASO-0109259	0.8265
ASO-0140920	0.8476
ASO-0101369	1.4933
ASO-0100157	1.8982
ASO-0136356	11.7063

Example 8. Identification of potential off-target sequences for provided oligonucleotides.

[0473] Base sequences of provided oligonucleotides were examined for potential targeting of off-target sequences comprising up to 2 mismatches. In brief, base sequences of oligonucleotides targeting DMD were aligned against the reference human genome version GRCh38.p13 (obtained from the National Center for Biotechnology Information (NCBI)) and against pre-mRNA sequences from the human transcriptome (obtained from reference sequences available from the NCBI). The bowtie software package was used to identify off-target hits. Algorithm parameters were set to identify all hits with up to 2 mismatches between the oligonucleotide base sequences and the reference human genome and transcriptome sequences. Transcriptomic off-target hits were prioritized for further analysis by identifying off-target hits which

overlapped with predicted RNA-binding protein (RBP) motifs. RBP motifs were identified using the BRIO (Beam RNA Interaction Motif) search tool. Certain off-target hits are presented in the table below. Determination of potential off-target genes and potential overlaps with RBP motifs in said genes can be used to assist in selection of base sequences for oligonucleotides.

Oligonucleotide	Off-Target Gene	Overlap with Motif
ASO-0116227	ST3GAL3	Y
ASO-0116227	METTL14	N
ASO-0116227	ATP5PF	N
ASO-0116227	MSR1	Y
ASO-0116227	GDNF-AS1	N
ASO-0116227	LINC02325	N
ASO-0116227	TNFAIP8L3	N
ASO-0116227	RNF13	N
ASO-0116227	CIBAR1-DT	N
ASO-0116227	CPNE5	N
ASO-0116227	PRPF3	Y
ASO-0116227	IQCM	N
ASO-0116227	MINDY2	N
ASO-0116227	ADAMTSL1	N
ASO-0116227	ZNF438	N
ASO-0116227	TARID	Y
ASO-0116227	PKN2-AS1	N
ASO-0116227	KAT6B	N

Oligonucleotide	Off-Target Gene	Overlap with Motif
ASO-0116109	PDE4B	N
ASO-0116456	VSTM4	N
ASO-0116456	ATP1A1	N
ASO-0116456	FOXO3	N
ASO-0116267	PHACTR1	Y
ASO-0116267	PRPF3	Y
ASO-0116267	MROH2A	N
ASO-0116267	ST3GAL3	Y
ASO-0116267	CNR2	N
ASO-0116267	EPB41L3	N
ASO-0116267	B3GALT1	Y
ASO-0116267	LINC02325	N
ASO-0116267	POLR2F	N
ASO-0116267	ATP5PF	N
ASO-0116234	TPMTP4	N
ASO-0116234	KLHL5	Y
ASO-0116234	RNF13	N

Example 9. Assessment of complement activation in vitro.

[0474] Provided oligonucleotides were examined using a serum-based assay to determine potential associated induction of complement activation. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed. As demonstrated herein, various oligonucleotides that can provide effective exon skipping showed low levels of complement activation in vitro. In some embodiments, a provided oligonucleotide or a composition thereof provides low toxicity (e.g., when induction of complement activation is assessed) compared to a reference oligonucleotide or composition and/or another provided oligonucleotide or a composition thereof.

[0475] Normal human serum was obtained from Quidel (San Diego, CA) and cynomolgus monkey serum was obtained from Innovative Research (Novi, MI). The complement activation assay was performed as summarized herein. In brief, serum was quickly thawed in a 37°C water bath and then maintained at 2-8°C. For activation, 75 µL of serum was added to wells of a 96-well silicone coated microtiter plate (Thermo Scientific™ Cat# 60180P334), followed by addition of 25 µL oligonucleotides at a final concentration of 0.3

μM, 1 μM, 3 μM, 10 μM, and 30 μM or controls (e.g., PBS) (each in duplicate per timepoint). C3a and Factor Bb were quantified using commercial ELISA kits from Quidel, San Diego, CA (cat #A031, and A027, respectively) per manufacturer's instruction. A standard curve was generated for C3a and Factor Bb as determined by the total number of points (with no more than 2 points masked, and no 2 points from the same standard masked), with acceptable correlation coefficient, slope, upper asymptote, and y-intercept, as set by the manufacturer and %CV ≤ 20% between replicates; %CV ≤ 25% for the lowest and the highest passing standards. Samples were accepted for analysis, if for each analyte, %CV between replicates ≤ 20%. Data analysis for complement split products was performed using SoftMax®Pro 7.0.3 GxP (Molecular Devices). Calibration curves were generated by plotting the mean absorbance values (y-axis) versus concentration (x-axis) and fitting the curve per kit instructions. Complement level and 'Fold change over PBS' were calculated. Certain data are presented in the table below. Determination of complement activation potential by oligonucleotides can be used to assist in selection of oligonucleotides for usage in, e.g., treatment of a condition, disorder, or disease. As confirmed, e.g., by the results below, various oligonucleotides showed low levels of complement activation.

Oligonucleotide	Complement Activation
ASO-0140873	-
ASO-0140878	-
ASO-0141048	-
ASO-0140920	-
ASO-0125921	-
ASO-0141056	-

In table above, "-" indicates fold change over PBS ≤ 2; "+" indicates 2 ≤ Fold change over PBS < 3; "++" indicates 3 ≤ Fold change over PBS < 4; "+++" indicates 4 ≤ Fold change over PBS.

Example 10. Assessment of cytokine response induction in vitro.

[0476] Provided oligonucleotides were examined using a cell-based assay to determine potential associated induction of cytokine responses. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed. As demonstrated herein, various oligonucleotides that can provide effective exon skipping showed low levels of cytokine responses. In some embodiments, a provided oligonucleotide or a composition thereof provides low toxicity (e.g., when cytokine response induction is assessed) compared to a reference oligonucleotide or composition and/or another provided oligonucleotide or a composition thereof.

[0477] Human peripheral blood mononuclear cells (PBMCs) were incubated at a density of 4 x 10^5 cells/well for 24 hours with test articles in a 96-well plate. PBMCs were treated with 1X PBS or oligonucleotides (0.1, 0.3, 1, 3, 10 or 30 μM). After 24 hours, the supernatant was transferred, and the presence of cytokines (IFNγ, IL-6, IL-10, IP-10, MCP-1, MIP-1β, and TNFα) was assessed using a commercial cytokine magnetic bead kit

(R&D Systems, catalog number FCSTM18B-07). The procedure was carried out in accordance with the manufacturer's instructions and the samples were acquired on the FLEXMAP 3D® system. Median fluorescent intensity was analyzed using a 5-parameter logistic curve-fitting method to calculate analyte concentrations in the test samples based on standard curves. Any test article concentrations below the limit of quantification were replaced with a zero value and any above were replaced with highest standard concentration for the given cytokine. Significant increases in cytokine concentrations above PBS and a negative oligonucleotide control (ASO-0104323) were used to determine inflammatory risk. Certain data are presented in the table below as the fold-change over negative oligonucleotide control values (concentration at which the fold-change was observed is indicated in parentheses). Determination of cytokine response induction potential by oligonucleotides can be used to assist in selection of oligonucleotides for usage in, e.g., treatment of a condition, disorder, or disease. As confirmed, e.g., by the results below, ASO-0140921 and ASO-0141048 showed low cytokine responses induction under various conditions.

Oligonucleotide	Donor 1	Donor 2	Donor 3
ASO-0140921	IL-10↑ 1.4X (10μM)	IL-6↑ 1.3X (3μM); MIP-1β↑ 2.4X (30μM); TNF-α↑ 1.3X (3μM)	MCP-1↑ 8.9X (10μM), 16.8X (30μM); slight increases of IL-6, MIP1β, and TNF-α
ASO-0141048	IP-10↑ 1.9X (30μM)	MCP-1↑ 2X (30μM); MIP-1β↑ 1.6X (30μM)	No significant increase for any analytes compared to PBS or negative oligonucleotide control

Example 11. Assessment of cytotoxicity in vitro.

[0478] Provided oligonucleotides were examined using a human liver spheroid-based assay to determine potential associated cytotoxicity. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed. As demonstrated herein, various oligonucleotides that can provide effective exon skipping showed low levels of cytotoxicity in vitro. In some embodiments, a provided oligonucleotide or a composition thereof provides low toxicity (e.g., when cytotoxicity is assessed) compared to a reference oligonucleotide or composition and/or another provided oligonucleotide or a composition thereof.

[0479] The in vitro cytotoxicity of oligonucleotides was assessed by monitoring the viability and cytosolic leakage of 3D Insight™ Human Liver Spheroid Model that consisted of 10 donor mixture of primary human hepatocytes and physiological ratio of primary non-parenchymal cells treated with the oligonucleotide. This cell-based assay was a combination of a full dose response hepatoxicity assay (with a top concentration of 100 μM, with half-log dilutions down to 0.1 μM) with two biomarker readout: i) Cellular ATP measurement, which informs on viability of the liver spheroids, was conducted at the conclusion of the assay (day 14); ii) LDH leakage measurement in the supernatant, which informs on cytoplasmic membrane damage. LDH leakage

measurement was performed at every cell culture media exchange, to provide a kinetic measurement of toxicity (day 0-5, day 5-9, day 9-14). Certain data are presented in the table below. Determination of cytotoxicity potential by oligonucleotides can be used to assist in selection of oligonucleotides for usage in, e.g., treatment of a condition, disorder, or disease. As confirmed, e.g., by the results below, various oligonucleotides showed low cytotoxicity.

Oligonucleotide	IC_{50}
ASO-0140921	IC_{50} > 100μM
ASO-0141048	IC_{50} > 100μM
ASO-0140873	IC_{50} > 100μM
ASO-0140878	IC_{50} > 100μM
ASO-0140920	IC_{50} > 100μM
ASO-0141056	IC_{50} > 100μM

Example 12. Assessment of effects on mitochondrial functioning in vitro.

[0480] Provided oligonucleotides were examined using a cell-based assay to determine potential associated effects on mitochondrial functioning. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed. As demonstrated herein, various oligonucleotides that can provide effective exon skipping showed low levels of effects on mitochondrial functioning in vitro. In some embodiments, a provided oligonucleotide or a composition thereof provides low toxicity (e.g., when effects on mitochondrial functioning is assessed) compared to a reference oligonucleotide or composition and/or another provided oligonucleotide or a composition thereof.

[0481] Mitochondrial respiration and acidification rate analysis was done using Seahorse XFe96 technology. Human hepatocellular carcinoma–HepG2cells (ATCC® HB-8065) were seeded (3500 cells) in Seahorse XFe96-well cell culture microplates (Agilent) and treated for 72h with oligonucleotides. The treatment was performed in the presence of DMEM 5mM glucose (Gibco) + FBS 10% (Fetal bovine serum; Gibco). After treatment, the assay was initiated by removing the growth medium from each well and replacing it with 160μL of Seahorse assay medium (Sigma D5030 non-buffered medium + 5mM Glucose, 2mM Glutamine, and 1mM sodium pyruvate) prewarmed to 37°C. The oxygen consumption rate (OCR) was measured following the manufacturer's run program and using injection Port A - 1 μM Oligomycin, injection port B – 1.5 μM FCCP, and injection port C – 1 μM Rotenone/2.5 μM Antimycin A. The number of cells was measured by sulforhodamine B (SRB) assay, as described previously by Skehan et al. (J Natl Cancer Inst. 1990;82(13):1107-1112), at the end of the run program. Final seahorse analysis was performed in the Seahorse assay medium for 2 hours in the absence of the tested oligonucleotides. Extracellular acidification rate (ECAR) was measured at the same conditions described for OCR measurements in the presence of non buffered medium to avoid interference with pH measurements. Seahorse data analysis was performed with Seahorse Wave Desktop analysis software (Agilent). Results were represented in pmoles/min/104 cells. Treatment with 2'-Fluoro-

cytidine (2'-FC) for 72h was used as a positive control.

[0482] Total and mitochondrial ATP content was measured using bioluminescent ATP methods (CellTiter-Glo® Luminescent Cell Viability Assay; Promega). Cells were seeded in 96-well plates (4000 cells/well). After attaching, the cells were treated for 72h of treatment with 1, 3, 10, 30 or 100 μM of oligonucleotides in the presence of glucose. Glycolytic ATP was evaluated after its specific and potent inhibition with a cocktail of mitochondrial ATP synthesis inhibitors (30 μM oligomycin, 30 μM Antimycin A, and 5 μM Rotenone) for 5 min. The mitochondrial ATP content was measured by the difference between total ATP – glycolytic ATP. Afterwards, cells were immediately lysed to release the intracellular ATP by adding a lysis buffer containing a luciferase reagent for 10 min at room temperature. ATP concentration was determined using the light-emitting luciferase-catalyzed oxidation of luciferin in presence of ATP. The bioluminescence was measured (1s integration time) on a luminometer (CLARIOstar, BMG LABTECH). Standardization was performed with known quantities of standard ATP measured under the same conditions. Number of cells was determined by SRB assay and values normalized by the number of cells. Treatment with 2'-Fluoro-cytidine (2'-FC) for 72h was used as a positive control.

[0483] Quantification of mitochondrial DNA / genomic DNA was measured using an Applied Biosystems 7500 Real Time PCR machine. For DNA extraction, cells were seeded in 6-well plates (150000 cells/well). After attaching, the cells were treated for 72h of treatment with 30 or 100 μM of oligonucleotides in the presence of glucose. Total DNA extraction was performed following the protocol described in Dneasy Blood & Tissue kit (Qiagen). Treatment with 2'-Fluoro-cytidine (2'-FC) for 72h was used as a positive control. Total DNA was quantified using a Nanodrop and 1 ug/ul of total DNA was used for amplification. The real time PCR was performed using the TaqMan probe system and RNR1 probe: Hs02596859_g1 (targeting mitochondrial DNA) and B2M probe: Hs06637353_s1 (targeting genomic DNA).

[0484] Glutathione/glutathione disulfide (GSH/GSSG) ratio was measured using a bioluminescent GSH/GSSG-Glo assay (Promega). Cells were seeded in 96-well plates (3000 cells/well). After attaching, the cells were treated for 72h with 1, 3, 10, 30 or 100 μM of oligonucleotides in the presence of glucose. The bioluminescence was measured (1s integration time) on a luminometer (CLARIOstar, BMG LABTECH). The GSH/GSSG ratio was calculated using the following formula: (Net total glutathione RLU – Net GSSG RLU/Net GSSG RLU/2). GSH levels were also calculated using a generated standard curve of GSH. The treatment with 0.2 mM buthionine sulphoximine (BSO) for 72h was used as a positive control for decreasing GSH levels.

[0485] Certain data of the above described assays are presented in the table below. Determination of effects on mitochondrial functioning by oligonucleotides can be used to assist in selection of oligonucleotides for usage in, e.g., treatment of a condition, disorder, or disease. As confirmed, e.g., by the results below, ASO-0140921 and ASO-0141048 showed no or low impact in various mitochondrial functioning assessments.

Oligonucleotide	Mitochondrial respiration and	Mitochondrial ATP/ total ATP	Mitochondrial DNA content	GSH/GSSG

	acidification rate			
ASO-0140921	Only increases uncoupled respiration at 3 and 100 μM without affecting routine respiration and ECAR	No impact	Increases more than 3-fold of PBS at 100 μM	No impact
ASO-0141048	No impact on OCR, only decreases ECAR at 3 μM	Slightly increases from 3 to 30 μM	Increases more than 3-fold of PBS at 30 μM but down to less than 3-fold at 100 μM	No impact

Example 13. Provided oligonucleotides can provide exon skipping and increased protein levels *in vivo*.

[0486] As demonstrated herein, provided technologies, among other things, can provide skipping of exons in target transcripts, e.g., DMD transcripts. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed including in mice models, and various results, e.g., those described below, confirmed such oligonucleotides can provide exon skipping *in vivo* in a variety of samples (e.g., heart, quadriceps, diaphragm, tibialis, etc.). Among other things, such results support that various provided oligonucleotides designed for exon skipping in human (e.g., ASO-0140921, ASO-0141048, etc.) can provide exon skipping in human.

[0487] MDX mice (male, 7-8 weeks old) were dosed intravenously on day 0 with oligonucleotides targeting DMD for skipping of exon 23 at 5, 10, 30, or 75 mpk or PBS (negative control). Animals were sacrificed at various timepoints after dosing as listed in the table below and tissues (e.g., heart, diaphragm, quadriceps, tibialis) collected. Fresh tissues were snap frozen in Liquid Nitrogen in 2-mL tubes and stored at -80C (on dry ice). For histology, quadriceps was postfixed in 10% formalin and processed to slides (paraffin embedded sections).

Oligonucleotide	Dose (mg/kg)	Timepoints for Sacrifice (day)
PBS	-	28, 56, 84
ASO-0116492	5	28
	10	28, 56
	30	28, 56
	75	28, 56
ASO-0126332	5	28
	10	28, 56
	30	28, 56
	75	28, 56, 84

[0488] Samples of frozen tissue were homogenized in trizol and RNA was collected. Collected RNA was transcribed to cDNA and diluted 1:4 with water, and 3 ul of the dilution was used to quantify exon skipping as described in Example 1. Additionally, samples of frozen tissue were homogenized in tissue homogenization buffer (125 mM Tris pH 8.0, 12% SDS, 4% glycerol, 50 mM DTT, 5 mM EDTA, 1X protease inhibitor

(Roche)) and spun down for 10 min at 14,000 rpm. Resulting supernatants were transferred to new tubes and total protein concentration was quantified using a Qubit protein assay kit (Thermo Fisher) according to manufacturer's protocol. Quantification of dystrophin protein in resulting supernatants was performed on a capillary immunoassay system (Wes; ProteinSimple) according to manufacturer's instructions and using the appropriate detection modules. In brief, supernatant samples were diluted to 0.5 ug/ul in 0.1x sample buffer, mixed with fluorescent master mix, denatured at 95°C for 5 min, and loaded together with blocking reagent, primary antibodies (1:50 anti-dystrophin antibody (Abcam; ab15277); 1:1000 anti-vinculin antibody (Thermo Fisher; MA5-11690)), and appropriate HRP-conjugated secondary antibodies. The instrument's default settings were used and a 7-point calibration curve was generated using pooled lysate from CB57BL10 mouse (healthy) tissue / mdx mouse (DMD) tissue. Chemiluminescence was quantified as area under the curve (AUC) of detected peaks using Compass software. Standard curves were generated by plotting percentage standard versus the ratio of AUC dystrophin / AUC vinculin. Dystrophin in treated samples was calculated using the standards. Certain data are presented in the tables below.

			% Exon skipping								
			ASO-0116492			ASO-0126332			PBS		
Tissue	Dose (mg/kg)	Time-point (day)	Mean	SD	N	Mean	SD	N	Mean	SD	N
Heart	5	28	0.0452	0.0248	5	0.415	0.107	5	0.008836	0.00453	5
	10		0.136	0.126	5	0.793	0.179	5			
	30		0.378	0.130	5	2.9	0.462	4			
	75		0.895	0.251	3	5.628	3.114	5			
	10	56	0.0227	0.00977	5	0.555	0.0962	5	0.008026	0.005144	4
	30		0.0788	0.0188	5	1.462	0.407	4			
	75		0.274	0.0755	5	2.109	0.844	5			
	75	84	-	-	-	1.426	0.371	5	0.009128	0.004648	4
Quad	5	28	0.1734	0.113	5	1.669	1.02	5	0	0	5
	10		0.1904	0.106	4	4.381	2.386	5			
	30		2.079	2.124	5	20.41	2.487	4			
	75		3.27	0.547	3	40.02	11.64	5			
	10	56	0.07934	0.0436	5	1.044	0.374	5	0	0	4
	30		0.3642	0.472	5	5.287	1.903	4			
	75		1.171	0.712	5	22.71	10.71	5			
	75	84	-	-	-	8.121	1.912	5	0	0	5
Diaphragm	5	28	0.0561	0.0173	5	1.563	0.584	5	0.006099	0.0003118	2
	10		0.08674	0.02458	5	3.97	1.42	5			
	30		0.7266	0.226	5	11.44	2.786	4			
	75		2.99	1.169	3	34.91	10.31	5			
	10	56	0.07908	0.08909	4	3.125	2.484	5	0.03439	0.05243	4
	30		0.4116	0.4748	5	12.67	7.455	4			

	75		1.055	0.7811	5	17.31	10.06	5			
	75	84	-	-	-	8.686	3.033	5	0.01587	0.02043	3
Tibialis	5	28	2.455	1.761	5	4.805	0.846	5	3.091	0.894	4
	10		2.915	1.561	5	6.805	0.9129	5			
	30		5.651	1.884	5	16.09	2.337	4			
	75		7.611	1.687	3	29.75	10.92	5			
	10	56	1.907	1.784	5	5.039	0.922	4	1.829	1.158	5
	30		2.378	1.153	5	11.64	6.55	4			
	75		4.597	0.6525	5	20.98	9.79	5			
	75	84	-	-	-	10.91	2.075	5	1.097	0.6886	5

In the table above, "-" indicates no data reported.

			% Protein Restoration								
			ASO-0116492			ASO-0126332			PBS		
Tissue	Dose (mg/kg)	Time-point (day)	Mean	SD	N	Mean	SD	N	Mean	SD	N
Heart	5	28	-	-	-	-	-	-	3.67E-12	1.60E-06	3
	10		0.012	0.022	5	0.074	0.056	4			
	30		0.034	0.035	5	0.208	0.010	4			
	75		0.129	0.052	3	0.991	0.297	5			
	10	56	-	-	-	-	-	-	4.00E-07	7.96E-02	5
	30		0.008	0.018	5	0.209	0.064	5			
	75		0.088	0.060	5	0.607	0.150	5			
	75	84	-	-	-	0.330	0.075	5	0	0	5
Quad	5	28	-	-	-	-	-	-	0	0	3
	10		0.443	0.475	5	3.236	0.854	5			
	30		2.380	0.972	5	6.934	2.621	4			
	75		3.450	0.267	3	18.540	3.072	5			
	10	56	0.854	0.457	5	2.816	1.213	5	2.80E-03	6.26E-03	5
	30		1.205	0.586	5	6.844	0.852	4			
	75		2.309	1.032	5	17.880	4.206	5			
	75	84	-	-	-	13.650	1.140	5	-1.00E-09	5.31E-02	3
Diaphragm	5	28	-	-	-	-	-	-	4.04E-02	9.20E-02	5
	10		0.255	0.371	5	1.719	0.453	5			
	30		0.247	0.217	5	6.698	1.224	4			
	75		1.131	0.376	3	18.850	3.578	5			
	10	56	0.012	0.027	5	1.589	1.522	5	1.39E-01	2.01E-01	4
	30		0.000	0.000	5	7.381	4.370	4			
	75		0.106	0.110	5	17.320	3.618	5			
	75	84	-	-	-	7.751	1.869	5	1.60E-01	3.58E-01	3
Tibialis	5	28	-	-	-	-	-	-	-8.00E-07	1.25E-01	5
	10		0.188	0.740	5	0.366	0.508	5			
	30		1.147	1.087	5	4.778	3.415	4			

	75		1.989	1.094	3	11.470	5.251	5			
	10	56	0.207	0.464	5	0.476	0.468	5	1.52E-01	2.10E-01	5
	30		0	0	5	2.490	1.274	4			
	75		0	0	5	14.920	6.303	5			
	75	84	-	-	-	7.509	2.822	5	9.25E-02	1.28E-01	5

In the table above, "-" indicates no data reported.

[0489] As depicted in the tables above, various oligonucleotides provided exon skipping in target transcripts, e.g., DMD transcripts *in vivo*. In some embodiments, oligonucleotides provided herein can provide skipping of exon 23 in DMD transcripts *in vivo*. In some embodiments, oligonucleotides provided herein can provide increased levels of proteins, e.g., dystrophin encoded by target transcripts, e.g., DMD transcripts *in vivo*. In some embodiments, oligonucleotides provided herein can provide exon skipping in target transcripts (e.g., skipping of exon 23 in DMD transcripts) in various target tissues, e.g., skeletal muscle (e.g., tibialis, quadriceps, diaphragm), cardiac muscle (e.g., heart) *in vivo*. In some embodiments, oligonucleotides provided herein can provide increased levels of proteins, e.g., dystrophin encoded by target transcripts, e.g., DMD transcripts in various target tissues, e.g., skeletal muscle (e.g., tibialis, quadriceps, diaphragm), cardiac muscle (e.g., heart) *in vivo*.

Example 14. Provided oligonucleotides can provide exon skipping and increased protein levels *in vivo*.

[0490] As demonstrated herein, provided technologies, among other things, can provide skipping of exons in target transcripts, e.g., DMD transcripts *in vivo*. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed including in mice models, and various results, e.g., those described below, confirmed such oligonucleotides can provide exon skipping and production of dystrophin *in vivo* in a variety of samples (e.g., heart, quadriceps, diaphragm, tibialis, etc.). Among other things, such results support that various provided oligonucleotides designed for exon skipping in human (e.g., ASO-0140921, ASO-0141048, etc.) can provide exon skipping in human.

[0491] Male and female dystrophin-utrophin double knockout (dko) mice were dosed subcutaneously with oligonucleotides targeting DMD for skipping of exon 23 using various dosing regimens indicated in the table below or PBS (negative control). Animals were weighed weekly and other physical changes (e.g., kyphosis) were recorded. Animals were sacrificed at various timepoints after dosing as listed in the table below and serum and tissues (brain, liver, kidney, heart, diaphragm, quadriceps, tibialis) collected. Fresh tissues were snap frozen in Liquid Nitrogen in 2-mL tubes and stored at -80C (on dry ice). For histology, quadriceps was postfixed in 10% formalin and processed to slides (paraffin embedded sections). Serum was used serum biomarker analysis (creatine kinase (CK), alanine transaminase (ALT), aspartate aminotransferase (AST), and glutamate dehydrogenase (GLDH)) as known in the art. In some experiments, suitable variants of this protocol were used.

Oligonucleotide	Dosing Regimen	Timepoint for Sacrifice (day)
ASO-0126332	6 x 10 mg/kg weekly, starting at day 10	47
	6 x 30 mg/kg weekly, starting at day 10	47
	6 x 75 mg/kg weekly, starting at day 10	47
	75 mg/kg at day 10	47
	75 mg/kg at day 38	75
	75 mg/kg at day 10 and day 38	75
ASO-0116492	6 x 30 mg/kg weekly, starting at day 10	47

[0492] Samples of frozen tissue were homogenized in trizol and RNA was collected. Collected RNA was transcribed to cDNA and diluted 1:4 with water, and 3 ul of the dilution was used to quantify exon skipping as described in Example 1. Additionally, samples of frozen tissue were homogenized in tissue homogenization buffer (125 mM Tris pH 8.0, 12% SDS, 4% glycerol, 50 mM DTT, 5 mM EDTA, 1X protease inhibitor (Roche)) and spun down for 10 min at 14,000 rpm. Resulting supernatants were transferred to new tubes and total protein concentration was quantified using a Qubit protein assay kit (Thermo Fisher) according to manufacturer's protocol. Quantification of dystrophin protein in resulting supernatants was performed on a capillary immunoassay system (Wes; ProteinSimple) according to manufacturer's instructions and using the appropriate detection modules. In brief, supernatant samples were diluted to 0.5 ug/ul in 0.1x sample buffer, mixed with fluorescent master mix, denatured at 95˚C for 5 min, and loaded together with blocking reagent, primary antibodies (1:50 anti-dystrophin antibody (Abcam; ab15277); 1:1000 anti-vinculin antibody (Thermo Fisher; MA5-11690)), and appropriate HRP-conjugated secondary antibodies. The instrument's default settings were used and a 7-point calibration curve was generated using pooled lysate from CB57BL10 mouse (healthy) tissue / mdx mouse (DMD) tissue. Chemiluminescence was quantified as area under the curve (AUC) of detected peaks using Compass software. Standard curves were generated by plotting percentage standard versus the ratio of AUC dystrophin / AUC vinculin. Dystrophin in treated samples was calculated using the standards. Certain data are presented in the tables below.

			% Exon Skipping								
			ASO-0126332			ASO-0116492			PBS		
Tissue	Dose (mg/kg)	Time-point (day)	Mean	SD	N	Mean	SD	N	Mean	SD	N
Heart	6x10 mg/kg weekly	47	4.716	0.8963	5	-	-	-	0.00562	0.00111	3
	6x30 mg/kg weekly	47	11.41	2.737	4	2.256	0.7835	5	-	-	-
	6x75 mg/kg weekly	47	20.86	2.728	4	-	-	-	-	-	-
	75 mg/kg at day 10	47	11.38	1.854	4	-	-	-	-	-	-
	75 mg/kg at day 38	75	23.93	7.43	4	-	-	-	-	-	-
	2x 75	75	28.22	12.45	5	-	-	-	-	-	-

	mg/kg (d10 and d38)										
Quad	6x10 mg/kg weekly	47	4.115	1.481	5	-	-	-	0.0310	0.0176	5
	6x30 mg/kg weekly	47	19.25	2.386	5	4.866	2.262	5	-	-	-
	6x75 mg/kg weekly	47	58	13.96	5	-	-	-	-	-	-
	75 mg/kg at day 10	47	9.555	6.788	4	-	-	-	-	-	-
	75 mg/kg at day 38	75	21.93	4.273	4	-	-	-	-	-	-
	2x 75 mg/kg (day 10 and 38)	75	26.38	13.09	4	-	-	-	-	-	-
Diaphragm	6x10 mg/kg weekly	47	5.784	1.991	5	-	-	-	0	0	5
	6x30 mg/kg weekly	47	43.21	17.13	5	10.01	3.419	5	-	-	-
	6x75 mg/kg weekly	47	90.68	16.11	5	-	-	-	-	-	-
	75 mg/kg at day 10	47	13.4	10.13	4	-	-	-	-	-	-
	75 mg/kg at day 38	75	4.724	1.567	4	-	-	-	-	-	-
	2x 75 mg/kg (day 10 and 38)	75	5.413	1.702	5	-	-	-	-	-	-
Tibialis	6x10 mg/kg weekly	47	17.01	4.144	5	-	-	-	0.0626	0.0284	2
	6x30 mg/kg weekly	47	67.47	15.13	5	11.57	3.947	4	-	-	-
	6x75 mg/kg weekly	47	106	4.128	4	-	-	-	-	-	-
	75 mg/kg at day 10	47	38.68	5.801	4	-	-	-	-	-	-
	75 mg/kg at day 38	75	39.6	6.377	4	-	-	-	-	-	-
	2x 75 mg/kg (day 10 and 38)	75	33.46	4.582	4	-	-	-	-	-	-

In the table above, "-" indicates no data reported.

			% Protein Restoration								
			ASO-0126332			ASO-0116492			PBS		
Tissue	Dose (mg/kg)	Time -point (day)	Mean	SD	N	Mean	SD	N	Mean	SD	N
Heart	6x10 mg/kg weekly	47	0.182	0.0909	5	-	-	-	0.002	0.4942	5
	6x30 mg/kg	47	1.85	0.490	5	-0.146	0.313	5	-	-	-

	weekly										
	6x75 mg/kg weekly	47	2.584	0.546	5	-	-	-	-	-	-
	75 mg/kg at day 10	47	2.598	1.816	4	-	-	-	-	-	-
	75 mg/kg at day 38	75	1.344	0.246	5	-	-	-	-	-	-
	2x 75 mg/kg (d10 and d38)	75	0.2275	0.506	4	-	-	-	-	-	-
Quad	6x10 mg/kg weekly	47	0.236	0.184	5	-	-	-	0.002	0.4876	5
	6x30 mg/kg weekly	47	1.544	1.299	5	-0.494	0.1292	5	-	-	-
	6x75 mg/kg weekly	47	3.218	1.243	5	-	-	-	-	-	-
	75 mg/kg at day 10	47	0.4	0.497	5	-	-	-	-	-	-
	75 mg/kg at day 38	75	6.21	2.373	5	-	-	-	-	-	-
	2x 75 mg/kg (day 10 and 38)	75	2.473	1.33	4	-	-	-	-	-	-
Diaphragm	6x10 mg/kg weekly	47	0.85	1.137	5	-	-	-	0	0.186	5
	6x30 mg/kg weekly	47	11.36	4.673	5	0.37	0.2729	5	-	-	-
	6x75 mg/kg weekly	47	18.69	8.484	5	-	-	-	-	-	-
	75 mg/kg at day 10	47	9.248	5.455	5	-	-	-	-	-	-
	75 mg/kg at day 38	75	17.51	3.699	5	-	-	-	-	-	-
	2x 75 mg/kg (day 10 and 38)	75	14.72	7.419	4	-	-	-	-	-	-
Tibialis	6x10 mg/kg weekly	47	0.254	0.212	5	-	-	-	0	0.1697	5
	6x30 mg/kg weekly	47	2.618	2.057	5	-0.012	0.1455	5	-	-	-
	6x75 mg/kg weekly	47	5.866	1.743	5	-	-	-	-	-	-
	75 mg/kg at day 10	47	0.044	0.424	5	-	-	-	-	-	-
	75 mg/kg at day 38	75	5.664	2.077	5	-	-	-	-	-	-
	2x 75 mg/kg (day 10 and 38)	75	1.335	1.417	4	-	-	-	-	-	-

In the table above, "-" indicates no data reported.

	CK			ALT			AST			GLDH		
Dose (mg/kg)	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
PBS	8069	3835	5	200.2	40.09	5	1016	265.6	5	51.07	11.38	5
6 x 10 mg/kg weekly	2422	1575	3	127.3	14.01	3	553.3	165.1	3	56.07	17.58	3
6 x 30 mg/kg weekly	1780	720.7	5	63.6	17.5	5	377	195.8	5	21.23	2.995	5
6 x 75 mg/kg weekly	917.8	537.9	5	50	19.85	5	185.4	86	5	26.35	6.84	5
75 mg/kg at day 10	4869	1244	5	172	130.1	3	522	156.1	3	28.52	9.682	3
75 mg/kg at day 38	2773	2181	3	62.33	20.53	3	332.3	117.4	3	25.19	5.142	3
2 x 75 mg/kg (day 10 and 38)	1205	1104	5	67	30.5	5	262	122.3	5	15.26	4.1	5

	ASO-0116492											
	CK			ALT			AST			GLDH		
Dose (mg/kg)	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
PBS	8069	3835	5	200.2	40.09	5	1016	265.6	5	51.07	11.38	5
6 x 30 mg/kg weekly	2546	571.8	5	149.8	27.48	5	611.6	88.9	5	42.4	7.134	5

	Kyphosis		
	No.	Mild	Severe
PBS	0/8	4/8	4/8
ASO-0126332, 6x10mg/kg weekly	0/5	3/5	2/5
ASO-0126332, 6x30mg/kg weekly	5/5	0/5	0/5
ASO-0126332 6x75mg/kg weekly	5/5	0/5	0/5
ASO-0126332 Single 75mg/kg @d10	4/5	1/5	0/5

		% Weight Change					
	Timepoint (Day)	10	17	24	31	38	45
PBS	Mean	100	129.687	156.002	221.764	305.659	327.247
	SD	0	9.492	18.915	46.942	50.855	68.365
	N	10	10	10	10	9	8
ASO-0126332, 6x10mg/kg weekly	Mean	100	138.099	189.715	292.146	363.638	369.504
	SD	0	4.241	8.486	32.782	52.228	84.969
	N	5	5	5	5	5	5
ASO-0126332, 6x30mg/kg weekly	Mean	100	141.320	202.139	334.998	411.741	459.505
	SD	0	29.243	40.775	41.406	69.597	91.665
	N	5	5	5	5	5	5
ASO-0126332, 6x75mg/kg weekly	Mean	100	134.204	201.939	318.903	395.168	438.292
	SD	0	9.154	25.852	19.303	50.612	61.966
	N	5	5	5	5	5	5

ASO-0116492, 6x30mg/kg weekly	Mean	100	131.650	155.712	262.301	390.303	441.020
	SD	0	11.472	20.268	35.570	26.541	28.110
	N	5	5	5	5	5	5
ASO-0126332, 75mg/kg 1X d0	Mean	100	140.699	194.648	278.976	377.075	420.637
	SD	0	9.206	19.506	26.911	55.776	59.149
	N	7	7	7	7	5	5

	% Weight change at Nx					
	PBS	ASO-0126332, 6x10mg/kg weekly	ASO-0126332, 6x30mg/kg weekly	ASO-0126332, 6x75mg/kg weekly	ASO-0116492, 6x30mg/kg weekly	ASO-0126332, single 75mg/kg
Mean	327.2	369.5	459.5	438.3	441	420.6
SD	68.36	84.97	91.67	61.97	28.11	59.15
N	8	5	5	5	5	5

[0493] As depicted in the tables above, various oligonucleotides provided exon skipping in target transcripts, e.g., DMD transcripts *in vivo*. In some embodiments, oligonucleotides provided herein can provide skipping of exon 23 in DMD transcripts *in vivo*. In some embodiments, oligonucleotides provided herein can provide increased levels of proteins, e.g., dystrophin encoded by target transcripts, e.g., DMD transcripts *in vivo*. In some embodiments, oligonucleotides provided herein can provide exon skipping in target transcripts (e.g., skipping of exon 23 in DMD transcripts) in various target tissues, e.g., skeletal muscle (e.g., tibialis, quadriceps, diaphragm), cardiac muscle (e.g., heart) *in vivo*. In some embodiments, oligonucleotides provided herein can provide increased levels of proteins, e.g., dystrophin encoded by target transcripts, e.g., DMD transcripts in various target tissues, e.g., skeletal muscle (e.g., tibialis, quadriceps, diaphragm), cardiac muscle (e.g., heart) *in vivo*. In some embodiments, oligonucleotides provided herein can provide decreased levels of one or more serum biomarkers, e.g., CK, ALT, AST, GLDH *in vivo*. In some embodiments, one or more of such serum biomarkers are useful for assessing oligonucleotides and compositions thereof for administration or delivery to human patients. In some embodiments, one or more of such serum biomarkers are useful for assessing or monitoring treatment of DMD in human patients.

Example 15. Assessment of stability *in vivo*.

[0494] Among other things, the present disclosure provides oligonucleotides having improved stability including *in vivo* stability (e.g., when compared to a reference oligonucleotide or another oligonucleotide described in the present disclosure). Provided oligonucleotides were examined *in vivo* including to determine quantity and identity of full-length and metabolite species. Oligonucleotides comprising various modifications, such as linkage modifications (e.g., PS, PN (e.g., phosphoryl guanidine linkages such as n001), etc.), sugar modifications (e.g., 2'-F, 2'-OMe, etc.), and stereochemistry and patterns thereof were designed and assessed. To assess, among other things, oligonucleotide distribution, half-life, and metabolite profile,

wild-type CD-1 mice were administered 30 mg/kg of various oligonucleotides by intravenous infusion. Mice were sacrificed and necropsied 1, 14, 28, 56, or 84 days after dosing. Among other things, the relative quantity of full-length and metabolites species was determined in tissue samples collected from heart, tibialis, liver, and kidney by mass spectrometry at days 14 and 28 after dosing. Certain oligonucleotides showed significantly higher levels of stability compared to others. For example, full-length ASO-0140921 were detected as a major oligonucleotide species at both day 14 and day 28: at day 14, the highest level of a detected metabolite was about 5% relative to full-length ASO-0140921; at day 28, the highest level of a detected metabolite was about 15% relative to full-length ASO-0140921. In contrast, no full-length ASO-0140920 were detected at day 14 or 28. Without the intention to be limited by theory, Applicant notes that a PN internucleotidic linkage between the first and second nucleosides can provide improved stability.

[0495] While various embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described in the present disclosure, and each of such variations and/or modifications is deemed to be included. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be example and that the actual parameters, dimensions, materials, and/or configurations may depend upon the specific application or applications for which the teachings of the present disclosure is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the embodiments of the present disclosure. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, claimed technologies may be practiced otherwise than as specifically described and claimed. In addition, any combination of two or more features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the scope of the present disclosure.

CLAIMS

1. An oligonucleotide, wherein the oligonucleotide is selected from

[fl2r](U)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A),

[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](C)[Ssp].m(A)[Ssp].[fl2r](A)[Ssp].m(A)p.m(U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](U),

[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G),

[fl2r](U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A),

[fl2r](C)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G),

[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[n001R].[fl2r](G),

[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C),

[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C),

[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C),

m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G),

m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].m(G),

m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A),

m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].m(A),

[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C), and

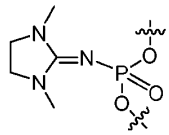
m(C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C),or a salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

2. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](U)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a salt thereof.

3. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a salt thereof.

4. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A), or a salt thereof.

5. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](C)[Ssp].m(A)[Ssp].[fl2r](A)[Ssp].m(A)p.m(U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](U), or a salt thereof.

6. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G), or a salt thereof.

7. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a salt thereof.

8. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a salt thereof.

9. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A), or a salt thereof.

10. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](C)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G), or a salt thereof.

11. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[n001R].[fl2r](G), or a salt thereof.

12. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a salt thereof.

13. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a salt thereof.

14. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C), or a salt thereof.

15. The oligonucleotide of claim 1, wherein the oligonucleotide is m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a salt thereof.

16. The oligonucleotide of claim 1, wherein the oligonucleotide is m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].m(G), or a salt thereof.

17. The oligonucleotide of claim 1, wherein the oligonucleotide is m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A), or a salt thereof.

18. The oligonucleotide of claim 1, wherein the oligonucleotide is m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].m(A), or a salt thereof.

19. The oligonucleotide of claim 1, wherein the oligonucleotide is [fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C), or a salt thereof.

20. The oligonucleotide of claim 1, wherein the oligonucleotide is m(C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C), or a salt thereof.

21. An oligonucleotide, wherein:

the base sequence of the oligonucleotide comprises 10 or more contiguous nucleobases of a complement of a DMD transcript; and

the oligonucleotide comprises 3 or more PN internucleotidic linkages.

22. The oligonucleotide of claim 21, wherein the base sequence of the oligonucleotide comprises 15, 20, or more contiguous nucleobases of a complement of a DMD transcript.

23. The oligonucleotide of any one of the preceding claims, wherein the base sequence of the oligonucleotide is the same as an equal length portion in a complement of a DMD transcript.

24. An oligonucleotide, wherein:

the base sequence of the oligonucleotide comprises 10 or more contiguous nucleobases that are complementary to an equal length portion in a DMD transcript; and

the oligonucleotide comprises 3 or more PN internucleotidic linkages.

25. The oligonucleotide of claim 24, wherein the base sequence of the oligonucleotide comprises 15, 20, or more contiguous nucleobases that are complementary to an equal length portion in a DMD transcript.

26. The oligonucleotide of any one of the preceding claims, wherein the base sequence of the oligonucleotide is complementary to the base sequence of an equal length portion in a DMD transcript.

27. An oligonucleotide, wherein:

the oligonucleotide can hybridize to an equal length portion in a DMD transcript; and

the oligonucleotide comprises 3 or more PN internucleotidic linkages.

28. An oligonucleotide, wherein the base sequence of the oligonucleotide comprises 15 or more contiguous nucleotides of a complement of a DMD transcript, wherein the oligonucleotide comprises 4 or more PN internucleotidic linkages, and wherein the oligonucleotide comprises a PN internucleotidic linkage between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and/or a PN internucleotidic linkage between the 3' terminal (N) nucleotide and the penultimate (N-1) nucleotide.

29. The oligonucleotide of claim 28, wherein the oligonucleotide comprises a PN internucleotidic linkage between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide, and wherein the oligonucleotide does not comprise a PN internucleotidic linkage between the 3' terminal (N) nucleotide and the penultimate (N-1) nucleotide.

30. The oligonucleotide of claim 28 or 29, wherein the PN internucleotidic linkage between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide is in the *R*p configuration.

31. The oligonucleotide of any one of claims 28-30, wherein all of the PN internucleotidic linkages are in the *R*p configuration.

32. The oligonucleotide of any one of claims 28-31, wherein the oligonucleotide comprises only 4 PN internucleotidic linkages.

33. The oligonucleotide of any one of claims 28-32, wherein the 5' terminal (+1) nucleotide, and/or the 3' terminal (N) nucleotide, of the oligonucleotide comprises a modified sugar moiety, optionally wherein the modified sugar moiety comprises a 2'-OMe modification.

34. The oligonucleotide of any one of claims 28-33, wherein the oligonucleotide comprises a PN internucleotidic linkage between the +3 nucleotide and the +4 nucleotide, a PN internucleotidic linkage between the +6 nucleotide and the +7 nucleotide, and/or a PN internucleotidic linkage between the +17 nucleotide and the +18 nucleotide.

35. The oligonucleotide of claim 34, wherein the PN internucleotidic linkage between the +3 nucleotide and the +4 nucleotide, the PN internucleotidic linkage between the +6 nucleotide and the +7 nucleotide, and/or the PN internucleotidic linkage between the +17 nucleotide and the +18 nucleotide, are in the *R*p configuration.

36. An oligonucleotide, wherein the base sequence of the oligonucleotide comprises 15 or more contiguous nucleotides of a complement of a DMD transcript, wherein the oligonucleotide comprises a PN internucleotidic linkage between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide in the *R*p configuration, a PN internucleotidic linkage between the +3 nucleotide and the +4 nucleotide in the *R*p configuration, a PN internucleotidic linkage between the +6 nucleotide and the +7 nucleotide in the *R*p configuration, and/or a PN internucleotidic linkage between the +17 nucleotide and the +18 nucleotide in the *R*p configuration.

37. The oligonucleotide of claim 36, wherein the sugar moiety of the 5' terminal (+1) nucleotide comprises a 2'-OMe modification or wherein the sugar moiety of the 5' terminal (+1) nucleotide comprises a 2'-F modification.

38. The oligonucleotide of any one of claims 36-37, wherein the oligonucleotide comprises a PS linkage between the +2 nucleotide and the +3 nucleotide, a PS linkage between the +4 nucleotide and the +5 nucleotide, a PS linkage between the +5 nucleotide and the +6 nucleotide, a PS linkage between the +8 nucleotide and the +9 nucleotide, a PS linkage between the +9 nucleotide and the +10 nucleotide, a PS linkage between the +10 nucleotide and the +11 nucleotide, a PS linkage between the +11 nucleotide and the +12 nucleotide, a PS linkage between the +13 nucleotide and the +14 nucleotide, a PS linkage between the +14 nucleotide and the +15 nucleotide, a PS linkage between the +15 nucleotide and the +16 nucleotide, a PS linkage between the +16 nucleotide and the +17 nucleotide, and/or a PS linkage between the +18 nucleotide and the +19 nucleotide.

39. The oligonucleotide of claim 38, wherein the oligonucleotide comprises a PS linkage between the 3' terminal (N) nucleotide and the penultimate (N-1) nucleotide.

40. The oligonucleotide of claim 38 or 39, wherein all of the PS linkages are in the *S*p configuration.

41. The oligonucleotide of any one of claims 36-40, wherein the sugar moiety of the +7 nucleotide comprises a 2'-OMe modification, the sugar moiety of the +10 nucleotide comprises a 2'-OMe modification,

the sugar moiety of the +12 nucleotide comprises a 2'-OMe modification, and/or the sugar moiety of the +13 nucleotide comprises a 2'-OMe modification.

42. The oligonucleotide of claim 41, wherein the sugar moiety of the 3' terminal (N) nucleotide, comprises a 2'-OMe modification.

43. The oligonucleotide of any one of the preceding claims, wherein the DMD transcript is a DMD mRNA, the DMD transcript or a wild-type version thereof encodes dystrophin, and/or the DMD transcript comprises a mutation that is amenable to exon 52 skipping.

44. The oligonucleotide of any one of the preceding claims, wherein each nucleobase is independently an optionally substituted nucleobase selected from A, T, C, G and U, or an optionally substituted tautomer of a nucleobase selected from A, T, C, G and U, optionally wherein each nucleobase is independently A, T, C, 5mC, G or U.

45. The oligonucleotide of any one of the preceding claims, wherein the length of the oligonucleotide is 20 or more nucleobases, optionally wherein the length of the oligonucleotide is 20 nucleobases.

46. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises 4, 5, 6 or more PN internucleotidic linkages.

47. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises a *R*p PN internucleotidic linkage, optionally wherein the oligonucleotide comprises 4, 5, 6 or more *R*p PN internucleotidic linkages.

48. The oligonucleotide of any one of the preceding claims, wherein each PN internucleotidic linkage is independently a phosphoryl guanidine internucleotidic linkage.

49. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises a phosphoryl guanidine internucleotidic linkage, optionally wherein the oligonucleotide comprises 4, 5, 6 or more phosphoryl guanidine internucleotidic linkages.

50. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises a *R*p phosphoryl guanidine internucleotidic linkage, optionally wherein the oligonucleotide comprises 4, 5, 6 or more *R*p phosphoryl guanidine internucleotidic linkages.

51. The oligonucleotide of any one of the preceding claims, wherein each phosphoryl guanidine internucleotidic linkage is independently a n001 internucleotidic linkage.

52. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises a n001 internucleotidic linkage, optionally wherein the oligonucleotide comprises 4, 5, 6 or more n001 internucleotidic linkages.

53. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises a *R*p n001 internucleotidic linkage, optionally wherein the oligonucleotide comprises 4, 5, 6 or more *R*p n001 internucleotidic linkages.

54. The oligonucleotide of any one of the preceding claims, wherein each n001 is *R*p, optionally wherein each phosphoryl guanidine internucleotidic linkage is *R*p, optionally wherein each PN internucleotidic linkage is *R*p.

55. The oligonucleotide of any one of the preceding claims, wherein the internucleotidic linkage between the 3rd and the 4th nucleosides, between the 6th and the 7th nucleosides, and/or between the 17th and the 18th nucleosides is a PN linkage (unless otherwise noted, 5' to 3' direction).

56. The oligonucleotide of any one of the preceding claims, wherein the internucleotidic linkage between the first and the second nucleosides is a PN linkage or wherein the internucleotidic linkage between the last two nucleosides is a PN linkage.

57. The oligonucleotide of any one of the preceding claims, wherein the number of *R*p n001 internucleotidic linkages, the number of n001 internucleotidic linkages, the number of *R*p phosphoryl guanidine internucleotidic linkages, the number of phosphoryl guanidine internucleotidic linkages, the number of *R*p PN internucleotidic linkages, and/or the number of PN internucleotidic linkages in the oligonucleotide is 4.

58. The oligonucleotide of claim 56, wherein the internucleotidic linkage between the last two nucleosides is a PN linkage.

59. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more PS internucleotidic linkages.

60. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more *S*p PS internucleotidic linkages.

61. The oligonucleotide of any one of the preceding claims, wherein each PS internucleotidic linkage is *S*p and/or wherein each PS internucleotidic linkage is independently a phosphorothioate internucleotidic linkage.

62. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more phosphorothioate internucleotidic linkages.

63. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more *S*p phosphorothioate internucleotidic linkages.

64. The oligonucleotide of any one of the preceding claims, wherein each phosphorothioate internucleotidic linkage is independently *S*p.

65. The oligonucleotide of any one of the preceding claims, wherein the internucleotidic linkage between the first and the second nucleosides, if it is not a PN linkage, is a PS internucleotidic linkage; and/or wherein the internucleotidic linkage between the last two nucleosides, if it is not a PN linkage, is a PS internucleotidic linkage.

66. The oligonucleotide of any one of the preceding claims, wherein the internucleotidic linkage between the 2nd and the 3rd nucleosides, between the 4th and the 5th nucleosides, between the 5th and the 6th nucleosides, between the 8th and the 9th nucleosides, between the 9th and the 10th nucleosides, between

the 10th and the 11th nucleosides, between the 11th and the 12th nucleosides, between the 13th and the 14th nucleosides, between the 14th and the 15th nucleosides, between the 15th and the 16th nucleosides, between the 16th and the 17th nucleosides, and/or between the 18th and the 19th nucleosides is a PS internucleotidic linkage.

67. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises 1, 2, 3, 4, 5 or more PO internucleotidic linkages.

68. The oligonucleotide of any one of the preceding claims, wherein each PO internucleotidic linkage is independently a natural phosphate linkage.

69. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises 1, 2, 3, 4, 5 or more natural phosphate internucleotidic linkages.

70. The oligonucleotide of any one of the preceding claims, wherein the internucleotidic linkage between the seventh and the eighth nucleoside is a PO internucleotidic linkage and/or wherein the internucleotidic linkage between the 12th and the 13th nucleoside is a PO internucleotidic linkage.

71. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises a sugar modification.

72. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more 2'-modified sugars.

73. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more 2'-F modified sugars.

74. The oligonucleotide of any one of the preceding claims, wherein the sugar of nucleosides 2, 3, 4, 5, 6, 8, 9, 11, 14, 15, 16, 17, 18, and 19 are 2'-F modified sugars; the sugar of nucleosides 2, 3, 4, 5, 6, 8, 9, 11, 14, 15, 16, 17, 18, 19 and 20 are 2'-F modified sugars; the sugar of nucleosides 1, 2, 3, 4, 5, 6, 8, 9, 11, 14, 15, 16, 17, 18, and 19 are 2'-F modified sugars; or the sugar of nucleosides 1, 2, 3, 4, 5, 6, 8, 9, 11, 14, 15, 16, 17, 18, 19 and 20 are 2'-F modified sugars.

75. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more 2'-OR modified sugars, wherein R is optionally substituted C_1-C_6 aliphatic.

76. The oligonucleotide of any one of the preceding claims, wherein the sugar of nucleosides 7, 10, 12 and 13 are independently 2'-OR modified sugars; the sugar of nucleosides 1, 7, 10, 12 and 13 are independently 2'-OR modified sugars; the sugar of nucleosides 1, 7, 10, 12, 13 and 20 are independently 2'-OR modified sugars; or the sugar of nucleosides 7, 10, 12, 13 and 20 are independently 2'-OR modified sugars;

wherein R is optionally substituted C_1-C_6 aliphatic.

77. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more 2'-OMe modified sugars.

78. The oligonucleotide of any one of the preceding claims, wherein each 2'-OR modified sugar is a 2'-OMe modified sugar.

79. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide can hybridize to a region of a DMD transcript.

80. The oligonucleotide of claim 79, wherein the region of the DMD transcript comprises CCAGUUGGAAGAACUCAUUA, CCCCAGUUGGAAGAACUCAU, UGCAGGAUUUGGAACAGAGG, AUGCAGGAUUUGGAACAGAG, CAGGAUUUGGAACAGAGGCG, or GGAAGAACUCAUUACCGCUG, wherein each U is optionally and independently replaced with T.

81. The oligonucleotide of any one of the preceding claims, wherein the base sequence comprises 10, 15, 20 or more contiguous nucleobases complementary to 10, 15, 20 or more contiguous nucleobases of a DMD transcript.

82. The oligonucleotide of any one of claims 21-81, wherein the base sequence comprises 10, 15, 20 or more contiguous nucleobases complementary to 10, 15, or 20 or more contiguous nucleobases of CCAGUUGGAAGAACUCAUUA, CCCCAGUUGGAAGAACUCAU, UGCAGGAUUUGGAACAGAGG, AUGCAGGAUUUGGAACAGAG, CAGGAUUUGGAACAGAGGCG, or GGAAGAACUCAUUACCGCUG, wherein each U is optionally and independently replaced with T.

83. The oligonucleotide of any one of the preceding claims, wherein the base sequence comprises 10, 15, or more contiguous nucleobases of UAAUGAGUUCUUCCAACUGG, AUGAGUUCUUCCAACUGGGG, CCUCUGUUCCAAAUCCUGCA, CUCUGUUCCAAAUCCUGCAU, CGCCUCUGUUCCAAAUCCUG, or CAGCGGUAAUGAGUUCUUCC, wherein each U is optionally and independently replaced with T.

84. The oligonucleotide of claim 83 wherein the base sequence comprises UAAUGAGUUCUUCCAACUGG, AUGAGUUCUUCCAACUGGGG, CCUCUGUUCCAAAUCCUGCA, CUCUGUUCCAAAUCCUGCAU, CGCCUCUGUUCCAAAUCCUG, or CAGCGGUAAUGAGUUCUUCC, wherein each T is optionally and independently replaced with U.

85. The oligonucleotide of claim 84, wherein the base sequence is UAAUGAGUUCUUCCAACUGG, AUGAGUUCUUCCAACUGGGG, CCUCUGUUCCAAAUCCUGCA, CUCUGUUCCAAAUCCUGCAU, CGCCUCUGUUCCAAAUCCUG, or CAGCGGUAAUGAGUUCUUCC.

86. The oligonucleotide of any one of the preceding claims, wherein each chiral internucleotidic linkage of the oligonucleotide independently has a diastereopurity of at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%.

87. The oligonucleotide of any one of the preceding claims, wherein the oligonucleotide is a pharmaceutically acceptable salt, optionally wherein the oligonucleotide is a sodium salt, optionally wherein the number of sodium ions in the sodium salt equals the total number of phosphorothioate internucleotidic linkages and natural phosphate internucleotidic linkages in the oligonucleotide.

88. A chirally controlled oligonucleotide composition comprising an oligonucleotide of any one of the preceding claims, wherein the composition is enriched, relative to a substantially racemic preparation of the oligonucleotide, for the oligonucleotide.

89. A chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides each of which is an oligonucleotide of any one of claims 1-87, wherein oligonucleotides of the plurality share the same constitution and at least 5% of all oligonucleotides in the composition that share the same constitution are oligonucleotides of the plurality.

90. A chirally controlled oligonucleotide composition comprising an oligonucleotide of any one of claims 1-87, wherein at least 5% of all oligonucleotides in the composition that share the same constitution as the oligonucleotide are the oligonucleotide.

91. The composition of any one of claims 89-90, wherein at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of all oligonucleotides in the composition that share the same constitution are oligonucleotides of the plurality; or

wherein at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of all oligonucleotides in the composition that share the same base sequence are oligonucleotides of the plurality.

92. An oligonucleotide composition comprising a plurality of oligonucleotides, wherein oligonucleotides of the plurality share:

1) a common base sequence; and

2) the same linkage phosphorus stereochemistry independently at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more chiral internucleotidic linkages;

wherein oligonucleotides of the plurality are an oligonucleotide of any one of claims 1-87; and

wherein at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the oligonucleotides in the composition that share the common base sequence are oligonucleotides of the plurality.

93. The oligonucleotide composition of claim 92, wherein oligonucleotides of plurality share the same linkage phosphorus stereochemistry independently at each chiral internucleotidic linkage.

94. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

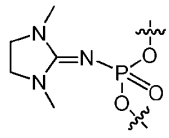
[fl2r](U)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

95. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

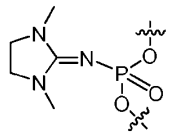
[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

96. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

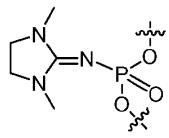
[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

97. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](C)[Ssp].m(A)[Ssp].[fl2r](A)[Ssp].m(A)p.m(U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](

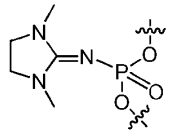
U)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](U), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

98. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

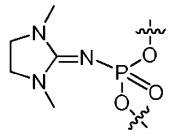
[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

99. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

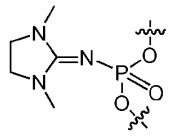
[fl2r](U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

100. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

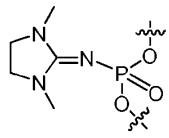
[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

101. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

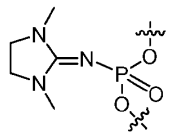
[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

102. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

[fl2r](C)[n001R].[fl2r](G)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[n001R].m(U)p.[fl2r](G)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2

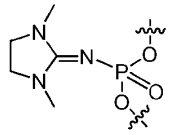
r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

103. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

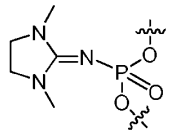
[fl2r](A)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](A)[Ssp].[fl2r](G)[Ssp].[fl2r](U)[n001R].m(U)p.[fl2r](C)[Ssp].[fl2r](U)[Ssp].m(U)[Ssp].[fl2r](C)[Ssp].m(C)p.m(A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].[fl2r](G)[Ssp].[fl2r](G)[n001R].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

104. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

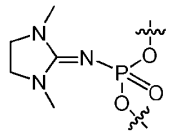
[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

105. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

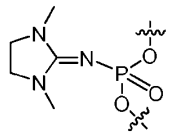
[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

106. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

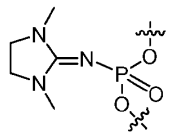
[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[n001R].[fl2r](C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

107. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](

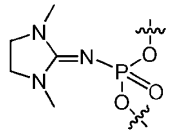
A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

108. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

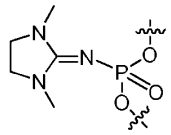
m(U)[n001R].[fl2r](A)[Ssp].[fl2r](A)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].[fl2r](A)[n001R].m(G)p.[fl2r](U)[Ssp].[fl2r](U)[Ssp].m(C)[Ssp].[fl2r](U)[Ssp].m(U)p.m(C)[Ssp].[fl2r](C)[Ssp].[fl2r](A)[Ssp].[fl2r](A)[Ssp].[fl2r](C)[n001R].[fl2r](U)[Ssp].[fl2r](G)[Ssp].m(G), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

109. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

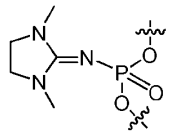
m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].[fl2r](A), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

110. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

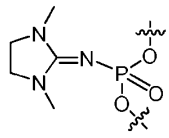
m(C)[n001R].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](C)[Ssp].[fl2r](U)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C)[Ssp].[fl2r](A)[Ssp].m(A)p.m(A)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](G)[Ssp].[fl2r](C)[Ssp].m(A), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

111. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

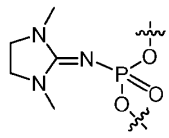
[fl2r](C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

112. An oligonucleotide composition, wherein a level of all oligonucleotides in the composition are oligonucleotides each independently having the structure of:

m(C)[n001R].[fl2r](A)[Ssp].[fl2r](G)[n001R].[fl2r](C)[Ssp].[fl2r](G)[Ssp].[fl2r](G)[n001R].m(U)p.[fl2r](A)[Ssp].[fl2r](A)[Ssp].m(U)[Ssp].[fl2r](G)[Ssp].m(A)p.m(G)[Ssp].[fl2r](U)[Ssp].[fl2r](U)[Ssp].[fl2r](

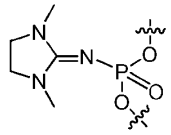
C)[Ssp].[fl2r](U)[n001R].[fl2r](U)[Ssp].[fl2r](C)[Ssp].m(C), or a pharmaceutically acceptable salt thereof, wherein:

[fl2r] represents a 2'-F modified nucleoside;

m represents a 2'-OMe modified nucleoside;

p represents a phosphodiester;

[Ssp] represents a phosphorothioate in the *S*p configuration; and

[n001R] represents  wherein the phosphorus is of the *R*p configuration.

113. The composition of any one of claims 94-112, wherein a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of all oligonucleotides that share the same base sequence as an oligonucleotide having the structure or a pharmaceutically acceptable salt thereof; or

wherein a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of all oligonucleotides that share the same constitution as an oligonucleotide having the structure or a pharmaceutically acceptable salt thereof; or

wherein a level is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% based on UV peak area at 260 nm.

114. The composition of any one of claims 88-112, wherein a level or percentage is about or at least about $(DS)^{nc}$, wherein DS is about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%, and nc is the number of chirally controlled internucleotidic linkages (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 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); or

wherein a level or percentage is about or at least about $(DS)^{nc}$, wherein DS is about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%, and nc is the number of chiral linkage phosphorus in an oligonucleotide of the plurality (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 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).

115. A pharmaceutical composition, comprising an oligonucleotide or oligonucleotide composition of any one of the preceding claims, and a pharmaceutically acceptable carrier.

116. The pharmaceutical composition of claim 115, wherein the pharmaceutical composition comprises one or more pharmaceutically acceptable salts of an oligonucleotide and/or the pharmaceutical composition is a solution.

117. A method for altering splicing of a DMD transcript in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition of any one of the preceding claims.

118. A method for providing DMD exon skipping in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition of any one of the preceding claims.

119. A method for increasing level of a DMD polypeptide in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition of any one of the preceding claims.

120. The method of claim 119, wherein the DMD polypeptide is a truncated dystrophin.

121. A method for increasing level of dystrophin in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition of any one of the preceding claims.

122. A method for increasing level of a dystrophin polypeptide encoded by exon 52-skipped DMD transcript in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition of any one of the preceding claims.

123. A method for increasing level of DMD activity in a system, comprising administering or delivering to the system an effective amount of an oligonucleotide or composition of any one of the preceding claims.

124. The method of any one of claims 117-123, wherein the system expresses DMD transcripts and/or the system comprises a mutation of the DMD gene, optionally wherein the mutation is amenable to exon 52 skipping.

125. The method of any one of claims 117-124, wherein the system comprises or is a cell, tissue, organ, animal, human or subject,

optionally wherein the cell comprises or is a skeletal muscle cell, a cardiac muscle cell, or a neuron; the tissue comprises or is a muscle tissue; and/or the animal comprises a mouse or a non-human primate.

126. A method for treating muscular dystrophy, comprising administering or delivering to a subject suffering therefrom an effective amount of an oligonucleotide or composition of any one of the preceding claims.

127. The method of any one of claims 125-126, wherein the subject is suffering from Duchenne's muscular dystrophy or Becker's muscular dystrophy and/or the subject has a mutation of the DMD gene that is amenable to exon 52 skipping.

128. The oligonucleotide, composition or method of any one of the preceding claims, wherein the mutation of the DMD gene is Δ2-51, Δ8-51, Δ20-51, Δ22-51, Δ51, Δ53, Δ53-55, Δ53-57, Δ53-59, Δ53-60, Δ53-67, Δ53-69, Δ53-75, or Δ53-78; optionally wherein the mutation comprises Δ51 or Δ53.

129. The method of any one of the preceding claims, wherein exon 52 is skipped in DMD mRNA.

130. The method of any one of the preceding claims, wherein level of exon 52-skipped DMD mRNA is increased as compared to absence of the oligonucleotide or composition and/or as compared to administering or delivering a reference oligonucleotide or composition.

131. The method of any one of the preceding claims, wherein a truncated DMD polypeptide is produced as compared to a wild-type DMD protein, optionally wherein the truncated DMD polypeptide performs one or more functions of wild-type dystrophin.

132. The method of any one of the preceding claims, wherein level of a truncated DMD polypeptide is increased as compared to absence of the oligonucleotide or composition and/or as compared to administering or delivering a reference oligonucleotide or composition.

133. The method of claim 130 or 132, wherein the reference oligonucleotide targets DMD or the reference composition comprises oligonucleotides targeting DMD.

134. An oligonucleotide or composition of any one of the preceding claims, for use in a method of any one of the preceding claims.

135. An oligonucleotide or composition of any one of the preceding claims, for use in manufacturing a medicament for a method of any one of the preceding claims.

136. A method for manufacturing an oligonucleotide or composition of any one of the preceding claims according to a method described in the specification.

137. An oligonucleotide, composition, method, or use of any one of Embodiments 1-292.