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(71) **Applicant** *(for all designated States except US)*: **WAVE LIFE SCIENCES LTD.** [SG/SG]; 8 Cross Street, #10-00, PWC Building, Singapore 048424 (SG).

(72) **Inventors; and**

(71) **Applicants** *(for US only)*: **VARGEESE, Chandra** [US/US]; c/o WAVE LIFE SCIENCES LTD., 733 Concord Avenue, Cambridge, Massachusetts 02138 (US). **., Meena** [IN/US]; c/o WAVE LIFE SCIENCES LTD., 733 Concord Avenue, Cambridge, Massachusetts 02138 (US). **SVRZIKAPA, Nenad** [US/US]; c/o WAVE LIFE SCIENCES LTD., 733 Concord Avenue, Cambridge, Massachusetts 02138 (US). **MOHAPATRA, Susovan** [IN/US]; c/o WAVE LIFE SCIENCES LTD., 733 Concord Avenue, Cambridge, Massachusetts 02138 (US). **FRANCIS, Christopher J.** [AU/US]; c/o WAVE LIFE SCIENCES LTD., 733 Concord Avenue, Cambridge, Massachusetts 02138 (US). **VERDINE, Gregory L.** [US/US]; c/o WAVE LIFE SCIENCES LTD., 733 Concord Avenue, Cambridge, Massachusetts 02138 (US). **SOKOLOVSKA, Anna** [UA/US]; c/o WAVE LIFE SCIENCES LTD., 733 Concord Avenue, Cambridge, Massachusetts 02138 (US).

(74) **Agents**: **LI, Xiaodong** et al.; Choate, Hall & Stewart LLP, Two International Place, Boston, Massachusetts 02110 (US).

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(54) **Title**: OLIGONUCLEOTIDE COMPOSITIONS AND METHODS THEREOF

(57) **Abstract**: Among other things, the present disclosure relates to chirally controlled oligonucleotides of select designs, chirally controlled oligonucleotide compositions, and methods of making and using the same. In some embodiments, a provided chirally controlled oligonucleotide composition provides different cleavage patterns of a nucleic acid polymer than a reference oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition provides single site cleavage within a complementary sequence of a nucleic acid polymer. In some embodiments, a chirally controlled oligonucleotide composition has any sequence of bases, and/or pattern or base modifications, sugar modifications, backbone modifications and/or stereochemistry, or combination of these elements, described herein.

OLIGONUCLEOTIDE COMPOSITIONS AND METHODS THEREOF

Cross-Reference to Related Applications

[0001] This application claims priority to United States Provisional Application Nos. 62/195,779, filed July 22, 2015, 62/236,847, filed October 2, 2015, and 62/331,960, filed May 4, 2016, the entirety of each of which is incorporated herein by reference.

Background

[0002] Oligonucleotides are useful in therapeutic, diagnostic, research and nanomaterials applications. The use of naturally occurring nucleic acids (*e.g.,* unmodified DNA or RNA) for therapeutics can be limited, for example, because of their instability against extra- and intracellular nucleases and/or their poor cell penetration and distribution. There is a need for new and improved oligonucleotides and oligonucleotide compositions, such as, *e.g.,* new antisense and siRNA oligonucleotides and oligonucleotide compositions.

Summary

[0003] Among other things, the present disclosure encompasses the recognition that structural elements of oligonucleotides, such as base sequence, chemical modifications (*e.g.,* modifications of sugar, base, and/or internucleotidic linkages, and patterns thereof), and/or stereochemistry (*e.g.,* stereochemistry of backbone chiral centers (chiral internucleotidic linkages), and/or patterns thereof), can have significant impact on properties, *e.g.,* activities, of oligonucleotides. In some embodiments, the present disclosure demonstrates that oligonucleotide compositions comprising oligonucleotides with controlled structural elements, *e.g.,* controlled chemical modification and/or controlled backbone stereochemistry patterns, provide unexpected properties, including but not limited to those described herein. In some embodiments, the present disclosure demonstrates that combinations of chemical modifications and stereochemistry can provide unexpected, greatly improved properties (*e.g.,* bioactivity, selectivity, *etc.*). In some embodiments, the present disclosure provides an oligonucleotide composition having a particular sequence of bases, and/or pattern of sugar modifications (e.g., 2'-OMe, 2'-F, 2'-MOE, etc.), and/or pattern or base modifications (e.g., 5-methylcytosine), and/or pattern of backbone modifications (phosphate or phosphorothioate), and/or pattern of

stereochemistry of backbone modifications (e.g., each phosphorothioate is *S*p or *R*p).

[0004] In some embodiments, modifications of internucleotidic linkages can convert phosphorus atoms in modified linkages into chiral centers. For example, in a phosphorothioate (PS) modification, one of the non-bridging oxygen (O) atoms bonded to a phosphorus (P) atom is replaced with a sulfur (S) atom. A consequence of using PS modification in oligonucleotide synthesis is that it creates a chiral center at phosphorus, which can have either an "Sp" or "Rp" configuration. For instance, a conventional stereorandom PS-modified oligonucleotide composition having 19 PS linkages [e.g., having 20 nucleotides in length, 19 PS modifications, each with two possible stereochemistries (*S*p or *R*p) at each PS modification] is a random mixture of over 500,000 (2^{19}) stereoisomers, each having the same nucleotide sequence (e.g., sequence of bases) but differing in the stereochemistry along their backbones; such a composition is a "stereorandom" oligonucleotide composition. In some embodiments, in contrast to stereorandom compositions, a chirally controlled oligonucleotide composition is a substantially pure preparation of a single oligonucleotide in that a predetermined level of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, some oligonucleotide compositions are stereopure (i.e., a chirally controlled oligonucleotide composition), wherein the stereochemistry at each PS is defined (*S*p or *R*p). In some embodiments, in a stereorandom compositions of oligonucleotides, the various oligonucleotides can have the same base sequence, same pattern of sugar modifications (e.g., 2'-OMe, 2'-F, 2'-OME, etc.), same pattern of base modifications (e.g., 5-methylcytosine), and same pattern of backbone modifications (phosphate or PS), but different patterns of backbone chiral centers, and their levels are random from non-stereocontrolled synthesis (not pre-determined as through stereocontrolled synthesis as certain methods exemplified herein using chiral auxilier). A chirally controlled oligonucleotide composition can be selected to have greater desired biological activity (e.g., greater activities, efficiency in RNA interference or RNAse H-mediated pathways, *etc.*) and decreased undesired activity (e.g., undesired immunogenicity, toxicity, *etc.*) than a stereorandom preparation of oligonucleotides of the same base sequence. In some embodiments, a chirally controlled oligonucleotide composition is better able to differentiate between a mutant (mu) and a wild-type (wt) HTT sequence (with a single nt difference).

[0005] Among other things, the present disclosure encompasses the recognition that

stereorandom oligonucleotide preparations contain a plurality of distinct chemical entities that differ from one another, *e.g.,* in the stereochemical structure of individual backbone chiral centers within the oligonucleotide chain. Without control of stereochemistry of backbone chiral centers, stereorandom oligonucleotide preparations provide uncontrolled compositions comprising undetermined levels of oligonucleotide stereoisomers. Even though these stereoisomers may have the same base sequence, they are different chemical entities at least due to their different backbone stereochemistry, and they can have, as demonstrated herein, different properties, *e.g.,* bioactivities. Among other things, the present disclosure provides new compositions that are or contain particular stereoisomers of oligonucleotides of interest. In some embodiments, a particular stereoisomer may be defined, for example, by its base sequence, its length, its pattern of backbone linkages, and its pattern of backbone chiral centers. As is understood in the art, in some embodiments, base sequence may refer to the identity and/or modification status of nucleoside residues (*e.g.,* of sugar and/or base components, relative to standard naturally occurring nucleotides such as adenine, cytosine, guanosine, thymine, and uracil) in an oligonucleotide and/or to the hybridization character (i.e., the ability to hybridize with particular complementary residues) of such residues.

[0006] The present disclosure demonstrates, among other things, that individual stereoisomers of a particular oligonucleotide can show different stability and/or activity (*e.g.,* functional and/or toxicity properties) from each other. Moreover, the present disclosure demonstrates that stability and/or activity improvements achieved through inclusion and/or location of particular chiral structures within an oligonucleotide can be comparable to, or even better than those achieved through use of particular backbone linkages, residue modifications, *etc.* (*e.g.,* through use of certain types of modified phosphates [*e.g.,* phosphorothioate, substituted phosphorothioate, *etc.*], sugar modifications [*e.g.,* 2'- modifications, *etc.*], and/or base modifications [*e.g.,* methylation, *etc.*]).

[0007] Among other things, the present disclosure recognizes that, in some embodiments, properties (*e.g.,* stability and/or activities) of an oligonucleotide can be adjusted by optimizing its pattern of backbone chiral centers, optionally in combination with adjustment/optimization of one or more other features (*e.g.,* linkage pattern, nucleoside modification pattern, *etc.*) of the oligonucleotide.

[0008] In some embodiments, the present disclosure provides compositions of

oligonucleotides, wherein the oligonucleotides have a common pattern of backbone chiral centers which, unexpectedly, greatly enhances the stability and/or biological activity of the oligonucleotides. In some embodiments, a pattern of backbone chiral centers provides increased stability. In some embodiments, a pattern of backbone chiral centers provides surprisingly increased activity. In some embodiments, a pattern of backbone chiral centers provides increased stability and activity. In some embodiments, when an oligonucleotide is utilized to cleave a nucleic acid polymer, a pattern of backbone chiral centers, surprisingly by itself, changes the cleavage pattern of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers effectively prevents cleavage at secondary sites. In some embodiments, a pattern of backbone chiral centers creates new cleavage sites. In some embodiments, a pattern of backbone chiral centers minimizes the number of cleavage sites. In some embodiments, a pattern of backbone chiral centers minimizes the number of cleavage sites so that a target nucleic acid polymer is cleaved at only one site within the sequence of the target nucleic acid polymer that is complementary to the oligonucleotide. In some embodiments, a pattern of backbone chiral centers enhances cleavage efficiency at a cleavage site. In some embodiments, a pattern of backbone chiral centers of the oligonucleotide improves cleavage of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers increases selectivity. In some embodiments, a pattern of backbone chiral centers minimizes off-target effect. In some embodiments, a pattern of backbone chiral centers increase selectivity, *e.g.,* cleavage selectivity between two target sequences differing only by a single nucleotide polymorphism (SNP). In some embodiments, a pattern of backbone chiral centers comprises, comprises one or more repeats of, or is $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$. In some embodiments described herein, m is 1-50; and n is 1-10; and t is 1-50. In some embodiments, a pattern of backbone chiral centers comprises or is (*S*p)m(*R*p)n, (*R*p)n(*S*p)m, (*N*p)t(*R*p)n(*S*p)m, or (*S*p)t(*R*p)n(*S*p)m. In some embodiments, a pattern of backbone chiral centers comprises or is $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein $m > 2$. In some embodiments, a pattern of backbone chiral centers is a sequence comprising at least 5, 6, 7, 8, 9, or 10 or more consecutive (*S*p) positions. In some embodiments, a pattern of backbone chiral centers is a sequence comprising at least 5 consecutive (*S*p) positions. In some embodiments, a pattern of backbone chiral centers is a sequence comprising at least 8 consecutive (*S*p) positions. In some embodiments, a pattern of backbone chiral centers is a

sequence comprising at least 10 consecutive (*S*p) positions. In some embodiments, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p). In some embodiments, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p) at or adjacent to the position of a SNP. In some embodiments, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format. In some embodiments, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein the wing on the 5' end is 1-9 nt long, the core is 1-15 nt long, and the wing on the 3' end is 1-9 nt long. In some embodiments, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein the wing on the 5' end is 5 nt long, the core is 1-15 nt long, and the wing on the 3' end is 5 nt long. In some embodiments, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein the wing on the 5' end is 1-9 nt long, the core is 10 nt long, and the wing on the 3' end is 1-9 nt long. In some embodiments, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein the wing on the 5' end is 5 nt long, the core is 10 nt long, and the wing on the 3' end is 5 nt long. In some embodiments, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein the wing on the 5' end is 5 nt long, the core is 10 nt long, and the wing on the 3' end is 5 nt long, and at least one wing comprises a nucleotide with a 2'-OMe modification. In some embodiments, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein each wing comprises at least one nucleotide with a 2'-OMe modification. In some embodiments, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein each nucleotide in both wings has a 2'-OMe modification. In some embodiments, a pattern of backbone chiral centers is a sequence consisting of all (*S*p) with a single (*R*p), wherein the molecule has a wing-core-wing format, wherein the wing on the 5' end is 5 nt long, the core is 10 nt long, and the wing on the 3' end is 5 nt long, and each nucleotide in each wing has a 2'-OMe modification. In some embodiments, the oligonucleotide is single-stranded and has a wing-core-wing format, wherein the wing on the 5' end of the molecule

comprises 4 to 8 nt, each of which has a 2'-OMe modification and wherein the nt at the 5' end of the molecule has a phosphorothioate in the Sp conformation; the core comprises 8 to 12 nt, each of which is DNA (2'-H), wherein each has a phosphorothioate in the Sp position except one nt which has the phosphorothioate in the Rp position; and wherein the wing on the 3' end of the molecule comprises 4 to 8 nt, each of which has a 2'-OMe modification, and wherein the nt at the 3' end of the molecule comprises a phosphorothioate in the Sp conformation. In some embodiments, the oligonucleotide is single-stranded and has a wing-core-wing format, wherein the wing on the 5' end of the molecule comprises 6 nt, each of which has a 2'-OMe modification and wherein the nt at the 5' end of the molecule has a phosphorothioate in the Sp conformation; the core comprises 10 nt, each of which is DNA (2'-H), wherein each has a phosphorothioate in the Sp position except one nt which has the phosphorothioate in the Rp position; and wherein the wing on the 3' end of the molecule comprises 6 nt, each of which has a 2'-OMe modification, and wherein the nt at the 3' end of the molecule comprises a phosphorothioate in the Sp conformation.

[0009] In some embodiments, the present disclosure recognizes that chemical modifications, such as modifications of nucleosides and internucleotidic linkages, can provide enhanced properties. In some embodiments, the present disclosure demonstrates that combinations of chemical modifications and stereochemistry can provide unexpected, greatly improved properties (*e.g.*, bioactivity, selectivity, *etc.*). In some embodiments, chemical combinations, such as modifications of sugars, bases, and/or internucleotidic linkages, are combined with stereochemistry patterns, *e.g.*, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, to provide oligonucleotides and compositions thereof with surprisingly enhanced properties. In some embodiments, a provided oligonucleotide composition is chirally controlled, and comprises a combination of 2'-modification of one or more sugar moieties, one or more natural phosphate linkages, one or more phosphorothioate linkages, and a stereochemistry pattern of $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein $m > 2$.

[0010] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which composition is a substantially pure preparation of a single oligonucleotide in that a predetermined level of the oligonucleotides

in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

[0011] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

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

[0012] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

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

[0013] Among other things, the present disclosure recognizes that combinations of oligonucleotide structural elements (*e.g.*, patterns of chemical modifications, backbone linkages, backbone chiral centers, and/or backbone phosphorus modifications) can provide surprisingly improved properties such as bioactivities. In some embodiments, the present disclosure provides an oligonucleotide composition comprising a predetermined level of oligonucleotides which comprise one or more wing regions and a common core region, wherein:

each wing region independently has a length of two or more bases, and independently and optionally comprises one or more chiral internucleotidic linkages;

the core region independently has a length of two or more bases, and independently comprises one or more chiral internucleotidic linkages, and the common core region has:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers.

[0014] In some embodiments, in an oligonucleotide comprising a wing-core-wing format, a "wing" is a portion of the oligonucleotide on the 5' or 3' end of the core, with the "core" (alternatively designated a "gap") between the two wings. In some embodiments, an oligonucleotide can have a single wing and a single core; in such cases, the wing is on the 5' or the 3' end of the oligonucleotide. A wing and core can be defined by any of several structural elements (e.g., modifications or patterns of modifications of sugar, base, backbone or backbone stereochemistry, *etc.*). In some embodiments, a wing and core is defined by nucleoside modifications, wherein a wing comprises a nucleoside modification that the core region does not have. In some embodiments, oligonucleotides in provided compositions have a wing-core structure of nucleoside modification. In some embodiments, oligonucleotides in provided compositions have a core-wing structure of nucleoside modification. In some embodiments, oligonucleotides in provided compositions have a wing-core-wing structure of nucleoside modification. In some embodiments, a wing and core is defined by modifications of the sugar moieties. In some embodiments, a wing and core is defined by modifications of the base moieties. In some embodiments, each sugar moiety in the wing region has the same 2'-modification which is not found in the core region. In some embodiments, each sugar moiety in the wing region has the same 2'-modification which is different than any sugar modifications in the core region. In some embodiments, each sugar moiety in the wing region has the same 2'-modification, and the core region has no 2'-modifications. In some embodiments, when two or more wings are present, each sugar moiety in a wing region has the same 2'-modification, yet the common 2'-modification in a first wing region can either be the same as or different from the common 2'-modification in a second wing region.

[0015] In some embodiments, each wing comprises at least one chiral internucleotidic linkage and at least one natural phosphate linkage. In some embodiments, each wing comprises at least one modified sugar moiety. In some embodiments, each wing sugar moiety is modified. In some embodiments, a wing sugar moiety is modified by a modification that is absent from the core region. In some embodiments, a wing region only has modified internucleotidic linkages at one or both of its ends. In some embodiments, a wing region only has a modified internucleotidic linkage at its 5'-end. In some embodiments, a wing region only has a modified

internucleotidic linkage at its 3'-end. In some embodiments, a wing region only has modified internucleotidic linkages at its 5'- and 3'-ends. In some embodiments, a wing is to the 5'-end of a core, and the wing only has a modified internucleotidic linkage at its 5'-end. In some embodiments, a wing is to the 5'-end of a core, and the wing only has a modified internucleotidic linkage at its 3'-end. In some embodiments, a wing is to the 5'-end of a core, and the wing only has modified internucleotidic linkages at both its 5'- and 3'-ends. In some embodiments, a wing is to the 3'-end of a core, and the wing only has a modified internucleotidic linkage at its 5'-end. In some embodiments, a wing is to the 3'-end of a core, and the wing only has a modified internucleotidic linkage at its 3'-end. In some embodiments, a wing is to the 3'-end of a core, and the wing only has modified internucleotidic linkages at both its 5'- and 3'-ends. In some embodiments, the modification(s) to the sugar moiety or internucleotidic linkage or other modifications in one wing can differ from those in another wing.

[0016] In some embodiments, each internucleotidic linkage within a core region is modified. In some embodiments, each internucleotidic linkage within a core region is chiral. In some embodiments, a core region has a pattern of backbone chiral centers of $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$. In some embodiments, a core region has a pattern of backbone chiral centers of $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein $m > 2$. Among other things, the present disclosure demonstrates that, in some embodiments, such patterns can provide or enhance controlled cleavage of a target sequence, *e.g.,* an RNA sequence.

[0017] In some embodiments, oligonucleotides in provided compositions have a common pattern of backbone phosphorus modifications. In some embodiments, a provided composition is an oligonucleotide composition that is chirally controlled in that the composition contains a predetermined level of oligonucleotides of an individual oligonucleotide type, wherein an oligonucleotide type is defined by:

1) base sequence;

2) pattern of backbone linkages;

3) pattern of backbone chiral centers; and

4) pattern of backbone phosphorus modifications.

[0018] As noted above and understood in the art, in some embodiments, the base sequence of an oligonucleotide may refer to the identity and/or modification status of nucleoside residues (*e.g.,* of sugar and/or base components, relative to standard naturally occurring

nucleotides such as adenine, cytosine, guanosine, thymine, and uracil) in the oligonucleotide and/or to the hybridization character (i.e., the ability to hybridize with particular complementary residues) of such residues.

[0019] In some embodiments, a particular oligonucleotide type may be defined by

1A) base identity;

1B) pattern of base modification;

1C) pattern of sugar modification;

2) pattern of backbone linkages;

3) pattern of backbone chiral centers; and

4) pattern of backbone phosphorus modifications.

Thus, in some embodiments, oligonucleotides of a particular type may share identical bases but differ in their pattern of base modifications and/or sugar modifications. In some embodiments, oligonucleotides of a particular type may share identical bases and pattern of base modifications (including, *e.g.,* absence of base modification), but differ in pattern of sugar modifications.

[0020] In some embodiments, oligonucleotides of a particular type are chemically identical in that they have the same base sequence (including length), the same pattern of chemical modifications to sugar and base moieties, the same pattern of backbone linkages (*e.g.,* pattern of natural phosphate linkages, phosphorothioate linkages, phosphorothioate triester linkages, and combinations thereof), the same pattern of backbone chiral centers (*e.g.,* pattern of stereochemistry (*R*p/*S*p) of chiral internucleotidic linkages), and the same pattern of backbone phosphorus modifications (*e.g.,* pattern of modifications on the internucleotidic phosphorus atom, such as $-S^-$, and $-L-R^1$ of formula I).

[0021] In some embodiments, the sequence of the oligonucleotide comprises or consists of the sequence of any oligonucleotide disclosed herein. In some embodiments, the sequence of the oligonucleotide comprises or consists of the sequence of any oligonucleotide selected from Tables N1, N2, N3, N4 and 8. In some embodiments, the sequence of the oligonucleotide comprises or consists of the sequence of any oligonucleotide selected from Tables N1A, N2A, N3A, N4A and 8. In some embodiments, the sequence of the oligonucleotide in a stereopure (chirally controlled) oligonucleotide composition comprises or consists of the sequence of WV-1092, WVE120101, WV-2603 or WV-2595. In some embodiments, a sequence of an oligonucleotide includes any one or more of: base sequence (including length); pattern of

chemical modifications to sugar and base moieties; pattern of backbone linkages; pattern of natural phosphate linkages, phosphorothioate linkages, phosphorothioate triester linkages, and combinations thereof; pattern of backbone chiral centers; pattern of stereochemistry (*R*p/*S*p) of chiral internucleotidic linkages; pattern of backbone phosphorus modifications; pattern of modifications on the internucleotidic phosphorus atom, such as $-S^-$, and $-L-R^1$ of formula I.

[0022] Among other things, the present disclosure recognizes the challenge of stereoselective (rather than stereorandom or racemic) preparation of oligonucleotides. Among other things, the present disclosure provides methods and reagents for stereoselective preparation of oligonucleotides comprising multiple (*e.g.,* more than 5, 6, 7, 8, 9, or 10) internucleotidic linkages, and particularly for oligonucleotides comprising multiple (*e.g.,* more than 5, 6, 7, 8, 9, or 10) chiral internucleotidic linkages. In some embodiments, in a stereorandom or racemic preparation of oligonucleotides, at least one chiral internucleotidic linkage is formed with less than 90:10, 95:5, 96:4, 97:3, or 98:2 diastereoselectivity. In some embodiments, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 90:10, 95:5, 96:4, 97:3, or 98:2 diastereoselectivity. In some embodiments, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 95:5 diastereoselectivity. In some embodiments, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 96:4 diastereoselectivity. In some embodiments, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 97:3 diastereoselectivity. In some embodiments, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 98:2 diastereoselectivity. In some embodiments, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 99:1 diastereoselectivity. In some embodiments, diastereoselectivity of a chiral internucleotidic linkage in an oligonucleotide may be measured through a model reaction, *e.g.* formation of a dimer under essentially the same or comparable conditions wherein the dimer has the same internucleotidic linkage as the chiral internucleotidic linkage, the 5'-nucleoside of the dimer is the same as the nucleoside to the 5'-end of the chiral internucleotidic linkage, and the 3'-nucleoside of the dimer is the same as the nucleoside to the 3'-end of the chiral internucleotidic linkage.

[0023] Among other things, it is surprisingly found that certain provided oligonucleotide compositions achieve unprecedented control of cleavage of target sequences, *e.g.,* cleavage of target RNA by RNase H. In some embodiments, the present disclosure demonstrates that precise control of chemical and stereochemical attributes of oligonucleotides achieves improved activity of oligonucleotide preparations as compared with otherwise comparable preparations for which stereochemical attributes are not controlled. Among other things, the present disclosure specifically demonstrates improved rate, degree, and or specificity of cleavage of nucleic acid targets to which provided oligonucleotides hybridize.

[0024] In some embodiments, the present disclosure provides various uses of oligonucleotide compositions. Among other things, the present disclosure demonstrates that by controlling structural elements of oligonucleotides, such as base sequence, chemical modifications, stereochemistry, *etc.*, properties of oligonucleotides can be greatly improved. For example, in some embodiments, the present disclosure provides methods for highly selective suppression of transcripts of a target nucleic acid sequence. In some embodiments, the present disclosure provides methods for treating a subject by suppressing transcripts from a disease-causing copy (*e.g.,* a disease-causing allele). In some embodiments, the present disclosure provides methods for designing and preparing oligonucleotide compositions with surprisingly enhanced activity and/or selectivity when suppressing a transcript of a target sequence. In some embodiments, the present disclosure provides methods for designing and/or preparing oligonucleotide compositions which provide allele-specific suppression of a transcript from a target nucleic acid sequence.

[0025] In some embodiments, the present disclosure provides a method for controlled cleavage of a nucleic acid polymer, the method comprising steps of:

contacting a nucleic acid polymer whose nucleotide sequence comprises a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a target sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

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

[0026] In some embodiments, the present disclosure provides a method for altering a cleavage pattern observed when a nucleic acid polymer whose nucleotide sequence includes a target sequence is contacted with a reference oligonucleotide composition that comprises oligonucleotides having a particular base sequence and length, which particular base sequence is or comprises a sequence that is complementary to the target sequence, the method comprising:

contacting the nucleic acid polymer with a chirally controlled oligonucleotide composition of oligonucleotides having the particular base sequence and length, which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of a single oligonucleotide type characterized by:

1) the particular base sequence and length;

2) a particular pattern of backbone linkages; and

3) a particular pattern of backbone chiral centers.

[0027] In some embodiments, the present disclosure provides a method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines the target nucleic acid sequence, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target nucleic acid sequence and a similar nucleic acid sequences, transcripts of the target nucleic acid sequence are suppressed at a greater level than a level of suppression observed for a similar nucleic acid sequence.

[0028] In some embodiments, the present disclosure provides a method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines the target nucleic acid sequence, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target nucleic acid sequence and a similar nucleic acid sequences, transcripts of the target nucleic acid sequence are suppressed at a greater level than a level of suppression observed for a similar nucleic acid sequence.

[0029] In some embodiments, transcripts of the target nucleic acid sequence are suppressed at a greater level than a level of suppression observed for any one of the similar nucleic acid sequence.

[0030] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target

allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

[0031] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

[0032] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0033] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0034] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that

defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0035] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid

sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

[0036] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

[0037] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0038] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that,

when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0039] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with an oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0040] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that

defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[0041] In some embodiments, a nucleotide characteristic sequence comprises a mutation that defines the target sequence relative to other similar sequences. In some embodiments, a nucleotide characteristic sequence comprises a point mutation that defines the target sequence relative to other similar sequences. In some embodiments, a nucleotide characteristic sequence comprises a SNP that defines the target sequence relative to other similar sequences.

[0042] In some embodiments, the present disclosure provides a method for preparing an oligonucleotide composition comprising oligonucleotides of a particular sequence, which composition provides selective suppression of a transcript of a target sequence, comprising providing a chirally controlled oligonucleotide composition comprising oligonucleotides of a

particular oligonucleotide type characterized by:

1) a common base sequence which is the same as the particular sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which pattern comprises $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein:

m is 1-50;

n is 1-10;

t is 1-50; and

each *N*p is independent *R*p or *S*p.

[0043] In general, activities of oligonucleotide compositions as described herein can be assessed using any appropriate assay. Relative activities for different compositions (*e.g.*, stereocontrolled vs non-stereocontrolled, and/or different stereocontrolled compositions) are typically desirably determined in the same assay, in some embodiments substantially simultaneously and in some embodiments with reference to historical results.

[0044] Those of skill in the art will be aware of and/or will readily be able to develop appropriate assays for particular oligonucleotide compositions. The present disclosure provides descriptions of certain particular assays, for example that may be useful in assessing one or more features of oligonucleotide composition behavior with respect to RNAse H cleavage of a target sequence.

[0045] For example, certain assays that may be useful in the assessment of one or more features (*e.g.*, rate, extent, and/or selectivity of cleavage) of RNase H cleavage may include an assay as described in any assay described and/or exemplified herein (*e.g.*, in one or more of Examples 4, 9-10, 12, 14, 17-20, *etc.*).

[0046] In some embodiments, the present disclosure recognizes that a base sequence can impact properties of oligonucleotides. The present disclosure demonstrates that chemical and stereochemical modifications, combined with designed base sequences, can provide oligonucleotide compositions with unexpectedly improved properties (*e.g.*, surprisingly higher activity, and/or selectivity, *etc.*). In some embodiments, oligonucleotides having a common base sequence complementary to a characteristic sequence element of a target nucleic acid sequence provide better activity compared to another common base sequence complementary to the characteristic sequence element of a target nucleic acid sequence. In some embodiments,

oligonucleotides having a common base sequence complementary to a characteristic sequence element of a target nucleic acid sequence provide better selectivity compared to another common base sequence complementary to the characteristic sequence element of a target nucleic acid sequence.

[0047] In some embodiments, a composition of oligonucleotides having a common base sequence complementary to a characteristic sequence element of a target nucleic acid sequence, when compared to another composition of oligonucleotides having another common base sequence complementary to the characteristic sequence element of the target nucleic acid sequence, provides higher cleavage rate of a transcript from the target nucleic acid sequence, and/or a cleavage pattern which has only one major cleavage site, and the major cleavage site is within or close to the nucleotide characteristic sequence. In some embodiments, a composition of oligonucleotides having a complementary common base sequence, when compared to another composition of oligonucleotides having another complementary common base sequence, provide higher cleavage rate of a transcript from the target nucleic acid sequence, and a cleavage pattern which has only one major cleavage site, and the major cleavage site is within or close to a nucleotide characteristic sequence. In some embodiments, greater than 50%, 60%, 70%, 80% or 90% of cleavage occurs at the one major cleavage site, for example, when measured by a suitable method, *e.g.,* an RNase H assay. In some embodiments, a composition of oligonucleotides having a complementary common base sequence, when compared to another composition of oligonucleotides having another complementary common base sequence, provides higher cleavage rate of a transcript from the target nucleic acid sequence, and a cleavage pattern which has only one major cleavage site, and the major cleavage site is within or close to a mutation or a SNP that defines the target sequence relative to other similar sequences. In some embodiments, a mutation is a point mutation. In some embodiments, a major cleavage site is next to a mutation or a SNP that defines the target sequence relative to other similar sequences. In some embodiments, each common base sequence is 100% complementary to the characteristic sequence element of the target nucleic acid sequence. In some embodiments, a major cleavage site is within less than 5, 4, 3, or 1 internucleotidic linkage from a mutation or a SNP that defines the target sequence relative to other similar sequences. In some embodiments, a major cleavage site is within less than 5, 4, 3, or 1 internucleotidic linkage from a mutation or a SNP that defines the target sequence relative to other similar sequences, and is within less than 5, 4, 3, or 1

internucleotidic linkage from a cleavage site when a stereorandom composition of oligonucleotides having the same common sequence, and/or a composition of DNA oligonucleotides having the same common sequence, is used. In some embodiments, a major cleavage site is a cleavage site when a stereorandom composition of oligonucleotides having the same common sequence is used. In some embodiments, a major cleavage site is a major cleavage site when a stereorandom composition of oligonucleotides having the same common sequence is used. In some embodiments, a major cleavage site is a cleavage site when a composition of DNA oligonucleotides having the same common sequence is used. In some embodiments, a major cleavage site is a major cleavage site when a composition of DNA oligonucleotides having the same common sequence is used.

[0048] In some embodiments, when comparing effects of a first and a second common base sequences, a stereorandom composition of oligonucleotides having a first common base sequence may be compared to a stereorandom composition of oligonucleotides having a second common base sequence. In some embodiments, a stereorandom composition is a composition of oligonucleotides having a common base sequence, a common pattern of nucleoside modifications, and a common pattern of backbone linkages. In some embodiments, a stereorandom composition is a composition of oligonucleotides having a common base sequence, a common pattern of nucleoside modifications, wherein each internucleotidic linkage is phosphorothioate. In some embodiments, when comparing effects of a first and a second common base sequences, a chirally controlled oligonucleotide composition of oligonucleotides having a first common base sequence may be compared to a chirally controlled oligonucleotide composition of oligonucleotides having a second common base sequence. In some embodiments, oligonucleotides in a chirally controlled oligonucleotide composition have a common base sequence, a common pattern of nucleoside modifications, a common pattern of backbone linkages, a common pattern of backbone chiral centers, and a common pattern of backbone phosphorus modifications. In some embodiments, each internucleotidic linkage is phosphorothioate.

[0049] In some embodiments, oligonucleotide compositions and technologies described herein are particularly useful in the treatment of Huntington's disease. For example, in some embodiments, the present disclosure defines stereochemically controlled oligonucleotide compositions that direct cleavage (*e.g.*, RNase H-mediated cleavage) of nucleic acids associated

with Huntington's disease. In some embodiments, such compositions direct preferential cleavage of a Huntington's disease-associated allele of a particular target sequence, relative to one or more (*e.g.*, all non-Huntington's disease-associated) other alleles of the sequence.

[0050] Huntington's disease is an inherited disease that can cause progressive degeneration of nerve cells in the brain and affect a subject's motor and cognitive abilities. In some embodiments, Huntington's disease is an autosomal dominant disorder. In some embodiments, it is caused by mutations in the Huntingtin gene. Normal HTT gene contains 10 to 35 CAG tri-nucleotide repeats. People with 40 or more repeats often develop the disorder. In some embodiments, the expanded CAG segment on the first exon of HTT gene leads to the production of an abnormally long version of the Huntingtin protein (expanded polyglutamine tract) which is cut into smaller, toxic fragments that bind together and accumulate in neurons, disrupting the normal functions of these cells. Warby et al. (Am J Hum Genet. 2009, 84(3), 351–366) reported many SNPs that are associated with disease chromosomes and have stronger linkage associations with CAG expansion than those reported before. Many SNPs highly associated with CAG expansion do not segregate independently and are in Linkage Disequilibrium with each other. Among other things, the present disclosure recognizes that strong association between specific SNPs and CAG expanded chromosomes provides an attractive therapeutic opportunity for the treatment of Huntington Disease, *e.g.*, through antisense therapy. Furthermore, the association of specific SNPs combined with high rates of heterozygosity in HD patients provides suitable targets for allele-specific knockdown of the mutant gene product. For example references, see Liu et al. Journal of Huntington's Disease 2, 2013, 491–500; Aronin, Neil and Pfister, Edith WO 2010/118263 A1; Pfister et al. Current Biology 2009,19, 774–778.

[0051] In some embodiments, a targeted SNP of the present disclosure has high frequency of heterozygosity in HD and has a particular variant associated with the mutant HTT allele. In some embodiments, a SNP is rs362307. In some embodiments, a SNP is rs7685686. In some embodiments, a SNP may not be linked but may have a high heterozygous frequency. In some embodiments, a SNP is rs362268 (3'-UTR region). In some embodiments, a SNP is rs362306 (3'-UTR region). In some embodiments, a SNP is rs2530595. In some embodiments, a SNP is rs362331.

[0052] In some embodiments, a provided method for treating or preventing Huntington's disease in a subject, comprising administering to the subject a provided oligonucleotide compositions. In some embodiments, a provided method for treating or preventing Huntington's disease in a subject, comprising administering to the subject a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

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

[0053] In some embodiments, a provided method ameliorates a symptom of Huntington's disease. In some embodiments, a provided method slows onset of Huntington's disease. In some embodiments, a provided method slows progression of Huntington's disease.

[0054] In some embodiments, the present disclosure provides methods for identifying patients for a given oligonucleotide composition. In some embodiments, the present disclosure provides methods for patient stratification. In some embodiments, a provided method comprises identifying a mutation and/or SNP associated with a disease-causing allele. For example, in some embodiments, a provided method comprises identifying in a subject a SNP associated with expanded CAG repeats that are associated with or causing Huntington's disease.

[0055] In some embodiments, a subject has a SNP in the subject's Huntingtin gene. In some embodiments, a subject has a SNP, wherein one allele is mutant Huntingtin associated with expanded CAG repeats. In some embodiments, a subject has a SNP selected from rs362307, rs7685686, rs362268, rs2530595, rs362331, or rs362306. In some embodiments, oligonucleotides of a provided composition have a sequence complementary to a sequence comprising a SNP from the disease-causing allele (mutant), and the composition selectively suppresses expression from the diseasing-causing allele.

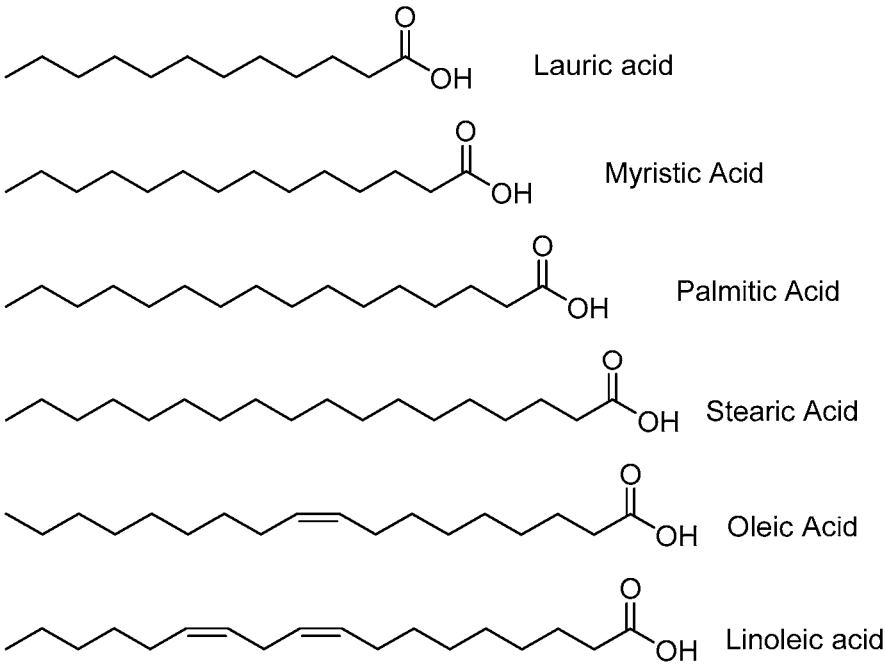
[0056] In some embodiments, the sequence of oligonucleotides in provided technologies (compounds, compositions, methods, *etc.*) comprises, consists of, or is the sequence of any oligonucleotide described herein. In some embodiments, a sequence is selected from Tables N1A, N2A, N3A, N4A or 8; or WV-1092, WVE120101, WV-2603 or WV-2595. In some

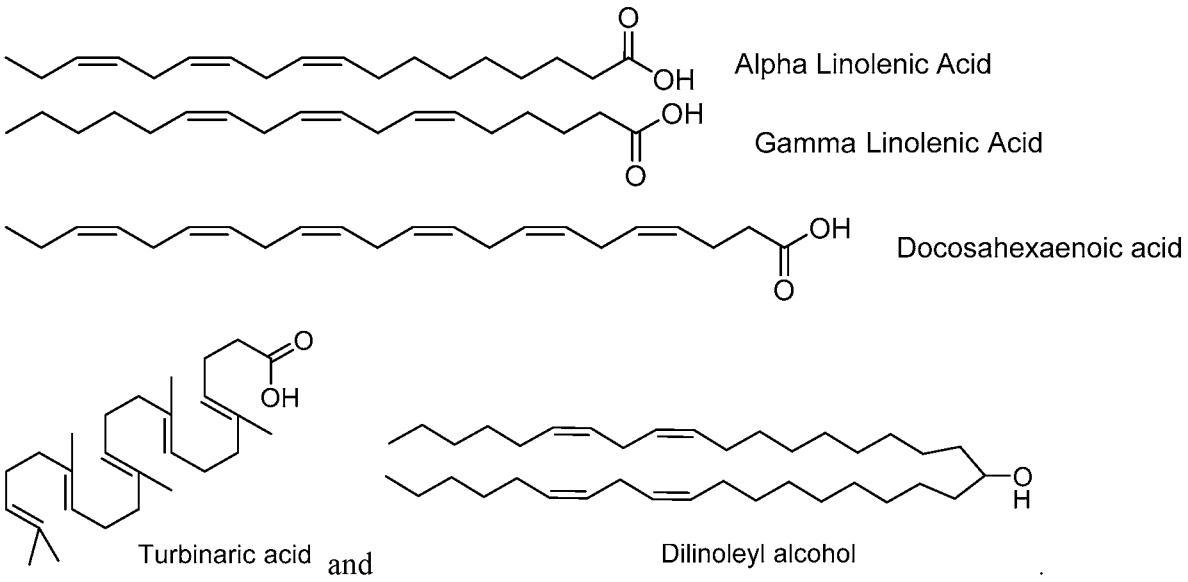
embodiments, a sequence is selected from the sequence of WV-1092, WVE120101, WV-2603 or WV-2595. In some embodiments, provided oligonucleotides are of the type defined by WV-1092, WVE120101, WV-2603 or WV-2595. In some embodiments, provided oligonucleotides are of the type defined by WV-1092. In some embodiments, provided oligonucleotides are of the type defined by WVE120101. In some embodiments, provided oligonucleotides are of the type defined by WV-2603. In some embodiments, provided oligonucleotides are of the type defined by WV-2595.

[0057] In some embodiments, provided oligonucleotide compositions comprises a lipid and an oligonucleotide. In some embodiments, a lipid is conjugated to an oligonucleotide.

[0058] In some embodiments, a composition comprises an oligonucleotide and a lipid selected from the list of: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, arachidonic acid, and dilinoleyl. In some embodiments, a composition comprises an oligonucleotide and a lipid selected from the list of: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, and dilinoleyl.

[0059] In some embodiments, a composition comprises an oligonucleotide and a lipid selected from:





[0060] In some embodiments, a composition comprises an oligonucleotide and a lipid, wherein the lipid comprises a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic group.

[0061] In some embodiments, an oligonucleotide composition comprises a plurality of oligonucleotides, which share:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone phosphorus modifications;

wherein one or more oligonucleotides of the plurality are individually conjugated to a lipid.

[0062] In some embodiments, a chirally controlled oligonucleotide composition comprises a plurality of oligonucleotides, which share:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone phosphorus modifications;

wherein:

the composition is chirally controlled in that the plurality of oligonucleotides share the same stereochemistry at one or more chiral internucleotidic linkages;

one or more oligonucleotides of the plurality are individually conjugated to a lipid; and

one or more oligonucleotides of the plurality are optionally and individually conjugated to a targeting compound or moiety.

[0063] In some embodiments, a method of delivering an oligonucleotide to a cell or tissue in a human subject, comprises:

(a) Providing a composition of any one of the embodiments described herein; and

(b) Administering the composition to the human subject such that the oligonucleotide is delivered to a cell or tissue in the subject.

[0064] In some embodiments, a method for delivering an oligonucleotide to a cell or tissue comprises preparing a composition according to any one of the embodiments described herein and contacting the cell or tissue with the composition.

[0065] In some embodiments, a method of modulating the level of a transcript or gene product of a gene in a cell, the method comprises the step of contacting the cell with a composition according to any one of the embodiments described herein, wherein the oligonucleotide is capable of modulating the level of the transcript or gene product.

[0066] In some embodiments, a method for inhibiting expression of a gene in a cell or tissue comprises preparing a composition according to any one of the embodiments described herein and treating the cell or tissue with the composition.

[0067] In some embodiments, a method for inhibiting expression of a gene in a cell or tissue in a mammal comprises preparing a composition according to any one of the embodiments described herein and administering the composition to the mammal.

[0068] In some embodiments, a method of treating a disease that is caused by the over-expression of one or several proteins in a cell or tissue in a subject, said method comprises the administration of a composition according to any one of the embodiments described herein to the subject.

[0069] In some embodiments, a method of treating a disease that is caused by a reduced, suppressed or missing expression of one or several proteins in a subject, said method comprises the administration of a composition according to any one of the embodiments described herein to the subject.

[0070] In some embodiments, a method for generating an immune response in a subject, said method comprises the administration of a composition according to any one of the

embodiments described herein to the subject, wherein the biologically active compound is an immunomodulating nucleic acid.

[0071] In some embodiments, a method for treating a sign and/or symptom of Huntington'sDisease by providing a composition of any one of the embodiments described herein and administering the composition to the subject.

[0072] In some embodiments, a method of modulating the amount of RNaseH-mediated cleavage in a cell, the method comprises the step of contacting the cell with a composition according to any one of the embodiments described herein, wherein the oligonucleotide is capable of modulating the amount of RNaseH-mediated cleavage.

[0073] In some embodiments, a method of administering an oligonucleotide to a subject in need thereof, comprises steps of providing a composition comprises the agent a lipid, and administering the composition to the subject, wherein the agent is any agent disclosed herein, and wherein the lipid is any lipid disclosed herein.

[0074] In some embodiments, a method of treating a disease in a subject, the method comprises steps of providing a composition comprises the agent a lipid, and administering a therapeutically effective amount of the composition to the subject, wherein the agent is any agent disclosed herein, and wherein the lipid is any lipid disclosed herein, and wherein the disease is any disease disclosed herein.

[0075] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{40} saturated or partially unsaturated aliphatic chain.

[0076] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[0077] In some embodiments, a lipid comprises a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic group.

[0078] In some embodiments, a lipid comprises an unsubstituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[0079] In some embodiments, a lipid comprises no more than one optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

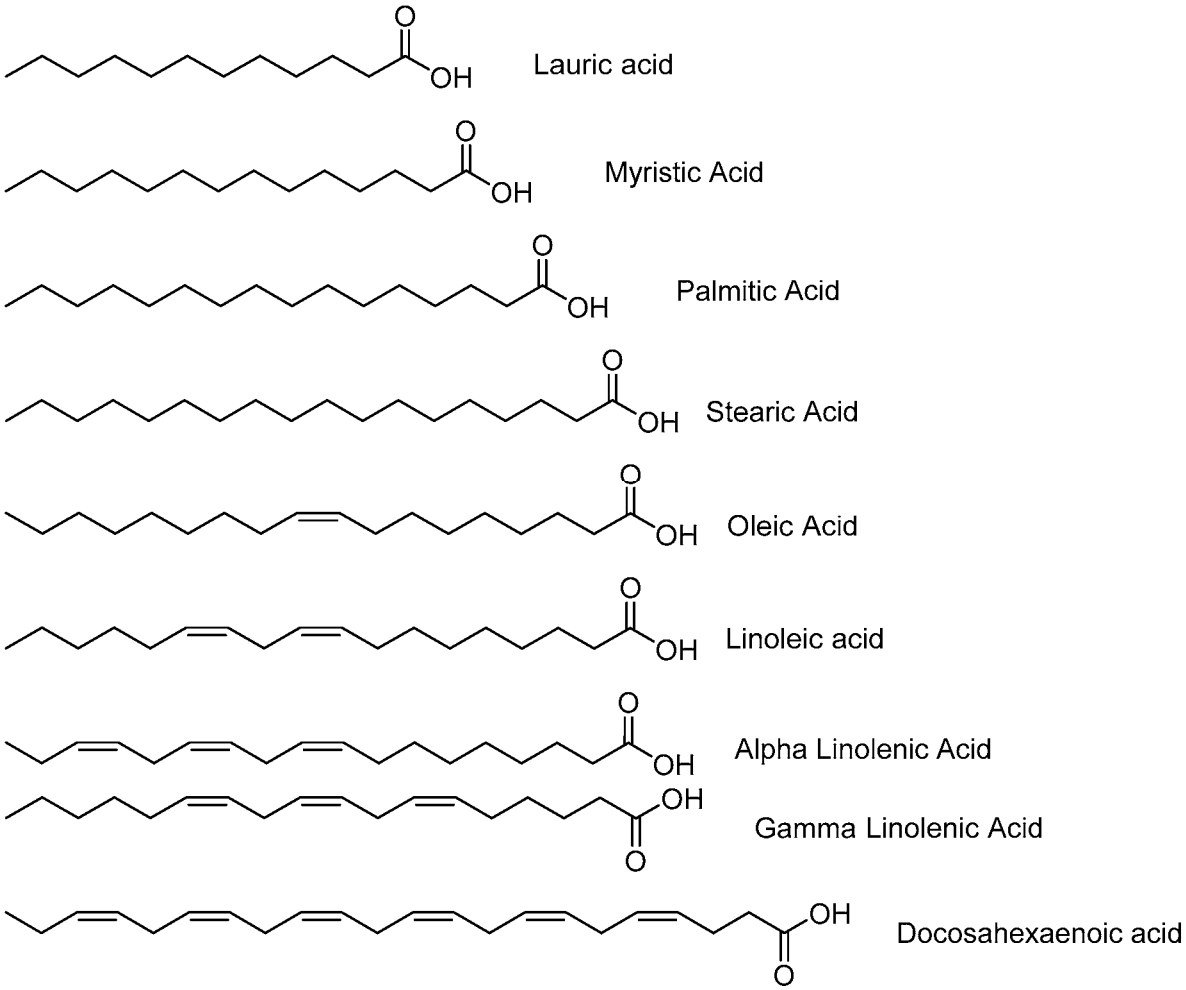
[0080] In some embodiments, a lipid comprises two or more optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

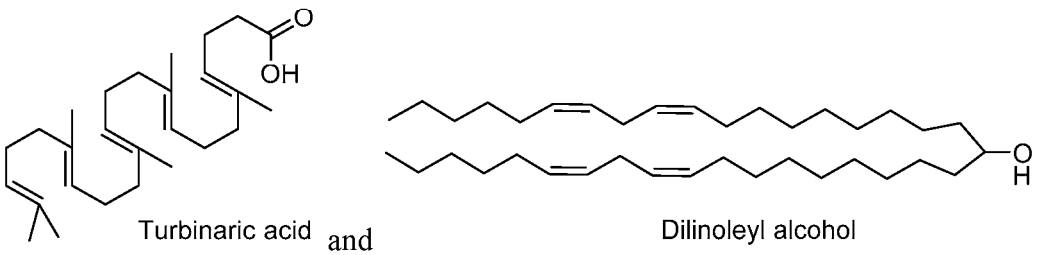
[0081] In some embodiments, a lipid comprises no tricyclic or polycyclic moiety.

[0082] In some embodiments, a lipid has the structure of R^1-COOH, wherein R^1 is an optionally substituted C_{10}-C_{40} saturated or partially unsaturated aliphatic chain.

[0083] The composition or method of any one of claim 16, wherein the lipid is conjugated through its carboxyl group.

[0084] The composition or method according to any one of the embodiments described herein, wherein the lipid is selected from:





[0085] In some embodiments, a lipid is conjugated to the oligonucleotide.

[0086] In some embodiments, a lipid is directly conjugated to the oligonucleotide.

[0087] In some embodiments, a lipid is conjugated to the oligonucleotide via a linker.

[0088] In some embodiments, a linker is selected from: an uncharged linker; a charged linker; a linker comprises an alkyl; a linker comprises a phosphate; a branched linker; an unbranched linker; a linker comprises at least one cleavage group; a linker comprises at least one redox cleavage group; a linker comprises at least one phosphate-based cleavage group; a linker comprises at least one acid-cleavage group; a linker comprises at least one ester-based cleavage group; and a linker comprises at least one peptide-based cleavage group.

[0089] In some embodiments, each oligonucleotide of the plurality is individually conjugated to the same lipid at the same location.

[0090] In some embodiments, a lipid is conjugated to an oligonucleotide through a linker.

[0091] In some embodiments, one or more oligonucleotides of the plurality are independently conjugated to a targeting compound or moiety.

[0092] In some embodiments, one or more oligonucleotides of the plurality are independently conjugated to a lipid and a targeting compound or moiety.

[0093] In some embodiments, one or more oligonucleotides of the plurality are independently conjugated to a lipid at one end and a targeting compound or moiety at the other.

[0094] In some embodiments, oligonucleotides of the plurality share the same chemical modification patterns.

[0095] In some embodiments, oligonucleotides of the plurality share the same chemical modification patterns comprises one or more base modifications.

[0096] In some embodiments, oligonucleotides of the plurality share the same chemical modification patterns comprises one or more sugar modifications.

[0097] In some embodiments, a common base sequence is capable of hybridizing with a transcript in a cell, which transcript contains a mutation that is linked to Huntington's Disease, or whose level, activity and/or distribution is linked to Huntington's Disease.

[0098] In some embodiments, an oligonucleotide is a nucleic acid.

[0099] In some embodiments, an oligonucleotide is an oligonucleotide.

[00100] In some embodiments, an oligonucleotide is an oligonucleotide which participates in RNaseH-mediated cleavage of a mutant Huntingtin gene mRNA.

[00101] In some embodiments, a disease or disorder is Huntington's Disease.

[00102] In some embodiments, a lipid comprises an optionally substituted, C_{10}-C_{80} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, –C(S)–, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, –SC(O)–, –C(O)S–, –OC(O)–, and $-C(O)O-$, wherein each variable is independently as defined and described herein.

[00103] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{80} saturated or partially unsaturated, aliphatic chain.

[00104] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain.

[00105] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{60} saturated or partially unsaturated, aliphatic chain.

[00106] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain.

[00107] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{40} saturated or partially unsaturated, aliphatic chain.

[00108] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[00109] In some embodiments, a lipid comprises an optionally substituted, C_{10}-C_{60} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, –C(S)–, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, –SC(O)–, –C(O)S–, –OC(O)–, and $-C(O)O-$, wherein each variable is independently as defined and described herein.

[00110] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{80} saturated or partially unsaturated, aliphatic chain.

[00111] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain.

[00112] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[00113] In some embodiments, a lipid comprises an optionally substituted, C_{10}-C_{40} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, –C(S)–, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, –SC(O)–, –C(O)S–, –OC(O)–, and $-C(O)O-$, wherein each variable is independently as defined and described herein.

[00114] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{40} saturated or partially unsaturated, aliphatic chain.

[00115] In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[00116] In some embodiments, a composition further comprises one or more additional components selected from: a polynucleotide, carbonic anhydrase inhibitor, a dye, an intercalating agent, an acridine, a cross-linker, psoralene, mitomycin C, a porphyrin, TPPC4, texaphyrin, Sapphyrin, a polycyclic aromatic hydrocarbon phenazine, dihydrophenazine, an artificial endonuclease, a chelating agent, EDTA, an alkylating agent, a phosphate, an amino, a mercapto, a PEG, PEG-40K, MPEG, $[MPEG]_2$, a polyamino, an alkyl, a substituted alkyl, a radiolabeled marker, an enzyme, a hapten biotin, a transport/absorption facilitator, aspirin, vitamin E, folic acid, a synthetic ribonuclease, a protein, a glycoprotein, a peptide, a molecule having a specific affinity for a co-ligand, an antibody, a hormone, a hormone receptor, a non-peptidic species, a lipid, a lectin, a carbohydrate, a vitamin, a cofactor, selectivity agent, or a drug. In some embodiments, a composition further comprises one or more additional components selected from: a polynucleotide, carbonic anhydrase inhibitor, a dye, an intercalating agent, an acridine, a cross-linker, psoralene, mitomycin C, a porphyrin, TPPC4, texaphyrin, Sapphyrin, a polycyclic aromatic hydrocarbon phenazine, dihydrophenazine, an artificial endonuclease, a chelating agent, EDTA, an alkylating agent, a phosphate, an amino, a mercapto, a PEG, PEG-40K, MPEG,

$[MPEG]_2$, a polyamino, an alkyl, a substituted alkyl, a radiolabeled marker, an enzyme, a hapten biotin, a transport/absorption facilitator, aspirin, vitamin E, folic acid, a synthetic ribonuclease, a protein, a glycoprotein, a peptide, a molecule having a specific affinity for a co-ligand, an antibody, a hormone, a hormone receptor, a non-peptidic species, a lipid, a lectin, a carbohydrate, a vitamin, a cofactor, or a drug.

[00117] In some embodiments, the present disclosure provides an oligonucleotide conjugated to a selectivity agent. In some embodiments, the present disclosure provides a composition comprising an oligonucleotide or oligonucleotide type comprising a selectivity agent. In some embodiments, a selectivity agent binds specifically to one or more neurotransmitter transporters selected from the group consisting of a dopamine transporter (DAT), a serotonin transporter (SERT), and a norepinephrine transporter (NET). In some embodiments, a selectivity agent is selected from the group consisting of a dopamine reuptake inhibitor (DRI), a selective serotonin reuptake inhibitor (SSRI), a noradrenaline reuptake inhibitor (NRI), a norepinephrine-dopamine reuptake inhibitor (NDRI), and a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI). In some embodiments, a selectivity agent is selected from the group consisting of a triple reuptake inhibitor, a noradrenaline dopamine double reuptake inhibitor, a serotonin single reuptake inhibitor, a noradrenaline single reuptake inhibitor, and a dopamine single reuptake inhibitor. In some embodiments, a selectivity agent is selected from the group consisting of a dopamine reuptake inhibitor (DRI), a Norepinephrine-Dopamine Reuptake Inhibitor (NDRI) and a serotonin-Norepinephrine-Dopamine Reuptake Inhibitor (SNDRI). In some embodiments, a selectivity agent is selected from the selectivity agents which are described in U.S. Pat. Nos. 9,084,825; and 9,193,969; and WO2011131693, WO2014064258.

[00118] In some embodiments, a lipid comprises a C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain.

[00119] In some embodiments, a composition further comprises a linker linking the oligonucleotide and the lipid, wherein the linker is selected from: an uncharged linker; a charged linker; a linker comprises an alkyl; a linker comprises a phosphate; a branched linker; an unbranched linker; a linker comprises at least one cleavage group; a linker comprises at least one redox cleavage group; a linker comprises at least one phosphate-based cleavage group; a linker

comprises at least one acid-cleavage group; a linker comprises at least one ester-based cleavage group; a linker comprises at least one peptide-based cleavage group.

[00120] In some embodiments, an oligonucleotide comprises or consists of or is an oligonucleotide or oligonucleotide composition or chirally controlled oligonucleotide composition.

[00121] In some embodiments, an oligonucleotide comprises or consists of or is an oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of any oligonucleotide described herein.

[00122] In some embodiments, an oligonucleotide comprises or consists of or is an oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of any oligonucleotide listed in Table 4.

[00123] In some embodiments, an oligonucleotide comprises or consists of or is an oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of a splice-switching oligonucleotide.

[00124] The composition or method of any of the embodiments described herein, wherein the oligonucleotide is a chirally controlled oligonucleotide composition.

[00125] The composition or method of any of the embodiments described herein, wherein the disease or disorder is Huntington's Disease.

[00126] The composition or method of any of the embodiments described herein, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a mutant Huntingtin gene mRNA.

[00127] The composition or method of any of the embodiments described herein, wherein the oligonucleotide comprises, consists of or is the sequence of any oligonucleotide disclosed herein.

[00128] The composition or method of any of the embodiments described herein, wherein the oligonucleotide is capable of differentiating between a wild-type and a mutant Huntingtin allele.

[00129] The composition or method of any of the embodiments described herein, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a mutant Huntingtin gene mRNA.

[00130] The composition or method of any of the embodiments described herein, wherein the oligonucleotide comprises, consists of or is the sequence of any oligonucleotide disclosed in Table 4.

[00131] In some embodiments, an oligonucleotide comprises or consists of or is an oligonucleotide or oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of any of: WV-1092, WV-2595, or WV-2603.

[00132] In some embodiments, a sequence of an oligonucleotide includes any one or more of: base sequence (including length); pattern of chemical modifications to sugar and base moieties; pattern of backbone linkages; pattern of natural phosphate linkages, phosphorothioate linkages, phosphorothioate triester linkages, and combinations thereof; pattern of backbone chiral centers; pattern of stereochemistry (Rp/Sp) of chiral internucleotidic linkages; pattern of backbone phosphorus modifications; pattern of modifications on the internucleotidic phosphorus atom, such as $-S^-$, and $-L-R^1$ of formula I.

[00133] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type, wherein the oligonucleotides target a mutant Huntingtin gene, and the length is from about 10 to about 50 nucleotides, wherein the backbone linkages comprise at least one phosphorothioate, and wherein the pattern of backbone chiral centers comprises at least one chiral center in a *R*p conformation and at least one chiral center in a *S*p conformation.

[00134] In some embodiments, the present disclosure provides a method for cleavage of a nucleic acid having a base sequence comprising a target sequence, the method comprising steps of:

(a) contacting a nucleic acid having a base sequence comprising a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to the target sequence in the nucleic acid;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type, wherein the oligonucleotide targets a mutant Huntingtin gene, and the length is from about 10 to about 50 nucleotides, wherein the backbone linkages comprise at least one phosphorothioate, and wherein the pattern of backbone chiral centers comprises at least one chiral center in a Rp conformation and at least one chiral center in a Sp conformation.

[00135] In some embodiments, the present disclosure provides a method for cleavage of a nucleic acid having a base sequence comprising a target sequence, the method comprising steps of:

(a) contacting a nucleic acid having a base sequence comprising a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to the target sequence in the nucleic acid;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type, wherein the oligonucleotide targets a mutant Huntingtin gene, and the length is from about 10 to about 50 nucleotides, wherein the

backbone linkages comprise at least one phosphorothioate, and wherein the pattern of backbone chiral centers comprises at least one chiral center in a Rp conformation and at least one chiral center in a Sp conformation; and

(b) cleavage of the nucleic acid mediated by a RNAseH or RNA interference mechanism.

[00136] In some embodiments, a provided composition further comprises a selectivity agent selected from: the group of compounds which binds specifically to one or more neurotransmitter transporters selected from the group consisting of a dopamine transporter (DAT), a serotonin transporter (SERT), and a norepinephrine transporter (NET); the group consisting of a dopamine reuptake inhibitor (DRI), a selective serotonin reuptake inhibitor (SSRI), a noradrenaline reuptake inhibitor (NRI), a norepinephrine-dopamine reuptake inhibitor (NDRI), and a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI); the group consisting of a triple reuptake inhibitor, a noradrenaline dopamine double reuptake inhibitor, a serotonin single reuptake inhibitor, a noradrenaline single reuptake inhibitor, and a dopamine single reuptake inhibitor; and the group consisting of a dopamine reuptake inhibitor (DRI), a Norepinephrine-Dopamine Reuptake Inhibitor (NDRI) and a serotonin-Norepinephrine-Dopamine Reuptake Inhibitor (SNDRI).

[00137] In some embodiments, a provided composition comprises oligonucleotides wherein the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of the oligonucleotides comprises or consists of the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of any of any oligonucleotide selected from Tables N1A, N2A, N3A, N4A and 8; and WV-1092, WV-2595, and WV-2603.

[00138] In some embodiments, a provided composition comprises oligonucleotides wherein the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of the oligonucleotides comprises or consists of the base sequence, and pattern of backbone linkages, and/or pattern of backbone chiral centers of any of any oligonucleotide selected from Tables N1A, N2A, N3A, N4A and 8; and WV-1092, WV-2595, and WV-2603.

[00139] In some embodiments, a provided composition comprises oligonucleotides wherein the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of the oligonucleotides comprises or consists of the base sequence, and pattern of backbone linkages, and pattern of backbone chiral centers of any of any oligonucleotide selected from Tables N1A, N2A, N3A, N4A and 8; and WV-1092, WV-2595, and WV-2603.

Definitions

[00140] *Aliphatic*: The term "aliphatic" or "aliphatic group", as used herein, means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation, or a monocyclic hydrocarbon or bicyclic or polycyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic (also referred to herein as "carbocycle" "cycloaliphatic" or "cycloalkyl"), that has a single point of attachment to the rest of the molecule. In some embodiments, aliphatic groups contain 1-50 aliphatic carbon atoms. Unless otherwise specified, aliphatic groups contain 1-10 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-6 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-5 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-4 aliphatic carbon atoms. In still other embodiments, aliphatic groups contain 1-3 aliphatic carbon atoms, and in yet other embodiments, aliphatic groups contain 1-2 aliphatic carbon atoms. In some embodiments, "cycloaliphatic" (or "carbocycle" or "cycloalkyl") refers to a monocyclic or bicyclic C_3-C_{10} hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule. In some embodiments, "cycloaliphatic" (or "carbocycle" or "cycloalkyl") refers to a monocyclic C_3–C_6 hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule. Suitable aliphatic groups include, but are not limited to, linear or branched, substituted or unsubstituted alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

[00141] *Alkylene*: The term "alkylene" refers to a bivalent alkyl group. An "alkylene chain" is a polymethylene group, i.e., $–(CH_2)_n–$, wherein n is a positive integer, preferably from 1 to 6, from 1 to 4, from 1 to 3, from 1 to 2, or from 2 to 3. A substituted alkylene chain is a polymethylene group in which one or more methylene hydrogen atoms are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

[00142] *Alkenylene*: The term "alkenylene" refers to a bivalent alkenyl group. A substituted alkenylene chain is a polymethylene group containing at least one double bond in which one or more hydrogen atoms are replaced with a substituent. Suitable substituents include those described below for a substituted aliphatic group.

[00143] *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.

[00144] *Approximately:* As used herein, the terms "approximately" or "about" in reference to a number are generally taken to include numbers that fall within a range of 5%, 10%, 15%, or 20% in either direction (greater than or less than) of the number unless otherwise stated or otherwise evident from the context (except where such number would be less than 0% or exceed 100% of a possible value). In some embodiments, use of the term "about" in reference to dosages means ± 5 mg/kg/day.

[00145] *Aryl:* The term "aryl" used alone or as part of a larger moiety as in "aralkyl," "aralkoxy," or "aryloxyalkyl," refers to monocyclic and bicyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic and wherein each ring in the system contains three to seven ring members. The term "aryl" may be used interchangeably with the term "aryl ring." In certain embodiments of the present disclosure, "aryl" refers to an aromatic ring system which includes, but not limited to, phenyl, biphenyl, naphthyl, anthracyl and the like, which may bear one or more substituents. Also included within the scope of the term "aryl," as it is used herein, is a group in which an aromatic ring is fused to one or more non–aromatic rings, such as indanyl, phthalimidyl, naphthimidyl, phenanthridinyl, or tetrahydronaphthyl, and the like.

[00146] *Characteristic portion*: As used herein, the phrase a "characteristic portion" of a protein or polypeptide is one that contains a continuous stretch of amino acids, or a collection of continuous stretches of amino acids, that together are characteristic of a protein or polypeptide. Each such continuous stretch generally will contain at least two amino acids. Furthermore, those of ordinary skill in the art will appreciate that typically at least 5, 10, 15, 20 or more amino acids are required to be characteristic of a protein. In general, a characteristic portion is one that, in addition to the sequence identity specified above, shares at least one functional characteristic

with the relevant intact protein.

[00147] *Characteristic sequence*: A "characteristic sequence" is a sequence that is found in all members of a family of polypeptides or nucleic acids, and therefore can be used by those of ordinary skill in the art to define members of the family.

[00148] *Characteristic structural element:* The term "characteristic structural element" refers to a distinctive structural element (*e.g.*, core structure, collection of pendant moieties, sequence element, *etc*) that is found in all members of a family of polypeptides, small molecules, or nucleic acids, and therefore can be used by those of ordinary skill in the art to define members of the family.

[00149] *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.

[00150] *Dosing regimen:* As used herein, a "dosing regimen" or "therapeutic regimen" refers to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regime comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount.

[00151] *Equivalent agents:* Those of ordinary skill in the art, reading the present disclosure, will appreciate that the scope of useful agents in the context of the present disclosure is not limited to those specifically mentioned or exemplified herein. In particular, those skilled in the art will recognize that active agents typically have a structure that consists of a core and attached pendant moieties, and furthermore will appreciate that simple variations of such core and/or pendant moieties may not significantly alter activity of the agent. For example, in some embodiments, substitution of one or more pendant moieties with groups of comparable three-dimensional structure and/or chemical reactivity characteristics may generate a substituted compound or portion equivalent to a parent reference compound or portion. In some embodiments, addition or removal of one or more pendant moieties may generate a substituted compound equivalent to a parent reference compound. In some embodiments, alteration of core structure, for example by addition or removal of a small number of bonds (typically not more than 5, 4, 3, 2, or 1 bonds, and often only a single bond) may generate a substituted compound equivalent to a parent reference compound. In many embodiments, equivalent compounds may be prepared by methods illustrated in general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional or provided synthesis procedures. In these reactions, it is also possible to make use of variants, which are in themselves known, but are not mentioned here.

[00152] *Equivalent Dosage:* The term "equivalent dosage" is used herein to compare dosages of different pharmaceutically active agents that effect the same biological result. Dosages of two different agents are considered to be "equivalent" to one another in accordance with the present disclosure if they achieve a comparable level or extent of the biological result. In some embodiments, equivalent dosages of different pharmaceutical agents for use in accordance with the present disclosure are determined using *in vitro* and/or *in vivo* assays as described herein. In some embodiments, one or more lysosomal activating agents for use in accordance with the present disclosure is utilized at a dose equivalent to a dose of a reference lysosomal activating agent; in some such embodiments, the reference lysosomal activating agent for such purpose is selected from the group consisting of small molecule allosteric activators (*e.g.,* pyrazolpyrimidines), imminosugars (*e.g.,* isofagomine), antioxidants (*e.g.,* n-acetyl-cysteine), and regulators of cellular trafficking (*e.g.,* Rab1a polypeptide).

[00153] *Heteroaliphatic*: The term "heteroaliphatic" refers to an aliphatic group wherein

one or more units selected from C, CH, CH_2, or CH_3 are independently replaced by a heteroatom. In some embodiments, a heteroaliphatic group is heteroalkyl. In some embodiments, a heteroaliphatic group is heteroalkenyl.

[00154] *Heteroaryl*: The terms "heteroaryl" and "heteroar–," used alone or as part of a larger moiety, *e.g.*, "heteroaralkyl," or "heteroaralkoxy," refer to groups having 5 to 10 ring atoms, preferably 5, 6, or 9 ring atoms; having 6, 10, or 14 π electrons shared in a cyclic array; and having, in addition to carbon atoms, from one to five heteroatoms. The term "heteroatom" refers to nitrogen, oxygen, or sulfur, and includes any oxidized form of nitrogen or sulfur, and any quaternized form of a basic nitrogen. Heteroaryl groups include, without limitation, thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, indolizinyl, purinyl, naphthyridinyl, and pteridinyl. The terms "heteroaryl" and "heteroar–," as used herein, also include groups in which a heteroaromatic ring is fused to one or more aryl, cycloaliphatic, or heterocyclyl rings, where the radical or point of attachment is on the heteroaromatic ring. Nonlimiting examples include indolyl, isoindolyl, benzothienyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4*H*–quinolizinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, and pyrido[2,3–b]–1,4–oxazin–3(4H)–one. A heteroaryl group may be mono– or bicyclic. The term "heteroaryl" may be used interchangeably with the terms "heteroaryl ring," "heteroaryl group," or "heteroaromatic," any of which terms include rings that are optionally substituted. The term "heteroaralkyl" refers to an alkyl group substituted by a heteroaryl, wherein the alkyl and heteroaryl portions independently are optionally substituted.

[00155] *Heteroatom*: The term "heteroatom" means one or more of oxygen, sulfur, nitrogen, phosphorus, boron, selenium, or silicon (including, any oxidized form of nitrogen, boron, selenium, sulfur, phosphorus, or silicon; the quaternized form of any basic nitrogen or; a substitutable nitrogen of a heterocyclic ring, for example N (as in 3,4-dihydro-2*H*-pyrrolyl), NH (as in pyrrolidinyl) or NR^+ (as in N-substituted pyrrolidinyl)).

[00156] *Heterocycle*: As used herein, the terms "heterocycle," "heterocyclyl," "heterocyclic radical," and "heterocyclic ring" are used interchangeably and refer to a stable 3– to 7–membered monocyclic or 7–10–membered bicyclic heterocyclic moiety that is either

saturated or partially unsaturated, and having, in addition to carbon atoms, one or more, preferably one to four, heteroatoms, as defined above. When used in reference to a ring atom of a heterocycle, the term "nitrogen" includes a substituted nitrogen. As an example, in a saturated or partially unsaturated ring having 0–3 heteroatoms selected from oxygen, sulfur or nitrogen, the nitrogen may be N (as in 3,4–dihydro–2*H*–pyrrolyl), NH (as in pyrrolidinyl), or $^{+}$NR (as in *N*–substituted pyrrolidinyl).

[00157] A heterocyclic ring can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted. Examples of such saturated or partially unsaturated heterocyclic radicals include, without limitation, tetrahydrofuranyl, tetrahydrothiophenyl pyrrolidinyl, piperidinyl, pyrrolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, dioxanyl, dioxolanyl, diazepinyl, oxazepinyl, thiazepinyl, morpholinyl, and quinuclidinyl. The terms "heterocycle," "heterocyclyl," "heterocyclyl ring," "heterocyclic group," "heterocyclic moiety," and "heterocyclic radical," are used interchangeably herein, and also include groups in which a heterocyclyl ring is fused to one or more aryl, heteroaryl, or cycloaliphatic rings, such as indolinyl, 3*H*–indolyl, chromanyl, phenanthridinyl, or tetrahydroquinolinyl, where the radical or point of attachment is on the heterocyclyl ring. A heterocyclyl group may be mono– or bicyclic. The term "heterocyclylalkyl" refers to an alkyl group substituted by a heterocyclyl, wherein the alkyl and heterocyclyl portions independently are optionally substituted.

[00158] *Intraperitoneal:* The phrases "intraperitoneal administration" and "administered intraperitonealy" as used herein have their art-understood meaning referring to administration of a compound or composition into the peritoneum of a subject.

[00159] *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).

[00160] *In vivo:* As used herein, the term "*in vivo*" refers to events that occur within an organism (*e.g.,* animal, plant, and/or microbe).

[00161] *Lower alkyl:* The term "lower alkyl" refers to a C_{1-4} straight or branched alkyl group. Example lower alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tert-butyl.

[00162] *Lower haloalkyl:* The term "lower haloalkyl" refers to a C_{1-4} straight or branched

alkyl group that is substituted with one or more halogen atoms.

[00163] *Optionally substituted:* As described herein, compounds of the disclosure may contain "optionally substituted" moieties. In general, the term "substituted," whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this disclosure are preferably those that result in the formation of stable or chemically feasible compounds. The term "stable," as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

[00164] Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group are independently halogen; $-(CH_2)_{0-4}R^\circ$; $-(CH_2)_{0-4}OR^\circ$; $-O(CH_2)_{0-4}R^\circ$, $-O-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}CH(OR^\circ)_2$; $-(CH_2)_{0-4}SR^\circ$; $-(CH_2)_{0-4}Ph$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; $-CH=CHPh$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}$-pyridyl which may be substituted with R°; $-NO_2$; $-CN$; $-N_3$; $-(CH_2)_{0-4}N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$; $-N(R^\circ)C(S)R^\circ$; $-(CH_2)_{0-4}N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)C(S)NR^\circ_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $-N(R^\circ)N(R^\circ)C(O)R^\circ$; $-N(R^\circ)N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)N(R^\circ)C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)R^\circ$; $-C(S)R^\circ$; $-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)SR^\circ$; $-(CH_2)_{0-4}C(O)OSiR^\circ_3$; $-(CH_2)_{0-4}OC(O)R^\circ$; $-OC(O)(CH_2)_{0-4}SR$, $-SC(S)SR^\circ$; $-(CH_2)_{0-4}SC(O)R^\circ$; $-(CH_2)_{0-4}C(O)NR^\circ_2$; $-C(S)NR^\circ_2$; $-C(S)SR^\circ$; $-SC(S)SR^\circ$, $-(CH_2)_{0-4}OC(O)NR^\circ_2$; $-C(O)N(OR^\circ)R^\circ$; $-C(O)C(O)R^\circ$; $-C(O)CH_2C(O)R^\circ$; $-C(NOR^\circ)R^\circ$; $-(CH_2)_{0-4}SSR^\circ$; $-(CH_2)_{0-4}S(O)_2R^\circ$; $-(CH_2)_{0-4}S(O)_2OR^\circ$; $-(CH_2)_{0-4}OS(O)_2R^\circ$; $-S(O)_2NR^\circ_2$; $-(CH_2)_{0-4}S(O)R^\circ$; $-N(R^\circ)S(O)_2NR^\circ_2$; $-N(R^\circ)S(O)_2R^\circ$; $-N(OR^\circ)R^\circ$; $-C(NH)NR^\circ_2$; $-P(O)_2R^\circ$; $-P(O)R^\circ_2$; $-OP(O)R^\circ_2$; $-OP(O)(OR^\circ)_2$; $-SiR^\circ_3$; $-(C_{1-4}$ straight or branched alkylene$)O-N(R^\circ)_2$; or $-(C_{1-4}$ straight or branched alkylene$)C(O)O-N(R^\circ)_2$, wherein each R° may be substituted as defined below and is independently hydrogen, C_{1-6} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, $-CH_2$-(5-6 membered heteroaryl ring), or a 5-6

membered saturated, partially unsaturated, or aryl ring having 0−4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3-12 membered saturated, partially unsaturated, or aryl mono− or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

[00165] Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $-(CH_2)_{0-2}R^{\bullet}$, −(haloR•), $-(CH_2)_{0-2}OH$, $-(CH_2)_{0-2}OR^{\bullet}$, $-(CH_2)_{0-2}CH(OR^{\bullet})_2$; -O(haloR•), −CN, $-N_3$, $-(CH_2)_{0-2}C(O)R^{\bullet}$, $-(CH_2)_{0-2}C(O)OH$, $-(CH_2)_{0-2}C(O)OR^{\bullet}$, $-(CH_2)_{0-2}SR^{\bullet}$, $-(CH_2)_{0-2}SH$, $-(CH_2)_{0-2}NH_2$, $-(CH_2)_{0-2}NHR^{\bullet}$, $-(CH_2)_{0-2}NR^{\bullet}_2$, $-NO_2$, $-SiR^{\bullet}_3$, $-OSiR^{\bullet}_3$, -C(O)SR•, −(C_{1-4} straight or branched alkylene)C(O)OR•, 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 5-6 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

[00166] Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: =O, =S, $=NNR^*_2$, =NNHC(O)R*, =NNHC(O)OR*, $=NNHS(O)_2R^*$, =NR*, =NOR*, $-O(C(R^*_2))_{2-3}O-$, or $-S(C(R^*_2))_{2-3}S-$, wherein each independent occurrence of R* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: $-O(CR^*_2)_{2-3}O-$, wherein each independent occurrence of R* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00167] Suitable substituents on the aliphatic group of R* include halogen, −R•, -(haloR•), -OH, −OR•, −O(haloR•), −CN, −C(O)OH, −C(O)OR•, $-NH_2$, −NHR•, $-NR^{\bullet}_2$, 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 5-6 membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00168] Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include $-R^{\dagger}$, $-NR^{\dagger}_2$, $-C(O)R^{\dagger}$, $-C(O)OR^{\dagger}$, $-C(O)C(O)R^{\dagger}$, $-C(O)CH_2C(O)R^{\dagger}$, $-S(O)_2R^{\dagger}$, $-S(O)_2NR^{\dagger}_2$, $-C(S)NR^{\dagger}_2$, $-C(NH)NR^{\dagger}_2$, or $-N(R^{\dagger})S(O)_2R^{\dagger}$; wherein each $R^{\dagger}$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted –OPh, or an unsubstituted 5-6 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^{\dagger}$, taken together with their intervening atom(s) form an unsubstituted 3-12 membered saturated, partially unsaturated, or aryl mono– or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00169] Suitable substituents on the aliphatic group of $R^{\dagger}$ are independently halogen, $-R^{\bullet}$, -(halo$R^{\bullet}$), $-OH$, $-OR^{\bullet}$, $-O$(halo$R^{\bullet}$), $-CN$, $-C(O)OH$, $-C(O)OR^{\bullet}$, $-NH_2$, $-NHR^{\bullet}$, $-NR^{\bullet}_2$, or $-NO_2$, wherein each $R^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5-6 membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00170] *Oral:* The phrases "oral administration" and "administered orally" as used herein have their art-understood meaning referring to administration by mouth of a compound or composition.

[00171] *Parenteral:* The phrases "parenteral administration" and "administered parenterally" as used herein have their art-understood meaning referring to modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticulare, subcapsular, subarachnoid, intraspinal, and intrasternal injection and infusion.

[00172] *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.

[00173] *Pharmaceutical composition:* As used herein, the term "pharmaceutical composition" refers to an active agent, formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.,* those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

[00174] *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.

[00175] *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.

[00176] *Pharmaceutically acceptable salt:* The term "pharmaceutically acceptable salt", as used herein, refers to salts of such compounds that are appropriate for use in pharmaceutical contexts, i.e., salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, et al. describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977). In some embodiments, pharmaceutically acceptable salt include, but are not limited to, nontoxic acid addition salts, which are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. In some embodiments, pharmaceutically acceptable salts include, but are not limited to, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. In some embodiments, pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl having from 1 to 6 carbon atoms, sulfonate and aryl sulfonate.

[00177] *Prodrug:* A general, a "prodrug," as that term is used herein and as is understood in the art, is an entity that, when administered to an organism, is metabolized in the body to deliver an active (*e.g.*, therapeutic or diagnostic) agent of interest. Typically, such metabolism involves removal of at least one "prodrug moiety" so that the active agent is formed. Various forms of "prodrugs" are known in the art. For examples of such prodrug moieties, see:

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

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

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

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

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

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

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

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

[00178] As with other compounds described herein, prodrugs may be provided in any of a variety of forms, *e.g.*, crystal forms, salt forms *etc*. In some embodiments, prodrugs are provided as pharmaceutically acceptable salts thereof.

[00179] *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.

[00180] 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.

[00181] Suitable hydroxyl protecting groups include methyl, methoxylmethyl (MOM), methylthiomethyl (MTM), *t*–butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), *p*–methoxybenzyloxymethyl (PMBM), (4–methoxyphenoxy)methyl (*p*–AOM), guaiacolmethyl (GUM), *t*–butoxymethyl, 4–pentenyloxymethyl (POM), siloxymethyl, 2–methoxyethoxymethyl (MEM), 2,2,2–trichloroethoxymethyl, bis(2–chloroethoxy)methyl, 2–(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3–bromotetrahydropyranyl, tetrahydrothiopyranyl, 1–methoxycyclohexyl, 4–methoxytetrahydropyranyl (MTHP), 4–methoxytetrahydrothiopyranyl, 4–methoxytetrahydrothiopyranyl S,S–dioxide, 1–[(2–chloro–4–methyl)phenyl]–4–methoxypiperidin–4–yl (CTMP), 1,4–dioxan–2–yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a–octahydro–7,8,8–trimethyl–4,7–methanobenzofuran–2–yl, 1–ethoxyethyl, 1–(2–chloroethoxy)ethyl, 1–methyl–1–methoxyethyl, 1–methyl–1–benzyloxyethyl, 1–methyl–1–benzyloxy–2–fluoroethyl, 2,2,2–trichloroethyl, 2–trimethylsilylethyl, 2–(phenylselenyl)ethyl, *t*–butyl, allyl, *p*–chlorophenyl, *p*–methoxyphenyl, 2,4–dinitrophenyl, benzyl, *p*–methoxybenzyl, 3,4–dimethoxybenzyl, *o*–nitrobenzyl, *p*–nitrobenzyl, *p*–halobenzyl, 2,6–dichlorobenzyl, *p*–

cyanobenzyl, *p*–phenylbenzyl, 2–picolyl, 4–picolyl, 3–methyl–2–picolyl *N*–oxido, diphenylmethyl, *p,p'*–dinitrobenzhydryl, 5–dibenzosuberyl, triphenylmethyl, α–naphthyldiphenylmethyl, *p*–methoxyphenyldiphenylmethyl, di(*p*–methoxyphenyl)phenylmethyl, tri(*p*–methoxyphenyl)methyl, 4–(4'–bromophenacyloxyphenyl)diphenylmethyl, 4,4',4''–tris(4,5–dichlorophthalimidophenyl)methyl, 4,4',4''–tris(levulinoyloxyphenyl)methyl, 4,4',4''–tris(benzoyloxyphenyl)methyl, 3–(imidazol–1–yl)bis(4',4''–dimethoxyphenyl)methyl, 1,1–bis(4–methoxyphenyl)–1'–pyrenylmethyl, 9–anthryl, 9–(9–phenyl)xanthenyl, 9–(9–phenyl–10–oxo)anthryl, 1,3–benzodithiolan–2–yl, benzisothiazolyl S,S–dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylthexylsilyl, *t*–butyldimethylsilyl (TBDMS), *t*–butyldiphenylsilyl (TBDPS), tribenzylsilyl, tri–*p*–xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), *t*–butylmethoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, *p*–chlorophenoxyacetate, 3–phenylpropionate, 4–oxopentanoate (levulinate), 4,4–(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantoate, crotonate, 4–methoxycrotonate, benzoate, *p*–phenylbenzoate, 2,4,6–trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9–fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2–trichloroethyl carbonate (Troc), 2–(trimethylsilyl)ethyl carbonate (TMSEC), 2–(phenylsulfonyl) ethyl carbonate (Psec), 2–(triphenylphosphonio) ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate alkyl allyl carbonate, alkyl *p*–nitrophenyl carbonate, alkyl benzyl carbonate, alkyl *p*–methoxybenzyl carbonate, alkyl 3,4–dimethoxybenzyl carbonate, alkyl *o*–nitrobenzyl carbonate, alkyl *p*–nitrobenzyl carbonate, alkyl *S*–benzyl thiocarbonate, 4–ethoxy–1–napththyl carbonate, methyl dithiocarbonate, 2–iodobenzoate, 4–azidobutyrate, 4–nitro–4–methylpentanoate, *o*–(dibromomethyl)benzoate, 2–formylbenzenesulfonate, 2–(methylthiomethoxy)ethyl, 4–(methylthiomethoxy)butyrate, 2–(methylthiomethoxymethyl)benzoate, 2,6–dichloro–4–methylphenoxyacetate, 2,6–dichloro–4–(1,1,3,3–tetramethylbutyl)phenoxyacetate, 2,4–bis(1,1–dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (*E*)–2–methyl–2–butenoate, *o*–(methoxycarbonyl)benzoate, α–naphthoate, nitrate, alkyl *N,N,N',N'*–tetramethylphosphorodiamidate, alkyl *N*–phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4–dinitrophenylsulfenate, sulfate, methanesulfonate (mesylate), benzylsulfonate, and

tosylate (Ts). For protecting 1,2– or 1,3–diols, the protecting groups include methylene acetal, ethylidene acetal, 1–*t*–butylethylidene ketal, 1–phenylethylidene ketal, (4–methoxyphenyl)ethylidene acetal, 2,2,2–trichloroethylidene acetal, acetonide, cyclopentylidene ketal, cyclohexylidene ketal, cycloheptylidene ketal, benzylidene acetal, *p*–methoxybenzylidene acetal, 2,4–dimethoxybenzylidene ketal, 3,4–dimethoxybenzylidene acetal, 2–nitrobenzylidene acetal, methoxymethylene acetal, ethoxymethylene acetal, dimethoxymethylene ortho ester, 1–methoxyethylidene ortho ester, 1–ethoxyethylidine ortho ester, 1,2–dimethoxyethylidene ortho ester, α–methoxybenzylidene ortho ester, 1–(*N,N*–dimethylamino)ethylidene derivative, α–(*N,N'*–dimethylamino)benzylidene derivative, 2–oxacyclopentylidene ortho ester, di–*t*–butylsilylene group (DTBS), 1,3–(1,1,3,3–tetraisopropyldisiloxanylidene) derivative (TIPDS), tetra–*t*–butoxydisiloxane–1,3–diylidene derivative (TBDS), cyclic carbonates, cyclic boronates, ethyl boronate, and phenyl boronate.

[00182] In some embodiments, a hydroxyl protecting group is acetyl, t-butyl, t-butoxymethyl, methoxymethyl, tetrahydropyranyl, 1 -ethoxyethyl, 1 -(2-chloroethoxy)ethyl, 2-trimethylsilylethyl, p-chlorophenyl, 2,4-dinitrophenyl, benzyl, benzoyl, p-phenylbenzoyl, 2,6-dichlorobenzyl, diphenylmethyl, p-nitrobenzyl, triphenylmethyl (trityl), 4,4'-dimethoxytrityl, trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triphenylsilyl, triisopropylsilyl, benzoylformate, chloroacetyl, trichloroacetyl, trifiuoroacetyl, pivaloyl, 9-fluorenylmethyl carbonate, mesylate, tosylate, triflate, trityl, monomethoxytrityl (MMTr), 4,4'-dimethoxytrityl, (DMTr) and 4,4',4''-trimethoxytrityl (TMTr), 2-cyanoethyl (CE or Cne), 2-(trimethylsilyl)ethyl (TSE), 2-(2-nitrophenyl)ethyl, 2-(4-cyanophenyl)ethyl 2-(4-nitrophenyl)ethyl (NPE), 2-(4-nitrophenylsulfonyl)ethyl, 3,5-dichlorophenyl, 2,4-dimethylphenyl, 2-nitrophenyl, 4-nitrophenyl, 2,4,6-trimethylphenyl, 2-(2-nitrophenyl)ethyl, butylthiocarbonyl, 4,4',4''-tris(benzoyloxy)trityl, diphenylcarbamoyl, levulinyl, 2-(dibromomethyl)benzoyl (Dbmb), 2-(isopropylthiomethoxymethyl)benzoyl (Ptmt), 9-phenylxanthen-9-yl (pixyl) or 9-(p-methoxyphenyl)xanthine-9-y1 (MOX). In some embodiments, each of the hydroxyl protecting groups is, independently selected from acetyl, benzyl, t- butyldimethylsilyl, t-butyldiphenylsilyl and 4,4'-dimethoxytrityl. In some embodiments, the hydroxyl protecting group is selected from the group consisting of trityl, monomethoxytrityl and 4,4'-dimethoxytrityl group.

[00183] In some embodiments, a phosphorus protecting group is a group attached to the

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

[00184] *Protein:* As used herein, the term "protein" refers to a polypeptide (*i.e.*, a string of at least two amino acids linked to one another by peptide bonds). In some embodiments, proteins include only naturally-occurring amino acids. In some embodiments, proteins include one or more non-naturally-occurring amino acids (*e.g.,* moieties that form one or more peptide bonds with adjacent amino acids). In some embodiments, one or more residues in a protein chain contain a non-amino-acid moiety (*e.g.,* a glycan, *etc*). In some embodiments, a protein includes more than one polypeptide chain, for example linked by one or more disulfide bonds or associated by other means. In some embodiments, proteins contain L-amino acids, D-amino acids, or both; in some embodiments, proteins contain one or more amino acid modifications or analogs known in the art. Useful modifications include, *e.g.,* terminal acetylation, amidation, methylation, *etc.* The term "peptide" is generally used to refer to a polypeptide having a length of less than about 100 amino acids, less than about 50 amino acids, less than 20 amino acids, or less than 10 amino acids. In some embodiments, proteins are antibodies, antibody fragments, biologically active portions thereof, and/or characteristic portions thereof.

[00185] *Sample*: A "sample" as used herein is a specific organism or material obtained therefrom. In some embodiments, a sample is a biological sample obtained or derived from a source of interest, as described herein. In some embodiments, a source of interest comprises an organism, such as an animal or human. In some embodiments, a biological sample comprises biological tissue or fluid. In some embodiments, a biological sample is or comprises any one or more of: bone marrow; blood; blood cells; ascites; tissue or fine needle biopsy samples; cell-

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

[00186] *Stereochemically isomeric forms:* The phrase "stereochemically isomeric forms," as used herein, refers to different compounds made up of the same atoms bonded by the same sequence of bonds but having different three-dimensional structures which are not interchangeable. In some embodiments of the disclosure, provided chemical compositions may be or include pure preparations of individual stereochemically isomeric forms of a compound; in some embodiments, provided chemical compositions may be or include mixtures of two or more stereochemically isomeric forms of the compound. In certain embodiments, such mixtures contain equal amounts of different stereochemically isomeric forms; in certain embodiments, such mixtures contain different amounts of at least two different stereochemically isomeric forms. In some embodiments, a chemical composition may contain all diastereomers and/or enantiomers of the compound. In some embodiments, a chemical composition may contain less than all diastereomers and/or enantiomers of a compound. In some embodiments, if a particular

enantiomer of a compound of the present disclosure is desired, it may be prepared, for example, by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, diastereomeric salts are formed with an appropriate optically-active acid, and resolved, for example, by fractional crystallization.

[00187] *Subject:* As used herein, the term "subject" or "test subject" refers to any organism to which a provided compound or composition is administered in accordance with the present disclosure *e.g.,* for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (*e.g.,* mammals such as mice, rats, rabbits, non-human primates, and humans; insects; worms; *etc.*) and plants. In some embodiments, a subject may be suffering from, and/or susceptible to a disease, disorder, and/or condition.

[00188] *Substantially:* As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and/or chemical phenomena.

[00189] *Suffering from*: An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with and/or displays one or more symptoms of a disease, disorder, and/or condition.

[00190] *Susceptible to*: An individual who is "susceptible to" a disease, disorder, and/or condition is one who has a higher risk of developing the disease, disorder, and/or condition than does a member of the general public. In some embodiments, an individual who is susceptible to a disease, disorder and/or condition may not have been diagnosed with the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition may not exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease,

disorder, and/or condition will not develop the disease, disorder, and/or condition.

[00191] *Systemic:* The phrases "systemic administration," "administered systemically," "peripheral administration," and "administered peripherally" as used herein have their art-understood meaning referring to administration of a compound or composition such that it enters the recipient's system.

[00192] *Tautomeric forms:* The phrase "tautomeric forms," as used herein, is used to describe different isomeric forms of organic compounds that are capable of facile interconversion. Tautomers may be characterized by the formal migration of a hydrogen atom or proton, accompanied by a switch of a single bond and adjacent double bond. In some embodiments, tautomers may result from prototropic tautomerism (*i.e.*, the relocation of a proton). In some embodiments, tautomers may result from valence tautomerism (*i.e.*, the rapid reorganization of bonding electrons). All such tautomeric forms are intended to be included within the scope of the present disclosure. In some embodiments, tautomeric forms of a compound exist in mobile equilibrium with each other, so that attempts to prepare the separate substances results in the formation of a mixture. In some embodiments, tautomeric forms of a compound are separable and isolatable compounds. In some embodiments of the disclosure, chemical compositions may be provided that are or include pure preparations of a single tautomeric form of a compound. In some embodiments of the disclosure, chemical compositions may be provided as mixtures of two or more tautomeric forms of a compound. In certain embodiments, such mixtures contain equal amounts of different tautomeric forms; in certain embodiments, such mixtures contain different amounts of at least two different tautomeric forms of a compound. In some embodiments of the disclosure, chemical compositions may contain all tautomeric forms of a compound. In some embodiments of the disclosure, chemical compositions may contain less than all tautomeric forms of a compound. In some embodiments of the disclosure, chemical compositions may contain one or more tautomeric forms of a compound in amounts that vary over time as a result of interconversion. In some embodiments of the disclosure, the tautomerism is keto-enol tautomerism. One of skill in the chemical arts would recognize that a keto-enol tautomer can be "trapped" (i.e., chemically modified such that it remains in the "enol" form) using any suitable reagent known in the chemical arts in to provide an enol derivative that may subsequently be isolated using one or more suitable techniques known in the art. Unless otherwise indicated, the present disclosure encompasses all tautomeric

forms of relevant compounds, whether in pure form or in admixture with one another.

[00193] *Therapeutic agent:* As used herein, the phrase "therapeutic agent" refers to any agent that, when administered to a subject, has a therapeutic effect and/or elicits a desired biological and/or pharmacological effect. In some embodiments, a therapeutic agent is any substance that can be used to alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition.

[00194] *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.

[00195] *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.

[00196] *Unsaturated:* The term "unsaturated," as used herein, means that a moiety has

one or more units of unsaturation.

[00197] *Unit dose*: The expression "unit dose" as used herein refers to an amount administered as a single dose and/or in a physically discrete unit of a pharmaceutical composition. In many embodiments, a unit dose contains a predetermined quantity of an active agent. In some embodiments, a unit dose contains an entire single dose of the agent. In some embodiments, more than one unit dose is administered to achieve a total single dose. In some embodiments, administration of multiple unit doses is required, or expected to be required, in order to achieve an intended effect. A unit dose may be, for example, a volume of liquid (*e.g.*, an acceptable carrier) containing a predetermined quantity of one or more therapeutic agents, a predetermined amount of one or more therapeutic agents in solid form, a sustained release formulation or drug delivery device containing a predetermined amount of one or more therapeutic agents, *etc*. It will be appreciated that a unit dose may be present in a formulation that includes any of a variety of components in addition to the therapeutic agent(s). For example, acceptable carriers (*e.g.*, pharmaceutically acceptable carriers), diluents, stabilizers, buffers, preservatives, *etc*., may be included as described infra. It will be appreciated by those skilled in the art, in many embodiments, a total appropriate daily dosage of a particular therapeutic agent may comprise a portion, or a plurality, of unit doses, and may be decided, for example, by the attending physician within the scope of sound medical judgment. In some embodiments, the specific effective dose level for any particular subject or organism may depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of specific active compound employed; specific composition employed; age, body weight, general health, sex and diet of the subject; time of administration, and rate of excretion of the specific active compound employed; duration of the treatment; drugs and/or additional therapies used in combination or coincidental with specific compound(s) employed, and like factors well known in the medical arts.

[00198] *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).

[00199] *Nucleic acid*: The term "nucleic acid" includes any nucleotides, modified variants

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

[00200] *Nucleotide:* The term "nucleotide" as used herein refers to a monomeric unit of a polynucleotide that consists of a heterocyclic base, a sugar, and one or more phosphate groups or phosphorus-containing internucleotidic linkages. The naturally occurring bases, (guanine, (G), adenine, (A), cytosine, (C), thymine, (T), and uracil (U)) are derivatives of purine or pyrimidine, though it should be understood that naturally and non-naturally occurring base analogs are also included. The naturally occurring sugar is the pentose (five-carbon sugar) deoxyribose (which forms DNA) or ribose (which forms RNA), though it should be understood that naturally and non-naturally occurring sugar analogs are also included. Nucleotides are linked via internucleotidic linkages to form nucleic acids, or polynucleotides. Many internucleotidic linkages are known in the art (such as, though not limited to, phosphate, phosphorothioates, boranophosphates and the like). Artificial nucleic acids include PNAs (peptide nucleic acids), phosphotriesters, phosphorothionates, *H*-phosphonates, phosphoramidates, boranophosphates,

methylphosphonates, phosphonoacetates, thiophosphonoacetates and other variants of the phosphate backbone of native nucleic acids, such as those described herein. Other analogs (e.g., artificial nucleic acids or components which can be incorporated into a nucleic acid or artificial nucleic acid) include: boranophosphate RNA, FANA, locked nucleic acids (LNA), Morpholinos, peptidic nucleic acids (PNA), threose nucleic acid (TNA), and glycol nucleic acid (GNA). These skilled in the art are aware of a variety of modified nucleotides or nucleotide analogs, including, for example, those 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 20070900071; WO 20070900071; or WO 2016/079181.

[00201] *Nucleoside*: The term "nucleoside" refers to a moiety wherein a nucleobase or a modified nucleobase is covalently bound to a sugar or modified sugar.

[00202] *Sugar:* The term "sugar" refers to a monosaccharide in closed and/or open form.

Sugars include, but are not limited to, ribose, deoxyribose, pentofuranose, pentopyranose, and hexopyranose moieties. As used herein, the term also encompasses structural analogs used in lieu of conventional sugar molecules, such as glycol, polymer of which forms the backbone of the nucleic acid analog, glycol nucleic acid ("GNA").

[00203] *Modified sugar*: The term "modified sugar" refers to a moiety that can replace a sugar. The modified sugar mimics the spatial arrangement, electronic properties, or some other physicochemical property of a sugar.

[00204] *Nucleobase*: The term "nucleobase" refers to the parts of nucleic acids that are involved in the hydrogen-bonding that binds one nucleic acid strand to another complementary strand in a sequence specific manner. The most common naturally-occurring nucleobases are adenine (A), guanine (G), uracil (U), cytosine (C), and thymine (T). In some embodiments, the naturally-occurring nucleobases are modified adenine, guanine, uracil, cytosine, or thymine. In some embodiments, the naturally-occurring nucleobases are methylated adenine, guanine, uracil, cytosine, or thymine. In some embodiments, a nucleobase is a "modified nucleobase," *e.g.*, a nucleobase other than adenine (A), guanine (G), uracil (U), cytosine (C), and thymine (T). In some embodiments, the modified nucleobases are methylated adenine, guanine, uracil, cytosine, or thymine. In some embodiments, the modified nucleobase mimics the spatial arrangement, electronic properties, or some other physicochemical property of the nucleobase and retains the property of hydrogen-bonding that binds one nucleic acid strand to another in a sequence specific manner. In some embodiments, a modified nucleobase can pair with all of the five naturally occurring bases (uracil, thymine, adenine, cytosine, or guanine) without substantially affecting the melting behavior, recognition by intracellular enzymes or activity of the oligonucleotide duplex.

[00205] *Chiral ligand*: The term "chiral ligand" or "chiral auxiliary" refers to a moiety that is chiral and can be incorporated into a reaction so that the reaction can be carried out with certain stereoselectivity.

[00206] *Condensing reagent*: In a condensation reaction, the term "condensing reagent" refers to a reagent that activates a less reactive site and renders it more susceptible to attack by another reagent. In some embodiments, such another reagent is a nucleophile.

[00207] *Blocking group*: The term "blocking group" refers to a group that masks the reactivity of a functional group. The functional group can be subsequently unmasked by

removal of the blocking group. In some embodiments, a blocking group is a protecting group.

[00208] *Moiety*: The term "moiety" refers to a specific segment or functional group of a molecule. Chemical moieties are often recognized chemical entities embedded in or appended to a molecule.

[00209] *Solid support*: The term "solid support" refers to any support which enables synthesis of nucleic acids. In some embodiments, the term refers to a glass or a polymer, that is insoluble in the media employed in the reaction steps performed to synthesize nucleic acids, and is derivatized to comprise reactive groups. In some embodiments, the solid support is Highly Cross-linked Polystyrene (HCP) or Controlled Pore Glass (CPG). In some embodiments, the solid support is Controlled Pore Glass (CPG). In some embodiments, the solid support is hybrid support of Controlled Pore Glass (CPG) and Highly Cross-linked Polystyrene (HCP).

[00210] *Linking moiety*: The term "linking moiety" refers to any moiety optionally positioned between the terminal nucleoside and the solid support or between the terminal nucleoside and another nucleoside, nucleotide, or nucleic acid.

[00211] *DNA molecule*: A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (*e.g.*, restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences can be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

[00212] *Coding sequence*: A DNA "coding sequence" or "coding region" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate expression control sequences. The boundaries of the coding sequence (the "open reading frame" or "ORF") are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (*e.g.*, mammalian) DNA, and synthetic DNA sequences. A polyadenylation signal and transcription termination sequence is, usually, be

located 3' to the coding sequence. The term "non-coding sequence" or "non-coding region" refers to regions of a polynucleotide sequence that are not translated into amino acids (e.g. 5′ and 3′ un-translated regions).

[00213] *Reading frame*: The term "reading frame" refers to one of the six possible reading frames, three in each direction, of the double stranded DNA molecule. The reading frame that is used determines which codons are used to encode amino acids within the coding sequence of a DNA molecule.

[00214] *Antisense*: As used herein, an "antisense" nucleic acid molecule comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule, complementary to an mRNA sequence or complementary to the coding strand of a gene. Accordingly, an antisense nucleic acid molecule can associate via hydrogen bonds to a sense nucleic acid molecule. In some embodiments, an antisense oligonucleotide is an oligonucleotide which participates in RNaseH-mediated cleavage; for example, an antisense oligonucleotide hybridizes in a sequence-specific manner to a portion of a target mRNA, thus targeting the mRNA for cleavage by RNaseH. In some embodiments, an antisense oligonucleotide is able to differentiate between a wild-type and a mutant allele of a target. In some embodiments, an antisense oligonucleotide significantly participates in RNaseH-mediated cleavage of a mutant allele but participates in RNaseH-mediated cleavage of a wild-type allele to a much less degree (e.g., does not significantly participate in RNaseH-mediated cleavage of the wild-type allele of the target).

[00215] *Wobble position*: As used herein, a "wobble position" refers to the third position of a codon. Mutations in a DNA molecule within the wobble position of a codon, in some embodiments, result in silent or conservative mutations at the amino acid level. For example, there are four codons that encode Glycine, i.e., GGU, GGC, GGA and GGG, thus mutation of any wobble position nucleotide, to any other nucleotide selected from A, U, C and G, does not result in a change at the amino acid level of the encoded protein and, therefore, is a silent substitution.

[00216] *Silent substitution*: a "silent substitution" or "silent mutation" is one in which a nucleotide within a codon is modified, but does not result in a change in the amino acid residue encoded by the codon. Examples include mutations in the third position of a codon, as well in the first position of certain codons such as in the codon "CGG" which, when mutated to AGG,

still encodes Arg.

[00217] *Gene*: The terms "gene," "recombinant gene" and "gene construct" as used herein, refer to a DNA molecule, or portion of a DNA molecule, that encodes a protein or a portion thereof. The DNA molecule can contain an open reading frame encoding the protein (as exon sequences) and can further include intron sequences. The term "intron" as used herein, refers to a DNA sequence present in a given gene which is not translated into protein and is found in some, but not all cases, between exons. It can be desirable for the gene to be operably linked to, (or it can comprise), one or more promoters, enhancers, repressors and/or other regulatory sequences to modulate the activity or expression of the gene, as is well known in the art.

[00218] *Complementary DNA*: As used herein, a "complementary DNA" or "cDNA" includes recombinant polynucleotides synthesized by reverse transcription of mRNA and from which intervening sequences (introns) have been removed.

[00219] *Homology*: "Homology" or "identity" or "similarity" refers to sequence similarity between two nucleic acid molecules. Homology and identity can each be determined by comparing a position in each sequence which can be aligned for purposes of comparison. When an equivalent position in the compared sequences is occupied by the same base, then the molecules are identical at that position; when the equivalent site occupied by the same or a similar nucleic acid residue (*e.g.*, similar in steric and/or electronic nature), then the molecules can be referred to as homologous (similar) at that position. Expression as a percentage of homology/similarity or identity refers to a function of the number of identical or similar nucleic acids at positions shared by the compared sequences. A sequence which is "unrelated" or "non-homologous" shares less than 40% identity, less than 35% identity, less than 30% identity, or less than 25% identity with a sequence described herein. In comparing two sequences, the absence of residues (amino acids or nucleic acids) or presence of extra residues also decreases the identity and homology/similarity.

[00220] In some embodiments, the term "homology" describes a mathematically based comparison of sequence similarities which is used to identify genes with similar functions or motifs. The nucleic acid sequences described herein can be used as a "query sequence" to perform a search against public databases, for example, to identify other family members, related sequences or homologs. In some embodiments, such searches can be performed using the

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

[00221] *Identity*: As used herein, "identity" means the percentage of identical nucleotide residues at corresponding positions in two or more sequences when the sequences are aligned to maximize sequence matching, i.e., taking into account gaps and insertions. Identity can be readily calculated by known methods, including but not limited to those described in (Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48: 1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., Nucleic Acids Research 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Altschul, S. F. et al., J. Molec. Biol. 215: 403-410 (1990) and Altschul et al. Nuc. Acids Res. 25: 3389-3402 (1997)). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, Md. 20894; Altschul, S., et al., J. Mol. Biol. 215: 403-410 (1990). The well-known Smith Waterman algorithm can also be used to determine identity.

[00222] *Heterologous*: A "heterologous" region of a DNA sequence is an identifiable segment of DNA within a larger DNA sequence that is not found in association with the larger sequence in nature. Thus, when the heterologous region encodes a mammalian gene, the gene

can usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example of a heterologous coding sequence is a sequence where the coding sequence itself is not found in nature (*e.g.*, a cDNA where the genomic coding sequence contains introns or synthetic sequences having codons or motifs different than the unmodified gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA as defined herein.

[00223] *Transition mutation:* The term "transition mutations" refers to base changes in a DNA sequence in which a pyrimidine (cytidine (C) or thymidine (T) is replaced by another pyrimidine, or a purine (adenosine (A) or guanosine (G) is replaced by another purine.

[00224] *Transversion mutation*: The term "transversion mutations" refers to base changes in a DNA sequence in which a pyrimidine (cytidine (C) or thymidine (T) is replaced by a purine (adenosine (A) or guanosine (G), or a purine is replaced by a pyrimidine.

[00225] *Oligonucleotide:* the term "oligonucleotide" refers to a polymer or oligomer of nucleotide monomers, containing any combination of nucleobases, modified nucleobases, sugars, modified sugars, phosphate bridges, or modified phosphorus atom bridges (also referred to herein as "internucleotidic linkage", defined further herein).

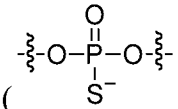
[00226] Oligonucleotides can be single-stranded or double-stranded. As used herein, the term "oligonucleotide strand" encompasses a single-stranded oligonucleotide. A single-stranded oligonucleotide can have double-stranded regions and a double-stranded oligonucleotide can have single-stranded regions. Example oligonucleotides include, but are not limited to structural genes, genes including control and termination regions, self-replicating systems such as viral or plasmid DNA, single-stranded and double-stranded siRNAs and other RNA interference reagents (RNAi agents or iRNA agents), shRNA, antisense oligonucleotides, ribozymes, microRNAs, microRNA mimics, supermirs, aptamers, antimirs, antagomirs, Ul adaptors, triplex-forming oligonucleotides, G-quadruplex oligonucleotides, RNA activators, immuno-stimulatory oligonucleotides, and decoy oligonucleotides.

[00227] Double-stranded and single-stranded oligonucleotides that are effective in inducing RNA interference are also referred to as siRNA, RNAi agent, or iRNA agent, herein. In some embodiments, these RNA interference inducing oligonucleotides associate with a cytoplasmic multi-protein complex known as RNAi-induced silencing complex (RISC). In many embodiments, single-stranded and double-stranded RNAi agents are sufficiently long that they

can be cleaved by an endogenous molecule, *e.g.,* by Dicer, to produce smaller oligonucleotides that can enter the RISC machinery and participate in RISC mediated cleavage of a target sequence, e.g. a target mRNA.

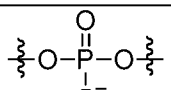
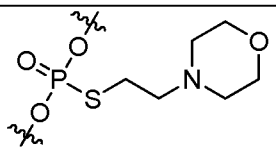
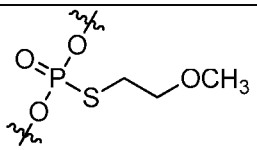
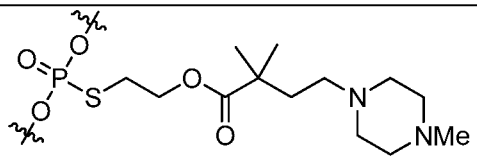
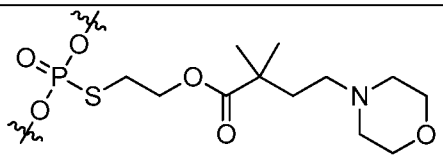
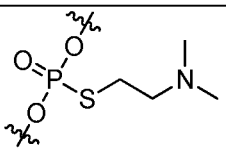
[00228] Oligonucleotides of the present disclosure can be of various lengths. In particular embodiments, oligonucleotides can range from about 2 to about 200 nucleotides in length. In various related embodiments, oligonucleotides, single-stranded, double-stranded, and triple-stranded, can range in length from about 4 to about 10 nucleotides, from about 10 to about 50 nucleotides, from about 20 to about 50 nucleotides, from about 15 to about 30 nucleotides, from about 20 to about 30 nucleotides in length. In some embodiments, the oligonucleotide is from about 9 to about 39 nucleotides in length. In some embodiments, the oligonucleotide is at least 4 nucleotides in length. In some embodiments, the oligonucleotide is at least 5 nucleotides in length. In some embodiments, the oligonucleotide is at least 6 nucleotides in length. In some embodiments, the oligonucleotide is at least 7 nucleotides in length. In some embodiments, the oligonucleotide is at least 8 nucleotides in length. In some embodiments, the oligonucleotide is at least 9 nucleotides in length. In some embodiments, the oligonucleotide is at least 10 nucleotides in length. In some embodiments, the oligonucleotide is at least 11 nucleotides in length. In some embodiments, the oligonucleotide is at least 12 nucleotides in length. In some embodiments, the oligonucleotide is at least 15 nucleotides in length. In some embodiments, the oligonucleotide is at least 20 nucleotides in length. In some embodiments, the oligonucleotide is at least 25 nucleotides in length. In some embodiments, the oligonucleotide is at least 30 nucleotides in length. In some embodiments, the oligonucleotide is a duplex of complementary strands of at least 18 nucleotides in length. In some embodiments, the oligonucleotide is a duplex of complementary strands of at least 21 nucleotides in length.

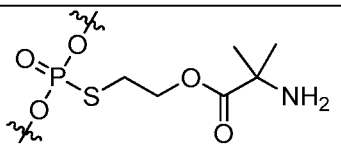
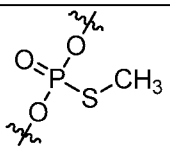
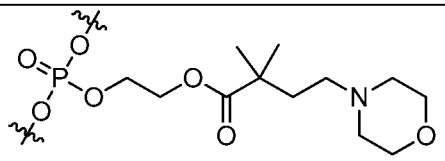
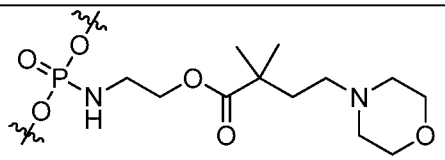
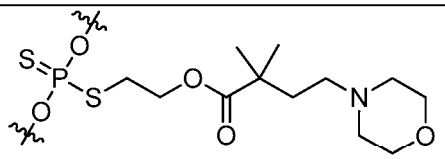
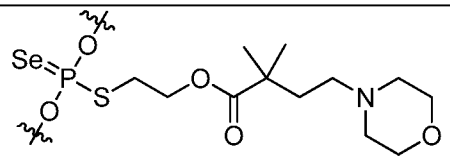
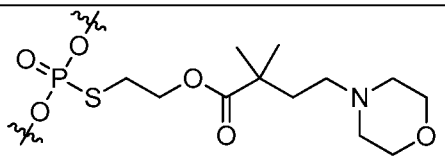
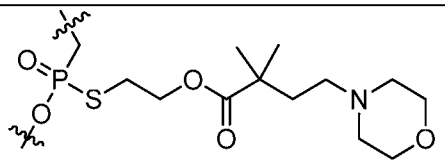
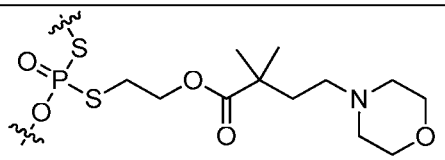
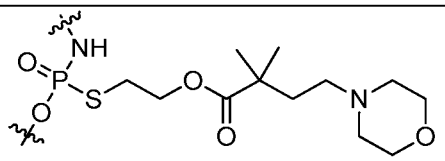
[00229] *Internucleotidic linkage:* As used herein, the phrase "internucleotidic linkage" refers generally to the phosphorus-containing linkage between nucleotide units of an oligonucleotide, and is interchangeable with "inter-sugar linkage" and "phosphorus atom bridge," as used above and herein. In some embodiments, an internucleotidic linkage is a phosphodiester linkage, as found in naturally occurring DNA and RNA molecules. In some embodiments, an internucleotidic linkage is a "modified internucleotidic linkage" wherein each oxygen atom of the phosphodiester linkage is optionally and independently replaced by an organic or inorganic moiety. In some embodiments, such an organic or inorganic moiety is

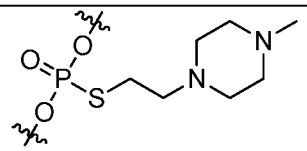
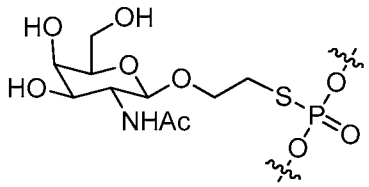
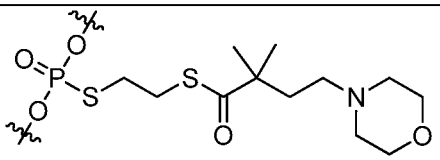
selected from but not limited to =S, =Se, =NR', –SR', –SeR', –N(R')$_2$, B(R')$_3$, –S–, –Se–, and –N(R')–, wherein each R' is independently as defined and described below. In some embodiments, an internucleotidic linkage is a phosphotriester linkage, phosphorothioate diester linkage (), or modified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

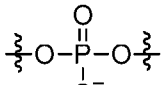
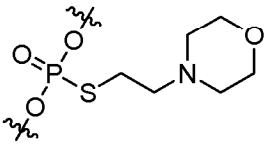
[00230] Unless otherwise specified, when used with an oligonucleotide sequence, each of s, s1, s2, s3, s4, s5, s6 and s7 independently represents the following modified internucleotidic linkage as illustrated in Table 1, below.

[00231] Table 1. Example Modified Internucleotidic Linkage.

Symbol	Modified Internucleotidic Linkage
s	 phosphorothioate ( )
s1	
s2	
s3	
s4	
s5	

s6	
s7	
s8	
s9	
s10	
s11	
s12	
s13	
s14	
s15	

s16	
s17	
s18	

[00232] For instance, (*R*p, *S*p)–ATsCs1GA has 1) a phosphorothioate internucleotidic linkage () between T and C; and 2) a phosphorothioate triester internucleotidic linkage having the structure of  between C and G. Unless otherwise specified, the *R*p/*S*p designations preceding an oligonucleotide sequence describe the configurations of chiral linkage phosphorus atoms in the internucleotidic linkages sequentially from 5' to 3' of the oligonucleotide sequence. For instance, in (*R*p, *S*p)–ATsCs1GA, the phosphorus in the "s" linkage between T and C has *R*p configuration and the phosphorus in "s1" linkage between C and G has *S*p configuration. In some embodiments, "All-(*R*p)" or "All-(*S*p)" is used to indicate that all chiral linkage phosphorus atoms in oligonucleotide have the same *R*p or *S*p configuration, respectively. For instance, All-(*R*p)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC indicates that all the chiral linkage phosphorus atoms in the oligonucleotide have *R*p configuration; All-(*S*p)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC indicates that all the chiral linkage phosphorus atoms in the oligonucleotide have *S*p configuration.

[00233] *Oligonucleotide type:* As used herein, the phrase "oligonucleotide type" is used to define an oligonucleotide that has a particular base sequence, pattern of backbone linkages (i.e., pattern of internucleotidic linkage types, for example, phosphate, phosphorothioate, *etc*), pattern

of backbone chiral centers (i.e. pattern of linkage phosphorus stereochemistry (*R*p/*S*p)), and pattern of backbone phosphorus modifications (*e.g.*, pattern of "−XLR^1" groups in formula **I**). Oligonucleotides of a common designated "type" are structurally identical to one another.

[00234] One of skill in the art will appreciate that synthetic methods of the present disclosure provide for a degree of control during the synthesis of an oligonucleotide strand such that each nucleotide unit of the oligonucleotide strand can be designed and/or selected in advance to have a particular stereochemistry at the linkage phosphorus and/or a particular modification at the linkage phosphorus, and/or a particular base, and/or a particular sugar. In some embodiments, an oligonucleotide strand is designed and/or selected in advance to have a particular combination of stereocenters at the linkage phosphorus. In some embodiments, an oligonucleotide strand is designed and/or determined to have a particular combination of modifications at the linkage phosphorus. In some embodiments, an oligonucleotide strand is designed and/or selected to have a particular combination of bases. In some embodiments, an oligonucleotide strand is designed and/or selected to have a particular combination of one or more of the above structural characteristics. The present disclosure provides compositions comprising or consisting of a plurality of oligonucleotide molecules (*e.g.*, chirally controlled oligonucleotide compositions). In some embodiments, all such molecules are of the same type (i.e., are structurally identical to one another). In many embodiments, however, provided compositions comprise a plurality of oligonucleotides of different types, typically in pre-determined relative amounts.

[00235] *Chiral control:* As used herein, "chiral control" refers to an ability to control the stereochemical designation of every chiral linkage phosphorus within an oligonucleotide strand. The phrase "chirally controlled oligonucleotide" refers to an oligonucleotide which exists in a single diastereomeric form with respect to the chiral linkage phosphorus. Chirally controlled oligonucleotides are prepared from chirally controlled oligonucleotide synthesis.

[00236] *Chirally controlled oligonucleotide composition:* As used herein, the phrase "chirally controlled oligonucleotide composition" refers to an oligonucleotide composition that contains predetermined levels of individual oligonucleotide types. For instance, in some embodiments a chirally controlled oligonucleotide composition comprises one oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide composition comprises more than one oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide

composition comprises a mixture of multiple oligonucleotide types. Example chirally controlled oligonucleotide compositions are described further herein.

[00237] *Chirally pure:* as used herein, the phrase "chirally pure" is used to describe a chirally controlled oligonucleotide composition in which all of the oligonucleotides exist in a single diastereomeric form with respect to the linkage phosphorus.

[00238] *Chirally uniform:* as used herein, the phrase "chirally uniform" is used to describe an oligonucleotide molecule or type in which all nucleotide units have the same stereochemistry at the linkage phosphorus. For instance, an oligonucleotide whose nucleotide units all have *R*p stereochemistry at the linkage phosphorus is chirally uniform. Likewise, an oligonucleotide whose nucleotide units all have *S*p stereochemistry at the linkage phosphorus is chirally uniform.

[00239] *Predetermined:* By predetermined is meant deliberately selected, for example as opposed to randomly occurring or achieved. Those of ordinary skill in the art, reading the present specification, will appreciate that the present disclosure provides new and surprising technologies that permit selection of particular oligonucleotide types for preparation and/or inclusion in provided compositions, and further permits controlled preparation of precisely the selected particular types, optionally in selected particular relative amounts, so that provided compositions are prepared. Such provided compositions are "predetermined" as described herein. Compositions that may contain certain individual oligonucleotide types because they happen to have been generated through a process that cannot be controlled to intentionally generate the particular oligonucleotide types is not a "predetermined" composition. In some embodiments, a predetermined composition is one that can be intentionally reproduced (*e.g.*, through repetition of a controlled process).

[00240] *Linkage phosphorus:* as defined herein, the phrase "linkage phosphorus" is used to indicate that the particular phosphorus atom being referred to is the phosphorus atom present in the internucleotidic linkage, which phosphorus atom corresponds to the phosphorus atom of a phosphodiester of an internucleotidic linkage as occurs in naturally occurring DNA and RNA. In some embodiments, a linkage phosphorus atom is in a modified internucleotidic linkage, wherein each oxygen atom of a phosphodiester linkage is optionally and independently replaced by an organic or inorganic moiety. In some embodiments, a linkage phosphorus atom is P* of formula **I**. In some embodiments, a linkage phosphorus atom is chiral. In some embodiments, a chiral linkage phosphorus atom is P* of formula **I**.

[00241] *P-modification:* as used herein, the term "P-modification" refers to any modification at the linkage phosphorus other than a stereochemical modification. In some embodiments, a P-modification comprises addition, substitution, or removal of a pendant moiety covalently attached to a linkage phosphorus. In some embodiments, the "P-modification" is –X–L–R^1 wherein each of X, L and R^1 is independently as defined and described herein and below.

[00242] *Blockmer:* the term "blockmer," as used herein, refers to an oligonucleotide strand whose pattern of structural features characterizing each individual nucleotide unit is characterized by the presence of at least two consecutive nucleotide units sharing a common structural feature at the internucleotidic phosphorus linkage. By common structural feature is meant common stereochemistry at the linkage phosphorus or a common modification at the linkage phosphorus. In some embodiments, the at least two consecutive nucleotide units sharing a common structure feature at the internucleotidic phosphours linkage are referred to as a "block".

[00243] In some embodiments, a blockmer is a "stereoblockmer," *e.g.,* at least two consecutive nucleotide units have the same stereochemistry at the linkage phosphorus. Such at least two consecutive nucleotide units form a "stereoblock." For instance, (*S*p, *S*p)-ATsCs1GA is a stereoblockmer because at least two consecutive nucleotide units, the Ts and the Cs1, have the same stereochemistry at the linkage phosphorus (both *S*p). In the same oligonucleotide (*S*p, *S*p)-ATsCs1GA, TsCs1 forms a block, and it is a stereoblock.

[00244] In some embodiments, a blockmer is a "P-modification blockmer," *e.g.,* at least two consecutive nucleotide units have the same modification at the linkage phosphorus. Such at least two consecutive nucleotide units form a "P-modification block". For instance, (*R*p, *S*p)-ATsCsGA is a P-modification blockmer because at least two consecutive nucleotide units, the Ts and the Cs, have the same P-modification (i.e., both are a phosphorothioate diester). In the same oligonucleotide of (*R*p, *S*p)-ATsCsGA, TsCs forms a block, and it is a P-modification block.

[00245] In some embodiments, a blockmer is a "linkage blockmer," *e.g.,* at least two consecutive nucleotide units have identical stereochemistry and identical modifications at the linkage phosphorus. At least two consecutive nucleotide units form a "linkage block". For instance, (*R*p, *R*p)-ATsCsGA is a linkage blockmer because at least two consecutive nucleotide units, the Ts and the Cs, have the same stereochemistry (both *R*p) and P-modification (both phosphorothioate). In the same oligonucleotide of (*R*p, *R*p)-ATsCsGA, TsCs forms a block, and

it is a linkage block.

[00246] In some embodiments, a blockmer comprises one or more blocks independently selected from a stereoblock, a P-modification block and a linkage block. In some embodiments, a blockmer is a stereoblockmer with respect to one block, and/or a P-modification blockmer with respect to another block, and/or a linkage blockmer with respect to yet another block. For instance, (*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p)–AAsTsCsGsAs1Ts1Cs1Gs1ATCG is a stereoblockmer with respect to the stereoblock AsTsCsGsAs1 (all *R*p at linkage phosphorus) or Ts1Cs1Gs1 (all *S*p at linkage phosphorus), a P-modification blockmer with respect to the P-modification block AsTsCsGs (all s linkage) or As1Ts1Cs1Gs1 (all s1 linkage), or a linkage blockmer with respect to the linkage block AsTsCsGs (all *R*p at linkage phosphorus and all s linkage) or Ts1Cs1Gs1 (all *S*p at linkage phosphorus and all s1 linkage).

[00247] *Altmer:* the term "altmer," as used herein, refers to an oligonucleotide strand whose pattern of structural features characterizing each individual nucleotide unit is characterized in that no two consecutive nucleotide units of the oligonucleotide strand share a particular structural feature at the internucleotidic phosphorus linkage. In some embodiments, an altmer is designed such that it comprises a repeating pattern. In some embodiments, an altmer is designed such that it does not comprise a repeating pattern.

[00248] In some embodiments, an altmer is a "stereoaltmer," *e.g.,* no two consecutive nucleotide units have the same stereochemistry at the linkage phosphorus. For instance, (*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC.

[00249] In some embodiments, an altmer is a "P-modification altmer" *e.g.,* no two consecutive nucleotide units have the same modification at the linkage phosphorus. For instance, All-(*S*p)-CAs1GsT, in which each linkage phosphorus has a different P-modification than the others.

[00250] In some embodiments, an altmer is a "linkage altmer," *e.g.,* no two consecutive nucleotide units have identical stereochemistry or identical modifications at the linkage phosphorus. For instance, (*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-GsCs1CsTs1CsAs1GsTs1CsTs1GsCs1TsTs2CsGs3CsAs4CsC.

[00251] *Unimer:* the term "unimer," as used herein, refers to an oligonucleotide strand whose pattern of structural features characterizing each individual nucleotide unit is such that all

nucleotide units within the strand share at least one common structural feature at the internucleotidic phosphorus linkage. By common structural feature is meant common stereochemistry at the linkage phosphorus or a common modification at the linkage phosphorus.

[00252] In some embodiments, a unimer is a "stereounimer," *e.g.,* all nucleotide units have the same stereochemistry at the linkage phosphorus. For instance, All-(*S*p)-CsAs1GsT, in which all the linkages have *S*p phosphorus.

[00253] In some embodiments, a unimer is a "P-modification unimer", *e.g.,* all nucleotide units have the same modification at the linkage phosphorus. For instance, (*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC, in which all the internucleotidic linkages are phosphorothioate diester.

[00254] In some embodiments, a unimer is a "linkage unimer," *e.g.,* all nucleotide units have the same stereochemistry and the same modifications at the linkage phosphorus. For instance, All-(*S*p)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC, in which all the internucleotidic linkages are phosphorothioate diester having *S*p linkage phosphorus.

[00255] *Gapmer*: as used herein, the term "gapmer" refers to an oligonucleotide strand characterized in that at least one internucleotidic phosphorus linkage of the oligonucleotide strand is a phosphate diester linkage, for example such as those found in naturally occurring DNA or RNA. In some embodiments, more than one internucleotidic phosphorus linkage of the oligonucleotide strand is a phosphate diester linkage such as those found in naturally occurring DNA or RNA. For instance, All-(*S*p)-CAs1GsT, in which the internucleotidic linkage between C and A is a phosphate diester linkage.

[00256] *Skipmer*: as used herein, the term "skipmer" refers to a type of gapmer in which every other internucleotidic phosphorus linkage of the oligonucleotide strand is a phosphate diester linkage, for example such as those found in naturally occurring DNA or RNA, and every other internucleotidic phosphorus linkage of the oligonucleotide strand is a modified internucleotidic linkage. For instance, All-(*S*p)-AsTCs1GAs2TCs3G.

[00257] For purposes of this disclosure, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, *Handbook of Chemistry and Physics*, 67th Ed., 1986-87, inside cover.

[00258] The methods and structures described herein relating to compounds and

compositions of the disclosure also apply to the pharmaceutically acceptable acid or base addition salts and all stereoisomeric forms of these compounds and compositions.

Brief Description of the Drawing

[00259] *Figure 1.* Reverse phase HPLCs after incubation with rat liver homogenate. Total amounts of oligonucleotides remaining when incubated with rat whole liver homogenate at 37°C at different days were measured. The *in-vitro* metabolic stability of ONT-154 was found to be similar to ONT-87, which has 2'-MOE wings, while both have much better stability than 2'-MOE gapmer which is stereorandom (ONT-41, Mipomersen). The amount of full length oligomer remaining was measured by reverse phase HPLC where peak area of the peak of interest was normalized with internal standard.

[00260] *Figure 2.* Degradation of various chirally pure analogues of Mipomersen (ONT-41) in rat whole liver homogenate. Total amounts of oligonucleotide remaining when incubated with rat whole liver homogenate at 37°C at different days were measured. The *in-vitro* metabolic stability of chirally pure diastereomers of human ApoB sequence ONT-41 (Mipomersen) was found to increase with increased *S*p internucleotidic linkages. The amount of full length oligomer remaining was measured by reverse phase HPLC where peak area of the peak of interest was normalized with internal standard. Compositions used include: ONT-41, ONT-75, ONT-77, ONT-80, ONT-81, ONT-87, ONT-88 and ONT-89.

[00261] *Figure 3.* Degradation of various chirally pure analogues of mouse ApoB sequence (ISIS 147764, ONT-83) in rat whole liver homogenate. Total amounts of oligonucleotide remaining when incubated with rat whole liver homogenate at 37°C at different days were measured. The *in-vitro* metabolic stability of chirally pure diastereomers of murine ApoB sequence (ONT-83, 2'-MOE gapmer, stereorandom phosphorothioate) was found to increase with increased *S*p internucleotidic linkages. The amount of full length oligomer remaining was measured by reverse phase HPLC where peak area of the peak of interest was normalized with internal standard. Compositions used include: ONT-82 to ONT-86.

[00262] *Figure 4.* Degradation of Mipomersen analogue ONT-75 in rat whole liver homogenate over a period of 24hrs. This figure illustrates stability of ONT-75 in rate whole liver homogenate.

[00263] *Figure 5.* Degradation of Mipomersen analogue ONT-81 in rat whole liver

homogenate over a period of 24hrs. This figure illustrates stability of ONT-81 in rate whole liver homogenate.

[00264] *Figure 6.* Durations of knockdown for ONT-87, ONT-88, and ONT-89. Stereoisomers can exhibit substantially different durations of knockdown. ONT-87 results in substantially more durable suppression than other stereoisomers. Increased duration of action of ONT-87 in multiple *in vivo* studies was observed. ONT-88 showed similar efficacy and recovery profile as ONT-41 (Mipomersen) in certain *in-vivo* studies. Hu ApoB transgenic mice, n=4, were dosed with 10 mpk IP bolus, 2X/week for three weeks. The mice were randomized to study groups, and dosed intraperitoneally (IP) at 10 mg/kg on Days 1, 4, 8, 11, 15, 18, and 22, based on individual mouse body weight measured prior to dosing on each dosing day. Blood was collected on days 0, 17, 24, 31, 38, 45 and 52 by submandibular (cheek) bleed and at sacrifice on Day 52 by cardiac puncture and then processed to serum. ApoB was measured by ELISA. Highlighted: 72% vs. 35% knock-down maintained at 3 weeks postdose.

[00265] *Figure 7.* HPLC profiles exhibiting the difference in metabolic stability determined in Human Serum for siRNA duplexes having several *R*p, *S*p or stereorandom phosphorothioate linkages. Compositions used include: ONT-114, ONT-116, ONT-109, ONT-107, ONT-108 and ONT-106.

[00266] *Figure 8.* Effect of stereochemistry on RNase H activity. Oligonucleotides were hybridized with RNA and then incubated with RNase H at 37°C in the presence of 1X RNase H buffer. From top to bottom at 120 min: ONT-89, ONT-77, ONT-81, ONT-80, ONT-75, ONT-41, ONT-88, ONT-154, ONT-87, with ONT-77/154 very close to each other.

[00267] *Figure 9.* Analysis of human RNase H1 cleavage of a 20-mer RNA when hybridized with different preparations of stereoisomers of phosphorothioate oligonucleotides targeting the same region of human ApoB mRNA. Specific sites of cleavage are strongly influenced by the distinct stereochemistries. Arrows represent position of cleavage (cleavage sites). Products were analyzed by UPLC/MS. The length of the arrow signifies the amount of products present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment (the larger the arrow, the more the detected cleavage products). (A): Legend for cleavage maps. (B) and (C): cleavage maps of oligonucleotides. In the figures: (┬) indicates that both RNase H1 cleavage fragments (5'-phosphate species as well as 5'-OH 3'-OH species) were identified in reaction mixtures. (┌)

indicates that only 5'-phosphate species was detected and (┐) indicates that 5'-OH 3'-OH component was detected in mass spectrometry analysis. Compositions used include: ONT-41, ONT-75, ONT-77, ONT-80, ONT-81, ONT-87, ONT-88, ONT-88 and ONT-154.

[00268] *Figure 10.* Cleavage maps of different oligonucleotide compositions ((A)-(C)). These three sequences target different regions in FOXO1 mRNA. Each sequence was studied with five different chemistries. Cleavage maps are derived from reaction mixtures obtained after 30 minutes of incubation of respective duplexes with RNase H1C in the presence of 1XPBS buffer at 37°C. Arrows indicate sites of cleavage. The length of the arrow signifies the amount of products present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment (the larger the arrow, the more the detectable cleavage products). Only in the cases where 5'-OH 3'-OH was not detected in the reaction mixture, 5'-phosphate species peak was used for quantification. Cleavage rates were determined by measuring amount of full length RNA remaining in the reaction mixtures by reverse phase HPLC. Reactions were quenched at fixed time points by 30mM Na_2EDTA. Compositions used include: ONT-316, ONT-355, ONT-361, ONT-367, ONT-373, ONT-302, ONT-352, ONT-358, ONT-364, ONT-370, ONT-315, ONT-354, ONT-360, ONT-366, and ONT-372.

[00269] *Figure 11.* Cleavage maps of oligonucleotide compositions having different common base sequences and lengths ((A)-(B)). The maps show a comparison of stereorandom DNA compositions (top panel) with three distinct and stereochemically pure oligonucleotide compositions. Data compare results of chirally controlled oligonucleotide compositions with two stereorandom phosphorothioate oligonucleotide compositions (ONT-366 and ONT-367) targeting different regions in FOXO1 mRNA. Each panel shows a comparison of stereorandom DNA (top panel) with three distinct and stereochemically pure oligonucleotide preparaitons. Cleavage maps were derived from reaction mixtures obtained after 30 minutes of incubation of respective duplexes with RNase H1C in the presence of 1XPBS buffer at 37°C. Arrows indicate sites of cleavage. The length of the arrow signifies the amount of metabolite present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment (the larger the arrow, the more the detectable cleavage products). Only in the cases where 5'-OH 3'-OH was not detected in the reaction mixture, 5'-phosphate species peak was used for quantification. Compositions used include: ONT-366, ONT-389, ONT-390, ONT-391, ONT-367, ONT-392, ONT-393, and ONT-394.

[00270] *Figure 12.* Effect of stereochemistry on RNase H activity. In two independent experiments, antisense oligonucleotides targeting an identical region of FOXO1 mRNA were hybridized with RNA and then incubated with RNase H at 37°C in the presence of 1X RNase H buffer. Disappearance of full length RNA was measured from its peak area at 254nm using RP-HPLC. (A): from top to bottom at 60 min: ONT-355, ONT-316, ONT-367, ONT-392, ONT-393 and ONT-394 (ONT-393 and ONT-394 about the same at 60 min; ONT-393 had higher %RNA substrate remaining at 5 min). (B): from top to bottom at 60 min: ONT-315, ONT-354, ONT-366, ONT-391, ONT-389 and ONT-390. Cleavage rates were determined by measuring amount of full length RNA remaining in the reaction mixtures by reverse phase HPLC. Reactions were quenched at fixed time points by 30mM Na_2EDTA.

[00271] *Figure 13.* Turnover of antisense oligonucleotides. The duplexes were made with each DNA strand concentration equal to 6 μM and RNA being 100 μM. These duplexes were incubated with 0.02 μM RNase H enzyme and disappearance of full length RNA was measured from its peak area at 254 nm using RP-HPLC. Cleavage rates were determined by measuring amount of full length RNA remaining in the reaction mixtures by reverse phase HPLC. Reactions were quenched at fixed time points by 30 mM Na_2EDTA. From top to bottom at 40 min: ONT-316, ONT-367 and ONT-392.

[00272] *Figure 14.* Cleavage map comparing a stereorandom phosphorothioate oligonucleotide with six distinct and stereochemically pure oligonucleotide preparations targeting the same FOXO1 mRNA region. Compositions used include: ONT-367, ONT-392, ONT-393, ONT-394, ONT-400, ONT-401, and ONT-406.

[00273] *Figure 15.* Effect of stereochemistry on RNase H activity. Antisense oligonucleotides were hybridized with RNA and then incubated with RNase H at 37°C in the presence of 1X RNase H buffer. Dependence of stereochemistry upon RNase H activity was observed. Also evident in comparing ONT-367 (stereorandom DNA) and ONT-316 (5-10-5 2'-MOE Gapmer) is the strong dependence of compositional chemistry upon RNase H activity. From top to bottom at 40 min: ONT-316, ONT-421, ONT-367, ONT-392, ONT-394, ONT-415, and ONT-422 (ONT-394/415/422 have similar levels at 40 min; at 5 min, ONT-422 > ONT-394 > ONT-415 in % RNA remaining in DNA/RNA duplex).

[00274] *Figure 16.* Effect of stereochemistry on RNase H activity. Antisense oligonucleotides targeting an identical region of FOXO1 mRNA were hybridized with RNA and

then incubated with RNase H at 37°C in the presence of 1X RNase H buffer. Dependence of stereochemistry upon RNase H activity was observed. Form top to bottom at 40 min: ONT-396, ONT-409, ONT-414, ONT-408 (ONT-396/409/414/408 have similar levels at 40 min), ONT-404, ONT-410, ONT-402 (ONT-404/410/408 have similar levels at 40 min), ONT-403, ONT-407, ONT-405, ONT-401, ONT-406 and ONT-400 (ONT-401/405/406/400 have similar levels at 40 min).

[00275] *Figure 17.* Effect of stereochemistry on RNase H activity. Antisense oligonucleotides targeting an identical region of FOXO1 mRNA were hybridized with RNA and then incubated with RNase H at 37°C in the presence of 1X RNase H buffer. Dependence of stereochemistry upon RNase H activity was observed. ONT-406 was observed to elicit cleavage of duplexed RNA at a rate in slight excess of that of the phosphodiester oligonucleotide ONT-415. From top to bottom at 40 min: ONT-396, ONT-421, ONT-392, ONT-394, ONT-415 ONT-406, and ONT-422 (ONT-394/415/406 have similar levels at 40 min; at 5 min, ONT-394 > ONT-415 > ONT-406 in % RNA remaining in DNA/RNA duplex).

[00276] *Figure 18.* Example UV chromatograms of RNA cleavage products obtained when RNA (ONT-388) was duplexed with stereorandom DNA, ONT-367 (top) and stereopure DNA with repeat triplet motif-3'-SSR-5', ONT-394 (bottom).). 2.35min: 7mer; 3.16min: 8mer and p-6mer; 4.48min: P-7mer; 5.83min: P-8mer; 6.88min: 12mer; 9.32min: 13mer; 10.13min: P-11mer; 11.0min: P-12mer and 14mer; 11.93min: P-13mer; 13.13min: P-14mer. ONT-394 (on the bottom) peak assignment: 4.55min: p-7mer; 4.97min: 10mer; 9.53min: 13mer.

[00277] *Figure 19.* Electrospray Ionization Spectrum of RNA cleavage products. RNA fragments obtained from the duplex ONT-387, RNA/ONT-354, (7-6-7, DNA-2'-OMe-DNA) on the top and ONT-387, RNA/ONT-315, (5-10-5,2'-MOE Gapmer) at the bottom when these duplexes were incubated with RNase H for 30min in the presence of 1X RNse H buffer.

[00278] *Figure 20.* UV Chromatogram and TIC of ONT-406 and ONT-388 duplex after 30 minutes of incubation with RNase H.

[00279] *Figure 21.* An example proposed cleavage. Provided chirally controlled oligonucleotide compositions are capable of cleaving targets as depicted.

[00280] *Figure 22.* Example allele specific cleavage targeting mutant Huntingtin mRNA. (A) and (B): example oligonucleotides. (C)-(E): cleavage maps. (F)-(H): RNA cleavage. Stereorandom and chirally controlled oligonucleotide compositions were prepared to target

single nucleotide polymorphisms for allele selective suppression of mutant Huntingtin. ONT-450 (stereorandom) targeting ONT-453 (muHTT) and ONT-454 (wtHTT) showed marginal differentiation in RNA cleavage and their cleavage maps. Chirally controlled ONT-451 with selective placement of 3'-SSR-5' motif in RNase H recognition site targeting ONT-453 (muHTT) and ONT-454 (wtHTT) showed large differentiation in RNA cleavage rate. From the cleavage map, it is notable that 3'-SSR-5' motif is placed to direct the cleavage between positions 8 and 9 which is after the mismatch if read from 5'-end of RNA. ONT-452 with selective placement of 3'-SSR-5' motif in RNase H recognition site targeting ONT-453 (muHTT) and ONT-454 (wtHTT) showed moderate differentiation in RNA cleavage rate. 3'-SSR-5' motif was placed to direct the cleavage at positions 7 and 8 which is before the mismatch if read from 5'-end of RNA. Example data illustrate significance of position in placement of 3'-SSR-5' motif to achieve enhanced discrimination for allele specific cleavage. All cleavage maps are derived from the reaction mixtures obtained after 5 minutes of incubation of respective duplexes with RNase H1C in the presence of 1XPBS buffer at 37°C. Arrows indicate sites of cleavage. The length of the arrow signifies the amount of metabolite present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment. Only in the cases where 5'-OH 3'-OH was not detected in the reaction mixture, 5'-phosphate species peak was used for quantification. Compositions used include: ONT-450 to ONT-454.

[00281] *Figure 23.* (A)-(C): example allele specific cleavage targeting FOXO1 mRNA.

[00282] *Figure 24. In vitro* dose response silencing of ApoB mRNA after treatment with ApoB oligonucleotides. Stereochemically pure diasetereomers with and without 2'-MOE wings show similar efficacy as ONT-41 (Mipomersen). Compositions used include: ONT-87, ONT-41, and ONT-154.

[00283] *Figure 25.* Comparison of RNase H cleavage maps (A) and RNA cleavage rates (B) for stereorandom composition (ONT-367) and chirally controlled oligonucleotide compositions (ONT-421, all *S*p and ONT-455, all *R*p) and DNA (ONT-415). These sequences target the same region in FOXO1 mRNA. Cleavage maps were derived from the reaction mixtures obtained after 5 minutes of incubation of respective duplexes with RNase H1C in the presence of 1XPBS buffer at 37°C. Arrows indicate sites of cleavage. The length of the arrow signifies the amount of metabolite present in the reaction mixture which was determined from

the ratio of UV peak area to theoretical extinction coefficient of that fragment. Only in the cases where 5'-OH 3'-OH was not detected in the reaction mixture, 5'-phosphate species peak was used for quantification. Cleavage rates were determined by measuring amount of full length RNA remaining in the reaction mixtures by reverse phase HPLC. Reactions are quenched at fixed time points by 30mM Na_2EDTA.

[00284] *Figure 26.* Comparison of cleavage maps of sequences containing one *R*p with change of position starting from 3'-end of DNA. Compositions used include: ONT-396 to ONT-414. These sequences target the same region in FOXO1 mRNA. Cleavage maps are derived from the reaction mixtures obtained after 5 minutes of incubation of respective duplexes with RNase H1C in the presence of 1 X RNase H buffer at 37°C. Arrows indicate sites of cleavage. The length of the arrow signifies the amount of metabolite present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment. Only in the cases where 5'-OH 3'-OH was not detected in the reaction mixture, 5'-phosphate species peak was used for quantification.

[00285] *Figure 27.* (A) Comparison of RNase H cleavage rates for stereopure oligonucleotides (ONT-406), (ONT-401), (ONT-404) and (ONT-408). All four sequences are stereopure phosphorothioates with one *R*p linkage. These sequences target the same region in FOXO1 mRNA. All duplexes were incubation with RNase H1C in the presence of 1 X RNase H buffer at 37°C. Reactions were quenched at fixed time points by 30mM Na_2EDTA. Cleavage rates were determined by measuring amount of full length RNA remaining in the reaction mixtures by reverse phase HPLC. ONT-406 and ONT-401 were found to have superior cleavage rates. (B) Correlation between %RNA cleaved in RNase H assay (10 μM oligonucleotide) and %mRNA knockdown in *in vitro* assay (20 nM oligonucleotide). All sequences target the same region of mRNA in the FOXO1 target. The quantity of RNA remaining is determined by UV peak area for RNA when normalized to DNA in the same reaction mixture. All of the above maps are derived from the reaction mixture obtained after 5 minutes of incubation of respective duplexes with RNase H1C in the presence of 1 X PBS buffer at 37°C. All sequences from ONT-396 to ONT-414 have one *R*p phosphorothioate and they vary in the position of *R*p. ONT-421 (All *S*p) phosphorothioate was inactive *in-vitro* assay. It relates poor cleavage rate of RNA in RNase H assay when ONT-421 is duplexed with complementary RNA.

[00286] *Figure 28.* Serum stability assay of single *R*p walk PS DNA (ONT-396-ONT-

414), stereorandom PS DNA(ONT-367), all-*S*p PS DNA (ONT-421) and all-*R*p PS DNA (ONT-455) in rat serum for 2 days. Note ONT-396 and ONT-455 decomposed at tested time point. Compositions used include: ONT-396 to ONT-414, ONT-367, ONT-421, and ONT-455.

[00287] *Figure 29.* Example oligonucleotides including hemimers. (A): cleavage maps. (B): RNA cleavage assay. (C): FOXO1 mRNA knockdown. ONT-440, ONT-441, and ONT-367 are used. In some embodiments, introduction of 2'-modifications on 5'-end of the sequences increases stability for binding to target RNA while maintaining RNase H activity. ONT-367 (stereorandom phosphorothioate DNA) and ONT-440 (5-15, 2'-F-DNA) have similar cleavage maps and similar rate of RNA cleavage in RNase H assay (10 μM oligonucleotide). In some embodiments, ONT-440 (5-11, 2'-F-DNA) sequence can have better cell penetration properties. In some embodiments, asymmetric 2'-modifications provide Tm advantage while maintaining RNase H activity. Introduction of RSS motifs can further enhance RNase H efficiency in the hemimers. Cleavage maps are derived from the reaction mixtures obtained after 5 minutes of incubation of respective duplexes with RNase H1C in the presence of 1 X RNase H buffer at 37 °C. Arrows indicate sites of cleavage. (┬) indicates that both fragments, 5'-phosphate species as well as 5'-OH 3'-OH species were identified in reaction mixtures. (┌) indicates that only 5'-phosphate species was detected and (┐) indicates that 5'-OH 3'-OH component was detected in mass spectrometry analysis. The length of the arrow signifies the amount of metabolite present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment. Only in the cases where 5'-OH 3'-OH was not detected in the reaction mixture, 5'-phosphate species peak was used for quantification.

[00288] *Figure 30.* Example mass spectrometry data of cleavage assay. Top: data for ONT-367: 2.35 min: 7 mer; 3.16 min: 8 mer and P-6 mer; 4.58 min: P-7 mer; 5.91 min: P-8 mer; 7.19 min: 12 mer; 9.55 min: 13 mer; 10.13 min: P-11 mer; 11.14 min: P-12 mer and 14 mer; 12.11 min: P-13 mer; 13.29 min: P-14 mer; 14.80 min: full length RNA (ONT-388) and 18.33 min: stereorandom DNA (ONT-367). Bottom: data for ONT-406: 4.72 min: p-rArUrGrGrCrUrA, 5'-phosphorylated 7 mer RNA; 9.46 min: 5'-rGrUrGrArGrCrArGrCrUrGrCrA, 5'-OH 3'-OH 13 mer RNA; 16.45 min: full length RNA (ONT-388); 19.48 and 19.49 min: stereopure DNA (ONT-406).

[00289] *Figure 31.* Example RNA cleavage rates. Duplexes were incubated with RNase H1C in the presence of 1 X RNase H buffer at 37 °C. Reactions were quenched at fixed time

points by addition of 30 mM Na_2EDTA. Cleavage rates were determined by measuring amount of full length RNA remaining in the reaction mixtures. Compositions used include: WV-944, WV-945, WV-936, WV-904, WV-937, WV-905, WV-938, WV-906, WV-939, WV-907, WV-940, WV-908, WV-941, and WV-909.

[00290] *Figure 32.* A-N: RNA cleavage rates in RNase H assay for certain compositions targeting rs362307. Some of these compositions are stereorandom and some chirally controlled. Compositions used include: WV-1085, WV-1086, WV-1087, WV-1088, WV-1089, WV-1090, WV-1091, WV-1092, WV-905, WV-944, WV-945, WV-911, WV-917, WV-931, WV-937, and WV-1497.

[00291] *Figure 33.* A: Example cleavage maps. Cleavage maps were derived from reaction mixtures obtained after 5 minutes of incubation of respective duplexes with RNase H1C in the presence of 1 X RNaseH buffer at 37 °C. B: Legend. Arrows indicate sites of cleavage. (┬) indicates that both fragments, 5'-phosphate species as well as 3'-OH species were identified. (┌) indicates that only 5'-OH 3'-OH species was detected and (┐) indicates that 5'-Phosphate component was detected. Length of an arrow signifies the amount of fragment present in the reaction mixture which was determined from the ratio of UV peak area to theoretical extinction coefficient of that fragment. Only in the cases where 5'-OH 3'-OH fragments were not detected in the reaction mixture, the 5'-phosphate species peak was used for quantification. Compositions used include: WV-944, WV-945, WV-904, WV-905, WV-906, WV-907, WV-908, and WV-909.

[00292] *Figure 34.* Example cleavage maps. Example cleavage maps. Cleavage maps were derived from reaction mixtures obtained after 30 minutes of incubation of respective duplexes with RNase H1C in the presence of 1 X RNase H buffer at 37 °C. For legend, see Figure 33. Compositions used include: WV-944, WV-945, WV-936, WV-937, WV-938, WV-939, WV-940, WV-941, WV-1085, WV-1086, WV-1087, WV-1088, WV-1089, WV-1090, WV-1091, and WV-1092.

[00293] *Figure 35.* Example cleavage maps. For legend, see Figure 33. Compositions used include: WV-944, WV-945, WV-905, WV-911, WV-917, WV-931, and WV-937.

[00294] *Figure 36.* Total ion chromatogram of RNase H cleavage reaction for WV-937 when duplexed with WT HTT RNA (WV-944, upper panel) or mu HTT RNA (WV-945, lower panel). Following quenching of the enzymatic reaction with disodium EDTA after 30 minutes, the RNase H cleavage products were chromatographically resolved and analyzed using an

Agilent 1290 UPLC coupled with an Agilent 6230 MS-TOF mass spectrometer. The high mass accuracy high resolution MS spectra for each identified peak was extracted and deconvoluted. Identification of the metabolites which led to determination of position of cleavage was done by comparing the deconvoluted average masses to masses of predicted RNA metabolites.

[00295] *Figure 37.* Illustration of the Luciferase Reporter-based screening.

[00296] *Figures 38A–38I.* Figures 38A-38I and 39A-39G show the activity of various HTT oligonucleotides. Dose-response curves for HTT silencing in reporter-based assay in COS7 cells after transfection of ASOs targeting rs362331_T or rs2530595_T SNPs. ASO specificity is increased with no significant loss of potency by addition of stereopure design (calculated IC50s specified). Data are representative of 2 independent experiments. Lines indicate fit curves, error bars indicate standard deviations. In the figures, the location of the SNP is indicated. Compositions tested in Figure 38 include: WV-2067, WV-2416, WV-2069, WV-2417, WV-2072, WV-2418, WV-2076, WV-2419, WV-2605, WV-2589, WV-2606, WV-2590, WV-2607, WV-2591, WV-2608, WV-2592, WV-2609, WV-2593, WV-2610, WV-2594, WV-2611, WV-2595, WV-2612, WV-2596, WV-2611, WV-2595, WV-2671, WV-2672, WV-2673, WV-2675, WV-2674, WV-2613, WV-2597, WV-2614, WV-2598, WV-2615, WV-2599, WV-2616, WV-2600, WV-2617, WV-2601, WV-2618, WV-2602, WV-2619, WV-2603, WV-2620, and WV-2604.

[00297] *Figures 39A-39G.* Dose-response curves for HTT silencing in reporter-based assay in COS7 cells after transfection of ASOs. Compositions tested include: WVE120101, WV-1092, WV-1497, WV-2619, WV-2603, WV-2611, and WV-2595. IC_{50} data is also shown.

[00298] *Figures 40A-40D.* Figures 40A-40D shows liquid chromatograph and mass spectra data for oligonucleotides: WV1092.22 (WV-1092), WV2595.01 (WV-2595) and WV2603.01 (WV-2603). The suffices (01), (02), .01, .02, .22, etc., as used herein, indicate batch numbers.

[00299] *Figure 41.* Figure 41 shows liquid chromatograph and mass spectra data for oligonucleotides: WV-1510, WV-2378 and WV-2380.

Detailed Description of Certain Embodiments

[00300] Synthetic oligonucleotides provide useful molecular tools in a wide variety of applications. For example, oligonucleotides are useful in therapeutic, diagnostic, research, and

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

[00301] Among other things, the present disclosure encompasses the recognition that structural elements of oligonucleotides, such as base sequence, chemical modifications (*e.g.*, modifications of sugar, base, and/or internucleotidic linkages, and patterns thereof), and/or stereochemistry (*e.g.*, stereochemistry of backbone chiral centers (chiral internucleotidic linkages), and/or patterns thereof), can have significant impact on properties, *e.g.*, activities, of oligonucleotides. In some embodiments, the present disclosure demonstrates that oligonucleotide compositions comprising oligonucleotides with controlled structural elements, *e.g.*, controlled chemical modification and/or controlled backbone stereochemistry patterns, provide unexpected properties, including but not limited to those described herein. In some embodiments, the present disclosure provide an oligonucleotide composition comprises a predetermined level of oligonucleotides of an individual oligonucleotide type which are chemically identical, *e.g.*, they have the same base sequence, the same pattern of nucleoside modifications (modifications to sugar and base moieties, if any), the same pattern of backbone chiral centers, and the same pattern of backbone phosphorus modifications.

[00302] Among other things, the present disclosure encompasses the recognition that stereorandom oligonucleotide preparations contain a plurality of distinct chemical entities that differ from one another, *e.g.*, in the stereochemical structure of individual backbone chiral centers within the oligonucleotide chain. Without control of stereochemistry of backbone chiral centers, stereorandom oligonucleotide preparations provide uncontrolled compositions comprising undetermined levels of oligonucleotide stereoisomers. Even though these

stereoisomers may have the same base sequence, they are different chemical entities at least due to their different backbone stereochemistry, and they can have, as demonstrated herein, different properties, *e.g.,* bioactivities. A stereopure (or "chirally controlled") oligonucleotide composition or preparation can have improved bioactivity compared to a stereorandom oligonucleotide preparation which is otherwise identical (e.g., both the stereopure and stereorandom versions have the same base sequence, pattern of base and sugar modifications, etc.). For example, stereorandom oligonucleotide WV-1497 composition and a stereopure oligonucleotide WV-1092 composition both have the same sequence of bases and identical patterns of sugar modifications and backbone linkages, differing only in stereochemistry. However, at higher concentrations, there was a marked difference in the ability of the stereopure WV-1092 composition and the stereorandom WV-1497 composition to differentiate between wt and mutant HTT (which differ in only one nt). At the high concentration, both knocked down the mutant HTT to a great degree, which is desirable; but stereopure WV-1092 showed only a small knock down of wildtype HTT, while WV-1497 showed significantly more knock down of wt HTT, which is less desirable in some instances.

[00303] Chirally controlled oligonucleotide compositions of both WVE120101 and WV-1092 were able to differentiate between wt and mutant versions of SNP rs362307, which differ by one nt; both WVE120101 and WV-1092 significantly knocked down the mutant allele but not the wt, while the stereorandom version, WV-1497, was not able to significantly differentiate between the wt and mutant alleles (see Figure 39D). The modified sequences of WVE120101 and WV-1092 are identical.

[00304] A chirally controlled oligonucleotide composition of WV-2595 was able to differentiate between the C and T alleles at SNP rs2530595, which also differ at only the one nt. Stereopure WV-2595 significantly knocked down the T allele but not the C allele, unlike the stereorandom oligonucleotide composition of WV-2611, which was not able to significantly differentiate the alleles (see Figure 39F). The sequence of WV-2595 is 5'-mG*mGmGmUmC*C*T*C*C*C*C*A*C*A*G*mAmGmGmG*mA -3' or 5'-mG*SmGmGmUmC*SC*ST*SC*SC*SC*SC*SA*SC*RA*SG*SmAmGmGmG*SmA -3' with certain stereochemistry information.

[00305] A stereopure oligonucleotide composition of WV-2603 was able to differentiate between the C and T alleles of SNP rs362331, which also differ at only the one nt. Stereopure

WV-2603 significantly knocked down the T allele but not the C allele, unlike the stereorandom oligonucleotide composition of WV-2619, which was not able to significantly differentiate between the alleles (see Figures 39A, 39B, 39C and 39E). The sequence of WV-2603 is 5'-mG*mUmGmCmA*C*A*C*A*G*T*A*G*A*T*mGmAmGmG*mG -3' or 5'-mG*SmUmGmCmA*SC*SA*SC*SA*SG*ST*SA*SG*RA*ST*SmGmAmGmG*SmG -3' with certain stereochemistry information.

[00306] In some embodiments, the sequence of the oligonucleotide in a stereopure (chirally controlled) oligonucleotide composition comprises or consists of the sequence of any oligonucleotide disclosed herein. In some embodiments, the sequence of the oligonucleotide in a stereopure (chirally controlled) oligonucleotide composition comprises or consists of the sequence of any oligonucleotide selected from Tables N1, N2, N3, N4 and 8. In some embodiments, the sequence of the oligonucleotide in a stereopure (chirally controlled) oligonucleotide composition comprises or consists of the sequence of any oligonucleotide selected from Tables N1A, N2A, N3A, N4A and 8. In some embodiments, the sequence of the oligonucleotide in a stereopure (chirally controlled) oligonucleotide composition comprises or consists of the sequence of WV-1092, WVE120101, WV-2603 or WV-2595.

[00307] Each oligonucleotide described herein comprising a HTT sequence represents an HTT oligonucleotide which was designed, constructed and tested in various assays, in some embodiments, one or more *in vitro* assays. Each HTT oligonucleotide listed in any of Tables N1A, N2A, N3A, N4A and 8, or described elsewhere herein, was designed, constructed and tested in various assays, in some embodiments, one or more *in vitro* assays. For example, HTT oligonucleotides described herein were tested in a dual luciferase reporter assay. In some embodiments, HTT oligonucleotides were tested in one or more other assays described in this disclosure and/or in the art in accordance with the present disclosure. In some embodiments, HTT oligonucleotides which were found to be particularly efficacious in the dual luciferase assay were tested in further *in vitro* and *in vivo* assays in accordance with the present disclosure.

[00308] In some embodiments, a sequence of an oligonucleotide in a stereopure (chirally controlled) oligonucleotide composition includes any one or more of: base sequence (including length); pattern of chemical modifications to sugar and base moieties; pattern of backbone linkages; pattern of natural phosphate linkages, phosphorothioate linkages, phosphorothioate triester linkages, and combinations thereof; pattern of backbone chiral centers; pattern of

stereochemistry (*R*p/*S*p) of chiral internucleotidic linkages; pattern of backbone phosphorus modifications; pattern of modifications on the internucleotidic phosphorus atom, such as $-S^-$, and $-L-R^1$ of formula I.

[00309] Among other things, the present disclosure provides new compositions that are or contain particular stereoisomers of oligonucleotides of interest. In some embodiments, a particular stereoisomer may be defined, for example, by its base sequence, its length, its pattern of backbone linkages, and its pattern of backbone chiral centers. As is understood in the art, in some embodiments, base sequence may refer to the identity and/or modification status of nucleoside residues (*e.g.*, of sugar and/or base components, relative to standard naturally occurring nucleotides such as adenine, cytosine, guanosine, thymine, and uracil) in an oligonucleotide and/or to the hybridization character (i.e., the ability to hybridize with particular complementary residues) of such residues. In some embodiments, oligonucleotides in provided compositions comprise sugar modifications, *e.g.*, 2'-modifications, at *e.g.*, a wing region. In some embodiments, oligonucleotides in provided compositions comprise a region in the middle, *e.g.*, a core region, that has no sugar modifications.

[00310] The present disclosure demonstrates, among other things, that individual stereoisomers of a particular oligonucleotide can show different stability and/or activity (*e.g.*, functional and/or toxicity properties) from each other. Moreover, the present disclosure demonstrates that stability and/or activity improvements achieved through inclusion and/or location of particular chiral structures within an oligonucleotide can be comparable to, or even better than those achieved through use of particular backbone linkages, residue modifications, *etc*. (*e.g.*, through use of certain types of modified phosphates [*e.g.*, phosphorothioate, substituted phosphorothioate, *etc*.], sugar modifications [*e.g.*, 2'-modifications, *etc*.], and/or base modifications [*e.g.*, methylation, *etc*.]).

[00311] Among other things, the present disclosure recognizes that, in some embodiments, properties (*e.g.*, stability and/or activities) of an oligonucleotide can be adjusted by optimizing its pattern of backbone chiral centers, optionally in combination with adjustment/optimization of one or more other features (*e.g.*, linkage pattern, nucleoside modification pattern, *etc*.) of the oligonucleotide. In some embodiments, the present disclosure provides oligonucleotide compositions wherein the oligonucleotides comprise nucleoside modifications, chiral internucleotidic linkages and natural phosphate linkages. For example, WV-1092 comprises 2'-

OMe modifications, phosphate linkages in its 5'- and 3'-wing regions, and phosphorothioate linkages in its core regions.

[00312] In some embodiments, the present disclosure demonstrates that stability improvements achieved through inclusion and/or location of particular chiral structures within an oligonucleotide can be comparable to, or even better than those achieved through use of modified backbone linkages, bases, and/or sugars (*e.g.*, through use of certain types of modified phosphates, 2'-modifications, base modifications, *etc.*). The present disclosure, in some embodiments, also demonstrates that activity improvements achieved through inclusion and/or location of particular chiral structures within an oligonucleotide can be comparable to, or even better than those achieved through use of modified backbone linkages, bases, and/or sugars (*e.g.*, through use of certain types of modified phosphates, 2'-modifications, base modifications, *etc.*).

[00313] In some embodiments, inclusion and/or location of particular chiral linkages within an oligonucleotide can surprisingly change the cleavage pattern of a nucleic acid polymer when such an oligonucleotide is utilized for cleaving said nucleic acid polymer. For example, in some embodiments, a pattern of backbone chiral centers provides unexpectedly high cleavage efficiency of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers provides new cleavage sites. In some embodiments, a pattern of backbone chiral centers provides fewer cleavage sites, for example, by blocking certain existing cleavage sites. Even more unexpectedly, in some embodiments, a pattern of backbone chiral centers provides cleavage at only one site of a target nucleic acid polymer within the sequence that is complementary to an oligonucleotide utilized for cleavage. In some embodiments, higher cleavage efficiency is achieved by selecting a pattern of backbone chiral centers to minimize the number of cleavage sites.

[00314] In some embodiments, the present disclosure provides compositions of oligonucleotides, wherein the oligonucleotides have a common pattern of backbone chiral centers which, unexpectedly, greatly enhances the stability and/or biological activity of the oligonucleotides. In some embodiments, a pattern of backbone chiral centers provides increased stability. In some embodiments, a pattern of backbone chiral centers provides surprisingly increased activity. In some embodiments, a pattern of backbone chiral centers provides increased stability and activity. In some embodiments, when an oligonucleotide is utilized to cleave a nucleic acid polymer, a pattern of backbone chiral centers, surprisingly by itself,

changes the cleavage pattern of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers effectively prevents cleavage at secondary sites. In some embodiments, a pattern of backbone chiral centers creates new cleavage sites. In some embodiments, a pattern of backbone chiral centers minimizes the number of cleavage sites. In some embodiments, a pattern of backbone chiral centers minimizes the number of cleavage sites so that a target nucleic acid polymer is cleaved at only one site within the sequence of the target nucleic acid polymer that is complementary to the oligonucleotide (*e.g.*, cleavage at other sites cannot be readily detected by a certain method; in some embodiments, greater than 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% cleavage occurs at such a site). In some embodiments, a pattern of backbone chiral centers enhances cleavage efficiency at a cleavage site. In some embodiments, a pattern of backbone chiral centers of the oligonucleotide improves cleavage of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers increases selectivity. In some embodiments, a pattern of backbone chiral centers minimizes off-target effect. In some embodiments, a pattern of backbone chiral centers increase selectivity, *e.g.*, cleavage selectivity between two target sequences differing only by a single nucleotide polymorphism (SNP). In some embodiments, a pattern of backbone chiral centers increase cleavage at a cleavage site of a stereorandom or DNA oligonucleotide composition. In some embodiments, a pattern of backbone chiral centers increase cleavage at a major cleavage site of a stereorandom or DNA oligonucleotide composition. In some embodiments, such a site is a major cleavage site of oligonucleotides having the pattern of backbone chiral centers. In some embodiments, a site is considered a major site if it is a site having the most, or the second, third, fourth or fifth most cleavage, or a site where greater than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% of cleavage occurs. In some embodiments, a pattern of backbone chiral centers comprises or is $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$. In some embodiments, a pattern of backbone chiral centers comprises or is $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein $m > 2$. In some embodiments, a pattern of backbone chiral centers comprises or is $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein n is 1, $t > 1$, and $m > 2$. In some embodiments, $m > 3$. In some embodiments, $m > 4$.

[00315] In some embodiments, the present disclosure recognizes that chemical modifications, such as modifications of nucleosides and internucleotidic linkages, can provide

enhanced properties. In some embodiments, the present disclosure demonstrates that combinations of chemical modifications and stereochemistry can provide unexpected, greatly improved properties (*e.g.,* bioactivity, selectivity, *etc.*). In some embodiments, chemical combinations, such as modifications of sugars, bases, and/or internucleotidic linkages, are combined with stereochemistry patterns, *e.g.,* $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, to provide oligonucleotides and compositions thereof with surprisingly enhanced properties. In some embodiments, a provided oligonucleotide composition is chirally controlled, and comprises a combination of 2'-modification of one or more sugar moieties, one or more natural phosphate linkages, one or more phosphorothioate linkages, and a stereochemistry pattern of $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein m > 2. In some embodiments, n is 1, t >1, and m > 2. In some embodiments, m > 3. In some embodiments, m > 4.

[00316] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

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

[00317] In some embodiments, a common base sequence and length may be referred to as a common base sequence. In some embodiments, oligonucleotides having a common base sequence may have the same pattern of nucleoside modifications, *e.g.*, sugar modifications, base modifications, *etc.* In some embodiments, a pattern of nucleoside modifications may be represented by a combination of locations and modifications. For example, for WV-1092, the pattern of nucleoside modifications is 5 x 2'-OMe (2'-OMe modification on sugar moieties)-DNA (no 2'-modifications on the sugar moiety)-5 x 2'-OMe from the 5'-end to the 3'-end. In some embodiments, a pattern of backbone linkages comprises locations and types （*e.g.,* phosphate, phosphorothioate, substituted phosphorothioate, *etc.*） of each internucleotidic linkages. In some embodiments, an oligonucleotide can have a specified pattern of backbone linkages. In some embodiments, an oligonucleotide has a pattern of backbone linkages of $_n$PS-$_n$PO-$_n$PS-$_n$PO-$_n$PS, wherein PO is phosphate (phosphorodiester), PS is phosphorothioate, and n is

1-15, and each occurrence of n can be the same or different. In some embodiments, at least one n is greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, at least one n for PS is greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, the n for the PS between the two PO is greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, n is greater than 5. In some embodiments, n is greater than 6. In some embodiments, n is greater than 7. In some embodiments, n is greater than 8. In some embodiments, n is greater than 9. In some embodiments, n is greater than 10. In some embodiments, n is greater than 11. In some embodiments, n is greater than 12. In some embodiments, n is greater than 13. In some embodiments, n is greater than 14. In some embodiments, n is greater than 15. In some embodiments, an oligonucleotide has a pattern of backbone linkages of 1-5PS - 1-7PO - 5-15PS- 1-7 PO- 1-5PS (meaning 1 to 5 phosphorothioates, 1 to 7 phosphates, 5 to 15 phosphorothioates, 1 to 7 phosphates, and 1 to 5 phosphorothioates). In some embodiments, the oligonucleotide has a pattern of backbone linkages, from 5' to 3', of 1PS-3PO-11PS-3PO-1PS (meaning 1 phosphorothioate, 3 phosphates, 11 phosphorothioates, 3 phosphates, and 1 phosphorothioate, and which can alternatively be represented as PS_1 PO_3 PS_{11} PO_3 PS_1). For example, for WV-1092, the pattern of backbone linkages is 1PS-3PO-11PS-3PO-1PS from the 5'-end to the 3'-end. In some embodiments, an oligonucleotide has a pattern of backbone linkages of 1-5PS - 1-7PO - 5-15PS - 1-7PO - 1-5PS, wherein each PS is *S*p except for one *R*p. In some embodiments, the oligonucleotide has a pattern of backbone linkages of 1-5PS - 1-7PO - 5-15PS - 1-7PO - 1-5PS, wherein each PS is *S*p except one PS at any position from the 5^{th} to 15^{th} PS is *R*p. In some embodiments, the oligonucleotide has a pattern of backbone linkages of 1-5PS - 1-7PO - 5-15PS - 1-7PO - 1-5PS, wherein each PS is *S*p except that the 10^{th} PS counting from the 5' end is *R*p. In some embodiments, the oligonucleotide has a pattern of backbone linkages of 1-5PS - 1-7PO - 5-15PS - 1-7PO - 1-5PS, wherein each PS is *S*p except that the 9^{th} counting from the 5' end PS is *R*p. In some embodiments, the oligonucleotide has a pattern of backbone linkages of 1-5PS - 1-7PO - 5-15PS - 1-7PO - 1-5PS, wherein each PS is *S*p except that the 11^{th} PS counting from the 5' end is *R*p. A pattern of backbone chiral centers of an oligonucleotide can be designated by a combination of linkage phosphorus stereochemistry (*R*p/*S*p) from 5' to 3'. For example, WV-1092 has a pattern of 1S-3PO (phosphate)-8S-1R-2S-3PO-1S, and WV-937 has a pattern of 12S-1R-6S. In some embodiments, all non-chiral linkages (*e.g.*, PO) may be omitted when

describing a pattern of backbone chiral centers. As exemplified above, locations of non-chiral linkages may be obtained, for example, from pattern of backbone linkages. Any sequence disclosed herein can be combined with any patterns of backbone linkages and/or any patterns of backbone chiral centers disclosed herein. Base sequences, patterns of backbone linkages, patterns of stereochemistry (e.g., *R*p or *S*p), patterns of base modifications, patterns of backbone chiral centers, *etc.* are presented in 5' to 3' direction unless otherwise indicated.

[00318] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

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

[00319] An example substantially racemic preparation of oligonucleotides is the preparation of phosphorothioate oligonucleotides through sulfurizing phosphite triesters from commonly used phosphoramidite oligonucleotide synthesis with either tetraethylthiuram disulfide or (TETD) or 3H-1, 2-bensodithiol-3-one 1, 1-dioxide (BDTD), a well-known process in the art. In some embodiments, substantially racemic preparation of oligonucleotides provides substantially racemic oligonucleotide compositions (or chirally uncontrolled oligonucleotide compositions).

[00320] As understood by a person having ordinary skill in the art, a stereorandom or racemic preparation of oligonucleotides is prepared by non-stereoselective and/or low-stereoselective coupling of nucleotide monomers, typically without using any chiral auxiliaries, chiral modification reagents, and/or chiral catalysts. In some embodiments, in a substantially racemic (or chirally uncontrolled) preparation of oligonucleotides, all or most coupling steps are not chirally controlled in that the coupling steps are not specifically conducted to provide enhanced stereoselectivity. An example substantially racemic preparation of oligonucleotides is the preparation of phosphorothioate oligonucleotides through sulfurizing phosphite triesters from commonly used phosphoramidite oligonucleotide synthesis with either tetraethylthiuram

disulfide or (TETD) or 3H-1, 2-bensodithiol-3-one 1, 1-dioxide (BDTD), a well-known process in the art. In some embodiments, substantially racemic preparation of oligonucleotides provides substantially racemic oligonucleotide compositions (or chirally uncontrolled oligonucleotide compositions). In some embodiments, at least one coupling of a nucleotide monomer has a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, at least two couplings of a nucleotide monomer have a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, at least three couplings of a nucleotide monomer have a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, at least four couplings of a nucleotide monomer have a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, at least five couplings of a nucleotide monomer have a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, in a stereorandom or racemic preparations, at least one internucleotidic linkage has a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, at least two internucleotidic linkages have a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, at least three internucleotidic linkages have a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, at least four internucleotidic linkages have a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, at least five internucleotidic linkages have a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, a diastereoselectivity is lower than about 60:40. In some embodiments, a diastereoselectivity is lower than about 70:30. In some embodiments, a diastereoselectivity is lower than about 80:20. In some embodiments, a diastereoselectivity is lower than about 90:10. In some embodiments, a diastereoselectivity is lower than about 91:9. In some embodiments, a diastereoselectivity is lower than about 92:8. In some embodiments, a diastereoselectivity is lower than about 93:7. In some embodiments, a diastereoselectivity is lower than about 94:6. In some embodiments, a diastereoselectivity is lower than about 95:5. In some embodiments, a diastereoselectivity is lower than about 96:4. In some embodiments, a diastereoselectivity is lower than about 97:3. In

some embodiments, a diastereoselectivity is lower than about 98:2. In some embodiments, a diastereoselectivity is lower than about 99:1. In some embodiments, at least one coupling has a diastereoselectivity lower than about 90:10. In some embodiments, at least two couplings have a diastereoselectivity lower than about 90:10. In some embodiments, at least three couplings have a diastereoselectivity lower than about 90:10. In some embodiments, at least four couplings have a diastereoselectivity lower than about 90:10. In some embodiments, at least five couplings have a diastereoselectivity lower than about 90:10. In some embodiments, at least one internucleotidic linkage has a diastereoselectivity lower than about 90:10. In some embodiments, at least two internucleotidic linkages have a diastereoselectivity lower than about 90:10. In some embodiments, at least three internucleotidic linkages have a diastereoselectivity lower than about 90:10. In some embodiments, at least four internucleotidic linkages have a diastereoselectivity lower than about 90:10. In some embodiments, at least five internucleotidic linkages have a diastereoselectivity lower than about 90:10.

[00321] As understood by a person having ordinary skill in the art, in some embodiments, diastereoselectivity of a coupling or a linkage can be assessed through the diastereoselectivity of a dimer formation under the same or comparable conditions, wherein the dimer has the same 5'- and 3'-nucleosides and internucleotidic linkage. For example, diastereoselectivity of the underlined coupling or linkage in WV-1092 mG*SmGmCmAmC*SA*SA*S**G*SG**SG*SC*SA*SC*RA*SG*SmAmCmUmU*SmC can be assessed from coupling two G moieties under the same or comparable conditions, *e.g.*, monomers, chiral auxiliaries, solvents, activators, temperatures, *etc*.

[00322] In some embodiments, the present disclosure provides chirally controlled (and/or stereochemically pure) oligonucleotide compositions comprising oligonucleotides defined by having:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

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

[00323] In some embodiments, the present disclosure provides chirally controlled

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

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers.

[00324] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

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

[00325] In some embodiments, oligonucleotides having a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers have a common pattern of backbone phosphorus modifications and a common pattern of base modifications. In some embodiments, oligonucleotides having a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers have a common pattern of backbone phosphorus modifications and a common pattern of nucleoside modifications. In some embodiments, oligonucleotides having a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers have identical structures.

[00326] In some embodiments, oligonucleotides of an oligonucleotide type have a common pattern of backbone phosphorus modifications and a common pattern of sugar modifications. In some embodiments, oligonucleotides of an oligonucleotide type have a common pattern of backbone phosphorus modifications and a common pattern of base

modifications. In some embodiments, oligonucleotides of an oligonucleotide type have a common pattern of backbone phosphorus modifications and a common pattern of nucleoside modifications. In some embodiments, oligonucleotides of an oligonucleotide type are identical.

[00327] In some embodiments, a chirally controlled oligonucleotide composition is a substantially pure preparation of an oligonucleotide type in that oligonucleotides in the composition that are not of the oligonucleotide type are impurities form the preparation process of said oligonucleotide type, in some case, after certain purification procedures.

[00328] In some embodiments, at least about 20% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 25% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 30% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 35% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 40% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 45% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 50% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 55% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 60% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 65% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 70% of

the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 75% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 80% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 85% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 90% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 92% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 94% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 95% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, greater than about 99% of the oligonucleotides in the composition have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers. In some embodiments, purity of a chirally controlled oligonucleotide composition of an oligonucleotide can be expressed as the percentage of oligonucleotides in the composition that have a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers.

[00329] In some embodiments, oligonucleotides having a common base sequence and length, a common pattern of backbone linkages, and a common pattern of backbone chiral centers have a common pattern of backbone phosphorus modifications. In some embodiments, oligonucleotides having a common base sequence and length, a common pattern of backbone

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

[00330] In some embodiments, oligonucleotides in provided compositions have a common pattern of backbone phosphorus modifications. In some embodiments, a common base sequence is a base sequence of an oligonucleotide type. In some embodiments, a provided composition is an oligonucleotide composition that is chirally controlled in that the composition contains a predetermined level of oligonucleotides of an individual oligonucleotide type, wherein an oligonucleotide type is defined by:

1) base sequence;

2) pattern of backbone linkages;

3) pattern of backbone chiral centers; and

4) pattern of backbone phosphorus modifications.

[00331] As noted above and understood in the art, in some embodiments, base sequence of an oligonucleotide may refer to the identity and/or modification status of nucleoside residues (*e.g.,* of sugar and/or base components, relative to standard naturally occurring nucleotides such as adenine, cytosine, guanosine, thymine, and uracil) in the oligonucleotide and/or to the hybridization character (i.e., the ability to hybridize with particular complementary residues) of such residues.

[00332] In some embodiments, a particular oligonucleotide type may be defined by

1A) base identity;

1B) pattern of base modification;

1C) pattern of sugar modification;

2) pattern of backbone linkages;

3) pattern of backbone chiral centers; and

4) pattern of backbone phosphorus modifications.

Thus, in some embodiments, oligonucleotides of a particular type may share identical bases but differ in their pattern of base modifications and/or sugar modifications. In some embodiments, oligonucleotides of a particular type may share identical bases and pattern of base modifications (including, *e.g.*, absence of base modification), but differ in pattern of sugar modifications.

[00333] In some embodiments, oligonucleotides of a particular type are identical in that they have the same base sequence (including length), the same pattern of chemical modifications to sugar and base moieties, the same pattern of backbone linkages (*e.g.*, pattern of natural phosphate linkages, phosphorothioate linkages, phosphorothioate triester linkages, and combinations thereof), the same pattern of backbone chiral centers (*e.g.*, pattern of stereochemistry (*R*p/*S*p) of chiral internucleotidic linkages), and the same pattern of backbone phosphorus modifications (*e.g.*, pattern of modifications on the internucleotidic phosphorus atom, such as $-S^-$, and $-L-R^1$ of formula I).

[00334] In some embodiments, purity of a chirally controlled oligonucleotide composition of an oligonucleotide type is expressed as the percentage of oligonucleotides in the composition that are of the oligonucleotide type. In some embodiments, at least about 10% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 20% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 30% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 40% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 50% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 60% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same oligonucleotide type. In some embodiments, at least about 70% of the oligonucleotides in a chirally controlled oligonucleotide composition are of the same

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

[00335] In some embodiments, purity of a chirally controlled oligonucleotide composition can be controlled by stereoselectivity of each coupling step in its preparation process. In some embodiments, a coupling step has a stereoselectivity (*e.g.,* diastereoselectivity) of 60% (60% of the new internucleotidic linkage formed from the coupling step has the intended stereochemistry). After such a coupling step, the new internucleotidic linkage formed may be referred to have a 60% purity. In some embodiments, each coupling step has a stereoselectivity of at least 60%. In some embodiments, each coupling step has a stereoselectivity of at least 70%. In some embodiments, each coupling step has a stereoselectivity of at least 80%. In some embodiments, each coupling step has a stereoselectivity of at least 85%. In some embodiments, each coupling step has a stereoselectivity of at least 90%. In some embodiments, each coupling step has a stereoselectivity of at least 91%. In some embodiments, each coupling step has a stereoselectivity of at least 92%. In some embodiments, each coupling step has a stereoselectivity of at least 93%. In some embodiments, each coupling step has a stereoselectivity of at least 94%. In some embodiments, each coupling step has a stereoselectivity of at least 95%. In some embodiments, each coupling step has a

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

[00336] Among other things, the present disclosure recognizes that combinations of oligonucleotide structural elements (*e.g.,* patterns of chemical modifications, backbone linkages, backbone chiral centers, and/or backbone phosphorus modifications) can provide surprisingly improved properties such as bioactivities.

[00337] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a predetermined level of oligonucleotides which comprise one or more wing regions and a common core region, wherein:

each wing region independently has a length of two or more bases, and independently and optionally comprises one or more chiral internucleotidic linkages;

the core region independently has a length of two or more bases, and independently comprises one or more chiral internucleotidic linkages, and the common core region has:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers.

[00338] In some embodiments, a wing region comprises a structural feature that is not in a core region. In some embodiments, a wing and core can be defined by any structural elements, *e.g.,* base modifications (*e.g.,* methylated/non-methylated, methylation at position 1/methylation at position 2, *etc.*), sugar modifications (*e.g.*, modified/non-modified, 2'-modification/another type of modification, one type of 2'-modification/another type of 2'-modification, *etc.*), backbone linkage types (*e.g.,* phosphate/phosphorothioate, phosphorothioate/substituted phosphorothioate, *etc.*), backbone chiral center stereochemistry(*e.g.,* all *S*p/all *R*p, (*Sp*Rp) repeats/all *R*p, *etc.*), backbone phosphorus modification types (*e.g.,* s1/s2, s1/s3, *etc.*), *etc*.

[00339] In some embodiments, a wing and core is defined by nucleoside modifications,

wherein a wing comprises a nucleoside modification that the core region does not have. In some embodiments, a wing and core is defined by sugar modifications, wherein a wing comprises a sugar modification that the core region does not have. In some embodiments, a sugar modification is a 2'-modification. In some embodiments, a sugar modification is 2'-OR^1. In some embodiments, a sugar modification is 2'-MOE. In some embodiments, a sugar modification is 2'-OMe. Additionally example sugar modifications are described in the present disclosure.

[00340] In some embodiments, oligonucleotides in provided compositions have a wing-core structure (hemimer). In some embodiments, oligonucleotides in provided compositions have a wing-core structure of nucleoside modifications. In some embodiments, oligonucleotides in provided compositions have a core-wing structure (another type of hemimer). In some embodiments, oligonucleotides in provided compositions have a core-wing structure of nucleoside modifications. In some embodiments, oligonucleotides in provided compositions have a wing-core-wing structure (gapmer). In some embodiments, oligonucleotides in provided compositions have a wing-core-wing structure of nucleoside modifications. In some embodiments, a wing and core is defined by modifications of the sugar moieties. In some embodiments, a wing and core is defined by modifications of the base moieties. In some embodiments, each sugar moiety in the wing region has the same 2'-modification which is not found in the core region. In some embodiments, each sugar moiety in the wing region has the same 2'-modification which is different than any sugar modifications in the core region. In some embodiments, a core region has no sugar modification. In some embodiments, each sugar moiety in the wing region has the same 2'-modification, and the core region has no 2'-modifications. In some embodiments, when two or more wings are present, each wing is defined by its own modifications. In some embodiments, each wing has its own characteristic sugar modification. In some embodiments, each wing has the same characteristic sugar modification differentiating it from a core. In some embodiments, each wing sugar moiety has the same modification. In some embodiments, each wing sugar moiety has the same 2'-modification. In some embodiments, each sugar moiety in a wing region has the same 2'-modification, yet the common 2'-modification in a first wing region can either be the same as or different from the common 2'-modification in a second wing region. In some embodiments, each sugar moiety in a wing region has the same 2'-modification, and the common 2'-modification in a first wing

region is the same as the common 2'-modification in a second wing region. In some embodiments, each sugar moiety in a wing region has the same 2'-modification, and the common 2'-modification in a first wing region is different from the common 2'-modification in a second wing region.

[00341] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are antisense oligonucleotides (*e.g.,* chiromersen). In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are siRNA oligonucleotides. In some embodiments, a provided chirally controlled oligonucleotide composition is of oligonucleotides that can be antisense oligonucleotide, antagomir, microRNA, pre-microRNs, antimir, supermir, ribozyme, Ul adaptor, RNA activator, RNAi agent, decoy oligonucleotide, triplex forming oligonucleotide, aptamer or adjuvant. In some embodiments, a chirally controlled oligonucleotide composition is of antisense oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of antagomir oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of microRNA oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of pre-microRNA oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of antimir oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of supermir oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of ribozyme oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of Ul adaptor oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of RNA activator oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of RNAi agent oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of decoy oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of triplex forming oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of aptamer oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of adjuvant oligonucleotides.

[00342] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides that include one or more modified backbone linkages, bases, and/or sugars.

[00343] In some embodiments, a provided oligonucleotide comprises one or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide comprises two or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide comprises three or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide comprises four or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide comprises five or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide comprises 1, 2, 3, 4, 5, 6, 7, 8,9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 5 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 6 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 7 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 8 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 9 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 10 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 11 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 12 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 13 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 14 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 15 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 16 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 17 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 18 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 19 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 20 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 21 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 22 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises

23 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 24 or more chiral, modified phosphate linkages. In some embodiments, a provided oligonucleotide type comprises 25 or more chiral, modified phosphate linkages.

[00344] In some embodiments, a provided oligonucleotide comprises at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% chiral, modified phosphate linkages. Example such chiral, modified phosphate linkages are described above and herein. In some embodiments, a provided oligonucleotide comprises at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% chiral, modified phosphate linkages in the *Sp* configuration.

[00345] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 80%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 85%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 90%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 91%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 92%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 93%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 94%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 95%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 96%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 97%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater than about 98%. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of a stereochemical purity of greater

than about 99%.

[00346] In some embodiments, a chiral, modified phosphate linkage is a chiral phosphorothioate linkage, i.e., phosphorothioate internucleotidic linkage. In some embodiments, a provided oligonucleotide comprises at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% chiral phosphorothioate internucleotidic linkages. In some embodiments, all chiral, modified phosphate linkages are chiral phosphorothioate internucleotidic linkages. In some embodiments, at least about 10, 20, 30, 40, 50, 60, 70, 80, or 90% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 10% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 20% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 30% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 40% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 50% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 60% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 70% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 80% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 90% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 95% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *S*p conformation. In some embodiments, at least about 10, 20, 30, 40, 50, 60, 70, 80, or 90% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 10% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 20% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *R*p conformation. In some embodiments, at least about 30% chiral

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

provided oligonucleotide are of the *R*p conformation. In some embodiments, a provided oligonucleotide has only one *R*p chiral phosphorothioate internucleotidic linkages. In some embodiments, a provided oligonucleotide has only one *R*p chiral phosphorothioate internucleotidic linkages, wherein all internucleotide linkages are chiral phosphorothioate internucleotidic linkages. In some embodiments, a chiral phosphorothioate internucleotidic linkage is a chiral phosphorothioate diester linkage. In some embodiments, each chiral phosphorothioate internucleotidic linkage is independently a chiral phosphorothioate diester linkage. In some embodiments, each internucleotidic linkage is independently a chiral phosphorothioate diester linkage. In some embodiments, each internucleotidic linkage is independently a chiral phosphorothioate diester linkage, and only one is *R*p.

[00347] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides that contain one or more modified bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides that contain no modified bases. Example such modified bases are described above and herein.

[00348] In some embodiments, oligonucleotides of provided compositions comprise at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least one natural phosphate linkage. In some embodiments, oligonucleotides of provided compositions comprise at least two natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least three natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least four natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least five natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least six natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least seven natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least eight natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least nine natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least ten natural phosphate linkages.

[00349] In some embodiments, oligonucleotides of provided compositions comprise 2, 3,

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

[00350] In some embodiments, oligonucleotides of provided compositions comprise at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least two consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least three consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least four consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least five consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least six consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least seven consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least eight consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least nine consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise at least ten consecutive natural phosphate linkages.

[00351] In some embodiments, oligonucleotides of provided compositions comprise 2, 3, 4, 5, 6, 7, 8, 9 or 10 consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise two consecutive natural phosphate linkages.

In some embodiments, oligonucleotides of provided compositions comprise three consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise four consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise five consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise six consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise seven consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise eight consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise nine consecutive natural phosphate linkages. In some embodiments, oligonucleotides of provided compositions comprise ten consecutive natural phosphate linkages.

[00352] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 8 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 9 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 10 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 11 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 12 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 13 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 14 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 15 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 16 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of at least 17 bases. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are of oligonucleotides having a common base sequence of

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

[00353] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations comprise oligonucleotides containing one or more residues which are modified at the sugar moiety. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations comprise oligonucleotides containing one or more residues which are modified at the 2' position of the sugar moiety (referred to herein as a "2'-modification"). Examples of such modifications are described above and herein and include, but are not limited to, 2'-OMe, 2'-MOE, 2'-LNA, 2'-F, FRNA, FANA, *S*-cEt, *etc*. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations comprise oligonucleotides containing one or more residues which are 2'-modified. For example, in some embodiments, provided oligonucleotides contain one or more residues which are 2'-O-methoxyethyl (2'-MOE)-modified residues. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations comprise oligonucleotides which do not contain any 2'-modifications. In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are oligonucleotides which do not contain any 2'-MOE residues. That is, in some embodiments, provided oligonucleotides are not MOE-modified. Additional example

sugar modifications are described in the present disclosure.

[00354] In some embodiments, provided oligonucleotides are of a general motif of wing-core or core-wing (hemimer, also represented herein generally as X-Y or Y-X, respectively). In some embodiments, provided oligonucleotides are of a general motif of wing-core-wing (gapmer, also represented herein generically as X-Y-X). In some embodiments, each wing independently contains one or more residues having a particular modification, which modification is absent from the core "Y" portion. In some embodiments, each wing independently contains one or more residues having a particular nucleoside modification, which modification is absent from the core "Y" portion. In some embodiments, each wing independently contains one or more residues having a particular base modification, which modification is absent from the core "Y" portion. In some embodiments, each wing independently contains one or more residues having a particular sugar modification, which modification is absent from the core "Y" portion. Example sugar modifications are widely known in the art. In some embodiments, a sugar modification is a modification selected from those modifications described in US9006198, which sugar modifications are incorporated herein by references. Additional example sugar modifications are described in the present disclosure. In some embodiment, each wing contains one or more residues having a 2' modification that is not present in the core portion. In some embodiments, a 2'-modification is 2'-OR^1, wherein R^1 is as defined and described in the present disclosure.

[00355] In some embodiments, provided oligonucleotides have a wing-core motif represented as X-Y, or a core-wing motif represented as Y-X, wherein the residues at the "X" portion are sugar modified residues of a particular type and the residues in the core "Y" portion are not sugar modified residues of the same particular type. In some embodiments, provided oligonucleotides have a wing-core-wing motif represented as X-Y-X, wherein the residues at each "X" portion are sugar modified residues of a particular type and the residues in the core "Y" portion are not sugar modified residues of the same particular type. In some embodiments, provided oligonucleotides have a wing-core motif represented as X-Y, or a core-wing motif represented as Y-X, wherein the residues at the "X" portion are 2'-modified residues of a particular type and the residues in the core "Y" portion are not 2'-modified residues of the same particular type. In some embodiments, provided oligonucleotides have a wing-core motif represented as X-Y, wherein the residues at the "X" portion are 2'-modified residues of a

particular type and the residues in the core "Y" portion are not 2'-modified residues of the same particular type. In some embodiments, provided oligonucleotides have a core-wing motif represented as Y-X, wherein the residues at the "X" portion are 2'-modified residues of a particular type and the residues in the core "Y" portion are not 2'-modified residues of the same particular type. In some embodiments, provided oligonucleotides have a wing-core-wing motif represented as X-Y-X, wherein the residues at each "X" portion are 2'-modified residues of a particular type and the residues in the core "Y" portion are not 2'-modified residues of the same particular type. In some embodiments, provided oligonucleotides have a wing-core motif represented as X-Y, wherein the residues at the "X" portion are 2'-modified residues of a particular type and the residues in the core "Y" portion are 2'-deoxyribonucleoside. In some embodiments, provided oligonucleotides have a core-wing motif represented as Y-X, wherein the residues at the "X" portion are 2'-modified residues of a particular type and the residues in the core "Y" portion are 2'-deoxyribonucleoside. In some embodiments, provided oligonucleotides have a wing-core-wing motif represented as X-Y-X, wherein the residues at each "X" portion are 2'-modified residues of a particular type and the residues in the core "Y" portion are 2'-deoxyribonucleoside. In some embodiments, provided oligonucleotides have a wing-core-wing motif represented as X-Y-X, wherein the residues at each "X" portion are 2'-modified residues of a particular type and the residues in the core "Y" portion are 2'-deoxyribonucleoside. For instance, in some embodiments, provided oligonucleotides have a wing-core-wing motif represented as X-Y-X, wherein the residues at each "X" portion are 2'-MOE-modified residues and the residues in the core "Y" portion are not 2'-MOE-modified residues. In some embodiments, provided oligonucleotides have a wing-core-wing motif represented as X-Y-X, wherein the residues at each "X" portion are 2'-MOE-modified residues and the residues in the core "Y" portion are 2'-deoxyribonucleoside. One of skill in the relevant arts will recognize that all such 2'-modifications described above and herein are contemplated in the context of such X-Y, Y-X and/or X-Y-X motifs.

[00356] In some embodiments, a wing has a length of one or more bases. In some embodiments, a wing has a length of two or more bases. In some embodiments, a wing has a length of three or more bases. In some embodiments, a wing has a length of four or more bases. In some embodiments, a wing has a length of five or more bases. In some embodiments, a wing has a length of six or more bases. In some embodiments, a wing has a length of seven or more

bases. In some embodiments, a wing has a length of eight or more bases. In some embodiments, a wing has a length of nine or more bases. In some embodiments, a wing has a length of ten or more bases. In some embodiments, a wing has a length of 11 or more bases. In some embodiments, a wing has a length of 12 or more bases. In some embodiments, a wing has a length of 13 or more bases. In some embodiments, a wing has a length of 14 or more bases. In some embodiments, a wing has a length of 15 or more bases. In some embodiments, a wing has a length of 16 or more bases. In some embodiments, a wing has a length of 17 or more bases. In some embodiments, a wing has a length of 18 or more bases. In some embodiments, a wing has a length of 19 or more bases. In some embodiments, a wing has a length of ten or more bases.

[00357] In some embodiments, a wing has a length of one base. In some embodiments, a wing has a length of two bases. In some embodiments, a wing has a length of three bases. In some embodiments, a wing has a length of four bases. In some embodiments, a wing has a length of five bases. In some embodiments, a wing has a length of six bases. In some embodiments, a wing has a length of seven bases. In some embodiments, a wing has a length of eight bases. In some embodiments, a wing has a length of nine bases. In some embodiments, a wing has a length of ten bases. In some embodiments, a wing has a length of 11 bases. In some embodiments, a wing has a length of 12 bases. In some embodiments, a wing has a length of 13 bases. In some embodiments, a wing has a length of 14 bases. In some embodiments, a wing has a length of 15 bases. In some embodiments, a wing has a length of 16 bases. In some embodiments, a wing has a length of 17 bases. In some embodiments, a wing has a length of 18 bases. In some embodiments, a wing has a length of 19 bases. In some embodiments, a wing has a length of ten bases.

[00358] In some embodiments, a wing comprises one or more chiral internucleotidic linkages. In some embodiments, a wing comprises one or more natural phosphate linkages. In some embodiments, a wing comprises one or more chiral internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, a wing comprises one or more chiral internucleotidic linkages and two or more natural phosphate linkages. In some embodiments, a wing comprises one or more chiral internucleotidic linkages and two or more natural phosphate linkages, wherein two or more natural phosphate linkages are consecutive. In some embodiments, a wing comprises no chiral internucleotidic linkages. In some embodiments, each wing linkage is a natural phosphate linkage. In some embodiments, a wing comprises no

phosphate linkages. In some embodiments, each wing is independently a chiral internucleotidic linkage.

[00359] In some embodiments, each wing independently comprises one or more chiral internucleotidic linkages. In some embodiments, each wing independently comprises one or more natural phosphate linkages. In some embodiments, each wing independently comprises one or more chiral internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, each wing independently comprises one or more chiral internucleotidic linkages and two or more natural phosphate linkages. In some embodiments, each wing independently comprises one or more chiral internucleotidic linkages and two or more natural phosphate linkages, wherein two or more natural phosphate linkages are consecutive.

[00360] In some embodiments, each wing independently comprises at least one chiral internucleotidic linkage. In some embodiments, each wing independently comprises at least two chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least three chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least four chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least five chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least six chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least seven chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least eight chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least nine chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least ten chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 11 chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 12 chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 13 chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 14 chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 15 chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 16 chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 17 chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 18 chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 19

chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 20 chiral internucleotidic linkages.

[00361] In some embodiments, each wing independently comprises one chiral internucleotidic linkage. In some embodiments, each wing independently comprises two chiral internucleotidic linkages. In some embodiments, each wing independently comprises three chiral internucleotidic linkages. In some embodiments, each wing independently comprises four chiral internucleotidic linkages. In some embodiments, each wing independently comprises five chiral internucleotidic linkages. In some embodiments, each wing independently comprises six chiral internucleotidic linkages. In some embodiments, each wing independently comprises seven chiral internucleotidic linkages. In some embodiments, each wing independently comprises eight chiral internucleotidic linkages. In some embodiments, each wing independently comprises nine chiral internucleotidic linkages. In some embodiments, each wing independently comprises ten chiral internucleotidic linkages. In some embodiments, each wing independently comprises 11 chiral internucleotidic linkages. In some embodiments, each wing independently comprises 12 chiral internucleotidic linkages. In some embodiments, each wing independently comprises 13 chiral internucleotidic linkages. In some embodiments, each wing independently comprises 14 chiral internucleotidic linkages. In some embodiments, each wing independently comprises 15 chiral internucleotidic linkages. In some embodiments, each wing independently comprises 16 chiral internucleotidic linkages. In some embodiments, each wing independently comprises 17 chiral internucleotidic linkages. In some embodiments, each wing independently comprises 18 chiral internucleotidic linkages. In some embodiments, each wing independently comprises 19 chiral internucleotidic linkages. In some embodiments, each wing independently comprises 20 chiral internucleotidic linkages.

[00362] In some embodiments, each wing independently comprises at least one consecutive natural phosphate linkage. In some embodiments, each wing independently comprises at least two consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least three consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least four consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least five consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least six consecutive chiral internucleotidic linkages. In some

embodiments, each wing independently comprises at least seven consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least eight consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least nine consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least ten consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 11 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 12 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 13 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 14 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 15 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 16 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 17 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 18 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 19 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises at least 20 consecutive chiral internucleotidic linkages.

[00363] In some embodiments, each wing independently comprises one consecutive natural phosphate linkage. In some embodiments, each wing independently comprises two consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises three consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises four consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises five consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises six consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises seven consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises eight consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises nine consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises ten consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises 11 consecutive chiral

internucleotidic linkages. In some embodiments, each wing independently comprises 12 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises 13 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises 14 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises 15 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises 16 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises 17 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises 18 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises 19 consecutive chiral internucleotidic linkages. In some embodiments, each wing independently comprises 20 consecutive chiral internucleotidic linkages.

[00364] In some embodiments, each wing independently comprises at least one natural phosphate linkage. In some embodiments, each wing independently comprises at least two natural phosphate linkages. In some embodiments, each wing independently comprises at least three natural phosphate linkages. In some embodiments, each wing independently comprises at least four natural phosphate linkages. In some embodiments, each wing independently comprises at least five natural phosphate linkages. In some embodiments, each wing independently comprises at least six natural phosphate linkages. In some embodiments, each wing independently comprises at least seven natural phosphate linkages. In some embodiments, each wing independently comprises at least eight natural phosphate linkages. In some embodiments, each wing independently comprises at least nine natural phosphate linkages. In some embodiments, each wing independently comprises at least ten natural phosphate linkages. In some embodiments, each wing independently comprises at least 11 natural phosphate linkages. In some embodiments, each wing independently comprises at least 12 natural phosphate linkages. In some embodiments, each wing independently comprises at least 13 natural phosphate linkages. In some embodiments, each wing independently comprises at least 14 natural phosphate linkages. In some embodiments, each wing independently comprises at least 15 natural phosphate linkages. In some embodiments, each wing independently comprises at least 16 natural phosphate linkages. In some embodiments, each wing independently comprises at least 17 natural phosphate linkages. In some embodiments, each wing independently comprises at least 18 natural phosphate linkages. In some embodiments, each

wing independently comprises at least 19 natural phosphate linkages. In some embodiments, each wing independently comprises at least 20 natural phosphate linkages.

[00365] In some embodiments, each wing independently comprises one natural phosphate linkage. In some embodiments, each wing independently comprises two natural phosphate linkages. In some embodiments, each wing independently comprises three natural phosphate linkages. In some embodiments, each wing independently comprises four natural phosphate linkages. In some embodiments, each wing independently comprises five natural phosphate linkages. In some embodiments, each wing independently comprises six natural phosphate linkages. In some embodiments, each wing independently comprises seven natural phosphate linkages. In some embodiments, each wing independently comprises eight natural phosphate linkages. In some embodiments, each wing independently comprises nine natural phosphate linkages. In some embodiments, each wing independently comprises ten natural phosphate linkages. In some embodiments, each wing independently comprises 11 natural phosphate linkages. In some embodiments, each wing independently comprises 12 natural phosphate linkages. In some embodiments, each wing independently comprises 13 natural phosphate linkages. In some embodiments, each wing independently comprises 14 natural phosphate linkages. In some embodiments, each wing independently comprises 15 natural phosphate linkages. In some embodiments, each wing independently comprises 16 natural phosphate linkages. In some embodiments, each wing independently comprises 17 natural phosphate linkages. In some embodiments, each wing independently comprises 18 natural phosphate linkages. In some embodiments, each wing independently comprises 19 natural phosphate linkages. In some embodiments, each wing independently comprises 20 natural phosphate linkages.

[00366] In some embodiments, each wing independently comprises at least one consecutive natural phosphate linkage. In some embodiments, each wing independently comprises at least two consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least three consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least four consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least five consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least six consecutive natural phosphate linkages. In some embodiments, each wing independently

comprises at least seven consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least eight consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least nine consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least ten consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least 11 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least 12 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least 13 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least 14 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least 15 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least 16 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least 17 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least 18 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least 19 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises at least 20 consecutive natural phosphate linkages.

[00367] In some embodiments, each wing independently comprises one consecutive natural phosphate linkage. In some embodiments, each wing independently comprises two consecutive natural phosphate linkages. In some embodiments, each wing independently comprises three consecutive natural phosphate linkages. In some embodiments, each wing independently comprises four consecutive natural phosphate linkages. In some embodiments, each wing independently comprises five consecutive natural phosphate linkages. In some embodiments, each wing independently comprises six consecutive natural phosphate linkages. In some embodiments, each wing independently comprises seven consecutive natural phosphate linkages. In some embodiments, each wing independently comprises eight consecutive natural phosphate linkages. In some embodiments, each wing independently comprises nine consecutive natural phosphate linkages. In some embodiments, each wing independently comprises ten consecutive natural phosphate linkages. In some embodiments, each wing independently comprises 11 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises 12 consecutive natural phosphate linkages. In some embodiments,

each wing independently comprises 13 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises 14 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises 15 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises 16 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises 17 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises 18 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises 19 consecutive natural phosphate linkages. In some embodiments, each wing independently comprises 20 consecutive natural phosphate linkages.

[00368] In some embodiments, a wing comprises only one chiral internucleotidic linkage. In some embodiments, a 5'-end wing comprises only one chiral internucleotidic linkage. In some embodiments, a 5'-end wing comprises only one chiral internucleotidic linkage at the 5'-end of the wing. In some embodiments, a 5'-end wing comprises only one chiral internucleotidic linkage at the 5'-end of the wing, and the chiral internucleotidic linkage is *R*p. In some embodiments, a 5'-end wing comprises only one chiral internucleotidic linkage at the 5'-end of the wing, and the chiral internucleotidic linkage is *S*p. In some embodiments, a 3'-end wing comprises only one chiral internucleotidic linkage at the 3'-end of the wing. In some embodiments, a 3'-end wing comprises only one chiral internucleotidic linkage at the 3'-end of the wing, and the chiral internucleotidic linkage is *R*p. In some embodiments, a 3'-end wing comprises only one chiral internucleotidic linkage at the 3'-end of the wing, and the chiral internucleotidic linkage is *S*p.

[00369] In some embodiments, a wing comprises two or more natural phosphate linkages. In some embodiments, all phosphate linkages within a wing are consecutive, and there are no non-phosphate linkages between any two phosphate linkages within a wing.

[00370] In some embodiments, a linkage connecting a wing and a core is considered part of the core when describing linkages, *e.g.*, linkage chemistry, linkage stereochemistry, *etc*. For example, in WV-1092, mG*SmGmCmAmC**<u>*S</u>A*SA*SG*SG*SG*SC*S A*SC*RA*SG<u>*S</u>**mAmCmUmU*SmC, the underlined linkages may be considered as part of the core (bold), its 5'-wing (having 2'-OMe on sugar moieties) has one single *S*p phosphorothioate linkages at its 5'- end, its 3'-wing (having 2'-OMe on sugar moieties) has one *S*p phosphorothioate linkage at its 3'-end, and its core has no 2'-modifications on sugar).

[00371] In some embodiments, a 5'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is a modified linkage. In some embodiments, a 5'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is a linkage having the structure of formula **I**. In some embodiments, a 5'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is phosphorothioate linkage. In some embodiments, a 5'-internucleotidic linkage connected to a sugar moiety without a 2'-modification is a substituted phosphorothioate linkage. In some embodiments, a 5'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is a phosphorothioate triester linkage. In some embodiments, each 5'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is a modified linkage. In some embodiments, each 5'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is a linkage having the structure of formula **I**. In some embodiments, each 5'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is phosphorothioate linkage. In some embodiments, each 5'-internucleotidic linkage connected to a sugar moiety without a 2'-modification is a substituted phosphorothioate linkage. In some embodiments, each 5'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is a phosphorothioate triester linkage.

[00372] In some embodiments, a 3'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is a modified linkage. In some embodiments, a 3'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is a linkage having the structure of formula **I**. In some embodiments, a 3'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is phosphorothioate linkage. In some embodiments, a 3'-internucleotidic linkage connected to a sugar moiety without a 2'-modification is a substituted phosphorothioate linkage. In some embodiments, a 3'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is a phosphorothioate triester linkage. In some embodiments, each 3'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is a modified linkage. In some embodiments, each 3'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is a linkage having the structure of formula **I**. In some embodiments, each 3'- internucleotidic linkage connected to a sugar moiety without a 2'-modification is phosphorothioate linkage. In some embodiments, each 3'-internucleotidic linkage connected to a sugar moiety without a 2'-modification is a substituted phosphorothioate linkage. In some embodiments, each 3'- internucleotidic linkage connected to

a sugar moiety without a 2'-modification is a phosphorothioate triester linkage.

[00373] In some embodiments, both internucleotidic linkages connected to a sugar moiety without a 2'-modification are modified linkages. In some embodiments, both internucleotidic linkages connected to a sugar moiety without a 2'-modification are linkage having the structure of formula **I**. In some embodiments, both internucleotidic linkages connected to a sugar moiety without a 2'-modification are phosphorothioate linkages. In some embodiments, both internucleotidic linkages connected to a sugar moiety without a 2'-modification are substituted phosphorothioate linkages. In some embodiments, both internucleotidic linkages connected to a sugar moiety without a 2'-modification are phosphorothioate triester linkages. In some embodiments, each internucleotidic linkage connected to a sugar moiety without a 2'-modification is a modified linkage. In some embodiments, each internucleotidic linkage connected to a sugar moiety without a 2'-modification is a linkage having the structure of formula **I**. In some embodiments, each internucleotidic linkage connected to a sugar moiety without a 2'-modification is phosphorothioate linkage. In some embodiments, each internucleotidic linkage connected to a sugar moiety without a 2'-modification is a substituted phosphorothioate linkage. In some embodiments, each internucleotidic linkage connected to a sugar moiety without a 2'-modification is a phosphorothioate triester linkage.

[00374] In some embodiments, a sugar moiety without a 2'-modification is a sugar moiety found in a natural DNA nucleoside.

[00375] In some embodiments, for a wing-core-wing structure, the 5'-end wing comprises only one chiral internucleotidic linkage. In some embodiments, for a wing-core-wing structure, the 5'-end wing comprises only one chiral internucleotidic linkage at the 5'-end of the wing. In some embodiments, for a wing-core-wing structure, the 3'-end wing comprises only one chiral internucleotidic linkage. In some embodiments, for a wing-core-wing structure, the 3'-end wing comprises only one chiral internucleotidic linkage at the 3'-end of the wing. In some embodiments, for a wing-core-wing structure, each wing comprises only one chiral internucleotidic linkage. In some embodiments, for a wing-core-wing structure, each wing comprises only one chiral internucleotidic linkage, wherein the 5'-end wing comprises only one chiral internucleotidic linkage at its 5'-end; and the 3'-end wing comprises only one chiral internucleotidic linkage at its 3'-end. In some embodiments, the only chiral internucleotidic linkage in the 5'-wing is *R*p. In some embodiments, the only chiral internucleotidic linkage in

the 5'-wing is *S*p. In some embodiments, the only chiral internucleotidic linkage in the 3'-wing is *R*p. In some embodiments, the only chiral internucleotidic linkage in the 3'-wing is *S*p. In some embodiments, the only chiral internucleotidic linkage in both the 5'- and the 3'-wings are *S*p. In some embodiments, the only chiral internucleotidic linkage in both the 5'- and the 3'-wings are *R*p. In some embodiments, the only chiral internucleotidic linkage in the 5'-wing is *S*p, and the only chiral internucleotidic linkage in the 3'-wing is *R*p. In some embodiments, the only chiral internucleotidic linkage in the 5'-wing is *R*p, and the only chiral internucleotidic linkage in the 3'-wing is *S*p.

[00376] In some embodiments, a wing comprises two chiral internucleotidic linkages. In some embodiments, a wing comprises only two chiral internucleotidic linkages, and one or more natural phosphate linkages. In some embodiments, a wing comprises only two chiral internucleotidic linkages, and two or more natural phosphate linkages. In some embodiments, a wing comprises only two chiral internucleotidic linkages, and two or more consecutive natural phosphate linkages. In some embodiments, a wing comprises only two chiral internucleotidic linkages, and two consecutive natural phosphate linkages. In some embodiments, a wing comprises only two chiral internucleotidic linkages, and three consecutive natural phosphate linkages. In some embodiments, a 5'-wing (to a core) comprises only two chiral internucleotidic linkages, one at its 5'-end and the other at its 3'-end, with one or more natural phosphate linkages in between. In some embodiments, a 5'-wing (to a core) comprises only two chiral internucleotidic linkages, one at its 5'-end and the other at its 3'-end, with two or more natural phosphate linkages in between. In some embodiments, a 3'-wing (to a core) comprises only two chiral internucleotidic linkages, one at its 3'-end and the other at its 3'-end, with one or more natural phosphate linkages in between. In some embodiments, a 3'-wing (to a core) comprises only two chiral internucleotidic linkages, one at its 3'-end and the other at its 3'-end, with two or more natural phosphate linkages in between.

[00377] In some embodiments, a 5'-wing comprises only two chiral internucleotidic linkages, one at its 5'-end and the other at its 3'-end, with one or more natural phosphate linkages in between, and the 3'-wing comprise only one internucleotidic linkage at its 3'-end. In some embodiments, a 5'-wing (to a core) comprises only two chiral internucleotidic linkages, one at its 5'-end and the other at its 3'-end, with two or more natural phosphate linkages in between, and the 3'-wing comprise only one internucleotidic linkage at its 3'-end. In some

embodiments, each chiral internucleotidic linkage independently has its own stereochemistry. In some embodiments, both chiral internucleotidic linkages in the 5'-wing have the same stereochemistry. In some embodiments, both chiral internucleotidic linkages in the 5'-wing have different stereochemistry. In some embodiments, both chiral internucleotidic linkages in the 5'-wing are *R*p. In some embodiments, both chiral internucleotidic linkages in the 5'-wing are *S*p. In some embodiments, chiral internucleotidic linkages in the 5'- and 3'-wings have the same stereochemistry. In some embodiments, chiral internucleotidic linkages in the 5'- and 3'-wings are *R*p. In some embodiments, chiral internucleotidic linkages in the 5'- and 3'-wings are *S*p. In some embodiments, chiral internucleotidic linkages in the 5'- and 3'-wings have different stereochemistry.

[00378] In some embodiments, a core region has a length of one or more bases. In some embodiments, a core region has a length of two or more bases. In some embodiments, a core region has a length of three or more bases. In some embodiments, a core region has a length of four or more bases. In some embodiments, a core region has a length of five or more bases. In some embodiments, a core region has a length of six or more bases. In some embodiments, a core region has a length of seven or more bases. In some embodiments, a core region has a length of eight or more bases. In some embodiments, a core region has a length of nine or more bases. In some embodiments, a core region has a length of ten or more bases. In some embodiments, a core region has a length of 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or more bases. In certain embodiments, a core region has a length of 11 or more bases. In certain embodiments, a core region has a length of 12 or more bases. In certain embodiments, a core region has a length of 13 or more bases. In certain embodiments, a core region has a length of 14 or more bases. In certain embodiments, a core region has a length of 15 or more bases. In certain embodiments, a core region has a length of 16 or more bases. In certain embodiments, a core region has a length of 17 or more bases. In certain embodiments, a core region has a length of 18 or more bases. In certain embodiments, a core region has a length of 19 or more bases. In certain embodiments, a core region has a length of 20 or more bases. In certain embodiments, a core region has a length of more than 20 bases. In certain embodiments, a core region has a length of 2 bases. In certain embodiments, a core region has a length of 3 bases. In certain embodiments, a core region has a length of 4 bases. In certain embodiments, a core region has a length of 5 bases. In certain embodiments, a core region has a length of 6 bases. In certain

embodiments, a core region has a length of 7 bases. In certain embodiments, a core region has a length of 8 bases. In certain embodiments, a core region has a length of 9 bases. In certain embodiments, a core region has a length of 10 bases. In certain embodiments, a core region has a length of 11 bases. In certain embodiments, a core region has a length of 12 bases. In certain embodiments, a core region has a length of 13 bases. In certain embodiments, a core region has a length of 14 bases. In certain embodiments, a core region has a length of 15 bases. In certain embodiments, a core region has a length of 16 bases. In certain embodiments, a core region has a length of 17 bases. In certain embodiments, a core region has a length of 18 bases. In certain embodiments, a core region has a length of 19 bases. In certain embodiments, a core region has a length of 20 bases.

[00379] In some embodiments, a core comprises one or more chiral internucleotidic linkages. In some embodiments, a core comprises one or more natural phosphate linkages. In some embodiments, a core independently comprises one or more chiral internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, a core comprises no phosphate linkages. In some embodiments, each core linkage is a chiral internucleotidic linkage.

[00380] In some embodiments, a core comprises at least one natural phosphate linkage. In some embodiments, a core comprises at least two chiral internucleotidic linkages. In some embodiments, a core comprises at least three chiral internucleotidic linkages. In some embodiments, a core comprises at least four chiral internucleotidic linkages. In some embodiments, a core comprises at least five chiral internucleotidic linkages. In some embodiments, a core comprises at least six chiral internucleotidic linkages. In some embodiments, a core comprises at least seven chiral internucleotidic linkages. In some embodiments, a core comprises at least eight chiral internucleotidic linkages. In some embodiments, a core comprises at least nine chiral internucleotidic linkages. In some embodiments, a core comprises at least ten chiral internucleotidic linkages. In some embodiments, a core comprises at least 11 chiral internucleotidic linkages. In some embodiments, a core comprises at least 12 chiral internucleotidic linkages. In some embodiments, a core comprises at least 13 chiral internucleotidic linkages. In some embodiments, a core comprises at least 14 chiral internucleotidic linkages. In some embodiments, a core comprises at least 15 chiral internucleotidic linkages. In some embodiments, a core comprises at least 16 chiral internucleotidic linkages. In some

embodiments, a core comprises at least 17 chiral internucleotidic linkages. In some embodiments, a core comprises at least 18 chiral internucleotidic linkages. In some embodiments, a core comprises at least 19 chiral internucleotidic linkages. In some embodiments, a core comprises at least 20 chiral internucleotidic linkages.

[00381] In some embodiments, a core comprises one natural phosphate linkage. In some embodiments, a core comprises two chiral internucleotidic linkages. In some embodiments, a core comprises three chiral internucleotidic linkages. In some embodiments, a core comprises four chiral internucleotidic linkages. In some embodiments, a core comprises five chiral internucleotidic linkages. In some embodiments, a core comprises six chiral internucleotidic linkages. In some embodiments, a core comprises seven chiral internucleotidic linkages. In some embodiments, a core comprises eight chiral internucleotidic linkages. In some embodiments, a core comprises nine chiral internucleotidic linkages. In some embodiments, a core comprises ten chiral internucleotidic linkages. In some embodiments, a core comprises 11 chiral internucleotidic linkages. In some embodiments, a core comprises 12 chiral internucleotidic linkages. In some embodiments, a core comprises 13 chiral internucleotidic linkages. In some embodiments, a core comprises 14 chiral internucleotidic linkages. In some embodiments, a core comprises 15 chiral internucleotidic linkages. In some embodiments, a core comprises 16 chiral internucleotidic linkages. In some embodiments, a core comprises 17 chiral internucleotidic linkages. In some embodiments, a core comprises 18 chiral internucleotidic linkages. In some embodiments, a core comprises 19 chiral internucleotidic linkages. In some embodiments, a core comprises 20 chiral internucleotidic linkages.

[00382] In some embodiments, a core region has a pattern of backbone chiral centers comprising $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein each of m, n, t and *N*p is independently as defined and described in the present disclosure. In some embodiments, a core region has a pattern of backbone chiral centers comprising $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$. In some embodiments, a core region has a pattern of backbone chiral centers comprising $(Sp)_m(Rp)_n$. In some embodiments, a core region has a pattern of backbone chiral centers comprising $(Sp)_m(Rp)_n$, wherein $m > 2$ and n is 1. In some embodiments, a core region has a pattern of backbone chiral centers comprising $(Rp)_n(Sp)_m$. In some embodiments, a core region has a pattern of backbone chiral centers comprising $(Rp)_n(Sp)_m$, wherein $m > 2$ and n is 1. In some embodiments, a core region has a

pattern of backbone chiral centers comprising $(Np)_t(Rp)_n(Sp)_m$. In some embodiments, a core region has a pattern of backbone chiral centers comprising $(Np)_t(Rp)_n(Sp)_m$, wherein m > 2 and n is 1. In some embodiments, a core region has a pattern of backbone chiral centers comprising $(Np)_t(Rp)_n(Sp)_m$, wherein t > 2, m > 2 and n is 1. In some embodiments, a core region has a pattern of backbone chiral centers comprising $(Sp)_t(Rp)_n(Sp)_m$. In some embodiments, a core region has a pattern of backbone chiral centers comprising $(Sp)_t(Rp)_n(Sp)_m$, wherein m > 2 and n is 1. In some embodiments, a core region has a pattern of backbone chiral centers comprising $(Sp)_t(Rp)_n(Sp)_m$, wherein t > 2, m > 2 and n is 1. Among other things, the present disclosure demonstrates that, in some embodiments, such patterns can provide and/or enhance controlled cleavage, improved cleavage rate, selectivity, *etc.*, of a target sequence, *e.g.,* an RNA sequence. Example patterns of backbone chiral centers are described in the present disclosure.

[00383] In some embodiments, at least 60% of the chiral internucleotidic linkages in the core region are *S*p. In some embodiments, at least 65% of the chiral internucleotidic linkages in the core region are *S*p. In some embodiments, at least 66% of the chiral internucleotidic linkages in the core region are *S*p. In some embodiments, at least 67% of the chiral internucleotidic linkages in the core region are *S*p. In some embodiments, at least 70% of the chiral internucleotidic linkages in the core region are *S*p. In some embodiments, at least 75% of the chiral internucleotidic linkages in the core region are *S*p. In some embodiments, at least 80% of the chiral internucleotidic linkages in the core region are *S*p. In some embodiments, at least 85% of the chiral internucleotidic linkages in the core region are *S*p. In some embodiments, at least 90% of the chiral internucleotidic linkages in the core region are *S*p. In some embodiments, at least 95% of the chiral internucleotidic linkages in the core region are *S*p.

[00384] In some embodiments, a wing-core-wing (i.e., X-Y-X) motif is represented numerically as, *e.g.,* 5-10-4, meaning the wing to the 5'-end of the core is 5 bases in length, the core region is 10 bases in length, and the wing region to the 3'-end of the core is 4-bases in length. In some embodiments, a wing-core-wing motif is any of, e.g. 2-16-2, 3-14-3, 4-12-4, 5-10-5, 2-9-6, 3-9-3, 3-9-4, 3-9-5, 4-7-4, 4-9-3, 4-9-4, 4-9-5, 4-10-5, 4-11-4, 4-11-5, 5-7-5, 5-8-6, 8-7-5, 7-7-6, 5-9-3, 5-9-5, 5-10-4, 5-10-5, 6-7-6, 6-8-5, and 6-9-2, *etc*. In certain embodiments, a wing-core-wing motif is 5-10-5. In certain embodiments, a wing-core-wing motif is 7-7-6. In certain embodiments, a wing-core-wing motif is 8-7-5.

[00385] In some embodiments, a wing-core motif is 5-15, 6-14, 7-13, 8-12, 9-12, *etc*. In

some embodiments, a core-wing motif is 5-15, 6-14, 7-13, 8-12, 9-12, *etc.*

[00386] In some embodiments, the internucleosidic linkages of provided oligonucleotides of such wing-core-wing (i.e., X-Y-X) motifs are all chiral, modified phosphate linkages. In some embodiments, the internucleosidic linkages of provided oligonucleotides of such wing-core-wing (i.e., X-Y-X) motifs are all chiral phosphorothioate internucleotidic linkages. In some embodiments, chiral internucleotidic linkages of provided oligonucleotides of such wing-core-wing motifs are at least about 10, 20, 30, 40, 50, 50, 70, 80, or 90% chiral, modified phosphate internucleotidic linkages. In some embodiments, chiral internucleotidic linkages of provided oligonucleotides of such wing-core-wing motifs are at least about 10, 20, 30, 40, 50, 60, 70, 80, or 90% chiral phosphorothioate internucleotidic linkages. In some embodiments, chiral internucleotidic linkages of provided oligonucleotides of such wing-core-wing motifs are at least about 10, 20, 30, 40, 50, 50, 70, 80, or 90% chiral phosphorothioate internucleotidic linkages of the *S*p conformation.

[00387] In some embodiments, each wing region of a wing-core-wing motif optionally contains chiral, modified phosphate internucleotidic linkages. In some embodiments, each wing region of a wing-core-wing motif optionally contains chiral phosphorothioate internucleotidic linkages. In some embodiments, each wing region of a wing-core-wing motif contains chiral phosphorothioate internucleotidic linkages. In some embodiments, the two wing regions of a wing-core-wing motif have the same internucleotidic linkage stereochemistry. In some embodiments, the two wing regions have different internucleotidic linkage stereochemistry. In some embodiments, each internucleotidic linkage in the wings is independently a chiral internucleotidic linkage.

[00388] In some embodiments, the core region of a wing-core-wing motif optionally contains chiral, modified phosphate internucleotidic linkages. In some embodiments, the core region of a wing-core-wing motif optionally contains chiral phosphorothioate internucleotidic linkages. In some embodiments, the core region of a wing-core-wing motif comprises a repeating pattern of internucleotidic linkage stereochemistry. In some embodiments, the core region of a wing-core-wing motif has a repeating pattern of internucleotidic linkage stereochemistry. In some embodiments, the core region of a wing-core-wing motif comprises repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is $(Sp)_mRp$ or $Rp(Sp)_m$, wherein m is 1-50. In some embodiments, the core region of a wing-core-wing motif comprises

repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is $(Sp)_mRp$ or $Rp(Sp)_m$, wherein m is 1-50. In some embodiments, the core region of a wing-core-wing motif comprises repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is $(Sp)_mRp$, wherein m is 1-50. In some embodiments, the core region of a wing-core-wing motif comprises repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is $Rp(Sp)_m$, wherein m is 1-50. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is $(Sp)_mRp$ or $Rp(Sp)_m$, wherein m is 1-50. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is $(Sp)_mRp$, wherein m is 1-50. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is $Rp(Sp)_m$, wherein m is 1-50. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a motif comprising at least 33% of internucleotidic linkage in the S conformation. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a motif comprising at least 50% of internucleotidic linkage in the S conformation. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a motif comprising at least 66% of internucleotidic linkage in the S conformation. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a repeating triplet motif selected from *Rp*Rp*Sp* and *Sp*Sp*Rp*. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a repeating *Rp*Rp*Sp*. In some embodiments, the core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a repeating *Sp*Sp*Rp*.

[00389] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises $(Sp)_mRp$ or $Rp(Sp)_m$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose

pattern of backbone chiral centers in the core region comprises *Rp*(*Sp*)$_m$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*Sp*)$_m$*Rp*. In some embodiments, m is 2. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises *Rp*(*Sp*)$_2$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*Sp*)$_2$*Rp*(*Sp*)$_2$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*Rp*)$_2$*Rp*(*Sp*)$_2$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises *RpSpRp*(*Sp*)$_2$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises *SpRpRp*(*Sp*)$_2$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises (*Sp*)$_2$*Rp*.

[00390] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*Sp*)$_m$*Rp* or *Rp*(*Sp*)$_m$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises *Rp*(*Sp*)$_m$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*Sp*)$_m$*Rp*. In some embodiments, m is 2. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises *Rp*(*Sp*)$_2$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises (*Sp*)$_2$*Rp*(*Sp*)$_2$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises

$(Rp)_2Rp(Sp)_2$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises $RpSpRp(Sp)_2$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises $SpRpRp(Sp)_2$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises $(Sp)_2Rp$.

[00391] As defined herein, m is 1-50. In some embodiments, m is 1. In some embodiments, m is 2-50. In some embodiments, m is 2, 3, 4, 5, 6, 7 or 8. In some embodiments, m is 3, 4, 5, 6, 7 or 8. In some embodiments, m is 4, 5, 6, 7 or 8. In some embodiments, m is 5, 6, 7 or 8. In some embodiments, m is 6, 7 or 8. In some embodiments, m is 7 or 8. In some embodiments, m is 2. In some embodiments, m is 3. In some embodiments, m is 4. In some embodiments, m is 5. In some embodiments, m is 6. In some embodiments, m is 7. In some embodiments, m is 8. In some embodiments, m is 9. In some embodiments, m is 10. In some embodiments, m is 11. In some embodiments, m is 12. In some embodiments, m is 13. In some embodiments, m is 14. In some embodiments, m is 15. In some embodiments, m is 16. In some embodiments, m is 17. In some embodiments, m is 18. In some embodiments, m is 19. In some embodiments, m is 20. In some embodiments, m is 21. In some embodiments, m is 22. In some embodiments, m is 23. In some embodiments, m is 24. In some embodiments, m is 25. In some embodiments, m is greater than 25.

[00392] In some embodiments, a repeating pattern is $(Sp)_m(Rp)_n$, wherein n is 1-10, and m is independently as defined above and described herein. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises $(Sp)_m(Rp)_n$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises $(Sp)_m(Rp)_n$. In some embodiments, a repeating pattern is $(Rp)_n(Sp)_m$, wherein n is 1-10, and m is independently as defined above and described herein. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises $(Rp)_n(Sp)_m$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an

oligonucleotide type whose pattern of backbone chiral centers in the core region comprises $(Rp)_n(Sp)_m$. In some embodiments, $(Rp)_n(Sp)_m$ is $(Rp)(Sp)_2$. In some embodiments, $(Sp)_n(Rp)_m$ is $(Sp)_2(Rp)$.

[00393] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises $(Sp)_m(Rp)_n(Sp)_t$. In some embodiments, a repeating pattern is $(Sp)_m(Rp)_n(Sp)_t$, wherein n is 1-10, t is 1-50, and m is as defined above and described herein. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises $(Sp)_m(Rp)_n(Sp)_t$. In some embodiments, a repeating pattern is $(Sp)_t(Rp)_n(Sp)_m$, wherein n is 1-10, t is 1-50, and m is as defined above and described herein. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises $(Sp)_t(Rp)_n(Sp)_m$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises $(Sp)_t(Rp)_n(Sp)_m$.

[00394] In some embodiments, a repeating pattern is $(Np)_t(Rp)_n(Sp)_m$, wherein n is 1-10, t is 1-50, Np is independently Rp or Sp, and m is as defined above and described herein. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises $(Np)_t(Rp)_n(Sp)_m$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises $(Np)_t(Rp)_n(Sp)_m$. In some embodiments, a repeating pattern is $(Np)_m(Rp)_n(Sp)_t$, wherein n is 1-10, t is 1-50, Np is independently Rp or Sp, and m is as defined above and described herein. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises $(Np)_m(Rp)_n(Sp)_t$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region comprises $(Np)_m(Rp)_n(Sp)_t$. In some embodiments, Np is Rp. In some embodiments, Np is Sp. In some embodiments, all Np are the same. In some embodiments, all Np are Sp. In some embodiments, at least one Np is different from the other

*N*p. In some embodiments, t is 2.

[00395] As defined herein, n is 1-10. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7 or 8. In some embodiments, n is 1. In some embodiments, n is 2, 3, 4, 5, 6, 7 or 8. In some embodiments, n is 3, 4, 5, 6, 7 or 8. In some embodiments, n is 4, 5, 6, 7 or 8. In some embodiments, n is 5, 6, 7 or 8. In some embodiments, n is 6, 7 or 8. In some embodiments, n is 7 or 8. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5. In some embodiments, n is 6. In some embodiments, n is 7. In some embodiments, n is 8. In some embodiments, n is 9. In some embodiments, n is 10.

[00396] As defined herein, t is 1-50. In some embodiments, t is 1. In some embodiments, t is 2-50. In some embodiments, t is 2, 3, 4, 5, 6, 7 or 8. In some embodiments, t is 3, 4, 5, 6, 7 or 8. In some embodiments, t is 4, 5, 6, 7 or 8. In some embodiments, t is 5, 6, 7 or 8. In some embodiments, t is 6, 7 or 8. In some embodiments, t is 7 or 8. In some embodiments, t is 2. In some embodiments, t is 3. In some embodiments, t is 4. In some embodiments, t is 5. In some embodiments, t is 6. In some embodiments, t is 7. In some embodiments, t is 8. In some embodiments, t is 9. In some embodiments, t is 10. In some embodiments, t is 11. In some embodiments, t is 12. In some embodiments, t is 13. In some embodiments, t is 14. In some embodiments, t is 15. In some embodiments, t is 16. In some embodiments, t is 17. In some embodiments, t is 18. In some embodiments, t is 19. In some embodiments, t is 20. In some embodiments, t is 21. In some embodiments, t is 22. In some embodiments, t is 23. In some embodiments, t is 24. In some embodiments, t is 25. In some embodiments, t is greater than 25.

[00397] In some embodiments, at least one of m and t is greater than 2. In some embodiments, at least one of m and t is greater than 3. In some embodiments, at least one of m and t is greater than 4. In some embodiments, at least one of m and t is greater than 5. In some embodiments, at least one of m and t is greater than 6. In some embodiments, at least one of m and t is greater than 7. In some embodiments, at least one of m and t is greater than 8. In some embodiments, at least one of m and t is greater than 9. In some embodiments, at least one of m and t is greater than 10. In some embodiments, at least one of m and t is greater than 11. In some embodiments, at least one of m and t is greater than 12. In some embodiments, at least one of m and t is greater than 13. In some embodiments, at least one of m and t is greater than 14. In some embodiments, at least one of m and t is greater than 15. In some embodiments, at least one

of m and t is greater than 16. In some embodiments, at least one of m and t is greater than 17. In some embodiments, at least one of m and t is greater than 18. In some embodiments, at least one of m and t is greater than 19. In some embodiments, at least one of m and t is greater than 20. In some embodiments, at least one of m and t is greater than 21. In some embodiments, at least one of m and t is greater than 22. In some embodiments, at least one of m and t is greater than 23. In some embodiments, at least one of m and t is greater than 24. In some embodiments, at least one of m and t is greater than 25.

[00398] In some embodiments, each one of m and t is greater than 2. In some embodiments, each one of m and t is greater than 3. In some embodiments, each one of m and t is greater than 4. In some embodiments, each one of m and t is greater than 5. In some embodiments, each one of m and t is greater than 6. In some embodiments, each one of m and t is greater than 7. In some embodiments, each one of m and t is greater than 8. In some embodiments, each one of m and t is greater than 9. In some embodiments, each one of m and t is greater than 10. In some embodiments, each one of m and t is greater than 11. In some embodiments, each one of m and t is greater than 12. In some embodiments, each one of m and t is greater than 13. In some embodiments, each one of m and t is greater than 14. In some embodiments, each one of m and t is greater than 15. In some embodiments, each one of m and t is greater than 16. In some embodiments, each one of m and t is greater than 17. In some embodiments, each one of m and t is greater than 18. In some embodiments, each one of m and t is greater than 19. In some embodiments, each one of m and t is greater than 20.

[00399] In some embodiments, the sum of m and t is greater than 3. In some embodiments, the sum of m and t is greater than 4. In some embodiments, the sum of m and t is greater than 5. In some embodiments, the sum of m and t is greater than 6. In some embodiments, the sum of m and t is greater than 7. In some embodiments, the sum of m and t is greater than 8. In some embodiments, the sum of m and t is greater than 9. In some embodiments, the sum of m and t is greater than 10. In some embodiments, the sum of m and t is greater than 11. In some embodiments, the sum of m and t is greater than 12. In some embodiments, the sum of m and t is greater than 13. In some embodiments, the sum of m and t is greater than 14. In some embodiments, the sum of m and t is greater than 15. In some embodiments, the sum of m and t is greater than 16. In some embodiments, the sum of m and t is greater than 17. In some embodiments, the sum of m and t is greater than 18. In some

embodiments, the sum of m and t is greater than 19. In some embodiments, the sum of m and t is greater than 20. In some embodiments, the sum of m and t is greater than 21. In some embodiments, the sum of m and t is greater than 22. In some embodiments, the sum of m and t is greater than 23. In some embodiments, the sum of m and t is greater than 24. In some embodiments, the sum of m and t is greater than 25.

[00400] In some embodiments, n is 1, and at least one of m and t is greater than 1. In some embodiments, n is 1 and each of m and t is independently greater than 1. In some embodiments, m>n and t>n. In some embodiments, $(Sp)_m(Rp)_n(Sp)_t$ is $(Sp)_2Rp(Sp)_2$. In some embodiments, $(Sp)_t(Rp)_n(Sp)_m$ is $(Sp)_2Rp(Sp)_2$. In some embodiments, $(Sp)_t(Rp)_n(Sp)_m$ is $SpRp(Sp)_2$. In some embodiments, $(Np)_t(Rp)_n(Sp)_m$ is $(Np)_tRp(Sp)_m$. In some embodiments, $(Np)_t(Rp)_n(Sp)_m$ is $(Np)_2Rp(Sp)_m$. In some embodiments, $(Np)_t(Rp)_n(Sp)_m$ is $(Rp)_2Rp(Sp)_m$. In some embodiments, $(Np)_t(Rp)_n(Sp)_m$ is $(Sp)_2Rp(Sp)_m$. In some embodiments, $(Np)_t(Rp)_n(Sp)_m$ is $RpSpRp(Sp)_m$. In some embodiments, $(Np)_t(Rp)_n(Sp)_m$ is $SpRpRp(Sp)_m$.

[00401] In some embodiments, $(Sp)_t(Rp)_n(Sp)_m$ is $SpRpSpSp$. In some embodiments, $(Sp)_t(Rp)_n(Sp)_m$ is $(Sp)_2Rp(Sp)_2$. In some embodiments, $(Sp)_t(Rp)_n(Sp)_m$ is $(Sp)_3Rp(Sp)_3$. In some embodiments, $(Sp)_t(Rp)_n(Sp)_m$ is $(Sp)_4Rp(Sp)_4$. In some embodiments, $(Sp)_t(Rp)_n(Sp)_m$ is $(Sp)_tRp(Sp)_5$. In some embodiments, $(Sp)_t(Rp)_n(Sp)_m$ is $SpRp(Sp)_5$. In some embodiments, $(Sp)_t(Rp)_n(Sp)_m$ is $(Sp)_2Rp(Sp)_5$. In some embodiments, $(Sp)_t(Rp)_n(Sp)_m$ is $(Sp)_3Rp(Sp)_5$. In some embodiments, $(Sp)_t(Rp)_n(Sp)_m$ is $(Sp)_4Rp(Sp)_5$. In some embodiments, $(Sp)_t(Rp)_n(Sp)_m$ is $(Sp)_5Rp(Sp)_5$.

[00402] In some embodiments, $(Sp)_m(Rp)_n(Sp)_t$ is $(Sp)_2Rp(Sp)_2$. In some embodiments, $(Sp)_m(Rp)_n(Sp)_t$ is $(Sp)_3Rp(Sp)_3$. In some embodiments, $(Sp)_m(Rp)_n(Sp)_t$ is $(Sp)_4Rp(Sp)_4$. In some embodiments, $(Sp)_m(Rp)_n(Sp)_t$ is $(Sp)_mRp(Sp)_5$. In some embodiments, $(Sp)_m(Rp)_n(Sp)_t$ is $(Sp)_2Rp(Sp)_5$. In some embodiments, $(Sp)_m(Rp)_n(Sp)_t$ is $(Sp)_3Rp(Sp)_5$. In some embodiments, $(Sp)_m(Rp)_n(Sp)_t$ is $(Sp)_4Rp(Sp)_5$. In some embodiments, $(Sp)_m(Rp)_n(Sp)_t$ is $(Sp)_5Rp(Sp)_5$.

[00403] In some embodiments, the core region comprises at least one *Rp* internucleotidic linkage. In some embodiments, the core region of a wing-core-wing motif comprises at least one *Rp* internucleotidic linkage. In some embodiments, a core region comprises at least one *Rp* phosphorothioate internucleotidic linkage. In some embodiments, the core region of a wing-core-wing motif comprises at least one *Rp* phosphorothioate internucleotidic linkage. In some embodiments, the core region of a wing-core-wing motif comprises only one *Rp*

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

[00404] In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each wing region are 2'-modified residues. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each wing region are 2'-OR^1-modified residues. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each wing region are 2'-MOE-modified residues. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each wing region are 2'-OMe-modified residues. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues in the core region are 2'-deoxyribonucleoside residues. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif, wherein all internucleotidic linkages are phosphorothioate linkages. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif, wherein all internucleotidic linkages are chiral phosphorothioate linkages. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each wing region are 2'-modified residues, the residues in the core region are 2'-deoxyribonucleoside residues, and all internucleotidic linkages in the core region are chiral phosphorothioate linkages. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each wing region are 2'-OR^1-modified residues, the residues in the core region are 2'-deoxyribonucleoside residues, and all internucleotidic

linkages in the core region are chiral phosphorothioate linkages. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each wing region are 2'-MOE-modified residues, the residues in the core region are 2'-deoxyribonucleoside residues, and all internucleotidic linkages in the core region are chiral phosphorothioate linkages. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each wing region are 2'-OMe-modified residues, the residues in the core region are 2'-deoxyribonucleoside residues, and all internucleotidic linkages in the core region are chiral phosphorothioate linkages.

[00405] In some embodiments, residues at the "X" wing region are not 2'-MOE-modified residues. In certain embodiments, a wing-core motif is a motif wherein the residues at the "X" wing region are not 2'-MOE-modified residues. In certain embodiments, a core-wing motif is a motif wherein the residues at the "X" wing region are not 2'-MOE-modified residues. In certain embodiments, a wing-core-wing motif is a motif wherein the residues at each "X" wing region are not 2'-MOE-modified residues. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each "X" wing region are not 2'-MOE-modified residues. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues in the core "Y" region are 2'-deoxyribonucleoside residues. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif, wherein all internucleotidic linkages are phosphorothioate internucleotidic linkages. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif, wherein all internucleotidic linkages are chiral phosphorothioate internucleotidic linkages. In certain embodiments, a wing-core-wing motif is a 5-10-5 motif wherein the residues at each "X" wing region are not 2'-MOE-modified residues, the residues in the core "Y" region are 2'-deoxyribonucleoside, and all internucleotidic linkages are chiral phosphorothioate internucleotidic linkages.

[00406] As understood by a person having ordinary skill in the art, provided oligonucleotides and compositions, among other things, can target a great number of nucleic acid polymers. For instance, in some embodiments, provided oligonucleotides and compositions may target a transcript of a nucleic acid sequence, wherein a common base sequence of oligonucleotides (*e.g.*, a base sequence of an oligonucleotide type) comprises or is a sequence complementary to a sequence of the transcript. In some embodiments, a common base sequence comprises a sequence complimentary to a sequence of a target. In some embodiments, a common base sequence is a sequence complimentary to a sequence of a target. In some

embodiments, a common base sequence comprises or is a sequence 100% complimentary to a sequence of a target. In some embodiments, a common base sequence comprises a sequence 100% complimentary to a sequence of a target. In some embodiments, a common base sequence is a sequence 100% complimentary to a sequence of a target. In some embodiments, a common base sequence in a core comprises or is a sequence complimentary to a sequence of a target. In some embodiments, a common base sequence in a core comprises a sequence complimentary to a sequence of a target. In some embodiments, a common base sequence in a core is a sequence % complimentary to a sequence of a target. In some embodiments, a common base sequence in a core comprises or is a sequence 100% complimentary to a sequence of a target. In some embodiments, a common base sequence in a core comprises a sequence 100% complimentary to a sequence of a target. In some embodiments, a common base sequence in a core is a sequence 100% complimentary to a sequence of a target.

[00407] In some embodiments, as described in this disclosure, provided oligonucleotides and compositions may provide new cleavage patterns, higher cleavage rate, higher cleavage degree, higher cleavage selectivity, *etc.* In some embodiments, provided compositions can selectively suppress (*e.g.,* cleave) a transcript from a target nucleic acid sequence which has one or more similar sequences exist within a subject or a population, each of the target and its similar sequences contains a specific nucleotidic characteristic sequence element that defines the target sequence relative to the similar sequences. In some embodiments, for example, a target sequence is a wild-type allele or copy of a gene, and a similar sequence is a sequence has very similar base sequence, *e.g.*, a sequence having SNP, mutations, *etc.*; In some embodiments, a characteristic sequence element defines that target sequence relative to the similar sequence: for example, when a target sequence is a Huntington's disease-associated allele with T at rs362307 (U in the corresponding RNA; C for the non-disease-associated allele), a characteristic sequence comprises this SNP.

[00408] In some embodiments, a similar sequence has greater than 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence homology with a target sequence. In some embodiments, a target sequence is a disease-causing copy of a nucleic acid sequence comprising one or more mutations and/or SNPs, and a similar sequence is a copy not causing the disease (wild type). In some embodiments, a target sequence comprises a mutation, wherein a similar sequence is the corresponding wild-type sequence. In some

embodiments, a target sequence is a mutant allele, while a similar sequence is a wild-type allele. In some embodiments, a target sequence comprises a SNP that is associated with a disease-causing allele, while a similar sequence comprises the same SNP that is not associated with the disease-causing allele. In some embodiments, the region of a target sequence that is complementary to a common base sequence of a provided oligonucleotide composition has greater than 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence homology with the corresponding region of a similar sequence. In some embodiments, the region of a target sequence that is complementary to a common base sequence of a provided oligonucleotide composition differs from the corresponding region of a similar sequence at less than 5, less than 4, less than 3, less than 2, or only 1 base pairs. In some embodiments, the region of a target sequence that is complementary to a common base sequence of a provided oligonucleotide composition differs from the corresponding region of a similar sequence only at a mutation site or SNP site. In some embodiments, the region of a target sequence that is complementary to a common base sequence of a provided oligonucleotide composition differs from the corresponding region of a similar sequence only at a mutation site. In some embodiments, the region of a target sequence that is complementary to a common base sequence of a provided oligonucleotide composition differs from the corresponding region of a similar sequence only at a SNP site.

[00409] In some embodiments, a common base sequence comprises or is a sequence complementary to a characteristic sequence element. In some embodiments, a common base sequence comprises a sequence complementary to a characteristic sequence element. In some embodiments, a common base sequence is a sequence complementary to a characteristic sequence element. In some embodiments, a common base sequence comprises or is a sequence 100% complementary to a characteristic sequence element. In some embodiments, a common base sequence comprises a sequence 100% complementary to a characteristic sequence element. In some embodiments, a common base sequence is a sequence 100% complementary to a characteristic sequence element. In some embodiments, a common base sequence in a core comprises or is a sequence complementary to a characteristic sequence element. In some embodiments, a common base sequence in a core comprises a sequence complementary to a characteristic sequence element. In some embodiments, a common base sequence in a core is a sequence complementary to a characteristic sequence element. In some embodiments, a

common base sequence in a core comprises or is a sequence 100% complementary to a characteristic sequence element. In some embodiments, a common base sequence in a core comprises a sequence 100% complementary to a characteristic sequence element. In some embodiments, a common base sequence in a core is a sequence 100% complementary to a characteristic sequence element.

[00410] In some embodiments, a characteristic sequence element comprises or is a mutation. In some embodiments, a characteristic sequence element comprises a mutation. In some embodiments, a characteristic sequence element is a mutation. In some embodiments, a characteristic sequence element comprises or is a point mutation. In some embodiments, a characteristic sequence element comprises a point mutation. In some embodiments, a characteristic sequence element is a point mutation. In some embodiments, a characteristic sequence element comprises or is a SNP. In some embodiments, a characteristic sequence element comprises a SNP. In some embodiments, a characteristic sequence element is a SNP.

[00411] In some embodiments, a common base sequence 100% matches a target sequence, which it does not 100% match a similar sequence of the target sequence. For example, in some embodiments, a common base sequence matches a mutation in the disease-causing copy or allele of a target nucleic acid sequence, but does not match a non-disease-causing copy or allele at the mutation site; in some other embodiments, a common base sequence matches a SNP in the disease-causing allele of a target nucleic acid sequence, but does not match a non-disease-causing allele at the corresponding site. In some embodiments, a common base sequence in a core 100% matches a target sequence, which it does not 100% match a similar sequence of the target sequence. For example, in WV-1092, its common base sequence (and its common base sequence in its core) matches the disease-associated U, but not the non-disease-associated (wild-type) C at rs362307.

[00412] Among other things, the present disclosure recognizes that a base sequence may have impact on oligonucleotide properties. In some embodiments, a base sequence may have impact on cleavage pattern of a target when oligonucleotides having the base sequence are utilized for suppressing a target, *e.g.,* through a pathway involving RNase H: for example, Figure 33 demonstrates that structurally similar (all phosphorothioate linkages, all stereorandom) oligonucleotides have different sequences may have different cleavage patterns. In some embodiments, a common base sequence of a non-stereorandom oligonucleotide compositions

(*e.g.*, certain oligonucleotide compositions provided in the present disclosure) is a base sequence that when applied to a DNA oligonucleotide composition (*e.g.*, ONT-415) or a stereorandom all-phosphorothioate oligonucleotide composition (*e.g.*, WV-905), cleavage pattern of the DNA (DNA cleavage pattern) and/or the stereorandom all-phosphorothioate (stereorandom cleavage pattern) composition has a cleavage site within or in the vicinity of a characteristic sequence element. In some embodiments, a cleavage site within or in the vicinity is within a sequence complementary to a core region of a common sequence. In some embodiments, a cleavage site within or in the vicinity is within a sequence 100% complementary to a core region of a common sequence.

[00413] In some embodiments, a common base sequence is a base sequence that has a cleavage site within or in the vicinity of a characteristic sequence element in its DNA cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site within a characteristic sequence element in its DNA cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a characteristic sequence element in its DNA cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a mutation or SNP of a characteristic sequence element in its DNA cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a mutation in its DNA cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a SNP in its DNA cleavage pattern.

[00414] In some embodiments, a common base sequence is a base sequence that has a cleavage site within or in the vicinity of a characteristic sequence element in its stereorandom cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site within a characteristic sequence element in its stereorandom cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a characteristic sequence element in its stereorandom cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a mutation or SNP of a characteristic sequence element in its stereorandom cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a mutation in its stereorandom cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a SNP in its

stereorandom cleavage pattern.

[00415] In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a mutation of a characteristic sequence element in its DNA and/or stereorandom cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a mutation in its DNA and/or stereorandom cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a mutation in its DNA cleavage pattern. In some embodiments, a cleavage site in the vicinity of a mutation is at a mutation, i.e., a cleavage site is at the internucleotidic linkage of a mutated nucleotide (*e.g.,* if a mutation is at A in the target sequence of GGG**A**CGTCTT, the cleavage is between A and C). In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 internucleotidic linkages away from a mutation, where 0 means cleavage at the mutation site (*e.g.,* if a mutation is at A in the target sequence of GGG**A**CGTCTT, the cleavage is between A and C for 0 internucleotidic linkage away; a cleavage site 1 internucleotidic linkage away from the mutation is between G and A to the 5' from the mutation or between C and G to the 3' from the mutation). In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away to the 5' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away to the 3' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away to the 5' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away to the 3' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, or 4 internucleotidic linkages away from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, or 4 internucleotidic linkages away to the 5' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, or 4 internucleotidic linkages away to the 3' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, or 3 internucleotidic linkages away from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, or 3 internucleotidic

linkages away to the 5' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, or 3 internucleotidic linkages away to the 3' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, or 2 internucleotidic linkages away from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, or 2 internucleotidic linkages away to the 5' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, or 2 internucleotidic linkages away to the 3' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0 or 1 internucleotidic linkage away from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0 or 1 internucleotidic linkage away to the 5' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0 or 1 internucleotidic linkage away to the 3' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site 0 internucleotidic linkage away from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site one internucleotidic linkage away from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site one internucleotidic linkage away to the 5' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site one internucleotidic linkage away to the 3' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site two internucleotidic linkages away from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site two internucleotidic linkages away to the 5' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site two internucleotidic linkages away to the 3' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site three internucleotidic linkages away from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site three internucleotidic linkages away to the 5' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site three internucleotidic linkages away to the 3' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site four internucleotidic linkages away from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site four internucleotidic linkages away to the 5' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site four internucleotidic linkages away to the 3' from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site five internucleotidic linkages away from a mutation. In some embodiments, a cleavage site in the vicinity is a cleavage site five internucleotidic linkages away to the 5' from a mutation. In

some embodiments, a cleavage site in the vicinity is a cleavage site five internucleotidic linkages away to the 3' from a mutation.

[00416] In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a SNP of a characteristic sequence element in its DNA and/or stereorandom cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a SNP in its DNA and/or stereorandom cleavage pattern. In some embodiments, a common base sequence is a base sequence that has a cleavage site in the vicinity of a SNP in its DNA cleavage pattern. In some embodiments, a cleavage site in the vicinity of a SNP is at a SNP, i.e., a cleavage site is at the internucleotidic linkage of a nucleotide at a SNP (*e.g.*, for the target of WV-905, G*G*C*A*C*A*A*G*G*G*C*A*C*A*G*A*C*T*T*C, which comprises rUrUrUrGrGrArArGrUrCrUrG**rU**rGrCrCrCrUrUrGrUrGrGrCrCrC (rs362307 bolded), the cleavage is between the bolded rU and the underlined rG immediately after it). In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 internucleotidic linkages away from a SNP, where 0 means cleavage at a SNP (*e.g.*, for the target of WV-905, G*G*C*A*C*A*A*G*G*G*C*A*C*A*G*A*C*T*T*C, which comprises rUrUrUrGrGrArArGrUrCrUrG**rU**rGrCrCrCrUrUrGrUrGrGrCrCrC (rs362307 bolded), the cleavage is between the bolded rU and the underlined rG immediately after it for 0 internucleotidic linkage away; a cleavage site 1 internucleotidic linkage away from a SNP is between the rG and rU to the 5' from the SNP (underlined: rUrUrUrGrGrArArGrUrCrU**rGrU**rGrCrCrCrUrUrGrUrGrGrCrCrC), or between rG and rC to the 3'-end of the SNP (underlined: rUrUrUrGrGrArArGrUrCrUrG**rU**rGrCrCrCrUrUrGrUrGrGrCrCrC)). In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away to the 5' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away to the 3' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away to the 5' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic

linkages away to the 3' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, or 4 internucleotidic linkages away from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, or 4 internucleotidic linkages away to the 5' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, or 4 internucleotidic linkages away to the 3' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, or 3 internucleotidic linkages away from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, or 3 internucleotidic linkages away to the 5' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, or 3 internucleotidic linkages away to the 3' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, or 2 internucleotidic linkages away from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, or 2 internucleotidic linkages away to the 5' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, or 2 internucleotidic linkages away to the 3' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0 or 1 internucleotidic linkage away from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0 or 1 internucleotidic linkage away to the 5' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0 or 1 internucleotidic linkage away to the 3' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site 0 internucleotidic linkage away from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site one internucleotidic linkage away from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site one internucleotidic linkage away to the 5' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site one internucleotidic linkage away to the 3' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site two internucleotidic linkages away from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site two internucleotidic linkages away to the 5' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site two internucleotidic linkages away to the 3' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site three internucleotidic linkages away from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site three internucleotidic linkages away to the 5' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site three internucleotidic linkages away to the 3' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site four internucleotidic

linkages away from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site four internucleotidic linkages away to the 5' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site four internucleotidic linkages away to the 3' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site five internucleotidic linkages away from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site five internucleotidic linkages away to the 5' from a SNP. In some embodiments, a cleavage site in the vicinity is a cleavage site five internucleotidic linkages away to the 3' from a SNP. For example, Figure 33 demonstrates that stereorandom cleavage pattern of the WV-905 sequence has cleavage sites at the SNP (between CUGU and GCCC), two internucleotidic linkages away (between GUCU and GUGC, and between GUGC and CCUU), three internucleotidic linkages away (between UGCC and CUUG); four internucleotidic linkages away (between GCCC and UUGU, and AAGU and CUGU), and five internucleotidic linkages away (between CCCU and UGUG).

[00417] In some embodiments, a cleavage site within or in the vicinity of a characteristic sequence element, *e.g.*, in the vicinity of a mutation, a SNP, *etc.*, is a major cleavage site of a DNA and/or stereorandom cleavage pattern. In some embodiments, a cleavage site within or in the vicinity of a characteristic sequence element is a major cleavage site of a DNA cleavage pattern. In some embodiments, a cleavage site within or in the vicinity of a characteristic sequence element is a major cleavage site of a stereorandom cleavage pattern. In some embodiments, a cleavage site in the vicinity of a mutation is a major cleavage site of a DNA cleavage pattern. In some embodiments, a cleavage site in the vicinity of a mutation is a major cleavage site of a stereorandom cleavage pattern. In some embodiments, a cleavage site in the vicinity of a SNP is a major cleavage site of a DNA cleavage pattern. In some embodiments, a cleavage site in the vicinity of a SNP is a major cleavage site of a stereorandom cleavage pattern. In some embodiments, a major cleavage site is within a sequence complementary to a core region of a common sequence. In some embodiments, a major cleavage site is within a sequence 100% complementary to a core region of a common sequence.

[00418] In some embodiments, a major cleavage site is a site having the most, or the second, third, fourth or fifth most cleavage. In some embodiments, a major cleavage site is a site having the most, or the second, third, or fourth most cleavage. In some embodiments, a major cleavage site is a site having the most, or the second, or third most cleavage. In some

embodiments, a major cleavage site is a site having the most or the second most cleavage. In some embodiments, a major cleavage site is a site having the most cleavage. In some embodiments, a major cleavage site is a site having the second most cleavage. In some embodiments, a major cleavage site is a site having the third most cleavage. In some embodiments, a major cleavage site is a site having the fourth most cleavage. In some embodiments, a major cleavage site is a site having the fifth most cleavage.

[00419] In some embodiments, a major cleavage site is a site wherein greater than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 5% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 10% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 15% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 20% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 25% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 30% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 35% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 40% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 45% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 50% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 55% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 60% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 65% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 70% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 75% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 80% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 85% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 90% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 91% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 92% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 93% of

cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 94% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 95% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 96% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 97% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 98% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein greater than 99% of cleavage occurs. In some embodiments, a major cleavage site is a site wherein 100% of cleavage occurs.

[00420] In some embodiments, a major cleavage site is a site wherein greater than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 5% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 10% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 15% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 20% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 25% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 30% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 35% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 40% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 45% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 50% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 55% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 60% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 65% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 70% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 75% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 80% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 85% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 90% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 91%

of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 92% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 93% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 94% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 95% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 96% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 97% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 98% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein greater than 99% of a target is cleaved. In some embodiments, a major cleavage site is a site wherein 100% of a target is cleaved. In some embodiments, a cleavage pattern may not have a major cleavage site as no site reaches an abosulte cleavage threshold level.

[00421] As a person having ordinary skill in the art understands, various methods may be useful for generating cleavage patterns and/or identify cleavage sites, including major cleavage site. In some embodiments, an example of such an assay is an RNase cleavage assay as described herein; for example results, see Figure 33, Figure 34, *etc*.

[00422] In some embodiments, the present disclosure recognizes location effects of a sequence motif complementary to a characteristic sequence element. In some embodiments, the present disclosure recognizes location effects of a sequence motif complementary to a mutation. In some embodiments, the present disclosure recognizes location effects of a sequence motif complementary to a SNP.

[00423] In some embodiments, position 11, 12 or 13 of a sequence as counted from its 5'-terminus aligns with a characteristic sequence element. In some embodiments, position 11 of a sequence as counted from its 5'-terminus aligns with a characteristic sequence element. In some embodiments, position 12 of a sequence as counted from its 5'-terminus aligns with a characteristic sequence element. In some embodiments, position 13 of a sequence as counted from its 5'-terminus aligns with a characteristic sequence element. In some embodiments, position 8, 9 or 10 of a sequence as counted from its 3'-terminus aligns with a characteristic sequence element. In some embodiments, position 8 of a sequence as counted from its 3'-terminus aligns with a characteristic sequence element. In some embodiments, position 9 of a sequence as counted from its 3'-terminus aligns with a characteristic sequence element. In some

embodiments, position 10 of a sequence as counted from its 3'-terminus aligns with a characteristic sequence element. In some embodiments, position 6, 7, or 8 of a core region as counted from the 5'-terminus of the core region aligns with a characteristic sequence element. In some embodiments, position 6 of a core region as counted from the 5'-terminus of the core region aligns with a characteristic sequence element. In some embodiments, position 7 of a core region as counted from the 5'-terminus of the core region aligns with a characteristic sequence element. In some embodiments, position 8 of a core region as counted from the 5'-terminus of the core region aligns with a characteristic sequence element. In some embodiments, position 3, 4, or 5 of a core region as counted from the 3'-terminus of the core region aligns with a characteristic sequence element. In some embodiments, position 3 of a core region as counted from the 3'-terminus of the core region aligns with a characteristic sequence element. In some embodiments, position 4 of a core region as counted from the 3'-terminus of the core region aligns with a characteristic sequence element. In some embodiments, position 5 of a core region as counted from the 3'-terminus of the core region aligns with a characteristic sequence element.

[00424] In some embodiments, position 11, 12 or 13 of a sequence as counted from its 5'-terminus aligns with a mutation. In some embodiments, position 11 of a sequence as counted from its 5'-terminus aligns with a mutation. In some embodiments, position 12 of a sequence as counted from its 5'-terminus aligns with a mutation. In some embodiments, position 13 of a sequence as counted from its 5'-terminus aligns with a mutation. In some embodiments, position 8, 9 or 10 of a sequence as counted from its 3'-terminus aligns with a mutation. In some embodiments, position 8 of a sequence as counted from its 3'-terminus aligns with a mutation. In some embodiments, position 9 of a sequence as counted from its 3'-terminus aligns with a mutation. In some embodiments, position 10 of a sequence as counted from its 3'-terminus aligns with a mutation. In some embodiments, position 6, 7, or 8 of a core region as counted from the 5'-terminus of the core region aligns with a mutation. In some embodiments, position 6 of a core region as counted from the 5'-terminus of the core region aligns with a mutation. In some embodiments, position 7 of a core region as counted from the 5'-terminus of the core region aligns with a mutation. In some embodiments, position 8 of a core region as counted from the 5'-terminus of the core region aligns with a mutation. In some embodiments, position 3, 4, or 5 of a core region as counted from the 3'-terminus of the core region aligns with a mutation. In some embodiments, position 3 of a core region as counted from the 3'-terminus of

the core region aligns with a mutation. In some embodiments, position 4 of a core region as counted from the 3'-terminus of the core region aligns with a mutation. In some embodiments, position 5 of a core region as counted from the 3'-terminus of the core region aligns with a mutation.

[00425] In some embodiments, position 11, 12 or 13 of a sequence as counted from its 5'-terminus aligns with a SNP. In some embodiments, position 11 of a sequence as counted from its 5'-terminus aligns with a SNP. In some embodiments, position 12 of a sequence as counted from its 5'-terminus aligns with a SNP. In some embodiments, position 13 of a sequence as counted from its 5'-terminus aligns with a SNP. In some embodiments, position 8, 9 or 10 of a sequence as counted from its 3'-terminus aligns with a SNP. In some embodiments, position 8 of a sequence as counted from its 3'-terminus aligns with a SNP. In some embodiments, position 9 of a sequence as counted from its 3'-terminus aligns with a SNP. In some embodiments, position 10 of a sequence as counted from its 3'-terminus aligns with a SNP. In some embodiments, position 6, 7, or 8 of a core region as counted from the 5'-terminus of the core region aligns with a SNP. In some embodiments, position 6 of a core region as counted from the 5'-terminus of the core region aligns with a SNP. In some embodiments, position 7 of a core region as counted from the 5'-terminus of the core region aligns with a SNP. In some embodiments, position 8 of a core region as counted from the 5'-terminus of the core region aligns with a SNP. In some embodiments, position 3, 4, or 5 of a core region as counted from the 3'-terminus of the core region aligns with a SNP. In some embodiments, position 3 of a core region as counted from the 3'-terminus of the core region aligns with a SNP. In some embodiments, position 4 of a core region as counted from the 3'-terminus of the core region aligns with a SNP. In some embodiments, position 5 of a core region as counted from the 3'-terminus of the core region aligns with a SNP.

[00426] In some embodiments, a common base sequence comprises or is a sequence complementary to a nucleic acid sequence. In some embodiments, a common base sequence comprises or is a sequence 100% complementary to a nucleic acid sequence. In some embodiments, a common base sequence comprises or is a sequence complementary to a disease-causing nucleic acid sequence. In some embodiments, a common base sequence comprises or is a sequence 100% complementary to a disease-causing nucleic acid sequence. In some embodiments, a common base sequence comprises or is a sequence complementary to a

characteristic sequence element of disease-causing nucleic acid sequence, which characteristic sequences differentiate a disease-causing nucleic acid sequence from a non-diseasing-causing nucleic acid sequence. In some embodiments, a common base sequence comprises or is a sequence 100% complementary to a characteristic sequence element of disease-causing nucleic acid sequence, which characteristic sequences differentiate a disease-causing nucleic acid sequence from a non-diseasing-causing nucleic acid sequence. In some embodiments, a common base sequence comprises or is a sequence complementary to a disease-associated nucleic acid sequence. In some embodiments, a common base sequence comprises or is a sequence 100% complementary to a disease-associated nucleic acid sequence. In some embodiments, a common base sequence comprises or is a sequence complementary to a characteristic sequence element of disease-associated nucleic acid sequence, which characteristic sequences differentiate a disease-associated nucleic acid sequence from a non-diseasing-associated nucleic acid sequence. In some embodiments, a common base sequence comprises or is a sequence 100% complementary to a characteristic sequence element of disease-associated nucleic acid sequence, which characteristic sequences differentiate a disease-associated nucleic acid sequence from a non-diseasing-associated nucleic acid sequence.

[00427] In some embodiments, a common base sequence comprises or is a sequence complementary to a gene. In some embodiments, a common base sequence comprises or is a sequence 100% complementary to a gene. In some embodiments, a common base sequence comprises or is a sequence complementary to a characteristic sequence element of a gene, which characteristic sequences differentiate the gene from a similar sequence sharing homology with the gene. In some embodiments, a common base sequence comprises or is a sequence 100% complementary to a characteristic sequence element of a gene, which characteristic sequences differentiate the gene from a similar sequence sharing homology with the gene. In some embodiments, a common base sequence comprises or is a sequence complementary to characteristic sequence element of a target gene, which characteristic sequences comprises a mutation that is not found in other copies of the gene, *e.g.*, the wild-type copy of the gene, another mutant copy the gene, *etc*. In some embodiments, a common base sequence comprises or is a sequence 100% complementary to characteristic sequence element of a target gene, which characteristic sequences comprises a mutation that is not found in other copies of the gene, *e.g.*, the wild-type copy of the gene, another mutant copy the gene, *etc*.

[00428] In some embodiments, a common base sequence comprises or is a sequence complementary to a sequence comprising a SNP. In some embodiments, a common base sequence comprises or is a sequence complementary to a sequence comprising a SNP, and the common base sequence is 100% complementary to the SNP that is associated with a disease. For example, in some embodiments, a common base sequence is 100% complementary to a SNP associated with a Huntington's disease-associated (or -causing) allele. In some embodiments, a common base sequence is that of WV-1092, which is 100% complementary to the disease-associated allele in many Huntington's disease patients at rs362307. In some embodiments, a SNP is rs362307. In some embodiments, a SNP is rs7685686. In some embodiments, a SNP is rs362268. In some embodiments, a SNP is rs362306. In some embodiments, a SNP is rs362331. In some embodiments, a SNP is rs2530595. In some embodiments, other example SNP site may be any of the Huntingtin site disclosed in the present disclosure.

[00429] In some embodiments, a common base sequence comprises a sequence found in GCCTCAGTCTGCTTCGCACC. In some embodiments, a common base sequence comprises a sequence found in GCCTCAGTCTGCTTCGCACC, wherein the sequence found in GCCTCAGTCTGCTTCGCACC comprises at least 15 nucleotides. In some embodiments, a common base sequence is GCCTCAGTCTGCTTCGCACC.

[00430] In some embodiments, a common base sequence comprises a sequence found in GAGCAGCTGCAACCTGGCAA. In some embodiments, a common base sequence comprises a sequence found in GAGCAGCTGCAACCTGGCAA, wherein the sequence found in GAGCAGCTGCAACCTGGCAA comprises at least 15 nucleotides. In some embodiments, a common base sequence is GAGCAGCTGCAACCTGGCAA. In some embodiments, a common base sequence is GGGCACAAGGGCACAGACTT. In some embodiments, a common base sequence is GAGCAGCTGCAACCTGGCAA. In some embodiments, a common base sequence is GCACAAGGGCACAGACTTCC. In some embodiments, a common base sequence is CACAAGGGCACAGACTTCCA. In some embodiments, a common base sequence is ACAAGGGCACAGACTTCCAA. In some embodiments, a common base sequence is CAAGGGCACAGACTTCCAAA. In some embodiments, a common base sequence comprises a sequence found in GAGCAGCTGCAACCTGGCAA. In some embodiments, a common base sequence comprises a sequence found in GAGCAGCTGCAACCTGGCAA, wherein the sequence found in GAGCAGCTGCAACCTGGCAA comprises at least 15 nucleotides. In some

embodiments, a common base sequence is GAGCAGCTGCAACCTGGCAA. In some embodiments, a common base sequence is GAGCAGCTGCAACCTGGCAA. In some embodiments, a common base sequence is AGCAGCTGCAACCTGGCAAC. In some embodiments, a common base sequence is GCAGCTGCAACCTGGCAACA. In some embodiments, a common base sequence is CAGCTGCAACCTGGCAACAA. In some embodiments, a common base sequence is AGCTGCAACCTGGCAACAAC. In some embodiments, a common base sequence is GCTGCAACCTGGCAACAACC. In some embodiments, a common base sequence comprises a sequence found in GGGCCAACAGCCAGCCTGCA. In some embodiments, a common base sequence comprises a sequence found in GGGCCAACAGCCAGCCTGCA, wherein the sequence found in GGGCCAACAGCCAGCCTGCAcomprises at least 15 nucleotides. In some embodiments, a common base sequence is GGGCCAACAGCCAGCCTGCA. In some embodiments, a common base sequence is GGGCCAACAGCCAGCCTGCA. In some embodiments, a common base sequence is GGCCAACAGCCAGCCTGCAG. In some embodiments, a common base sequence is GCCAACAGCCAGCCTGCAGG. In some embodiments, a common base sequence is CCAACAGCCAGCCTGCAGGA. In some embodiments, a common base sequence is CAACAGCCAGCCTGCAGGAG. In some embodiments, a common base sequence is AACAGCCAGCCTGCAGGAGG. In some embodiments, a common base sequence comprises a sequence found in ATTAATAAATTGTCATCACC. In some embodiments, a common base sequence comprises a sequence found in ATTAATAAATTGTCATCACC, wherein the sequence found in ATTAATAAATTGTCATCACC comprises at least 15 nucleotides. In some embodiments, a common base sequence is ATTAATAAATTGTCATCACC. In some embodiments, a common base sequence is ATTAATAAATTGTCATCACC.

[00431] In some embodiments, the present disclosure provides stereochemical design parameters for oligonucleotides. That is, among other things, the present disclosure demonstrates impact of stereochemical structure at different positions along an oligonucleotide chain, for example on stability and/or activity of the oligonucleotide, including on interaction of the oligonucleotide with a cognate ligand and/or with a processing enzyme. The present disclosure specifically provides oligonucleotides whose structure incorporates or reflects the design parameters. Such oligonucleotides are new chemical entities relative to stereorandom preparations having the same base sequence and length.

[00432] In some embodiments, the present disclosure provides stereochemical design parameters for antisense oligonucleotides. In some embodiments, the present disclosure specifically provides design parameter for oligonucleotides that may be bound and/or cleaved by RNaseH. In ome embodiments, the present disclosure provides stereochemical design parameters for siRNA oligonucleotides. In some embodiments, the present disclosure specifically provides design parameters for oligonucleotides that may be bound and/or cleaved by, *e.g.,* DICER, Argonaute proteins (*e.g.,* Argonaute-1 and Argonaute-2), *etc.*

[00433] In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral. In some embodiments, at least two of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least three of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least four of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least five of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least six of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least seven of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least eight of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least nine of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, one of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral. In some embodiments, two of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, three of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, four of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, five of the first, second, third, fifth,

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

[00434] In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral. In some embodiments, at least two of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least three of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least four of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least five of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least six of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, at least seven of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, one of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral. In some embodiments, two of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, three of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, four of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, five of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In some embodiments, six of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral. In

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

[00435] In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral, and at least one internucleotidic linkage is achiral. In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral, and at least one internucleotidic linkage is achiral. In some embodiments, at least two internucleotidic linkages are achiral. In some embodiments, at least three internucleotidic linkages are achiral. In some embodiments, at least four internucleotidic linkages are achiral. In some embodiments, at least five internucleotidic linkages are achiral. In some embodiments, at least six internucleotidic linkages are achiral. In some embodiments, at least seven internucleotidic linkages are achiral. In some embodiments, at least eight internucleotidic linkages are achiral. In some embodiments, at least nine internucleotidic linkages are achiral. In some embodiments, at least 10 internucleotidic linkages are achiral. In some embodiments, at least 11 internucleotidic linkages are achiral. In some embodiments, at least 12 internucleotidic linkages are achiral. In some embodiments, at least 13 internucleotidic linkages are achiral. In some embodiments, at least 14 internucleotidic linkages are achiral. In some embodiments, at least 15 internucleotidic linkages are achiral. In some embodiments, at least 16 internucleotidic linkages are achiral. In some embodiments, at least 17 internucleotidic linkages are achiral. In some embodiments, at least 18 internucleotidic linkages are achiral. In some embodiments, at least 19 internucleotidic linkages are achiral. In some embodiments, at least 20 internucleotidic linkages are achiral. In some embodiments, one internucleotidic linkage is achiral. In some embodiments, two internucleotidic linkages are achiral. In some embodiments, three internucleotidic linkages are achiral. In some embodiments, four internucleotidic linkages are achiral. In some embodiments, five internucleotidic linkages are achiral. In some embodiments, six internucleotidic linkages are achiral. In some embodiments, seven internucleotidic linkages are achiral. In some embodiments, eight internucleotidic linkages are achiral. In some embodiments, nine internucleotidic linkages are achiral. In some embodiments, 10 internucleotidic linkages are

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

[00436] In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral, and at least one internucleotidic linkage is phosphate. In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral, and at least one internucleotidic linkage is phosphate. In some embodiments, at least two internucleotidic linkages are phosphate. In some embodiments, at least three internucleotidic linkages are phosphate. In some embodiments, at least four internucleotidic linkages are phosphate. In some embodiments, at least five internucleotidic linkages are phosphate. In some embodiments, at least six internucleotidic linkages are phosphate. In some embodiments, at least seven internucleotidic linkages are phosphate. In some embodiments, at least eight internucleotidic linkages are phosphate. In some embodiments, at least nine internucleotidic linkages are phosphate. In some embodiments, at least 10 internucleotidic linkages are phosphate. In some embodiments, at least 11 internucleotidic linkages are phosphate. In some embodiments, at least 12 internucleotidic linkages are phosphate. In some embodiments, at least 13 internucleotidic linkages are phosphate. In some embodiments, at least 14 internucleotidic linkages are phosphate. In some embodiments, at least 15 internucleotidic linkages are phosphate. In some embodiments, at least 16 internucleotidic linkages are phosphate. In some embodiments, at least 17 internucleotidic linkages are phosphate. In some embodiments, at least 18 internucleotidic linkages are phosphate. In some embodiments, at least 19 internucleotidic linkages are

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

[00437] In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighth, ninth, eighteenth, nineteenth and twentieth internucleotidic linkages are chiral, and at least 10% of all the internucleotidic linkages in the region is achiral. In some embodiments, a single oligonucleotide of a provided composition comprises a region in which at least one of the first, second, third, fifth, seventh, eighteenth, nineteenth and twentieth internucleotidic linkages is chiral, and at least 10% of all the internucleotidic linkages in the region are achiral. In some embodiments, at least 20% of all the internucleotidic linkages in the region are achiral. In some embodiments, at least 30% of all the internucleotidic linkages in the region are achiral. In some embodiments, at least 40% of all the internucleotidic linkages in the region are achiral. In some embodiments, at least 50% of all the internucleotidic linkages in the region are achiral. In some

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

[00438] In some embodiments, the first internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the first internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the second internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the second internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the third internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the third internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the fifth internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the fifth internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the seventh internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the seventh internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the eighth internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the eighth internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the ninth internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the ninth internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the eighteenth internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the eighteenth internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the nineteenth internucleotidic linkage of the region is an *S*p modified internucleotidic linkage. In some embodiments, the nineteenth internucleotidic linkage of the region is an *R*p modified internucleotidic linkage. In some embodiments, the twentieth internucleotidic linkage of the region is an *S*p modified

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

[00439] In some embodiments, the region has a length of at least 21 bases. In some embodiments, the region has a length of 21 bases. In some embodiments, a single oligonucleotide in a provided composition has a length of at least 21 bases. In some embodiments, a single oligonucleotide in a provided composition has a length of 21 bases.

[00440] In some embodiments, a chiral internucleotidic linkage has the structure of formula **I**. In some embodiments, a chiral internucleotidic linkage is phosphorothioate. In some embodiments, each chiral internucleotidic linkage in a single oligonucleotide of a provided composition independently has the structure of formula **I**. In some embodiments, each chiral internucleotidic linkage in a single oligonucleotide of a provided composition is a phosphorothioate.

[00441] In some embodiments, oligonucleotides of the present disclosure comprise one or more modified sugar moieties. In some embodiments, oligonucleotides of the present disclosure comprise one or more modified base moieties. As known by a person of ordinary skill in the art and described in the disclosure, various modifications can be introduced to a sugar and/or moiety. For example, in some embodiments, a modification is a modification described in US9006198 and WO2014/012081, the sugar and base modifications of each of which are incorporated herein by reference.

[00442] In some embodiments, a sugar modification is a 2'-modification. Commonly used 2'-modifications include but are not limited to 2'–OR^1, wherein R^1 is not hydrogen. In some embodiments, a modification is 2'−OR, wherein R is optionally substituted aliphatic. In some embodiments, a modification is 2'−OMe. In some embodiments, a modification is 2'-MOE. In some embodiments, the present disclosure demonstrates that inclusion and/or location of particular chirally pure internucleotidic linkages can provide stability improvements comparable to or better than those achieved through use of modified backbone linkages, bases, and/or sugars. In some embodiments, a provided single oligonucleotide of a provided composition has no modifications on the sugars. In some embodiments, a provided single oligonucleotide of a provided composition has no modifications on 2'-positions of the sugars (i.e., the two groups at the 2'-position are either −H/−H or −H/−OH). In some embodiments, a provided single oligonucleotide of a provided composition does not have any 2'-MOE

modifications.

[00443] In some embodiments, a 2'-modification is −O−L− or −L− which connects the 2'-carbon of a sugar moiety to another carbon of a sugar moiety. In some embodiments, a 2'-modification is −O−L− or −L− which connects the 2'-carbon of a sugar moiety to the 4'-carbon of a sugar moiety. In some embodiments, a 2'-modification is *S*-cEt. In some embodiments, a modified sugar moiety is an LNA moiety.

[00444] In some embodiments, a 2'-modification is −F. In some embodiments, a 2'-modification is FANA. In some embodiments, a 2'-modification is FRNA.

[00445] In some embodiments, a sugar modification is a 5'-modification, *e.g.*, *R*-5'-Me, *S*-5'-Me, *etc*.

[00446] 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.

[00447] In some embodiments, a single oligonucleotide in a provided composition is a better substrate for Argonaute proteins (*e.g.*, hAgo-1 and hAgo-2) compared to stereorandom oligonucleotide compositions. Selection and/or location of chirally pure linkages as described in the present closure are useful design parameters for oligonucleotides that interacting with such proteins, such as siRNA.

[00448] In some embodiments, a single oligonucleotide in a provided composition has at least about 25% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 30% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 35% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 40% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 45% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 50% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 55% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 60% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 65% of its

internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 70% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 75% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 80% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 85% of its internucleotidic linkages in *S*p configuration. In some embodiments, a single oligonucleotide in a provided composition has at least about 90% of its internucleotidic linkages in *S*p configuration.

[00449] In some embodiments, oligonucleotides in a provided composition is not an oligonucleotide selected from : $T_kT_k{}^mC_kAGT^mCATGA^mCT_kT^mC_k{}^mC_k$, wherein each nucleoside followed by a subscript 'k' indicates a (S)-cEt modification, R is *R*p phosphorothioate linkage, S is *S*p phosphorothioate linkage, each ^{m}C is a 5-methylcytosine modified nucleoside, and all internucleoside linkages are phosphorothioates (PS) with stereochemistry patterns selected from RSSSRSRRRS, RSSSSSSSSS, SRRSRSSSSR, SRSRSSRSSR, RRRSSSRSSS, RRRSRSSRSR, RRSSSRSRSR, SRSSSRSSSS, SSRRSSRSRS, SSSSSSRRSS, RRRSSRRRSR, RRRRSSSSRS, SRRSRRRRRR, RSSRSSRRRR, RSRRSRRSRR, RRSRSSRSRS, SSRRRRRSRR, RSRRSRSSSR, RRSSRSRRRR, RRSRSRRSSS, RRSRSSSRRR, RSRRRRSRSR, SSRSSSRRRS, RSSRSRSRSR, RSRSRSSRSS, RRRSSRRSRS, SRRSSRRSRS, RRRRSRSRRR, SSSSRRRRSR, RRRRRRRRRR and SSSSSSSSSS.

[00450] In some embodiments, a single oligonucleotide in a provided composition is not an oligonucleotide selected from : $T_kT_k{}^mC_kAGT^mCATGA^mCTT_k{}^mC_k{}^mC_k$, wherein each nucleoside followed by a subscript 'k' indicates a (S)-cEt modification, R is *R*p phosphorothioate linkage, S is *S*p phosphorothioate linkage, each ^{m}C is a 5-methylcytosine modified nucleoside and all core internucleoside linkages are phosphorothioates (PS) with stereochemistry patterns selected from: RSSSRSRRRS, RSSSSSSSSS, SRRSRSSSSR, SRSRSSRSSR, RRRSSSRSSS, RRRSRSSRSR, RRSSSRSRSR, SRSSSRSSSS, SSRRSSRSRS, SSSSSSRRSS, RRRSSRRRSR, RRRRSSSSRS, SRRSRRRRRR, RSSRSSRRRR, RSRRSRRSRR, RRSRSSRSRS, SSRRRRRSRR, RSRRSRSSSR, RRSSRSRRRR, RRSRSRRSSS, RRSRSSSRRR, RSRRRRSRSR, SSRSSSRRRS, RSSRSRSRSR, RSRSRSSRSS, RRRSSRRSRS, SRRSSRRSRS, RRRRSRSRRR, SSSSRRRRSR, RRRRRRRRRR and

SSSSSSSSSS.

Chirally Controlled Oligonucleotides and Chirally Controlled Oligonucleotide Compositions

[00451] The present disclosure provides chirally controlled oligonucleotides, and chirally controlled oligonucleotide compositions which are of high crude purity and of high diastereomeric purity. In some embodiments, the present disclosure provides chirally controlled oligonucleotides, and chirally controlled oligonucleotide compositions which are of high crude purity. In some embodiments, the present disclosure provides chirally controlled oligonucleotides, and chirally controlled oligonucleotide compositions which are of high diastereomeric purity.

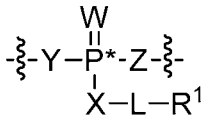
[00452] In some embodiments, a chirally controlled oligonucleotide composition is a substantially pure preparation of an oligonucleotide type in that oligonucleotides in the composition that are not of the oligonucleotide type are impurities form the preparation process of said oligonucleotide type, in some case, after certain purification procedures.

[00453] In some embodiments, the present disclosure provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages with respect to the chiral linkage phosphorus. In some embodiments, the present disclosure provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages having the structure of formula **I**. In some embodiments, the present disclosure provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages with respect to the chiral linkage phosphorus, and one or more phosphate diester linkages. In some embodiments, the present disclosure provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages having the structure of formula **I**, and one or more phosphate diester linkages. In some embodiments, the present disclosure provides oligonucleotides comprising one or more diastereomerically pure internucleotidic linkages having the structure of formula **I-c**, and one or more phosphate diester linkages. In some embodiments, such oligonucleotides are prepared by using stereoselective oligonucleotide synthesis, as described in this application, to form pre-designed diastereomerically pure internucleotidic linkages with respect to the chiral linkage phosphorus. For instance, in one example oligonucleotide of (*R*p/*S*p, *R*p/*S*p, *R*p/*S*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGs1Cs1As1CsC], the first three

internucleotidic linkages are constructed using traditional oligonucleotide synthesis method, and the diastereomerically pure internucleotidic linkages are constructed with stereochemical control as described in this application. Example internucleotidic linkages, including those having structures of formula **I**, are further described below.

[00454] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different stereochemistry and/or different P-modifications relative to one another. In certain embodiments, the present disclosure provides a chirally controlled oligonucleotide, wherein at least two individual internucleotidic linkages within the oligonucleotide have different P-modifications relative to one another. In certain embodiments, the present disclosure provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different P-modifications relative to one another, and wherein the chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage. In certain embodiments, the present disclosure provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different P-modifications relative to one another, and wherein the chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least one phosphorothioate diester internucleotidic linkage. In certain embodiments, the present disclosure provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different P-modifications relative to one another, and wherein the chirally controlled oligonucleotide comprises at least one phosphorothioate triester internucleotidic linkage. In certain embodiments, the present disclosure provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different P-modifications relative to one another, and wherein the chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least one phosphorothioate triester internucleotidic linkage.

[00455] In certain embodiments, a modified internucleotidic linkages has the structure of formula **I**:



(I)

wherein each variable is as defined and described below. In some embodiments, a linkage of formula **I** is chiral. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising one or more modified internucleotidic linkages of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising one or more modified internucleotidic linkages of formula **I**, and wherein individual internucleotidic linkages of formula **I** within the oligonucleotide have different P-modifications relative to one another. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising one or more modified internucleotidic linkages of formula **I**, and wherein individual internucleotidic linkages of formula **I** within the oligonucleotide have different $–X–L–R^1$ relative to one another. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising one or more modified internucleotidic linkages of formula **I**, and wherein individual internucleotidic linkages of formula **I** within the oligonucleotide have different X relative to one another. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising one or more modified internucleotidic linkages of formula **I**, and wherein individual internucleotidic linkages of formula **I** within the oligonucleotide have different $–L–R^1$ relative to one another. In some embodiments, a chirally controlled oligonucleotide is an oligonucleotide in a provided composition that is of the particular oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide is an oligonucleotide in a provided composition that has the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

[00456] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different stereochemistry and/or different P-modifications relative to one another. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different stereochemistry relative to one another, and wherein at least a portion of the structure of the chirally controlled oligonucleotide is characterized by a repeating pattern of alternating stereochemisty.

[00457] In some embodiments, the present disclosure provides a chirally controlled

oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different P-modifications relative to one another, in that they have different X atoms in their $-XLR^1$ moieties, and/or in that they have different L groups in their $-XLR^1$ moieties, and/or that they have different R^1 atoms in their $-XLR^1$ moieties.

[00458] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide, wherein at least two of the individual internucleotidic linkages within the oligonucleotide have different stereochemistry and/or different P-modifications relative to one another and the oligonucleotide has a structure represented by the following formula:

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

wherein:

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

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

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

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

[00459] In some such embodiments, each n has the same value; in some embodiments, each even n has the same value as each other even n; in some embodiments, each odd n has the same value each other odd n; in some embodiments, at least two even ns have different values from one another; in some embodiments, at least two odd ns have different values from one another.

[00460] In some embodiments, at least two adjacent ns are equal to one another, so that a provided oligonucleotide includes adjacent blocks of S stereochemistry linkages and R stereochemistry linkages of equal lengths. In some embodiments, provided oligonucleotides include repeating blocks of S and R stereochemistry linkages of equal lengths. In some

embodiments, provided oligonucleotides include repeating blocks of S and R stereochemistry linkages, where at least two such blocks are of different lengths from one another; in some such embodiments each S stereochemistry block is of the same length, and is of a different length from each R stereochemistry length, which may optionally be of the same length as one another.

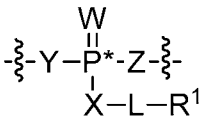
[00461] In some embodiments, at least two skip-adjacent ns are equal to one another, so that a provided oligonucleotide includes at least two blocks of linkages of a first steroechemistry that are equal in length to one another and are separated by a block of linkages of the other stereochemistry, which separating block may be of the same length or a different length from the blocks of first steroechemistry.

[00462] In some embodiments, ns associated with linkage blocks at the ends of a provided oligonucleotide are of the same length. In some embodiments, provided oligonucleotides have terminal blocks of the same linkage stereochemistry. In some such embodiments, the terminal blocks are separated from one another by a middle block of the other linkage stereochemistry.

[00463] In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ is a stereoblockmer. In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ is a stereoskipmer. In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ is a stereoaltmer. In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ is a gapmer.

[00464] In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ is of any of the above described patterns and further comprises patterns of P-modifications. For instance, in some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ and is a stereoskipmer and P-modification skipmer. In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ and is a stereoblockmer and P-modification altmer. In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ and is a stereoaltmer and P-modification blockmer.

[00465] In some embodiments, a provided oligonucleotide of formula $[S^{B}n1R^{B}n2S^{B}n3R^{B}n4...S^{B}nxR^{B}ny]$ is a chirally controlled oligonucleotide comprising one or more modified internuceotidic linkages independently having the structure of formula I:



(I)

wherein:

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

W is O, S or Se;

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

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

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

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

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

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

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

each –≋– independently represents a connection to a nucleoside.

[00466] In some embodiments, L is a covalent bond or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and

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

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

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

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

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

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

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

each $-\overset{}{\underset{}{\lesssim}}-$ independently represents a connection to a nucleoside.

[00467] In some embodiments, a chirally controlled oligonucleotide comprises one or more modified internucleotidic phosphorus linkages. In some embodiments, a chirally controlled oligonucleotide comprises, *e.g.,* a phosphorothioate or a phosphorothioate triester linkage. In some embodiments, a chirally controlled oligonucleotide comprises a phosphorothioate triester linkage. In some embodiments, a chirally controlled oligonucleotide comprises at least two phosphorothioate triester linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least three phosphorothioate triester linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least four phosphorothioate triester linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least five phosphorothioate triester linkages. Examples of such modified internucleotidic phosphorus linkages are described further herein.

[00468] In some embodiments, a chirally controlled oligonucleotide comprises different internucleotidic phosphorus linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least one modified internucleotidic linkage. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least one phosphorothioate triester linkage. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least two phosphorothioate triester linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least three phosphorothioate triester linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least four phosphorothioate triester linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least five phosphorothioate triester linkages. Examples of such modified internucleotidic phosphorus linkages are described further herein.

[00469] In some embodiments, a phosphorothioate triester linkage comprises a chiral auxiliary, which, for example, is used to control the stereoselectivity of a reaction. In some embodiments, a phosphorothioate triester linkage does not comprise a chiral auxiliary. In some embodiments, a phosphorothioate triester linkage is intentionally maintained until and/or during the administration to a subject.

[00470] In some embodiments, a chirally controlled oligonucleotide is linked to a solid support. In some embodiments, a chirally controlled oligonucleotide is cleaved from a solid support.

[00471] In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least two consecutive modified internucleotidic linkages. In some embodiments, a chirally controlled oligonucleotide comprises at least one phosphate diester internucleotidic linkage and at least two consecutive phosphorothioate triester internucleotidic linkages.

[00472] In some embodiments, a chirally controlled oligonucleotide is a blockmer. In some embodiments, a chirally controlled oligonucleotide is a stereoblockmer. In some embodiments, a chirally controlled oligonucleotide is a P-modification blockmer. In some

embodiments, a chirally controlled oligonucleotide is a linkage blockmer.

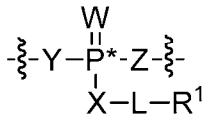
[00473] In some embodiments, a chirally controlled oligonucleotide is an altmer. In some embodiments, a chirally controlled oligonucleotide is a stereoaltmer. In some embodiments, a chirally controlled oligonucleotide is a P-modification altmer. In some embodiments, a chirally controlled oligonucleotide is a linkage altmer.

[00474] In some embodiments, a chirally controlled oligonucleotide is a unimer. In some embodiments, a chirally controlled oligonucleotide is a stereounimer. In some embodiments, a chirally controlled oligonucleotide is a P-modification unimer. In some embodiments, a chirally controlled oligonucleotide is a linkage unimer.

[00475] In some embodiments, a chirally controlled oligonucleotide is a gapmer.

[00476] In some embodiments, a chirally controlled oligonucleotide is a skipmer.

[00477] In some embodiments, the present disclosure provides oligonucleotides comprising one or more modified internucleotidic linkages independently having the structure of formula **I**:



(I)

wherein:

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

W is O, S or Se;

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

L is a covalent bond or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$ $-SC(O)-$, $-C(O)S-$, $-OC(O)-$, and $-C(O)O-$;

R^1 is halogen, R, or an optionally substituted C_1–C_{50} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected

from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C— , a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, –C(S)–, $-C(NR')-$, –C(O)N(R′)–, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, –S(O)–, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$ –SC(O)–, –C(O)S–, –OC(O)–, and $-C(O)O-$

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

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

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

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

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

[00478] In some embodiments, L is a covalent bond or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C— , $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, –C(S)–, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, –S(O)–, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, –SC(O)–, –C(O)S–, –OC(O)–, or $-C(O)O-$;

R^1 is halogen, R, or an optionally substituted C_1–C_{50} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, –C(S)–, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, –S(O)–, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, –SC(O)–, –C(O)S–, –OC(O)–, or $-C(O)O-$;

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

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

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

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

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

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

[00479] In some embodiments, P* is an asymmetric phosphorus atom and is either *R*p or *S*p. In some embodiments, P* is *R*p. In other embodiments, P* is *S*p. In some embodiments, an oligonucleotide comprises one or more internucleotidic linkages of formula **I** wherein each P* is independently *R*p or *S*p. In some embodiments, an oligonucleotide comprises one or more internucleotidic linkages of formula **I** wherein each P* is *R*p. In some embodiments, an oligonucleotide comprises one or more internucleotidic linkages of formula **I** wherein each P* is *S*p. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein P* is *R*p. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein P* is *S*p. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein P* is *R*p, and at least one internucleotidic linkage of formula **I** wherein P* is *S*p.

[00480] In some embodiments, W is O, S, or Se. In some embodiments, W is O. In some embodiments, W is S. In some embodiments, W is Se. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein W is O. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein W is S. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein W is Se.

[00481] In some embodiments, each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl.

[00482] In some embodiments, R is hydrogen. In some embodiments, R is an optionally substituted group selected from C_1–C_6 aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl.

[00483] In some embodiments, R is an optionally substituted C_1–C_6 aliphatic. In some embodiments, R is an optionally substituted C_1–C_6 alkyl. In some embodiments, R is optionally substituted, linear or branched hexyl. In some embodiments, R is optionally substituted, linear or branched pentyl. In some embodiments, R is optionally substituted, linear or branched butyl. In some embodiments, R is optionally substituted, linear or branched propyl. In some

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

[00484] In some embodiments, R is optionally substituted phenyl. In some embodiments, R is substituted phenyl. In some embodiments, R is phenyl.

[00485] In some embodiments, R is optionally substituted carbocyclyl. In some embodiments, R is optionally substituted C_3-C_{10} carbocyclyl. In some embodiments, R is optionally substituted monocyclic carbocyclyl. In some embodiments, R is optionally substituted cycloheptyl. In some embodiments, R is optionally substituted cyclohexyl. In some embodiments, R is optionally substituted cyclopentyl. In some embodiments, R is optionally substituted cyclobutyl. In some embodiments, R is an optionally substituted cyclopropyl. In some embodiments, R is optionally substituted bicyclic carbocyclyl.

[00486] In some embodiments, R is an optionally substituted aryl. In some embodiments, R is an optionally substituted bicyclic aryl ring.

[00487] In some embodiments, R is an optionally substituted heteroaryl. In some embodiments, R is an optionally substituted 5-6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, sulfur, or oxygen. In some embodiments, R is a substituted 5-6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an unsubstituted 5-6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, sulfur, or oxygen.

[00488] In some embodiments, R is an optionally substituted 5 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen or sulfur. In some embodiments, R is an optionally substituted 6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00489] In some embodiments, R is an optionally substituted 5-membered monocyclic heteroaryl ring having 1 heteroatom selected from nitrogen, oxygen, or sulfur. In some embodiments, R is selected from pyrrolyl, furanyl, or thienyl.

[00490] In some embodiments, R is an optionally substituted 5-membered heteroaryl ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R is an optionally substituted 5-membered heteroaryl ring having 1 nitrogen atom, and an additional heteroatom selected from sulfur or oxygen. Example R groups include

optionally substituted pyrazolyl, imidazolyl, thiazolyl, isothiazolyl, oxazolyl or isoxazolyl.

[00491] In some embodiments, R is a 6-membered heteroaryl ring having 1–3 nitrogen atoms. In other embodiments, R is an optionally substituted 6-membered heteroaryl ring having 1–2 nitrogen atoms. In some embodiments, R is an optionally substituted 6-membered heteroaryl ring having 2 nitrogen atoms. In certain embodiments, R is an optionally substituted 6-membered heteroaryl ring having 1 nitrogen. Example R groups include optionally substituted pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, or tetrazinyl.

[00492] In certain embodiments, R is an optionally substituted 8–10 membered bicyclic heteroaryl ring having 1–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted 5,6–fused heteroaryl ring having 1–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, R is an optionally substituted 5,6–fused heteroaryl ring having 1–2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R is an optionally substituted 5,6–fused heteroaryl ring having 1 heteroatom independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted indolyl. In some embodiments, R is an optionally substituted azabicyclo[3.2.1]octanyl. In certain embodiments, R is an optionally substituted 5,6–fused heteroaryl ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted azaindolyl. In some embodiments, R is an optionally substituted benzimidazolyl. In some embodiments, R is an optionally substituted benzothiazolyl. In some embodiments, R is an optionally substituted benzoxazolyl. In some embodiments, R is an optionally substituted indazolyl. In certain embodiments, R is an optionally substituted 5,6–fused heteroaryl ring having 3 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00493] In certain embodiments, R is an optionally substituted 6,6–fused heteroaryl ring having 1–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted 6,6–fused heteroaryl ring having 1–2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, R is an optionally substituted 6,6–fused heteroaryl ring having 1 heteroatom independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted quinolinyl. In some embodiments, R is an optionally substituted isoquinolinyl. According to one aspect, R is an optionally substituted 6,6–fused heteroaryl ring having 2 heteroatoms

independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is a quinazoline or a quinoxaline.

[00494] In some embodiments, R is an optionally substituted heterocyclyl. In some embodiments, R is an optionally substituted 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is a substituted 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an unsubstituted 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00495] In some embodiments, R is an optionally substituted heterocyclyl. In some embodiments, R is an optionally substituted 6 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted 6 membered partially unsaturated heterocyclic ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted 6 membered partially unsaturated heterocyclic ring having 2 oxygen atom.

[00496] In certain embodiments, R is a 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R is oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, oxepaneyl, aziridineyl, azetidineyl, pyrrolidinyl, piperidinyl, azepanyl, thiiranyl, thietanyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, thiepanyl, dioxolanyl, oxathiolanyl, oxazolidinyl, imidazolidinyl, thiazolidinyl, dithiolanyl, dioxanyl, morpholinyl, oxathianyl, piperazinyl, thiomorpholinyl, dithianyl, dioxepanyl, oxazepanyl, oxathiepanyl, dithiepanyl, diazepanyl, dihydrofuranonyl, tetrahydropyranonyl, oxepanonyl, pyrolidinonyl, piperidinonyl, azepanonyl, dihydrothiophenonyl, tetrahydrothiopyranonyl, thiepanonyl, oxazolidinonyl, oxazinanonyl, oxazepanonyl, dioxolanonyl, dioxanonyl, dioxepanonyl, oxathiolinonyl, oxathianonyl, oxathiepanonyl, thiazolidinonyl, thiazinanonyl, thiazepanonyl, imidazolidinonyl, tetrahydropyrimidinonyl, diazepanonyl, imidazolidinedionyl, oxazolidinedionyl, thiazolidinedionyl, dioxolanedionyl, oxathiolanedionyl, piperazinedionyl, morpholinedionyl, thiomorpholinedionyl, tetrahydropyranyl, tetrahydrofuranyl, morpholinyl, thiomorpholinyl,

piperidinyl, piperazinyl, pyrrolidinyl, tetrahydrothiophenyl, or tetrahydrothiopyranyl. In some embodiments, R is an optionally substituted 5 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00497] In certain embodiments, R is an optionally substituted 5–6 membered partially unsaturated monocyclic ring having 1–2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R is an optionally substituted tetrahydropyridinyl, dihydrothiazolyl, dihydrooxazolyl, or oxazolinyl group.

[00498] In some embodiments, R is an optionally substituted 8-10 membered bicyclic saturated or partially unsaturated heterocyclic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R is an optionally substituted indolinyl. In some embodiments, R is an optionally substituted isoindolinyl. In some embodiments, R is an optionally substituted 1, 2, 3, 4-tetrahydroquinoline. In some embodiments, R is an optionally substituted 1, 2, 3, 4-tetrahydroisoquinoline.

[00499] In some embodiments, each R′ is independently –R, -C(O)R, -CO_2R, or –SO_2R, or:

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

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

[00500] In some embodiments, R’ is –R, -C(O)R, -CO_2R, or –SO_2R, wherein R is as defined above and described herein.

[00501] In some embodiments, R’ is –R, wherein R is as defined and described above and herein. In some embodiments, R’ is hydrogen.

[00502] In some embodiments, R’ is –C(O)R, wherein R is as defined above and described herein. In some embodiments, R’ is –CO_2R, wherein R is as defined above and described herein. In some embodiments, R’ is –SO_2R, wherein R is as defined above and described herein.

[00503] In some embodiments, two R′ on the same nitrogen are taken together with their intervening atoms to form an optionally substituted heterocyclic or heteroaryl ring. In some embodiments, two R′ on the same carbon are taken together with their intervening atoms to form

an optionally substituted aryl, carbocyclic, heterocyclic, or heteroaryl ring.

[00504] In some embodiments, –Cy– is an optionally substituted bivalent ring selected from phenylene, carbocyclylene, arylene, heteroarylene, or heterocyclylene.

[00505] In some embodiments, –Cy– is optionally substituted phenylene. In some embodiments, –Cy– is optionally substituted carbocyclylene. In some embodiments, –Cy– is optionally substituted arylene. In some embodiments, –Cy– is optionally substituted heteroarylene. In some embodiments, –Cy– is optionally substituted heterocyclylene.

[00506] In some embodiments, each of X, Y and Z is independently –O–, –S–, –N(–L–R^1)–, or L, wherein each of L and R^1 is independently as defined above and described below.

[00507] In some embodiments, X is –O–. In some embodiments, X is –S–. In some embodiments, X is –O– or –S–. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein X is –O–. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein X is –S–. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein X is –O–, and at least one internucleotidic linkage of formula **I** wherein X is –S–. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage of formula **I** wherein X is –O–, and at least one internucleotidic linkage of formula **I** wherein X is –S–, and at least one internucleotidic linkage of formula **I** wherein L is an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C— , –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$– , –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–.

[00508] In some embodiments, X is –N(–L–R^1)–. In some embodiments, X is –N(R^1)–. In some embodiments, X is –N(R')–. In some embodiments, X is –N(R)–. In some embodiments, X is –NH–.

[00509] In some embodiments, X is L. In some embodiments, X is a covalent bond. In some embodiments, X is or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –

N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –$S(O)_2$–, –$S(O)_2$N(R′)–, –N(R′)$S(O)_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–. In some embodiments, X is an optionally substituted C_1–C_{10} alkylene or C_1–C_{10} alkenylene. In some embodiments, X is methylene.

[00510] In some embodiments, Y is –O–. In some embodiments, Y is –S–.

[00511] In some embodiments, Y is –N(–L–R^1)–. In some embodiments, Y is –N(R^1)–. In some embodiments, Y is –N(R')–. In some embodiments, Y is –N(R)–. In some embodiments, Y is –NH–.

[00512] In some embodiments, Y is L. In some embodiments, Y is a covalent bond. In some embodiments, Y is or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –$S(O)_2$–, –$S(O)_2$N(R′)–, –N(R′)$S(O)_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–. In some embodiments, Y is an optionally substituted C_1–C_{10} alkylene or C_1–C_{10} alkenylene. In some embodiments, Y is methylene.

[00513] In some embodiments, Z is –O–. In some embodiments, Z is –S–.

[00514] In some embodiments, Z is –N(–L–R^1)–. In some embodiments, Z is –N(R^1)–. In some embodiments, Z is –N(R')–. In some embodiments, Z is –N(R)–. In some embodiments, Z is –NH–.

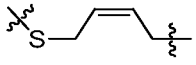
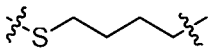
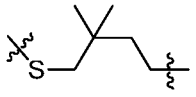
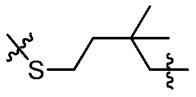
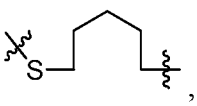
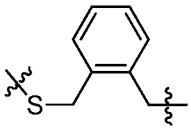
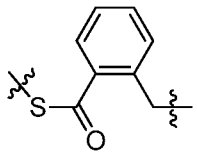
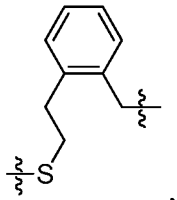
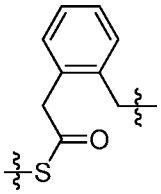
[00515] In some embodiments, Z is L. In some embodiments, Z is a covalent bond. In some embodiments, Z is or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –$S(O)_2$–, –$S(O)_2$N(R′)–, –N(R′)$S(O)_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–. In some embodiments, Z is an optionally substituted C_1–C_{10} alkylene or C_1–C_{10} alkenylene. In some embodiments, Z is methylene.

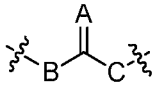
[00516] In some embodiments, L is a covalent bond or an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C— , –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –

C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –$S(O)_2$–, –$S(O)_2$N(R′)–, –N(R′)$S(O)_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–.

[00517] In some embodiments, L is a covalent bond. In some embodiments, L is an optionally substituted, linear or branched C_1–C_{10} alkylene, wherein one or more methylene units of L are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –$C(R')_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –$S(O)_2$–, –$S(O)_2$N(R′)–, –N(R′)$S(O)_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–.

[00518] In some embodiments, L has the structure of –L^1–V–, wherein:

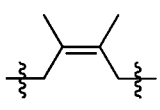
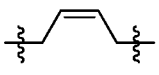
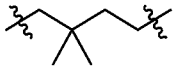
L^1 is an optionally substituted group selected from  ,  ,  ,  ,  ,  ,  ,  ,  , C$_1$–C$_6$ alkylene, C$_1$-C$_6$ alkenylene, carbocyclylene, arylene, C$_1$–C$_6$ heteroalkylene, heterocyclylene, and heteroarylene;

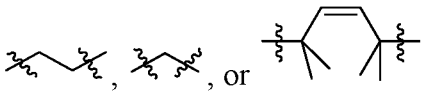
V is selected from –O–, –S–, –NR'–, $C(R')_2$, –S–S–, –B–S–S–C–,  , or an optionally substituted group selected from C$_1$–C$_6$ alkylene, arylene, C$_1$–C$_6$ heteroalkylene, heterocyclylene, and heteroarylene;

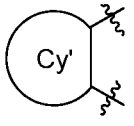
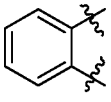
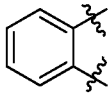
A is =O, =S, =NR', or $=C(R')_2$;

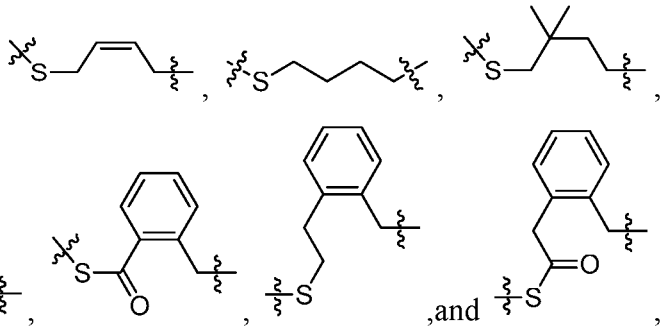
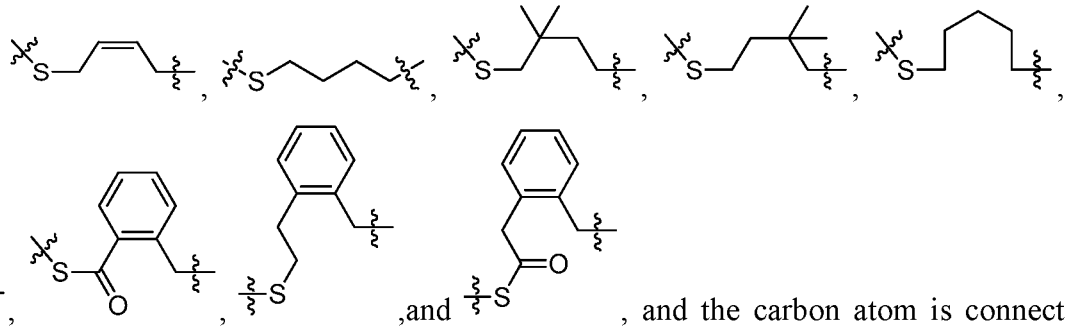
each of B and C is independently –O–, –S–, –NR'–, $–C(R')_2$–, or an optionally substituted group selected from C$_1$–C$_6$ alkylene, carbocyclylene, arylene, heterocyclylene, or heteroarylene; and

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

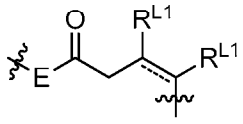
[00519] In some embodiments, L^1 is  ,  ,  ,  ,



[00520] In some embodiments, L^1 is  , wherein Ring Cy' is an optionally substituted arylene, carbocyclylene, heteroarylene, or heterocyclylene. In some embodiments, L^1 is optionally substituted  . In some embodiments, L^1 is  .

[00521] In some embodiments, L^1 is connected to X. In some embodiments, L^1 is an optionally substituted group selected from  , and the sulfur atom is connect to V. In some embodiments, L^1 is an optionally substituted group selected from  , and the carbon atom is connect to X.

[00522] In some embodiments, L has the structure of:



wherein:

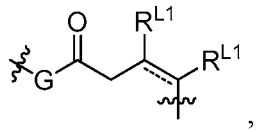
E is –O–, –S–, –NR'– or $–C(R')_2–$;

==== is a single or double bond;

the two R^{L1} are taken together with the two carbon atoms to which they are bound to form an

optionally substituted aryl, carbocyclic, heteroaryl or heterocyclic ring; and each R' is independently as defined above and described herein.

[00523] In some embodiments, L has the structure of:



,

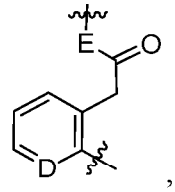
wherein:

G is –O–, –S–, or –NR';

⁼⁼⁼ is a single or double bond; and

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

[00524] In some embodiments, L has the structure of:



,

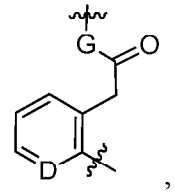
wherein:

E is –O–, –S–, –NR'– or –C(R')$_2$–;

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

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

[00525] In some embodiments, L has the structure of:



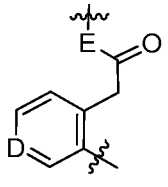
,

wherein:

G is –O–, –S–, or –NR';

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

[00526] In some embodiments, L has the structure of:



,

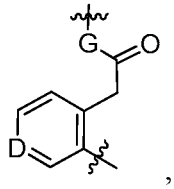
wherein:

E is –O–, –S–, –NR'– or $-C(R')_2-$;

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

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

[00527] In some embodiments, L has the structure of:



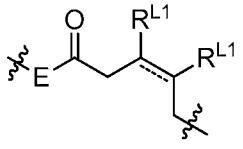
,

wherein:

G is O , S , or NR';

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

[00528] In some embodiments, L has the structure of:



,

wherein:

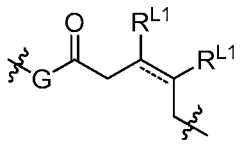
E is –O–, –S–, –NR'– or $-C(R')_2-$;

⁼⁼⁼ is a single or double bond;

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

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

[00529] In some embodiments, L has the structure of:



,

wherein:

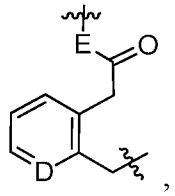
G is –O–, –S–, or –NR’;

⎓ is a single or double bond;

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

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

[00530] In some embodiments, L has the structure of:



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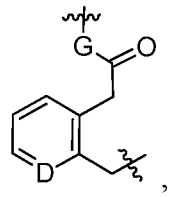
wherein:

E is –O–, –S–, –NR’– or –C(R’)$_2$–;

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

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

[00531] In some embodiments, L has the structure of:



,

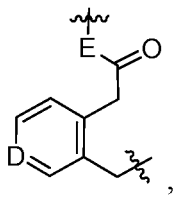
wherein:

G is –O–, –S–, or –NR’;

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

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

[00532] In some embodiments, L has the structure of:



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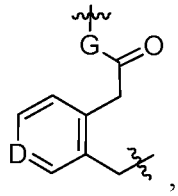
wherein:

E is –O–, –S–, –NR'– or $–C(R')_2–$;

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

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

[00533] In some embodiments, L has the structure of:



,

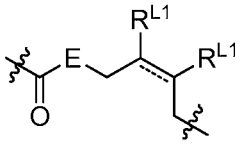
wherein:

G is –O–, –S–, or –NR';

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

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

[00534] In some embodiments, L has the structure of:



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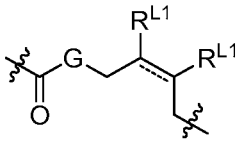
wherein:

E is –O–, –S–, –NR'– or $–C(R')_2–$;

⎓ is a single or double bond;

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

[00535] In some embodiments, L has the structure of:



,

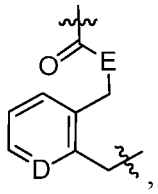
wherein:

G is –O–, –S–, or –NR';

⎓ is a single or double bond;

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

[00536] In some embodiments, L has the structure of:



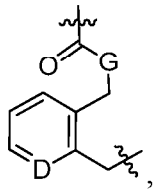
wherein:

E is –O–, –S–, –NR'– or $–C(R')_2–$;

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

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

[00537] In some embodiments, L has the structure of:



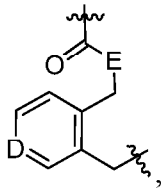
wherein:

G is –O–, –S–, or –NR';

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

R' is as defined above and described herein.

[00538] In some embodiments, L has the structure of:



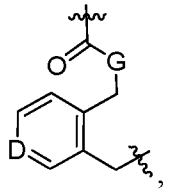
wherein:

E is –O–, –S–, –NR'– or $–C(R')_2–$;

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

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

[00539] In some embodiments, L has the structure of:



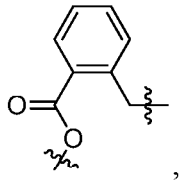
wherein:

G is –O–, –S–, or –NR';

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

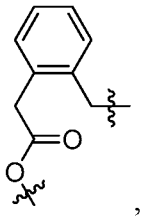
R' is as defined above and described herein.

[00540] In some embodiments, L has the structure of:



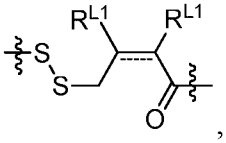
wherein the phenyl ring is optionally substituted. In some embodiments, the phenyl ring is not substituted. In some embodiments, the phenyl ring is substituted.

[00541] In some embodiments, L has the structure of:



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

[00542] In some embodiments, L has the structure of:



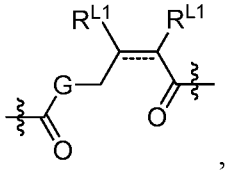
wherein:

⎓ is a single or double bond; and

the two R^{L1} are taken together with the two carbon atoms to which they are bound to form an

optionally substituted aryl, C_3-C_{10} carbocyclic, heteroaryl or heterocyclic ring.

[00543] In some embodiments, L has the structure of:



,

wherein:

G is –O–, –S–, or –NR';

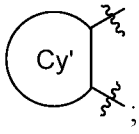
═══ is a single or double bond; and

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

[00544] In some embodiments, E is –O–, –S–, –NR'– or –C(R')$_2$–, wherein each R' independently as defined above and described herein. In some embodiments, E is –O–, –S–, or –NR'–. In some embodiments, E is –O–, –S–, or –NH–. In some embodiments, E is –O–. In some embodiments, E is –S–. In some embodiments, E is –NH–.

[00545] In some embodiments, G is –O–, –S–, or –NR', wherein each R' independently as defined above and described herein. In some embodiments, G is –O–, –S–, or –NH–. In some embodiments, G is –O–. In some embodiments, G is –S–. In some embodiments, G is –NH–.

[00546] In some embodiments, L is –L^3–G–, wherein:

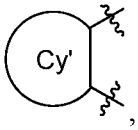
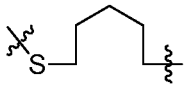
L^3 is an optionally substituted C_1–C_5 alkylene or alkenylene, wherein one or more methylene units are optionally and independently replaced by –O–, –S–,–N(R')–, –C(O)–, –C(S)–, –C(NR')–, –S(O)–, –S(O)$_2$–, or 

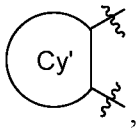
; and

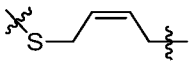
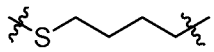
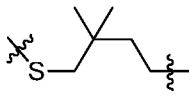
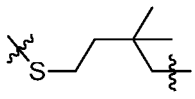
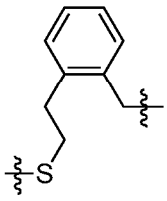
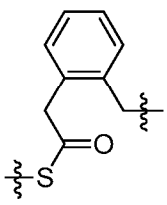
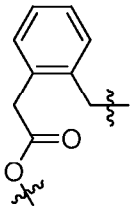
wherein each of G, R' and Ring Cy' is independently as defined above and described herein.

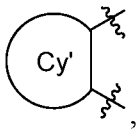
[00547] In some embodiments, L is –L^3–S–, wherein L^3 is as defined above and described herein. In some embodiments, L is –L^3–O–, wherein L^3 is as defined above and described herein. In some embodiments, L is –L^3–N(R')–, wherein each of L^3 and R' is independently as defined above and described herein. In some embodiments, L is –L^3–NH–, wherein each of L^3 and R' is independently as defined above and described herein.

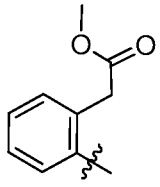
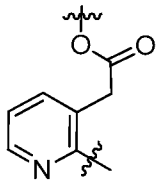
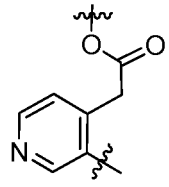
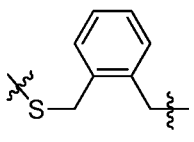
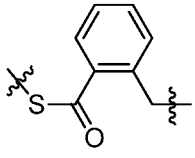
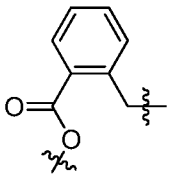
[00548] In some embodiments, L^3 is an optionally substituted C_5 alkylene or alkenylene, wherein one or more methylene units are optionally and independently replaced by –O–, –S–,–

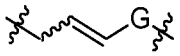
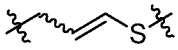
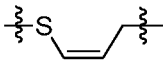
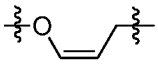
N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –S(O)–, –S(O)$_2$–, or  , and each of R' and Ring Cy' is independently as defined above and described herein. In some embodiments, L^3 is an optionally substituted C_5 alkylene. In some embodiments, –L^3–G– is  .

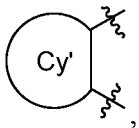
[00549] In some embodiments, L^3 is an optionally substituted C_4 alkylene or alkenylene, wherein one or more methylene units are optionally and independently replaced by –O–, –S–,–N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –S(O)–, –S(O)$_2$–, or  , and each of R' and Cy' is independently as defined above and described herein.

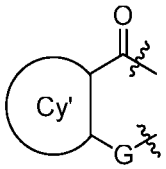
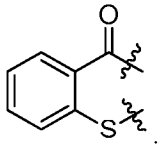
[00550] In some embodiments, –L^3–G– is  ,  ,  ,  ,  ,  , or  .

[00551] In some embodiments, L^3 is an optionally substituted C_3 alkylene or alkenylene, wherein one or more methylene units are optionally and independently replaced by –O–, –S–,–N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –S(O)–, –S(O)$_2$–, or  , and each of R' and Cy' is independently as defined above and described herein.

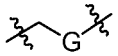
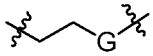
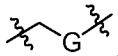
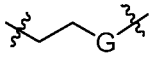
[00552] In some embodiments, –L^3–G– is  ,  ,  ,  ,  , or  .

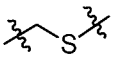
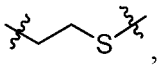
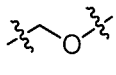
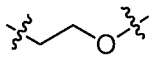
[00553] In some embodiments, L is  . In some embodiments, L is  or  . In some embodiments, L is  or  .

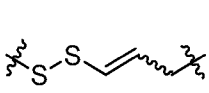
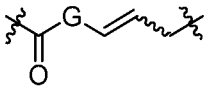
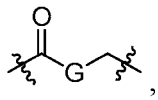
[00554] In some embodiments, L^3 is an optionally substituted C_2 alkylene or alkenylene, wherein one or more methylene units are optionally and independently replaced by –O–, –S–,–N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –S(O)–, –$S(O)_2$–, or  , and each of R' and Cy' is independently as defined above and described herein.

[00555] In some embodiments, –L^3–G– is  , wherein each of G and Cy' is independently as defined above and described herein. In some embodiments, L is  .

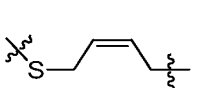
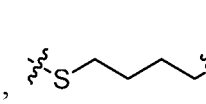
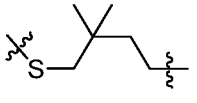
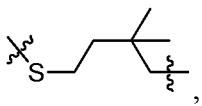
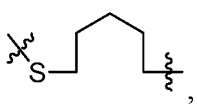
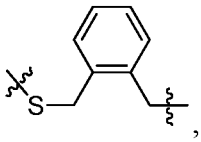
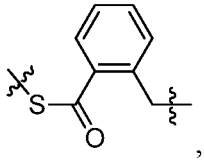
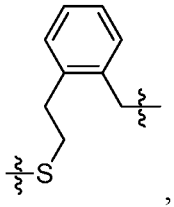
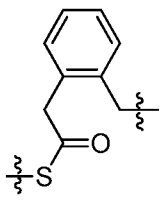
[00556] In some embodiments, L is –L^4–G–, wherein L^4 is an optionally substituted C_1–C_2 alkylene; and G is as defined above and described herein. In some embodiments, L is –L^4–G–, wherein L^4 is an optionally substituted C_1–C_2 alkylene; G is as defined above and described herein; and G is connected to R^1. In some embodiments, L is –L^4–G–, wherein L^4 is an optionally substituted methylene; G is as defined above and described herein; and G is connected to R^1. In some embodiments, L is –L^4–G–, wherein L^4 is methylene; G is as defined above and described herein; and G is connected to R^1. In some embodiments, L is –L^4–G–, wherein L^4 is an optionally substituted –$(CH_2)_2$–; G is as defined above and described herein; and G is connected to R^1. In some embodiments, L is –L^4–G–, wherein L^4 is –$(CH_2)_2$–; G is as defined above and described herein; and G is connected to R^1.

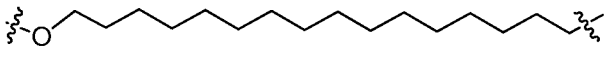
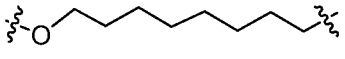
[00557] In some embodiments, L is  , or  , wherein G is as defined above and described herein, and G is connected to R^1. In some embodiments, L is  , wherein G is as defined above and described herein, and G is connected to R^1. In some embodiments, L is  , wherein G is as defined above and described herein, and G is

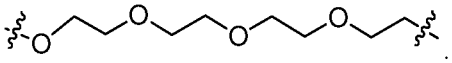
connected to R^1. In some embodiments, L is  , or  , wherein the sulfur atom is connected to R^1. In some embodiments, L is  , or  , wherein the oxygen atom is connected to R^1.

[00558] In some embodiments, L is  ,  , or  , wherein G is as defined above and described herein.

[00559] In some embodiments, L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$, wherein R^{L3} is an optionally substituted, linear or branched, $C_1–C_9$ alkylene, wherein one or more methylene units are optionally and independently replaced by an optionally substituted $C_1–C_6$ alkylene, $C_1–C_6$ alkenylene, $—C\equiv C—$, $–C(R')_2–$, –Cy–, –O–, –S–, –S–S–, $–N(R')–$, $–C(O)–$, $–C(S)–$, $–C(NR')–$, $–C(O)N(R')–$, $–N(R')C(O)N(R')-$, $–N(R')C(O)–$, $–N(R')C(O)O–$, $–OC(O)N(R')-$, $–S(O)–$, $–S(O)_2–$, $–S(O)_2N(R')–$, $–N(R')S(O)_2–$, $–SC(O)–$, $–C(O)S–$, $–OC(O)–$, or $–C(O)O–$, wherein each of R' and –Cy– is independently as defined above and described herein. In some embodiments, L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$, wherein R^{L3} is an optionally substituted $C_1–C_6$ alkylene. In some embodiments, L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$, wherein R^{L3} is an optionally substituted $C_1–C_6$ alkenylene. In some embodiments, L is $–S–R^{L3}–$ or $–S–C(O)–R^{L3}–$, wherein R^{L3} is an optionally substituted C_1.C_6 alkylene wherein one or more methylene units are optionally and independently replaced by an optionally substituted $C_1–C_6$ alkenylene, arylene, or heteroarylene. In some embodiments, In some embodiments, R^{L3} is an optionally substituted $–S–(C_1–C_6$ alkenylene$)–$, $–S–(C_1–C_6$ alkylene$)–$, $–S–(C_1–C_6$ alkylene$)–$arylene$–(C_1–C_6$ alkylene$)–$, $–S–CO–$arylene$–(C_1–C_6$ alkylene$)–$, or $–S–CO–(C_1–C_6$ alkylene$)–$arylene$–(C_1–C_6$ alkylene$)–$.

[00560] In some embodiments, L is  ,  ,  ,  ,  ,  ,  ,  , or  .

[00561] In some embodiments, L is  . In some embodiments, L is  . In some embodiments,



[00562] In some embodiments, the sulfur atom in the L embodiments described above and herein is connected to X. In some embodiments, the sulfur atom in the L embodiments described above and herein is connected to R^1.

[00563] In some embodiments, R^1 is halogen, R, or an optionally substituted C_1–C_{50} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, wherein each variable is independently as defined above and described herein. In some embodiments, R^1 is halogen, R, or an optionally substituted C_1–C_{10} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, wherein each variable is independently as defined above and described herein.

[00564] In some embodiments, R^1 is hydrogen. In some embodiments, R^1 is halogen. In some embodiments, R^1 is –F. In some embodiments, R^1 is –Cl. In some embodiments, R^1 is –Br. In some embodiments, R^1 is –I.

[00565] In some embodiments, R^1 is R wherein R is as defined above and described herein.

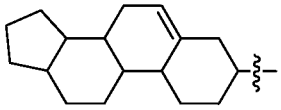
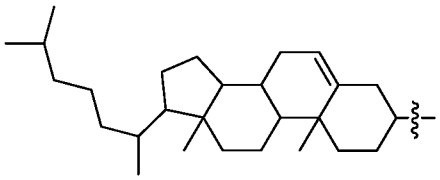
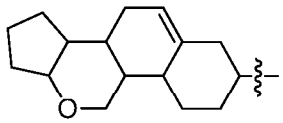
[00566] In some embodiments, R^1 is hydrogen. In some embodiments, R^1 is an optionally substituted group selected from C_1–C_{50} aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl.

[00567] In some embodiments, R^1 is an optionally substituted C_1–C_{50} aliphatic. In some embodiments, R^1 is an optionally substituted C_1–C_{10} aliphatic. In some embodiments, R^1 is an optionally substituted C_1–C_6 aliphatic. In some embodiments, R^1 is an optionally substituted C_1–C_6 alkyl. In some embodiments, R^1 is optionally substituted, linear or branched hexyl. In some embodiments, R^1 is optionally substituted, linear or branched pentyl. In some embodiments, R^1 is optionally substituted, linear or branched butyl. In some embodiments, R^1 is optionally

substituted, linear or branched propyl. In some embodiments, R^1 is optionally substituted ethyl. In some embodiments, R^1 is optionally substituted methyl.

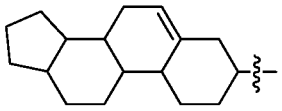
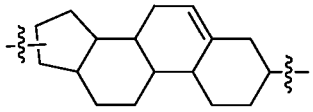
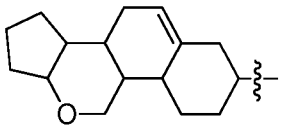
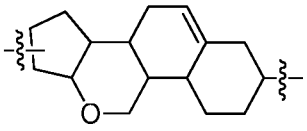
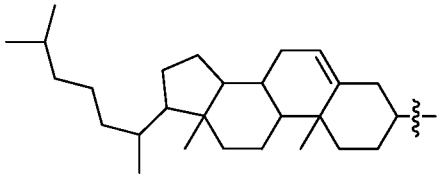
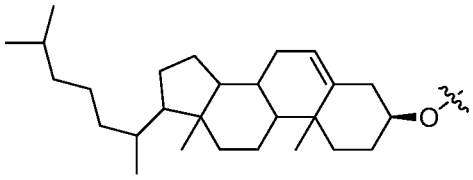
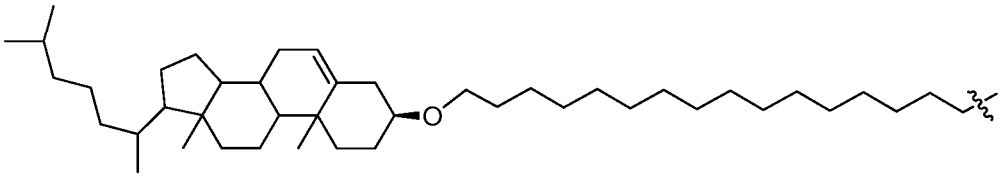
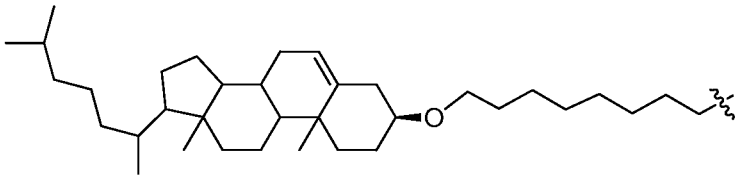
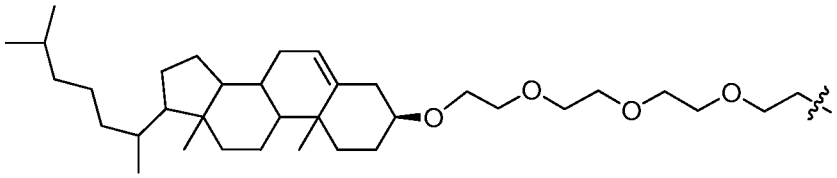
[00568] In some embodiments, R^1 is optionally substituted phenyl. In some embodiments, R^1 is substituted phenyl. In some embodiments, R^1 is phenyl.

[00569] In some embodiments, R^1 is optionally substituted carbocyclyl. In some embodiments, R^1 is optionally substituted C_3-C_{10} carbocyclyl. In some embodiments, R^1 is optionally substituted monocyclic carbocyclyl. In some embodiments, R^1 is optionally substituted cycloheptyl. In some embodiments, R^1 is optionally substituted cyclohexyl. In some embodiments, R^1 is optionally substituted cyclopentyl. In some embodiments, R^1 is optionally substituted cyclobutyl. In some embodiments, R^1 is an optionally substituted cyclopropyl. In some embodiments, R^1 is optionally substituted bicyclic carbocyclyl.

[00570] In some embodiments, R^1 is an optionally substituted C_1–C_{50} polycyclic hydrocarbon. In some embodiments, R^1 is an optionally substituted C_1–C_{50} polycyclic hydrocarbon wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, wherein each variable is independently as defined above and described herein. In some embodiments, R^1 is optionally substituted  . In some embodiments, R^1 is  . In some embodiments, R^1 is optionally substituted  .

[00571] In some embodiments, R^1 is an optionally substituted C_1–C_{50} aliphatic comprising one or more optionally substituted polycyclic hydrocarbon moieties. In some embodiments, R^1 is an optionally substituted C_1–C_{50} aliphatic comprising one or more optionally substituted polycyclic hydrocarbon moieties, wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C— , –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –

C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –$S(O)_2$–, –$S(O)_2N(R')$–, –$N(R')S(O)_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, wherein each variable is independently as defined above and described herein. In some embodiments, R^1 is an optionally substituted C_1–C_{50} aliphatic comprising one or more optionally substituted

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

[00572] In some embodiments, R^1 is an optionally substituted aryl. In some embodiments, R^1 is an optionally substituted bicyclic aryl ring.

[00573] In some embodiments, R^1 is an optionally substituted heteroaryl. In some embodiments, R^1 is an optionally substituted 5-6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, sulfur, or oxygen. In some embodiments, R^1 is a substituted 5-6 membered monocyclic heteroaryl ring having 1-3 heteroatoms

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

[00574] In some embodiments, R^1 is an optionally substituted 5 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen or sulfur. In some embodiments, R^1 is an optionally substituted 6 membered monocyclic heteroaryl ring having 1-3 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00575] In some embodiments, R^1 is an optionally substituted 5-membered monocyclic heteroaryl ring having 1 heteroatom selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is selected from pyrrolyl, furanyl, or thienyl.

[00576] In some embodiments, R^1 is an optionally substituted 5-membered heteroaryl ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R^1 is an optionally substituted 5-membered heteroaryl ring having 1 nitrogen atom, and an additional heteroatom selected from sulfur or oxygen. Example R^1 groups include optionally substituted pyrazolyl, imidazolyl, thiazolyl, isothiazolyl, oxazolyl or isoxazolyl.

[00577] In some embodiments, R^1 is a 6-membered heteroaryl ring having 1–3 nitrogen atoms. In other embodiments, R^1 is an optionally substituted 6-membered heteroaryl ring having 1–2 nitrogen atoms. In some embodiments, R^1 is an optionally substituted 6-membered heteroaryl ring having 2 nitrogen atoms. In certain embodiments, R^1 is an optionally substituted 6-membered heteroaryl ring having 1 nitrogen. Example R^1 groups include optionally substituted pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, or tetrazinyl.

[00578] In certain embodiments, R^1 is an optionally substituted 8–10 membered bicyclic heteroaryl ring having 1–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted 5,6–fused heteroaryl ring having 1–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, R^1 is an optionally substituted 5,6–fused heteroaryl ring having 1–2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R^1 is an optionally substituted 5,6–fused heteroaryl ring having 1 heteroatom independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted indolyl. In some embodiments, R^1 is an optionally substituted azabicyclo[3.2.1]octanyl. In certain embodiments, R^1 is an optionally substituted 5,6–fused heteroaryl ring having 2 heteroatoms independently

selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted azaindolyl. In some embodiments, R^1 is an optionally substituted benzimidazolyl. In some embodiments, R^1 is an optionally substituted benzothiazolyl. In some embodiments, R^1 is an optionally substituted benzoxazolyl. In some embodiments, R^1 is an optionally substituted indazolyl. In certain embodiments, R^1 is an optionally substituted 5,6–fused heteroaryl ring having 3 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00579] In certain embodiments, R^1 is an optionally substituted 6,6–fused heteroaryl ring having 1–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted 6,6–fused heteroaryl ring having 1–2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, R^1 is an optionally substituted 6,6–fused heteroaryl ring having 1 heteroatom independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted quinolinyl. In some embodiments, R^1 is an optionally substituted isoquinolinyl. According to one aspect, R^1 is an optionally substituted 6,6–fused heteroaryl ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is a quinazoline or a quinoxaline.

[00580] In some embodiments, R^1 is an optionally substituted heterocyclyl. In some embodiments, R^1 is an optionally substituted 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is a substituted 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an unsubstituted 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00581] In some embodiments, R^1 is an optionally substituted heterocyclyl. In some embodiments, R^1 is an optionally substituted 6 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted 6 membered partially unsaturated heterocyclic ring having 2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted 6 membered partially unsaturated heterocyclic ring having 2 oxygen atoms.

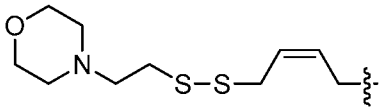
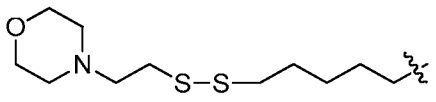
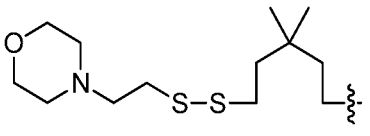
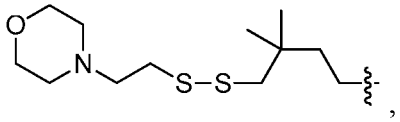
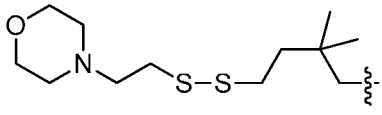
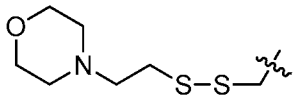
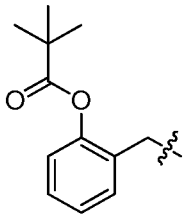
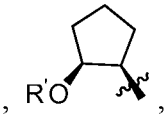
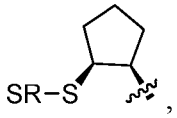
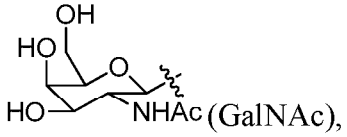
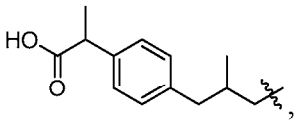
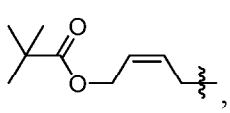
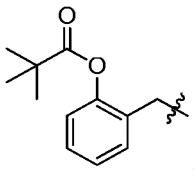
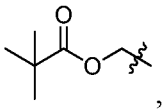
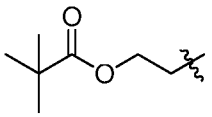
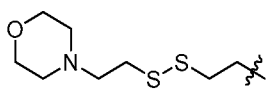
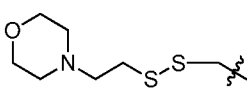
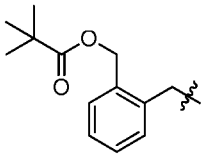
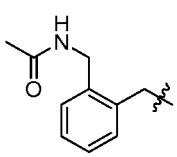
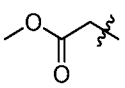
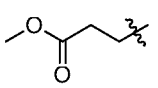
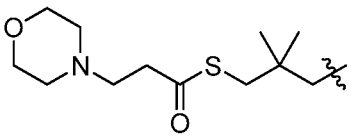
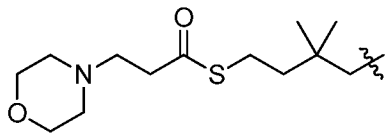
[00582] In certain embodiments, R^1 is a 3-7 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R^1 is oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, oxepaneyl, aziridineyl, azetidineyl, pyrrolidinyl, piperidinyl, azepanyl, thiiranyl, thietanyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, thiepanyl, dioxolanyl, oxathiolanyl, oxazolidinyl, imidazolidinyl, thiazolidinyl, dithiolanyl, dioxanyl, morpholinyl, oxathianyl, piperazinyl, thiomorpholinyl, dithianyl, dioxepanyl, oxazepanyl, oxathiepanyl, dithiepanyl, diazepanyl, dihydrofuranonyl, tetrahydropyranonyl, oxepanonyl, pyrolidinonyl, piperidinonyl, azepanonyl, dihydrothiophenonyl, tetrahydrothiopyranonyl, thiepanonyl, oxazolidinonyl, oxazinanonyl, oxazepanonyl, dioxolanonyl, dioxanonyl, dioxepanonyl, oxathiolinonyl, oxathianonyl, oxathiepanonyl, thiazolidinonyl, thiazinanonyl, thiazepanonyl, imidazolidinonyl, tetrahydropyrimidinonyl, diazepanonyl, imidazolidinedionyl, oxazolidinedionyl, thiazolidinedionyl, dioxolanedionyl, oxathiolanedionyl, piperazinedionyl, morpholinedionyl, thiomorpholinedionyl, tetrahydropyranyl, tetrahydrofuranyl, morpholinyl, thiomorpholinyl, piperidinyl, piperazinyl, pyrrolidinyl, tetrahydrothiophenyl, or tetrahydrothiopyranyl. In some embodiments, R^1 is an optionally substituted 5 membered saturated or partially unsaturated heterocyclic ring having 1-2 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

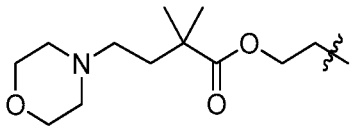
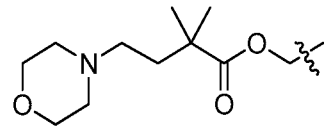
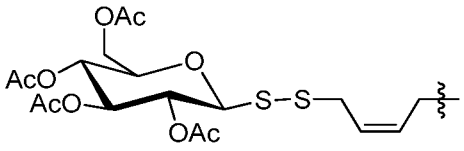
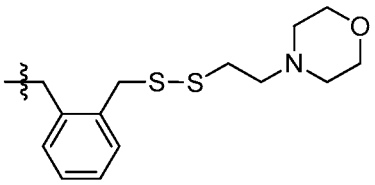
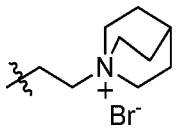
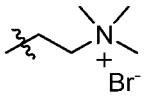
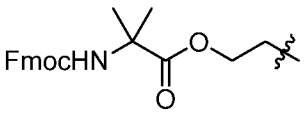
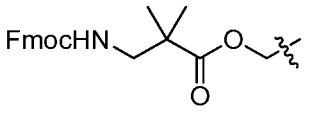
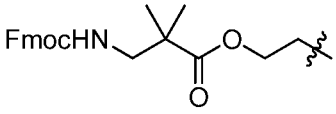
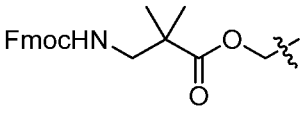
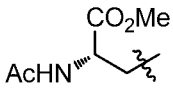
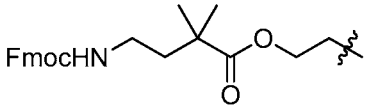
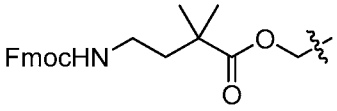
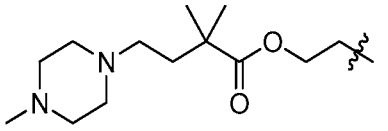
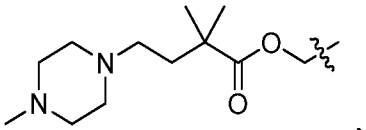
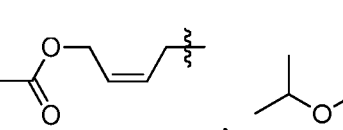
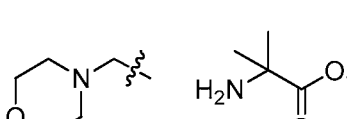
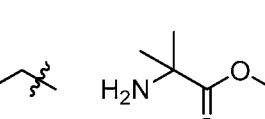
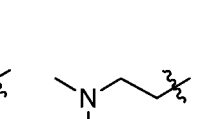
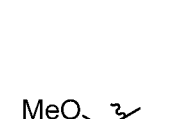
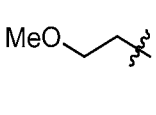
[00583] In certain embodiments, R^1 is an optionally substituted 5–6 membered partially unsaturated monocyclic ring having 1–2 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, R^1 is an optionally substituted tetrahydropyridinyl, dihydrothiazolyl, dihydrooxazolyl, or oxazolinyl group.

[00584] In some embodiments, R^1 is an optionally substituted 8-10 membered bicyclic saturated or partially unsaturated heterocyclic ring having 1-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In some embodiments, R^1 is an optionally substituted indolinyl. In some embodiments, R^1 is an optionally substituted isoindolinyl. In some embodiments, R^1 is an optionally substituted 1, 2, 3, 4-tetrahydroquinoline. In some embodiments, R^1 is an optionally substituted 1, 2, 3, 4-tetrahydroisoquinoline.

[00585] In some embodiments, R^1 is an optionally substituted C_1–C_{10} aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R′)$_2$–, –Cy–, –O–, –S–, –S–S–, –

N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, $–S(O)_2–$, $–S(O)_2N(R′)–$, $–N(R′)S(O)_2–$, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, wherein each variable is independently as defined above and described herein. In some embodiments, R^1 is an optionally substituted $C_1–C_{10}$ aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally–Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, $–S(O)_2–$, $–S(O)_2N(R′)–$, $–N(R′)S(O)_2–$, –OC(O)–, or –C(O)O–, wherein each R' is independently as defined above and described herein. In some embodiments, R^1 is an optionally substituted $C_1–C_{10}$ aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally–Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –OC(O)–, or –C(O)O–, wherein each R' is independently as defined above and described herein.

[00586] In some embodiments, R^1 is  ,  ,  ,  ,  ,  ,  ,  ,  ,  , (GalNAc),  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,


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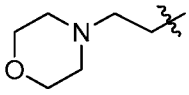
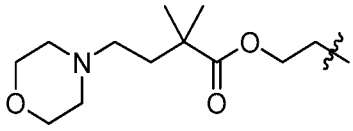
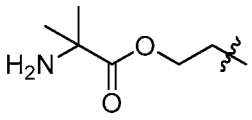
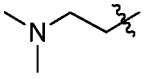
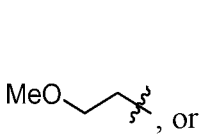
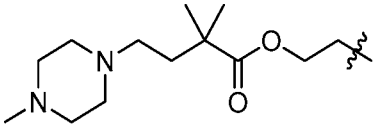
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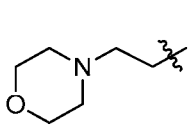
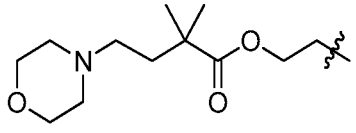
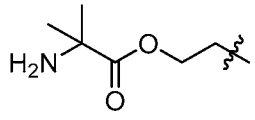
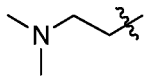
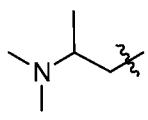
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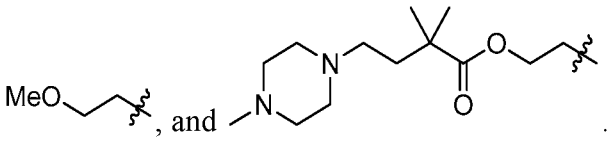
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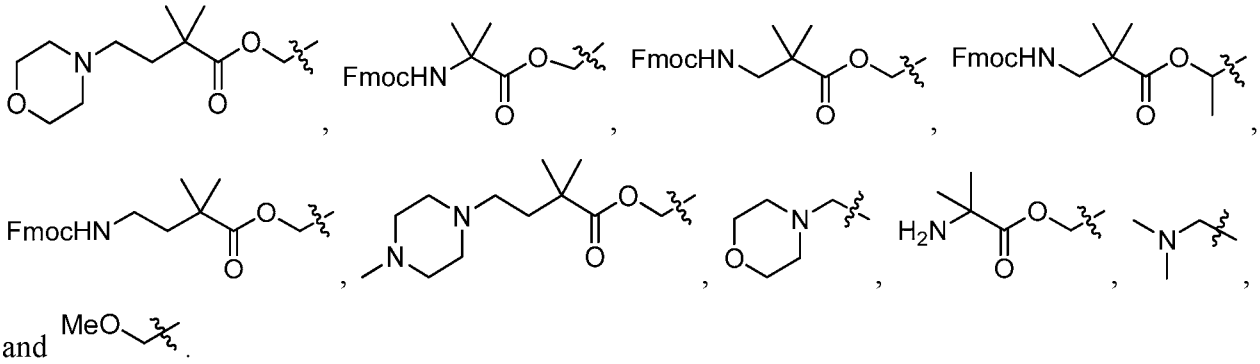
[00587] In some embodiments, R^1 is CH_3–,

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[00588] In some embodiments, R^1 comprises a terminal optionally substituted $–(CH_2)_2–$ moiety which is connected to L. Examples of such R^1 groups are depicted below:


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[00589] In some embodiments, R^1 comprises a terminal optionally substituted $–(CH_2)–$ moiety which is connected to L. Example such R^1 groups are depicted below:

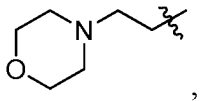
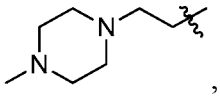
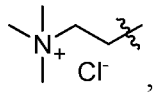
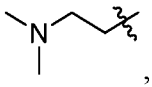
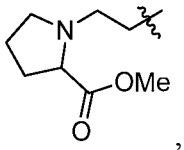
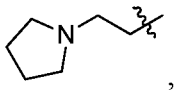
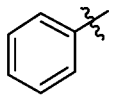


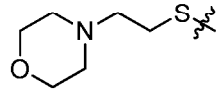
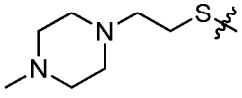
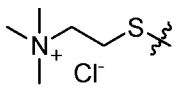
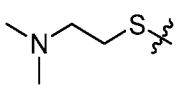
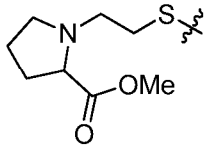
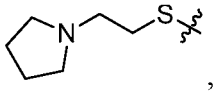
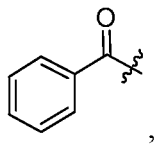
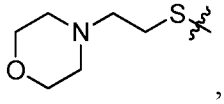
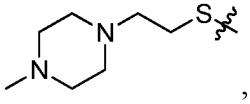
[00590] In some embodiments, R^1 is $–S–R^{L2}$, wherein R^{L2} is an optionally substituted $C_1–C_9$ aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted $C_1–C_6$ alkylene, $C_1–C_6$ alkenylene, —C≡C—, $–C(R')_2–$, –Cy–, –O–, –S–, –S–S–, $–N(R')–$, $–C(O)–$, $–C(S)–$, $–C(NR')–$, $–C(O)N(R')–$, $–N(R')C(O)N(R')-$, $–N(R')C(O)–$, $–N(R')C(O)O–$, $–OC(O)N(R')-$, $–S(O)–$, $–S(O)_2–$, $–S(O)_2N(R')–$, $–N(R')S(O)_2–$, $–SC(O)–$, $–C(O)S–$, $–OC(O)–$, or $–C(O)O–$, and each of R' and –Cy– is independently as defined above and described herein. In some embodiments, R^1 is $–S–R^{L2}$, wherein the sulfur atom is connected with the sulfur atom in L group.

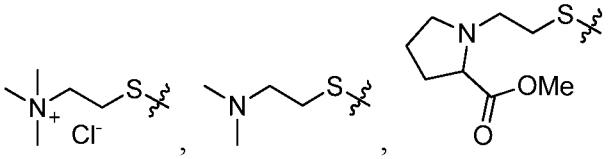
[00591] In some embodiments, R^1 is $–C(O)–R^{L2}$, wherein R^{L2} is an optionally substituted $C_1–C_9$ aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted $C_1–C_6$ alkylene, $C_1–C_6$ alkenylene, —C≡C—, $–C(R')_2–$, –Cy–, –O–, –S–, –S–S–, $–N(R')–$, $–C(O)–$, $–C(S)–$, $–C(NR')–$, $–C(O)N(R')–$, $–N(R')C(O)N(R')-$, $–N(R')C(O)–$, $–N(R')C(O)O–$, $–OC(O)N(R')-$, $–S(O)–$, $–S(O)_2–$, $–S(O)_2N(R')–$, $–N(R')S(O)_2–$, $–SC(O)–$, $–C(O)S–$, $–OC(O)–$, or $–C(O)O–$, and each of R' and –Cy– is independently as defined above and described herein. In some embodiments, R^1 is $–C(O)–R^{L2}$, wherein the carbonyl group is connected with G in L group. In some embodiments, R^1 is $–C(O)–R^{L2}$, wherein the carbonyl group is connected with the sulfur atom in L group.

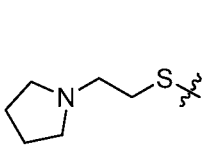
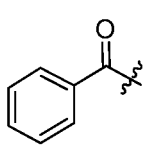
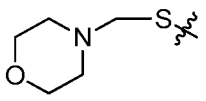
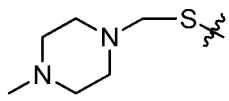
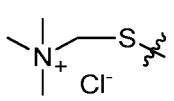
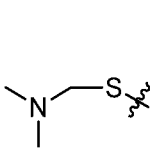
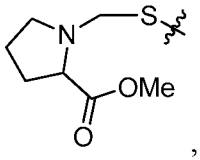
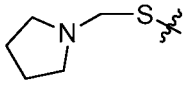
[00592] In some embodiments, R^{L2} is optionally substituted $C_1–C_9$ aliphatic. In some embodiments, R^{L2} is optionally substituted $C_1–C_9$ alkyl. In some embodiments, R^{L2} is optionally

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

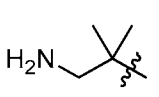
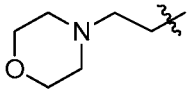
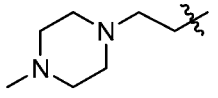
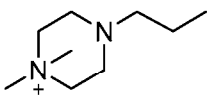
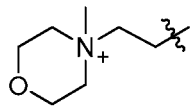
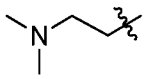
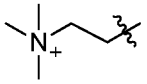
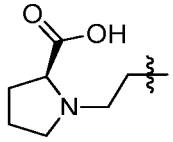
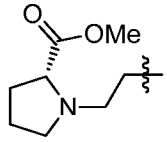
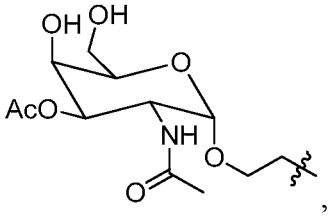
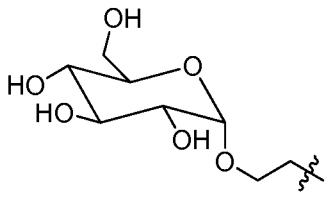
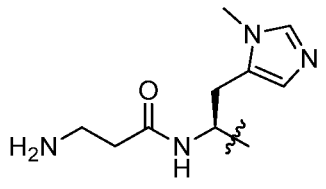

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[00593] In some embodiments, R^1 is hydrogen, or an optionally substituted group selected from

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, –S–(C_1–C_{10} aliphatic), C_1–C_{10} aliphatic, aryl, C_1–C_6 heteroalkyl, heteroaryl and heterocyclyl. In some embodiments, R^1 is

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





, or

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[00594] In some embodiments, R^1 is an optionally substituted group selected from –S–(C_1-C_6 aliphatic), C_1-C_{10} aliphatic, C_1-C_6 heteroaliphatic, aryl, heterocyclyl and heteroaryl.

[00595] In some embodiments, R^1 is












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[00596] In some embodiments, the sulfur atom in the R^1 embodiments described above and herein is connected with the sulfur atom, G, E, or –C(O)– moiety in the L embodiments described above and herein. In some embodiments, the –C(O)– moiety in the R^1 embodiments described above and herein is connected with the sulfur atom, G, E, or –C(O)– moiety in the L embodiments described above and herein.

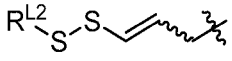
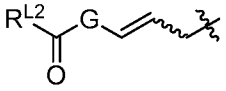
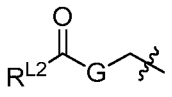
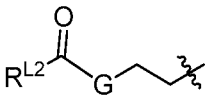
[00597] In some embodiments, –L–R^1 is any combination of the L embodiments and R^1 embodiments described above and herein.

[00598] In some embodiments, –L–R^1 is –L^3–G–R^1 wherein each variable is independently as defined above and described herein.

[00599] In some embodiments, –L–R^1 is –L^4–G–R^1 wherein each variable is independently as defined above and described herein.

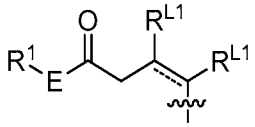
[00600] In some embodiments, –L–R^1 is –L^3–G–S–R^{L2}, wherein each variable is independently as defined above and described herein.

[00601] In some embodiments, –L–R^1 is –L^3–G–C(O)–R^{L2}, wherein each variable is independently as defined above and described herein.

[00602] In some embodiments, –L–R^1 is  ,  ,  , or  , wherein R^{L2} is an optionally substituted C_1–C_9 aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, –C(R')$_2$–, –Cy–, –O–, –S–, –S–S–, –N(R′)–, –C(O)–, –C(S)–, –C(NR′)–, –C(O)N(R′)–, –N(R′)C(O)N(R′)-, –N(R′)C(O)–, –N(R′)C(O)O–, –OC(O)N(R′)-, –S(O)–, –S(O)$_2$–, –S(O)$_2$N(R′)–, –N(R′)S(O)$_2$–, –SC(O)–, –C(O)S–, –OC(O)–, or –C(O)O–, and each G is independently as defined above and described herein.

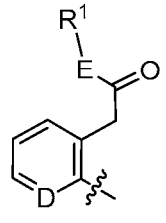
[00603] In some embodiments, –L–R^1 is –R^{L3}–S–S–R^{L2}, wherein each variable is independently as defined above and described herein. In some embodiments, –L–R^1 is –R^{L3}–C(O)–S–S–R^{L2}, wherein each variable is independently as defined above and described herein.

[00604] In some embodiments, –L–R^1 has the structure of:

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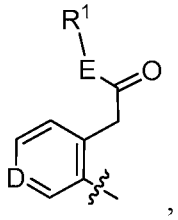
wherein each variable is independently as defined above and described herein.

[00605] In some embodiments, –L–R^1 has the structure of:

 ,

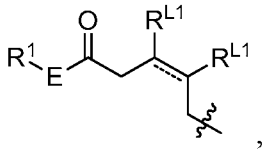
wherein each variable is independently as defined above and described herein.

[00606] In some embodiments, –L–R^1 has the structure of:



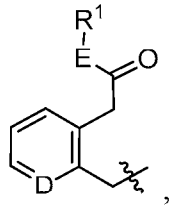
wherein each variable is independently as defined above and described herein.

[00607] In some embodiments, –L–R^1 has the structure of:



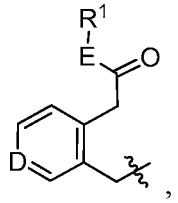
wherein each variable is independently as defined above and described herein.

[00608] In some embodiments, –L–R^1 has the structure of:



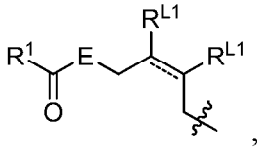
wherein each variable is independently as defined above and described herein.

[00609] In some embodiments, –L–R^1 has the structure of:



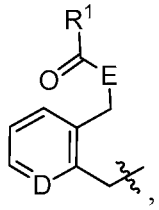
wherein each variable is independently as defined above and described herein.

[00610] In some embodiments, –L–R^1 has the structure of:



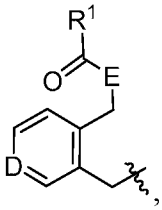
wherein each variable is independently as defined above and described herein.

[00611] In some embodiments, –L–R^1 has the structure of:



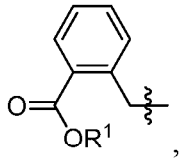
wherein each variable is independently as defined above and described herein.

[00612] In some embodiments, –L–R^1 has the structure of:



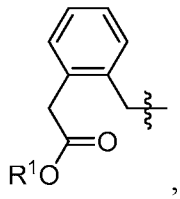
wherein each variable is independently as defined above and described herein.

[00613] In some embodiments, –L–R^1 has the structure of:



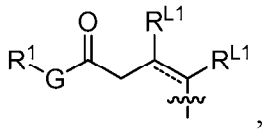
wherein each variable is independently as defined above and described herein.

[00614] In some embodiments, –L–R^1 has the structure of:



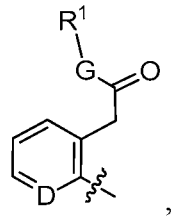
wherein each variable is independently as defined above and described herein.

[00615] In some embodiments, –L–R^1 has the structure of:



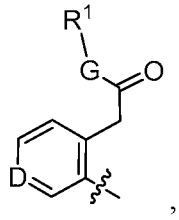
wherein each variable is independently as defined above and described herein.

[00616] In some embodiments, –L–R^1 has the structure of:



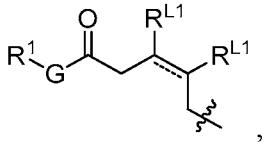
wherein each variable is independently as defined above and described herein.

[00617] In some embodiments, –L–R^1 has the structure of:



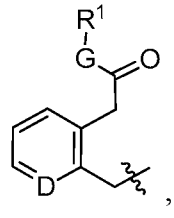
wherein each variable is independently as defined above and described herein.

[00618] In some embodiments, –L–R^1 has the structure of:



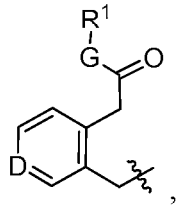
wherein each variable is independently as defined above and described herein.

[00619] In some embodiments, –L–R^1 has the structure of:



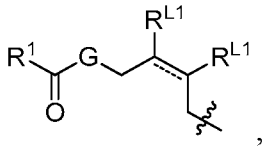
wherein each variable is independently as defined above and described herein.

[00620] In some embodiments, –L–R^1 has the structure of:



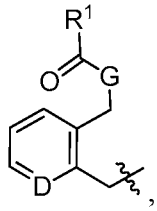
wherein each variable is independently as defined above and described herein.

[00621] In some embodiments, –L–R^1 has the structure of:



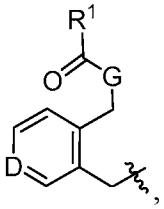
wherein each variable is independently as defined above and described herein.

[00622] In some embodiments, –L–R^1 has the structure of:



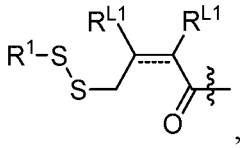
wherein each variable is independently as defined above and described herein.

[00623] In some embodiments, –L–R^1 has the structure of:



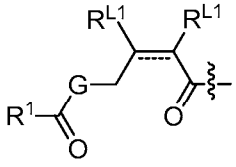
wherein each variable is independently as defined above and described herein.

[00624] In some embodiments, –L–R^1 has the structure of:



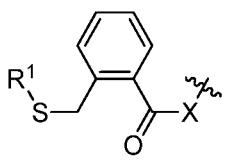
wherein each variable is independently as defined above and described herein.

[00625] In some embodiments, L has the structure of:



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

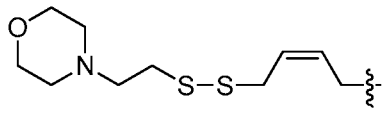
[00626] In some embodiments, –X–L–R^1 has the structure of:

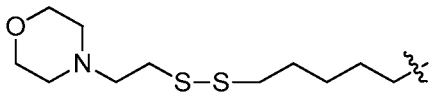
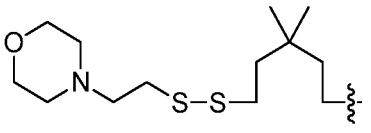
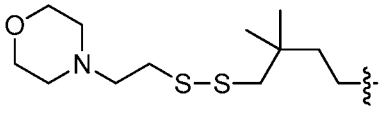


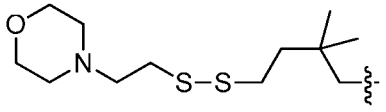
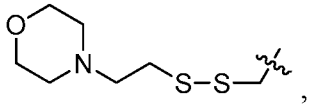
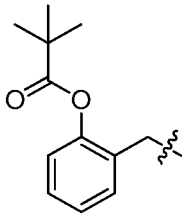
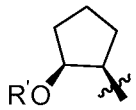
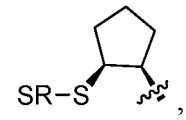
wherein:

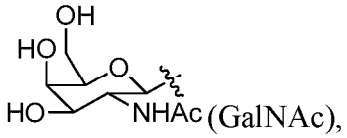
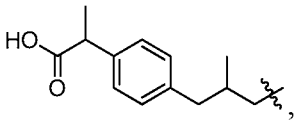
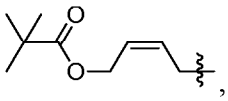
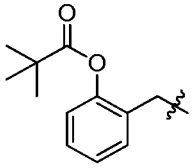
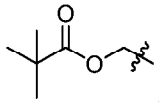
the phenyl ring is optionally substituted, and

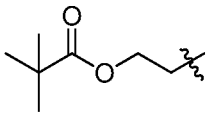
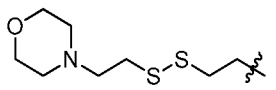
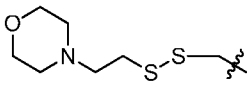
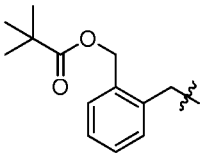
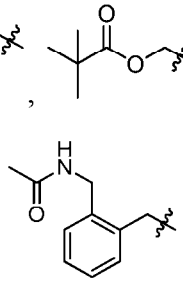
each of R^1 and X is independently as defined above and described herein.

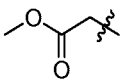
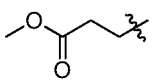
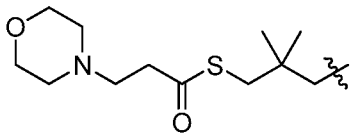
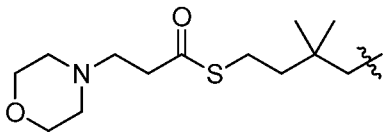
[00627] In some embodiments, –L–R^1 is  ,

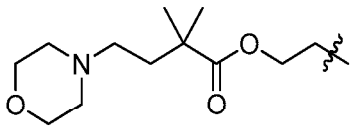
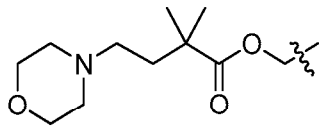
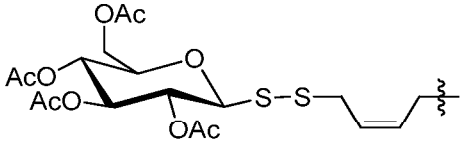
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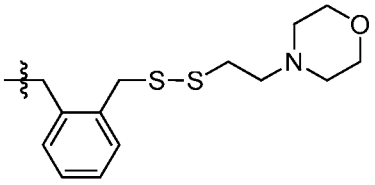
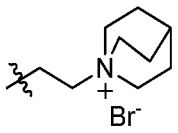
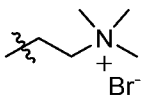
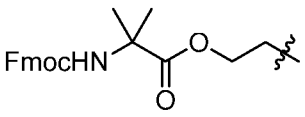
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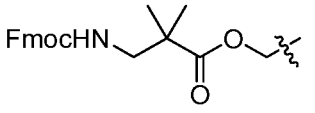
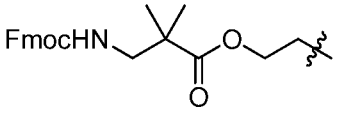
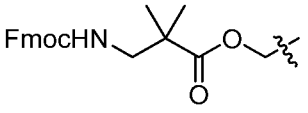
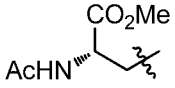
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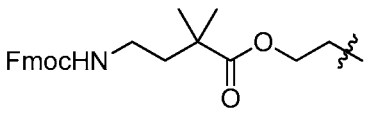
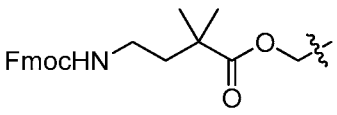
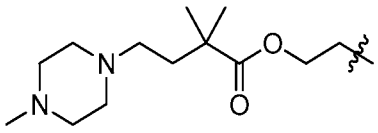
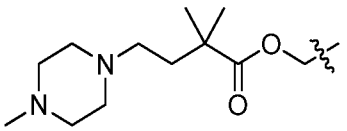
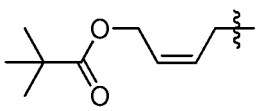
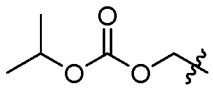
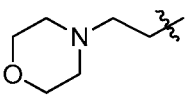
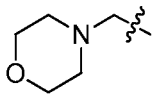
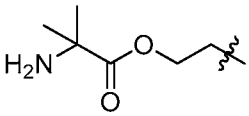
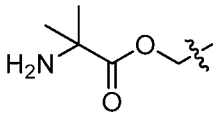
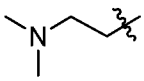
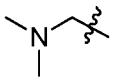
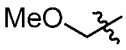
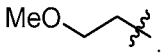
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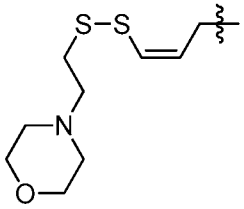
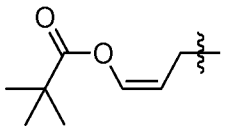
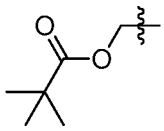
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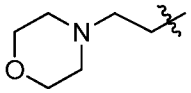
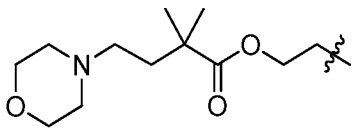
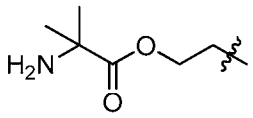
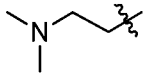
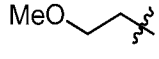
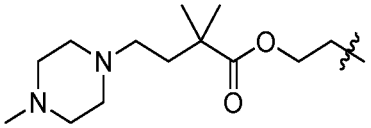
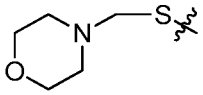
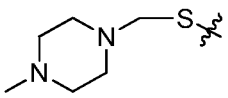
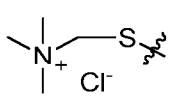
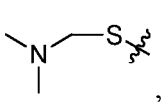
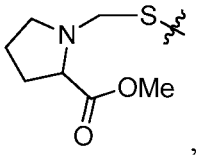
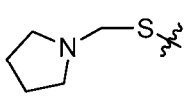
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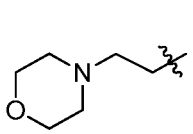
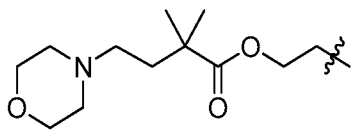
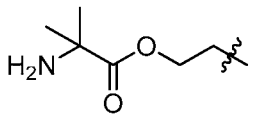
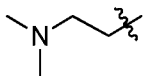
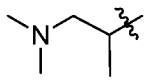















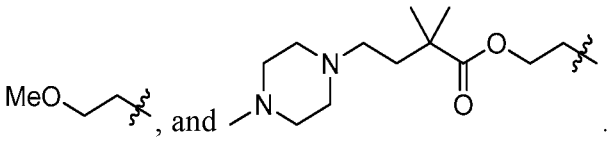
[00628] In some embodiments, –L–R^1 is:

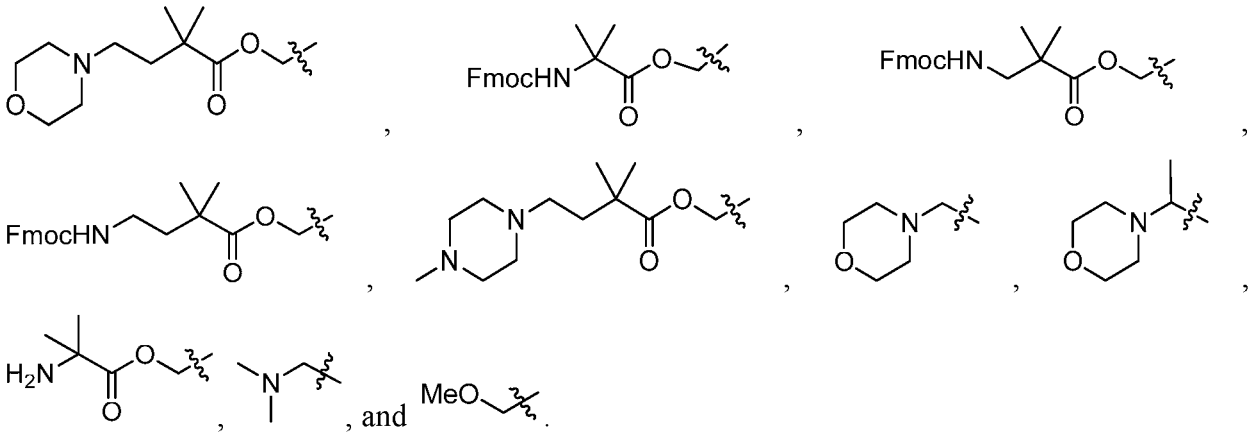
[00629] In some embodiments, –L–R^1 is CH_3–,  ,




 . In some embodiments, –L–R^1 is  ,




 .

[00630] In some embodiments, –L–R^1 comprises a terminal optionally substituted –$(CH_2)_2$– moiety which is connected to X. In some embodiments, –L–R^1 comprises a terminal –$(CH_2)_2$– moiety which is connected to X. Examples of such –L–R^1 moieties are depicted below:

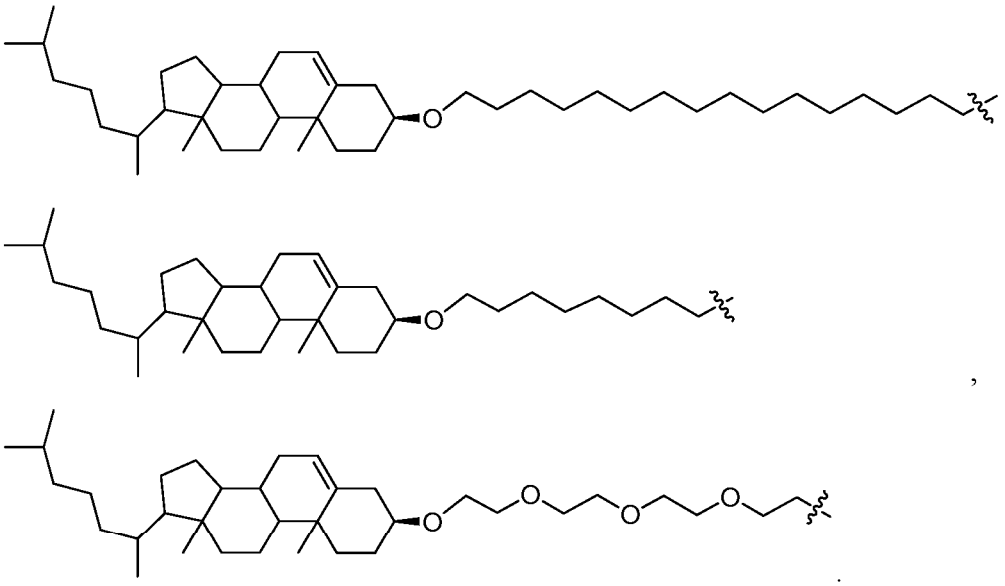








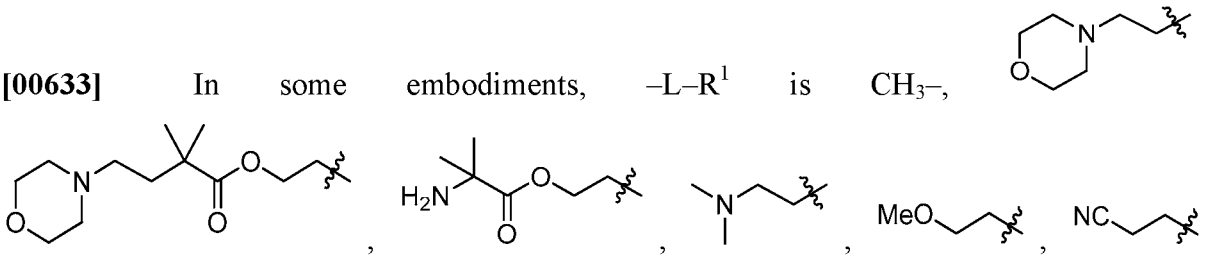
[00631] In some embodiments, –L–R^1 comprises a terminal optionally substituted –(CH_2)– moiety which is connected to X. In some embodiments, –L–R^1 comprises a terminal –(CH_2)– moiety which is connected to X. Examples of such –L–R^1 moieties are depicted below:

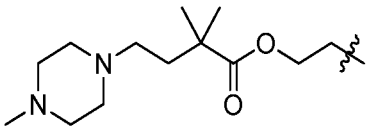


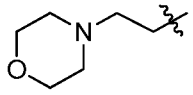
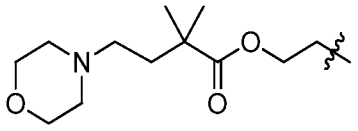
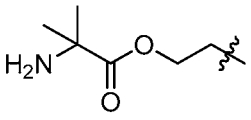
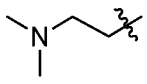
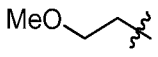
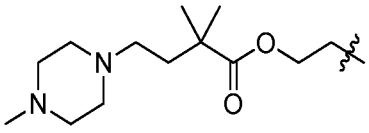
[00632] In some embodiments, –L–R^1 is

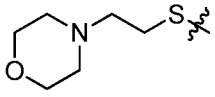
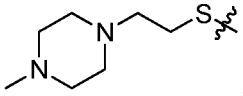
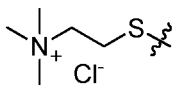
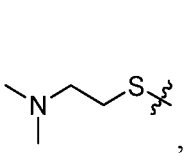
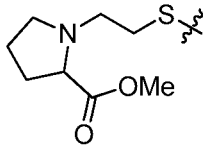
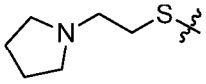
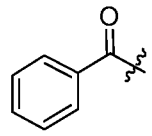


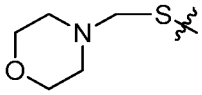
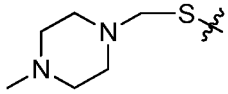
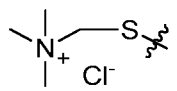
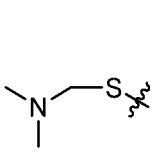
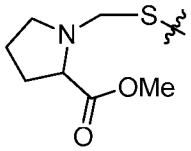
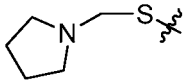
[00633] In some embodiments, –L–R^1 is CH_3–,

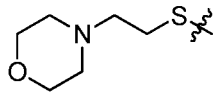
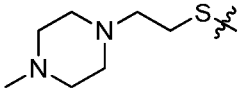
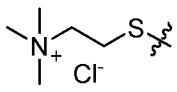
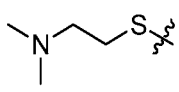
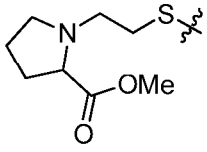
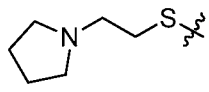
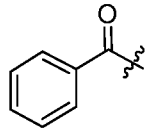


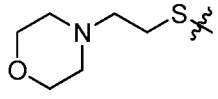

; and X is –S–.

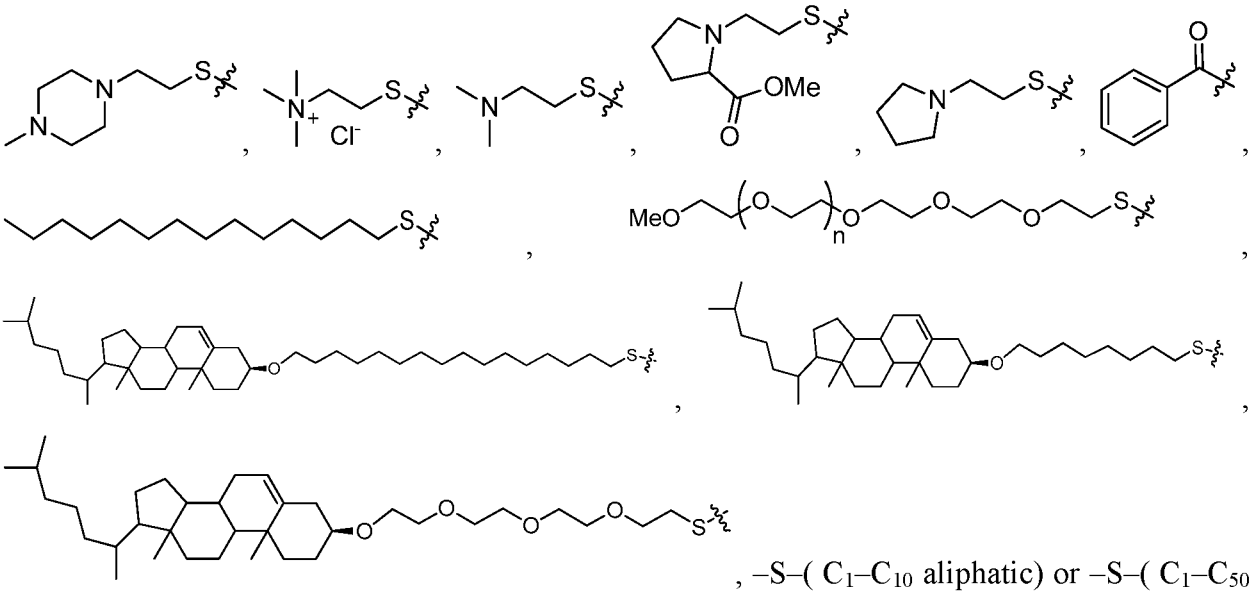
[00634] In some embodiments, –L–R^1 is CH_3–,  ,  ,  ,  ,  , or  , X is –S–, W is O, Y is –O–, and Z is –O–.

[00635] In some embodiments, R^1 is  ,  ,  ,  ,  ,  ,  , or –S–(C_1–C_{10} aliphatic).

[00636] In some embodiments, R^1 is  ,  ,  ,  ,  , or  .

[00637] In some embodiments, X is –O– or –S–, and R^1 is  ,  ,  ,  ,  ,  ,  , or –S–(C_1–C_{10} aliphatic).

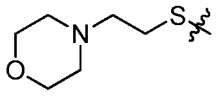
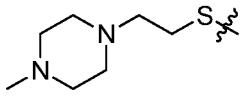
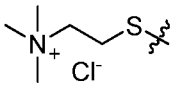
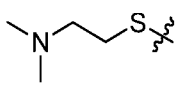
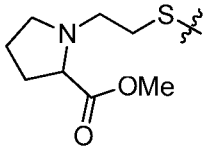
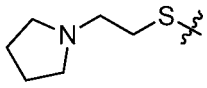
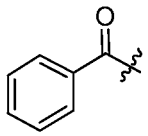
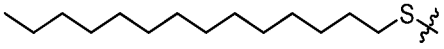
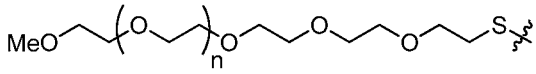
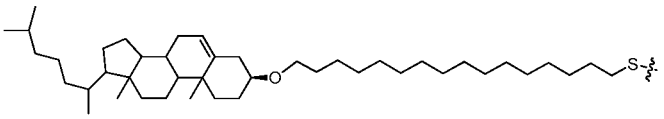
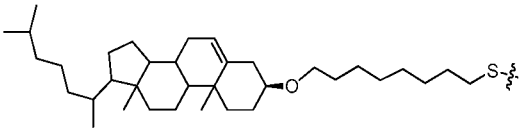
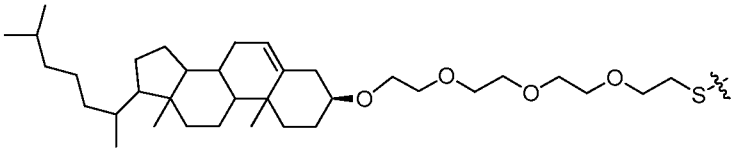
[00638] In some embodiments, X is –O– or –S–, and R^1 is  ,

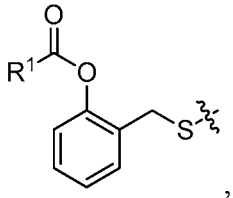
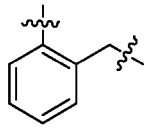
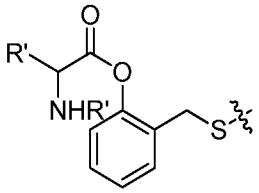
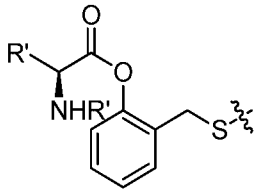
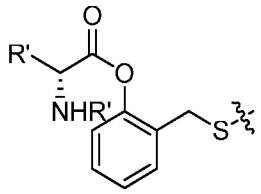
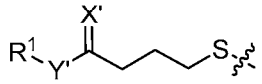
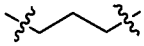
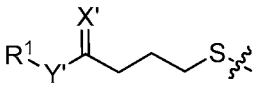
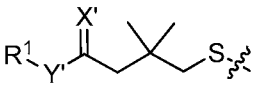
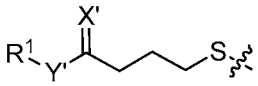
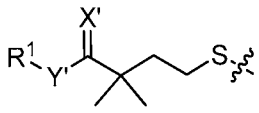
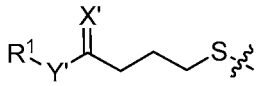
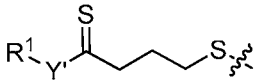
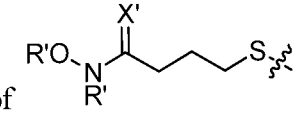
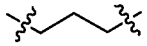
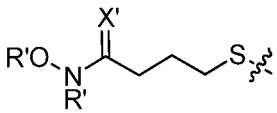
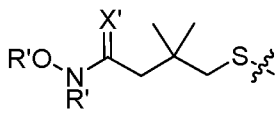
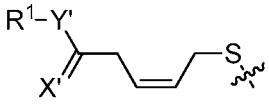
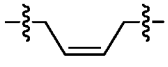


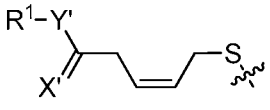
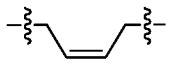
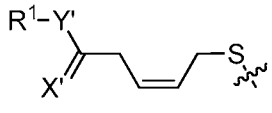
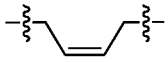
, –S–(C_1–C_{10} aliphatic) or –S–(C_1–C_{50} aliphatic).

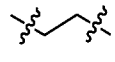
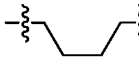
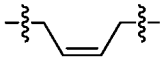
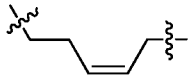
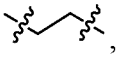
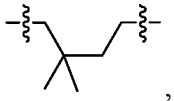
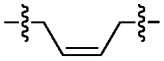
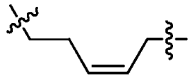
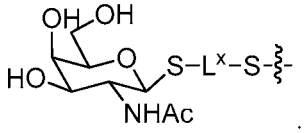
[00639] In some embodiments, L is a covalent bond and –L–R^1 is R^1.

[00640] In some embodiments, –L–R^1 is not hydrogen.

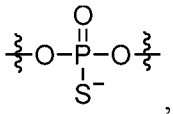
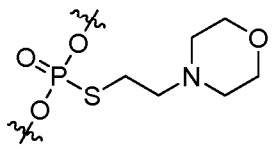
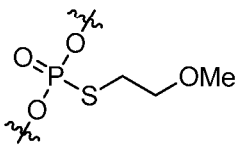
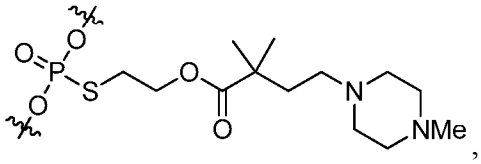
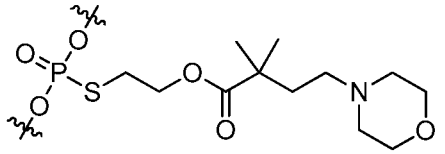
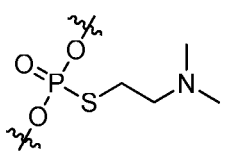
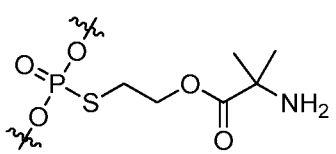
[00641] In some embodiments, –X–L–R^1 is R^1 is  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , –S–(C_1–C_{10} aliphatic) or –S–(C_1–C_{50} aliphatic).

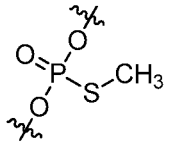
[00642] In some embodiments, –X–L–R^1 has the structure of  , wherein the  moiety is optionally substituted. In some embodiments, –X–L–R^1 is  . In some embodiments, –X–L–R^1 is  . In some embodiments, –X–L–R^1 is  . In some embodiments, –X–L–R^1 has the structure of  , wherein X' is O or S, Y' is –O–, –S– or –NR'–, and the  moiety is optionally substituted. In some embodiments, Y' is –O–, –S– or –NH–. In some embodiments,  is  . In some embodiments,  is  . In some embodiments,  is  . In some embodiments, –X–L–R^1 has the structure of  , wherein X' is O or S, and the  moiety is optionally substituted. In some embodiments,  is  . In some embodiments, –X–L–R^1 is  , wherein the  is optionally substituted. In some embodiments,

–X–L–R^1 is  , wherein the  is substituted. In some embodiments, –X–L–R^1 is  , wherein the  is unsubstituted.

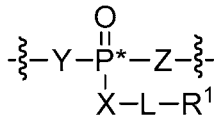
[00643] In some embodiments, –X–L–R^1 is R^1–C(O)–S–L^x–S–, wherein L^x is an optionally substituted group selected from  ,  ,  ,  , and  . In some embodiments, L^x is  ,  ,  ,  , and  . In some embodiments, –X–L–R^1 is $(CH_3)_3C$–S–S–L^x–S–. In some embodiments, –X–L–R^1 is R^1–C(=X')–Y'–$C(R)_2$–S–L^x–S–. In some embodiments, –X–L–R^1 is R–C(=X')–Y'–CH_2–S–L^x–S–. In some embodiments, –X–L–R^1 is  .

[00644] As will be appreciated by a person skilled in the art, many of the –X–L–R^1 groups described herein are cleavable and can be converted to $–X^-$ after administration to a subject. In some embodiments, –X–L–R^1 is cleavable. In some embodiments, –X–L–R^1 is –S–L–R^1, and is converted to $–S^-$ after administration to a subject. In some embodiments, the conversion is promoted by an enzyme of a subject. As appreciated by a person skilled in the art, methods of determining whether the –S–L–R^1 group is converted to $–S^-$ after administration is widely known and practiced in the art, including those used for studying drug metabolism and pharmacokinetics.

[00645] In some embodiments, the internucleotidic linkage having the structure of formula **I** is  ,  ,  ,  ,  ,  ,  , or



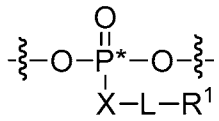
[00646] In some embodiments, the internucleotidic linkage of formula **I** has the structure of formula **I-a**:



(I-a)

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

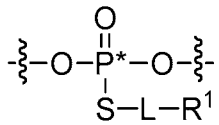
[00647] In some embodiments, the internucleotidic linkage of formula **I** has the structure of formula **I-b**:



(I-b)

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

[00648] In some embodiments, the internucleotidic linkage of formula **I** is an phosphorothioate triester linkage having the structure of formula **I-c**:



(I-c)

wherein:

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

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

R^1 is halogen, R, or an optionally substituted C_1–C_{50} aliphatic wherein one or more methylene

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

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

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

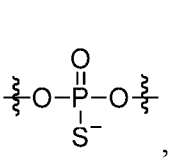
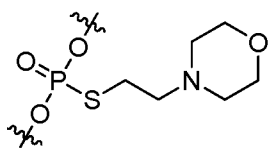
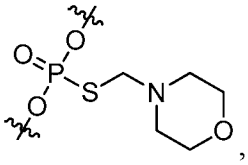
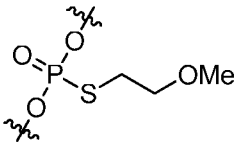
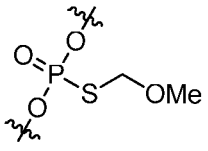
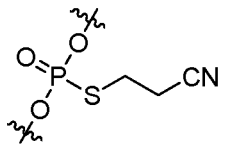
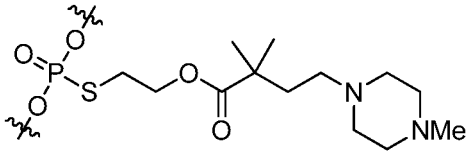
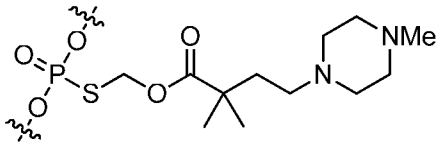
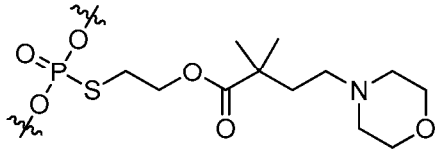
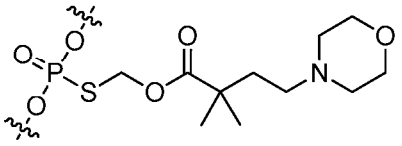
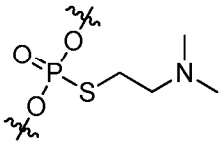
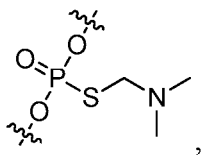
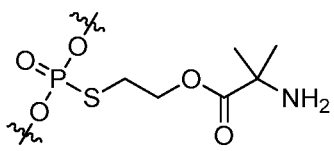
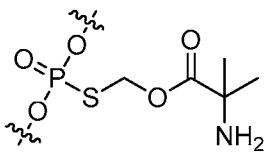
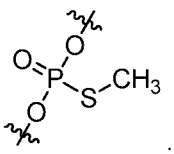
two R′ on the same carbon are taken together with their intervening atoms to form an optionally substituted aryl, carbocyclic, heterocyclic, or heteroaryl ring;

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

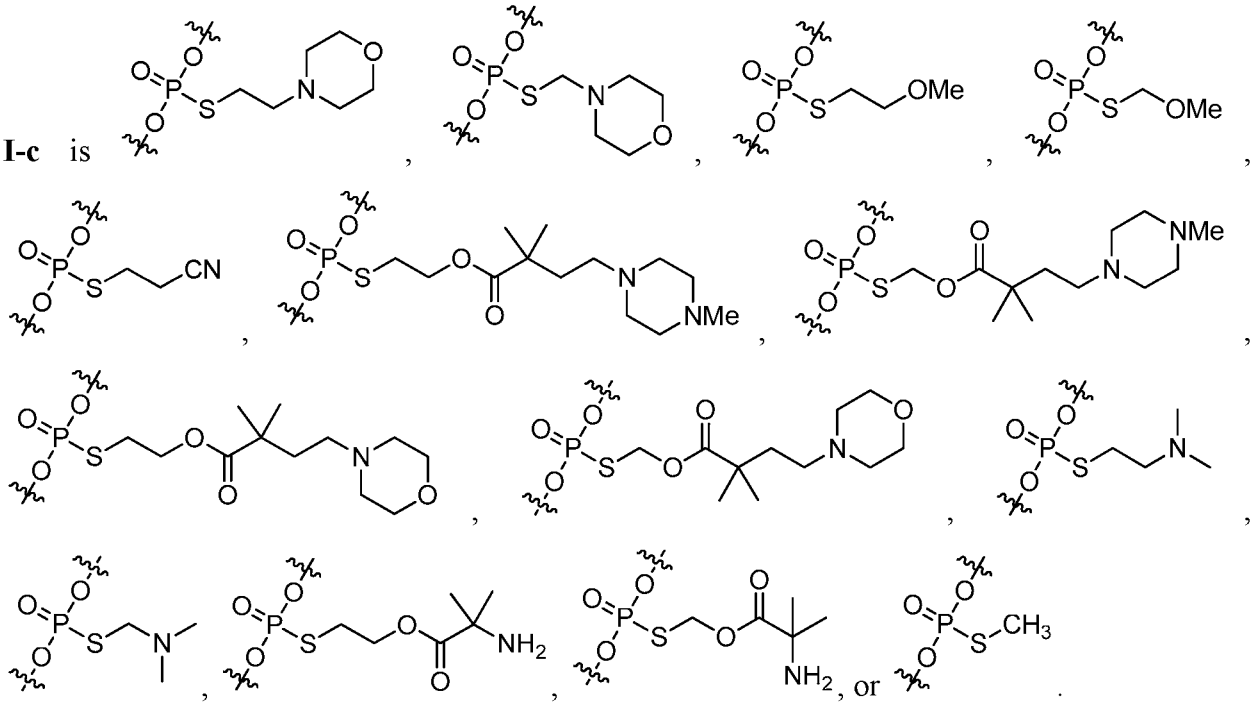
each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, phenyl, carbocyclyl, aryl, heteroaryl, or heterocyclyl;

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

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

[00649] In some embodiments, the internucleotidic linkage having the structure of formula **I** is  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , or  .

[00650] In some embodiments, the internucleotidic linkage having the structure of formula

I-c is 

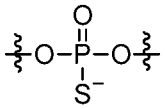
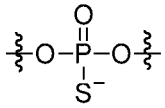
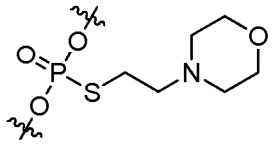
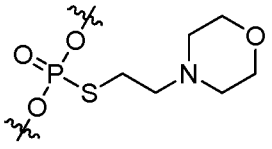
[00651] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising one or more phosphate diester linkages, and one or more modified internucleotide linkages having the formula of **I-a**, **I-b**, or **I-c**.

[00652] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising at least one phosphate diester internucleotidic linkage and at least one phosphorothioate triester linkage having the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising at least one phosphate diester internucleotidic linkage and at least two phosphorothioate triester linkages having the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising at least one phosphate diester internucleotidic linkage and at least three phosphorothioate triester linkages having the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising at least one phosphate diester internucleotidic linkage and at least four phosphorothioate triester linkages having the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising at least one phosphate diester internucleotidic linkage and at least five phosphorothioate triester linkages having the structure of formula **I-c**.

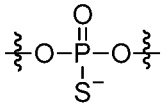
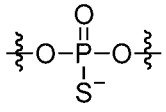
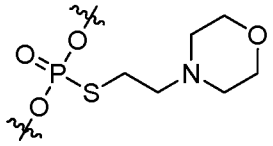
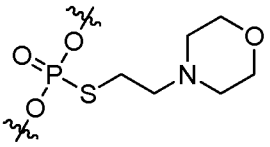
[00653] In some embodiments, the present disclosure provides a chirally controlled

oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein the said sequence has over 50% identity with GGCACAAGGGCACAGACTTC. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein the said sequence has over 60% identity with GGCACAAGGGCACAGACTTC. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein the said sequence has over 70% identity with GGCACAAGGGCACAGACTTC. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein the said sequence has over 80% identity with GGCACAAGGGCACAGACTTC. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein the said sequence has over 90% identity with GGCACAAGGGCACAGACTTC. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein the said sequence has over 95% identity with GGCACAAGGGCACAGACTTC. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising the sequence of GGCACAAGGGCACAGACTTC. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC.

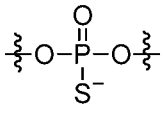
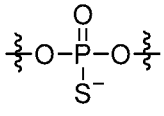
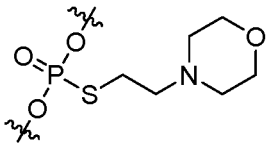
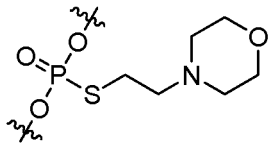
[00654] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage has a chiral linkage phosphorus. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage has the structure of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein each internucleotidic linkage has the structure of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in

GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein each internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein each internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence found in GGCACAAGGGCACAGACTTC, wherein each internucleotidic linkage is  .

[00655] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising the sequence of GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage has a chiral linkage phosphorus. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising the sequence of GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage has the structure of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising the sequence of GGCACAAGGGCACAGACTTC, wherein each internucleotidic linkage has the structure of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising the sequence of GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage has the structure

of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising the sequence of GGCACAAGGGCACAGACTTC, wherein each internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising the sequence of GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising the sequence of GGCACAAGGGCACAGACTTC, wherein each internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising the sequence of GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising the sequence of GGCACAAGGGCACAGACTTC, wherein each internucleotidic linkage is  .

[00656] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage has a chiral linkage phosphorus. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage has the structure of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein each internucleotidic linkage has the structure of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled

oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein each internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein each internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein at least one internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein each internucleotidic linkage is  .

[00657] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein at least one linkage phosphorus is *R*p. It is understood by a person of ordinary skill in the art that in certain embodiments wherein the chirally controlled oligonucleotide comprises an RNA sequence, each T is independently and optionally replaced with U. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein each linkage phosphorus is *R*p. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein at least one linkage phosphorus is *S*p. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein each linkage phosphorus is *S*p. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide

having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a blockmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a stereoblockmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a P-modification blockmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a linkage blockmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is an altmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a stereoaltmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a P-modification altmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a linkage altmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a unimer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a stereounimer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a P-modification unimer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a linkage unimer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a gapmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein the oligonucleotide is a skipmer.

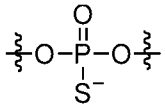
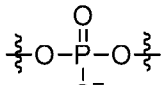
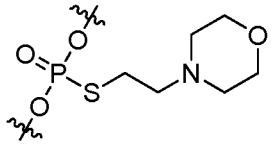
[00658] In some embodiments, the present disclosure provides a chirally controlled

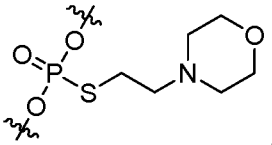
oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein each cytosine is optionally and independently replaced by 5-methylcytosine. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of GGCACAAGGGCACAGACTTC, wherein at least one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

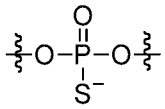
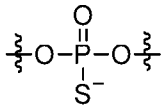
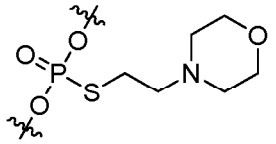
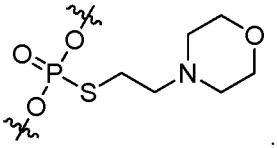
[00659] In some embodiments, a chirally controlled oligonucleotide is designed such that one or more nucleotides comprise a phosphorus modification prone to "autorelease" under certain conditions. That is, under certain conditions, a particular phosphorus modification is designed such that it self-cleaves from the oligonucleotide to provide, *e.g.,* a phosphate diester such as those found in naturally occurring DNA and RNA. In some embodiments, such a phosphorus modification has a structure of $–O–L–R^1$, wherein each of L and R^1 is independently as defined above and described herein. In some embodiments, an autorelease group comprises a morpholino group. In some embodiments, an autorelease group is characterized by the ability to deliver an agent to the internucleotidic phosphorus linker, which agent facilitates further modification of the phosphorus atom such as, *e.g.,* desulfurization. In some embodiments, the agent is water and the further modification is hydrolysis to form a phosphate diester as is found in naturally occurring DNA and RNA.

[00660] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein (including, as non-limiting examples, any sequence disclosed in any Table). In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence having over 50% identity with any sequence disclosed herein. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence having over 60% identity with any sequence disclosed herein. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence having over 70% identity with any sequence disclosed herein. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence having over 80% identity with any sequence disclosed herein. In some embodiments, the present disclosure provides a chirally controlled

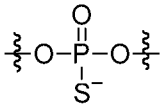
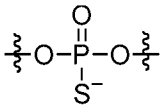
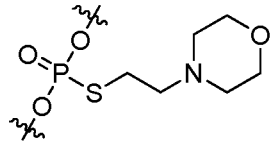
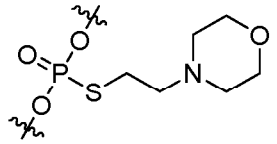
oligonucleotide comprising a sequence having over 90% identity with any sequence disclosed herein. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising a sequence having over 95% identity with any sequence disclosed herein. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein.

[00661] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein at least one internucleotidic linkage has a chiral linkage phosphorus. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein at least one internucleotidic linkage has the structure of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein each internucleotidic linkage has the structure of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein at least one internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein each internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein at least one internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein each internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein at least one internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed

herein, wherein each internucleotidic linkage is  .

[00662] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein at least one internucleotidic linkage has a chiral linkage phosphorus. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein at least one internucleotidic linkage has the structure of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein each internucleotidic linkage has the structure of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein at least one internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein each internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein at least one internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein each internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein at least one internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide comprising any sequence disclosed herein, wherein each internucleotidic linkage is  .

[00663] In some embodiments, the present disclosure provides a chirally controlled

oligonucleotide having any sequence disclosed herein, wherein at least one internucleotidic linkage has a chiral linkage phosphorus. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein at least one internucleotidic linkage has the structure of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein each internucleotidic linkage has the structure of formula **I**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein at least one internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein each internucleotidic linkage has the structure of formula **I-c**. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein at least one internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein each internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein at least one internucleotidic linkage is  . In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein each internucleotidic linkage is  .

[00664] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein at least one linkage phosphorus is *R*p. It is understood by a person of ordinary skill in the art that in certain embodiments wherein the chirally controlled oligonucleotide comprises an RNA sequence, each T is independently and optionally replaced with U. In some embodiments, the present disclosure provides a chirally

controlled oligonucleotide having any sequence disclosed herein, wherein each linkage phosphorus is *R*p. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein at least one linkage phosphorus is *S*p. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein each linkage phosphorus is *S*p. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is a blockmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is a stereoblockmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is a P-modification blockmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is a linkage blockmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is an altmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is a stereoaltmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is a P-modification altmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is a linkage altmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is a unimer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is a stereounimer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is a P-modification unimer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is a linkage unimer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein the oligonucleotide is a gapmer. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed

herein, wherein the oligonucleotide is a skipmer.

[00665] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein each cytosine is optionally and independently replaced by 5-methylcytosine. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein at least one cytosine is optionally and independently replaced by 5-methylcytosine. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having any sequence disclosed herein, wherein each cytosine is optionally and independently replaced by 5-methylcytosine.

[00666] In various embodiments, any sequence disclosed herein can be combined with one or more of the following as disclosed herein or known in the art: pattern of backbone linkages; pattern of backbone chiral centers; and pattern of backbone P-modifications; pattern of base modification; pattern of sugar modification; pattern of backbone linkages; pattern of backbone chiral centers; and pattern of backbone P-modifications.

[00667] In some embodiments, a chirally controlled oligonucleotide is designed such that the resulting pharmaceutical properties are improved through one or more particular modifications at phosphorus. It is well documented in the art that certain oligonucleotides are rapidly degraded by nucleases and exhibit poor cellular uptake through the cytoplasmic cell membrane [Poijarvi-Virta *et al.*, Curr. Med. Chem. (2006), 13(28);3441-65; Wagner *et al.*, Med. Res. Rev. (2000), 20(6):417-51; Peyrottes *et al.*, Mini Rev. Med. Chem. (2004), 4(4):395-408; Gosselin *et al.*, (1996), 43(1):196-208; Bologna *et al.*, (2002), Antisense & Nucleic Acid Drug Development 12:33-41]. For instance, Vives *et al.*, Nucleic Acids Research (1999), 27(20):4071-76, found that tert-butyl SATE pro-oligonucleotides displayed markedly increased cellular penetration compared to the parent oligonucleotide.

[00668] In some embodiments, a modification at a linkage phosphorus is characterized by its ability to be transformed to a phosphate diester, such as those present in naturally occurring DNA and RNA, by one or more esterases, nucleases, and/or cytochrome P450 enzymes, including but not limited to, those listed in Table 1A, below.

Table 1A. Example enzymes.

Family	Gene

CYP1	CYP1A1, CYP1A2, CYP1B1
CYP2	CYP2A6, CYP2A7, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2R1, CYP2S1, CYP2U1, CYP2W1
CYP3	CYP3A4, CYP3A5, CYP3A7, CYP3A43
CYP4	CYP4A11, CYP4A22, CYP4B1, CYP4F2, CYP4F3, CYP4F8, CYP4F11, CYP4F12, CYP4F22, CYP4V2, CYP4X1, CYP4Z1
CYP5	CYP5A1
CYP7	CYP7A1, CYP7B1
CYP8	CYP8A1 (prostacyclin synthase), CYP8B1 (bile acid biosynthesis)
CYP11	CYP11A1, CYP11B1, CYP11B2
CYP17	CYP17A1
CYP19	CYP19A1
CYP20	CYP20A1
CYP21	CYP21A2
CYP24	CYP24A1
CYP26	CYP26A1, CYP26B1, CYP26C1
CYP27	CYP27A1 (bile acid biosynthesis), CYP27B1 (vitamin D3 1-alpha hydroxylase, activates vitamin D3), CYP27C1 (unknown function)
CYP39	CYP39A1
CYP46	CYP46A1
CYP51	CYP51A1 (lanosterol 14-alpha demethylase)

[00669] In some embodiments, a modification at phosphorus results in a P-modification moiety characterized in that it acts as a pro-drug, *e.g.*, the P-modification moiety facilitates delivery of an oligonucleotide to a desired location prior to removal. For instance, in some embodiments, a P-modification moiety results from PEGylation at the linkage phosphorus. One

of skill in the relevant arts will appreciate that various PEG chain lengths are useful and that the selection of chain length will be determined in part by the result that is sought to be achieved by PEGylation. For instance, in some embodiments, PEGylation is effected in order to reduce RES uptake and extend *in vivo* circulation lifetime of an oligonucleotide.

[00670] In some embodiments, a PEGylation reagent for use in accordance with the present disclosure is of a molecular weight of about 300 g/mol to about 100,000 g/mol. In some embodiments, a PEGylation reagent is of a molecular weight of about 300 g/mol to about 10,000 g/mol. In some embodiments, a PEGylation reagent is of a molecular weight of about 300 g/mol to about 5,000 g/mol. In some embodiments, a PEGylation reagent is of a molecular weight of about 500 g/mol. In some embodiments, a PEGylation reagent of a molecular weight of about 1000 g/mol. In some embodiments, a PEGylation reagent is of a molecular weight of about 3000 g/mol. In some embodiments, a PEGylation reagent is of a molecular weight of about 5000 g/mol.

[00671] In certain embodiments, a PEGylation reagent is PEG500. In certain embodiments, a PEGylation reagent is PEG1000. In certain embodiments, a PEGylation reagent is PEG3000. In certain embodiments, a PEGylation reagent is PEG5000.

[00672] In some embodiments, a P-modification moiety is characterized in that it acts as a PK enhancer, *e.g.,* lipids, PEGylated lipids, *etc.*

[00673] In some embodiments, a P-modification moiety is characterized in that it acts as an agent which promotes cell entry and/or endosomal escape, such as a membrane-disruptive lipid or peptide.

[00674] In some embodiments, a P-modification moiety is characterized in that it acts as a targeting agent. In some embodiments, a P-modification moiety is or comprises a targeting agent. The phrase "targeting agent," as used herein, is an entity that is associates with a payload of interest (*e.g.,* with an oligonucleotide or oligonucleotide composition) and also interacts with a target site of interest so that the payload of interest is targeted to the target site of interest when associated with the targeting agent to a materially greater extent than is observed under otherwise comparable conditions when the payload of interest is not associated with the targeting agent. A targeting agent may be, or comprise, any of a variety of chemical moieties, including, for example, small molecule moieties, nucleic acids, polypeptides, carbohydrates, *etc.* Targeting agents are described further by Adarsh et al., "Organelle Specific Targeted Drug Delivery – A

Review," International Journal of Research in Pharmaceutical and Biomedical Sciences, 2011, p. 895.

[00675] Examples of such targeting agents include, but are not limited to, proteins (e.g. Transferrin), oligopeptides (*e.g.,* cyclic and acylic RGD-containing oligopedptides), antibodies (monoclonal and polyclonal antibodies, e.g. IgG, IgA, IgM, IgD, IgE antibodies), sugars / carbohydrates (*e.g.,* monosaccharides and/or oligosaccharides (mannose, mannose-6-phosphate, galactose, and the like)), vitamins (*e.g.,* folate), or other small biomolecules. In some embodiments, a targeting moiety is a steroid molecule (*e.g.,* bile acids including cholic acid, deoxycholic acid, dehydrocholic acid; cortisone; digoxigenin; testosterone; cholesterol; cationic steroids such as cortisone having a trimethylaminomethyl hydrazide group attached via a double bond at the 3-position of the cortisone ring, *etc.*). In some embodiments, a targeting moiety is a lipophilic molecule (*e.g.,* alicyclic hydrocarbons, saturated and unsaturated fatty acids, waxes, terpenes, and polyalicyclic hydrocarbons such as adamantine and buckminsterfullerenes). In some embodiments, a lipophilic molecule is a terpenoid such as vitamin A, retinoic acid, retinal, or dehydroretinal. In some embodiments, a targeting moiety is a peptide.

[00676] In some embodiments, a P-modification moiety is a targeting agent of formula –-X-L-R^1 wherein each of X, L, and R^1 are as defined in Formula **I** above.

[00677] In some embodiments, a P-modification moiety is characterized in that it facilitates cell specific delivery.

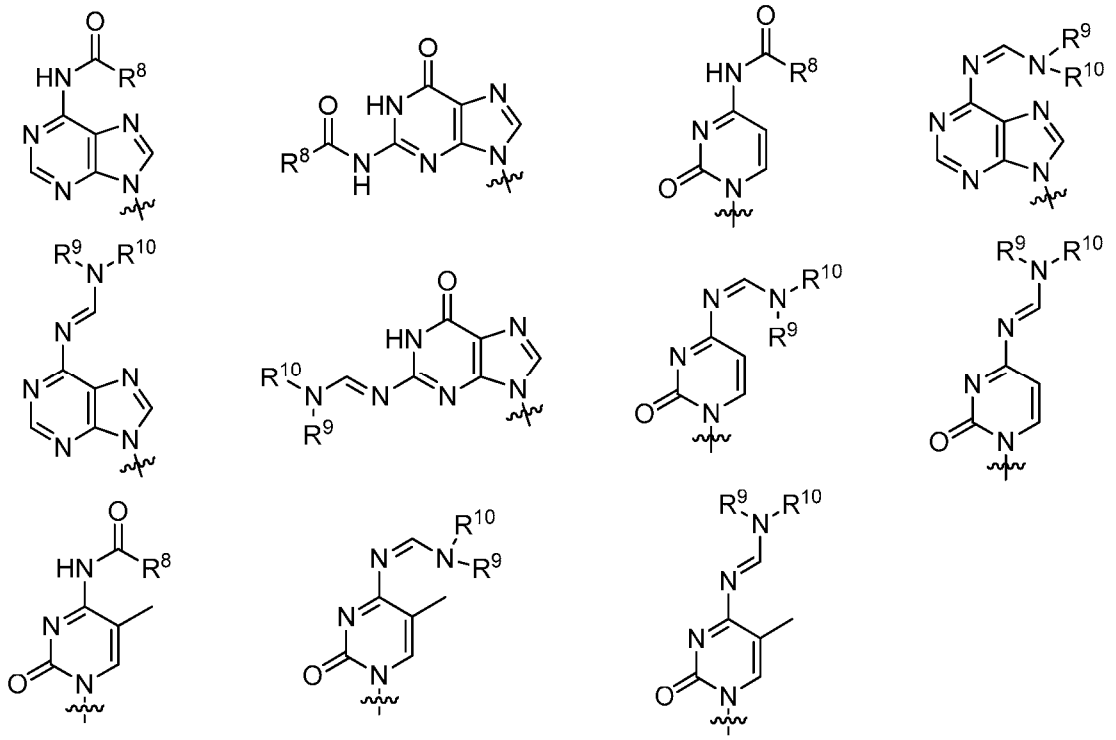
[00678] In some embodiments, a P-modification moiety is characterized in that it falls into one or more of the above-described categories. For instance, in some embodiments, a P-modification moiety acts as a PK enhancer and a targeting ligand. In some embodiments, a P-modification moiety acts as a pro-drug and an endosomal escape agent. One of skill in the relevant arts would recognize that numerous other such combinations are possible and are contemplated by the present disclosure.

Nucleobases

[00679] In some embodiments, a nucleobase present in a provided oligonucleotide is a natural nucleobase or a modified nucleobase derived from a natural nucleobase. Examples include, but are not limited to, uracil, thymine, adenine, cytosine, and guanine having their respective amino groups protected by acyl protecting groups, 2-fluorouracil, 2-fluorocytosine, 5-

bromouracil, 5-iodouracil, 2,6-diaminopurine, azacytosine, pyrimidine analogs such as pseudoisocytosine and pseudouracil and other modified nucleobases such as 8-substituted purines, xanthine, or hypoxanthine (the latter two being the natural degradation products). Example 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.

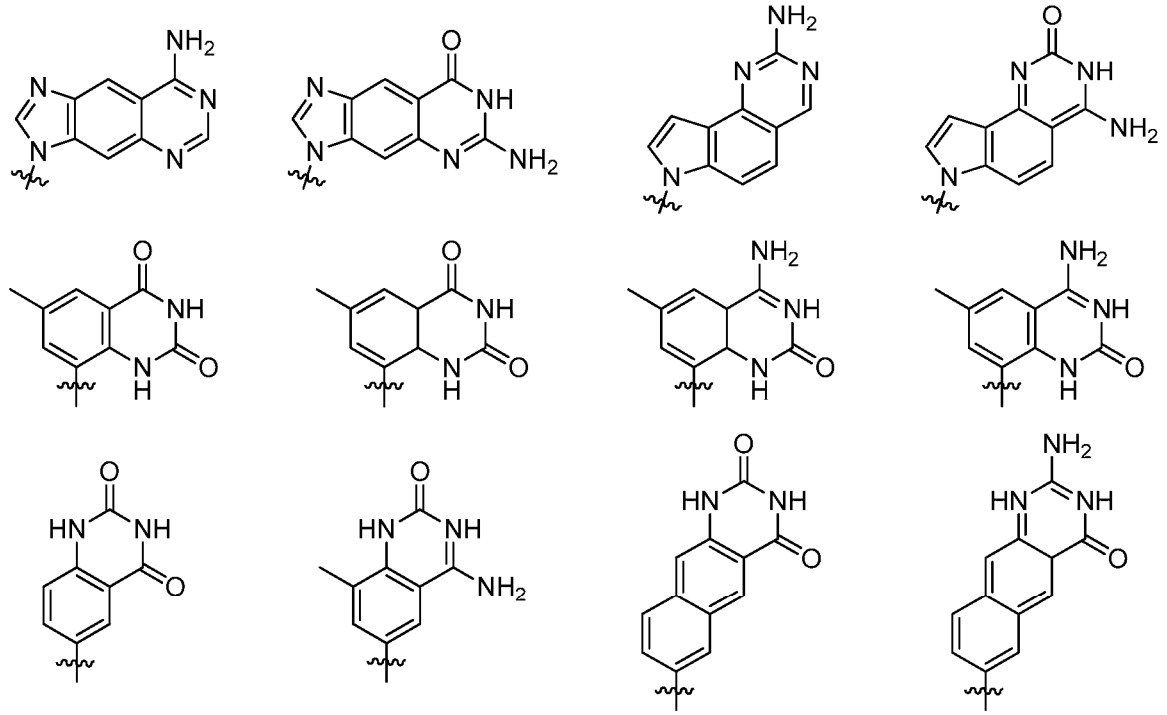
[00680] Compounds represented by the following general formulae are also contemplated as modified nucleobases:



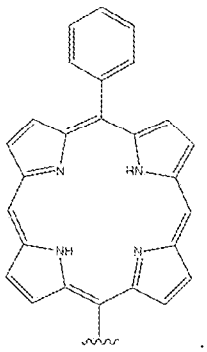
wherein R^8 is an optionally substituted, linear or branched group selected from aliphatic, aryl, aralkyl, aryloxylalkyl, carbocyclyl, heterocyclyl or heteroaryl group having 1 to 15 carbon atoms, including, by way of example only, a methyl, isopropyl, phenyl, benzyl, or phenoxymethyl group; and each of R^9 and R^{10} is independently an optionally substituted group selected from linear or branched aliphatic, carbocyclyl, aryl, heterocyclyl and heteroaryl.

[00681] Modified nucleobases also include expanded-size nucleobases in which one or more aryl rings, such as phenyl rings, have been added. Nucleic base replacements described in the Glen Research catalog (www.glenresearch.com); Krueger AT *et al*, *Acc. Chem. Res.*, **2007**, *40*, 141-150; Kool, ET, *Acc. Chem. Res.*, **2002**, *35*, 936-943; Benner S.A., *et al.*, *Nat. Rev.*

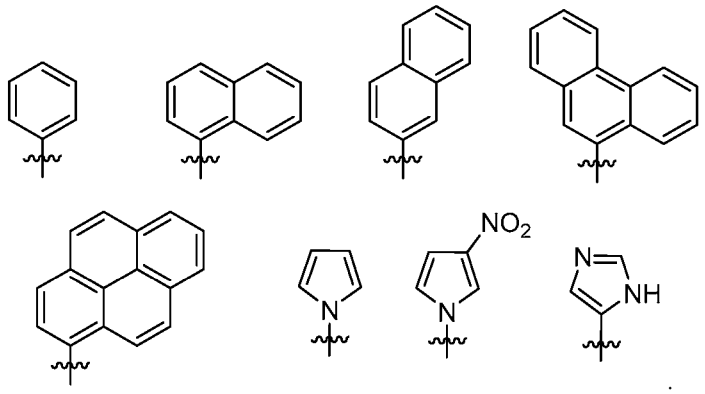
Genet., **2005**, *6*, 553-543; Romesberg, F.E., *et al.*, *Curr. Opin. Chem. Biol.*, **2003**, *7*, 723-733; Hirao, I., *Curr. Opin. Chem. Biol.*, **2006**, *10*, 622-627, are contemplated as useful for the synthesis of the nucleic acids described herein. Some examples of these expanded-size nucleobases are shown below:



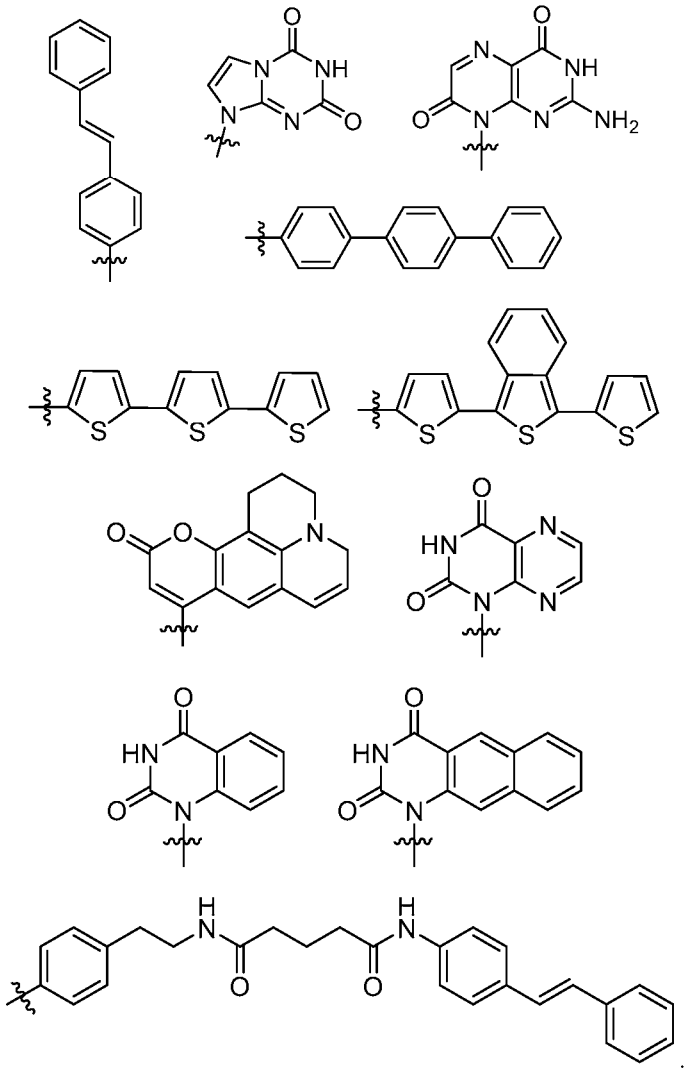
[00682] Herein, modified nucleobases also encompass structures that are not considered nucleobases but are other moieties such as, but not limited to, corrin- or porphyrin-derived rings. Porphyrin-derived base replacements have been described in Morales-Rojas, H and Kool, ET, *Org. Lett.*, 2002, *4*, 4377-4380. Shown below is an example of a porphyrin-derived ring which can be used as a base replacement:



[00683] In some embodiments, modified nucleobases are of any one of the following structures, optionally substituted:



[00684] 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, and naphtho-uracil, as shown below:



[00685] In some embodiments, a modified nucleobase is unsubstituted. In some embodiments, a modified nucleobase is substituted. In some embodiments, a modified nucleobase is substituted such that it contains, *e.g.*, heteroatoms, alkyl groups, or linking moieties connected to fluorescent moieties, biotin or avidin moieties, or other protein or peptides. In some embodiments, a modified nucleobase is a "universal base" that is not a nucleobase in the most classical sense, but that functions similarly to a nucleobase. One representative example of such a universal base is 3-nitropyrrole.

[00686] In some embodiments, other nucleosides can also be used in the process disclosed herein and include nucleosides that incorporate modified nucleobases, or nucleobases covalently bound to modified sugars. Some examples of nucleosides that incorporate modified nucleobases include 4-acetylcytidine; 5-(carboxyhydroxylmethyl)uridine; 2′-*O*-methylcytidine; 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyluridine; dihydrouridine; 2′-*O*-methylpseudouridine; beta,D-galactosylqueosine; 2′-*O*-methylguanosine; N^6-isopentenyladenosine; 1-methyladenosine; 1-methylpseudouridine; 1-methylguanosine; 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.

[00687] In some embodiments, nucleosides include 6′-modified bicyclic nucleoside analogs that have either (*R*) or (*S*)-chirality at the 6′-position and include the analogs described in US Patent No. 7,399,845. In other embodiments, nucleosides include 5′-modified bicyclic nucleoside analogs that have either (*R*) or (*S*)-chirality at the 5′-position and include the analogs described in US Patent Application Publication No. 20070287831.

[00688] In some embodiments, a nucleobase or modified nucleobase comprises one or more biomolecule binding moieties such as *e.g.*, antibodies, antibody fragments, biotin, avidin,

streptavidin, receptor ligands, or chelating moieties. In other embodiments, a nucleobase or modified nucleobase is 5-bromouracil, 5-iodouracil, or 2,6-diaminopurine. In some embodiments, a nucleobase or modified nucleobase is modified by substitution with a fluorescent or biomolecule binding moiety. In some embodiments, the substituent on a nucleobase or modified nucleobase is a fluorescent moiety. In some embodiments, the substituent on a nucleobase or modified nucleobase is biotin or avidin.

[00689] Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,457,191; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, each of which is herein incorporated by reference in its entirety.

[00690] In some embodiments, a base is optionally substituted A, T, C, G or U, wherein one or more $-NH_2$ are independently and optionally replaced with $-C(-L-R^1)_3$, one or more $-NH-$ are independently and optionally replaced with $-C(-L-R^1)_2-$, one or more $=N-$ are independently and optionally replaced with $-C(-L-R^1)-$, one or more $=CH-$ are independently and optionally replaced with $=N-$, and one or more $=O$ are independently and optionally replaced with $=S$, $=N(-L-R^1)$, or $=C(-L-R^1)_2$, wherein two or more $-L-R^1$ are optionally taken together with their intervening atoms to form a 3-30 membered bicyclic or polycyclic ring having 0-10 heteroatom ring atoms. In some embodiments, a modified base is optionally substituted A, T, C, G or U, wherein one or more $-NH_2$ are independently and optionally replaced with $-C(-L-R^1)_3$, one or more $-NH-$ are independently and optionally replaced with $-C(-L-R^1)_2-$, one or more $=N-$ are independently and optionally replaced with $-C(-L-R^1)-$, one or more $=CH-$ are independently and optionally replaced with $=N-$, and one or more $=O$ are independently and optionally replaced with $=S$, $=N(-L-R^1)$, or $=C(-L-R^1)_2$, wherein two or more $-L-R^1$ are optionally taken together with their intervening atoms to form a 3-30 membered bicyclic or polycyclic ring having 0-10 heteroatom ring atoms, wherein the modified base is different than the natural A, T, C, G and U. 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.

[00691] In some embodiments, a modified nucleotide or nucleotide analog is any modified nucleotide or nucleotide analog 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 20070900071; WO 20070900071; or WO 2016/079181.

Sugars

[00692] In some embodiments, provided oligonucleotides comprise one or more modified sugar moieties beside the natural sugar moieties.

[00693] The most common naturally occurring nucleotides are comprised of ribose sugars linked to the nucleobases adenosine (A), cytosine (C), guanine (G), and thymine (T) or uracil (U). Also contemplated are modified nucleotides wherein a phosphate group or linkage

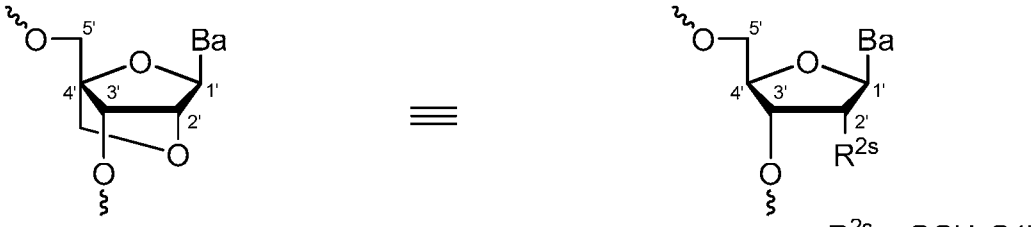
phosphorus in the nucleotides can be linked to various positions of a sugar or modified sugar. As non-limiting examples, the phosphate group or linkage phosphorus can be linked to the 2′, 3′, 4′ or 5′ hydroxyl moiety of a sugar or modified sugar. Nucleotides that incorporate modified nucleobases as described herein are also contemplated in this context. In some embodiments, nucleotides or modified nucleotides comprising an unprotected –OH moiety are used in accordance with methods of the present disclosure.

[00694] Other modified sugars can also be incorporated within a provided oligonucleotide. In some embodiments, a modified sugar contains one or more substituents at the 2′ position including one of the following: –F; $–CF_3$, –CN, $–N_3$, –NO, $–NO_2$, –OR', –SR', or $–N(R')_2$, wherein each R' is independently as defined above and described herein; $–O–(C_1–C_{10}$ alkyl), $–S–(C_1–C_{10}$ alkyl), $–NH–(C_1–C_{10}$ alkyl), or $–N(C_1–C_{10}$ alkyl)$_2$; $–O–(C_2–C_{10}$ alkenyl), $–S–(C_2–C_{10}$ alkenyl), $–NH–(C_2–C_{10}$ alkenyl), or $–N(C_2–C_{10}$ alkenyl)$_2$; $–O–(C_2–C_{10}$ alkynyl), $–S–(C_2–C_{10}$ alkynyl), $–NH–(C_2–C_{10}$ alkynyl), or $–N(C_2–C_{10}$ alkynyl)$_2$; or $–O—(C_1–C_{10}$ alkylene)$–O—(C_1–C_{10}$ alkyl), $–O–(C_1–C_{10}$ alkylene)$–NH–(C_1–C_{10}$ alkyl) or $–O–(C_1–C_{10}$ alkylene)$–NH(C_1–C_{10}$ alkyl)$_2$, $–NH–(C_1–C_{10}$ alkylene)$–O–(C_1–C_{10}$ alkyl), or $–N(C_1–C_{10}$ alkyl)$–(C_1–C_{10}$ alkylene)$–O–(C_1–C_{10}$ alkyl), wherein the alkyl, alkylene, alkenyl and alkynyl may be substituted or unsubstituted. Examples of substituents include, and are not limited to, $–O(CH_2)_nOCH_3$, and $–O(CH_2)_nNH_2$, wherein n is from 1 to about 10, MOE, DMAOE, DMAEOE. Also contemplated herein are modified sugars described in WO 2001/088198; and Martin *et al.*, *Helv. Chim. Acta*, **1995**, *78*, 486-504. In some embodiments, a modified sugar comprises one or more groups selected from a substituted silyl group, an RNA cleaving group, a reporter group, a fluorescent label, an intercalator, a group for improving the pharmacokinetic properties of a nucleic acid, a group for improving the pharmacodynamic properties of a nucleic acid, or other substituents having similar properties. In some embodiments, modifications are made at one or more of the the 2′, 3′, 4′, 5′, or 6′ positions of the sugar or modified sugar, including the 3′ position of the sugar on the 3′-terminal nucleotide or in the 5′ position of the 5′-terminal nucleotide.

[00695] In some embodiments, the 2'-OH of a ribose is replaced with a substituent including one of the following: –H, –F; $–CF_3$, –CN, $–N_3$, –NO, $–NO_2$, –OR', –SR', or $–N(R')_2$, wherein each R' is independently as defined above and described herein; $–O–(C_1–C_{10}$ alkyl), $–S–(C_1–C_{10}$ alkyl), $–NH–(C_1–C_{10}$ alkyl), or $–N(C_1–C_{10}$ alkyl)$_2$; $–O–(C_2–C_{10}$ alkenyl), $–S–(C_2–C_{10}$ alkenyl), $–NH–(C_2–C_{10}$ alkenyl), or $–N(C_2–C_{10}$ alkenyl)$_2$; $–O–(C_2–C_{10}$ alkynyl), $–S–(C_2–C_{10}$

alkynyl), –NH–(C_2–C_{10} alkynyl), or –N(C_2–C_{10} alkynyl)$_2$; or –O—(C_1–C_{10} alkylene)–O—(C_1–C_{10} alkyl), –O–(C_1–C_{10} alkylene)–NH–(C_1–C_{10} alkyl) or –O–(C_1–C_{10} alkylene)–NH(C_1–C_{10} alkyl)$_2$, –NH–(C_1–C_{10} alkylene)–O–(C_1–C_{10} alkyl), or –N(C_1–C_{10} alkyl)–(C_1–C_{10} alkylene)–O–(C_1–C_{10} alkyl), wherein the alkyl, alkylene, alkenyl and alkynyl may be substituted or unsubstituted. In some embodiments, the 2'–OH is replaced with –H (deoxyribose). In some embodiments, the 2'–OH is replaced with –F. In some embodiments, the 2'–OH is replaced with –OR'. In some embodiments, the 2'–OH is replaced with –OMe. In some embodiments, the 2'–OH is replaced with –OCH_2CH_2OMe.

[00696] Modified sugars also include locked nucleic acids (LNAs). In some embodiments, two substituents on sugar carbon atoms are taken together to form a bivalent moiety. In some embodiments, two substituents are on two different sugar carbon atoms. In some embodiments, a formed bivalent moiety has the structure of –L– as defined herein. In some embodiments, –L– is –O–CH_2–, wherein –CH_2– is optionally substituted. In some embodiments, –L– is –O–CH_2–. In some embodiments, –L– is –O–CH(Et)–. In some embodiments, –L– is between C2 and C4 of a sugar moiety. In some embodiments, a locked nucleic acid has the structure indicated below. A locked nucleic acid of the structure below is indicated, wherein Ba represents a nucleobase or modified nucleobase as described herein, and wherein R^{2s} is –OCH_2C4'–.



C2'OCH_2C4' = LNA (Locked Nucleic Acid)

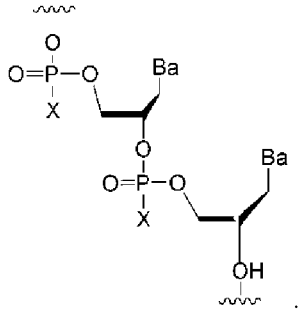
R^{2s} = OCH_2C4'

[00697] In some embodiments, a modified sugar is an ENA such as those described in, *e.g.,* Seth et al., J Am Chem Soc. 2010 October 27; 132(42): 14942–14950. In some embodiments, a modified sugar is any of those found in an XNA (xenonucleic acid), for instance, arabinose, anhydrohexitol, threose, 2'fluoroarabinose, or cyclohexene.

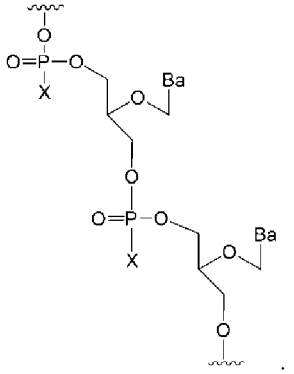
[00698] Modified sugars include sugar mimetics such as cyclobutyl or cyclopentyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, US Patent Nos.: 4,981,957; 5,118,800; 5,319,080 ; and 5,359,044. Some modified sugars that are contemplated

include sugars in which the oxygen atom within the ribose ring is replaced by nitrogen, sulfur, selenium, or carbon. In some embodiments, a modified sugar is a modified ribose wherein the oxygen atom within the ribose ring is replaced with nitrogen, and wherein the nitrogen is optionally substituted with an alkyl group (*e.g.*, methyl, ethyl, isopropyl, *etc*).

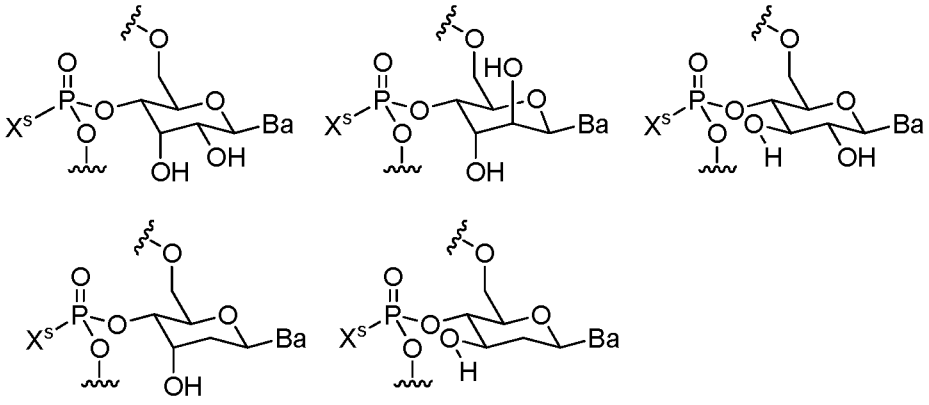
[00699] Non-limiting examples of modified sugars include glycerol, which form glycerol nucleic acid (GNA) analogues. One example of a GNA analogue is shown below and is described in Zhang, R *et al.*, *J. Am. Chem. Soc.*, 2008, *130*, 5846-5847; Zhang L, *et al.*, *J. Am. Chem. Soc.*, 2005, *127*, 4174-4175 and Tsai CH *et al.*, *PNAS*, 2007, 14598-14603 (X = O^-):



[00700] Another example of a GNA derived analogue, flexible nucleic acid (FNA) based on the mixed acetal aminal of formyl glycerol, is described in Joyce GF *et al.*, *PNAS*, 1987, *84*, 4398-4402 and Heuberger BD and Switzer C, *J. Am. Chem. Soc.*, 2008, *130*, 412-413, and is shown below:

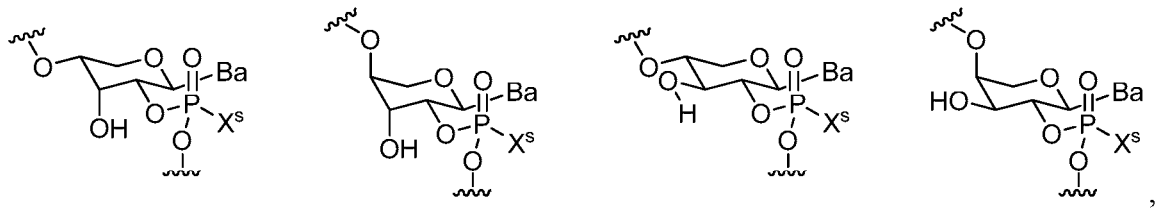


[00701] Additional non-limiting examples of modified sugars include hexopyranosyl (6' to 4'), pentopyranosyl (4' to 2'), pentopyranosyl (4' to 3'), or tetrofuranosyl (3' to 2') sugars. In some embodiments, a hexopyranosyl (6' to 4') sugar is of any one in the following formulae:



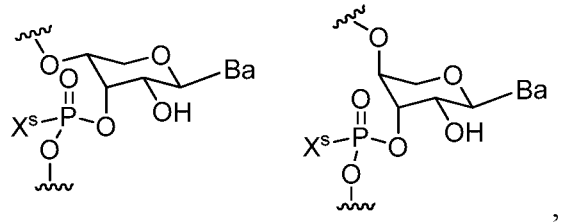
wherein X^s corresponds to the P-modification group "-XLR^1" described herein and Ba is as defined herein.

[00702] In some embodiments, a pentopyranosyl (4' to 2') sugar is of any one in the following formulae:



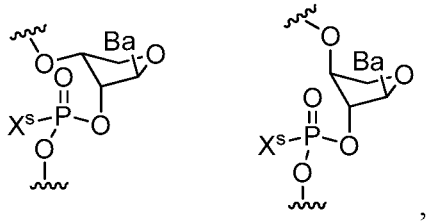
wherein X^s corresponds to the P-modification group "-XLR^1" described herein and Ba is as defined herein.

[00703] In some embodiments, a pentopyranosyl (4' to 3') sugar is of any one in the following formulae:



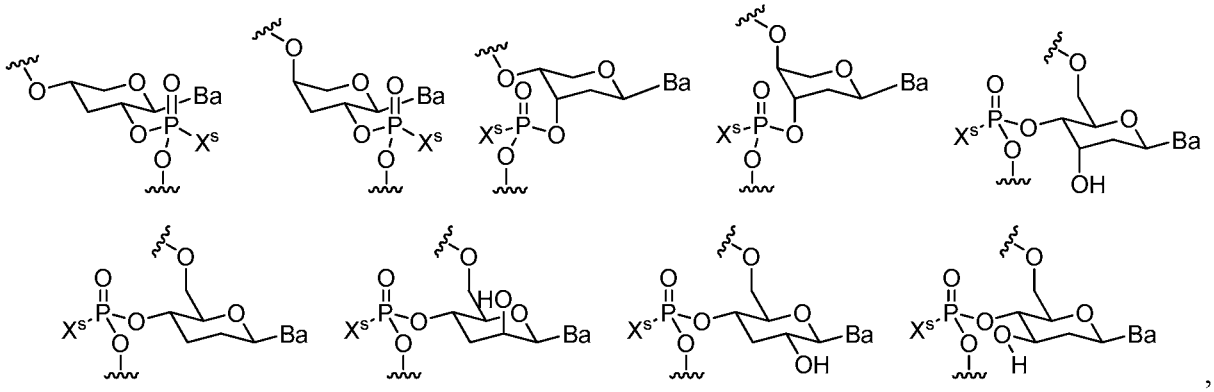
wherein X^s corresponds to the P-modification group "-XLR^1" described herein and Ba is as defined herein.

[00704] In some embodiments, a tetrofuranosyl (3' to 2') sugar is of either in the following formulae:



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

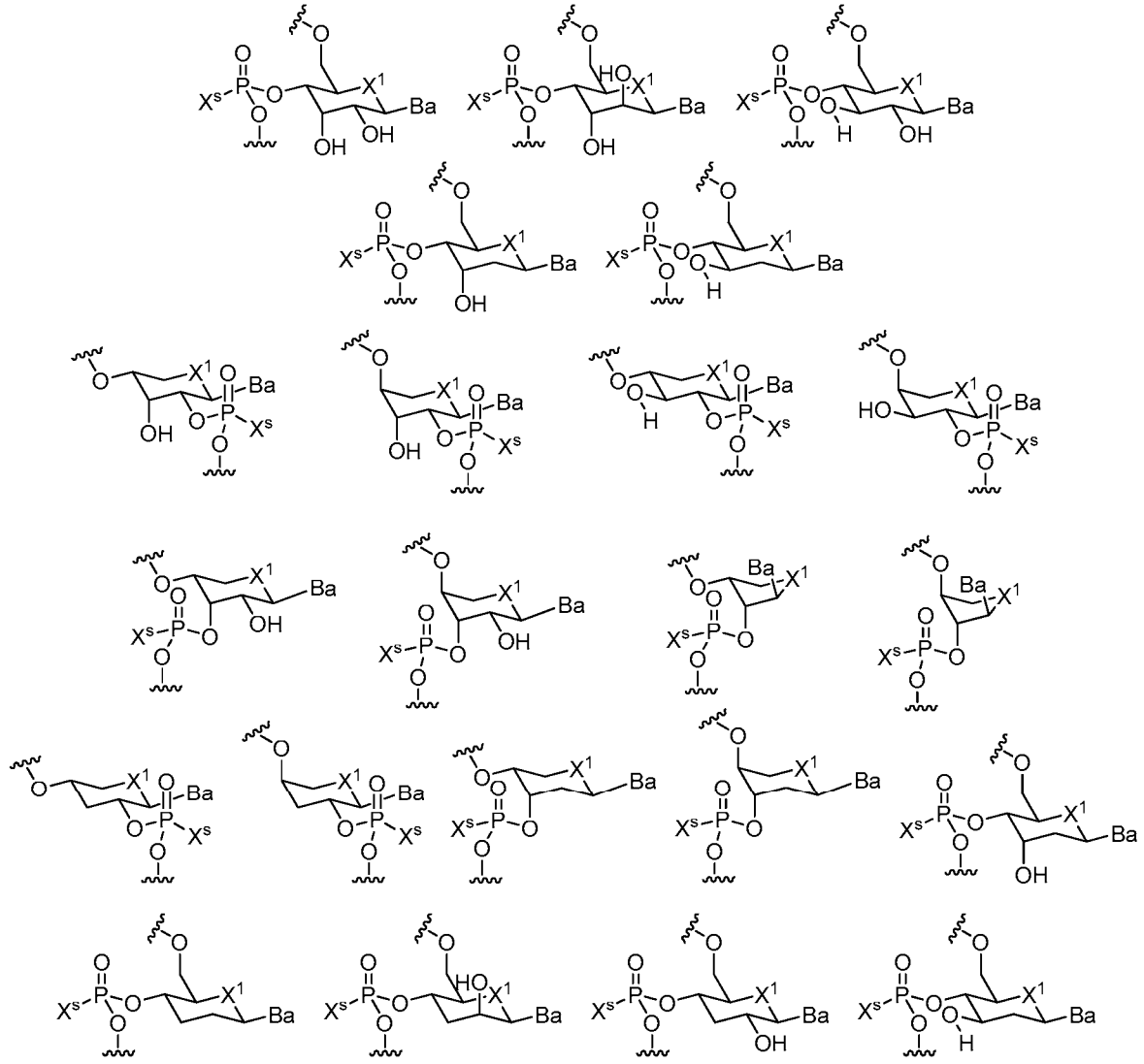
[00705] In some embodiments, a modified sugar is of any one in the following formulae:



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

[00706] In some embodiments, one or more hydroxyl group in a sugar moiety is optionally and independently replaced with halogen, R' $-N(R')_2$, $-OR'$, or $-SR'$, wherein each R' is independently as defined above and described herein.

[00707] In some embodiments, a sugar mimetic is as illustrated below, wherein X^s corresponds to the P-modification group "-XLR^1" described herein, Ba is as defined herein, and X^1 is selected from –S–, –Se–, $-CH_2-$, –NMe–, –NEt– or –N*i*Pr–.



[00708] In some embodiments, at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50% or more (*e.g.*, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more), inclusive, of the sugars in a chirally controlled oligonucleotide composition are modified. In some embodiments, only purine residues are modified (*e.g.*, about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50% or more [*e.g.*, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more] of the purine residues

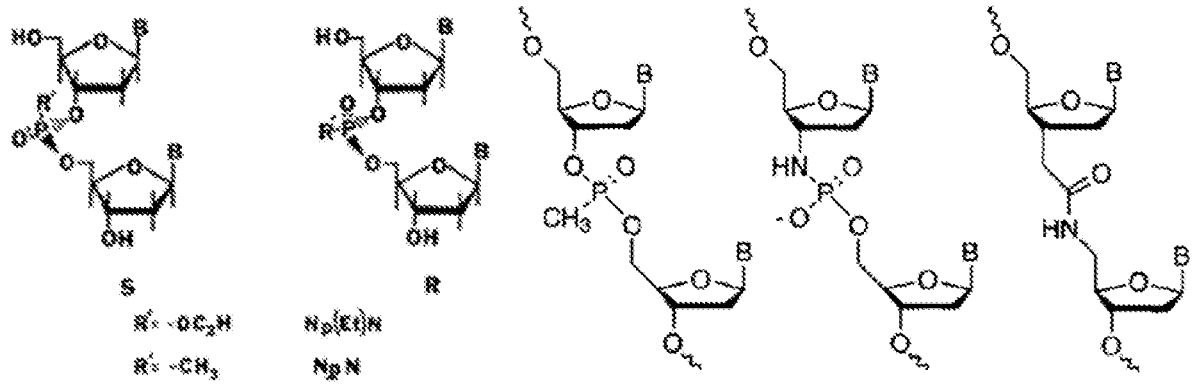
are modified). In some embodiments, only pyrimidine residues are modified (*e.g.*, about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50% or more [*e.g.*, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more] of the pyridimine residues are modified). In some embodiments, both purine and pyrimidine residues are modified.

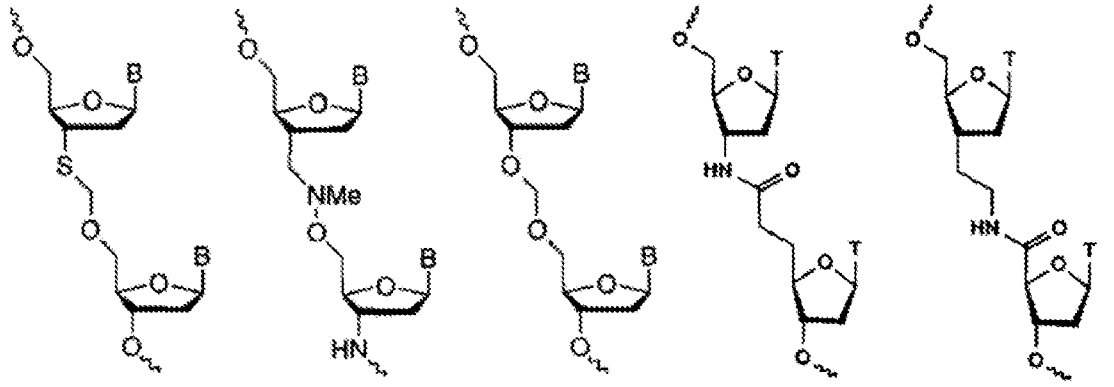
[00709] Modified sugars and sugar mimetics can be prepared by methods known in the art, including, but not limited to: A. Eschenmoser, Science (1999), 284:2118; M. Bohringer *et al,* Helv. Chim. Acta (1992), 75:1416-1477; M. Egli *et al*, J. Am. Chem. Soc. (2006), 128(33):10847-56; A. Eschenmoser in *Chemical Synthesis: Gnosis to Prognosis,* C. Chatgilialoglu and V. Sniekus, Ed., (Kluwer Academic, Netherlands, 1996), p.293; K.-U. Schoning *et al,* Science (2000), 290:1347-1351; A. Eschenmoser *et al,* Helv. Chim. Acta (1992), 75:218; J. Hunziker *et al,* Helv. Chim. Acta (1993), 76:259; G. Otting *et al,* Helv. Chim. Acta (1993), 76:2701; K. Groebke *et al,* Helv. Chim. Acta (1998), 81:375; and A. Eschenmoser, Science (1999), 284:2118. Modifications to the 2' modifications can be found in Verma, S. *et al. Annu. Rev. Biochem.* **1998,** 67, 99-134 and all references therein. Specific modifications to the ribose can be found in the following references: 2'-fluoro (Kawasaki et. al., *J. Med. Chem.,* **1993,** 36, 831- 841), 2'-MOE (Martin, P. *Helv. Chim. Acta* **1996,** 79, 1930-1938), "LNA" (Wengel, J. *Acc. Chem. Res.* **1999,** *32,* 301-310). In some embodiments, a modified sugar is any of those described in PCT Publication No. WO2012/030683, incorporated herein by reference, and depicted in the Figures 26-30 of the present application. In some embodiments, a modified sugar is any modified sugar 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 20070900071; WO 20070900071; or WO 2016/079181.

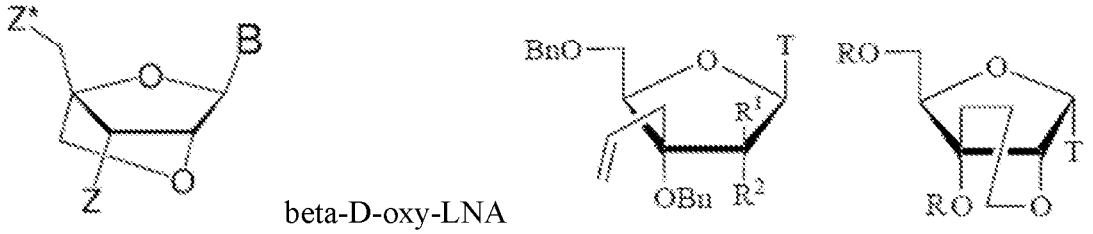
[00710] In some embodiments, a modified sugar moiety is an optionally substituted pentose or hexose moiety. In some embodiments, a modified sugar moiety is an optionally substituted pentose moiety. In some embodiments, a modified sugar moiety is an optionally substituted hexose moiety. In some embodiments, a modified sugar moiety is an optionally substituted ribose or hexitol moiety. In some embodiments, a modified sugar moiety is an optionally substituted ribose moiety. In some embodiments, a modified sugar moiety is an optionally substituted hexitol moiety.

[00711] In some embodiments, an example modified internucleotidic linkage and/or sugar is selected from:

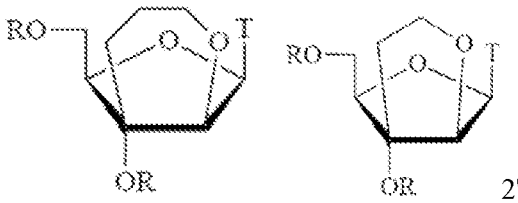




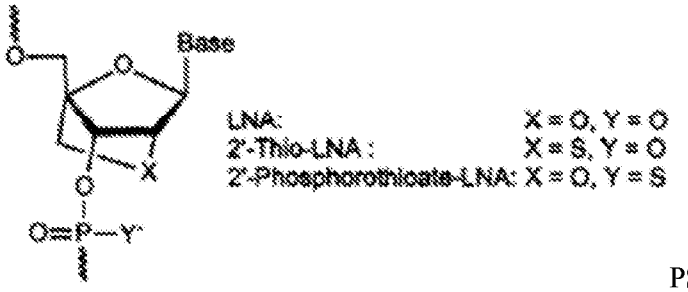
HNA PNA 2'-Fluoro N3'-P5'-phosphoramidate



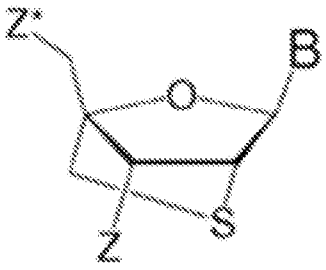
LNA beta-D-oxy-LNA



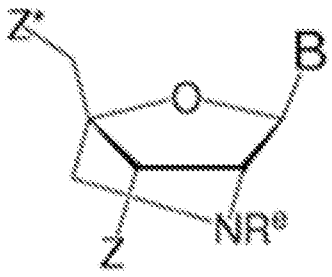
2'-O,3'-C-linked bicyclic



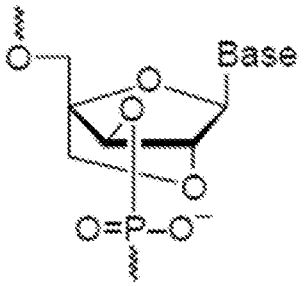
PS-LNA



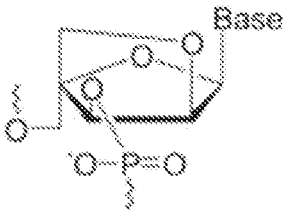
beta-



D-thio-LNA



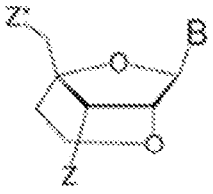
beta-D-amino-LNA



xylo-LNA [c]

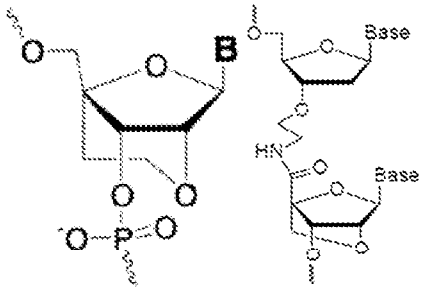


alpha-L-LNA

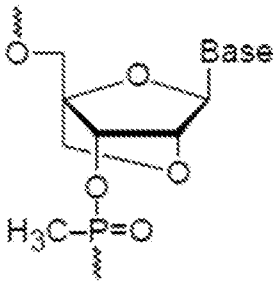


ENA

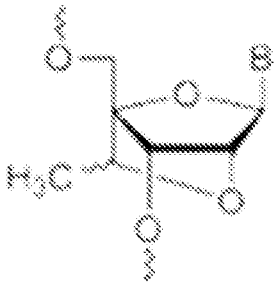
beta-D-ENA



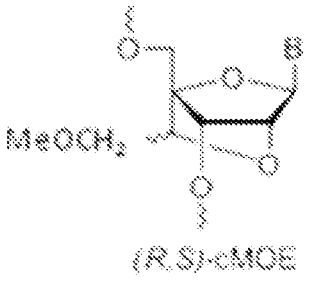
amide-linked LNA



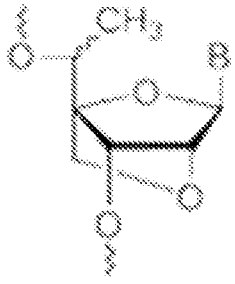
methylphosphonate-LNA



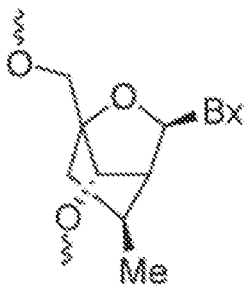
(*R, S*)-cEt



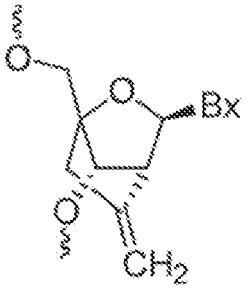
(*R, S*)-cMOE



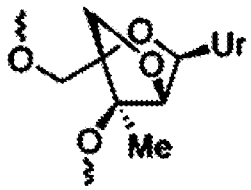
(*R, S*)-5'-Me-LNA



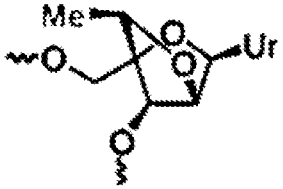
S-Me cLNA



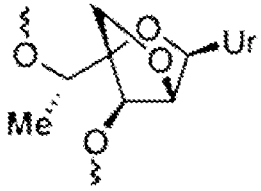
Methylene-cLNA



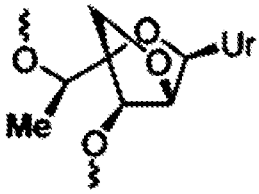
3'-Me-alpha-L-LNA



R-6'-Me-alpha-L-LNA



S-5'-Me-alpha-L-LNA



R-5'-Me-alpha-L-LNA

In some embodiments, R^1 is R as defined and described. In some embodiments, R^2 is R. In some embodiments, R^e is R. In some embodiments, R^e is H, CH_3, Bn, $COCF_3$, benzoyl, benzyl, pyren-1-ylcarbonyl, pyren-1-ylmethyl, 2-aminoethyl. In some embodiments, an example modified internucleotidic linkage and/or sugar is selected from those described in Ts'o et al. Ann. N. Y. Acad. Sci. **1988**, 507, 220; Gryaznov, S.; Chen, J.-K. J. Am. Chem. Soc. **1994**, 116, 3143; Mesmaeker et al. Angew. Chem., Int. Ed. Engl. **1994**, 33, 226; Jones et al. J. Org. Chem. **1993**, 58, 2983; Vasseur et al. J. Am. Chem. Soc. **1992**, 114, 4006; Van Aerschot et al. **1995** Angew. Chem. Int. Ed. Engl. 34: 1338; Hendrix et al. 1997 Chem. Eur. J. 3: 110; Koshkin et al. 1998 Tetrahedron 54: 3607-3630; Hyrup et al. **1996** Bioorg. Med. Chem. 4: 5; Nielsen et al. 1997 Chem. Soc. Rev. 73; Schultz et al. **1996** Nucleic Acids Res. 24: 2966; Obika et al. **1997** Tetrahedron Lett. 38 (50): 8735–8; Obika et al. 1998 Tetrahedron Lett. 39: 5401-5404; Singh et al. 1998 Chem. Comm. 1247-1248; Kumar et al. 1998 Bioo. Med. Chem. Let. 8: 2219-2222; Nielsen et al. **1997** J. Chem. Soc. Perkins Transl. 1: 3423-3433; Singh et al. **1998** J. Org. Chem. 63: 6078-6079; Seth et al. 2010 J. Org. Chem. 75: 1569-1581; Singh et al. **1998** J. Org. Chem. 63: 10035-39; Sorensen 2003 Chem. Comm. 2130-2131; Petersen et al. 2003 TRENDS Biotech. 21: 74-81; Rajwanshi et al. **1999** Chem. Commun. 1395-1396; Jepsen et al. 2004 Oligo. 14: 130-146; 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; Koizumi et al. 2003 Nuc. Acids Res. 12: 3267-3273; Lauritsen et al. **2002** Chem. Comm. 5: 530-531; Lauritsen et al. **2003** Bioo. Med. Chem. Lett. 13: 253-256; WO 20070900071; Seth et al., Nucleic Acids Symposium Series (2008), 52(1), 553-554; Seth et al. 2009 J.

Med. Chem. 52: 10-13; Seth et al. 2012 Mol. Ther-Nuc. Acids. 1, e47; Pallan et al. 2012 Chem. Comm. 48: 8195-8197; Seth et al. **2010** J. Med. Chem. 53: 8309-8318; Seth et al. 2012 Bioo. Med. Chem. Lett. 22: 296-299; WO 2016/079181; US 6,326,199; US 6,066,500; and US 6,440,739, the base and sugar modifications of each of which is herein incorporated by reference.

Oligonucleotides

[00712] In some embodiments, the present disclosure provides oligonucleotides and oligonucleotide compositions that are chirally controlled. For instance, in some embodiments, a provided composition contains predetermined levels of one or more individual oligonucleotide types, wherein an oligonucleotide type is defined by: 1) base sequence; 2) pattern of backbone linkages; 3) pattern of backbone chiral centers; and 4) pattern of backbone P-modifications. In some embodiments, a particular oligonucleotide type may be defined by 1A) base identity; 1B) pattern of base modification; 1C) pattern of sugar modification; 2) pattern of backbone linkages; 3) pattern of backbone chiral centers; and 4) pattern of backbone P-modifications. In some embodiments, oligonucleotides of the same oligonucleotide type are identical.

[00713] As described herein, the present disclosure provides various oligonucleotides. In some embodiments, the present disclosure provides oligonucleotides comprising a sequence that shares greater than about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% identity with a sequence found in a provided example oligonucleotide, such as those listed in various tables. In some embodiments, a provided oligonucleotide is WV-1092. In some embodiments, a provided oligonucleotide is WV-2595. In some embodiments, a provided oligonucleotide is WV-2603. In some embodiments, the present disclosure provides oligonucleotides comprising or consisting of a sequence found in a provided example oligonucleotide. In some embodiments, the present disclosure provides oligonucleotides comprising or consisting of a sequence found in WV-1092. In some embodiments, the present disclosure provides oligonucleotides comprising or consisting of a sequence found in WV-2595. In some embodiments, the present disclosure provides oligonucleotides comprising or consisting of a sequence found in WV-2603. In some embodiments, a provided oligonucleotide further comprises one or more natural phosphate linkages and one or more modified internucleotidic linkages. In some embodiments, a provided oligonucleotide comprises two or more natural phosphate linkages. In some embodiments, a provided oligonucleotide comprises two or more consecutive natural phosphate linkages. In some embodiments, a provided oligonucleotide comprises two or more modified internucleotidic

linkages. In some embodiments, a provided oligonucleotide comprises two or more consecutive modified internucleotidic linkages. In some embodiments, a provided oligonucleotide comprises 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more consecutive modified internucleotidic linkages. In some embodiments, a provided oligonucleotide comprises 5 or more consecutive modified internucleotidic linkages. In some embodiments, a provided oligonucleotide comprises 5 or more consecutive modified internucleotidic linkages. In some embodiments, a provided oligonucleotide comprises 6 or more consecutive modified internucleotidic linkages. In some embodiments, a provided oligonucleotide comprises 7 or more consecutive modified internucleotidic linkages. In some embodiments, a provided oligonucleotide comprises 8 or more consecutive modified internucleotidic linkages. In some embodiments, a provided oligonucleotide comprises 9 or more consecutive modified internucleotidic linkages. In some embodiments, a provided oligonucleotide comprises 10 or more consecutive modified internucleotidic linkages. In some embodiments, at least one of the modified internucleotidic linkages is a chirally controlled internucleotidic linkage in that oligonucleotides having the same sequence and chemical modifications within a composition share the same configuration, either *R*p or *S*p, at the chiral phosphorus atom of the modified internucleotidic linkage. In some embodiments, at least two modified internucleotidic linkages are chirally controlled. In some embodiments, at least one modified internucleotidic linkage within a consecutive modified internucleotidic linkage region is chirally controlled. In some embodiments, at least two modified internucleotidic linkages within a consecutive modified internucleotidic linkage region are chirally controlled. In some embodiments, each modified internucleotidic linkage within a consecutive modified internucleotidic linkage region is chirally controlled. In some embodiments, each modified internucleotidic linkage is chirally controlled. In some embodiments, a provided oligonucleotide comprises a (*S*p)x*R*p(*S*p)y pattern, wherein each of x and y is independently 1-20, and the sum of x and y is 1-50. In some embodiments, each of x and y is independently 2-20. In some embodiments, at least one of x and y is greater than 5, 6, 7, 8, 9, or 10. In some embodiments, the sum of x and y is greater than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In some embodiments, a provided oligonucleotide comprises one or more chemical modifications as presented in a provided example oligonucleotide. In some embodiments, a provided oligonucleotide comprises one or more base modifications as presented in a provided example oligonucleotide. In some embodiments, a

provided oligonucleotide comprises one or more sugar modifications as presented in a provided example oligonucleotide. In some embodiments, a sugar modification is a 2'-modification. In some embodiments, a sugar modification is LNA. In some embodiments, a sugar modification is ENA. In some embodiments, a provided oligonucleotide is a chirally controlled oligonucleotide. In some embodiments, the present disclosure provides an oligonucleotide composition comprising a provided oligonucleotide. In some embodiments, a provided oligonucleotide composition is a chirally controlled oligonucleotide composition.

[00714] In some embodiments, a provided oligonucleotide is a unimer. In some embodiments, a provided oligonucleotide is a P-modification unimer. In some embodiments, a provided oligonucleotide is a stereounimer. In some embodiments, a provided oligonucleotide is a stereounimer of configuration *R*p. In some embodiments, a provided oligonucleotide is a stereounimer of configuration *S*p.

[00715] In some embodiments, a provided oligonucleotide is an altmer. In some embodiments, a provided oligonucleotide is a P-modification altmer. In some embodiments, a provided oligonucleotide is a stereoaltmer.

[00716] In some embodiments, a provided oligonucleotide is a blockmer. In some embodiments, a provided oligonucleotide is a P-modification blockmer. In some embodiments, a provided oligonucleotide is a stereoblockmer.

[00717] In some embodiments, a provided oligonucleotide is a gapmer.

[00718] In some embodiments, a provided oligonucleotide is a skipmer.

[00719] In some embodiments, a provided oligonucleotide is a hemimer. In some embodiments, a hemimer is an oligonucleotide wherein the 5'-end or the 3'-end has a sequence that possesses a structure feature that the rest of the oligonucleotide does not have. In some embodiments, the 5'-end or the 3'-end has or comprises 2 to 20 nucleotides. In some embodiments, a structural feature is a base modification. In some embodiments, a structural feature is a sugar modification. In some embodiments, a structural feature is a P-modification. In some embodiments, a structural feature is stereochemistry of the chiral internucleotidic linkage. In some embodiments, a structural feature is or comprises a base modification, a sugar modification, a P-modification, or stereochemistry of the chiral internucleotidic linkage, or combinations thereof. In some embodiments, a hemimer is an oligonucleotide in which each sugar moiety of the 5'-end sequence shares a common modification. In some embodiments, a

hemimer is an oligonucleotide in which each sugar moiety of the 3'-end sequence shares a common modification. In some embodiments, a common sugar modification of the 5' or 3' end sequence is not shared by any other sugar moieties in the oligonucleotide. In some embodiments, an example hemimer is an oligonucleotide comprising a sequence of substituted or unsubstituted 2'-O-alkyl sugar modified nucleosides, bicyclic sugar modified nucleosides, β-D-ribonucleosides or β-D- deoxyribonucleosides (for example 2'-MOE modified nucleosides, and LNA™ or ENA™ bicyclic syugar modified nucleosides) at one terminus and a sequence of nucleosides with a different sugar moiety (such as a substituted or unsubstituted 2'-O-alkyl sugar modified nucleosides, bicyclic sugar modified nucleosides or natural ones) at the other terminus. In some embodiments, a provided oligonucleotide is a combination of one or more of unimer, altmer, blockmer, gapmer, hemimer and skipmer. In some embodiments, a provided oligonucleotide is a combination of one or more of unimer, altmer, blockmer, gapmer, and skipmer. For instance, in some embodiments, a provided oligonucleotide is both an altmer and a gapmer. In some embodiments, a provided nucleotide is both a gapmer and a skipmer. One of skill in the chemical and synthetic arts will recognize that numerous other combinations of patterns are available and are limited only by the commercial availability and / or synthetic accessibility of constituent parts required to synthesize a provided oligonucleotide in accordance with methods of the present disclosure. In some embodiments, a hemimer structure provides advantageous benefits, as exemplified by Figure 29. In some embodiments, provided oligonucleotides are 5'-hemmimers that comprises modified sugar moieties in a 5'-end sequence. In some embodiments, provided oligonucleotides are 5'-hemmimers that comprises modified 2'-sugar moieties in a 5'-end sequence.

[00720] In some embodiments, a provided oligonucleotide comprises one or more optionally substituted nucleotides. In some embodiments, a provided oligonucleotide comprises one or more modified nucleotides. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted nucleosides. In some embodiments, a provided oligonucleotide comprises one or more modified nucleosides. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted LNAs.

[00721] In some embodiments, a provided oligonucleotide comprises one or more optionally substituted nucleobases. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted natural nucleobases. In some embodiments, a provided

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

[00722] In some embodiments, a provided oligonucleotide comprises one or more optionally substituted sugars. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted sugars found in naturally occurring DNA and RNA. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted ribose or deoxyribose. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted ribose or deoxyribose, wherein one or more hydroxyl groups of the ribose or deoxyribose moiety is optionally and independently replaced by halogen, R', $-N(R')_2$, $-OR'$, or $-SR'$, wherein each R' is independently as defined above and described herein. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with halogen, R', $-N(R')_2$, $-OR'$, or $-SR'$, wherein each R' is independently as defined above and described herein. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with halogen. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with one or more –F. halogen. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with $-OR'$, wherein each R' is independently as defined above and described herein. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with $-OR'$, wherein each R' is independently an optionally substituted C_1–C_6 aliphatic. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with $-OR'$, wherein each R' is independently an optionally substituted C_1–C_6 alkyl. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and

independently substituted with –OMe. In some embodiments, a provided oligonucleotide comprises one or more optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally and independently substituted with –O–methoxyethyl.

[00723] In some embodiments, a provided oligonucleotide is single-stranded oligonucleotide.

[00724] In some embodiments, a provided oligonucleotide is a hybridized oligonucleotide strand. In certain embodiments, a provided oligonucleotide is a partially hydridized oligonucleotide strand. In certain embodiments, a provided oligonucleotide is a completely hydridized oligonucleotide strand. In certain embodiments, a provided oligonucleotide is a double-stranded oligonucleotide. In certain embodiments, a provided oligonucleotide is a triple-stranded oligonucleotide (*e.g.*, a triplex).

[00725] In some embodiments, a provided oligonucleotide is chimeric. For example, in some embodiments, a provided oligonucleotide is DNA-RNA chimera, DNA-LNA chimera, *etc*.

[00726] In some embodiments, any one of the structures comprising an oligonucleotide depicted in WO2012/030683 can be modified in accordance with methods of the present disclosure to provide chirally controlled variants thereof. For example, in some embodiments the chirally controlled variants comprise a stereochemical modification at any one or more of the linkage phosphorus and/or a P-modification at any one or more of the linkage phosphorus. For example, in some embodiments, a particular nucleotide unit of an oligonucleotide of WO2012/030683 is preselected to be stereochemically modified at the linkage phosphorus of that nucleotide unit and/or P-modified at the linkage phosphorus of that nucleotide unit. In some embodiments, a chirally controlled oligonucleotide is of any one of the structures depicted in Figures 26-30. In some embodiments, a chirally controlled oligonucleotide is a variant (*e.g.*, modified version) of any one of the structures depicted in Figures 26-30. The disclosure of WO2012/030683 is herein incorporated by reference in its entirety.

[00727] In some embodiments, a provided oligonucleotide is a therapeutic agent.

[00728] In some embodiments, a provided oligonucleotide is an antisense oligonucleotide.

[00729] In some embodiments, a provided oligonucleotide is an antigene oligonucleotide.

[00730] In some embodiments, a provided oligonucleotide is a decoy oligonucleotide.

[00731] In some embodiments, a provided oligonucleotide is part of a DNA vaccine.

[00732] In some embodiments, a provided oligonucleotide is an immunomodulatory

oligonucleotide, *e.g.,* immunostimulatory oligonucleotide and immunoinhibitory oligonucleotide.

[00733] In some embodiments, a provided oligonucleotide is an adjuvant.

[00734] In some embodiments, a provided oligonucleotide is an aptamer.

[00735] In some embodiments, a provided oligonucleotide is a ribozyme.

[00736] In some embodiments, a provided oligonucleotide is a deoxyribozyme (DNAzymes or DNA enzymes).

[00737] In some embodiments, a provided oligonucleotide is an siRNA.

[00738] In some embodiments, a provided oligonucleotide is a microRNA, or miRNA.

[00739] In some embodiments, a provided oligonucleotide is a ncRNA (non-coding RNAs), including a long non-coding RNA (lncRNA) and a small non-coding RNA, such as piwi-interacting RNA (piRNA).

[00740] In some embodiments, a provided oligonucleotide is complementary to a structural RNA, *e.g.,* tRNA.

[00741] In some embodiments, a provided oligonucleotide is a nucleic acid analog, *e.g.,* GNA, LNA, PNA, TNA, GNA, ANA, FANA, CeNA, HNA, UNA, ZNA, or Morpholino.

[00742] In some embodiments, a provided oligonucleotide is a P-modified prodrug.

[00743] In some embodiments, a provided oligonucleotide is a primer. In some embodiments, a primers is for use in polymerase-based chain reactions (i.e., PCR) to amplify nucleic acids. In some embodiments, a primer is for use in any known variations of PCR, such as reverse transcription PCR (RT-PCR) and real-time PCR.

[00744] In some embodiments, a provided oligonucleotide is characterized as having the ability to modulate RNase H activation. For example, in some embodiments, RNase H activation is modulated by the presence of stereocontrolled phosphorothioate nucleic acid analogs, with natural DNA/RNA being more or equally susceptible than the *R*p stereoisomer, which in turn is more susceptible than the corresponding *S*p stereoisomer.

[00745] In some embodiments, a provided oligonucleotide is characterized as having the ability to indirectly or directly increase or decrease activity of a protein or inhibition or promotion of the expression of a protein. In some embodiments, a provided oligonucleotide is characterized in that it is useful in the control of cell proliferation, viral replication, and/or any other cell signaling process.

[00746] In some embodiments, a provided oligonucleotide is from about 2 to about 200

nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 180 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 160 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 140 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 120 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 100 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 90 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 80 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 70 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 60 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 50 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 40 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 30 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 29 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 28 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 27 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 26 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 25 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 24 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 23 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 22 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 21 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 2 to about 20 nucleotide units in length.

[00747] In some embodiments, a provided oligonucleotide is from about 4 to about 200 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 180 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 160 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 140 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 120 nucleotide units in length. In some

embodiments, a provided oligonucleotide is from about 4 to about 100 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 90 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 80 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 70 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 60 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 50 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 40 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 30 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 29 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 28 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 27 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 26 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 25 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 24 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 23 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 22 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 21 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 4 to about 20 nucleotide units in length.

[00748] In some embodiments, a provided oligonucleotide is from about 5 to about 10 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 10 to about 30 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 15 to about 25 nucleotide units in length. In some embodiments, a provided oligonucleotide is from about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotide units in length.

[00749] In some embodiments, an oligonucleotide is at least 2 nucleotide units in length. In some embodiments, an oligonucleotide is at least 3 nucleotide units in length. In some embodiments, an oligonucleotide is at least 4 nucleotide units in length. In some embodiments, an oligonucleotide is at least 5 nucleotide units in length. In some embodiments, an oligonucleotide is at least 6 nucleotide units in length. In some embodiments, an oligonucleotide

is at least 7 nucleotide units in length. In some embodiments, an oligonucleotide is at least 8 nucleotide units in length. In some embodiments, an oligonucleotide is at least 9 nucleotide units in length. In some embodiments, an oligonucleotide is at least 10 nucleotide units in length. In some embodiments, an oligonucleotide is at least 11 nucleotide units in length. In some embodiments, an oligonucleotide is at least 12 nucleotide units in length. In some embodiments, an oligonucleotide is at least 13 nucleotide units in length. In some embodiments, an oligonucleotide is at least 14 nucleotide units in length. In some embodiments, an oligonucleotide is at least 15 nucleotide units in length. In some embodiments, an oligonucleotide is at least 16 nucleotide units in length. In some embodiments, an oligonucleotide is at least 17 nucleotide units in length. In some embodiments, an oligonucleotide is at least 18 nucleotide units in length. In some embodiments, an oligonucleotide is at least 19 nucleotide units in length. In some embodiments, an oligonucleotide is at least 20 nucleotide units in length. In some embodiments, an oligonucleotide is at least 21 nucleotide units in length. In some embodiments, an oligonucleotide is at least 22 nucleotide units in length. In some embodiments, an oligonucleotide is at least 23 nucleotide units in length. In some embodiments, an oligonucleotide is at least 24 nucleotide units in length. In some embodiments, an oligonucleotide is at least 25 nucleotide units in length. In some other embodiments, an oligonucleotide is at least 30 nucleotide units in length. In some other embodiments, an oligonucleotide is a duplex of complementary strands of at least 18 nucleotide units in length. In some other embodiments, an oligonucleotide is a duplex of complementary strands of at least 21 nucleotide units in length.

[00750] In some embodiments, the 5'-end and/or the 3'-end of a provided oligonucleotide is modified. In some embodiments, the 5'-end and/or the 3'-end of a provided oligonucleotide is modified with a terminal cap moiety. Examples of such modifications, including terminal cap moieties are extensively described herein and in the art, for example but not limited to those described in US Patent Application Publication US 2009/0023675A1.

[00751] In some embodiments, oligonucleotides of an oligonucleotide type characterized by 1) a common base sequence and length, 2) a common pattern of backbone linkages, and 3) a common pattern of backbone chiral centers, have the same chemical structure. For example, they have the same base sequence, the same pattern of nucleoside modifications, the same

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

Example Oligonucleotides and Compositions

[00752] In some embodiments, a provided chirally controlled oligonucleotide comprises the sequence of, or part of the sequence of mipomersen. Mipomersen is based on the following base sequence GCCT/UCAGT/UCT/UGCT/UT/UCGCACC. In some embodiments, one or more of any of the nucleotide or linkages may be modified in accordance of the present disclosure. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide having the sequence of G*-C*-C*-U*-C*-dA-dG-dT-dC-dT-dG-dmC-dT-dT-dmC-G*-C*-A*-C*-C* [d = 2'-deoxy, * = 2'-O-(2-methoxyethyl)] with 3'→5' phosphorothioate linkages. Example modified mipomersen sequences are described throughout the application, including but not limited to those in Table 2.

[00753] In certain embodiments, a provided oligonucleotide is a mipomersen unimer. In certain embodiments, a provided oligonucleotide is a mipomersen unimer of configuration *R*p. In certain embodiments, a provided oligonucleotide is a mipomersen unimer of configuration *S*p.

[00754] Exempary chirally controlled oligonucleotides comprising the sequence of, or part of the sequence of mipomersen is depicted in Table 2, below.

[00755] Table 2. Example oligonucleotides.

Oligo	Stereochemistry/Sequence	Description
101	All-(*R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	All-R
102	All-(*S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	All-S
103	(*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	5R-9S-5R
104	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	5S-9R-5S
105	(*S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p, *S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	1S-17R-1S
106	(*R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	1R-17S-1R
107	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	$(R/S)_9R$
108	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	$(S/R)_9S$

109	(*Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp* *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*)d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	3S-13R-3S
110	(*Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp* *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	3R-13S-3R
111	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp* *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	18S/R[19]
112	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp* *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	18S/R[9]
113	(*Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp* *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	18S/R[2]
114	(*Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp* *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	$(RRS)_6$-R
115	(*Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp* *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	S-$(RRS)_6$
116	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp* *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp* *Rp*)d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]	RS-$(RRS)_5$-RR
122	All-(*Rp*)-d[Gs1Cs1Cs1Ts1Cs1As1Gs1Ts1Cs1Ts1Gs1Cs1Ts1Ts1Cs1Gs1Cs1As1Cs1C]	All-R
123	(*Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp* *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*)-d[Gs1Cs1Cs1Ts1Cs1As1Gs1Ts1Cs1Ts1Gs1Cs1Ts1Ts1Cs1Gs1Cs1As1Cs1C]	1S-17R-1S
124	All-(*Sp*)-d[Gs1Cs1Cs1Ts1Cs1As1Gs1Ts1Cs1Ts1Gs1Cs1Ts1Ts1Cs1Gs1Cs1As1Cs1C]	All-S
126	All-(*Rp*)-d[Cs2As2Gs2T]	All-R
127	All-(*Rp*)-d[Cs3As3Gs3T]	All-R
128	All-(*Sp*)-d[Cs4As4Gs4T]	All-S
129	All-(*Sp*)-d[Cs5As5Gs5T]	All-S
130	All-(*Sp*)-d[Cs6As6Gs6T]	All-S
131	All-(*Rp*)-d[Gs7Cs7Cs7Ts7Cs7As7Gs7Ts7Cs7Ts7Gs7Cs7Ts7Ts7Cs7Gs7Cs7As7Cs7C]	All-R
132	All-(*Sp*)-d[Gs7Cs7Cs7Ts7Cs7As7Gs7Ts7Cs7Ts7Gs7Cs7Ts7Ts7Cs7Gs7Cs7As7Cs7C]	All-S
133	(*Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp* *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*)-d[Gs15mCs15mCs1Ts15mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1Gs15mCs1As15mCs15mC]	5R-9S-5R
134	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp* *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[Gs15mCs15mCs1Ts15mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1Gs15mCs1As15mCs15mC]	5S-9R-5S
135	All-(*Rp*)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	All-R
136	All-(*Sp*)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	All-S
137	(*Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	1S-9R-1S
138	(*Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	2S-7R-2S
139	(*Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*)-	1R-9S-1R

	d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	
140	(*Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	2R-7S-2R
141	(*Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	3S-5R-3S
142	(*Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	3R-5S-3R
143	(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	$(SSR)_3$-SS
144	(*Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G]	$(RRS)_3$-RR
145	All-(*Rp*)-d[5mCs1Ts15mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1Gs15mC]	All-R
146	All-(*Rp*)-d[Gs15mCs1Ts1G]	All-R
147	All-(*Rp*)-d[5mCs1As1Gs1T]	All-R
148	All-(*Rp*)-d[5mCs2As2Gs2Ts25mCs2Ts2Gs25mCs2Ts2Ts25mCs2G]	All-R
149	All-(*Rp*)-d[5mCs4As4Gs4Ts45mCs4Ts4Gs45mCs4Ts4Ts45mCs4G]	All-R
151	All-(*Sp*)-d[Cs1AsGs1T]	All-S
152	All-(*Sp*)-d[Cs1AGs1T]	All-S
153	All-(*Sp*)-d[CAs1GsT]	All-S
157	All-(*Sp*)-d[5mCs1As1Gs1T]	All-S
158	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCs1GsCsACsC]	5S-9R-4S
159	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[Gs1Cs1Cs1Ts1CsAsGsTsCsTsGsCsTsTsCs1GsCs2As2Cs2C]	5S-9R-5S
160	All-(*Rp*)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	All-R
161	All-(*Sp*)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	All-S
162	(*Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	5R-9S-5R
163	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	5S-9R-5S
164	(*Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	1S-17R-1S
165	(*Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*)-$(Gs5mCs5mCsTs5mCs)_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs]$(Gs5mCsAs5mCs5mC)_{MOE}$	1R-17S-1R
166	(*Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*,	$(R/S)_9R$

	Rp)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	
167	(*Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	(S/R)$_9$S
168	(*Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*)(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	3S-13R-3S
169	(*Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	3R-13S-3R
170	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	18S/R^{19}
171	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	18S/R^{9}
172	(*Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	18S/R^{2}
173	(*Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	(RRS)$_6$-R
174	(*Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	S-(RRS)$_6$
175	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*)(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	RS-(RRS)$_5$-RR
176	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*)(Gs15mCs15mCs1Ts15mCs1)$_{MOE}$d[As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1] (Gs15mCs1As15mCs15mC)$_{MOE}$	RS-(RRS)$_5$-RR
177	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*)(Gs15mCs15mCs1Ts15mCs1)$_{MOE}$d[AGT5mCTG5mCTT5mC] (Gs25mCs2As25mCs25mC)$_{MOE}$	RS-(RRS)$_5$-RR
178	(*Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTsTs5mCs] (Gs5mCsAs5mCs5mC)$_F$ (F: 2-fluorodeoxyribose)	S-(RRS)$_6$
179	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*)d[Gs8Cs8Cs8Ts8Cs8As8Gs8Ts8Cs8Ts8Gs8Cs8Ts8Ts8Cs8Gs8Cs8As8Cs8C]	RS-(RRS)$_5$-RR
180	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*)d[Gs9Cs9Cs9Ts9Cs9As9Gs9Ts9Cs9Ts9Gs9Cs9Ts9Ts9Cs9Gs9Cs9As9Cs9C]	RS-(RRS)$_5$-RR
181	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*)d[Gs10Cs10Cs10Ts10Cs10As10Gs10Ts10Cs10Ts10Gs10Cs10Ts1	RS-(RRS)$_5$-RR

	0Ts10Cs10Gs10Cs10As10Cs10C]	
182	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*)d[Gs11Cs11Cs11Ts11Cs11As11Gs11Ts11Cs11Ts11Gs11Cs11Ts11Ts11Cs11Gs11Cs11As11Cs11C]	RS-(RRS)$_5$-RR
183	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*)d[Gs12Cs12Cs12Ts12Cs12As12Gs12Ts12Cs12Ts12Gs12Cs12Ts12Ts12Cs12Gs12Cs12As12Cs12C]	RS-(RRS)$_5$-RR
184	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*)d[Gs13Cs13Cs13Ts13Cs13As13Gs13Ts13Cs13Ts13Gs13Cs13Ts13Ts13Cs13Gs13Cs13As13Cs13C]	RS-(RRS)$_5$-RR
185	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*)d[Gs14Cs14Cs14Ts14Cs14As14Gs14Ts14Cs14Ts14Gs14Cs14Ts14Ts14Cs14Gs14Cs14As14Cs14C]	RS-(RRS)$_5$-RR
186	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*)d[Gs15Cs15Cs15Ts15Cs15As15Gs15Ts15Cs15Ts15Gs15Cs15Ts15Ts15Cs15Gs15Cs15As15Cs15C]	RS-(RRS)$_5$-RR
187	(*Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp Rp*)d[GsCsCs1TsCsAs]GsUs2CsUsGsd[CsTs3TsCsGs]CsAs4CsC	RS-(RRS)$_5$-RR
188	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsACsC]	5S-9R-4S
189	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[Gs1Cs1Cs1Ts1Cs1As1Gs1Ts1Cs1Ts1Gs1Cs1Ts1Ts1Cs1Gs1CsACs1C]	5S-9R-4S
190	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[Gs8Cs8Cs8Ts8Cs8As8Gs8Ts8Cs8Ts8Gs8Cs8Ts8Ts8Cs8Gs8Cs1ACs8C]	5S-9R-4S
191	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[Gs9Cs9Cs9Ts9Cs9As9Gs9Ts9Cs9Ts9Gs9Cs9Ts9Ts9Cs9Gs9Cs1ACs9C]	5S-9R-4S
192	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[Gs10Cs10Cs10Ts10Cs10As10Gs10Ts10Cs10Ts10Gs10Cs10Ts10Ts10Cs10Gs10Cs1ACs10C]	5S-9R-4S
193	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[Gs11Cs11Cs11Ts11Cs11As11Gs11Ts11Cs11Ts11Gs11Cs11Ts11Ts11Cs11Gs11Cs1ACs11C]	5S-9R-4S
194	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[Gs12Cs12Cs12Ts12Cs12As12Gs12Ts12Cs12Ts12Gs12Cs12Ts12Ts12Cs12Gs12Cs1ACs12C]	5S-9R-4S
195	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[Gs13Cs13Cs13Ts13Cs13As13Gs13Ts13Cs13Ts13Gs13Cs13Ts13Ts13Cs13Gs13Cs1ACs13C]	5S-9R-4S
196	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[Gs14Cs14Cs14Ts14Cs14As14Gs14Ts14Cs14Ts14Gs14Cs14Ts14Ts14Cs14Gs14Cs1ACs14C]	5S-9R-4S
197	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[Gs15Cs15Cs15Ts15Cs15As15Gs15Ts15Cs15Ts15Gs15Cs15Ts15Ts	5S-9R-4S

	15Cs15Gs15Cs1ACs15C]	
198	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-GsCsCsUsCsAsGsUsCsUsGsCsUsUsCsGsCsACsC	5S-9R-4S
199	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-Gs1Cs1Cs1Us1Cs1As1Gs1Us1Cs1Us1Gs1Cs1Us1Us1Cs1Gs1CsACs1C	5S-9R-4S
200	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-Gs8Cs8Cs8Us8Cs8As8Gs8Us8Cs8Us8Gs8Cs8Us8Us8Cs8Gs8Cs1ACs8C	5S-9R-4S
201	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-Gs9Cs9Cs9Us9Cs9As9Gs9Us9Cs9Us9Gs9Cs9Us9Us9Cs9Gs9Cs1ACs9C	5S-9R-4S
202	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-Gs10Cs10Cs10Us10Cs10As10Gs10Us10Cs10Us10Gs10Cs10Us10Us10Cs10Gs10Cs1ACs10C	5S-9R-4S
203	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-Gs11Cs11Cs11Us11Cs11As11Gs11Us11Cs11Us11Gs11Cs11Us11Us11Cs11Gs11Cs1ACs11C	5S-9R-4S
204	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-Gs12Cs12Cs12Us12Cs12As12Gs12Us12Cs12Us12Gs12Cs12Us12Us12Cs12Gs12Cs1ACs12C	5S-9R-4S
205	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-Gs13Cs13Cs13Us13Cs13As13Gs13Us13Cs13Us13Gs13Cs13Us13Us13Cs13Gs13Cs1ACs13C	5S-9R-4S
206	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-Gs14Cs14Cs14Us14Cs14As14Gs14Us14Cs14Us14Gs14Cs14Us14Us14Cs14Gs14Cs1ACs14C	5S-9R-4S
207	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-Gs15Cs15Cs15Us15Cs15As15Gs15Us15Cs15Us15Gs15Cs15Us15Us15Cs15Gs15Cs1ACs15C	5S-9R-4S

[00756] In some embodiments, the present disclosure provides oligonucleotides and/or oligonucleotide compositions that are useful for treating Huntington's disease, for example, selected from:

	Table N1. Example sequences targeting rs362307		
WV-904	G*G*G*C*A*C*A*A*G*G*G*C*A*C*A*G*A*C*T*T	rs362307	P13
WV-905	G*G*C*A*C*A*A*G*G*G*C*A*C*A*G*A*C*T*T*C	rs362307	P12
WV-906	G*C*A*C*A*A*G*G*G*C*A*C*A*G*A*C*T*T*C*C	rs362307	P11
WV-907	C*A*C*A*A*G*G*G*C*A*C*A*G*A*C*T*T*C*C*A	rs362307	P10
WV-908	A*C*A*A*G*G*G*C*A*C*A*G*A*C*T*T*C*C*A*A	rs362307	P9
WV-909	C*A*A*G*G*G*C*A*C*A*G*A*C*T*T*C*C*A*A*A	rs362307	P8
WV-910	mG*mG*mG*mC*mA*C*A*A*G*G*G*C*A*C*A*G*A*C*T*T	rs362307	P13
WV-911	mG*mG*mC*mA*mC*A*A*G*G*G*C*A*C*A*G*A*C*T*T*C	rs362307	P12
WV-912	mG*mC*mA*mC*mA*A*G*G*G*C*A*C*A*G*A*C*T*T*C*C	rs362307	P11
WV-913	mC*mA*mC*mA*mA*G*G*G*C*A*C*A*G*A*C*T*T*C*C*A	rs362307	P10
WV-914	mA*mC*mA*mA*mG*G*G*C*A*C*A*G*A*C*T*T*C*C*A*A	rs362307	P9
WV-915	mC*mA*mA*mG*mG*G*C*A*C*A*G*A*C*T*T*C*C*A*A*A	rs362307	P8
WV-916	mG*mG*mG*mC*mA*C*A*A*G*G*G*C*A*C*A*mG*mA*mC*mU*mU	rs362307	P13
WV-917	mG*mG*mC*mA*mC*A*A*G*G*G*C*A*C*A*G*mA*mC*mU*mU*mC	rs362307	P12
WV-918	mG*mC*mA*mC*mA*A*G*G*G*C*A*C*A*G*A*mC*mU*mU*mC*mC	rs362307	P11
WV-919	mC*mA*mC*mA*mA*G*G*G*C*A*C*A*G*A*C*mU*mU*mC*mC*mA	rs362307	P10
WV-920	mA*mC*mA*mA*mG*G*G*C*A*C*A*G*A*C*T*mU*mC*mC*mA*mA	rs362307	P9
WV-921	mC*mA*mA*mG*mG*G*C*A*C*A*G*A*C*T*T*mC*mC*mA*mA*mA	rs362307	P8

WV-922	mG*mC*mA*mC*mA*mA*mG*mG*G*C*A*C*A*G*A*mC*mU*mU*mC*mC	rs362307	P11
WV-923	mC*mA*mC*mA*mA*mG*mG*G*C*A*C*A*G*A*mC*mU*mU*mC*mC*mA	rs362307	P10
WV-924	mA*mC*mA*mA*mG*mG*G*C*A*C*A*G*A*mC*mU*mU*mC*mC*mA*mA	rs362307	P9
WV-925	mC*mA*mA*mG*mG*G*C*A*C*A*G*A*mC*mU*mU*mC*mC*mA*mA*mA	rs362307	P8
WV-926	mGmCmAmCmAmAmGmG*G*C*A*C*A*G*A*mCmUmUmUmCmC	rs362307	P11
WV-927	mCmAmCmAmAmGmG*G*C*A*C*A*G*A*mCmUmUmUmCmCmA	rs362307	P10
WV-928	mAmCmAmAmGmG*G*C*A*C*A*G*A*mCmUmUmUmCmCmAmA	rs362307	P9
WV-929	mCmAmAmGmG*G*C*A*C*A*G*A*mCmUmUmUmCmCmAmAmA	rs362307	P8
WV-930	mGmGmGmCmA*C*A*A*G*G*G*C*A*C*A*mGmAmCmUmU	rs362307	P13
WV-931	mGmGmCmAmC*A*A*G*G*G*C*A*C*A*G*mAmCmUmUmC	rs362307	P12
WV-932	mGmCmAmCmA*A*G*G*G*C*A*C*A*G*A*mCmUmUmCmC	rs362307	P11
WV-933	mCmAmCmAmA*G*G*G*C*A*C*A*G*A*C*mUmUmCmCmA	rs362307	P10
WV-934	mAmCmAmAmG*G*C*A*C*A*G*A*C*T*mUmCmCmAmA	rs362307	P9
WV-935	mCmAmAmGmG*G*C*A*C*A*G*A*C*T*T*mCmCmAmAmA	rs362307	P8
WV-936	G*SG*SG*SC*SA*SC*SA*SA*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST*ST*SC	rs362307	P13
WV-937	G*SG*SC*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST*ST*SC	rs362307	P12
WV-938	G*SC*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST*ST*SC*SC	rs362307	P11
WV-939	C*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST*ST*SC*SC*SA	rs362307	P10
WV-940	A*SC*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST*ST*SC*SC*SA*SA	rs362307	P9

WV-941	C*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST*ST*SC*SC*SA*SA*SA	rs362307	P8
WV-1085	mG*SmG*SmC*SmC*SmA*SmC*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SmA*SmC*SmU*SmU*SmC	rs362307	P12
WV-1086	mG*ℝmG*ℝmC*ℝmA*ℝmC*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SmA*ℝmC*ℝmU*ℝmU*ℝmC	rs362307	P12
WV-1087	mGmGmCmAmC*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SmAmCmUmUmC	rs362307	P12
WV-1088	mG*SmG*SmC*SmA*SmC*SmA*SmA*SmG*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST*ST*SC	rs362307	P12
WV-1089	mG*ℝmG*ℝmC*ℝmA*ℝmA*ℝmC*ℝmA*ℝmG*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST*ST*SC	rs362307	P12
WV-1090	mGmGmCmAmCmAmAmG*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST*ST*SC	rs362307	P12
WV-1091	mG*ℝmGmCmAmC*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SmAmCmUmU*ℝmC	rs362307	P12
WV-1092	mG*SmGmCmAmC*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SmAmCmUmU*SmC	rs362307	P12
WV-982	G*SC*SA*SG*SG*SG*SC*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SA	rs362307	P16
WV-983	C*SA*SG*SG*SG*SC*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SA*SC	rs362307	P15
WV-984	A*SG*SG*SG*SC*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST	rs362307	P14
WV-985	A*SA*SG*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST*ST*SC*SC*SA*SA*SA*SG	rs362307	P7
WV-986	A*SG*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST*ST*SC*SC*SA*SA*SA*SG*SG	rs362307	P6
WV-987	G*SG*SG*SC*SA*SC*ℝA*SG*SA*SC*ST*ST*SC*SC*SA*SA*SA*SG*SG*SC	rs362307	P5
WV-1234	mG*mG*mC*mA*mC*A*A*G*G*G*C*A*C*A*G*mA*mC*mU*BrdU*mC	rs362307	P12
WV-1235	mG*mG*mC*mA*mC*A*A*G*G*G*C*A*C*A*G*mA*mC*BrdU*BrdU*mC	rs362307	P12
WV-1067	G*G*G*C*A*C*A*A*G*G*G*C*d2AP*C*A*G*A*C*T*T	rs362307	P13

WV-1068	G*G*C*A*C*A*A*G*G*G*C*d2AP*C*A*G*A*C*T*T*C	rs362307	P12
WV-1069	G*C*A*C*A*A*G*G*C*d2AP*C*A*G*A*C*T*T*C*C	rs362307	P11
WV-1070	G*G*G*C*A*C*A*A*G*G*G*C*dDAP*C*A*G*A*C*T*T	rs362307	P13
WV-1071	G*G*C*A*C*A*A*G*G*G*C*dDAP*C*A*G*A*C*T*T*C	rs362307	P12
WV-1072	G*C*A*C*A*A*G*G*G*C*dDAP*C*A*G*A*C*T*T*C*C	rs362307	P11
WV-1510	G*SmGmCmAmC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SmAmCmUmU*SC	rs362307	P12
WV-1511	G*mGmCmAmC*A*A*G*G*G*C*A*C*A*G*mAmCmUmU*C	rs362307	P12
WV-1497	mG*mGmCmAmC*A*A*G*G*C*A*C*A*G*mAmCmUmU*mC	rs362307	P12
WV-1655	Geo*Geom5CeoAeom5Ceo*A*A*G*G*C*A*C*A*G*Aeom5CeoTeoTeo*m5Ceo	rs362307	P12
	Table N2. Example sequences targeting rs362306		
WV-1001	G*A*G*C*A*G*C*T*G*C*A*A*C*C*T*G*G*C*A*A	rs362306	P10
WV-1002	A*G*C*A*G*C*T*G*C*A*A*C*C*T*G*G*C*A*A*C	rs362306	P9
WV-1003	G*C*A*G*C*T*G*C*A*A*C*C*T*G*G*C*A*A*C*A	rs362306	P8
WV-1004	C*A*G*C*T*G*C*A*A*C*C*T*G*G*C*A*A*C*A*A	rs362306	P7
WV-1005	A*G*C*T*G*C*A*A*C*C*T*G*C*A*A*C*A*A*C	rs362306	P6
WV-1006	G*C*T*G*C*A*A*C*C*T*G*G*C*A*A*C*A*A*C*C	rs362306	P5
WV-1007	mG*mA*mG*mC*mA*G*C*T*G*C*A*A*C*C*T*G*G*C*A*A	rs362306	P10
WV-1008	mA*mG*mC*mA*mG*C*T*G*C*A*A*C*C*T*G*G*C*A*A*C	rs362306	P9
WV-1009	mG*mC*mA*mG*mC*T*G*C*A*A*C*C*T*G*G*C*A*A*C*A	rs362306	P8

WV-1010	mC*mA*mG*mC*mU*G*C*A*A*C*C*T*G*G*C*A*A*C*A*A	rs362306	P7
WV-1011	mA*mG*mC*mU*mG*C*A*A*C*C*T*G*G*C*A*A*C*A*A*C	rs362306	P6
WV-1012	mG*mC*mU*mG*mC*A*A*C*C*T*G*G*C*A*A*A*C*A*A*C*C	rs362306	P5
WV-1013	mG*mA*mG*mC*mA*G*C*T*G*C*A*A*C*C*T*mG*mG*mC*mA*mA	rs362306	P10
WV-1014	mA*mG*mC*mA*mG*C*T*G*C*A*A*C*C*T*G*mG*mC*mA*mA*mC	rs362306	P9
WV-1015	mG*mC*mA*mG*mC*T*G*C*A*A*C*C*T*G*G*mC*mA*mA*mC*mA	rs362306	P8
WV-1016	mC*mA*mG*mC*mU*G*C*A*A*C*C*T*G*G*C*mA*mA*mC*mA*mA	rs362306	P7
WV-1017	mA*mG*mC*mU*mG*C*A*A*C*C*T*G*G*C*A*mA*mC*mA*mA*mC	rs362306	P6
WV-1018	mG*mC*mU*mG*mC*A*A*C*C*T*G*G*C*A*A*mC*mA*mA*mC*mC	rs362306	P5
WV-1019	mG*mA*mG*mC*mA*mG*mC*T*G*C*A*A*C*C*mU*mG*mG*mC*mA*mA	rs362306	P10
WV-1020	mGmAmGmCmAmGmC*T*G*C*A*A*C*C*mUmGmGmCmAmA	rs362306	P10
WV-1021	mA*mG*mC*mA*mG*mC*T*G*C*A*A*C*C*T*G*mG*mC*mA*mA*mC	rs362306	P9
WV-1022	mAmGmCmAmGmC*T*G*C*A*A*C*C*T*G*mGmCmAmAmC	rs362306	P9
WV-1023	mG*mC*mA*mG*mC*T*G*C*A*A*C*C*mU*mG*mG*mC*mA*mA*mC*mA	rs362306	P8
WV-1024	mGmCmAmGmC*T*G*C*A*A*C*C*mUmGmGmCmAmAmCmA	rs362306	P8
WV-1025	mGmAmGmCmA*G*C*T*G*C*A*A*C*C*T*mGmGmCmAmA	rs362306	P10
WV-1026	mAmGmCmAmG*C*T*G*C*A*A*C*C*T*G*mGmCmAmAmC	rs362306	P9
WV-1027	mGmCmAmGmC*T*G*C*A*A*C*C*T*G*mCmAmAmCmA	rs362306	P8
WV-1028	mCmAmGmCmU*G*C*A*A*C*C*T*G*G*C*mAmAmCmAmA	rs362306	P7

WV-1029	mAmGmCmUmG*C*A*A*C*C*T*G*G*C*A*mAmCmAmAmC	rs362306	P6
WV-1030	mGmCmUmGmC*A*A*C*C*T*G*G*C*A*A*mCmAmAmCmC	rs362306	P5
WV-952	G*SA*SG*SC*SA*SG*SC*ST*SG*SC*SA*RA*SC*SC*ST*SG*SG*SC*SA*SA	rs362306	P10
WV-953	A*SG*SC*SA*SG*SC*ST*SG*SC*SA*RA*SC*SC*ST*SG*SG*SC*SA*SA*SC	rs362306	P9
WV-954	G*SC*SA*SG*SC*ST*SG*SC*SA*RA*SC*SC*ST*SG*SG*SC*SA*SA*SC*SA	rs362306	P8
WV-955	C*SA*SG*SC*ST*SG*SC*SA*RA*SC*SC*ST*SG*SG*SC*SA*SA*SC*SA*SA	rs362306	P7
WV-956	A*SG*SC*ST*SG*SC*SA*RA*SC*SC*ST*SG*SG*SC*SA*SA*SC*SA*SA*SC	rs362306	P6
WV-957	G*SC*ST*SG*SC*SA*RA*SC*SC*ST*SG*SG*SC*SA*SA*SC*SA*SA*SC*SC	rs362306	P5
	Table N3. Example sequences targeting rs362268		
WV-1031	G*G*G*C*C*A*A*C*A*G*C*C*A*G*C*C*T*G*C*A	rs362268	P10
WV-1032	G*G*C*C*A*A*C*A*G*C*C*A*G*C*C*T*G*C*A*G	rs362268	P9
WV-1033	G*C*C*A*A*C*A*G*C*C*A*G*C*C*T*G*C*A*G*G	rs362268	P8
WV-1034	C*C*A*A*C*A*G*C*C*C*A*G*C*C*T*G*C*A*G*G*A	rs362268	P7
WV-1035	C*A*A*C*A*G*C*C*A*G*C*C*T*G*C*A*G*G*A*G	rs362268	P6
WV-1036	A*A*C*A*G*C*C*A*G*C*C*T*G*C*A*G*G*A*G*G	rs362268	P5
WV-1037	mG*mG*mG*mC*mC*A*A*C*A*G*C*C*C*A*G*C*C*T*G*C*A	rs362268	P10
WV-1038	mG*mG*mC*mC*mA*A*C*A*G*C*C*C*A*G*C*C*T*G*C*A*G	rs362268	P9
WV-1039	mG*mC*mC*mA*mA*C*A*G*C*C*A*G*C*C*T*G*C*A*G*G	rs362268	P8
WV-1040	mC*mC*mA*mA*mC*A*G*C*C*A*G*C*C*T*G*C*A*G*G*A	rs362268	P7

WV-1041	mC*mA*mA*mC*mA*mG*C*C*C*A*G*C*C*T*mG*C*A*G*G*A*G	rs362268	P6
WV-1042	mA*mA*mC*mA*mG*C*C*A*G*C*C*T*G*C*A*G*G*A*G*G	rs362268	P5
WV-1043	mG*mG*mG*mC*mC*A*A*C*A*G*C*C*C*A*G*C*C*mC*mU*mG*mC*mA	rs362268	P10
WV-1044	mG*mG*mC*mC*mA*A*C*A*G*C*C*C*A*G*C*C*mU*mG*mC*mC*mA*mG	rs362268	P9
WV-1045	mG*mC*mC*mA*mA*mA*C*A*G*C*C*C*A*G*C*C*C*T*mG*mC*mC*mA*mG*mG	rs362268	P8
WV-1046	mC*mC*mA*mA*mA*mC*A*G*C*C*C*A*G*C*C*C*T*G*mC*mC*mA*mG*mG*mA	rs362268	P7
WV-1047	mC*mA*mA*mA*mC*mA*G*C*C*C*A*G*C*C*C*T*G*C*C*mA*mG*mG*mG*mA*mG	rs362268	P6
WV-1048	mA*mA*mA*mC*mA*mG*C*C*C*A*G*C*C*C*T*G*C*C*A*mG*mG*mA*mG*mG	rs362268	P5
WV-1049	mG*mG*mG*mG*mC*mC*mC*mA*mA*C*C*A*G*C*C*C*C*A*G*mC*C*mC*mU*mG*mC*C*mA	rs362268	P10
WV-1050	mGmGmGmGmCmCmAmA*C*C*A*G*C*C*C*A*G*mCmCmCmUmGmGmCmA	rs362268	P10
WV-1051	mG*mG*mG*mC*mC*mA*mA*C*C*A*G*C*C*C*C*A*G*C*C*C*mU*mG*mC*C*mA*mG	rs362268	P9
WV-1052	mGmGmGmCmCmAmA*C*C*A*G*C*C*C*A*G*C*C*C*mUmGmGmCmAmG	rs362268	P9
WV-1053	mG*mC*mC*mC*mA*mA*C*C*A*G*C*C*C*A*G*C*C*C*A*G*mC*mC*mU*mG*mC*mA*mG*mG	rs362268	P8
WV-1054	mGmCmCmCmAmA*C*C*A*G*C*C*C*A*G*C*C*C*A*G*mCmCmCmUmGmGmCmAmGmG	rs362268	P8
WV-1055	mGmGmGmGmCmC*A*C*A*C*C*A*G*C*C*C*A*G*C*C*mCmCmUmGmGmCmA	rs362268	P10
WV-1056	mGmGmGmCmCmA*A*C*A*G*C*C*C*A*G*C*C*C*A*G*C*C*C*mUmGmGmCmAmG	rs362268	P9
WV-1057	mGmCmCmCmAmA*C*C*A*G*C*C*C*A*G*C*C*C*T*C*T*mGmCmCmAmGmG	rs362268	P8
WV-1058	mCmCmAmAmAmC*C*A*G*C*C*C*C*A*G*C*C*C*T*G*C*mCmAmGmGmGmA	rs362268	P7
WV-1059	mCmAmAmAmCmA*G*C*C*C*A*G*C*C*C*T*G*C*C*mAmGmGmAmG	rs362268	P6

WV-1060	mAmAmCmAmG*C*C*A*G*C*C*T*G*C*A*mGmGmAmGmG	rs362268	P5
WV-960	G*SG*SG*SC*SC*SA*SA*SC*SA*SG*SC*RC*SA*SG*SC*SC*ST*SG*SC*SA	rs362268	P10
WV-961	G*SG*SC*SC*SA*SA*SC*SA*SG*SC*RC*SA*SG*SC*SC*ST*SG*SC*SA*SG	rs362268	P9
WV-962	G*SC*SC*SA*SA*SC*SA*SG*SC*RC*SA*SG*SC*SC*ST*SG*SC*SA*SG*SG	rs362268	P8
WV-963	C*SC*SA*SA*SC*SA*SG*SC*RC*SA*SG*SC*SC*ST*SG*SC*SA*SG*SG*SA	rs362268	P7
WV-964	C*SA*SA*SC*SA*SG*SC*RC*SA*SG*SC*SC*ST*SG*SC*SA*SG*SG*SA*SG	rs362268	P6
WV-965	A*SA*SC*SA*SG*SC*RC*SA*SG*SC*SC*ST*SG*SC*SA*SG*SG*SA*SG*SG	rs362268	P5
	Table N4. Example sequences targeting rs7685686		
ONT-450	A*T*T*A*A*T*A*A*A*T*T*G*T*C*A*T*C*A*C*C	rs7685686	P13
ONT-451	A*ST*ST*SA*SA*ST*SA*SA*ST*ST*SG*ST*SC*RA*ST*SC*SA*SC*SC	rs7685686	P13
ONT-452	A*ST*ST*SA*SA*ST*SA*SA*ST*ST*SG*ST*SC*SA*RT*SC*SA*SC*SC	rs7685686	P13
WV-1077	mA*SmU*SmU*SmA*SmA*SmU*SA*SA*SA*ST*ST*SG*ST*SC*RA*ST*SmC*SmA*SmC*SmC	rs7685686	P13
WV-1078	mA*RmU*RmU*RmA*RmU*SA*SA*SA*ST*ST*SG*ST*SC*RA*ST*SmC*RmA*RmC*RmC	rs7685686	P13
WV-1079	mA*SmU*SmU*SmA*SmA*SmU*SmA*SmA*SA*ST*ST*SG*ST*SC*RA*ST*SC*SA*SC*SC	rs7685686	P13
WV-1080	mA*RmU*RmU*RmA*RmA*RmU*RmA*RmA*SA*ST*ST*SG*ST*SC*RA*ST*SC*SA*SC*SC	rs7685686	P13
WV-1081	mAmUmUmAmAmUmAmA*SA*ST*ST*SG*ST*SC*RA*ST*SC*SA*SC*SC	rs7685686	P13
WV-1082	mAmUmUmAmAmU*SA*SA*SA*ST*ST*SG*ST*SC*RA*ST*SmCmAmCmC	rs7685686	P13
WV-1083	mA*SmUmUmAmAmU*SA*SA*SA*ST*ST*SG*ST*SC*RA*ST*SmCmAmC*SmC	rs7685686	P13

WV-1084	mA*ⅈmUmUmAmAmU*ⅆA*ⅆA*ⅆA*ⅆT*ⅆT*ⅆG*ⅆT*ⅆC*ⅈA*ⅆT*ⅆmCmAmC*ⅈmC	rs7685686	P13
WV-1508	A*ⅆmUmUmAmAmU*ⅆA*ⅆA*ⅆA*ⅆT*ⅆT*ⅆG*ⅆT*ⅆC*ⅈA*ⅆT*ⅆmCmAmC*ⅆC	rs7685686	P13
WV-1509	A*mUmUmAmAmU*A*A*A*T*T*G*T*C*A*T*mCmAmC*C	rs7685686	P13
WV-2023	T*G*T*C*A*T*C*A*C*C*A*G*A*A*A*mA*mA*mG*mU*mC	rs7685686	P3
WV-2024	mU*T*G*T*C*A*T*C*A*C*C*A*G*A*A*A*mA*mA*mA*mG*mU	rs7685686	P4
WV-2025	T*T*G*T*C*A*T*C*A*C*C*C*A*G*A*A*A*mA*mA*mA*mG*mU	rs7685686	P4
WV-2026	mA*mU*T*G*T*C*A*T*C*A*C*C*C*A*G*A*A*mA*mA*mA*mA*mA*mG	rs7685686	P5
WV-2027	mA*T*T*G*T*C*A*T*C*A*C*C*C*A*G*A*A*mA*mA*mA*mA*mG	rs7685686	P5
WV-2028	mA*mA*mU*T*G*T*C*A*T*C*A*C*C*C*A*G*mA*mA*mA*mA*mA*mA	rs7685686	P6
WV-2029	mA*mA*T*T*G*T*C*A*T*C*A*C*C*C*A*G*mA*mA*mA*mA*mA*mA	rs7685686	P6
WV-2030	mA*mA*mA*T*T*G*T*C*A*T*C*A*C*C*C*A*A*mG*mA*mA*mA*mA*mA	rs7685686	P7
WV-2031	mA*mA*mA*mU*T*G*T*C*A*T*C*A*C*C*C*A*A*mG*mA*mA*mA*mA*mA	rs7685686	P7
WV-2032	mU*mA*mA*mA*mA*mU*T*G*T*C*A*T*C*A*C*C*C*A*A*mG*mA*mA*mA*mA	rs7685686	P8
WV-2033	mU*mA*mA*mA*mA*mU*T*G*T*C*A*T*C*A*C*C*C*A*A*mG*mA*mA*mA*mA	rs7685686	P8
WV-2034	mA*mU*mA*mA*mA*T*T*G*T*C*A*T*C*A*C*C*C*A*A*mG*mA*mA*mA	rs7685686	P9
WV-2035	mA*mU*mA*mA*mA*T*T*G*T*C*A*T*C*A*C*C*mC*mA*mA*mG*mA*mA	rs7685686	P9
WV-2036	mA*mA*mU*mA*mA*mA*A*T*T*G*T*C*A*T*C*A*C*C*C*mA*mA*mG*mA	rs7685686	P10
WV-2037	mA*mA*mU*mA*mA*A*T*T*G*T*C*A*T*C*A*C*C*mC*mA*mA*mG*mA	rs7685686	P10
WV-2038	mA*mA*mU*mA*mA*A*T*T*G*T*C*A*T*C*A*C*C*mC*mC*mA*mA*mG*mA	rs7685686	P10

WV-2039	mU*mA*mA*mU*mA*A*A*T*T*G*T*C*A*T*C*A*C*C*mA*mG	rs7685686	P11
WV-2040	mU*mA*mA*mU*mA*A*A*T*T*G*T*C*A*T*C*A*C*mC*mA*mG	rs7685686	P11
WV-2041	mU*mA*mA*mU*mA*A*A*T*T*G*T*C*A*T*C*A*T*C*A*mC*mC*mA*mG	rs7685686	P11
WV-2042	mU*mA*mA*mU*mA*A*A*T*T*G*T*C*A*T*C*mA*mC*mC*mA*mG	rs7685686	P11
WV-2043	mU*mU*mA*mA*mU*A*A*A*T*T*G*T*C*A*T*C*A*C*C*mA	rs7685686	P12
WV-2044	mU*mU*mA*mA*mU*A*A*A*T*T*G*T*C*A*T*C*A*C*mC*mA	rs7685686	P12
WV-2045	mU*mU*mA*mA*mU*A*A*A*T*T*G*T*C*A*T*C*A*mC*mC*mA	rs7685686	P12
WV-2046	mU*mU*mA*mA*mU*A*A*A*T*T*G*T*C*A*T*C*mA*mC*mC*mA	rs7685686	P12
WV-2047	mA*mU*mC*mA*mA*T*A*A*A*T*T*G*T*C*A*T*C*A*C*C	rs7685686	P13
WV-2048	mA*mU*mC*mA*mA*T*A*A*A*T*T*G*T*C*A*T*C*A*C*mC	rs7685686	P13
WV-2049	mA*mU*mC*mA*mA*T*A*A*A*T*T*G*T*C*A*T*C*A*mC*mC	rs7685686	P13
WV-2050	mA*mU*mC*mA*mA*T*A*A*A*T*T*G*T*C*A*T*C*mA*mC*mC	rs7685686	P13
WV-2051	mU*mA*mC*mU*mA*A*T*A*A*A*T*T*G*T*C*A*T*C*A*C	rs7685686	P14
WV-2052	mU*mA*mC*mU*mA*A*T*A*A*A*T*T*G*T*C*A*T*C*A*mC	rs7685686	P14
WV-2053	mU*mA*mC*mU*mA*A*T*A*A*A*T*T*G*T*C*A*T*C*mA*mC	rs7685686	P14
WV-2054	mC*mU*mA*mU*mU*A*A*T*A*A*A*T*T*G*T*C*A*T*C*A	rs7685686	P15
WV-2055	mC*mU*mA*mU*mU*A*A*T*A*A*A*T*T*G*T*C*A*T*C*mA	rs7685686	P15
WV-2056	mA*mC*mU*mA*mU*T*A*A*T*A*A*A*T*T*G*T*C*A*T*C	rs7685686	P16
WV-2057	T*G*T*C*A*T*C*A*C*C*A*G*A*A*A*A*mAmAmGmU*mC	rs7685686	P3

WV-2058	mU*T*G*T*C*A*T*C*A*C*C*A*G*A*A*mAmAmAmG*mU	rs7685686	P4
WV-2059	T*T*G*T*C*A*T*C*A*C*C*A*G*A*A*mAmAmAmG*mU	rs7685686	P4
WV-2060	mA*mU*T*G*T*C*A*T*C*A*C*C*A*G*A*mAmAmAmA*mG	rs7685686	P5
WV-2061	mA*T*T*G*T*C*A*T*C*A*C*C*A*G*A*mAmAmAmA*mG	rs7685686	P5
WV-2062	mA*mAmU*T*G*T*C*A*T*C*A*C*C*A*G*mAmAmAmA*mA	rs7685686	P6
WV-2063	mA*mA*T*T*G*T*C*A*T*C*A*C*C*A*G*mAmAmAmA*mA	rs7685686	P6
WV-2064	mA*mAmA*T*T*G*T*C*A*T*C*A*C*C*A*mGmAmAmA*mA	rs7685686	P7
WV-2065	mA*mAmAmU*T*G*T*C*A*T*C*A*C*C*A*mGmAmAmA*mA	rs7685686	P7
WV-2066	mU*mAmAmAmU*T*G*T*C*A*T*C*A*C*C*C*A*mGmAmA*mA	rs7685686	P8
WV-2067	mU*mAmAmAmU*T*G*T*C*A*T*C*A*C*C*C*mAmGmAmA*mA	rs7685686	P8
WV-2068	mA*mUmAmAmA*T*T*G*T*C*A*T*C*A*C*C*C*mAmGmA*mA	rs7685686	P9
WV-2069	mA*mUmAmAmA*T*T*G*T*C*A*T*C*A*C*C*mCmAmGmA*mA	rs7685686	P9
WV-2070	mA*mAmUmAmA*A*T*T*G*T*C*A*T*C*A*C*C*C*mAmG*mA	rs7685686	P10
WV-2071	mA*mAmUmAmA*A*T*T*G*T*C*A*T*C*A*C*C*mCmAmG*mA	rs7685686	P10
WV-2072	mA*mAmUmAmA*A*T*T*G*T*C*A*T*C*A*C*mCmCmAmG*mA	rs7685686	P10
WV-2073	mU*mAmAmUmA*A*A*T*T*G*T*C*A*T*C*A*C*C*C*mA*mG	rs7685686	P11
WV-2074	mU*mAmAmUmA*A*A*T*T*G*T*C*A*T*C*A*C*C*mCmA*mG	rs7685686	P11
WV-2075	mU*mAmAmUmA*A*A*T*T*G*T*C*A*T*C*C*A*mCmCmCmA*mG	rs7685686	P11
WV-2076	mU*mAmAmUmA*A*A*T*T*G*T*C*A*T*C*C*mAmCmCmCmA*mG	rs7685686	P11

WV-2077	mU*mUmAmAmU*A*A*A*T*T*G*T*C*A*T*C*A*C*C*mA	rs7685686	P12
WV-2078	mU*mUmAmAmU*A*A*A*T*T*G*T*C*A*T*C*A*C*mC*mA	rs7685686	P12
WV-2079	mU*mUmAmAmU*A*A*A*T*T*G*T*C*A*T*C*A*mCmC*mA	rs7685686	P12
WV-2080	mU*mUmAmAmU*A*A*A*T*T*G*T*C*A*T*C*mAmCmC*mA	rs7685686	P12
WV-2081	mA*mUmUmAmA*T*A*A*A*T*T*G*T*C*A*T*C*A*C*C	rs7685686	P13
WV-2082	mA*mUmUmAmA*T*A*A*A*T*T*G*T*C*A*T*C*A*C*mC	rs7685686	P13
WV-2083	mA*mUmUmAmA*T*A*A*A*T*T*G*T*C*A*T*C*A*mC*mC	rs7685686	P13
WV-2084	mA*mUmUmAmA*T*A*A*A*T*T*G*T*C*A*T*C*mAmC*mC	rs7685686	P13
WV-2085	mU*mAmUmUmA*A*T*A*A*A*T*T*G*T*C*A*T*C*A*C	rs7685686	P14
WV-2086	mU*mAmUmUmA*A*T*A*A*A*T*T*G*T*C*A*T*C*A*mC	rs7685686	P14
WV-2087	mU*mAmUmUmA*A*T*A*A*A*T*T*G*T*C*A*T*C*mA*mC	rs7685686	P14
WV-2088	mC*mUmAmUmU*A*A*T*A*A*A*T*T*G*T*C*A*T*C*A	rs7685686	P15
WV-2089	mC*mUmAmUmU*A*A*T*A*A*A*T*T*G*T*C*A*T*C*mA	rs7685686	P15
WV-2090	mA*mCmUmAmU*T*A*A*T*A*A*A*T*T*G*T*C*A*T*C	rs7685686	P16

	Table N1. Example sequences targeting rs362307 - continued (certain features)		
WV-904	All DNA, stereorandom PS	rs362307	P13
WV-905	All DNA, stereorandom PS	rs362307	P12
WV-906	All DNA, stereorandom PS	rs362307	P11

WV-907	All DNA, stereorandom PS	rs362307	P10
WV-908	All DNA, stereorandom PS	rs362307	P9
WV-909	All DNA, stereorandom PS	rs362307	P8
WV-910	5-15 (2'-OMe-DNA), stereorandom PS	rs362307	P13
WV-911	5-15 (2'-OMe-DNA), stereorandom PS	rs362307	P12
WV-912	5-15 (2'-OMe-DNA), stereorandom PS	rs362307	P11
WV-913	5-15 (2'-OMe-DNA), stereorandom PS	rs362307	P10
WV-914	5-15 (2'-OMe-DNA), stereorandom PS	rs362307	P9
WV-915	5-15 (2'-OMe-DNA), stereorandom PS	rs362307	P8
WV-916	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362307	P13
WV-917	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362307	P12
WV-918	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362307	P11
WV-919	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362307	P10
WV-920	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362307	P9
WV-921	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362307	P8
WV-922	8-7-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362307	P11
WV-923	7-7-6 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362307	P10
WV-924	6-7-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362307	P9
WV-925	5-7-8 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362307	P8

WV-926	8-7-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362307	P11
WV-927	7-7-6 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362307	P10
WV-928	6-7-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362307	P9
WV-929	5-7-8 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362307	P8
WV-930	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362307	P13
WV-931	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362307	P12
WV-932	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362307	P11
WV-933	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362307	P10
WV-934	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362307	P9
WV-935	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362307	P8
WV-936	All DNA, stereopure, One Rp	rs362307	P13
WV-937	All DNA, stereopure, One Rp	rs362307	P12
WV-938	All DNA, stereopure, One Rp	rs362307	P11
WV-939	All DNA, stereopure, One Rp	rs362307	P10
WV-940	All DNA, stereopure, One Rp	rs362307	P9
WV-941	All DNA, stereopure, One Rp	rs362307	P8
WV-1085	5-10-5 (2'-OMe-DNA-2'-OMe) Gapmer, Stereopure, One Rp in DNA	rs362307	P12
WV-1086	5-10-5 (2'-OMe-DNA-2'-OMe) Gapmer, Stereopure, One Rp in DNA and Rp wings	rs362307	P12
WV-1087	5-10-5 (2'-OMe-DNA-2'-OMe) Gapmer, Stereopure, One Rp in DNA, PO wings	rs362307	P12

WV-1088	8-12 (2'-OMe-DNA) hemimer, Stereopure, One Rp in DNA, Sp wing	rs362307	P12
WV-1089	8-12 (2'-OMe-DNA) hemimer, Stereopure, One Rp in DNA and Rp wing	rs362307	P12
WV-1090	8-12 (2'-OMe-DNA) hemimer, Srereopure, One Rp in DNA and PO wing	rs362307	P12
WV-1091	5-10-5 (2'-OMe-DNA-2'-OMe) gapmer, Stereopure, One Rp in DNA, First and last PS as Rp and rest PO wing	rs362307	P12
WV-1092	5-10-5 (2'-OMe-DNA-2'-OMe) gapmer, Stereopure, One Rp in DNA, First and last PS as Sp and rest PO wing	rs362307	P12
WV-982	All DNA, stereopure, One Rp	rs362307	P16
WV-983	All DNA, stereopure, One Rp	rs362307	P15
WV-984	All DNA, stereopure, One Rp	rs362307	P14
WV-985	All DNA, stereopure, One Rp	rs362307	P7
WV-986	All DNA, stereopure, One Rp	rs362307	P6
WV-987	All DNA, stereopure, One Rp	rs362307	P5
WV-1234	5-10-5 (2'-OMe-DNA-2'-OMe) Gapmer, Stereorandom, One Br-dU	rs362307	P12
WV-1235	5-10-5 (2'-OMe-DNA-2'-OMe) Gapmer, Stereorandom, Two Br-dU	rs362307	P12
WV-1067	All DNA, stereorandom PS, one 2-amino purine	rs362307	P13
WV-1068	All DNA, stereorandom PS, one 2-amino purine	rs362307	P12
WV-1069	All DNA, stereorandom PS, one 2-amino purine	rs362307	P11
WV-1070	All DNA, stereorandom PS, one 2,6-diamino purine	rs362307	P13
WV-1071	All DNA, stereorandom PS, one 2,6-diamino purine	rs362307	P12

WV-1072	All DNA, stereorandom PS, one 2,6-diamino purine	rs362307	P11
WV-1510	1-4-10-4-1 (DNA/2'-OMe) gapmer, Stereopure, one Rp in the DNA, first and last nucletotide is DNA and first and last PS are Sp	rs362307	P12
WV-1511	1-4-10-4-1 (DNA/2'-OMe) gapmer, Stereorandom, 1st and last PS, rest of the wing is PO	rs362307	P12
WV-1497	5-10-5 (2'-OMe-DNA-2'-OMe) gapmer, Stereorandom, First and last PS and rest PO wing	rs362307	P12
WV-1655	5-10-5 (2'-MOE-DNA-2'-MOE) Gapmer, Stereorandom, PO wings with One PS on each end	rs362307	P12
	Table N2. Example sequences targeting rs362306 - continued (certain features)		
WV-1001	All DNA, stereorandom PS	rs362306	P10
WV-1002	All DNA, stereorandom PS	rs362306	P9
WV-1003	All DNA, stereorandom PS	rs362306	P8
WV-1004	All DNA, stereorandom PS	rs362306	P7
WV-1005	All DNA, stereorandom PS	rs362306	P6
WV-1006	All DNA, stereorandom PS	rs362306	P5
WV-1007	5-15 (2'-OMe-DNA), stereorandom PS	rs362306	P10
WV-1008	5-15 (2'-OMe-DNA), stereorandom PS	rs362306	P9
WV-1009	5-15 (2'-OMe-DNA), stereorandom PS	rs362306	P8
WV-1010	5-15 (2'-OMe-DNA), stereorandom PS	rs362306	P7
WV-1011	5-15 (2'-OMe-DNA), stereorandom PS	rs362306	P6
WV-1012	5-15 (2'-OMe-DNA), stereorandom PS	rs362306	P5
WV-1013	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362306	P10

WV-1014	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362306	P9
WV-1015	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362306	P8
WV-1016	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362306	P7
WV-1017	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362306	P6
WV-1018	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362306	P5
WV-1019	7-7-6 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362306	P10
WV-1020	7-7-6 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in wings	rs362306	P10
WV-1021	6-7-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362306	P9
WV-1022	6-7-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362306	P9
WV-1023	5-7-8 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362306	P8
WV-1024	5-7-8 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362306	P8
WV-1025	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362306	P10
WV-1026	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362306	P9
WV-1027	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362306	P8
WV-1028	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362306	P7
WV-1029	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362306	P6
WV-1030	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362306	P5
WV-952	All DNA, stereopure, One Rp	rs362306	P10
WV-953	All DNA, stereopure, One Rp	rs362306	P9

WV-954	All DNA, stereopure, One Rp	rs362306	P8
WV-955	All DNA, stereopure, One Rp	rs362306	P7
WV-956	All DNA, stereopure, One Rp	rs362306	P6
WV-957	All DNA, stereopure, One Rp	rs362306	P5
	Table N3. Example sequences targeting rs362268 - continued (certain features)		
WV-1031	All DNA, stereorandom PS	rs362268	P10
WV-1032	All DNA, stereorandom PS	rs362268	P9
WV-1033	All DNA, stereorandom PS	rs362268	P8
WV-1034	All DNA, stereorandom PS	rs362268	P7
WV-1035	All DNA, stereorandom PS	rs362268	P6
WV-1036	All DNA, stereorandom PS	rs362268	P5
WV-1037	5-15 (2'-OMe-DNA), stereorandom PS	rs362268	P10
WV-1038	5-15 (2'-OMe-DNA), stereorandom PS	rs362268	P9
WV-1039	5-15 (2'-OMe-DNA), stereorandom PS	rs362268	P8
WV-1040	5-15 (2'-OMe-DNA), stereorandom PS	rs362268	P7
WV-1041	5-15 (2'-OMe-DNA), stereorandom PS	rs362268	P6
WV-1042	5-15 (2'-OMe-DNA), stereorandom PS	rs362268	P5
WV-1043	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362268	P10
WV-1044	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362268	P9

WV-1045	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362268	P8
WV-1046	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362268	P7
WV-1047	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362268	P6
WV-1048	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362268	P5
WV-1049	7-7-6 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362268	P10
WV-1050	7-7-6 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in wings	rs362268	P10
WV-1051	6-7-5 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362268	P9
WV-1052	6-7-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362268	P9
WV-1053	5-7-8 (2'-OMe-DNA-2'-OMe), stereorandom PS	rs362268	P8
WV-1054	5-7-8 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362268	P8
WV-1055	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362268	P10
WV-1056	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362268	P9
WV-1057	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362268	P8
WV-1058	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362268	P7
WV-1059	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362268	P6
WV-1060	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings	rs362268	P5
WV-960	All DNA, stereopure, One Rp	rs362268	P10
WV-961	All DNA, stereopure, One Rp	rs362268	P9
WV-962	All DNA, stereopure, One Rp	rs362268	P8

WV-963	All DNA, stereopure, One Rp	rs362268	P7
WV-964	All DNA, stereopure, One Rp	rs362268	P6
WV-965	All DNA, stereopure, One Rp	rs362268	P5
	Table N4. Example sequences targeting rs7685686 - continued (certain features)		
ONT-450	All DNA, stereorandom PS	rs7685686	P13
ONT-451	All DNA, stereopure, One Rp in DNA between position 14 and 15	rs7685686	P13
ONT-452	All DNA, stereopure, One Rp in DNA between position 15 and 16	rs7685686	P13
WV-1077	6-10-4 (2'-OMe-DNA-2'-OMe) Gapmer, stereopure with one Rp in DNA between position 14 and 15	rs7685686	P13
WV-1078	6-10-4 (2'-OMe-DNA-2'-OMe) Gapmer, stereopure with one Rp in DNA between position 14 and 15 and Rp wings	rs7685686	P13
WV-1079	8-12 (2'-OMe-DNA) Hemimer, stereopure with one Rp in DNA between position 14 and 15 and Sp wing	rs7685686	P13
WV-1080	8-12 (2'-OMe-DNA) Hemimer, stereopure with one Rp in DNA between position 14 and 15 and Rp wing	rs7685686	P13
WV-1081	8-12 (2'-OMe-DNA) Hemimer, stereopure with one Rp in DNA between position 14 and 15 and PO wing	rs7685686	P13
WV-1082	6-10-4 (2'-OMe-DNA-2'-OMe), stereopure with one Rp in DNA between position 14 and 15 and PO wings	rs7685686	P13
WV-1083	6-10-4 (2'-OMe-DNA-2'-OMe), stereopure with one Rp in DNA between position 14 and 15, first and last PS Sp and rest PO wing	rs7685686	P13
WV-1084	6-10-4 (2'-OMe-DNA-2'-OMe), stereopure with one Rp in DNA between position 14 and 15, first and last PS Rp and rest PO wing	rs7685686	P13
WV-1508	1-5-10-3-1 (DNA/2'-OMe) Gapmer, Stereopure, one Rp in the core, first and last PS is Sp, rest is PO in the wing	rs7685686	P13
WV-1509	1-5-10-3-1 (DNA/2'-OMe) Gapmer, Stereorandom, first and last PS, rest is PO in the wing	rs7685686	P13

In Table N1-N4, * only represents a stereorandom phosphorothioate linkage; *S represents an *Sp* phosphorothioate linkage, *R represents an *Rp* phosphorothioate linkage, all non-labeled linkage is a natural phosphate linkage, m preceding a base represents 2'-OMe, d2AP represents a 2-amino purine, and dDAP represents a 2,6-diamino purine.

[00757] In some embodiments, the present disclosure provides oligonucleotides and/or oligonucleotide compositions that are useful for treating Huntington's disease, for example, selected from:

	Table N1A. Example sequences targeting rs362307
WV-936	G*SG*SG*SC*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST*ST
WV-937	G*SG*SC*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST*ST*SC
WV-938	G*SC*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST*ST*SC*SC
WV-939	C*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST*ST*SC*SC*SA
WV-940	A*SC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST*ST*SC*SC*SA*SA
WV-941	C*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST*ST*SC*SC*SA*SA*SA
WV-1085	mG*SmG*SmC*SmA*SmC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SmA*SmC*SmU*SmU*SmC
WV-1086	mG*RmG*RmC*RmA*RmC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SmA*RmC*RmU*RmU*RmC
WV-1087	mGmGmCmAmC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SmAmCmUmUmC
WV-1088	mG*SmG*SmC*SmA*SmC*SmA*SmA*SmG*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST*ST*SC
WV-1089	mG*RmG*RmC*RmA*RmC*RmA*RmA*RmG*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST*ST*SC
WV-1090	mGmGmCmAmCmAmAmG*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST*ST*SC
WV-1091	mG*RmGmCmAmC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SmAmCmUmU*RmC
WV-1092	mG*SmGmCmAmC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SmAmCmUmU*SmC
WV-982	G*SC*SA*SG*SG*SG*SC*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SA
WV-983	C*SA*SG*SG*SG*SC*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SA*SC
WV-984	A*SG*SG*SG*SC*SA*SC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST
WV-985	A*SA*SG*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST*ST*SC*SC*SA*SA*SA*SG
WV-986	A*SG*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST*ST*SC*SC*SA*SA*SA*SG*SG
WV-987	G*SG*SG*SC*SA*SC*RA*SG*SA*SC*ST*ST*SC*SC*SA*SA*SA*SG*S

	G*SC
WV-1510	G*SmGmCmAmC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SmAmCmUmU*SC
	Table N2A. Example sequences targeting rs362306
WV-952	G*SA*SG*SC*SA*SG*SC*ST*SG*SC*SA*RA*SC*SC*ST*SG*SG*SC*SA*SA
WV-953	A*SG*SC*SA*SG*SC*ST*SG*SC*SA*RA*SC*SC*ST*SG*SG*SC*SA*SA*SC
WV-954	G*SC*SA*SG*SC*ST*SG*SC*SA*RA*SC*SC*ST*SG*SG*SC*SA*SA*SC*SA
WV-955	C*SA*SG*SC*ST*SG*SC*SA*RA*SC*SC*ST*SG*SG*SC*SA*SA*SC*SA*SA
WV-956	A*SG*SC*ST*SG*SC*SA*RA*SC*SC*ST*SG*SG*SC*SA*SA*SC*SA*SA*SC
WV-957	G*SC*ST*SG*SC*SA*RA*SC*SC*ST*SG*SG*SC*SA*SA*SC*SA*SA*SC*SC
	Table N3A. Example sequences targeting rs362268
WV-960	G*SG*SG*SC*SC*SA*SA*SC*SA*SG*SC*RC*SA*SG*SC*SC*ST*SG*SC*SA
WV-961	G*SG*SC*SC*SA*SA*SC*SA*SG*SC*RC*SA*SG*SC*SC*ST*SG*SC*SA*SG
WV-962	G*SC*SC*SA*SA*SC*SA*SG*SC*RC*SA*SG*SC*SC*ST*SG*SC*SA*SG*SG
WV-963	C*SC*SA*SA*SC*SA*SG*SC*RC*SA*SG*SC*SC*ST*SG*SC*SA*SG*SG*SA
WV-964	C*SA*SA*SC*SA*SG*SC*RC*SA*SG*SC*SC*ST*SG*SC*SA*SG*SG*SA*SG
WV-965	A*SA*SC*SA*SG*SC*RC*SA*SG*SC*SC*ST*SG*SC*SA*SG*SG*SA*SG*SG
	Table N4A. Example sequences targeting rs7685686
ONT-450	A*T*T*A*A*T*A*A*A*T*T*G*T*C*A*T*C*A*C*C
ONT-451	A*ST*ST*SA*SA*ST*SA*SA*SA*ST*ST*SG*ST*SC*RA*ST*SC*SA*SC*SC
ONT-452	A*ST*ST*SA*SA*ST*SA*SA*SA*ST*ST*SG*ST*SC*SA*RT*SC*SA*SC*SC
WV-1077	mA*SmU*SmU*SmA*SmA*SmU*SA*SA*SA*ST*ST*SG*ST*SC*RA*ST*SmC*SmA*SmC*SmC
WV-1078	mA*RmU*RmU*RmA*RmA*RmU*SA*SA*SA*ST*ST*SG*ST*SC*RA*ST*SmC*RmA*RmC*RmC
WV-1079	mA*SmU*SmU*SmA*SmA*SmU*SmA*SmA*SA*ST*ST*SG*ST*SC*RA*ST*SC*SA*SC*SC

WV-1080	mA*ℝmU*ℝmU*ℝmA*ℝmA*ℝmU*ℝmA*ℝmA*𝕊A*𝕊T*𝕊T*𝕊G*𝕊T*𝕊C*ℝA*𝕊T*𝕊C*𝕊A*𝕊C*𝕊C
WV-1081	mAmUmUmAmAmUmAmA*𝕊A*𝕊T*𝕊T*𝕊G*𝕊T*𝕊C*ℝA*𝕊T*𝕊C*𝕊A*𝕊C*𝕊C
WV-1082	mAmUmUmAmAmU*𝕊A*𝕊A*𝕊A*𝕊T*𝕊T*𝕊G*𝕊T*𝕊C*ℝA*𝕊T*𝕊mCmAmCmC
WV-1083	mA*𝕊mUmUmAmAmU*𝕊A*𝕊A*𝕊A*𝕊T*𝕊T*𝕊G*𝕊T*𝕊C*ℝA*𝕊T*𝕊mCmAmC*𝕊mC
WV-1084	mA*ℝmUmUmAmAmU*𝕊A*𝕊A*𝕊A*𝕊T*𝕊T*𝕊G*𝕊T*𝕊C*ℝA*𝕊T*𝕊mCmAmC*ℝmC
WV-1508	A*𝕊mUmUmAmAmU*𝕊A*𝕊A*𝕊A*𝕊T*𝕊T*𝕊G*𝕊T*𝕊C*ℝA*𝕊T*𝕊mCmAmC*𝕊C

	Table N1A. Example sequences targeting rs362307 - continued
WV-936	All DNA, stereopure, One Rp
WV-937	All DNA, stereopure, One Rp
WV-938	All DNA, stereopure, One Rp
WV-939	All DNA, stereopure, One Rp
WV-940	All DNA, stereopure, One Rp
WV-941	All DNA, stereopure, One Rp
WV-1085	5-10-5 (2'-OMe-DNA-2'-OMe) Gapmer, Stereopure, One Rp in DNA
WV-1086	5-10-5 (2'-OMe-DNA-2'-OMe) Gapmer, Stereopure, One Rp in DNA and Rp wings
WV-1087	5-10-5 (2'-OMe-DNA-2'-OMe) Gapmer, Stereopure, One Rp in DNA, PO wings
WV-1088	8-12 (2'-OMe-DNA) hemimer, Stereopure, One Rp in DNA, Sp wing
WV-1089	8-12 (2'-OMe-DNA) hemimer, Stereopure, One Rp in DNA and Rp wing
WV-1090	8-12 (2'-OMe-DNA) hemimer, Srereopure, One Rp in DNA and PO wing
WV-1091	5-10-5 (2'-OMe-DNA-2'-OMe) gapmer, Stereopure, One Rp in DNA, First and last PS as Rp and rest PO wing
WV-1092	5-10-5 (2'-OMe-DNA-2'-OMe) gapmer, Stereopure, One Rp in DNA, First and last PS as Sp and rest PO wing
WV-982	All DNA, stereopure, One Rp
WV-983	All DNA, stereopure, One Rp

WV-984	All DNA, stereopure, One Rp
WV-985	All DNA, stereopure, One Rp
WV-986	All DNA, stereopure, One Rp
WV-987	All DNA, stereopure, One Rp
WV-1510	1-4-10-4-1 (DNA/2'-OMe) gapmer, Stereopure, one Rp in the DNA, first and last nucletotide is DNA and first and last PS are Sp
	Table N2A. Example sequences targeting rs362306 - continued
WV-952	All DNA, stereopure, One Rp
WV-953	All DNA, stereopure, One Rp
WV-954	All DNA, stereopure, One Rp
WV-955	All DNA, stereopure, One Rp
WV-956	All DNA, stereopure, One Rp
WV-957	All DNA, stereopure, One Rp
	Table N3A. Example sequences targeting rs362268 - continued
WV-960	All DNA, stereopure, One Rp
WV-961	All DNA, stereopure, One Rp
WV-962	All DNA, stereopure, One Rp
WV-963	All DNA, stereopure, One Rp
WV-964	All DNA, stereopure, One Rp
WV-965	All DNA, stereopure, One Rp
	Table N4A. Example sequences targeting rs7685686 - continued
ONT-451	All DNA, stereopure, One Rp in DNA between position 14 and 15
ONT-452	All DNA, stereopure, One Rp in DNA between position 15 and 16
WV-1077	6-10-4 (2'-OMe-DNA-2'-OMe) Gapmer, stereopure with one Rp in DNA between position 14 and 15
WV-1078	6-10-4 (2'-OMe-DNA-2'-OMe) Gapmer, stereopure with one Rp in DNA between position 14 and 15 and Rp wings
WV-1079	8-12 (2'-OMe-DNA) Hemimer, stereopure with one Rp in DNA between position 14 and 15 and Sp wing

WV-1080	8-12 (2'-OMe-DNA) Hemimer, stereopure with one Rp in DNA between position 14 and 15 and Rp wing
WV-1081	8-12 (2'-OMe-DNA) Hemimer, stereopure with one Rp in DNA between position 14 and 15 and PO wing
WV-1082	6-10-4 (2'-OMe-DNA-2'-OMe), stereopure with one Rp in DNA between position 14 and 15 and PO wings
WV-1083	6-10-4 (2'-OMe-DNA-2'-OMe), stereopure with one Rp in DNA between position 14 and 15, first and last PS Sp and rest PO wing
WV-1084	6-10-4 (2'-OMe-DNA-2'-OMe), stereopure with one Rp in DNA between position 14 and 15, first and last PS Rp and rest PO wing
WV-1508	1-5-10-3-1 (DNA/2'-OMe) Gapmer, Stereopure, one Rp in the core, first and last PS is Sp, rest is PO in the wing

In Table N1A-N4A, * only represents a stereorandom phosphorothioate linkage; *S represents an *S*p phosphorothioate linkage, *R represents an *R*p phosphorothioate linkage, all non-labeled linkage is a natural phosphate linkage, m preceding a base represents 2'-OMe, d2AP represents a 2-amino purine, and dDAP represents a 2,6-diamino purine.

[00758] In some embodiments, a provided oligonucleotide composition is a chirally controlled oligonucleotide composition of an oligonucleotide type listed in Table N1A, Table N2A, Table N3A, and Table N4A. In some embodiments, a provided composition is of WV-1092. Each oligonucleotide described herein comprising a HTT sequence represents an HTT oligonucleotide which was designed, constructed and tested in various assays, for example, *in vitro* assays, in accordance with the present disclosure. For example, each HTT oligonucleotide listed in any of Tables N1A, N2A, N3A, N4A and 8, or described elsewhere herein were designed, constructed and tested *in vitro* in accordance with the present disclosure. Among others, every HTT oligonucleotide described herein was tested in a dual luciferase reporter assay. In some embodiments, HTT oligonucleotides which were found to be particularly efficacious in the dual luciferase assay were tested in further *in vitro* and *in vivo*. In some embodiments, a provided composition is selected from: WVE120101; WV-2603; WV-2595; WV-1510; WV-2378; and WV-2380; each of which was found to be highly efficacious, for example, as demonstrated *in vitro* in the dual luciferase reporter assay in accordance with the present disclosure. In some embodiments, a provided composition is selected from: WV-1092; WV-1497; WV-1085; WV-1086; ONT-905; and WV-2623; each of which was found to be highly efficacious, for example, as demonstrated *in vitro* in the dual luciferase reporter assay in accordance with the present disclosure. Various additional HTT oligonucleotides were also

shown to be particularly efficacious. In some embodiments, a provided composition is of WV-1092. In some embodiments, a provided composition is of WV-1497. In some embodiments, a provided composition is of WV-1085. In some embodiments, a provided composition is of WV-1086. In some embodiments, a provided composition is of ONT-905. In some embodiments, a provided composition is of WV-2623.

[00759] The present disclosure provides compositions comprising or consisting of a plurality of provided oligonucleotides (*e.g.,* chirally controlled oligonucleotide compositions). In some embodiments, all such provided oligonucleotides are of the same type, i.e., all have the same base sequence, pattern of backbone linkages (i.e., pattern of internucleotidic linkage types, for example, phosphate, phosphorothioate, *etc*), pattern of backbone chiral centers (i.e. pattern of linkage phosphorus stereochemistry (*R*p/*S*p)), and pattern of backbone phosphorus modifications (*e.g.,* pattern of "-XLR^1" groups in formula **I**). In some embodiments, all oligonucleotides of the same type are identical. In many embodiments, however, provided compositions comprise a plurality of oligonucleotides types, typically in pre-determined relative amounts.

[00760] In some embodiments, a provided composition comprises a predetermined level of an oligonucleotide selected from a Table. In some embodiments, a provided composition comprises a predetermined level of an oligonucleotide selected from Tables N1-N4. In some embodiments, a provided composition comprises a predetermined level of WV-1092. In some embodiments, a provided composition comprises a predetermined level of WV-2595. In some embodiments, a provided composition comprises a predetermined level of WV-2603. In some embodiments, a provided composition comprises a predetermined level of mG*SmGmCmAmC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SmAmCmUmU*SmC, wherein the oligonucleotide has a free 5'-OH and 3'-OH, m preceding a base represents 2'-OMe modification in the nucleoside containing the base, *S represents an *S*p phosphorothioate linkage, *R represents an *R*p phosphorothioate linkage, and all non-labeled linkage is a natural phosphate linkage. In some embodiments, a provided composition comprises a predetermined level of mG*SmGmGmUmC*SC*ST*SC*SC*SC*SC*SA*SC*RA*SG*SmAmGmGmG*SmA, wherein the oligonucleotide has a free 5'-OH and 3'-OH, m preceding a base represents 2'-OMe modification in the nucleoside containing the base, *S represents an *S*p phosphorothioate linkage, *R represents an *R*p phosphorothioate linkage, and all non-labeled linkage is a natural phosphate linkage. In some embodiments, a provided composition comprises a predetermined

level of mG*SmUmGmCmA*SC*SA*SC*SA*SG*ST*SA*SG*RA*ST*SmGmAmGmG*S mG, wherein the oligonucleotide has a free 5'-OH and 3'-OH, m preceding a base represents 2'-OMe modification in the nucleoside containing the base, *S represents an *S*p phosphorothioate linkage, *R represents an *R*p phosphorothioate linkage, and all non-labeled linkage is a natural phosphate linkage.

[00761] In some embodiments, a provided chirally controlled oligonucleotide composition is a chirally pure mipomersen composition. That is to say, in some embodiments, a provided chirally controlled oligonucleotide composition provides mipomersen as a single diastereomer with respect to the configuration of the linkage phosphorus. In some embodiments, a provided chirally controlled oligonucleotide composition is a chirally uniform mipomersen composition. That is to say, in some embodiments, every linkage phosphorus of mipomersen is in the *R*p configuration or every linkage phosphorus of mipomersen is in the *S*p configuration.

[00762] In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of one or more provided oligonucleotide types. One of skill in the chemical and medicinal arts will recognize that the selection and amount of each of the one or more types of provided oligonucleotides in a provided composition will depend on the intended use of that composition. That is to say, one of skill in the relevant arts would design a provided chirally controlled oligonucleotide composition such that the amounts and types of provided oligonucleotides contained therein cause the composition as a whole to have certain desirable characteristics (*e.g.*, biologically desirable, therapeutically desirable, *etc.*).

[00763] In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of two or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of three or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of four or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of five or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of six or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of seven or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition

comprises a combination of eight or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of nine or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of ten or more provided oligonucleotide types. In some embodiments, a provided chirally controlled oligonucleotide composition comprises a combination of fifteen or more provided oligonucleotide types.

[00764] In some embodiments, a provided chirally controlled oligonucleotide composition is a combination of an amount of chirally uniform mipomersen of the *R*p configuration and an amount of chirally uniform mipomersen of the *S*p configuration.

[00765] In some embodiments, a provided chirally controlled oligonucleotide composition is a combination of an amount of chirally uniform mipomersen of the *R*p configuration, an amount of chirally uniform mipomersen of the *S*p configuration, and an amount of one or more chirally pure mipomersen of a desired diastereomeric form.

[00766] In some embodiments, a provided oligonucleotide type is selected from those described in PCT/US2013/050407, which is incorporated herein by reference. In some embodiments, a provided chirally controlled oligonucleotide composition comprises oligonucleotides of an oligonucleotide type selected from those described in PCT/US2013/050407.

Example Methods for Preparing Oligomucleotides and Compositions

[00767] The present disclosure provides methods for making chirally controlled oligonucleotides and chirally controlled compositions comprising one or more specific nucleotide types. In some embodiments, the phrase "oligonucleotide type," as used herein, defines an oligonucleotide that has a particular base sequence, pattern of backbone linkages, pattern of backbone chiral centers, and pattern of backbone phosphorus modifications (*e.g.*, "-XLR^1" groups). Oligonucleotides of a common designated "type" are structurally identical to one another with respect to base sequence, pattern of backbone linkages, pattern of backbone chiral centers, and pattern of backbone phosphorus modifications. In some embodiments, oligonucleotides of an oligonucleotide type are identical.

[00768] In some embodiments, a provided chirally controlled oligonucleotide in the

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

[00769] One of skill in the chemical and synthetic arts will appreciate that synthetic methods of the present disclosure provide for a degree of control during each step of the synthesis of a provided oligonucleotide such that each nucleotide unit of the oligonucleotide can be designed and/or selected in advance to have a particular stereochemistry at the linkage phosphorus and/or a particular modification at the linkage phosphorus, and/or a particular base, and/or a particular sugar. In some embodiments, a provided oligonucleotide is designed and/or selected in advance to have a particular combination of stereocenters at the linkage phosphorus of the internucleotidic linkage.

[00770] In some embodiments, a provided oligonucleotide made using methods of the present disclosure is designed and/or determined to have a particular combination of linkage phosphorus modifications. In some embodiments, a provided oligonucleotide made using methods of the present disclosure is designed and/or determined to have a particular combination

of bases. In some embodiments, a provided oligonucleotide made using methods of the present disclosure is designed and/or determined to have a particular combination of sugars. In some embodiments, a provided oligonucleotide made using methods of the present disclosure is designed and/or determined to have a particular combination of one or more of the above structural characteristics.

[00771] Methods of the present disclosure exhibit a high degree of chiral control. For instance, methods of the present disclosure facilitate control of the stereochemical configuration of every single linkage phosphorus within a provided oligonucleotide. In some embodiments, methods of the present disclosure provide an oligonucleotide comprising one or more modified internucleotidic linkages independently having the structure of formula **I**.

[00772] In some embodiments, methods of the present disclosure provide an oligonucleotide which is a mipomersen unimer. In some embodiments, methods of the present disclosure provide an oligonucleotide which is a mipomersen unimer of configuration *R*p. In some embodiments, methods of the present disclosure provide an oligonucleotide which is a mipomersen unimer of configuration *S*p.

[00773] In some embodiments, methods of the present disclosure provide a chirally controlled oligonucleotide composition, i.e., an oligonucleotide composition that contains predetermined levels of individual oligonucleotide types. In some embodiments a chirally controlled oligonucleotide composition comprises one oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide composition comprises more than one oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide composition comprises a plurality of oligonucleotide types. Example chirally controlled oligonucleotide compositions made in accordance with the present disclosure are described herein.

[00774] In some embodiments, methods of the present disclosure provide chirally pure mipomersen compositions with respect to the configuration of the linkage phosphorus. That is to say, in some embodiments, methods of the present disclosure provide compositions of mipomersen wherein mipomersen exists in the composition in the form of a single diastereomer with respect to the configuration of the linkage phosphorus.

[00775] In some embodiments, methods of the present disclosure provide chirally uniform mipomersen compositions with respect to the configuration of the linkage phosphorus. That is to say, in some embodiments, methods of the present disclosure provide compositions of

mipomersen in which all nucleotide units therein have the same stereochemistry with respect to the configuration of the linkage phosphorus, *e.g.,* all nucleotide units are of the *R*p configuration at the linkage phosphorus or all nucleotide units are of the *S*p configuration at the linkage phosphorus.

[00776] In some embodiments, a provided chirally controlled oligonucleotide is over 50% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 55% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 60% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 65% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 70% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 75% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 80% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 85% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 90% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 91% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 92% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 93% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 94% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 95% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 96% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 97% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 98% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 99% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 99.5% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 99.6% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 99.7% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 99.8% pure. In some embodiments, a provided chirally controlled oligonucleotide is over about 99.9% pure. In some embodiments, a provided chirally controlled oligonucleotide is over at least about 99% pure.

[00777] In some embodiments, a chirally controlled oligonucleotide composition is a composition designed to comprise a single oligonucleotide type. In certain embodiments, such

compositions are about 50% diastereomerically pure. In some embodiments, such compositions are about 50% diastereomerically pure. In some embodiments, such compositions are about 50% diastereomerically pure. In some embodiments, such compositions are about 55% diastereomerically pure. In some embodiments, such compositions are about 60% diastereomerically pure. In some embodiments, such compositions are about 65% diastereomerically pure. In some embodiments, such compositions are about 70% diastereomerically pure. In some embodiments, such compositions are about 75% diastereomerically pure. In some embodiments, such compositions are about 80% diastereomerically pure. In some embodiments, such compositions are about 85% diastereomerically pure. In some embodiments, such compositions are about 90% diastereomerically pure. In some embodiments, such compositions are about 91% diastereomerically pure. In some embodiments, such compositions are about 92% diastereomerically pure. In some embodiments, such compositions are about 93% diastereomerically pure. In some embodiments, such compositions are about 94% diastereomerically pure. In some embodiments, such compositions are about 95% diastereomerically pure. In some embodiments, such compositions are about 96% diastereomerically pure. In some embodiments, such compositions are about 97% diastereomerically pure. In some embodiments, such compositions are about 98% diastereomerically pure. In some embodiments, such compositions are about 99% diastereomerically pure. In some embodiments, such compositions are about 99.5% diastereomerically pure. In some embodiments, such compositions are about 99.6% diastereomerically pure. In some embodiments, such compositions are about 99.7% diastereomerically pure. In some embodiments, such compositions are about 99.8% diastereomerically pure. In some embodiments, such compositions are about 99.9% diastereomerically pure. In some embodiments, such compositions are at least about 99% diastereomerically pure.

[00778] Among other things, the present disclosure recognizes the challenge of stereoselective (rather than stereorandom or racemic) preparation of oligonucleotides. Among other things, the present disclosure provides methods and reagents for stereoselective preparation of oligonucleotides comprising multiple (*e.g.*, more than 5, 6, 7, 8, 9, or 10) internucleotidic linkages, and particularly for oligonucleotides comprising multiple (*e.g.*, more than 5, 6, 7, 8, 9,

or 10) chiral internucleotidic linkages. In some embodiments, in a stereorandom or racemic preparation of oligonucleotides, at least one chiral internucleotidic linkage is formed with less than 90:10, 95:5, 96:4, 97:3, or 98:2 diastereoselectivity. In some embodiments, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 90:10, 95:5, 96:4, 97:3, or 98:2 diastereoselectivity. In some embodiments, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 95:5 diastereoselectivity. In some embodiments, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 96:4 diastereoselectivity. In some embodiments, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 97:3 diastereoselectivity. In some embodiments, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 98:2 diastereoselectivity. In some embodiments, for a stereoselective or chirally controlled preparation of oligonucleotides, each chiral internucleotidic linkage is formed with greater than 99:1 diastereoselectivity. In some embodiments, diastereoselectivity of a chiral internucleotidic linkage in an oligonucleotide may be measured through a model reaction, *e.g.* formation of a dimer under essentially the same or comparable conditions wherein the dimer has the same internucleotidic linkage as the chiral internucleotidic linkage, the 5'-nucleoside of the dimer is the same as the nucleoside to the 5'-end of the chiral internucleotidic linkage, and the 3'-nucleoside of the dimer is the same as the nucleoside to the 3'-end of the chiral internucleotidic linkage.

[00779] In some embodiments, a chirally controlled oligonucleotide composition is a composition designed to comprise multiple oligonucleotide types. In some embodiments, methods of the present disclosure allow for the generation of a library of chirally controlled oligonucleotides such that a pre-selected amount of any one or more chirally controlled oligonucleotide types can be mixed with any one or more other chirally controlled oligonucleotide types to create a chirally controlled oligonucleotide composition. In some embodiments, the pre-selected amount of an oligonucleotide type is a composition having any one of the above-described diastereomeric purities.

[00780] In some embodiments, the present disclosure provides methods for making a chirally controlled oligonucleotide comprising steps of:

(1) coupling;

(2) capping;

(3) modifying;

(4) deblocking; and

(5) repeating steps (1) – (4) until a desired length is achieved.

[00781] When describing the provided methods, the word "cycle" has its ordinary meaning as understood by a person of ordinary skill in the art. In some embodiments, one round of steps (1)-(4) is referred to as a cycle.

[00782] In some embodiments, the present disclosure provides methods for making chirally controlled oligonucleotide compositions, comprising steps of:

(a) providing an amount of a first chirally controlled oligonucleotide; and

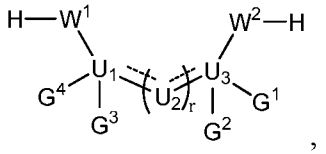
(b) optionally providing an amount of one or more additional chirally controlled oligonucleotides.

[00783] In some embodiments, a first chirally controlled oligonucleotide is an oligonucleotide type, as described herein. In some embodiments, a one or more additional chirally controlled oligonucleotide is a one or more oligonucleotide type, as described herein.

[00784] One of skill in the relevant chemical and synthetic arts will recognize the degree of versatility and control over structural variation and stereochemical configuration of a provided oligonucleotide when synthesized using methods of the present disclosure. For instance, after a first cycle is complete, a subsequent cycle can be performed using a nucleotide unit individually selected for that subsequent cycle which, in some embodiments, comprises a nucleobase and/or a sugar that is different from the first cycle nucleobase and/or sugar. Likewise, the chiral auxiliary used in the coupling step of the subsequent cycle can be different from the chiral auxiliary used in the first cycle, such that the second cycle generates a phosphorus linkage of a different stereochemical configuration. In some embodiments, the stereochemistry of the linkage phosphorus in the newly formed internucleotidic linkage is controlled by using stereochemically pure phosphoramidites. Additionally, the modification reagent used in the modifying step of a subsequent cycle can be different from the modification reagent used in the first or former cycle. The cumulative effect of this iterative assembly approach is such that each component of a provided oligonucleotide can be structurally and configurationally tailored to a high degree. An additional advantage to this approach is that the step of capping minimizes the formation of "n-

1" impurities that would otherwise make isolation of a provided oligonucleotide extremely challenging, and especially oligonucleotides of longer lengths.

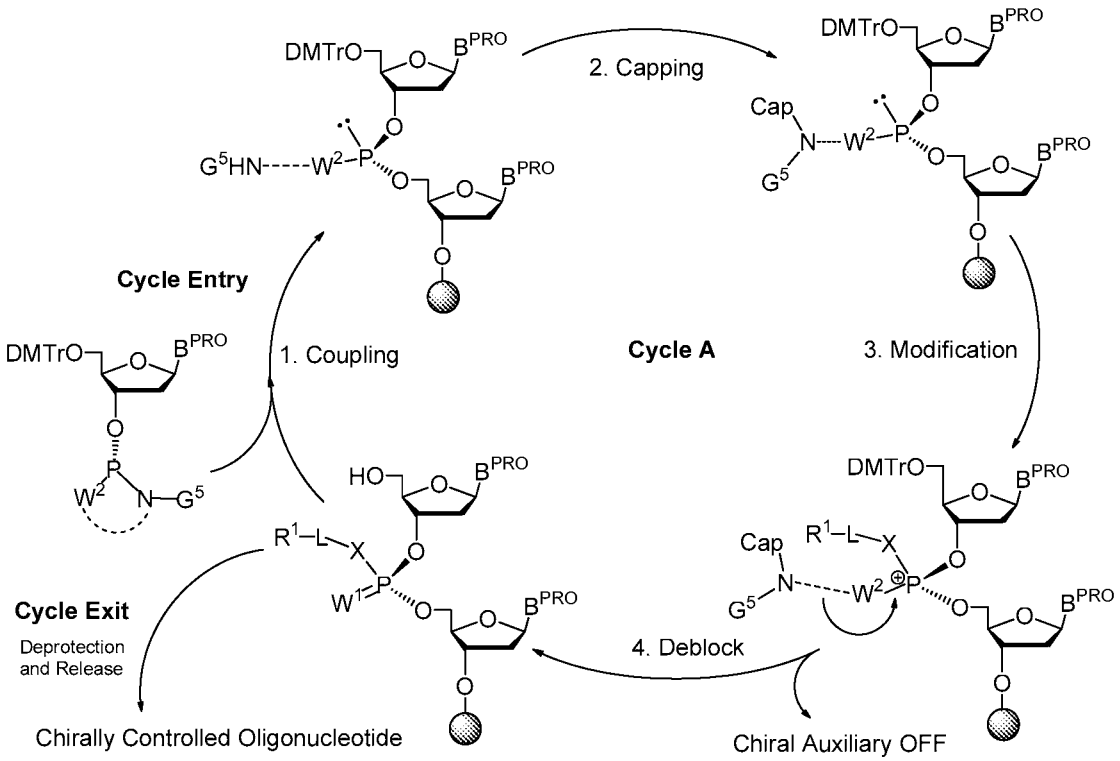
[00785] In some embodiments, an example cycle of the method for making chirally controlled oligonucleotides is illustrated in example schemes described in the present disclosure. In some embodiments, an example cycle of the method for making chirally controlled oligonucleotides is illustrated in Scheme I. In some embodiments,  represents the solid support, and optionally a portion of the growing chirally controlled oligonucleotide attached to the solid support. The chiral auxiliary exemplified has the structure of formula 3-I:



Formula 3-I

which is further described below. "Cap" is any chemical moiety introduced to the nitrogen atom by the capping step, and in some embodiments, is an amino protecting group. One of ordinary skill in the art understands that in the first cycle, there may be only one nucleoside attached to the solid support when started, and cycle exit can be performed optionally before deblocking. As understood by a person of skill in the art, B^{PRO} is a protected base used in oligonucleotide synthesis. Each step of the above-depicted cycle of Scheme I is described further below.

[00786] **Scheme I.** Synthesis of chirally controlled oligonucleotide.



Synthesis on solid support

[00787] In some embodiments, the synthesis of a provided oligonucleotide is performed on solid phase. In some embodiments, reactive groups present on a solid support are protected. In some embodiments, reactive groups present on a solid support are unprotected. During oligonucleotide synthesis a solid support is treated with various reagents in several synthesis cycles to achieve the stepwise elongation of a growing oligonucleotide chain with individual nucleotide units. The nucleoside unit at the end of the chain which is directly linked to the solid support is termed "the first nucleoside" as used herein. A first nucleoside is bound to a solid support via a linker moiety, *i.e.* a diradical with covalent bonds between either of a CPG, a polymer or other solid support and a nucleoside. The linker stays intact during the synthesis cycles performed to assemble the oligonucleotide chain and is cleaved after the chain assembly to liberate the oligonucleotide from the support.

[00788] Solid supports for solid-phase nucleic acid synthesis include the supports described in, *e.g.,* US patents 4,659,774, 5,141,813, 4,458,066; Caruthers U.S. Pat. Nos. 4,415,732, 4,458,066, 4,500,707, 4,668,777, 4,973,679, and 5,132,418; Andrus *et al.* U.S. Pat. Nos. 5,047,524, 5,262,530; and Koster U.S. Pat. Nos. 4,725,677 (reissued as RE34,069). In

some embodiments, a solid phase is an organic polymer support. In some embodiments, a solid phase is an inorganic polymer support. In some embodiments, an organic polymer support is polystyrene, aminomethyl polystyrene, a polyethylene glycol-polystyrene graft copolymer, polyacrylamide, polymethacrylate, polyvinylalcohol, highly cross-linked polymer (HCP), or other synthetic polymers, carbohydrates such as cellulose and starch or other polymeric carbohydrates, or other organic polymers and any copolymers, composite materials or combination of the above inorganic or organic materials. In some embodiments, an inorganic polymer support is silica, alumina, controlled polyglass (CPG), which is a silica-gel support, or aminopropyl CPG. Other useful solid supports include fluorous solid supports (see *e.g.*, WO/2005/070859), long chain alkylamine (LCAA) controlled pore glass (CPG) solid supports (see *e.g.*, S. P. Adams, K. S. Kavka, E. J. Wykes, S. B. Holder and G. R. Galluppi, *J. Am. Chem. Soc.*, **1983**, *105*, 661-663; G. R. Gough, M. J. Bruden and P. T. Gilham, *Tetrahedron Lett.*, **1981**, *22*, 4177-4180). Membrane supports and polymeric membranes (see *e.g.* Innovation and Perspectives in Solid Phase Synthesis, Peptides, Proteins and Nucleic Acids, ch 21 pp 157-162, 1994, Ed. Roger Epton and U.S. Pat. No. 4,923,901) are also useful for the synthesis of nucleic acids. Once formed, a membrane can be chemically functionalized for use in nucleic acid synthesis. In addition to the attachment of a functional group to the membrane, the use of a linker or spacer group attached to the membrane is also used in some embodiments to minimize steric hindrance between the membrane and the synthesized chain.

[00789] Other suitable solid supports include those generally known in the art to be suitable for use in solid phase methodologies, including, for example, glass sold as PrimerTM 200 support, controlled pore glass (CPG), oxalyl-controlled pore glass (see, *e.g.*, Alul, *et al.*, *Nucleic Acids Research*, **1991**, *19*, 1527), TentaGel Support-an aminopolyethyleneglycol derivatized support (see, *e.g.*, Wright, *et al.*, *Tetrahedron Lett.*, **1993**, *34*, 3373), and Poros-a copolymer of polystyrene/divinylbenzene.

[00790] Surface activated polymers have been demonstrated for use in synthesis of natural and modified nucleic acids and proteins on several solid supports mediums. A solid support material can be any polymer suitably uniform in porosity, having sufficient amine content, and sufficient flexibility to undergo any attendant manipulations without losing integrity. Examples of suitable selected materials include nylon, polypropylene, polyester, polytetrafluoroethylene, polystyrene, polycarbonate, and nitrocellulose. Other materials can serve as a solid support,

depending on the design of the investigator. In consideration of some designs, for example, a coated metal, in particular gold or platinum can be selected (see *e.g.*, US publication No. 20010055761). In one embodiment of oligonucleotide synthesis, for example, a nucleoside is anchored to a solid support which is functionalized with hydroxyl or amino residues. Alternatively, a solid support is derivatized to provide an acid labile trialkoxytrityl group, such as a trimethoxytrityl group (TMT). Without being bound by theory, it is expected that the presence of a trialkoxytrityl protecting group will permit initial detritylation under conditions commonly used on DNA synthesizers. For a faster release of oligonucleotide material in solution with aqueous ammonia, a diglycoate linker is optionally introduced onto the support.

[00791] In some embodiments, a provided oligonucleotide alternatively is synthesized from the 5′ to 3′ direction. In some embodiments, a nucleic acid is attached to a solid support through its 5′ end of the growing nucleic acid, thereby presenting its 3′ group for reaction, i.e. using 5′-nucleoside phosphoramidites or in enzymatic reaction (*e.g.* ligation and polymerization using nucleoside 5′-triphosphates). When considering the 5’ to 3’ synthesis the iterative steps of the present disclosure remain unchanged (i.e. capping and modification on the chiral phosphorus).

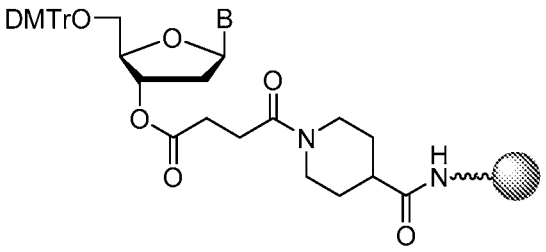
Linking moiety

[00792] A linking moiety or linker is optionally used to connect a solid support to a compound comprising a free nucleophilic moiety. Suitable linkers are known such as short molecules which serve to connect a solid support to functional groups (*e.g.*, hydroxyl groups) of initial nucleosides molecules in solid phase synthetic techniques. In some embodiments, the linking moiety is a succinamic acid linker, or a succinate linker (-CO-CH_2-CH_2-CO-), or an oxalyl linker (-CO-CO-). In some embodiments, the linking moiety and the nucleoside are bonded together through an ester bond. In some embodiments, a linking moiety and a nucleoside are bonded together through an amide bond. In some embodiments, a linking moiety connects a nucleoside to another nucleotide or nucleic acid. Suitable linkers are disclosed in, for example, *Oligonucleotides And Analogues A Practical Approach*, Ekstein, F. Ed., IRL Press, N.Y., **1991**, Chapter 1 and Solid-Phase Supports for Oligonucleotide Synthesis, Pon, R. T., *Curr. Prot. Nucleic Acid Chem.*, **2000**, 3.1.1-3.1.28.

[00793] A linker moiety is used to connect a compound comprising a free nucleophilic

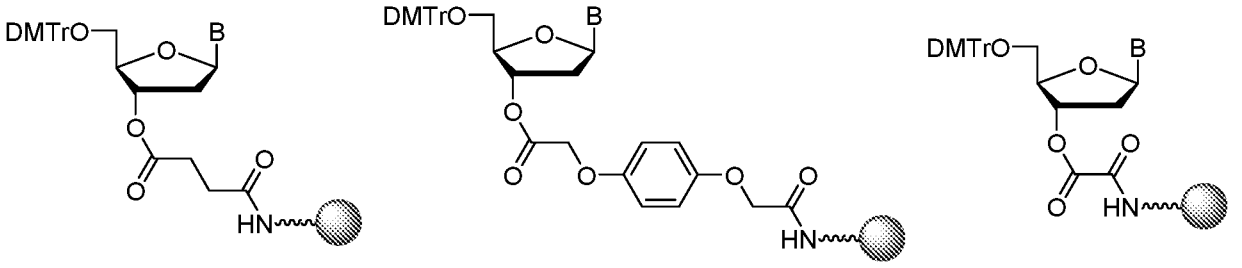
moiety to another nucleoside, nucleotide, or nucleic acid. In some embodiments, a linking moiety is a phosphodiester linkage. In some embodiments, a linking moiety is an *H*-phosphonate moiety. In some embodiments, a linking moiety is a modified phosphorus linkage as described herein. In some embodiments, a universal linker (UnyLinker) is used to attached the oligonucleotide to the solid support (Ravikumar et al., *Org. Process Res. Dev.*, **2008**, *12* (3), 399–410). In some embodiments, other universal linkers are used (Pon, R. T., *Curr. Prot. Nucleic Acid Chem.*, **2000**, 3.1.1-3.1.28). In some embodiments, various orthogonal linkers (such as disulfide linkers) are used (Pon, R. T., *Curr. Prot. Nucleic Acid Chem.*, **2000**, 3.1.1-3.1.28).

[00794] Among other things, the present disclosure recognizes that a linker can be chosen or designed to be compatible with a set of reaction conditions employed in oligonucleotide synthesis. In some embodiments, to avoid degradation of oligonucleotides and to avoid desulfurization, auxiliary groups are selectively removed before de-protection. In some embodiments, DPSE group can selectively be removed by F^- ions. In some embodiments, the present disclosure provides linkers that are stable under a DPSE de-protection condition, *e.g.*, 0.1M TBAF in MeCN, 0.5M HF-Et_3N in THF or MeCN, *etc*. In some embodiments, a provided linker is the SP linker. In some embodiments, the present disclosure demonstrates that the SP linker is stable under a DPSE de-protection condition, *e.g.*, 0.1M TBAF in MeCN, 0.5M HF-Et_3N in THF or MeCN, *etc*.; they are also stable, *e.g.*, under anhydrous basic conditions, such as om1M DBU in MeCN.



succinyl-piperidine (SP) linker

In some embodiments, an example linker is:



succinyl linker Q-linker oxalyl linker

In some embodiments, the succinyl linker, Q-linker or oxalyl linker is not stable to one or more DPSE-deprotection conditions using F^-.

General conditions - solvents for synthesis

[00795] Syntheses of provided oligonucleotides are generally performed in aprotic organic solvents. In some embodiments, a solvent is a nitrile solvent such as, *e.g.*, acetonitrile. In some embodiments, a solvent is a basic amine solvent such as, *e.g.*, pyridine. In some embodiments, a solvent is an ethereal solvent such as, *e.g.*, tetrahydrofuran. In some embodiments, a solvent is a halogenated hydrocarbon such as, *e.g.*, dichloromethane. In some embodiments, a mixture of solvents is used. In certain embodiments a solvent is a mixture of any one or more of the above-described classes of solvents.

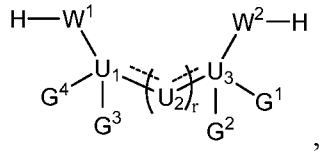
[00796] In some embodiments, when an aprotic organic solvent is not basic, a base is present in the reacting step. In some embodiments where a base is present, the base is an amine base such as, *e.g.*, pyridine, quinoline, or *N*,*N*-dimethylaniline. Examples of other amine bases include pyrrolidine, piperidine, *N*-methyl pyrrolidine, pyridine, quinoline, *N*,*N*-dimethylaminopyridine (DMAP), or *N*,*N*-dimethylaniline.

[00797] In some embodiments, a base is other than an amine base.

[00798] In some embodiments, an aprotic organic solvent is anhydrous. In some embodiments, an anhydrous aprotic organic solvent is freshly distilled. In some embodiments, a freshly distilled anhydrous aprotic organic solvent is a basic amine solvent such as, *e.g.*, pyridine. In some embodiments, a freshly distilled anhydrous aprotic organic solvent is an ethereal solvent such as, *e.g.*, tetrahydrofuran. In some embodiments, a freshly distilled anhydrous aprotic organic solvent is a nitrile solvent such as, *e.g.*, acetonitrile.

Chiral reagent/chiral auxiliary

[00799] In some embodiments, chiral reagents are used to confer stereoselectivity in the production of chirally controlled oligonucleotides. Many different chiral reagents, also referred to by those of skill in the art and herein as chiral auxiliaries, may be used in accordance with methods of the present disclosure. Examples of such chiral reagents are described herein and in Wada I, II and III, referenced above. In certain embodiments, a chiral reagent is as described by Wada I. In some embodiments, a chiral reagent for use in accordance with the methods of the present disclosure are of Formula **3-I**, below:



,

Formula 3-I

wherein W^1 and W^2 are any of -O-, -S-, or $-NG^5-$, U_1 and U_3 are carbon atoms which are bonded to U_2 if present, or to each other if r is 0, via a single, double or triple bond. U_2 is -C-, $-CG^8-$, $-CG^8G^8-$, $-NG^8-$, -N-, -O-, or -S- where r is an integer of 0 to 5 and no more than two heteroatoms are adjacent. When any one of U_2 is C, a triple bond must be formed between a second instance of U_2, which is C, or to one of U_1 or U_3. Similarly, when any one of U_2 is CG^8, a double bond is formed between a second instance of U_2 which is $-CG^8-$ or -N-, or to one of U_1 or U_3.

[00800] In some embodiments, $-U_1(G_3G_4)-(U_2)_r-U_3(G_1G_2)-$ is $-CG^3G^4-CG^1G^2-$. In some embodiments, $-U_1-(U_2)_r-U_3-$ is $-CG^3=CG^1-$. In some embodiments, $-U_1-(U_2)_r-U_3-$ is -C≡C-. In some embodiments, $-U_1-(U_2)_r-U_3-$ is $-CG^3=CG^8-CG^1G^2-$. In some embodiments, $-U_1-(U_2)_r-U_3-$ is $-CG^3G^4-O-CG^1G^2-$. In some embodiments, $-U_1-(U_2)_r-U_3-$ is $-CG^3G^4-NG^8-CG^1G^2-$. In some embodiments, $-U_1-(U_2)_r-U_3-$ is $-CG^3G^4-N-CG^2-$. In some embodiments, $-U_1-(U_2)_r-U_3-$ is $-CG^3G^4-N=C\ G^8-CG^1G^2-$.

[00801] As defined herein, G^1, G^2, G^3, G^4, G^5, and G^8 are independently hydrogen, or an optionally substituted group selected from alkyl, aralkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heteroaryl, and aryl; or two of G^1, G^2, G^3, G^4, and G^5 are G^6 (taken together to form an optionally substituted, saturated, partially unsaturated or unsaturated carbocyclic or heteroatom-containing ring of up to about 20 ring atoms which is monocyclic or polycyclic, and is fused or unfused). In some embodiments, a ring so formed is substituted by oxo, thioxo, alkyl, alkenyl, alkynyl, heteroaryl, or aryl moieties. In some embodiments, when a ring formed by

taking two G^6 together is substituted, it is substituted by a moiety which is bulky enough to confer stereoselectivity during the reaction.

[00802] In some embodiments, a ring formed by taking two of G^6 together is optionally substituted cyclopentyl, pyrrolyl, cyclopropyl, cyclohexenyl, cyclopentenyl, tetrahydropyranyl, or piperazinyl. In some embodiments, a ring formed by taking two of G^6 together is optionally substituted cyclopentyl, pyrrolyl, cyclopropyl, cyclohexenyl, cyclopentenyl, tetrahydropyranyl, pyrrolidinyl, or piperazinyl.

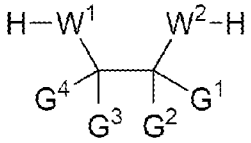
[00803] In some embodiments, G^1 is optionally substituted phenyl. In some embodiments, G^1 is phenyl. In some embodiments, G^2 is methyl or hydrogen. In some embodiments, G^1 is optionally substituted phenyl and G^2 is methyl. In some embodiments, G^1 is phenyl and G^2 is methyl.

[00804] In some embodiments, r is 0.

[00805] In some embodiments, W^1 is $-NG^5-$. In some embodiments, one of G^3 and G^4 is taken together with G^5 to form an optionally substituted pyrrolidinyl ring. In some embodiments, one of G^3 and G^4 is taken together with G^5 to form a pyrrolidinyl ring.

[00806] In some embodiments, W^2 is $-O-$.

[00807] In some embodiments, a chiral reagent is a compound of Formula 3-AA:

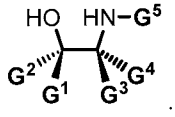
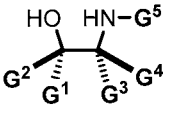
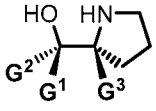
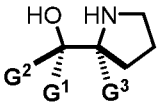
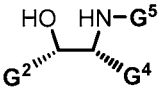
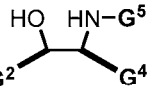
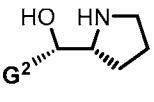
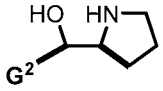


Formula 3-AA

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

[00808] In some embodiments of Formula 3AA, W^1 and W^2 are independently $-NG^5-$, -O-, or -S-; G^1, G^2, G^3, G^4, and G^5 are independently hydrogen, or an optionally substituted group selected from alkyl, aralkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heteroaryl, or aryl; or two of G^1, G^2, G^3, G^4, and G^5 are G^6 (taken together to form an optionally substituted saturated, partially unsaturated or unsaturated carbocyclic or heteroatom-containing ring of up to about 20 ring atoms which is monocyclic or polycyclic, fused or unfused), and no more than four of G^1, G^2, G^3, G^4, and G^5 are G^6. Similarly to the compounds of Formula 3-I, any of G^1, G^2, G^3, G^4, or G^5 are optionally substituted by oxo, thioxo, alkyl, alkenyl, alkynyl, heteroaryl, or aryl moieties. In some embodiments, such substitution induces stereoselectivity in chirally controlled

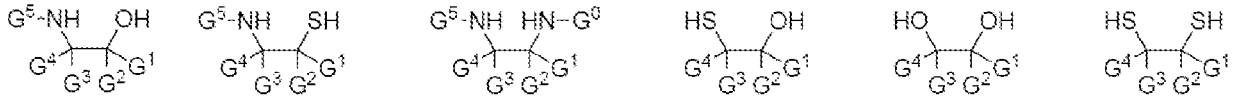
oligonucleotide production.

[00809] In some embodiments, a provided chiral reagent has the structure of  . In some embodiments, a provided chiral reagent has the structure of  . In some embodiments, a provided chiral reagent has the structure of  . In some embodiments, a provided chiral reagent has the structure of  . In some embodiments, a provided chiral reagent has the structure of  . In some embodiments, a provided chiral reagent has the structure of  . In some embodiments, a provided chiral reagent has the structure of  . In some embodiments, a provided chiral reagent has the structure of  .

[00810] In some embodiments, W^1 is $-NG^5$, W^2 is O, each of G^1 and G^3 is independently hydrogen or an optionally substituted group selected from C_{1-10} aliphatic, heterocyclyl, heteroaryl and aryl, G^2 is $-C(R)_2Si(R)_3$, and G^4 and G^5 are taken together to form an optionally substituted saturated, partially unsaturated or unsaturated heteroatom-containing ring of up to about 20 ring atoms which is monocyclic or polycyclic, fused or unfused. In some embodiments, each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, carbocyclyl, aryl, heteroaryl, and heterocyclyl. In some embodiments, G^2 is $-C(R)_2Si(R)_3$, wherein $-C(R)_2-$ is optionally substituted $-CH_2-$, and each R of $-Si(R)_3$ is independently an optionally substituted group selected from C_{1-10} aliphatic, heterocyclyl, heteroaryl and aryl. In some embodiments, at least one R of $-Si(R)_3$ is independently optionally substituted C_{1-10} alkyl. In some embodiments, at least one R of $-Si(R)_3$ is independently optionally substituted phenyl. In some embodiments, one R of $-Si(R)_3$ is independently

optionally substituted phenyl, and each of the other two R is independently optionally substituted C_{1-10} alkyl. In some embodiments, one R of $-Si(R)_3$ is independently optionally substituted C_{1-10} alkyl, and each of the other two R is independently optionally substituted phenyl. In some embodiments, G^2 is optionally substituted $-CH_2Si(Ph)(Me)_2$. In some embodiments, G^2 is optionally substituted $-CH_2Si(Me)(Ph)_2$. In some embodiments, G^2 is $-CH_2Si(Me)(Ph)_2$. In some embodiments, G^4 and G^5 are taken together to form an optionally substituted saturated 5-6 membered ring containing one nitrogen atom (to which G^5 is attached). In some embodiments, G^4 and G^5 are taken together to form an optionally substituted saturated 5-membered ring containing one nitrogen atom. In some embodiments, G^1 is hydrogen. In some embodiments, G^3 is hydrogen. In some embodiments, both G^1 and G^3 are hydrogen.

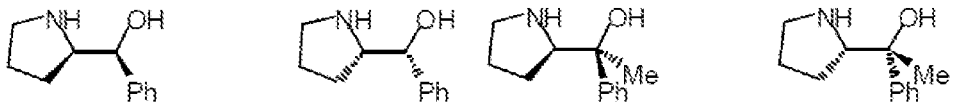
[00811] In some embodiments, a chiral reagent has one of the following formulae:



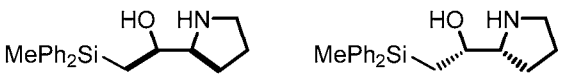
Formulae 3-AB 3-BB 3-CC 3-DD 3-EE 3-FF

[00812] In some embodiments, a chiral reagent is an aminoalcohol. In some embodiments, a chiral reagent is an aminothiol. In some embodiments, a chiral reagent is an aminophenol. In some embodiments, a chiral reagent is (*S*)- and (*R*)-2-methylamino-1-phenylethanol, (1*R*, 2*S*)-ephedrine, or (1*R*, 2*S*)-2-methylamino-1,2-diphenylethanol.

[00813] In some embodiments of the disclosure, a chiral reagent is a compound of one of the following formulae:



Formula O Formula P Formula Q Formula R



DPSE

[00814] As demonstrated herein, when used for preparing a chiral internucleotidic linkage, to obtain stereoselectivity generally stereochemically pure chiral reagents are utilized. Among other things, the present disclosure provides stereochemically pure chiral reagents, including those having structures described.

[00815] The choice of chiral reagent, for example, the isomer represented by Formula Q or its stereoisomer, Formula R, permits specific control of chirality at a linkage phosphorus. Thus, either an *R*p or *S*p configuration can be selected in each synthetic cycle, permitting control of the overall three dimensional structure of a chirally controlled oligonucleotide. In some embodiments, a chirally controlled oligonucleotide has all *R*p stereocenters. In some embodiments of the disclosure, a chirally controlled oligonucleotide has all *S*p stereocenters. In some embodiments of the disclosure, each linkage phosphorus in the chirally controlled oligonucleotide is independently *R*p or *S*p. In some embodiments of the disclosure, each linkage phosphorus in the chirally controlled oligonucleotide is independently *R*p or *S*p, and at least one is *R*p and at least one is *S*p. In some embodiments, the selection of *R*p and *S*p centers is made to confer a specific three dimensional superstructure to a chirally controlled oligonucleotide. Examples of such selections are described in further detail herein.

[00816] In some embodiments, a chiral reagent for use in accordance with the present disclosure is selected for its ablility to be removed at a particular step in the above-depicted cycle. For example, in some embodiments it is desirable to remove a chiral reagent during the step of modifying the linkage phosphorus. In some embodiments, it is desirable to remove a chiral reagent before the step of modifying the linkage phosphorus. In some embodiments, it is desirable to remove a chiral reagent after the step of modifying the linkage phosphorus. In some embodiments, it is desirable to remove a chiral reagent after a first coupling step has occurred but before a second coupling step has occurred, such that a chiral reagent is not present on the growing oligonucleotide during the second coupling (and likewise for additional subsequent coupling steps). In some embodiments, a chiral reagent is removed during the "deblock" reaction that occurs after modification of the linkage phosphorus but before a subsequent cycle begins. Example methods and reagents for removal are described herein.

[00817] In some embodiments, removal of chiral auxiliary is achieved when performing the modification and/or deblocking step, as illustrated in Scheme I. It can be beneficial to combine chiral auxiliary removal together with other transformations, such as modification and deblocking. A person of ordinary skill in the art would appreciate that the saved steps/transformation could improve the overall efficiency of synthesis, for instance, with respect to yield and product purity, especially for longer oligonucleotides. One example wherein the chiral auxiliary is removed during modification and/or deblocking is illustrated in Scheme I.

[00818] In some embodiments, a chiral reagent for use in accordance with methods of the present disclosure is characterized in that it is removable under certain conditions. For instance, in some embodiments, a chiral reagent is selected for its ability to be removed under acidic conditions. In certain embodiments, a chiral reagent is selected for its ability to be removed under mildly acidic conditions. In certain embodiments, a chiral reagent is selected for its ability to be removed by way of an E1 elimination reaction (*e.g.,* removal occurs due to the formation of a cation intermediate on the chiral reagent under acidic conditons, causing the chiral reagent to cleave from the oligonucleotide). In some embodiments, a chiral reagent is characterized in that it has a structure recognized as being able to accommodate or facilitate an E1 elimination reaction. One of skill in the relevant arts will appreciate which structures would be envisaged as being prone toward undergoing such elimination reactions.

[00819] In some embodiments, a chiral reagent is selected for its ability to be removed with a nucleophile. In some embodiments, a chiral reagent is selected for its ability to be removed with an amine nucleophile. In some embodiments, a chiral reagent is selected for its ability to be removed with a nucleophile other than an amine.

[00820] In some embodiments, a chiral reagent is selected for its ability to be removed with a base. In some embodiments, a chiral reagent is selected for its ability to be removed with an amine. In some embodiments, a chiral reagent is selected for its ability to be removed with a base other than an amine.

[00821] Additional chiral auxiliaries and their use can be found in *e.g.,* Wada I (JP4348077; WO2005/014609; WO2005/092909), Wada II (WO2010/064146), Wada III (WO2012/039448), Chiral Control (WO2010/064146), *etc*.

Activation

[00822] An achiral *H*-phosphonate moiety is treated with the first activating reagent to form the first intermediate. In one embodiment, the first activating reagent is added to the reaction mixture during the condensation step. Use of the first activating reagent is dependent on reaction conditions such as solvents that are used for the reaction. Examples of the first activating reagent are phosgene, trichloromethyl chloroformate, bis(trichloromethyl)carbonate (BTC), oxalyl chloride, Ph_3PCl_2, $(PhO)_3PCl_2$, *N,N'*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BopCl), 1,3-dimethyl-2-(3-nitro-1,2,4-triazol-1-yl)-2-pyrrolidin-1-yl-1,3,2-

diazaphospholidinium hexafluorophosphate (MNTP), or 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyNTP).

[00823] The example of achiral *H*-phosphonate moiety is a compound shown in the above Scheme. DBU represents 1,8-diazabicyclo[5.4.0]undec-7-ene. H^+DBU may be, for example, ammonium ion, alkylammonium ion, heteroaromatic iminium ion, or heterocyclic iminium ion, any of which is primary, secondary, tertiary or quaternary, or a monovalent metal ion.

Reacting with Chiral Reagent

[00824] After the first activation step, the activated achiral *H*-phosphonate moiety reacts with a chiral reagent, which is represented by formula (Z-I) or (Z-I'), to form a chiral intermediate of formula (Z-Va), (Z-Vb), (Z-Va'), or (Z-Vb').

Stereospecific Condensation Step

[00825] A chiral intermediate of Formula Z-Va ((Z-Vb), (Z-Va'), or (Z-Vb')) is treated with the second activating reagent and a nucleoside to form a condensed intermediate. The nucleoside may be on solid support. Examples of the second activating reagent are 4,5-dicyanoimidazole (DCI), 4,5-dichloroimidazole, 1-phenylimidazolium triflate (PhIMT), benzimidazolium triflate (BIT), benztriazole, 3-nitro-l,2,4-triazole (NT), tetrazole, 5-ethylthiotetrazole (ETT), 5-benzylthiotetrazole (BTT), 5-(4-nitrophenyl)tetrazole, *N*-cyanomethylpyrrolidinium triflate (CMPT), *N*-cyanomethylpiperidinium triflate, *N*-cyanomethyldimethylammonium triflate. A chiral intermediate of Formula Z-Va ((Z-Vb), (Z-Va'), or (Z-Vb')) may be isolated as a monomer. Usually, the chiral intermediate of Z-Va ((Z-Vb), (Z-Va'), or (Z-Vb')) is not isolated and undergoes a reaction in the same pot with a nucleoside or modified nucleoside to provide a chiral phosphite compound, a condensed intermediate. In other embodiments, when the method is performed via solid phase synthesis, the solid support comprising the compound is filtered away from side products, impurities, and/or reagents.

Capping Step

[00826] If the final nucleic acid is larger than a dimer, the unreacted -OH moiety is capped with a blocking group and the chiral auxiliary in the compound may also be capped with a

blocking group to form a capped condensed intermediate. If the final nucleic acid is a dimer, then the capping step is not necessary.

Modifying Step

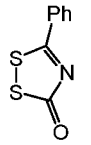
[00827] The compound is modified by reaction with an electrophile. The capped condensed intermediate may be executed modifying step. In some embodiments, the modifying step is performed using a sulfur electrophile, a selenium electrophile or a boronating agent. Examples of modifying steps are step of oxidation and sulfurization.

[00828] In some embodiments of the method, the sulfur electrophile is a compound having one of the following formulas:

S_8 (Formula Z-B), Z^{z1}-S-S-Z^{z2}, or Z^{z1}-S-V^z-Z^{z2};

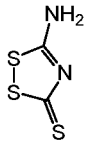
wherein Z^{z1} and Z^{z2} are independently alkyl, aminoalkyl, cycloalkyl, heterocyclic, cycloalkylalkyl, heterocycloalkyl, aryl, heteroaryl, alkyloxy, aryloxy, heteroaryloxy, acyl, amide, imide, or thiocarbonyl, or Z^{z1} and Z^{z2} are taken together to form a 3 to 8 membered alicyclic or heterocyclic ring, which may be substituted or unsubstituted; V^z is SO_2, O, or NR^f; and R^f is hydrogen, alkyl, alkenyl, alkynyl, or aryl.

[00829] In some embodiments of the method, the sulfur electrophile is a compound of following Formulae Z-A, Z-B, Z-C, Z-D, Z-E, or Z-F:

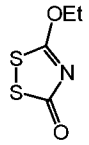


Formula Z-A

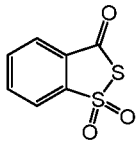
Formula Z-B



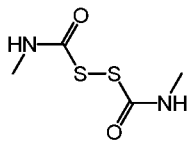
Formula Z-C



Formula Z-D



Formula Z-E



Formula Z-F

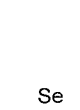
[00830] In some embodiments, a sulfurization reagent is 3-phenyl-1,2,4-dithiazolin-5-one.

[00831] In some embodiments, the selenium electrophile is a compound having one of the following formulae:

Se (Formula Z-G), Z^{z3}-Se-Se-Z^{z4}, or Z^{z3}-Se-V^z-Z^{z4};

wherein Z^{z3} and Z^{z4} are independently alkyl, aminoalkyl, cycloalkyl, heterocyclic, cycloalkylalkyl, heterocycloalkyl, aryl, heteroaryl, alkyloxy, aryloxy, heteroaryloxy, acyl, amide, imide, or thiocarbonyl, or Z^{z3} and Z^{z4} are taken together to form a 3 to 8 membered alicyclic or heterocyclic ring, which may be substituted or unsubstituted; V^z is SO_2, S, O, or NR^f; and R^f is hydrogen, alkyl, alkenyl, alkynyl, or aryl.

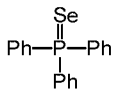
[00832] In some embodiments, the selenium electrophile is a compound of Formula Z-G, Z-H, Z-I, Z-J, Z-K, or Z-L.



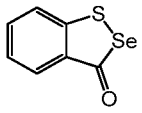
Formula Z-G



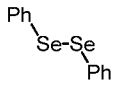
Formula Z-H



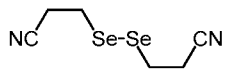
Formula Z-I



Formula Z-J



Formula Z-K



Formula Z-L

[00833] In some embodiments, the boronating agent is borane-*N*,*N*-diisopropylethylamine (BH_3 DIPEA), borane-pyridine (BH_3 Py), borane-2-chloropyridine (BH_3 CPy), borane-aniline (BH_3 An), borane-tetrahydrofiirane (BH_3 THF), or borane-dimethylsulfide (BH_3 Me_2S).

[00834] In some embodiments, after the modifying step, a chiral auxiliary group falls off from the growing oligonucleotide chain. In some embodiments, after the modifying step, a chiral auxiliary group remains connected to the internucleotidic phosphorus atom.

[00835] In some embodiments of the method, the modifying step is an oxidation step. In some embodiments of the method, the modifying step is an oxidation step using similar conditions as described above in this application. In some embodiments, an oxidation step is as disclosed in, *e.g.,* JP 2010-265304 A and WO2010/064146.

Chain Elongation Cycle and De-protection Step

[00836] The capped condensed intermediate is deblocked to remove the blocking group at the 5'-end of the growing nucleic acid chain to provide a compound. The compound is optionally allowed to re-enter the chain elongation cycle to form a condensed intermediate, a capped condensed intermediate, a modified capped condensed intermediate, and a 5'-deprotected modified capped intermediate. Following at least one round of chain elongation cycle, the 5'-deprotected modified capped intermediate is further deblocked by removal of the chiral auxiliary ligand and other protecting groups for, *e.g.,* nucleobase, modified nucleobase, sugar and modified sugar protecting groups, to provide a nucleic acid. In other embodiments, the nucleoside comprising a 5'-OH moiety is an intermediate from a previous chain elongation cycle as described herein. In yet other embodiments, the nucleoside comprising a 5'-OH moiety is an intermediate obtained from another known nucleic acid synthetic method. In embodiments where a solid support is used, the phosphorus-atom modified nucleic acid is then cleaved from the solid support. In certain embodiments, the nucleic acids is left attached on the solid support for purification purposes and then cleaved from the solid support following purification.

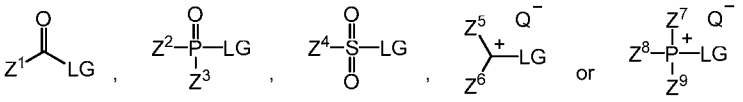
[00837] In yet other embodiments, the nucleoside comprising a 5'-OH moiety is an intermediate obtained from another known nucleic acid synthetic method. In yet other embodiments, the nucleoside comprising a 5'-OH moiety is an intermediate obtained from another known nucleic acid synthetic method as described in this application. In yet other embodiments, the nucleoside comprising a 5'-OH moiety is an intermediate obtained from another known nucleic acid synthetic method comprising one or more cycles illustrated in Scheme I. In yet other embodiments, the nucleoside comprising a 5'-OH moiety is an intermediate obtained from another known nucleic acid synthetic method comprising one or more cycles illustrated in Scheme I-b, I-c or I-d.

[00838] In some embodiments, the present disclosure provides oligonucleotide synthesis methods that use stable and commercially available materials as starting materials. In some embodiments, the present disclosure provides oligonucleotide synthesis methods to produce stereocontrolled phosphorus atom-modified oligonucleotide derivatives using an achiral starting material.

[00839] In some embodiments, the method of the present disclosure does not cause degradations under the de-protection steps. Further the method does not require special capping agents to produce phosphorus atom-modified oligonucleotide derivatives.

Condensing reagent

[00840] Condensing reagents (C_R) useful in accordance with methods of the present disclosure are of any one of the following general formulae:



C_R1 C_R2 C_R3 C_R4 C_R5

wherein Z^1, Z^2, Z^3, Z^4, Z^5, Z^6, Z^7, Z^8, and Z^9 are independently optionally substituted group selected from alkyl, aminoalkyl, cycloalkyl, heterocyclic, cycloalkylalkyl, heterocycloalkyl, aryl, heteroaryl, alkyloxy, aryloxy, or heteroaryloxy, or wherein any of Z^2 and Z^3, Z^5 and Z^6, Z^7 and Z^8, Z^8 and Z^9, Z^9 and Z^7, or Z^7 and Z^8 and Z^9 are taken together to form a 3 to 20 membered alicyclic or heterocyclic ring; Q^- is a counter anion; and LG is a leaving group.

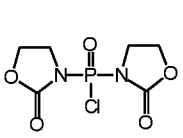
[00841] In some embodiments, a counter ion of a condensing reagent C_R is Cl^-, Br^-, BF_4^-,

PF_6^-, TfO^-, Tf_2N^-, AsF_6^-, ClO_4^-, or SbF_6^-, wherein Tf is CF_3SO_2. In some embodiments, a leaving group of a condensing reagent C_R is F, Cl, Br, I, 3-nitro-1,2,4-triazole, imidazole, alkyltriazole, tetrazole, pentafluorobenzene, or 1-hydroxybenzotriazole.

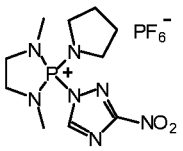
[00842] Examples of condensing reagents used in accordance with methods of the present disclosure include, but are not limited to, pentafluorobenzoyl chloride, carbonyldiimidazole (CDI), 1-mesitylenesulfonyl-3-nitrotriazole (MSNT), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDCI-HCl), benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (PyBOP), *N,N'*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BopCl), 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), and *O*-benzotriazole-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU), DIPCDI; *N,N'*-bis(2-oxo-3-oxazolidinyl)phosphinic bromide (BopBr), 1,3-dimethyl-2-(3-nitro-1,2,4-triazol-1-yl)-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium hexafluorophosphate (MNTP), 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyNTP), bromotripyrrolidinophosphonium hexafluorophosphate (PyBrOP); *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU); and tetramethylfluoroformamidinium hexafluorophosphate (TFFH). In certain embodiments, a counter ion of the condensing reagent C_R is Cl^-, Br^-, BF_4^-, PF_6^-, TfO^-, Tf_2N^-, AsF_6^-, ClO_4^-, or SbF_6^-, wherein Tf is CF_3SO_2.

[00843] In some embodiments, a condensing reagent is 1-(2,4,6-triisopropylbenzenesulfonyl)-5-(pyridin-2-yl) tetrazolide, pivaloyl chloride, bromotrispyrrolidinophosphonium hexafluorophosphate, *N,N'*-bis(2-oxo-3-oxazolidinyl) phosphinic chloride (BopCl), or 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane. In some embodiment, a condensing reagent is *N,N'*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BopCl). In some embodiments, a condensing reagent is selected from those described in WO/2006/066260).

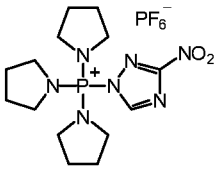
[00844] In some embodiments, a condensing reagent is 1,3-dimethyl-2-(3-nitro-1,2,4-triazol-1-yl)-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium hexafluorophosphate (MNTP), or 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyNTP):



BopCl



MNTP

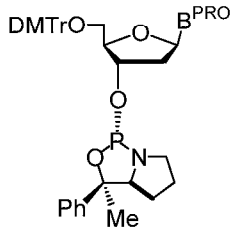


PyNTP

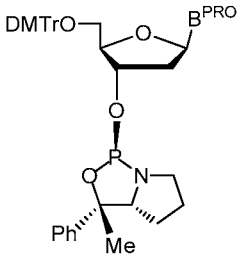
Selection of base and sugar of nucleoside coupling partner

[00845] As described herein, nucleoside coupling partners for use in accordance with methods of the present disclosure can be the same as one another or can be different from one another. In some embodiments, nucleoside coupling partners for use in the synthesis of a provided oligonucleotide are of the same structure and/or stereochemical configuration as one another. In some embodiments, each nucleoside coupling partner for use in the synthesis of a provided oligonucleotide is not of the same structure and/or stereochemical configuration as certain other nucleoside coupling partners of the oligonucleotide. Example nucleobases and sugars for use in accordance with methods of the present disclosure are described herein. One of skill in the relevant chemical and synthetic arts will recognize that any combination of nucleobases and sugars described herein are contemplated for use in accordance with methods of the present disclosure.

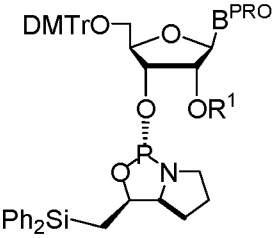
Coupling step

[00846] Example coupling procedures and chiral reagents and condensing reagents for use in accordance with the present disclosure are outlined in, *inter alia*, Wada I (JP4348077; WO2005/014609; WO2005/092909), Wada II (WO2010/064146), Wada III (WO2012/039448), and Chiral Control (WO2010/064146). Chiral nucleoside coupling partners for use in accordance with the present disclosure are also referred to herein as "Wada amidites." In some embodiments, a coupling partner has the structure of 

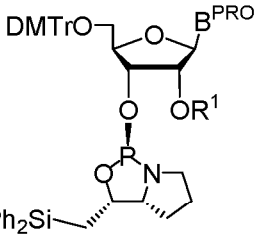
, wherein B^{PRO} is a protected nucleobase. In some embodiments, a coupling partner has the structure of



, wherein B^{PRO} is a protected nucleobase. In some embodiments, a coupling partner has the structure of

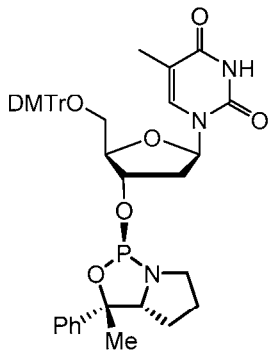


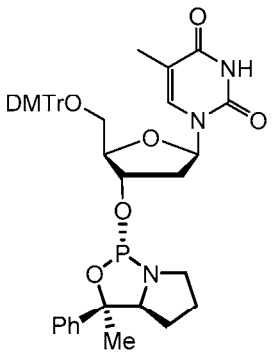
, wherein B^{PRO} is a protected nucleobase, and R^1 is as defined and described herein.. In some embodiments, a coupling partner has the structure of

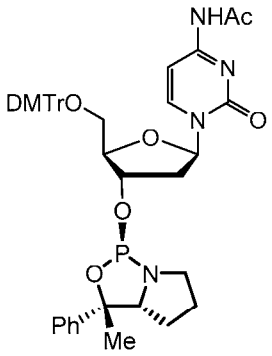


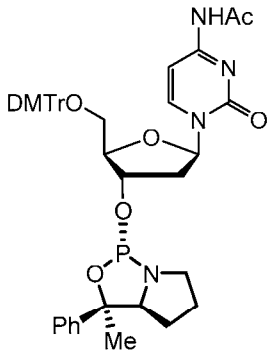
, wherein B^{PRO} is a protected nucleobase, and R^1 is as defined and described herein. In some embodiments, R^1 is optionally substituted C_{1-6} alkyl. In some embodiments, R^1 is Me.

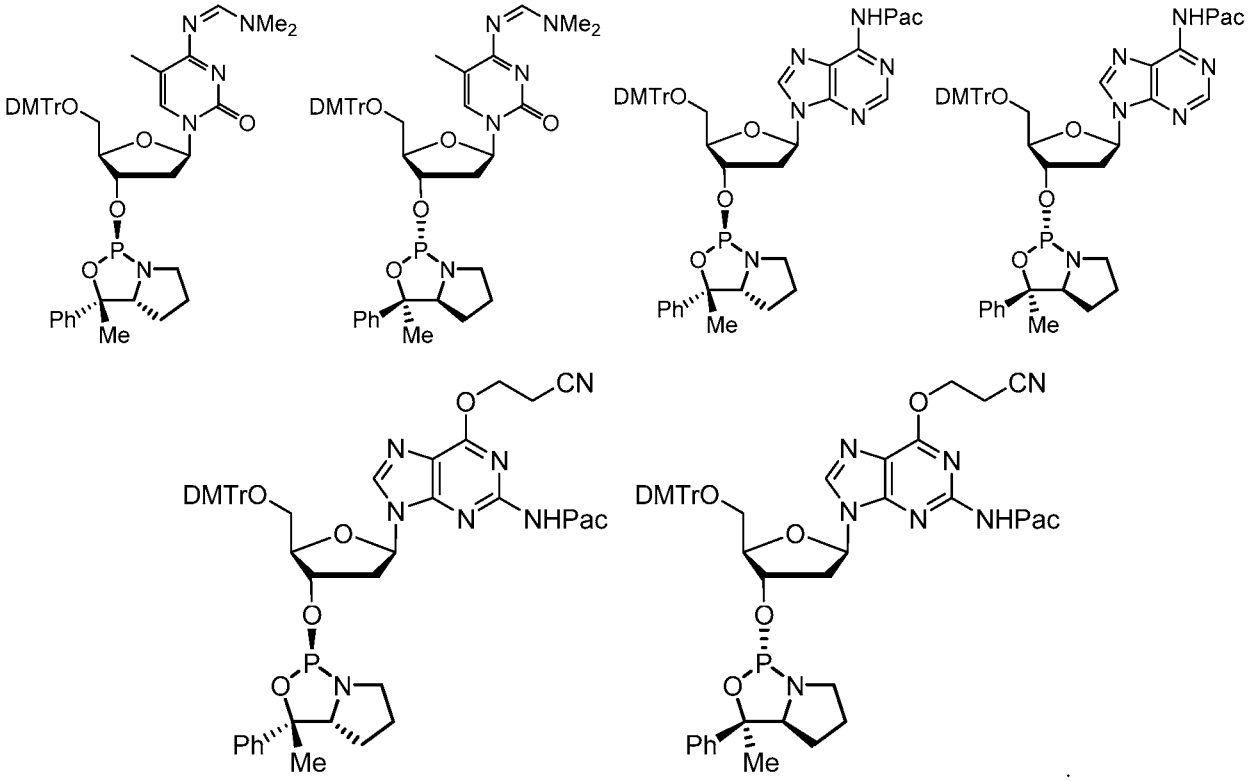
[00847] Example chiral phosphoramidites as coupling partner are depicted below:







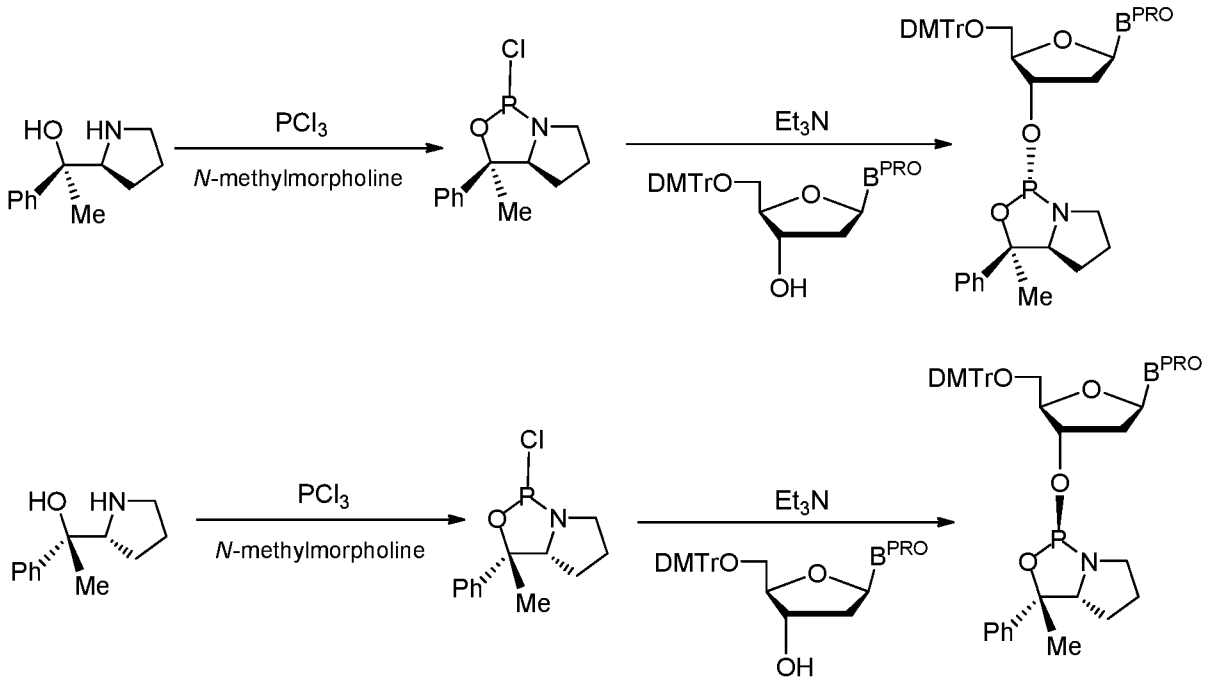




Additional examples are described in Chiral Control (WO2010/064146).

[00848] One of the methods used for synthesizing the coupling partner is depicted in Scheme II, below.

Scheme II. Example synthesis of coupling partner.



[00849] In some embodiments, the step of coupling comprises reacting a free hydroxyl group of a nucleotide unit of an oligonucleotide with a nucleoside coupling partner under suitable conditions to effect the coupling. In some embodiments, the step of coupling is preceded by a step of deblocking. For instance, in some embodiments, the 5' hydroxyl group of the growing oligonucleotide is blocked (i.e., protected) and must be deblocked in order to subsequently react with a nucleoside coupling partner.

[00850] Once the appropriate hydroxyl group of the growing oligonucleotide has been deblocked, the support is washed and dried in preparation for delivery of a solution comprising a chiral reagent and a solution comprising an activator. In some embodiments, a chiral reagent and an activator are delivered simultaneously. In some embodiments, co-delivery comprises delivering an amount of a chiral reagent in solution (*e.g.,* a phosphoramidite solution) and an amount of activator in a solution (*e.g.,* a CMPT solution) in a polar aprotic solvent such as a nitrile solvent (*e.g.,* acetonitrile).

[00851] In some embodiments, the step of coupling provides a crude product composition in which the chiral phosphite product is present in a diastereomeric excess of > 95%. In some embodiments, the chiral phosphite product is present in a diastereomeric excess of > 96%. In some embodiments, the chiral phosphite product is present in a diastereomeric excess of > 97%. In some embodiments, the chiral phosphite product is present in a diastereomeric excess of > 98%. In some embodiments, the chiral phosphite product is present in a diastereomeric excess of > 99%.

Capping step:

[00852] Provided methods for making chirally controlled oligonucleotides comprise a step of capping. In some embodiments, a step of capping is a single step. In some embodiments, a step of capping is two steps. In some embodiments, a step of capping is more than two steps.

[00853] In some embodiments, a step of capping comprises steps of capping the free amine of the chiral auxiliary and capping any residual unreacted 5' hydroxyl groups. In some embodiments, the free amine of the chiral auxiliary and the unreacted 5' hydroxyl groups are capped with the same capping group. In some embodiments, the free amine of the chiral auxiliary and the unreacted 5' hydroxyl groups are capped with different capping groups. In certain embodiments, capping with different capping groups allows for selective removal of one

capping group over the other during synthesis of the oligonucleotide. In some embodiments, the capping of both groups occurs simultaneously. In some embodiments, the capping of both groups occurs iteratively.

[00854] In certain embodiments, capping occurs iteratively and comprises a first step of capping the free amine followed by a second step of capping the free 5' hydroxyl group, wherein both the free amine and the 5' hydroxyl group are capped with the same capping group. For instance, in some embodiments, the free amine of the chiral auxiliary is capped using an anhydride (*e.g.,* phenoxyacetic anhydride, i.e., Pac_2O) prior to capping of the 5' hydroxyl group with the same anhydride. In certain embodiments, the capping of the 5' hydroxyl group with the same anhydride occurs under different conditions (*e.g.,* in the presence of one or more additional reagents). In some embodiments, capping of the 5' hydroxyl group occurs in the presence of an amine base in an etherial solvent (*e.g.,* NMI (N-methylimidazole) in THF). The phrase "capping group" is used interchangeably herein with the phrases "protecting group" and "blocking group".

[00855] In some embodiments, an amine capping group is characterized in that it effectively caps the amine such that it prevents rearrangement and/or decomposition of the intermediate phosphite species. In some embodiments, a capping group is selected for its ability to protect the amine of the chiral auxiliary in order to prevent intramolecular cleavage of the internucleotide linkage phosphorus.

[00856] In some embodiments, a 5' hydroxyl group capping group is characterized in that it effectively caps the hydroxyl group such that it prevents the occurrence of "shortmers," *e.g.,* "n-m" (m and n are integers and $m<n$; n is the number of bases in the targeted oligonucleotide) impurities that occur from the reaction of an oligonucleotide chain that fails to react in a first cycle but then reacts in one or more subsequent cycles. The presence of such shortmers, especially "n-1", has a deleterious effect upon the purity of the crude oligonucleotide and makes final purification of the oligonucleotide tedious and generally low-yielding.

[00857] In some embodiments, a particular cap is selected based on its tendency to facilitate a particular type of reaction under particular conditions. For instance, in some embodiments, a capping group is selected for its ability to facilitate an E1 elimination reaction, which reaction cleaves the cap and/or auxiliary from the growing oligonucleotide. In some embodiments, a capping group is selected for its ability to facilitate an E2 elimination reaction, which reaction cleaves the cap and/or auxiliary from the growing oligonucleotide. In some

embodiments, a capping group is selected for its ability to facilitate a β-elimination reaction, which reaction cleaves the cap and/or auxiliary from the growing oligonucleotide.

Modifying step:

[00858] As used herein, the phrase "modifying step", "modification step" and "P-modification step" are used interchangeably and refer generally to any one or more steps used to install a modified internucleotidic linkage. In some embodiments, the modified internucleotidic linkage having the structure of formula I. A P-modification step of the present disclosure occurs during assembly of a provided oligonucleotide rather than after assembly of a provided oligonucleotide is complete. Thus, each nucleotide unit of a provided oligonucleotide can be individually modified at the linkage phosphorus during the cycle within which the nucleotide unit is installed.

[00859] In some embodiments, a suitable P-modification reagent is a sulfur electrophile, selenium electrophile, oxygen electrophile, boronating reagent, or an azide reagent.

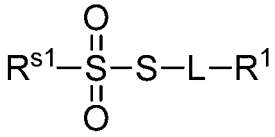
[00860] For instance, in some embodiments, a selemium reagent is elemental selenium, a selenium salt, or a substituted diselenide. In some embodiments, an oxygen electrophile is elemental oxygen, peroxide, or a substituted peroxide. In some embodiments, a boronating reagent is a borane-amine (*e.g.*, *N*,*N*-diisopropylethylamine ($BH_3 \cdot$DIPEA), borane-pyridine ($BH_3 \cdot$Py), borane-2-chloropyridine ($BH_3 \cdot$CPy), borane-aniline ($BH_3 \cdot$An)), a borane-ether reagent (*e.g.*, borane-tetrahydrofuran ($BH_3 \cdot$THF)), a borane-dialkylsulfide reagent (*e.g.*, $BH_3 \cdot Me_2S$), aniline-cyanoborane, or a triphenylphosphine-carboalkoxyborane. In some embodiments, an azide reagent is comprises an azide group capable of undergoing subsequent reduction to provide an amine group.

[00861] In some embodiments, a P-modification reagent is a sulfurization reagent as described herein. In some embodiments, a step of modifying comprises sulfurization of phosphorus to provide a phosphorothioate linkage or phosphorothioate triester linkage. In some embodiments, a step of modifying provides an oligonucleotide having an internucleotidic linkage of formula I.

[00862] In some embodiments, the present disclosure provides sulfurizing reagents, and methods of making, and use of the same.

[00863] In some embodiments, such sulfurizing reagents are thiosulfonate reagents. In

some embodiments, a thiosulfonate reagent has a structure of formula **S-I**:



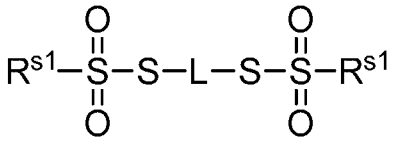
S-I

wherein:

R^{s1} is R; and

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

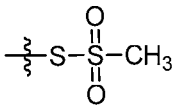
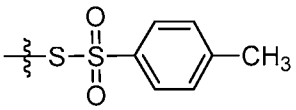
[00864] In some embodiments, the sulfurizing reagent is a bis(thiosulfonate) reagent. In some embodiments, the bis(thiosulfonate) reagent has the structure of formula **S-II**:

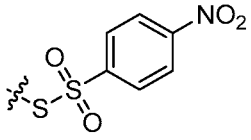
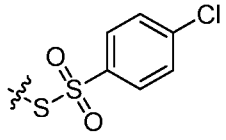
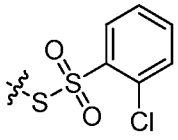
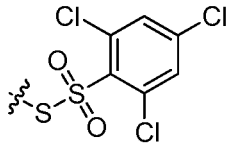
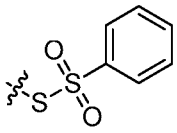
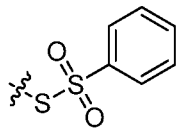
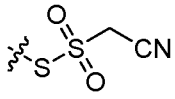
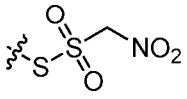
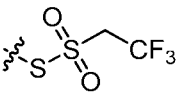
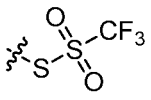
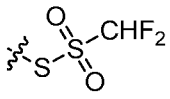
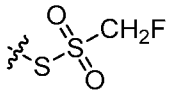


S-II

wherein each of R^{s1} and L is independently as defined and described above and herein.

[00865] As defined generally above, R^{s1} is R, wherein R is as defined and described above and herein. In some embodiments, R^{s1} is optionally substituted aliphatic, aryl, heterocyclyl or heteroaryl. In some embodiments, R^{s1} is optionally substituted alkyl. In some embodiments, R^{s1} is optionally substituted alkyl. In some embodiments, R^{s1} is methyl. In some embodiments, R^{s1} is cyanomethyl. In some embodiments, R^{s1} is nitromethyl. In some embodiments, R^{s1} is optionally substituted aryl. In some embodiments, R^{s1} is optionally substituted phenyl. In some embodiments, R^{s1} is phenyl. In some embodiments, R^{s1} is *p*-nitrophenyl. In some embodiments, R^{s1} is *p*-methylphenyl. In some embodiments, R^{s1} is *p*-chlorophenyl. In some embodiments, R^{s1} is *o*-chlorophenyl. In some embodiments, R^{s1} is 2,4,6-trichlorophenyl. In some embodiments, R^{s1} is pentafluorophenyl. In some embodiments, R^{s1} is optionally substituted heterocyclyl. In some embodiments, R^{s1} is optionally substituted heteroaryl.

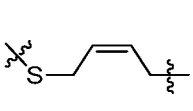
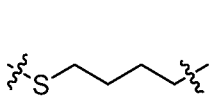
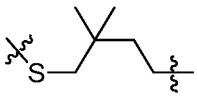
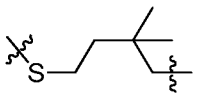
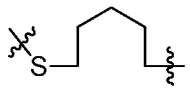
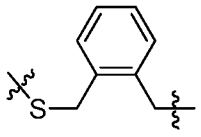
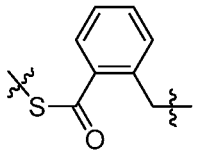
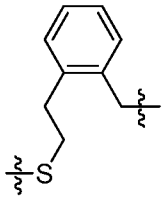
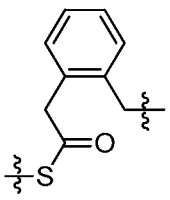
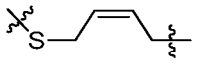
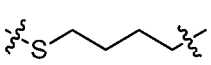
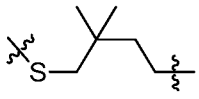
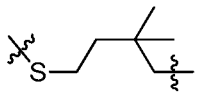
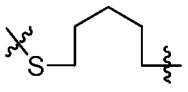
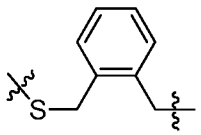
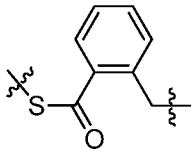
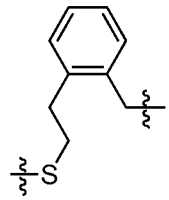
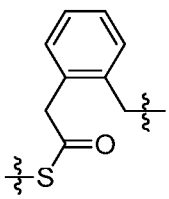
[00866] In some embodiments, R^{s1}–$S(O)_2S$– is  (MTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (TTS). In some embodiments, R^{s1}–

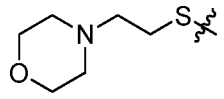
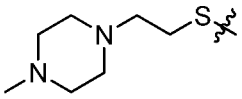
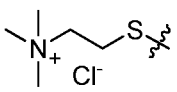
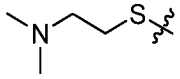
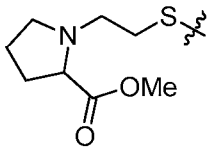
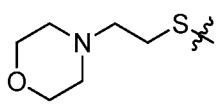
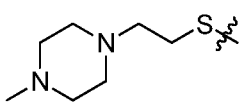
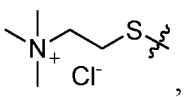
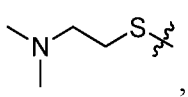
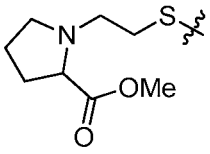
$S(O)_2S$– is  (NO_2PheTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (p-ClPheTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (o-ClPheTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (2,4,6-TriClPheTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (PheTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (PFPheTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (a-CNMTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (a-NO_2MTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (a-CF_3MTS). In some embodiments, R^{s1}–$S(O)_2S$– is  (a-CF_3TS). In some embodiments, R^{s1}–$S(O)_2S$– is  (a-CHF_2TS). In some embodiments, R^{s1}–$S(O)_2S$– is  (a-CH_2FTS).

[00867] In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein L is –S–R^{L3}– or –S–C(O)–R^{L3}–. In some embodiments, L is –S–R^{L3}– or –S–C(O)–R^{L3}–, wherein R^{L3} is an optionally substituted C_1–C_6 alkylene. In some embodiments, L is –S–R^{L3}– or –S–C(O)–R^{L3}–, wherein R^{L3} is an optionally substituted C_1–C_6 alkenylene. In some embodiments, L is –S–R^{L3}– or –S–C(O)–R^{L3}–, wherein R^{L3} is an optionally substituted C_1.C_6 alkylene wherein one or more methylene units are optionally and independently replaced by an optionally substituted C_1–C_6 alkenylene, arylene, or heteroarylene. In some embodiments, In some embodiments, R^{L3} is an optionally substituted –S–(C_1–C_6 alkenylene)–, –S–(C_1–C_6

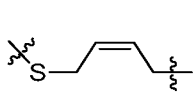
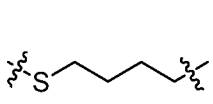
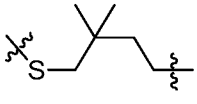
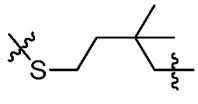
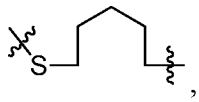
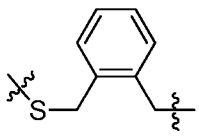
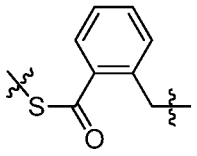
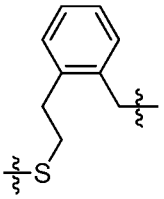
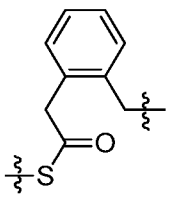
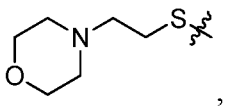
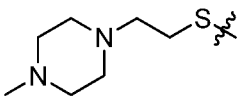
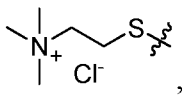
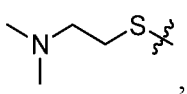
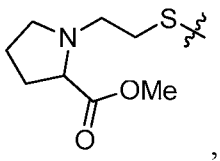
alkylene)–, –S–(C_1–C_6 alkylene)–arylene–(C_1–C_6 alkylene)–, –S–CO–arylene–(C_1–C_6 alkylene)–, or –S–CO–(C_1–C_6 alkylene)–arylene–(C_1–C_6 alkylene)–. In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein L is –S–R^{L3}– or –S–C(O)–R^{L3}–, and the sulfur atom is connected to R^1.

[00868] In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein L is alkylene, alkenylene, arylene or heteroarylene.

[00869] In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein L is  ,  ,  ,  ,  ,  ,  ,  , or  . In some embodiments, L is  ,  ,  ,  ,  ,  ,  ,  , or  , wherein the sulfur atom is connected to R^1.

[00870] In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein R^1 is  ,  ,  ,  , or  . In some embodiments, R^1 is  ,  ,  ,  , or  , wherein the sulfur atom is connected to L.

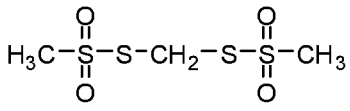
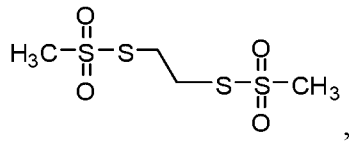
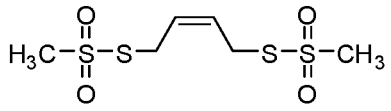
[00871] In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**,

wherein L is  ,  ,  ,  ,  ,  ,  ,  , or  , wherein the sulfur atom is connected to R^1; and R^1 is  ,  ,  ,  , or  , wherein the sulfur atom is connected to L.

[00872] In some embodiments, the sulfurizing reagent has the structure of **S-I** or **S-II**, wherein R^1 is $–S–R^{L2}$, wherein R^{L2} is as defined and described above and herein. In some embodiments, R^{L2} is an optionally substituted group selected from $–S–(C_1–C_6$ alkylene)–heterocyclyl, $–S–(C_1–C_6$ alkenylene)–heterocyclyl, $–S–(C_1–C_6$ alkylene)$–N(R')_2$, $–S–(C_1–C_6$ alkylene)$–N(R')_3$, wherein each R' is as defined above and described herein.

[00873] In some embodiments, $–L–R^1$ is $–R^{L3}–S–S–R^{L2}$, wherein each variable is independently as defined above and described herein. In some embodiments, $–L–R^1$ is $–R^{L3}–C(O)–S–S–R^{L2}$, wherein each variable is independently as defined above and described herein.

[00874] Example bis(thiosulfonate) reagents of formula **S-II** are depicted below:

 ,  ,  .

[00875] In some embodiments, the sulfurization reagent is a compound having one of the following formulae:

S_8, $R^{s2}–S–S–R^{s3}$, or $R^{s2}–S–X^s–R^{s3}$,

wherein:

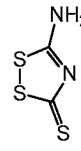
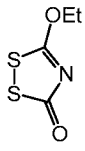
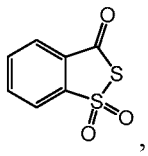
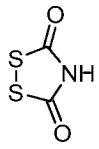
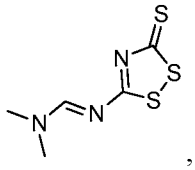
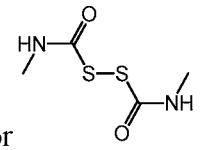
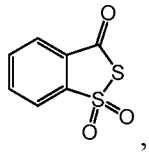
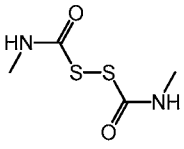
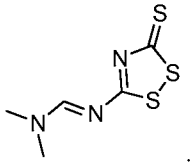
each of R^{s2} and R^{s3} is independently an optionally substituted group selected from aliphatic, aminoalkyl, carbocyclyl, heterocyclyl, heterocycloalkyl, aryl, heteroaryl, alkyloxy, aryloxy, heteroaryloxy, acyl, amide, imide, or thiocarbonyl; or

R^{s2} and R^{s3} are taken together with the atoms to which they are bound to form an

optionally substituted heterocyclic or heteroaryl ring;

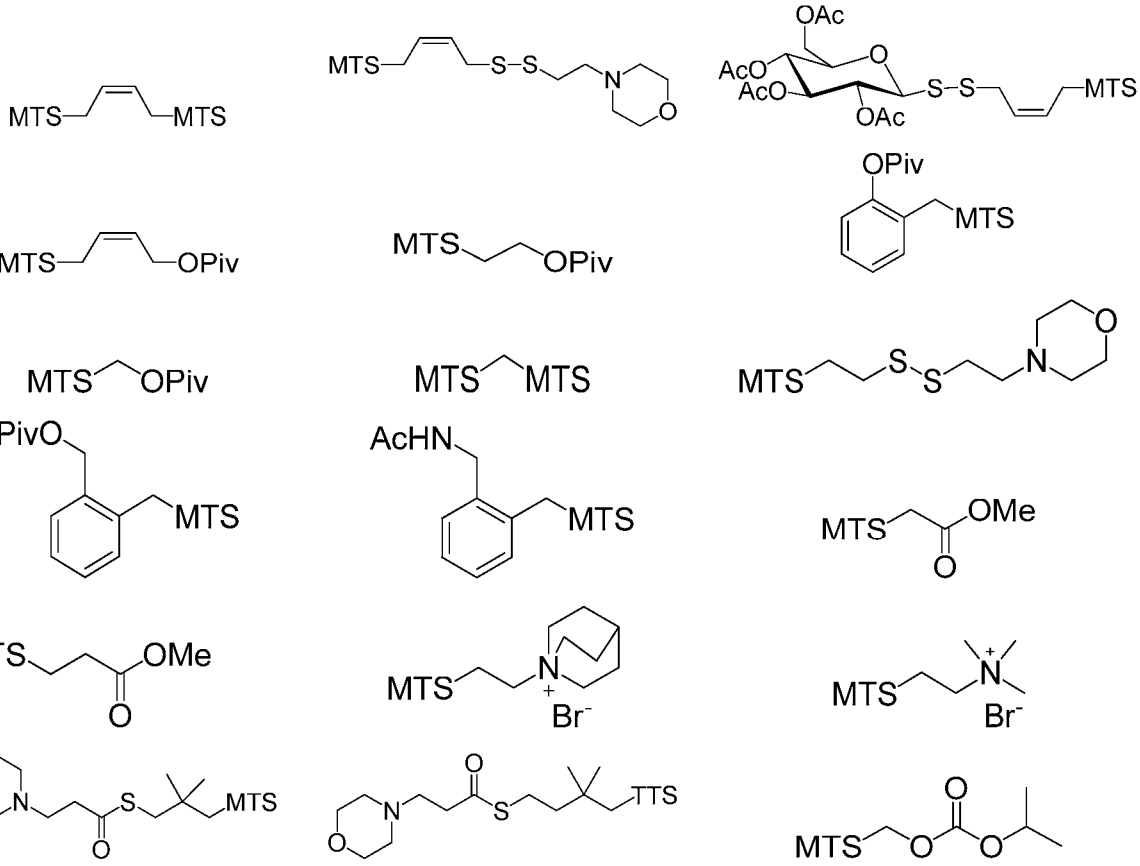
X^s is $–S(O)_2–$, –O–, or –N(R')–; and

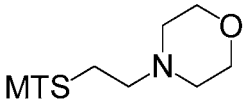
R' is as defined and described above and herein.

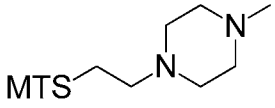
[00876] In some embodiments, the sulfurization reagent is S_8,  ,  ,  ,  ,  , or  . In some embodiments, the sulfurization reagent is S_8,  , or  . In some embodiments, the sulfurization reagent is  .

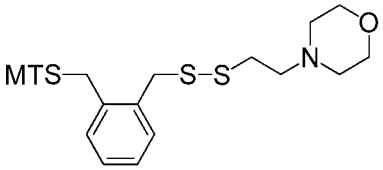
[00877] Example sulfuring reagents are depicted in Table 5 below.

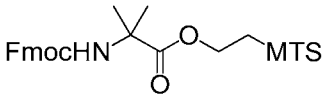
Table 5. Example sulfurization reagents.

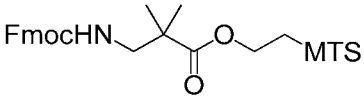


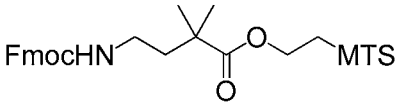

MTS
N
O

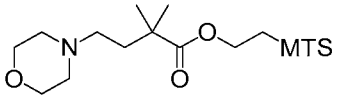

MTS
N
N

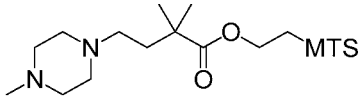

MTS
S–S
N
O

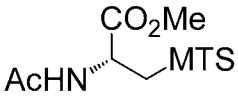

FmocHN
O
MTS
O

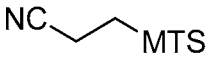

FmocHN
O
MTS
O

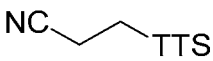

FmocHN
O
MTS
O


N
O
MTS
O

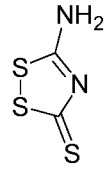

N
O
MTS
O
N

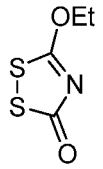

CO2Me
AcHN
MTS

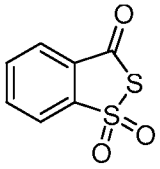

NC
MTS

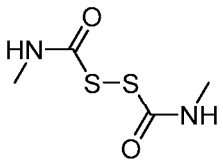

NC
TTS

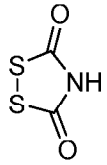

S8

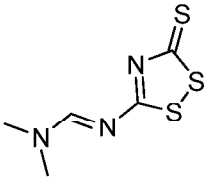

NH2
S
S
N
S

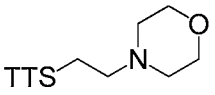

OEt
S
S
N
O

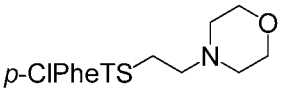

O
S
S
O
O

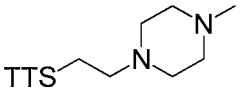

HN
O
S–S
NH
O

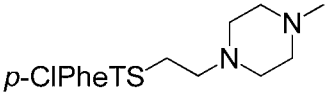

O
S
NH
S
O

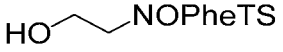

S
N
S
S
N
N

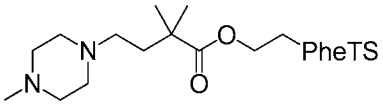

TTS
N
O

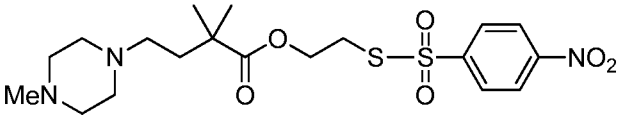

p-ClPheTS
N
O

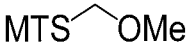

TTS
N
N

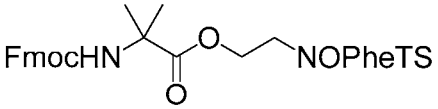

p-ClPheTS
N
N

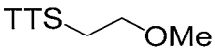

HO
NOPheTS

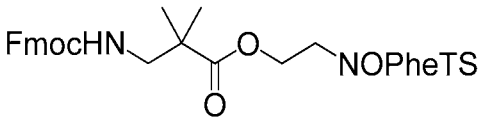

N
N
O
O
PheTS

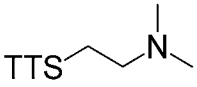

MeN
N
O
O
S
S
O
O
NO2

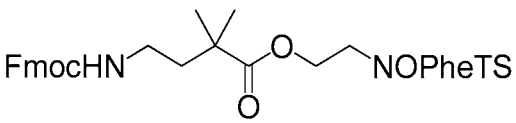

MTS
OMe

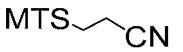

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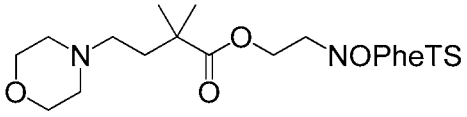

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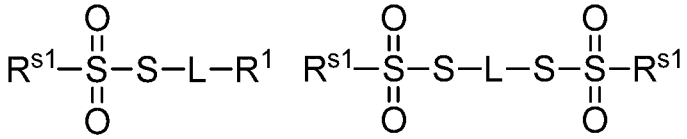

FmocHN
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POS

[00878] In some embodiments, a provided sulfurization reagent is used to modify an H-phosphonate. For instance, in some embodiments, an H-phosphonate oligonucleotide is synthesized using, *e.g.,* a method of Wada I or Wada II, and is modified using a sulfurization reagent of formula **S-I** or **S-II**:



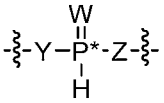
S-I S-II

,

wherein each of R^{S1}, L, and R^1 are as described and defined above and herein.

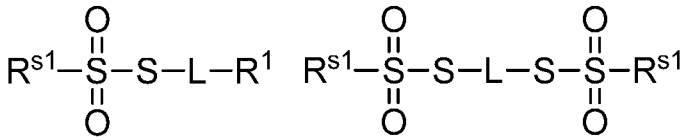
[00879] In some embodiments, the present disclosure provides a process for synthesizing a phosphorothioate triester, comprising steps of:

i) reacting an H-phosphonate of structure:



wherein each of W, Y, and Z are as described and defined above and herein, with a silylating reagent to provide a silyloxyphosphonate; and

ii) reacting the silyloxyphosphonate with a sulfurization reagent of structure **S-I** or **S-II**:



S-I S-II

,

to provide a phosphorothiotriester.

[00880] In some embodiments, a selenium electrophile is used instead of a sulfurizing reagent to introduce modification to the internucleotidic linkage. In some embodiments, a selenium electrophile is a compound having one of the following formulae:

Se, R^{s2}–Se–Se– R^{s3}, or R^{s2}–Se–X^s–R^{s3},

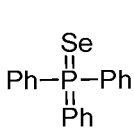
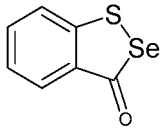
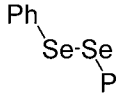
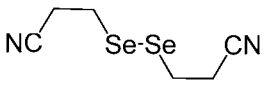
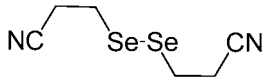
wherein:

each of R^{s2} and R^{s3} is independently an optionally substituted group selected from aliphatic, aminoalkyl, carbocyclyl, heterocyclyl, heterocycloalkyl, aryl, heteroaryl, alkyloxy, aryloxy, heteroaryloxy, acyl, amide, imide, or thiocarbonyl; or

R^{s2} and R^{s3} are taken together with the atoms to which they are bound to form an optionally substituted heterocyclic or heteroaryl ring;

X^s is $-S(O)_2-$, $-O-$, or $-N(R')-$; and

R' is as defined and described above and herein.

[00881] In other embodiments, the selenium electrophile is a compound of Se, KSeCN,  ,  ,  , or  . In some embodiments, the selenium electrophile is Se or  .

[00882] In some embodiments, a sulfurization reagent for use in accordance with the present disclosure is characterized in that the moiety transferred to phosphorus during sulfurization is a substituted sulfur (*e.g.*, -SR) as opposed to a single sulfur atom (*e.g.*, $-S^-$ or =S).

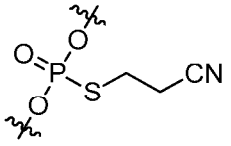
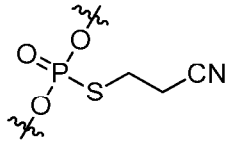
[00883] In some embodiments, a sulfurization reagent for use in accordance with the present disclosure is characterized in that the activity of the reagent is tunable by modifying the reagent with a certain electron withdrawing or donating group.

[00884] In some embodiments, a sulfurization reagent for use in accordance with the present disclosure is characterized in that it is crystalline. In some embodiments, a sulfurization reagent for use in accordance with the present disclosure is characterized in that it has a high degree of crystallinity. In certain embodiments, a sulfurization reagent for use in accordance with the present disclosure is characterized by ease of purification of the reagent via, *e.g.*, recrystallization. In certain embodiments, a sulfurization reagent for use in accordance with the present disclosure is characterized in that it is substantially free from sulfur-containing impurities. In some embodiments, sulfurization reagents which are substantially free from sulfur-containing impurities show increased efficiency.

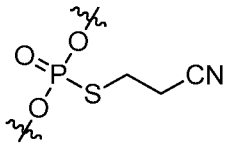
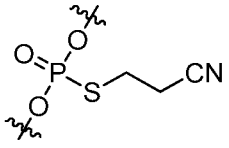
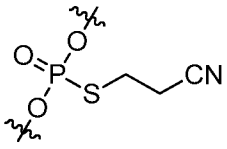
[00885] In some embodiments, the provided chirally controlled oligonucleotide comprises one or more phosphate diester linkages. To synthesize such chirally controlled oligonucleotides, one or more modifying steps are optionally replaced with an oxidation step to install the corresponding phosphate diester linkages. In some embodiments, the oxidation step is

performed in a fashion similar to ordinary oligonucleotide synthesis. In some embodiments, an oxidation step comprises the use of I_2. In some embodiments, an oxidation step comprises the use of I_2 and pyridine. In some embodiments, an oxidation step comprises the use of 0.02 M I_2 in a THF/pyridine/water (70:20:10 – v/v/v) co-solvent system. An example cycle is depicted in Scheme I-c.

[00886] In some embodiments, a phosphorothioate is directly formed through sulfurization by a sulfurization reagents, *e.g.,* 3-phenyl-1,2,4-dithiazolin-5-one. In some embodiments, after a direct installation of a phosphorothioate, a chiral auxiliary group remains attached to the internucleotidic phosphorus atom. In some embodiments, an additional de-protecting step is required to remove the chiral auxiliary (*e.g.,* for DPSE-type chiral auxiliary, using TBAF, HF-Et_3N, *etc.*).

[00887] In some embodiments, a phosphorothioate precursor is used to synthesize chirally controlled oligonucleotides comprising phosphorothioate linkages. In some embodiments, such a phosphorothioate precursor is  . In some embodiments,  is converted into phosphorothioate diester linkages during standard deprotection/release procedure after cycle exit. Examples are further depicted below.

[00888] In some embodiments, the provided chirally controlled oligonucleotide comprises one or more phosphate diester linkages and one or more phosphorothioate diester linkages. In some embodiments, the provided chirally controlled oligonucleotide comprises one or more phosphate diester linkages and one or more phosphorothioate diester linkages, wherein at least one phosphate diester linkage is installed after all the phosphorothioate diester linkages when synthesized from 3' to 5'. To synthesize such chirally controlled oligonucleotides, in some embodiments, one or more modifying steps are optionally replaced with an oxidation step to install the corresponding phosphate diester linkages, and a phosphorothioate precursor is installed for each of the phosphorothioate diester linkages. In some embodiments, a phosphorothioate precursor is converted to a phosphorothioate diester linkage after the desired oligonucleotide length is achieved. In some embodiments, the deprotection/release step during or after cycle exit converts the phosphorothioate precursors into phosphorothioate diester linkages. In some embodiments, a phosphorothioate precursor is characterized in that it has the

ability to be removed by a beta-elimination pathway. In some embodiments, a phosphorothioate precursor is  . As understood by one of ordinary skill in the art, one of the benefits of using a phosphorothioate precursor, for instance,  , during synthesis is that  is more stable than phosphorothioate in certain conditions.

[00889] In some embodiments, a phosphorothioate precursor is a phosphorus protecting group as described herein, *e.g.,* 2-cyanoethyl (CE or Cne), 2-trimethylsilylethyl, 2-nitroethyl, 2-sulfonylethyl, methyl, benzyl, *o*-nitrobenzyl, 2-(*p*-nitrophenyl)ethyl (NPE or Npe), 2-phenylethyl, 3-(*N*-*tert*-butylcarboxamido)-1-propyl, 4-oxopentyl, 4-methylthio-l-butyl, 2-cyano-1,1-dimethylethyl, 4-*N*-methylaminobutyl, 3-(2-pyridyl)-1-propyl, 2-[*N*-methyl-*N*-(2-pyridyl)]aminoethyl, 2-(*N*-formyl,*N*-methyl)aminoethyl, 4-[*N*-methyl-*N*-(2,2,2-trifluoroacetyl)amino]butyl. Examples are further depicted below.

[00890] Methods for synthesizing a desired sulfurization reagent are described herein and in the examples section.

[00891] As noted above, in some embodiments, sulfurization occurs under conditions which cleave the chiral reagent from the growing oligonucleotide. In some embodiments, sulfurization occurs under conditions which do not cleave the chiral reagent from the growing oligonucleotide.

[00892] In some embodiments, a sulfurization reagent is dissolved in a suitable solvent and delivered to the column. In certain embodiments, the solvent is a polar aprotic solvent such as a nitrile solvent. In some embodiments, the solvent is acetonitrile. In some embodiments, a solution of sulfurization reagent is prepared by mixing a sulfurization reagent (*e.g.,* a thiosulfonate derivative as described herein) with BSTFA (N,O-bis-trimethylsilyl-trifluoroacetamide) in a nitrile solvent (*e.g.,* acetonitrile). In some embodiments, BSTFA is not included. For example, the present inventors have found that relatively more reactive sulfurization reagents of general formula R^{s2}–S–$S(O)_2$–R^{s3} can often successfully participate in

sulfurization reactions in the absence of BSTFA. To give but one example, the inventors have demonstrated that where R^{s2} is *p*-nitrophenyl and R^{s3} is methyl then no BSTFA is required. In light of this disclosure, those skilled in the art will readily be able to determine other situations and/or sulfurization reagents that do not require BSTFA.

[00893] In some embodiments, the sulfurization step is performed at room temperature. In some embodiments, the sulfurization step is performed at lower temperatures such as about 0 °C, about 5 °C, about 10 °C, or about 15 °C. In some embodiments, the sulfurization step is performed at elevated temperatures of greater than about 20 °C.

[00894] In some embodiments, a sulfurization reaction is run for about 1 minute to about 120 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 90 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 60 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 30 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 25 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 20 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 15 minutes. In some embodiments, a sulfurization reaction is run for about 1 minute to about 10 minutes. In some embodiments, a sulfurization reaction is run for about 5 minute to about 60 minutes.

[00895] In some embodiments, a sulfurization reaction is run for about 5 minutes. In some embodiments, a sulfurization reaction is run for about 10 minutes. In some embodiments, a sulfurization reaction is run for about 15 minutes. In some embodiments, a sulfurization reaction is run for about 20 minutes. In some embodiments, a sulfurization reaction is run for about 25 minutes. In some embodiments, a sulfurization reaction is run for about 30 minutes. In some embodiments, a sulfurization reaction is run for about 35 minutes. In some embodiments, a sulfurization reaction is run for about 40 minutes. In some embodiments, a sulfurization reaction is run for about 45 minutes. In some embodiments, a sulfurization reaction is run for about 50 minutes. In some embodiments, a sulfurization reaction is run for about 55 minutes. In some embodiments, a sulfurization reaction is run for about 60 minutes.

[00896] It was unexpectedly found that certain of the sulfurization modification products made in accordance with methods of the present disclosure are unexpectedly stable. In some embodiments, it the unexpectedly stable products are phosphorothioate triesters. In some

embodiments, the unexpectedly stable products are chirally controlled oligonucleotides comprising one or more internucleotidic linkages having the structure of formula I-c.

[00897] One of skill in the relevant arts will recognize that sulfurization methods described herein and sulfurization reagents described herein are also useful in the context of modifying H-phosphonate oligonucleotides such as those described in Wada II (WO2010/064146).

[00898] In some embodiments, the sulfurization reaction has a stepwise sulfurization efficiency that is at least about 80%, 85%, 90%, 95%, 96%, 97%, or 98%. In some embodiments, the sulfurization reaction provides a crude dinucleotide product compositon that is at least 98% pure. In some embodiments, the sulfurization reaction provides a crude tetranucleotide product compositon that is at least 90% pure. In some embodiments, the sulfurization reaction provides a crude dodecanucleotide product compositon that is at least 70% pure. In some embodiments, the sulfurization reaction provides a crude icosanucleotide product compositon that is at least 50% pure.

[00899] Once the step of modifying the linkage phosphorus is complete, the oligonucleotide undergoes another deblock step in preparation for re-entering the cycle. In some embodiments, a chiral auxiliary remains intact after sulfurization and is deblocked during the subsequent deblock step, which necessarily occurs prior to re-entering the cycle. The process of deblocking, coupling, capping, and modifying, are repeated until the growing oligonucleotide reaches a desired length, at which point the oligonucleotide can either be immediately cleaved from the solid support or left attached to the support for purification purposes and later cleaved. In some embodiments, one or more protecting groups are present on one or more of the nucleotide bases, and cleaveage of the oligonucleotide from the support and deprotection of the bases occurs in a single step. In some embodiments, one or more protecting groups are present on one or more of the nucleotide bases, and cleaveage of the oligonucleotide from the support and deprotection of the bases occurs in more than one step. In some embodiments, deprotection and cleavage from the support occurs under basic conditions using, *e.g.,* one or more amine bases. In certain embodiments, the one or more amine bases comprise propyl amine. In certain embodiments, the one or more amine bases comprise pyridine.

[00900] In some embodiments, cleavage from the support and/or deprotection occurs at elevated temperatures of about 30 °C to about 90 °C. In some embodiments, cleavage from the

support and/or deprotection occurs at elevated temperatures of about 40 °C to about 80 °C. In some embodiments, cleavage from the support and/or deprotection occurs at elevated temperatures of about 50 °C to about 70 °C. In some embodiments, cleavage from the support and/or deprotection occurs at elevated temperatures of about 60 °C. In some embodiments, cleavage from the support and/or deprotection occurs at ambient temperatures.

[00901] Example purification procedures are described herein and/or are known generally in the relevant arts.

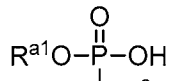
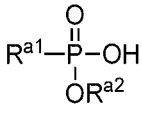
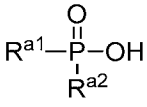
[00902] Noteworthy is that the removal of the chiral auxiliary from the growing oligonucleotide during each cycle is beneficial for at least the reasons that (1) the auxiliary will not have to be removed in a separate step at the end of the oligonucleotide synthesis when potentially sensitive functional groups are installed on phosphorus; and (2) unstable phosphorus-auxiliary intermediates prone to undergoing side reactions and/or interfering with subsequent chemistry are avoided. Thus, removal of the chiral auxiliary during each cycle makes the overall synthesis more efficient.

[00903] While the step of deblocking in the context of the cycle is described above, additional general methods are included below.

Deblocking step

[00904] In some embodiments, the step of coupling is preceded by a step of deblocking. For instance, in some embodiments, the 5' hydroxyl group of the growing oligonucleotide is blocked (i.e., protected) and must be deblocked in order to subsequently react with a nucleoside coupling partner.

[00905] In some embodiments, acidification is used to remove a blocking group. In some embodiments, the acid is a Brønsted acid or Lewis acid. Useful Brønsted acids are carboxylic acids, alkylsulfonic acids, arylsulfonic acids, phosphoric acid and its derivatives, phosphonic acid and its derivatives, alkylphosphonic acids and their derivatives, arylphosphonic acids and their derivatives, phosphinic acid, dialkylphosphinic acids, and diarylphosphinic acids which have a pKa (25 °C in water) value of -0.6 (trifluoroacetic acid) to 4.76 (acetic acid) in an organic solvent or water (in the case of 80% acetic acid). The concentration of the acid (1 to 80%) used in the acidification step depends on the acidity of the acid. Consideration to the acid strength must be taken into account as strong acid conditions will result in depurination/depyrimidination,

wherein purinyl or pyrimidinyl bases are cleaved from ribose ring and or other sugar ring. In some embodiments, an acid is selected from $R^{a1}COOH$, $R^{a1}SO_3H$, $R^{a3}SO_3H$,  ,  , or  , wherein each of R^{a1} and R^{a2} is independently hydrogen or an optionally substituted alkyl or aryl, and R^{a3} is an optionally substituted alkyl or aryl.

[00906] In some embodiments, acidification is accomplished by a Lewis acid in an organic solvent. Examples of such useful Lewis acids are $Zn(X^a)_2$ wherein X^a is Cl, Br, I, or CF_3SO_3.

[00907] In some embodiments, the step of acidifying comprises adding an amount of a Brønsted or Lewis acid effective to remove a blocking group without removing purine moieties from the condensed intermediate.

[00908] Acids that are useful in the acidifying step also include, but are not limited to 10% phosphoric acid in an organic solvent, 10% hydrochloric acid in an organic solvent, 1% trifluoroacetic acid in an organic solvent, 3% dichloroacetic acid or trichloroacetic acid in an organic solvent or 80% acetic acid in water. The concentration of any Brønsted or Lewis acid used in this step is selected such that the concentration of the acid does not exceed a concentration that causes cleavage of a nucleobase from a sugar moiety.

[00909] In some embodiments, acidification comprises adding 1% trifluoroacetic acid in an organic solvent. In some embodiments, acidification comprises adding about 0.1% to about 8% trifluoroacetic acid in an organic solvent. In some embodiments, acidification comprises adding 3% dichloroacetic acid or trichloroacetic acid in an organic solvent. In some embodiments, acidification comprises adding about 0.1% to about 10% dichloroacetic acid or trichloroacetic acid in an organic solvent. In some embodiments, acidification comprises adding 3% trichloroacetic acid in an organic solvent. In some embodiments, acidification comprises adding about 0.1% to about 10% trichloroacetic acid in an organic solvent. In some embodiments, acidification comprises adding 80% acetic acid in water. In some embodiments, acidification comprises adding about 50% to about 90%, or about 50% to about 80%, about 50% to about 70%, about 50% to about 60%, about 70% to about 90% acetic acid in water. In some embodiments, the acidification comprises the further addition of cation scavengers to an acidic

solvent. In certain embodiments, the cation scavengers can be triethylsilane or triisopropylsilane. In some embodiments, a blocking group is deblocked by acidification, which comprises adding 1% trifluoroacetic acid in an organic solvent. In some embodiments, a blocking group is deblocked by acidification, which comprises adding 3% dichloroacetic acid in an organic solvent. In some embodiments, a blocking group is deblocked by acidification, which comprises adding 3% trichloroacetic acid in an organic solvent. In some embodiments, a blocking group is deblocked by acidification, which comprises adding 3% trichloroacetic acid in dichloromethane.

[00910] In certain embodiments, methods of the present disclosure are completed on a synthesizer and the step of deblocking the hydroxyl group of the growing oligonucleotide comprises delivering an amount solvent to the synthesizer column, which column contains a solid support to which the oligonucleotide is attached. In some embodiments, the solvent is a halogenated solvent (*e.g.,* dichloromethane). In certain embodiments, the solvent comprises an amount of an acid. In some embodiments, the solvent comprises an amount of an organic acid such as, for instance, trichloroacetic acid. In certain embodiments, the acid is present in an amount of about 1% to about 20% w/v. In certain embodiments, the acid is present in an amount of about 1% to about 10% w/v. In certain embodiments, the acid is present in an amount of about 1% to about 5% w/v. In certain embodiments, the acid is present in an amount of about 1 to about 3% w/v. In certain embodiments, the acid is present in an amount of about 3% w/v. Methods for deblocking a hydroxyl group are described further herein. In some embodiments, the acid is present in 3% w/v is dichloromethane.

[00911] In some embodiments, the chiral auxiliary is removed before the deblocking step. In some embodiments, the chiral auxiliary is removed during the deblocking step.

[00912] In some embodiments, cycle exit is performed before the deblocking step. In some embodiments, cycle exit is preformed after the deblocking step.

General conditions for blocking group/protecting group removal

[00913] Functional groups such as hydroxyl or amino moieties which are located on nucleobases or sugar moieties are routinely blocked with blocking (protecting) groups (moieties) during synthesis and subsequently deblocked. In general, a blocking group renders a chemical functionality of a molecule inert to specific reaction conditions and can later be removed from

such functionality in a molecule without substantially damaging the remainder of the molecule (see *e.g.,* Green and Wuts, Protective Groups in Organic Synthesis, 2nd Ed., John Wiley & Sons, New York, 1991). For example, amino groups can be blocked with nitrogen blocking groups such as phthalimido, 9-fludrenylmethoxycarbonyl (FMOC), triphenylmethylsulfenyl, *t*-BOC, 4,4'-dimethoxytrityl (DMTr), 4-methoxytrityl (MMTr), 9-phenylxanthin-9-yl (Pixyl), trityl (Tr), or 9-(*p*-methoxyphenyl)xanthin-9-yl (MOX). Carboxyl groups can be protected as acetyl groups. Hydroxy groups can be protected such as tetrahydropyranyl (THP), *t*-butyldimethylsilyl (TBDMS), 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (Ctmp), 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp), 1-(2-chloroethoxy)ethyl, 3-methoxy-1,5-dicarbomethoxypentan-3-yl (MDP), bis(2-acetoxyethoxy)methyl (ACE), triisopropylsilyloxymethyl (TOM), 1-(2-cyanoethoxy)ethyl (CEE), 2-cyanoethoxymethyl (CEM), [4-(*N*-dichloroacetyl-*N*-methylamino)benzyloxy]methyl, 2-cyanoethyl (CN), pivaloyloxymethyl (PivOM), levunyloxymethyl (ALE). Other representative hydroxyl blocking groups have been described (see *e.g.,* Beaucage *et al.*, *Tetrahedron*, **1992**, *46*, 2223). In some embodiments, hydroxyl blocking groups are acid-labile groups, such as the trityl, monomethoxytrityl, dimethoxytrityl, trimethoxytrityl, 9-phenylxanthin-9-yl (Pixyl) and 9-(p-methoxyphenyl)xanthin-9-yl (MOX). Chemical functional groups can also be blocked by including them in a precursor form. Thus an azido group can be considered a blocked form of an amine as the azido group is easily converted to the amine. Further representative protecting groups utilized in nucleic acid synthesis are known (see e.g. Agrawal et al., Protocols for Oligonucleotide Conjugates, Eds., Humana Press, New Jersey, 1994, Vol. 26, pp. 1-72).

[00914] Various methods are known and used for removal of blocking groups from nucleic acids. In some embodiments, all blocking groups are removed. In some embodiments, a portion of blocking groups are removed. In some embodiments, reaction conditions can be adjusted to selectively remove certain blocking groups.

[00915] In some embodiments, nucleobase blocking groups, if present, are cleavable with an acidic reagent after the assembly of a provided oligonucleotide. In some embodiment, nucleobase blocking groups, if present, are cleavable under neither acidic nor basic conditions, *e.g.* cleavable with fluoride salts or hydrofluoric acid complexes. In some embodiments, nucleobase blocking groups, if present, are cleavable in the presence of base or a basic solvent after the assembly of a provided oligonucleotide. In certain embodiments, one or more of the

nucleobase blocking groups are characterized in that they are cleavable in the presence of base or a basic solvent after the assembly of a provided oligonucleotide but are stable to the particular conditions of one or more earlier deprotection steps occurring during the assembly of the provided oligonucleotide.

[00916] In some embodiments, blocking groups for nucleobases are not required. In some embodiments, blocking groups for nucleobases are required. In some embodiments, certain nucleobases require one or more blocking groups while other nucleobases do not require one or more blocking groups.

[00917] In some embodiments, the oligonucleotide is cleaved from the solid support after synthesis. In some embodiments, cleavage from the solid support comprises the use of propylamine. In some embodiments, cleavage from the solid support comprises the use of propylamine in pyridine. In some embodiments, cleavage from the solid support comprises the use of 20% propylamine in pyridine. In some embodiments, cleavage from the solid support comprises the use of propylamine in anhydrous pyridine. In some embodiments, cleavage from the solid support comprises the use of 20% propylamine in anhydrous pyridine. In some embodiments, cleavage from the solid support comprises use of a polar aprotic solvent such as acetonitrile, NMP, DMSO, sulfone, and/or lutidine. In some embodiments, cleavage from the solid support comprises use of solvent, *e.g.,* a polar aprotic solvent, and one or more primary amines (*e.g.,* a C_{1-10} amine), and/or one or more of methoxylamine, hydrazine, and pure anhydrous ammonia.

[00918] In some embodiments, deprotection of oligonucleotide comprises the use of propylamine. In some embodiments, deprotection of oligonucleotide comprises the use of propylamine in pyridine. In some embodiments, deprotection of oligonucleotide comprises the use of 20% propylamine in pyridine. In some embodiments deprotection of oligonucleotide comprises the use of propylamine in anhydrous pyridine. In some embodiments, deprotection of oligonucleotide comprises the use of 20% propylamine in anhydrous pyridine.

[00919] In some embodiments, the oligonucleotide is deprotected during cleavage.

[00920] In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about room temperature. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at elevated temperature. In some embodiments, cleavage of oligonucleotide from solid support,

or deprotection of oligonucleotide, is performed at above about 30 °C, 40 °C, 50 °C, 60 °C, 70°C, 80 °C 90 °C or 100 °C. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about 30 °C, 40 °C, 50 °C, 60 °C, 70°C, 80 °C 90 °C or 100 °C. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about 40-80 °C. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about 50-70 °C. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about 60 °C.

[00921] In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for more than 0.1 hr, 1 hr, 2 hrs, 5 hrs, 10 hrs, 15 hrs, 20 hrs, 24 hrs, 30 hrs, or 40 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 0.1-5 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 3-10 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 5-15 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 10-20 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 15-25 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 20-40 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 2 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 5 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 10 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 15 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 18 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed for about 24 hrs.

[00922] In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at room temperature for more than 0.1 hr, 1 hr, 2

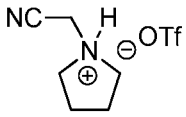
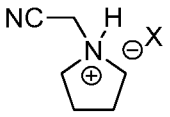
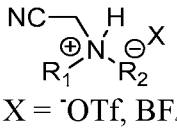
hrs, 5 hrs, 10 hrs, 15 hrs, 20 hrs, 24 hrs, 30 hrs, or 40 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at room temperature for about 5-48 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at room temperature for about 10-24 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at room temperature for about 18 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at elevated temperature for more than 0.1 hr, 1 hr, 2 hrs, 5 hrs, 10 hrs, 15 hrs, 20 hrs, 24 hrs, 30 hrs, or 40 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at elevated temperature for about 0.5-5 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about 60 °C for about 0.5-5 hrs. In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide, is performed at about 60 °C for about 2 hrs.

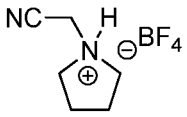
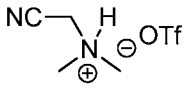
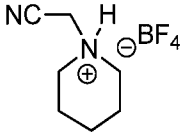
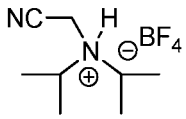
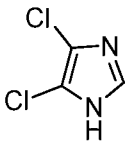
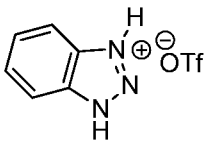
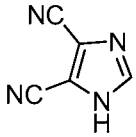
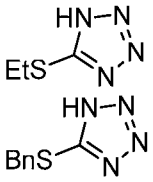
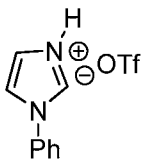
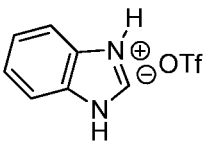
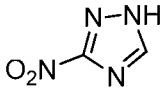
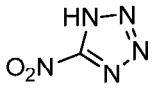
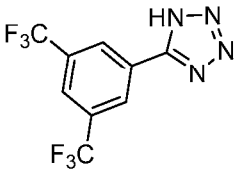
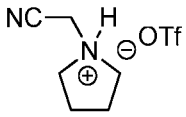
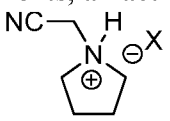
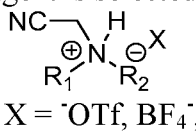
[00923] In some embodiments, cleavage of oligonucleotide from solid support, or deprotection of oligonucleotide comprises the use of propylamine and is performed at room temperature or elevated temperature for more than 0.1 hr, 1 hr, 2 hrs, 5 hrs, 10 hrs, 15 hrs, 20 hrs, 24 hrs, 30 hrs, or 40 hrs. Example conditions are 20% propylamine in pyridine at room temperature for about 18 hrs, and 20% propylamine in pyridine at 60 °C for about 18 hrs,

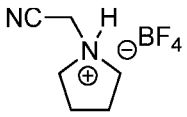
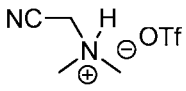
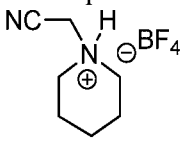
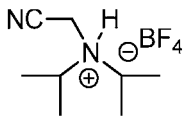
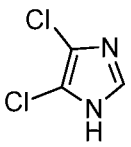
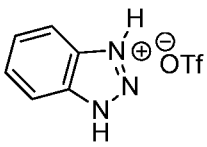
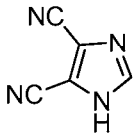
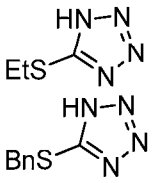
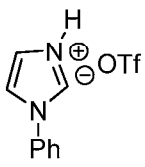
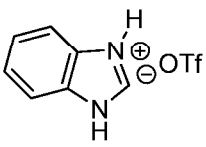
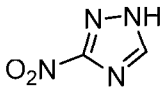
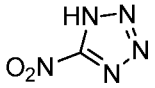
[00924] In some embodiments, prior to cleavage from solid support, a step is performed to remove a chiral auxiliary group, if one is still attached to an internucleotidic phosphorus atom. In some embodiments, for example, one or more DPSE type chiral auxiliary groups remain attached to internucleotidic phosphorus atoms during the oligonucleotide synthesis cycle. Suitable conditions for removing remaining chiral auxiliary groups are widely known in the art, *e.g.,* those described in Wada I, Wada II, Wada III, Chiral Control, *etc*. In some embodiments, a condition for removing DPSE type chiral auxiliary is TBAF or HF-Et_3N, *e.g.,* 0.1M TBAF in MeCN, 0.5M HF-Et_3N in THF or MeCN, *etc*. In some embodiments, the present disclosure recognizes that a linker may be cleaved during the process of removing a chiral auxiliary group. In some embodiments, the present disclosure provides linkers, such as the SP linker, that provides better stability during chiral auxiliary group removal. Among other things, certain linkers provided by the present disclosure provided improved yield and/or purity.

[00925] In some embodiments, an activator is a "Wada" activator, i.e., the activator is from any one of Wada I, II, or III documents cited above.

[00926] Example activating groups are depicted below:

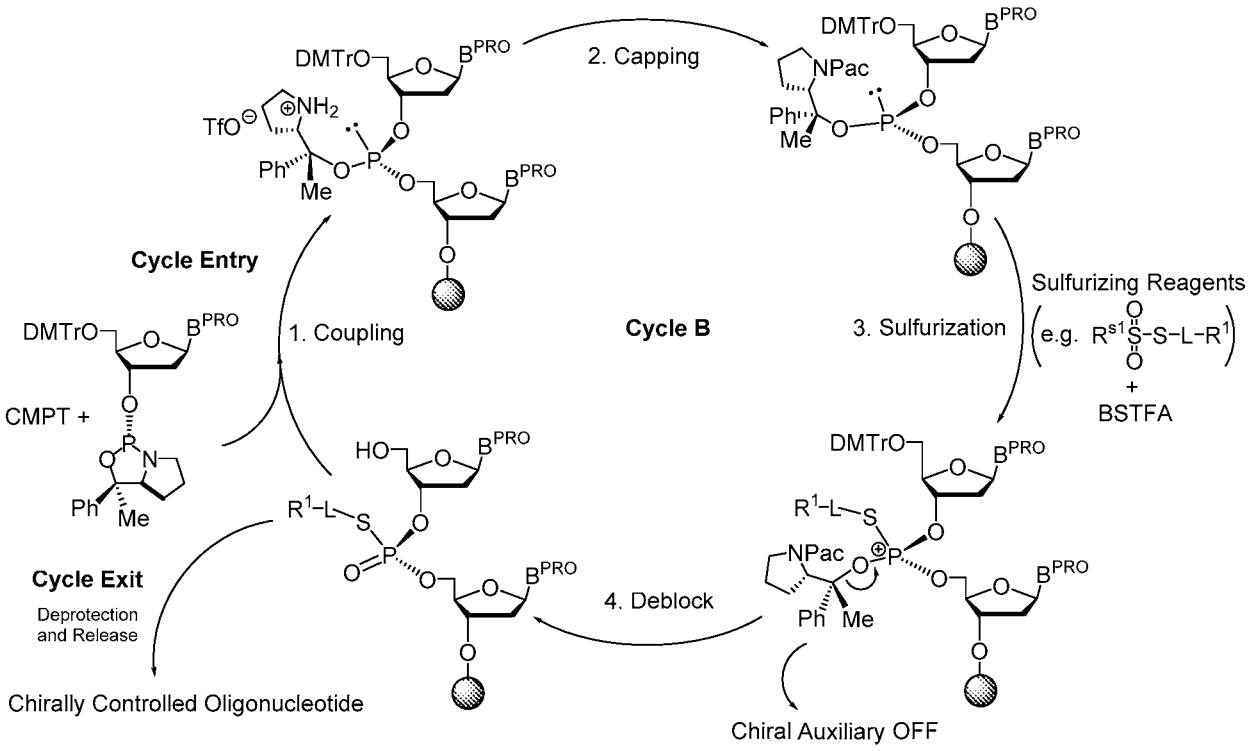


X = non nucleophilic anion

X = $^-$OTf, BF_4^-, $PF6^-$, TfN^-














In some embodiments, an activating reagent is selected from


X = non nucleophilic anion

X = $^-$OTf, BF_4^-, $PF6^-$, TfN^-

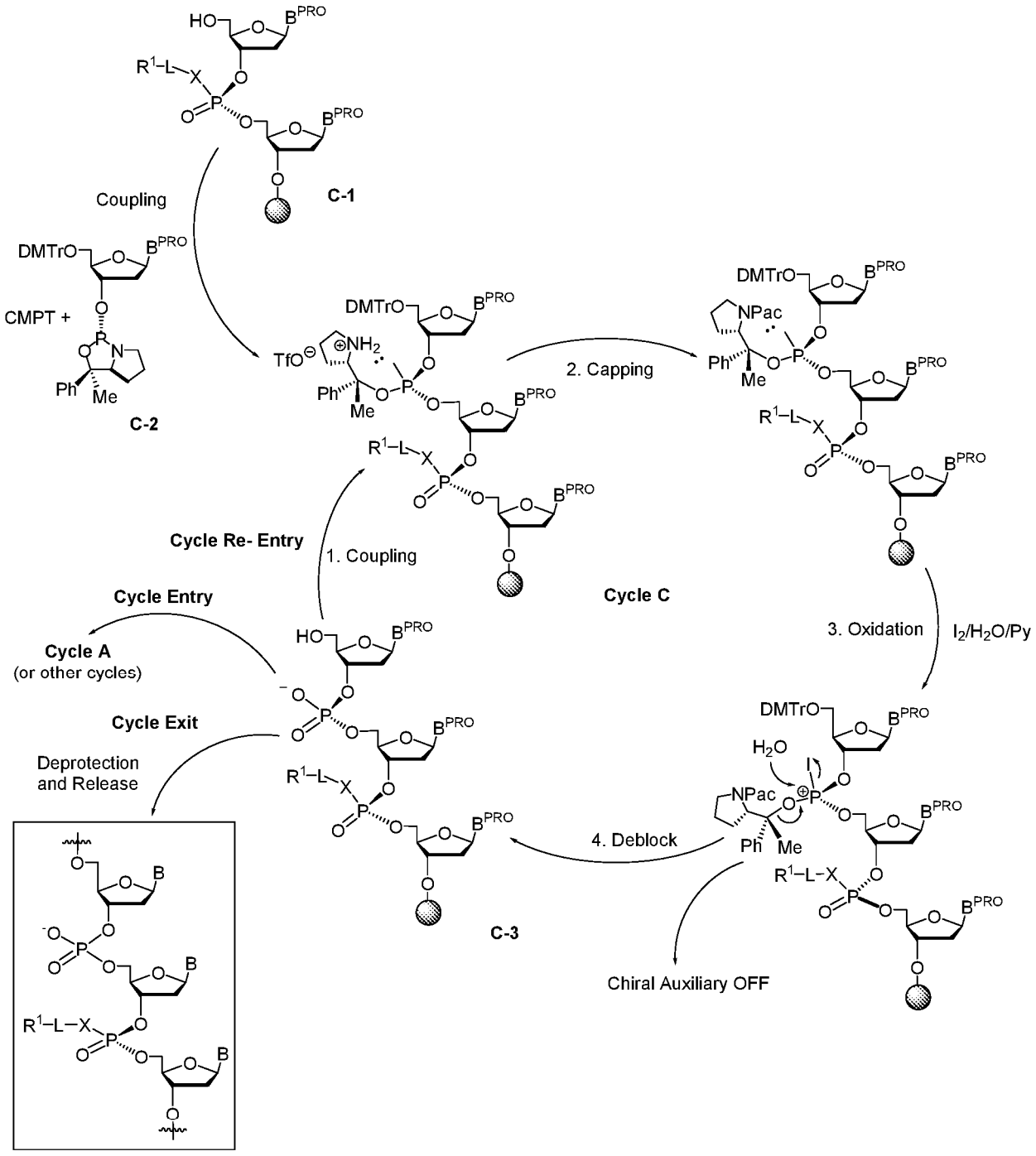
[00927] In some embodiments, an example cycle is depicted in Scheme I-b.

Scheme I-b. Installation of phosphorothioate linkages.



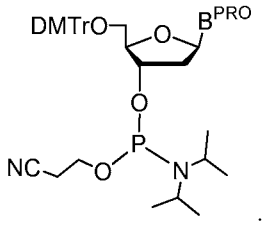
[00928] In some embodiments, an example cycle is illustrated in Scheme I-c.

Scheme I-c. Installation of both phosphate diester and modified internucleotidic linkages in a chirally controlled oligonucleotide.

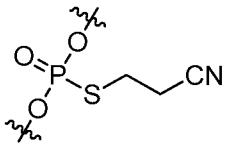


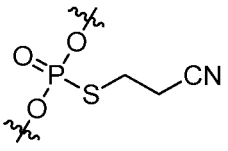
[00929] In Scheme I-c, oligonucleotide (or nucleotide, or oligonucleotide with modified internucleotidic linkage) on solid support (C-1) is coupled with phosphoramidite C-2. After coupling and capping, an oxidation step is performed. After deblocking, a phosphate diester linkage is formed. The cycle product C-3 can either re-enter cycle C to install more phosphate diester linkage, or enter other cycles to install other types of internucleotidic linkages, or go to cycle exit.

[00930] In some embodiments, non-chirally pure phosphoramidite can be used instead of C-2 in Scheme I-c. In some embodiments, β-cyanoethylphosphoramidites protected with DMTr is used. In some embodiments, the phosphoramidite being used has the structure of



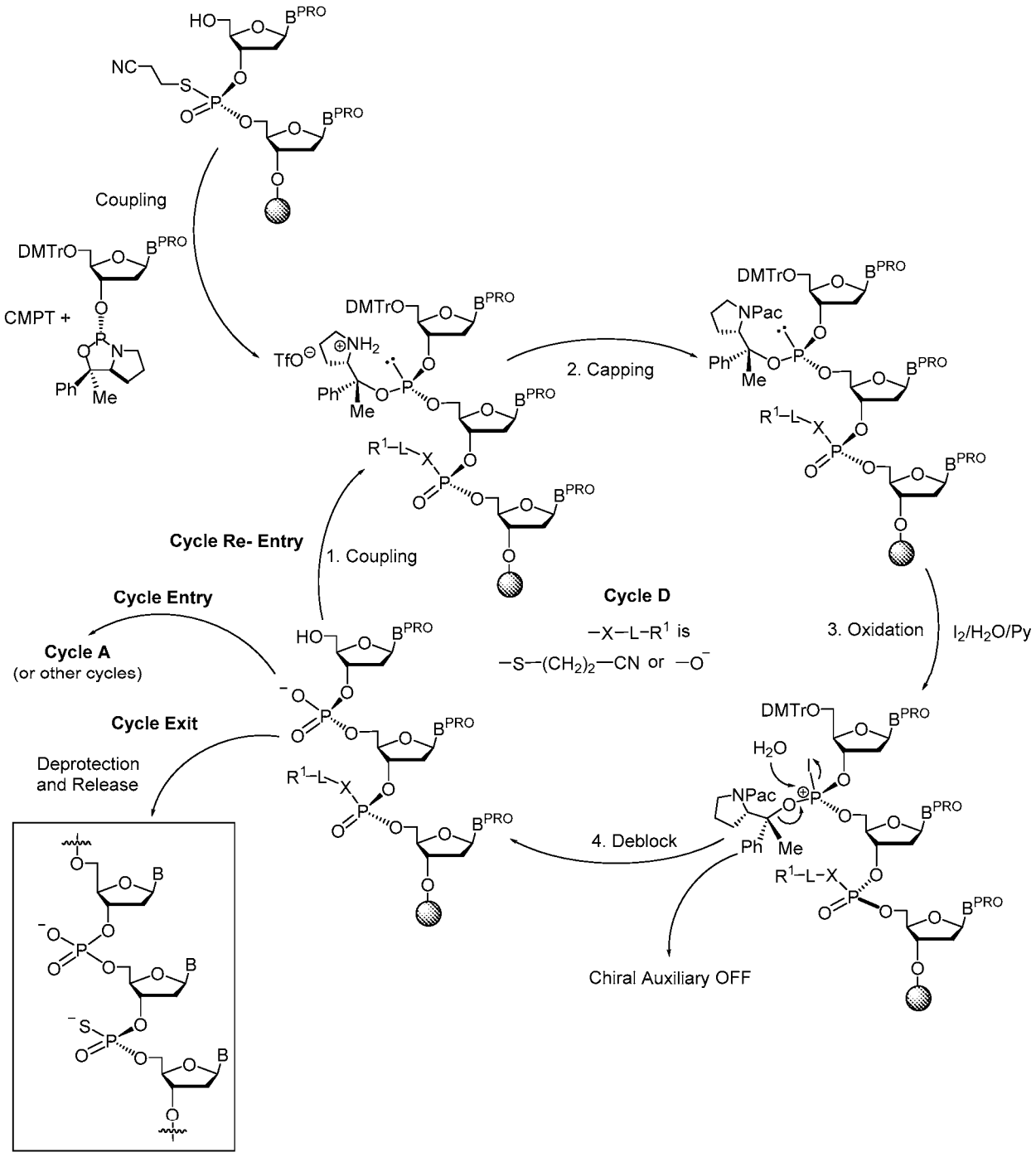
[00931] In some embodiments, the use of a phosphorothioate diester precursor increases the stability of oligonucleotide during synthesis. In some embodiments, the use of a phosphorothioate diester precursor improves the efficiency of chirally controlled oligonucleotide synthesis. In some embodiments, the use of a phosphorothioate diester precursor improves the yield of chirally controlled oligonucleotide synthesis. In some embodiments, the use of a phosphorothioate diester precursor improves the product purity of chirally controlled oligonucleotide synthesis.

[00932] In some embodiments, the phosphorothioate diester precursor in the above-mentioned methods is 

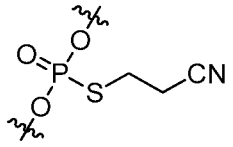
. In some embodiments, 

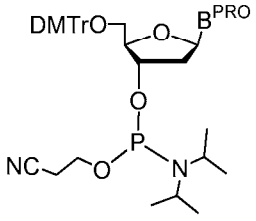
is converted to a phosphorothioate diester linkage during deprotection/release. In some embodiments, an example cycle is depicted in Scheme I-d. More examples are depicted below.

Scheme I-d. Phosphorothioate diester precursor in chirally controlled oligonucleotide synthesis.



[00933] As illustrated in Scheme I-d, both phosphorothioate and phosphate diester linkages can be incorporated into the same chirally controlled oligonucleotide. As understood by a person of ordinary skill in the art, the provided methods do not require that the phosphorothioate diester and the phosphate diester to be consecutive – other internucleotidic linkages can form between them using a cycle as described above. In Scheme I-d,

phosphorothioate diester precursors,  , are installed in place of the phosphorothioate diester linkages. In some embodiments, such replacement provided increased synthesis efficiency during certain steps, for instance, the oxidation step. In some embodiments, the use of phosphorothioate diester precursors generally improve the stability of chirally controlled oligonucleotides during synthesis and/or storage. After cycle exit, during deprotection/release, the phosphorothioate diester precursor is converted to phosphorothioate diester linkage. In some embodiments, it is benefical to use phosphorothioate diester precursor even when no phosphate diester linkage is present in the chirally controlled oligonucleotide, or no oxidation step is required during synthesis.

[00934] As in Scheme I-c, in some embodiments, non-chirally pure phosphoramidite can be used for cycles comprising oxidation steps. In some embodiments, β-cyanoethylphosphoramidites protected with DMTr is used. In some embodiments, the phosphoramidite being used has the structure of 

[00935] In some embodiments, methods of the present disclosure provide chirally controlled oligonucleotide compositions that are enriched in a particular oligonucleotide type.

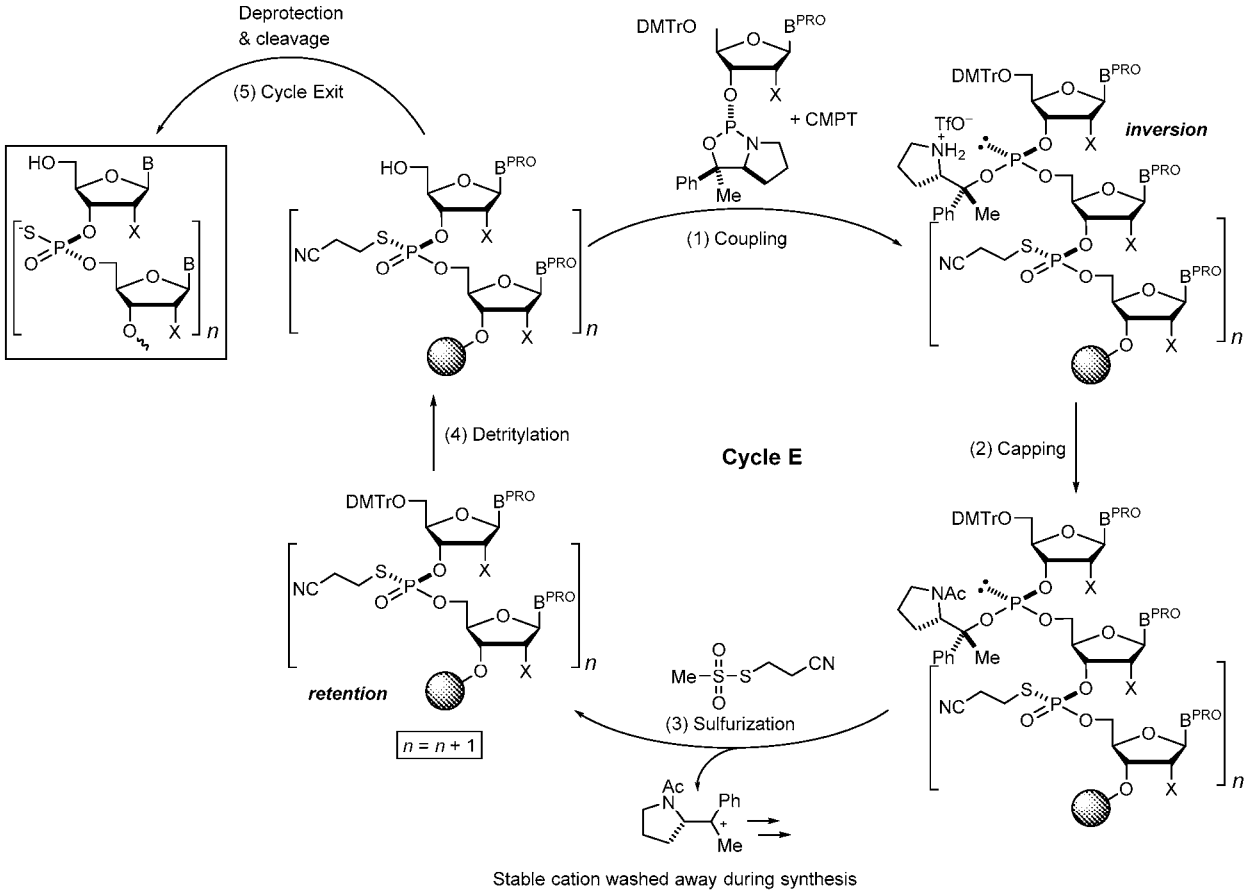
[00936] In some embodiments, at least about 10% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 20% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 30% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 40% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 50% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 60% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 70% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 80% of a provided crude composition is of a particular oligonucleotide type. In some embodiments, at least about 90% of a provided crude composition is of a particular

oligonucleotide type. In some embodiments, at least about 95% of a provided crude composition is of a particular oligonucleotide type.

[00937] In some embodiments, at least about 1% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 2% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 3% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 4% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 5% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 10% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 20% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 30% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 40% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 50% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 60% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 70% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 80% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 90% of a provided composition is of a particular oligonucleotide type. In some embodiments, at least about 95% of a provided composition is of a particular oligonucleotide type.

[00938] In some embodiments, an example cycle is depicted in Scheme I-e, below.

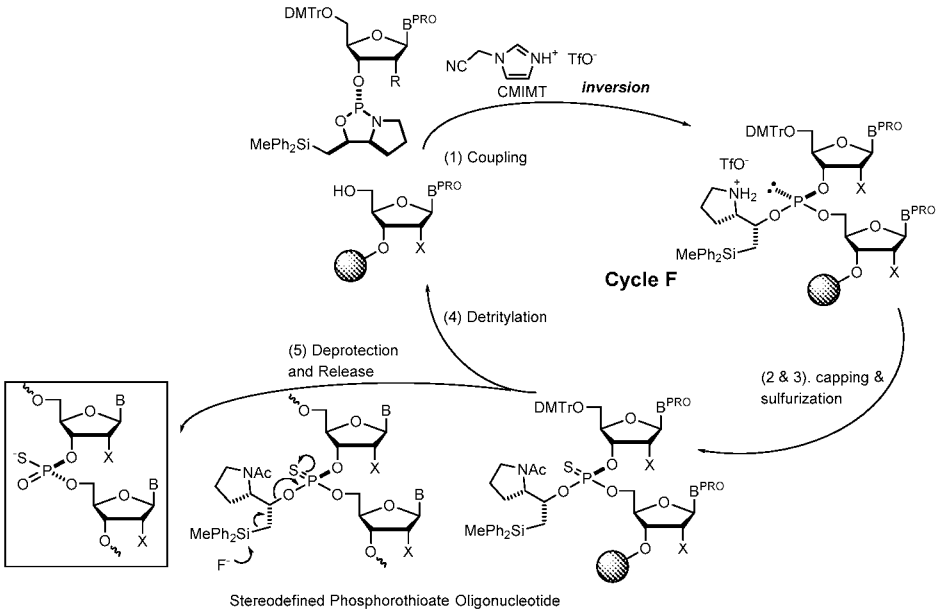
Scheme I-e. Example cycle using PhMe chiral auxiliary.



[00939] In some embodiments, X is H or a 2'-modification. In some embodiments, X is H or $-OR^1$, wherein R^1 is not hydrogen. In some embodiments, X is H or $-OR^1$, wherein R^1 is optionally substituted C_{1-6} alkyl. In some embodiments, X is H. In some embodiments, X is $-OMe$. In some embodiments, X is $-OCH_2CH_2OCH_3$. In some embodiments, X is $-F$.

[00940] In some embodiments, an example cycle is depicted in Scheme I-f.

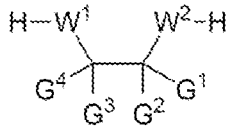
Scheme I-e. Example cycle using DPSE chiral auxiliary.



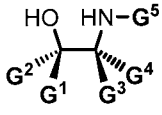
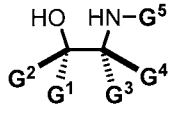
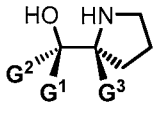
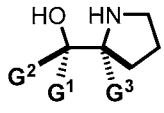
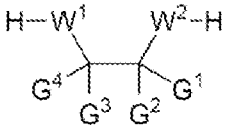
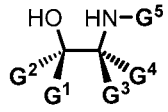
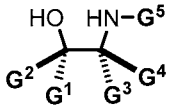
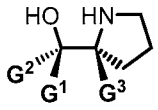
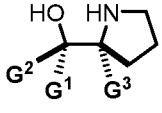
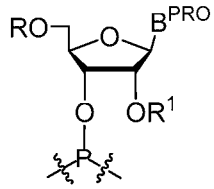
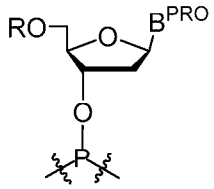
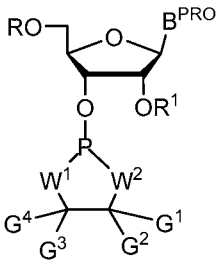
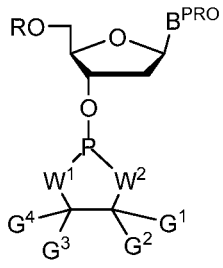
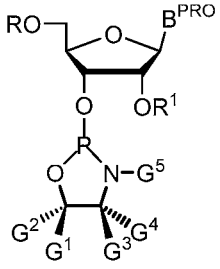
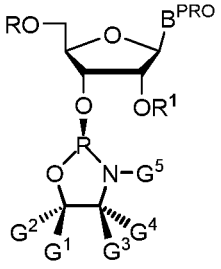
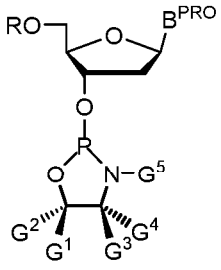
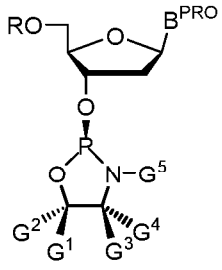
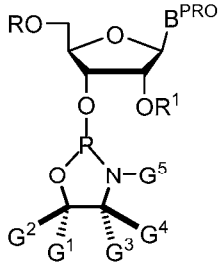
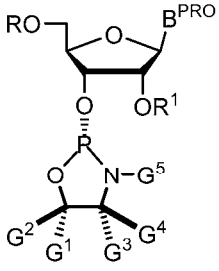
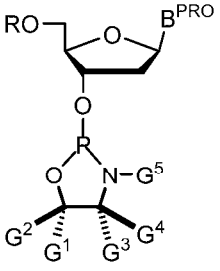
[00941] In some embodiments, X is H or a 2'-modification. In some embodiments, X is H or $-OR^1$, wherein R^1 is not hydrogen. In some embodiments, X is H or $-OR^1$, wherein R^1 is optionally substituted C_{1-6} alkyl. In some embodiments, X is H. In some embodiments, X is −OMe. In some embodiments, X is $-OCH_2CH_2OCH_3$. In some embodiments, X is −F.

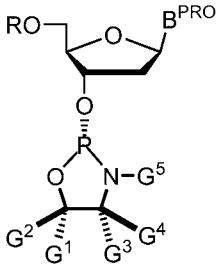
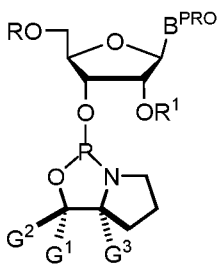
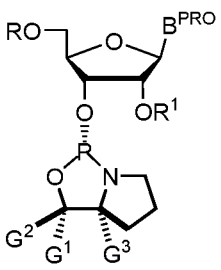
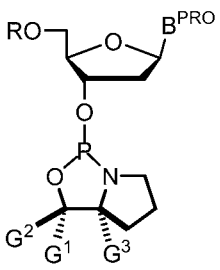
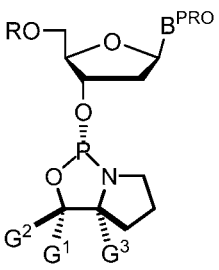
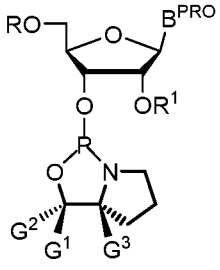
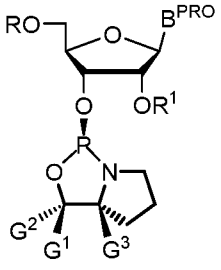
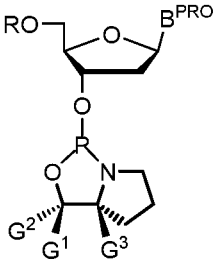
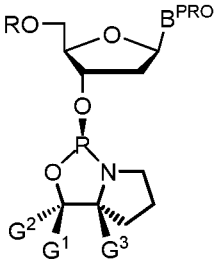
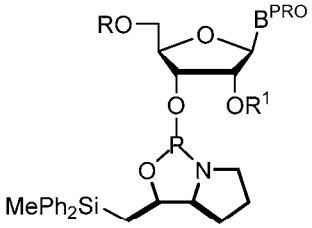
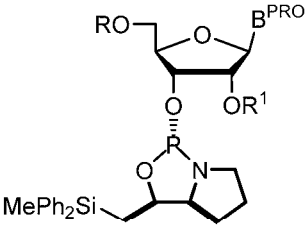
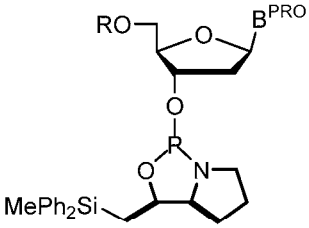
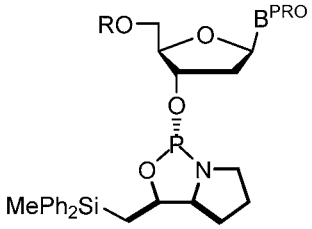
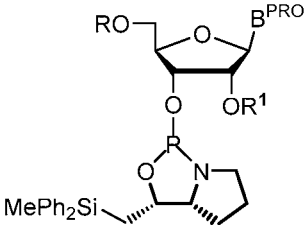
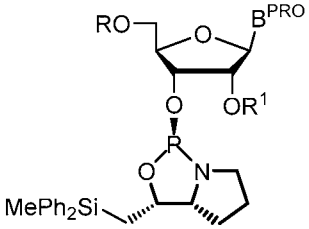
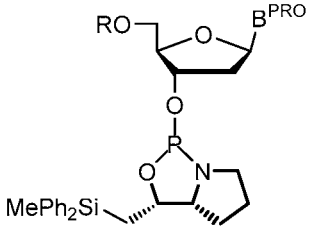
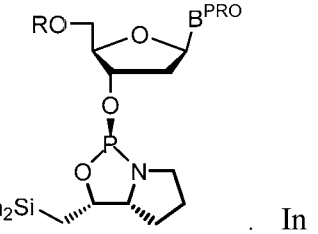
[00942] It is understood by a person having ordinary skill in the art that different types of cycles may be combined to provide complete control of the chemical modifications and stereochemistry of oligonucleotides. In some embodiments, for example, an oligonucleotide synthesis process may contain one or more Cycles A-F. In some embodiments, a provided method comprises at least one cyle using a DPSE-type chiral auxiliary.

[00943] In some embodiments, the present disclosure provides methods for preparing provided oligonucleotide and oligonucleotide compositions. In some embodiments, a provide methods comprising providing a provided chiral reagent having the structure of



, wherein W^1 is $-NG^5$, W^2 is O, each of G^1 and G^3 is independently hydrogen or an optionally substituted group selected from C_{1-10} aliphatic, heterocyclyl, heteroaryl and aryl, G^2 is $-C(R)_2Si(R)_3$, and G^4 and G^5 are taken together to form an optionally substituted saturated, partially unsaturated or unsaturated heteroatom-containing ring of up to about 20 ring atoms which is monocyclic or polycyclic, fused or unfused, wherein each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, carbocyclyl, aryl, heteroaryl,

and heterocyclyl. In some embodiments, a provided chiral reagent has the structure of  ,  ,  , or  . In some embodiments, a provided methods comprises providing a phosphoramidite comprising a moiety from a chiral reagent having the structure of  ,  ,  ,  , or  , wherein $-W^1H$ and $-W^2H$, or the hydroxyl and amino groups, form bonds with the phosphorus atom of the phosphoramidite. In some embodiments, $-W^1H$ and $-W^2H$, or the hydroxyl and amino groups, form bonds with the phosphorus atom of the phosphoramidite, *e.g.*, in  or  . In some embodiments, a phosphoramidite has the structure of  ,  ,  ,  ,  ,  ,  ,  ,  ,


















, or

. In some embodiments, R is a protection group. In some embodiments, R is DMTr. In some embodiments, G^2 is $-C(R)_2Si(R)_3$, wherein $-C(R)_2-$ is optionally substituted $-CH_2-$, and each R of $-Si(R)_3$ is independently an optionally substituted group selected from C_{1-10} aliphatic, heterocyclyl, heteroaryl and aryl. In some embodiments, at least one R of $-Si(R)_3$ is independently optionally substituted C_{1-10} alkyl. In some embodiments, at least one R of $-Si(R)_3$ is independently optionally substituted phenyl. In some embodiments, one R of $-Si(R)_3$ is independently optionally substituted phenyl, and each of

the other two R is independently optionally substituted C_{1-10} alkyl. In some embodiments, one R of $-Si(R)_3$ is independently optionally substituted C_{1-10} alkyl, and each of the other two R is independently optionally substituted phenyl. In some embodiments, G^2 is optionally substituted $-CH_2Si(Ph)(Me)_2$. In some embodiments, G^2 is optionally substituted $-CH_2Si(Me)(Ph)_2$. In some embodiments, G^2 is $-CH_2Si(Me)(Ph)_2$. In some embodiments, G^4 and G^5 are taken together to form an optionally substituted saturated 5-6 membered ring containing one nitrogen atom (to which G^5 is attached). In some embodiments, G^4 and G^5 are taken together to form an optionally substituted saturated 5-membered ring containing one nitrogen atom. In some embodiments, G^1 is hydrogen. In some embodiments, G^3 is hydrogen. In some embodiments, both G^1 and G^3 are hydrogen. In some embodiments, both G^1 and G^3 are hydrogen, G^2 is $-C(R)_2Si(R)_3$, wherein $-C(R)_2-$ is optionally substituted $-CH_2-$, and each R of $-Si(R)_3$ is independently an optionally substituted group selected from C_{1-10} aliphatic, heterocyclyl, heteroaryl and aryl, and G^4 and G^5 are taken together to form an optionally substituted saturated 5-membered ring containing one nitrogen atom. In some embodiments, a provided method further comprises providing a fluoro-containing reagent. In some embodiments, a provided fluoro-containing reagent removes a chiral reagent, or a product formed from a chiral reagent, from oligonucleotides after synthesis. Various known fluoro-containing reagents, including those F^- sources for removing $-SiR_3$ groups, can be utilized in accordance with the present disclosure, for example, TBAF, HF_3-Et_3N *etc*. In some embodiments, a fluoro-containing reagent provides better results, for example, shorter treatment time, lower temperature, less de-sulfurization, *etc*, compared to traditional methods, such as concentrated ammonia. In some embodiments, for certain fluoro-containing reagent, the present disclosure provides linkers for improved results, for example, less cleavage of oligonucleotides from support during removal of chiral reagent (or product formed therefrom during oligonucleotide synthesis). In some embodiments, a provided linker is an SP linker. In some embodiments, the present disclosure demonstrated that a HF-base complex can be utilized, such as $HF\text{-}NR_3$, to control cleavage during removal of chiral reagent (or product formed therefrom during oligonucleotide synthesis). In some embodiments, $HF\text{-}NR_3$ is $HF\text{-}NEt_3$. In some embodiments, $HF\text{-}NR_3$ enables use of traditional linkers, *e.g.*, succinyl linker.

Biological applications and example of use

[00944] Among other things, the present disclosure recognizes that properties and activities of an oligonucleotide can be adjusted by optimizing its pattern of backbone chiral centers through the use of provided chirally controlled oligonucleotide compositions. In some embodiments, the present disclosure provides chirally controlled oligonucleotide compositions, wherein the oligonucleotides have a common pattern of backbone chiral centers which enhances their stability and/or biological activity. In some embodiments, a pattern of backbone chiral centers provides unexpectedly increased stability. In some embodiments, a pattern of backbone chiral centers, surprisingly, provides greatly increased activity. In some embodiments, a pattern of backbone chiral centers provides both increased stability and activity. In some embodiments, when an oligonucleotide is utilized to cleave a nucleic acid polymer, a pattern of backbone chiral centers of the oligonucleotide, surprisingly by itself, changes the cleavage pattern of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers effectively prevents cleavage at secondary sites. In some embodiments, a pattern of backbone chiral centers creates new cleavage sites. In some embodiments, a pattern of backbone chiral centers minimizes the number of cleavage sites. In some embodiments, a pattern of backbone chiral centers minimizes the number of cleavage sites so that a target nucleic acid polymer is cleaved at only one site within the sequence of the target nucleic acid polymer that is complementary to the oligonucleotide. In some embodiments, a pattern of backbone chiral centers enhances cleavage efficiency at a cleavage site. In some embodiments, a pattern of backbone chiral centers of the oligonucleotide improves cleavage of a target nucleic acid polymer. In some embodiments, a pattern of backbone chiral centers increases selectivity. In some embodiments, a pattern of backbone chiral centers minimizes off-target effect. In some embodiments, a pattern of backbone chiral centers increase selectivity, *e.g.*, cleavage selectivity among target sequences differing by point mutations or single nucleotide polymorphisms (SNPs). In some embodiments, a pattern of backbone chiral centers increase selectivity, *e.g.*, cleavage selectivity among target sequences differing by only one point mutation or single nucleotide polymorphism (SNP).

[00945] Among other things, it is surprisingly found that certain provided oligonucleotide compositions achieve unprecedented control of cleavage of target sequences, *e.g.*, cleavage of target RNA by RNase H. In some embodiments, the present disclosure demonstrates that precise control of chemical and stereochemical attributes of oligonucleotides achieves improved activity of oligonucleotide preparations as compared with otherwise comparable preparations for which

stereochemical attributes are not controlled. Among other things, the present disclosure specifically demonstrates improved rate, degree, and or specificity of cleavage of nucleic acid targets to which provided oligonucleotides hybridize.

[00946] In some embodiments, the present disclosure provides various uses of oligonucleotide compositions. Among other things, the present disclosure demonstrates that by controlling structural elements of oligonucleotides, such as base sequence, chemical modifications, stereochemistry, *etc.*, properties of oligonucleotides can be greatly improved. For example, in some embodiments, the present disclosure provides methods for highly selective suppression of transcripts of a target nucleic acid sequence. In some embodiments, the present disclosure provides methods for treating a subject by suppressing transcripts from a diseasing-causing copy (*e.g.,* a disease-causing allele). In some embodiments, the present disclosure provides methods for designing and preparing oligonucleotide compositions with surprisingly enhanced activity and/or selectivity when suppressing a transcript of a target sequence. In some embodiments, the present disclosure provides methods for designing and/or preparing oligonucleotide compositions which provide allele-specific suppression of a transcript from a target nucleic acid sequence.

[00947] In some embodiments, the present disclosure provides a method for controlled cleavage of a nucleic acid polymer, the method comprising steps of:

contacting a nucleic acid polymer whose nucleotide sequence comprises a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a target sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

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

[00948] In some embodiments, the present disclosure provides a method for altering a cleavage pattern observed when a nucleic acid polymer whose nucleotide sequence includes a

target sequence is contacted with a reference oligonucleotide composition that comprises oligonucleotides having a particular base sequence and length, which particular base sequence is or comprises a sequence that is complementary to the target sequence, the method comprising:

contacting the nucleic acid polymer with a chirally controlled oligonucleotide composition of oligonucleotides having the particular base sequence and length, which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of a single oligonucleotide type characterized by:

1) the particular base sequence and length;

2) a particular pattern of backbone linkages; and

3) a particular pattern of backbone chiral centers.

[00949] In some embodiments, the present disclosure provides a method for controlled cleavage of a nucleic acid polymer, comprising providing a chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages;

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

wherein the nucleic acid polymer is cleaved in a cleavage pattern that is different than the cleavage pattern when chirally uncontrolled oligonucleotide composition is provided.

[00950] As used herein, a cleavage pattern of a nucleic acid polymer is defined by the number of cleavage sites, the locations of the cleavage sites, and the percentage of cleavage at each sites. In some embodiments, a cleavage pattern has multiple cleavage sites, and the percentage of cleavage at each site is different. In some embodiments, a cleavage pattern has multiple cleavage sites, and the percentage of cleavage at each site is the same. In some embodiments, a cleavage pattern has only one cleavage site. In some embodiments, cleavage patterns differ from each other in that they have different numbers of cleavage sites. In some

embodiments, cleavage patterns differ from each other in that at least one cleavage location is different. In some embodiments, cleavage patterns differ from each other in that the percentage of cleavage at at least one common cleavage site is different. In some embodiments, cleavage patterns differ from each other in that they have different numbers of cleavage sites, and/or at least one cleavage location is different, and/or the percentage of cleavage at at least one common cleavage site is different.

[00951] In some embodiments, the present disclosure provides a method for controlled cleavage of a nucleic acid polymer, the method comprising steps of:

contacting a nucleic acid polymer whose nucleotide sequence comprises a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a target sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type, the contacting being performed under conditionsso that cleavage of the nucleic acid polymer occurs.

[00952] In some embodiments, the present disclosure provides a method for changing a first cleavage pattern of a nucleic acid polymer resulted from using a first oligonucleotide composition, comprising providing a second chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages;

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

linkages, and the common pattern of backbone chiral centers; and

wherein the nucleic acid polymer is cleaved in a cleavage pattern that is different than the first cleavage pattern.

[00953] In some embodiments, the present disclosure provides a method for altering a cleavage pattern observed when a nucleic acid polymer whose nucleotide sequence includes a target sequence is contacted with a reference oligonucleotide composition that comprises oligonucleotides having a particular base sequence and length, which particular base sequence is or comprises a sequence that is complementary to the target sequence, the method comprising:

contacting the nucleic acid polymer with a chirally controlled oligonucleotide composition of oligonucleotides having the particular base sequence and length, which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of a single oligonucleotide type characterized by:

1) the particular base sequence and length;

2) a particular pattern of backbone linkages; and

3) a particular pattern of backbone chiral centers,

the contacting being performed under conditions so that cleavage of the nucleic acid polymer occurs.

[00954] In some embodiments, a provided chirally controlled oligonucleotide composition reduces the number of cleavage sites within the target sequence. In some embodiments, a provided chirally controlled oligonucleotide composition provides single-site cleavage within the target sequence. In some embodiments, a chirally controlled oligonucleotide composition provides enhanced cleavage rate at a cleavage site within the target sequence. In some embodiments, a chirally controlled oligonucleotide composition provides enhanced efficiency at a cleavage site within the target sequence. In some embodiments, a chirally controlled oligonucleotide composition provides increased turn-over in cleaving a target nucleic acid polymer. In some embodiments, a chirally controlled oligonucleotide composition increase percentage of cleavage at a site within or in the vicinity of a characteristic sequence element. In some embodiments, a chirally controlled oligonucleotide composition increase percentage of cleavage at a site in the vicinity of a mutation. In some embodiments, a chirally controlled oligonucleotide composition increase percentage of cleavage at a site in the vicinity of a SNP.

Example embodiments of a site within or in the vicinity of a characteristic sequence element, in the vicinity of a mutation, in the vicinity of a SNP, are described in the present disclosure. For example, in some embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away from a mutation; in some other embodiments, a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away from a SNP.

[00955] In some embodiments, cleavage occurs with a cleavage pattern differs from a reference cleavage pattern. In some embodiments, a reference cleavage pattern is one observed when a nucleic acid polymer is contacted under comparable conditions with a reference oligonucleotide composition. In some embodiments, a reference oligonucleotide composition is a chirally uncontrolled (*e.g.,* stereorandom) oligonucleotide composition of oligonucleotides that share the common base sequence and length of a chirally controlled oligonucleotide composition. In some embodiments, a reference oligonucleotide composition is a substantially racemic preparation of oligonucleotides that share the common sequence and length.

[00956] In some embodiments, a nucleic acid polymer is RNA. In some embodiments, a nucleic acid polymer is an oligonucleotide. In some embodiments, a nucleic acid polymer is an RNA oligonucleotide. In some embodiments, a nucleic acid polymer is a transcript. In some embodiments, oligonucleotides of a provided chirally controlled oligonucleotide composition form duplexes with a nucleic acid polymer to be cleaved.

[00957] In some embodiments, a nucleic acid polymer is cleaved by an enzyme. In some embodiments, an enzyme cleaves a duplex formed by a nucleic acid polymer. In some embodiments, an enzyme is RNase H. In some embodiments, an enzyme is Dicer. In some embodiments, an enzyme is an Argonaute protein. In some embodiments, an enzyme is Ago2. In some embodiments, an enzyme is within a protein complex. An example protein complex is RNA-induced silencing complex (RISC).

[00958] In some embodiments, a provided chirally controlled oligonucleotide composition comprising oligonucleotides with a common pattern of backbone chiral centers provides unexpectedly high selectivity so that nucleic acid polymers that have only small sequence variations within a target region can be selectively targeted. In some embodiments, a nucleic acid polymer is a transcript from an allele. In some embodiments, transcripts from different alleles can be selectively targeted by provided chirally controlled oligonucleotide compositions.

[00959] In some embodiments, provided chirally controlled oligonucleotide compositions

and methods thereof enables precise control of cleavage sites within a target sequence. In some embodiments, a cleavage site is around a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is upstream of and near a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 5 base pairs upstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 4 base pairs upstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 3 base pairs upstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 2 base pairs upstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 1 base pair upstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is downstream of and near a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 5 base pairs downstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 4 base pairs downstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 3 base pairs downstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 2 base pairs downstream of a sequence of *R*p*S*p*S*p backbone chiral centers. In some embodiments, a cleavage site is within 1 base pair downstream of a sequence of *R*p*S*p*S*p backbone chiral centers. Among other things, the present disclosure therefore provides control of cleavage sites with in a target sequence. In some embodiments, an example cleavage is depicted in Figure 21. In some embodiments, cleavage depicted in Figure 21 is designated as cleavage at a site two base pairs downstream a sequence of *R*p*S*p*S*p backbone chiral centers. As extensively described in the present disclosure, a sequence of *R*p*S*p*S*p backbone chiral centers can be found in a single or repeating units of $(Np)_m(Rp)_n(Sp)_t$, $(Np)_t(Rp)_n(Sp)_m$, $(Sp)_m(Rp)_n(Sp)_t$, $(Sp)_t(Rp)_n(Sp)_m$, $(Rp)_n(Sp)_m$, $(Rp)_m(Sp)_n$, $(Sp)_mRp$ and/or $Rp(Sp)_m$, each of which is independently as defined above and described herein. In some embodiments, a provided chirally controlled oligonucleotide composition creates a new cleavage site 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule (*e.g.,* see Figure 21), wherein said new cleavage site does not exist if a reference (*e.g.,* chirally uncontrolled) oligonucleotide composition is used (cannot be detected). In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a cleavage site 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule (*e.g.,* see Figure 21), wherein cleavage at such a site occurs at

a higher percentage than when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, cleavage at such a site by a provided chirally controlled oligonucleotide composition is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500 or 1000 fold of that by a reference oligonucleotide composition (for example, when measured by percentage of cleavage at a site). In some embodiments, a provided chirally controlled oligonucleotide composition provides accelerated cleavage at a cleavage site 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule (*e.g.*, see Figure 21), compared to when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, cleavage by a provided chirally controlled oligonucleotide composition is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500 or 1000 fold faster than that by a reference oligonucleotide composition. In some embodiments, a cleavage site of a provided chirally controlled oligonucleotide composition 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule (*e.g.*, see Figure 21) is a cleavage site when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, a cleavage site of a provided chirally controlled oligonucleotide composition 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule (*e.g.*, see Figure 21) is within one base pair of a cleavage site when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, a cleavage site of a provided chirally controlled oligonucleotide composition 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule (*e.g.*, see Figure 21) is within 2 base pairs of a cleavage site when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, it is within 3 base pairs. In some embodiments, it is within 4 base pairs. In some embodiments, it is within 5 base pairs. In some embodiments, a cleavage site of a provided chirally controlled oligonucleotide composition 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule is one of the major cleavage sites when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, such a site is the cleavage site with the highest cleavage percentage when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some embodiments, a cleavage site of a provided chirally controlled oligonucleotide composition 2 base pairs downstream of *R*p*S*p*S*p backbone chiral centers in a target molecule is one of the cleavage sites with higher cleavage rate when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used. In some

embodiments, such a site is the cleavage site with the highest cleavage rate when a reference (*e.g.*, chirally uncontrolled) oligonucleotide composition is used.

[00960] In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at one or more sites, *e.g.*, relative to a reference (*e.g.*, chirally uncontrolled/stereorandom) oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition selectively enhances cleavage at a single site relative to a reference (*e.g.*, chirally uncontrolled/stereorandom) composition. In some embodiments, a chirally controlled oligonucleotide composition enhances cleavage at a site by providing a higher cleavage rate. In some embodiments, a chirally controlled oligonucleotide composition enhances cleavage at a site by providing a higher percentage of cleavage at said site. Percentage of cleavage at a site can be determined by various methods widely known and practiced in the art. In some embodiments, percentage of cleavage at a site is determined by analysis of cleavage products, for example, as by HPLC-MS as illustrated in Figure 18, Figure 19 and Figure 30; see also example cleavage maps such as Figure 9, Figure 10, Figure 11, Figure 14, Figure 22, Figure 25 and Figure 26. In some embodiments, enhancement is relative to a reference oligonucleotide composition. In some embodiments, enhancement is relative to another cleavage site. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site that is a preferred cleavage site of a reference oligonucleotide composition. In some embodiments, a preferred cleavage site, or a group of preferred cleavage sites, is a site or sites that have relatively higher percentage of cleavage compared to one or more other cleavage sites. In some embodiments, preferred cleavage sites can indicate preference of an enzyme. For example, for RNase H, when a DNA oligonucleotide is used, resulting cleavage sites may indicate preference of RNase H. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site that is a preferred cleavage site of an enzyme. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site that is not a preferred cleavage site of a reference oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site that is not a cleavage site of a reference oligonucleotide composition, effectively creating a new cleavage site which does not exist when a reference oligonucleotide composition is used. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site within 5 base pairs from a

targeted mutation or SNP, thereby increasing selective cleavage of the undesired target oligonucleotide. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site within 4 base pairs from a targeted mutation or SNP, thereby increasing selective cleavage of the undesired target oligonucleotide. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site within 3 base pairs from a targeted mutation or SNP, thereby increasing selective cleavage of the undesired target oligonucleotide. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site within 2 base pairs from a targeted mutation or SNP, thereby increasing selective cleavage of the undesired target oligonucleotide. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage at a site immediately upstream or downstream targeted mutation or SNP, thereby increasing selective cleavage of the undesired target oligonucleotide (*e.g.,* Figure 22, Panel D, muRNA).

[00961] In some embodiments, a provided chirally controlled oligonucleotide composition suppresses cleavage at one or more sites, *e.g.,* relative to a reference (*e.g.,* chirally uncontrolled/stereorandom) oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition selectively suppresses cleavage at a single site relative to a reference (*e.g.,* chirally uncontrolled/stereorandom) composition. In some embodiments, a chirally controlled oligonucleotide composition suppresses cleavage at a site by providing a lower cleavage rate. In some embodiments, a chirally controlled oligonucleotide composition suppresses cleavage at a site by providing a lower percentage of cleavage at said site. In some embodiments, suppression is relative to a reference oligonucleotide composition. In some embodiments, suppression is relative to another cleavage site. In some embodiments, a provided chirally controlled oligonucleotide composition suppresses cleavage at a site that is a preferred cleavage site of a reference oligonucleotide composition. In some embodiments, a preferred cleavage site, or a group of preferred cleavage sites, is a site or sites that have relatively higher percentage of cleavage compared to one or more other cleavage sites. In some embodiments, preferred cleavage sites can indicate preference of an enzyme. For example, for RNase H, when a DNA oligonucleotide is used, resulting cleavage sites may indicate preference of RNase H. In some embodiments, a provided chirally controlled oligonucleotide composition suppresses cleavage at a site that is a preferred cleavage site of an enzyme. In some

embodiments, a provided chirally controlled oligonucleotide composition suppresses cleavage at a site that is not a preferred cleavage site of a reference oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition suppresses all cleavage sites of a reference oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition generally enhances cleavage of target oligonucleotides. In some embodiments, a provided chirally controlled oligonucleotide composition generally suppresses cleavage of non-target oligonucleotides. In some embodiments, a provided chirally controlled oligonucleotide composition enhances cleavage of target oligonucleotides and suppresses cleavage of non-target oligonucleotides. Using Figure 22, Panel D, as an example, a target oligonucleotide for cleavage is muRNA, while a non-target oligonucleotide is wtRNA. In a subject comprising a diseased tissue comprising a mutation or SNP, a target oligonucleotide for cleavage can be transcripts with a mutation or SNP, while a non-target oligonucleotide can be normal transcripts without a mutation or SNP, such as those expressed in healthy tissues.

[00962] In some embodiments, a reference oligonucleotide composition is a stereorandom oligonucleotide composition. In some embodiments, a reference oligonucleotide composition is a stereorandom composition of oligonucleotides of which all internucleotidic linkages are phosphorothioate. In some embodiments, a reference oligonucleotide composition is a DNA oligonucleotide composition with all phosphate linkages.

[00963] In some embodiments, besides patterns of backbone chiral centers described herein, provided oligonucleotides optionally comprises modified bases, modified sugars, modified backbone linkages and any combinations thereof. In some embodiments, a provided oligonucleotide is a unimer, altmer, blockmer, gapmer, hemimer and skipmer. In some embodiments, a provided oligonucleotide comprises one or more unimer, altmer, blockmer, gapmer, hemimer or skipmer moieties, or any combinations thereof. In some embodiments, besides patterns of backbone chiral centers herein, a provided oligonucleotide is a hemimer. In some embodiments, besides patterns of backbone chiral centers herein, a provided oligonucleotide is a 5'-hemimer with modified sugar moieties. In some embodiments, a provided oligonucleotide is 5'-hemimer with 2'-modified sugar moieties. Suitable modifications are widely known in the art, *e.g.,* those described in the present application. In some embodiments, a modification is 2'−F. In some embodiments, a modification is 2'−MOE. In some embodiments, a modification is s-cEt.

[00964] In some embodiments, the present disclosure provides a method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines the target nucleic acid sequence, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target nucleic acid sequence and a similar nucleic acid sequences, transcripts of the target nucleic acid sequence are suppressed at a greater level than a level of suppression observed for a similar nucleic acid sequence.

[00965] In some embodiments, the present disclosure provides a method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines the target nucleic acid sequence, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target nucleic acid sequence and a similar nucleic acid sequences, transcripts of the target nucleic acid sequence are suppressed at a greater level than a level of suppression observed for a similar nucleic acid sequence.

[00966] In some embodiments, a common base sequence is or comprises a sequence that is 100% complementary to the characteristic sequence elements. In some embodiments, suppression can be assessed through various suitable assays as known by a person having ordinary skill in the art. In some embodiments, an assay is a RNase H assay as described in the present disclosure, which can assess suppression by evaluating cleavage of a sequence found in a transcript of a target nucleic acid sequence comprising the characteristic sequence element and cleavage of a sequence found in a transcript of a similar sequence. In some embodiments, transcripts of the target nucleic acid sequence are suppressed at a greater level than a level of suppression observed for any one of the similar nucleic acid sequence. In some embodiments, example target and similar sequences are described in the present disclosure.

[00967] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

[00968] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a

chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

[00969] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with

a system comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence,
the contacting being performed under conditions determined to permit the composition to suppress transcripts of the particular allele.

[00970] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

[00971] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

[00972] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide

type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence,

the contacting being performed under conditions determined to permit the composition to suppress transcripts of the particular allele.

[00973] In some embodiments, a transcript is suppressed by cleavage of said transcript. In some embodiments, a specific nucleotide characteristic sequence element is in an intron. In some embodiments, a specific nucleotide characteristic sequence element is in an exon. In some embodiments, a specific nucleotide characteristic sequence element is partially in an exon and partially in an intron. In some embodiments, a specific nucleotide characteristic sequence element comprises a mutation that differentiates an allele from other alleles. In some embodiments, a mutation is a deletion. In some embodiments, a mutation is an insertion. In some embodiments, a mutation is a point mutation. In some embodiments, a specific nucleotide characteristic sequence element comprises at least one single nucleotide polymorphism (SNP) that differentiates an allele from other alleles.

[00974] In some embodiments, a target nucleic acid sequence is a target gene.

[00975] In some embodiments, the present disclosure provides a method for allele-specific suppression of a gene whose sequence comprises at least one single nucleotide polymorphism (SNP), comprising providing a chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is completely complementary to a sequence found in a transcript from the first allele but not to the corresponding sequence found in a transcript from the second allele, wherein the sequence found in the transcripts comprises a SNP site;

2) a common pattern of backbone linkages;

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

wherein the transcript from the first allele is suppressed at least five folds more than that from the second allele.

[00976] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[00977] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[00978] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene,

the contacting being performed under conditions determined to permit the composition to

suppress transcripts of the particular allele.

[00979] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene,

the contacting being performed under conditions determined to permit the composition to suppress expression of the particular allele.

[00980] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to

the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[00981] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the

same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

[00982] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene,

the contacting being performed under conditions determined to permit the composition to suppress transcripts of the particular allele.

[00983] In some embodiments, the present disclosure provides a method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene,

the contacting being performed under conditions determined to permit the composition to suppress expression of the particular allele.

[00984] As described herein, in some embodiments, in a provided method contacting is performed under conditions determined to permit a composition to suppress transcripts of a particular allele. In some embodiments, contacting is performed under conditions determined to permit a composition to suppress expression of a particular allele.

[00985] In some embodiments, suppression of transcripts of a particular allele is at a level that is greater than when the composition is absent. In some embodiments, suppression of transcripts of a particular allele is at a level that is at least 1.1 fold relative to when the composition is absent, in that transcripts from the particular allele are detected in amounts that are at least 1.1 fold lower when the composition is present relative to when it is absent. In some embodiments, a level is at least 1.2 fold. In some embodiments, a level is at least 1.3 fold. In some embodiments, a level is at least 1.4 fold. In some embodiments, a level is at least 1.5 fold. In some embodiments, a level is at least 1.6 fold. In some embodiments, a level is at least 1.7 fold. In some embodiments, a level is at least 1.8 fold. In some embodiments, a level is at least 1.9 fold. In some embodiments, a level is at least 2 fold. In some embodiments, a level is at least 3 fold. In some embodiments, a level is at least 4 fold. In some embodiments, a level is at least 5 fold. In some embodiments, a level is at least 6 fold. In some embodiments, a level is at least 7 fold. In some embodiments, a level is at least 8 fold. In some embodiments, a level is at least 9 fold. In some embodiments, a level is at least 10 fold. In some embodiments, a level is at least 11 fold. In some embodiments, a level is at least 12 fold. In some embodiments, a level is at least 13 fold. In some embodiments, a level is at least 14 fold. In some embodiments, a level is at least 15 fold. In some embodiments, a level is at least 20 fold. In some embodiments, a level is at least 30 fold. In some embodiments, a level is at least 40 fold. In some embodiments, a level is at least 50 fold. In some embodiments, a level is at least 75 fold. In some embodiments, a level is at least 100 fold. In some embodiments, a level is at least 150 fold. In some embodiments, a level is at least 200 fold. In some embodiments, a level is at least 300 fold. In some embodiments, a level is at least 400 fold. In some embodiments, a level is at least 500 fold. In some embodiments, a level is at least 750 fold. In some embodiments, a level is at least 1000 fold. In some embodiments, a level is at least 5000 fold.

[00986] In some embodiments, suppression of transcripts of a particular allele is at a level that is greater than a level of suppression observed for another allele of the same nucleic acid sequence. In some embodiments, suppression of transcripts of a particular allele is at a level that is at least 1.1 fold greater than a level of suppression observed for another allele of the same nucleic acid sequence. In some embodiments, a level is at least 1.2 fold. In some embodiments, a level is at least 1.3 fold. In some embodiments, a level is at least 1.4 fold. In some embodiments, a level is at least 1.5 fold. In some embodiments, a level is at least 1.6 fold. In

some embodiments, a level is at least 1.7 fold. In some embodiments, a level is at least 1.8 fold. In some embodiments, a level is at least 1.9 fold. In some embodiments, a level is at least 2 fold. In some embodiments, a level is at least 3 fold. In some embodiments, a level is at least 4 fold. In some embodiments, a level is at least 5 fold. In some embodiments, a level is at least 6 fold. In some embodiments, a level is at least 7 fold. In some embodiments, a level is at least 8 fold. In some embodiments, a level is at least 9 fold. In some embodiments, a level is at least 10 fold. In some embodiments, a level is at least 11 fold. In some embodiments, a level is at least 12 fold. In some embodiments, a level is at least 13 fold. In some embodiments, a level is at least 14 fold. In some embodiments, a level is at least 15 fold. In some embodiments, a level is at least 20 fold. In some embodiments, a level is at least 30 fold. In some embodiments, a level is at least 40 fold. In some embodiments, a level is at least 50 fold. In some embodiments, a level is at least 75 fold. In some embodiments, a level is at least 100 fold. In some embodiments, a level is at least 150 fold. In some embodiments, a level is at least 200 fold. In some embodiments, a level is at least 300 fold. In some embodiments, a level is at least 400 fold. In some embodiments, a level is at least 500 fold. In some embodiments, a level is at least 750 fold. In some embodiments, a level is at least 1000 fold. In some embodiments, a level is at least 5000 fold.

[00987] In some embodiments, suppression of transcripts of a particular allele is at a level that is greater than when the composition is absent, and at a level that is greater than a level of suppression observed for another allele of the same nucleic acid sequence. In some embodiments, suppression of transcripts of a particular allele is at a level that is at least 1.1 fold relative to when the composition is absent, and at least 1.1 fold greater than a level of suppression observed for another allele of the same nucleic acid sequence. In some embodiments, each fold is independently as described above.

[00988] In some embodiments, a system is a composition comprising a transcript. In some embodiments, a system is a composition comprising transcripts from different alleles. In some embodiments, a system can be *in vivo* or *in vitro*, and in either way can comprise one or more cells, tissues, organs or organisms. In some embodiments, a system comprises one or more cells. In some embodiments, a system comprises one or more tissues. In some embodiments, a system comprises one or more organs. In some embodiments, a system comprises one or more organisms. In some embodiments, a system is a subject.

[00989] In some embodiments, suppression of a transcript, or suppression of expression of

an allele from which a transcript is transcribed, can be measured in *in vitro* assay. In some embodiments, a sequence from a transcript and comprising a specific nucleotide characteristic sequence element is usned in assays instead of the full-length transcript. In some embodiments, an assay is a biochemical assay. In some embodiments, an assay is a biochemical assay wherein a nucleic acid polymer, for example, a transcript or a sequence from a transcript and comprising a specific nucleotide characteristic sequence element, is tested for cleavage by an enzyme in the presence of a chirally controlled oligonucleotide composition.

[00990] In some embodiments, a provided chirally controlled oligonucleotide composition is administered to a subject. In some embodiments, a subject is an animal. In some embodiments, a subject is a plant. In some embodiments, a subject is a human.

[00991] In some embodiments, for allele-specific suppression of transcripts from a particular allele, transcripts are cleaved at a site near a sequence difference, for example a mutation, within a specific nucleotide characteristic sequence element, which sequence difference differentiates transcripts from a particular allele from transcripts from the other alleles. In some embodiments, transcripts are selectively cleaved at a site near such a sequence difference. In some embodiments, transcripts are cleaved at a higher percentage at a site near such a sequence difference that when a chirally uncontrolled oligonucleotide composition is used. In some embodiments, transcripts are cleaved at the site of a sequence difference. In some embodiments, transcripts are cleaved only at the site of a sequence difference within a specific nucleotide characteristic sequence element. In some embodiments, transcripts are cleaved at a site within 5 base pairs downstream or upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 4 base pairs downstream or upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 3 base pairs downstream or upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 2 base pairs downstream or upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 1 base pair downstream or upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 5 base pairs downstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 4 base pairs downstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 3 base pairs downstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 2 base pairs downstream a sequence difference. In some embodiments, transcripts

are cleaved at a site within 1 base pair downstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 5 base pairs upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 4 base pairs upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 3 base pairs upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 2 base pairs upstream a sequence difference. In some embodiments, transcripts are cleaved at a site within 1 base pair upstream a sequence difference. Such precise control of cleavage patterns, and the resulting highly selective suppression of transcripts from a particular allele, would not be possible without chirally controlled oligonucleotide compositions and methods thereof provided by Applicant in this disclosure.

[00992] In some embodiments, the present disclosure provides methods for treating a subject, or preventing a disease in a subject, by specifically suppress transcripts from a particular allele, for example, an allele that causes or may cause a disease. In some embodiments, the present disclosure provides methods for treating a subject suffering from a disease, comprising administering to the subject a pharmaceutical composition comprising a chirally controlled oligonucleotide composition, wherein transcripts from an allele that causes or contributes to the disease is selectively suppressed. In some embodiments, the present disclosure provides methods for treating a subject suffering from a disease, comprising administering to the subject a pharmaceutical composition comprising a chirally controlled oligonucleotide composition, wherein transcripts from an allele that causes the disease is selectively suppressed. In some embodiments, the present disclosure provides methods for treating a subject suffering from a disease, comprising administering to the subject a pharmaceutical composition comprising a chirally controlled oligonucleotide composition, wherein transcripts from an allele that contributes to the disease is selectively suppressed. In some embodiments, the present disclosure provides methods for treating a subject suffering from a disease, comprising administering to the subject a pharmaceutical composition comprising a chirally controlled oligonucleotide composition, wherein transcripts from an allele that is related to the disease is selectively suppressed. In some embodiments, the present disclosure provides methods for preventing a disease in a subject, by specifically suppress transcripts from a particular allele that may cause a disease. In some embodiments, the present disclosure provides methods for preventing a disease in a subject, by specifically suppress transcripts from a particular allele that increases risk of a

disease in the subject. In some embodiments, a provided method comprises administering to the subject a pharmaceutical composition comprising a chirally controlled oligonucleotide composition. In some embodiments, a pharmaceutical composition further comprises a pharmaceutical carrier.

[00993] In some embodiments, a nucleotide characteristic sequence comprises a mutation that defines the target sequence relative to other similar sequences. In some embodiments, a nucleotide characteristic sequence comprises a point mutation that defines the target sequence relative to other similar sequences. In some embodiments, a nucleotide characteristic sequence comprises a SNP that defines the target sequence relative to other similar sequences.

[00994] In some embodiments, the present disclosure provides a method for preparing an oligonucleotide composition for selective suppression of a transcript of a target nucleic acid sequence, comprising providing an oligonucleotide composition comprising a predetermined level of oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which pattern comprises $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein each of m, n, t, *N*p is independently as defined and described herein;

wherein the target nucleic acid sequence comprises a characteristic sequence element that defines the target nucleic acid sequence relative to a similar nucleic acid sequence;

wherein the common base sequence is a sequence whose DNA cleavage pattern and/or stereorandom cleavage pattern has a cleavage site within or in the vicinity of the characteristic sequence element.

[00995] In some embodiments, the present disclosure provides a method for preparing an oligonucleotide composition for selective suppression of a transcript of a target nucleic acid sequence, comprising providing an oligonucleotide composition comprising a predetermined level of oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which pattern comprises $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein each of m, n, t, *N*p is independently as

defined and described herein;

wherein the target nucleic acid sequence comprises a characteristic sequence element that defines the target nucleic acid sequence relative to a similar nucleic acid sequence;

wherein the common base sequence is a sequence whose DNA cleavage pattern and/or stereorandom cleavage pattern has a major cleavage site within or in the vicinity of characteristic sequence element.

[00996] In some embodiments, a common pattern of backbone chiral centers comprises $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$ as described above. In some embodiments, a common pattern of backbone chiral centers comprises $(Sp)_m(Rp)_n$ as described above. In some embodiments, a common pattern of backbone chiral centers comprises $(Rp)_n(Sp)_m$ as described above. In some embodiments, a common pattern of backbone chiral centers comprises $(Np)_t(Rp)_n(Sp)_m$ as described above. In some embodiments, a common pattern of backbone chiral centers comprises $(Sp)_t(Rp)_n(Sp)_m$ as described above. In some embodiments, n is 1. In some embodiments, $m > 2$. In some embodiments, n is 1 and $m > 2$. In some embodiments, $t > 2$. In some embodiments, n is 1, $m > 2$, and $t > 2$.

[00997] In some embodiments, oligonucleotides of the particular oligonucleotide type have a wing-core structure. In some embodiments, oligonucleotides of the particular oligonucleotide type have a core-wing structure. In some embodiments, oligonucleotides of the particular oligonucleotide type have a wing-core-wing structure. In some embodiments, each sugar moieties in the wing regions has a sugar modification. In some embodiments, each sugar moiety in the wing regions has a 2'-modification. In some embodiments, each sugar moieties in the wing regions has a 2'-modification, wherein the 2'-modification is 2'-OR^1, wherein R^1 is optionally substituted C_{1-6} alkyl. In some embodiments, each sugar moiety in the wing regions has 2'-OMe. In some embodiments, each wing independently comprises a chiral internucleotidic linkage and a natural phosphate linkage. In some embodiments, a chiral internucleotidic linkage is phosphorothioate. In some embodiments, for a wing-core-wing structure, like in WV-1092, the 5'-wing has an *Sp* internucleotidic linkage at each of its 5'- and 3'-end, and phosphate linkages in between, and 3'-wing has an *Sp* internucleotidic linkage at its 3'-end, and the rest of its internucleotidic linkages are phosphate. Additional embodiments for the wing and/or core, *e.g.,* sugar modification, stereochemistry, *etc.*, are described in the present disclosure.

[00998] Common base sequences which are sequences whose DNA cleavage patterns

and/or stereorandom cleavage patterns have cleavage sites within or in the vicinity of the target nucleic acid sequence are extensively described in the present disclose. In some embodiments, a cleavage site within or in the vicinity of the target nucleic acid sequence is a cleavage site in the vicinity of a mutation which defines the target sequence from its similar sequences. In some embodiments, a cleavage site within or in the vicinity of the target nucleic acid sequence is a cleavage site in the vicinity of a SNP which defines the target sequence from its similar sequences. In some embodiments, as described above, in the vicinity of a mutation or a SNP is 0, 1, 2, 3, 4, 5 internucleotidic linkages away from the mutation or SNP. Additional embodiments are described above in the present disclosure.

[00999] In some embodiments, a common base sequence is a sequence whose DNA cleavage pattern and/or stereorandom cleavage pattern has a major cleavage site within or in the vicinity of the target nucleic acid sequence. In some embodiments, a major cleavage site is defined by absolute cleavage at that site (% of cleavage at that site over total target sequence). Additional example embodiments of a major cleavage site are described in the present disclosure. In some embodiments, as exemplified by Figure 33, a common base sequence (P12) may be identified by comparing cleavage maps of different sequences complementary to the characteristic sequence element.

[001000] In some embodiments, the present disclosure provides a method for preparing an oligonucleotide composition comprising oligonucleotides of a particular sequence, which composition provides selective suppression of a transcript of a target sequence, comprising providing a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence which is the same as the particular sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which pattern comprises $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein:

each n and t is independently 1, 2, 3, 4, 5, 6, 7 or 8;

m is 2, 3, 4, 5, 6, 7 or 8, and

each *N*p is independent *R*p or *S*p.

[001001] Diseases that involves disease-causing alleles are widely known in the art, including but not limited to those described in Hohjoh, *Pharmaceuticals* **2013**, *6*, 522-535; US

patent application publication US 2013/0197061; Østergaard *et al.*, *Nucleic Acids Research* **2013**, *41*(21), 9634–9650; and Jiang *et al.*, *Science* **2013**, *342*, 111-114. In some embodiments, a disease is Huntington's disease. In some embodiments, a disease is human hypertrophic cardiomyopathy (HCM). In some embodiments, a disease is dilated cardiomyopathy. In some embodiments, a disease-causing allele is an allele of myosin heavy chains (MHC). In some embodiments, an example disease is selected from:

Disease	Target gene	Target variation
Familial Alzheimer's disease	Amyloid precursor protein (APP)	K670N-M671L (Swedish mutant)
	Amyloid precursor protein (APP)	K670N-M671L (Swedish mutant)
	Amyloid precursor protein (APP)	V717F (London mutant)
	Amyloid precursor protein (APP)	V717I (London mutant)
	Presenilin 1 (PSEN1)	L392V
Amyotrophic lateral sclerosis (ALS)	Superoxide dismutase (SOD1)	G93A
	Superoxide dismutase (SOD1)	G85R
Slow channel congenital myasthenic syndrome (SCCMS)	Acetylcholine receptor (AChR)	aS226F
Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17)	Microtubule-associated protein TAU (MAPT)	V337M
Ehlers-Danlos syndrome (vEDS)	Procollagen type III (COL3A1)	G252V
Sickle cell anemia	Hemoglobin-beta locus (HBB)	E6V
Familial amyloidotic polyneuropathy (FAP)	Transthyretin (TTR)	V30M
Fibrodysplasia ossificans progressiva (FOP)	Activin A receptor type I (ACVR1)	R206H, G356D
	Activin A receptor type I (ACVR1)	R206H
Tumors	Phosphomosiitide-3-kinase, catalytic, alpha polypeptide (PIK3CA)	1633G -> A 3140A -> G
Spinocerebellar ataxia type 1 (SCA1)	Ataxin-1 (ATXN1)	flanking region of expanded CAG repeat

Disease	Target gene	Target variation
Machado-Joseph disease/spinocerebellar ataxia type 3 (MJD/SCA3)	ATAXIN3/MJD1	SNPs linked to expanded CAG repeat
Spinocerebellar ataxia type 7 (SCA7)	Ataxin-7 (ATXN7)	SNP linked to expanded CAG repeat
Parkinson's disease	Leucine-rich repeat kinase 2 (LRRK2)	R1441G, R1441C
	Leucine-rich repeat kinase 2 (LRRK2)	G2019S
	alpha-synuclein	A30P
Huntington disease	Huntingtin (HTT)	SNPs linked to expanded CAG repeat
Hypertrophic cardiomyopathy	MYH7	R403Q

[001002] In some embodiments, example targets of, and diseases that can be treated by, provided chirally controlled oligonucleotide compositions and methods, comprises:

Disease	Target gene	Target variation
Familial Alzheimer's disease	Amyloid precursor protein (APP)	K670N-M671L (Swedish mutant)
	Amyloid precursor protein (APP)	K670N-M671L (Swedish mutant)
	Amyloid precursor protein (APP)	V717F (London mutant)
	Amyloid precursor protein (APP)	V717I (London mutant)
	Preseniline 1 (PSEN1)	L392V
Amyotrophic lateral sclerosis (ALS)	Superoxide dismutase (SOD1)	G93A
	Superoxide dismutase (SOD1)	G85R
Slow channel congenital myasthenic syndrome (SCCMS)	Acetylcholine receptor (AChR)	aS226F, aT254I, aS269I

Disease	Target gene	Target variation
Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17)	Microtubule-associated protein TAU (MAPT)	V337M
Ehlers-Danlos syndrome (vEDS)	Procollagen type III (COL3A1)	G252V
Sickle cell anemia	Hemoglobin-beta locus (HBB)	E6V
Familial amyloidotic polyneuropathy (FAP)	Transthyretin (TTR)	V30M
Fibrodysplasia ossificans progressiva (FOP)	Activin A receptor type I (ACVR1)	R206H, G356D
	Activin A receptor type I (ACVR1)	R206H
Tumors	KRAS	G12V, G12D, G13D
Tumors	Phosphoinositide-3-kinase, catalytic, alpha polypeptide (PIK3CA)	G1633A, A3140G
Spinocerebellar ataxia type 1 (SCA1)	Ataxin-1 (ATXN1)	SNPs linked to expanded CAG repeat
Spinocerebellar ataxia type 7 (SCA7)	Ataxin-7 (ATXN7)	SNPs linked to expanded CAG repeat
Spinocerebellar Ataxia Type 3 (SCA3) /Machado-Joseph Disease	Ataxin-3 (ATXN3)	SNPs linked to expanded CAG repeat
Parkinson's disease	Leucine-rich repeat kinase 2 (LRRK2)	R1441G, R1441C
	Leucine-rich repeat kinase 2 (LRRK2)	G20195S
	Alpha-synuclein (SNCA)	A30P, A53T, E46K
Huntington's disease	Huntingtin (HTT)	SNPs linked to expanded CAG repeat
Huntington's disease-like 2	JPH3	SNPs linked to expanded CTG repeat
Friedreich's ataxia	FXN	SNPs linked to expanded GAA repeat
Fragile X mental retardation syndrome/fragile X tremor ataxia syndrome	FMR1	SNPs linked to expanded CGG repeat
Myotonic Dystophy (DM1)	DMPK	SNPs linked to expanded CTG repeat
Myotonic Dystophy (DM2)	ZNF9	SNPs linked to expanded CTG repeat
Spinal-Bulbar Muscular Atrophy	AR	SNPs linked to expanded CAG repeat

Disease	Target gene	Target variation
Hypertrophic cardiomyopathy	MHY7	R403Q

[001003] In some embodiments, oligonucleotide compositions and technologies described herein are particularly useful in the treatment of Huntington's disease. For example, in some embodiments, the present disclosure defines stereochemically controlled oligonucleotide compositions that direct cleavage (*e.g.*, RNAse H-mediated cleavage) of nucleic acids associated with Huntington's disease. In some embodiments, such compositions direct preferential cleavage of a Huntington's disease-associated allele of a particular target sequence, relative to one or more (*e.g.*, all non-Huntington's disease-associated) other alleles of the sequence.

[001004] In some embodiments, a provided method for treating or preventing Huntington's disease in a subject, comprising administering to the subject a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

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

[001005] SNPs related to Huntington's disease are widely known in the art. In some embodiments, a common base sequence is complementary to a nucleic acid sequence comprising a SNP related to Huntington's disease. In some embodiments, a provided composition selectively suppresses transcripts from the disease-causing allele. In some embodiments, a provided composition selectively cleaves transcripts from the disease-causing allele. Example SNPs that can be targeted by a provided composition (target Huntingtin sites) are described herein.

[001006] In some embodiments, a target Huntingtin site is selected from rs9993542_C, rs362310_C, rs362303_C, rs10488840_G, rs363125_C, rs363072_A, rs7694687_C, rs363064_C, rs363099_C, rs363088_A, rs34315806_C, rs2298967_T, rs362272_G,

rs362275_C, rs362306_G, rs3775061_A, rs1006798_A, rs16843804_C, rs3121419_C, rs362271_G, rs362273_A, rs7659144_C, rs3129322_T, rs3121417_G, rs3095074_G, rs362296_C, rs108850_C, rs2024115_A, rs916171_C, rs7685686_A, rs6844859_T, rs4690073_G, rs2285086_A, rs362331_T, rs363092_C, rs3856973_G, rs4690072_T, rs7691627_G, rs2298969_A, rs2857936_C, rs6446723_T, rs762855_A, rs1263309_T, rs2798296_G, rs363096_T, rs10015979_G, rs11731237_T, rs363080_C, rs2798235_G and rs362307_T. In some embodiments, a target Huntingtin site is selected from rs34315806_C, rs362273_A, rs362331_T, rs363099_C, rs7685686_A, rs362306_G, rs363064_C, rs363075_G, rs2276881_G, rs362271_G, rs362303_C, rs362322_A, rs363088_A, rs6844859_T, rs3025838_C, rs363081_G, rs3025849_A, rs3121419_C, rs2298967_T, rs2298969_A, rs16843804_C, rs4690072_T, rs362310_C, rs3856973_G, rs2530595_C, rs2530595_T, and rs2285086_A. In some embodiments, a target Huntingtin site is selected from rs34315806_C, rs362273_A, rs362331_T, rs363099_C, rs7685686_A, rs362306_G, rs363064_C, rs363075_G, rs2276881_G, rs362271_G, rs362303_C, rs362322_A, rs363088_A, rs6844859_T, rs3025838_C, rs363081_G, rs3025849_A, rs3121419_C, rs2298967_T, rs2298969_A, rs16843804_C, rs4690072_T, rs362310_C, rs3856973_G, and rs2285086_A. In some embodiments, a target Huntingtin site is selected from rs362331_T, rs7685686_A, rs6844859_T, rs2298969_A, rs4690072_T, rs2024115_A, rs3856973_G, rs2285086_A, rs363092_C, rs7691627_G, rs10015979_G, rs916171_C, rs6446723_T, rs11731237_T, rs362272_G, rs4690073_G, and rs363096_T. In some embodiments, a target Huntingtin site is selected from rs362267, rs6844859, rs1065746, rs7685686, rs362331, rs362336, rs2024115, rs362275, rs362273, rs362272, rs3025805, rs3025806, rs35892913, rs363125, rs17781557, rs4690072, rs4690074, rs1557210, rs363088, rs362268, rs362308, rs362307, rs362306, rs362305, rs362304, rs362303, rs362302, rs363075, rs2530595, and rs2298969. In some embodiments, a target Huntingtin site is selected from rs362267, rs6844859, rs1065746, rs7685686, rs362331, rs362336, rs2024115, rs362275, rs362273, rs362272, rs3025805, rs3025806, rs35892913, rs363125, rs17781557, rs4690072, rs4690074, rs1557210, rs363088, rs362268, rs362308, rs362307, rs362306, rs362305, rs362304, rs362303, rs362302, rs363075 and rs2298969. In some embodiments, a target Huntingtin site is selected from:

Frequency of Heterozygosity for 24 SNP Sites in the Huntingtin mRNA		
Location in	Reference	Percent Heterozygosity

mRNA (Position, nt)	Number	Controls	HD Patients
ORF, exon 20 (2822)	rs363075	G/A, 10.3% (G/G, 89.7%)	G/A, 12.8% (G/G, 86.2%; A/A, 0.9%)
ORF, exon 25 (3335)	rs35892913	G/A, 10.3% (G/G, 89.7%)	G/A, 13.0% (G/G, 86.1%; A/A, 0.9%)
ORF, exon 25 (3389)	rs1065746	G/C, 0% (G/G, 100%)	G/C, 0.9% (G/G, 99.1%)
ORF, exon 25 (3418)	rs17781557	T/G, 12.9% (T/T, 87.1%)	T/G, 1.9% (T/T, 98.1%)
ORF, exon 29 (3946)	rs4690074	C/T, 37.9% (C/C, 50.9%; T/T, 11.2)	C/T, 35.8% (C/C, 59.6%; T/T, 4.6%)
ORF, exon 39 (5304)	rs363125	C/A, 17.5% (C/C, 79.0%; A/A, 3.5%)	C/A, 11.0% (C/C, 87.2%; A/A, 1.8%)
ORF, exon 44 (6150)	exon 44	G/A, 0% (G/G, 100%)	G/A, 2.8% (G/G, 97.2%)
ORF, exon 48 (6736)	rs362336	G/A, 38.7% (G/G, 49.6%; A/A, 11.7%)	G/A, 37.4% (G/G, 57.9%; A/A, 4.7%)
ORF, exon 50 (7070)	rs362331	T/C, 45.7% (T/T, 31.0%; C/C, 23.3%)	T/C, 39.4% (T/T, 49.5%; C/C, 11.0%)
ORF, exon 57 (7942)	rs362273	A/G, 40.3% (A/A, 48.2%; G/G, 11.4%)	A/G, 35.2% (A/A, 60.2%; G/G, 4.6%)
ORF, exon 61 (8501)	rs362272	G/A, 37.1% (G/G, 51.7%; A/A, 11.2%)	G/A, 36.1% (G/G, 59.3%; A/A, 4.6%)
ORF, exon 65 (9053)	rs3025806	A/T, 0% (C/C, 100%)	A/T, 0% (C/C, 100%)
ORF, exon 65 (9175)	exon 65	G/A, 2.3% (G/G, 97.7%)	G/A, 0% (G/G, 100%)
ORF, exon 67 (9523)	rs362308	T/C, 0% (T/T, 100%)	T/C, 0% (T/T, 100%)
3′UTR, exon 67 (9633)	rs362307	C/T, 13.0% (C/C, 87.0%)	C/T, 48.6% (C/C, 49.5%; T/T, 1.9%)
3′UTR, exon 67 (9888)	rs362306	G/A, 36.0% (G/G, 52.6%; A/A, 11.4%)	G/A, 35.8% (G/G, 59.6%; A/A, 4.6%)
3′UTR, exon 67 (9936)	rs362268	C/G, 36.8% (C/C, 50.0%; G/G 13.2%)	C/G, 35.8% (C/C, 59.6%; G/G, 4.6%)
3′UTR, exon 67 (9948)	rs362305	C/G, 20.2% (C/C, 78.1%; G/G 1.8%)	C/G, 11.9% (C/C, 85.3%; G/G, 2.8%)
3′UTR, exon 67 (10060)	rs362304	C/A, 22.8% (C/C, 73.7%; A/A, 3.5%)	C/A, 11.9% (C/C, 85.3%; AA, 2.8%)
3′UTR, exon 67 (10095)	rs362303	C/T, 18.4% (C/C, 79.8%; T/T, 1.8%)	C/A, 11.9% (C/C, 85.3%; T/T, 2.8%)
3′UTR, exon 67 (10704)	rs1557210	C/T, 0% (C/C, 100%)	C/T, 0% (C/C, 100%)
3′UTR, exon 67 (10708)	rs362302	C/T, 4.3% (C/C, 95.7%)	C/T, 0% (C/C, 100%)

3′UTR, exon 67 (10796)	rs3025805	G/T, 0% (G/G, 100%)	G/T, 0% (G/G, 100%)
3′UTR, exon 67 (11006)	rs362267	C/T, 36.2% (C/C, 52.6%; T/T, 11.2%)	C/T, 35.5% (C/C, 59.8%; T/T, 4.7%)

[001007] In some embodiments, a chirally controlled oligonucleotide composition targets two or more sites. In some embodiments, targeted two or more sites are selected from sited listed herein. In some embodiments, a targeted SNP is rs362307, rs7685686, rs362268, rs2530595, rs362331, or rs362306. In some embodiments, a targeted SNP is rs362307, rs7685686, rs362268 or rs362306. In some embodiments, a targeted SNP is rs362307. In some embodiments, a targeted SNP is rs7685686. In some embodiments, a targeted SNP is not rs7685686. In some embodiments, a targeted SNP is rs362268. In some embodiments, a targeted SNP is rs362306.

[001008] In some embodiments, a chirally controlled oligonucleotide composition is able to differentiate between two alleles of a particular SNP.

[001009] A chirally controlled oligonucleotide compositions of both WVE120101 and WV-1092 were able to differentiate between wt and mutant versions of SNP rs362307, which differ by one nt; both WVE120101 and WV-1092 chirally controlled oligonucleotide compositions significantly knocked down the mutant allele but not the wt, while the stereorandom oligonucleotide composition, WV-1497, was not able to significantly differentiate between the wt and mutant alleles (see Figure 39D).

[001010] A chirally controlled oligonucleotide composition of WV-2595 was also able to differentiate between the C and T alleles at SNP rs2530595, which also differ at only the one nt. A chirally controlled WV-2595 oligonucleotide composition significantly knocked down the T allele but not the C allele, unlike the stereorandom oligonucleotide composition of WV-2611, which was not able to significantly differentiate the alleles (see Figure 39F).

[001011] A chirally controlled oligonucleotide composition of WV-2603 was able to differentiate between the C and T alleles of SNP rs362331, which also differ at only the one nt. A chirally controlled WV-2603 oligonucleotide composition significantly knocked down the T allele but not the C allele, unlike the stereorandom oligonucleotide composition of WV-2619, which was not able to significantly differentiate between the alleles (see Figures 39A, 39B, 39C and 39E).

[001012] In some embodiments, a provided composition for treating Huntington's disease is selected from Tables N1, N2, N3 or N4. In some embodiments, a provided composition for treating Huntington's disease is selected from Table N1. In some embodiments, a provided composition for treating Huntington's disease is selected from Table N2. In some embodiments, a provided composition for treating Huntington's disease is selected from Table N3. In some embodiments, a provided composition for treating Huntington's disease is selected from Table N4. In some embodiments, a provided composition for treating Huntington's disease is selected from Tables N1A, N2A, N3A or N4A. In some embodiments, a provided composition for treating Huntington's disease is selected from Table N1A. In some embodiments, a provided composition for treating Huntington's disease is selected from Table N2A. In some embodiments, a provided composition for treating Huntington's disease is selected from Table N3A. In some embodiments, a provided composition for treating Huntington's disease is selected from Table N4A. In some embodiments, a provided composition is WV-1092. In some embodiments, a provided composition is selected from: an oligonucleotide having a sequence of WVE120101; WV-2603; WV-2595; WV-1510; WV-2378; and WV-2380; each of these was constructed and found to be very effective, for example, as demonstrated *in vitro* in the dual luciferase reporter assay. In some embodiments, a provided composition is a chirally controlled oligonucleotide composition of WVE120101, WV-2603, WV-2595, WV-1510, WV-2378, or WV-2380. In some embodiments, a provided composition is a chirally controlled oligonucleotide composition of WV-937. In some embodiments, a provided composition is a chirally controlled oligonucleotide composition of WV-1087. In some embodiments, a provided composition is a chirally controlled oligonucleotide composition of WV-1090. In some embodiments, a provided composition is a chirally controlled oligonucleotide composition of WV-1091. In some embodiments, a provided composition is a chirally controlled oligonucleotide composition of WV-1092. In some embodiments, a provided composition is a chirally controlled oligonucleotide composition of WV-1510. In some embodiments, a provided composition is a chirally controlled oligonucleotide composition of WV-2378. In some embodiments, a provided composition is a chirally controlled oligonucleotide composition of WV-2380. In some embodiments, a provided composition is a chirally controlled oligonucleotide composition of WV-2595. In some embodiments, a provided composition is a chirally controlled oligonucleotide composition of WV-2603. In some embodiments, provided

oligonucleotides comprise base sequence, pattern of backbone linkages, pattern or backbone chiral centers, and/or pattern of chemical modifications (*e.g.*, base modifications, sugar modifications, *etc.*) of any oligonucleotide disclosed herein.

[001013] In some embodiments, a provided composition is not a composition of ONT-451, ONT-452 or ONT-450. In some embodiments, a provided composition is not a composition of ONT-451. In some embodiments, a provided composition is not a composition of ONT-452. In some embodiments, a provided composition is not a composition of ONT-450. In some embodiments, a composition does not contain a pre-determined level of ONT-451 or ONT-452. In some embodiments, a composition does not contain a pre-determined level of ONT-451. In some embodiments, a composition does not contain a pre-determined level of ONT-452. In some embodiments, an oligonucleotide type is not ONT-451 or ONT-452. In some embodiments, an oligonucleotide type is not ONT-451. In some embodiments, an oligonucleotide type is not ONT-452. In some embodiments, a composition is not a chirally controlled oligonucleotide composition of ONT-451 or ONT-452. In some embodiments, a composition is not a chirally controlled oligonucleotide composition of ONT-451. In some embodiments, a composition is not a chirally controlled oligonucleotide composition of ONT-452.

[001014] In some embodiments, a provided method ameliorates a symptom of Huntington's disease. In some embodiments, a provided method slows onset of Huntington's disease. In some embodiments, a provided method slows progression of Huntington's disease. In some embodiments, a provided method stops progression of Huntington's disease. In some embodiments, a provided method cures Huntington's disease according to a clinical standard.

[001015] In some embodiments, the present disclosure provides methods for identifying patients for a given oligonucleotide composition. In some embodiments, the present disclosure provides methods for patient stratification. In some embodiments, a provided method comprises identifying a mutation and/or SNP associated with a disease-causing allele. For example, in some embodiments, a provided method comprises identifying in a subject a SNP associated with expanded CAG repeats that are associated with or causing Huntington's disease. In some embodiments, a provided method comprises identifying in a subject a SNP associated with more than 35 CAG repeats in Huntingtin. In some embodiments, a provided method comprises identifying in a subject a SNP associated with more than 36 CAG repeats in Huntingtin. In some

embodiments, a provided method comprises identifying in a subject a SNP associated with more than 37 CAG repeats in Huntingtin. In some embodiments, a provided method comprises identifying in a subject a SNP associated with more than 38 CAG repeats in Huntingtin. In some embodiments, a provided method comprises identifying in a subject a SNP associated with more than 39 CAG repeats in Huntingtin. In some embodiments, a provided method comprises identifying in a subject a SNP associated with more than 40 CAG repeats in Huntingtin.

[001016] In some embodiments, a subject has a SNP in the subject's Huntingtin gene. In some embodiments, a subject has a SNP, wherein one allele is mutant Huntingtin associated with expanded CAG repeats. In some embodiments, a subject has a SNP as described herein. In some embodiments, a subject has a SNP selected from rs362307, rs7685686, rs362268, rs2530595, rs362331, or rs362306. In some embodiments, a subject has a SNP selected from rs362307, rs7685686, rs362268, or rs362306. In some embodiments, a subject has a SNP selected from rs362307. In some embodiments, a subject has a SNP selected from rs7685686. In some embodiments, a subject has a SNP selected from rs362268. In some embodiments, a subject has a SNP selected from rs362306.

[001017] In some embodiments, oligonucleotides of a provided composition have a sequence complementary to a sequence comprising a SNP from the disease-causing allele (mutant), and the composition selectively suppresses expression from the diseasing-causing allele. In some embodiments, a SNP is rs362307, rs7685686, rs362268, rs2530595, rs362331, or rs362306. In some embodiments, a SNP is rs362307, rs7685686, rs362268, or rs362306. In some embodiments, a SNP is rs362307. In some embodiments, a SNP is rs7685686. In some embodiments, a SNP is rs362268. In some embodiments, a SNP is rs362306. In some embodiments, a SNP is rs2530595. In some embodiments, a SNP is rs362331.

[001018] As understood by a person having ordinary skill in the art, various methods may be used to monitor a treatment process. In some embodiments, mutant HTT (mHTT) may be assessed from cerebrospinal fluid (Wild *et al.*, Quantification of mutant Huntingtin protein in cerebrospinal fluid from Huntington's disease patients, *J Clin Invest.* 2015; 125 (5): 1979-86), and may be used to monitor a treatment. In some embodiments, this approach may be used to determined and/or optimize a regimen, monitor pharmacodynamic endpoints, and/or determine dosage and frequency for administration, *etc.*

[001019] It is understood by a person having ordinary skill in the art that provided methods

apply to any similar targets containing a mismatch. In some embodiments, a mismatch is between a maternal and paternal gene. Additional example targets for suppression and/or knockdown, including allele-specific suppression and/or knockdown, can be any genetic abnormalties, *e.g.,* mutations, related to any diseases. In some embodiments, a target, or a set of targets, is selected from genetic determinants of diseases, *e.g.,* as disclosed in Xiong, *et al.*, The human splicing code reveals new insights into the genetic determinants of disease. *Science* Vol. 347 no. 6218 DOI: 10.1126/science.1254806. In some embodiments, a mismatch is between a mutant and a wild type.

[001020] In some embodiments, provided chirally controlled oligonucleotide compositions and methods are used to selectively suppress oligonucleotides with a mutation in a disease. In some embodiments, a disease is cancer. In some embodiments, provided chirally controlled oligonucleotide compositions and methods are used to selectively suppress transcripts with mutations in cancer. In some embodiments, provided chirally controlled oligonucleotide compositions and methods are used to suppress transcripts of KRAS. Example target KRAS sites comprises G12V = GGU -> GUU Position 227 G->U, G12D = GGU->GAU Position 227 G->A and G13D = GGC -> GAC Position 230 G->A.

[001021] In some embodiments, provided chirally controlled oligonucleotide compositions and methods provide allele-specific suppression of a transcript in an organism. In some embodiments, an organism comprises a target gene for which two or more alleles exist. For example, a subject has a wild type gene in its normal tissues, while the same gene is mutated in diseased tissues such as in a tumor. In some embodiments, the present disclosure provides chirally controlled oligonucleotide compositions and methods that selectively suppress one allele, for example, one with a mutation or SNP. In some embodiments, the present disclosure provides treatment with higher efficacy and/or low toxicity, and/or other benefits as described in the application.

[001022] In some embodiments, provided chirally controlled oligonucleotide compositions comprises oligonucleotides of one oligonucleotide type. In some embodiments, provided chirally controlled oligonucleotide compositions comprises oligonucleotides of only one oligonucleotide type. In some embodiments, provided chirally controlled oligonucleotide compositions has oligonucleotides of only one oligonucleotide type. In some embodiments, provided chirally controlled oligonucleotide compositions comprises oligonucleotides of two or

more oligonucleotide types. In some embodiments, using such compositions, provided methods can target more than one target. In some embodiments, a chirally controlled oligonucleotide composition comprising two or more oligonucleotide types targets two or more targets. In some embodiments, a chirally controlled oligonucleotide composition comprising two or more oligonucleotide types targets two or more mismatches. In some embodiments, a single oligonucleotide type targets two or more targets, *e.g.*, mutations. In some embodiments, a target region of oligonucleotides of one oligonucleotide type comprises two or more "target sites" such as two mutations or SNPs.

[001023] In some embodiments, oligonucleotides in a provided chirally controlled oligonucleotide composition optionally comprise modified bases or sugars. In some embodiments, a provided chirally controlled oligonucleotide composition does not have any modified bases or sugars. In some embodiments, a provided chirally controlled oligonucleotide composition does not have any modified bases. In some embodiments, oligonucleotides in a provided chirally controlled oligonucleotide composition comprise modified bases and sugars. In some embodiments, oligonucleotides in a provided chirally controlled oligonucleotide composition comprise a modified base. In some embodiments, oligonucleotides in a provided chirally controlled oligonucleotide composition comprise a modified sugar. Modified bases and sugars for oligonucleotides are widely known in the art, including but not limited in those described in the present disclosure. In some embodiments, a modified base is 5-mC. In some embodiments, a modified sugar is a 2'-modified sugar. Suitable 2'-modification of oligonucleotide sugars are widely known by a person having ordinary skill in the art. In some embodiments, 2'-modifications include but are not limited to 2'–OR^1, wherein R^1 is not hydrogen. In some embodiments, a 2'-modification is 2'–OR^1, wherein R^1 is optionally substituted C_{1-6} aliphatic. In some embodiments, a 2'-modification is 2'-MOE.In some embodiments, a modification is 2'-halogen. In some embodiments, a modification is 2'–F. In some embodiments, modified bases or sugars may further enhance activity, stability and/or selectivity of a chirally controlled oligonucleotide composition, whose common pattern of backbone chiral centers provides unexpected activity, stability and/or selectivity.

[001024] In some embodiments, a provided chirally controlled oligonucleotide composition does not have any modified sugars. In some embodiments, a provided chirally controlled oligonucleotide composition does not have any 2'- modified sugars. In some embodiments, the

present disclosure surprisingly found that by using chirally controlled oligonucleotide compositions, modified sugars are not needed for stability, activity, and/or control of cleavage patterns. Furthermore, in some embodiments, the present disclosure surprisingly found that chirally controlled oligonucleotide compositions of oligonucleotides without modified sugars deliver better properties in terms of stability, activity, turn-over and/or control of cleavage patterns. For example, in some embodiments, it is surprisingly found that chirally controlled oligonucleotide compositions of oligonucleotides having no modified sugars dissociates much faster from cleavage products and provide significantly increased turn-over than compositions of oligonucleotides with modified sugars.

[001025] In some embodiments, oligonucleotides of provided chirally controlled oligonucleotide compositions useful for provided methods have structures as extensively described in the present disclosure. In some embodiments, an oligonucleotide has a wing-core-wing structure as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_mRp$ as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_2Rp$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_m(Rp)_n$ as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Rp)_n(Sp)_m$ as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $Rp(Sp)_m$ as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $Rp(Sp)_2$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_m(Rp)_n(Sp)_t$ as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_mRp(Sp)_t$ as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_t(Rp)_n(Sp)_m$ as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_tRp(Sp)_m$ as described. In some embodiments, the common pattern of backbone chiral centers of a provided chirally

controlled oligonucleotide composition comprises *Sp*Rp*Sp*Sp. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_2Rp(Sp)_2$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_3Rp(Sp)_3$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_4Rp(Sp)_4$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_5Rp(Sp)_5$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises *Sp*Rp$(Sp)_5$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_2Rp(Sp)_5$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_3Rp(Sp)_5$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_4Rp(Sp)_5$. In some embodiments, the common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises $(Sp)_5Rp(Sp)_5$. In some embodiments, a common pattern of backbone chiral centers has only one *R*p, and each of the other internucleotidic linkages is *S*p. In some embodiments, a common base length is greater than 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 35, 40, 45 or 50 as described in the present disclosure. In some embodiments, a common base length is greater than 10. In some embodiments, a common base length is greater than 11. In some embodiments, a common base length is greater than 12. In some embodiments, a common base length is greater than 13. In some embodiments, a common base length is greater than 14. In some embodiments, a common base length is greater than 15. In some embodiments, a common base length is greater than 16. In some embodiments, a common base length is greater than 17. In some embodiments, a common base length is greater than 18. In some embodiments, a common base length is greater than 19. In some embodiments, a common base length is greater than 20. In some embodiments, a common base length is greater than 21. In some embodiments, a common base length is greater than 22. In some embodiments, a common base length is greater than 23. In some embodiments, a common base length is greater than 24. In some embodiments, a common base length is greater than 25. In some embodiments, a common

base length is greater than 26. In some embodiments, a common base length is greater than 27. In some embodiments, a common base length is greater than 28. In some embodiments, a common base length is greater than 29. In some embodiments, a common base length is greater than 30. In some embodiments, a common base length is greater than 31. In some embodiments, a common base length is greater than 32. In some embodiments, a common base length is greater than 33. In some embodiments, a common base length is greater than 34. In some embodiments, a common base length is greater than 35.

[001026] In some embodiments, a provided chirally controlled oligonucleotide composition provides higher turn-over. In some embodiments, cleavage products from a nucleic acid polymer dissociate from oligonucleotides of a provided chirally controlled oligonucleotide composition at a faster rate than from oligonucleotides of a reference oligonucleotide composition, for example, a chirally uncontrolled oligonucleotide composition. In some embodiments, a provided chirally controlled oligonucleotide composition can be administered in lower unit dosage, and/or total dosage, and/or fewer doses than chirally uncontrolled oligonucleotide composition.

[001027] In some embodiments, a chirally controlled oligonucleotide composition provides fewer cleavage sites in the sequence of a nucleic acid polymer that is complementary to its common base sequence or a sequence within its common base sequence when compared to a reference oligonucleotide composition. In some embodiments, a chirally controlled oligonucleotide composition provides fewer cleavage sites in the sequence of a nucleic acid polymer that is complementary to its common base sequence. In some embodiments, a nucleic acid polymer is selectively cleaved at a single site within the sequence that is complementary to the common base sequence, or a sequence within the common base sequence, of a chirally controlled oligonucleotide composition. In some embodiments, a chirally controlled oligonucleotide composition provides higher cleavage percentage at a cleavage site within the sequence that is complementary to the common base sequence, or a sequence within the common base sequence, of the chirally controlled oligonucleotide composition. In some embodiments, a chirally controlled oligonucleotide composition provides higher cleavage percentage at a cleavage site within the sequence that is complementary to the common base sequence of the chirally controlled oligonucleotide composition. In some embodiments, a site having a higher cleavage percentage is a cleavage site when a reference oligonucleotide composition is used. In

some embodiments, a site having a higher cleavage percentage is a cleavage site that is not present when a reference oligonucleotide composition is used.

[001028] It is surprisingly found that with reduced number of cleavage sites in the complementary sequence, cleavage rate can be unexpectedly increased and/or higher cleavage percentage can be achieved. As demonstrated in the examples of this disclosure, provided chirally controlled oligonucleotide compositions that produce fewer cleavage sites, especially those that provide single-site cleavage, within the complementary sequences of target nucleic acid polymers provide much higher cleavage rates and much lower levels of remaining un-cleaved nucleic acid polymers. Such results are in sharp contrast to general teachings in the art in which more cleavage sites have been pursued in order to increase the cleavage rate.

[001029] In some embodiments, a chirally controlled oligonucleotide composition increases cleavage rate by 1.5 fold compared to a reference oligonucleotide composition. In some embodiments, cleavage rate is increased by at least 2 fold. In some embodiments, cleavage rate is increased by at least 3 fold. In some embodiments, cleavage rate is increased by at least 4 fold. In some embodiments, cleavage rate is increased by at least 5 fold. In some embodiments, cleavage rate is increased by at least 6 fold. In some embodiments, cleavage rate is increased by at least 7 fold. In some embodiments, cleavage rate is increased by at least 8 fold. In some embodiments, cleavage rate is increased by at least 9 fold. In some embodiments, cleavage rate is increased by at least 10 fold. In some embodiments, cleavage rate is increased by at least 11 fold. In some embodiments, cleavage rate is increased by at least 12 fold. In some embodiments, cleavage rate is increased by at least 13 fold. In some embodiments, cleavage rate is increased by at least 14 fold. In some embodiments, cleavage rate is increased by at least 15 fold. In some embodiments, cleavage rate is increased by at least 20 fold. In some embodiments, cleavage rate is increased by at least 30 fold. In some embodiments, cleavage rate is increased by at least 40 fold. In some embodiments, cleavage rate is increased by at least 50 fold. In some embodiments, cleavage rate is increased by at least 60 fold. In some embodiments, cleavage rate is increased by at least 70 fold. In some embodiments, cleavage rate is increased by at least 80 fold. In some embodiments, cleavage rate is increased by at least 90 fold. In some embodiments, cleavage rate is increased by at least 100 fold. In some embodiments, cleavage rate is increased by at least 200 fold. In some embodiments, cleavage rate is increased by at least 300 fold. In some embodiments, cleavage rate is increased by at least

400 fold. In some embodiments, cleavage rate is increased by at least 500 fold. In some embodiments, cleavage rate is increased by at least more than 500 fold.

[001030] In some embodiments, a chirally controlled oligonucleotide composition provides a lower level of remaining, un-cleaved target nucleic acid polymer compared to a reference oligonucleotide composition. In some embodiments, it is 1.5 fold lower. In some embodiments, it is at least 2 fold lower. In some embodiments, it is at least 3 fold lower. In some embodiments, it is at least 4 fold lower. In some embodiments, it is at least 5 fold lower. In some embodiments, it is at least 6 fold lower. In some embodiments, it is at least 7 fold lower. In some embodiments, it is at least 8 fold lower. In some embodiments, it is at least 9 fold lower. In some embodiments, it is at least 10 fold lower. In some embodiments, it is at least 11 fold lower. In some embodiments, it is at least 12 fold lower. In some embodiments, it is at least 13 fold lower. In some embodiments, it is at least 14 fold lower. In some embodiments, it is at least 15 fold lower. In some embodiments, it is at least 20 fold lower. In some embodiments, it is at least 30 fold lower. In some embodiments, it is at least 40 fold lower. In some embodiments, it is at least 50 fold lower. In some embodiments, it is at least 60 fold lower. In some embodiments, it is at least 70 fold lower. In some embodiments, it is at least 80 fold lower. In some embodiments, it is at least 90 fold lower. In some embodiments, it is at least 100 fold lower. In some embodiments, it is at least 200 fold lower. In some embodiments, it is at least 300 fold lower. In some embodiments, it is at least 400 fold lower. In some embodiments, it is at least 500 fold lower. In some embodiments, it is at least 1000 fold lower.

[001031] As discussed in detail herein, the present disclosure provides, among other things, a chirally controlled oligonucleotide composition, meaning that the composition contains a plurality of oligonucleotides of at least one type. Each oligonucleotide molecule of a particular "type" is comprised of preselected (*e.g.,* predetermined) structural elements with respect to: (1) base sequence; (2) pattern of backbone linkages; (3) pattern of backbone chiral centers; and (4) pattern of backbone P-modification moieties. In some embodiments, provided oligonucloetide compositions contain oligonucleotides that are prepared in a single synthesis process. In some embodiments, provided compositions contain oligonucloetides having more than one chiral configuration within a single oligonucleotide molecule (*e.g.,* where different residues along the oligonucleotide have different stereochemistry); in some such embodiments, such oligonucleotides may be obtained in a single synthesis process, without the need for secondary

conjugation steps to generate individual oligonucleotide molecules with more than one chiral configuration.

[001032] Oligonucleotide compositions as provided herein can be used as agents for modulating a number of cellular processes and machineries, including but not limited to, transcription, translation, immune responses, epigenetics, *etc.* In addition, oligonucleotide compositions as provided herein can be used as reagents for research and/or diagnostic purposes. One of ordinary skill in the art will readily recognize that the present disclosure disclosure herein is not limited to particular use but is applicable to any situations where the use of synthetic oligonucleitides is desirable. Among other things, provided compositions are useful in a variety of therapeutic, diagnostic, agricultural, and/or research applications.

[001033] In some embodiments, provided oligonucloetide compositions comprise oligonucleotides and/or residues thereof that include one or more structural modifications as described in detail herein. In some embodiments, provided oligonucleotide compositions comprise oligonucleoties that contain one or more nucleic acid analogs. In some embodiments, provided oligonucleotide compositions comprise oligonucleotides that contain one or more artificial nucleic acids or residues (e.g., a nucleotide analog), including but not limited to: a peptide nucleic acid (PNA), locked nucleic acid (LNA), morpholino, threose nucleic acid (TNA), glycol nucleic acid (GNA), arabinose nucleic acid (ANA), 2'-fl uoroarabinose nucleic acid (FANA), cyclohexene nucleic acid (CeNA), anhydrohexitol nucleic acid (HNA), and/or unlocked nucleic acid (UNA), threose nucleic acids (TNA), and/or Xeno nucleic acids (XNA), and any combination thereof.

[001034] In any of the embodiments, the disclosure is useful for oligonucleotide-based modulation of gene expression, immune response, *etc.* Accordingly, stereo-defined,oligonucleotide compositions of the disclosure, which contain oligonucleotides of predetermined type (i.e., which are chirally controlled, and optionally chirally pure), can be used in lieu of conventional stereo-random or chirally impure counterparts. In some embodiments, provided compositions show enhanced intended effects and/or reduced unwanted side effects. Certain embodimetns of biological and clinical/therapeutic applications of the disclosure are discussed explicitly below.

[001035] Various dosing regimens can be utilized to administer provided chirally controlled oligonucleotide compositions. In some embodiments, multiple unit doses are administered,

separated by periods of time. In some embodiments, a given composition has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second (or subsequent) dose amount that is same as or different from the first dose (or another prior dose) amount. In some embodiments, a dosing regimen comprises administering at least one unit dose for at least one day. In some embodiments, a dosing regimen comprises administering more than one dose over a time period of at least one day, and sometimes more than one day. In some embodiments, a dosing regimen comprises administering multiple doses over a time period of at least week. In some embodiments, the time period is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 or more (*e.g.,* about 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more) weeks. In some embodiments, a dosing regimen comprises administering one dose per week for more than one week. In some embodiments, a dosing regimen comprises administering one dose per week for 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 or more (*e.g.,* about 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more) weeks. In some embodiments, a dosing regimen comprises administering one dose every two weeks for more than two week period. In some embodiments, a dosing regimen comprises administering one dose every two weeks over a time period of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 or more (*e.g.,* about 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more) weeks. In some embodiments, a dosing regimen comprises administering one dose per month for one month. In some embodiments, a dosing regimen comprises administering one dose per month for more than one month. In some embodiments, a dosing regimen comprises administering one dose per month for 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,

or more months. In some embodiments, a dosing regimen comprises administering one dose per week for about 10 weeks. In some embodiments, a dosing regimen comprises administering one dose per week for about 20 weeks. In some embodiments, a dosing regimen comprises administering one dose per week for about 30 weeks. In some embodiments, a dosing regimen comprises administering one dose per week for 26 weeks. In some embodiments, a chirally controlled oligonucleotide composition is administered according to a dosing regimen that differs from that utilized for a chirally uncontrolled (*e.g.,* stereorandom) oligonucleotide composition of the same sequence, and/or of a different chirally controlled oligonucleotide composition of the same sequence. In some embodiments, a chirally controlled oligonucleotide composition is administered according to a dosing regimen that is reduced as compared with that of a chirally uncontrolled (*e.g.,* sterorandom) oligonucleotide composition of the same sequence in that it achieves a lower level of total exposure over a given unit of time, involves one or more lower unit doses, and/or includes a smaller number of doses over a given unit of time. In some embodiments, a chirally controlled oligonucleotide composition is administered according to a dosing regimen that extends for a longer period of time than does that of a chirally uncontrolled (*e.g.,* stereorandom) oligonucleotide composition of the same sequence Without wishing to be limited by theory, Applicant notes that in some embodiments, the shorter dosing regimen, and/or longer time periods between doses, may be due to the improved stability, bioavailability, and/or efficacy of a chirally controlled oligonucleotide composition. In some embodiments, a chirally controlled oligonucleotide composition has a longer dosing regimen compared to the corresponding chirally uncontrolled oligonucleotide composition. In some embodiments, a chirally controlled oligonucleotide composition has a shorter time period between at least two doses compared to the corresponding chirally uncontrolled oligonucleotide composition. Without wishing to be limited by theory, Applicant notes that in some embodiments longer dosing regimen, and/or shorter time periods between doses, may be due to the improved safety of a chirally controlled oligonucleotide composition.

[001036] A single dose can contain various amounts of a type of chirally controlled oligonucleotide, as desired suitable by the application. In some embodiments, a single dose contains about 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300 or more (*e.g.,* about 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more) mg of a type of chirally

controlled oligonucleotide. In some embodiments, a single dose contains about 1 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 5 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 10 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 15 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 20 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 50 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 100 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 150 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 200 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 250 mg of a type of chirally controlled oligonucleotide. In some embodiments, a single dose contains about 300 mg of a type of chirally controlled oligonucleotide. In some embodiments, a chirally controlled oligonucleotide is administered at a lower amount in a single dose, and/or in total dose, than a chirally uncontrolled oligonucleotide. In some embodiments, a chirally controlled oligonucleotide is administered at a lower amount in a single dose, and/or in total dose, than a chirally uncontrolled oligonucleotide due to improved efficacy. In some embodiments, a chirally controlled oligonucleotide is administered at a higher amount in a single dose, and/or in total dose, than a chirally uncontrolled oligonucleotide. In some embodiments, a chirally controlled oligonucleotide is administered at a higher amount in a single dose, and/or in total dose, than a chirally uncontrolled oligonucleotide due to improved safety.

Biologically Active Oligonucleotides

[001037] A provided oligonucleotide composition as used herein may comprise single stranded and/or multiply stranded oligonucleotides. In some embodiments, single-stranded oligonucleotides contain self-complementary portions that may hybridize under relevant conditions so that, as used, even single-stranded oligonucleotides may have at least partially double-stranded character. In some embodiments, an oligonucleotide included in a provided composition is single-stranded, double-stranded, or triple-stranded. In some embodiments, an oligonucleotide included in a provided composition comprises a single-stranded portion and a multiple-stranded portion within the oligonucleotide. In some embodiments, as noted above, individual single-stranded oligonucleotides can have double-stranded regions and single-stranded

regions.

[001038] In some embodiments, provided compositions include one or more oligonucleotides fully or partially complementary to strand of: structural genes, genes control and/or termination regions, and/or self-replicating systems such as viral or plasmid DNA. In some embodiments, provided compositions include one or more oligonucleotides that are or act as siRNAs or other RNA interference reagents (RNAi agents or iRNA agents), shRNA, antisense oligonucleotides, self-cleaving RNAs, ribozymes, fragment thereof and/or variants thereof (such as Peptidyl transferase 23S rRNA, RNase P, Group I and Group II introns, GIR1 branching ribozymes, Leadzyme, Hairpin ribozymes, Hammerhead ribozymes, HDV ribozymes, Mammalian CPEB3 ribozyme, VS ribozymes, glmS ribozymes, CoTC ribozyme, *etc.*), microRNAs, microRNA mimics, supermirs, aptamers, antimirs, antagomirs, Ul adaptors, triplex-forming oligonucleotides, RNA activators, long non-coding RNAs, short non-coding RNAs (*e.g.*, piRNAs), immunomodulatory oligonucleotides (such as immunostimulatory oligonucleotides, immunoinhibitory oligonucleotides), GNA, LNA, ENA, PNA, TNA, HNA, TNA, XNA, HeNA, CeNA, morpholinos, G-quadruplex (RNA and DNA), antiviral oligonucleotides, and decoy oligonucleotides.

[001039] In some embodiments, provided compositions include one or more hybrid (*e.g.*, chimeric) oligonucleotides. In the context of the present disclosure, the term "hybrid" broadly refers to mixed structural components of oligonucloetides. Hybrid oligonucleotides may refer to, for example, (1) an oligonucleotide molecule having mixed classes of nucleotides, *e.g.*, part DNA and part RNA within the single molecule (*e.g.*, DNA-RNA); (2) complementary pairs of nucleic acids of different classes, such that DNA:RNA base pairing occurs either intramolecularly or intermolecularly; or both; (3) an oligonucleotide with two or more kinds of the backbone or internucleotide linkages.

[001040] In some embodiments, provided compositions include one or more oligonucleotide that comprises more than one classes of nucleic acid residues within a single molecule. For example, in any of the embodiments described herein, an oligonucleotide may comprise a DNA portion and an RNA portion. In some embodiments, an oligonucleotide may comprise a unmodified portion and modified portion.

[001041] Provided oligonucleotide compositions can include oligonucleotides containing any of a variety of modifications, for example as described herein. In some embodiments,

particular modifications are selected, for example, in light of intended use. In some embodiments, it is desirable to modify one or both strands of a double-stranded oligonucleotide (or a double-stranded portion of a single-stranded oligonucleotie). In some embodiments, the two strands (or portions) include different modifications. In some embodiments, the two strands include the same modificatinons. One of skill in the art will appreciate that the degree and type of modifications enabled by methods of the present disclosure allow for numerous permutations of modifications to be made. Examples of such modifications are described herein and are not meant to be limiting.

[001042] The phrase "antisense strand" as used herein, refers to an oligonucleotide that is substantially or 100% complementary to a target sequence of interest. The phrase "antisense strand" includes the antisense region of both oligonucleotides that are formed from two separate strands, as well as unimolecular oligonucleotides that are capable of forming hairpin or dumbbell type structures. The terms "antisense strand" and "guide strand" are used interchangeably herein.

[001043] The phrase "sense strand" refers to an oligonucleotide that has the same nucleoside sequence, in whole or in part, as a target sequence such as a messenger RNA or a sequence of DNA. The terms "sense strand" and "passenger strand" are used interchangeably herein.

[001044] By "target sequence" is meant any nucleic acid sequence whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA, such as endogenous DNA or RNA, viral DNA or viral RNA, or other RNA encoded by a gene, virus, bacteria, fungus, mammal, or plant. In some embodiments, a target sequence is associated with a disease or disorder. In some embodiments, a target sequence is or comprises a portion of the Huntingtin gene. In some embodiments, a target sequence is or comprises a portion of the Huntingtin gene comprising a SNP.

[001045] By "specifically hybridizable" and "complementary" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non- traditional types. In reference to the nucleic molecules of the present disclosure, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, *e.g.,* RNAi activity. Determination of binding free energies for nucleic acid molecules is well known in the art (see, *e.g.,* Turner et al, 1987, *CSH Symp. Quant. Biol.* LIT pp.123-133; Frier et al., 1986, *Proc. Nat.*

Acad. Sci. USA83:9373-9377; Turner et al., 1987, /. *Ain. Chem. Soc.* 109:3783-3785)

[001046] A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (*e.g.,* Watson-Crick base pairing) with a second nucleic acid sequence (*e.g.,* 5, 6, 7, 8, 9,10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" or 100% complementarity means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. Less than perfect complementarity refers to the situation in which some, but not all, nucleoside units of two strands can hydrogen bond with each other. "Substantial complementarity" refers to polynucleotide strands exhibiting 90% or greater complementarity, excluding regions of the polynucleotide strands, such as overhangs, that are selected so as to be noncomplementary. Specific binding requires a sufficient degree of complementarity to avoid non-specific binding of the oligomeric compound to non-target sequences under conditions in which specific binding is desired, *e.g.,* under physiological conditions in the case of *in vivo* assays or therapeutic treatment, or in the case of *in vitro* assays, under conditions in which the assays are performed. In some embodiments, non-target sequences differ from corresponding target sequences by at least 5 nucleotides.

[001047] When used as therapeutics, a provided oligonucleotide is administered as a pharmaceutical composition. In some embodiments, the pharmaceutical composition comprises a therapeutically effective amount of a provided oligonucleotide comprising, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable inactive ingredient selected from pharmaceutically acceptable diluents, pharmaceutically acceptable excipients, and pharmaceutically acceptable carriers. In another embodiment, the pharmaceutical composition is formulated for intravenous injection, oral administration, buccal administration, inhalation, nasal administration, topical administration, ophthalmic administration, intrathecal administration, or otic administration. In another embodiment, the pharmaceutical composition is formulated for intravenous injection, oral administration, buccal administration, inhalation, nasal administration, topical administration, ophthalmic administration or otic administration. In further embodiments, the pharmaceutical composition is a tablet, a pill, a capsule, a liquid, an inhalant, a nasal spray solution, a suppository, a suspension, a gel, a colloid, a dispersion, a suspension, a solution, an emulsion, an ointment, a

lotion, an eye drop, an ear drop, or a preparation comprising artificial cerebrospinal fluid. In further embodiments, the pharmaceutical composition is a tablet, a pill, a capsule, a liquid, an inhalant, a nasal spray solution, a suppository, a suspension, a gel, a colloid, a dispersion, a suspension, a solution, an emulsion, an ointment, a lotion, an eye drop or an ear drop. In some embodiments, a pharmaceutical composition comprises cerebrospinal fluid. In some embodiments, a pharmaceutical composition comprises artificial cerebrospinal fluid. In some embodiments, a pharmaceutical composition comprises an oligonucleotide, wherein the sequence of the oligonucleotide comprises a sequence which targets a portion of the Huntingtin gene. In some embodiments, the sequence targets a portion of the Huntingtin gene comprising a SNP. In some embodiments, the base sequence, pattern of backbone linkages, pattern of backbone chiral centers, and/or pattern of sugar modifications of the oligonucleotide is or comprises the base sequence, pattern of backbone linkages, pattern of backbone chiral centers, and/or pattern of sugar modifications of any oligonucleotide disclosed herein, and the oligonucleotide is comprised in a pharmaceutical composition comprising any component of a pharmaceutical composition disclosed herein. In some embodiments, the oligonucleotide targets the Huntingtin gene (as a non-limiting example, a SNP in the Huntingtin gene), and the base sequence, pattern of backbone linkages, pattern of backbone chiral centers, and/or pattern of sugar modifications of the oligonucleotide is or comprises the base sequence, pattern of backbone linkages, pattern of backbone chiral centers, and/or pattern of sugar modifications of any oligonucleotide disclosed herein, and the oligonucleotide is comprised in a pharmaceutical composition comprising artificial cerebrospinal fluid, and the pharmaceutical composition is administered via intrathecal administration.

Pharmaceutical compositions

[001048] When used as therapeutics, a provided oligonucleotide or oligonucleotide composition described herein is administered as a pharmaceutical composition. In some embodiments, the pharmaceutical composition comprises a therapeutically effective amount of a provided oligonucleotides, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable inactive ingredient selected from pharmaceutically acceptable diluents, pharmaceutically acceptable excipients, and pharmaceutically acceptable carriers. In some embodiments, the pharmaceutical composition is formulated for intravenous injection, oral

administration, buccal administration, inhalation, nasal administration, topical administration, ophthalmic administration, intrathecal administration, or otic administration. 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, an ear drop, or a preparation comprising artificial cerebrospinal fluid. 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. In some embodiments, a provided composition comprises cerebrospinal fluid. In some embodiments, a provided composition comprises artificial cerebrospinal fluid.

[001049] In some embodiments, the present disclosure provides a pharmaceutical composition comprising chirally controlled oligonucleotide, or composition thereof, in admixture with a pharmaceutically acceptable excipient. One of skill in the art will recognize that the pharmaceutical compositions include the pharmaceutically acceptable salts of the chirally controlled oligonucleotide, or composition thereof, described above.

[001050] A variety of supramolecular nanocarriers can be used to deliver nucleic acids. Example nanocarriers include, but are not limited to liposomes, cationic polymer complexes and various polymeric. Complexation of nucleic acids with various polycations is another approach for intracellular delivery; this includes use of PEGlyated polycations, polyethyleneamine (PEI) complexes, cationic block co-polymers, and dendrimers. Several cationic nanocarriers, including PEI and polyamidoamine dendrimers help to release contents from endosomes. Other approaches include use of polymeric nanoparticles, polymer micelles, quantum dots and lipoplexes.

[001051] Additional nucleic acid delivery strategies are known in addition to the example delivery strategies described herein.

[001052] In therapeutic and/or diagnostic applications, the compounds of the disclosure can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remington, The Science and Practice of Pharmacy, (20th ed. 2000).

[001053] Provided oligonucleotides, and compositions thereof, are effective over a wide dosage range. For example, in the treatment of adult humans, dosages from about 0.01 to about 1000 mg, from about 0.5 to about 100 mg, from about 1 to about 50 mg per day, and from about 5 to about 100 mg per day are examples of dosages that may be used. The exact dosage will depend upon the route of administration, the form in which the compound is administered, the subject to be treated, the body weight of the subject to be treated, and the preference and experience of the attending physician.

[001054] Pharmaceutically acceptable salts are generally well known to those of ordinary skill in the art, and may include, by way of example but not limitation, acetate, benzenesulfonate, besylate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, carnsylate, carbonate, citrate, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, mucate, napsylate, nitrate, pamoate (embonate), pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, or teoclate. Other pharmaceutically acceptable salts may be found in, for example, Remington, The Science and Practice of Pharmacy (20th ed. 2000). Preferred pharmaceutically acceptable salts include, for example, acetate, benzoate, bromide, carbonate, citrate, gluconate, hydrobromide, hydrochloride, maleate, mesylate, napsylate, pamoate (embonate), phosphate, salicylate, succinate, sulfate, or tartrate.

[001055] 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.

[001056] In some embodiments of a method or composition of the present disclosure, a composition comprising an oligonucleotide is administered via intrathecal administration. In some embodiments of a method or composition of the present disclosure, a composition comprising an oligonucleotide comprises artificial cerebrospinal fluid and is administered via intrathecal administration. In some embodiments of a method or composition of the present disclosure, a composition comprising an oligonucleotide comprises one or more components of artificial cerebrospinal fluid (for example, NaCl, $NaHCO_3$, KCl, NaH_2PO_4, $MgCl_2$ and glucose) and is administered via intrathecal administration. In some embodiments of a method or composition of the present disclosure, a composition comprising an oligonucleotide comprises one or more components of artificial cerebrospinal fluid (for example, NaCl, $NaHCO_3$, KCl, NaH_2PO_4, $MgCl_2$ and glucose) and is administered via intrathecal administration, wherein the sequence of the oligonucleotide comprises a sequence which targets a portion of the Huntingtin gene. In some embodiments of a method or composition of the present disclosure, a composition comprising an oligonucleotide comprises two or more components of artificial cerebrospinal fluid (for example, NaCl, $NaHCO_3$, KCl, NaH_2PO_4, $MgCl_2$ and glucose) and is administered via intrathecal administration, wherein the sequence of the oligonucleotide comprises a sequence which targets a portion of the Huntingtin gene. In some embodiments of a method or composition of the present disclosure, a composition comprising an oligonucleotide comprises three or more components of artificial cerebrospinal fluid (for example, NaCl, $NaHCO_3$, KCl, NaH_2PO_4, $MgCl_2$ and glucose) and is administered via intrathecal administration, wherein the sequence of the oligonucleotide comprises a sequence which targets a portion of the Huntingtin gene. In some embodiments, provided oligonucleotides comprise base sequence, pattern of backbone linkages, pattern or backbone chiral centers, and/or pattern of chemical modifications (*e.g.*, base modifications, sugar modifications, *etc.*) of any oligonucleotide disclosed herein. In some embodiments, the sequence targets a portion of the Huntingtin gene comprising a SNP.

[001057] 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.

[001058] Use of pharmaceutically acceptable inert carriers to formulate the compounds

herein disclosed for the practice of the disclosure into dosages suitable for systemic administration is within the scope of the disclosure. With proper choice of carrier and suitable manufacturing practice, the compositions of the present disclosure, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection.

[001059] The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the 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.

[001060] 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.

[001061] In certain embodiments, oligonucleotides and compositions are delivered to the CNS. In certain embodiments, oligonucleotides and compositions are delivered to the cerebrospinal fluid. In certain embodiments, oligonucleotides and compositions are administered to the brain parenchyma. In certain embodiments, oligonucleotides and compositions are delivered to an animal/subject by intrathecal administration, or intracerebroventricular administration. Broad distribution of oligonucleotides and compositions, described herein, within the central nervous system may be achieved with intraparenchymal administration, intrathecal administration, or intracerebroventricular administration.

[001062] In certain embodiments, parenteral administration is by injection, by, *e.g.*, a syringe, a pump, *etc*. In certain embodiments, the injection is a bolus injection. In certain embodiments, the injection is administered directly to a tissue, such as striatum, caudate, cortex, hippocampus and cerebellum.

[001063] In certain embodiments, methods of specifically localizing a pharmaceutical agent, such as by bolus injection, decreases median effective concentration (EC50) by a factor of 20, 25, 30, 35, 40, 45 or 50. In certain embodiments, the pharmaceutical agent in an antisense compound as further described herein. In certain embodiments, the targeted tissue is brain tissue. In certain embodiments the targeted tissue is striatal tissue. In certain embodiments, decreasing EC50 is desirable because it reduces the dose required to achieve a pharmacological result in a patient in need thereof.

[001064] In certain embodiments, an antisense oligonucleotide is delivered by injection or infusion once every month, every two months, every 90 days, every 3 months, every 6 months, twice a year or once a year.

[001065] Pharmaceutical compositions suitable for use in the present disclosure include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[001066] In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions.

[001067] Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethyl-cellulose (CMC), and/or polyvinylpyrrolidone (PVP: povidone). If desired, disintegrating agents may be added, such as the cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[001068] 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.

[001069] Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin, and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium

stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols (PEGs). In addition, stabilizers may be added.

[001070] Depending upon the particular condition, or disease state, to be treated or prevented, additional therapeutic agents, which are normally administered to treat or prevent that condition, may be administered together with oligonucleotides of this disclosure. For example, chemotherapeutic agents or other anti-proliferative agents may be combined with the oligonucleotides of this disclosure to treat proliferative diseases and cancer. Examples of known chemotherapeutic agents include, but are not limited to, adriamycin, dexamethasone, vincristine, cyclophosphamide, fluorouracil, topotecan, taxol, interferons, and platinum derivatives.

[001071] The function and advantage of these and other embodiments of the present disclosure will be more fully understood from the examples described below. The following examples are intended to illustrate the benefits of the present disclosure, but do not exemplify the full scope of the disclosure.

Lipids

[001072] In some embodiments, provided oligonucleotide compositions further comprise one or more lipids. In some embodiments, the lipids are conjugated to provided oligonucleotides in the compositions. In some embodiments, two or more same or different lipids can be conjugated to one oligonucleotide, through either the same or differently chemistry and/or locations. In some embodiments, a composition can comprise an oligonucleotide disclosed herein (as non-limiting examples, a chirally controlled oligonucleotide composition, or a chirally controlled oligonucleotide composition wherein the sequence of the oligonucleotide comprises, consists of or is the sequence of any oligonucleotide disclosed herein, or a chirally controlled oligonucleotide composition wherein the sequence of the oligonucleotide comprises, consists of or is the sequence of any oligonucleotide disclosed in Table 8 or any other Table herein, etc.) and a lipid. In some embodiments, a provided oligonucleotide comprises base sequence, pattern of backbone linkages, pattern or backbone chiral centers, and/or pattern of chemical modifications (*e.g.*, base modifications, sugar modifications, *etc.*) of any oligonucleotide disclosed herein, and is conjugated to a lipid. In some embodiments, a provided composition comprises an oligonucleotide disclosed herein and a lipid, wherein the lipid is conjugated to the

oligonucleotide.

[001073] In some embodiments, the present disclosure provides a composition comprising an oligonucleotide amd a lipid. Many lipids can be utilized in provided technologies in accordance with the present disclosure.

[001074] In some embodiments, a lipid comprises an R^{LD} group, wherein R^{LD} is an optionally substituted, C_{10}-C_{80} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, –S(O)–, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$ –SC(O)–, –C(O)S–, –OC(O)–, and $-C(O)O-$, wherein:

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

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

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

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

[001075] In some embodiments, a lipid comprises an R^{LD} group, wherein R^{LD} is an optionally substituted, C_{10}-C_{60} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, –S(O)–, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$ –SC(O)–, –C(O)S–, –OC(O)–, and $-C(O)O-$, wherein:

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

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

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

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

[001076] In some embodiments, a lipid comprises an R^{LD} group, wherein R^{LD} is an optionally substituted, C_{10}-C_{40} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, –S(O)–, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$ –SC(O)–, $-C(O)S-$, $-OC(O)-$, and $-C(O)O-$, wherein:

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

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

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

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

[001077] In some embodiments, R^{LD} is an optionally substituted, C_{10}-C_{80} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, and –Cy–. In some embodiments, R^{LD} is an optionally substituted, C_{10}-C_{60} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, and –Cy–. In some embodiments, R^{LD} is a hydrocarbon goup consisting carbon and hydrogen atoms.

[001078] In some embodiments, R^{LD} is an optionally substituted, C_{10}-C_{60} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, and –Cy–. In some embodiments, R^{LD} is an optionally substituted, C_{10}-C_{60} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced

by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, and –Cy–. In some embodiments, R^{LD} is a hydrocarbon goup consisting carbon and hydrogen atoms.

[001079] In some embodiments, R^{LD} is an optionally substituted, C_{10}-C_{40} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C— , a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, and –Cy–. In some embodiments, R^{LD} is an optionally substituted, C_{10}-C_{60} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, and –Cy–. In some embodiments, R^{LD} is a hydrocarbon goup consisting carbon and hydrogen atoms.

[001080] The aliphatic group of R^{LD} can be a variety of suitable length. In some embodiments, it is C_{10}-C_{80}. In some embodiments, it is C_{10}-C_{75}. In some embodiments, it is C_{10}-C_{70}. In some embodiments, it is C_{10}-C_{65}. In some embodiments, it is C_{10}-C_{60}. In some embodiments, it is C_{10}-C_{50}. In some embodiments, it is C_{10}-C_{40}. In some embodiments, it is C_{10}-C_{35}. In some embodiments, it is C_{10}-C_{30}. In some embodiments, it is C_{10}-C_{25}. In some embodiments, it is C_{10}-C_{24}. In some embodiments, it is C_{10}-C_{23}. In some embodiments, it is C_{10}-C_{22}. In some embodiments, it is C_{10}-C_{21}. In some embodiments, it is C_{12}-C_{22}. In some embodiments, it is C_{13}-C_{22}. In some embodiments, it is C_{14}-C_{22}. In some embodiments, it is C_{15}-C_{22}. In some embodiments, it is C_{16}-C_{22}. In some embodiments, it is C_{17}-C_{22}. In some embodiments, it is C_{18}-C_{22}. In some embodiments, it is C_{10}-C_{20}. In some embodiments, the lower end of the range is C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, or C_{18}. In some embodiments, the higher end of the range is C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29}, C_{30}, C_{35}, C_{40}, C_{45}, C_{50}, C_{55}, or C_{60}. In some embodiments, it is C_{10}. In some embodiments, it is C_{11}. In some embodiments, it is C_{12}. In some embodiments, it is C_{13}. In some embodiments, it is C_{14}. In some embodiments, it is C_{15}. In some embodiments, it is C_{16}. In some embodiments, it is C_{17}. In some embodiments, it is C_{18}. In some embodiments, it is C_{19}. In some embodiments, it is C_{20}. In some embodiments, it is C_{21}. In some embodiments, it is C_{22}. In some embodiments, it is C_{23}. In some embodiments, it is C_{24}. In some embodiments, it is C_{25}. In some embodiments, it is C_{30}.

In some embodiments, it is C_{35}. In some embodiments, it is C_{40}. In some embodiments, it is C_{45}. In some embodiments, it is C_{50}. In some embodiments, it is C_{55}. In some embodiments, it is C_{60}.

[001081] In some embodiments, a lipid comprises no more than one R^{LD} group. In some embodiments, a lipid comprises two or more R^{LD} groups.

[001082] In some embodiments, a lipid is conjugated to a biologically active agent, optionally through a linker, as a moiety comprising an R^{LD} group. In some embodiments, a lipid is conjugated to a biologically active agent, optionally through a linker, as a moiety comprising no more than one R^{LD} group. In some embodiments, a lipid is conjugated to a biologically active agent, optionally through a linker, as an R^{LD} group. In some embodiments, a lipid is conjugated to a biologically active agent, optionally through a linker, as a moiety comprising two or more R^{LD} groups.

[001083] In some embodiments, R^{LD} is an optionally substituted, C_{10}-C_{40} saturated or partially unsaturated, aliphatic chain. In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{40} saturated or partially unsaturated, aliphatic chain.

[001084] In some embodiments, R^{LD} is an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain. In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[001085] In some embodiments, R^{LD} is a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic groups. In some embodiments, a lipid comprises a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic groups. In some embodiments, R^{LD} is a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-2} aliphatic groups. In some embodiments, a lipid comprises a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-2} aliphatic groups. In some embodiments, R^{LD} is a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more methyl groups. In some embodiments, a lipid comprises a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more methyl groups.

[001086] In some embodiments, R^{LD} is an unsubstituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain. In some embodiments, a lipid comprises an unsubstituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[001087] In some embodiments, a lipid comprises no more than one optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain. In some embodiments, a lipid comprises two or more optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

[001088] In some embodiments, R^{LD} is an optionally substituted, C_{10}-C_{60} saturated or partially unsaturated, aliphatic chain. In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{60} saturated or partially unsaturated, aliphatic chain.

[001089] In some embodiments, R^{LD} is an optionally substituted C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain. In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain.

[001090] In some embodiments, R^{LD} is a C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic groups. In some embodiments, a lipid comprises a C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic groups. In some embodiments, R^{LD} is a C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-2} aliphatic groups. In some embodiments, a lipid comprises a C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-2} aliphatic groups. In some embodiments, R^{LD} is a C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more methyl groups. In some embodiments, a lipid comprises a C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more methyl groups.

[001091] In some embodiments, R^{LD} is an unsubstituted C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain. In some embodiments, a lipid comprises an unsubstituted C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain.

[001092] In some embodiments, a lipid comprises no more than one optionally substituted C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain. In some embodiments, a lipid comprises two or more optionally substituted C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain.

[001093] In some embodiments, R^{LD} is an optionally substituted, C_{10}-C_{80} saturated or partially unsaturated, aliphatic chain. In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{80} saturated or partially unsaturated, aliphatic chain.

[001094] In some embodiments, R^{LD} is an optionally substituted C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain. In some embodiments, a lipid comprises an optionally substituted C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain.

[001095] In some embodiments, R^{LD} is a C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic groups. In some embodiments, a lipid comprises a C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic groups. In some embodiments, R^{LD} is a C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-2} aliphatic groups. In some embodiments, a lipid comprises a C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-2} aliphatic groups. In some embodiments, R^{LD} is a C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more methyl groups. In some embodiments, a lipid comprises a C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more methyl groups.

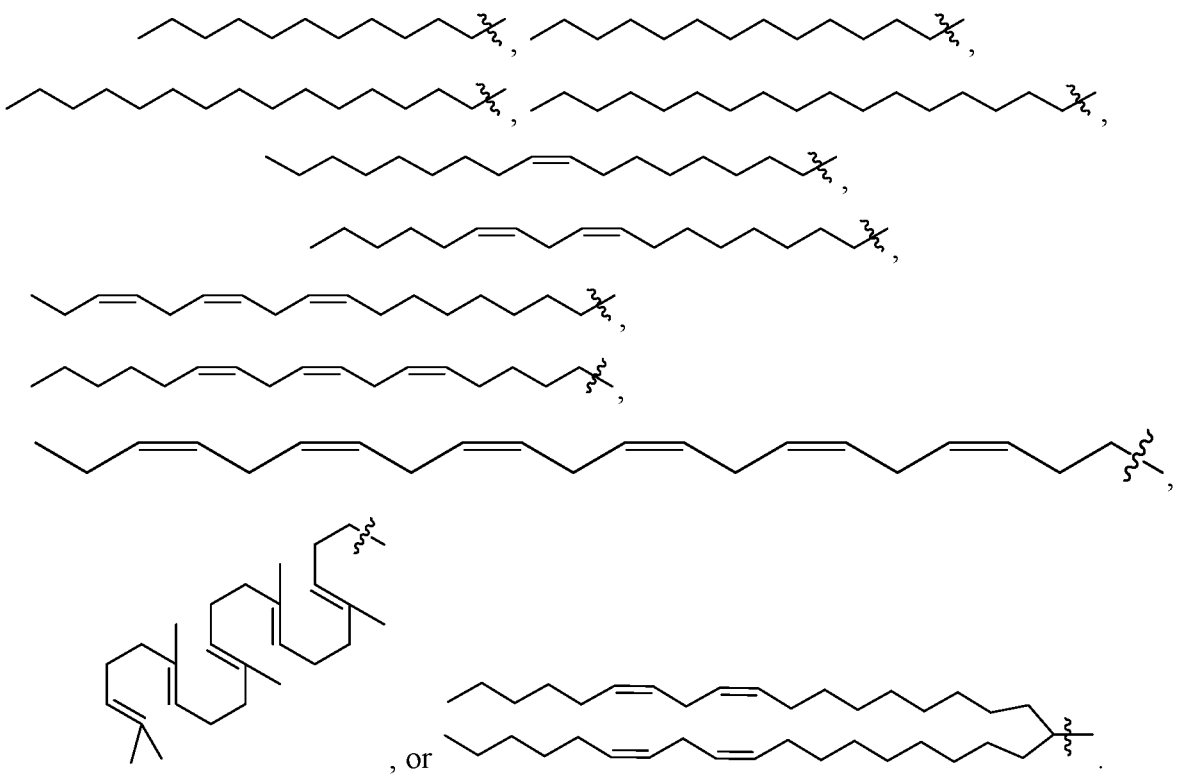
[001096] In some embodiments, R^{LD} is an unsubstituted C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain. In some embodiments, a lipid comprises an unsubstituted C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain.

[001097] In some embodiments, a lipid comprises no more than one optionally substituted C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain. In some embodiments, a lipid comprises two or more optionally substituted C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain.

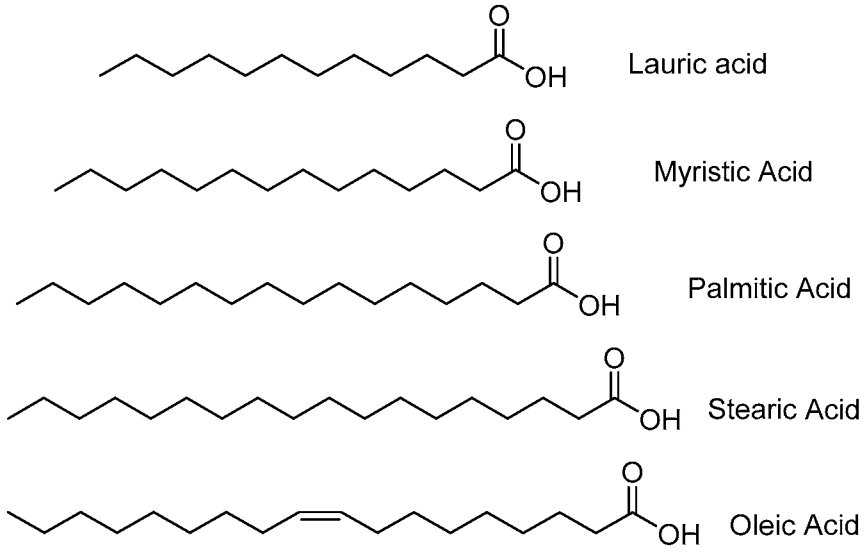
[001098] In some embodiments, R^{LD} is or comprises a C_{10} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{10} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{11} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{11} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{12} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{12} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{13} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{13} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{14} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{14} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{15} saturated

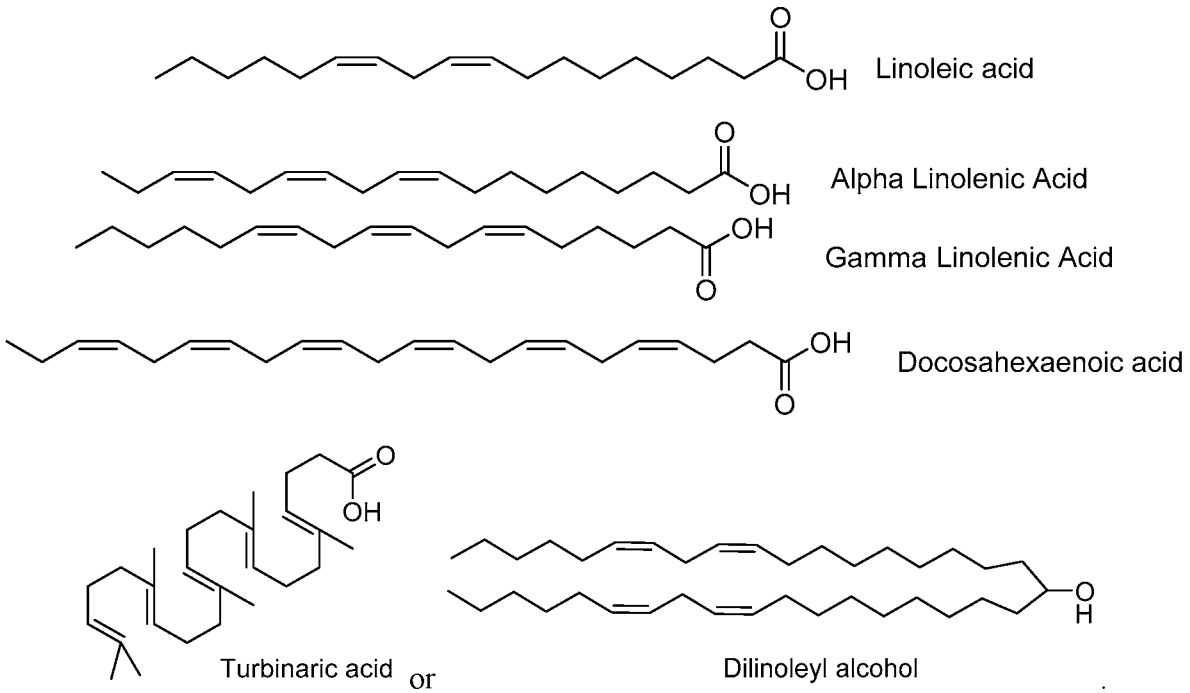
linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{15} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{16} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{16} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{17} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{17} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{18} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{18} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{19} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{19} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{20} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{20} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{21} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{21} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{22} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{22} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{23} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{23} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{24} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{24} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{25} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{25} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{26} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{26} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{27} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{27} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{28} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{28} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{29} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{29} partially unsaturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{30} saturated linear aliphatic chain. In some embodiments, R^{LD} is or comprises a C_{30} partially unsaturated linear aliphatic chain.

[001099] In some embodiments, a lipid has the structure of R^{LD}–OH. In some embodiments, a lipid has the structure of R^{LD}–C(O)OH. In some embodiments, R^{LD} is



In some embodiments, a lipid is lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (DHA or cis-DHA), turbinaric acid, arachidonic acid, and dilinoleyl. In some embodiments, a lipid is lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (DHA or cis-DHA), turbinaric acid, and dilinoleyl. In some embodiments, a lipid has a structure of:





[001100] In some embodiments, a lipid is, comprises or consists of any of: an at least partially hydrophobic or amphiphilic molecule, a phospholipid, a triglyceride, a diglyceride, a monoglyceride, a fat-soluble vitamin, a sterol, a fat and a wax. In some embodiments, a lipid is any of: a fatty acid, glycerolipid, glycerophospholipid, sphingolipid, sterol lipid, prenol lipid, saccharolipid, polyketide, and other molecule.

[001101] Lipids can be incorporated into provided technologies through many types of methods in accordance with the present disclosure. In some embodiments, lipids are physically mixed with provided oligonucleotides to form provided compositions. In some embodiments, lipids are chemically conjugated with oligonucleotides.

[001102] In some embodiments, provided compositions comprise two or more lipids. In some embodiments, provided oligonucleotides comprise two or more conjugated lipids. In some embodiments, the two or more conjugated lipids are the same. In some embodiments, the two or more conjugated lipids are different. In some embodiments, provided oligonucleotides comprise no more than one lipid. In some embodiments, oligonucleotides of a provided composition comprise different types of conjugated lipids. In some embodiments, oligonucleotides of a provided composition comprise the same type of lipids.

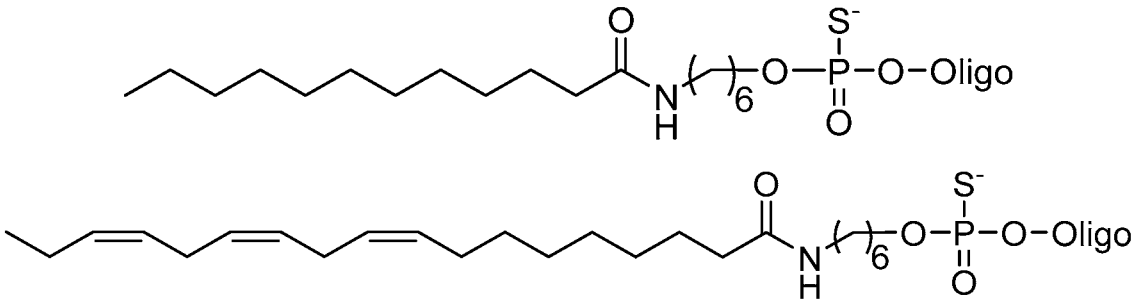
[001103] Lipids can be conjugated to oligonucleotides optionally through linkers. Various types of linkers in the art can be utilized in accordance of the present disclosure. In some

embodiments, a linker comprise a phosphate group, which can, for example, be used for conjugating lipids through chemistry similar to those employed in oligonucleotide synthesis. In some embodiments, a linker comprises an amide, ester, or ether group. In some embodiments, a linker has the structure of −L−. In some embodiments, after conjugation to oligonucleotides, a lipid forms a moiety having the structure of $-L-R^{LD}$, wherein each of L and R^{LD} is independently as defined and described herein.

[001104] In some embodiments, −L− comprises a bivalent aliphatic chain. In some embodiments, −L− comprises a phosphate group. In some embodiments, −L− comprises a phosphorothioate group. In some embodiments, −L− has the structure of $-C(O)NH-(CH_2)_6-OP(=O)(S^-)-$.

[001105] Lipids, optionally through linkers, can be conjugated to oligonucleotides at various suitable locations. In some embodiments, lipids are conjugated through the 5'-OH group. In some embodiments, lipids are conjugated through the 3'-OH group. In some embodiments, lipids are conjugated through one or more sugar moieties. In some embodiments, lipids are conjugated through one or more bases. In some embodiments, lipids are incorporated through one or more internucleotidic linkages. In some embodiments, an oligonucleotide may contain multiple conjugated lipids which are independently conjugated through its 5'-OH, 3'-OH, sugar moieties, base moieties and/or internucleotidic linkages.

[001106] In some embodiments, a lipid is conjugated to an oligonucleotide optionally through a linker moiety. A person having ordinary skill in the art appreciates that various technologies can be utilized to conjugate lipids to an oligonucleotide in accordance with the present disclosure. For example, for lipids comprising carboxyl groups, such lipids can be conjugated through the carboxyl groups. In some embodiments, a lipid is conjugated through a linker having the structure of −L−, wherein L is as defined and described in formula I. In some embodiments, L comprises a phosphate diester or modified phosphate diester moiety. In some embodiments, a compound formed by lipid conjugation has the structure of $(R^{LD}-L-)_x-$(oligonucleotide), wherein x is 1 or an integer greater than 1, and each of R^{LD} and L is independently as defined and described herein. In some embodiments, x is 1. In some embodiments, x is greater than 1. In some embodiments, an oligonucleotide is an oligonucleotide. For example, in some embodiments, a conjugate has the following structures:



[001107] In some embodiments, a linker is selected from: an uncharged linker; a charged linker; a linker comprising an alkyl; a linker comprising a phosphate; a branched linker; an unbranched linker; a linker comprising at least one cleavage group; a linker comprising at least one redox cleavage group; a linker comprising at least one phosphate-based cleavage group; a linker comprising at least one acid-cleavage group; a linker comprising at least one ester-based cleavage group; and a linker comprising at least one peptide-based cleavage group.

[001108] In some embodiments, a lipid is not conjugated to an oligonucleotide.

[001109] In some embodiments, the present disclosure pertains to compositions and methods related to a composition comprising an oligonucleotide and a lipid comprising a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, wherein the lipid is conjugated to the biologically active agent. In some embodiments, the present disclosure pertains to compositions and methods related to a composition comprising an oligonucleotide and a lipid comprising a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic group, wherein the lipid is conjugated to the biologically active agent.

[001110] In some embodiments, the present disclosure pertains to compositions and methods related to a composition comprising an oligonucleotide and a lipid comprising a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, wherein the lipid is not conjugated to the biologically active agent. In some embodiments, the present disclosure pertains to compositions and methods related to a composition comprising an oligonucleotide and a lipid comprising a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic group, wherein the lipid is not conjugated to the biologically active agent.

[001111] In some embodiments, a composition comprises an oligonucleotide and a lipid selected from: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid,

arachidonic acid, and dilinoleyl, wherein the lipid is not conjugated to the biologically active agent. In some embodiments, a composition comprises an oligonucleotide and a lipid selected from: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, and dilinoleyl, wherein the lipid is not conjugated to the biologically active agent.

[001112] In some embodiments, a composition comprises an oligonucleotide and a lipid selected from: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, arachidonic acid, and dilinoleyl, wherein the lipid is conjugated to the biologically active agent. In some embodiments, a composition comprises an oligonucleotide and a lipid selected from: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, and dilinoleyl, wherein the lipid is conjugated to the biologically active agent.

[001113] In some embodiments, a composition comprises an oligonucleotide and a lipid selected from: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, arachidonic acid, and dilinoleyl, wherein the lipid is directly conjugated to the biologically active agent (without a linker interposed between the lipid and the biologically active agent). In some embodiments, a composition comprises an oligonucleotide and a lipid selected from: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, and dilinoleyl, wherein the lipid is directly conjugated to the biologically active agent (without a linker interposed between the lipid and the biologically active agent).

[001114] In some embodiments, a composition comprises an oligonucleotide and a lipid selected from: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, arachidonic acid, and dilinoleyl, wherein the lipid is indirectly conjugated to the biologically active agent (with a linker interposed between the lipid and the biologically active agent). In some embodiments, a composition comprises an oligonucleotide and a lipid selected from: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, and dilinoleyl, wherein

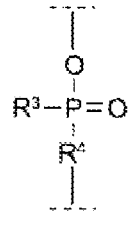
the lipid is indirectly conjugated to the biologically active agent (with a linker interposed between the lipid and the biologically active agent).

[001115] A linker is a moiety that connects two parts of a composition; as a non-limiting example, a linker physically connects an oligonucleotide to a lipid.

[001116] Non-limiting examples of suitable linkers include: an uncharged linker; a charged linker; a linker comprising an alkyl; a linker comprising a phosphate; a branched linker; an unbranched linker; a linker comprising at least one cleavage group; a linker comprising at least one redox cleavage group; a linker comprising at least one phosphate-based cleavage group; a linker comprising at least one acid- cleavage group; a linker comprising at least one ester-based cleavage group; a linker comprising at least one peptide-based cleavage group.

[001117] In some embodiments, a linker comprises an uncharged linker or a charged linker.

[001118] In some embodiments, a linker comprises an alkyl.

[001119] In some embodiments, a linker comprises a phosphate. In various embodiments, a phosphate can also be modified by replacement of bridging oxygen, (i.e. oxygen that links the phosphate to the nucleoside), with nitrogen (bridged phosphoroamidates), sulfur (bridged phosphorothioates) and carbon (bridged methylenephosphonates). The replacement can occur at the either linking oxygen or at both the linking oxygens. When the bridging oxygen is the 3'-oxygen of a nucleoside, replacement with carbon can be done. When the bridging oxygen is the 5'-oxygen of a nucleoside, replacement with nitrogen can be done. In various embodiments, the linker comprising a phosphate comprises any one or more of: a phosphorodithioate, phosphoramidate, boranophosphonoate, or a compound of formula (I):  (I), where R^3 is selected from OH, SH, NH_2, BH_3, CH_3, C_{1-6} alkyl, C_{6-10} aryl, C_{1-6} alkoxy and C_{6-10} aryl-oxy, wherein C_{1-6} alkyl and C_{6-10} aryl are unsubstituted or optionally independently substituted with 1 to 3 groups independently selected from halo, hydroxyl and NH_2; and R^4 is selected from O, S, NH, or CH_2.

[001120] In some embodiments, a linker comprises a direct bond or an atom such as oxygen or sulfur, a unit such as NR^1, C(O), C(O)NH, SO, SO_2, SO_2NH or a chain of atoms, such

as substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, where one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, $N(R_1)_2$, C(O), cleavable linking group, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted heterocyclic; where R^1 is hydrogen, acyl, aliphatic or substituted aliphatic.

[001121] In some embodiments, a linker is a branched linker. In some embodiments, a branchpoint of the branched linker may be at least trivalent, but may be a tetravalent, pentavalent or hexavalent atom, or a group presenting such multiple valencies. In some embodiments, a branchpoint is --N, --N(Q)-C, --O--C, --S--C, --SS--C, --C(O)N(Q)-C, --OC(O)N(Q)-C, --N(Q)C(O)--C, or --N(Q)C(O)O--C; wherein Q is independently for each occurrence H or optionally substituted alkyl. In other embodiment, the branchpoint is glycerol or glycerol derivative.

[001122] In one embodiment, a linker comprises at least one cleavable linking group.

[001123] As a non-limiting example, a cleavable linking group can be sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. As a non-limiting example, a cleavable linking group is cleaved at least 10 times or more, at least 100 times faster in the target cell or under a first reference condition (which can, e.g., be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, e.g., be selected to mimic or represent conditions found in the blood or serum). Cleavable linking groups are susceptible to

cleavage agents, e.g., pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, e.g., oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, e.g., those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

[001124] As a non-limiting example, a cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a desired pH, thereby releasing the cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

[001125] As a non-limiting example, a linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, liver targeting ligands can be linked to the cationic lipids through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

[001126] As a non-limiting example, a linker can contain a peptide bond, which can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

[001127] As a non-limiting example, suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, e.g., blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue

culture, or in whole animals. It may be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. As a non-limiting example, useful candidate compounds are cleaved at least 2, 4, 10 or 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood or serum (or under in vitro conditions selected to mimic extracellular conditions).

[001128] In some embodiments, a linker comprises a redox cleavable linking group. As a non-limiting example, one class of cleavable linking groups are redox cleavable linking groups that are cleaved upon reduction or oxidation. A non-limiting example of reductively cleavable linking group is a disulphide linking group (--S--S--). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. As a non-limiting example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents know in the art, which mimic the rate of cleavage which would be observed in a cell, e.g., a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. As a non-limiting example, candidate compounds are cleaved by at most 10% in the blood. As a non-limiting example, useful candidate compounds are degraded at least 2, 4, 10 or 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood (or under in vitro conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

[001129] In some embodiments, a linker comprises a phosphate-based cleavable linking groups are cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are --O--P(O)(ORk)-O--, --O--P(S)(ORk)-O--, --O--P(S)(SRk)-O--, --S--P(O)(ORk)-O--, --O--P(O)(ORk)-S--, --S--P(O)(ORk)-S--, --O--P(S)(ORk)-S--, --S--P(S)(ORk)-O--, --O--P(O)(Rk)-O--, --O--P(S)(Rk)-O--, --S--P(O)(Rk)-O--, --S--P(S)(Rk)-O--, --S--P(O)(Rk)-S--, --O--P(S)(Rk)-S--. Additional non-limiting examples are --O--P(O)(OH)--O--, --O--P(S)(OH)--O--, --O--P(S)(SH)--O--, --S--P(O)(OH)--O--, --O--P(O)(OH)--S--, --S--P(O)(OH)--S--, --O--P(S)(OH)--S--, --S--P(S)(OH)--O--, --O--P(O)(H)--O--, --O--

P(S)(H)--O--, --S--P(O)(H)--O--, --S--P(S)(H)--O--, --S--P(O)(H)--S--, --O--P(S)(H)--S--. An additional non-limiting examples is --O--P(O)(OH)--O--.

[001130] In some embodiments, a linker comprises an acid cleavable linking groups are linking groups that are cleaved under acidic conditions. As a non-limiting example, acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (e.g., about 6.0, 5.5, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula --C=NN--, C(O)O, or --OC(O). In an additional non-limiting example, when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl.

[001131] In some embodiments, a linker comprises an ester-based linking groups. As a non-limiting example, ester-based cleavable linking groups are cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula --C(O)O--, or --OC(O)--. These candidates can be evaluated using methods analogous to those described above.

[001132] In some embodiments, a linker comprises a peptide-based cleaving group. Peptide-based cleavable linking groups are cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (e.g., dipeptides, tripeptides etc.) and polypeptides. As a non-limiting example, peptide-based cleavable groups do not include the amide group (--C(O)NH--). The amide group can be formed between any alkylene, alkenylene or alkynylene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. As a non-limiting example, a peptide based cleavage group can be limited to the peptide bond (i.e., the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. As a non-limiting example, a peptide-based cleavable linking groups can have the general formula --NHCHR^{A}C(O)NHCHR^{B}C(O)--, where R^{A} and R^{B} are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

[001133] Any linker reported in the art can be used, including, as non-limiting examples, those described in: U.S. Pat. App. No. 20150265708.

[001134] A non-limiting example of a method of conjugating a lipid and an oligonucleotide is presented in Example 1.

[001135] A non-limiting example of a linker is a C6 amino linker.

Target Components

[001136] In some embodiments, a provided composition further comprises a targeting component (targeting compound or moiety). A target component can be either conjugated or not conjugated to a lipid or a biologically active agent. In some embodiments, a target component is conjugated to a biologically active agent. In some embodiments, a biologically active agent is conjugated to both a lipid and a targeting component. As described in here, in some embodiments, a biologically active agent is a provided oligonucleotide. Thus, in some embodiments, a provided oligonucleotide compostion further comprises, besides a lipid and oligonucleotides, a target elements. Various targeting components can be used in accordance with the present disclosure, *e.g.*, lipids, antibodies, peptides, carbohydrates, *etc.*

[001137] Target components can be incorporated into provided technologies through many types of methods in accordance with the present disclosure. In some embodiments, target components are physically mixed with provided oligonucleotides to form provided compositions. In some embodiments, target components are chemically conjugated with oligonucleotides.

[001138] In some embodiments, provided compositions comprise two or more target components. In some embodiments, provided oligonucleotides comprise two or more conjugated target components. In some embodiments, the two or more conjugated target components are the same. In some embodiments, the two or more conjugated target components are different. In some embodiments, provided oligonucleotides comprise no more than one target component. In some embodiments, oligonucleotides of a provided composition comprise different types of conjugated target components. In some embodiments, oligonucleotides of a provided composition comprise the same type of target components.

[001139] Target components can be conjugated to oligonucleotides optionally through linkers. Various types of linkers in the art can be utilized in accordance of the present disclosure. In some embodiments, a linker comprise a phosphate group, which can, for example, be used for

conjugating target components through chemistry similar to those employed in oligonucleotide synthesis. In some embodiments, a linker comprises an amide, ester, or ether group. In some embodiments, a linker has the structure of −L−. Target components can be conjugated through either the same or different linkers compared to lipids.

[001140] Target components, optionally through linkers, can be conjugated to oligonucleotides at various suitable locations. In some embodiments, target components are conjugated through the 5'-OH group. In some embodiments, target components are conjugated through the 3'-OH group. In some embodiments, target components are conjugated through one or more sugar moieties. In some embodiments, target components are conjugated through one or more bases. In some embodiments, target components are incorporated through one or more internucleotidic linkages. In some embodiments, an oligonucleotide may contain multiple conjugated target components which are independently conjugated through its 5'-OH, 3'-OH, sugar moieties, base moieties and/or internucleotidic linkages. Target components and lipids can be conjugated either at the same, neighboring and/or separated locations. In some embodiments, a target component is conjugated at one end of an oligonucleotide, and a lipid is conjugated at the other end.

[001141] In some embodiments, the oligonucleotide or oligonucleotides in a chirally controlled oligonucleotide composition is or are antisense oligonucleotide or oligonucleotides. In some embodiments, the sequence of the oligonucleotide(s) comprises or consists of the sequence of any oligonucleotide disclosed herein. In some embodiments, provided oligonucleotides comprise base sequence, pattern of backbone linkages, pattern or backbone chiral centers, and/or pattern of chemical modifications (*e.g.*, base modifications, sugar modifications, *etc.*) of any oligonucleotide disclosed herein. In some embodiments, the sequence of the oligonucleotide(s) comprises or consists of the sequence of any oligonucleotide disclosed in Table 8.

[001142] In some embodiments, an antisense oligonucleotide is an oligonucleotide which participates in RNaseH-mediated cleavage; for example, an antisense oligonucleotide hybridizes in a sequence-specific manner to a portion of a target mRNA, thus targeting the mRNA for cleavage by RNaseH. In some embodiments, an antisense oligonucleotide is able to differentiate between different alleles of the same gene or target. In some embodiments, an antisense oligonucleotide is able to differentiate between a wild-type and a mutant allele of a target. In

some embodiments, an antisense oligonucleotide significantly participates in RNaseH-mediated cleavage of a mutant allele but participates in RNaseH-mediated cleavage of a wild-type allele to a much less degree (e.g., does not significantly participate in RNaseH-mediated cleavage of the wild-type allele of the target). In some embodiments, an antisense oligonucleotide is capable of participating in RNAseH-mediated cleavage of a nucleic acid comprising a mutation. In some embodiments, an antisense oligonucleotide targets a mutant allele. In some embodiments, an antisense oligonucleotide targets a mutant allele of the Huntingtin gene.

[001143] In some embodiments, an antisense oligonucleotide is able to differentiate between a wild-type and a mutant allele of a target in the Huntingtin gene.

[001144] In some embodiments, the present disclosure pertains to:

[001145] A method for inhibiting expression of a mutant Huntingtin gene in a mammal comprising preparing a composition comprising a lipid and an oligonucleotide (as a non-limiting example, an antisense oligonucleotide that targets a mutant allele of the Huntingtin gene) and administering the composition to the mammal.

[001146] A method of treating a disease that is caused by the over-expression of a mutant Huntingtin gene in a subject, said method comprising the administration of a composition comprising a lipid and an oligonucleotide (as a non-limiting example, an antisense oligonucleotide that targets a mutant allele of the Huntingtin gene).

[001147] A method of treating Huntington's Disease, said method comprising the administration of a composition comprising a lipid and an oligonucleotide (as a non-limiting example, an antisense oligonucleotide that targets a mutant allele of the Huntingtin gene).

[001148] A method for treating a sign and/or symptom of Huntington's Disease in a subject by providing a composition comprising a lipid and an oligonucleotide (as a non-limiting example, an antisense oligonucleotide that targets a mutant allele of the Huntingtin gene) and administering a therapeutically effective amount of the composition to the subject.

[001149] A method of administering an oligonucleotide to a subject in need thereof, comprising steps of providing a composition comprising an oligonucleotide and a lipid, and administering the composition to the subject, wherein the biologically active compound is an oligonucleotide (as a non-limiting example, an antisense oligonucleotide that targets a mutant allele of the Huntingtin gene), and wherein the lipid is any lipid disclosed herein.

[001150] A method of treating a disease in a subject, the method comprising steps of providing a composition comprising an oligonucleotide and a lipid, and administering a therapeutically effective amount of the composition to the subject, wherein the biologically active compound is an oligonucleotide (as a non-limiting example, an antisense oligonucleotide that targets a mutant allele of the Huntingtin gene), and wherein the lipid is any lipid disclosed herein, and wherein the disease is any disease disclosed herein.

[001151] A method for inhibiting expression of a mutant Huntingtin gene in a mammal, the method comprising steps of preparing a composition comprising a lipid and an oligonucleotide (as a non-limiting example, an antisense oligonucleotide that targets a mutant allele of the Huntingtin gene) and administering the composition to the mammal.

[001152] A method of administering a biologically active agent to a subject in need thereof, comprising steps of providing a composition comprising a biologically active agent and a lipid, and administering the composition to the subject, wherein the biologically active compound is an oligonucleotide (as a non-limiting example, an antisense oligonucleotide that targets a mutant allele of the Huntingtin gene), and wherein the lipid is any lipid disclosed herein.

[001153] A method of treating Huntington's Disease in a subject, the method comprising steps of providing a composition comprising a biologically active agent and a lipid, and administering a therapeutically effective amount of the composition to the subject, wherein the biologically active compound is an oligonucleotide (as a non-limiting example, an antisense oligonucleotide that targets a mutant allele of the Huntingtin gene), and wherein the lipid is any lipid disclosed herein.

[001154] A method for mediating RNAseH-mediated cleavage of a nucleic acid comprising a mutant Huntingtin gene in a mammal, the method comprising steps of preparing a composition comprising a lipid and an antisense oligonucleotide and administering the composition to the mammal.

[001155] A method of treating a disease that is caused by a mutation in a Huntingtin gene, said method comprising the administration of a composition comprising a lipid and an antisense oligonucleotide, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a nucleic acid comprising the mutation.

[001156] A method for treating a sign and/or symptom of Huntington's Disease in a subject by providing a composition comprising a lipid and an oligonucleotide (as a non-limiting

example, an antisense oligonucleotide that targets a mutant allele of the Huntingtin gene) and administering a therapeutically effective amount of the composition to the subject.

[001157] A method of administering an oligonucleotide to a subject in need thereof, comprising steps of providing a composition comprising an oligonucleotide and a lipid, and administering the composition to the subject, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a nucleic acid comprising a mutation, and wherein the lipid is any lipid disclosed herein.

[001158] A method of treating Huntington's Disease in a subject, wherein the disease or disorder is related to a mutation in a gene, the method comprising steps of providing a composition comprising an oligonucleotide and a lipid, and administering a therapeutically effective amount of the composition to the subject, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a nucleic acid comprising the mutation, and wherein the lipid is any lipid disclosed herein.

[001159] A method for mediating RNAseH-mediated cleavage of a nucleic acid comprising a mutant Huntingtin gene in a mammal, the method comprising steps of preparing a composition comprising a lipid and an antisense oligonucleotide and administering the composition to the mammal.

[001160] A method of treating a disease related to a mutation in the Huntingtin gene, said method comprising the administration of a composition comprising a lipid and an antisense oligonucleotide, wherein the antisense oligonucleotide is capable of participating in RNaseH-mediated cleavage of a nucleic acid comprising the mutation.

[001161] A method of treating a disease that is caused by a mutation in the Huntingtin gene, said method comprising the administration of a composition comprising a lipid and an oligonucleotide, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a nucleic acid comprising the mutation.

[001162] A method for treating a sign and/or symptom of Huntington's Disease in a subject by providing a composition comprising a lipid and an oligonucleotide (as a non-limiting example, an antisense oligonucleotide that targets a mutant allele of the Huntingtin gene) and administering a therapeutically effective amount of the composition to the subject.

[001163] A method of administering an oligonucleotide to a subject in need thereof, comprising steps of providing a composition comprising an oligonucleotide and a lipid, and

administering the composition to the subject, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a nucleic acid comprising the mutation, and wherein the lipid is any lipid disclosed herein.

[001164] A method of treating Huntington's Disease in a subject, wherein the Huntington's Disease is related to a mutation in the Huntingtin gene, the method comprising steps of providing a composition comprising an oligonucleotide and a lipid, and administering a therapeutically effective amount of the composition to the subject, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a nucleic acid comprising the mutation, and wherein the lipid is any lipid disclosed herein.

[001165] A method for mediating RNAseH-mediated cleavage of a nucleic acid comprising a mutant Huntingtin gene in a mammal, the method comprising steps of preparing a composition comprising a lipid and an antisense oligonucleotide and administering the composition to the mammal, wherein the lipid is any lipid disclosed herein, and wherein the sequence of the antisense oligonucleotide comprises or consists of the sequence of any antisense oligonucleotide disclosed herein (e.g., in Table 8). In some embodiments, provided oligonucleotides comprise base sequence, pattern of backbone linkages, pattern or backbone chiral centers, and/or pattern of chemical modifications (*e.g.*, base modifications, sugar modifications, *etc.*) of any oligonucleotide disclosed herein (e.g., in Table 8).

[001166] A method of treating a disease related to a mutation in the Huntingtin gene, said method comprising the administration of a composition comprising a lipid and an antisense oligonucleotide, wherein the antisense oligonucleotide is capable of participating in RNaseH-mediated cleavage of a nucleic acid comprising the mutation, wherein the lipid is any lipid disclosed herein, and wherein the sequence of the antisense oligonucleotide comprises or consists of the sequence of any antisense oligonucleotide disclosed herein (e.g., in Table 8).

[001167] A method of treating a disease that is caused by a mutation in the Huntingtin gene, said method comprising the administration of a composition comprising a lipid and an oligonucleotide, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a nucleic acid comprising the mutation, wherein the lipid is any lipid disclosed herein, and wherein the oligonucleotide comprises or consists of the sequence of any antisense oligonucleotide disclosed herein (e.g., in Table 8).

[001168] A method for treating a sign and/or symptom of Huntington's Disease in a subject by providing a composition comprising a lipid and an oligonucleotide (as a non-limiting example, an antisense oligonucleotide that targets a mutant allele of the Huntingtin gene) and administering a therapeutically effective amount of the composition to the subject, wherein the lipid is any lipid disclosed herein, and wherein the sequence of the oligonucleotide comprises or consists of the sequence of any antisense oligonucleotide disclosed herein (e.g., in Table 8).

[001169] A method of administering an oligonucleotide to a subject in need thereof, comprising steps of providing a composition comprising an oligonucleotide and a lipid, and administering the composition to the subject, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a nucleic acid comprising the mutation, and wherein the lipid is any lipid disclosed herein, wherein the lipid is any lipid disclosed herein, and wherein the sequence of the oligonucleotide comprises or consists of the sequence of any antisense oligonucleotide disclosed herein (e.g., in Table 8).

[001170] A method of treating Huntington's Disease in a subject, wherein the Huntington's Disease is related to a mutation in the Huntingtin gene, the method comprising steps of providing a composition comprising an oligonucleotide and a lipid, and administering a therapeutically effective amount of the composition to the subject, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a nucleic acid comprising the mutation, and wherein the lipid is any lipid disclosed herein, and wherein the oligonucleotide comprises or consists of the sequence of any antisense oligonucleotide disclosed herein (e.g., in Table 8).

[001171] In some embodiments, provided oligonucleotides comprise base sequence, pattern of backbone linkages, pattern or backbone chiral centers, and/or pattern of chemical modifications (*e.g.*, base modifications, sugar modifications, *etc.*) of any oligonucleotide disclosed herein.

[001172] In some embodiments, an oligonucleotide composition comprises a plurality of oligonucleotides, which share:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone phosphorus modifications;

wherein one or more oligonucleotides of the plurality are individually conjugated to a lipid.

[001173] In some embodiments, a chirally controlled oligonucleotide composition comprises a plurality of oligonucleotides, which share:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone phosphorus modifications;

wherein:

the composition is chirally controlled in that the plurality of oligonucleotides share the same stereochemistry at one or more chiral internucleotidic linkages;

one or more oligonucleotides of the plurality are individually conjugated to a lipid; and

one or more oligonucleotides of the plurality are optionally and individually conjugated to a targeting compound or moiety.

[001174] In some embodiments, an antisense oligonucleotide is in a chirally controlled oligonucleotide composition. In some embodiments, an oligonucleotide is in a chirally controlled oligonucleotide composition.

[001175] Various oligonucleotides are listed in Table 8. Many of these are capable of participating in RNaseH-mediated claeavage of the human Huntingtin gene, as shown in data presented in U.S. Pat. Application No. 62/195,779, filed 7/22/15, and U.S. Pat. Application No. 62/331,960, filed 5/4/16, which are incorporated by reference in its entirety; and in data shown here.

[001176] Various oligonucleotides particularly capable of participating in RNaseH-mediated cleavage of human Huntingtin gene target or a mutant variant thereof include: WV-1092, WVE120101, WV-2603 or WV-2595, or any other nucleic acid disclosed herein (including, but not limited to, those listed in Table 8).

[001177] Any oligonucleotide or chirally controlled oligonucleotide composition can be used in combination with any method or composition (e.g., any pharmaceutical composition, modification, and/or method of use and/or manufacture) disclosed herein.

[001178] In some embodiments, the present disclosure provides the following embodiments:

1. A chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

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

2. A chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

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

2a. A chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type, wherein the oligonucleotides target a mutant Huntingtin gene, and the length is from about 10 to about 50 nucleotides, wherein the backbone linkages comprise at least one phosphorothioate, and wherein the pattern of backbone chiral centers comprises at least one chiral center in a Rp conformation and at least one chiral center in a Sp conformation.

3. A chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which composition is a substantially pure preparation of a single oligonucleotide in that at least about 10% of the oligonucleotides in

the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers.

4. A composition of any one of the preceding embodiments, wherein the oligonucleotides comprise one or more wing regions and a common core region, wherein:

each wing region independently has a length of two or more bases, and independently and optionally comprises one or more chiral internucleotidic linkages; and

the core region independently has a length of two or more bases and independently comprises one or more chiral internucleotidic linkages.

5. An oligonucleotide composition comprising a predetermined level of oligonucleotides which comprise one or more wing regions and a common core region, wherein:

each wing region independently has a length of two or more bases, and independently and optionally comprises one or more chiral internucleotidic linkages;

the core region independently has a length of two or more bases, and independently comprises one or more chiral internucleotidic linkages, and the common core region has:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers.

6. An oligonucleotide composition comprising a predetermined level of oligonucleotides which comprise one or more wing regions and a common core region, wherein:

each wing region independently has a length of two or more bases, and independently and optionally comprises one or more chiral internucleotidic linkages;

the core region independently has a length of two or more bases, and independently comprises one or more chiral internucleotidic linkages, and the core region has:

1) a common base sequence;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers; and

4) a common pattern of backbone phosphorus modifications.

6a. A composition of any one of the preceding embodiments, wherein oligonucleotides of the oligonucleotide type comprises at least one wing region and a core region, wherein:

each wing region independently has a length of two or more bases, and independently and optionally comprises one or more chiral internucleotidic linkages;

the core region independently has a length of two or more bases, and independently comprises one or more chiral internucleotidic linkages; and

wherein at least one nucleotide in a wing region differs from at least one nucleotide of the core region, wherein the difference is in one or more of:

1) backbone linkage;

2) pattern of backbone chiral centers;

3) sugar modification .

7. A composition of any one of the preceding embodiments, wherein the oligonucleotides are defined by having a common pattern of backbone phosphorus modifications.

8. A composition of any one of the preceding embodiments, wherein the composition contains a predetermined level of oligonucleotides of an individual oligonucleotide type, wherein an oligonucleotide type is defined by:

1) base sequence;

2) pattern of backbone linkages;

3) pattern of backbone chiral centers; and

4) pattern of backbone phosphorus modifications.

9. A composition of any one of the preceding embodiments, wherein oligonucleotides having a common base sequence is of the same oligonucleotide type characterized by base sequence, pattern of backbone linkages, pattern of backbone chiral centers, and pattern of backbone phosphorus modifications.

10. A composition of any one of the preceding embodiments, wherein the composition contains predetermined levels of oligonucleotides of two or more individual oligonucleotide types, wherein an oligonucleotide type is defined by:

1) base sequence;

2) pattern of backbone linkages;

3) pattern of backbone chiral centers; and

4) pattern of backbone phosphorus modifications.

11. An oligonucleotide composition that is chirally controlled in that the composition contains a predetermined level of oligonucleotides of an individual oligonucleotide type, wherein an oligonucleotide type is defined by:

1) base sequence;

2) pattern of backbone linkages;

3) pattern of backbone chiral centers; and

4) pattern of backbone phosphorus modifications.

12. A composition of any one of the preceding embodiments, wherein the composition comprises two or more individual oligonucleotide types.

13. A composition of any one of the preceding embodiments, wherein an oligonucleotide type is defined by base identity, pattern of base modification, pattern of sugar modification, pattern of backbone linkages, pattern of backbone chiral centers, and pattern of backbone phosphorus modifications.

14. A composition of any one of the preceding embodiments, wherein oligonucleotides having a common sequence have identical structure.

15. A composition of any one of the preceding embodiments, wherein oligonucleotides of the same oligonucleotide type have identical structure.

16. A composition of any one of the preceding embodiments, wherein the oligonucleotides have one wing.

17. A composition of any one of the preceding embodiments, wherein the oligonucleotides are hemimers having the structure of wing-core.

18. A composition of any one of the preceding embodiments, wherein the oligonucleotides are hemimers having the structure of core-wing.

19. A composition of any one of embodiments 1-15, wherein the oligonucleotides have two wings.

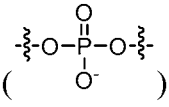
20. A composition of any one of embodiments 1-15, wherein the oligonucleotides are gapmers having the structure of wing-core-wing.

21. A composition of any one of the preceding embodiments, wherein a wing comprises a chiral internucleotidic linkage.

22. A composition of any one of the preceding embodiments, wherein each wing independently comprises a chiral internucleotidic linkage.

23. A composition of any one of embodiments 1-17 and 19-22, wherein a wing to the 5'-end of the core comprises a chiral internucleotidic linkage at the 5'-end of the wing.

24. A composition of any one of embodiments 1-16 and 18-22, wherein a wing to the 3'-end of the core comprises a chiral internucleotidic linkage at the 3'-end of the wing.

25. A composition of any one the preceding embodiments, wherein a wing has only one chiral internucleotidic linkage, and each of the other internucleotidic linkages of the wing is a natural phosphate linkage ().

26. A composition of any one of the preceding embodiments, wherein the chiral internucleotidic linkage has the structure of formula I.

27. A composition of any one of the preceding embodiments, wherein a chiral internucleotidic linkage has the structure of formula I, and wherein X is S, and Y and Z are O.

28. A composition of any one of the preceding embodiments, wherein a chiral internucleotidic linkage is a phosphorothioate linkage.

29. A composition of any one of the preceding embodiments, wherein a chiral internucleotidic linkage is *S*p.

30. A composition of any one of the preceding embodiments, wherein each chiral internucleotidic linkage is *S*p.

31. A composition of any one of embodiments 1-29, wherein a chiral internucleotidic linkage is *R*p.

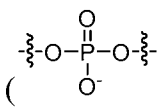
32. A composition of any one of embodiments 1-28, wherein each chiral internucleotidic linkage is *R*p.

33. A composition of any one of embodiments 1-31, wherein a wing comprises an *S*p phosphorothioate linkage.

34. A composition of any one of embodiments 1-31, wherein each wing independently comprises an *S*p phosphorothioate linkage.

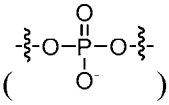
35. A composition of any one of embodiments 1-17, 19-31, and 33-34, wherein a wing is to the 5'-end of the core, and the wing has an *S*p phosphorothioate linkage.

36. A composition of any one of embodiments 1-17, 19-31, and 33-35, wherein a wing is to the 5'-end of the core, and the wing has an *S*p phosphorothioate linkage at the 5'-end of the wing.

37. A composition of any one of embodiments 1-17, 19-31, and 33-36, wherein a wing is to the 5'-end of the core, the wing has an *S*p phosphorothioate linkage at the 5'-end of the wing, and each of the other internucleotidic linkages of the wing is a natural phosphate linkage ().

38. A composition of any one of embodiments 1-16, 18-31 and 33-34, wherein a wing is to the 3'-end of the core, and the wing has an *S*p phosphorothioate linkage at the 3'-end of the wing.

39. A composition of any one of embodiments 1-16, 18-31, 33-34 and 38, wherein a wing is to the 3'-end of the core, and the wing has an *S*p phosphorothioate linkage at the 3'-end of the wing.

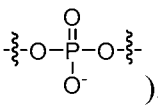
40. A composition of any one of embodiments 1-16, 18-31, 33-34 and 38-39, wherein one wing is to the 3'-end of the common core, the wing has an *S*p phosphorothioate linkage at the 3'-end of the wing, and each of the other internucleotidic linkages of the wing is a natural phosphate linkage ().

41. A composition of any one of embodiments 1-29 and 31-40, wherein a wing comprises an *R*p phosphorothioate linkage.

42. A composition of any one of embodiments 1-29 and 31-40, wherein each wing independently comprises an *R*p phosphorothioate linkage.

43. A composition of any one of embodiments 1-17, 19-29 and 31-42, wherein a wing is to the 5'-end of the core, and the wing has an *R*p phosphorothioate linkage.

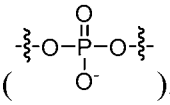
44. A composition of any one of embodiments 1-17, 19-29 and 31-43, wherein a wing is to the 5'-end of the core, and the wing has an *R*p phosphorothioate linkage at the 5'-end of the wing.

45. A composition of any one of embodiments 1-17, 19-29 and 31-44, wherein a wing is to the 5'-end of the core, the wing has an *R*p phosphorothioate linkage at the 5'-end of the wing, and each of the other internucleotidic linkages of the wing is a natural phosphate linkage ().

46. A composition of any one of embodiments 1-16, 18-29 and 31-42, wherein a wing is to the 3'-end of the core, and the wing has an *R*p phosphorothioate.

47. A composition of any one of embodiments 1-16, 18-29 and 31-42, wherein a wing is to the 3'-end of the core, and the wing has an *R*p phosphorothioate linkage at the 3'-end of the wing.

48. A composition of any one of embodiments 1-16, 18-29 and 31-42, wherein one wing is to the 3'-end of the common core, the wing has an *R*p phosphorothioate linkage at the 3'-end of the

wing, and each of the other internucleotidic linkages of the wing is a natural phosphate linkage ().

49. A composition of any one of embodiments 1-28, wherein a wing is to the 5'-end of a core, and its 5'-end internucleotidic linkage is a chiral internucleotidic linkage.

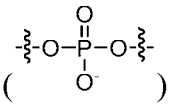
50. A composition of any one of embodiments 1-28, wherein a wing is to the 5'-end of a core, and its 5'-end internucleotidic linkage is an *S*p chiral internucleotidic linkage.

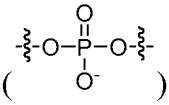
51. A composition of any one of embodiments 1-28, wherein a wing is to the 5'-end of a core, and its 5'-end internucleotidic linkage is an *R*p chiral internucleotidic linkage.

52. A composition of any one of embodiments 1-28 and 49-51, wherein a wing is to the 3'-end of a core, and its 3'-end internucleotidic linkage is a chiral internucleotidic linkage.

53. A composition of any one of embodiments 1-28 and 49-51, wherein a wing is to the 3'-end of a core, and its 3'-end internucleotidic linkage is an *S*p chiral internucleotidic linkage.

54. A composition of any one of embodiments 1-28 and 49-51, wherein a wing is to the 3'-end of a core, and its 3'-end internucleotidic linkage is an *R*p chiral internucleotidic linkage.

55. A composition of any one of the preceding embodiments, wherein each wing independently comprises a natural phosphate linkage ().

56. A composition of any one of the preceding embodiments, wherein each wing independently comprises two or more natural phosphate linkages ().

57. A composition of any one of the preceding embodiments, wherein each wing independently comprises two or more natural phosphate linkages, and all natural phosphate linkages are consecutive.

58. A composition of any one of the preceding embodiments, wherein a wing has a length of three or more bases.

59. A composition of any one of the preceding embodiments, wherein one wing has a length of four or more bases.

60. A composition of any one of the preceding embodiments, wherein one wing has a length of five or more bases.

61. A composition of any one of the preceding embodiments, wherein one wing has a length of six or more bases.

62. A composition of any one of the preceding embodiments, wherein one wing has a length of seven or more bases.

63. A composition of any one of the preceding embodiments, wherein one wing has a length of eight or more bases.

64. A composition of any one of the preceding embodiments, wherein one wing has a length of nine or more bases.

65. A composition of any one of the preceding embodiments, wherein one wing has a length of ten or more bases.

66. A composition of any one of the preceding embodiments, wherein each wing independently has a length of three or more bases.

67. A composition of any one of the preceding embodiments, wherein each wing independently has a length of four or more bases.

68. A composition of any one of the preceding embodiments, wherein each wing independently has a length of five or more bases.

69. A composition of any one of the preceding embodiments, wherein each wing independently has a length of six or more bases.

70. A composition of any one of the preceding embodiments, wherein each wing independently has a length of seven or more bases.

71. A composition of any one of the preceding embodiments, wherein each wing independently has a length of eight or more bases.

72. A composition of any one of the preceding embodiments, wherein each wing independently has a length of nine or more bases.

73. A composition of any one of the preceding embodiments, wherein each wing independently has a length of ten or more bases.

74. A composition of any one of embodiments 1-57, wherein a wing has a length of two bases.

75. A composition of any one of embodiments 1-57, wherein a wing has a length of three bases.

76. A composition of any one of embodiments 1-57, wherein a wing has a length of four bases.

77. A composition of any one of embodiments 1-57, wherein a wing has a length of five bases.

78. A composition of any one of embodiments 1-57, wherein a wing has a length of six bases.

79. A composition of any one of embodiments 1-57, wherein a wing has a length of seven bases.

80. A composition of any one of embodiments 1-57, wherein a wing has a length of eight bases.

81. A composition of any one of embodiments 1-57, wherein a wing has a length of nine bases.

82. A composition of any one of embodiments 1-57, wherein a wing has a length of ten bases.

83. A composition of any one of embodiments 1-57, wherein a wing has a length of 11 bases.

84. A composition of any one of embodiments 1-57, wherein a wing has a length of 12 bases.

85. A composition of any one of embodiments 1-57, wherein a wing has a length of 13 bases.

86. A composition of any one of embodiments 1-57, wherein a wing has a length of 14 bases.

87. A composition of any one of embodiments 1-57, wherein a wing has a length of 15 bases.

88. A composition of any one of embodiments 1-11 and 15-76, wherein each wing has the same length.

89. A composition of any one of the preceding embodiments, wherein a wing is defined by sugar modifications relative to a core.

90. A composition of any one of the preceding embodiments, wherein each wing independently comprises a modified sugar moiety.

91. A composition of any one of the preceding embodiments, wherein each wing sugar moiety is independently a modified sugar moiety.

92. A composition of any one of the preceding embodiments, wherein a modified sugar moiety comprises a high-affinity sugar modification.

93. A composition of any one of the preceding embodiments, wherein a modified sugar moiety has a 2'-modification.

94. A composition of any one of the preceding embodiments, wherein a modified sugar moiety comprises a bicyclic sugar modification.

95. A composition of any one of the preceding embodiments, wherein a modified sugar moiety comprises a bicyclic sugar modification having a −L− or −O−L− bridge connecting two ring carbon atoms.

96. A composition of any one of the preceding embodiments, wherein a modified sugar moiety comprises a bicyclic sugar modification having a 4'-$CH(CH_3)$-O-2' bridge.

97. A composition of any one of embodiments 1-93, wherein a modified sugar moiety comprises a 2'-modification, wherein a 2'-modification is 2'-OR^1.

98. A composition of any one of embodiments 1-93, wherein a modified sugar moiety comprises a 2'-modification, wherein a 2'-modification is 2'-OR^1, wherein R^1 is optionally substituted C_{1-6} alkyl.

99. A composition of any one of embodiments 1-93, wherein a modified sugar moiety comprises a 2'-modification, wherein a 2'-modification is 2'-MOE.

100. A composition of any one of embodiments 1-93, wherein a modified sugar moiety comprises a 2'-modification, wherein a 2'-modification is 2'-OMe.

101. A composition of any one of embodiments 1-96, wherein a modified sugar moiety comprises a 2'-modification, wherein the 2'-modification is *S*-cEt.

102. A composition of any one of embodiments 1-93, wherein a modified sugar moiety comprises a 2'-modification, wherein the 2'-modification is FANA.

103. A composition of any one of embodiments 1-93, wherein a modified sugar moiety comprises a 2'-modification, wherein the 2'-modification is FRNA.

104. A composition of any one of embodiments 1-92, wherein a modified sugar moiety has a 5'-modification.

105. A composition of any one of embodiments 1-92, wherein a modified sugar moiety is *R*-5'-Me-DNA.

106. A composition of any one of embodiments 1-92, wherein a modified sugar moiety is *S*-5'-Me-DNA.

107. A composition of any one of embodiments 1-92, wherein a modified sugar moiety is FHNA.

108. A composition of any one of the preceding embodiments, wherein each wing sugar moiety is modified.

109. A composition of any one of the preceding embodiments, wherein all modified wing sugar moieties within a wing have the same modification.

110. A composition of any one of the preceding embodiments, wherein all modified wing sugar moieties have the same modification.

111. A composition of any one of embodiments 1-108, wherein at least one modified wing sugar moiety is different than another modified wing sugar moiety.

112. A composition of any one of the preceding embodiments, wherein a wing comprises a modified base.

113. A composition of any one of the preceding embodiments, wherein a wing comprises a 2S-dT.

114. A composition of any one of the preceding embodiments, wherein the core region has a length of five or more bases.

115. A composition of any one of the preceding embodiments, wherein the core region has a length of six or more bases.

116. A composition of any one of the preceding embodiments, wherein the core region has a length of seven or more bases.

117. A composition of any one of the preceding embodiments, wherein the core region has a length of eight or more bases.

118. A composition of any one of the preceding embodiments, wherein the core region has a length of nine or more bases.

119. A composition of any one of the preceding embodiments, wherein the core region has a length of ten or more bases.

120. A composition of any one of the preceding embodiments, wherein the core region has a length of 11 or more bases.

121. A composition of any one of the preceding embodiments, wherein the core region has a length of 12 or more bases.

122. A composition of any one of the preceding embodiments, wherein the core region has a length of 13 or more bases.

123. A composition of any one of the preceding embodiments, wherein the core region has a length of 14 or more bases.

124. A composition of any one of the preceding embodiments, wherein the core region has a length of 15 or more bases.

125. A composition of any one of 1-113, wherein the core region has a length of five bases.

126. A composition of any one of 1-113, wherein the core region has a length of six bases.

127. A composition of any one of 1-113, wherein the core region has a length of seven bases.

128. A composition of any one of 1-113, wherein the core region has a length of eight bases.

129. A composition of any one of 1-113, wherein the core region has a length of nine bases.

130. A composition of any one of 1-113, wherein the core region has a length of ten bases.

131. A composition of any one of 1-113, wherein the core region has a length of 11 bases.

132. A composition of any one of 1-113, wherein the core region has a length of 12 bases.

133. A composition of any one of 1-113, wherein the core region has a length of 13 bases.

134. A composition of any one of 1-113, wherein the core region has a length of 14 bases.

135. A composition of any one of 1-113, wherein the core region has a length of 15 bases.

136. A composition of any one of the preceding embodiments, wherein the core region does not have any 2'-modification.

137. A composition of any one of the preceding embodiments, wherein each core sugar moiety is not modified.

138. A composition of any one of the preceding embodiments, wherein each sugar moiety of the core region is the natural DNA sugar moiety.

139. A composition of any one of the preceding embodiments, wherein the core region comprises a chiral internucleotidic linkage.

140. A composition of any one of the preceding embodiments, wherein each internucleotidic linkage of the core region is a chiral internucleotidic linkage.

141. A composition of any one of the preceding embodiments, wherein each internucleotidic linkage of the core region is a chiral internucleotidic linkage having the structure of formula I.

142. A composition of any one of the preceding embodiments, wherein each internucleotidic linkage of the core region is a chiral internucleotidic linkage having the structure of formula I, and wherein X is S, and Y and Z are O.

143. A composition of any one of the preceding embodiments, wherein each internucleotidic linkage of the core region is a chiral internucleotidic linkage having the structure of formula I, and wherein one $-L-R^1$ is not $-H$.

144. A composition of any one of embodiments 1-142, wherein each internucleotidic linkage of the core region is a phosphorothioate linkage.

145. A composition of any one of the preceding embodiments, wherein the core region has a pattern of backbone chiral center comprises $(Sp)_m(Rp)_n$, wherein m is 1-50, and n is 1-10.

146. A composition of any one of the preceding embodiments, wherein the core region has a pattern of backbone chiral center comprises $(Sp)_m(Rp)_n$, wherein m is 1-50, n is 1-10, and m>n.

147. A composition of any one of the preceding embodiments, wherein the core region has a pattern of backbone chiral center comprises $(Sp)_m(Rp)_n$, wherein m is 2, 3, 4, 5, 6, 7 or 8, and n is 1.

148. A composition of any one of embodiments 1-144, wherein the core region has a pattern of backbone chiral centers comprising $(Rp)_n(Sp)_m$, wherein m is 1-50 and n is 1-10.

149. A composition of any one of embodiments 1-144 and 148, wherein the core region has a pattern of backbone chiral centers comprising $Rp(Sp)_m$, wherein m is 2, 3, 4, 5, 6, 7 or 8.

150. A composition of any one of embodiments 1-144 and 148-149, wherein the core region has a pattern of backbone chiral centers comprising $Rp(Sp)_2$.

151. A composition of any one of embodiments 1-144, wherein the core region has a pattern of backbone chiral centers comprising $(Np)_t(Rp)_n(Sp)_m$, wherein t is 1-10, n is 1-10, m is 1-50, and each *N*p is independent *R*p or *S*p.

152. A composition of any one of embodiments 1-144 and 151, wherein the core region has a pattern of backbone chiral centers comprising $(Sp)_t(Rp)_n(Sp)_m$, wherein t is 1-10, n is 1-10, m is 1-50.

153. A composition of any one of embodiments 1-144 and 151-152, wherein n is 1.

154. A composition of any one of embodiments 1-144 and 151-153, wherein t is 2, 3, 4, 5, 6, 7 or 8.

155. A composition of any one of embodiments 1-144 and 151-154, wherein m is 2, 3, 4, 5, 6, 7 or 8.

156. A composition of any one of embodiments 1-144 and 151-155, wherein at least one of t and m is greater than 5.

157. A composition of any one of the preceding embodiments, wherein the core region has a pattern of backbone chiral centers comprising *S*p*S*p*R*p*S*p*S*p.

158. A composition of any one of the preceding embodiments, wherein 50% or more of the chiral internucleotidic linkages in the core region have *S*p configuration.

159. A composition of any one of the preceding embodiments, wherein 60% or more of the chiral internucleotidic linkages in the core region have *S*p configuration.

160. A composition of any one of the preceding embodiments, wherein 70% or more of the chiral internucleotidic linkages in the core region have *S*p configuration.

161. A composition of any one of the preceding embodiments, wherein 80% or more of the chiral internucleotidic linkages in the core region have *S*p configuration.

162. A composition of any one of the preceding embodiments, wherein 90% or more of the chiral internucleotidic linkages in the core region have *S*p configuration.

163. A composition of any one of the preceding embodiments, wherein each internucleotidic linkage in the core region is chiral, the core region has only one *R*p, and each of the other internucleotidic linkages in the core region is *S*p.

164. A composition of any one of the preceding embodiments, wherein each base moiety in the core is not modified.

165. A composition of any one of embodiments 1-163, wherein the core region comprises a modified base.

166. A composition of any one of embodiments 1-163, wherein the core region comprises a modified base, wherein a modified base is substituted A, T, C or G.

167. A composition of any one of embodiments 1-164, wherein each base moiety in the core region is independently selected from A, T, C and G.

168. A composition of any one of embodiments 1-163, wherein the core region is a DNA sequence whose phosphate linkages are independently replaced with phosphorothioate linkages.

169. A composition of any one of the preceding embodiments, wherein the oligonucleotides are single stranded.

170. A composition of any one of the preceding embodiments, wherein the oligonucleotides are antisense oligonucleotide, antagomir, microRNA, pre-microRNs, antimir, supermir, ribozyme, Ul adaptor, RNA activator, RNAi agent, decoy oligonucleotide, triplex forming oligonucleotide, aptamer or adjuvant.

171. A composition of any one of the preceding embodiments, wherein the oligonucleotides are antisense oligonucleotides.

172. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 10 bases.

173. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 11 bases.

174. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 12 bases.

175. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 13 bases.

176. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 14 bases.

177. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 15 bases.

178. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 16 bases.

179. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 17 bases.

180. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 18 bases.

181. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 19 bases.

182. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 20 bases.

183. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 21 bases.

184. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 22 bases.

185. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 23 bases.

186. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 24 bases.

187. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of greater than 25 bases.

188. A composition of any one of the preceding embodiments, wherein the oligonucleotides have a length of less than about 200 bases.

189. A composition of any one of the preceding embodiments, wherein the oligonucleotides have a length of less than about 150 bases.

190. A composition of any one of the preceding embodiments, wherein the oligonucleotides have a length of less than about 100 bases.

191. A composition of any one of the preceding embodiments, wherein the oligonucleotides have a length of less than about 50 bases.

192. A composition of any one of the preceding embodiments, wherein the oligonucleotides have a length of less than about 40 bases.

193. A composition of any one of the preceding embodiments, wherein the oligonucleotides have a length of less than about 30 bases.

194. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 10 bases.

195. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 11 bases.

196. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 12 bases.

197. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 13 bases.

198. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 14 bases.

199. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 15 bases.

200. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 16 bases.

201. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 17 bases.

202. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 18 bases.

203. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 19 bases.

204. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 20 bases.

205. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 21 bases.

206. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 22 bases.

207. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 23 bases.

208. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 24 bases.

209. A composition of any one of embodiments 1-171, wherein the oligonucleotides have a length of 25 bases.

210. A composition of any one of the preceding embodiments, wherein the oligonucleotide type is not (*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G] or (*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p)-Gs5mCs5mCsTs5mCsAsGsTs5mCsTsGs5mCsTsTs5mCsGs5mCsAs5mCs5mC (5R-(SSR)3-5R), wherein in the underlined nucleotide are 2'-MOE modified.

211. A composition of any one of the preceding embodiments, wherein the oligonucleotide is not an oligonucleotide selected from: (*S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p)-d[5mCs1As1Gs1Ts15mCs1Ts1Gs15mCs1Ts1Ts15mCs1G] or (*R*p, *R*p, *R*p, *R*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *R*p, *R*p, *R*p, *R*p, *R*p)-Gs5mCs5mCsTs5mCsAsGsTs5mCsTsGs5mCsTsTs5mCsGs5mCsAs5mCs5mC (5R-(SSR)3-5R), wherein in the underlined nucleotide are 2'-MOE modified.

212. A composition of any one of the preceding embodiments, wherein the oligonucleotide is not an oligonucleotide selected from:

ONT-106	(*R*p)- uucuAGAccuGuuuuGcuudT*s*dT	PCSK9 sense

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

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

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

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

PCSK9 (7)	(All (*R*p))- AsAsGscsAsAsAsAscsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (8)	(All (*S*p))- AsAsGscsAsAsAsAscsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (9)	(All (*R*p))- AsAGcAAAAcsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (10)	(All (*S*p))- AsAGcAAAAcsAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (11)	(All (*R*p))- AAsGscsAsAsAsAscAGGUCuAGAAdTsdT
PCSK9 (12)	(All (*S*p))- AAsGscsAsAsAsAscAGGUCuAGAAdTsdT
PCSK9 (13)	(All (*R*p))- AsAsGscAsAsAsAscAsGsGsUsCsuAsGsAsAsdTsdT
PCSK9 (14)	(All (*S*p))- AsAsGscAsAsAsAscAsGsGsUsCsuAsGsAsAsdTsdT
PCSK9 (15)	(All (*R*p))- AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT

PCSK9 (16)	(All (*S*p))- AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (17)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)- AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT
PCSK9 (18)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)- AsAGcAAAsAscAsGsGsUsCsusAsGsAsAsdTsdT

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

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

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

PCSK9 (25)	(All (*R*p))- asAfsgsCfsasAfsasAfscsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (26)	(All (*S*p))- asAfsgsCfsasAfsasAfscsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (27)	(All (*R*p))- asAfgCfaAfaAfcsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (28)	(All (*S*p))- asAfgCfaAfaAfcsAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (29)	(All (*R*p))- asAfsgCfsaAfsaAfscAfsgGfsuCfsuAfsgAfsadTsdT
PCSK9 (30)	(All (*S*p))- asAfsgCfsaAfsaAfscAfsgGfsuCfsuAfsgAfsadTsdT
PCSK9 (31)	(*R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p)- asAfgCfaAfasAfscAfsgsGfsusCfsusAfsgsAfsasdTsdT
PCSK9 (32)	(*S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p, *S*p, *R*p)- asAfgCfaAfasAfscAfsgsGfsusCfsusAfsgsAfsasdTsdT

213. A composition of any one of the preceding embodiments, wherein the oligonucleotide is not an oligonucleotide selected from:

d[$A_RC_SA_RC_SA_RC_SA_RC_SA_RC$], d[$C_SC_SC_SC_RC_RC_SC_SC_SC_SC$], d[$C_SC_SC_SC_SC_SC_SC_RC_RC_SC$] and d[$C_SC_SC_SC_SC_SC_RC_RC_SC_SC$], wherein R is *R*p phosphorothioate linkage, and S is *S*p phosphorothioate linkage.

214. A composition of any one of the preceding embodiments, wherein the oligonucleotide is not an oligonucleotide selected from: $GGA_RT_SG_RT_ST_R{}^mC_STCGA$, $GGA_RT_RG_ST_ST_R{}^mC_RTCGA$, $GGA_ST_SG_RT_RT_S{}^mC_STCGA$, wherein R is *R*p phosphorothioate linkage, S is *S*p phosphorothioate linkage, all other linkages are PO, and each ^mC is a 5-methylcytosine modified nucleoside.

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

215a. A composition of any one of the preceding embodiments, wherein the common pattern of backbone chiral centers comprises SSR, RSS, SSRSS, SSRSSR, RSSSRSRRRS, RSSSSSSSSS, SRRSRSSSSR, SRSRSSRSSR, RRRSSSRSSS, RRRSRSSRSR, RRSSSRSRSR, SRSSSRSSSS, SSRRSSRSRS, SSSSSSRRSS, RRRSSRRRSR, RRRRSSSSRS, SRRSRRRRRR, RSSRSSRRRR, RSRRSRRSRR, RRSRSSRSRS, SSRRRRRSRR, RSRRSRSSSR, RRSSRSRRRR, RRSRSRRSSS, RRSRSSSRRR, RSRRRRSRSR, SSRSSSRRRS, RSSRSRSRSR, RSRSRSSRSS, RRRSSRRSRS, SRRSSRRSRS, RRRRSRSRRR, or SSSSRRRRSR.

215b. A composition of any one of the preceding embodiments, wherein the common pattern of backbone chiral centers comprises SSRSS, SSRSSR, RSSSRSRRRS, RSSSSSSSSS, SRRSRSSSSR, SRSRSSRSSR, RRRSSSRSSS, RRRSRSSRSR, RRSSSRSRSR, SRSSSRSSSS, SSRRSSRSRS, SSSSSSRRSS, RRRSSRRRSR, RRRRSSSSRS, SRRSRRRRRR,

RSSRSSRRRR, RSRRSRRSRR, RRSRSSRSRS, SSRRRRRSRR, RSRRSRSSSR, RRSSRSRRRR, RRSRSRRSSS, RRSRSSSRRR, RSRRRRSRSR, SSRSSSRRRS, RSSRSRSRSR, RSRSRSSRSS, RRRSSRRSRS, SRRSSRRSRS, RRRRSRSRRR, or SSSSRRRRSR.

215c. A composition of any one of the preceding embodiments, wherein the common pattern of backbone chiral centers comprises RSSSRSRRRS, RSSSSSSSSS, SRRSRSSSSR, SRSRSSRSSR, RRRSSSRSSS, RRRSRSSRSR, RRSSSRSRSR, SRSSSRSSSS, SSRRSSRSRS, SSSSSSRRSS, RRRSSRRRSR, RRRRSSSSRS, SRRSRRRRRR, RSSRSSRRRR, RSRRSRRSRR, RRSRSSRSRS, SSRRRRRSRR, RSRRSRSSSR, RRSSRSRRRR, RRSRSRRSSS, RRSRSSSRRR, RSRRRRSRSR, SSRSSSRRRS, RSSRSRSRSR, RSRSRSSRSS, RRRSSRRSRS, SRRSSRRSRS, RRRRSRSRRR, or SSSSRRRRSR.

216. A composition of any one of the preceding embodiments, wherein the oligonucleotide is not an oligonucleotide selected from: $T_kT_k{}^mC_k\underline{AGT{}^mCATGA{}^mCT}T_k{}^mC_k{}^mC_k$, wherein each nucleoside followed by a subscript 'k' indicates a (S)-cEt modification, R is *R*p phosphorothioate linkage, S is *S*p phosphorothioate linkage, each ^mC is a 5-methylcytosine modified nucleoside and all internucleoside linkages in the underlined core are phosphorothioates (PS) with stereochemistry patterns selected from: RSSSRSRRRS, RSSSSSSSSS, SRRSRSSSSR, SRSRSSRSSR, RRRSSSRSSS, RRRSRSSRSR, RRSSSRSRSR, SRSSSRSSSS, SSRRSSRSRS, SSSSSSRRSS, RRRSSRRRSR, RRRRSSSSRS, SRRSRRRRRR, RSSRSSRRRR, RSRRSRRSRR, RRSRSSRSRS, SSRRRRRSRR, RSRRSRSSSR, RRSSRSRRRR, RRSRSRRSSS, RRSRSSSRRR, RSRRRRSRSR, SSRSSSRRRS, RSSRSRSRSR, RSRSRSSRSS, RRRSSRRSRS, SRRSSRRSRS, RRRRSRSRRR, SSSSRRRRSR, RRRRRRRRRR and SSSSSSSSSS.

217. A composition of embodiment 215 or 216, wherein each phosphorothioate moiety of each nucleotide comprising (S)-cEt modification is stereorandom.

218. A composition of any one of the preceding embodiments, wherein the base sequence is or comprises a sequence that is complementary to a target sequence, wherein when contacted with a nucleic acid polymer comprising the target sequence, the composition provides an altered cleavage pattern than a reference cleavage pattern from a reference oligonucleotide composition.

219. A composition of any one of the preceding embodiments, wherein the nucleic acid polymer is RNA, and a reference oligonucleotide composition is a substantially racemic preparation of oligonucleotides that share the common sequence and length.

220. A composition of any one of the preceding embodiments, wherein the nucleic acid polymer is RNA, and a reference oligonucleotide composition is a chirally uncontrolled oligonucleotide composition of oligonucleotides that share the common sequence and length.

221. A composition of any one of the preceding embodiments, wherein the altered cleavage pattern has fewer cleavage sites than the reference cleavage pattern.

222. A composition of any one of the preceding embodiments, wherein the altered cleavage pattern has only one cleavage site within the target sequence, and the reference cleavage pattern has two or more cleavage sites within the target sequence.

223. A composition of any one of the preceding embodiments, wherein the base sequence for the oligonucleotides is or comprises a sequence that is complementary to a characteristic sequence element that defines a particular allele of a target gene relative to other alleles of the same target gene that exist in a population, the composition being characterized in that, when it is contacted with a system expressing transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

224. A composition of any one of the preceding embodiments, wherein the base sequence for the oligonucleotides is or comprises a sequence that is complementary to a characteristic sequence element that defines a particular allele of a target gene relative to other alleles of the same target gene that exist in a population, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

225. A composition of any one of the preceding embodiments, wherein the base sequence comprises a sequence that is complementary to a characteristic sequence element of a target, wherein a characteristic sequence element defines that target sequence relative to a similar sequence.

226. A composition of any one of the preceding embodiments, wherein the base sequence of a core region comprises a sequence that is complementary to a characteristic sequence element of a target, wherein a characteristic sequence element defines that target sequence relative to a similar sequence.

227. A composition of any one of the preceding embodiments, wherein a target sequence is a sequence comprising a mutation, and a similar sequence is the wild-type sequence.

228. A composition of any one of the preceding embodiments, wherein a characteristic sequence element defines a particular allele of a target sequence relative to other alleles of the same target sequence.

229. A composition of any one of the preceding embodiments, wherein a characteristic sequence element defines a particular allele of a target gene relative to other alleles of the same target gene.

230. A composition of any one of the preceding embodiments, wherein the sequence is 100% complementary to a characteristic sequence element.

231. A composition of any one of the preceding embodiments, wherein position 11, 12, or 13 of the oligonucleotides as counted from the 5'-terminus of the oligonucleotides aligns with a characteristic sequence element.

232. A composition of any one of embodiments 1-230, wherein position 11 of the oligonucleotides as counted from the 5'-terminus of the oligonucleotides aligns with a characteristic sequence element.

233. A composition of any one of embodiments 1-230, wherein position 12 of the oligonucleotides as counted from the 5'-terminus of the oligonucleotides aligns with a characteristic sequence element.

234. A composition of any one of embodiments 1-230, wherein position 13 of the oligonucleotides as counted from the 5'-terminus of the oligonucleotides aligns with a characteristic sequence element.

235. A composition of any one of embodiments 1-230, wherein position 8, 9 or 10 of the oligonucleotides as counted from the 3'-terminus of the oligonucleotides aligns with a characteristic sequence element.

236. A composition of any one of embodiments 1-230, wherein position 8 of the oligonucleotides as counted from the 3'-terminus of the oligonucleotides aligns with a characteristic sequence element.

237. A composition of any one of embodiments 1-230, wherein position 9 of the oligonucleotides as counted from the 3'-terminus of the oligonucleotides aligns with a characteristic sequence element.

238. A composition of any one of embodiments 1-230, wherein position 10 of the oligonucleotides as counted from the 3'-terminus of the oligonucleotides aligns with a characteristic sequence element.

239. A composition of any one of embodiments 1-230, wherein position 6, 7 or 8 of the core region as counted from the 5'-terminus of the core region aligns with a characteristic sequence element.

240. A composition of any one of embodiments 1-230, wherein position 6 of the core region as counted from the 5'-terminus of the core region aligns with a characteristic sequence element.

241. A composition of any one of embodiments 1-230, wherein position 7 of the core region as counted from the 5'-terminus of the core region aligns with a characteristic sequence element.

242. A composition of any one of embodiments 1-230, wherein position 8 of the core region as counted from the 5'-terminus of the core region aligns with a characteristic sequence element.

243. A composition of any one of embodiments 1-230, wherein position 3, 4 or 5 of the core region as counted from the 3'-terminus of the core region aligns with a characteristic sequence element.

244. A composition of any one of embodiments 1-230, wherein position 3 of the core region as counted from the 3'-terminus of the core region aligns with a characteristic sequence element.

245. A composition of any one of embodiments 1-230, wherein position 4 of the core region as counted from the 3'-terminus of the core region aligns with a characteristic sequence element.

246. A composition of any one of embodiments 1-230, wherein position 5 of the core region as counted from the 3'-terminus of the core region aligns with a characteristic sequence element.

247. A composition of any one of the preceding embodiments, wherein a common base sequence or a base sequence of an oligonucleotide type is a sequence whose DNA cleavage pattern has a cleavage site within or in the vicinity of a characteristic sequence element of a target nucleic acid sequence.

248. A composition of any one of the preceding embodiments, wherein the DNA cleavage pattern is the cleavage pattern of an oligonucleotide composition of DNA oligonucleotides having the sequence, wherein each oligonucleotide in the composition has the same structure.

249. A composition of any one of the preceding embodiments, wherein a common base sequence or a base sequence of an oligonucleotide type is a sequence whose stereorandom cleavage pattern has a cleavage site within or in the vicinity of a characteristic sequence element of a target nucleic acid sequence.

250. A composition of any one of the preceding embodiments, wherein the stereorandom cleavage pattern is the cleavage pattern of a stereorandom composition of oligonucleotides having the sequence, wherein each internucleotidic linkage is phosphorothioate.

251. A composition of any one of the preceding embodiments, wherein the cleavage site with or in the vicinity of a characteristic sequence element of a target nucleic acid sequence is in a core region.

252. A composition of any one of the preceding embodiments, wherein the cleavage site is in the vicinity of a characteristic sequence element of a target nucleic acid sequence.

253. A composition of any one of the preceding embodiments, wherein a cleavage site in the vicinity is a cleavage site 0, 1, 2, 3, 4, or 5 internucleotidic linkages away from the characteristic sequence element.

254. A composition of any one of the preceding embodiments, wherein a cleavage site in the vicinity is a cleavage site 0 internucleotidic linkages away from the characteristic sequence element.

255. A composition of any one of the preceding embodiments, wherein a cleavage site in the vicinity is a cleavage site 1 internucleotidic linkage away from the characteristic sequence element.

256. A composition of any one of the preceding embodiments, wherein a cleavage site in the vicinity is a cleavage site 2 internucleotidic linkages away from the characteristic sequence element.

257. A composition of any one of the preceding embodiments, wherein a cleavage site in the vicinity is a cleavage site 3 internucleotidic linkages away from the characteristic sequence element.

258. A composition of any one of the preceding embodiments, wherein a cleavage site in the vicinity is a cleavage site 4 internucleotidic linkages away from the characteristic sequence element.

259. A composition of any one of the preceding embodiments, wherein a cleavage site in the vicinity is a cleavage site 5 internucleotidic linkages away from the characteristic sequence element.

260. A composition of any one of the preceding embodiments, wherein a cleavage site in the vicinity is a cleavage site is 5' to the cleavage site.

261. A composition of any one of the preceding embodiments, wherein a cleavage site in the vicinity is a cleavage site is 3' to the cleavage site.

261a. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is a major cleavage site.

262. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is a relative major cleavage site.

263. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is a relative major cleavage site, wherein greater than 40% of total cleavage occurs at the site.

264. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is a relative major cleavage site, wherein greater than 50% of total cleavage occurs at the site.

265. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is a relative major cleavage site, wherein greater than 60% of total cleavage occurs at the site.

266. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is a relative major cleavage site, wherein greater than 70% of total cleavage occurs at the site.

267. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is a relative major cleavage site, wherein greater than 80% of total cleavage occurs at the site.

268. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is a relative major cleavage site, wherein greater than 90% of total cleavage occurs at the site.

269. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is a relative major cleavage site, wherein greater than 95% of total cleavage occurs at the site.

270. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is a relative major cleavage site, wherein greater than 100% of total cleavage occurs at the site.

271. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 5 % of total target is cleaved at the site.

272. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 10 % of total target is cleaved at the site.

273. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 15 % of total target is cleaved at the site.

274. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 20 % of total target is cleaved at the site.

275. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 25 % of total target is cleaved at the site.

276. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 30 % of total target is cleaved at the site.

277. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 35 % of total target is cleaved at the site.

278. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 40 % of total target is cleaved at the site.

279. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 45 % of total target is cleaved at the site.

280. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 50 % of total target is cleaved at the site.

281. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 60 % of total target is cleaved at the site.

282. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 70 % of total target is cleaved at the site.

283. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 80 % of total target is cleaved at the site.

284. A composition of any one of the preceding embodiments, wherein a cleavage site within or in the vicinity of a characteristic sequence element is an absolute major cleavage site, wherein greater than 90 % of total target is cleaved at the site.

285. A composition of any one the preceding embodiments, wherein a relative or absolute major cleavage site is determined by RNase H assay.

286. A composition of any one of the preceding embodiments, wherein the characteristic sequence element comprises a single nucleotide polymorphism (SNP) or a mutation.

287. A composition of any one of the preceding embodiments, wherein the characteristic sequence element comprises a single nucleotide polymorphism.

288. A composition of any one of the preceding embodiments, wherein the characteristic sequence element is a single nucleotide polymorphism.

289. A composition of any one of the preceding embodiments, wherein the single nucleotide polymorphism is a single nucleotide polymorphism associated with Huntington's disease.

290. A composition of any one of the preceding embodiments, wherein the single nucleotide polymorphism is a single nucleotide polymorphism found in the Huntingtin gene.

291. A composition of any one of the preceding embodiments, wherein the single nucleotide polymorphism is selected from rs362307, rs7685686, rs362268, rs2530595, rs362331, or rs362306.

291a. A composition of any one of the preceding embodiments, wherein the single nucleotide polymorphism is selected from rs362307, rs7685686, rs362268, or rs362306.

292. A composition of any one of the preceding embodiments, wherein the single nucleotide polymorphism is rs362307.

293. A composition of any one of the preceding embodiments, wherein the single nucleotide polymorphism is rs7685686.

294. A composition of any one of the preceding embodiments, wherein the single nucleotide polymorphism is rs362268.

295. A composition of any one of the preceding embodiments, wherein the single nucleotide polymorphism is rs362306.

295a. A composition of any one of the preceding embodiments, wherein the single nucleotide polymorphism is rs2530595.

295b. A composition of any one of the preceding embodiments, wherein the single nucleotide polymorphism is rs362331.

296. A composition of any one of embodiments 1-290, wherein the single nucleotide polymorphism is in an exon.

297. A composition of any one of embodiments 1-290, wherein the single nucleotide polymorphism is in an intron.

298. A composition of any one of embodiments 1-290, wherein the composition is selected from Tables N1, N2, N3, N4 and 8.

298a. A composition of any one of embodiments 1-290, wherein the composition is selected from Tables N1, N2, N3 and N4.

299. A composition of any one of embodiments 1-290, wherein the composition is selected from Tables N1A, N2A, N3A, N4A and 8; and WV-1092, WVE120101, WV-2603 and WV-2595.

299a. A composition of any one of embodiments 1-290, wherein the composition is selected from Tables N1A, N2A, N3A and N4A.

300. A composition of any one of embodiments 1-290, wherein the composition is WV-1092.

300a. A composition of any one of embodiments 1-290, wherein the composition is WVE120101.

300b. A composition of any one of embodiments 1-290, wherein the composition is WV-2603.

300c. A composition of any one of embodiments 1-290, wherein the composition is WV-2595.

301. A composition of any one of embodiments 1-290, wherein the composition is not ONT-450, ONT-451, or ONT-452.

302. A composition of any one of the preceding embodiments, wherein the characteristic sequence element comprises a mutation.

303. A composition of any one of the preceding embodiments, wherein the characteristic sequence element is a mutation.

304. A composition of any one of the preceding embodiments, wherein the oligonucleotides are at least 95% complementary to a mutant allele.

305. A composition of any one of the preceding embodiments, wherein the oligonucleotides are 100% complementary to a mutant allele.

306. A composition of any one of the preceding embodiments, wherein the oligonucleotides are at least 95% complementary to a target sequence comprising a SNP, wherein the SNP is associated with a disease.

307. A composition of any one of the preceding embodiments, wherein the oligonucleotides are 100% complementary to a target sequence comprising a SNP, wherein the SNP is associated with a disease.

307a. A composition of any one of the preceding embodiments, wherein the oligonucleotides selectively reduce RNA level of a mutant allele.

308. A pharmaceutical composition, comprising a composition of any one of the preceding embodiments, and a pharmaceutical carrier.

308a. A composition of any one of the preceding embodiments, further comprising cerebrospinal fluid.

309. A composition of any one of the preceding embodiments, further comprising artificial cerebrospinal fluid.

310. A method for controlled cleavage of a nucleic acid polymer, the method comprising steps of:

contacting a nucleic acid polymer whose nucleotide sequence comprises a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a target sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

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

310a. A method for cleavage of a nucleic acid having a base sequence comprising a target sequence, the method comprising steps of:

(a) contacting a nucleic acid having a base sequence comprising a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to the target sequence in the nucleic acid;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type, wherein the oligonucleotide targets a mutant Huntingtin gene, and the length is from about 10 to about 50 nucleotides, wherein the backbone linkages comprise at least one phosphorothioate, and wherein the pattern of backbone

chiral centers comprises at least one chiral center in a Rp conformation and at least one chiral center in a Sp conformation.

310b. A method for cleavage of a nucleic acid having a base sequence comprising a target sequence, the method comprising steps of:

(a) contacting a nucleic acid having a base sequence comprising a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to the target sequence in the nucleic acid;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type, wherein the oligonucleotide targets a mutant Huntingtin gene, and the length is from about 10 to about 50 nucleotides, wherein the backbone linkages comprise at least one phosphorothioate, and wherein the pattern of backbone chiral centers comprises at least one chiral center in a Rp conformation and at least one chiral center in a Sp conformation; and

(b) cleavage of the nucleic acid mediated by a RNAseH or RNA interference mechanism.

311. A method of embodiment 310, wherein the contacting being performed under conditions so that cleavage of the nucleic acid polymer occurs.

312. A method of any one of embodiments 310-311, wherein the cleavage occurs with a cleavage pattern that differs from a reference cleavage pattern observed when the nucleic acid polymer is contacted under comparable conditions with a reference oligonucleotide composition.

313. A method for altering a cleavage pattern observed when a nucleic acid polymer whose nucleotide sequence includes a target sequence is contacted with a reference oligonucleotide composition that comprises oligonucleotides having a particular base sequence and length, which particular base sequence is or comprises a sequence that is complementary to the target sequence, the method comprising:

contacting the nucleic acid polymer with a chirally controlled oligonucleotide composition of oligonucleotides having the particular base sequence and length, which

composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of a single oligonucleotide type characterized by:

1) the particular base sequence and length;

2) a particular pattern of backbone linkages; and

3) a particular pattern of backbone chiral centers.

314. A method of embodiment 313, wherein the contacting being performed under conditions so that cleavage of the nucleic acid polymer occurs.

315. A method of any one of embodiments 312-314, wherein the reference oligonucleotide composition is a substantially racemic preparation of oligonucleotides that share the common sequence and length.

316. A method of any one of embodiments 312-314, wherein the reference oligonucleotide composition is a chirally uncontrolled oligonucleotide composition of oligonucleotides that share the common sequence and length.

317. A method of any one of embodiments 312-316, wherein the cleavage pattern provided by the chirally controlled oligonucleotide composition differs from a reference cleavage pattern in that it has fewer cleavage sites within the target sequence found in the nucleic acid polymer than the reference cleavage pattern.

318. A method of embodiment 317, wherein the cleavage pattern provided by the chirally controlled oligonucleotide composition has a single cleavage site within the target sequence found in the nucleic acid polymer than the reference cleavage pattern.

319. A method of embodiment 318, wherein the single cleavage site is a cleavage site in the reference cleavage pattern.

320. A method of embodiment 318, wherein the single cleavage site is a cleavage site not in the reference cleavage pattern.

321. A method of any one of embodiments 312-316, wherein the cleavage pattern provided by the chirally controlled oligonucleotide composition differs from a reference cleavage pattern in that it increases cleavage percentage at a cleavage site.

322. A method of embodiment 321, wherein the cleavage site with increased cleavage percentage is a cleavage site in the reference cleavage pattern.

323. A method of embodiment 321, wherein the cleavage site with increased cleavage percentage is a cleavage site not in the reference cleavage pattern.

324. A method of any one of embodiments 310-323, wherein the chirally controlled oligonucleotide composition provides a higher cleavage rate of the target nucleic acid polymer than a reference oligonucleotide composition.

325. A method of any one of embodiments 310-324, where the cleavage rate is at least 5 fold higher.

326. A method of any one of embodiments 310-325, wherein the chirally controlled oligonucleotide composition provides a lower level of remaining un-cleaved target nucleic acid polymer than a reference oligonucleotide composition.

327. A method of any one of embodiments 310-326, wherein the remaining un-cleaved target nucleic acid polymer is at least 5 fold lower.

328. A methods of any one of embodiments 310-327, wherein the cleavage products from the nucleic acid polymer dissociate from oligonucleotides of the particular oligonucleotide type in the chirally controlled oligonucleotide composition at a faster rate than from oligonucleotides of the reference oligonucleotide composition.

329. A method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines the target nucleic acid sequence, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target nucleic acid sequence and a similar nucleic acid sequences, transcripts of the target nucleic acid sequence are suppressed at a greater level than a level of suppression observed for a similar nucleic acid sequence.

330. A method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines the target nucleic acid sequence, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target nucleic acid sequence and a similar nucleic acid sequences, transcripts of the target nucleic acid sequence are suppressed at a greater level than a level of suppression observed for a similar nucleic acid sequence.

331. A method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular target sequence relative to its similar sequences, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

332. A method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and

similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular target sequence relative to its similar sequences, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

333. A method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular target sequence relative to its similar sequences, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular target sequence at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for a similar ; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for a similar sequence.

334. A method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular target sequence relative to its similar sequences, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular target sequence at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for a similar ; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for a similar sequence.

335. A method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular target nucleic acid sequence, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target nucleic acid sequence, it shows suppression of expression of transcripts of the particular target nucleic acid sequence at a level that is:

a) at least 2 fold in that transcripts from the particular target nucleic acid sequence are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for a similar sequence; or

c) both at least 2 fold in that transcripts from the particular target nucleic acid sequence are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for a similar sequence.

336. A method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular target nucleic acid sequence, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target nucleic acid sequence, it shows suppression of expression of transcripts of the particular target nucleic acid sequence at a level that is:

a) at least 2 fold in that transcripts from the particular target nucleic acid sequence are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for a similar sequence; or

c) both at least 2 fold in that transcripts from the particular target nucleic acid sequence are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for a similar sequence.

337. A method of any one of the preceding embodiments, wherein a target sequence is a sequence comprising a mutation, and a similar sequence is the wild-type sequence.

338. A method of any one of the preceding embodiments, wherein a characteristic sequence element defines a particular allele of a target sequence relative to other alleles of the same target sequence.

339. A method of any one of the preceding embodiments, wherein a characteristic sequence element defines a particular allele of a target gene relative to other alleles of the same target gene.

340. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

341. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

342. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

343. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

344. A method of embodiment 340 or 342, the contacting being performed under conditions determined to permit the composition to suppress transcripts of the particular allele.

345. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of both the target allele and another allele of the same gene,

transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

346. A method of embodiment 345, wherein the contacting being performed under conditions determined to permit the composition to suppress expression of the particular allele.

347. A method of any one of embodiments 340-346, wherein transcripts of the particular allele are suppressed at a level at least 5, 10, 20, 50, 100, 200 or 500 fold greater than a level of suppression observed for another allele of the same gene.

348. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

349. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

350. A method for controlled cleavage of a nucleic acid polymer, the method comprising contacting a nucleic acid polymer whose nucleotide sequence comprises a target sequence with an oligonucleotide or an oligonucleotide composition of any one of embodiments 540-574.

351. A method for suppression of a transcript from a target nucleic acid sequence for which one or more similar nucleic acid sequences exist within a population, each of the target and similar sequences contains a specific nucleotide characteristic sequence element that defines the target sequence relative to the similar sequences, the method comprising contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide or an oligonucleotide composition of any one of embodiments 540-574, wherein the base sequence of the oligonucleotide is or comprises a sequence that is complementary to the characteristic sequence element that defines the target nucleic acid sequence.

352. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising contacting a sample comprising

transcripts of the target nucleic acid sequence with an oligonucleotide or an oligonucleotide composition of any one of embodiments 540-574, wherein the base sequence of the oligonucleotide is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele.

353. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide or an oligonucleotide composition of any one of embodiments 540-574, wherein the base sequence of the oligonucleotide is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the oligonucleotide or oligonucleotide composition being characterized in that, when it is contacted with a system comprising transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

354. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide or an oligonucleotide composition of any one of embodiments 540-574, wherein the base sequence of the oligonucleotide is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the oligonucleotide or oligonucleotide composition being characterized in that, when it is contacted with a system expressing transcripts of both the target allele and another allele of the same gene, transcripts of the particular allele are suppressed at a level at least 2 fold greater than a level of suppression observed for another allele of the same gene.

355. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the

same target nucleic acid sequence, the method comprising contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide or an oligonucleotide composition of any one of embodiments 540-574, wherein the base sequence of the oligonucleotide is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the oligonucleotide or oligonucleotide composition being characterized in that, when it is contacted with a system comprising transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

356. A method for allele-specific suppression of a transcript from a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising contacting a sample comprising transcripts of the target nucleic acid sequence with an oligonucleotide or an oligonucleotide composition of any one of embodiments 540-574, wherein the base sequence of the oligonucleotide is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the oligonucleotide or oligonucleotide composition being characterized in that, when it is contacted with a system expressing transcripts of the same target nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

b) greater than a level of suppression observed for another allele of the same nucleic acid sequence; or

c) both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.

357. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages;

wherein the common base sequence is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that, when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

358. A method for allele-specific suppression of a transcript from a target gene for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target gene, the method comprising steps of:

contacting a sample comprising transcripts of the target gene with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages;

3) a common pattern of backbone chiral centers;

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

wherein the common base sequence for the oligonucleotides of the particular oligonucleotide type is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, the composition being characterized in that,

when it is contacted with a system expressing transcripts of the target gene, it shows suppression of expression of transcripts of the particular allele at a level that is:

a) at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent;

b) at least 2 fold greater than a level of suppression observed for another allele of the same gene; or

c) both at least 2 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent, and at least 2 fold greater than a level of suppression observed for another allele of the same gene.

359. A method of any one of the preceding embodiments, wherein transcripts from the particular allele are detected in amounts that are 2 fold or more when the composition is absent relative to when it is present.

360. A method of any one of the preceding embodiments, wherein the level of transcripts of another allele of the same gene is at least 2 fold greater than the level of transcripts of the particular allele.

361. A method of any one of the preceding embodiments, wherein transcripts from the particular allele are detected in amounts that are 2 fold or more when the composition is absent relative to when it is present, and the level of transcripts of another allele of the same gene is at least 2 fold greater than the level of transcripts of the particular allele.

362. A method of any one of the preceding embodiments, the contacting being performed under conditions determined to permit the composition to suppress transcripts of the particular allele.

363. A method of any one of the preceding embodiments, wherein the contacting being performed under conditions determined to permit the composition to suppress expression of the particular allele.

364. A method of any one of the preceding embodiments, wherein transcripts of the particular allele are suppressed at a level that is at least 5, 10, 20, 50, 100, 200 or 500 fold in that transcripts from the particular allele are detected in amounts that are 2 fold lower when the composition is present relative to when it is absent.

365. A method of any one of the preceding embodiments, wherein transcripts of the particular allele are suppressed at a level that is at least 5, 10, 20, 50, 100, 200 or 500 fold greater than a level of suppression observed for another allele of the same gene.

366. A method of any one of the preceding embodiments, wherein the system is an *in vitro* or *in vivo* system.

366a A method of any one of the preceding embodiments, wherein the method is performed *in vitro* or *in vivo*.

367. A method of any one of the preceding embodiments, wherein the system comprises one or more cells, tissues or organs.

368. A method of any one of the preceding embodiments, wherein the system comprises one or more organisms.

369. A method of any one of the preceding embodiments, wherein the system comprises one or more subjects.

370. A method of any one of the preceding embodiments, wherein transcripts of the particular allele are cleaved.

371. A method of any one of the preceding embodiments, wherein the specific nucleotide characteristic sequence element is present within an intron of the target nucleic acid sequence or gene.

372. A method of any one of the preceding embodiments, wherein the specific nucleotide characteristic sequence element is present within an exon of the target nucleic acid sequence or gene.

373. A method of any one of the preceding embodiments, wherein the specific nucleotide characteristic sequence element spans an exon and an intron of the target nucleic acid sequence or gene.

374. A method of any one of the preceding embodiments, wherein the specific nucleotide characteristic sequence element comprises a mutation.

375. A method of any one of the preceding embodiments, wherein the specific nucleotide characteristic sequence element is a mutation.

376. A method of any one of the preceding embodiments, wherein the specific nucleotide characteristic sequence element comprises a SNP.

377. A method of any one of the preceding embodiments, wherein the specific nucleotide characteristic sequence element is a SNP.

378. A method of any one of the preceding embodiments, wherein the oligonucleotide composition is administered to a subject.

379. A method of any one of the preceding embodiments, wherein the target nucleic acid polymer or transcripts are oligonucleotides.

380. A method of any one of the preceding embodiments, wherein the target nucleic acid polymer or transcripts are RNA.

381. A method of any one of the preceding embodiments, wherein the target nucleic acid polymer or transcripts are newly transcribed RNA.

382. A method of any one of the preceding embodiments, wherein oligonucleotides of the particular oligonucleotide type in the chirally controlled oligonucleotide composition form duplexes with the nucleic acid polymer or transcripts.

383. A method of any one of the preceding embodiments, wherein the nucleic acid polymer or transcripts are cleaved by an enzyme.

384. A method of any one of the preceding embodiments, wherein the enzyme is RNase H.

385. A method of any one of the preceding embodiments, wherein the SNP is a SNP related to Huntington's disease.

386. A method of any one of the preceding embodiments, wherein the SNP is a SNP found in the Huntingtin gene.

387. A method of any one of the preceding embodiments, wherein the SNP is selected from rs362307, rs7685686, rs362268, or rs362306.

388. A method of embodiments 310-387, wherein the SNP is rs362307.

389. A method of embodiments 310-387, wherein the single nucleotide polymorphism is rs7685686.

390. A method of embodiments 310-387, wherein the single nucleotide polymorphism is rs362268.

391. A method of embodiments 310-387, wherein the single nucleotide polymorphism is rs362306.

392. A method of embodiments 310-391, wherein position 11 of the oligonucleotides as counted from the 5'-terminus of the oligonucleotides aligns with a single nucleotide polymorphism.

393. A method of embodiments 310-391, wherein position 12 of the oligonucleotides as counted from the 5'-terminus of the oligonucleotides aligns with a single nucleotide polymorphism.

394. A method of embodiments 310-391, wherein position 13 of the oligonucleotides as counted from the 5'-terminus of the oligonucleotides aligns with a single nucleotide polymorphism.

395. A method of embodiments 310-391, wherein position 8 of the oligonucleotides as counted from the 3'-terminus of the oligonucleotides aligns with a single nucleotide polymorphism.

396. A method of embodiments 310-391, wherein position 9 of the oligonucleotides as counted from the 3'-terminus of the oligonucleotides aligns with a single nucleotide polymorphism.

397. A method of embodiments 310-391, wherein position 10 of the oligonucleotides as counted from the 3'-terminus of the oligonucleotides aligns with a single nucleotide polymorphism.

398. A method of any one of the preceding embodiments, wherein the oligonucleotides comprise one or more wing regions and a common core region, wherein:

each wing region independently has a length of two or more bases, and independently and optionally comprises one or more chiral internucleotidic linkages; and

the core region independently has a length of two or more bases and independently comprises one or more chiral internucleotidic linkages.

399. A method of any one of embodiments 310-398, wherein position 6 of the core region as counted from the 5'-terminus of the core region aligns with a single nucleotide polymorphism.

400. A method of any one of embodiments 310-398, wherein position 7 of the core region as counted from the 5'-terminus of the core region aligns with a single nucleotide polymorphism.

401. A method of any one of embodiments 310-398, wherein position 8 of the core region as counted from the 5'-terminus of the core region aligns with a single nucleotide polymorphism.

402. A method of any one of embodiments 310-398, wherein position 3 of the core region as counted from the 3'-terminus of the core region aligns with a single nucleotide polymorphism.

403. A method of any one of embodiments 310-398, wherein position 4 of the core region as counted from the 3'-terminus of the core region aligns with a single nucleotide polymorphism.

404. A method of any one of embodiments 310-398, wherein position 5 of the core region as counted from the 3'-terminus of the core region aligns with a single nucleotide polymorphism.

405. A method of any one of the preceding embodiments, wherein:

each wing region independently has a length of two or more bases, and independently comprises one or more chiral internucleotidic linkages and one or more natural phosphate linkage; and

the core region independently has a length of two or more bases, wherein each internucleotidic linkage in the core region is chiral, only one of internucleotidic linkage in the core region is *R*p, and each of the other internucleotidic linkages in the core region is *S*p.

406. A method of any one of the preceding embodiments, wherein the oligonucleotides are hemimers having the structure of wing-core.

407. A method of any one of embodiments 310-405, wherein the oligonucleotides are hemimers having the structure of core-wing.

408. A method of any one of embodiments 310-405, wherein the oligonucleotides are gapmers having the structure of wing-core-wing.

409. A method of any one of the preceding embodiments, wherein level of transcripts from a disease-causing allele is selectively suppressed.

410. A method of any one of the preceding embodiments, wherein level of a protein translated from transcripts from a disease-causing allele are suppressed.

411. A method for treating or preventing Huntington's Disease in a subject, comprising administering to the subject an oligonucleotide composition comprising oligonucleotides having:

1) a common base sequence and length; and

2) a common pattern of backbone linkages.

412. A method for treating or preventing Huntington's Disease in a subject, comprising administering to the subject a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

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

413. A method of embodiment 411 or 412, wherein the oligonucleotides comprise one or more wing regions and a common core region, wherein:

each wing region independently has a length of two or more bases, and independently and optionally comprises one or more chiral internucleotidic linkages; and

the core region independently has a length of two or more bases and independently comprises one or more chiral internucleotidic linkages.

414. A method of any one of embodiments 411-4113, wherein:

each wing region independently has a length of two or more bases, and independently comprises one or more chiral internucleotidic linkages and one or more natural phosphate linkage; and

the core region independently has a length of two or more bases, wherein each internucleotidic linkage in the core region is chiral, only one of internucleotidic linkage in the core region is *R*p, and each of the other internucleotidic linkages in the core region is *S*p.

415. A method of any one of embodiments 411-414, wherein the oligonucleotides are hemimers having the structure of wing-core.

416. A method of any one of embodiments 411-414, wherein the oligonucleotides are hemimers having the structure of core-wing.

417. A method of any one of embodiments 411-414, wherein the oligonucleotides are gapmers having the structure of wing-core-wing.

418. A method of any one of embodiments 411-417, wherein position 11 of the oligonucleotides as counted from the 5'-terminus of the oligonucleotides aligns with a single nucleotide polymorphism.

419. A method of any one of embodiments 411-417, wherein position 12 of the oligonucleotides as counted from the 5'-terminus of the oligonucleotides aligns with a single nucleotide polymorphism.

420. A method of any one of embodiments 411-417, wherein position 13 of the oligonucleotides as counted from the 5'-terminus of the oligonucleotides aligns with a single nucleotide polymorphism.

421. A method of any one of embodiments 411-417, wherein position 8 of the oligonucleotides as counted from the 3'-terminus of the oligonucleotides aligns with a single nucleotide polymorphism.

422. A method of any one of embodiments 411-417, wherein position 9 of the oligonucleotides as counted from the 3'-terminus of the oligonucleotides aligns with a single nucleotide polymorphism.

423. A method of any one of embodiments 411-417, wherein position 10 of the oligonucleotides as counted from the 3'-terminus of the oligonucleotides aligns with a single nucleotide polymorphism.

424. A method of any one of embodiments 411-417, wherein position 6 of the core region as counted from the 5'-terminus of the core region aligns with a single nucleotide polymorphism.

425. A method of any one of embodiments 411-417, wherein position 7 of the core region as counted from the 5'-terminus of the core region aligns with a single nucleotide polymorphism.

426. A method of any one of embodiments 411-417, wherein position 8 of the core region as counted from the 5'-terminus of the core region aligns with a single nucleotide polymorphism.

427. A method of any one of embodiments 411-417, wherein position 3 of the core region as counted from the 3'-terminus of the core region aligns with a single nucleotide polymorphism.

428. A method of any one of embodiments 411-417, wherein position 4 of the core region as counted from the 3'-terminus of the core region aligns with a single nucleotide polymorphism.

429. A method of any one of embodiments 411-417, wherein position 5 of the core region as counted from the 3'-terminus of the core region aligns with a single nucleotide polymorphism.

430. A method of any one of embodiments 411-429, wherein the method ameliorating a symptom of Huntington's Disease.

431. A method of any one of embodiments 411-429, wherein the method slowing onset of Huntington's Disease.

432. A method of any one of embodiments 411-429, wherein the method slowing progression of Huntington's Disease.

433. A method of any one of embodiments 411-432, wherein the subject has a SNP related to Huntington's Disease.

434. A method of any one of embodiments 411-433, wherein the subject has a SNP in the subject's Huntingtin gene.

435. A method of any one of embodiments 411-434, wherein the subject has a SNP, wherein one allele is mutant Huntingtin associated with expanded CAG repeats.

436. A method of any one of embodiments 411-435, wherein the subject has a SNP selected from rs362307, rs7685686, rs362268, rs2530595, rs362331, or rs362306.

436a. A method of any one of embodiments 411-435, wherein the subject has a SNP selected from rs362307, rs7685686, rs362268, or rs362306.

437. A method of any one of embodiments 411-436, wherein the subject has the SNP rs362307.

438. A method of any one of embodiments 411-436, wherein the subject has the SNP rs7685686.

439. A method of any one of embodiments 411-436, wherein the subject has the SNP rs362268.

440. A method of any one of embodiments 411-436, wherein the subject has the SNP rs362306.

440a. A method of any one of embodiments 411-436, wherein the subject has the SNP rs2530595.

440b. A method of any one of embodiments 411-436, wherein the subject has the SNP rs362331.

441. A composition of any of embodiments 1-309, wherein a substantially racemic preparation of oligonucleotides is prepared by non-stereoselective preparation.

442. A composition of any one of embodiments 1-309 and 441, wherein a substantially racemic preparation of oligonucleotides is prepared by non-stereoselective preparation, wherein a chiral auxiliary is not used for formation of a chiral internucleotidic linkage.

443. A composition of any one of embodiments 1-309 and 441-442, wherein a substantially racemic preparation of oligonucleotides is prepared by non-stereoselective preparation, wherein at least one chiral internucleotidic linkage is formed with less than 80:20 diastereomeric selectivity.

444. A composition of any one of embodiments 1-309 and 441-443, wherein a substantially racemic preparation of oligonucleotides is prepared by non-stereoselective preparation, wherein at least one chiral internucleotidic linkage is formed with less than 90:10 diastereomeric selectivity.

445. A composition of any one of embodiments 1-309 and 441-444, wherein a substantially racemic preparation of oligonucleotides is prepared by non-stereoselective preparation, wherein at least one chiral internucleotidic linkage is formed with less than 95:5 diastereomeric selectivity.

446. A composition of any one of embodiments 1-309 and 441-445, wherein a substantially racemic preparation of oligonucleotides is prepared by non-stereoselective preparation, wherein at least one chiral internucleotidic linkage is formed with less than 97:3 diastereomeric selectivity.

447. A composition of any one of embodiments 1-309, wherein each chiral internucleotidic linkage is formed with greater than 90:10 diastereomeric selectivity.

448. A composition of any one of embodiments 1-309, wherein each chiral internucleotidic linkage is formed with greater than 95:5 diastereomeric selectivity.

449. A composition of any one of embodiments 1-309, wherein each chiral internucleotidic linkage is formed with greater than 96:4 diastereomeric selectivity.

450. A composition of any one of embodiments 1-309, wherein each chiral internucleotidic linkage is formed with greater than 97:3 diastereomeric selectivity.

451. A composition of any one of embodiments 1-309, wherein each chiral internucleotidic linkage is formed with greater than 98:2 diastereomeric selectivity.

452. A composition of any one of embodiments 1-309, wherein each chiral internucleotidic linkage is formed with greater than 98:2 diastereomeric selectivity.

453. A composition of any one of embodiments 443-452, wherein the diastereomeric selectivity for forming a chiral internucleotidic linkage is measured by forming a dimeric oligonucleotide comprising the chiral internucleotidic linkage and the nucleosides to both sides of the chiral internucleotidic linkage under the same or comparable reaction conditions.

454. A method for preparing an oligonucleotide composition for selective suppression of a transcript of a target nucleic acid sequence, comprising providing an oligonucleotide

composition comprising a predetermined level of oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which pattern comprises $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Np)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein:

m is 1-50;

n is 1-10;

t is 1-50;

each *N*p is independently *R*p or *S*p;

wherein the target nucleic acid sequence comprises a characteristic sequence element that defines the target nucleic acid sequence from a similar nucleic acid sequence;

wherein the common base sequence is a sequence whose DNA cleavage pattern and/or stereorandom cleavage pattern has a cleavage site within or in the vicinity of the target nucleic acid sequence.

455. A method of embodiment 454, wherein the pattern comprises $(Sp)_m(Rp)_n$.

456. A method of embodiment 454, wherein the pattern comprises $(Rp)_n(Sp)_m$.

457. A method of embodiment 454, wherein the pattern comprises $(Np)_t(Rp)_n(Sp)_m$.

458. A method of embodiment 454, wherein the pattern comprises $(Sp)_t(Rp)_n(Sp)_m$.

459. A method of embodiment 454, wherein the pattern is a pattern in any one of embodiments 145-157.

460. A method of any one of embodiments 454-458, wherein a cleavage site is in any one of embodiments 247-285.

461. A method of any one of the preceding embodiments, wherein the oligonucleotide composition is a composition of any one of embodiments 1-309 and 441-453.

462. A composition or method of any one of the preceding embodiments, wherein the sequence of the oligonucleotide in a chirally controlled oligonucleotide composition comprises, consists of, or is the sequence of any oligonucleotide described herein, or selected from Tables N1A, N2A, N3A, N4A or 8; or WV-1092, WVE120101, WV-2603 or WV-2595.

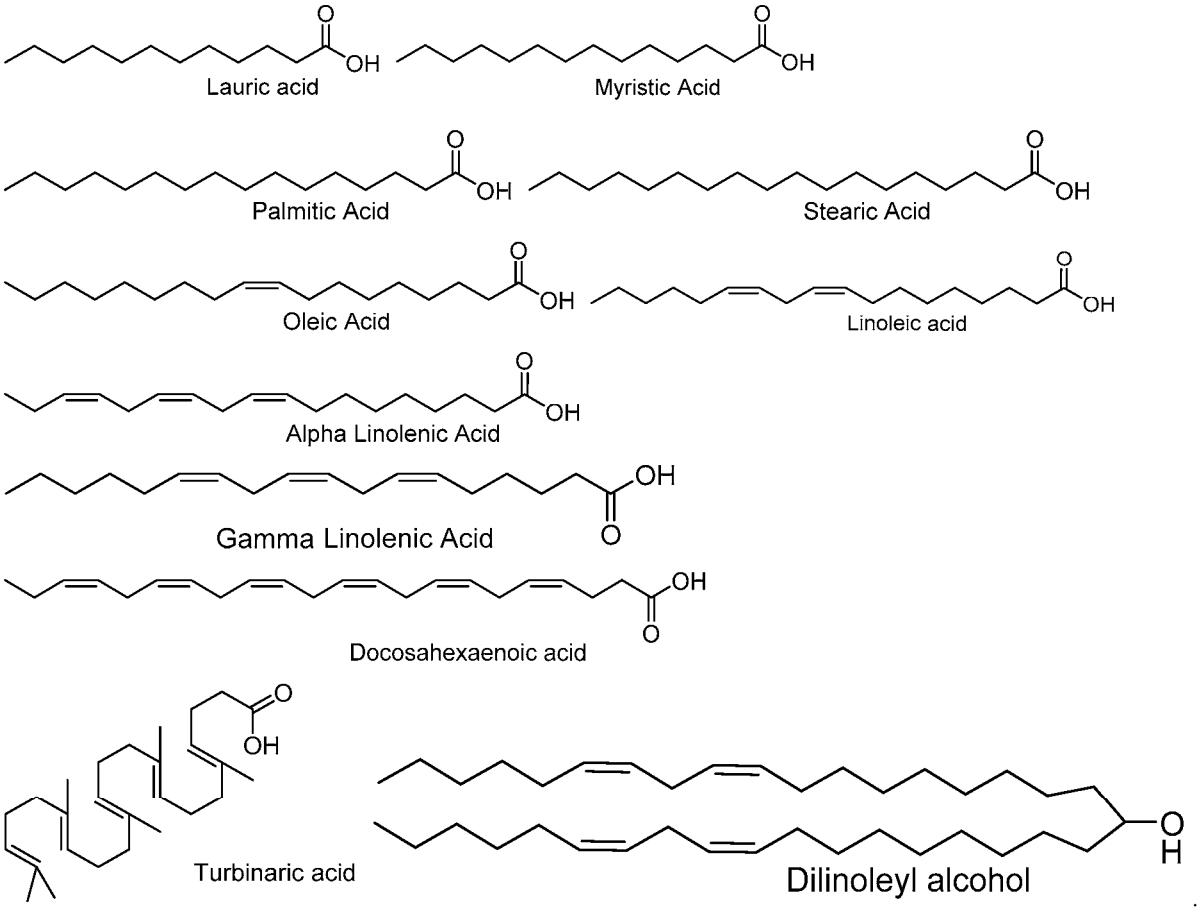
463. A composition comprising a lipid and an oligonucleotide.

463a. The composition of any one of the preceding embodiments, wherein the composition comprises one or more lipids conjugated with one or more oligonucleotides in the composition.

464. A composition comprising an oligonucleotide and a lipid selected from the list of: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, arachidonic acid, and dilinoleyl.

464a. A composition comprising an oligonucleotide and a lipid selected from the list of: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid, and dilinoleyl.

465. A composition comprising an oligonucleotide and a lipid selected from:



466. A composition comprising an oligonucleotide and a lipid,

wherein the lipid comprises a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic group.

467. An oligonucleotide composition comprising a plurality of oligonucleotides, which share:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone phosphorus modifications;

wherein one or more oligonucleotides of the plurality are individually conjugated to a lipid.

468. A chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides, which share:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone phosphorus modifications;

wherein:

the composition is chirally controlled in that the plurality of oligonucleotides share the same stereochemistry at one or more chiral internucleotidic linkages;

one or more oligonucleotides of the plurality are individually conjugated to a lipid; and

one or more oligonucleotides of the plurality are optionally and individually conjugated to a targeting compound or moiety.

469. A method of delivering an oligonucleotide to a cell or tissue in a human subject, comprising:

(a) providing a composition of any one of the preceding embodiments; and

(b) Administering the composition to the human subject such that the oligonucleotide is delivered to a cell or tissue in the subject.

470. A method for delivering an oligonucleotide to a cell or tissue comprising preparing a composition according to any one of the preceding embodiments and treating [contacting] the cell or tissue with the composition.

471. A method of modulating the level of a transcript or gene product of a gene in a cell, the method comprising the step of contacting the cell with a composition according to any one of the preceding embodiments, wherein the oligonucleotide is capable of modulating the level of the transcript or gene product.

472. A method for inhibiting expression of a gene in a cell or tissue comprising preparing a composition according to any one of the preceding embodiments and treating the cell or tissue with the composition.

473. A method for inhibiting expression of a gene in a cell or tissue in a mammal comprising preparing a composition according to any one of the preceding embodiments and administering the composition to the mammal.

474. A method of treating a disease that is caused by the over-expression of one or several proteins in a cell or tissue in a subject, said method comprising the administration of a composition according to any one of the preceding embodiments to the subject.

475. A method of treating a disease that is caused by a reduced, suppressed or missing expression of one or several proteins in a subject, said method comprising the administration of a composition according to any one of the preceding embodiments to the subject.

476. A method for generating an immune response in a subject, said method comprising the administration of a composition according to any one of the preceding embodiments to the subject, wherein the biologically active compound is an immunomodulating nucleic acid.

477. A method for treating a sign and/or symptom of Huntington'sDisease by providing a composition of any one of the preceding embodiments and administering the composition to the subject.

478. A method of modulating the amount of RNaseH-mediated cleavage in a cell, the method comprising the step of contacting the cell with a composition according to any one of the preceding embodiments, wherein the oligonucleotide is capable of modulating the amount of RNaseH-mediated cleavage.

479. A method of administering an oligonucleotide to a subject in need thereof, comprising steps of providing a composition comprising the agent a lipid, and administering the composition to the subject, wherein the agent is any agent disclosed herein, and wherein the lipid is any lipid disclosed herein.

480. A method of treating a disease in a subject, the method comprising steps of providing a composition comprising the agent a lipid, and administering a therapeutically effective amount of the composition to the subject, wherein the agent is any agent disclosed herein, and wherein the lipid is any lipid disclosed herein, and wherein the disease is any disease disclosed herein.

481. The composition or method of any one of the preceding embodiments, wherein a lipid comprises an optionally substituted C_{10}-C_{40} saturated or partially unsaturated aliphatic chain.

482. The composition or method of any one of the preceding embodiments, wherein a lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

483. The composition or method of any one of the preceding embodiments, wherein a lipid comprises a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic group.

484. The composition or method of any one of the preceding embodiments, wherein a lipid comprises an unsubstituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

485. The composition or method of any one of the preceding embodiments, wherein a lipid comprises no more than one optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

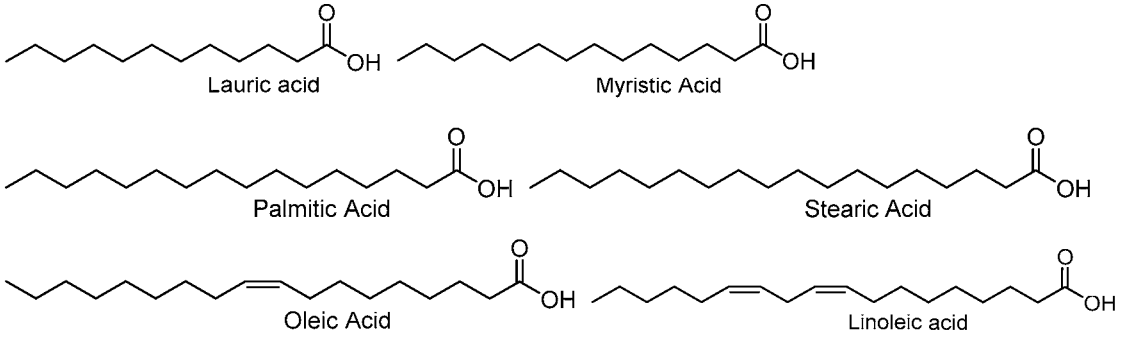
486. The composition or method of any one of the preceding embodiments, wherein a lipid comprises two or more optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

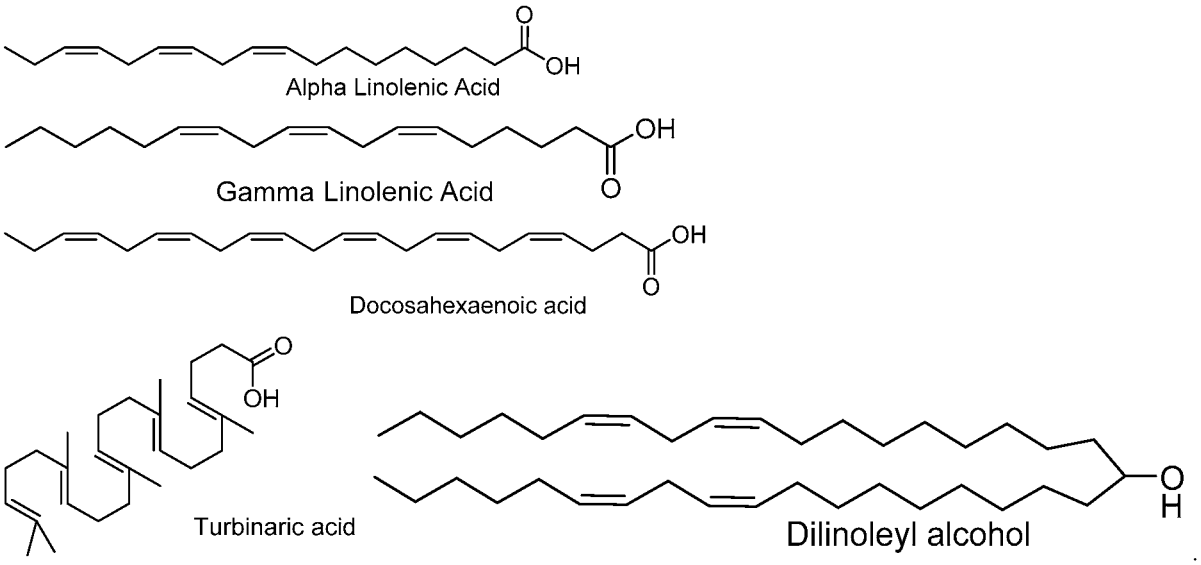
487. The composition or method of any one of the preceding embodiments, wherein a lipid comprises no tricyclic or polycyclic moiety.

488. The composition or method of any one of the preceding embodiments, wherein a lipid has the structure of R^1-COOH, wherein R^1 is an optionally substituted C_{10}-C_{40} saturated or partially unsaturated aliphatic chain.

489. The composition or method of any one of embodiment 16, wherein the lipid is conjugated through its carboxyl group.

490. The composition or method according to any one of the preceding embodiments, wherein the lipid is selected from:





491. The composition or method of any one of the preceding embodiments, wherein the lipid is conjugated to the oligonucleotide.

492. The composition or method of any one of the preceding embodiments, wherein the lipid is directly conjugated to the oligonucleotide.

493. The composition or method of any one of the preceding embodiments, wherein the lipid is conjugated to the oligonucleotide via a linker.

494. The composition or method of any one of the preceding embodiments, wherein the linker is selected from: an uncharged linker; a charged linker; a linker comprising an alkyl; a linker comprising a phosphate; a branched linker; an unbranched linker; a linker comprising at least one cleavage group; a linker comprising at least one redox cleavage group; a linker comprising at least one phosphate-based cleavage group; a linker comprising at least one acid-cleavage group; a linker comprising at least one ester-based cleavage group; and a linker comprising at least one peptide-based cleavage group.

495. The composition or method of any one of the preceding embodiments, wherein each oligonucleotide of the plurality is individually conjugated to the same lipid at the same location.

496. The composition or method of any one of the preceding embodiments, wherein a lipid is conjugated to an oligonucleotide through a linker.

497. The composition or method of any one of the preceding embodiments, wherein one or more oligonucleotides of the plurality are independently conjugated to a targeting compound or moiety.

498. The composition or method of any one of the preceding embodiments, wherein one or more oligonucleotides of the plurality are independently conjugated to a lipid and a targeting compound or moiety.

499. The composition or method of any one of the preceding embodiments, wherein one or more oligonucleotides of the plurality are independently conjugated to a lipid at one end and a targeting compound or moiety at the other.

500. The composition or method of any one of the preceding embodiments, wherein oligonucleotides of the plurality share the same chemical modification patterns.

501. The composition or method of any one of the preceding embodiments, wherein oligonucleotides of the plurality share the same chemical modification patterns comprising one or more base modifications.

502. The composition or method of any one of the preceding embodiments, wherein oligonucleotides of the plurality share the same chemical modification patterns comprising one or more sugar modifications.

503. The composition or method of any one of the preceding embodiments, wherein the common base sequence is capable of hybridizing with a transcript in a cell, which transcript contains a mutation that is linked to a muscle disease, or whose level, activity and/or distribution is linked to a muscle disease.

504. The composition or method of any one of the preceding embodiments, wherein the oligonucleotide is a nucleic acid.

505. The composition or method of any one of the preceding embodiments, wherein the oligonucleotide is an oligonucleotide.

506. The composition or method of any one of the preceding embodiments, wherein the oligonucleotide is an oligonucleotide which mediates exon skipping.

507. The composition or method of any one of the preceding embodiments, wherein the oligonucleotide is a stereodefined oligonucleotide which mediates exon skipping.

508. The composition or method of any one of the preceding embodiments, wherein the disease or disorder is a muscle-related disease or disorder.

509. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted, C_{10}-C_{80} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally

substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, –S(O)–, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, –SC(O)–, –C(O)S–, –OC(O)–, and $-C(O)O-$, wherein each variable is independently as defined and described herein.

510. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted C_{10}-C_{80} saturated or partially unsaturated, aliphatic chain.

511. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain.

512. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted C_{10}-C_{60} saturated or partially unsaturated, aliphatic chain.

513. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain.

514. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted C_{10}-C_{40} saturated or partially unsaturated, aliphatic chain.

515. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

516. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted, C_{10}-C_{60} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, –S(O)–, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, –SC(O)–, –C(O)S–, –OC(O)–, and $-C(O)O-$, wherein each variable is independently as defined and described herein.

517. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted C_{10}-C_{80} saturated or partially unsaturated, aliphatic chain.

518. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted C_{10}-C_{60} linear, saturated or partially unsaturated, aliphatic chain.

519. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

520. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted, C_{10}-C_{40} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_1–C_6 alkylene, C_1–C_6 alkenylene, —C≡C—, a C_1–C_6 heteroaliphatic moiety, $-C(R')_2-$, –Cy–, –O–, –S–, –S–S–, $-N(R')-$, $-C(O)-$, –C(S)–, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, –S(O)–, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, –SC(O)–, –C(O)S–, –OC(O)–, and $-C(O)O-$, wherein each variable is independently as defined and described herein.

521. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted C_{10}-C_{40} saturated or partially unsaturated, aliphatic chain.

522. The composition or method of any one of the preceding embodiments, wherein the lipid comprises an optionally substituted C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain.

523. The composition or method of any one of the preceding embodiments, wherein the composition further comprises one or more additional components selected from: a polynucleotide, carbonic anhydrase inhibitor, a dye, an intercalating agent, an acridine, a cross-linker, psoralene, mitomycin C, a porphyrin, TPPC4, texaphyrin, Sapphyrin, a polycyclic aromatic hydrocarbon phenazine, dihydrophenazine, an artificial endonuclease, a chelating agent, EDTA, an alkylating agent, a phosphate, an amino, a mercapto, a PEG, PEG-40K, MPEG, $[MPEG]_2$, a polyamino, an alkyl, a substituted alkyl, a radiolabeled marker, an enzyme, a hapten biotin, a transport/absorption facilitator, aspirin, vitamin E, folic acid, a synthetic ribonuclease, a protein, a glycoprotein, a peptide, a molecule having a specific affinity for a co-ligand, an antibody, a hormone, a hormone receptor, a non-peptidic species, a lipid, a lectin, a carbohydrate, a vitamin, a cofactor, or a drug.

524. The composition or method of any one of the preceding embodiments, wherein the lipid comprises a C_{10}-C_{80} linear, saturated or partially unsaturated, aliphatic chain.

525. The composition or method of any one of the preceding embodiments, wherein the composition further comprises a linker linking the oligonucleotide and the lipid, wherein the linker is selected from: an uncharged linker; a charged linker; a linker comprising an alkyl; a linker comprising a phosphate; a branched linker; an unbranched linker; a linker comprising at least one cleavage group; a linker comprising at least one redox cleavage group; a linker comprising at least one phosphate-based cleavage group; a linker comprising at least one acid-cleavage group; a linker comprising at least one ester-based cleavage group; a linker comprising at least one peptide-based cleavage group.

526. The composition or method of any one of the preceding embodiments, wherein the oligonucleotide comprises or consists of or is an oligonucleotide or oligonucleotide composition or chirally controlled oligonucleotide composition.

527. The composition or method of any one of the preceding embodiments, wherein the oligonucleotide comprises or consists of or is an oligonucleotide or oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of any oligonucleotide described herein.

528. The composition or method of any one of the preceding embodiments, wherein the oligonucleotide comprises or consists of or is an oligonucleotide or oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of any oligonucleotide listed in Table 4.

529. The composition or method of any one of the preceding embodiments, wherein the oligonucleotide comprises or consists of or is an oligonucleotide or oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of a splice-switching oligonucleotide.

530. The composition or method of any one of the preceding embodiments, wherein the oligonucleotide comprises or consists of or is an oligonucleotide or oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of an oligonucleotide capable of skipping or mediating skipping of an exon in the dystrophin gene.

531. The composition or method of any of the preceding embodiments, wherein the oligonucleotide is a chirally controlled oligonucleotide composition.

532. The composition or method of any of the preceding embodiments, wherein the disease or disorder is Huntington's Disease.

533. The composition or method of any of the preceding embodiments, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a mutant Huntingtin gene mRNA.

534. The composition or method of any of the preceding embodiments, wherein the oligonucleotide comprises, consists of or is the sequence of any oligonucleotide disclosed herein.

535. The composition or method of any of the preceding embodiments, wherein the oligonucleotide is capable of differentiating between a wild-type and a mutant Huntingtin allele.

536. The composition or method of any of the preceding embodiments, wherein the oligonucleotide is capable of participating in RNaseH-mediated cleavage of a mutant Huntingtin gene mRNA.

537. The composition or method of any of the preceding embodiments, wherein the oligonucleotide comprises, consists of or is the sequence of any oligonucleotide disclosed in Table 4.

538. The composition or method of any one of the preceding embodiments, wherein the oligonucleotide comprises or consists of or is an oligonucleotide or oligonucleotide composition or chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of any of: WV-1092, WV-2595, or WV-2603.

539. The composition or method of any one of the preceding embodiments, wherein the sequence of an oligonucleotide includes any one or more of: base sequence (including length); pattern of chemical modifications to sugar and base moieties; pattern of backbone linkages; pattern of natural phosphate linkages, phosphorothioate linkages, phosphorothioate triester linkages, and combinations thereof; pattern of backbone chiral centers; pattern of stereochemistry (Rp/Sp) of chiral internucleotidic linkages; pattern of backbone phosphorus modifications; pattern of modifications on the internucleotidic phosphorus atom, such as $-S^-$, and $-L-R^1$ of formula I.

540. An oligonucleotide comprising a sequence that shares greater than about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% identity with a sequence found in a provided example oligonucleotide.

540a. The oligonucleotide of embodiment 540, wherein the sequence of the oligonucleotide is the sequence of a provided example oligonucleotide.

540b. The oligonucleotide of embodiment 540 or 540a, wherein the provided example oligonucleotide is an oligonucleotide selected from Table N1A, N2A, N3A, N4A or 8.

541. The oligonucleotide of embodiment 540, wherein the provided example oligonucleotide is WV-1092.

542. The oligonucleotide of embodiment 540, wherein the provided example oligonucleotide is WV-2595.

543. The oligonucleotide of embodiment 540, wherein the provided example oligonucleotide is WV-2603

544. The oligonucleotide of any one of the preceding embodiments, wherein the oligonucleotide comprises the sequence found in the provided example oligonucleotide.

545. The oligonucleotide of any one of the preceding embodiments, wherein the oligonucleotide consists of the sequence found in the provided example oligonucleotide.

546. An oligonucleotide of any one of the preceding embodiments, wherein the oligonucleotide comprises one or more natural phosphate linkages and one or more modified internucleotidic linkages.

547. The oligonucleotide of embodiment 546, wherein the oligonucleotide is an oligonucleotide of any one of embodiments 540-545.

548. The oligonucleotide of any one of the preceding embodiments, wherein the oligonucleotide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, or more natural phosphate linkages.

549. The oligonucleotide of any one of the preceding embodiments, wherein the oligonucleotide comprises one or more modified internucleotidic linkages.

550. The oligonucleotide of any one of the preceding embodiments, wherein the oligonucleotide comprises two or more modified internucleotidic linkages.

551. The oligonucleotide of any one of the preceding embodiments, wherein the oligonucleotide comprises 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more modified internucleotidic linkages.

552. The oligonucleotide of any one of the preceding embodiments, wherein the oligonucleotide comprises 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more modified internucleotidic linkages.

553. The oligonucleotide of any one of the preceding embodiments, wherein the oligonucleotide comprises 10 or more modified internucleotidic linkages.

554. The oligonucleotide of any one of the preceding embodiments, wherein at least one of the modified internucleotidic linkages is a chirally controlled internucleotidic linkage in that oligonucleotides having the same sequence and chemical modifications within a composition share the same configuration, either *R*p or *S*p, at the chiral phosphorus atom of the modified internucleotidic linkage.

555. The oligonucleotide of any one of the preceding embodiments, wherein at least two modified internucleotidic linkages are chirally controlled.

556. The oligonucleotide of any one of the preceding embodiments, wherein at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 modified internucleotidic linkages are chirally controlled.

557. The oligonucleotide of any one of the preceding embodiments, wherein at least one modified internucleotidic linkage within a consecutive modified internucleotidic linkage region is chirally controlled.

558. The oligonucleotide of any one of the preceding embodiments, wherein at least two modified internucleotidic linkages within a consecutive modified internucleotidic linkage region are chirally controlled.

559. The oligonucleotide of any one of the preceding embodiments, wherein at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 modified internucleotidic linkages within a consecutive modified internucleotidic linkage region are chirally controlled.

560. The oligonucleotide of any one of the preceding embodiments, wherein each modified internucleotidic linkage within a consecutive modified internucleotidic linkage region is chirally controlled.

561. The oligonucleotide of any one of the preceding embodiments, wherein each modified internucleotidic linkage is chirally controlled.

562. The oligonucleotide of any one of the preceding embodiments, wherein a provided oligonucleotide comprises a (*S*p)x*R*p(*S*p)y pattern, wherein each of x and y is independently 1-20, and the sum of x and y is 1-50.

563. The oligonucleotide of any one of the preceding embodiments, wherein each of x and y is independently 2-20.

564. The oligonucleotide of any one of the preceding embodiments, wherein at least one of x and y is greater than 5, 6, 7, 8, 9, or 10.

565. The oligonucleotide of any one of the preceding embodiments, wherein the sum of x and y is greater than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20.

566. The oligonucleotide of any one of the preceding embodiments, wherein a provided oligonucleotide comprises one or more chemical modifications.

567. The oligonucleotide of any one of the preceding embodiments, wherein a provided oligonucleotide comprises one or more base modifications.

568. The oligonucleotide of any one of the preceding embodiments, wherein a provided oligonucleotide comprises one or more sugar modifications.

569. The oligonucleotide of any one of the preceding embodiments, wherein a sugar modification is a 2'-modification.

570. The oligonucleotide of any one of the preceding embodiments, wherein a sugar modification is LNA.

571. The oligonucleotide of any one of the preceding embodiments, wherein a provided oligonucleotide is a chirally controlled oligonucleotide.

572. The oligonucleotide of any one of the preceding embodiments, wherein the oligonucleotide is conjugated to a targeting component.

573. A oligonucleotide composition, comprising an oligonucleotide of any one of the preceding embodiments.

573a. The composition of embodiment 573, wherein the composition is a chirally controlled oligonucleotide composition comprising a predetermined level of the oligonucleotide.

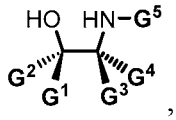
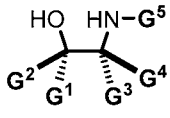
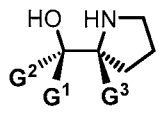
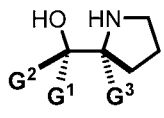
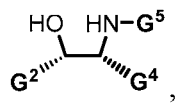
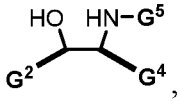
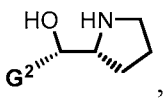
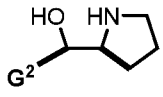
574. The composition of any one of the preceding embodiments, or the composition in the method of any one of the preceding embodiments, further comprising a selectivity agent selected from: the group of compounds which binds specifically to one or more neurotransmitter transporters selected from the group consisting of a dopamine transporter (DAT), a serotonin

transporter (SERT), and a norepinephrine transporter (NET); the group consisting of a dopamine reuptake inhibitor (DRI), a selective serotonin reuptake inhibitor (SSRI), a noradrenaline reuptake inhibitor (NRI), a norepinephrine-dopamine reuptake inhibitor (NDRI), and a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI); the group consisting of a triple reuptake inhibitor, a noradrenaline dopamine double reuptake inhibitor, a serotonin single reuptake inhibitor, a noradrenaline single reuptake inhibitor, and a dopamine single reuptake inhibitor; and the group consisting of a dopamine reuptake inhibitor (DRI), a Norepinephrine-Dopamine Reuptake Inhibitor (NDRI) and a serotonin-Norepinephrine-Dopamine Reuptake Inhibitor (SNDRI).

575. A method for treating or preventing Huntington's Disease in a subject, comprising administering to the subject an oligonucleotide or a composition of any one of the preceding embodiments.

575a. A method of any one of the preceding embodiments, wherein the oligonucleotide or composition is administered via intrathecal administration.

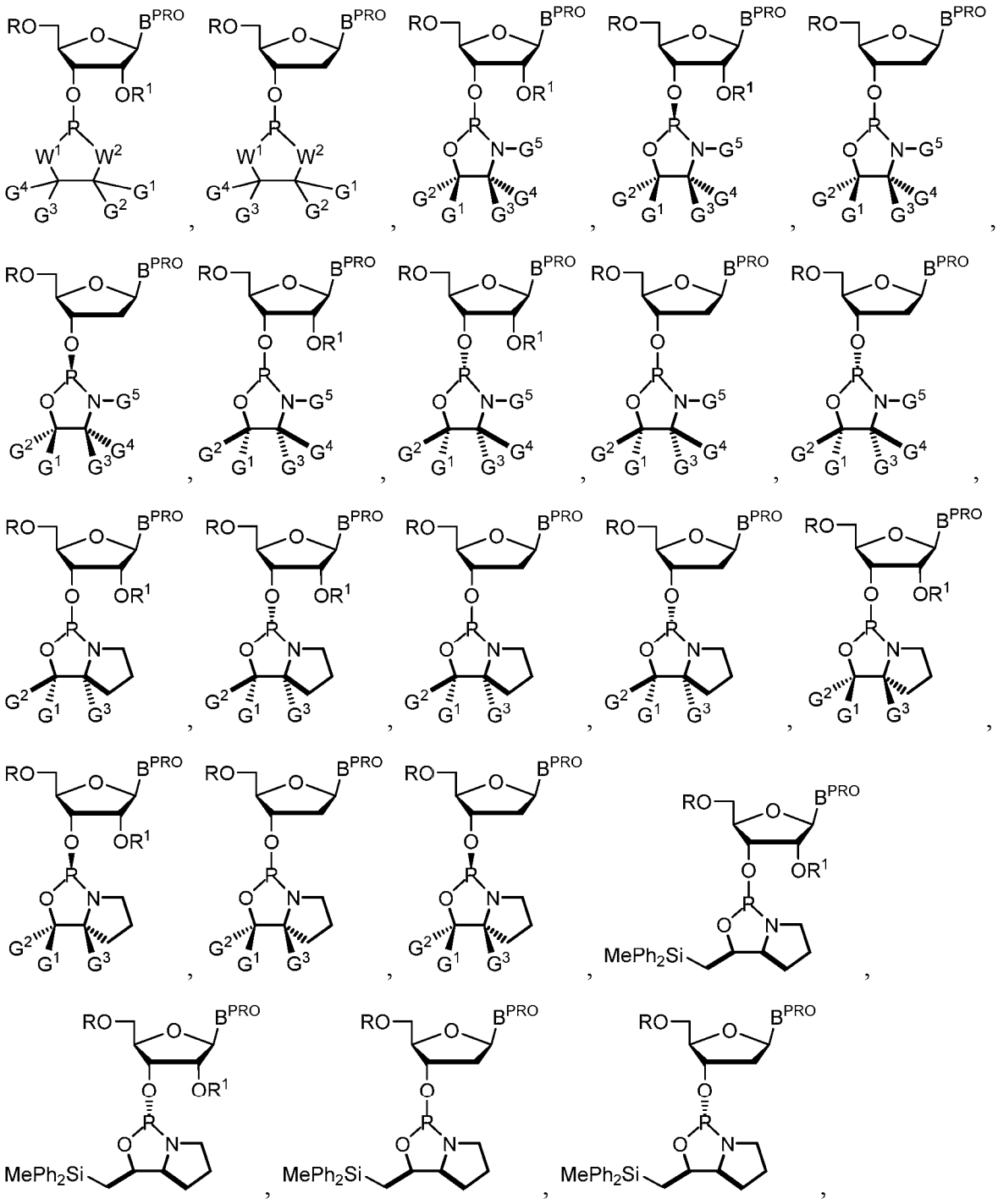
576. A method for preparing an oligonucleotide, comprising providing a chiral reagent having the structure of Formula 3-AA.

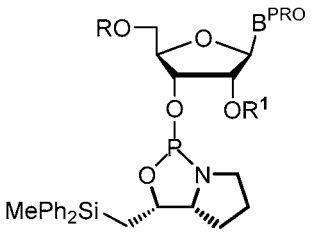
577. A method for preparing an oligonucleotide, comprising providing a chiral reagent having the structure of  ,  ,  ,  ,  ,  ,  , or  .

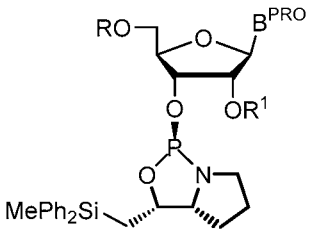
578. The method of any one of the preceding embodiments, wherein the chiral reagent is chirally pure.

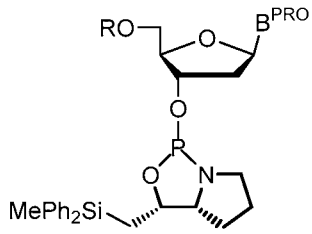
579. A method for preparing an oligonucleotide, comprising providing a compound comprising a moiety from a chiral reagent having the structure of any one of the preceding embodiments, wherein $-W^1H$ and $-W^2H$, or the hydroxyl and amino groups, form bonds with the phosphorus atom of the phosphoramidite.

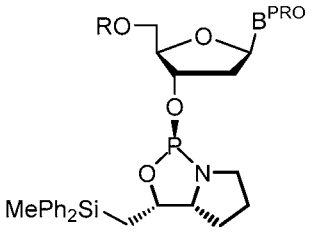
580. The method of embodiment 579, wherein the compound has the structure of





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, or


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581. The method of embodiment 580, wherein R connected to the 5'-O is a hydroxyl protecting group.

582. The method of embodiment 581, wherein the hydroxyl protecting group is DMTr.

583. The method of embodiment 582, wherein B^{PRO} is a protected nucleobase.

584. The method of embodiment 583, wherein the nucleobase is an optionally substituted nucleobase selected from A, T, C and G.

585. The method of any one of the preceding embodiments, wherein W^1 is $-NG^5$, W^2 is O.

586. The method of any one of the preceding embodiments, wherein each of G^1 and G^3 is independently hydrogen or an optionally substituted group selected from C_{1-10} aliphatic, heterocyclyl, heteroaryl and aryl, G^2 is $-C(R)_2Si(R)_3$, and G^4 and G^5 are taken together to form an optionally substituted saturated, partially unsaturated or unsaturated heteroatom-containing ring of up to about 20 ring atoms which is monocyclic or polycyclic, fused or unfused.

587. The method of any one of the preceding embodiments, wherein each R is independently hydrogen, or an optionally substituted group selected from C_1–C_6 aliphatic, carbocyclyl, aryl, heteroaryl, and heterocyclyl.

588. The method of any one of the preceding embodiments, wherein G^1 is hydrogen.

589. The method of any one of the preceding embodiments, wherein G^2 is $-C(R)_2Si(R)_3$, wherein $-C(R)_2-$ is optionally substituted $-CH_2-$, and each R of $-Si(R)_3$ is independently an optionally substituted group selected from C_{1-10} aliphatic, heterocyclyl, heteroaryl and aryl.

590. The method of any one of the preceding embodiments, wherein at least one R of $-Si(R)_3$ is independently optionally substituted C_{1-10} alkyl.

591. The method of any one of the preceding embodiments, wherein at least one R of $-Si(R)_3$ is independently optionally substituted phenyl.

592. The method of any one of the preceding embodiments, wherein one R of $-Si(R)_3$ is independently optionally substituted C_{1-10} alkyl, and each of the other two R is independently optionally substituted phenyl.

593. The method of any one of the preceding embodiments, wherein G^2 is optionally substituted $-CH_2Si(Me)(Ph)_2$.

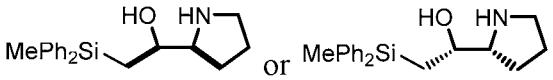
594. The method of any one of the preceding embodiments, wherein G^2 is $-CH_2Si(Me)(Ph)_2$.

595. The method of any one of the preceding embodiments, wherein G^3 is hydrogen.

596. The method of any one of the preceding embodiments, wherein G^4 and G^5 are taken together to form an optionally substituted saturated 5-6 membered ring containing one nitrogen atom.

597. The method of any one of the preceding embodiments, wherein G^4 and G^5 are taken together to form an optionally substituted saturated 5-membered ring containing one nitrogen atom.

598. The method of any one of the preceding embodiments, wherein the chiral reagent is



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599. The method of any one of the preceding embodiments, comprising providing a fluoro-containing reagent.

600. The method of any one of the preceding embodiments, wherein the fluoro-containing reagent is TBAF.

601. The method of any one of the preceding embodiments, comprising using a linker that is stable to a TBAF condition for removing the chiral reagent.

602. The method of any one of the preceding embodiments, wherein the linker is an SP linker.

603. The method of any one of embodiments 576-599, wherein the fluoro-containing reagent is $HF\text{-}NR_3$.

604. The method of embodiment 603, wherein the fluoro-containing reagent is $HF\text{-}NEt_3$.

605. The method of embodiment 603 or 604, comprising using a linker that is stable to a $HF\text{-}NR_3$ condition for removing the chiral reagent.

606. The method of any one of embodiments 603-605, wherein the linker is a succinyl linker.

Examples

[001179] The foregoing has been a description of certain non–limiting embodiments of the disclosure. Accordingly, it is to be understood that the embodiments of the disclosure herein described are merely illustrative of the application of the principles of the disclosure. Reference herein to details of the illustrated embodiments is not intended to limit the scope of the claims.

Example 1. In Vitro Metabolic Stabilities of human Chiromersens in preincubated rat whole liver homogenates

[001180] The present Example describes comparisons of *in vitro* whole rat liver homogenate stability of Mipomersen (stereochemical mixture) with chirally controlled oligonucleotide compositions of Mipomersen ("chiromersens"). The method, among other things, is useful in screening compounds to predict *in vivo* half lives.

[001181] As is known in the art, Mipomersen (previously ISIS 301012, sold under the trade name Kynamro) is a 20mer oligonucleotide whose base sequence is antisense to a portion of the apolipoprotein B gene. Mipomersen inhibits apolipoprotein B gene expression, presumably by targeting mRNA. Mipomersen has the following structure:

G*-C*-C*-U*-C*-dA-dG-dT-dC-dT-dG-dmC-dT-dT-dmC-G*-C*-A*-C*-C*

[d = 2'-deoxy, * = 2'-O-(2-methoxyethyl)]

with 3'→5' phosphorothioate linkages. Thus, Mipomersen has 2'-O-methoxyethyl-modified ribose residues at both ends, and deoxyribose residues in the middle.

[001182] Tested chirally pure Mipomersen analogs described in this Example included 3'→5' phosphorothioate linkages. In some embodiments, tested analogs include one or more 2'-O-(2-methoxyethyl)-modified residues; in some embodiments, tested analogs include only 2'-deoxy residues. Particular tested analogs had the structures set forth below in Tables 3 and 4.

[001183] **Protocol:** We used the protocol reported by Geary et al. (Oligonucleotides, Volume 20, Number 6, 2010) with some modifications.

[001184] **Test system:** Six male Sprague-Dawley rats (Rattus norvegicus) were supplied by Charles River Laboratories, Inc., (Hollister, CA), and were received at SNBL USA.

[001185] **Tissue Collection:** Animals were acclimated to the study room for two days prior to tissue collection. At the time of tissue collection, animals were anesthetized with an

intraperitoneal (IP) injection of sodium pentobarbital solution. Liver perfusion was performed using 500 mL of chilled saline/animal, administered via the hepatic portal vein. After perfusion, the livers were dissected and maintained on ice. Livers were minced into small pieces then weighed.

[001186] Liver Homogenate Preparation: The minced pieces of liver tissues were transferred to tared 50 mL centrifuge tubes and weighed. Chilled homogenization buffer (100 mM Tris pH 8.0, 1 mM magnesium acetate, with antibiotic-antimycotic agents) was added to each tube, such that the tube(s) contained 5 mL of buffer per gram of tissue. Using a QIAGEN TissueRuptor tissue homogenizer, the liver/buffer mixture was homogenized while maintaining the tube on ice. The protein concentration of the liver homogenate pool was determined using a Pierce BCA protein assay. Liver homogenates were divided into 5 mL aliquots, transferred to appropriately sized labeled cryovials and stored at -60 °C.

[001187] Incubation Conditions: 5ml aliquots of frozen liver homogenate (protein concentration = 22.48mg/ml) were thawed and incubated at 37 ºC for 24 hrs. Six eppendorf tubes (2ml) were taken for each oligomer in table 1and 450ul of homogenate was added in each tube. 50ul ASO (200uM) was added to each tube. Immediately after mixing, 125ul of (5X) stop buffer (2.5% IGEPAL, 0.5M NaCl, 5mM EDTA, 50mM Tris, pH=8.0) and 12.5 ul of 20 mg/ml Proteinase K (Ambion, # AM2546) was added to one tube for 0 hour time point. The remaining reaction mixtures were incubated at 37 ºC with shaking at 400 rpm on VWR Incubating Microplate shaker. After incubation for a designated period (1, 2, 3, 4, and 5 days), each mixture was treated with 125ul of (5X) stop buffer (2.5% IGEPAL, 0.5M NaCl, 5mM EDTA, 50mM Tris, pH=8.0) and 12.5ul of 20mg/ml Proteinase K (Ambion, # AM2546).

[001188] Work up and Bioanalysis: ISIS 355868 (5'-GCGTTTGCTCTTCTTCTTGCGTTTTTT-3'), a 27-mer oligonucleotide (underlined bases are MOE modified) was used as the internal standard for quantitation of chiromersens. 50 ul of internal standard (200uM) was added to each tube followed by addition of 250 ul of 30% ammonium hydroxide, 800 ul of Phenol: Chloroform: isoamyl alcohol (25:24:1). After mixing and centrifugation at 600 rpg, the aqueous layer was evaporated on speed vac to 100 ul and loaded on Sep Pak column (C18, 1g, WAT 036905). All the aqueous washings (2x20ml) of Sep pak column were tested with quick Ion Exchange method to ensure that no product was found there. 50% ACN (3.5ml) was used to elute the oligonucleotide and metabolites and the column

was further washed with 70% CAN (3.5) ensure that there was nothing left on the column. Five fractions were collected for each sequence. Water wash1, 2, 3, ACN1 and 2 using Visiprep system (Sigma, part number: 57031-U).

Ion Exchange method

	Time	Flow (ml/min)	%A	%B	Curve
	Time	1.0	95	5	
1	2	1.0	95	5	1
2	3	1.0	75	25	6
3	10	1.0	35	65	6
4	10.1	1.0	95	5	6
5	12.5	1.0	95	5	1

Buffer A = 10mM Tris HCl, 50%ACN, pH=8.0

Buffer B = A+800mM NaClO4

Column = DNA pac 100

Column Temperature 60°C

Wash method was used after each run (Described in M9-Exp21) using the same buffers as above and 50:50 (methanol:water) in buffer line C.

	Time	Flow (ml/min)	%A	%B	%C	Curve
	Time	1.0	0	0	100	
1	5.5	1.0	0	0	100	1
2	5.6	1.0	100	0	0	6
3	7.5	1.0	100	0	0	6
4	7.6	1.0	95	5	0	6
5	12.5	1.0	95	5	0	1

Acetonitrile eluate was concentrated to dryness and dissolved in 100ul water to be analyzed using RPHPIPC.

Eluant A = 10mM Tributylammonium acetate, pH=7.0

Eluant B= ACN (HPLC grade, B& J)

Coulmn : XTerra MS C18, 3.5um, 4.6x50mm, Part number: 186000432

Guard column from Phenomenex, part number: KJ0-4282

Column Temperature = 60°C

HPLC Gradient:

	Time	Flow (ml/min)	%A	%B	Curve
1		1.0	65	35	
2	5.0	1.0	65	35	1
3	30.0	1.0	40	60	6
4	35.0	1.0	5	90	6
5	36.0	1.0	65	35	6
6	40.0	1.0	65	35	1

For Analytical RP HPLC, 10 ul of this stock solution was added to 40 ul water and 40 ul was injected.

Table 3.

S.NO.	Sequence	Description
ONT-41	Gs5mCs5mCs Ts5mCsAs GsTs5mCs TsGs5mCs TsTs5mCs Gs5mCsAs 5mCs5mC	Mipomersen
ONT-87	Gs5mCs5mCsTs5mCsAsGsTs5mCsTsGs5mCsTsTs5mCsGs5mCsAs5mCs5mC	MOE-wing-core-wing design – (human) RNAse H substrate 1 5R-(SSR)$_3$-5R
ONT-154	Gs5mCs5mCsTs5mCsAsGsTs5mCsTsGs5mCsTsTs5mCsGs5mCsAs5mCs5mC	All deoxy, (5S-(SSR)$_3$-5S)
ONT-70	Gs5mCsGsTsTsTsGs5mCsTs5mCsTsTs5mCsTsTs5mCsTsTsGs5mCGsTsTsTsTsT	ISIS 355868 internal standard for quantitation of Mipomersen

[001189] **Discussion:** 2′ modifications in antisense and siRNAs are predicted to stabilize these molecules and increase their the persistence in plasma and tissues compared with wild-type DNAs and siRNAs.

[001190] 2'-MOE wing-core-wing design in Mipomersen. The first generation antisense oligonucleotides employed in the first antisense clinical trials had 2'-deoxy ribonucleotide residues and phosphorothioate internucleoside linkages. Subsequently, second generation antisense oligonucleotides were developed, which were typically of what is referred to herein as "5-10-5 2'-MOE wing-core-wing design", in that five (5) residues at each end were 2'-O-methoxyethyl (2'-MOE)-modified residues and ten (10) residues in the middle were 2'-deoxy ribonucleotides; the internucleotide linkages of such oligonucleotides were phosphorothioates. Such "5-10-5 2'-MOE wing-core-wing" oligonucleotides exhibited marked improvement in potency over first generation (PCT/US2005/033837). Similar wing-core-wing motifs like 2-16-2, 3-14-3, 4-12-4, or 5-10-5 were designed to improve the stability of oligonucleotides to nucleases, while at the same time maintaining enough DNA structure for RNase activity.

[001191] Chirally pure oligonucleotides. The present disclosure provides chirally pure oligonucleotides and demonstrates, among other things, that selection of stereochemistry in and of itself can improve oligonucleotide stability (i.e., independent of residue modification such as 2'MOE modification). Indeed, the present disclosure demonstrates that chirally pure phosphorothioate oligonucleotides can provide same or better stability than corresponding 2'-modified stereorandom phosphorothioate compounds.

[001192] In some embodiments, tested chirally pure oligonucleotides are of the general structure X-Y-X with respect to stereochemistry in that they contain wing "X" regions (typically about 1-10 residues long) where all residues have the same stereochemistry flanking a core "Y" region in which stereochemistry varies. In many embodiments, about 20-50% of the nucleotide analogs in tested such oligonucleotides are not substrates for RNase H. The ability to control the stereochemistry of phosphorothioates in DNA enables us to protect the oligomers from degradation by nucleases while maintaining the RNase active sites. One of these designs is ONT-154 where wings of the oligonucleotide have been stabilized by *S*p phosphorothioate chemistry with retention of few Rp phosphorothioates which are better substrates for RNase H (Molecular Cell, 2007). The crystal structure of human RNase H complexed with DNA/RNA duplex shows that the Phosphate-binding pocket of the enzyme makes contacts with four contiguous

phosphates of DNA. The first three contacts seem stronger than fourth one and they prefer Pro-R/Pro-R/Pro-S oxygen atoms of each of these three phosphates. Combining the stability advantage coming from Sp stereochemistry with RNase H active sites, several sequences can be designed to compete with/or improve upon 2'-modifications. From rat whole liver homogenate stability experiment comparing Mipomersen (ONT-41) with our rational (chiral control) design with and without 2'-modifications (ONT-87 and ONT-154) (Table 1 and Figure 1), it is evident that through removal of the 2'-modifications and careful chiral control with *Rp* and *Sp* phosphorothioates, we can improve the stability of these oligonucleotides which later affect the efficacy *in vivo*.

Table 4. Hu chiromersens studied for rat whole live homogenate stability

Sequence	Description	Target	Tm (°C)
ONT-41	Gs5mCs5mCs Ts5mCsAs GsTs5mCs TsGs5mCs TsTs5mCs Gs5mCsAs 5mCs5mC	Hu ApoB	80.7
ONT-75	**G§5mC§5mC§T§5mC**sAsGsTs5mCsTsGs5mCsTsTs 5mCs**G§5mC§A§5mC§5mC**	Hu ApoB	85.0
ONT-77	**G§5mC§5mC§T§5mC**sAsGsTs5mCsTsGs5mCsTsTs 5mCs**G§5mC§A§5mC§5mC**	Hu ApoB	79.9
ONT-80	**G§5mC§5mC§T§5mC§**AsGsTs5mCsTsGs5mCsTsTs5 mCs**G§5mC§A§5mC§5mC**	Hu ApoB	75.8
ONT-81	**G§5mC§5mC§T§5mC§**AsGsTs5mCsTsGs5mCsTsTs5 mCs**G§5mC§A§5mC§5mC**	Hu ApoB	80.7
ONT-87	**G§5mC§5mC§T§5mC§**AsGsTs5mCsTsGs5mCsTsTs5 mCs**G§5mC§A§5mC§5mC**	Hu ApoB	82.4
ONT-88	**G§5mC§5mC§T§5mC§**AsGsTs5mCsTsGs5mCsTsTs5 mCs**G§5mC§A§5mC§5mC**	Hu ApoB	78.9
ONT-89	**G§5mC§5mC§T§5mC§**AsGsTs5mCsTsGs5mCsTsTs5 mCs**G§5mC§A§5mC§5mC**	Hu ApoB	80.9
ONT-70	Gs5mCsGsTsTsTsGs5mCsTs5mCsTsTs5mCsTsTs5m CsTsTsGs5mCGsTsTsTsTsT	ISIS 355868 internal standard	

Table 5. Mouse chiromersens studied for rat whole live homogenate stability

Sequence	Description	Target
ONT-83	**GsTs5mCs5mCs5mCs**TsGsAsAsGsAsTsGsTs5mCs**AsAsTsGs5mC**	Mouse

		ApoB
ONT-82	**G§T§5mC§5mC§5mC§**TsGsAsAsGsAsTsGsTs5mCs**A§A§T§G§5mC**	Mouse ApoB
ONT-84	**G§T§5mC§5mC§5mC§**TsGsAsAsGsAsTsGsTs5mCs**A§A§T§G§5mC**	Mouse ApoB
ONT-85	**G§T§5mC§5mC§5mC§**TsGsAsAsGsAsTsGsTs5mCs**A§A§T§G§5mC**	Mouse ApoB
ONT-86	**G§T§5mC§5mC§5mC§**TsGsAsAsGsAsTsGsTs5mCs**A§A§T§G§5mC**	Mouse ApoB

Example 2. Example Chirally Controlled siRNA Molecules

Table 1. Summary of Phosphodiester Polar interactions with h-Ago-2 and h-Ago-1

Science 2012 hAgo-2

Phosphate*	Residue	Length/Å	Config.
2	Asn551	2.7	Pro(S)
	Gln548	2.9	Pro(S)
3	Lys566	3.1	Pro(R)
	Arg792	3.4	Pro(R)
4	Tyr790	2.6	Pro(R)
	Arg792	3.0	Pro(R)
		2.8	Pro(R)
		3.4	Pro(S)
5	Ser798	2.7	Pro(R)
		2.9	Pro(R)
	Tyr804	2.8	Pro(S)
6	Lys709	3.0	Pro(S)
	Arg761	2.9	Pro(R)
	His753	2.8	Pro(R)
7	Arg714	2.9	Pro(R)
		3.0	Pro(R)
	Arg761	3.0	Pro(S)

*Phosphate No. from 5'-end

Cell 2012 hAgo-2

Phosphate	Residue	Length/Å	Config.
2	Asn551	2.7	Pro(S)
	Gln548	3.1	Pro(S)
	Gln548	2.9	Pro(R)
3	Lys566	2.9	Pro(R)
	Arg792	3.3	Pro(R)
4	Tyr790	2.8	Pro(R)
	Arg792	2.8	Pro(R)
5	Ser798	2.6	Pro(R)
		2.9	Pro(R)
	Tyr804	2.5	Pro(S)
6	Lys709	3.2	Pro(S)
	Arg761	2.8	Pro(R)
	His753	3.0	Pro(R)
7	Arg714	2.8	Pro(R)
		3.1	Pro(R)
	Arg761	2.8	Pro(S)
8	Arg761	2.4	Pro(S)
	Ala221	3.5	Pro(R)
9	Arg351	2.2	Pro(R)
10	Arg710	2.5	Pro(R)
18	No contacts		
19	Tyr311	3.1	Pro(R)
	Arg315	2.8	Pro(R)
20	His271	3.1	Pro(R)
	His319	3.4	Pro(S)
	Tyr311	2.2	Pro(S)

Cell Rep 2013, h-Ago-1†

Phosphate	Residue	Length/Å	Config.
2	Asn549	2.7	Pro(S)
	Gln546	2.9	Pro(S)
		2.8	Pro(R)
2	Lys564	2.9	Pro(R)
	Arg790	3.4	Pro(R)
		3.3	Pro(R)
4	Tyr788	2.7	Pro(R)
	Arg790	3.3	Pro(R)
5	Ser796	2.5	Pro(R)
		2.8	Pro(R)
	Tyr802	2.6	Pro(S)
6	Lys707	2.8	Pro(S)
	Arg759	2.7	Pro(R)
	His751	3.0	Pro(R)
7	Arg712	3.1	Pro(S)
		3.3	Pro(S)
	Arg373	3.4	Pro(R)
	Thr757	2.8	Pro(R)
	Arg759	2.2	Pro(S)
8	His710	3.4	Pro(R)
	Ser218	2.7	Pro(R)
9	Arg349	3.5	Pro(R)
	Arg708	2.9	Pro(S)
10	Arg708	3.2	Pro(R)
		2.9	Pro(R)
21	Tyr309	2.6	Pro(S)
	Tyr314	2.6	Pro(S)
	His369	3.0	Pro(R)

†Complexed with h-let-7 22mer

[001193] The present disclosure, despite teachings in the art to the contrary, recognizes that stereochemistry of internucleotidic linkages can be utilized to increase stability and activity of oligonucleotides through chirally controlled oligonucleotide compositions. Such chirally controlled oligonucleotide compositions can provide much better results than chirally uncontrolled oligonucleotide compositions as demonstrated in this disclosure.

[001194] There are two reported crystal structures of RNA complexed with human Argonaute-2 protein (hAgo2): The Crystal Structure of Human Argonaute-2, Science, 2012 (PDB-4ei3); and The Structure of Human Argonaute-2 in Complex with miR-20a Cell, 2012 PDB-4f3t). In addition, there is one reported crystal structure of Let-7 RNA complexed with human Argonaute-1 protein (hAgo-1): The Making of a Slicer: Activation of Human Argonaute-1, Cell Rep. 2013 (PDB-4krf).

[001195] Based upon the information contained in these publications, it was anticipated that some judgments could be made about advantageous preferences for stereochemistry at the internucleotidic phosphate linkage if the phosphodiester bonds were to be replaced by phosphorothioate diester bonds. These advantages could relate to significantly improved potency, stability and other pharmacological properties. With this in mind, the computer program Pymol was used to locate all polar interactions between the protein and the internucleotidic phosphodiester linkage of the crystallized RNA for all three structures. Polar interactions at a distance of more than 3.5 Å were ignored.

[001196] The results of this analysis are summarized in Table 1. A particular phosphorus atom from the phosphodiester backbone on the RNA was assigned a Pro(R) or a Pro(S) configuration based upon the assumption that in the phosphorothioate diester analog the quite similar bond would be made between the polar group on an amino acid residue and the respectful phosphate oxygen atom. The sulfur substitution, instead of non-bridging oxygen would therefore confer a unique stereochemistry (either (Sp) or (Rp) absolute configuration) on the phosphorus atom within that motif.

[001197] Of note is the extraordinarily good agreement between the two structures of hAgo-2 in complex with RNA. Also, there is an excellent agreement between the structures of hAgo-1 and hAgo-2 in complex with RNA, indicating that the conformation that the RNA molecule adopts is highly conserved between these two proteins. Any conclusions or rules which are formed based upon the results of this analysis are likely, therefore, to be valid for both

protein molecules.

[001198] As can be seen, there is usually more than one polar interaction at any one phospodiester group, with the exception of those between the phosphodiesters at phosphate positions 9 and 10 and hAgo-2 (Cell 2012) which adopt exclusively Pro(Rp) preference through bonding with Arg351 and Arg710 respectively.

[001199] However, shorter distances (corresponding to stronger interactions) as well as the number of bonds per oxygen can suggest a predominant interaction for the Pro(Rp) or the Pro(Sp) oxygens: henceresulting in several interactions which are predominantly of one stereochemical type or the other. Within this group are the interactions between the phosphodiesters at phosphate positions 2 (Sp), 3 (Rp), 4 (Rp), 6 (Rp), 8 (Sp), 19 (Rp), 20 (Sp) and 21 (Sp).

[001200] Of the remaining interactions, there does not appear to be a preference for one particular stereochemistry to be adopted over the other, so the preferred stereochemistry could be either (Sp) or (Rp).

[001201] Within this category are the interactions formed between the phosphodiesters at phosphate positions 5 (Rp or Sp) and 7 (Rp or Sp).

[001202] For interactions at the other phosphate backbone, there is no crystal structure information, so stereochemistry at these positions can similarly be either (Rp) or (Sp) until empirical data shows otherwise.

[001203] To this end, Table 6 contains several non-limiting example siRNA general constructs which can be conceived to take advantage of this preference for stereochemistry at individual phosphorothioate diester motifs.

Table 6. Example general siRNA constructs

PS*	Chirally Controlled Antisense Strand Construct					
2	(Sp)	(Rp)	(Sp)	(Rp)	(Sp)	(Rp)
3	(Rp)	(Sp)	(Rp)	(Sp)	(Rp)	(Sp)
4	(Rp)	(Sp)	PO	PO	(Rp)	(Sp)
5	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
6	(Rp)	(Sp)	PO	PO	(Rp)	(Sp)
7	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
8	(Sp)	(Rp)	PO	PO	(Sp)	(Rp)
9	(Rp)	(Sp)	PO	PO	(Rp)	(Sp)
10	(Rp)	(Sp)	PO	PO	(Rp)	(Sp)
11	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO

12	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
13	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
14	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
15	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
16	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
17	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
18	(Rp) or (Sp)	(Sp) or (Rp)	PO	PO	PO	PO
19	(Rp)	(Sp)	PO	PO	(Rp)	(Sp)
20	(Sp)	(Rp)	(Sp)	(Rp)	(Sp)	(Rp)
21	(Sp)	(Rp)	(Sp)	(Rp)	(Sp)	(Rp)

*The number indicates the phosphate position from the 5′ end of the antisense strand of the siRNA, (e.g. #2 is located between nucleotides 1 and 2 and #21 is located between nucleotides 20 and 21). (Sp) and (Rp) designates stereochemistry of phosphorus atom on phosphorothioate (PS) diester internucleotidic linkage at the indicated position. PO designates a phosphodiester internucleotidic linkage at the indicated position.

[001204] Example siRNAs include but are not limited to siRNAs having a Sp configuration for a chiral phosphorothioate at the 3'end and at the 5'end of the antisense strand of the siRNA duplex, which confers unprecedentedly increased stability in human serum or biological fluids. That same Sp configuration for the chiral phosphorothioate at the 3'end and at the 5'end of the antisense strand of the siRNA duplex confers unprecedentedly increased biological potency caused by increased affitnity to the Ago2 protein leading to increased activity within the RISC RNAi silencing complex.

[001205] In one embodiment, a single chiral phosphorothioate motif is introduced independently at each position along the antisense or sense strand of the siRNA molecule. For a 21mer, this provides 80 unique sequences, with either an (Sp) or an (Rp) chirally controlled phosphorothioate group. When duplexed independently, 1600 unique combinations of siRNAs are prepared.

siRNA Transfection of Chiral siRNA Molecules

[001206] Hep3B, or HeLa cells are reverse transfected at a density of 2.0×10^4 cells/well in 96-well plates. Transfection of siRNA is carried out with lipofectamine RNAiMax (Life Technologies, cat. No. 13778-150) using the manufacturer's protocol, except with a decreased amount of Lipofectamine RNAiMax of 0.2ul per well. Twelve, 1:3 siRNA duplex dilutions are created starting at 1uM. 10ul of 10x siRNA duplex is then lipoplexed with a prepared mixture of 9.8ul of serum-free medium and 0.2ul of Lipofectamine RNAiMax per well. After a 10-15

minute incubation, 2.0×10^4 cells in 80ul of EMEM cell growing media (ATCC, 30-2003) is added to bring the final volume to 100ul per well. Two separate transfection events are performed for each dose.

[001207] 24 hours after transfection Hep3B or HeLa cells are lysed and mRNA against which the siRNA is targeted is purified using MagMAX™-96 Total RNA Isolation Kit (Life Technologies, AM1830); 15ul of cDNA is synthesized with High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Life Technologies, 4374967). Gene expression is evaluated by Real-Time PCR on a Lightcycler 480(Roche) using a Probes Master Mix(Roche, 04 707 494 001) according to manufacturer's protocol.

IC50s and Data Analysis

[001208] Delta delta Ct method is used to calculate values. Samples are normalized to hGAPDH and calibrated to mock transfected and untreated samples. A stereo-random molecule is used as a control. The data is represented as a mean of 2 biological replicates using Graphpad Prism. A four-parameter linear regression curve is fitted to the data and the bottom and top are constrained to a 0 and 100 constants respectively in order to calculate a relative IC50.

[001209] The present Example demonstrates successful inhibition of target gene expression using siRNA agents comprised of chirally controlled oligonucleotides as described herein. Specifically, this Example describes hybridization of individual oligonucleotide strands prepared through chirally controlled synthesis as described herein, so that double-stranded chirally controlled siRNA oligonucleotide compositions are provided. This Example further demonstrates successful transfection of cells with such agents and, moreover, successful inhibition of target gene expression.

In Vitro Metabolic Stabilities of Human PCSK9 siRNA Duplexes Having Stereocontrolled Phosphorothioate Diester Linkages in Human Serum.

[001210] 10 μM siRNA duplexes were incubated in 90% human serum (50 μL, Sigma, H4522) at 37 °C for 24 hours. A 0 min time point (50 μL) was prepared as well as a PBS control incubation time point (50 μL), where the 10 μM siRNA duplex was incubated in 90% 1×PBS (50 μL at 37 °C for 24 hours. After completion of the incubation, to each time point, were added 10 μL of Stop-Solution (0.5 M NaCl, 50 mM TRIS, 5 mM EDTA, 2.5% IGEPAL), followed by 3.2 μL of Proteinase K (20 mg/mL, Ambion). The samples were incubated at 60 °C for 20 min, and then centrifuged at 2000 rpm for 15 min. The final reaction mixtures were directly analyzed

in denaturing IEX HPLC (injection volume 50 μL). The ratio of integrated area at 24 h and 0 min was used to determine the % of degradation for each siRNA.

[001211] It was observed that the stereochemistry configuration of the single phosphorothioate at position 21 (3'end) of both the antisense strand and the sense strand of the siRNA had a crucial impact on the stability of the duplex upon incubation in Human Serum (Figure 1). As illustrated in the Figure 1 and as determined following the integration ratio of the degradation pattern, an (Rp, Rp) siRNA duplex exhibited a significant 55.0% degradation after 24 h. The stereorandom mixture of phosphorothioates in the stereorandom siRNA showed 25.2% degradation after 24 h. The (Sp/Sp) siRNA showed only minor 7.3% degradation after 24 h. This illustrates the drastic impact that phosphorothioate stereochemistry confers to therapeutic siRNAs. Additional example data were presented in Figure 2, Figure 3, Figure 4 and Figure 5.

[001212] It is observed that each of the stereopure constructs show different potency (IC_{50} values) dependent on the position of the phosphorothioate motif along the backbone. It is also observered that different IC_{50} values are obtained dependent upon whether the phosphorothioate motif at any single position is (Sp) or (Rp). The impact of stereochemistry upon stability is likewise clear and differentiating, using either Human Serum described above, or Human Hepatic Cytosol extract or Snake Venom Phosphodiesterase, or isolated endonuclease or isolated exonuclease.

[001213] Certain design rules may be formulated based upon data obtained in the above example. These design information can be applied for the introduction of multiple chiral phosphorothioate linkages within the antisense and/or sense strand of the siRNA as exemplified below. The present disclosure recognizes that an increased amount of chiral phosphorothioate within the antisense and/or sense strand of the siRNA, introduced at the right positions and having the right stereochemistry configuration leads to greatly improved siRNA constructs in terms of potency and metabolic stability in vitro – translating into greatly pharmacologically enhanced therapeutic siRNAs.

Example Chirally Controlled siRNA Oligonucleotides Targeting PCSK9

[001214] Proprotein convertase subtilisin/kexin type 9 (PCSK9), is an enzyme involved in cholesterol metabolism. PCSK9 binds to the receptor for low density lipoprotein (LDL), triggering its destruction. Although LDL associated with the receptor is also eliminated when the receptor is destroyed, the net effect of PCSK9 binding in fact increases LDL levels, as the

receptor would otherwise cycle back to the cell surface and remove more cholesterol.

[001215] Several companies are developing therapeutic agents that target PCSK9. Of particular relevance to the present disclosure, each of Isis Pharmaceuticals, Santaris Pharma, and Alnylam Pharmaceuticals is developing a nucleic acid agent that inhibits PCSK9. The Isis Pharmaceuticals product, an antisense oligonucleotide, has been shown to increase expression of the LDLR and decrease circulating total cholesterol levels in mice (Graham et al "Antisense inhibition of proprotein convertase subtilisin/kexin type 9 reduces serum LDL in hyperlipidemic mice". *J. Lipid Res.* **48** (4): 763–7, April 2007). Initial clinical trials with the Alnylam Pharmaceuticals product, ALN-PCS, reveal that RNA interference offers an effective mechanism for inhibiting PCSK9 (Frank-Kamenetsky et al "Therapeutic RNAi targeting PCSK9 acutely lowers plasma cholesterol in rodents and LDL cholesterol in nonhuman primates". *Proc. Natl. Acad. Sci. U.S.A.* **105** (33): 11915–20, August 2008).

[001216] In some embodiments, despite known results to the contrary, the present disclosure recognizes that phosphorothioate motifs of one stereochemical conformation or another can be rationally designed to take advantage of increased potency, stability and other pharmacological qualities through chirally controlled oligonucleotide compositions. To reinforce this concept, table 3 contains example stereochemically pure constructs based on an siRNA sequence which targets PCSK9 messenger RNA.

[001217] In this example embodiment, a single chiral phosphorothioate motif is introduced independently at each position along the antisense or sense strand of the siRNA molecule. For a 21mer, this provides 80 unique sequences, with either an (Sp) or an (Rp) chirally controlled phosphorothioate group. When duplexed independently, 1600 unique combinations of siRNAs are prepared.

[001218] In other example embodiments, a single chiral phosphorothioate motif is introduced independently at each position along the antisense or sense strand of the siRNA molecule, while a 3'-(Sp) phosphorothioate linkage is conserved. For a 21mer, this provides another additional 80 unique sequences, with either an (Sp) or an (Rp) chirally controlled phosphorothioate group. When duplexed independently, 1600 unique combinations of siRNAs are prepared.

[001219] In other example embodiments, multiple chiral phosphorothioate motifs are introduced independently at several positions along the antisense or sense strand of the siRNA

molecule, following the codes described in Table 7, while a 3'-(Sp) phosphorothioate linkage is conserved.

Table 7. Example of PCSK-9 Sense and Antisense RNAs

	PCSK9 siRNA Sense Strands
PCSK9 (1)	(*R*p)- uucuAGAccuGuuuuGcuudT*s*dT
PCSK9 (2)	(*S*p)- uucuAGAccuGuuuuGcuudT*s*dT
PCSK9 (3)	(*R*p)- uucuAGAccuGuuuuGcuusdTdT
PCSK9 (4)	(*S*p)- uucuAGAccuGuuuuGcuusdTdT
PCSK9 (5)	(*R*p)- uucuAGAccuGuuuuGcusudTdT
PCSK9 (6)	(*S*p)- uucuAGAccuGuuuuGcusudTdT
PCSK9 (7)	(*R*p)- uucuAGAccuGuuuuGcsuudTdT
PCSK9 (8)	(*S*p)- uucuAGAccuGuuuuGcsuudTdT
PCSK9 (9)	(*R*p)- uucuAGAccuGuuuuGscuudTdT
PCSK9 (10)	(*S*p)- uucuAGAccuGuuuuGscuudTdT
PCSK9 (11)	(*R*p)- uucuAGAccuGuuuusGcuudTdT
PCSK9 (12)	(*S*p)- uucuAGAccuGuuuusGcuudTdT
PCSK9 (13)	(*R*p)- uucuAGAccuGuuusuGcuudTdT
PCSK9 (14)	(*S*p)- uucuAGAccuGuuusuGcuudTdT
PCSK9 (15)	(*R*p)- uucuAGAccuGuusuuGcuudTdT
PCSK9 (16)	(*S*p)- uucuAGAccuGuusuuGcuudTdT
PCSK9 (17)	(*R*p)- uucuAGAccuGusuuuGcuudTdT
PCSK9 (18)	(*S*p)- uucuAGAccuGusuuuGcuudTdT
PCSK9 (19)	(*R*p)- uucuAGAccuGsuuuuGcuudTdT
PCSK9 (20)	(*S*p)- uucuAGAccuGsuuuuGcuudTdT
PCSK9 (21)	(*R*p)- uucuAGAccusGuuuuGcuudTdT
PCSK9 (22)	(*S*p)- uucuAGAccusGuuuuGcuudTdT
PCSK9 (23)	(*R*p)- uucuAGAccsuGuuuuGcuudTdT
PCSK9 (24)	(*S*p)- uucuAGAccsuGuuuuGcuudTdT
PCSK9 (25)	(*R*p)- uucuAGAcscuGuuuuGcuudTdT
PCSK9 (26)	(*S*p)- uucuAGAcscuGuuuuGcuudTdT

PCSK9 (27)	(*R*p)- uucuAGAsccuGuuuuGcuudTdT
PCSK9 (28)	(*S*p)- uucuAGAsccuGuuuuGcuudTdT
PCSK9 (29)	(*R*p)- uucuAGsAccuGuuuuGcuudTdT
PCSK9 (30)	(*S*p)- uucuAGsAccuGuuuuGcuudTdT
PCSK9 (31)	(*R*p)- uucuAsGAccuGuuuuGcuudTdT
PCSK9 (32)	(*S*p)- uucuAsGAccuGuuuuGcuudTdT
PCSK9 (33)	(*R*p)- uucusAGAccuGuuuuGcuudTdT
PCSK9 (34)	(*S*p)- uucusAGAccuGuuuuGcuudTdT
PCSK9 (35)	(*R*p)- uucsuAGAccuGuuuuGcuudTdT
PCSK9 (36)	(*S*p)- uucsuAGAccuGuuuuGcuudTdT
PCSK9 (37)	(*R*p)- uuscuAGAccuGuuuuGcuudTdT
PCSK9 (38)	(*S*p)- uuscuAGAccuGuuuuGcuudTdT
PCSK9 (38)	(*R*p)- usucuAGAccuGuuuuGcuudTdT
PCSK9 (40)	(*S*p)- usucuAGAccuGuuuuGcuudTdT

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

[001220] Synthesis examples for Human PCSK9 siRNA Antisense Strands having several chiral phosphorothioate internucleotide linkages and full chiral phosphorothioate internucleotide linkages.

	Human PCSK9 siRNA Antisense Strands
PCSK9 (41)	(*R*p)- AAGcAAAAcAGGUCuAGAAdTsdT
PCSK9 (42)	(*S*p)- AAGcAAAAcAGGUCuAGAAdTsdT
PCSK9 (43)	(*R*p)- AAGcAAAAcAGGUCuAGAAsdTdT
PCSK9 (44)	(*S*p)- AAGcAAAAcAGGUCuAGAAsdTdT
PCSK9 (45)	(*R*p)- AAGcAAAAcAGGUCuAGAsAdTdT
PCSK9 (46)	(*S*p)- AAGcAAAAcAGGUCuAGAsAdTdT
PCSK9 (47)	(*R*p)- AAGcAAAAcAGGUCuAGsAAdTdT
PCSK9 (48)	(*S*p)- AAGcAAAAcAGGUCuAGsAAdTdT
PCSK9 (49)	(*R*p)- AAGcAAAAcAGGUCuAsGAAdTdT

PCSK9 (50)	(*Sp*)- AAGcAAAAcAGGUCuAsGAAdTdT
PCSK9 (51)	(*Rp*)- AAGcAAAAcAGGUCusAGAAdTdT
PCSK9 (52)	(*Sp*)- AAGcAAAAcAGGUCusAGAAdTdT
PCSK9 (53)	(*Rp*)- AAGcAAAAcAGGUCsuAGAAdTdT
PCSK9 (54)	(*Sp*)- AAGcAAAAcAGGUCsuAGAAdTdT
PCSK9 (55)	(*Rp*)- AAGcAAAAcAGGUsCuAGAAdTdT
PCSK9 (56)	(*Sp*)- AAGcAAAAcAGGUsCuAGAAdTdT
PCSK9 (57)	(*Rp*)- AAGcAAAAcAGGsUCuAGAAdTdT
PCSK9 (58)	(*Sp*)- AAGcAAAAcAGGsUCuAGAAdTdT
PCSK9 (59)	(*Rp*)- AAGcAAAAcAGsGUCuAGAAdTdT
PCSK9 (60)	(*Sp*)- AAGcAAAAcAGsGUCuAGAAdTdT
PCSK9 (61)	(*Rp*)- AAGcAAAAcAsGGUCuAGAAdTdT
PCSK9 (62)	(*Sp*)- AAGcAAAAcAsGGUCuAGAAdTdT
PCSK9 (63)	(*Rp*)- AAGcAAAAcsAGGUCuAGAAdTdT
PCSK9 (64)	(*Sp*)- ΛΛGcΛΛΛΛcsΛGGUCuΛGΛΛdTdT
PCSK9 (65)	(*Rp*)- AAGcAAAAscAGGUCuAGAAdTdT
PCSK9 (66)	(*Sp*)- AAGcAAAAscAGGUCuAGAAdTdT
PCSK9 (67)	(*Rp*)- AAGcAAAsAcAGGUCuAGAAdTdT
PCSK9 (68)	(*Sp*)- AAGcAAAsAcAGGUCuAGAAdTdT
PCSK9 (69)	(*Rp*)- AAGcAAsAAcAGGUCuAGAAdTdT
PCSK9 (70)	(*Sp*)- AAGcAAsAAcAGGUCuAGAAdTdT
PCSK9 (71)	(*Rp*)- AAGcAsAAAcAGGUCuAGAAdTdT
PCSK9 (72)	(*Sp*)- AAGcAsAAAcAGGUCuAGAAdTdT
PCSK9 (73)	(*Rp*)- AAGcsAAAAcAGGUCuAGAAdTdT
PCSK9 (74)	(*Sp*)- AAGcsAAAAcAGGUCuAGAAdTdT
PCSK9 (75)	(*Rp*)- AAGscAAAAcAGGUCuAGAAdTdT
PCSK9 (76)	(*Sp*)- AAGscAAAAcAGGUCuAGAAdTdT
PCSK9 (77)	(*Rp*)- AAsGcAAAAcAGGUCuAGAAdTdT
PCSK9 (78)	(*Sp*)- AAsGcAAAAcAGGUCuAGAAdTdT
PCSK9 (77)	(*Rp*)- AsAGcAAAAcAGGUCuAGAAdTdT

PCSK9 (78)	(*Sp*)- AsAGcAAAAcAGGUCuAGAAdTdT
PCSK9 (79)	(*Rp*, *Sp*)- AAGcAAAAcAGGUCuAGAAsdTsdT
PCSK9 (80)	(*Sp*, *Sp*)- AAGcAAAAcAGGUCuAGAAsdTsdT
PCSK9 (81)	(*Rp*, *Sp*)- AAGcAAAAcAGGUCuAGAsAdTsdT
PCSK9 (82)	(*Sp*, *Sp*)- AAGcAAAAcAGGUCuAGAsAdTsdT
PCSK9 (83)	(*Rp*, *Sp*)- AAGcAAAAcAGGUCuAGsAAdTsdT
PCSK9 (84)	(*Sp*, *Sp*)- AAGcAAAAcAGGUCuAGsAAdTsdT
PCSK9 (85)	(*Rp*, *Sp*)- AAGcAAAAcAGGUCuAsGAAdTsdT
PCSK9 (86)	(*Sp*, *Sp*)- AAGcAAAAcAGGUCuAsGAAdTsdT
PCSK9 (87)	(*Rp*, *Sp*)- AAGcAAAAcAGGUCusAGAAdTsdT
PCSK9 (88)	(*Sp*, *Sp*)- AAGcAAAAcAGGUCusAGAAdTsdT
PCSK9 (89)	(*Rp*, *Sp*)- AAGcAAAAcAGGUCsuAGAAdTsdT
PCSK9 (90)	(*Sp*, *Sp*)- AAGcAAAAcAGGUCsuAGAAdTsdT
PCSK9 (91)	(*Rp*, *Sp*)- AAGcAAAAcAGGUsCuAGAAdTsdT
PCSK9 (92)	(*Sp*, *Sp*)- ΛΛGcΛΛΛΛcΛGGUsCuΛGΛΛdTsdT
PCSK9 (93)	(*Rp*, *Sp*)- AAGcAAAAcAGGsUCuAGAAdTsdT
PCSK9 (94)	(*Sp*, *Sp*)- AAGcAAAAcAGGsUCuAGAAdTsdT
PCSK9 (95)	(*Rp*, *Sp*)- AAGcAAAAcAGsGUCuAGAAdTsdT
PCSK9 (96)	(*Sp*, *Sp*)- AAGcAAAAcAGsGUCuAGAAdTsdT
PCSK9 (97)	(*Rp*, *Sp*)- AAGcAAAAcAsGGUCuAGAAdTsdT
PCSK9 (98)	(*Sp*, *Sp*)- AAGcAAAAcAsGGUCuAGAAdTsdT
PCSK9 (99)	(*Rp*, *Sp*)- AAGcAAAAcsAGGUCuAGAAdTsdT
PCSK9 (100)	(*Sp*, *Sp*)- AAGcAAAAcsAGGUCuAGAAdTsdT
PCSK9 (101)	(*Rp*, *Sp*)- AAGcAAAAscAGGUCuAGAAdTsdT
PCSK9 (102)	(*Sp*, *Sp*)- AAGcAAAAscAGGUCuAGAAdTsdT
PCSK9 (103)	(*Rp*, *Sp*)- AAGcAAAsAcAGGUCuAGAAdTsdT
PCSK9 (104)	(*Sp*, *Sp*)- AAGcAAAsAcAGGUCuAGAAdTsdT
PCSK9 (105)	(*Rp*, *Sp*)- AAGcAAsAAcAGGUCuAGAAdTsdT
PCSK9 (106)	(*Sp*, *Sp*)- AAGcAAsAAcAGGUCuAGAAdTsdT
PCSK9 (107)	(*Rp*, *Sp*)- AAGcAsAAAcAGGUCuAGAAdTsdT

PCSK9 (108)	(*S*p, *S*p)- AAGcAsAAAcAGGUCuAGAAdTsdT
PCSK9 (109)	(*R*p, *S*p)- AAGcsAAAAcAGGUCuAGAAdTsdT
PCSK9 (110)	(*S*p, *S*p)- AAGcsAAAAcAGGUCuAGAAdTsdT
PCSK9 (111)	(*R*p, *S*p)- AAGscAAAAcAGGUCuAGAAdTsdT
PCSK9 (112)	(*S*p, *S*p)- AAGscAAAAcAGGUCuAGAAdTsdT
PCSK9 (113)	(*R*p, *S*p)- AAsGcAAAAcAGGUCuAGAAdTsdT
PCSK9 (114)	(*S*p, *S*p)- AAsGcAAAAcAGGUCuAGAAdTsdT
PCSK9 (115)	(*R*p, *S*p)- AsAGcAAAAcAGGUCuAGAAdTsdT
PCSK9 (116)	(*S*p, *S*p)- AsAGcAAAAcAGGUCuAGAAdTsdT
PCSK9 (117)	(*R*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *R*p, *R*p)- AsAsGscAsAAsAscsAGGUCuAGAsAsdTsdT
PCSK9 (118)	(*S*p, *R*p, *R*p, *R*p, *S*p, *R*p, *R*p, *R*p, *S*p, *S*p)- AsAsGscAsAAsAscsAGGUCuAGAsAsdTsdT

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

Example 3. Stereopure FOXO-1 antisense analogs.

Rational Design − Chirally Controlled Antisense Oligonucleotide Compositions

[001221] The unprecedented nuclease stability determined *in vivo* and in a whole rat liver homogenate model of the Sp-chiral phosphorothioate internucleotide linkage is applied in the novel design of new types of RNaseH substrate gapmers, whereby the external flanks are composed of unmodified DNA and the internal gap core is modified with 2' chemical modifications (2'OMe, 2'MOE, 2'LNA, 2'F, *etc*). Eventually this design is extended to fully unmodified DNA therapeutic oligonucleotides wherein careful chiral control of the phosphorothioate backbone confers the desired pharmacological properties of the RNaseH therapeutic oligonucleotide.

[001222] The application of the triplet-phosphate repeating motif designed after studying the crystal structure of human RNaseH has been employed as well. The crystal structure of RNaseH has been previously published (Structure of Human RNase H1 Complexed with an RNA/DNA Hybrid: Insight into HIV Reverse Transcription, Nowotny et al., Molecular Cell, Volume 28, Issue 2, 264-276, 2007, pdb file: 2qkb). Among other things, the present disclosure recognizes the importance of internucleotidic linkage stereochemistry of oligonucleotides, for

example, in settings herein. Upon performing *in silico* analysis upon this structure using the program Pymol, Applicant found that the phosphate-binding pocket of Human RNase H1 makes polar contacts with three contiguous phosphates of the complexed DNA, and interacts preferentially with the Pro-R/Pro-R/Pro-S (or with the Pro-S/Pro-S/Pro-R) respective oxygen atoms of each of these three phosphates. Based on this observation we designed two chiral architectures with repeating (RRS) and (SSR) triplet phosphorothioates motifs as designed RNase H substrates. Applicant also designed other internucleotidic linkage stereochemical patterns. As demonstrated by example results provide herein, provided chirally controlled oligonucleotide compositions of oligonucleotide types that comprises certain backbone internucleotidic linkage patterns (patterns backbone chiral centers) provides significantly increased activity and/or kinetics. Among others, a sequence of 5'-RSS-3' backbone chiral centers is particularly useful and delivers unexpected results as described in the present disclosure.

[001223] The combination of increased Sp chiral backbone (for enzymatic stability and other pharmacologically advantageous properties) and (RRS) or (SSR) repeating triplet chiral backbone motifs (for enhancing the property as RNase H substrate) are also utilized in the novel designs.; "S" represents Sp-phosphorothioate linkage and "R" represents Rp-phosphorothioate linkage.

[001224] Another alternative design is based on the increased amount of *S*p chiral phosphorothioate backbone in extended repeating motifs such as:

$(SSSR)_n$, $SR(SSSR)_n$, $SSR(SSSR)_n$, $SSR(SSSR)_n$;

$(SSSSR)_n$, $SR(SSSSR)_n$, $SSR(SSSSR)_n$, $SSR(SSSSR)_n$, $SSSR(SSSSR)_n$;

$(SSSSSR)_n$; $SR(SSSSSR)_n$, $SSR(SSSSSR)_n$, $SSR(SSSSSR)_n$, $SSSR(SSSSSR)_n$, $SSSSR(SSSSSR)_n$; *etc.*, where n = 0-50, depending on the number of respective internucleotide linkages.; "S" represents *S*p-phosphorothioate linkage and "R" represents *R*p-phosphorothioate linkage. In some embodiments, n is 0. In some embodiments, R is 1-50. In some embodiments, R is 1. In some embodiments, a common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises a motif described herein. In some embodiments, a motif is in the core region. In some embodiments, n is 0. In some embodiments, R is 1-50. In some embodiments, R is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5.

[001225] Another alternative design is based on the "invert" architecture design of the stereo backbone ("stereo invert-mers"). These result from positioning the stereochemistry of the chiral phosphorothioate in a inverting manner, exposing some *S*p-rich motifs at the 5' and 3' end extremities of the oligonucleotide as well as the middle portion of the oligonucleotide and having the repeating stereochemistry motifs positioned in a invert image manner on both sides, such as:

$SS(SSR)_n(SSS)(RSS)_nSS$;

$SS(SSR)_n(SRS)(RSS)_nSS$;

$SS(SSR)_n(SSR)(RSS)_nSS$;

$SS(SSR)_n(RSS)(RSS)_nSS$;

$SS(RSS)_n(SSS)(SSR)_nSS$;

$SS(RSS)_n(SRS)(SSR)_nSS$;

$SS(RSS)_n(SSR)(SSR)_nSS$;

$SS(RSS)_n(RSS)(SSR)_nSS$; *etc.*,

where n = 0-50, depending on the number of respective internucleotide linkages. "S" represents Sp-phosphorothioate linkage and "R" represents Rp-phosphorothioate linkage. In some embodiments, a common pattern of backbone chiral centers of a provided chirally controlled oligonucleotide composition comprises a motif described herein. In some embodiments, a motif is in the core region. In some embodiments, n is 0. In some embodiments, n is 1. In some embodiments, n is 1-50. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5.

Initial screen

Synthesis: Summary for Oligonucleotide Synthesis on a DNA/RNA Synthesizer MerMade-12 (2'-deoxy and 2'-OMe cycle)

step	reaction	reagent	delivery volume (mL)	wait time (sec)
1	detritylation	3% TCA in DCM	4 x 1	N. A.
2	coupling	0.15 M phosphoramidite in ACN + 0.45 M ETT in	2 x 0.5 mL	60 + 60 (DNA), 300 + 300 (2'-OMe RNA)

		ACN		
3	capping	5% Pac_2O in THF/2,6-lutidine + 16% NMI in THF	1	60
4	oxidation	0.02 Iodine in water/pyridine	1	240

Stereorandom PS oligonucleotides having DNA-2'-OMe-DNA (7-6-7) design:

ONT-141 d(CsCsCsTsCsTsGs)gsaststsgsasd(GsCsAsTsCsCsA)
ONT-142 d(AsAsGsCsTsTsTs)gsgststsgsgsd(GsCsAsAsCsAsC)
ONT-143 d(AsGsTsCsAsCsTs)tsgsgsgsasgsd(CsTsTsCsTsCsC)
ONT-144 d(CsAsCsTsTsGsGs)gsasgscststsd(CsTsCsCsTsGsG)
ONT-145 d(AsTsAsGsCsCsAs)tstsgscsasgsd(CsTsGsCsTsCsA)
ONT-146 d(TsGsGsAsTsTsGs)asgscsastscsd(CsAsCsCsAsAsG)
ONT-147 d(CsCsAsTsAsGsCs)csaststsgscsd(AsGsCsTsGsCsT)
ONT-148 d(GsTsCsAsCsTsTs)gsgsgsasgscsd(TsTsCsTsCsCsT)
ONT-149 d(CsCsAsGsGsGsCs)ascstscsastsd(CsTsGsCsAsTsG)
ONT-150 d(GsCsCsAsTsCsCs)asasgstscsasd(CsTsTsGsGsGsA)
ONT-151 d(GsAsAsGsCsTsTs)tsgsgststsgsd(GsGsCsAsAsCsA)
ONT-152 d(CsTsGsGsAsTsTs)gsasgscsastsd(CsCsAsCsCsAsA)
ONT-183 d(CsAsAsGsTsCsAs)cststsgsgsgsd(AsGsCsTsTsCsT)
ONT-184 d(AsTsGsCsCsAsTs)cscsasasgstsd(CsAsCsTsTsGsG)
ONT-185 d(AsTsGsAsGsAsTs)gscscstsgsgsd(CsTsGsCsCsAsT)
ONT-186 d(TsTsGsGsGsAsGs)cststscstscsd(CsTsGsGsTsGsG)
ONT-187 d(TsGsGsGsAsGsCs)tstscstscscsd(TsGsGsTsGsGsA)
ONT-188 d(TsTsAsTsGsAsGs)astsgscscstsd(GsGsCsTsGsCsC)
ONT-189 d(GsTsTsAsTsGsAs)gsastsgscscsd(TsGsGsCsTsGsC)
ONT-190 d(CsCsAsAsGsTsCs)ascststsgsgsd(GsAsGsCsTsTsC)
ONT-191 d(AsGsCsTsTsTsGs)gststsgsgsgsd(CsAsAsCsAsCsA)
ONT-192 d(TsAsTsGsAsGsAs)tsgscscstsgsd(GsCsTsGsCsCsA)
ONT-193 d(TsGsTsTsAsTsGs)asgsastsgscsd(CsTsGsGsCsTsG)
ONT-194 d(AsTsCsCsAsAsGs)tscsascststsd(GsGsGsAsGsCsT)
ONT-195 d(GsGsGsAsAsGsCs)stststsgsgstsd(TsGsGsGsCsAsA)
ONT-196 d(CsTsCsCsAsTsCs)csastsgsasgsd(GsTsCsAsTsTsC)
ONT-197 d(AsAsGsTsCsAsCs)tstsgsgsgsasd(GsCsTsTsCsTsC)
ONT-198 d(CsCsAsTsCsCsAs)asgstscsascsd(TsTsGsGsGsAsG)
ONT-199 d(TsCsCsAsAsGsTs)csascststsgsd(GsGsAsGsCsTsT)
ONT-200 d(CsCsTsCsTsGsGs)aststsgsasgsd(CsAsTsCsCsAsC)
ONT-201 d(AsCsTsTsGsGsGs)asgscststscsd(TsCsCsTsGsGsT)
ONT-202 d(CsTsTsGsGsGsAs)gscststscstsd(CsCsTsGsGsTsG)

ONT-203	d(CsAsTsGsCsCsAs)tscscsasasgsd(TsCsAsCsTsTsG)
ONT-204	d(TsGsCsCsAsTsCs)csasasgstscsd(AsCsTsTsGsGsG)
ONT-205	d(TsCsCsAsTsCsCs)astsgsasgsgsd(TsCsAsTsTsCsC)
ONT-206	d(AsGsGsGsCsAsCs)tscsastscstsd(GsCsAsTsGsGsG)
ONT-207	d(CsCsAsGsTsTsCs)cststscsastsd(TsCsTsGsCsAsC)
ONT-208	d(CsAsTsAsGsCsCs)aststsgscsasd(GsCsTsGsCsTsC)
ONT-209	d(TsCsTsGsGsAsTs)tsgsasgscsasd(TsCsCsAsCsCsA)
ONT-210	d(GsGsAsTsTsGsAs)gscsastscscsd(AsCsCsAsAsGsA)

Biology *in vitro* data in HepG2 cells for the initial DNA-2'-OMe-DNA (7-6-7) design: (d Upper case) = DNA; lower case = 2'-OMe; s = phosphorothioate.

FOXO1

Levels at 20 nM	(%)	SD
ONT-141	89	6
ONT-142	45	1
ONT-143	98	2
ONT-144	89	1
ONT-145	46	5
ONT-146	99	1
ONT-147	66	6
ONT-148	101	2
ONT-149	95	6
ONT-150	58	4
ONT-151	41	5
ONT-152	84	5
ONT-183	95	2
ONT-184	58	4
ONT-185	42	2
ONT-186	96	4
ONT-187	92	3
ONT-188	47	5
ONT-189	63	5
ONT-190	83	2
ONT-191	58	4
ONT-192	46	2
ONT-193	58	2
ONT-194	76	1
ONT-195	66	0
ONT-196	77	2
ONT-197	90	6
ONT-198	42	4
ONT-199	68	1
ONT-200	89	6
ONT-201	91	2
ONT-202	94	2

ONT-203	86	1
ONT-204	58	2
ONT-205	75	3
ONT-206	94	5
ONT-207	96	0
ONT-208	54	0
ONT-209	87	4
ONT-210	92	4

FOXO1

Levels at 200 nM	(%)	SD
ONT-141	37	4
ONT-142	45	4
ONT-143	46	2
ONT-144	42	5
ONT-145	53	4
ONT-146	31	2
ONT-147	28	8
ONT-148	45	4
ONT-149	29	5
ONT-150	32	6
ONT-151	38	4
ONT-152	30	5
ONT-183	60	5
ONT-184	34	2
ONT-185	50	2
ONT-186	86	3
ONT-187	76	6
ONT-188	50	5
ONT-189	38	2
ONT-190	51	1
ONT-191	43	5
ONT-192	54	7
ONT-193	41	6
ONT-194	50	1
ONT-195	43	6
ONT-196	33	7
ONT-197	57	4
ONT-198	40	5
ONT-199	50	5
ONT-200	28	9
ONT-201	46	6
ONT-202	57	9
ONT-203	27	7
ONT-204	36	6
ONT-205	29	5

ONT-206	81	0
ONT-207	37	4
ONT-208	43	3
ONT-209	35	4
ONT-210	40	4

Stereorandom PS oligonucleotides having 2'-OMe-DNA-2'OMe (3-14-3) design: (d Upper case) = DNA; lower case = 2'-OMe; s = phosphorothioate.

ONT-129	cscscsd(TsCsTsGsGsAsTsTsGsAsGsCsAsTs)cscsa
ONT-130	asasgsd(CsTsTsTsGsGsTsTsGsGsGsCsAsAs)csasc
ONT-131	asgstsd(CsAsCsTsTsGsGsGsAsGsCsTsTsCs)tscsc
ONT-132	csascsd(TsTsGsGsGsAsGsCsTsTsCsTsCsCs)tsgsg
ONT-133	astsasd(GsCsCsAsTsTsGsCsAsGsCsTsGsCs)tscsa
ONT-134	tsgsgsd(AsTsTsGsAsGsCsAsTsCsCsAsCsCs)asasg
ONT-135	cscsasd(TsAsGsCsCsAsTsTsGsCsAsGsCsTs)gscst
ONT-136	gstscsd(AsCsTsTsGsGsGsAsGsCsTsTsCsTs)cscst
ONT-137	cscsasd(GsGsGsCsAsCsTsCsAsTsCsTsGsCs)astsg
ONT-138	gscscsd(AsTsCsCsAsAsGsTsCsAsCsTsTsGs)gsgsa
ONT-139	gsasasd(GsCsTsTsTsGsGsTsTsGsGsGsCsAs)ascsa
ONT-140	cstsgsd(GsAsTsTsGsAsGsCsAsTsCsCsAsCs)csasa
ONT-155	csasasd(GsTsCsAsCsTsTsGsGsGsAsGsCsTs)tscst
ONT-156	astsgsd(CsCsAsTsCsCsAsAsGsTsCsAsCsTs)tsgsg
ONT-157	astsgsd(AsGsAsTsGsCsCsTsGsGsCsTsGsCs)csast
ONT-158	tstsgsd(GsGsAsGsCsTsTsCsTsCsCsTsGsGs)tsgsg
ONT-159	tsgsgsd(GsAsGsCsTsTsCsTsCsCsTsGsGsTs)gsgsa
ONT-160	tstsasd(TsGsAsGsAsTsGsCsCsTsGsGsCsTs)gscsc
ONT-161	gststsd(AsTsGsAsGsAsTsGsCsCsTsGsGsCs)tsgsc
ONT-162	cscsasd(AsGsTsCsAsCsTsTsGsGsGsAsGsCs)tstsc
ONT-163	asgscsd(TsTsTsGsGsTsTsGsGsGsCsAsAsCs)ascsa
ONT-164	tsastsd(GsAsGsAsTsGsCsCsTsGsGsCsTsGs)cscsa
ONT-165	tsgstsd(TsAsTsGsAsGsAsTsGsCsCsTsGsGs)cstsg
ONT-166	astscsd(CsAsAsGsTsCsAsCsTsTsGsGsGsAs)gscst
ONT-167	gsgsgsd(AsAsGsCsTsTsTsGsGsTsTsGsGsGs)csasa
ONT-168	cstscsd(CsAsTsCsCsAsTsGsAsGsGsTsCsAs)tstsc
ONT-169	asasgsd(TsCsAsCsTsTsGsGsGsAsGsCsTsTs)cstsc
ONT-170	cscsasd(TsCsCsAsAsGsTsCsAsCsTsTsGsGs)gsasg
ONT-171	tscscsd(AsAsGsTsCsAsCsTsTsGsGsGsAsGs)cstst
ONT-172	cscstsd(CsTsGsGsAsTsTsGsAsGsCsAsTsCs)csasc
ONT-173	ascstsd(TsGsGsGsAsGsCsTsTsCsTsCsCsTs)gsgst
ONT-174	cststsd(GsGsGsAsGsCsTsTsCsTsCsCsTsGs)gstsg
ONT-175	csastsd(GsCsCsAsTsCsCsAsAsGsTsCsAsCs)tstsg
ONT-176	tsgscsd(CsAsTsCsCsAsAsGsTsCsAsCsTsTs)gsgsg
ONT-177	tscscsd(AsTsCsCsAsTsGsAsGsGsTsCsAsTs)tscsc
ONT-178	asgsgsd(GsCsAsCsTsCsAsTsCsTsGsCsAsTs)gsgsg
ONT-179	cscsasd(GsTsTsCsCsTsTsCsAsTsTsCsTsGs)csasc

ONT-180	csastsd(AsGsCsCsAsTsTsGsCsAsGsCsTsGs)cstsc
ONT-181	tscstsd(GsGsAsTsTsGsAsGsCsAsTsCsCsAs)cscsa
ONT-182	gsgsasd(TsTsGsAsGsCsAsTsCsCsAsCsCsAs)asgsa

Biology *in vitro* data in HepG2 cells for the 2'-OMe-DNA-2'-OMe (3-14-3) design:

FOXO1

	Levels at 20 nM (%)	SD
ONT-129	82	5
ONT-130	49	4
ONT-131	92	3
ONT-132	91	2
ONT-133	58	3
ONT-134	73	2
ONT-135	65	5
ONT-136	92	2
ONT-137	94	2
ONT-138	78	1
ONT-139	61	1
ONT-140	82	4
ONT-155	95	2
ONT-156	74	1
ONT-157	56	2
ONT-158	93	1
ONT-159	94	1
ONT-160	71	1
ONT-161	67	1
ONT-162	89	1
ONT-163	55	7
ONT-164	68	4
ONT-165	70	1
ONT-166	89	4
ONT-167	81	0
ONT-168	81	0
ONT-169	94	0
ONT-170	88	1
ONT-171	92	4
ONT-172	86	2
ONT-173	90	1
ONT-174	93	2
ONT-175	84	1
ONT-176	80	2
ONT-177	83	2
ONT-178	95	2
ONT-179	93	8
ONT-180	68	7

ONT-181	85	5
ONT-182	80	5

FOXO1

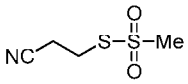
Levels at 200 nM	(%)	SD
ONT-129	27	1
ONT-130	46	4
ONT-131	53	9
ONT-132	53	2
ONT-133	48	6
ONT-134	35	9
ONT-135	45	15
ONT-136	40	7
ONT-137	50	4
ONT-138	80	3
ONT-139	40	3
ONT-140	33	13
ONT-155	52	2
ONT-156	35	4
ONT-157	39	2
ONT-158	87	6
ONT-159	89	5
ONT-160	33	10
ONT-161	40	11
ONT-162	60	7
ONT-163	42	8
ONT-164	34	10
ONT-165	38	1
ONT-166	62	9
ONT-167	64	1
ONT-168	38	2
ONT-169	67	3
ONT-170	74	8
ONT-171	65	5
ONT-172	33	18
ONT-173	72	15
ONT-174	65	15
ONT-175	38	21
ONT-176	48	8
ONT-177	28	5
ONT-178	97	11
ONT-179	47	6
ONT-180	56	12
ONT-181	45	26
ONT-182	33	17

Hit selection:

ONT-151	d(GsAsAsGsCsTsTs)tsgsgststsgsd(GsGsCsAsAsCsA)
ONT-198	d(CsCsAsTsCsCsAs)asgstscsascsd(TsTsGsGsGsAsG)
ONT-185	d(AsTsGsAsGsAsTs)gscscstsgsgsd(CsTsGsCsCsAsT)
ONT-142	d(AsAsGsCsTsTsTs)gsgststsgsgsd(GsCsAsAsCsAsC)
ONT-145	d(AsTsAsGsCsCsAs)tstsgscsasgsd(CsTsGsCsTsCsA)
ONT-192	d(TsAsTsGsAsGsAs)tsgscscstsgsd(GsCsTsGsCsCsA)
ONT-188	d(TsTsAsTsGsAsGs)astsgscscstsd(GsGsCsTsGsCsC)

Secondary screen. Chemistry and Stereochemistry Screen

Summary for Oligonucleotide Synthesis on a DNA/RNA Synthesizer MerMade-12 (stereodefined phosphorothioate 2'-deoxy and 2'-OMe cycle)

step	reaction	reagent	delivery vol (mL)	wait time (sec)
1	detritylation	3% TCA in DCM	4 x 1	N. A.
2	coupling	0.15 M chiral phosphoramidite in ACN + 2 M CMPT in ACN	2 x 0.5	2 x 450 (2'-OMe RNA) 2 x 300 (DNA)
3	capping 1	5% Pac_2O in THF/2,6-lutidine	1	60
4	capping 2	5% Pac_2O in THF/2,6-lutidine + 16% NMI in THF	1	60
5	sulfurization	0.3 M *S*-(2-cyanoethyl) methylthiosulfonate  in ACN/BSTFA	1	600

Examples applied on the FOXO1 Hit sequences:

Examples include but are not limited to:

(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*)d[GsAsAsGsCsTsTsTsGsGsTsTsGsGsGsCsAsAsCsA]

(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)d[GsAsAsGsCsTsTsTsGsGsTsTsGsGsGsCsAsAsCsA]

(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)d[GsAsAsGsCsTsTsTsGsGsTsTsGsGsGsCsAsAsCsA]

(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*)d[CsCsAsTsCsCsAs]$\underline{(AsGsTsCsAsCs)_{OMe}}$d[TsTsGsGsGsAsG]

(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*)d[AsTsGsAsGsAsTs]$\underline{(GsCsCsTsGsGs)_{OMe}}$d[CsTsGsCsCsAsT]

(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*)d[CsCsAsTsCsCsAs]$\underline{(AsGsTsCsAsCs)_{LNA}}$d[TsTsGsGsGsAsG]

(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*)d[AsTsGsAsGsAsTs]$\underline{(GsCsCsTsGsGs)_{LNA}}$d[CsTsGsCsCsAsT]

(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*,

Sp)d[CsCsAsTsCsCsAs](AsGsTsCsAsCs)$_{MOE}$d[TsTsGsGsGsAsG]
(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*,
Sp)d[AsTsGsAsGsAsTs](GsCsCsTsGsGs)$_{MOE}$d[CsTsGsCsCsAsT]
(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*,
Sp)(CsCsAs)$_{OMe}$d[TsCsCsAsAsGsTsCsAsCsTsTsGsGs](GsAsG)$_{OMe}$
(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*,
Sp)(AsTsGs)$_{MOE}$d[AsGsAsTsGsCsCsTsGsGsCsTsGsCs](CsAsT)$_{MOE}$
(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*,
Sp)(CsCsAs)$_{LNA}$d[TsCsCsAsAsGsTsCsAsCsTsTsGsGs](GsAsG)$_{LNA}$
(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*,
Sp)(AsTsGs)$_{OMe}$d[AsGsAsTsGsCsCsTsGsGsCsTsGsCs](CsAsT)$_{OMe}$
(*Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*,
Sp)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]
(*Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*,
Sp)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]
(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)
d[GsAsAsGsCsTsTsTsGsGsTsTsGsGsGsCsAsAsCsA]
(*Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*,
Sp)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]
(*Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*,
Sp)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]
(*Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*,
Sp)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]
(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*,
Sp)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]
(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*,
Sp)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)d[AsTsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsT]

(*Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)d[CsCsAsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGsAsG]

(*Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)(CsCs)$_{OMe}$d[AsTsCsCsAsAs](GsTsCs)$_{OMe}$d[AsCsTsTsGsGsGs](AsG)$_{OMe}$

(*Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)(CsCs)$_{LNA}$d[AsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGs](AsG)$_{LNA}$

(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*)(CsCs)$_{MOE}$d[AsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGs](AsG)$_{MOE}$

(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*)(CsCs)$_{OMe}$d[AsTsCsCsAsAsGsTsCsAsCsTsTsGsGsGs](AsG)$_{OMe}$

Example 4. Suppression of nucleic acid polymer

[001226] Among other things, the present disclosure provides chirally controlled oligonucleotide compositions and methods thereof that deliver unexpected results when, *e.g.*, used for suppressing nucleic acid polymers through, in some cases, cleavage of such nucleic acid polymers. Examples include but are not limited to those presented herein.

[001227] **RNase H Assay**

[001228] Cleavage rate of nucleic acid polymers by nucleases, for example, RNA by RNase H, is important with respect to the use of oligonucleotides in therapeutic technologies such as

antisense technology. Using our assay, we investigated the cleavage rates and analyzed the metabolites for chirally controlled oligonucleotide compositions of particular oligonucleotide types (*P*-diastereomers) when oligonucleotides of the particular oligonucleotide types are bound to complementary RNA. Results below also illustrate the importance of cleavage patterns recognized by the present disclosure.

[001229] RNase H used herein is a ubiquitously expressed endonuclease that hydrolyses the RNA strand of a RNA/DNA hybrid. It plays an important role in the mode of action of antisense oligonucleotides. In some embodiments, RNase H cleavage rate is significantly reduced when the RNA substrate is structured (Lima, W. F., Venkatraman, M., Crooke, S.T. The Influence of Antisense Oligonucleotide-induced RNA Structure on Escherichia coli RNase H1 Activity *The Journal Of Biological Chemistry* **272**, No. 29, 18191–18199, (1997)). Furthermore, the 2'-MOE gapmer designs (5-10-5) offer higher affinities for RNA targets leading to minimal turnover of the antisense strand. Presence of 2'-MOE modifications in the wings also reduce the number of RNase H cleavage sites.

[001230] To study the RNA cleavage rate, the present disclosure provides a simple assay to quantify the length of RNA remaining after incubation with RNase H. The provided method, among other things, provides the relative rates of RNase H cleavage for stereorandom 2'-modified gapmers, stereorandom DNA oligonucleotide compositions and chirally pure *P*-diastereomers (chirally controlled oligonucleotide compositions of a corresponding oligonucleotide type) for various oligomers for different targets. Changing the stereochemistry at 2'-modified regions and the DNA core provides information with respect to how stereochemistry in these regions affects the interaction of RNase H to its substrates. RNase H reaction mixtures at different time points were analyzed by LCMS to determine the cleavage pattern. The present disclosure, among other things, provides nucleic acid polymer, for example RNA, cleavage rates and cleavage patterns (maps) that are critical to design stereochemical nucleic acid architectures for optimal activity, *e.g.*, antisense activity.

[001231] **Equipment :**

[001232] Alliance HPLC, 2489 – TUV, 2695E - Equipped with autosampler

[001233] Cary100 (Agilent Technologies)

[001234] **Methods:**

[001235] **DNA/RNA Duplex Preparation**: Oligonucleotide concentrations were

determined by measuring the absorbance in water at 260nm. DNA/RNA duplexes were prepared by mixing equimolar solutions oligonucleotides with each strand concentration of 10uM. The mixtures were heated at 90°C for 2 minutes in water bath and were cooled down slowly over several hours.

[001236] Human RNase H Protein Expression and Purification: Human RNase HC clone was obtained from Prof. Wei Yang's laboratory at NIH Bethesda. The protocol for obtaining this human RNase HC (residues 136-286) has been described (Nowotny, M. et al. Structure of Human RNase H1 Complexed with an RNA/DNA Hybrid: Insight into HIV Reverse Transcription. *Molecular Cell* 28, 264–276, (2007). The protein expression was carried out by following reported protocol with the exception that the resulting protein had an N-terminal His6 tag. BL21(DE3) *E.coli* cells in LB medium were used for protein expression. Cells were grown at 37°C till OD600 reached around 0.7. The cultures were then cooled and 0.4 mM IPTG was added to induce protein expression overnight at 16°C. *E.coli* extract was prepared by sonication in buffer A (40 mM NaH_2PO_4 (pH 7.0), 1 M NaCl, 5% glycerol, 2.8 mM β-mercaptoethanol and 10 mM imidazole) with the addition of protease inhibitors (Sigma-Aldrich). The extract was purified by Ni affinity column using buffer A plus 60 mM imidazole. The protein was eluted with a linear gradient of 60 to 300 mM imidazole. The protein peak was collected and was further purified on a Mono S column (GE Healthcare) with a 100 mM - 500mM gradient of NaCl in buffer B. Fractions containing RNase HC were concentrated to 0.3 mg/ml in the storage buffer (20 mM HEPES (pH 7.0), 100mM NaCl, 5% glycerol, 0.5 mM EDTA, 2 mM DTT) and stored at -20°C. 0.3mg/ml enzyme concentration corresponds to 17.4uM based on its reported extinction coefficient (32095 $cm^{-1}M^{-1}$) and MW (18963.3 Da units).

[001237] RNase H assay: In a 96-well plate, to 25 µL DNA/RNA duplex (10 µM) was added 5 µL of 10X RNase H buffer followed by 15 µL water. The mixture was incubated at 37°C for a few minutes and then 5 µL of 0.1 µM stock solution of enzyme was added to give total volume of 50 µL with final substrate/enzyme concentration 5 µM/0.01 µM (500:1) and was further incubated at 37°C. Various ratios of the DNA/RNA duplex: RNase H protein were studied using these conditions to find an optimal ratio to study the kinetics. The reactions were quenched at different time points using 10 µL of 500 mM EDTA disodium solution in water. For zero min time point, EDTA was added to the reaction mixture before the addition of enzyme. Controls were run to ensure that EDTA was able to successfully inhibit the enzyme activity

completely. After all the reactions were quenched 10 µL of each reaction mixture was injected on to analytical HPLC column (XBridge C18, 3.5um, 4.6x150mm, Waters Part# 186003034). Kcat/Km can be measured by a number of methods, such as FRET (Fluorescence Resonance Energy Transfer) dependent RNase H assay using dual labeled RNA and monitored by SpectraMax.

[001238] Solid Phase Extraction Protocol for sample preparation for LCMS: 96 well plate (Waters part# 186002321) was used to clean the RNase H reaction mixture before running LCMS. 500 µL of acetonitrile followed by water was used to equilibrate the plate under mild vacuum with the help of manifold (Millipore part# MSV MHTS00). Precaution was taken not to let the plate dry. About 50-100 µL of RNase H reaction mixture was loaded in each well followed by water washings (2 mL) under mild vacuum. 2x500 µL of 70% ACN/Water was used to recover the sample. The recovered samples were transferred to 2 mL centrifuge tubes and were concentrated to dryness in speed vac. Each dry sample was reconstituted in 100 µL water and 10 µL was injected on Acquity UPLC@OST C18 1.7um, 2.1x50mm (part# 186003949) for LCMS analysis.

[001239] For mass spectrometry analysis, the reaction mixtures after quenching were cleaned using C_{18} 96 well plate (Waters). The oligomers were eluted in 70% Acetonitrile/Water. The Acetonitrile was evaporated using speedvac and the resulting residue was reconstituted in water for injection.

Eluent A = 50mM Triethyl ammonium acetate

Eluent B= Acetonitrile

Column Temperature = 60°C

UV was recorded at 254nm and 280nm

RP-HPLC Gradient Method

	Time (min)	Flow (ml/min)	%A	%B	Curve
1	0.0	1.00	95.0	5.0	
2	2.00	1.00	95.0	5.0	1
3	22.00	1.00	80.0	20.0	6
4	25.00	1.00	5.0	95.0	6
5	25.5	1.00	95.0	5.0	1
6	30	1.00	95.0	5.0	1

[001240] On HPLC chromatograms, peak areas corresponding to full length RNA oligomer (ONT-28) were integrated, normalized using the DNA peak and were plotted against time (Figure 8). ONT-87 demonstrated superior cleavage for complementary RNA when in duplex form, in comparison to the other product candidates and Mipomersen. Since all the diastereomers in this panel have 2'-MOE modified wings that do not activate RNase H enzyme, without the intention to be limited by theory, Applicant notes that activity is likely dictated by the stereochemistry in the DNA core. Heteroduplexes with ONT-77 to ONT-81 including Mipomersen in the antisense strand show very similar RNA cleavage rates. ONT-89 with alternating Sp/Rp stereochemistry showed the least activity in the tested time frame under the tested conditions. Among the tested oligonucleotides with MOE modifications, ONT-87 and ONT-88 units in the antisense strand exhibited increased in activity in comparison to the rest of the heteroduplexes. Particularly, ONT-87 provided surprisingly high cleavage rate and unexpected low level of remaining target RNA. Additional example data were illustrated in Figure 6 and Figure 24.

[001241] ***In Vitro*** **Oligonucleotide Transfection Assay:** Transfection assays are widely known and practiced by persons having ordinary skill in the art. An example protocol is described herein. Hep3B cells are reverse transfected with Lipofectamine 2000 (Life Technologies, Cat. No. 11668-019) at 18×10^3 cells/well density in 96-well plates using the manufacturer's protocol. For dose response curves eight 1/3 serial dilutions are used starting from 60-100 nM. 25 µL of 6x oligonucleotide concentration is mixed with a prepared mixture of 0.4 µL Lipofectamine 2000 with 25 µL of serum-free medium Opti-MEM medium (Gibco, Cat. No. 31985-062) per well. After a 20min minute incubation, 100 µL of 180×10^3 cells/ml suspended in 10% FBS in DMEM cell culture media (Gibco, Cat. No. 11965-092) is added to bring the final volume to 150 µL per well. 24-48 hours post transfection Hep3B cells are lysed by adding 75 µL of Lysis Mixture with 0.5mg/ml Proteinase K using QuantiGene Sample Processing Kit for Cultured Cells (Affymetrix, Cat. No. QS0103). The Target mRNA and GAPDH mRNA expression levels in cell lysates are measured using Affymetrix QuantiGene 2.0 Assay Kit (Cat. No. QS0011) according to the manufacturer's protocol. The Target mRNA expression is normalized to GAPDH mRNA expression from the same sample; and relative Target/GAPDH levels are compared to transfections using Lipofectamine 2000 only (no

oligonucleotide) control. Dose response curves are generated by GraphPad Prism 6 using nonlinear regression log (inhibitor) vs. response curve fit with variable slope (4 parameters). For example results, see Figure 24, Figure 27 and Figure 29.

Example 5. Provided compositions and methods provide control of cleavage patterns

[001242] The present disclosure surprisingly found that internucleotidic linkage stereochemistry pattern has unexpected impact on cleavage patterns of nucleic acid polymers. By changing common patterns of backbone chiral centers of chirally controlled oligonucleotide compositions, numbers of cleavage sites, cleavage percentage at a cleavage site, and/or locations of cleavage sits can be surprisingly altered, both independently and in combination. As described in the example herein, provided compositions and methods can provide control over cleavage patterns of nucleic acid polymers.

[001243] Using similar assay conditions, various chirally controlled oligonucleotide compositions of different oligonucleotide types were tested. Example cleavage patterns of the target RNA sequence is presented in Figure 9. Certain pattern of backbone chiral centers, such as that in ONT-87 and ONT-154, surprisingly produces only one cleavage site in the target sequence. Moreover, it is surprisingly found that oligonucleotides providing single cleavage site, such as ONT-87 and ONT-154, provide unexpectedly high cleavage rate and low level of remaining target nucleic acid polymer. See also Figure 8, Figure 10 and Figure 11.

Example 6. Example cleavage of FOXO1 mRNA

[001244] Oligonucleotide compositions targeting different regions of FOXO1 mRNA were tested in cleavage assays as described above. In each case, chirally controlled oligonucleotide compositions were shown to be capable of providing altered cleavage patterns relative to reference cleavage patterns from chirally uncontrolled oligonucleotide compositions sharing the same common base sequence and length. For example results, see Figure 10 and Figure 11. As shown in Figure 12, example chirally controlled oligonucleotide compositions provide both significantly faster cleavage rates and unexpectedly low levels of remaining substrates when compared to reference chirally uncontrolled oligonucleotide compositions. In some embodiments, as shown in Figure 11, the cleavage sites are associated with *R*p*S*p*S*p backbone chiral center sequence. In some embodiments, cleavage sites are two base pairs upstream of

*Rp*Sp*Sp*.

[001245] Example oligonucleotide compositions are listed below.

Oligo	Sequence	Description	Tm (°C)
ONT-366	dTsdGsdAsdGsdAsdTsdGsdCsdCsdTsdGsdGsdCsdTsdGsdCsdCsdAsdTsdA	All DNA	66.5
ONT-389	dTsdGsdAsdGsdAsdTsdGsdCsdCsdTsdGsdGsdCsdTsdGsdCsdCsdAsdTsdA	$S_7RSSRSSRS_5$	64.3
ONT-390	dTsdGsdAsdGsdAsdTsdGsdCsdCsdTsdGsdGsdCsdTsdGsdCsdCsdAsdTsdA	$S_6RSSRSSRS_6$	64.6
ONT-391	dTsdGsdAsdGsdAsdTsdGsdCsdCsdTsdGsdGsdCsdTsdGsdCsdCsdAsdTsdA	$S_5RSSRSSRS_7$	64.3
ONT-387	rUrArUrGrGrCrArGrCrCrArGrGrCrArUrCrUrCrA	complementary RNA	
ONT-367	dTsdAsdGsdCsdCsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGsdCsdTsdCsdAsdC	All DNA	62.9
ONT-392	dTsdAsdGsdCsdCsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGsdCsdTsdCsdAsdC	$S_7RSSRSSRS_5$	59.5
ONT-393	dTsdAsdGsdCsdCsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGsdCsdTsdCsdAsdC	$S_6RSSRSSRS_6$	60
ONT-394	dTsdAsdGsdCsdCsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGsdCsdTsdCsdAsdC	$S_5RSSRSSRS_7$	59.5
ONT-388	rGrUrGrArGrCrArGrCrUrGrCrArArUrGrGrCrUrA	complementary RNA	

Example 7. Example chirally controlled oligonucleotide compositions provide higher turn-over

[001246] In cases where the Tm of cleaved nucleic acid polymer fragments, for example RNA fragments, to oligonucleotides is greater than a physiological temperature, product dissociation may be inhibited and oligonucleotides may not be able to dissociate and find other target strands to form duplexes and cause the target strands to be cleaved. The Tm of ONT-316 (5-10-5 2'-MOE Gapmer) to complementary RNA is 76°C. After a cut or a few cuts in the RNA sequence complementary to the oligonucleotides, the 2'-MOE fragments may remain bound to RNA and thus cannot cause the other target molecules to be cleaved. Thermal melting temperatures of DNA strands generally are much lower when duplexed to RNA, for example, ONT-367 (63°C) and ONT-392 60°C). Additionally, thermal stability in DNA sequences is often relatively uniformly distributed compared to 2'-MOE modified oligonucleotides. In some

embodiments, oligonucleotides in provided chirally controlled oligonucleotide compositions do not contain 2'-modifications such as 2'-MOE. In some embodiments, oligonucleotides in provided chirally controlled oligonucleotide compositions, which do not contain 2'-modifications such as 2'-MOE, more easily dissociate from nucleic acid polymer cleavage fragments, and have higher turn-over than oligonucleotides having 2'-modifications such as 2'-MOE. In some embodiments, the present disclosure provides an all DNA designs, in which oligonucleotides do not have 2'-modifications. In some embodiments, chirally controlled oligonucleotide compositions wherein oligonucleotides having no 2'-modification provides higher turn-over of a nuclease such as RNase H. In some embodiments, after cleavage RNase H dissociates more easily from duplex formed by RNA and oligonucleotides of provided chirally controlled oligonucleotide compositions. Using similar protocols as described above, turn-over of two example chirally controlled oligonucleotide compositions of oligonucleotide type ONT-367 and ONT-392 indeed showed higher turn-over rate than reference chirally uncontrolled oligonucleotide compositions (see Figure 13).

Example 8. Example cleavage of FOXO1 mRNA

[001247] As exemplified in Figure 14, chirally controlled oligonucleotide compositions and methods thereof in the present disclosure can provide controlled cleavage of nucleic acid polymers. In some embodiments, chirally controlled oligonucleotide compositions of the present disclosure produces altered cleavage pattern in terms of number of cleavage sites, location of cleavage sites, and/or relative cleavage percentage of cleavage sites. In some embodiments, as exemplified by ONT-401 and ONT-406, chirally controlled oligonucleotide compositions provide single site cleavage.

[001248] In some embodiments, only one component from RNA cleavage was detected. Without the intention to be limited by theory, Applicant notes that such observation could be due to the processive nature of RNase H enzyme which could make multiple cuts on the same duplex resulting in much shorter 5'-OH 3'-OH fragments.

[001249] Additional chirally controlled oligonucleotide compositions were further tested. As described above, provided chirally controlled oligonucleotide compositions provides unexpected results, for example, in terms of cleavage rate and %RNA remaining in DNA/RNA duplex. See Figures 15-17. Exemple analytical data were presented in Figures 18-20. Without

the intention to be limited by theory, Applicant notes that in some embodiments, cleavage may happen as depicted in Figure 21. In Figure 17, it is noted ONT-406 was observed to elicit cleavage of duplexed RNA at a rate in slight excess of that of the natural DNA oligonucleotide ONT-415 having the same base sequence and length. Applicant notes that chirally controlled oligonucleotide compositions of ONT-406, and other chirally controlled oligonucleotide compositions provided in this disclosure, have other preferred properties that an ONT-415 composition does not have, for example, better stability profiles *in vitro* and/or *in vivo*. Additional example data were presented in Figure 25. Also, as will be appreciated by those skilled in the art, example data illustrated in Figure 26 and Figure 27 confirm that provided example chirally controlled oligonucleotide compositions, especially when so designed to control the cleavage patterns through patterns of backbone chiral centers, produced much better results than reference oligonucleotide compositions, *e.g.,* a stereorandom oligonucleotide composition. As exemplified in Figure 26, controlled patterns of backbone chiral centers, among other things, can selectively increase and/or decrease cleavage at existing cleavage site when a DNA oligonucleotide is used, or creates entirely new cleavage sites that do not exist when a DNA oligonucleotide is used (see Figure 25, ONT-415). In some embodiments, cleavage sites from a DNA oligonucleotide indicate endogenous cleavage preference of RNase H. As confirmed by Figure 27, provided chirally controlled oligonucleotide compositions are capable of modulating target cleavage rate. In some embodiments, approximately 75% of the variance in cellular activity is accounted for by differences in cleavage rate which can be controlled through patterns of backbone chiral centers. As provided in this Application, further structural features such as base modifications and their patterns, sugar modification and their patterns, internucleotidic linkage modifications and their patterns, and/or any combinations thereof, can be combined with patterns of backbone chiral centers to provide desired oligonucleotide properties.

Example 9. Example allele-specific suppression of mHTT

[001250] In some embodiments, the present disclosure provides chirally controlled oligonucleotide compositions and methods thereof for allele-specific suppression of transcripts from one particular allele with selectivity over the others. In some embodiments, the present disclosure provides allele-specific suppression of mHTT.

[001251] Figure 22 illustrates example chirally controlled oligonucleotide compositions

that specifically suppress transcripts from one allele but not the others. Oligonucleotides 451 and 452 were tested with transcripts from both exemplified alleles using biochemical assays described above. Allele-specific suppression is also tested in cells and animal models using similar procedures as described in Hohjoh, *Pharmaceuticals* **2013**, *6*, 522-535; US patent application publication US 2013/0197061; and Østergaard *et al.*, *Nucleic Acids Research*, **2013**, *41*(21), 9634–9650. In all cases, transcripts from the target allele are selectively suppressed over those from the other alleles. As will be appreciated by those skilled in the art, example data illustrated in Figure 22 confirm that provided example chirally controlled oligonucleotide compositions, especially when so designed to control the cleavage patterns through stereochemistry, produced much better results than reference oligonucleotide compositions, in this case, a stereorandom oligonucleotide composition. As confirmed by Figure 22, patterns of backbone chiral centers can dramatically change cleavage patterns (Figure 22 C-E), and stereochemistry patterns can be employed to position cleavage site at the mismatch site(Figure 22 C-E), and/or can dramatically improve selectivity between the mutant and wild type (Figure 22 G-H). In some embodiments, chirally controlled oligonucleotide compositions are incubated with wtRNA and muRNA of a target and both the duplexes are incubated with RNase H.

[001252] Huntingtin Allele Tm

Mutant Huntingtin Allele ONT-453/ONT-451	38.8 °C
Wild Type Huntingtin Allele ONT-454/ONT-451	37.3 °C
Mutant Huntingtin Allele ONT-453/ONT-452	38.8 °C
Wild Type Huntingtin Allele ONT-454/ONT-452	36.5 °C
Mutant Huntingtin Allele ONT-453/ONT-450	40.3 °C
Wild Type Huntingtin Allele ONT-454/ONT-450	38.8 °C

Example 10. Example allele-specific suppression of FOXO1

[001253] In some embodiments, the present disclosure provides allele-specific suppression of FOXO1.

[001254] Figure 23 illustrates example chirally controlled oligonucleotide compositions that specifically suppress transcripts from one allele but not the others. Oligonucleotides ONT-400, ONT-402 and ONT-406 were tested with transcripts from both exemplified alleles using biochemical assays described above. Allele-specific suppression is also tested in cells and

animal models using similar procedures as described in Hohjoh, *Pharmaceuticals* **2013**, *6*, 522-535; US patent application publication US 2013/0197061; Østergaard *et al.*, *Nucleic Acids Research* **2013**, *41*(21), 9634–9650; and Jiang *et al.*, *Science* **2013**, *342*, 111-114. Transcripts from the target allele are selectively suppressed over those from the other alleles. In some cases, two RNAs with mismatch ONT-442 (A/G, position 7th) and ONT-443 (A/G, position 13th) from ONT-388 are synthesized and are duplexed with ONT-396 to ONT-414. RNase H assay are performed to obtain cleavage rates and cleavage maps.

Example 11. Certain example oligonucleotides and oligonucleotide compositions

[001255] Stereorandom oligonucleotides with different 2' substitution chemistries targeting three distinct regions of FOXO1 mRNA with the thermal melting temperatures when duplexed with complementary RNA. The concentration of each strand was 1uM in 1X PBS buffer.

Oligo	Sequence	Description	Tm(°C)
ONT-316	TeosAeosGeos5mCeos5mCeosdAsdTsdTsdGs5mdCsdAsdGs5mdCsdTsdGs5mCeosTeos5mCeosAeos5mCeo	5-10-5 (2'-MOE Gapmer)	76.7
ONT-355	dTsdAsdGsdCsdCsdAsdTstsgscsasgscsdTsdGsdCsdTsdCsdAsdC	7-6-7 (DNA-2'-OMe-DNA) Gapmer	71.2
ONT-361	tsasgsdCsdCsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGsdCsdTscsascs	3-14-3 (2'-OMe-DNA-2'-OMe) Gapmer	65.8
ONT-367	dTsdAsdGsdCsdCsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGsdCsdTsdCsdAsdC	All DNA	62.9
ONT-373	tsasgscscsdAsdTsdTsdGsdCsdAsdGsdCsdTsdGscstscsasc	5-10-5 (2'-OMe Gapmer)	71.8
ONT-388	rGrUrGrArGrCrArGrCrUrGrCrArArUrGrGrCrUrA	Complementary RNA	
ONT-302	Teos5mCeos5mCeosAeosGeosdTsdTs5mdCs5mdCsdTsdTs5mdCsdAsdTsdTs5mCeosTeosGeos5mCeosAeo	5-10-5 (2'-MOE Gapmer)	72.5
ONT-352	dTsdCsdCsdAsdGsdTsdTscscststscsasdTsdTsdCsdTsdGsdCsdA	7-6-7 (DNA-2'-OMe-DNA) Gapmer	65.4
ONT-358	tscscsdAsdGsdTsdTsdCsdCsdTsdTsdCsdAsdTsdTsdCsdTsgscsas	3-14-3 (2'-OMe-DNA-2'-OMe) Gapmer	62.6
ONT-364	dTsdCsdCsdAsdGsdTsdTsdCsdCsdTsdTsdCsdAsdTsdTsdCsdTsdGsdCsdA	All DNA	58.4
ONT-370	tscscsasgsdTsdTsdCsdCsdTsdTsdCsdAsdTsdTscstsgscsa	5-10-5 (2'-OMe Gapmer)	68
ONT-386	rUrGrCrArGrArArUrGrArArGrGrArArCrUrGrGrA	Complementary	

Oligo	Sequence	Description	Tm(°C)
		RNA	
ONT-315	TeosGeosAeosGeosAeosdTsdGs5mdCs5mdCsdTsdGsdGs5mdCsdTsdGs5mCeos5mCeosAeosTeosAeo	5-10-5 (2'-MOE Gapmer)	77.5
ONT-354	dTsdGsdAsdGsdAsdTsdGscscstsgsgscsdTsdGsdCsdCsdAsdTsdA	7-6-7 (DNA-2'-OMe-DNA) Gapmer	75.5
ONT-360	tsgsasdGsdAsdTsdGsdCsdCsdTsdGsdGsdCsdTsdGsdCsdCsastsas	3-14-3 (2'-OMe-DNA-2'-OMe) Gapmer	69
ONT-366	dTsdGsdAsdGsdAsdTsdGsdCsdCsdTsdGsdGsdCsdTsdGsdCsdCsdCsdAsdTsdA	All DNA	66.5
ONT-372	tsgsasgsasdTsdGsdCsdCsdTsdGsdGsdCsdTsdGscscsastsa	5-10-5 (2'-OMe Gapmer)	74.4
ONT-387	rUrArUrGrGrCrArGrCrCrArGrGrCrArUrCrUrCrA	Complementary RNA	

[001256] Additional example stereorandom oligonucleotide compositions are listed below.

Oligo	Sequence (5′ to 3′)
ONT-41	$(Gs5mCs5mCsTs5mCs)_{MOE}d[AsGsTs5mCsTsGs5mCsTsTs5mCs](Gs5mCsAs5mCs5mC)_{MOE}$
ONT-70	$(Gs5mCs)_{MOE}d[GsTsTsTsGs5mCsTs5mCsTsTs5mCsTsTs](5mCsTsTsGs5mCGs)_{MOE}d[TsTsTsTs](TsT)_{MOE}$
ONT-83	$(GsTs5mCs5mCs5mCs)_{MOE}d(TsGsAsAsGsAsTsGsTs5mCs](AsAsTsGs5mC)_{MOE}$
ONT-302	$(Ts5mCs5mCsAsGs)_{MOE}d[TsTs5mCs5mCsTsTs5mCsAsTsTs](5mCsTsGs5mCsA)_{MOE}$
ONT-315	$(TsGsAsGsAs)_{MOE}d[TsGs5mCs5mCsTsGsGs5mCsTsGs](5mCs5mCsAsTsA)_{MOE}$
ONT-316	$(TsAsGs5mCs5mCs)_{MOE}d[AsTsTsGs5mCsAsGs5mCsTsGs5m](CsTs5mCsAs5mC)_{MOE}$
ONT-352	$[TsCsCsAsGsTsTs](cscststscsas)_{OMe}d[TsTsCsTsGsCsA]$
ONT-354	$[TsGsAsGsAsTsGs](CsCsTsGsGsCs)_{OMe}d[TsGsCsCsAsTsA]$
ONT-355	$[TsAsGsCsCsAsTs](TsGsCsAsGsCs)_{OMe}d[TsGsCsTsCsAsC]$
ONT-358	$(TsCsCs)_{OMe}d[AsGsTsTsCsCsTsTsCsAsTsTsCsTs](GsCsA)_{OMe}$
ONT-360	$(TsGsAs)_{OMe}d[GsAsTsGsCsCsTsGsGsCsTsGsCsCs](AsTsA)_{OMe}$
ONT-361	$(TsAsGs)_{OMe}d[CsCsAsTsTsGsCsAsGsCsTsGsCsTs](CsAsC)_{OMe}$
ONT-364	[TsCsCsAsGsTsTsCsCsTsTsCsAsTsTsCsTsGsCsA]
ONT-366	[TsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsTsA]
ONT-367	[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]
ONT-370	$(TsCsCsAsGs)_{OMe}d[TsTsCsCsTsTsCsAsTsTs](CsTsGsCsA)_{OMe}$
ONT-372	$(TsGsAsGsAs)_{OMe}d[TsGsCsCsTsGsGsCsTsGs](CsCsAsTsA)_{OMe}$
ONT-373	$(TsAsGsCsCs)_{OMe}d[AsTsTsGsCsAsGsCsTsGs](CsTsCsAsC)_{OMe}$
ONT-440	$(UsAsGsCsCs)_{F}d[AsTsTsGsCsAsGsCsTsGsCsTsCsAsC]$
ONT-441	$(UsAsGsCsCs)_{F}d[AsTsTsGsCsAsGsCsTsGsC]$

Oligo	Sequence (5′ to 3′)
ONT-460	$(TsAsGsCsCs)_{OMe}$d[AsTsTsGsCsAsGsCsTsGsCsTsCsAsC]
ONT-450	[AsTsTsAsAsTsAsAsAsTsTsGsTsCsAsTsCsAsCsC]

[001257] Example RNA and DNA oligonucleotides are listed below.

Oligo	Sequence (5′ to 3′)
ONT-28	rGrGrUrGrCrGrArArGrCrArGrArCrUrGrArGrGrC
ONT-386	rUrGrCrArGrArArUrGrArArGrGrArArCrUrGrGrA
ONT-387	rUrArUrGrGrCrArGrCrCrArGrGrCrArUrCrUrCrA
ONT-388	rGrUrGrArGrCrArGrCrUrGrCrArArUrGrGrCrUrA
ONT-415	d[TAGCCATTGCAGCTGCTCAC]
ONT-442	rGrUrGrArGrCrGrGrCrUrGrCrArArUrGrGrCrUrA
ONT-443	rGrUrGrArGrCrArGrCrUrGrCrGrArUrGrGrCrUrA
ONT-453	rGrGrUrGrArUrGrArCrArArUrUrUrArUrUrArArU
ONT-454	rGrGrUrGrArUrGrGrCrArArUrUrUrArUrUrArArU

[001258] Example chirally pure oligonucleotides are presented below. In some embodiments, the present disclosure provides corresponding chirally controlled oligonucleotide compositions of each of the following example oligonucleotides.

Oligo	Stereochemistry/Sequence (5′ to 3′)	Description
ONT-389	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsTsA]	7S-$(RSS)_3$-3S
ONT-390	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsTsA]	6S-$(RSS)_3$-4S
ONT-391	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsGsAsGsAsTsGsCsCsTsGsGsCsTsGsCsCsAsTsA]	5S-$(RSS)_3$-5S
ONT-392	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	7S-$(RSS)_3$-3S
ONT-393	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	6S-$(RSS)_3$-4S
ONT-394	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	5S-$(RSS)_3$-5S
ONT-396	(*Sp*, *Rp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	18S-1R
ONT-397	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	17S-RS
ONT-398	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	16S-(RSS)
ONT-399	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	15S-(RSS)-1S
ONT-400	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	14S-(RSS)-2S

Oligo	Stereochemistry/Sequence (5′ to 3′)	Description
ONT-401	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	13S-(RSS)-3S
ONT-402	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	12S-(RSS)-4S
ONT-403	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	11S-(RSS)-5S
ONT-404	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	10S-(RSS)-6S
ONT-405	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	9S-(RSS)-7S
ONT-406	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	8S-(RSS)-8S
ONT-407	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	7S-(RSS)-9S
ONT-408	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	6S-(RSS)-10S
ONT-409	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	5S-(RSS)-11S
ONT-410	(*Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	4S-(RSS)-12S
ONT-411	(*Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	3S-(RSS)-13S
ONT-412	(*Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	2S-(RSS)-14S
ONT-413	(*Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	S-(RSS)-15S
ONT-414	(*Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	(RSS)-16S
ONT-421	All-(*Sp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	All S
ONT-422	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-C6-amino-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	8S-(RSS)-3S-(RSS)-2S
ONT-455	All-(*Rp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	All R
ONT-451	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[AsTsTsAsAsTsAsAsAsTsTsGsTsCsAsTsCsAsCsC]	13S-(RSS)-3S
ONT-452	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*)-d[AsTsTsAsAsTsAsAsAsTsTsGsTsCsAsTsCsAsCsC]	14S-(RSS)-2S
ONT-75	All-(*Rp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs 5mCsTsTs5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	All R
ONT-76	(*Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Rp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCs TsTs5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	SRRSR-11S-RSR
ONT-77	(*Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTs Ts5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	5R-10S-4R

Oligo	Stereochemistry/Sequence (5′ to 3′)	Description
ONT-80	All-(*Sp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTs Ts5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	All S
ONT-81	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCs TsTs5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	5S-10R-4S
ONT-82	All-(*Rp*)-(GsTs5mCs5mCs5mCs)$_{MOE}$d[TsGsAsAsGsAsTsGsTs 5mCs](AsAsTsGs5mC)$_{MOE}$	All R
ONT-84	All-(*Sp*)-(GsTs5mCs5mCs5mCs)$_{MOE}$d[TsGsAsAsGsAsTsGsTs 5mCs](AsAsTsGs5mC)$_{MOE}$	All S
ONT-85	(*Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*)-(GsTs5mCs5mCs5mCs)$_{MOE}$d[TsGsAsAsGsAsTsGsTs 5mCs](AsAsTsGs5mC)$_{MOE}$	5R-10S-4R
ONT-86	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*)-(GsTs5mCs5mCs5mCs)$_{MOE}$d[TsGsAsAsGsAsTsGsTs 5mCs](AsAsTsGs5mC)$_{MOE}$	5S-10R-4S
ONT-87	(*Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*, *Rp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCs TsTs5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	5R-2S-(RSS)$_2$-6R
ONT-88	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Rp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCsTs Ts5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	5S-(RRS)$_3$-5S
ONT-89	(*Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCs TsTs5mCs](Gs5mCsAs5mCs5mC)$_{MOE}$	(SR)$_9$S
ONT-154	(*Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Rp*, *Sp*, *Sp*, *Sp*, *Sp*, *Sp*)-d[Gs5mCs5mCsTs5mCsAsGsTs5mCsTsGs5mCsTsTs 5mCsGs5mCsAs5mCs5mC]	7S-(RSS)$_3$-3S
ONT-75	All-(*Rp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCs TsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	All-R
ONT-80	All-(*Sp*)-(Gs5mCs5mCsTs5mCs)$_{MOE}$d[AsGsTs5mCsTsGs5mCs TsTs5mCs] (Gs5mCsAs5mCs5mC)$_{MOE}$	All-S

[001259] Additonal example oligonucleotides targeting FOXO1 with Tm are presented below. In some embodiments, the present disclosure provides corresponding chirally controlled oligonucleotide compositions of each of the following example oligonucleotides.

Oligo	Sequence (5’ to 3’)	Tm (°C)
ONT-439	[UsAsGs]$_F$d[CsCsAsTsTsGsCsAsGsCsTsGsCsTs][CsAsC]$_F$	68.3
ONT-440	[UsAsGsCsCs]$_F$d[AsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	70.0
ONT-441	[UsAsGsCsCs]$_F$d[AsTsTsGsCsAsGsCsTsGsC]	65.5
ONT-455	All-(*Rp*)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	66.8
ONT-316	[TsAsGs5mCs5mCs]$_{MOE}$d[AsTsTsGs5mCsAsGs5mCsTsGs][5mCsTs5	76.9

	mCsAs5mC]$_{MOE}$	
ONT-367	d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	62.8
ONT-415	d[TAGCCATTGCAGCTGCTCAC]	72.6
ONT-416	[TsAsGsCsCsAsTsTsGsCsAsGsCs]$_{OMe}$d[TsGsCsTsCsAsC]	78.4
ONT-421	All-(*S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	59.2
ONT-394	(*S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	60.0
ONT-406	(*S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *R*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p, *S*p)-d[TsAsGsCsCsAsTsTsGsCsAsGsCsTsGsCsTsCsAsC]	58.5

Example 12. Example additional controlled cleavage by provided chirally controlled oligonucleotide compositions

[001260] As will be appreciated by those skilled in the art, example data illustrated in Figure 26 confirm that provided chirally controlled oligonucleotide compositions and methods thereof provided unexpected results compared to reference compositions, such as stereorandom oligonucleotide compositions. Among other things, chirally controlled oligonucleotide compositions can produce controlled cleavage patterns, including but not limited to controlling of positions of cleavage sites, numbers of cleavage sites, and relative cleavage percentage of cleavage sites. See also example data presented in Figure 27.

Example 13. Stability of chirally controlled oligonucleotide compositions

[001261] As will be appreciated by those skilled in the art, example data illustrated in Figure 26 confirm that stability of provided chirally controlled oligonucleotide compositions can be adjusted by varying patterns of backbone chiral centers. For example data, see Figure 7 and Figure 28. An example protocol for performing serum stability experiment is described below.

[001262] **Protocol:** P-stereochemically pure PS DNA (ONT-396-ONT-414 (single Rp walk from 3'end to 5'end)), stereorandom PS DNA (ONT-367), all-Sp PS DNA (ONT-421) and all-Rp PS DNA (ONT-455) were incubated in Rat serum (Sigma, R9759) (0 h and 48 h) and analyzed by IEX-HPLC.

[001263] **Incubation Method:** 5 µL of 250 µM of each DNA solutions and 45 µL of Rat serum were mixed and incubated at 37 °C for each time points (0 h and 48 h). At each time points, reaction was stopped by adding 25 µL of 150 mM EDTA solution, 30 µL of Lysis buffer (erpicentre, MTC096H) and 3 µL of Proteinase K solution (20 mg/mL). The mixture was incubated at 60 °C for 20 min then 20 µL of the mixture was injected to IEX-HPLC and analyzed.

[001264] **Incubation Control Sample:** Mixture of 5 μL of 250 μM of each DNA solutions and 103 μL of 1x PBS buffer were prepared and 20 μL of the mixture was analyzed by IEX-HPLC as controls in order to check the absolute quantification.

[001265] **Example Analytical Method:**

IEx-HPLC

A: 10 mM TrisHCl, 50%ACN (pH 8.0)

B: 10 mM TrisHCl, 800 mM NaCl, 50%ACN (pH 8.0)

C: Water-ACN (1:1, v/v)

Temp : 60 °C

Column: DIONEX DNAPac PA-100, 250x4 mm

Gradient:

	Time	Flow	%A	%B	%C	%D	Curve
1	0.00	1.00	95.0	5.0	0.0	0.0	6
2	1.00	1.00	95.0	5.0	0.0	0.0	1
3	2.00	1.00	75.0	25.0	0.0	0.0	6
4	10.00	1.00	5.0	95.0	0.0	0.0	6
5	10.10	1.00	95.0	5.0	0.0	0.0	6
6	12.50	1.00	95.0	5.0	0.0	0.0	1

Washing:

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	1.00	0.0	0.0	100.0	0.0	6
2	5.50	1.00	0.0	0.0	100.0	0.0	1
3	5.60	1.00	0.0	100.0	0.0	0.0	6
4	7.50	1.00	0.0	100.0	0.0	0.0	1
5	7.60	1.00	95.0	5.0	0.0	0.0	6
6	12.50	1.00	95.0	5.0	0.0	0.0	1

Column Temperature: 60 °C.

Washing was performed every after the sample run.

Percentage of remained PS DNA was calculated by the analysis of the ratio from the 0 h to 48 h using the area of integration of HPLC chromatogram.

Example 14. Example analytical results (Figure 19)

[001266] Peak assignments for Fig. 19 (Top panel, M12-Exp11 B10, ONT-354, 30min)

Retention time (minutes)	$(M-2)^{2-}$	$(M-3)^{3-}$	$(M-4)^{4-}$	$(M-5)^{5-}$	$(M-6)^{6-}$
2.34	1100.6	733.7			
11.91		1390.6	1042.6		

13.07		1500.08	1125.5		750.73
		1805.29	1354.19		
13.58		1603.39	1202.2	961.35	801.15
14.80			1589.9	1271.4	1059.5
18.59			1653.3	1323.3	1101.6

Retention time (minutes)	Observed MW	Assignment based on mass match		
		5'-p-RNA fragment	3'-OH and 5'-OH, RNA	DNA
2.34	2203.2		7mer	
11.91	4176	13mer		
13.07	4505.7	14mer		
	5418.87		17mer	
13.58	4812.8	15mer		
14.80	6362.5		20mer, ONT-387	
18.59	6615.4			ONT-354

[001267] Peak assignments for Fig. 19 (Bottom panel, M12-Exp11 A10, ONT-315, 30min)

Retention time (minutes)	$(M-2)^{2-}$	$(M-3)^{3-}$	$(M-4)^{4-}$	$(M-5)^{5-}$	$(M-6)^{6-}$
4.01	1425.33	950.15			
4.4	1100.83	733.69			
4.94	1578.34	1051.54			
6.21	1741.91	1161.89	870.37		
	1445.42	963.31	722.97		
8.48	1610	1073.3			
9.15		1391.2	1043.1		
9.93	1763.4	1174.7			
11.8		1602.3	1201.7		
14.82					
20.73			1809.94	1447.82	1205.9

Retention time (minutes)	Observed MW	Assignment based on mass match		
		5'-p-RNA fragment	3'-OH and 5'-OH, RNA	DNA
4.01	2853.45		9mer	
4.4	2203.66	7mer		
4.94	3158.47		10mer	
6.21	3487.52		11mer	
	2892.84	9mer		
8.48	3220.94	10mer		
9.15	4177		13mer	

9.93	3528.88	11mer		
11.8	4810		15mer	
14.82			20mer, ONT-387	
20.73	7244.3			ONT-315

Example 15. Example analytical results (Figure 30)

[001268] Peak assignments for Fig. 30 (Top panel, M12-Exp11 D2, ONT-367, 30min)

Retention time (minutes)	$(M-2)^{2-}$	$(M-3)^{3-}$	$(M-4)^{4-}$	$(M-5)^{5-}$	$(M-6)^{6-}$
2.36	1120.28	746.25			
3.15	1292.41	861.32			
4.04	975.92				
4.49	1140.6	759.78			
5.83	1305.21	869.65	652.31		
6.88	1923.23	1281.69	961.28		
9.32		1390.76	1043.29	833.72	
9.96	1783.85	1187.98	891.6	712.94	
11.01	1936.14	1289.93			
		1501.52	1125.4	899.89	
11.93		1405.25	1053.78	842.84	
13.15		1514.72	1135.72		
14.81			1609.95	1287.53	1072.58
18.33			1587.9	1270.2	1058.3

Retention time (minutes)	Observed MW	Assignment based on mass match		
		5'-p-RNA fragment	3'-OH and 5'-OH, RNA	DNA
2.36	2242.56		7mer	
3.15	2586.82		8mer	
4.04	1953.84	6mer		
4.49	2283.2	7mer		
5.83	2612.42	8mer		
6.88	3849.14		12mer	
9.32	4175.28		13mer	
9.96	3569.7	11mer		
11.01	3874.28	12mer		
	4507.56		14mer	
11.93	4218.75	13mer		
13.15	4547.16	14mer		
14.81	6441.8		20mer, ONT-388	
18.33	6355.6			ONT-367

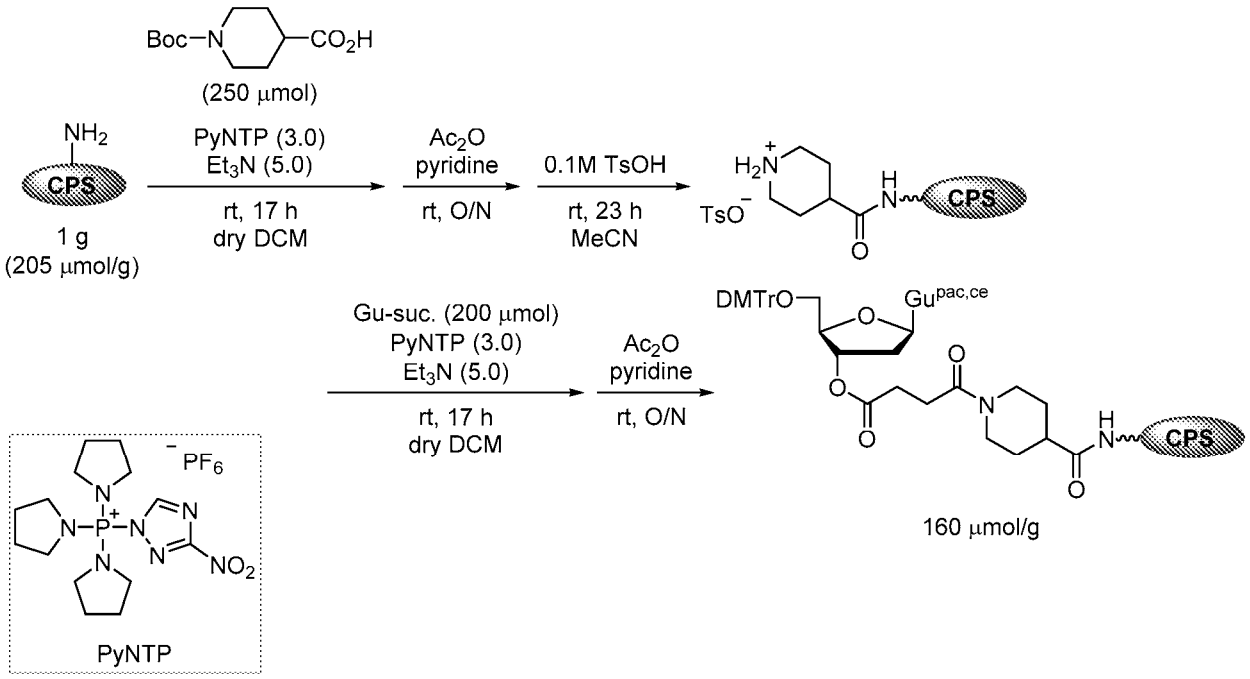
[001269] Peak assignments for Fig. 30 (Bottom panel, M12-Exp21 NM Plate1 (pool) F11 ONT-406 30min

Retention time (minutes)	$(M-2)^{2-}$	$(M-3)^{3-}$	$(M-4)^{4-}$	$(M-5)^{5-}$	$(M-6)^{6-}$
4.72	1140.6	759.78			
9.46		1390.76	1043.29	833.72	
16.45			1609.95	1287.53	1072.58
19.48			1588.1	1270.4	1058.4

Retention time (minutes)	Observed MW	Assignment based on mass match		
		5'-p-RNA fragment	3'-OH and 5'-OH, RNA	DNA
4.72	2203.2	2283.2	7mer	
9.46	4176	4175.28		13mer
16.45	6362.5	6441.8		20mer, ONT-388
19.48	6615.4	6355.9		

Example 16. Example preparation of linkers

[001270] In some embodiments, the SP linker was prepared following the scheme below:

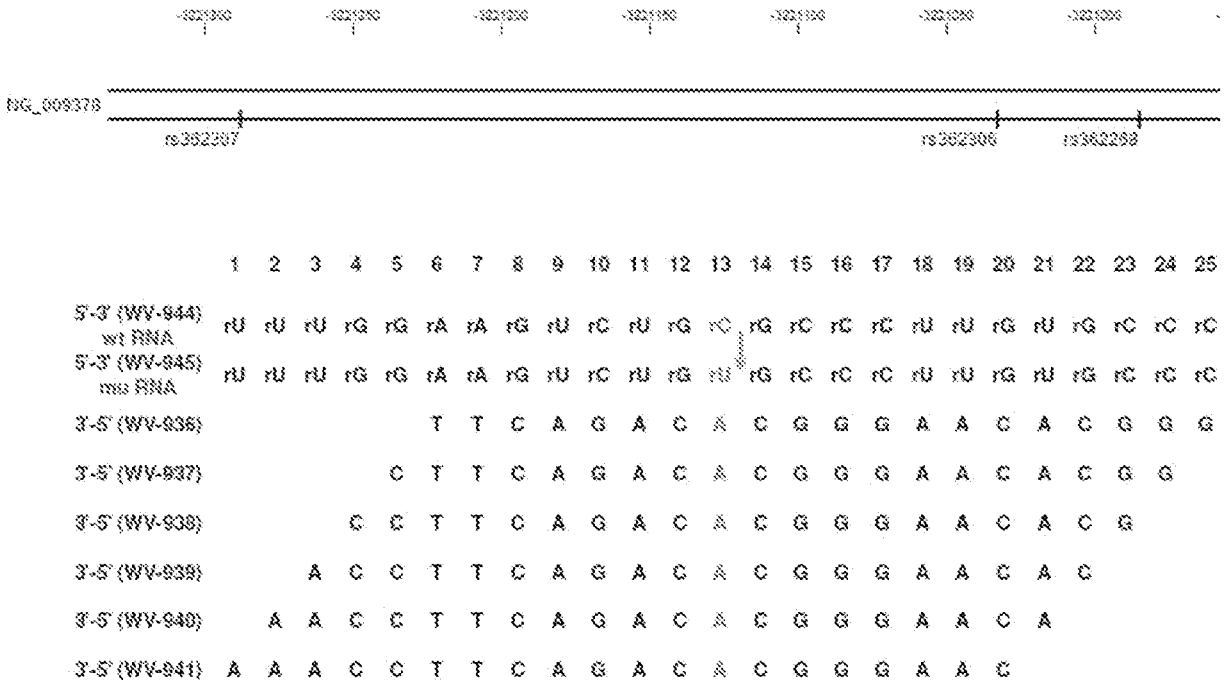


Example 17. Example designs of base sequence

[001271] As described in the present disclosure, the present disclosure recognizes the

importance of base sequence, *e.g.*, for provided chirally controlled oligonucleotide composition. In some embodiments, the present disclosure, as exemplified herein, provides methods for designing base sequence for oligonucleotides, such as antisense oligonucleotides.

[001272] In some embodiments, among other things, bioinformatics is used to design a sequence for a target, *e.g.*, a disease-associated mutant allele of Huntington's disease. The present example describes example steps that may be used for design antisense oligonucleotides for, *e.g.*, rs362268, rs362306, rs2530595, rs362331, rs362307, *etc.* In some embodiments, a provided methods comprising a step of examining sequence features for off-target, binding affinity with target, contiguous Gs, and paliandromic moieties. In some embodiments, a provided methods comprising a step of examining off-target effects in the presence of mismatches. In some embodiments, a sequence found in a target comprising a characteristic sequence element, *e.g.*, a mutation, a SNP, *etc.*, and having a length of about 10-1000, *e.g.*, about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 100, about 110, about 120, about 130, about 140, about 150, about 200, about 250, about 300, about 400, about 500, about 600, about 700, about 800, about 900, about 1000, about 2000, about 3000, about 4000, about 5000, *etc.*, nucleotides are used in assays, *e.g.*, RNase H assay, reporter assay, *etc.* In some embodiments, as in the present example, 40-bp flanking sequences for a SNP, *e.g.*, rs362268, rs362306, rs2530595, rs362331, rs362307, *etc.*, were used. A number of such sequences, for example 6 to 12, could be readily assessed by provided methods. Example tested sequences are listed below:



[001273] As described in the present disclosure and understood by a person having ordinary skill in the art, in some embodiments, assays, for example, RNase cleavage assay described herein, are useful in the assessment of one or more features (e.g., rate, extent, and/or selectivity of cleavage). In some embodiments, an RNase cleavage assay provides a cleavage pattern of an oligonucleotide composition. In some embodiments, a composition of DNA oligonucleotides having the same sequence is used, an RNase H assay may provide a DNA cleavage pattern of the sequence. In some embodiments, for generating a DNA cleavage pattern, all DNA oligonucleotides in the composition are identical. In some embodiments, when a stereorandom composition of all-phosphorothioate oligonucleotides having the same sequence is used, an RNase H assay may provide a stereorandom cleavage pattern of the sequence. In some embodiments, for generating stereorandom cleavage pattern, all oligonucleotides in the stereorandom composition are identical. In some embodiments, when a chirally controlled oligonucleotide composition is used, an RNase H assay may provide a stereorandom cleavage pattern of the chirally controlled oligonucleotide composition. In some embodiments, for generating cleavage pattern of a chirally controlled oligonucleotide composition, all oligonucleotides in the chirally controlled oligonucleotide composition are identical. In some embodiments, an RNase H assay provides cleavage rate information. In some embodiments, an RNase H assay provides relative cleavage extent, *e.g.*, (cleavage at a site) / (all cleavage). In

some embodiments, an RNase H assay provides absolute cleavage extent, (cleaved target at a site) / (all target both cleaved and non-cleaved). In some embodiments, an RNase H assay provides selectivity. In some embodiments, an RNase H assay provides suppression level information.

[001274] In some embodiments, as exemplified herein, an RNase H assay provides cleavage rates. For example results, see Figure 31. P represents position of mismatch in oligonucleotides from the 5'-end.

[001275] Analysis of human RNase H1 cleavage of a 25-mer RNA when hybridized with different phosphorothioate oligonucleotides targeting rs362307 SNP was performed. WV-944 and WV-945 are 25mer RNAs which include WT and mutant variant of rs362307, respectively. WV-936 to WV-941 are stereopure DNAs while WV-904 to WV-909 are all stereorandom DNAs. All duplexes were incubated with RNase H1C in the presence of 1X RNase H buffer at 37°C. Reactions were quenched at fixed time points by 30mM Na_2EDTA. One tenth of this reaction mixture was injected on Reverse Phase HPLC and peak areas were measured for full length RNA remaining in the reaction mixtures at different time points. Cleavage rates were determined by plotting these peak areas with respective time points. In some embodiments, differentiation between rates of cleavage of WT RNA vs. mu RNA was observed.

[001276] In some embodiments, as shown in Figure 31, when position 11, 12 or 13 of a sequence as counted from its 5'-terminus aligns with a SNP, or position 8, 9 or 10 of a sequence as counted from its 3'-terminus aligns with a SNP, better cleavage selectivity was observed.

Example 18. Example wing, core, wing-core, core-wing and wing-core-wing designs

[001277] Among other things, the present disclosure provides various embodiments of wing, core, wing-core, core-wing, and wing-core-wing structures. In some embodiments, it was surprisingly found that oligonucleotides with wings comprising phosphate linkages and cores comprising phosphorothioate linkages provided unexpectedly increased cleavage efficiency and selectivity. For example, see Figure 32 C, F, G, H, *etc.*

[001278] Analysis of human RNase H1 cleavage of a 25-mer RNA when hybridized with different chirally controlled oligonucleotide compositions targeting rs362307 SNP. WV-944 and WV-945 are 25mer RNAs which include WT and mutant variant of rs362307, respectively. WV-1085 to WV-1092 are all stereopure 2'-OMe/DNAs with mixed PO/PS backbone. All duplexes were incubated with RNase H1C in the presence of 1 X RNase H buffer at 37 °C.

Reactions were quenched at fixed time points by 30 mM Na_2EDTA. One tenth of this reaction mixture was injected on Reverse Phase HPLC and peak areas were measured for full length RNA remaining in the reaction mixtures at different time points. Cleavage rates were determined by plotting these peak areas with respective time points.

[001279] In some embodiments, 2'-OMe phosphate wings change cleavage rate and/or selectivity. In some embodiments, 2'-OMe phosphate wings change cleavage rate and selectivity. In some embodiments, 2'-OMe phosphate wings change cleavage rate or selectivity. In some embodiments, 2'-OMe phosphate wing change cleavage rate. In some embodiments, 2'-OMe phosphate wing change cleavage rates of both the mutant and the wild-type allele. In some embodiments, 2'-OMe phosphate wing change cleavage selectivity. In some embodiments, 2'-OMe phosphate wing change cleavage pattern.

[001280] In some embodiments, incorporation of a phosphate internucleotidic linkage surprisingly improves cleavage rate and/or selectivity. In some embodiments, incorporation of a phosphate internucleotidic linkage surprisingly improves cleavage rate and selectivity. In some embodiments, incorporation of a phosphate internucleotidic linkage surprisingly improves cleavage rate or selectivity. In some embodiments, incorporation of a phosphate internucleotidic linkage surprisingly improves cleavage rate. In some embodiments, a phosphate internucleotidic linkage improves cleavage rates of both the mutant and the wild-type allele, but at a greater level for the mutant than for the wild-type allele. In some embodiments, incorporation of a phosphate internucleotidic linkage surprisingly improves cleavage selectivity.

[001281] In some embodiments, as demonstrated by data exemplified herein, stereopure oligonucleotide compositions provided surprisingly high cleavage rate and/or selectivity compared to corresponding stereorandom compositions; for examples, see stereopure WV-1497/stereorandom WV-1092, 905/937, 931/1087, *etc*.

Example 19. Example cleavage maps

[001282] As described herein, in some embodiments, an assay, such as RNase H assay, provides cleavage maps for stereorandom or chirally controlled oligonucleotide compositions. Example cleavage maps are illustrated in Figure 33, which exemplifies stereorandom cleavage patterns of multiple base sequences. Additional cleavage maps are presented in Figure 35, which exemplifies, among other things, stereorandom cleavage patterns of base sequence having no nucleoside modifications (WV-905), as well as base sequence having nucleoside modifications.

[001283] Example cleavage patterns of chirally controlled stereopure oligonucleotide compositions are presented in Figure 34. As described in the present disclosure, major cleavage sites may be identified through assays such RNase H assay from cleavage patterns. For example, for WV-937, the relative major cleavage site, as assessed by (cleavage at the site/total cleavage) and reflected by the lengths of the arrows, are between GCGC and CCUU for the wild-type (2 internucleotidic linkages away from the SNP), and between CUGU and GCCC for the mutant (at the SNP site, 0 internucleotidic linkage away from the SNP). In some embodiments, a relative major cleavage site is not necessarily an absolute major cleavage site, which requires certain percentage of the total target, in this case, RNA, is cleaved at the site. For example, in some embodiments, the site between GCGC and CCUU for WV-937/wild type is not a major cleavage site if over 20% of total target needs to be cleaved at a site for a site to qualify as a major cleavage site (see Figure 32, M); the site between CUGU and GCCC for WV-937/mutant remains a major cleavage site if the threshold for a major site is 20% of total target being cleaved at that site.

[001284] In some embodiments, different oligonucleotide compositions have different cleave rates. In some embodiments, cleavage maps are generated at different time points. For example, for an oligonucleotide composition having a faster cleavage rate, its cleavage map can be generated at an earlier time point (*e.g.*, 5 minutes, 10 minutes, 15 minutes, *etc.*) than an oligonucleotide composition having a slower cleavage rate (*e.g.*, 30 minutes, 45 minutes, 60 minutes, *etc.*).

[001285] In some embodiments, when cleavage products at only one site can be identified by an analytical methods, *e.g.*, HPLC, HPLC-MS, *etc.*, the corresponding cleavage pattern is considered to have a single cleavage site. In some embodiments, when greater than about 90%, greater than about 91%, greater than about 92%, greater than about 93%, greater than about 94%, greater than about 95%, greater than about 96%, greater than about 97%, greater than about 98%, greater than about 99%, or greater than about 99.5% of total cleavage occurs at a site, the corresponding cleavage pattern may be considered to have a single cleavage site. In some embodiments, as understood by a person having ordinary skill in the art, selectivity in *e.g.*, cells, tissues, organs, subjects, *etc.*, may be higher than that observed in an RNase H assay. In some embodiments, a site having greater than about 90%, greater than about 91%, greater than about 92%, greater than about 93%, greater than about 94%, greater than about 95%, greater than about

96%, greater than about 97%, greater than about 98%, greater than about 99%, or greater than about 99.5% of total cleavage in an RNase H assay may have higher selectivity in cells, tissues, organs, or subjects. In some embodiments, a site having greater than about 90% of total cleavage in an RNase H assay may be the only cleavage site (*e.g.*, greater than about 99%, greater than about 99.5%, 100%, *etc.*) in cells, tissues, organs, or subjects.

[001286] In some embodiments, selectivity may be assessed by comparing absolute values of remaining transcripts (or representative sequences thereof, such as RNA sequences used in examples described herein) of a target sequence and a similar sequence, *e.g.*, RNA, or representative synthetic sequences, of a mutant allele as a target, and a wild-type allele as a similar sequence. In some embodiments, selectivity may be assessed by comparing absolute amounts of remaining transcripts (or representative sequences thereof, such as RNA sequences used in examples described herein) of a target sequence and a similar sequence, when the starting amounts are the same. In some embodiments, selectivity may be assessed by percentages of cleavage of transcripts (or representative sequences thereof, such as RNA sequences used in examples described herein) of a target sequence and a similar sequence. In some embodiments, selectivity may be assessed by comparing ratios of cleaved and non-cleaved transcripts (or representative sequences thereof, such as RNA sequences used in examples described herein).

[001287] In some embodiments, selectivity can be assessed by one or more assays exemplified herein. In some embodiments, selectivity can be measured by an RNase H cleavage assay. For example, selective cleavage of a target (*e.g.*, RNA from a mutant allele) can be measured by a biochemical RNase H cleavage assay, wherein cleavage of a mutant target sequence is compared to that of a wild-type RNA sequence, and the selectivity can be represented by either the rate of cleavage, ratio of cleaved mutant RNA and wild-type RNA at a time point, or ratio of remaining mutant RNA and wild-type RNA at a time point, or combinations thereof. In some embodiments, a time point is 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more minutes. In some embodiments, a time point is 10 minutes. In some embodiments, a time point is 15 minutes. In some embodiments, a time point is 20 minutes. In some embodiments, a time point is 25 minutes. In some embodiments, a time point is 30 minutes. In some embodiments, a time point is 35 minutes. In some embodiments, a time point is 40 minutes. In some embodiments, a time point is 45 minutes. In some embodiments, a time point is 50 minutes. In some embodiments, a time point is 55 minutes. In some embodiments, a

time point is 60 minutes. In some embodiments, a time point is 60 or minutes. A person having ordinary skill in the art understands how to choose a time point, for example, for cleavage shown in Figure 32, one or more time points of 5, 10, 15, 20, 20, 45 and 60 minutes can be chosen to assess selectivity. In some embodiments, selectivity can be measured by ratios of IC_{50} for target (*e.g.*, mutant) and non-target (*e.*g, wild-type) sequences, *e.g.*, from cell-based assays or animal models.

[001288] Example HPLC-MS traces are presented in Figure 36. In some embodiments, example RNase H assay conditions were described below.

[001289] DNA/RNA Duplex Preparation: Oligonucleotide concentrations were determined by measuring the absorbance in water at 260 nm. DNA/RNA duplexes were prepared by mixing equimolar solutions oligonucleotides with each strand concentration of 20 μM. The mixtures were heated at 90 °C for 2 minutes in water bath and were cooled down slowly over several hours.

[001290] Human RNase H Protein Expression and Purification: Human RNase HC clone was obtained from Prof. Wei Yang's laboratory at NIH Bethesda. The protocol for obtaining this human RNase HC (residues 136-286) has been described (Nowotny, M. et al. Structure of Human RNase H1 Complexed with an RNA/DNA Hybrid: Insight into HIV Reverse Transcription. *Molecular Cell* 28, 264–276, (2007)). The protein expression was carried out by following reported protocol with the exception that the resulting protein had an N-terminal His6 tag. BL21(DE3) *E.coli* cells in LB medium were used for protein expression. Cells were grown at 37 °C till OD_{600nm} reached around 0.7. The cultures were then cooled and 0.4 mM IPTG was added to induce protein expression overnight at 16 °C. *E.coli* extract was prepared by sonication in buffer A (40 mM NaH_2PO_4 (pH 7.0), 1 M NaCl, 5% glycerol, 2.8 mM β-mercaptoethanol and 10 mM imidazole) with the addition of protease inhibitors (Sigma-Aldrich). The extract was purified by Ni affinity column using buffer A plus 60 mM imidazole. The protein was eluted with a linear gradient of 60 to 300 mM imidazole. The protein peak was collected and was further purified on a Mono S column (GE Healthcare) with a 100 mM - 500mM gradient of NaCl in buffer B. Fractions containing RNase HC were concentrated to 0.3 mg/mL in the storage buffer (20 mM HEPES (pH 7.0), 100mM NaCl, 5% glycerol, 0.5 mM EDTA, 2 mM DTT) and stored at -20 °C. 0.3mg/mL enzyme concentration corresponds to 17.4 μM based on its reported extinction coefficient (32095 $cm^{-1}M^{-1}$) and MW (18963.3 Da units).

[001291] In a 96-well plate, to 50 µL DNA/RNA duplex (20 µM) was added 10 µL of 10 X RNase H buffer followed by 30 µL water. The mixture was incubated at 37 °C for a few minutes and then 10 µL of 0.2 µM stock solution of enzyme was added to give a total volume of 100 µL with final substrate/enzyme concentration 10 µM/0.02 µM (500:1) and was further incubated at 37 °C. Various ratios of the DNA/RNA duplex to RNase H protein were previously studied using these conditions to find this optimal ratio (500:1) to study the kinetics. The reactions were quenched at different time points using 7 µL of 500 mM EDTA disodium solution in water. For zero min time point, EDTA was added to the reaction mixture before the addition of enzyme. Controls were run to ensure that EDTA was able to inhibit the enzyme activity completely. After all the reactions were quenched 10 µL or 20 µL of each reaction mixture was injected on to LCMS-TOF using analytical column (Agilent Poroshell 120 EC-C18 2.7 micron, 2.1 x 150 mm, Part# 699775-902). Ratio of peak area of remaining full length RNA to DNA in each reaction mixture was normalized against this ratio at zero point reaction to obtain the % of full length RNA remaining.

[001292] In some embodiments, an example HPLC condition is:

Eluant A = 8mM TEA, 200mM HFIP in Water

Eluant B= 50:50 (Eluant A: Methanol)

Column Temperature = 50°C

Auto sampler temperature = 4°C

UV was recorded at 254nm and 280nm

LC Gradient Method

	Time (min)	Flow (mL/min)	%A	%B
1	0.0	0.2	90	10
2	15.0	0.2	65	35
3	22.0	0.2	40	60
4	25.0	0.2	5.0	95.0
5	25.5	0.2	90	10
6	30	0.2	90	10

Example 20. Example assays for assessing oligonucleotides

[001293] In some embodiments, the present disclosure provides reporter assays for assessing properties of provided oligonucleotides and compositions. In some embodiments, a

provided reporter assay is a dual-luciferase assay, *e.g.*, as described below.

[001294] Determination of mRNA inhibition by oligonucleotides using Dual Glo Luciferase System: The psiCheck2 vector system from Promega is a commercially available vector which encodes both *Photinus pyralis* and *Renilla Reniformis* luciferase genes on a single plasmid with a multiple cloning site in the 3′ UTR of *Renilla* luciferase for insertion of oligonucleotides encoding the miRNA target sites (or other cloned regulatory sequences, such as target 3′UTRs). A 250 base pairs fragment containing the targeting region of interest and its reverse complement and having appropriate overhanging bases corresponding to the restriction enzyme(s) used to digest the psiCheck vector was cloned into the psiCHECK-2 vector (Promega, C8021) between NotI and XhoI restriction enzyme sites. The vector containing the insert was sequenced to confirm correct orientation of insert, expanded and purified. Multiple vectors were generated using above design for SNPs of interest. In a typical co-transfection experiment, after the cells were at the correct density (30 to 40% confluency), oligonucleotides and vector were reverse-transfected using Lipofectamine 2000 (Life Technologies). Effects of oligonucleotides on target mRNA can be seen as early as 24 h post-transfection and were still present after 48 h. 24 hour or 48 hours after transfection of psiCheck vectors, cells were assayed for luciferase activity. Briefly, cells are washed with PBS, lysed in passive lysis buffer, luciferin reagents were added, and samples were read on a Spectramax M5 instrument (Molecular Devices). Measurements were taken at vector concentrations of 20 ng per well of a 96-well plate. The experiments were performed at various oligonucleotide concentrations (30, 10 and 3.3 nM) and two time points (24 and 48 hours). The relative levels of *Renilla* luciferase vs. Firefly were measured for untreated cells and cells which were treated with oligonucleotides targeting Renilla (WV-975) to measure the maximum Renilla knockdown. Control oligonucleotides (*e.g.*, WV-437, WV-993, *etc.*) were chosen to normalize the R/F levels. In some embodiments, dual luciferase reporter assay was used to assess oligonucleotides in Cos7 cell line. In some embodiments, the cell line was cotransfected for 24hrs with oligonucleotides and either of the psiCHECK2 plasmids, including rs362307 (T) or rs362307 (C) SNP. In some embodiments, rs362307 (T) and rs362307 (C) are referred as mu and wt.

[001295] Various chirally controlled and stereorandom oligonucleotide compositions were tested at 30 nM using the dual-luciferase assay. For oligonucleotides with mismatch at the same position (*e.g.*, positions 8, 9, 10, 11, 12 and 13 relative to the 5'-end), chirally controlled

compositions maintained high levels of wide-type measurements.

[001296] In some embodiments, when tested at 30 nM, WV-1092 selectively suppressed expression of the mutant sequence at 24 and/or 48 hours as shown by the dual luciferase reporter assay. In some embodiments, the observed selectivity for WV-1092 at 30 nM 24 hours and/or 48 hours was several fold more than other oligonucleotide compositions, *e.g.*, WV-917, WV-1497, certain P12 stereopure oligonucleotides, *etc.* In some embodiments, at 30 nM, 48 hours, WV-1092 maintained over 90% wild-type, and decreased the mutant to about 30%, while WV-917 decreased the wild-type to about 60%, and mutant to about 30%. Oligonucleotides are tested at multiple conditions (*e.g.*, concentrations, time points, *etc.*), and show improved properties, *e.g.*, activity, selectivity, *etc.*

[001297] As understood by a person having ordinary skill in the art, oligonucleotide properties, *e.g.*, activity, selectivity, *etc.*, may be assessed by many other assays, such as cell-based assays, animal models, *etc.* In some embodiments, allele-specific suppression may be tested in cells and animal models using similar procedures as described in Hohjoh, *Pharmaceuticals* **2013**, *6*, 522-535; US patent application publication US 2013/0197061; Østergaard *et al.*, *Nucleic Acids Research* **2013**, *41*(21), 9634–9650; Jiang *et al.*, *Science* **2013**, *342*, 111-114; and US9006198. In some embodiments, selectivity can be assessed by IC_{50} values for the wild-type and the mutant allele. Provided compositions, including those targeting SNPs associated with Huntington's disease, suppress disease-associated alleles selectively over wild type alleles.

Example 21. Example methods for preparing oligonucleotides and compositions

[001298] *Abbreviations*

AMA: conc. NH_3 - 40% $MeNH_2$ in H_2O (1:1, v/v)

CMIMT: *N*-cyanomethylimidazolium triflate

DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene

DCA: dichloroacetic acid

DCM: dichloromethane, CH_2Cl_2

DMTr: 4,4′-dimethoxytrityl

DVB: divinylbenzene

HCP: highly cross-linked polystyrene (contains 50% DVB, non-swelling polystyrene)

MeIm: *N*-methylimidazole

MQ: water obtained from "Milli-Q Reference"

PhIMT: *N*-phenylimidazolium triflate

POS: 3-phenyl-1,2,4-dithiazolin-5-one

PS200: primer support 200, commercially available from GE Healthcare

PS5G: primer support 5G, commercially available from GE Healthcare

TBAF: tetrabutylammonium fluoride

TBHP: *tert*-butylhydroperoxide

TEAA: triethylammonium aceate

[001299] Solid support: Various types of solid support (varied nucleosides loading) were tested. In some embodiments, HCP > PS5G ≈ PS200 ≥ CPG. In some embodiments, a solid support is HCP. In some embodiments, a solid support is PS5G. In some embodiments, a solid support is PS200. In some embodiments, a solid support is CPG. For nucleosides loading, various range (30~300 μmol/g) were tested. In some embodiments, 70~80 μmol/g loading performed than others. In some embodiments, nucleoside loading is 70~80 μmol/g. CPG was purchased from various suppliers (GlenReseach, LinkTechnologies, ChemGenes, PrimeSynthesis, and 3-Prime).

[001300] Various linkers were tested and can be used. In some embodiments, during preparation of chirally controlled oligonucleotide compositions by using DPSE-type chemistry, SP-linker was used.

[001301] Various activators were prepared and/or purchased, and evaluated. In some embodiments, for DPSE-type chemistry, CMIMT was used.

[001302] Example Analytical conditions:

1) RP-UPLC-MS
   - ❖ System: Waters, Aquity UPLC I-Class, Xevo G2-Tof
   - ❖ Column: Waters, BEH C18, 1.7 μm, 2.1 x 150 mm
   - ❖ Temp. & Flow rate: 55 °C, 0.3 mL/min
   - ❖ Buffer: A: 0.1M TEAA; B: MeCN
   - ❖ Gradient: %B: 1-30% / 30 min
2) AEX-HPLC
   - ❖ System: Waters, Alliance e2695
   - ❖ Column: Thermo, DNAPac PA-200, 4 x 250 mm

- ❖ Temp. & Flow rate: 50 °C, 1 mL/min
- ❖ Buffer: A: 20mM NaOH; B: A + 1M $NaClO_4$
- ❖ Gradient: %B: 10-50% / 30 min

[001303] Example procedure for the synthesis of chrial-oligos (1 μmol scale):

[001304] Automated solid-phase synthesis of chiral-oligos was performed according to example cycles shown herein. After the synthesis cycles, the resin was treated with 0.1M TBAF in MeCN (1 mL) for 2 h (30 min usually enough) at room temperature, washed with MeCN, dried, and add AMA (1 mL) for 30 min at 45 °C. The mixture was cooled to room temperature and the resin was removed by membrane filtration. The filtrate was concentrated under reduced pressure to about 1 mL. The residue was diluted with 1 mL of H_2O and analyzed by AEX-HPLC and RP-UPLC-MS (example conditions: refer to the analytical conditions).

step	operation	reagents and solvent	volume	waiting time
1	detritylation	3% DCA in toluene	10 mL	65 s
2	coupling	0.15M monomer in ^{i}PrCN + 0.5M CMIMT in MeCN	0.5 mL	5 min
3	capping	20% Ac_2O, 30% 2,6-lutidine in MeCN + 20% MeIm in MeCN	1.2 mL	60 s
4	oxidation or sulfurization	1.1M TBHP in DCM-decane or 0.1M POS in MeCN	1.0 mL	300 s

[001305] As described, TBAF treatement can provide better results, for example, less desulfurization. In some embodiments, SP linker provided better yields and/or purity through, without the intention to be limited by theory, better stability during chiral auxiliary removal as described. In some embodiments, fluoro-containing reagents such as HF-NR_3 (*e.g.*, HF-TEA (triethylamine)), provided better yields and/or purity when succinyl linker was used by, without the intention to be limited by theory, less cleavage during chiral auxiliary removal. In some embodiments, after synthesis, the resin was treated with 1M TEA-HF in DMF-H_2O (3:1, v/v; 1 mL) for 2 h at 50°C. PS5G support was washed with MeCN, H_2O, and add AMA (conc. NH_3 - 40%$MeNH_2$ (1:1, v/v)) (1 mL) for 45 min at 50°C. The mixture was cooled to room temperature and the resin was removed by membrane filtration (washed with H_2O for 2mL). The filtrate was concentrated under reduced pressure until it becomes about 1 mL. The residue was diluted with

1 mL of H_2O and analyzed by AEX-HPLC and RP-UPLC-MS (conditions: refer to the analytical conditions section).

[001306] Example procedure for the purification of chrial-oligos (1 μmol scale): in some embodiments, crude oligos were purified by AEX-MPLC according to the following example conditions:

- System: AKTA Purifier-10
- Column: TOHSOH, DNA STAT, 4.6 x 100 mm
- Temp. & Flow rate: 60 °C, 0.5 mL/min
- Buffer: A: 20mM Tris-HCl (pH 9.0) + 20% MeCN, B: A + 1.5M NaCl
- Gradient: %B: 20-70% / 25CV (2%/CV)

[001307] All fractions were analyzed by analytical AEX-HPLC, and fractions containg chiral oligo more than 80% purity were corrected and desalted by Sep-Pak Plus tC18 (WAT036800) using example conditions below:

1. Conditionning Sep-Pak Plus with 15 mL of MeCN.
2. Rinse cartridge with 15 mL of 50% MeCN/MQ.
3. Equilibrate cartridge with 30 mL of MQ.
4. Load sample, and wash with 40 mL of MQ.
5. Elute chiral oligos with 10 mL of 50% MeCN/MQ.

[001308] Eluted sample were evapolated under reduced pressure to remove MeCN, and lyophilized. The product were dissolved in MQ (1 mL), filtered by 0.2 μm mesh syringe filter, and analyzed. After yield calculation by UV absorbance, the preparation was lyophilized again.

[001309] Example methods, conditions and reagents were described in, *e.g.*, JP 2002-33436, WO2005/092909, WO2010/064146, WO2012/039448, WO2011/108682, WO2014/010250, WO2014/010780, WO2014/012081, *etc.*, and may be useful for preparing provided oligonucleotides and/or compositions.

[001310] Additional example oligonucleotides are listed below. In some embodiments, one or more of the oligonucleotides below are used as controls. In some embodiments, one or more of the oligonucleotides below are RNA sequences as cleavage targets in one or more assays.

	Table 5N. Example Control Oligonucleotides.
WV-975	G*T*A*G*G*A*G*T*A*G*T*G*A*A*A*G*G*C*C*A
WV-1061	mG*mU*mA*mG*mG*A*G*T*A*G*T*G*A*A*A*mG*mG*mC*mC*mA
WV-1062	mGmUmAmGmG*A*G*T*A*G*T*G*A*A*A*mGmGmCmCmA

WV-1063	mG*mU*mA*mG*mG*A*G*T*A*G*T*G*A*A*A*G*G*C*C*A
WV-1064	mC*mU*mC*mU*mU*A*C*T*G*T*G*C*T*G*T*mG*mG*mA*mC*mA
WV-1065	mCmUmCmUmU*A*C*T*G*T*G*C*T*G*T*mGmGmAmCmA
WV-1066	mC*mU*mC*mU*mU*A*C*T*G*T*G*C*T*G*T*G*G*A*C*A
WV-993	mC*mC*mU*mU*mC*C*C*T*G*A*A*G*G*T*T*mC*mC*mU*mC*mC
WV-975	All DNA, Stereorandom PS, positive control for Renilla luciferase in psiCHECK2 plasmid
WV-1061	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, +ve Luciferase control for psiCHECK2
WV-1062	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings: +ve Luciferase control for psiCHECK2
WV-1063	5-15 (2'-OMe-DNA), stereorandom PS, +ve Luciferase control for psiCHECK2
WV-1064	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, Negative Luciferase control for psiCHECK2
WV-1065	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, PO in the wings: Negative Luciferase control for psiCHECK2
WV-1066	5-15 (2'-OMe-DNA), stereorandom PS, Negative Luciferase control for psiCHECK2
WV-993	5-10-5 (2'-OMe-DNA-2'-OMe), stereorandom PS, Negative Luciferase control for psiCHECK2

	Table 6N. Example RNA Sequences.	
WV-944	rUrUrUrGrGrArArGrUrCrUrGrCrGrCrCrCrUrUrGrUrGrCrCrC	
WV-945	rUrUrUrGrGrArArGrUrCrUrGrUrGrCrCrCrUrUrGrUrGrCrCrC	
WV-1073	rGrArGrCrCrUrUrUrGrGrArArGrUrCrUrGrCrGrCrCrCrUrUrGrUrGrCrCrCr UrGrCrCrU	
WV-1074	rGrArGrCrCrUrUrUrGrGrArArGrUrCrUrGrUrGrCrCrCrUrUrGrUrGrCrCrCr UrGrCrCrU	
WV-950	rGrGrUrUrGrUrUrGrCrCrArGrGrUrUrArCrArGrCrUrGrCrUrC	
WV-951	rGrGrUrUrGrUrUrGrCrCrArGrGrUrUrGrCrArGrCrUrGrCrUrC	
WV-958	rCrCrUrCrCrUrGrCrArGrGrCrUrGrGrGrUrGrUrUrGrGrCrCrC	
WV-959	rCrCrUrCrCrUrGrCrArGrGrCrUrGrGrCrUrGrUrUrGrGrCrCrC	
ONT-453	rGrGrUrGrArUrGrArCrArArUrUrUrArUrUrArArU	
ONT-454	rGrGrUrGrArUrGrGrCrArArUrUrUrArUrUrArArU	
WV-944	rs362307	WT
WV-945	rs362307	mu
WV-1073	rs362307	WT
WV-1074	rs362307	mu
WV-950	rs362306	WT
WV-951	rs362306	mu
WV-958	rs362268	WT
WV-959	rs362268	mu
ONT-453	rs7685686	WT
ONT-454	rs7685686	mu

Example 22. Example oligonucleotides.

Additional example oligonucleotides are listed below in Table 8.

Table 8. HTT Oligonucleotides.

SEQ ID	Naked Sequence	Modified Sequence	Stereochemistry	Comment 1	Comment 2
ONT-450	ATTAATAAATTGTCATCACC	A * T * T * A * A * T * A * A * A * T * T * G * T * C * A * T * C * A * C * C	XXXXXXXXXXXXXXXXXXX	Stereorandom Htt sequence	Htt SNP rs7685686
ONT-451	ATTAATAAATTGTCATCACC	A * ST * ST * SA * SA * ST * SA * SA * SA * ST * ST * SG * ST * SC * RA * ST * SC * SA * SC * SC	SSSSSSSSSSSSSRSSSSS	Stereopure Htt sequence I	Htt SNP rs7685686
ONT-452	ATTAATAAATTGTCATCACC	A * ST * ST * SA * SA * ST * SA * SA * SA * ST * ST * SG * ST * SC * SA * RT * SC * SA * SC * SC	SSSSSSSSSSSSSSRSSSS	Stereopure Htt sequence II	Htt SNP rs7685686
ONT-453	GGUGAUGACAAUUUAUUAAU	rGrGrUrGrArUrGrArCrArArUrUrUrArUrUrArArU	OOOOOOOOOOOOOOOOO	RNA against Htt sequence Mutant	Htt SNP rs7685686
ONT-454	GGUGAUGGCAAUUUAUUAAU	rGrGrUrGrArUrGrGrCrArArUrUrUrArUrUrArArU	OOOOOOOOOOOOOOOOO	RNA against Htt sequence Wild Type	Htt SNP rs7685686
WV-902	UUUGGAAGUCUGCGCCCUUGUGCCC	rUrUrUrGrGrArArGrUrCrUrGrCrGrCrCrCrUrUrGrUrGrCrCrC	OOOOOOOOOOOOOOOOOOOOOOOOO	wtRNA	muHTT SNP 362307
WV-903	UUUGGAAGUCUGUGCCCUUGUGCCC	rUrUrUrGrGrArArGrUrCrUrGrUrGrCrCrCrUrUrGrUrGrCrCrC	OOOOOOOOOOOOOOOOOOOOOOOOO	mRNA	muHTT SNP 362307
WV-904	GGGCACAAGGGCACAGACTT	G * G * G * C * A * C * A * A * G * G * G * C * A * C * A * G * A * C * T * T	XXXXXXXXXXXXXXXXXXX	ASO1 All DNA; stereorandom PS	muHTT SNP 362307
WV-905	GGCACAAGGGCACAGACTTC	G * G * C * A * C * A * A * G * G * G * C * A * C * A * G * A * C * T * T * C	XXXXXXXXXXXXXXXXXXX	ASO2 All DNA; stereorandom PS	muHTT SNP 362307

WV-906	GCACAAGGGCACA GACTTCC	G * C * A * C * A * A * G * G * G * C * A * C * A * G * A * C * T * T * C * C	XXXXXXXXXXXXX XXXXXXXX	ASO3 All DNA; stereorandom PS	muHTT SNP 362307
WV-907	CACAAGGGCACAG ACTTCCA	C * A * C * A * A * G * G * G * G * C * A * C * A * G * A * C * T * T * C * C * A	XXXXXXXXXXXXX XXXXXXXX	ASO4 All DNA; stereorandom PS	muHTT SNP 362307
WV-908	ACAAGGGCACAGA CTTCCAA	A * C * A * A * G * G * G * C * A * A * C * A * G * A * C * T * T * C * C * A * A	XXXXXXXXXXXXX XXXXXXXX	ASO5 All DNA; stereorandom PS	muHTT SNP 362307
WV-909	CAAGGGCACAGAC TTCCAAA	C * A * A * G * G * G * C * A * C * A * A * G * A * C * T * T * C * C * A * A * A	XXXXXXXXXXXXX XXXXXXXX	ASO6 All DNA; stereorandom PS	muHTT SNP 362307
WV-910	GGGCACAAGGGCA CAGACTT	mG * mG * mG * mC * mA * C * A * A * G * G * G * C * A * C * A * G * A * C * T * T	XXXXXXXXXXXXX XXXXXXXX	ASO7 5-15 (2'-OMe-DNA); stereorandom PS	muHTT SNP 362307
WV-911	GGCACAAGGGCAC AGACTTC	mG * mG * mC * mA * mC * A * A * G * G * G * C * A * C * A * G * A * C * T * T * C	XXXXXXXXXXXXX XXXXXXXX	ASO8 5-15 (2'-OMe-DNA); stereorandom PS	muHTT SNP 362307
WV-912	GCACAAGGGCACA GACTTCC	mG * mC * mA * mC * mA * A * G * G * G * C * A * C * A * G * A * C * T * T * C * C	XXXXXXXXXXXXX XXXXXXXX	ASO9 5-15 (2'-OMe-DNA); stereorandom PS	muHTT SNP 362307
WV-913	CACAAGGGCACAG ACTTCCA	mC * mA * mC * mA * mA * G * G * G * C * A * C * A * G * A * C * T * T * C * C * A	XXXXXXXXXXXXX XXXXXXXX	ASO10 5-15 (2'-OMe-DNA); stereorandom PS	muHTT SNP 362307
WV-914	ACAAGGGCACAGA CTTCCAA	mA * mC * mA * mA * mG * G * G * C * A * C * A * G * A * C * T * T * C * C * A * A	XXXXXXXXXXXXX XXXXXXXX	ASO11 5-15 (2'-OMe-DNA); stereorandom PS	muHTT SNP 362307
WV-915	CAAGGGCACAGAC TTCCAAA	mC * mA * mA * mG * mG * G * C * A * C * A * G * A * A * C * T * T * C * C * A * A * A	XXXXXXXXXXXXX XXXXXXXX	ASO12 5-15 (2'-OMe-DNA); stereorandom PS	muHTT SNP 362307
WV-916	GGGCACAAGGGCA CAGACUU	mG * mG * mG * mC * mA * C * A * A * G * G * G * C * A * C * A * mG * mA * mC *	XXXXXXXXXXXXX XXXXXXXX	ASO13 5-10-5 (2'-OMe-DNA-2'-OMe);	muHTT SNP 362307

		mU * mU		stereorandom PS	
WV-917	GGCACAAGGGCAC AGACUUC	mG * mG * mC * mA * mC * A * A * G * G * C * A * C * A * G * mA * mC * mU * mU * mC	XXXXXXXXXXXXX XXXXXXXX	ASO14 5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	muHTT SNP 362307
WV-918	GCACAAGGGCACA GACUUCC	mG * mC * mA * mC * mA * A * G * G * G * C * A * C * A * G * A * mC * mU * mU * mC * mC	XXXXXXXXXXXXX XXXXXXXX	ASO15 5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	muHTT SNP 362307
WV-919	CACAAGGGCACAG ACUUCCA	mC * mA * mC * mA * mA * G * G * G * C * A * C * A * G * A * C * mU * mU * mC * mC * mA	XXXXXXXXXXXXX XXXXXXXX	ASO16 5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	muHTT SNP 362307
WV-920	ACAAGGGCACAGA CTUCCAA	mA * mC * mA * mA * mG * G * G * C * A * C * A * G * A * C * T * mU * mC * mC * mA * mA	XXXXXXXXXXXXX XXXXXXXX	ASO17 5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	muHTT SNP 362307
WV-921	CAAGGGCACAGAC TTCCAAA	mC * mA * mA * mG * mG * G * C * A * C * A * G * A * C * T * T * mC * mC * mA * mA * mA	XXXXXXXXXXXXX XXXXXXXX	ASO18 5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	muHTT SNP 362307
WV-922	GCACAAGGGCACA GACUUCC	mG * mC * mA * mC * mA * mA * mG * mG * G * C * A * C * A * G * A * mC * mU * mU * mC * mC	XXXXXXXXXXXXX XXXXXXXX	ASO19 8-7-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	muHTT SNP 362307
WV-923	CACAAGGGCACAG ACUUCCA	mC * mA * mC * mA * mA * mG * mG * G * C * A * C * A * G * A * mC * mU * mU * mC * mC * mA	XXXXXXXXXXXXX XXXXXXXX	ASO20 7-7-6 (2'-OMe-DNA-2'-OMe); stereorandom PS	muHTT SNP 362307
WV-924	ACAAGGGCACAGA CUUCCAA	mA * mC * mA * mA * mG * mG * G * C * A * C * A * G * A * mC * mU * mU * mC * mC * mA * mA	XXXXXXXXXXXXX XXXXXXXX	ASO21 6-7-5 (2'-OMe-DNA-2'-OMe); stereorandom PS;	muHTT SNP 362307

				PO in the wings	
WV-925	CAAGGGCACAGAC UUCCAAA	mC * mA * mA * mG * mG * G * C * A * C * A * G * A * mC * mU * mU * mC * mC * mA * mA * mA	XXXXXXXXXXXX XXXXXXXX	ASO22 5-7-8 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	muHTT SNP 362307
WV-926	GCACAAGGGCACA GACUUCC	mGmCmAmCmAmAmGmG * G * C * A * C * A * G * A * mCmUmUmCmC	OOOOOOOXXXX XXXXOOOO	ASO23 8-7-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	muHTT SNP 362307
WV-927	CACAAGGGCACAG ACUUCCA	mCmAmCmAmAmGmG * G * C * A * C * A * G * A * mCmUmUmCmCmA	OOOOOOXXXXX XXXOOOOO	ASO24 7-7-6 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	muHTT SNP 362307
WV-928	ACAAGGGCACAGA CUUCCAA	mAmCmAmAmGmG * G * C * A * C * A * G * A * mCmUmUmCmCmAmA	OOOOOXXXXXX XXOOOOOO	ASO25 6-7-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	muHTT SNP 362307
WV-929	CAAGGGCACAGAC UUCCAAA	mCmAmAmGmG * G * C * A * C * A * G * A * mCmUmUmCmCmAmAmA	OOOOXXXXXXX XOOOOOOO	ASO26 5-7-8 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	muHTT SNP 362307
WV-930	GGGCACAAGGGCA CAGACUU	mGmGmGmCmA * C * A * A * G * G * C * A * C * A * mGmAmCmUmU	OOOOXXXXXXX XXXXOOOO	ASO27 5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	muHTT SNP 362307
WV-931	GGCACAAGGGCAC AGACUUC	mGmGmCmAmC * A * A * G * G * G * C * A * C * A * G *	OOOOXXXXXXX XXXXOOOO	ASO28 5-10-5 (2'-OMe-DNA-2'-	muHTT SNP 362307

		mAmCmUmUmC		OMe); stereorandom PS; PO in the wings	
WV-932	GCACAAGGGCACA GACUUCC	mGmCmAmCmA * A * G * G * G * C * A * C * A * G * A * mCmUmUmCmC	OOOOXXXXXXX XXXXOOOO	ASO29 5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	muHTT SNP 362307
WV-933	CACAAGGGCACAG ACUUCCA	mCmAmCmAmA * G * G * G * C * A * C * A * G * A * C * mUmUmCmCmA	OOOOXXXXXXX XXXXOOOO	ASO30 5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	muHTT SNP 362307
WV-934	ACAAGGGCACAGA CTUCCAA	mAmCmAmAmG * G * G * C * A * C * A * G * A * C * T * mUmCmCmAmA	OOOOXXXXXXX XXXXOOOO	ASO31 5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	muHTT SNP 362307
WV-935	CAAGGGCACAGAC TTCCAAA	mCmAmAmGmG * G * C * A * C * A * G * A * C * T * T * mCmCmAmAmA	OOOOXXXXXXX XXXXOOOO	ASO32 5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	muHTT SNP 362307
WV-936	GGGCACAAGGGCA CAGACTT	G * SG * SG * SC * SA * SC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SA * SC * ST * ST	SSSSSSSSSSSSR SSSSS	ASO33 Stereopure DNA; One Rp; position 14	muHTT SNP 362307
WV-937	GGCACAAGGGCAC AGACTTC	G * SG * SC * SA * SC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SA * SC * ST * ST * SC	SSSSSSSSSSSRS SSSSS	ASO34 Stereopure DNA; One Rp; position 13	muHTT SNP 362307
WV-938	GCACAAGGGCACA GACTTCC	G * SC * SA * SC * SA * SA * SG * SG * SG * SC * SA * SC	SSSSSSSSSSRSS SSSSS	ASO35 Stereopure DNA; One Rp;	muHTT SNP 362307

		* RA * SG * SA * SC * ST * ST * SC * SC		position 12	
WV-939	CACAAGGGCACAG ACTTCCA	C * SA * SC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SA * SC * ST * ST * SC * SC * SA	SSSSSSSSSSRSSS SSSSS	ASO36 Stereopure DNA; One Rp; position 11	muHTT SNP 362307
WV-940	ACAAGGGCACAGA CTTCCAA	A * SC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SA * SC * ST * ST * SC * SC * SA * SA	SSSSSSSSSRSSSS SSSSS	ASO37 Stereopure DNA; One Rp; position 10	muHTT SNP 362307
WV-941	CAAGGGCACAGAC TTCCAAA	C * SA * SA * SG * SG * SG * SG * SC * SA * SC * RA * SG * SA * SC * ST * ST * SC * SC * SA * SA * SA	SSSSSSSSRSSSSS SSSSS	ASO38 Stereopure DNA; One Rp; position 9	muHTT SNP 362307
WV-944	UUUGGAAGUCUGC GCCCUUGUGCCC	rUrUrUrGrGrGrArArArGrUrCrUrG rCrGrCrCrCrCrUrUrGrUrGrCrCr C	OOOOOOOOOOO OOOOOOOOOOO OO	HTT-rs362307 human	Huntington
WV-945	UUUGGAAGUCUG UGCCCUUGUGCCC	rUrUrUrGrGrGrArArArGrUrCrUrG rUrGrCrCrCrCrUrUrGrUrGrCrCr C	OOOOOOOOOOO OOOOOOOOOOO OO	HTT-rs362307 human	Huntington
WV-948	GAGCAGCTGCAAC CTGGCAA	G * A * G * C * A * G * C * T * G * C * A * A * C * C * T * G * G * C * A * A	XXXXXXXXXXX XXXXXXXX	HTT-rs362306	HTT-rs362306
WV-949	GGGCCAACAGCCA GCCTGCA	G * G * G * C * C * A * A * C * A * G * C * C * C * A * G * C * C * T * G * C * A	XXXXXXXXXXX XXXXXXXX	HTT-rs362268	HTT-rs362268
WV-950	GGUUGUUGCCAGG UUACAGCUGCUC	rGrGrUrUrGrUrUrGrCrCrArGr GrUrUrArCrArGrCrUrGrCrUr C	OOOOOOOOOOO OOOOOOOOOOO OO		HTT-rs362306
WV-951	GGUUGUUGCCAGG UUGCAGCUGCUC	rGrGrUrUrGrUrUrGrCrCrArGr GrUrUrGrCrArGrCrUrGrCrUr C	OOOOOOOOOOO OOOOOOOOOOO OO		HTT-rs362306
WV-952	GAGCAGCTGCAAC	G * SA * SG * SC * SA * SG *	SSSSSSSSSRSSS	Stereopure PS	HTT-

	CTGGCAA	SC * ST * SG * SC * SA * RA * SC * SC * ST * SG * SG * SC * SA * SA	SSSSS	DNA; One Rp at position 11	rs362306
WV-953	AGCAGCTGCAACC TGGCAAC	A * SG * SC * SA * SG * SC * ST * SG * SC * SA * RA * SC * SC * ST * SG * SG * SC * SA * SA * SC	SSSSSSSSSRSSSS SSSSS	Stereopure PS DNA; One Rp at position 10	HTT-rs362306
WV-954	GCAGCTGCAACCT GGCAACA	G * SC * SA * SG * SC * ST * SG * SC * SA * RA * SC * SC * ST * SG * SG * SC * SA * SA * SC * SA	SSSSSSSSRSSSSS SSSSS	Stereopure PS DNA; One Rp at position 9	HTT-rs362306
WV-955	CAGCTGCAACCTG GCAACAA	C * SA * SG * SC * ST * SG * SC * SA * RA * SC * SC * ST * SG * SG * SC * SA * SA * SA * SC * SA	SSSSSSSRSSSSSS SSSSS	Stereopure PS DNA; One Rp at position 8	HTT-rs362306
WV-956	AGCTGCAACCTGG CAACAAC	A * SG * SC * ST * SG * SC * SA * RA * SC * SC * ST * SG * SG * SC * SA * SA * SC * SA * SC	SSSSSSRSSSSSSS SSSSS	Stereopure PS DNA; One Rp at position 7	HTT-rs362306
WV-957	GCTGCAACCTGGC AACAACC	G * SC * ST * SG * SC * SA * RA * SC * SC * ST * SG * SG * SC * SA * SA * SC * SA * SC	SSSSSRSSSSSSSS SSSSS	Stereopure PS DNA; One Rp at position 6	HTT-rs362306
WV-958	CCUCCUGCAGGCU GGGUGUUGGCCC	rCrCrUrCrCrUrGrCrArGrGrCrUrGrGrGrUrGrUrUrGrGrCrCrC	OOOOOOOOOOOOOOOOOOOO		HTT-rs362268
WV-959	CCUCCUGCAGGCU GGCUGUUGGCCC	rCrCrUrCrCrUrGrCrArGrGrCrUrGrGrGrCrUrGrUrUrGrGrCrCrC	OOOOOOOOOOOOOOOOOOOO		HTT-rs362268
WV-960	GGGCCAACAGCCA GCCTGCA	G * SG * SG * SC * SC * SA * SA * SC * SA * SG * SC * RC * SA * SG * SC * SC * ST * SG * SC * SA	SSSSSSSSSRSSS SSSSS	Stereopure PS DNA; One Rp at position 11	HTT-rs362268

WV-961	GGCCAACAGCCAG CCTGCAG	G * SG * SC * SC * SA * SA * SC * SA * SG * SC * RC * SA * SG * SC * SC * ST * SG * SC * SA * SG	SSSSSSSSRSSSS SSSSS	Stereopure PS DNA; One Rp at position 10	HTT-rs362268
WV-962	GCCAACAGCCAGC CTGCAGG	G * SC * SC * SA * SA * SC * SA * SG * SC * RC * SA * SG * SC * SC * ST * SG * SC * SA * SG * SG	SSSSSSSRSSSSS SSSSS	Stereopure PS DNA; One Rp at position 9	HTT-rs362268
WV-963	CCAACAGCCAGCC TGCAGGA	C * SC * SA * SA * SC * SA * SG * SC * RC * SA * SG * SC * SC * ST * SG * SC * SA * SG * SG * SA	SSSSSSSRSSSSSS SSSSS	Stereopure PS DNA; One Rp at position 8	HTT-rs362268
WV-964	CAACAGCCAGCCT GCAGGAG	C * SA * SA * SC * SA * SG * SC * RC * SA * SG * SC * SC * ST * SG * SC * SA * SG * SG * SA * SG	SSSSSSRSSSSSS SSSSS	Stereopure PS DNA; One Rp at position 7	HTT-rs362268
WV-965	AACAGCCAGCCTG CAGGAGG	A * SA * SC * SA * SG * SC * RC * SA * SG * SC * SC * ST * SG * SC * SA * SG * SG * SA * SG	SSSSSRSSSSSSS SSSSS	Stereopure PS DNA; One Rp at position 6	HTT-rs362268
WV-973	GGCCUUUCACUAC UCCUACTT	rGrGrCrCrUrUrUrCrArCrUrArCrUrCrCrUrArCTT	OOOOOOOOOOO OOOOOOOOO	siRNA (+control for Renilla luciferase in psiCHECK2 plasmid) antisense strand	Htt
WV-974	GUAGGAGUAGUG AAAGGCCTT	rGrUrArGrGrArGrUrArGrUrG rArArArGrGrCrCTT	OOOOOOOOOOO OOOOOOOOO	siRNA (+control for Renilla luciferase in psiCHECK2 plasmid) sense strand	Htt SNP rs362268
WV-975	GTAGGAGTAGTGA	G * T * A * G * G * A * G * T	XXXXXXXXXXX	ASO (+control for	Htt SNP

	AAGGCCA	* A * G * T * G * A * A * A * G * G * C * C * A	XXXXXXXX	Renilla luciferase in psiCHECK2 plasmid)	rs362268
WV-982	GCAGGGCACAAGG GCACAGA	G * SC * SA * SG * SG * SG * SC * SA * SC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SA	SSSSSSSSSSSSS SSRSS	Htt seq 307 expanding 3 nt towards 3' example 3	Htt rs362307
WV-983	CAGGGCACAAGGG CACAGAC	C * SA * SG * SG * SG * SC * SA * SC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SA * SC	SSSSSSSSSSSSS SRSSS	Htt seq 307 expanding 3 nt towards 3' example 2	Htt rs362307
WV-984	AGGGCACAAGGGC ACAGACT	A * SG * SG * SG * SC * SC * SA * SC * SA * SA * SG * SG * SG * SG * SC * SA * SC * RA * SG * SA * SC * ST	SSSSSSSSSSSSS RSSSS	Htt seq 307 expanding 3 nt towards 3' example 1	Htt rs362307
WV-985	AAGGGCACAGACT TCCAAAG	A * SA * SG * SG * SG * SC * SA * SC * RA * SG * SA * SC * ST * ST * SC * SC * SA * SA * SA * SG	SSSSSSSRSSSSSS SSSSS	Htt seq 307 expanding 3 nt towards 5' example 1	Htt rs362307
WV-986	AGGGCACAGACTT CCAAAGG	A * SG * SG * SG * SC * SC * SA * SC * RA * SG * SA * SC * ST * ST * SC * SC * SA * SA * SA * SG	SSSSSSRSSSSSSS SSSSS	Htt seq 307 expanding 3 nt towards 5' example 2	Htt rs362307
WV-987	GGGCACAGACTTC CAAAGGC	G * SG * SG * SC * SC * SA * SC * RA * SG * SA * SC * ST * ST * SC * SC * SA * SA * SA * SG * SG * SC	SSSSSRSSSSSSSS SSSSS	Htt seq 307 expanding 3 nt towards 5' example 3	Htt rs362307
WV-1001	GAGCAGCTGCAAC CTGGCAA	G * A * G * C * C * A * G * C * T * G * C * A * A * C * C * T * G * G * C * A * A	XXXXXXXXXXX XXXXXXXX	All DNA; stereorandom PS	HTT-rs362306
WV-1002	AGCAGCTGCAACC TGGCAAC	A * G * C * C * A * G * C * T * G * C * A * A * C * C * T * G * G * C * A * A * C	XXXXXXXXXXX XXXXXXXX	All DNA; stereorandom PS	HTT-rs362306

WV-1003	GCAGCTGCAACCT GGCAACA	G * C * A * G * C * T * G * C * A * A * C * C * T * G * G * C * A * A * C * A	XXXXXXXXXXXX XXXXXXXX	All DNA; stereorandom PS	HTT-rs362306
WV-1004	CAGCTGCAACCTG GCAACAA	C * A * G * C * T * G * C * A * A * C * C * T * G * G * C * A * A * C * A * A	XXXXXXXXXXXX XXXXXXXX	All DNA; stereorandom PS	HTT-rs362306
WV-1005	AGCTGCAACCTGG CAACAAC	A * G * C * T * G * C * A * A * C * C * T * G * G * C * A * A * C * A * A * C	XXXXXXXXXXXX XXXXXXXX	All DNA; stereorandom PS	HTT-rs362306
WV-1006	GCTGCAACCTGGC AACAACC	G * C * T * G * C * A * A * C * C * T * G * G * C * A * A * C * C * A * A * C	XXXXXXXXXXXX XXXXXXXX	All DNA; stereorandom PS	HTT-rs362306
WV-1007	GAGCAGCTGCAAC CTGGCAA	mG * mA * mG * mC * mA * G * C * T * G * C * A * A * C * C * T * G * G * C * A * A	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS	HTT-rs362306
WV-1008	AGCAGCTGCAACC TGGCAAC	mA * mG * mC * mA * mG * C * T * G * C * A * A * C * C * T * G * G * C * A * A * C	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS	HTT-rs362306
WV-1009	GCAGCTGCAACCT GGCAACA	mG * mC * mA * mG * mC * T * G * C * A * A * C * C * T * G * G * C * A * A * C * A	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS	HTT-rs362306
WV-1010	CAGCUGCAACCTG GCAACAA	mC * mA * mG * mC * mU * G * C * A * A * C * C * T * G * G * C * A * A * C * A * A	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS	HTT-rs362306
WV-1011	AGCUGCAACCTGG CAACAAC	mA * mG * mC * mU * mG * C * A * A * C * C * T * G * G * C * A * A * C * A * A * C	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS	HTT-rs362306
WV-1012	GCUGCAACCTGGC AACAACC	mG * mC * mU * mG * mC * A * A * C * C * T * G * G * C * A * A * C * A * A * C * C	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS	HTT-rs362306
WV-1013	GAGCAGCTGCAAC CTGGCAA	mG * mA * mG * mC * mA * G * C * T * G * C * A * A * C * C * T * mG * mG * mC *	XXXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362306

		mA * mA			
WV-1014	AGCAGCTGCAACC TGGCAAC	mA * mG * mC * mA * mG * C * T * G * C * A * A * C * C * T * G * mG * mC * mA * mA * mC	XXXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362306
WV-1015	GCAGCTGCAACCT GGCAACA	mG * mC * mA * mG * mC * T * G * C * A * A * C * C * T * G * G * mC * mA * mA * mC * mA	XXXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362306
WV-1016	CAGCUGCAACCTG GCAACAA	mC * mA * mG * mC * mU * G * C * A * A * C * C * T * G * G * C * mA * mA * mC * mA * mA	XXXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362306
WV-1017	AGCUGCAACCTGG CAACAAC	mA * mG * mC * mU * mG * C * A * A * C * C * T * G * G * C * A * mA * mC * mA * mC	XXXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362306
WV-1018	GCUGCAACCTGGC AACAACC	mG * mC * mU * mG * mC * A * A * C * C * T * G * G * C * A * A * mC * mA * mA * mC * mC	XXXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362306
WV-1019	GAGCAGCTGCAAC CUGGCAA	mG * mA * mG * mC * mA * mG * mC * T * G * C * A * A * C * C * mU * mG * mG * mC * mA * mA	XXXXXXXXXXXX XXXXXXXX	7-7-6 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362306
WV-1020	GAGCAGCTGCAAC CUGGCAA	mGmAmGmCmAmGmC * T * G * C * A * A * C * C * mUmGmGmCmAmA	OOOOOOXXXXX XXXOOOOO	7-7-6 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in wings	HTT-rs362306
WV-1021	AGCAGCTGCAACC TGGCAAC	mA * mG * mC * mA * mG * mC * T * G * C * A * A * C * C * T * G * mG * mC * mA * mA * mC	XXXXXXXXXXXX XXXXXXXX	6-7-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362306

WV-1022	AGCAGCTGCAACC TGGCAAC	mAmGmCmAmGmC * T * G * C * A * A * C * C * T * G * mGmCmAmAmC	OOOOOXXXXXX XXXXOOOO	6-7-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362306
WV-1023	GCAGCTGCAACCU GGCAACA	mG * mC * mA * mG * mC * T * G * C * A * A * C * C * mU * mG * mG * mC * mA * mA * mC * mA	XXXXXXXXXXX XXXXXXXX	5-7-8 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362306
WV-1024	GCAGCTGCAACCU GGCAACA	mGmCmAmGmC * T * G * C * A * A * C * C * mUmGmGmCmAmAmCmA	OOOOXXXXXXX XOOOOOOO	5-7-8 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362306
WV-1025	GAGCAGCTGCAAC CTGGCAA	mGmAmGmCmA * G * C * T * G * C * A * A * C * C * T * mGmGmCmAmA	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362306
WV-1026	AGCAGCTGCAACC TGGCAAC	mAmGmCmAmG * C * T * G * C * A * A * C * C * T * G * mGmCmAmAmC	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362306
WV-1027	GCAGCTGCAACCT GGCAACA	mGmCmAmGmCT * G * C * A * A * C * C * T * G * G * mCmAmAmCmA	OOOOOXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362306
WV-1028	CAGCUGCAACCTG GCAACAA	mCmAmGmCmU * G * C * A * A * C * C * T * G * G * C * mAmAmCmAmA	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362306
WV-1029	AGCUGCAACCTGG CAACAAC	mAmGmCmUmG * C * A * A * C * C * T * G * G * C * A * mAmCmAmAmC	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362306
WV-1030	GCUGCAACCTGGC	mGmCmUmGmC * A * A * C	OOOOXXXXXXX	5-10-5 (2'-OMe-	HTT-

	AACAACC	* C * T * G * G * C * A * A * mCmAmAmCmC	XXXXOOOO	DNA-2'-OMe); stereorandom PS; PO in the wings	rs362306
WV-1031	GGGCCAACAGCCA GCCTGCA	G * G * G * C * C * A * A * C * A * G * C * C * C * A * G * C * C * T * G * C * A	XXXXXXXXXXXX XXXXXXXX	All DNA; stereorandom PS	HTT-rs362268
WV-1032	GGCCAACAGCCAG CCTGCAG	G * G * C * C * A * A * C * A * G * C * C * C * A * G * C * C * T * G * C * A * G	XXXXXXXXXXXX XXXXXXXX	All DNA; stereorandom PS	HTT-rs362268
WV-1033	GCCAACAGCCAGC CTGCAGG	G * C * C * A * A * C * A * G * C * C * C * A * G * G * C * C * T * G * C * A * G * G	XXXXXXXXXXXX XXXXXXXX	All DNA; stereorandom PS	HTT-rs362268
WV-1034	CCAACAGCCAGCC TGCAGGA	C * C * A * A * C * A * G * C * C * T * G * C * A * G * G * C * C * A * G * G * A	XXXXXXXXXXXX XXXXXXXX	All DNA; stereorandom PS	HTT-rs362268
WV-1035	CAACAGCCAGCCT GCAGGAG	C * A * A * C * A * G * C * C * C * T * G * C * A * G * G * C * C * T * G * C * A * G	XXXXXXXXXXXX XXXXXXXX	All DNA; stereorandom PS	HTT-rs362268
WV-1036	AACAGCCAGCCTG CAGGAGG	A * A * C * A * G * C * C * A * G * C * C * C * T * G * C * A * G * G * A * G * G	XXXXXXXXXXXX XXXXXXXX	All DNA; stereorandom PS	HTT-rs362268
WV-1037	GGGCCAACAGCCA GCCTGCA	mG * mG * mG * mC * mC * A * A * C * A * G * C * C * C * A * G * C * C * T * G * C * A	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS	HTT-rs362268
WV-1038	GGCCAACAGCCAG CCTGCAG	mG * mG * mC * mC * mA * A * C * C * A * G * C * C * C * T * G * C * C * A * G	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS	HTT-rs362268
WV-1039	GCCAACAGCCAGC CTGCAGG	mG * mC * mC * mA * mA * C * A * G * C * C * C * A * G * G * C * C * T * G * C * A * G * G	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS	HTT-rs362268
WV-1040	CCAACAGCCAGCC TGCAGGA	mC * mC * mA * mA * mC * A * G * C * C * C * A * G * G * C * C * T * G * C * A * G * G * A	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS	HTT-rs362268

WV-1041	CAACAGCCAGCCT GCAGGAG	mC * mA * mA * mC * mA * G * C * C * A * G * C * C * C * T * G * C * A * G * G * A * G	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS	HTT-rs362268
WV-1042	AACAGCCAGCCTG CAGGAGG	mA * mA * mC * mA * mG * C * C * A * G * C * C * C * T * G * C * A * G * G * A * G * G	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS	HTT-rs362268
WV-1043	GGGCCAACAGCCA GCCUGCA	mG * mG * mG * mC * mC * A * A * C * A * G * C * C * C * A * G * C * mC * mU * mG * mC * mA	XXXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362268
WV-1044	GGCCAACAGCCAG CCUGCAG	mG * mG * mC * mC * mA * A * C * A * G * C * C * C * A * G * C * C * mU * mG * mC * mA * mG	XXXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362268
WV-1045	GCCAACAGCCAGC CTGCAGG	mG * mC * mC * mA * mA * C * A * G * C * C * C * A * G * C * * C * T * mG * mC * mA * mG * mG	XXXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362268
WV-1046	CCAACAGCCAGCC TGCAGGA	mC * mC * mA * mA * mC * A * G * C * C * C * A * G * C * C * * T * G * mC * mA * mG * mG * mA	XXXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362268
WV-1047	CAACAGCCAGCCT GCAGGAG	mC * mA * mA * mC * mA * G * C * C * A * G * C * C * C * T * G * C * mA * mG * mG * mA * mG	XXXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362268
WV-1048	AACAGCCAGCCTG CAGGAGG	mA * mA * mC * mA * mG * C * C * A * G * C * C * C * T * G * C * A * mG * mG * mA * mG * mG	XXXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362268
WV-1049	GGGCCAACAGCCA GCCUGCA	mG * mG * mG * mC * mC * mA * mA * C * A * G * C * C * * A * G * mC * mC * mU *	XXXXXXXXXXXX XXXXXXXX	7-7-6 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362268

		mG * mC * mA			
WV-1050	GGGCCAACAGCCA GCCUGCA	mGmGmGmCmCmAmA * C * A * G * C * C * A * G * mCmCmUmGmCmA	OOOOOOXXXXX XXXOOOOO	7-7-6 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in wings	HTT-rs362268
WV-1051	GGCCAACAGCCAG CCUGCAG	mG * mG * mC * mC * mA * mA * C * A * G * C * C * A * G * C * C * mU * mG * mC * mA * mG	XXXXXXXXXXX XXXXXXXXX	6-7-5 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362268
WV-1052	GGCCAACAGCCAG CCUGCAG	mGmGmCmCmAmA * C * A * G * C * C * A * G * C * C * C * mUmGmCmAmG	OOOOOXXXXXX XXXXOOOOO	6-7-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362268
WV-1053	GCCAACAGCCAGC CUGCAGG	mG * mC * mC * mA * mA * C * A * G * C * C * A * G * mC * mC * mU * mG * mC * mA * mG * mG	XXXXXXXXXXX XXXXXXXXX	5-7-8 (2'-OMe-DNA-2'-OMe); stereorandom PS	HTT-rs362268
WV-1054	GCCAACAGCCAGC CUGCAGG	mGmCmCmAmA * C * A * G * C * C * A * G * mCmCmUmGmCmAmGmG	OOOOXXXXXXX XOOOOOOO	5-7-8 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362268
WV-1055	GGGCCAACAGCCA GCCUGCA	mGmGmGmCmC * A * A * C * A * G * C * C * A * G * C * C * mCmUmGmCmA	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362268
WV-1056	GGCCAACAGCCAG CCUGCAG	mGmGmCmCmA * A * C * A * G * C * C * A * G * C * C * C * mUmGmCmAmG	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362268
WV-1057	GCCAACAGCCAGC CTGCAGG	mGmCmCmAmA * C * A * G * C * C * A * G * C * C * T * mGmCmAmGmG	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362268

WV-1058	CCAACAGCCAGCC TGCAGGA	mCmCmAmAmC * A * G * C * C * A * G * C * C * T * G * mCmAmGmGmA	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362268
WV-1059	CAACAGCCAGCCT GCAGGAG	mCmAmAmCmA * G * C * C * A * G * C * C * T * G * C * mAmGmGmAmG	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings	HTT-rs362268
WV-1060	AACAGCCAGCCTG CAGGAGG	mAmAmCmAmG * C * C * A * G * C * C * T * G * C * A * mGmGmAmGmG	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings: HTT-rs362268	HTT-rs362268
WV-1061	GUAGGAGTAGTGA AAGGCCA	mG * mU * mA * mG * mG * A * G * T * A * G * T * G * A * A * A * mG * mC * mC * mA	XXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS: +ve Luciferase control for psiCHECK2; WV-975 analogue	HTT-rs362268
WV-1062	GUAGGAGTAGTGA AAGGCCA	mGmUmAmGmG * A * G * T * A * G * A * A * A * mGmGmCmCmA	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings: +ve Luciferase control for psiCHECK2; WV-975 analogue	HTT-control
WV-1063	GUAGGAGTAGTGA AAGGCCA	mG * mU * mA * mG * mG * A * G * T * A * G * T * G * A * A * A * G * C * C * A	XXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS: +ve Luciferase control for	HTT-control

				psiCHECK2; WV-975 analogue	
WV-1064	CUCUUACTGTGCT GTGGACA	mC * mU * mC * mU * mU * A * C * T * G * T * G * C * T * G * T * mG * mG * mA * mC * mA	XXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS: Negative Luciferase control for psiCHECK2; ONT-67 analogue	HTT-control
WV-1065	CUCUUACTGTGCT GTGGACA	mCmUmCmUmU * A * C * T * G * T * G * C * T * G * T * mGmGmAmCmA	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); stereorandom PS; PO in the wings: Negative Luciferase control for psiCHECK2; ONT-67 analogue	HTT-control
WV-1066	CUCUUACTGTGCT GTGGACA	mC * mU * mC * mU * mU * A * C * T * G * T * G * C * T * G * T * G * A * C * A	XXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA); stereorandom PS: Negative Luciferase control for psiCHECK2; ONT-67 analogue	HTT-control
WV-1067	GGGCACAAGGGCA CAGACTT	G * G * G * C * A * C * A * A * G * G * G * C * d2AP * C * A * G * A * C * T * T	XXXXXXXXXXX XXXXXXXX	All DNA stereorandom; P13 (2-aminopurine): rs362307; WV-904 analogue	HTT-control
WV-1068	GGCACAAGGGCAC AGACTTC	G * G * C * A * C * A * A * G * G * G * C * d2AP * C * A * G * A * C * T * T * C	XXXXXXXXXXX XXXXXXXX	All DNA stereorandom; P12 (2-aminopurine): rs362307; WV-905	rs362307

WV-1069	GCACAAGGGCACA GACTTCC	G * C * A * C * A * A * G * G * G * C * d2AP * C * A * G * A * C * T * T * C * C	XXXXXXXXXXX XXXXXXXX	All DNA stereorandom; P11 (2-aminopurine): rs362307; WV-906 analogue	rs362307
WV-1070	GGGCACAAGGGCA CAGACTT	G * G * G * C * A * C * A * A * G * G * G * C * dDAP * C * A * G * A * C * T * T	XXXXXXXXXXX XXXXXXXX	All DNA stereorandom; P13 (2;6- diaminopurine): rs362307; WV-904 analogue	rs362307
WV-1071	GGCACAAGGGCAC AGACTTC	G * G * C * A * C * A * A * G * G * G * C * dDAP * C * A * G * A * C * T * T * C	XXXXXXXXXXX XXXXXXXX	All DNA stereorandom; P12 (2;6- diaminopurine): rs362307; WV-905 analogue	rs362307
WV-1072	GCACAAGGGCACA GACTTCC	G * C * A * C * A * A * G * G * G * C * dDAP * C * A * G * A * C * T * T * C * C	XXXXXXXXXXX XXXXXXXX	All DNA stereorandom; P12 (2;6- diaminopurine): rs362307; WV-906 analogue	rs362307
WV-1073	GAGCCUUUGGAAG UCUGCGCCCUUGU GCCCUGCCU	rGrArGrCrCrUrUrUrGrGrArArAr GrUrCrUrGrCrGrCrCrUrUr GrUrGrCrCrCrUrGrCrCrU	OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO O	wtRNA	rs362307
WV-1074	GAGCCUUUGGAAG UCUGUGCCCUUGU GCCCUGCCU	rGrArGrCrCrUrUrUrGrGrArArAr GrUrCrUrGrUrGrCrCrCrUrUr GrUrGrCrCrCrUrGrCrCrU	OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO O	muRNA	rs362307
WV-1075	CACACGGGCACAG	rCrArCrArCrGrGrGrCrArCrAr	OOOOOOOOOO	Antisense strand:	rs362307

	ACUUCCAA	GrArCrUrUrCrCrArA	OOOOOOOOO	Positive control; Curr. Bio. Vol 19 No 9; 776	
WV-1076	GGAAGUCUGUGCC CGUGUGCC	rGrGrArArGrUrCrUrGrUrGrCr CrCrGrUrGrUrGrGrCrC	OOOOOOOOOOO OOOOOOOOO	Sense strand: Positive control; Curr. Bio. Vol 19 No 9; 777: Note: incorrectly added as rGrGrArArGrUrCr UrGrUrGrGrCrCrCrG rUrGrUrUrUrCrC in earlier versions of databse	rs362307
WV-1077	AUUAAUAAATTGT CATCACC	mA * SmU * SmU * SmA * SmA * SmU * SA * SA * SA * ST * ST * SG * ST * SC * RA * ST * SmC * SmA * SmC * SmC	SSSSSSSSSSSSR SSSSS	6-10-4 (2'-OMe-DNA-2'-OMe) Gapmer: Analogue of WV-451	HTT rs7685686
WV-1078	AUUAAUAAATTGT CATCACC	mA * RmU * RmU * RmA * RmA * RmU * SA * SA * SA * ST * ST * SG * ST * SC * RA * ST * SmC * RmA * RmC * RmC	RRRRRSSSSSSSS RSSRRR	6-10-4 (2'-OMe-DNA-2'-OMe) Gapmer: Analogue of WV-451	HTT rs7685686
WV-1079	AUUAAUAAATTGT CATCACC	mA * SmU * SmU * SmA * SmA * SmU * SmA * SA * ST * ST * SG * ST * SC * RA * ST * SC * SA * SC * SC	SSSSSSSSSSSSR SSSSS	8-12 (2'-OMe-DNA) hemimer: Analogue of WV-451	HTT rs7685686
WV-1080	AUUAAUAAATTGT CATCACC	mA * RmU * RmU * RmA * RmA * RmU * RmA * RmA * SA * ST * ST * SG * ST * SC * RA * ST * SC * SA * SC * SC	RRRRRRSSSSSS RSSSSS	8-12 (2'-OMe-DNA) hemimer: Analogue of WV-451	HTT rs7685686

WV-1081	AUUAAUAAATTGT CATCACC	mAmUmUmAmAmUmAmA * SA * ST * ST * SG * ST * SC * RA * ST * SC * SA * SC * SC	OOOOOOOSSSSS SRSSSSS	8-12 (2'-OMe-DNA) hemimer; PO wing: Analogue of WV-451	HTT rs7685686
WV-1082	AUUAAUAAATTGT CATCACC	mAmUmUmAmAmAmU * SA * SA * SA * ST * ST * SG * ST * SC * RA * ST * SmCmAmCmC	OOOOOSSSSSSSS RSSOOO	6-10-4 (2'-OMe-DNA-2'-OMe); PO wings: Analogue of WV-451	HTT rs7685686
WV-1083	AUUAAUAAATTGT CATCACC	mA * SmUmUmAmAmAmU * SA * SA * SA * ST * ST * SG * ST * SC * RA * ST * SmCmAmAmC * SmC	SOOOOSSSSSSSS RSSOOS	6-10-4 (2'-OMe-DNA-2'-OMe) Gapmer: Analogue of WV-451	HTT rs7685686
WV-1084	AUUAAUAAATTGT CATCACC	mA * RmUmUmAmAmAmU * SA * SA * SA * ST * ST * SG * ST * SC * RA * ST * SmCmAmAmC * RmC	ROOOOSSSSSSSS RSSOOR	6-10-4 (2'-OMe-DNA-2'-OMe) Gapmer: Analogue of WV-451	HTT rs7685686
WV-1085	GGCACAAGGGCAC AGACUUC	mG * SmG * SmC * SmA * SmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmA * SmC * SmU * SmU * SmC	SSSSSSSSSSSSRS SSSSS	5-10-5 (2'-OMe-DNA-2'-OMe) Gapmer: Analogue of WV-905 and WV-937	HTT rs362307
WV-1086	GGCACAAGGGCAC AGACUUC	mG * RmG * RmC * RmA * RmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmA * RmC * RmU * RmU * RmC	RRRRSSSSSSSSR SSRRRR	5-10-5 (2'-OMe-DNA-2'-OMe) Gapmer: Analogue of WV-905 and WV-937	HTT rs362307
WV-1087	GGCACAAGGGCAC AGACUUC	mGmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmUmUmC	OOOOSSSSSSSSR SSOOOO	5-10-5 (2'-OMe-DNA-2'-OMe); PO wings: Analogue of WV-905 and WV-937	HTT rs362307
WV-1088	GGCACAAGGGCAC AGACTTC	mG * SmG * SmC * SmA * SmC * SmA * SmA * SmG *	SSSSSSSSSSSRS SSSSS	8-12 (2'-OMe-DNA) hemimer;	HTT rs362307

		SG * SG * SC * SA * SC * RA * SG * SA * SC * ST * ST * SC		Analogue of WV-905 and WV-937	
WV-1089	GGCACAAGGGCAC AGACTTC	mG * RmG * RmC * RmA * RmC * RmA * RmA * RmG * SG * SG * SC * SA * SC * RA * SG * SA * SC * ST * ST * SC	RRRRRRRSSSSS RSSSSSS	8-12 (2'-OMe-DNA) hemimer: Analogue of WV-905 and WV-937	HTT rs362307
WV-1090	GGCACAAGGGCAC AGACTTC	mGmGmCmAmCmAmAmG * SG * SG * SC * SA * SC * RA * SG * SA * SC * ST * ST * SC	OOOOOOOSSSSS RSSSSSS	8-12 (2'-OMe-DNA) hemimer; PO wing: Analogue of WV-905 and WV-937	HTT rs362307
WV-1091	GGCACAAGGGCAC AGACUUC	mG * RmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmUmU * RmC	ROOOSSSSSSSSR SSOOOR	8-12 (2'-OMe-DNA) gapmer PO wing: Analogue of WV-905 and WV-937: incorrectly added as gsSgcacsSdAsSdAs SdGsSdGsSdGsSd CsSdAsSdCsRdAs SdGsSacuusSc in earlier version of database	HTT rs362307
WV-1092	GGCACAAGGGCAC AGACUUC	mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmUmU * SmC	SOOOSSSSSSSSR SSOOOS	8-12 (2'-OMe-DNA) gapmer PO wing: Analogue of WV-905 and WV-937	HTT rs362307
WV-1183	GCAGGGCACAAGG GCACAGA	G * C * A * G * G * G * C * A * C * A * A * G * G * C * A * C * A * G * A	XXXXXXXXXXXX XXXXXXXX	Phosphorothioate DNA; Stereorandom	Huntington rs362307

WV-1184	GCAGGGCACAAGG GCACAGA	mG * mC * mA * mG * mG * G * C * A * C * A * A * G * G * G * C * A * C * A * G * A	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA) Hemimer	Huntington rs362307
WV-1185	GCAGGGCACAAGG GCACAGA	mGmCmAmGmG * G * C * A * C * A * A * G * G * G * C * A * C * A * G * A	OOOOXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA) Hemimer; PO wing	Huntington rs362307
WV-1186	GCAGGGCACAAGG GCACAGA	mG * mC * mA * mG * mG * mG * mC * A * C * A * A * G * G * G * C * A * C * A * G * A	XXXXXXXXXXXX XXXXXXXX	7-13 (2'-OMe-DNA) Hemimer	Huntington rs362307
WV-1187	GCAGGGCACAAGG GCACAGA	mGmCmAmGmGmGmC * A * C * A * A * G * G * G * C * A * C * A * G * A	OOOOOOXXXXXX XXXXXXXX	7-13 (2'-OMe-DNA) Hemimer; PO wing	Huntington rs362307
WV-1188	CAGGGCACAAGGG CACAGAC	C * A * G * G * G * C * A * C * A * A * G * G * G * C * A * C * A * G * A * C	XXXXXXXXXXXX XXXXXXXX	Phosphorothioate DNA; Stereorandom	Huntington rs362307
WV-1189	CAGGGCACAAGGG CACAGAC	mC * mA * mG * mG * mG * C * A * C * A * A * G * G * G * C * A * C * A * G * A * C	XXXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA) Hemimer	Huntington rs362307
WV-1190	CAGGGCACAAGGG CACAGAC	mCmAmGmGmG * C * A * C * A * A * G * G * G * C * A * C * A * G * A * C	OOOOXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA) Hemimer; PO wing	Huntington rs362307
WV-1191	CAGGGCACAAGGG CACAGAC	mC * mA * mG * mG * mG * mC * mA * C * A * A * G * G * G * C * A * mC * mA * mG * mA * mC	XXXXXXXXXXXX XXXXXXXX	7-13 (2'-OMe-DNA) Hemimer	Huntington rs362307
WV-1192	CAGGGCACAAGGG CACAGAC	mCmAmGmGmGmCmA * C * A * A * G * G * G * C * A * mCmAmGmAmC	OOOOOOXXXXXX XXXXOOOO	7-13 (2'-OMe-DNA) Hemimer; PO wing	Huntington rs362307
WV-1193	AGGGCACAAGGGC ACAGACT	A * G * G * G * C * A * C * A * * A * G * G * G * C * A * C * A * G * A * C * T	XXXXXXXXXXXX XXXXXXXX	Phosphorothioate DNA; Stereorandom	Huntington rs362307
WV-1194	AGGGCACAAGGGC	mA * mG * mG * mG * mC *	XXXXXXXXXXXX	5-15 (2'-OMe-	Huntington

	ACAGACT	A * C * A * A * G * G * G * C * A * C * A * G * A * C * T	XXXXXXXX	DNA) Hemimer	rs362307
WV-1195	AGGGCACAAGGGC ACAGACT	mAmGmGmGmC * A * C * A * A * G * G * G * C * A * C * A * G * A * C * T	OOOOXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA) Hemimer; PO wing	Huntington rs362307
WV-1196	AGGGCACAAGGGC ACAGACU	mA * mG * mG * mG * mC * mA * mC * A * A * G * G * G * C * A * C * A * G * A * C * mU	XXXXXXXXXXX XXXXXXXX	7-12-1 (2'-OMe-DNA-2'-DNA) Gapmer	Huntington rs362307
WV-1197	AGGGCACAAGGGC ACAGACU	mAmGmGmGmCmAmC * A * A * G * G * G * C * A * C * A * G * A * C * mU	OOOOOOXXXXX XXXXXXXX	7-12-1 (2'-OMe-DNA-2'-DNA) Gapmer; PO wings	Huntington rs362307
WV-1198	AAGGGCACAGACT TCCAAAG	A * A * G * G * G * C * A * C * A * A * G * G * A * C * C * T * T * C * C * A * A * A * G	XXXXXXXXXXX XXXXXXXX	Phosphorothioate DNA; Stereorandom	Huntington rs362307
WV-1199	AAGGGCACAGACT TCCAAAG	mA * mA * mG * mG * mG * C * A * C * A * G * A * C * T * T * C * C * A * A * A * G	XXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA) Hemimer	Huntington rs362307
WV-1200	AAGGGCACAGACT TCCAAAG	mAmAmGmGmG * C * A * C * A * G * A * C * C * T * T * C * C * A * A * A * G	OOOOXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA) Hemimer; PO wing	Huntington rs362307
WV-1201	AAGGGCACAGACT TCCAAAG	mA * mA * mG * mG * mG * C * A * C * A * G * A * C * T * T * C * mC * mA * mA * mA * mG	XXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-DNA) Gapmer	Huntington rs362307
WV-1202	AAGGGCACAGACT TCCAAAG	mAmAmGmGmG * C * A * C * A * G * A * C * C * T * T * C * mCmAmAmAmG	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-DNA) Gapmer; PO wings	Huntington rs362307
WV-1203	AAGGGCACAGACT TCCAAAG	mA * mA * mG * mG * G * C * A * C * A * G * A * C * T * T * mC * mC * mA * mA * mA * mG	XXXXXXXXXXX XXXXXXXX	4-10-6 (2'-OMe-DNA-2'-DNA) Gapmer	Huntington rs362307
WV-1204	AAGGGCACAGACT	mAmAmGmGG * C * A * C *	OOOOXXXXXXX	4-10-6 (2'-OMe-	Huntington

	TCCAAAG	A * G * A * C * T * T * mCmCmAmAmAmG	XXXOOOOO	DNA-2'-DNA) Gapmer; PO wings	rs362307
WV-1205	AGGGCACAGACTT CCAAAGG	A * G * G * G * C * A * C * A * G * A * C * T * T * C * C * A * A * A * G * G	XXXXXXXXXXX XXXXXXXX	Phosphorothioate DNA; Stereorandom	Huntington rs362307
WV-1206	AGGGCACAGACTT CCAAAGG	mA * mG * mG * mG * mC * A * C * A * G * A * C * T * T * C * C * A * A * A * G * G	XXXXXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA) Hemimer	Huntington rs362307
WV-1207	AGGGCACAGACTT CCAAAGG	mAmGmGmGmC * A * C * A * G * A * C * T * T * C * C * A * A * A * G * G	OOOOXXXXXXX XXXXXXXX	5-15 (2'-OMe-DNA) Hemimer; PO wing	Huntington rs362307
WV-1208	AGGGCACAGACTT CCAAAGG	mA * mG * mG * mG * mC * A * C * A * G * A * C * T * T * C * C * mA * mA * mA * mG * mG	XXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-DNA) Gapmer	Huntington rs362307
WV-1209	AGGGCACAGACTT CCAAAGG	mAmGmGmGmC * A * C * A * G * A * C * T * T * C * C * mAmAmAmGmG	OOOOXXXXXXX XXXXOOOO	5-10-5 (2'-OMe-DNA-2'-DNA) Gapmer; PO wings	Huntington rs362307
WV-1210	AGGGCACAGACTT CCAAAGG	mA * mG * mG * mG * C * A * C * A * G * A * C * T * T * C * mC * mA * mA * mA * mG * mG	XXXXXXXXXXX XXXXXXXX	4-10-6 (2'-OMe-DNA-2'-DNA) Gapmer	Huntington rs362307
WV-1211	AGGGCACAGACTT CCAAAGG	mAmGmGmG * C * A * C * A * G * A * C * T * T * C * mCmAmAmAmGmG	OOOXXXXXXXX XXXOOOOO	4-10-6 (2'-OMe-DNA-2'-DNA) Gapmer; PO wings	Huntington rs362307
WV-1212	GGGCACAGACTTC CAAAGGC	G * G * G * C * A * C * A * G * A * C * T * T * C * C * A * A * A * G * G * C	XXXXXXXXXXX XXXXXXXX	Phosphorothioate DNA; Stereorandom	Huntington rs362307
WV-1213	GGGCACAGACTTC CAAAGGC	mG * mG * mG * mC * mA * C * A * G * A * C * T * T * C * C * A * A * A * G * G * C	XXXXXXXXXXX XXXXXXXX	4-16 (2'-OMe-DNA) Hemimer	Huntington rs362307
WV-1214	GGGCACAGACTTC CAAAGGC	mGmGmGmCmA * C * A * G * A * C * T * T * C * C * A *	OOOOXXXXXXX XXXXXXXX	4-16 (2'-OMe-DNA) Hemimer;	Huntington rs362307

		A * A * G * G * C		PO wing	
WV-1215	GGGCACAGACTTC CAAAGGC	mG * mG * mG * mC * mA * C * A * G * A * C * T * T * C * C * A * mA * mA * mG * mG * mC	XXXXXXXXXXX XXXXXXXX	4-10-6 (2'-OMe-DNA-2'-DNA) Gapmer	Huntington rs362307
WV-1216	GGGCACAGACTTC CAAAGGC	mGmGmGmCmA * C * A * G * A * C * T * T * C * C * A * mAmAmGmGmC	OOOOXXXXXXX XXXXOOOO	4-10-6 (2'-OMe-DNA-2'-DNA) Gapmer; PO wings	Huntington rs362307
WV-1234	GGCACAAGGGCAC AGACUTC	mG * mG * mC * mA * mC * A * A * G * G * G * C * A * C * A * G * mA * mC * mU * BrdU * mC	XXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe) Gapmer; One Br-dU	HTT-rs362307
WV-1235	GGCACAAGGGCAC AGACTTC	mG * mG * mC * mA * mC * A * A * G * G * G * C * A * C * A * G * mA * mC * BrdU * BrdU * mC	XXXXXXXXXXX XXXXXXXX	5-10-5 (2'-OMe-DNA-2'-OMe) Gapmer; two Br-dU	HTT-rs362307
WV-1497	GGCACAAGGGCAC AGACUUC	mG * mGmCmAmC * A * A * G * G * G * C * A * C * A * G * mAmCmUmU * mC	XOOOXXXXXXX XXXXOOOX	stereo random version of WV-1092	HTT rs362307
WV-1508	AUUAAUAAATTGT CATCACC	A * SmUmUmAmAmU * SA * SA * SA * ST * ST * SG * ST * SC * RA * ST * SmCmAmC * SC	SOOOOSSSSSSSS RSSOOS	1-5-10-3-1 (DNA/2'-OMe) Gapmer: : Analogue of WV-1083	HTT rs7685686
WV-1509	AUUAAUAAATTGT CATCACC	A * mUmUmAmAmU * A * A * A * T * T * G * T * C * A * T * mCmAmC * C	XOOOOXXXXXX XXXXXOOX	1-5-10-3-1 (DNA/2'-OMe) Gapmer; 1st and last PS: : Analogue of WV-1083	HTT rs7685686
WV-1510	GGCACAAGGGCAC AGACUUC	G * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmUmU * SC	SOOOSSSSSSSSR SSOOOS	1-4-10-4-1 (DNA/2'-OMe) gapmer: : Analogue of WV-1092	HTT rs362307

WV-1511	GGCACAAGGGCAC AGACUUC	G * mGmCmAmC * A * A * G * G * G * C * A * C * A * G * mAmCmUmU * C	XOOOXXXXXXX XXXXOOOX	1-4-10-4-1 (DNA/2'-OMe) gapmer; 1st and last PS: : Analogue of WV-1092	HTT rs362307
WV-1654	GGCACAAGGGCAC AGACTTC	Geo * Geo * m5Ceo * Aeo * m5Ceo * A * A * G * G * G * C * A * C * A * G * Aeo * m5Ceo * Teo * Teo * m5Ceo	XXXXXXXXXXX XXXXXXXX	5-10-5; 2'-OMOE gapmer; All PS	HTT rs362307
WV-1655	GGCACAAGGGCAC AGACTTC	Geo * Geom5CeoAeom5Ceo * A * A * G * G * G * C * A * C * A * G * Aeom5CeoTeoTeo * m5Ceo	XOOOXXXXXXX XXXXOOOX	5-10-5; 2'-OMOE gapmer; 1st and last PS in the wing; rest of the wing is PO	HTT rs362307
WV-1656	CTCAGTAACATTG ACACCAC	m5Ceo * Teo * m5Ceo * Aeo * Geo * T * A * A * C * A * T * T * G * A * C * Aeo * m5Ceo * m5Ceo * Aeo * m5Ceo	XXXXXXXXXXX XXXXXXXX	5-10-5; 2'-OMOE gapmer; All PS	Huntington
WV-1657	CUCAGTAACATTG ACACCAC	mC * mU * mC * mA * mG * T * A * A * C * A * T * T * G * A * C * mA * mC * mC * mA * mC	XXXXXXXXXXX XXXXXXXX	5-10-5; 2'-OMe gapmer; All PS	Huntington
WV-1788	GGCACAAGGGCAC AGACUTC	mG * mGmCmAmC * A * A * G * G * G * C * A * C * A * G * mAmCmU * BrdU * mC	XOOOXXXXXXX XXXXOOXX	5/10/5 2'Ome Gapmer BrdU PO wings	HTT
WV-1789	CTCAGTAACATTG ACACCAC	mC * BrdU * mC * mA * mG * T * A * A * C * A * T * T * G * A * C * mA * mC * mC * mA * mC	XXXXXXXXXXX XXXXXXXX	5/10/5 2'Ome Gapmer BrdU	HTT
WV-1790	CTCAGTAACATTG ACACCAC	mC * BrdU * mCmAmG * T * A * A * C * A * T * T * G * A * C * mAmCmCmA * mC	XXOOXXXXXXX XXXXOOOX	5/10/5 2'Ome Gapmer BrdU PO wings	HTT
WV-1799	GAAGUCUGUGCCC UUGUGCC	rGrArArGrUrCrUrGrUrGrCrCr CrUrUrGrUrGrCrC	OOOOOOOOOOO OOOOOOO	RNA complementary to	HTT

				WV1092	
WV-2022	GGCACAAGGGCAC AGACUTC	mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SmAmCmU * SBrdU * SmC	SOOOSSSSSSSSR SSOOSS	BrdU version of WV-1092	HTT rs362307
WV-2023	TGTCATCACCAGA AAAAGUC	T * G * T * C * A * T * C * A * C * C * A * G * A * A * A * A * mA * mA * mG * mU * mC	XXXXXXXXXXXX XXXXXXXX	15-5 hemimer full PS	rs7685686 (A/G)
WV-2024	UTGTCATCACCAG AAAAAGU	mU * T * G * T * C * A * T * C * A * C * C * A * G * A * A * mA * mA * mG * mU	XXXXXXXXXXXX XXXXXXXX	1-14-5 gapmer full PS	rs7685686 (A/G)
WV-2025	TTGTCATCACCAG AAAAAGU	T * T * G * T * C * A * T * C * A * C * C * A * G * A * A * mA * mA * mG * mU	XXXXXXXXXXXX XXXXXXXX	15-5 hemimer full PS	rs7685686 (A/G)
WV-2026	AUTGTCATCACCA GAAAAAG	mA * mU * T * G * T * C * A * T * C * A * C * C * A * G * A * mA * mA * mA * mG	XXXXXXXXXXXX XXXXXXXX	2-13-5 gapmer full PS	rs7685686 (A/G)
WV-2027	ATTGTCATCACCA GAAAAAG	mA * T * T * G * T * C * A * T * C * A * C * C * A * G * A * mA * mA * mA * mG	XXXXXXXXXXXX XXXXXXXX	1-14-5 gapmer full PS	rs7685686 (A/G)
WV-2028	AAUTGTCATCACC AGAAAAA	mA * mA * mU * T * G * T * C * A * T * C * A * C * C * A * G * mA * mA * mA * mA	XXXXXXXXXXXX XXXXXXXX	3-12-5 gapmer full PS	rs7685686 (A/G)
WV-2029	AATTGTCATCACC AGAAAAA	mA * mA * T * T * G * T * C * A * T * C * A * C * C * A * G * mA * mA * mA * mA	XXXXXXXXXXXX XXXXXXXX	2-13-5 gapmer full PS	rs7685686 (A/G)
WV-2030	AAATTGTCATCAC CAGAAAA	mA * mA * mA * T * T * G * T * C * A * T * C * A * C * C * A * mG * mA * mA * mA	XXXXXXXXXXXX XXXXXXXX	3-12-5 gapmer full PS	rs7685686 (A/G)

WV-2031	AAAUTGTCATCAC CAGAAAA	mA * mA * mA * mU * T * G * T * C * A * T * C * A * C * C * A * mG * mA * mA * mA * mA	XXXXXXXXXXXX XXXXXXXX	4-11-5 gapmer full PS	rs7685686 (A/G)
WV-2032	UAAAUTGTCATCA CCAGAAA	mU * mA * mA * mA * mU * T * G * T * C * A * T * C * A * C * C * A * mG * mA * mA * mA	XXXXXXXXXXXX XXXXXXXX	5-11-4 gapmer full PS	rs7685686 (A/G)
WV-2033	UAAAUTGTCATCA CCAGAAA	mU * mA * mA * mA * mU * T * G * T * C * A * T * C * A * C * C * C * mA * mG * mA * mA * mA	XXXXXXXXXXXX XXXXXXXX	5-10-5 gapmer full PS	rs7685686 (A/G)
WV-2034	AUAAATTGTCATC ACCAGAA	mA * mU * mA * mA * mA * T * T * G * T * C * A * T * C * A * C * C * mA * mG * mA * mA	XXXXXXXXXXXX XXXXXXXX	5-11-4 gapmer full PS	rs7685686 (A/G)
WV-2035	AUAAATTGTCATC ACCAGAA	mA * mU * mA * mA * mA * T * T * G * T * C * A * T * C * A * C * mC * mA * mG * mA * mA	XXXXXXXXXXXX XXXXXXXX	5-10-5 gapmer full PS	rs7685686 (A/G)
WV-2036	AAUAAATTGTCAT CACCAGA	mA * mA * mU * mA * mA * A * T * T * G * T * C * A * T * C * A * C * C * mA * mG * mA	XXXXXXXXXXXX XXXXXXXX	5-12-3 gapmer full PS	rs7685686 (A/G)
WV-2037	AAUAAATTGTCAT CACCAGA	mA * mA * mU * mA * mA * A * T * T * G * T * C * A * T * C * A * C * mC * mA * mG * mA	XXXXXXXXXXXX XXXXXXXX	5-11-4 gapmer full PS	rs7685686 (A/G)
WV-2038	AAUAAATTGTCAT CACCAGA	mA * mA * mU * mA * mA * A * T * T * G * T * C * A * T * C * A * mC * mC * mA * mG * mA	XXXXXXXXXXXX XXXXXXXX	5-10-5 gapmer full PS	rs7685686 (A/G)
WV-2039	UAAUAAATTGTCA	mU * mA * mA * mU * mA *	XXXXXXXXXXXX	5-13-2 gapmer full	rs7685686

	TCACCAG	A * A * T * T * G * T * C * A * T * C * A * C * C * mA * mG	XXXXXXXX	PS	(A/G)
WV-2040	UAAUAAATTGTCA TCACCAG	mU * mA * mA * mU * mA * A * A * T * T * G * T * C * A * T * C * A * C * mC * mA * mG	XXXXXXXXXXX XXXXXXXX	5-12-3 gapmer full PS	rs7685686 (A/G)
WV-2041	UAAUAAATTGTCA TCACCAG	mU * mA * mA * mU * mA * A * A * T * T * G * T * C * A * T * C * A * mC * mC * mA * mG	XXXXXXXXXXX XXXXXXXX	5-11-4 gapmer full PS	rs7685686 (A/G)
WV-2042	UAAUAAATTGTCA TCACCAG	mU * mA * mA * mU * mA * A * A * T * T * G * T * C * A * T * C * mA * mC * mC * mA * mG	XXXXXXXXXXX XXXXXXXX	5-10-5 gapmer full PS	rs7685686 (A/G)
WV-2043	UUAAUAAATTGTC ATCACCA	mU * mU * mA * mA * mU * A * A * A * T * T * G * T * C * A * T * C * A * C * C * mA	XXXXXXXXXXX XXXXXXX	5-14-1 gapmer full PS	rs7685686 (A/G)
WV-2044	UUAAUAAATTGTC ATCACCA	mU * mU * mA * mA * mU * A * A * A * T * T * G * T * C * A * T * C * A * C * mC * mA	XXXXXXXXXXX XXXXXXX	5-13-2 gapmer full PS	rs7685686 (A/G)
WV-2045	UUAAUAAATTGTC ATCACCA	mU * mU * mA * mA * mU * A * A * A * T * T * G * T * C * A * T * C * A * mC * mC * mA	XXXXXXXXXXX XXXXXXX	5-12-3 gapmer full PS	rs7685686 (A/G)
WV-2046	UUAAUAAATTGTC ATCACCA	mU * mU * mA * mA * mU * A * A * A * T * T * G * T * C * A * T * C * mA * mC * mC * mA	XXXXXXXXXXX XXXXXXX	5-11-4 gapmer full PS	rs7685686 (A/G)
WV-2047	AUUAATAAATTGT CATCACC	mA * mU * mU * mA * mA * T * A * A * A * T * T * G * T * C * A * T * C * A * C * C	XXXXXXXXXXX XXXXXXX	5-15 hemimer full PS	rs7685686 (A/G)

WV-2048	AUUAATAAATTGT CATCACC	mA * mU * mU * mA * mA * T * A * A * A * T * T * G * T * C * A * T * C * A * C * mC	XXXXXXXXXXXX XXXXXXXX	5-14-1 gapmer full PS	rs7685686 (A/G)
WV-2049	AUUAATAAATTGT CATCACC	mA * mU * mU * mA * mA * T * A * A * A * T * T * G * T * C * A * T * C * A * mC * mC	XXXXXXXXXXXX XXXXXXXX	5-13-2 gapmer full PS	rs7685686 (A/G)
WV-2050	AUUAATAAATTGT CATCACC	mA * mU * mU * mA * mA * T * A * A * A * T * T * G * T * C * A * T * C * mA * mC * mC	XXXXXXXXXXXX XXXXXXXX	5-12-3 gapmer full PS	rs7685686 (A/G)
WV-2051	UAUUAATAAATTG TCATCAC	mU * mA * mU * mU * mA * A * T * A * A * A * T * T * G * T * C * A * T * C * A * C	XXXXXXXXXXXX XXXXXXXX	5-15 hemimer full PS	rs7685686 (A/G)
WV-2052	UAUUAATAAATTG TCATCAC	mU * mA * mU * mU * mA * A * T * A * A * A * T * T * G * T * C * A * T * C * A * mC	XXXXXXXXXXXX XXXXXXXX	5-14-1 gapmer full PS	rs7685686 (A/G)
WV-2053	UAUUAATAAATTG TCATCAC	mU * mA * mU * mU * mA * A * T * A * A * A * T * T * G * T * C * A * T * C * mA * mC	XXXXXXXXXXXX XXXXXXXX	5-13-2 gapmer full PS	rs7685686 (A/G)
WV-2054	CUAUUAATAAATT GTCATCA	mC * mU * mA * mU * mU * A * A * T * A * A * A * T * T * G * T * C * A * T * C * A	XXXXXXXXXXXX XXXXXXXX	5-15 hemimer full PS	rs7685686 (A/G)
WV-2055	CUAUUAATAAATT GTCATCA	mC * mU * mA * mU * mU * A * A * T * A * A * A * T * T * G * T * C * A * T * C * mA	XXXXXXXXXXXX XXXXXXXX	5-14-1 gapmer full PS	rs7685686 (A/G)
WV-2056	ACUAUTAATAAAT TGTCATC	mA * mC * mU * mA * mU * T * A * A * T * A * A * A * T * T * G * T * C * A * T * C	XXXXXXXXXXXX XXXXXXXX	5-15 hemimer full PS	rs7685686 (A/G)
WV-2057	TGTCATCACCAGA AAAAGUC	T * G * T * C * A * T * C * A * C * C * A * G * A * A * A * mAmAmGmU * mC	XXXXXXXXXXXX XXXXOOOX	15-5 hemimer 1 PS on each end and between dN-mN	rs7685686 (A/G)

WV-2058	UTGTCATCACCAG AAAAAGU	mU * T * G * T * C * A * T * C * A * C * C * A * G * A * A * mAmAmAmG * mU	XXXXXXXXXXX XXXXOOOX	1-14-5 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2059	TTGTCATCACCAG AAAAAGU	T * T * G * T * C * A * T * C * A * C * C * A * G * A * A * mAmAmAmG * mU	XXXXXXXXXXX XXXXOOOX	15-5 hemimer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2060	AUTGTCATCACCA GAAAAAG	mA * mU * T * G * T * C * A * T * C * A * C * C * A * G * A * mAmAmAmA * mG	XXXXXXXXXXX XXXXOOOX	2-13-5 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2061	ATTGTCATCACCA GAAAAAG	mA * T * T * G * T * C * A * T * C * A * C * C * A * G * A * mAmAmAmA * mG	XXXXXXXXXXX XXXXOOOX	1-14-5 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2062	AAUTGTCATCACC AGAAAAA	mA * mAmU * T * G * T * C * A * T * C * A * C * C * A * G * mAmAmAmA * mA	XOXXXXXXXXX XXXXOOOX	3-12-5 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2063	AATTGTCATCACC AGAAAAA	mA * mA * T * T * G * T * C * A * T * C * A * C * C * A * G * mAmAmAmA * mA	XXXXXXXXXXX XXXXOOOX	2-13-5 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2064	AAATTGTCATCAC CAGAAAA	mA * mAmA * T * T * G * T * C * A * T * C * A * C * C * A * mGmAmAmA * mA	XOXXXXXXXXX XXXXOOOX	3-12-5 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2065	AAAUTGTCATCAC CAGAAAA	mA * mAmAmU * T * G * T * C * A * T * C * A * C * C * A * mGmAmAmA * mA	XOOXXXXXXXX XXXXOOOX	4-11-5 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)

WV-2066	UAAAUTGTCATCA CCAGAAA	mU * mAmAmAmU * T * G * T * C * A * T * C * A * C * C * A * mGmAmA * mA	XOOOXXXXXXX XXXXXOOX	5-11-4 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2067	UAAAUTGTCATCA CCAGAAA	mU * mAmAmAmU * T * G * T * C * A * T * C * A * C * C * mAmGmAmA * mA	XOOOXXXXXXX XXXXOOOX	5-10-5 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2068	AUAAATTGTCATC ACCAGAA	mA * mUmAmAmA * T * T * G * T * C * A * T * C * A * C * C * mAmGmA * mA	XOOOXXXXXXX XXXXXOOX	5-11-4 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2069	AUAAATTGTCATC ACCAGAA	mA * mUmAmAmA * T * T * G * T * C * A * T * C * A * C * C * mCmAmGmA * mA	XOOOXXXXXXX XXXXOOOX	5-10-5 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2070	AAUAAATTGTCAT CACCAGA	mA * mAmUmAmA * A * T * T * G * T * C * A * T * C * A * C * C * mAmG * mA	XOOOXXXXXXX XXXXXXOX	5-12-3 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2071	AAUAAATTGTCAT CACCAGA	mA * mAmUmAmA * A * T * T * G * T * C * A * T * C * A * C * C * mCmAmG * mA	XOOOXXXXXXX XXXXXOOX	5-11-4 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2072	AAUAAATTGTCAT CACCAGA	mA * mAmUmAmA * A * T * T * G * T * C * A * T * C * A * C * C * mCmCmAmG * mA	XOOOXXXXXXX XXXXOOOX	5-10-5 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2073	UAAUAAATTGTCA TCACCAG	mU * mAmAmUmA * A * A * T * T * G * T * C * A * T * C * A * C * C * mA * mG	XOOOXXXXXXX XXXXXXXX	5-13-2 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2074	UAAUAAATTGTCA	mU * mAmAmUmA * A * A *	XOOOXXXXXXX	5-12-3 gapmer 1	rs7685686

	TCACCAG	T * T * G * T * C * A * T * C * A * C * mCmA * mG	XXXXXXOX	PS on each end and between dN-mN and dN-dN	(A/G)
WV-2075	UAAUAAATTGTCA TCACCAG	mU * mAmAmUmA * A * A * T * T * G * T * C * A * T * C * A * mCmCmA * mG	XOOOXXXXXXX XXXXXOOX	5-11-4 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2076	UAAUAAATTGTCA TCACCAG	mU * mAmAmUmA * A * A * T * T * G * T * C * A * T * C * mAmCmCmA * mG	XOOOXXXXXXX XXXXOOOX	5-10-5 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2077	UUAAUAAATTGTC ATCACCA	mU * mUmAmAmU * A * A * A * T * T * G * T * C * A * T * C * A * C * C * mA	XOOOXXXXXXX XXXXXXXX	5-14-1 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2078	UUAAUAAATTGTC ATCACCA	mU * mUmAmAmU * A * A * A * T * T * G * T * C * A * T * C * A * C * mC * mA	XOOOXXXXXXX XXXXXXX	5-13-2 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2079	UUAAUAAATTGTC ATCACCA	mU * mUmAmAmU * A * A * A * T * T * G * T * C * A * T * C * A * mCmC * mA	XOOOXXXXXXX XXXXXXOX	5-12-3 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2080	UUAAUAAATTGTC ATCACCA	mU * mUmAmAmU * A * A * A * T * T * G * T * C * A * T * C * mAmCmC * mA	XOOOXXXXXXX XXXXXOOX	5-11-4 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2081	AUUAATAAATTGT CATCACC	mA * mUmUmAmA * T * A * A * A * T * T * G * T * C * A * T * C * A * C * C	XOOOXXXXXXX XXXXXXXX	5-15 hemimer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2082	AUUAATAAATTGT CATCACC	mA * mUmUmAmA * T * A * A * A * T * T * G * T * C * A	XOOOXXXXXXX XXXXXXXX	5-14-1 gapmer 1 PS on each end and	rs7685686 (A/G)

		* T * C * A * C * mC	XOOOXXXXXXX XXXXXXXXX	between dN-mN and dN-dN	rs7685686 (A/G)
WV-2083	AUUAATAAATTGT CATCACC	mA * mUmUmAmA * T * A * A * A * T * T * G * T * C * A * T * C * A * mC * mC		5-13-2 gapmer 1 PS on each end and between dN-mN and dN-dN	
WV-2084	AUUAATAAATTGT CATCACC	mA * mUmUmAmA * T * A * A * A * T * T * G * T * C * A * T * C * mAmC * mC	XOOOXXXXXXX XXXXXXOX	5-12-3 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2085	UAUUAATAAATTG TCATCAC	mU * mAmUmUmA * A * T * A * A * A * T * T * G * T * C * A * T * C * A * C	XOOOXXXXXXX XXXXXXXXX	5-15 hemimer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2086	UAUUAATAAATTG TCATCAC	mU * mAmUmUmA * A * T * A * A * A * T * T * G * T * C * A * T * C * A * mC	XOOOXXXXXXX XXXXXXXXX	5-14-1 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2087	UAUUAATAAATTG TCATCAC	mU * mAmUmUmA * A * T * A * A * A * T * T * G * T * C * A * T * C * mA * mC	XOOOXXXXXXX XXXXXXXXX	5-13-2 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2088	CUAUUAATAAATT GTCATCA	mC * mUmAmUmU * A * A * T * A * A * A * T * T * T * G * T * C * A * T * C * A	XOOOXXXXXXX XXXXXXXXX	5-15 hemimer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2089	CUAUUAATAAATT GTCATCA	mC * mUmAmUmU * A * A * T * A * A * A * T * T * T * G * T * C * A * T * C * mA	XOOOXXXXXXX XXXXXXXXX	5-14-1 gapmer 1 PS on each end and between dN-mN and dN-dN	rs7685686 (A/G)
WV-2090	ACUAUTAATAAAT TGTCATC	mA * mCmUmAmU * T * A * A * T * A * A * A * T * T * T * G * T * C * A * T * C	XOOOXXXXXXX XXXXXXXXX	5-15 hemimer 1 PS on each end and between dN-mN	rs7685686 (A/G)

				and dN-dN	
WV-2163	GACUUUUUCUGGU GAUGGCAAUUUA UUAAUAG	rGrArCrUrUrUrUrUrCrUrGrGr UrGrArUrGrGrCrArArUrUrUrUr ArUrUrArArUrArG	OOOOOOOOOOO OOOOOOOOOOO OOOOOOOOO	HTT rs7685686	HTT rs7685686
WV-2164	GACUUUUUCUGGU GAUGACAAUUUA UUAAUAG	rGrArCrUrUrUrUrUrCrUrGrGr UrGrArUrGrArCrArArUrUrUrUr ArUrUrArArUrArG	OOOOOOOOOOO OOOOOOOOOOO OOOOOOOOO	HTT rs7685686	HTT rs7685686
WV-2269	UAAAUTGTCATCA CCAGAAA	mU * SmAmAmAmU * ST * SG * ST * SC * SA * RT * SC * SA * SC * SC * SmAmGmAmA * SmA	SOOOSSSSSRSSS SSOOOS	5-10-5 2' OMe-DNA-2'-OMe Gapmer 1-3-11-3-1 (PS/PO)	HTT rs7685686
WV-2270	AUAAATTGTCATC ACCAGAA	mA * SmUmAmAmA * ST * ST * SG * ST * SC * SA * RT * SC * SA * SC * SmCmAmGmA * SmA	SOOOSSSSSSRSS SSOOOS	5-10-5 2' OMe-DNA-2'-OMe Gapmer 1-3-11-3-1 (PS/PO)	HTT rs7685686
WV-2271	AAUAAATTGTCAT CACCAGA	mA * SmAmUmAmA * SA * ST * ST * SG * ST * SC * SA * RT * SC * SA * SmCmCmAmG * SmA	SOOOSSSSSSSRS SSOOOS	5-10-5 2' OMe-DNA-2'-OMe Gapmer 1-3-11-3-1 (PS/PO)	HTT rs7685686
WV-2272	UAAUAAATTGTCA TCACCAG	mU * SmAmAmUmA * SA * SA * ST * ST * SG * ST * SC * SA * RT * SC * SmAmCmCmA * SmG	SOOOSSSSSSSSR SSOOOS	5-10-5 2' OMe-DNA-2'-OMe Gapmer 1-3-11-3-1 (PS/PO)	HTT rs7685686
WV-2374	AAUAAATTGTCAT CACCAGA	mA * SmAmUmAmA * SA * ST * ST * SG * ST * SC * SA * RT * SC * SA * SC * SmCmAmG * SmA	SOOOSSSSSSSRS SSSOOS	P10 stereopure analogue of WV-2071 5-11-4 2'-OMe-DNA-2'-OMe Gapmer 1-3-12-2-1 (PS/PO)	HTT rs7685686
WV-2375	UAAUAAATTGTCA TCACCAG	mU * SmAmAmUmA * SA * SA * ST * ST * SG * ST * SC * SA * RT * SC * SA * SmCmCmA * SmG	SOOOSSSSSSSSR SSSOOS	P11 stereopure analogue of WV-20755-11-4 2'-OMe-DNA-2'-OMe	HTT rs7685686

				Gapmer 1-3-12-2-1 (PS/PO)	
WV-2377	GCACAAGGGCACA GACUUCC	mG * mCmAmCmA * A * G * G * G * C * A * C * A * G * A * mCmUmUmC * mC	XOOOXXXXXXX XXXXOOOX	P11 stereorandom analogue of WV-932 5-10-5 2'-OMe-DNA-2'-OMe Gapmer and 1-3-11-3-1 (PS/PO)	HTT rs362307
WV-2378	GCACAAGGGCACA GACUUCC	mG * SmCmAmCmA * SA * SG * SG * SG * SC * SA * SC * RA * SG * SA * SmCmUmUmC * SmC	SOOOSSSSSSSRS SSOOOS	P11 stereorandom analogue of WV-932 5-10-5 2'-OMe-DNA-2'-OMe Gapmer and 1-3-11-3-1 (PS/PO)	HTT rs362307
WV-2379	CACAAGGGCACAG ACUUCCA	mC * mAmCmAmA * G * G * G * C * A * C * A * G * A * C * mUmUmCmC * mA	XOOOXXXXXXX XXXXOOOX	P10 sereorandom analogue of WV-933 5-10-5 2'-OMe-DNA-2'-OMe Gapmer and 1-3-11-3-1 (PS/PO)	HTT rs362307
WV-2380	CACAAGGGCACAG ACUUCCA	mC * SmAmCmAmA * SG * SG * SG * SC * SA * SC * RA * SG * SA * SC * SmUmUmCmC * SmA	SOOOSSSSSSRSS SSOOOS	P10 stereopure analogue of WV-933 5-10-5 2'-OMe-DNA-2'-OMe Gapmer and 1-3-11-3-1 (PS/PO)	HTT rs362307
WV-2416	UAAAUTGTCATCA CCAGAAA	mU * SmAmAmAmU * ST * SG * ST * SC * RA * ST * SC * SA * SC * SC * SmAmGmAmA * SmA	SOOOSSSSRSSSS SSOOOS	P8 5-10-5 2' OMe-DNA-2'-OMe Gapmer 1-3-11-3-1 (PS/PO)	HTT rs7685686
WV-2417	AUAAATTGTCATC ACCAGAA	mA * SmUmAmAmA * ST * ST * SG * ST * SC * RA * ST * SC * SA * SC *	SOOOSSSSSRSSS SSOOOS	P9 5-10-5 2' OMe-DNA-2'-OMe Gapmer 1-3-11-3-1	HTT rs7685686

		SmCmAmGmA * SmA		(PS/PO)	
WV-2418	AAUAAATTGTCAT CACCAGA	mA * SmAmUmAmA * SA * ST * ST * SG * ST * SC * RA * ST * SC * SA * SmCmCmAmG * SmA	SOOOSSSSSSRSS SSOOOS	P10 5-10-5 2' OMe-DNA-2'-OMe Gapmer 1-3-11-3-1 (PS/PO)	HTT rs7685686
WV-2419	UAAUAAATTGTCA TCACCAG	mU * SmAmAmUmA * SA * SA * ST * ST * SG * ST * SC * RA * ST * SC * SmAmCmCmA * SmG	SOOOSSSSSSSRS SSOOOS	P11 5-10-5 2' OMe-DNA-2'-OMe Gapmer 1-3-11-3-1 (PS/PO)	HTT rs7685686
WV-2589	UCCCCACAGAGGG AGGAAGC	mU * SmCmCmCmC * SA * SC * RA * SG * SA * SG * SG * SG * SA * SG * SmGmAmAmG * SmC	SOOOSSRSSSSSS SSOOOS	P6 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT rs2530595 (C/T)
WV-2590	CUCCCCACAGAGG GAGGAAG	mC * SmUmCmCmC * SC * SA * SC * RA * SG * SA * SG * SG * SG * SA * SmGmGmAmA * SmG	SOOOSSSRSSSSS SSOOOS	P7 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT rs2530595 (C/T)
WV-2591	CCUCCCCACAGAG GGAGGAA	mC * SmCmUmCmC * SC * SC * SA * SC * RA * SG * SA * SG * SG * SG * SmAmGmGmA * SmA	SOOOSSSSRSSSS SSOOOS	P8 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT rs2530595 (C/T)
WV-2592	UCCUCCCCACAGA GGGAGGA	mU * SmCmCmUmC * SC * SC * SA * SC * RA * SG * SA * SG * SG * SmGmAmGmG * SmA	SOOOSSSSSRSSS SSOOOS	P9 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT rs2530595 (C/T)
WV-2593	GUCCUCCCCACAG AGGGAGG	mG * SmUmCmCmU * SC * SC * SC * SA * SC * RA * SG * SA * SG * SmGmGmAmG * SmG	SOOOSSSSSSRSS SSOOOS	P10 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT rs2530595 (C/T)
WV-2594	GGUCCTCCCCACA GAGGGAG	mG * SmGmUmCmC * ST * SC * SC * SC * SC * SA * SC * RA * SG * SA * SmGmGmGmA * SmG	SOOOSSSSSSSRS SSOOOS	P11 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT rs2530595 (C/T)

WV-2595	GGGUCCTCCCCAC AGAGGGA	mG * SmGmGmUmC * SC * ST * SC * SC * SC * SC * SA * SC * RA * SG * SmAmGmGmG * SmA	SOOOSSSSSSSSR SSOOOS	P12 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT rs2530595 (C/T)
WV-2596	CGGGUCCTCCCCA CAGAGGG	mC * SmGmGmGmU * SC * SC * ST * SC * SC * SC * SC * SA * SC * RA * SmGmAmGmG * SmG	SOOOSSSSSSSSS RSOOOS	P13 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT rs2530595 (C/T)
WV-2597	ACAGUAGATGAGG GAGCAGG	mA * SmCmAmGmU * SA * SG * RA * ST * SG * SA * SG * SG * SG * SA * SmGmCmAmG * SmG	SOOOSSRSSSSSS SSOOOS	P6 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT (rs362331) (C/T)
WV-2598	CACAGTAGATGAG GGAGCAG	mC * SmAmCmAmG * ST * SA * SG * RA * ST * SG * SA * SG * SG * SG * SmAmGmCmA * SmG	SOOOSSSRSSSSS SSOOOS	P7 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT (rs362331) (C/T)
WV-2599	ACACAGTAGATGA GGGAGCA	mA * SmCmAmCmA * SG * ST * SA * SG * RA * ST * SG * SA * SG * SG * SmGmAmGmC * SmA	SOOOSSSSRSSSS SSOOOS	P8 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT (rs362331) (C/T)
WV-2600	CACACAGTAGATG AGGGAGC	mC * SmAmCmAmC * SA * SG * ST * SA * SG * RA * ST * SG * SA * SG * SmGmGmAmG * SmC	SOOOSSSSSRSSS SSOOOS	P9 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT (rs362331) (C/T)
WV-2601	GCACACAGTAGAT GAGGGAG	mG * SmCmAmCmA * SC * SA * SG * ST * SA * SG * RA * ST * SG * SA * SmGmGmGmA * SmG	SOOOSSSSSSRSS SSOOOS	P10 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT (rs362331) (C/T)
WV-2602	UGCACACAGTAGA TGAGGGA	mU * SmGmCmAmC * SA * SC * SA * SG * ST * SA * SG * RA * ST * SG * SmAmGmGmG * SmA	SOOOSSSSSSSRS SSOOOS	P11 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT (rs362331) (C/T)
WV-2603	GUGCACACAGTAG	mG * SmUmGmCmA * SC *	SOOOSSSSSSSSR	P12 5-10-5 (2'-	HTT

	ATGAGGG	SA * SC * SA * SG * ST * SA * SG * RA * ST * SmGmAmGmG * SmG	SSOOOS	OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	(rs362331) (C/T)
WV-2604	AGUGCACACAGTA GAUGAGG	mA * SmGmUmGmC * SA * SC * SA * SC * SA * SG * ST * SA * SG * RA * SmUmGmAmG * SmG	SOOOSSSSSSSSS RSOOOS	P13 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (PS/PO) Gapmer	HTT (rs362331) (C/T)
WV-2605	UCCCCACAGAGGG AGGAAGC	mU * mCmCmCmC * A * C * A * G * A * G * G * G * A * G * mGmAmAmG * mC	XOOOXXXXXXX XXXXOOOX	P6 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT r2530595 (C/T)
WV-2606	CUCCCCACAGAGG GAGGAAG	mC * mUmCmCmC * C * A * C * A * G * A * G * G * G * A * mGmGmAmA * mG	XOOOXXXXXXX XXXXOOOX	P7 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT r2530595 (C/T)
WV-2607	CCUCCCCACAGAG GGAGGAA	mC * mCmUmCmC * C * C * A * C * A * G * A * G * G * G * mAmGmGmA * mA	XOOOXXXXXXX XXXXOOOX	P8 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT r2530595 (C/T)
WV-2608	UCCUCCCCACAGA GGAGGA	mU * mCmCmUmC * C * C * C * A * C * A * G * A * G * G * mGmAmGmG * mA	XOOOXXXXXXX XXXXOOOX	P9 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT r2530595 (C/T)
WV-2609	GUCCUCCCCACAG AGGGAGG	mG * mUmCmCmU * C * C * C * C * A * C * A * G * A * G * mGmGmAmG * mG	XOOOXXXXXXX XXXXOOOX	P10 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT r2530595 (C/T)
WV-2610	GGUCCTCCCCACA GAGGGAG	mG * mGmUmCmC * T * C * C * C * C * A * C * A * G * A * mGmGmGmA * mG	XOOOXXXXXXX XXXXOOOX	P11 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT r2530595 (C/T)
WV-2611	GGGUCCTCCCCAC AGAGGGA	mG * mGmGmUmC * C * T * C * C * C * C * A * C * A * G	XOOOXXXXXXX XXXXOOOX	P12 5-10-5 (2'-OMe-DNA-2'-	HTT r2530595

		* mAmGmGmG * mA		OMe) 1-3-11-3-1 (P/PO) Gapmer	(C/T)
WV-2612	CGGGUCCTCCCCA CAGAGGG	mC * mGmGmGmU * C * C * T * C * C * C * C * A * C * A * mGmAmGmG * mG	XOOOXXXXXXX XXXXOOOX	P13 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT r2530595 (C/T)
WV-2613	ACAGUAGATGAGG GAGCAGG	mA * mCmAmGmU * A * G * A * T * G * A * G * G * G * A * mGmCmAmG * mG	XOOOXXXXXXX XXXXOOOX	P6 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT (r362331) (C/T)
WV-2614	CACAGTAGATGAG GGAGCAG	mC * mAmCmAmG * T * A * G * A * T * G * A * G * G * G * mAmGmCmA * mG	XOOOXXXXXXX XXXXOOOX	P7 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT (r362331) (C/T)
WV-2615	ACACAGTAGATGA GGGAGCA	mA * mCmAmCmA * G * T * A * G * A * T * G * A * G * G * mGmAmGmC * mA	XOOOXXXXXXX XXXXOOOX	P8 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT (r362331) (C/T)
WV-2616	CACACAGTAGATG AGGGAGC	mC * mAmCmAmC * A * G * T * A * G * A * T * G * A * G * mGmGmAmG * mC	XOOOXXXXXXX XXXXOOOX	P9 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT (r362331) (C/T)
WV-2617	GCACACAGTAGAT GAGGGAG	mG * mCmAmCmA * C * A * G * T * A * G * A * T * G * A * mGmGmGmA * mG	XOOOXXXXXXX XXXXOOOX	P10 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT (r362331) (C/T)
WV-2618	UGCACACAGTAGA TGAGGGA	mU * mGmCmAmC * A * C * A * G * T * A * G * A * T * G * mAmGmGmG * mA	XOOOXXXXXXX XXXXOOOX	P11 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT (r362331) (C/T)
WV-2619	GUGCACACAGTAG ATGAGGG	mG * mUmGmCmA * C * A * C * A * G * T * A * G * A * T * mGmAmGmG * mG	XOOOXXXXXXX XXXXOOOX	P12 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT (r362331) (C/T)

				(P/PO) Gapmer	
WV-2620	AGUGCACACAGTA GAUGAGG	mA * mGmUmGmC * A * C * A * C * A * G * T * A * G * A * mUmGmAmG * mG	XOOOXXXXXXX XXXXOOOX	P13 5-10-5 (2'-OMe-DNA-2'-OMe) 1-3-11-3-1 (P/PO) Gapmer	HTT (r362331) (C/T)
WV-2623	GGCACAAGGGCAC AGACTTC	GGCACAAGGGCACAGACT TC	OOOOOOOOOOO OOOOOOO	DNA version of WV-1092	HTT rs362307 (C/T)
WV-2659	GGCACAAGGGCAC AGACUUC	mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * SA * SG * SmAmCmUmU * SmC	SOOOSSSSSSSSS SSOOOS	WV-1092 analogue with All Sp stereochemistry	rs362307 Human HTT
WV-2671	GGGUCCTCCCCAC AGAGGGA	mG * SmG * SmGmUmC * SC * ST * SC * SC * SC * SC * SA * SC * RA * SG * SmAmGmG * SmG * SmA	SSOOSSSSSSSSR SSOOSS	P12 5-10-5 (2'-OMe-DNA-2'-OMe) 2-2-11-2-2 (PS/PO) Gapmer with Sp wings	HTT rs2530595 (C/T)
WV-2672	GGGUCCTCCCCAC AGAGGGA	mG * RmG * RmGmUmC * SC * ST * SC * SC * SC * SC * SA * SC * RA * SG * SmAmGmG * RmG * RmA	RROOSSSSSSSSR SSOORR	P12 5-10-5 (2'-OMe-DNA-2'-OMe) 4-11-4 (PS/PO) Gapmer with Rp wings	HTT rs2530595 (C/T)
WV-2673	GGGUCCTCCCCAC AGAGGGA	mG * SmG * SmG * SmU * SmC * SC * ST * SC * SC * SC * SC * SA * SC * RA * SG * SmA * SmG * SmG * SmG * SmA	SSSSSSSSSSSRS SSSSS	P12 5-10-5 (2'-OMe-DNA-2'-OMe) 2-2-11-2-2 (PS/PO) Gapmer with Sp wings	HTT rs2530595 (C/T)
WV-2674	GGGUCCTCCCCAC AGAGGGA	mG * RmG * RmG * RmU * RmC * SC * ST * SC * SC * SC * SC * SA * SC * RA * SG * SmA * RmG * RmG * RmG * RmA	RRRRSSSSSSSSR SSRRRR	P12 5-10-5 (2'-OMe-DNA-2'-OMe) 2-2-11-2-2 (PS/PO) Gapmer with Rp wings	HTT rs2530595 (C/T)
WV-2675	GGGUUCTCCCCAC	mG * SmGmGmUmU * SC *	SOOOSSSSSSSSR	P12 analogue of	HTT

	AGAGGGA	ST * SC * SC * SC * SC * SA * SC * RA * SG * SmAmGmGmG * SmA	SSOOOS	WV-2595 with G:U mismatch at position 5	rs2530595 (C/T)
WV-2676	GGCACAAGGGCAC AGACUUC	mG * RmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * SA * SG * SmAmCmUmU * SmC	ROOOSSSSSSSS SSOOOS	WV-1092 analogue for CMC	rs362307 Human HTT
WV-2682	GGCACAAGGGCAC AGACUUC	mG * SmGmCmAmC * RA * SA * SG * SG * SG * SC * SA * SC * SA * SG * SmAmCmUmU * SmC	SOOORSSSSSSSS SSOOOS	WV-1092 analogue for CMC	rs362307 Human HTT
WV-2683	GGCACAAGGGCAC AGACUUC	mG * SmGmCmAmC * SA * RA * SG * SG * SG * SC * SA * SC * SA * SG * SmAmCmUmU * SmC	SOOOSRSSSSSSS SSOOOS	WV-1092 analogue for CMC	rs362307 Human HTT
WV-2684	GGCACAAGGGCAC AGACUUC	mG * SmGmCmAmC * SA * SA * RG * SG * SG * SC * SA * SC * SA * SG * SmAmCmUmU * SmC	SOOOSSRSSSSSS SSOOOS	WV-1092 analogue for CMC	rs362307 Human HTT
WV-2685	GGCACAAGGGCAC AGACUUC	mG * SmGmCmAmC * SA * SA * SG * RG * SG * SC * SA * SC * SA * SG * SmAmCmUmU * SmC	SOOOSSSRSSSSS SSOOOS	WV-1092 analogue for CMC	rs362307 Human HTT
WV-2686	GGCACAAGGGCAC AGACUUC	mG * SmGmCmAmC * SA * SA * SG * SG * RG * SC * SA * SC * SA * SG * SmAmCmUmU * SmC	SOOOSSSSRSSSS SSOOOS	WV-1092 analogue for CMC	rs362307 Human HTT
WV-2687	GGCACAAGGGCAC AGACUUC	mG * SmGmCmAmC * SA * SA * SG * SG * RC * SA * SC * SA * SG * SmAmCmUmU * SmC	SOOOSSSSSRSSS SSOOOS	WV-1092 analogue for CMC	rs362307 Human HTT
WV-2688	GGCACAAGGGCAC AGACUUC	mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * RA	SOOOSSSSSSRSS SSOOOS	WV-1092 analogue for CMC	rs362307 Human HTT

		* SC * SA * SG * SmAmCmUmU * SmC			
WV-2689	GGCACAAGGGCACAGACUUC	mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * RC * SA * SG * SmAmCmUmU * SmC	SOOOSSSSSSSRSSSOOOS	WV-1092 analogue for CMC	rs362307 Human HTT
WV-2690	GGCACAAGGGCACAGACUUC	mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * SA * RG * SmAmCmUmU * SmC	SOOOSSSSSSSSSRSOOOS	WV-1092 analogue for CMC	rs362307 Human HTT
WV-2691	GGCACAAGGGCACAGACUUC	mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * SA * SG * RmAmCmUmU * SmC	SOOOSSSSSSSSSSROOOS	WV-1092 analogue for CMC	rs362307 Human HTT
WV-2692	GGCACAAGGGCACAGACUUC	mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * SA * SC * SA * SG * SmAmCmUmU * RmC	SOOOSSSSSSSSSSSOOOR	WV-1092 analogue for CMC	rs362307 Human HTT
WV-2728	GGCAC	mG * SmGmCmAmC	SOOO	WV-1092 fragment for CMC	rs362307 Human HTT
WV-2729	GGCAC	mG * RmGmCmAmC	ROOO	WV-1092 fragment for CMC	rs362307 Human HTT
WV-2730	ACUUC	mAmCmUmU * SmC	OOOS	WV-1092 fragment for CMC	rs362307 Human HTT
WV-2731	ACUUC	mAmCmUmU * RmC	OOOR	WV-1092 fragment for CMC	rs362307 Human HTT
WV-2732	GGCACAAGGGCACAGACUUC	mG * SmGmCmAmC * SA * SA * SG * SG * SG * SC * RA * SC * RA * SG * SmAmCmUmU * SmC	SOOOSSSSSSRSRSSOOOS	WV-1092 for CM	rs362307 Human HTT

Abbreviations:

2\': 2'

3\': 3'

5\': 5'

307: SNP rs362307

C6: C6 amino linker

F, f: 2'-F

Htt, HTT: Huntingtin gene or Huntington's Disease

Lauric, Myristic, Palmitic, Stearic, Oleic, Linoleic, alpha-Linoleic, gamma-Linoleic, DHA, Turbinaric, Dilinoleic: Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Oleic acid, Linoleic acid, alpha-Linoleic acid, gamma-Linoleic acid, docosahexaenoic acid, Turbinaric acid, Dilinoreic acid, respectively.

muHtt or muHTT: mutant Huntingtin gene or gene product

OMe: 2'-OMe

O, PO: phoshodiester (phosphate)

*, PS: Phosphorothioate

R, Rp: Phosphorothioate in Rp conformation

S, Sp: Phosphorothioate in Sp conformation

WV: WV-

WV-: WV

X: Phosphorothioate, stereorandom

Equivalents

[001311] Having described some illustrative embodiments of the disclosure, it should be apparent to those skilled in the art that the foregoing is merely illustrative and not limiting, having been presented by way of example only. Numerous modifications and other illustrative embodiments are within the scope of one of ordinary skill in the art and are contemplated as falling within the scope of the disclosure. In particular, although many of the examples presented herein involve specific combinations of method acts or system elements, it should be understood that those acts and those elements may be combined in other ways to accomplish the same objectives. Acts, elements, and features discussed only in connection with one embodiment are not intended to be excluded from a similar role in other embodiments. Further, for the one or more means-plus-function limitations recited in the following claims, the means are not intended to be limited to the means disclosed herein for performing the recited function, but are intended to cover in scope any means, known now or later developed, for performing the recited function.

[001312] Use of ordinal terms such as "first", "second", "third", *etc.*, in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements. Similarly, use of a), b), *etc.*, or i), ii), *etc.* does not by itself connote any priority, precedence, or order of steps in the claims. Similarly, the use of these terms in the specification does not by itself connote any required priority, precedence, or order.

[001313] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the disclosure. The present disclosure is not to be limited in scope by examples provided, since the examples are intended as a single illustration of one aspect of the disclosure and other functionally equivalent embodiments are within the scope of the disclosure. Various modifications of the disclosure in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The advantages and objects of the disclosure are not necessarily encompassed by each embodiment of the disclosure.

Claims

1. A chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type, wherein the oligonucleotides target a mutant Huntingtin gene, and the length is from about 10 to about 50 nucleotides, wherein the backbone linkages comprise at least one phosphorothioate, and wherein the pattern of backbone chiral centers comprises at least one *R*p chiral center and at least one *S*p chiral center.

2. A chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which composition is a substantially pure preparation of a single oligonucleotide in that a predetermined level of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers; or

a chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:

1) a common base sequence and length;

2) a common pattern of backbone linkages; and

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

3. The composition of claim 1, wherein the oligonucleotides comprise one or more wing regions and a common core region, wherein:

each wing region independently has a length of two or more bases, and independently and optionally comprises one or more chiral internucleotidic linkages; and

the core region independently has a length of two or more bases and independently comprises one or more chiral internucleotidic linkages.

4. The composition of claim 1, wherein oligonucleotides of the oligonucleotide type comprises at least one wing region and a core region, wherein:

each wing region independently has a length of two or more bases, and independently and optionally comprises one or more chiral internucleotidic linkages;

the core region independently has a length of two or more bases, and independently comprises one or more chiral internucleotidic linkages; and

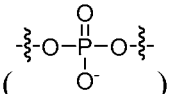
wherein at least one nucleotide in a wing region differs from at least one nucleotide of the core region, wherein the difference is in one or more of:

1) backbone linkage;

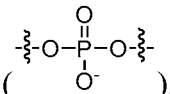
2) pattern of backbone chiral centers;

3) sugar modification.

5. The composition of claim 1, wherein oligonucleotides of the same oligonucleotide type have identical structure.

6. The composition of claim 1, wherein the oligonucleotides comprise one or more natural phosphate linkages () and one or more phosphorothioate linkages.

7. The composition of claim 1, wherein the oligonucleotides comprise a structure of wing-core-wing.

8. The composition claim 7, wherein a wing comprises a chiral internucleotidic linkage and a natural phosphate linkage ().

9. The composition of claim 8, wherein the core comprises one or more phosphorothioate linkages.

10. The composition of claim 6, wherein each of the oligonucleotides comprises a modified sugar moiety.

11. The composition of claim 10, wherein the modified sugar moiety comprises a high-affinity sugar modification.

12. The composition of claim 10, wherein the modified sugar moiety has a 2'-modification.

13. The composition of claim 10, wherein the modified sugar moiety comprises a bicyclic sugar modification.

14. The composition of claim 10, wherein the modified sugar moiety comprises a 2'-modification, wherein a 2'-modification is 2'-OR^1, wherein R^1 is optionally substituted C_{1-6} alkyl.

15. The composition of claim 10, wherein the modified sugar moiety comprises a 2'-modification, wherein a 2'-modification is 2'-MOE.

16. The composition of claim 10, wherein the modified sugar moiety comprises a 2'-modification, wherein a 2'-modification is 2'-OMe.

17. The composition of claim 10, wherein the modified sugar moiety comprises a 2'-modification, wherein the 2'-modification is *S*-cEt.

18. The composition of claim 10, wherein the modified sugar moiety comprises a 2'-modification, wherein the 2'-modification is FANA.

19. The composition of claim 10, wherein the modified sugar moiety comprises a 2'-modification, wherein the 2'-modification is FRNA.

20. The composition of claim 10, wherein the modified sugar moiety has a 5'-modification.

21. The composition of claim 1 or 2, wherein the oligonucleotides comprise one or more natural phosphate linkages, and a pattern of backbone chiral centers comprising $(Sp)_t(Rp)_n(Sp)_m$, wherein t is 2-10, n is 1, and m is 2-10, and at least one of t and m is greater than 5.

22. The composition of claim 6, wherein the oligonucleotides comprise a pattern of backbone chiral centers comprising SSR, RSS, SSRSS, SSRSSR, RSSSRSRRRS, RSSSSSSSSS, SRRSRSSSSR, SRSRSSRSSR, RRRSSSRSSS, RRRSRSSRSR, RRSSSRSRSR, SRSSSRSSSS, SSRRSSRSRS, SSSSSSRRSS, RRRSSRRRSR, RRRRSSSSRS, SRRSRRRRRR, RSSRSSRRRR, RSRRSRRSRR, RRSRSSRSRS, SSRRRRRSRR, RSRRSRSSSR, RRSSRSRRRR, RRSRSRRSSS, RRSRSSSRRR, RSRRRRSRSR, SSRSSSRRRS, RSSRSRSRSR, RSRSRSSRSS, RRRSSRRSRS, SRRSSRRSRS, RRRRSRSRRR, or SSSSRRRRSR.

23. The composition of claim 22, wherein the oligonucleotides target a mutant Huntingtin gene comprising a single nucleotide polymorphism (SNP).

24. The composition of claim 23, wherein the single nucleotide polymorphism is selected from rs362307, rs7685686, rs362268, rs2530595, rs362331, and rs362306.

25. The composition of claim 1, wherein the oligonucleotides have a structure selected from Tables N1A, N2A, N3A, N4A and 8; and WV-1092, WV-2595 and WV-2603.

26. The composition of claim 1, wherein the oligonucleotides are WV-1092.

27. The composition of claim 1, wherein the oligonucleotides are WV-2595.

28. The composition of claim 1, wherein the oligonucleotides are WV-2603.

29. A method for controlled cleavage of a nucleic acid polymer, the method comprising:

contacting a nucleic acid polymer whose nucleotide sequence comprises a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to a target sequence found in the nucleic acid polymer;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

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

30. A method for cleavage of a nucleic acid having a base sequence comprising a target sequence, the method comprising steps of:

(a) contacting a nucleic acid having a base sequence comprising a target sequence with a chirally controlled oligonucleotide composition comprising oligonucleotides of a particular oligonucleotide type characterized by:

1) a common base sequence and length, wherein the common base sequence is or comprises a sequence that is complementary to the target sequence in the nucleic acid;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the particular base sequence and length, for oligonucleotides of the particular oligonucleotide type, wherein the oligonucleotide targets a mutant Huntingtin gene, and the length is from about 10 to about 50 nucleotides, wherein the backbone linkages comprise at least one phosphorothioate, and wherein the pattern of backbone

chiral centers comprises at least one chiral center in a Rp conformation and at least one chiral center in a Sp conformation; and

(b) cleavage of the nucleic acid mediated by a RNAseH or RNA interference mechanism.

31. The method of claim 30, wherein the method is performed *in vitro* or *in vivo*.

32. The composition of claim 1, or the method of claim 30, wherein the composition further comprises one or more additional components selected from: a polynucleotide, carbonic anhydrase inhibitor, a dye, an intercalating agent, an acridine, a cross-linker, psoralene, mitomycin C, a porphyrin, TPPC4, texaphyrin, Sapphyrin, a polycyclic aromatic hydrocarbon phenazine, dihydrophenazine, an artificial endonuclease, a chelating agent, EDTA, an alkylating agent, a phosphate, an amino, a mercapto, a PEG, PEG-40K, MPEG, [MPEG]2, a polyamino, an alkyl, a substituted alkyl, a radiolabeled marker, an enzyme, a hapten biotin, a transport/absorption facilitator, aspirin, vitamin E, folic acid, a synthetic ribonuclease, a protein, a glycoprotein, a peptide, a molecule having a specific affinity for a co-ligand, an antibody, a hormone, a hormone receptor, a non-peptidic species, a lipid, a lectin, a carbohydrate, a vitamin, a cofactor, or a drug.

33. The composition of claim 1, or the method of claim 20, wherein the oligonucleotides are capable of participating in RNaseH-mediated cleavage of a mutant Huntingtin gene mRNA.

34. The composition of claim 1, or the method of claim 20, wherein the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of the oligonucleotides comprises or consists of the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of any of any oligonucleotide selected from Tables N1A, N2A, N3A, N4A and 8; and WV-1092, WV-2595, and WV-2603.

35. The composition of claim 1, or the method of claim 20, wherein the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of the oligonucleotides comprises or consists of the base sequence, and pattern of backbone linkages, and/or pattern of backbone chiral centers of any of any oligonucleotide selected from Tables N1A, N2A, N3A, N4A and 8; and WV-1092, WV-2595, and WV-2603.

36. The composition of claim 1, or the method of claim 20, wherein the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of the oligonucleotides comprises or consists of the base sequence, and pattern of backbone linkages, and pattern of

backbone chiral centers of any of any oligonucleotide selected from Tables N1A, N2A, N3A, N4A and 8; and WV-1092, WV-2595, and WV-2603.

37. The composition of claim 1, or the method of claim 20, wherein the base sequence, pattern of backbone linkages and pattern of backbone chiral centers of the oligonucleotides comprises or consists of the base sequence, pattern of backbone linkages and/or pattern of backbone chiral centers of any of WV-1092, WV-2595, and WV-2603.

38. A composition comprising the composition of claim 1 and a selectivity agent selected from: the group of compounds which binds specifically to one or more neurotransmitter transporters selected from the group consisting of a dopamine transporter (DAT), a serotonin transporter (SERT), and a norepinephrine transporter (NET); the group consisting of a dopamine reuptake inhibitor (DRI), a selective serotonin reuptake inhibitor (SSRI), a noradrenaline reuptake inhibitor (NRI), a norepinephrine-dopamine reuptake inhibitor (NDRI), and a serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI); the group consisting of a triple reuptake inhibitor, a noradrenaline dopamine double reuptake inhibitor, a serotonin single reuptake inhibitor, a noradrenaline single reuptake inhibitor, and a dopamine single reuptake inhibitor; and the group consisting of a dopamine reuptake inhibitor (DRI), a Norepinephrine-Dopamine Reuptake Inhibitor (NDRI) and a serotonin-Norepinephrine-Dopamine Reuptake Inhibitor (SNDRI).

39. A method for preventing and/or treating Huntington's disease in a subject, comprising administering to the subject a composition of claim 1.

40. The composition of any one of the preceding claims, further comprising artificial cerebrospinal fluid.

41. An oligonucleotide, an oligonucleotide composition, or a method selected from embodiments 1-606.

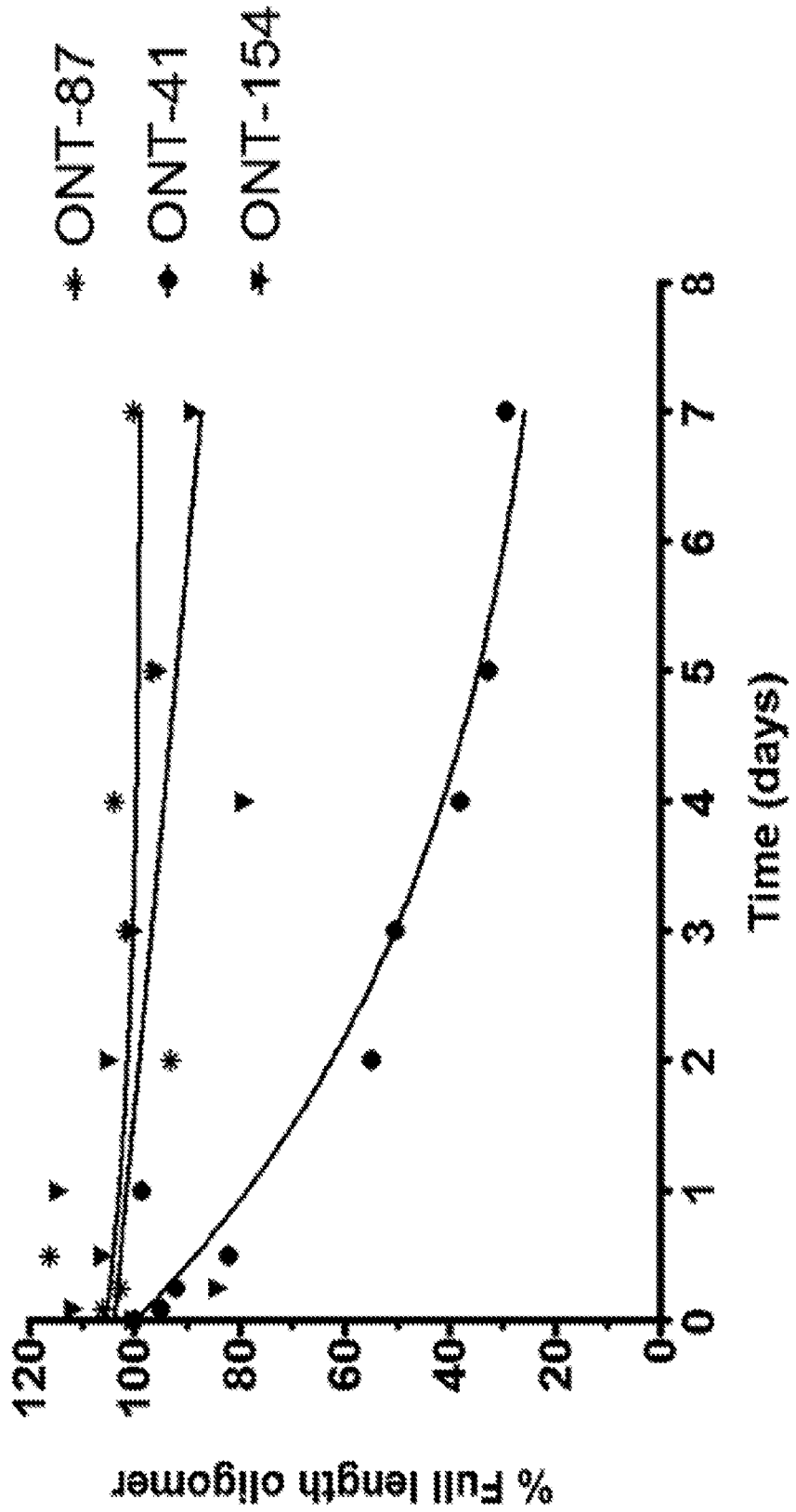

ONT-87
ONT-41
ONT-154
% Full length oligomer
120
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
Time (days)

FIG. 1

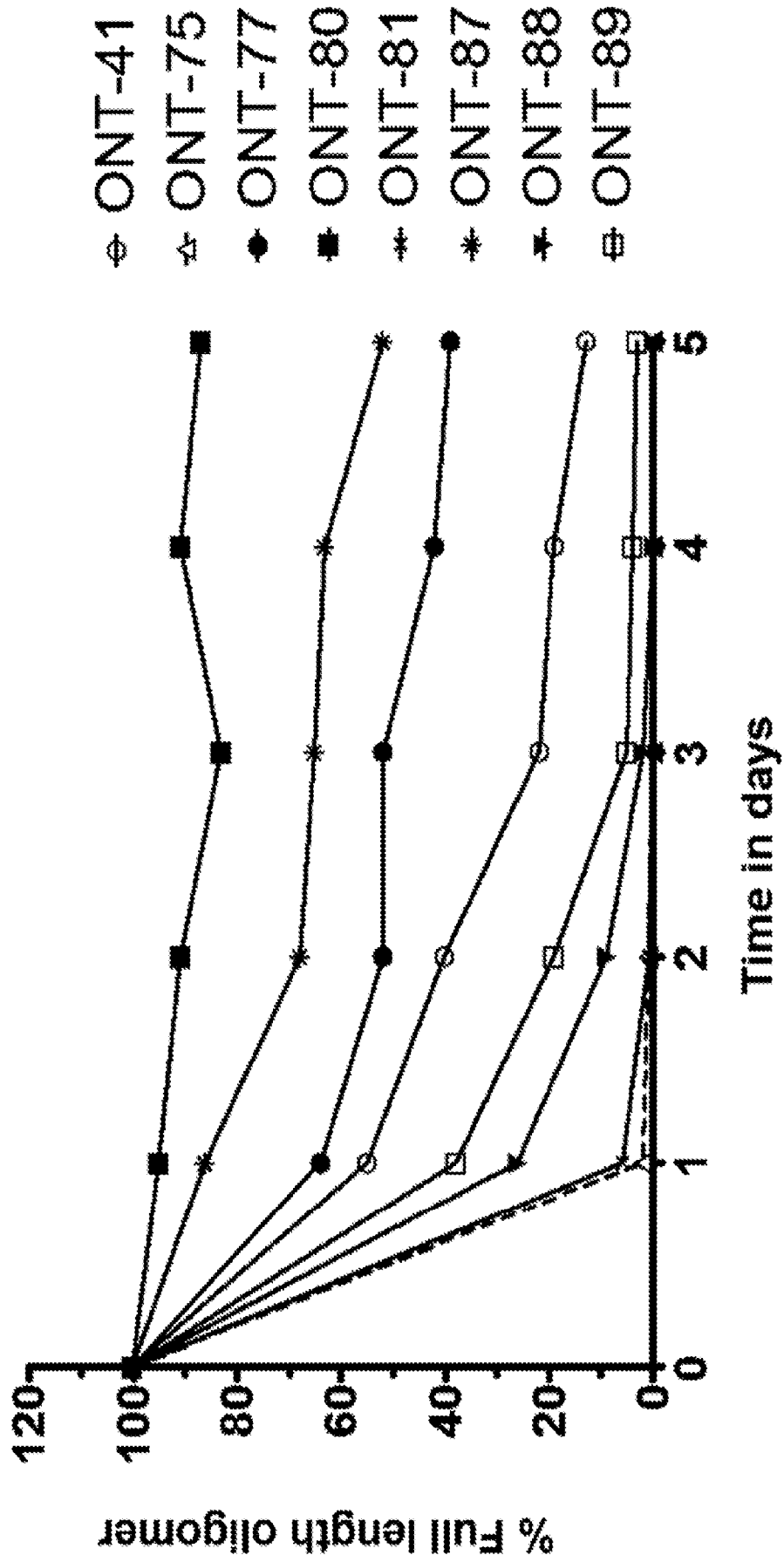

ONT-41
ONT-75
ONT-77
ONT-80
ONT-81
ONT-87
ONT-88
ONT-89
% Full length oligomer
120
100
80
60
40
20
0
0
1
2
3
4
5
Time in days

FIG. 2

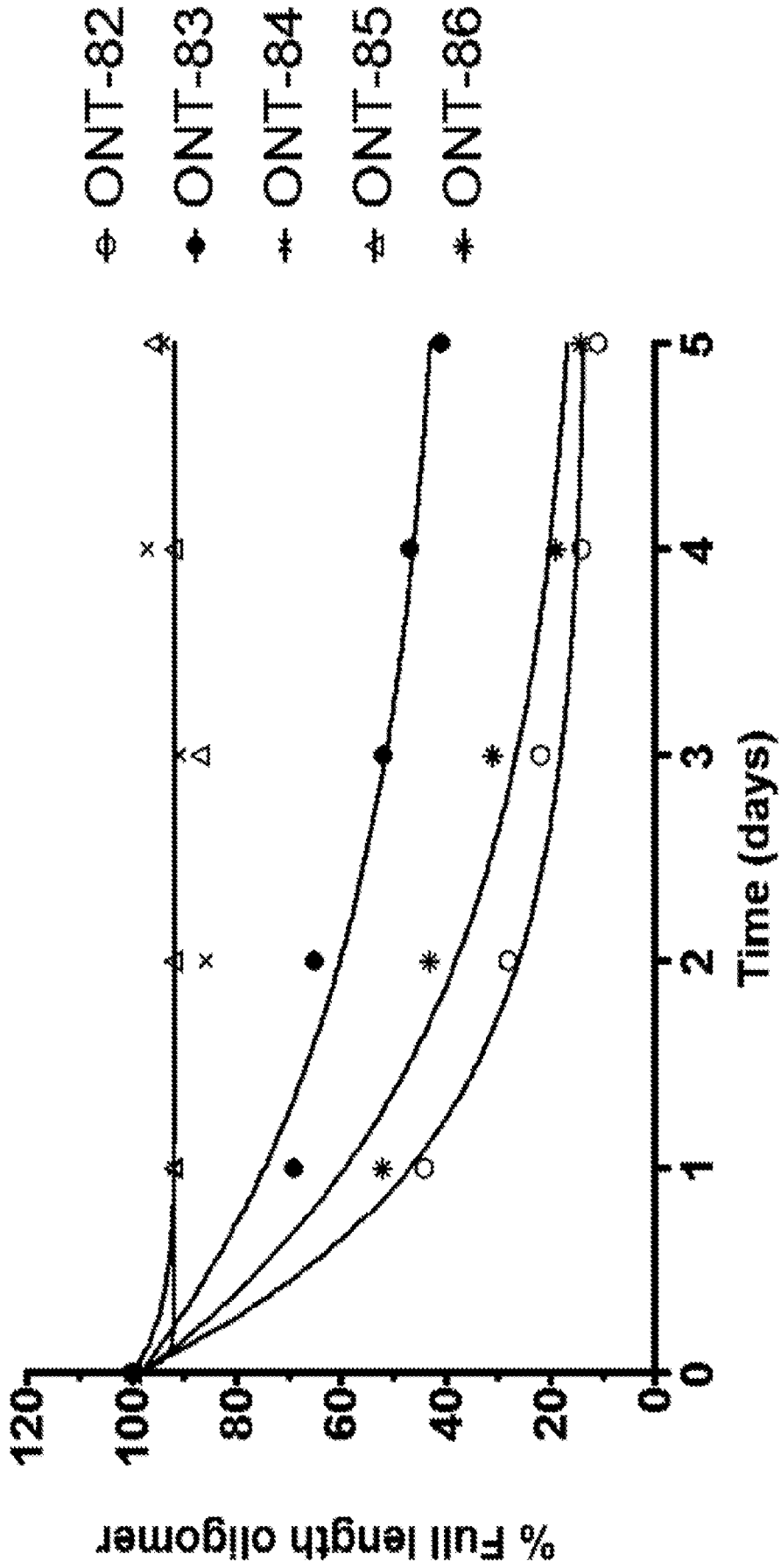

ONT-82
ONT-83
ONT-84
ONT-85
ONT-86
% Full length oligomer
120
100
80
60
40
20
0
0
1
2
3
4
5
Time (days)

FIG. 3

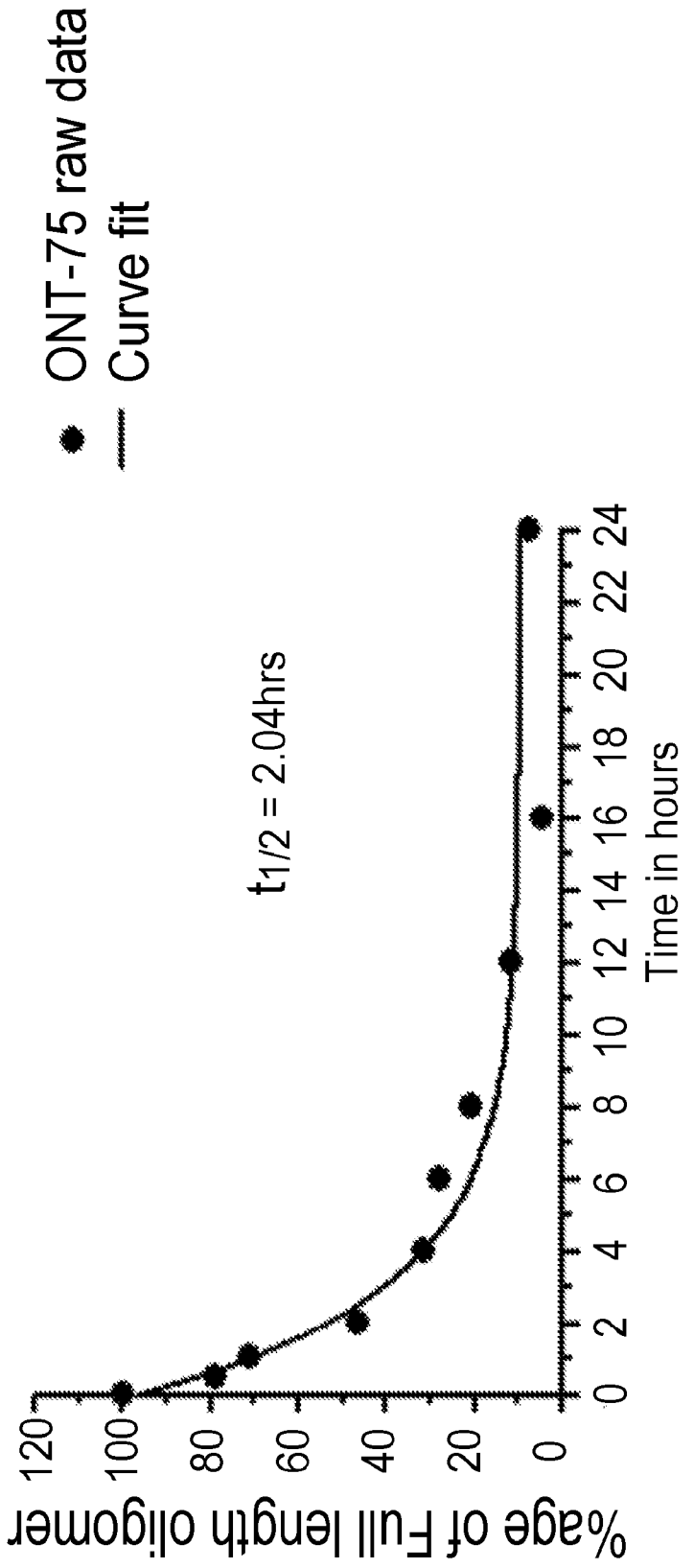

ONT-75 raw data
Curve fit
$t_{1/2}$ = 2.04hrs
%age of Full length oligomer
120
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Time in hours

FIG. 4

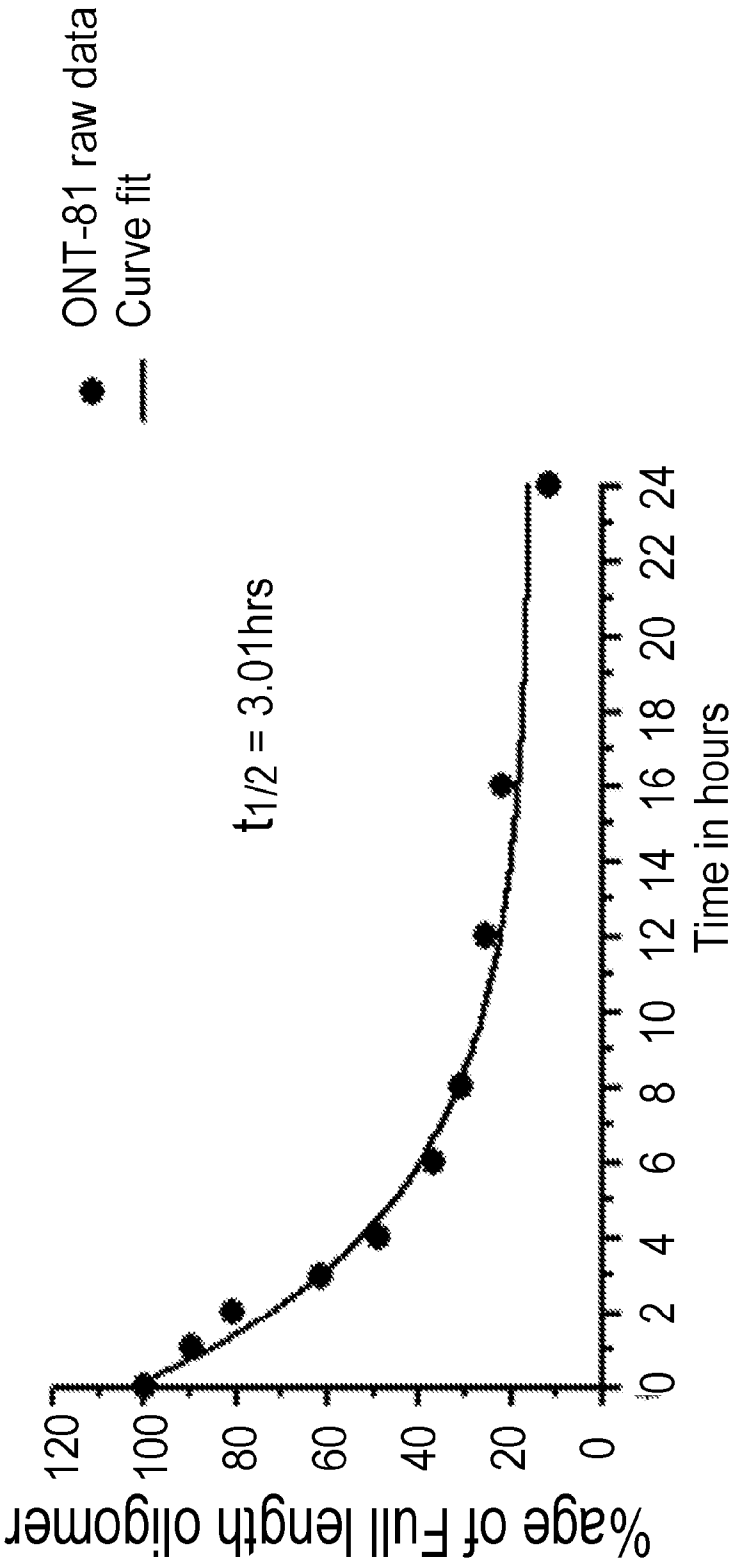

ONT-81 raw data
Curve fit
t1/2 = 3.01hrs
%age of Full length oligomer
120
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24
Time in hours

FIG. 5

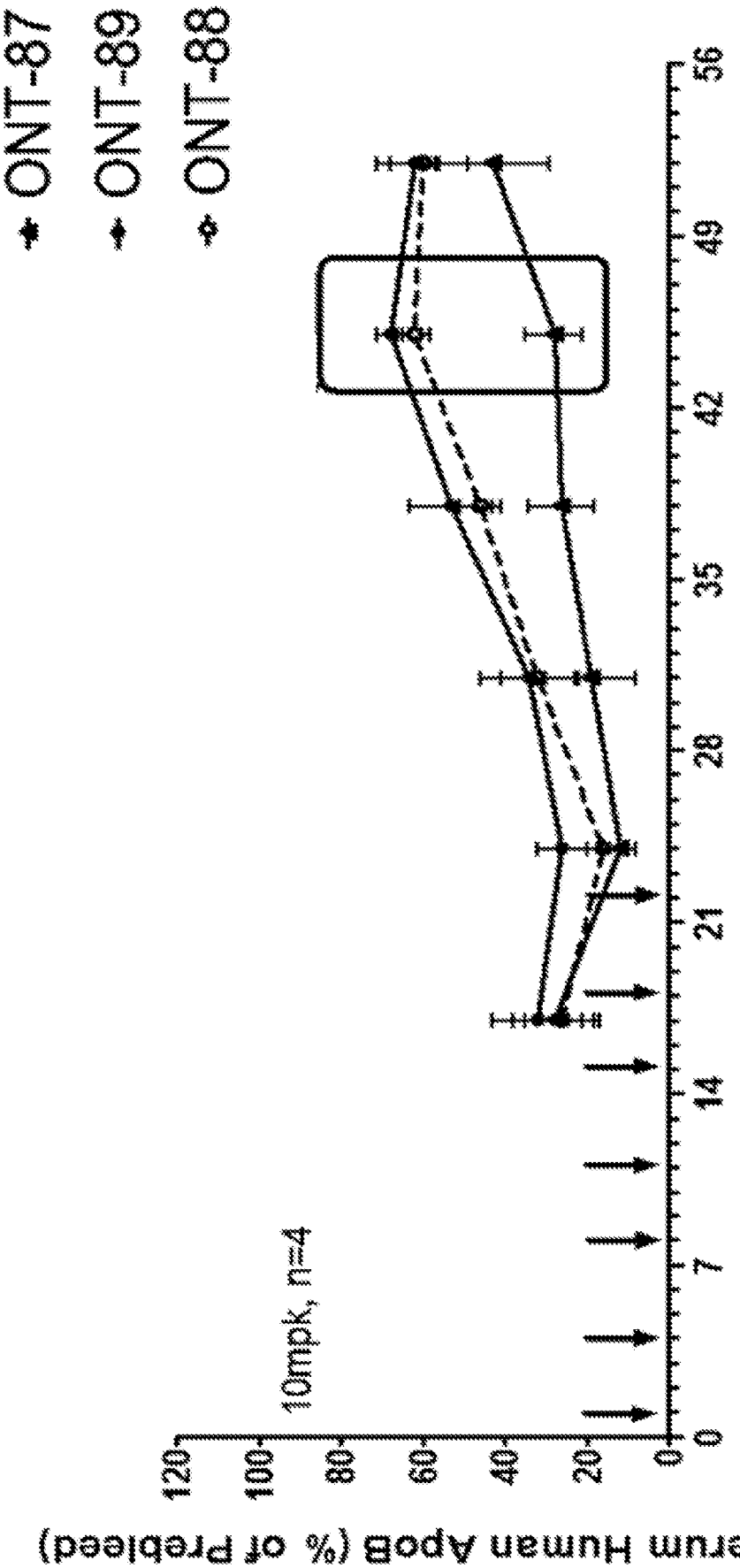

ONT-87
ONT-89
ONT-88
10mpk, n=4
Serum Human ApoB (% of Prebleed)
120
100
80
60
40
20
0
0
7
14
21
28
35
42
49
56
Day

FIG. 6

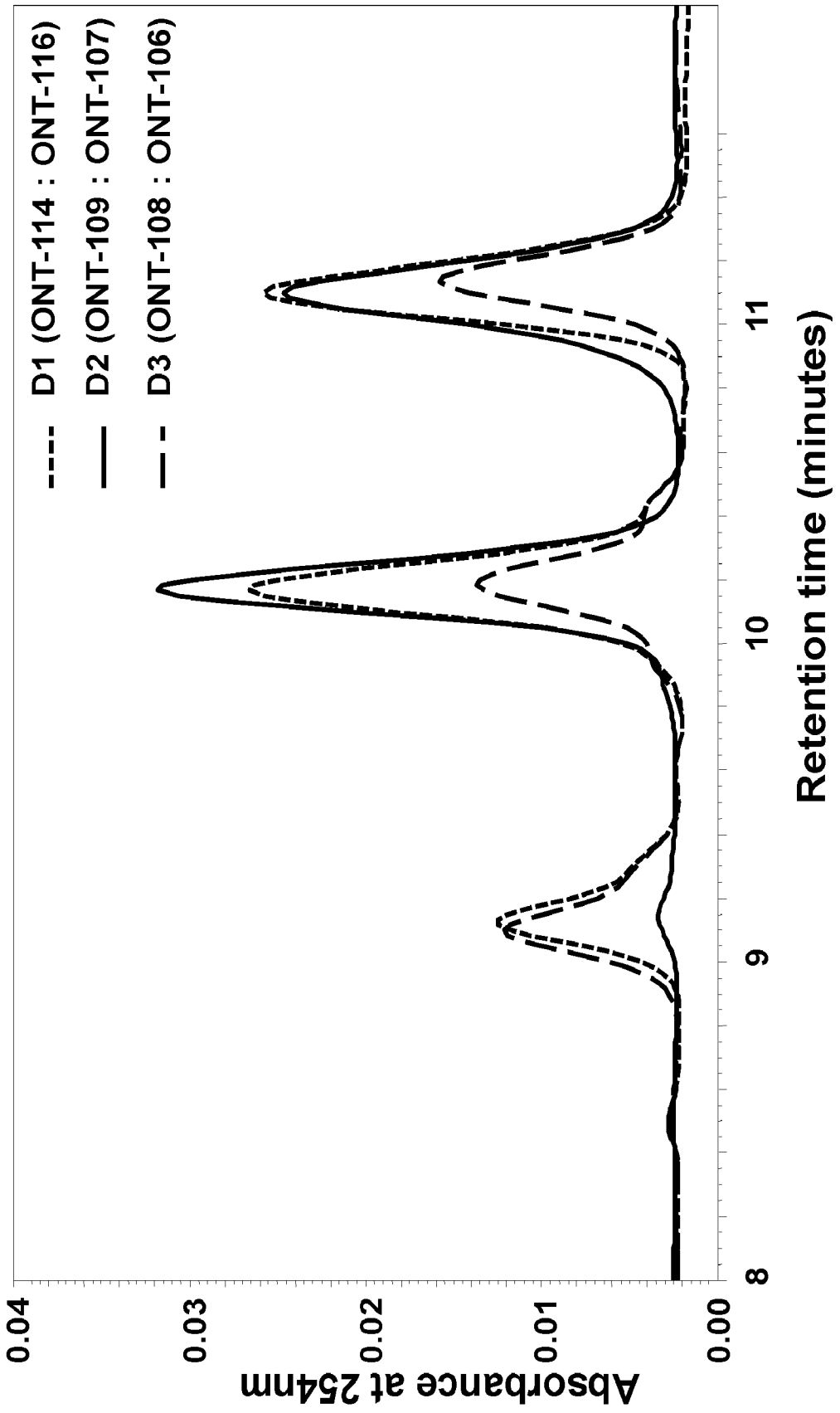

D1 (ONT-114 : ONT-116)
D2 (ONT-109 : ONT-107)
D3 (ONT-108 : ONT-106)
Absorbance at 254nm
0.04
0.03
0.02
0.01
0.00
8
9
10
11
Retention time (minutes)

FIG. 7

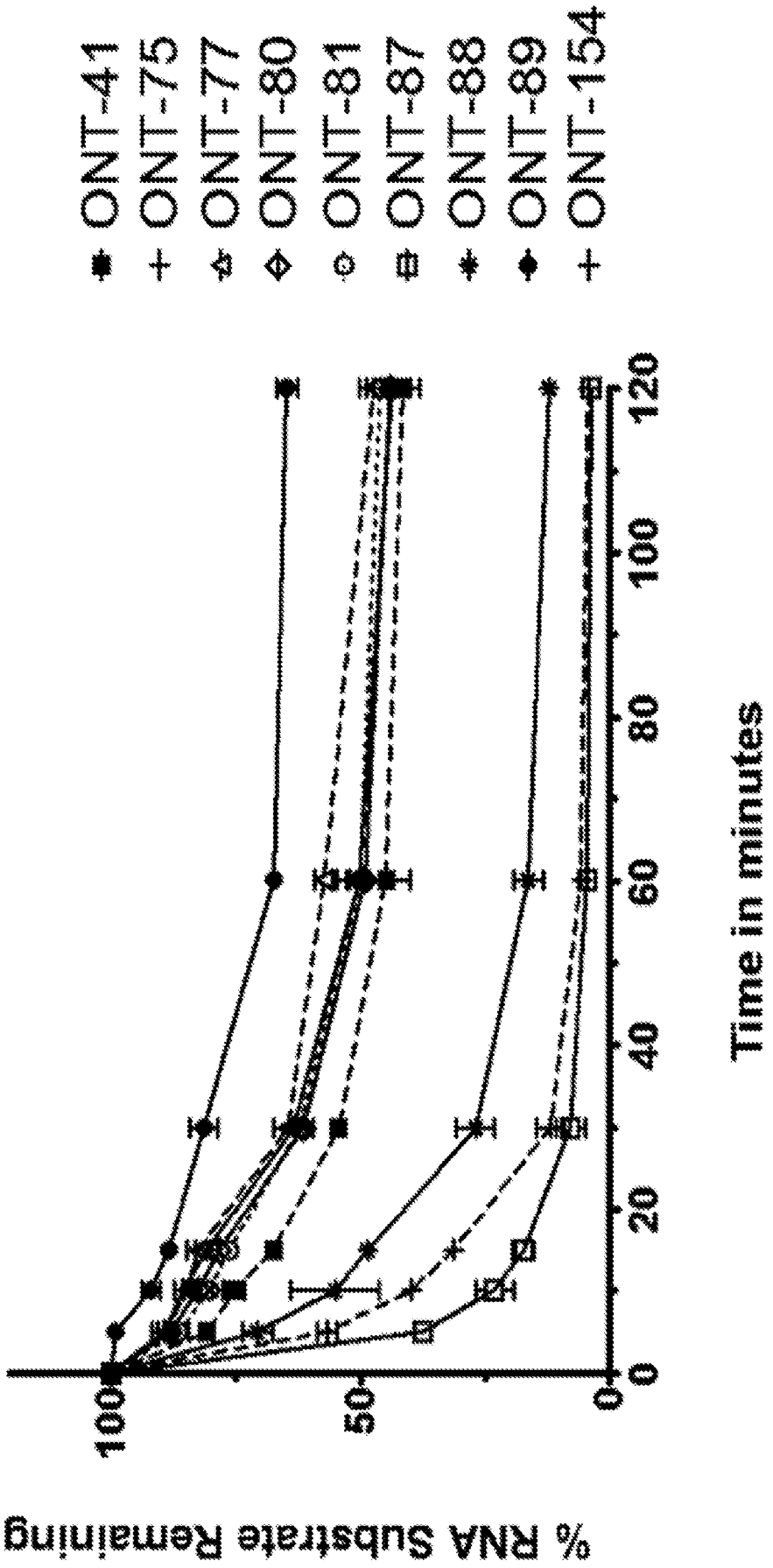
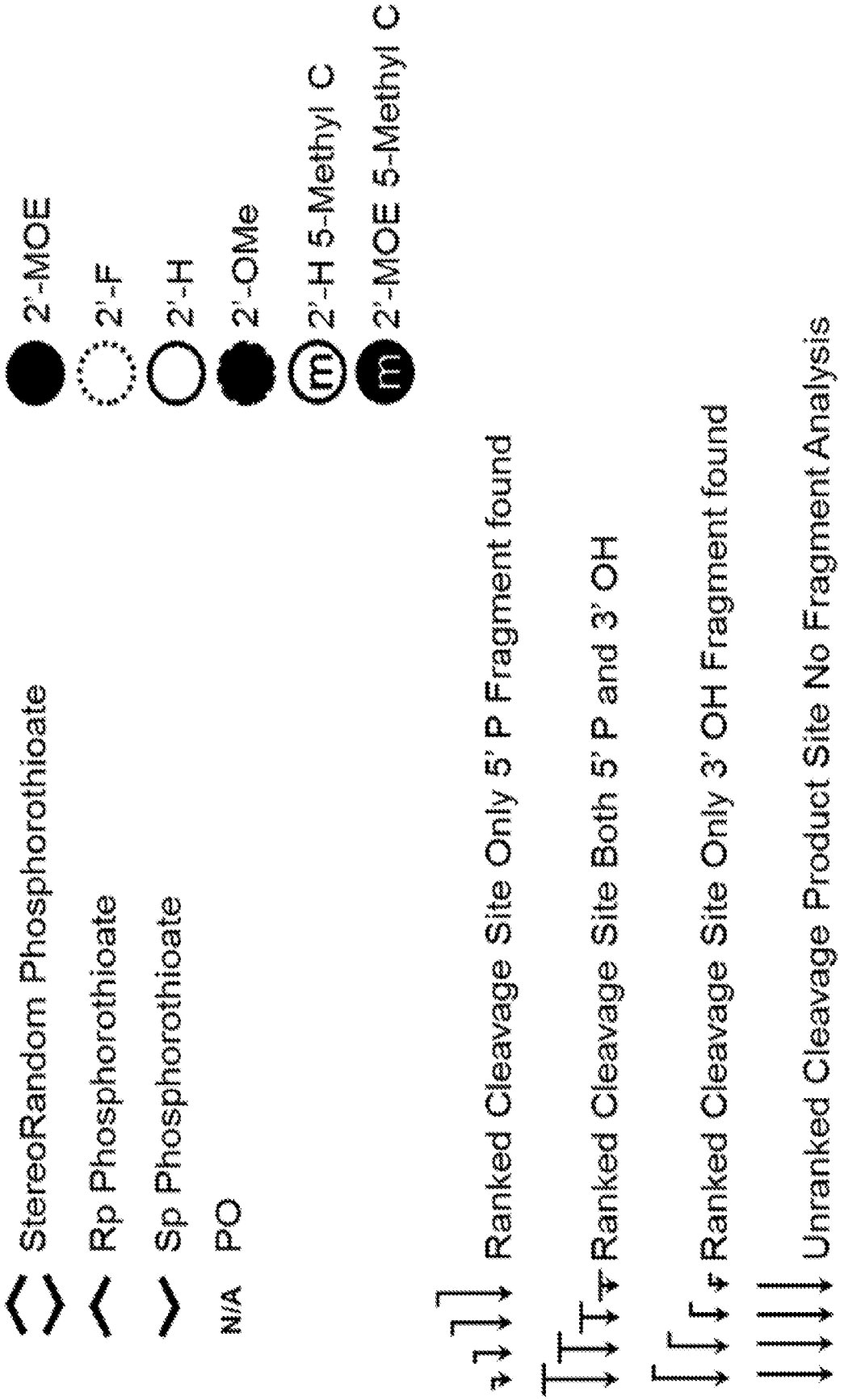

ONT-41
ONT-75
ONT-77
ONT-80
ONT-81
ONT-87
ONT-88
ONT-89
ONT-154
% RNA Substrate Remaining
100
50
0
0
20
40
60
80
100
120
Time in minutes

FIG. 8


StereoRandom Phosphorothioate
Rp Phosphorothioate
Sp Phosphorothioate
N/A PO
2'-MOE
2'-F
2'-H
2'-OMe
m 2'-H 5-Methyl C
m 2'-MOE 5-Methyl C
Ranked Cleavage Site Only 5' P Fragment found
Ranked Cleavage Site Both 5' P and 3' OH
Ranked Cleavage Site Only 3' OH Fragment found
Unranked Cleavage Product Site No Fragment Analysis

(PANEL A)
FIG. 9

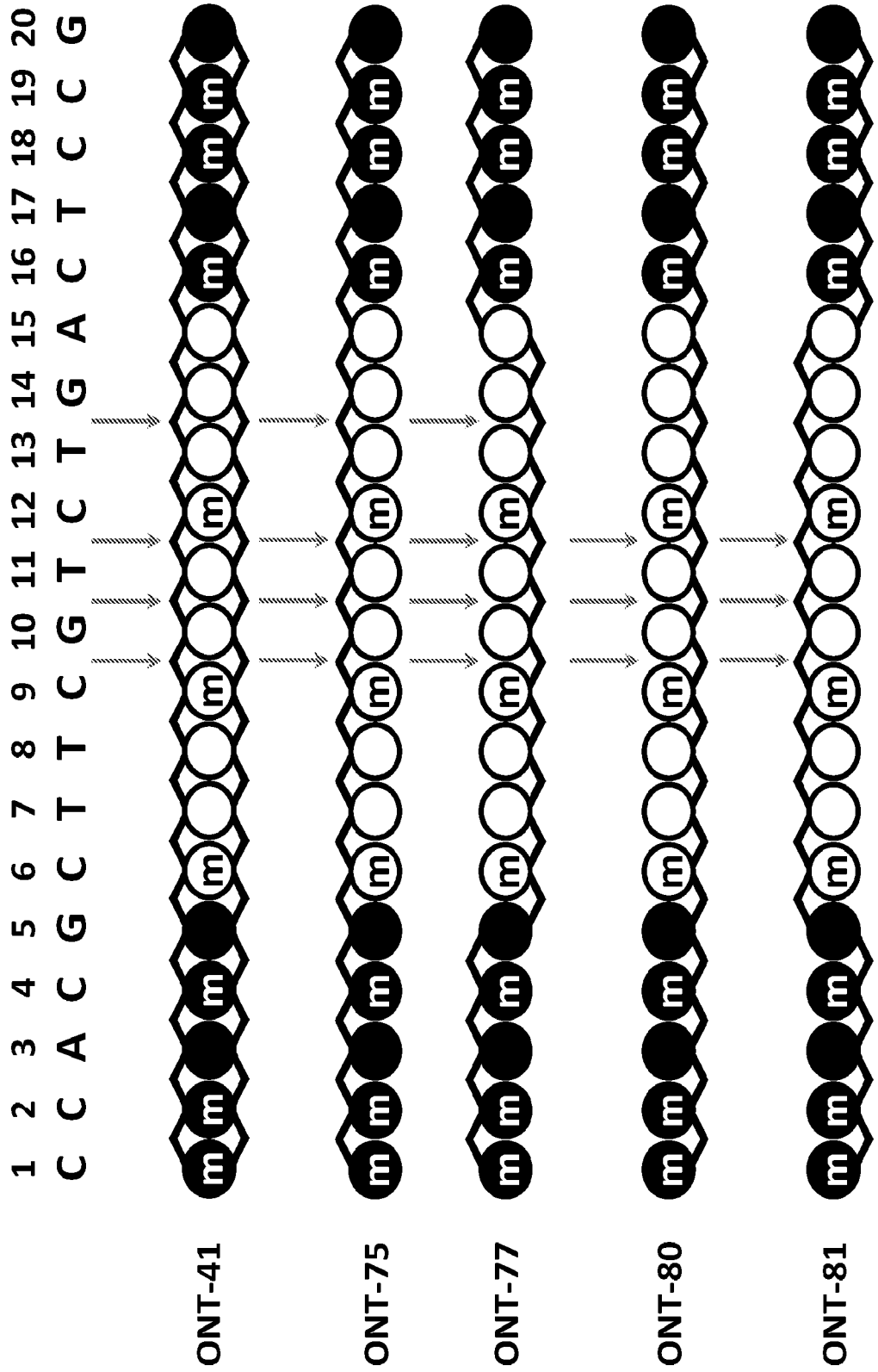
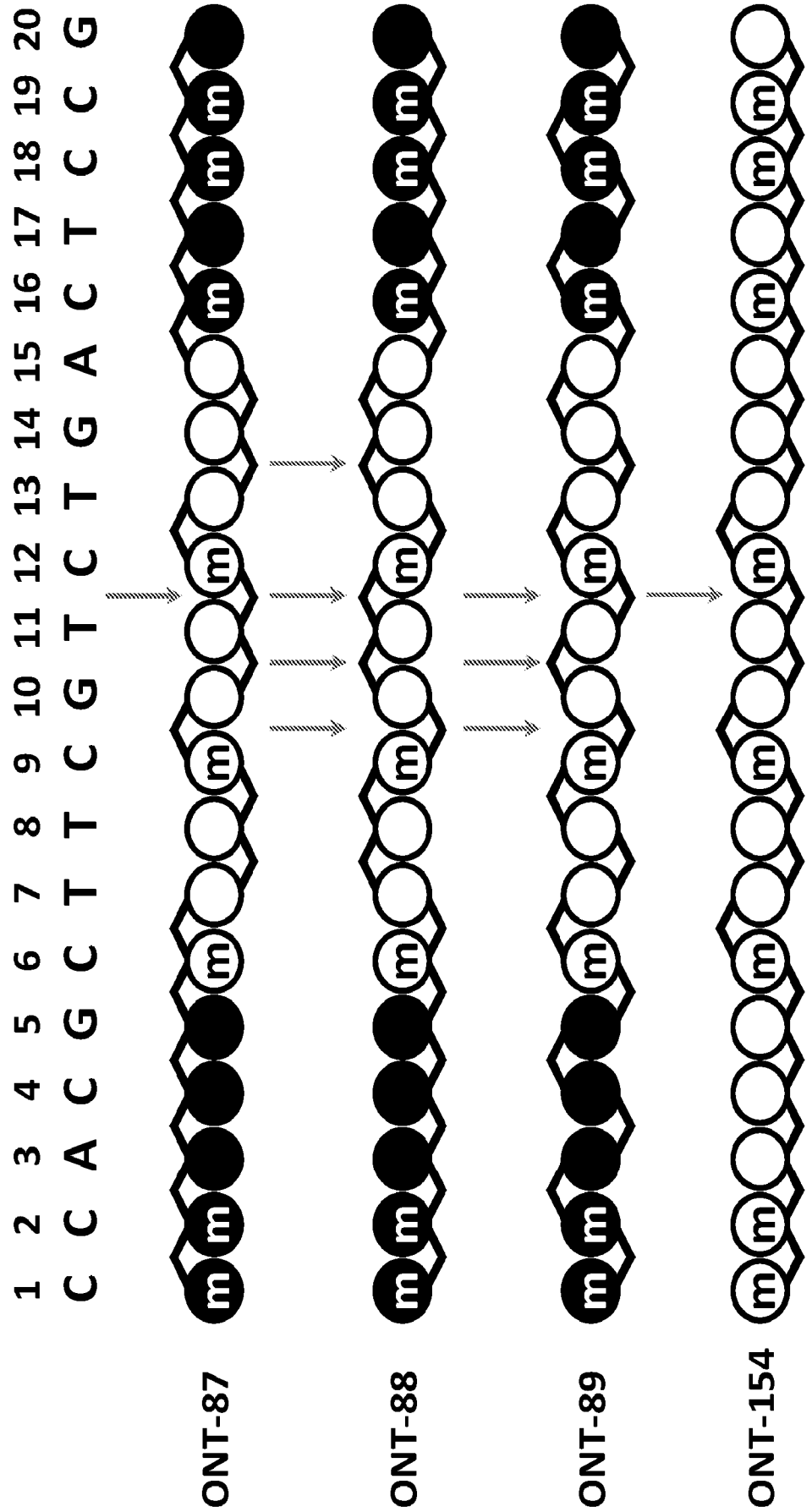
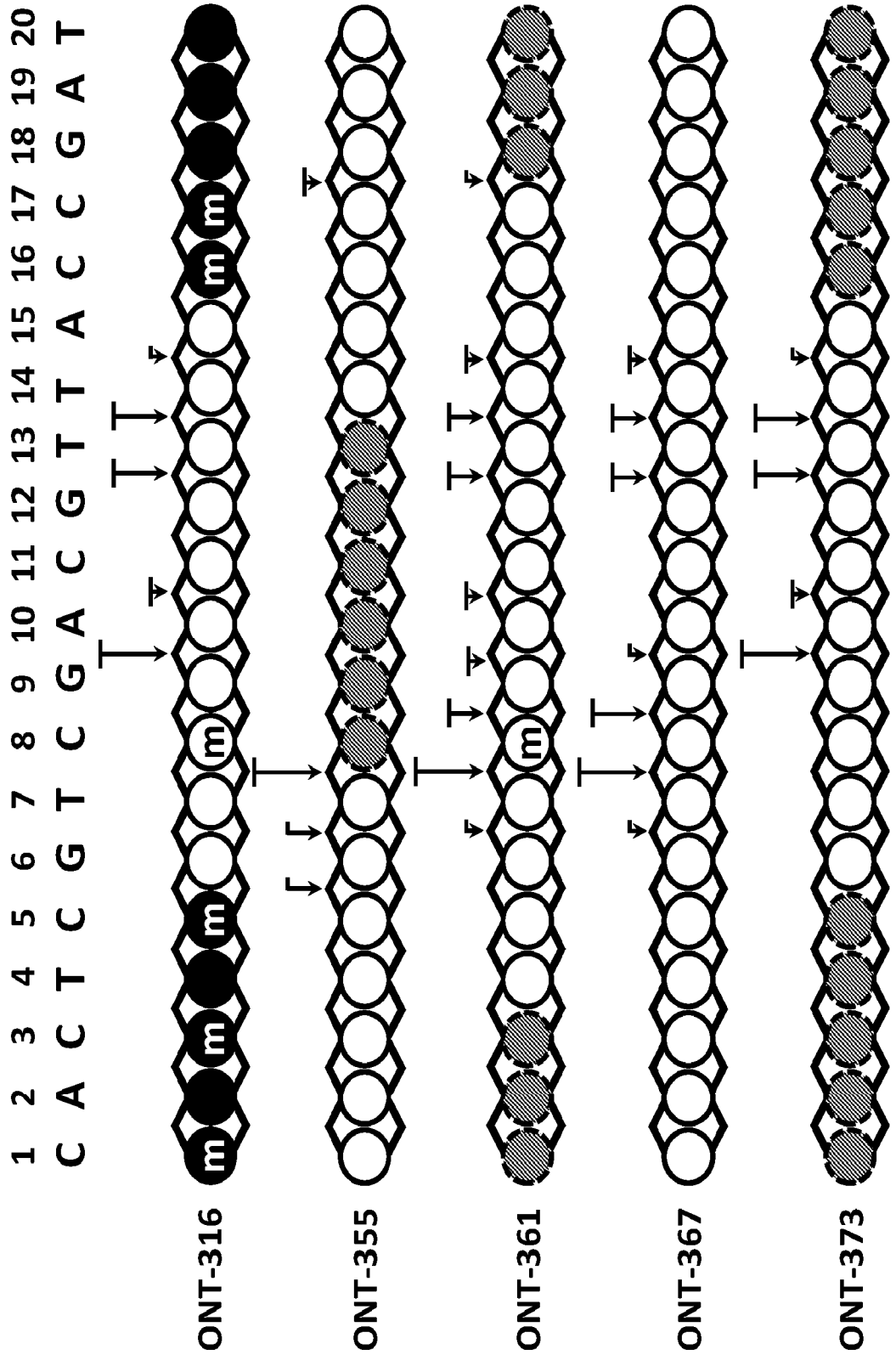


FIG. 9 (CONTINUED)
(PANEL B)

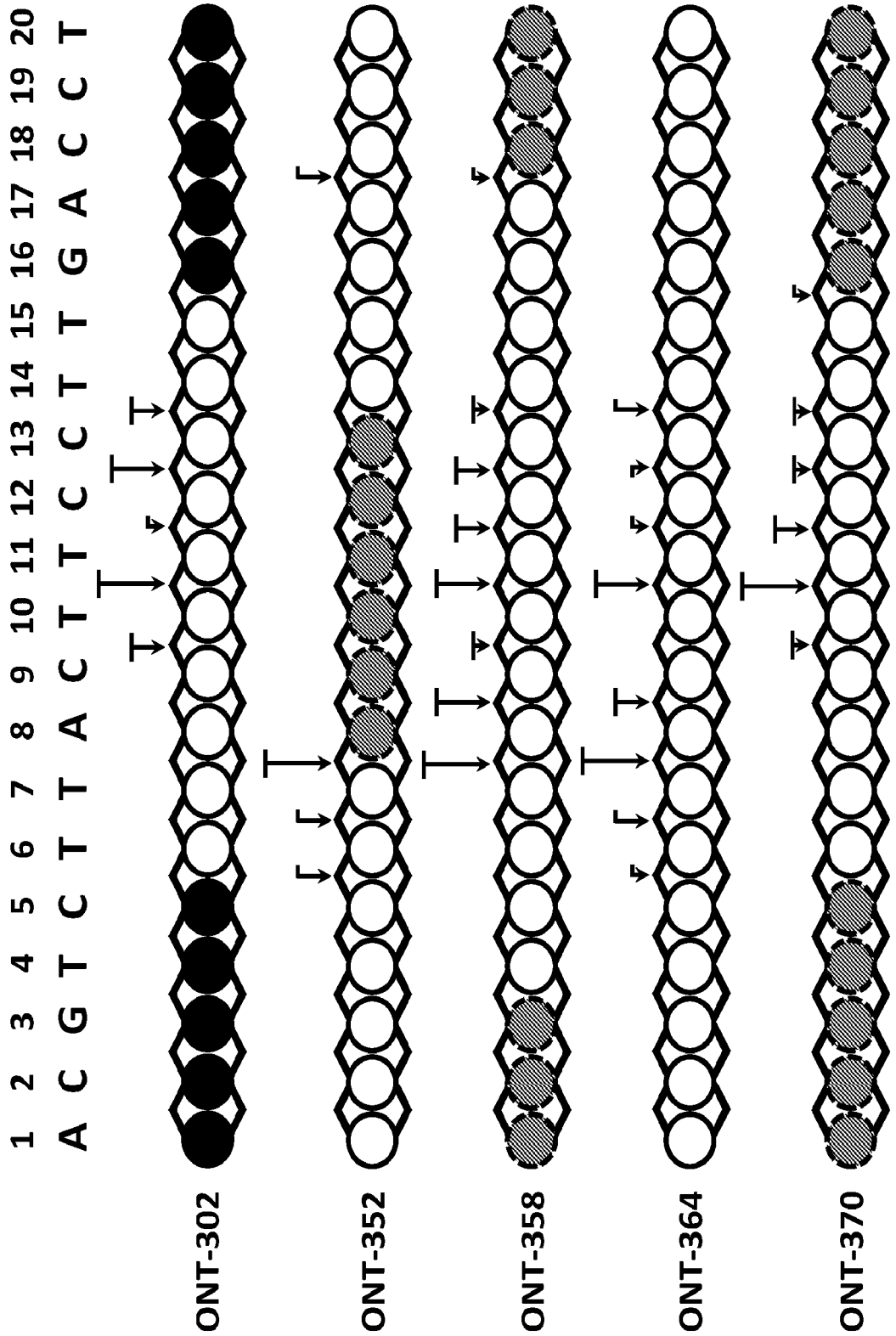


(PANEL C)

FIG. 9 (CONTINUED)

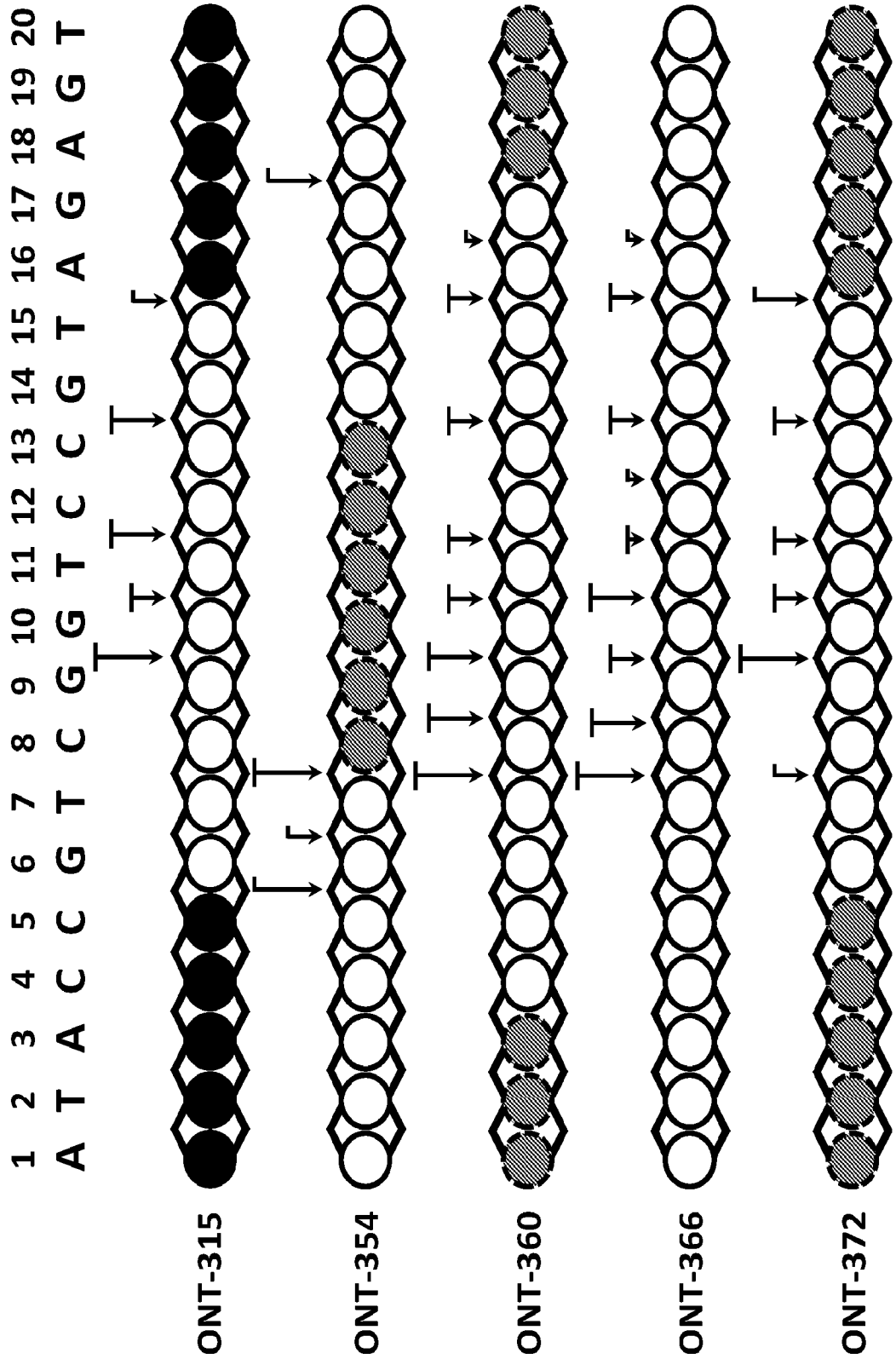


(PANEL A)
FIG. 10



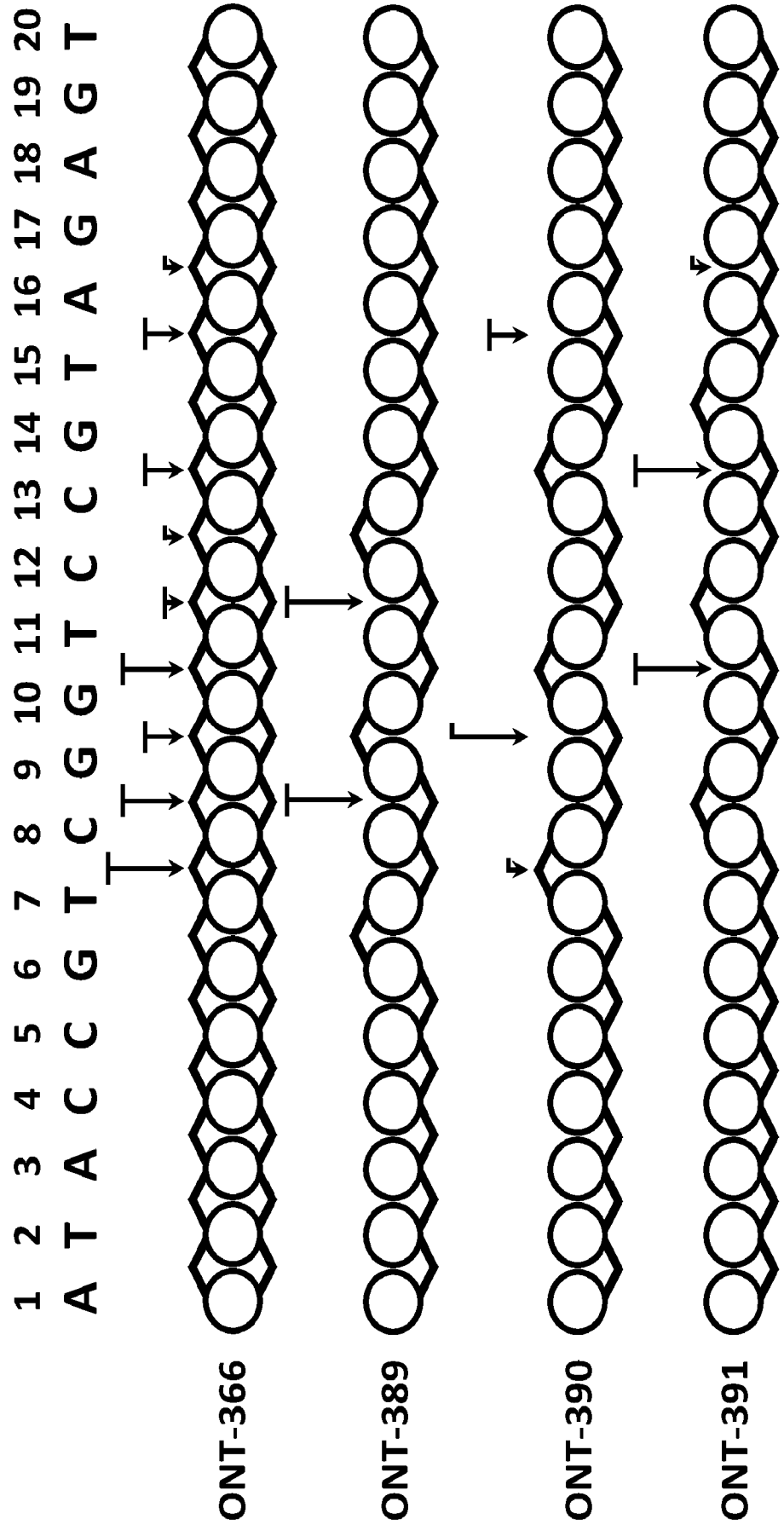
(PANEL B)

FIG. 10 (CONTINUED)

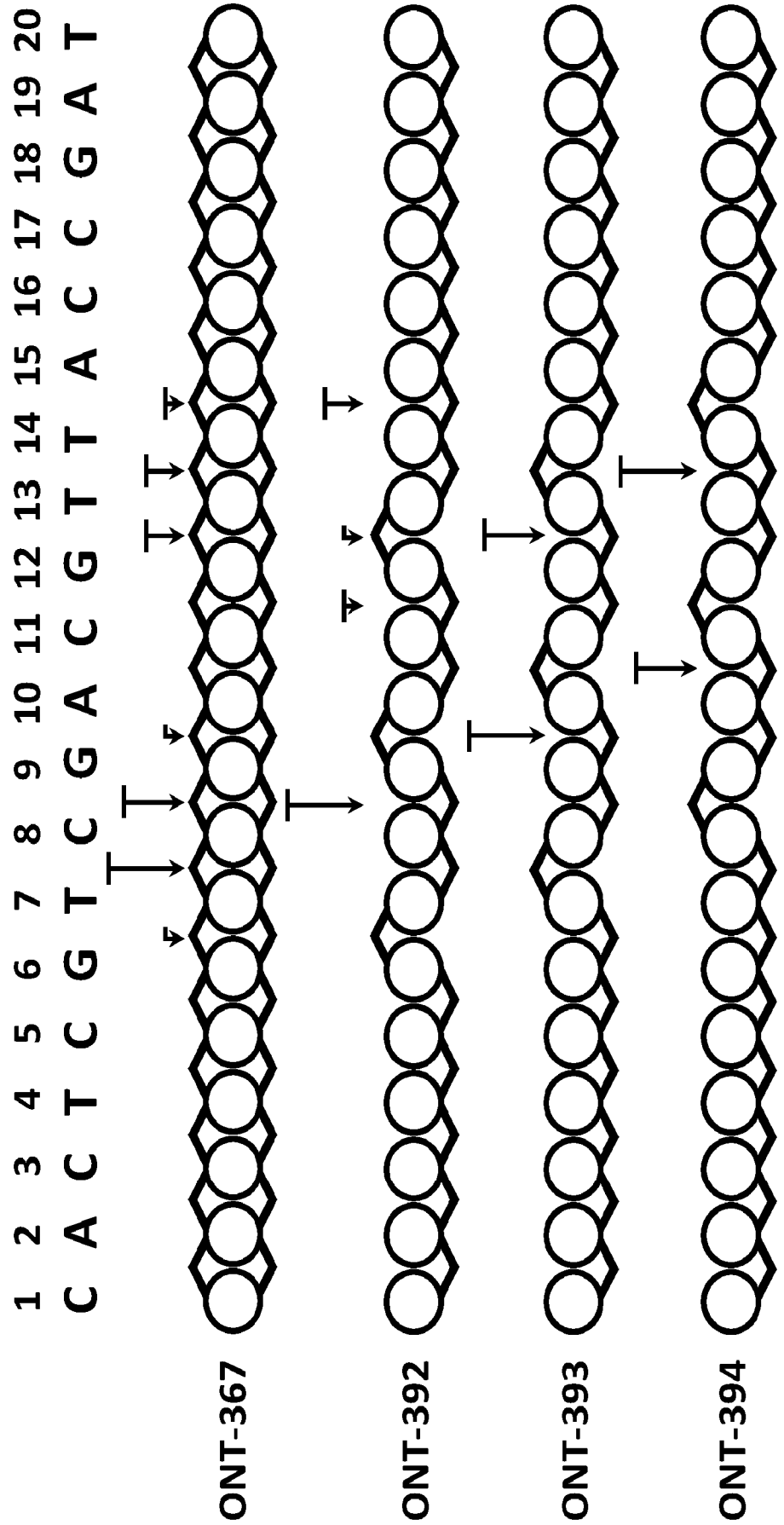


(PANEL C)

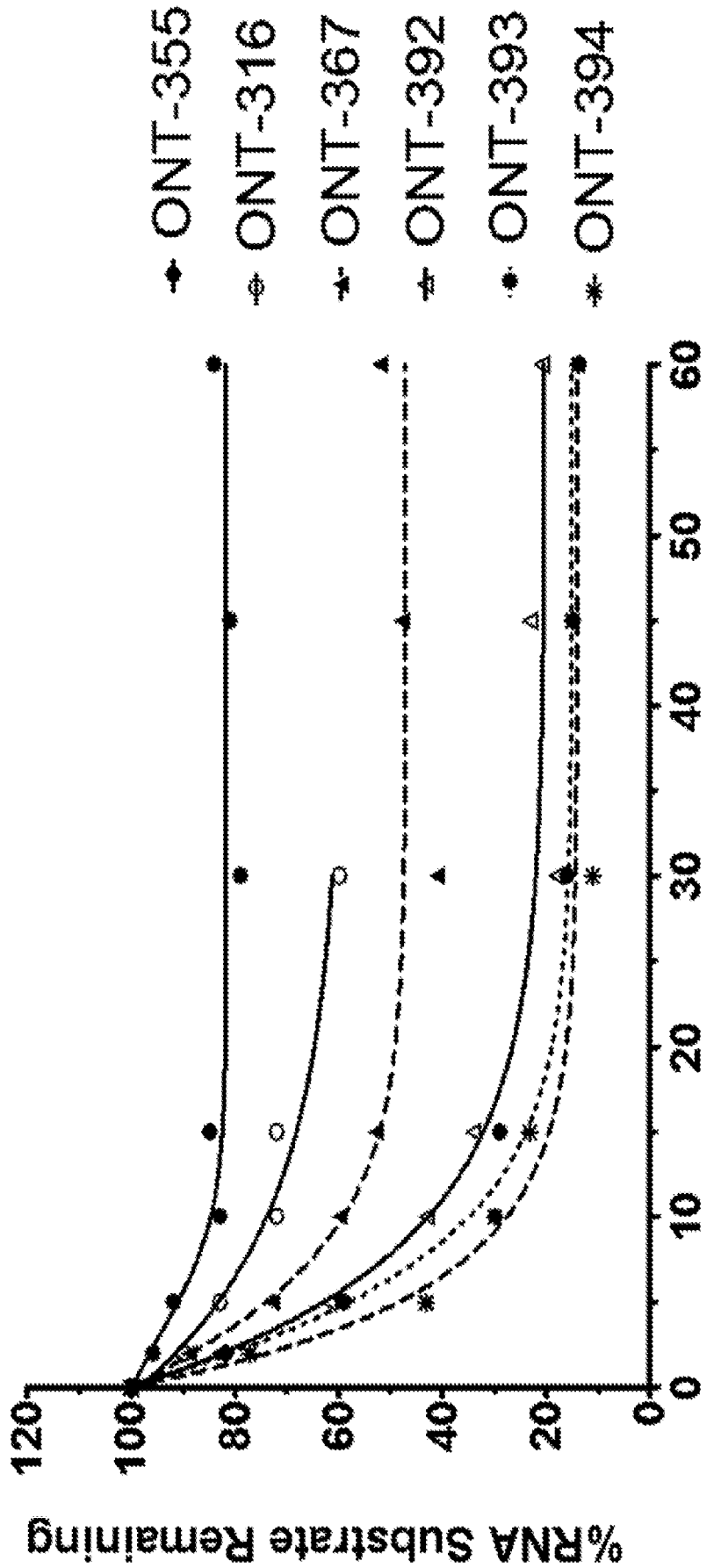
FIG. 10 (CONTINUED)



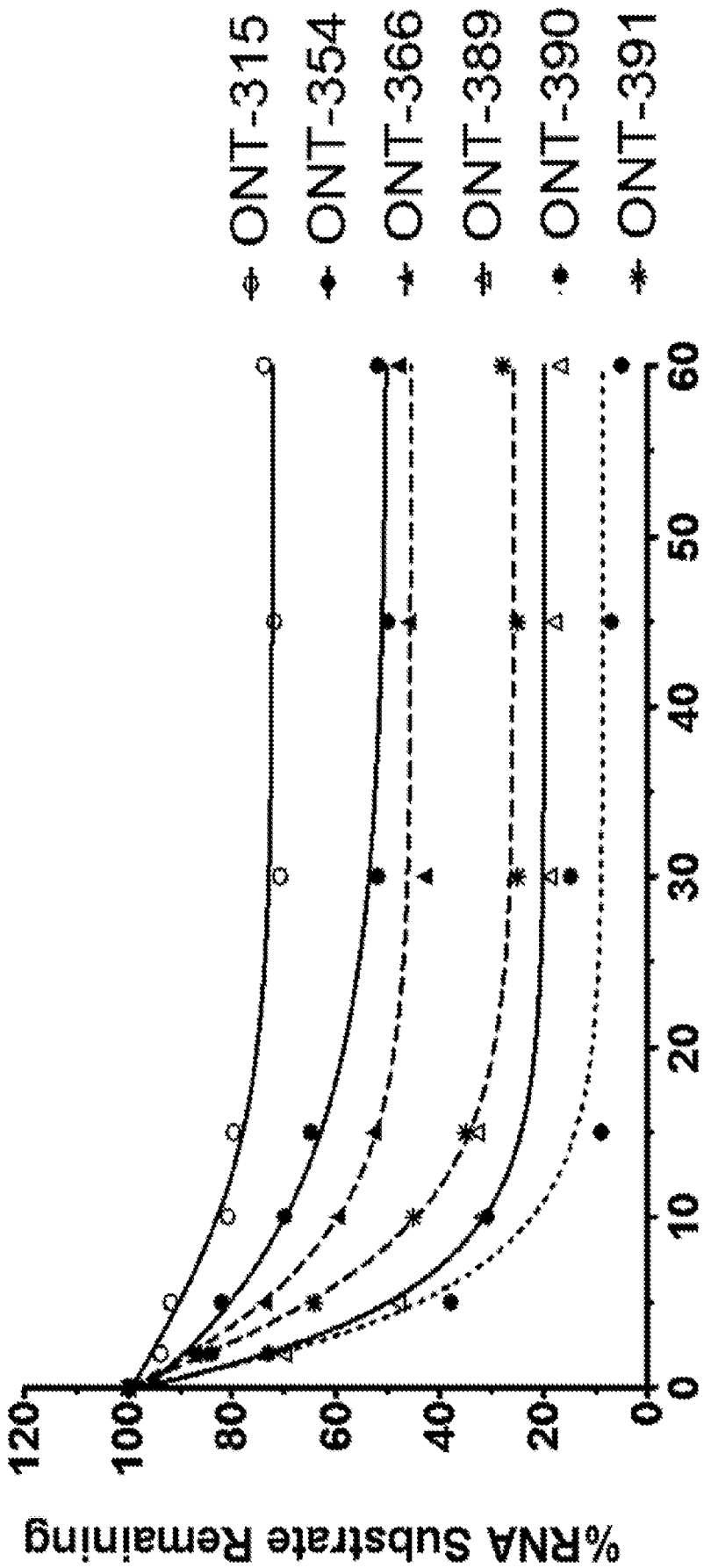
(PANEL A)
FIG. 11



(PANEL B)
FIG. 11 (CONTINUED)



(PANEL A)
FIG. 12



(PANEL B)
FIG. 12 (CONTINUED)

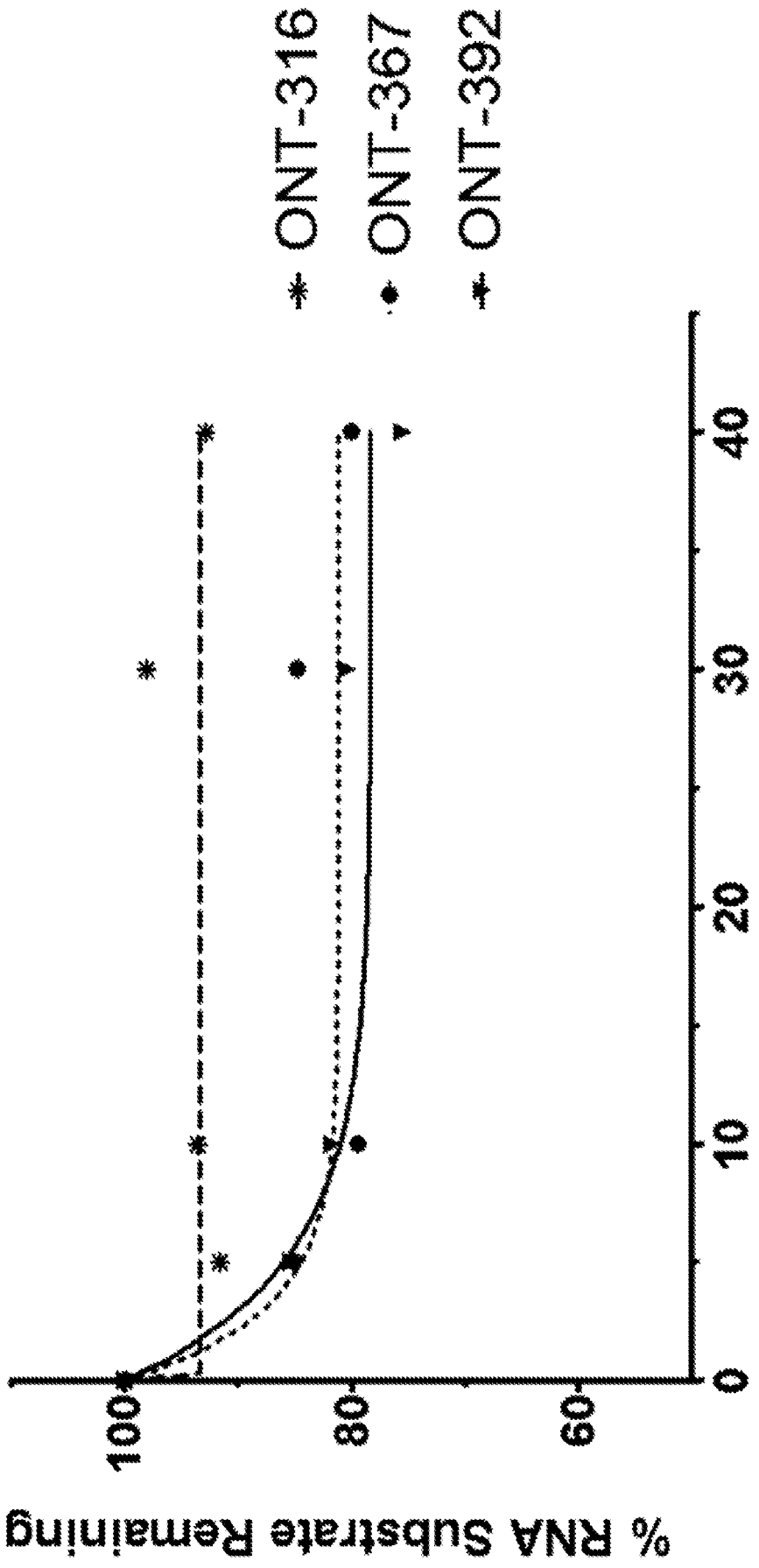

ONT-316
ONT-367
ONT-392
% RNA Substrate Remaining
100
80
60
0
10
20
30
40
Time (minutes)

FIG. 13

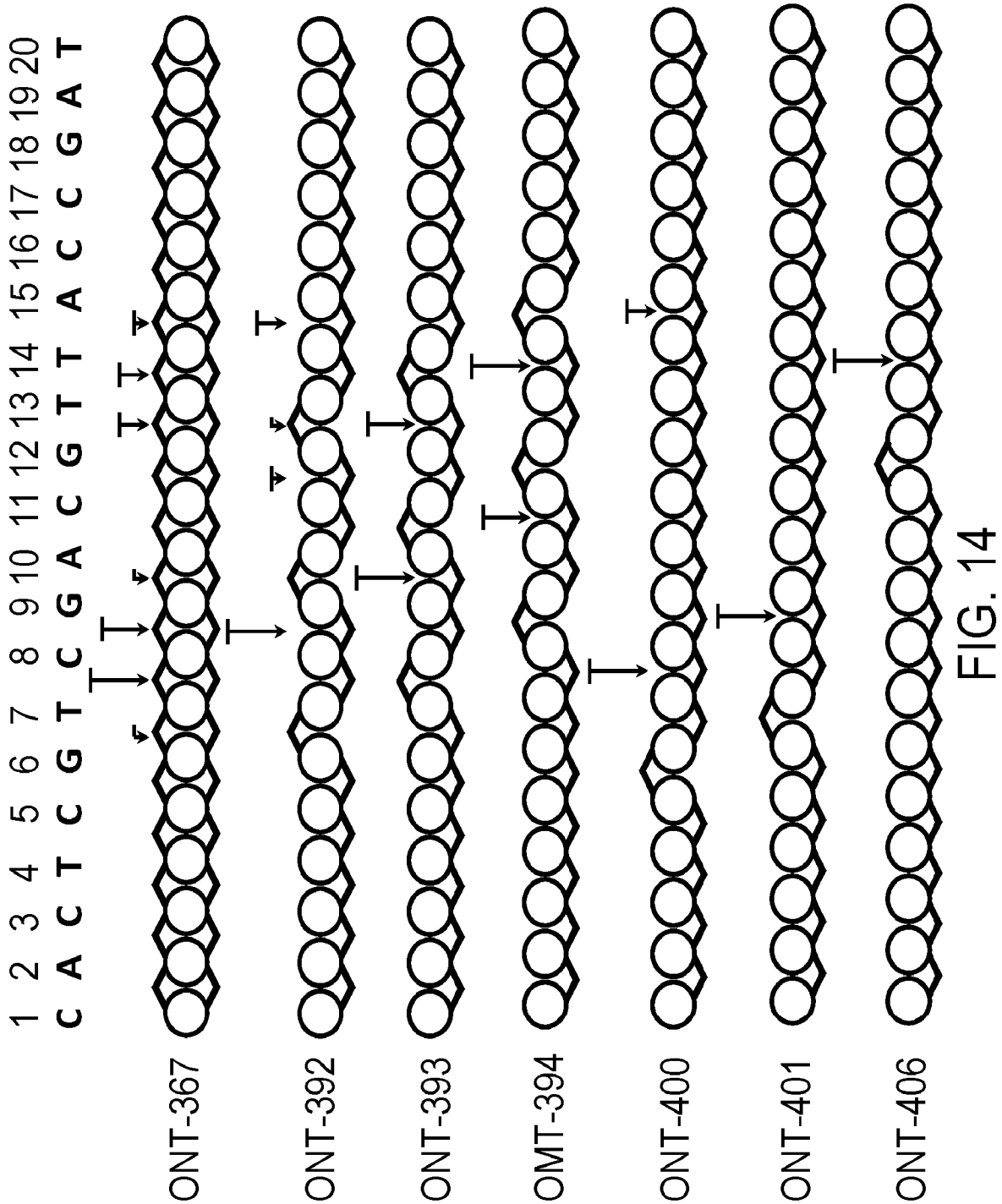

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
C A C T C G T C G A C G T T A C C G A T
ONT-367
ONT-392
ONT-393
OMT-394
ONT-400
ONT-401
ONT-406

FIG. 14

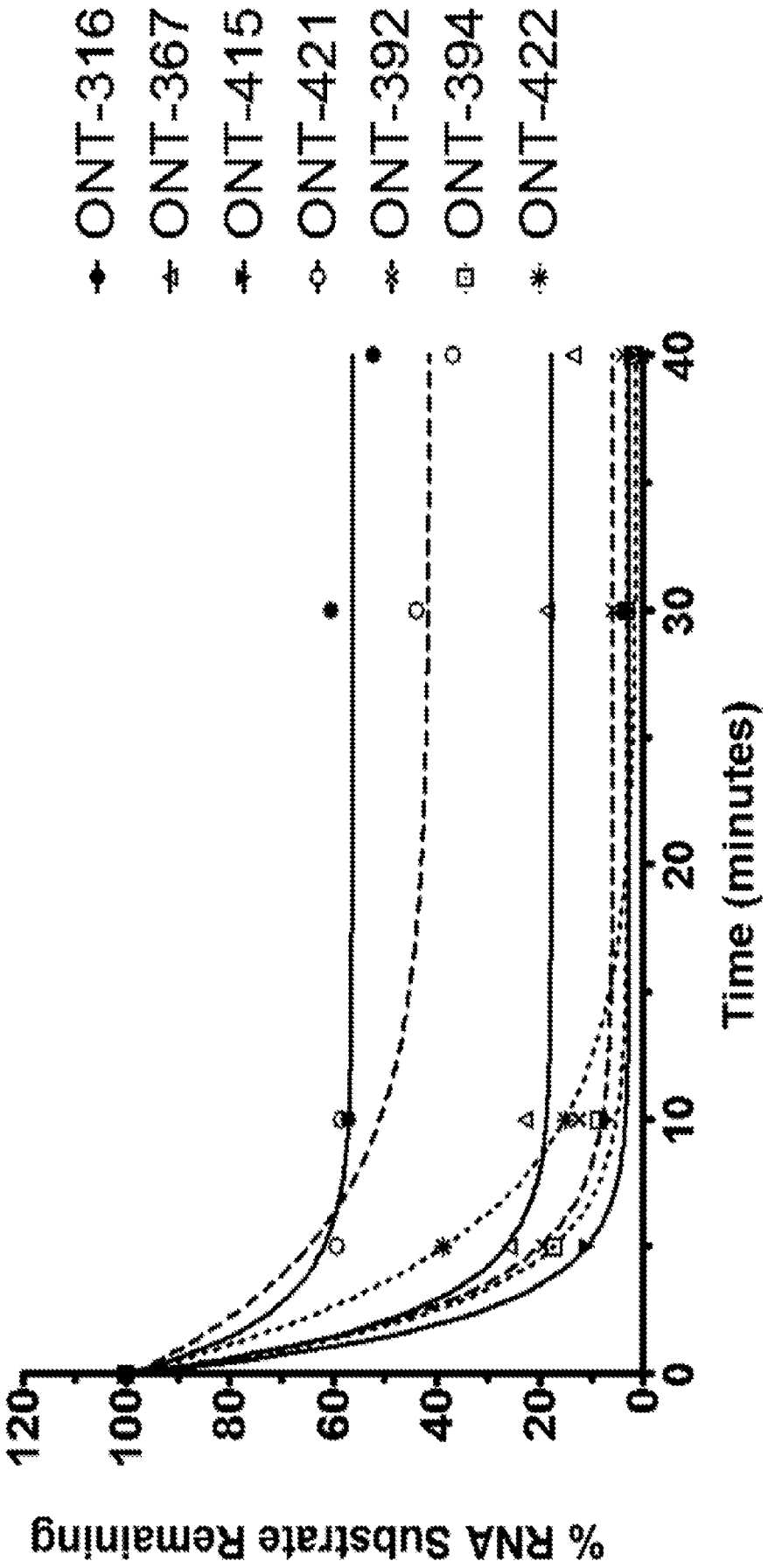

ONT-316
ONT-367
ONT-415
ONT-421
ONT-392
ONT-394
ONT-422
% RNA Substrate Remaining
120
100
80
60
40
20
0
0
10
20
30
40
Time (minutes)

FIG. 15

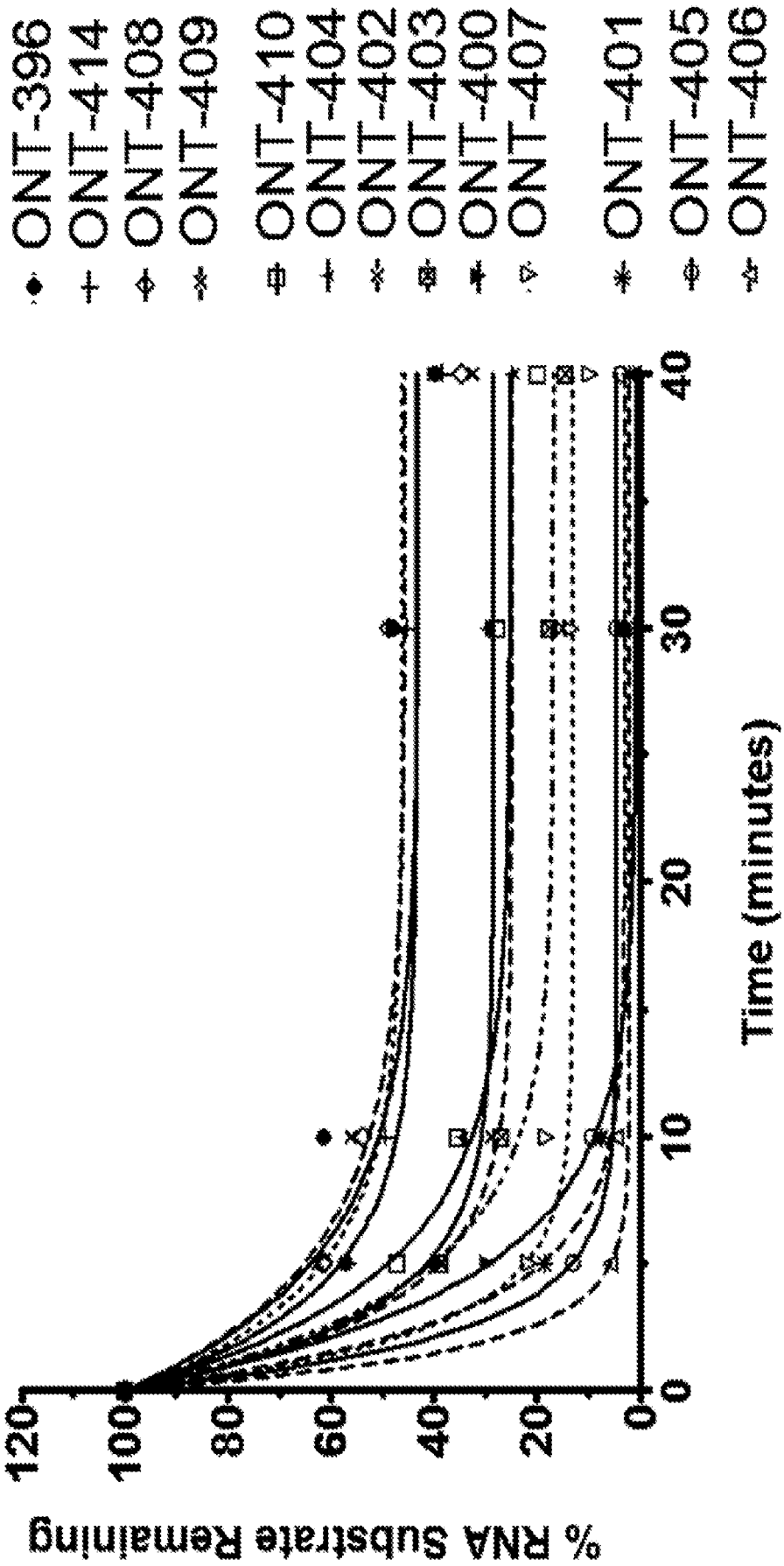

ONT-396
ONT-414
ONT-408
ONT-409
ONT-410
ONT-404
ONT-402
ONT-403
ONT-400
ONT-407
ONT-401
ONT-405
ONT-406
% RNA Substrate Remaining
120
100
80
60
40
20
0
0
10
20
30
40
Time (minutes)

FIG. 16

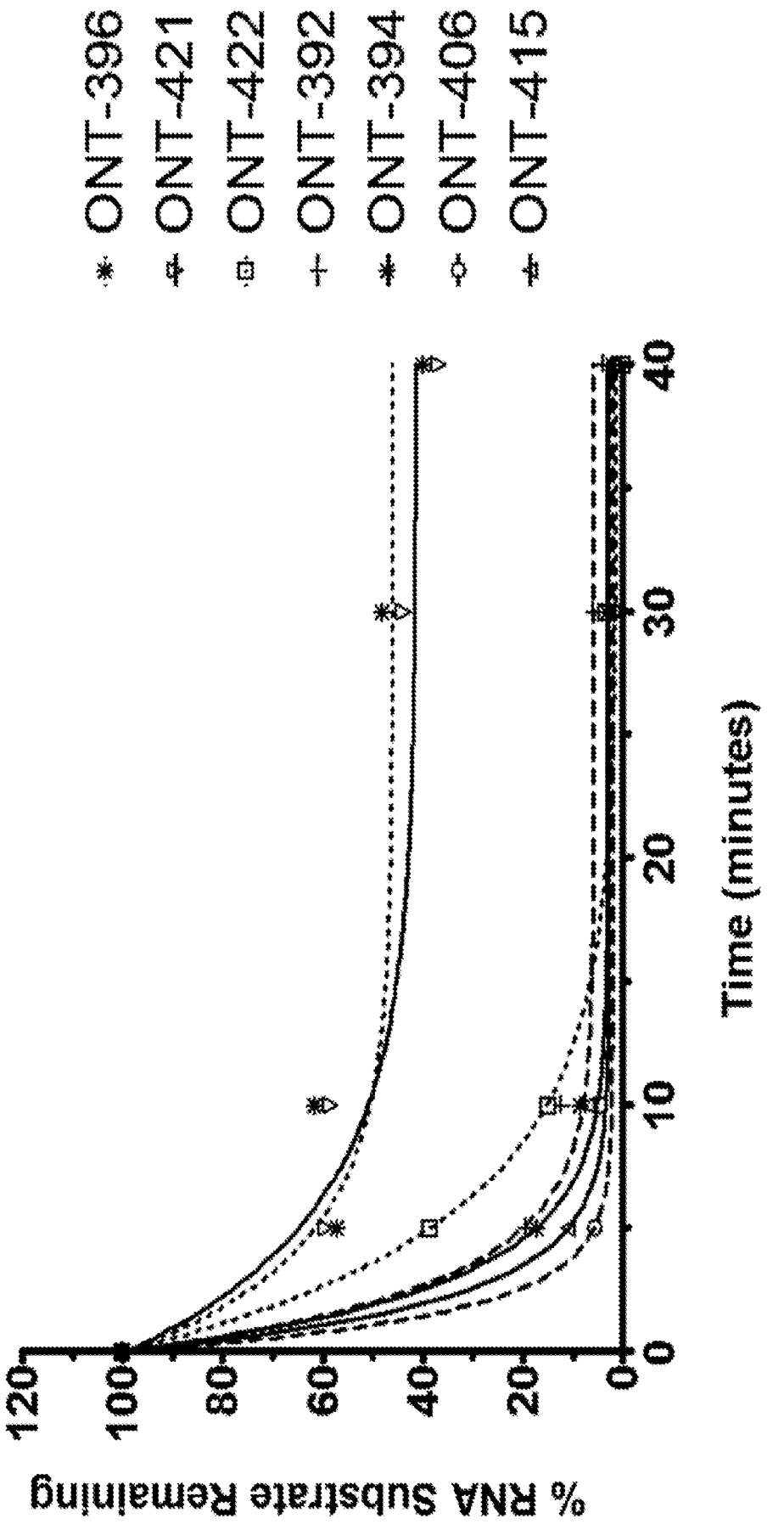

ONT-396
ONT-421
ONT-422
ONT-392
ONT-394
ONT-406
ONT-415
% RNA Substrate Remaining
120
100
80
60
40
20
0
0
10
20
30
40
Time (minutes)

FIG. 17

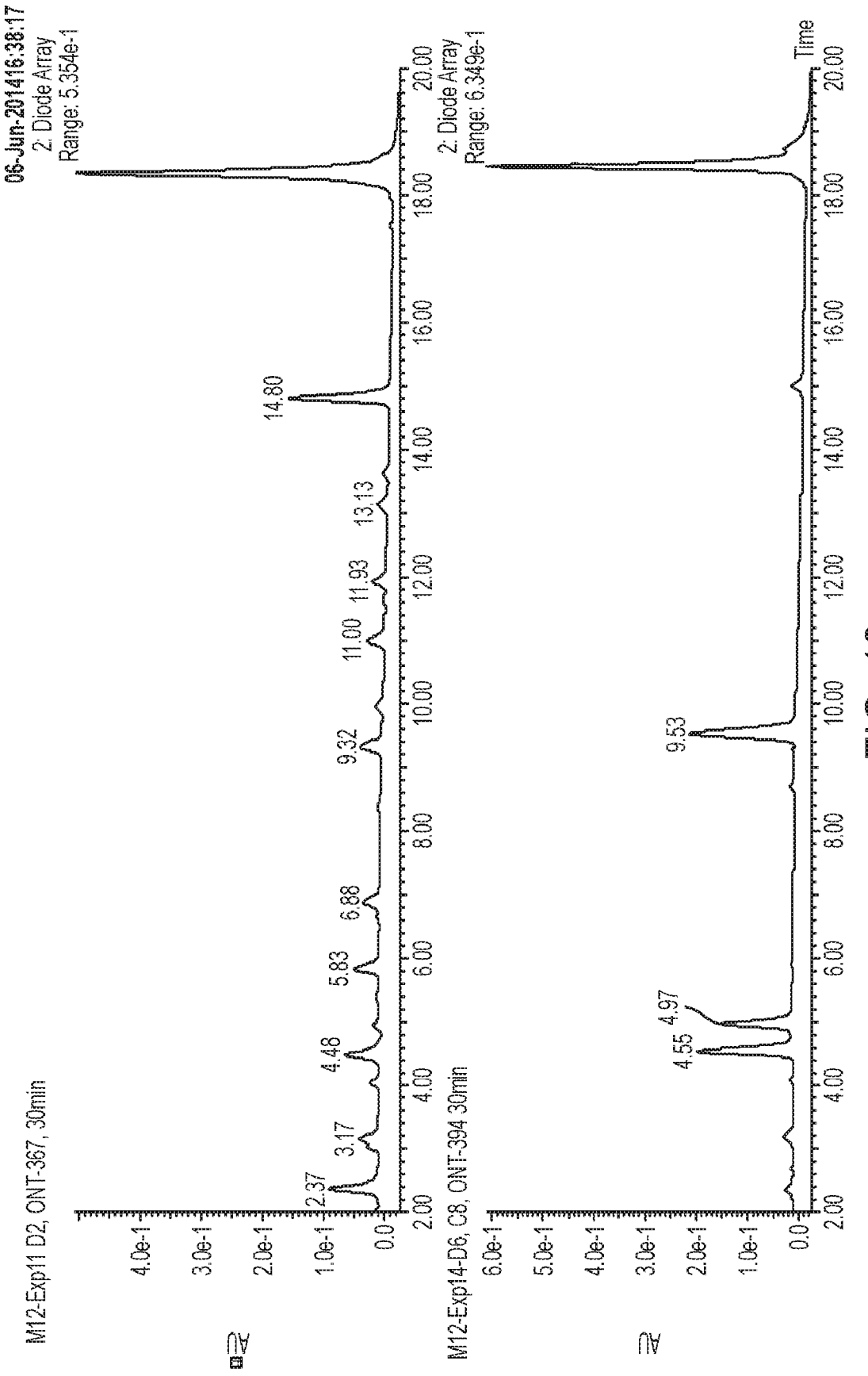

06-Jun-201416:38:17
2: Diode Array
Range: 5.354e-1
M12-Exp11 D2, ONT-367, 30min
2: Diode Array
Range: 6.349e-1
M12-Exp14-D6, C8, ONT-394 30min
Time
2.37
3.17
4.48
5.83
6.88
9.32
11.00
11.93
13.13
14.80
4.55
4.97
9.53
4.0e-1
3.0e-1
2.0e-1
1.0e-1
0.0
6.0e-1
5.0e-1
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00

FIG. 18

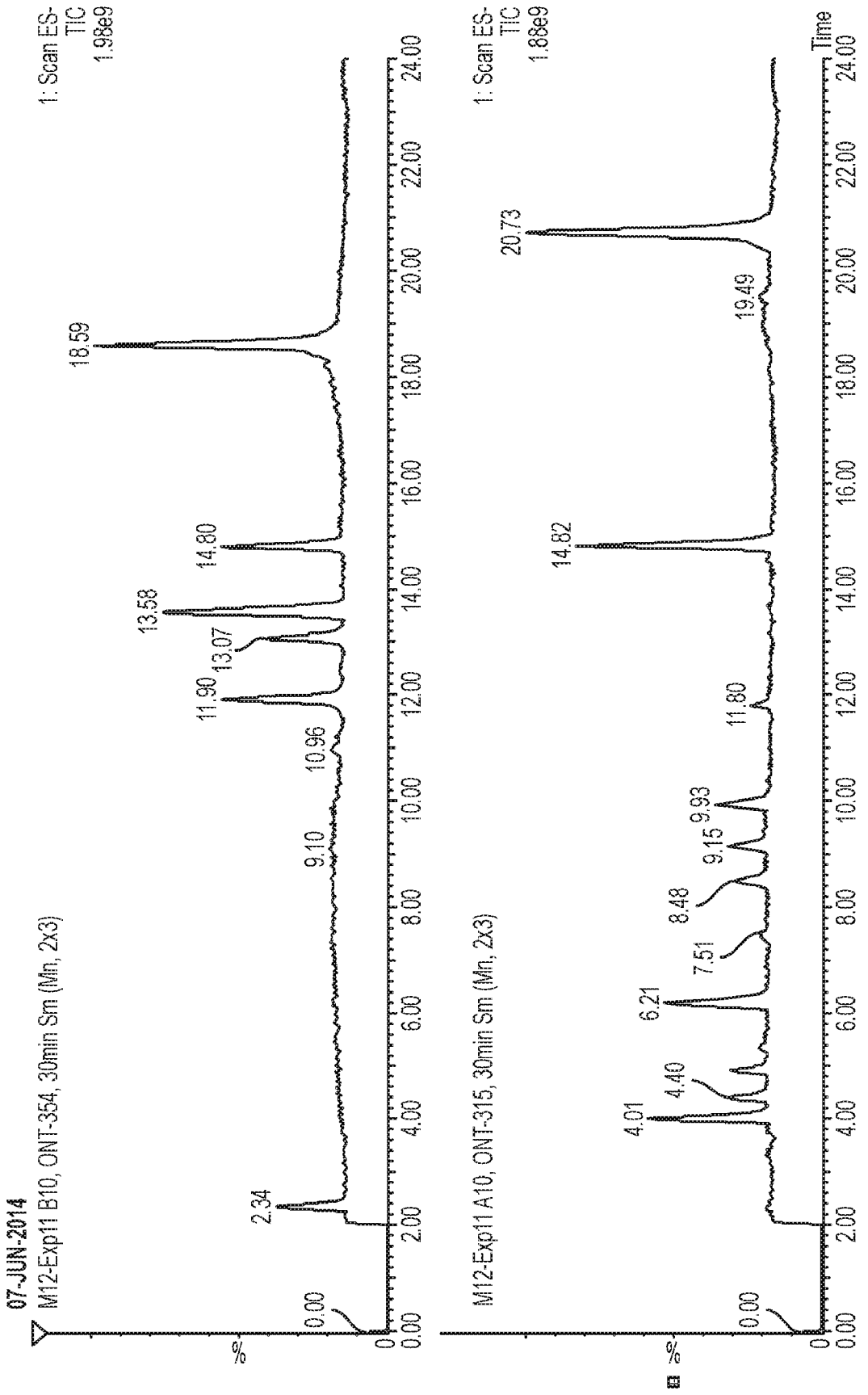

07-JUN-2014
M12-Exp11 B10, ONT-354, 30min Sm (Mn, 2x3)
1: Scan ES-
TIC
1.98e9
18.59
14.80
13.58
13.07
11.90
10.96
9.10
2.34
0.00
%
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
M12-Exp11 A10, ONT-315, 30min Sm (Mn, 2x3)
1: Scan ES-
TIC
1.88e9
20.73
14.82
19.49
11.80
9.93
9.15
8.48
7.51
6.21
4.40
4.01
0.00
%
Time

FIG. 19

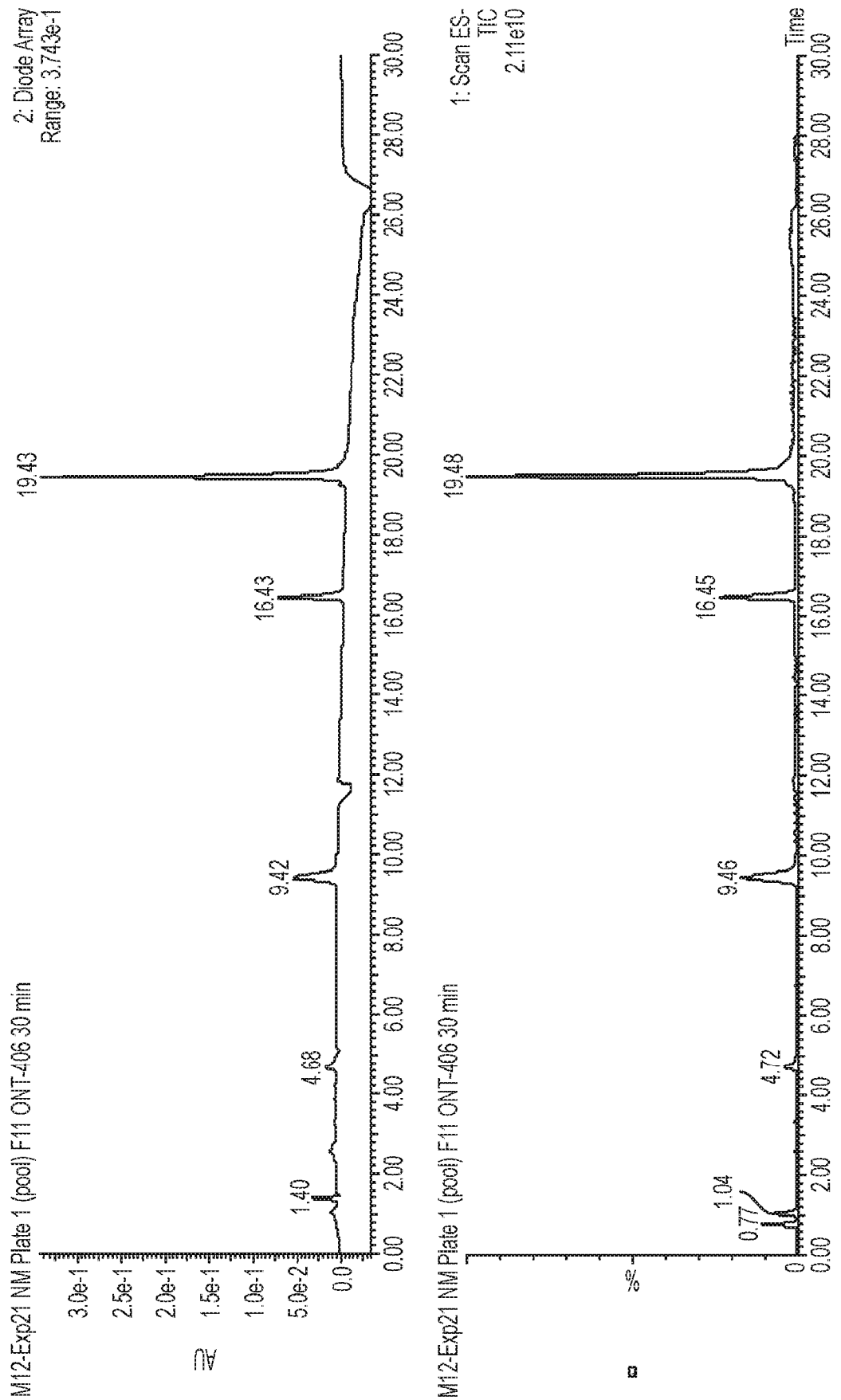

2: Diode Array
Range: 3.743e-1
M12-Exp21 NM Plate 1 (pool) F11 ONT-406 30 min
AU
3.0e-1
2.5e-1
2.0e-1
1.5e-1
1.0e-1
5.0e-2
0.0
19.43
16.43
9.42
4.68
1.40
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
1: Scan ES-
TIC
2.11e10
M12-Exp21 NM Plate 1 (pool) F11 ONT-406 30 min
%
19.48
16.45
9.46
4.72
1.04
0.77
0
Time

FIG. 20

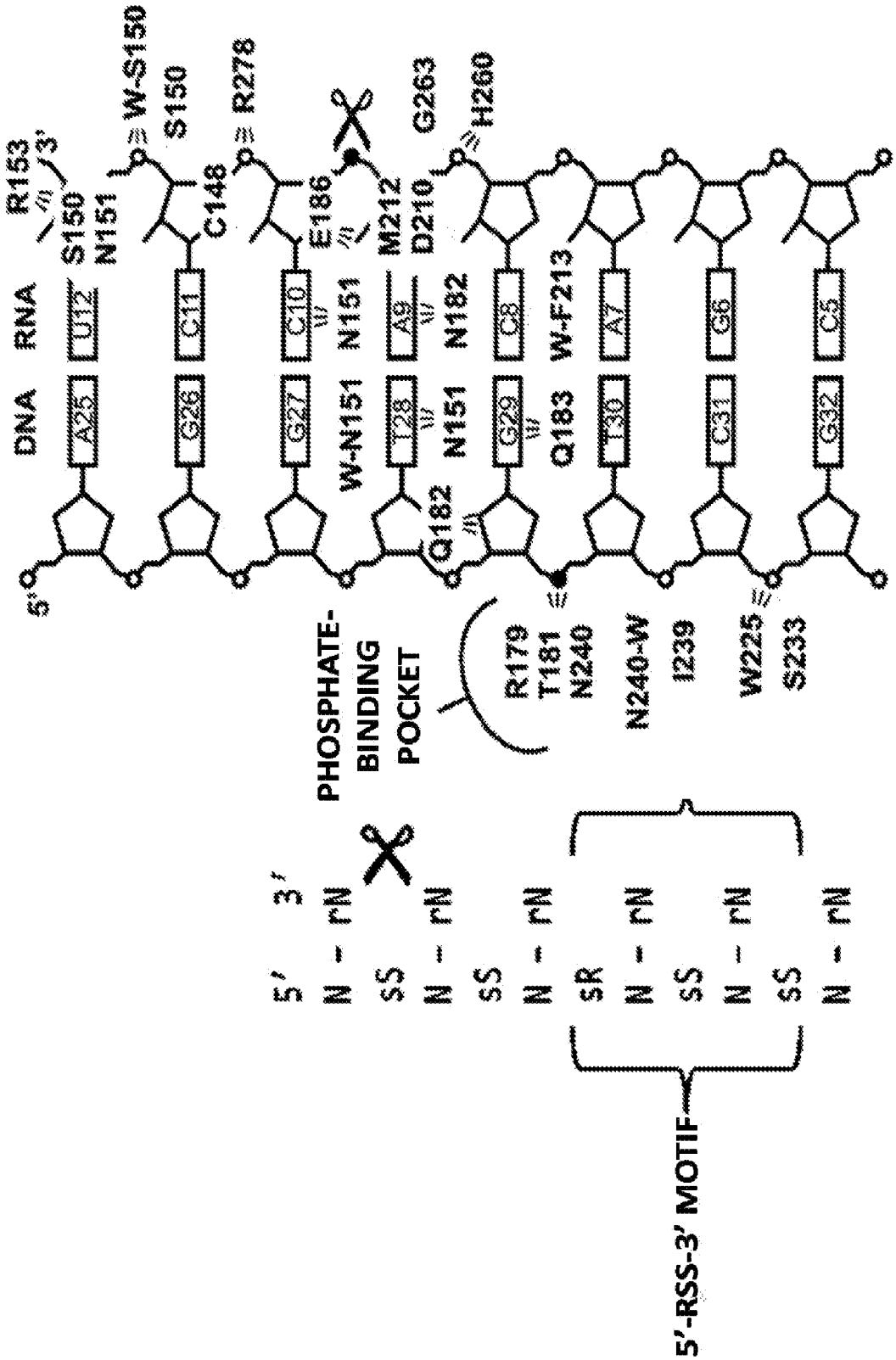
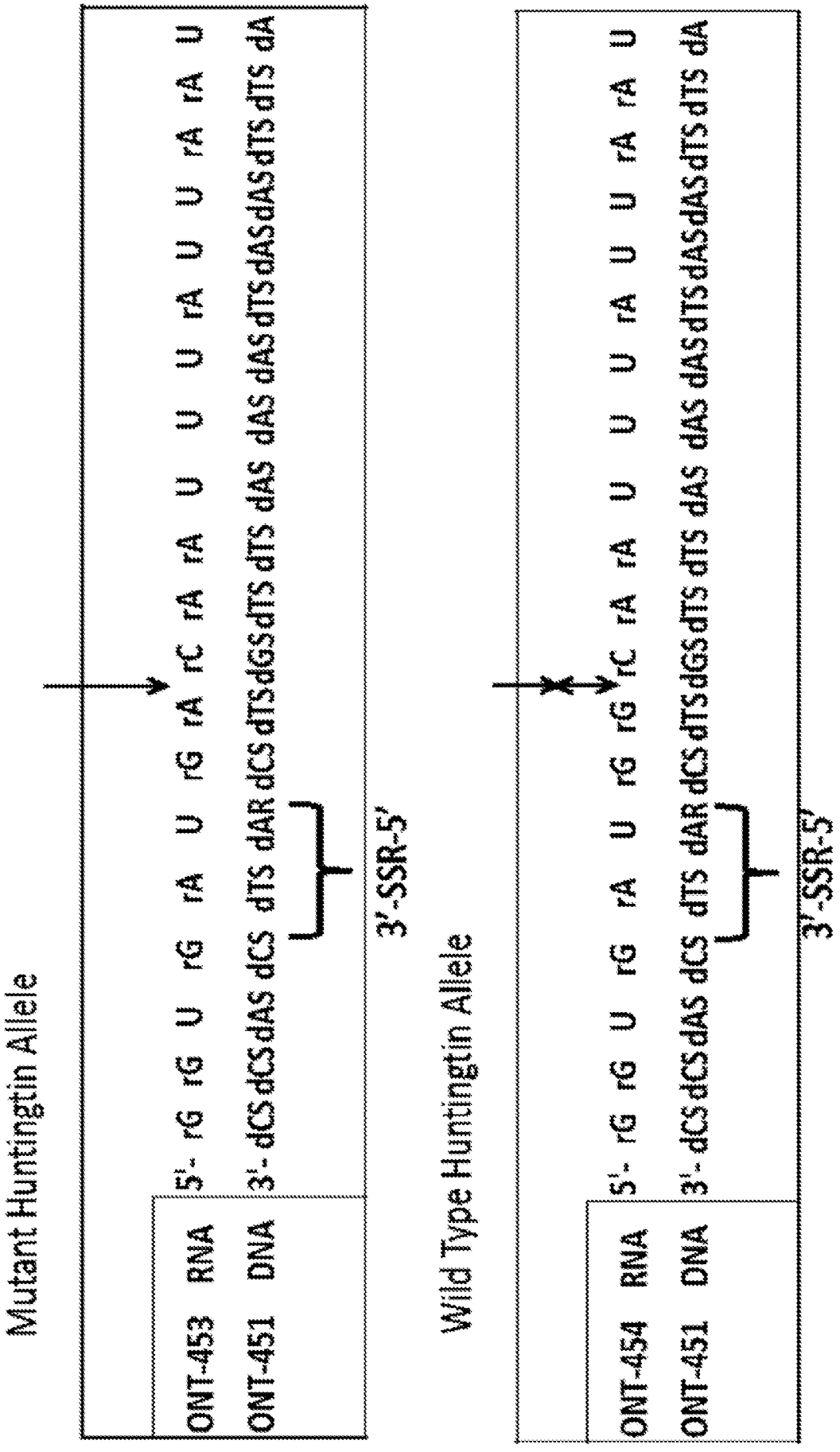
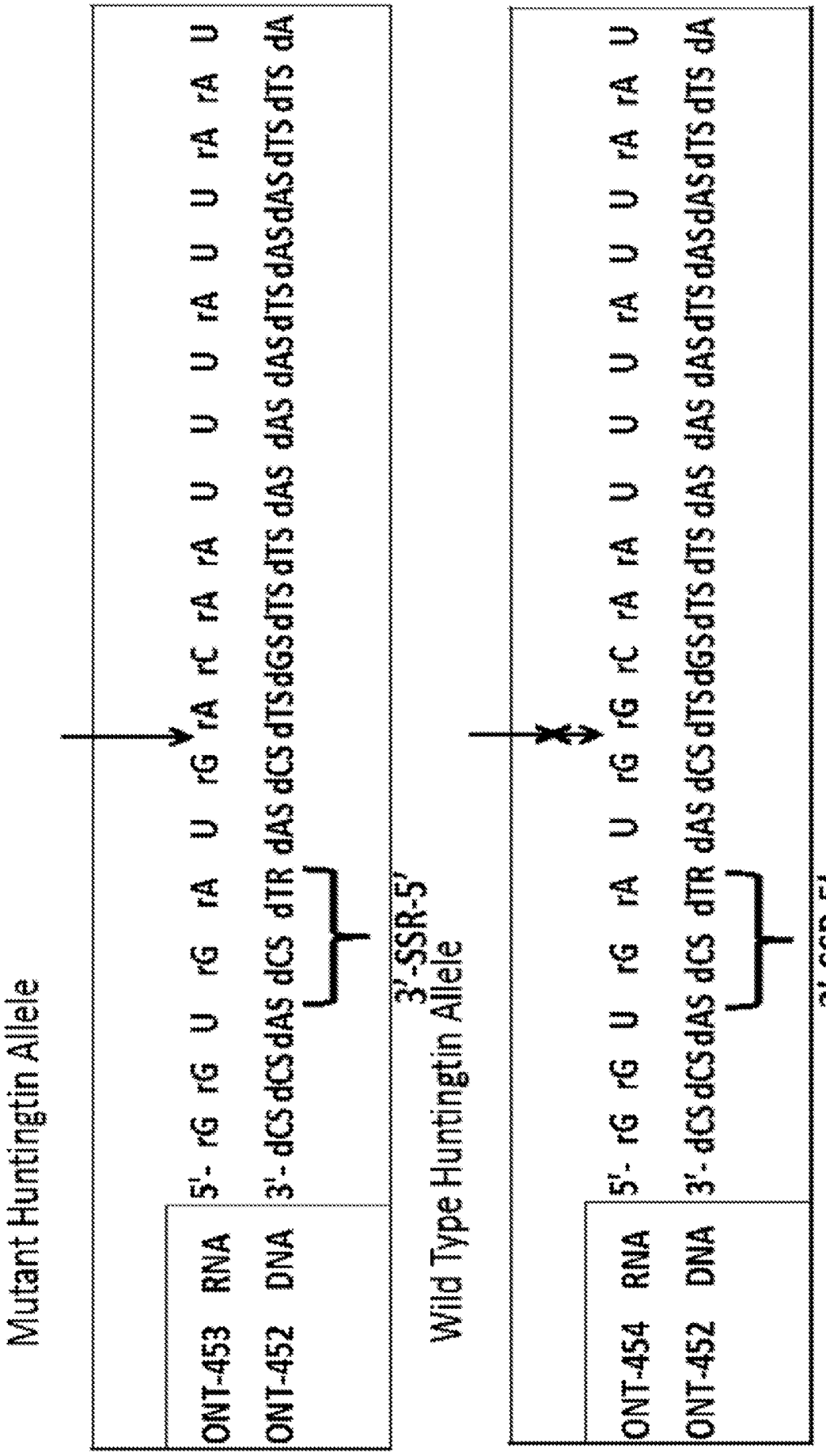

DNA
RNA
R153
3'
S150
N151
W-S150
S150
C148
R278
E186
M212
G263
D210
H260
N182
W-F213
A25
U12
G26
C11
G27
C10
W-N151
N151
T28
A9
N151
Q182
G29
C8
Q183
T30
A7
C31
G6
G32
C5
5'
PHOSPHATE-
BINDING
POCKET
R179
T181
N240
N240-W
I239
W225
S233
5'
3'
N - rN
sS
N - rN
sS
N - rN
sR
N - rN
sS
N - rN
sS
N - rN
5'-RSS-3' MOTIF

FIG. 21



(PANEL A)
FIG. 22



(PANEL B)

FIG. 22 (CONTINUED)

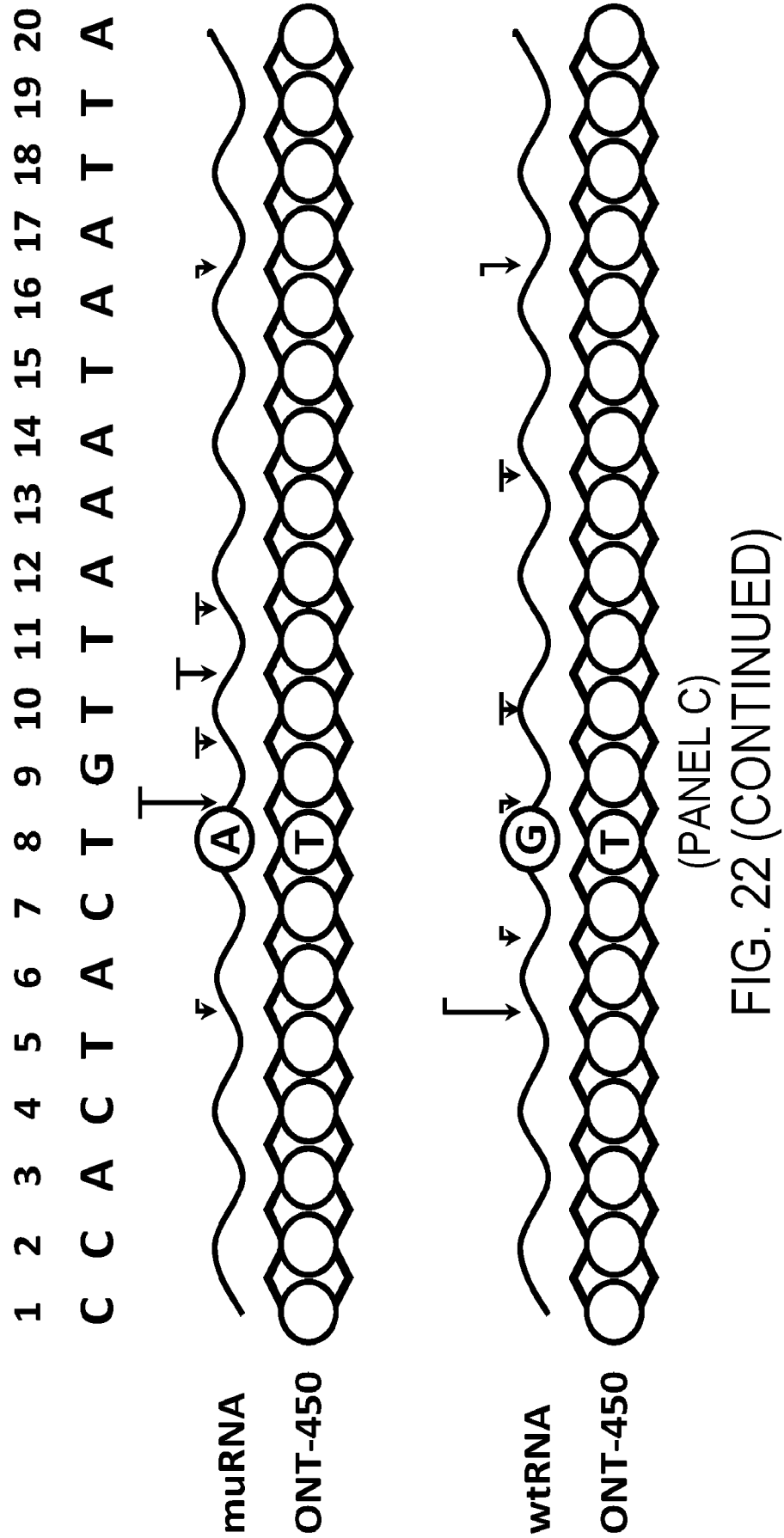
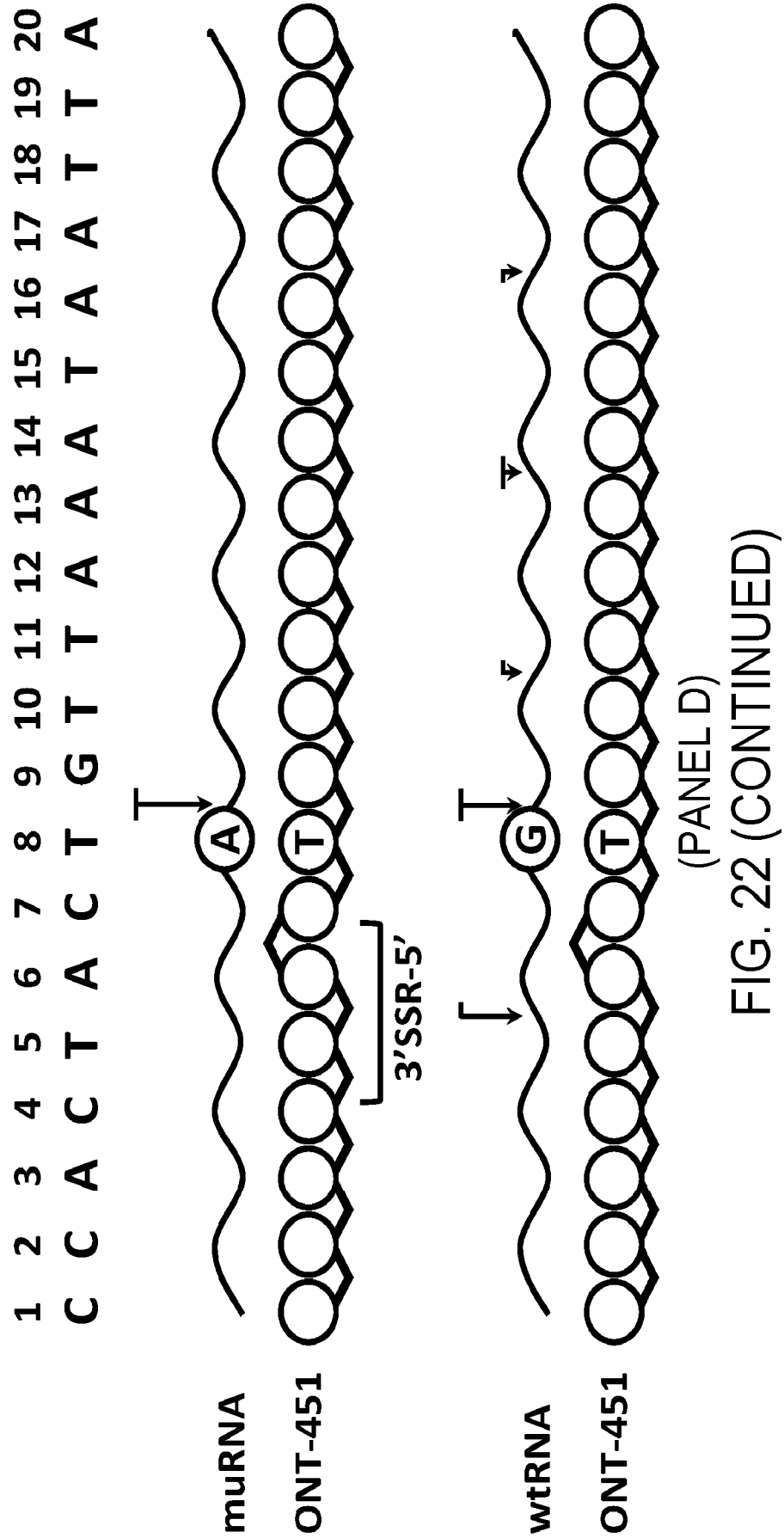


FIG. 22 (CONTINUED)
(PANEL C)



(PANEL D)

FIG. 22 (CONTINUED)

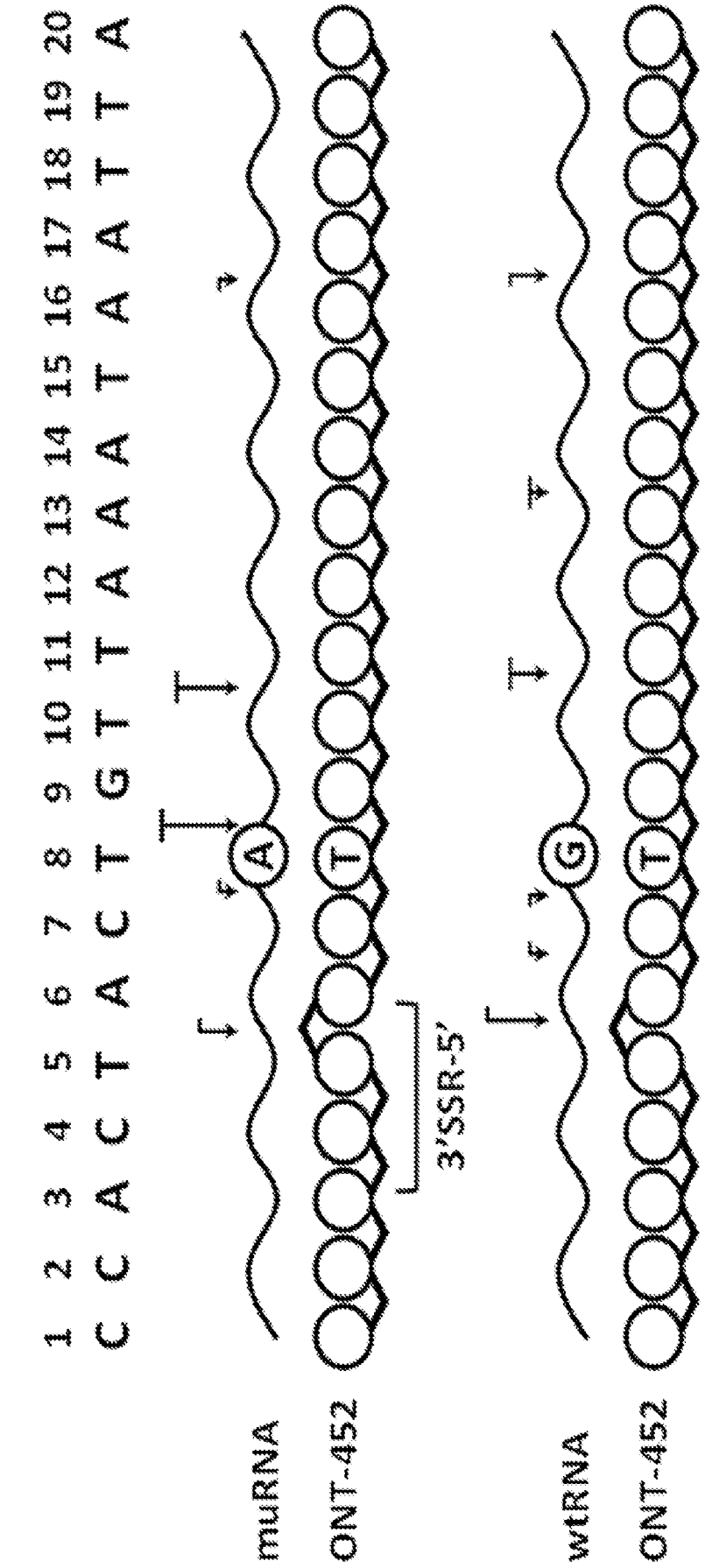
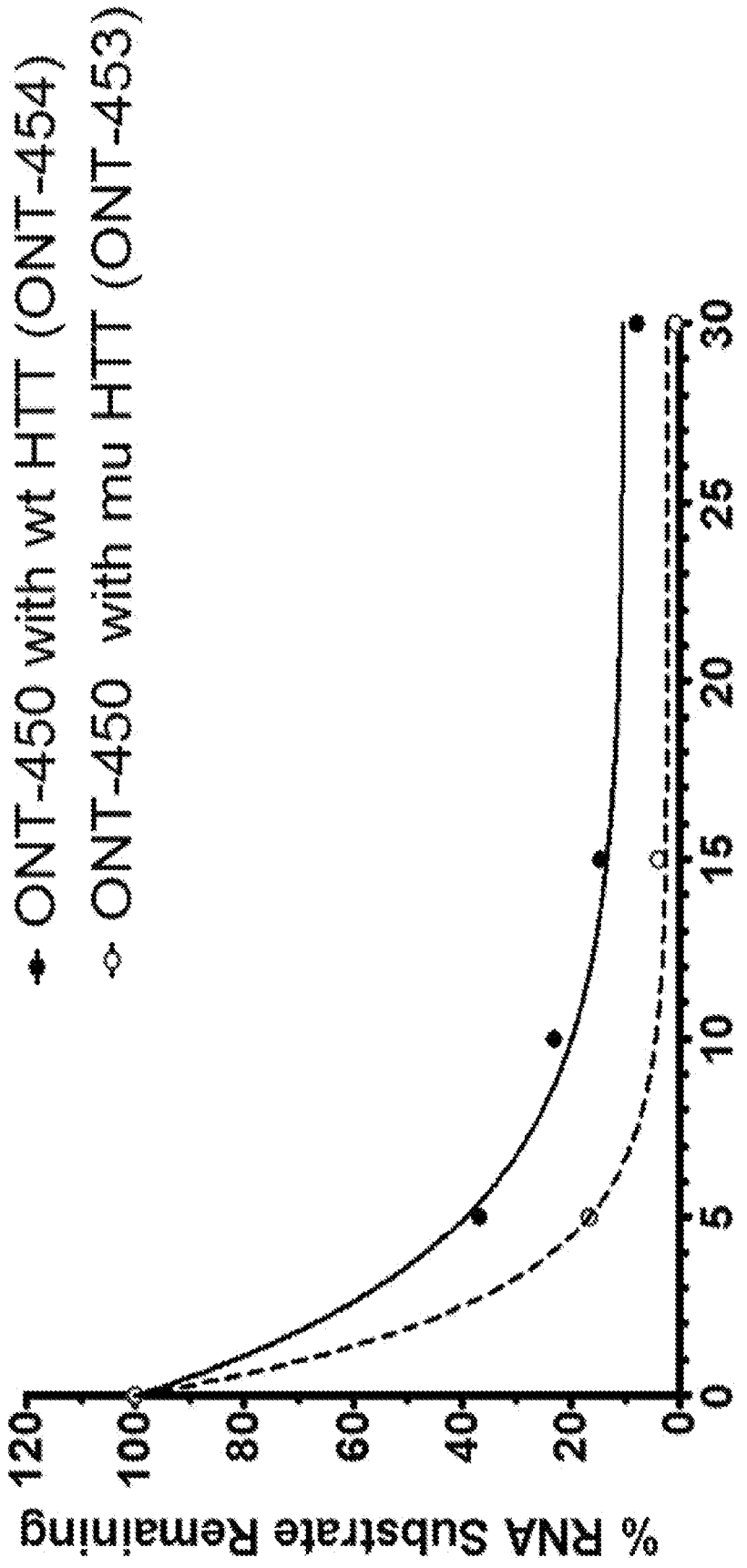
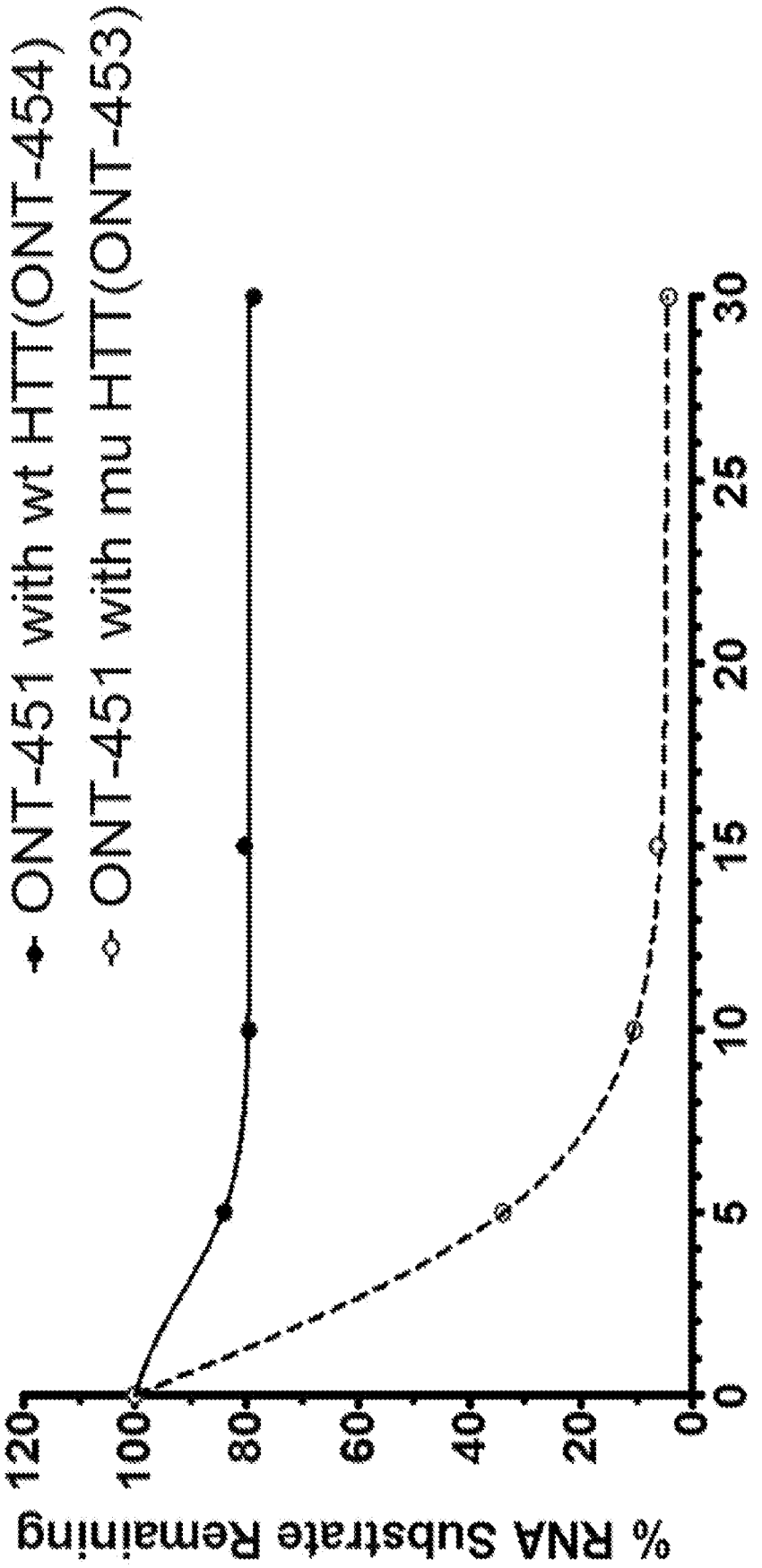


FIG. 22 (CONTINUED)
(PANEL E)

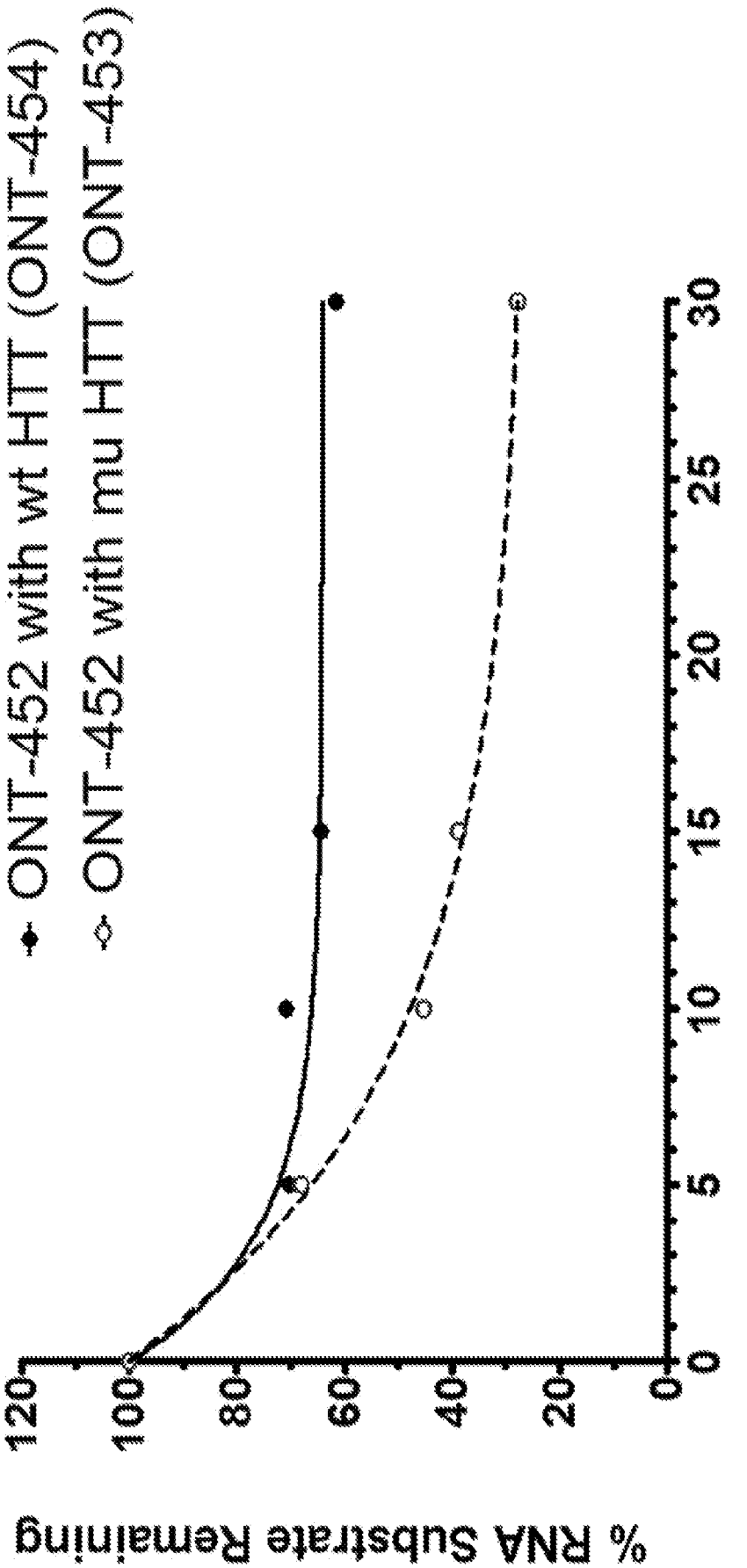


(PANEL F)

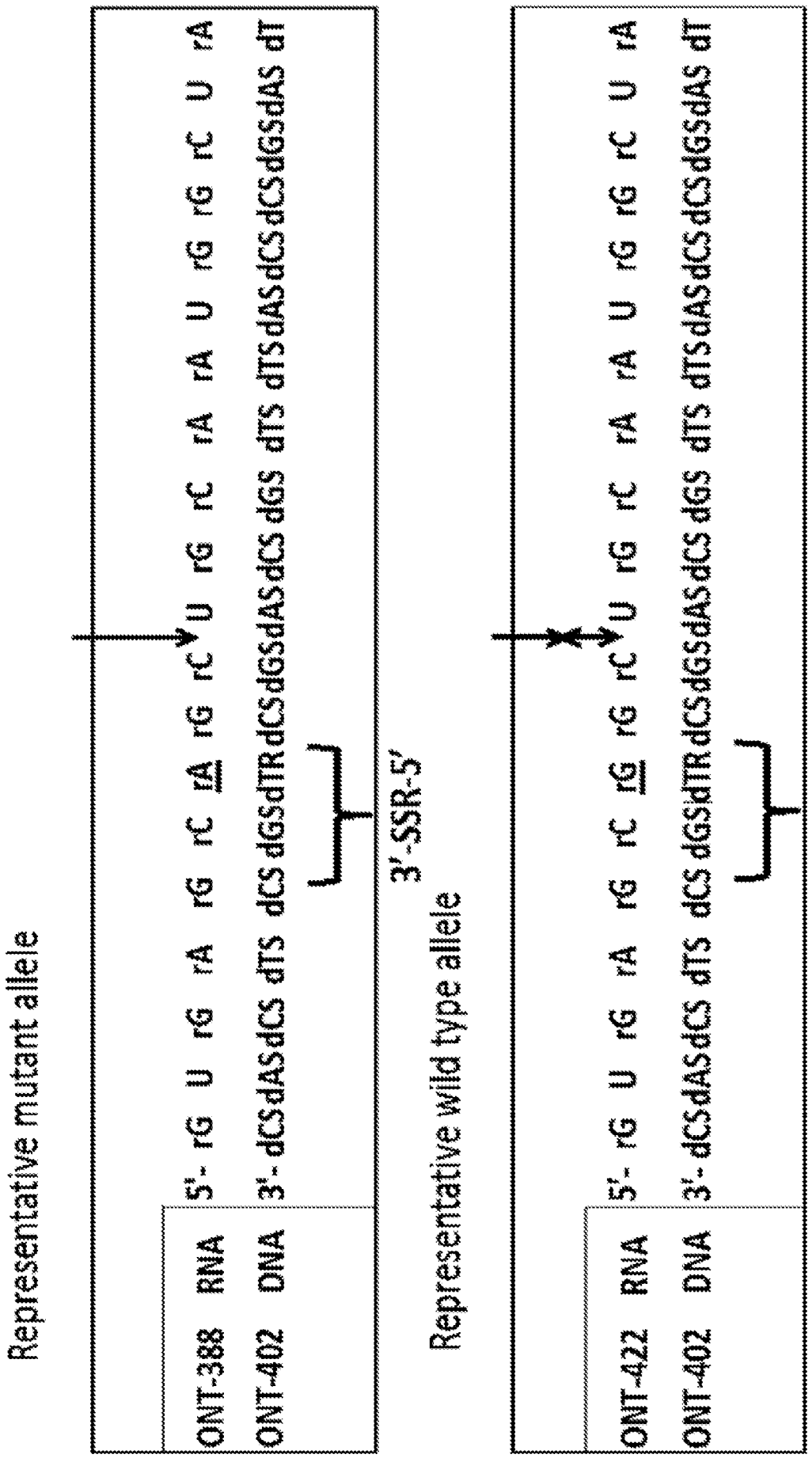
FIG. 22 (CONTINUED)



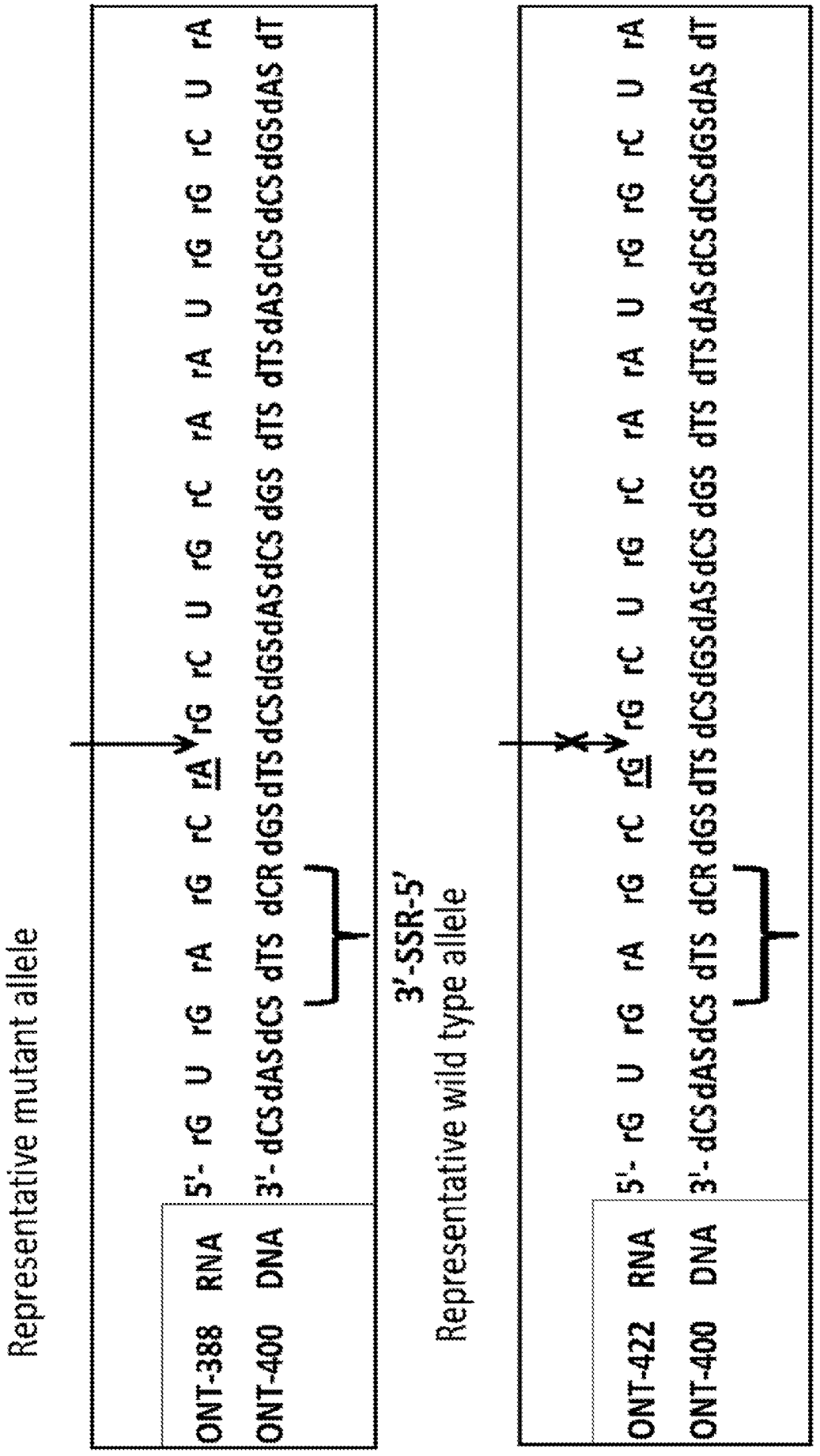
(PANEL G)
FIG. 22 (CONTINUED)



(PANEL H)
FIG. 22 (CONTINUED)

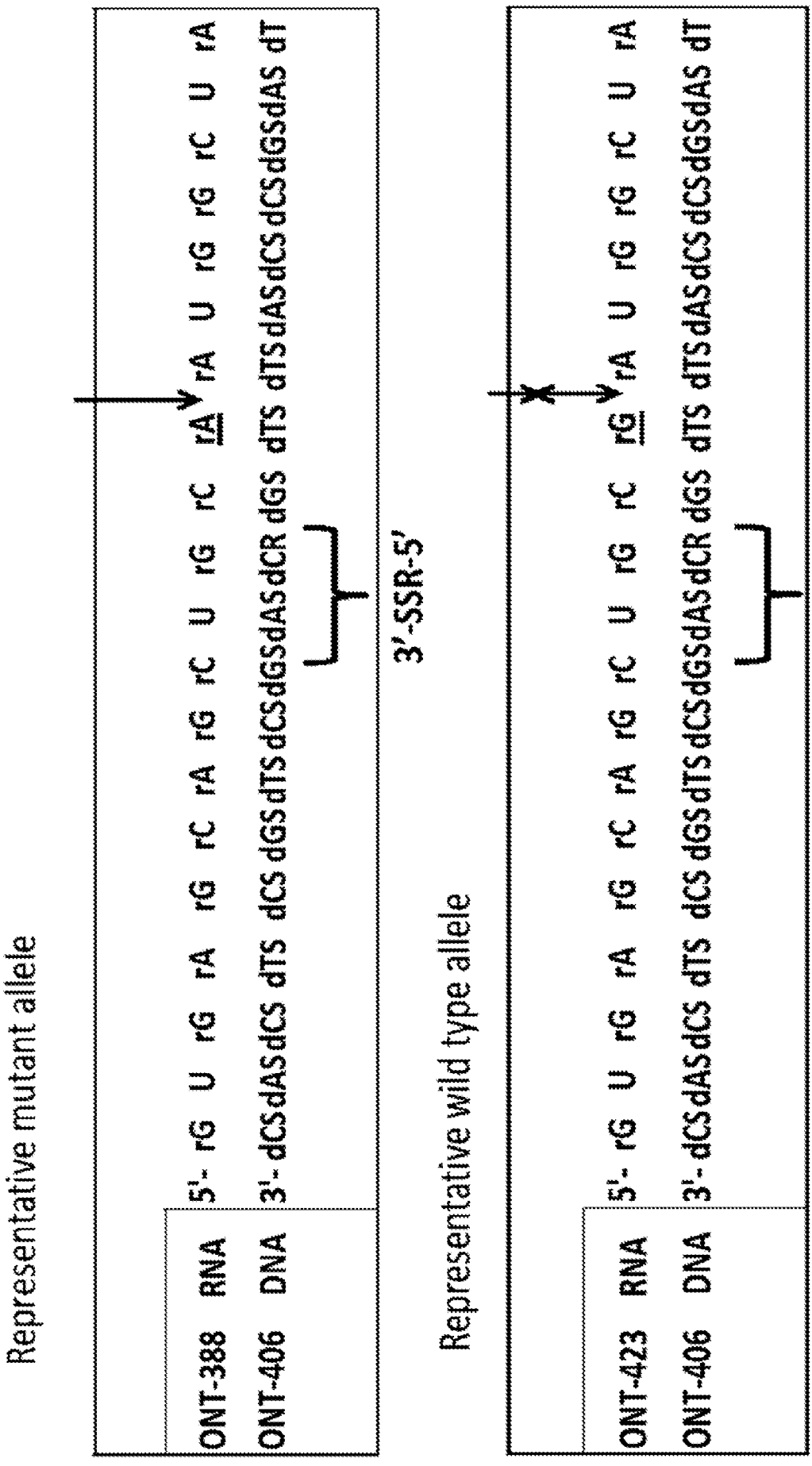


(PANEL A)
FIG. 23



(PANEL B)

FIG. 23 (CONTINUED)



(PANEL C)

FIG. 23 (CONTINUED)

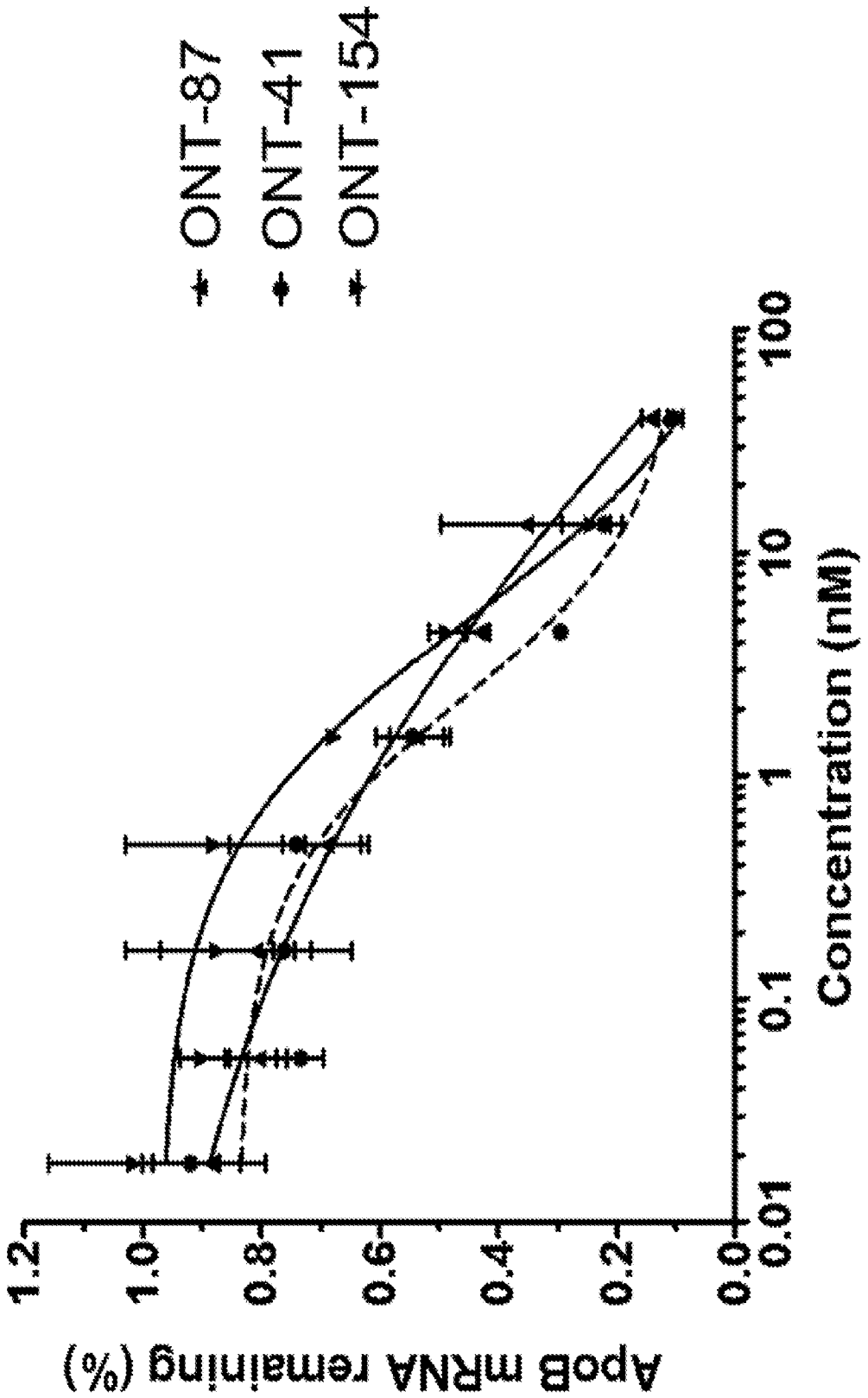
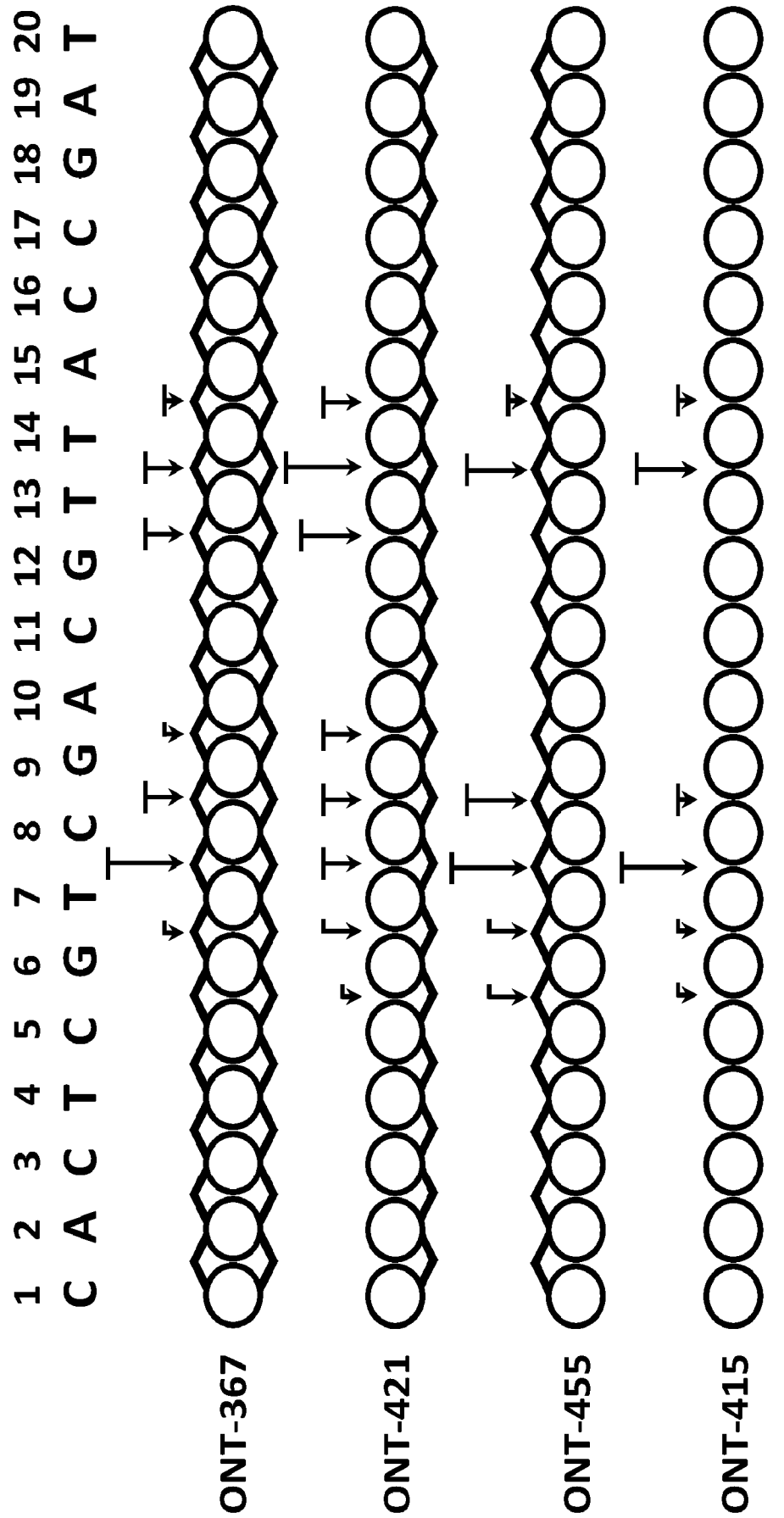
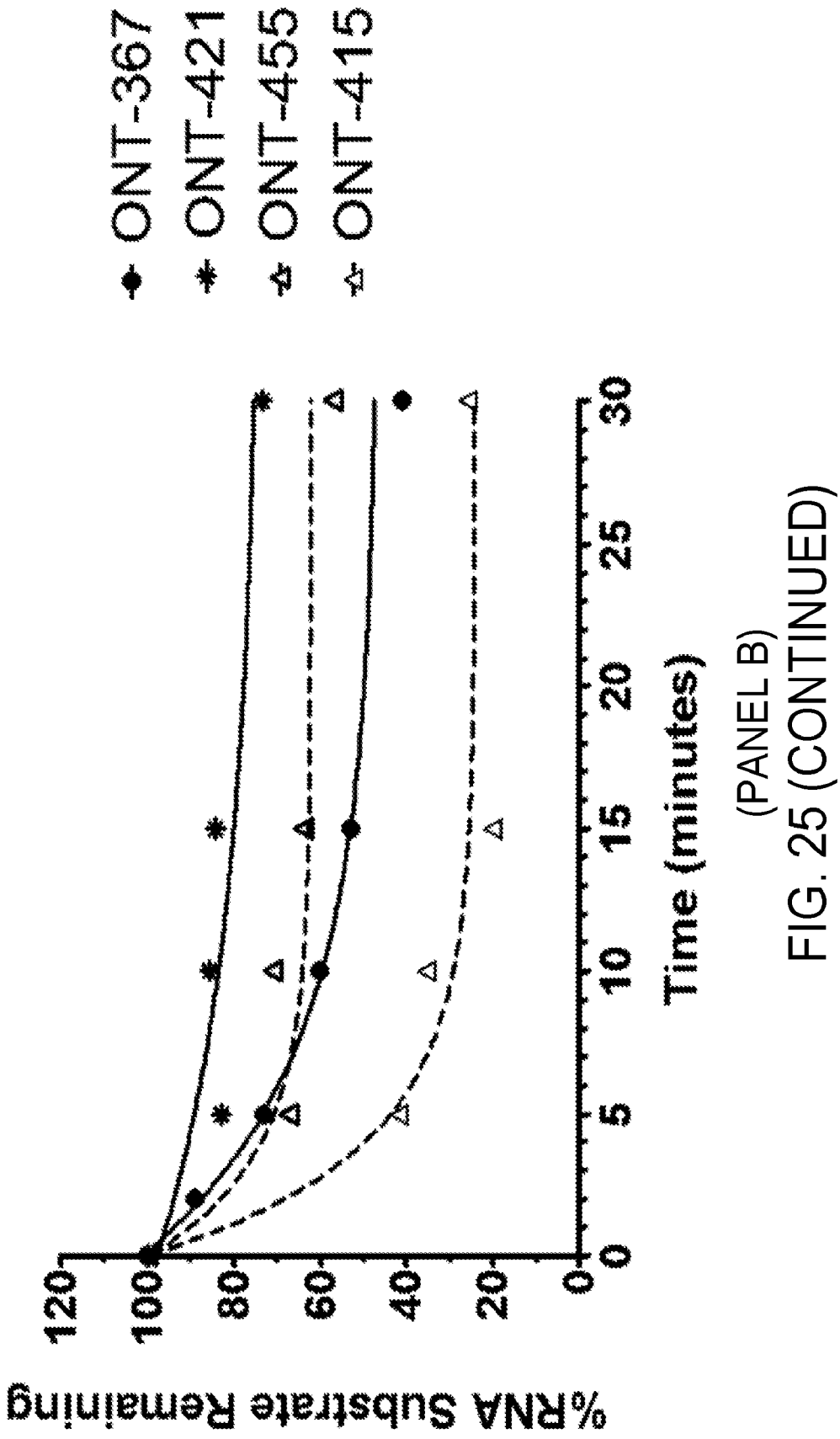

ApoB mRNA remaining (%)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.01
0.1
1
10
100
Concentration (nM)
ONT-87
ONT-41
ONT-154

FIG. 24

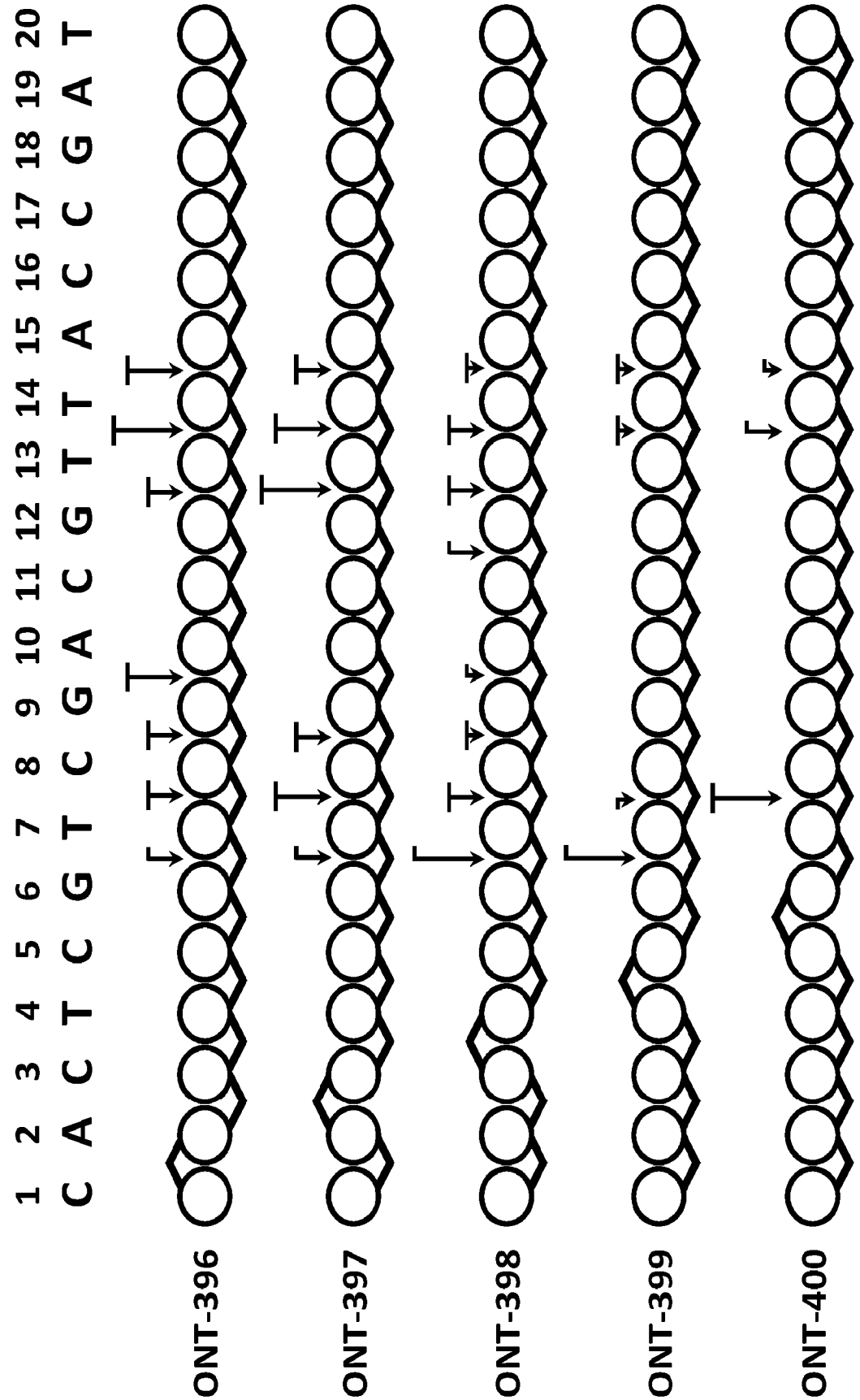


(PANEL A)
FIG. 25



(PANEL B)

FIG. 25 (CONTINUED)



(PANEL A)
FIG. 26

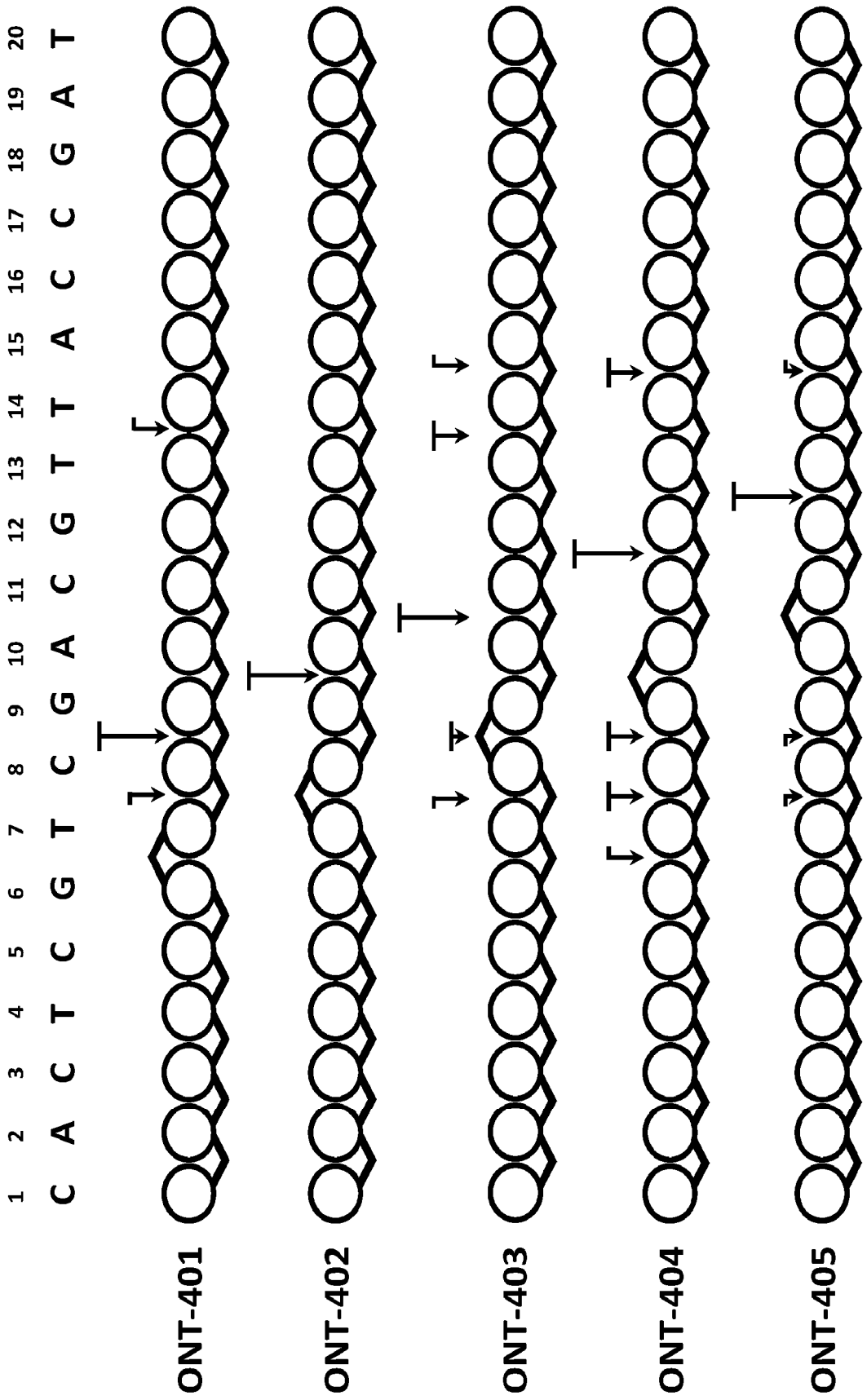
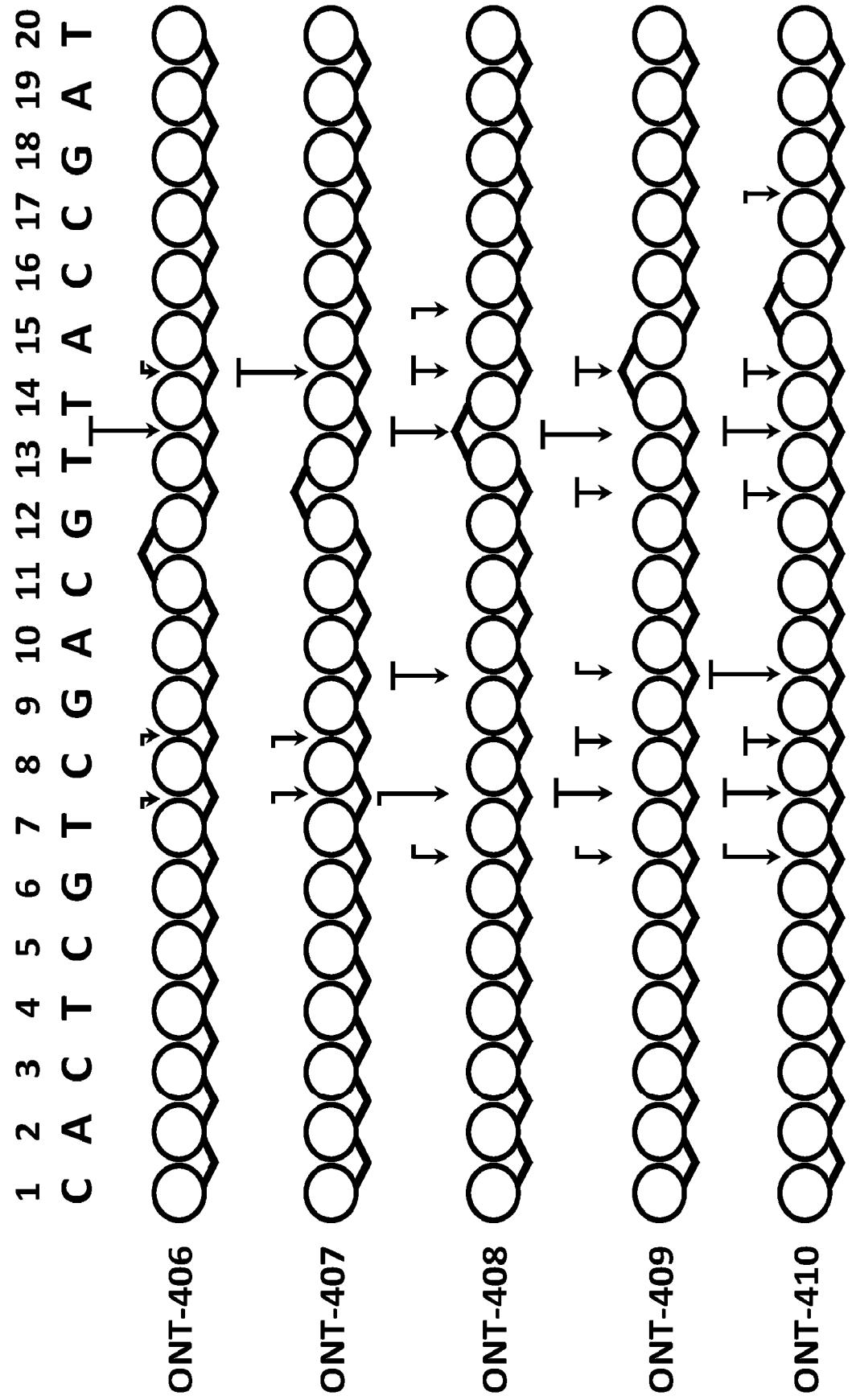
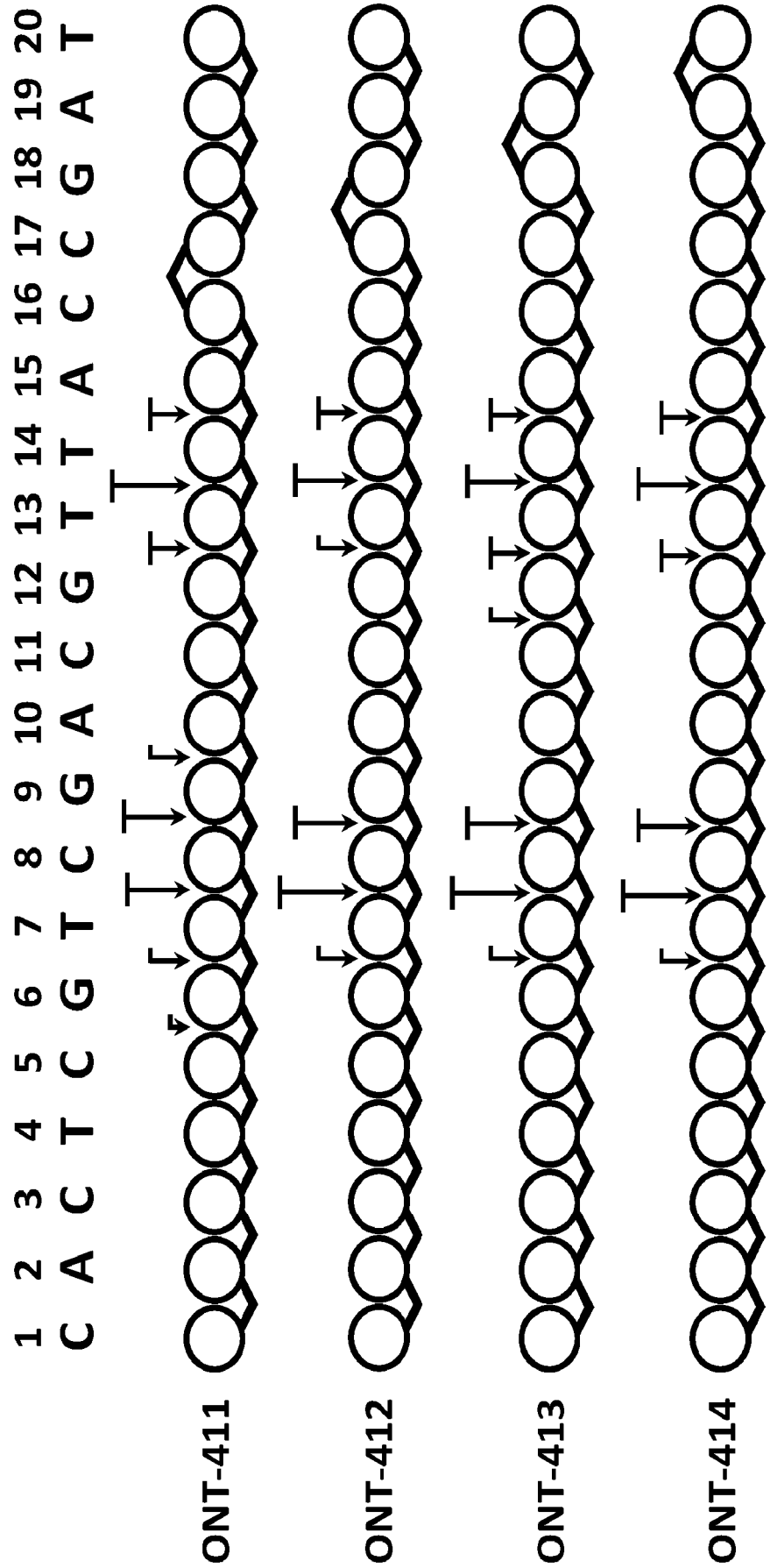


FIG. 26 (CONTINUED)
(PANEL B)

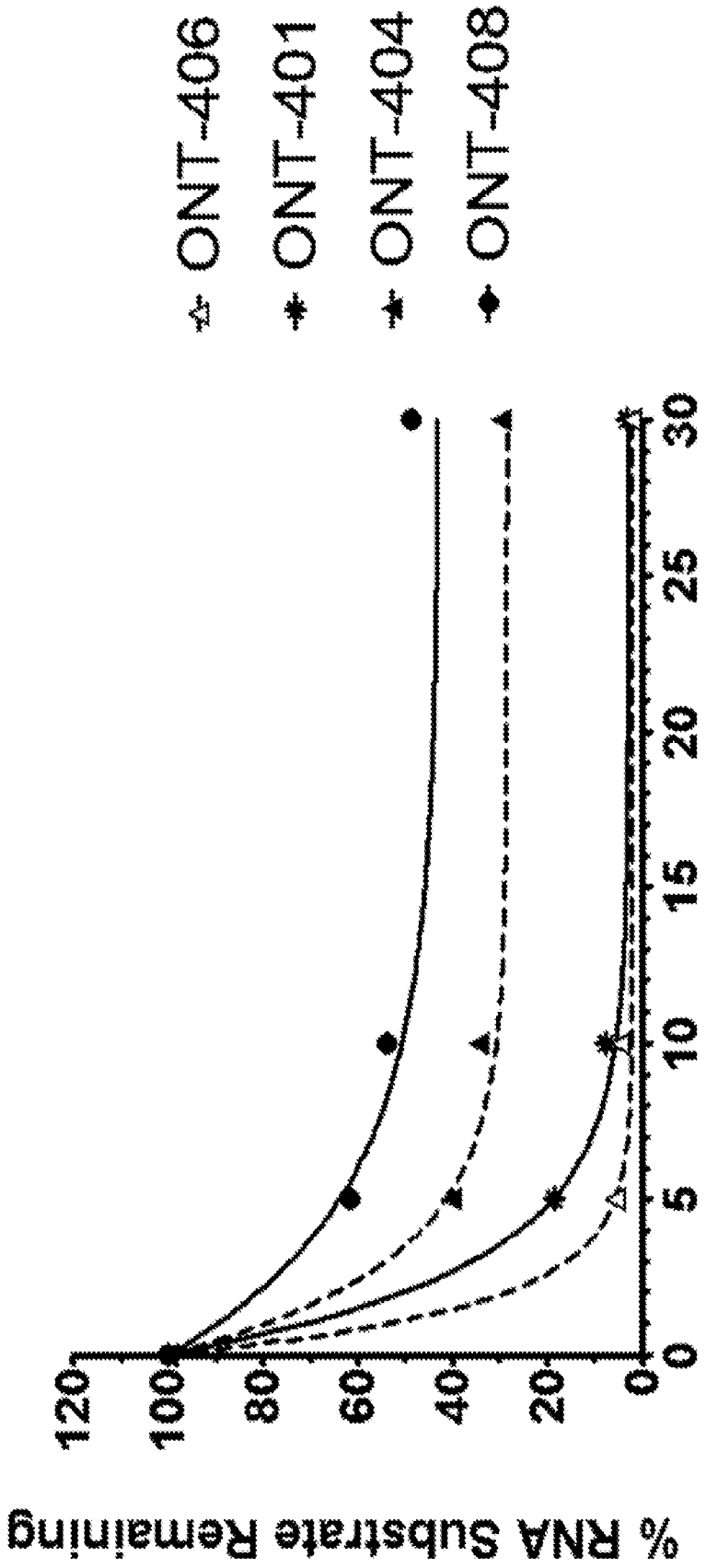


(PANEL C)

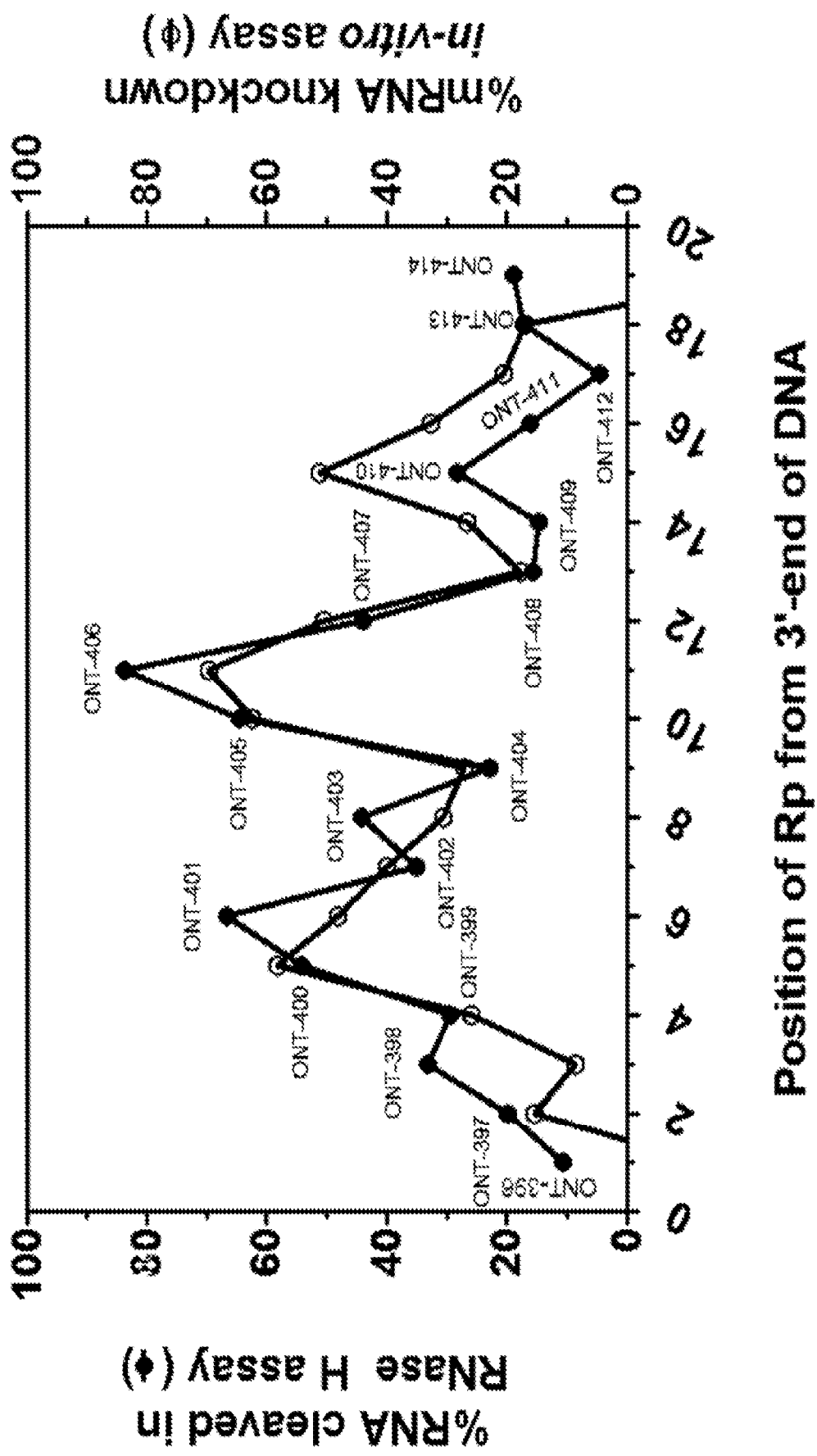
FIG. 26 (CONTINUED)



(PANEL D)
FIG. 26 (CONTINUED)



(PANEL A)
FIG. 27



(PANEL B)

FIG. 27 (CONTINUED)

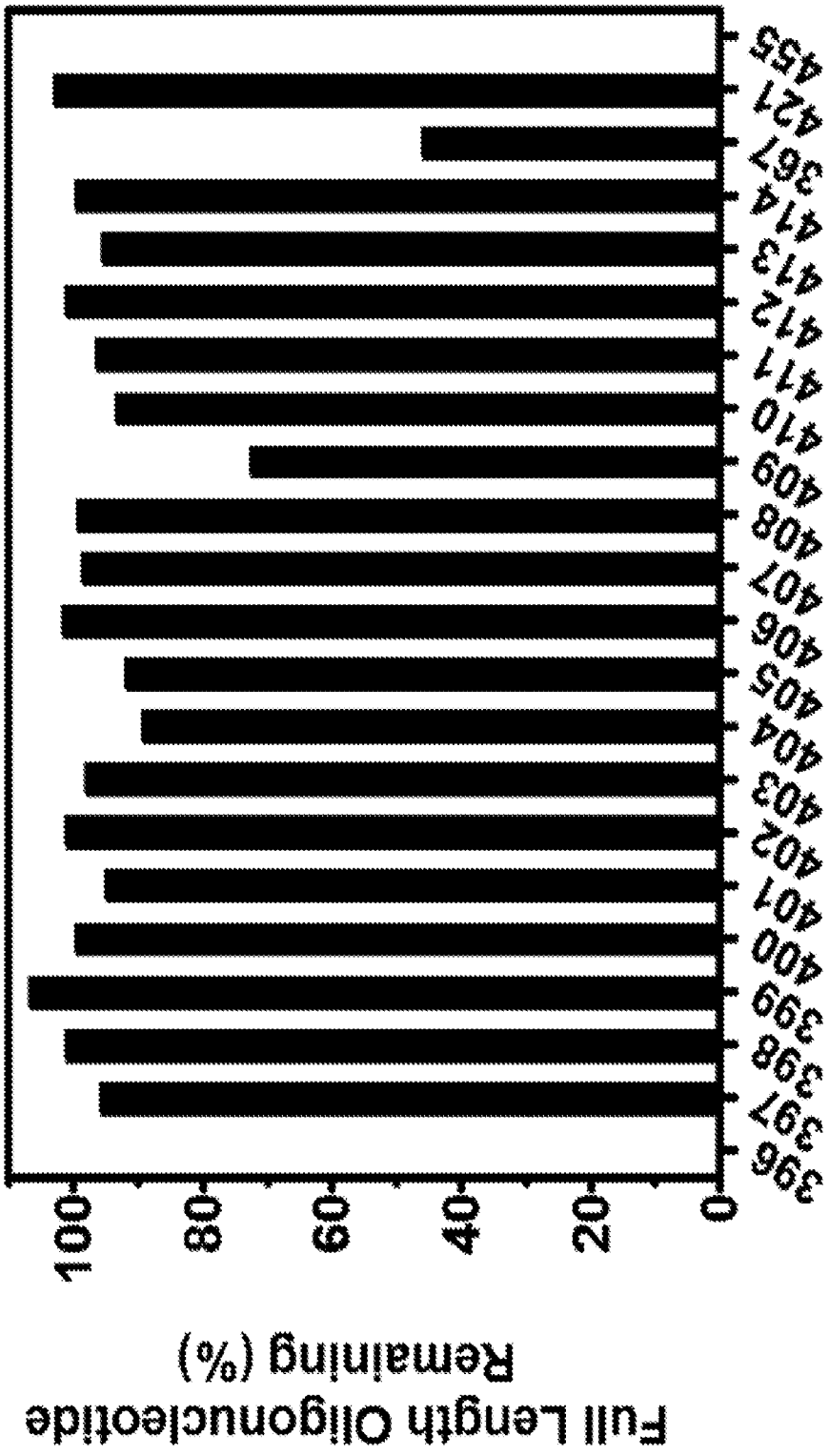
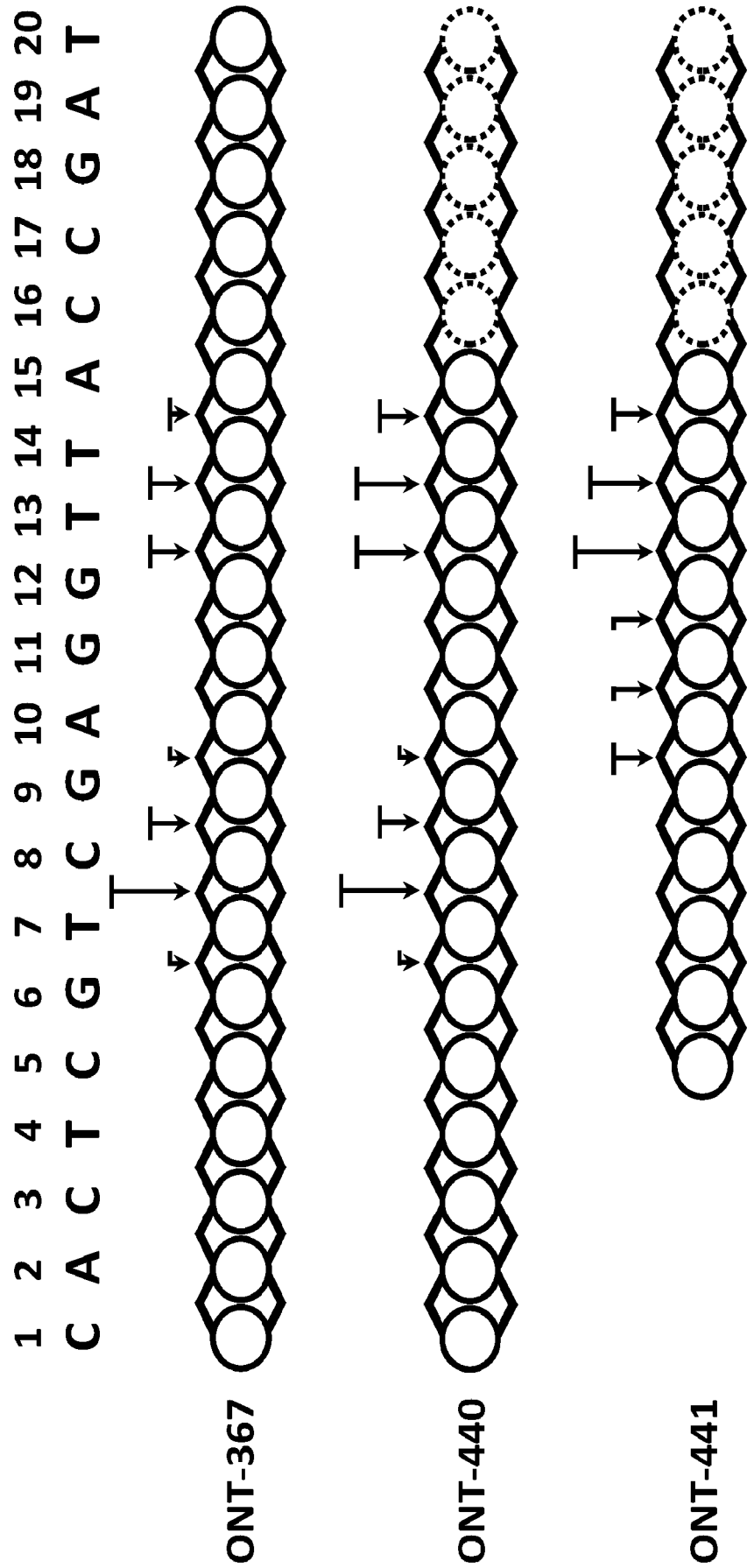
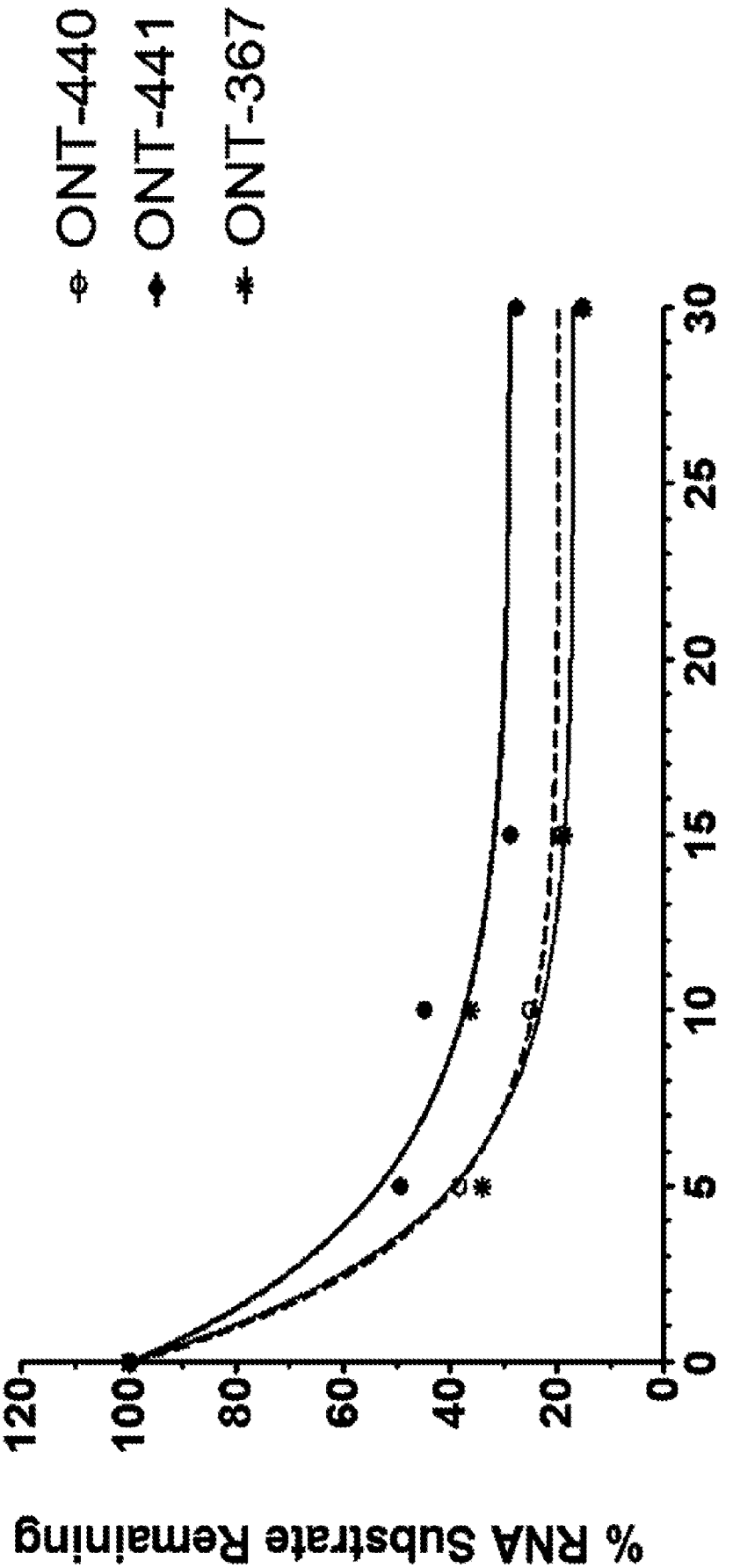

Full Length Oligonucleotide Remaining (%)
0
20
40
60
80
100
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
367
421
455

FIG. 28



(PANEL A)
FIG. 29



(PANEL B)
FIG. 29 (CONTINUED)

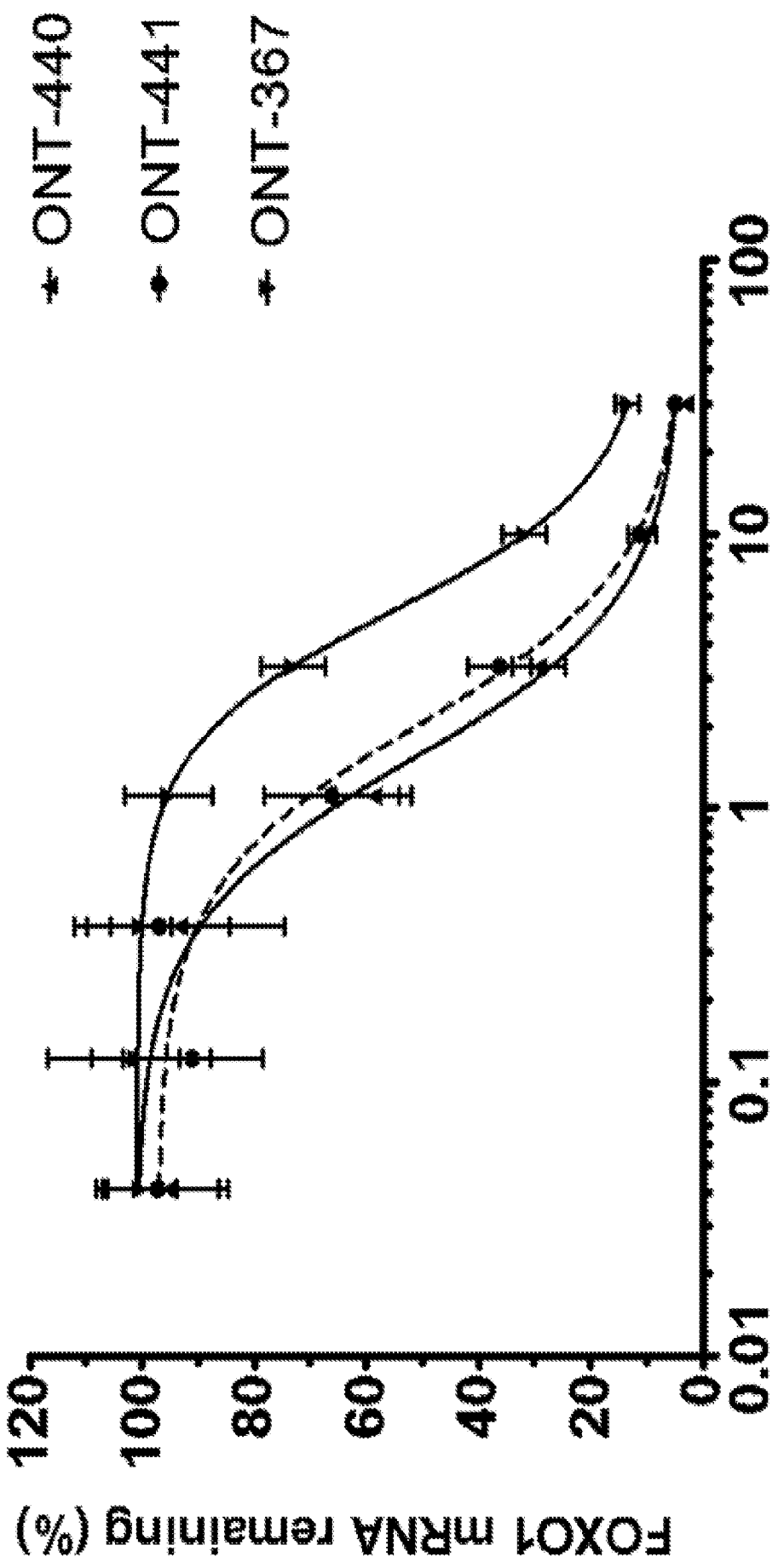


FIG. 29 (CONTINUED)
(PANEL C)

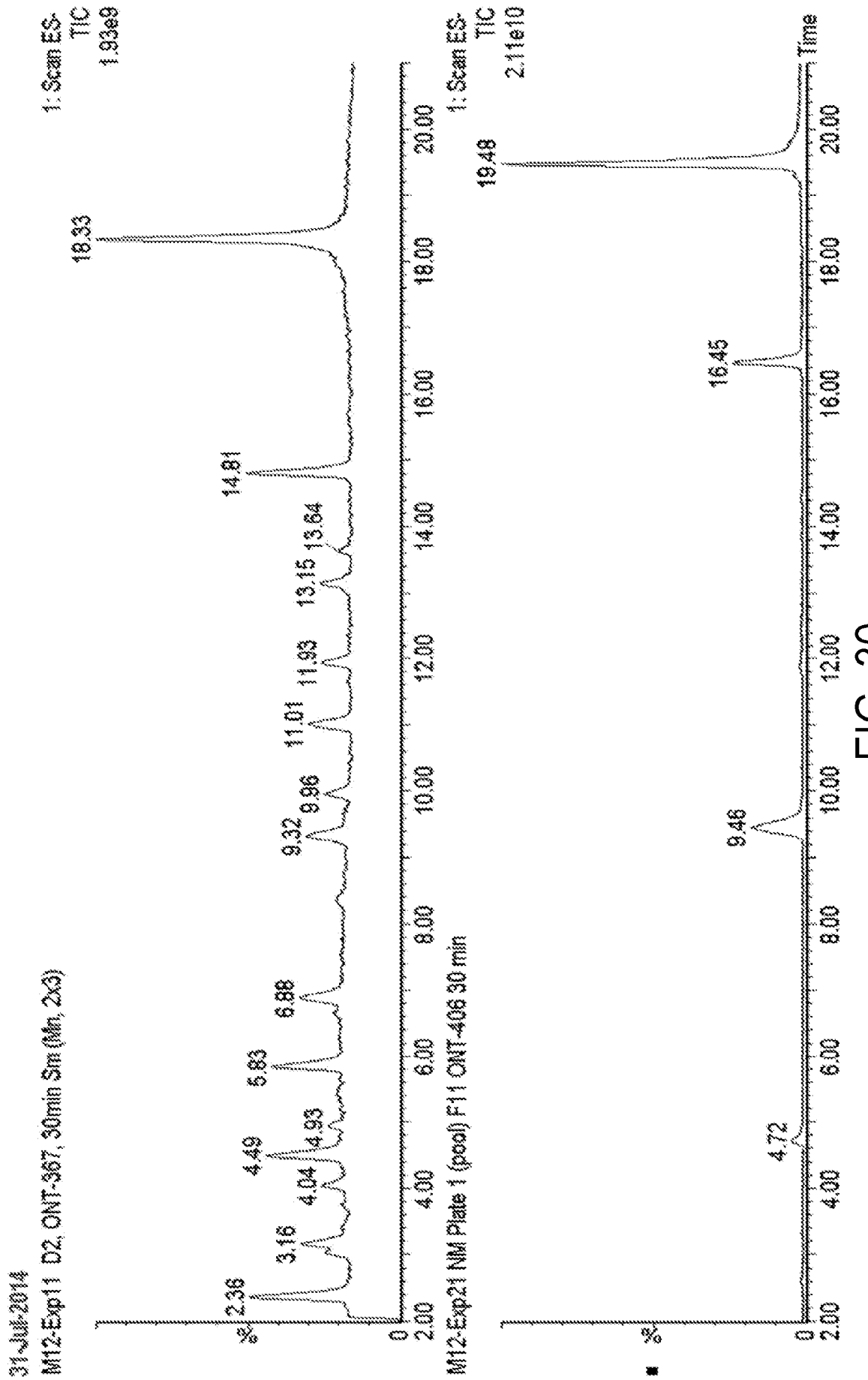
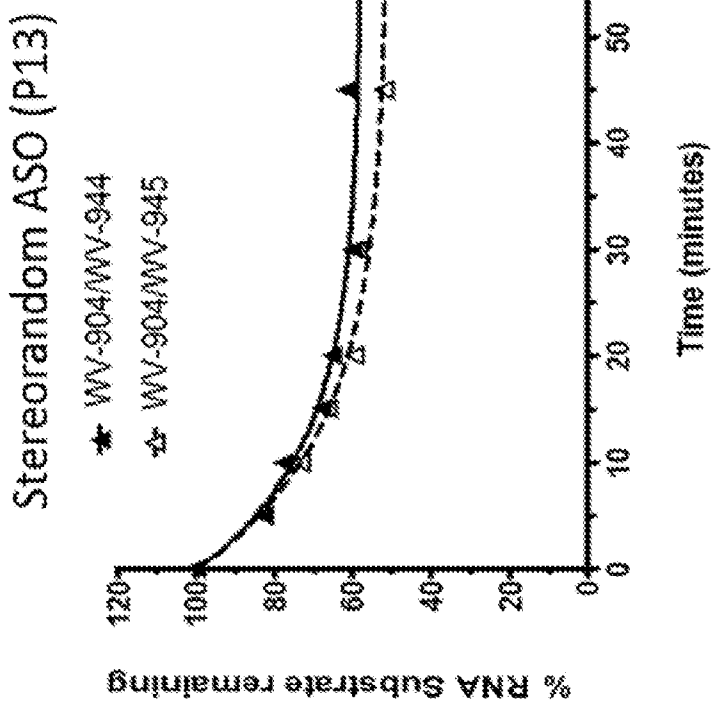
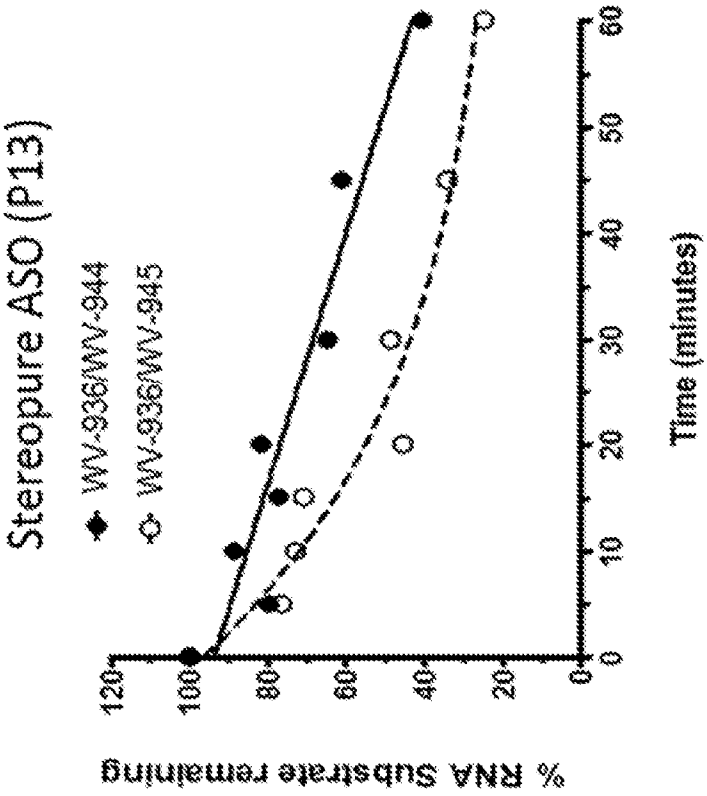

31-Jul-2014
M12-Exp11 D2, ONT-367, 30min Sm (Mn, 2x3)
1: Scan ES-
TIC
1.93e9
18.33
14.81
13.64
13.15
11.93
11.01
9.96
9.32
6.88
5.83
4.93
4.49
4.04
3.16
2.36
%
0
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
M12-Exp21 NM Plate 1 (pool) F11 ONT-406 30 min
1: Scan ES-
TIC
2.11e10
19.48
16.45
9.48
4.72
Time

FIG. 30





5'-3' (**wtRNA**) WV-944 rU rU rU rG rG rA rA rG rU rC rU rG rC rC rG rC rC rU rU rG rG rC rC rC

5'-3' (**muRNA**) WV-945 rU rU rU rG rG rA rA rG rU rC rU rG rU rG rC rC rC rU rU rG rG rC rC rC

3'-5' (**WV-904, WV-936**) T T C A G A C A C G G G G A A C A C G G G

(PANEL A)
FIG. 31

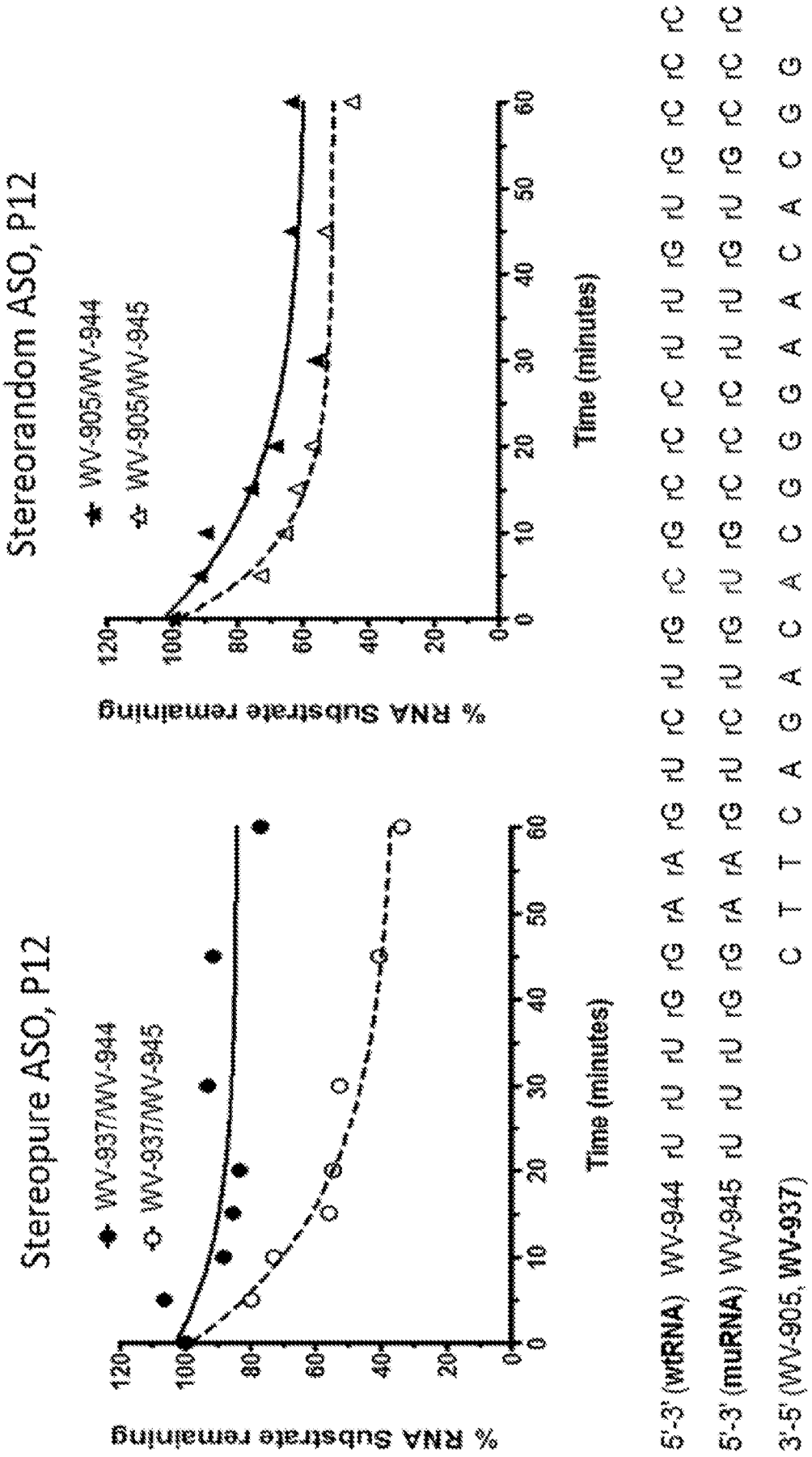


FIG. 31 (CONTINUED)
(PANEL B)

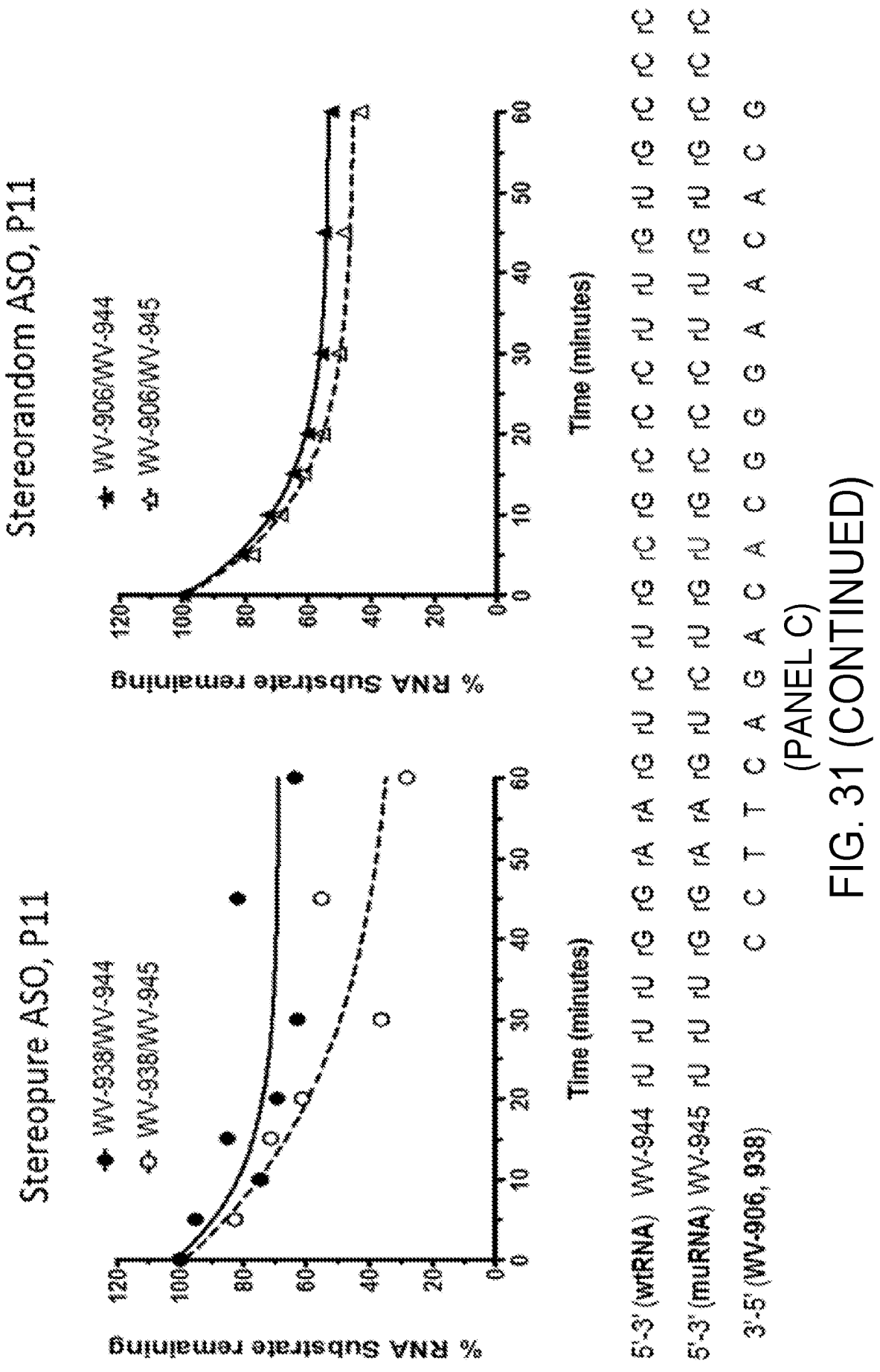
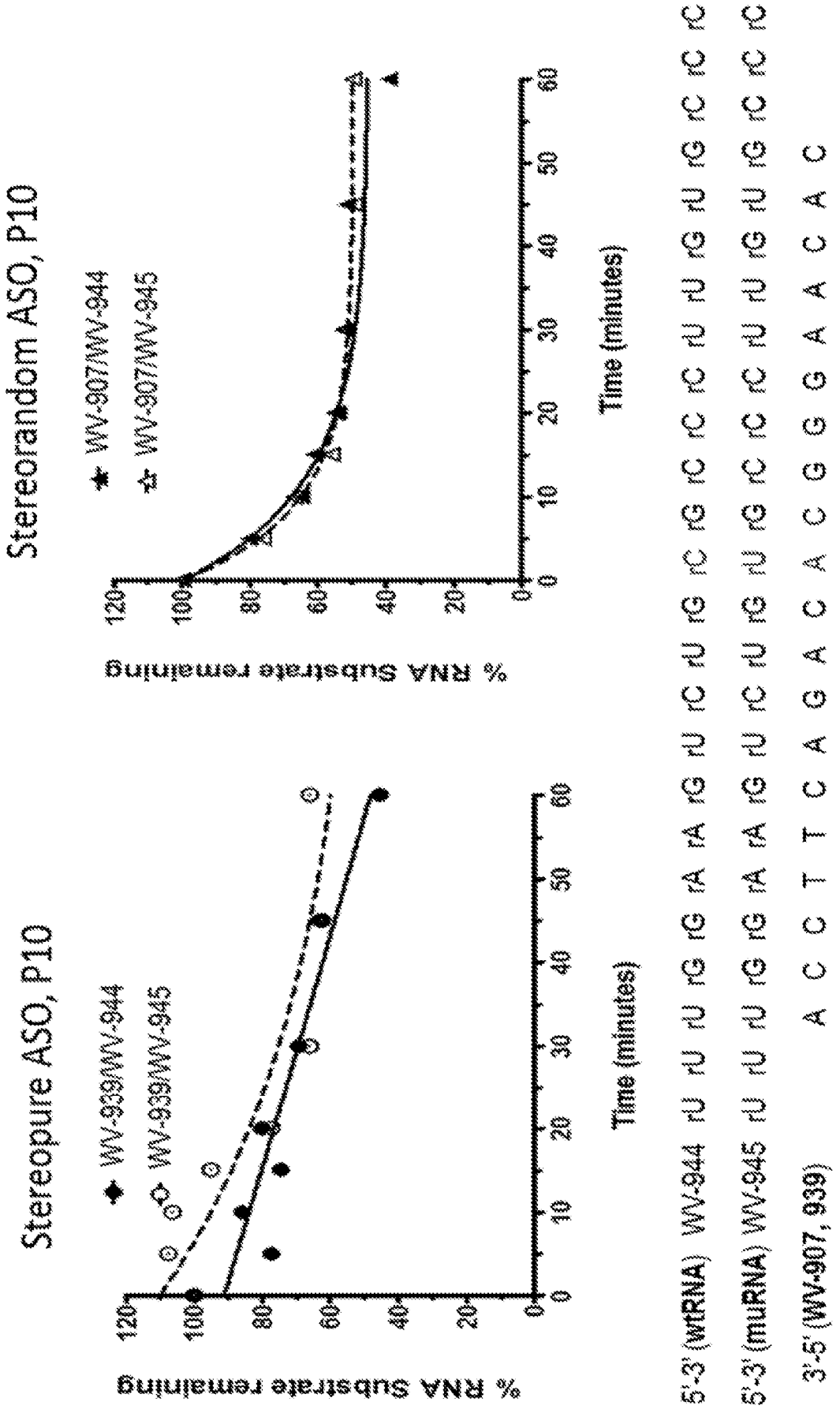


FIG. 31 (CONTINUED)
(PANEL C)



(PANEL D)

FIG. 31 (CONTINUED)

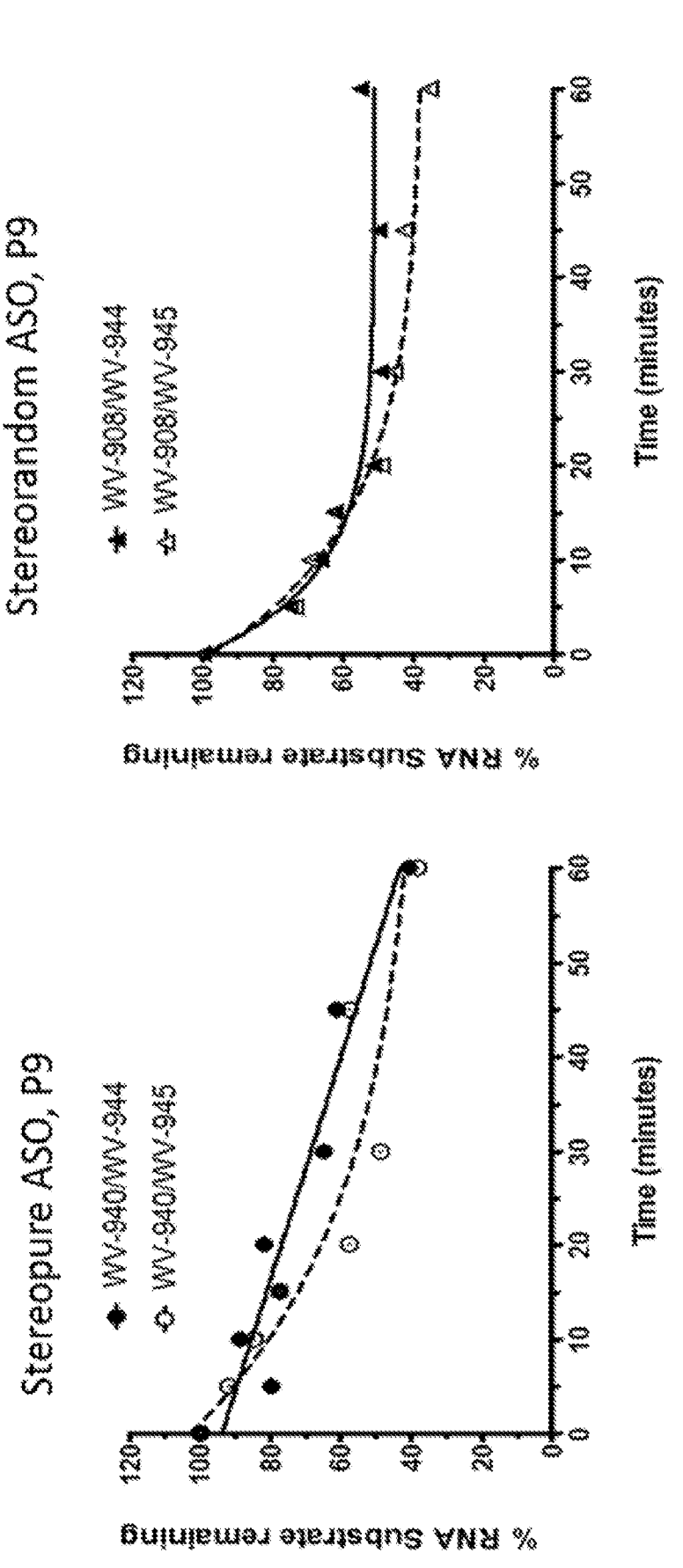


FIG. 31 (CONTINUED)
(PANEL E)

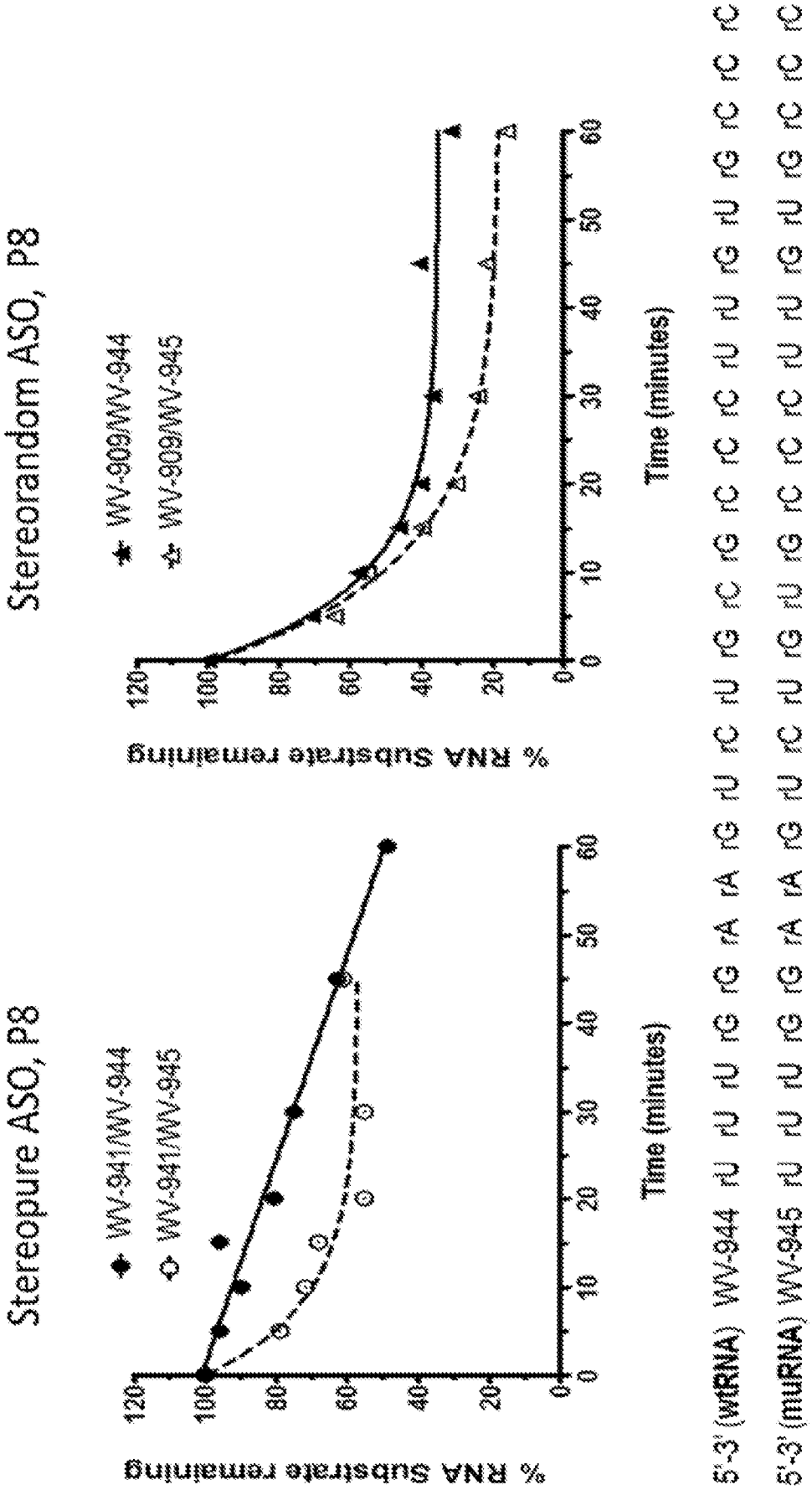
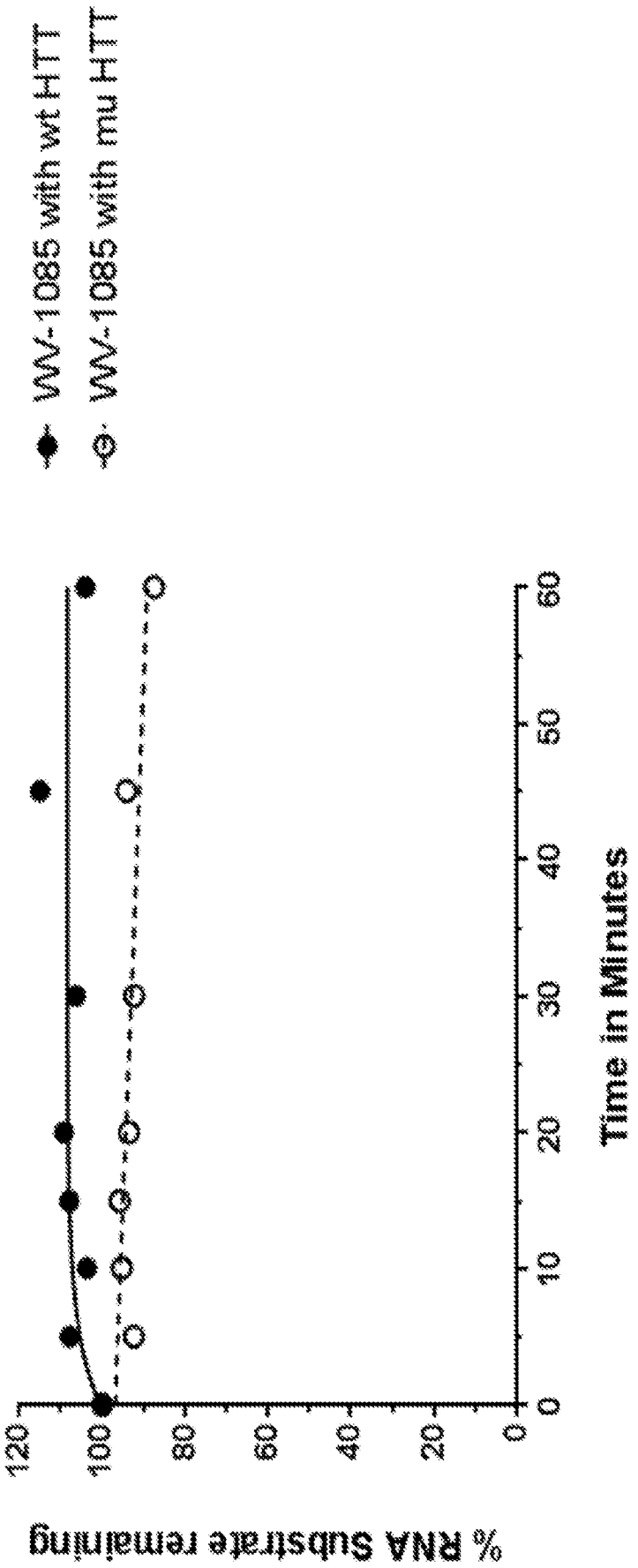
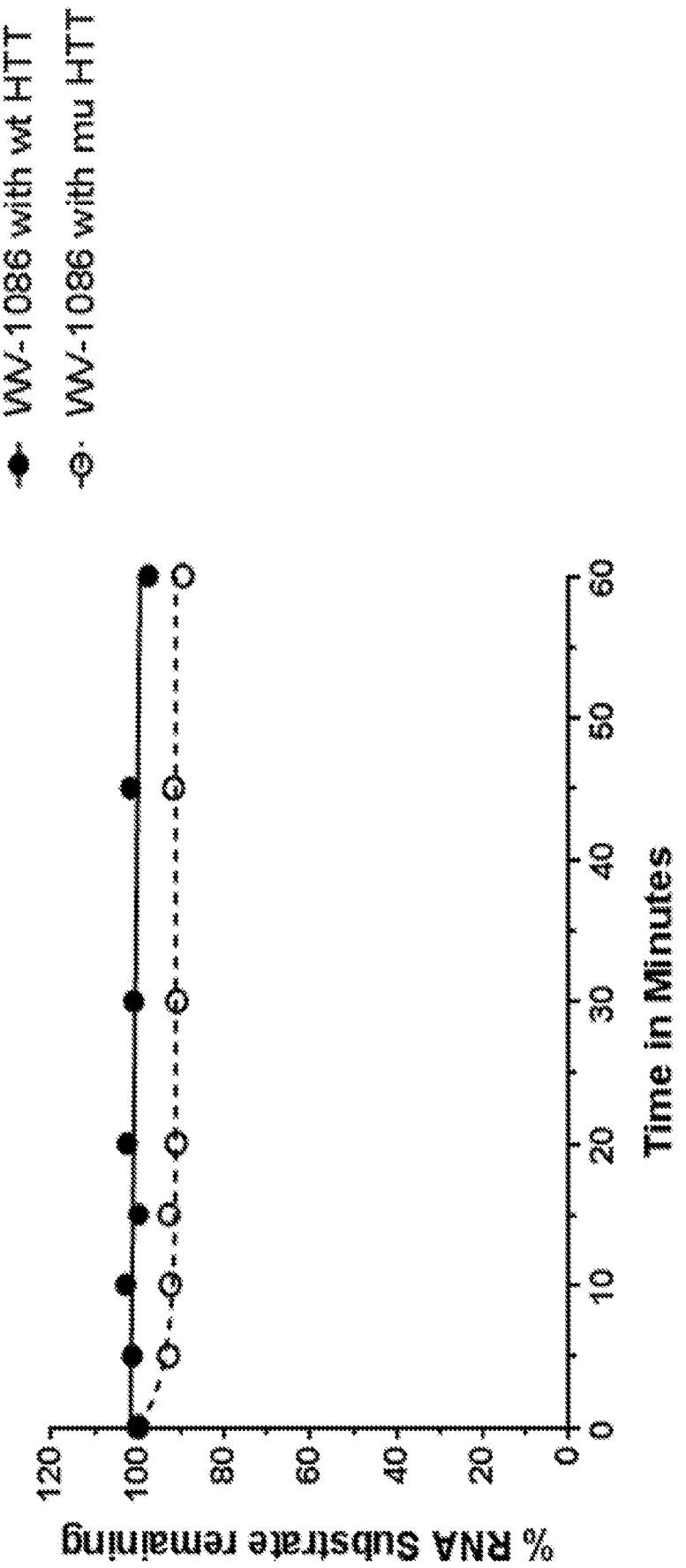


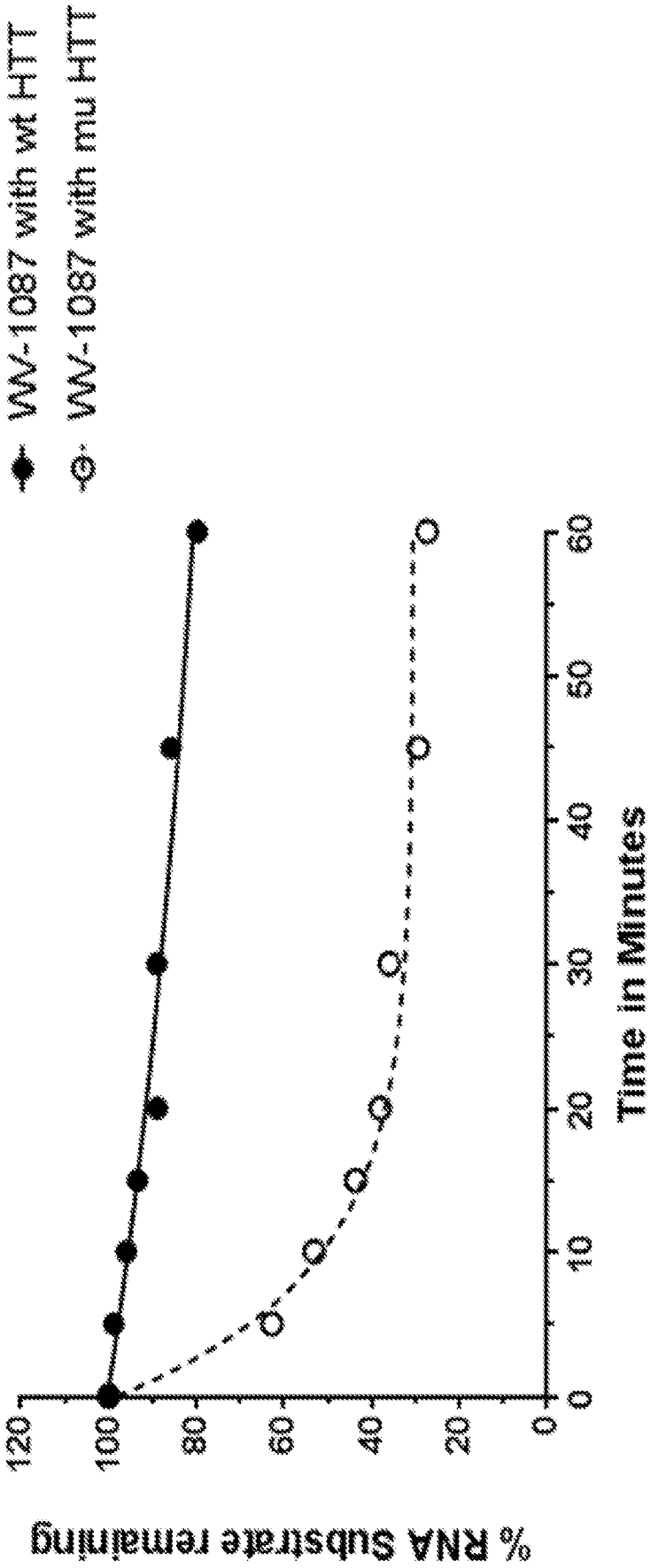
FIG. 31 (CONTINUED)
(PANEL F)



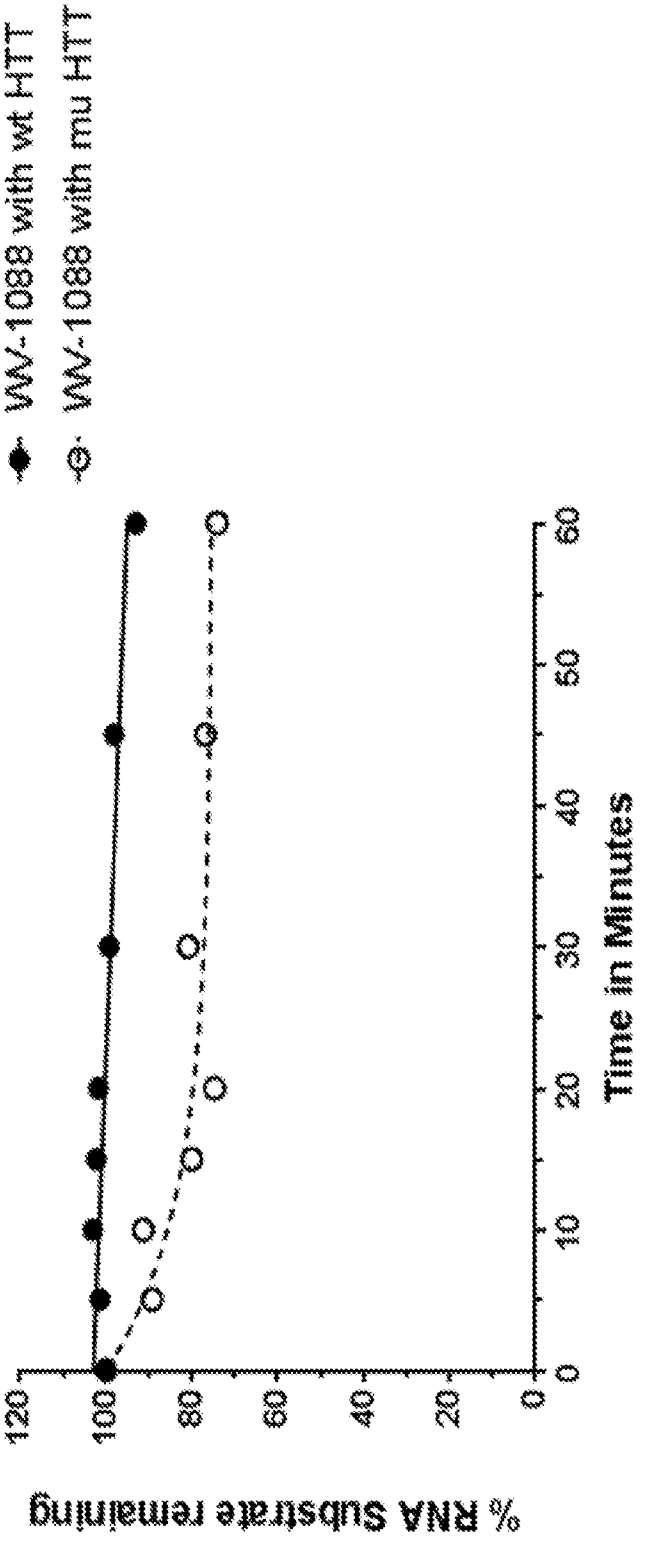
(PANEL A)
FIG. 32



(PANEL B)
FIG. 32 (CONTINUED)



(PANEL C)
FIG. 32 (CONTINUED)



(PANEL D)
FIG. 32 (CONTINUED)

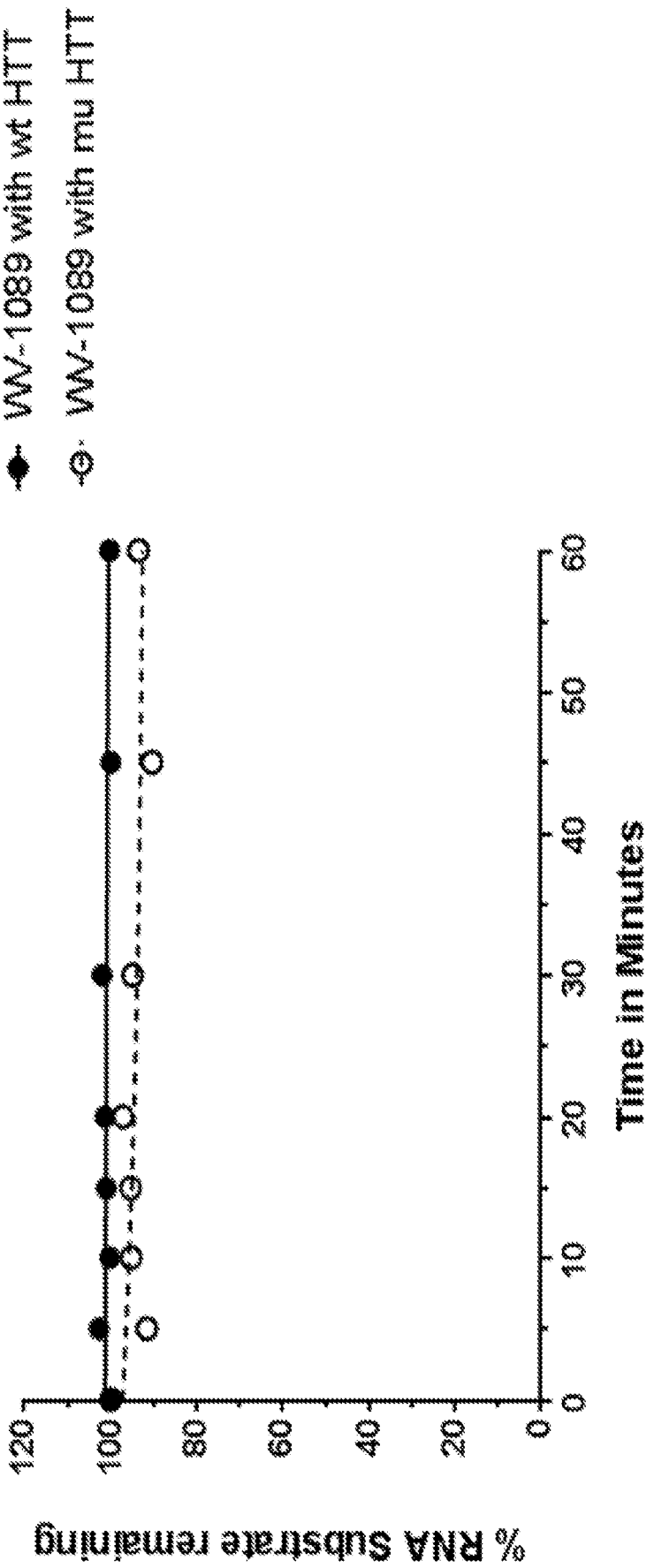
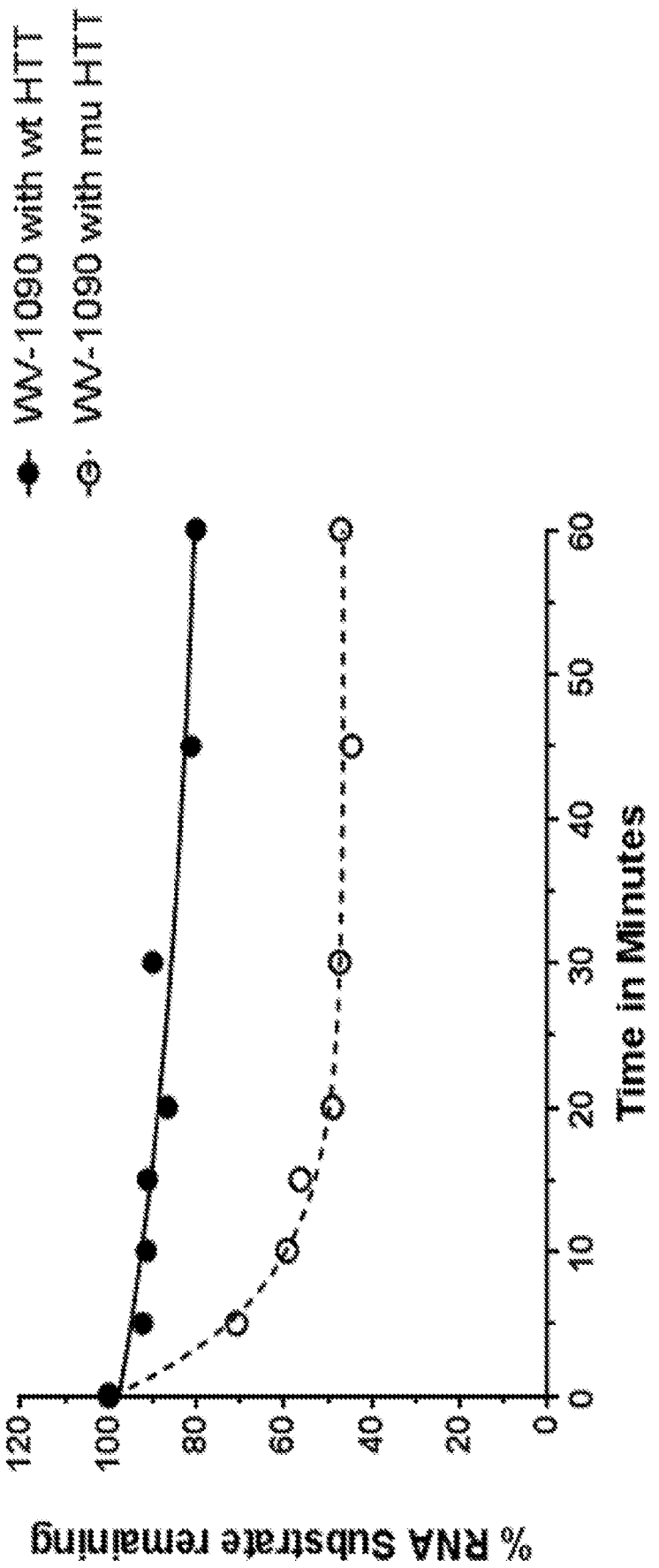
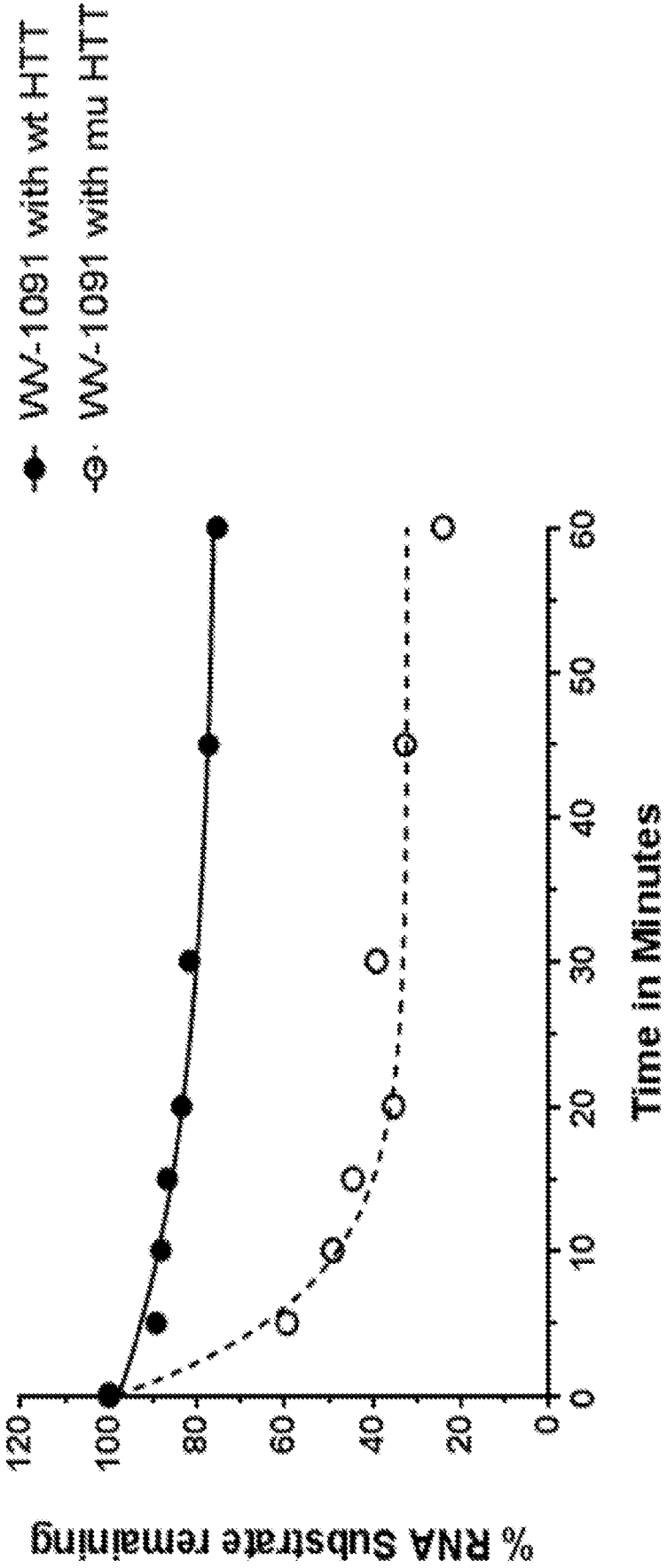


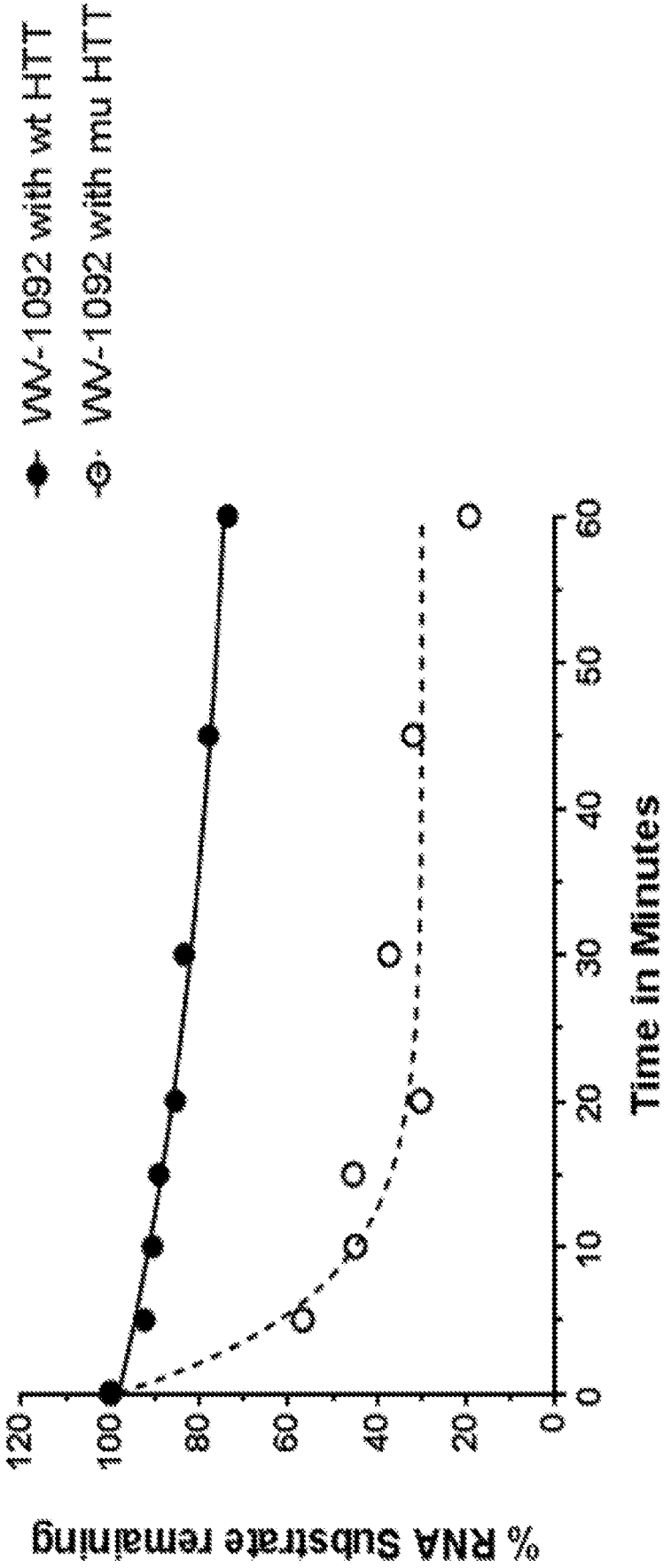
FIG. 32 (CONTINUED)
(PANEL E)



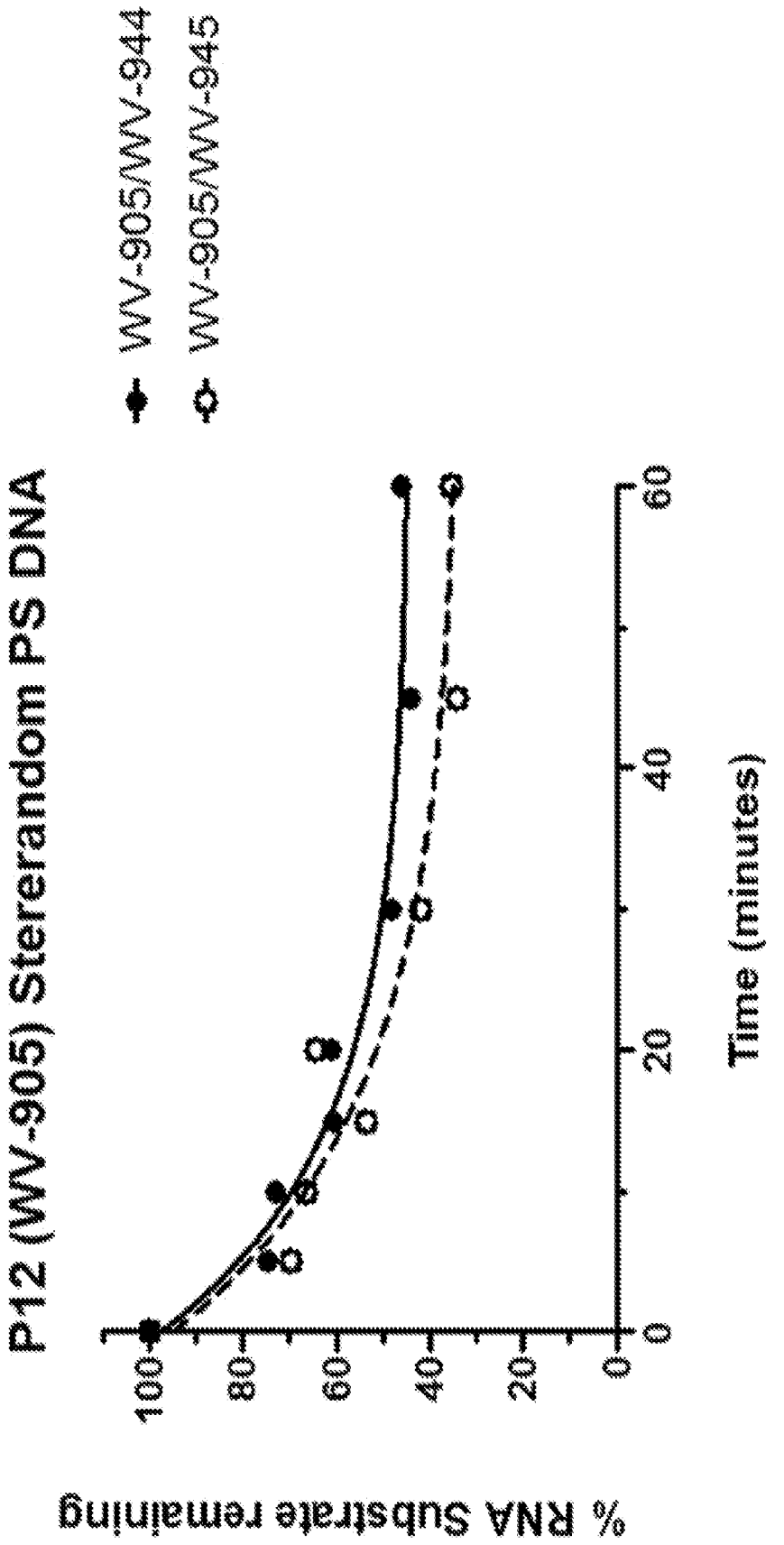
(PANEL F)
FIG. 32 (CONTINUED)



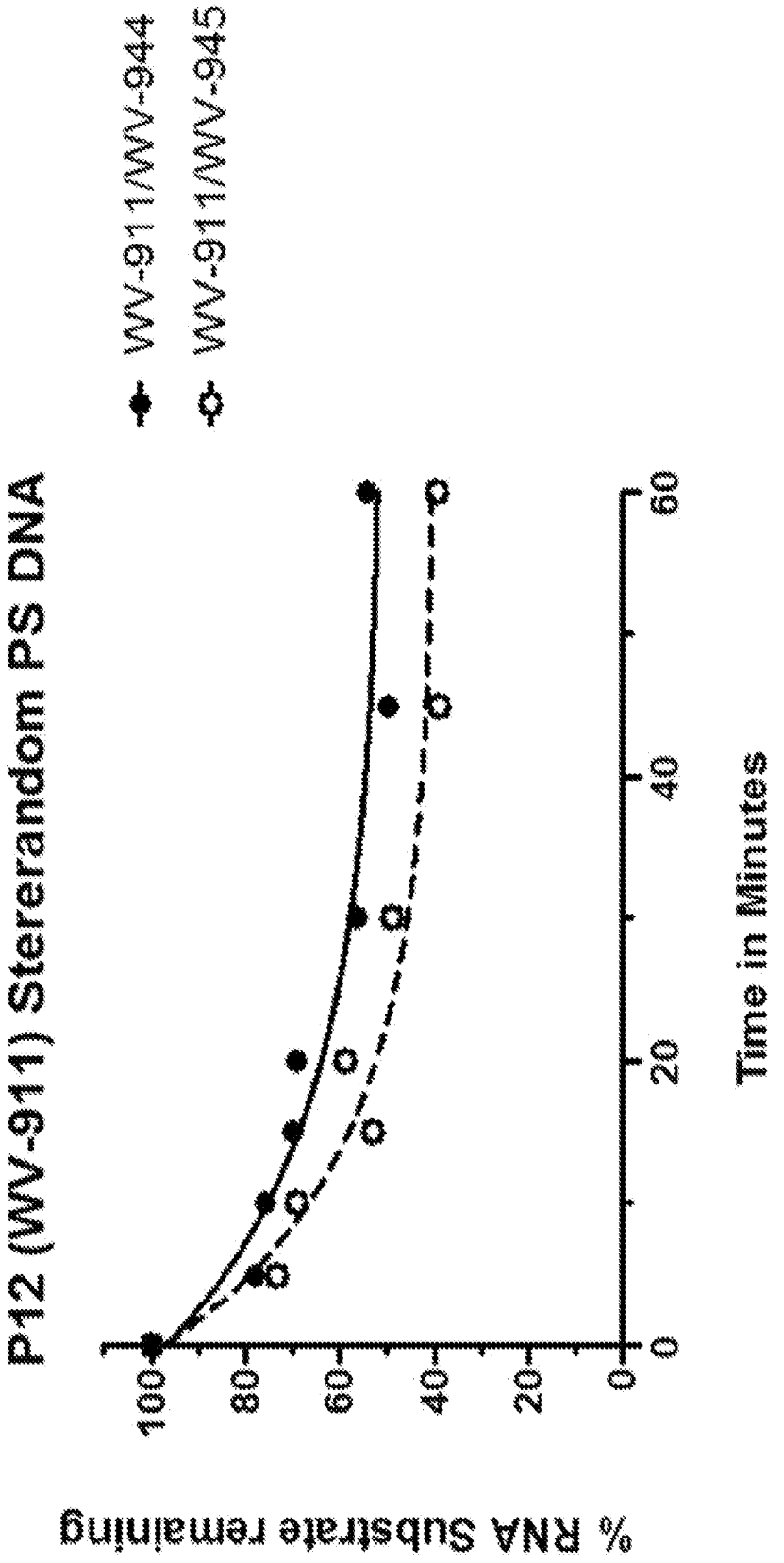
(PANEL G)
FIG. 32 (CONTINUED)



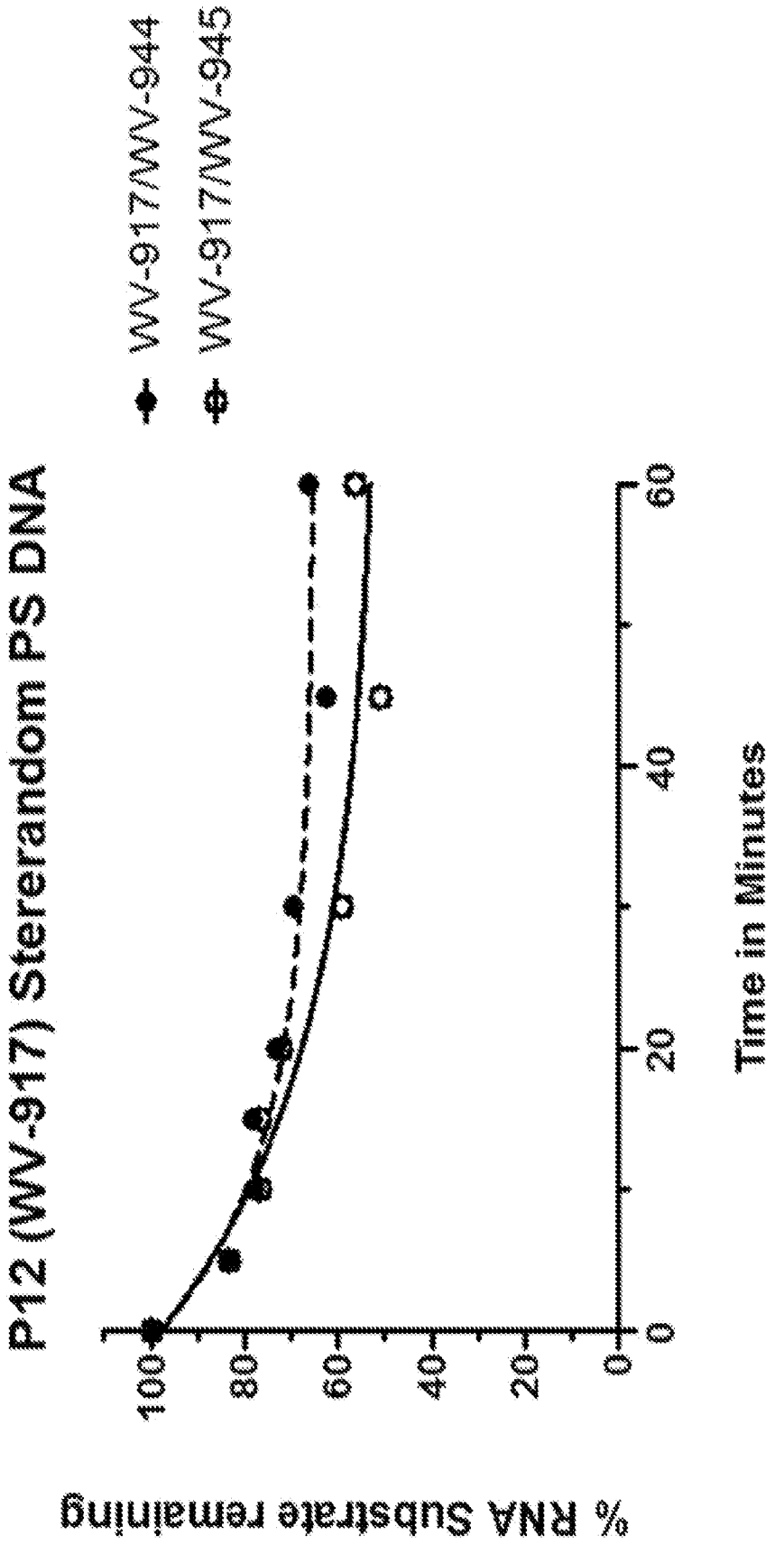
(PANEL H)
FIG. 32 (CONTINUED)



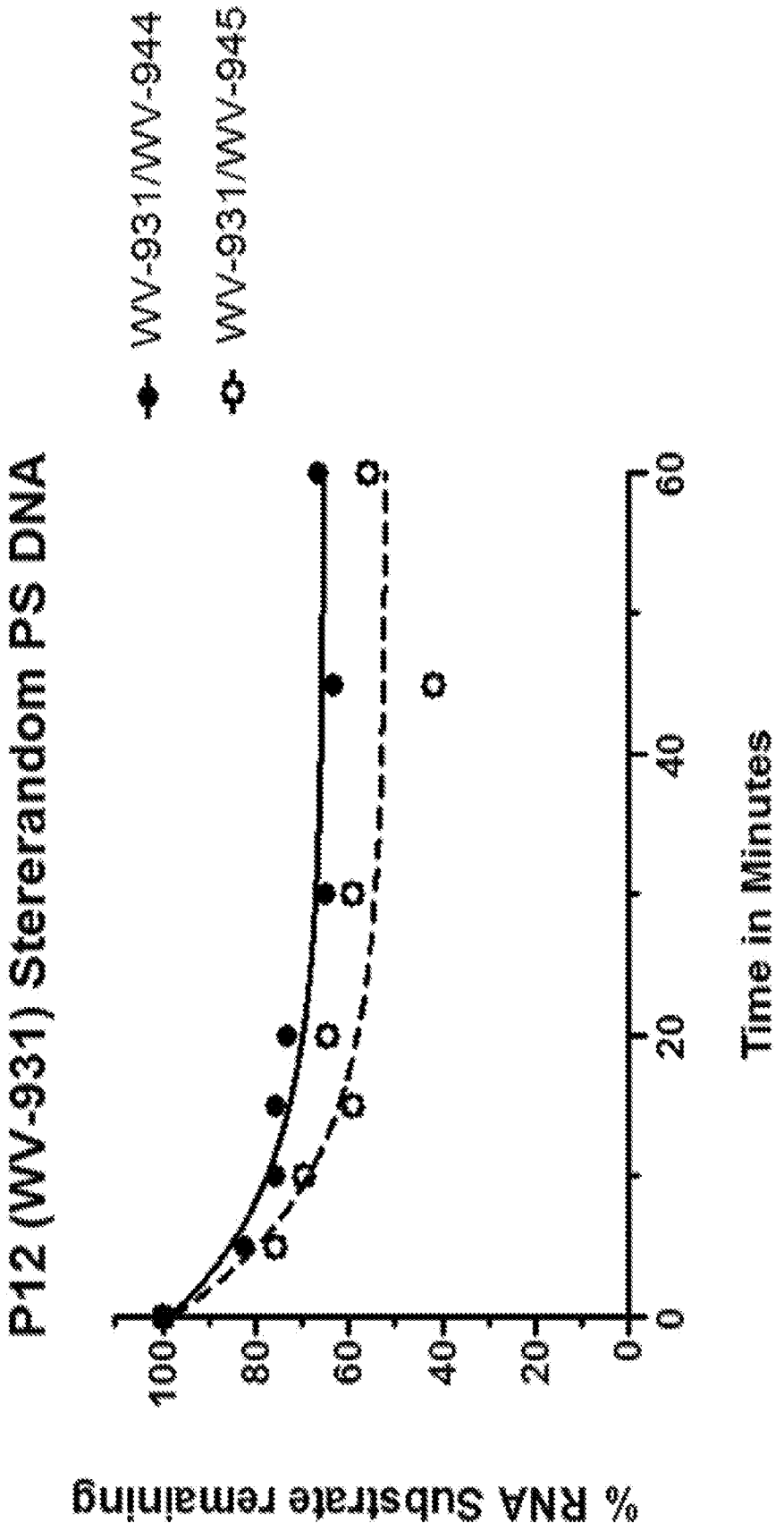
(PANEL I)
FIG. 32 (CONTINUED)



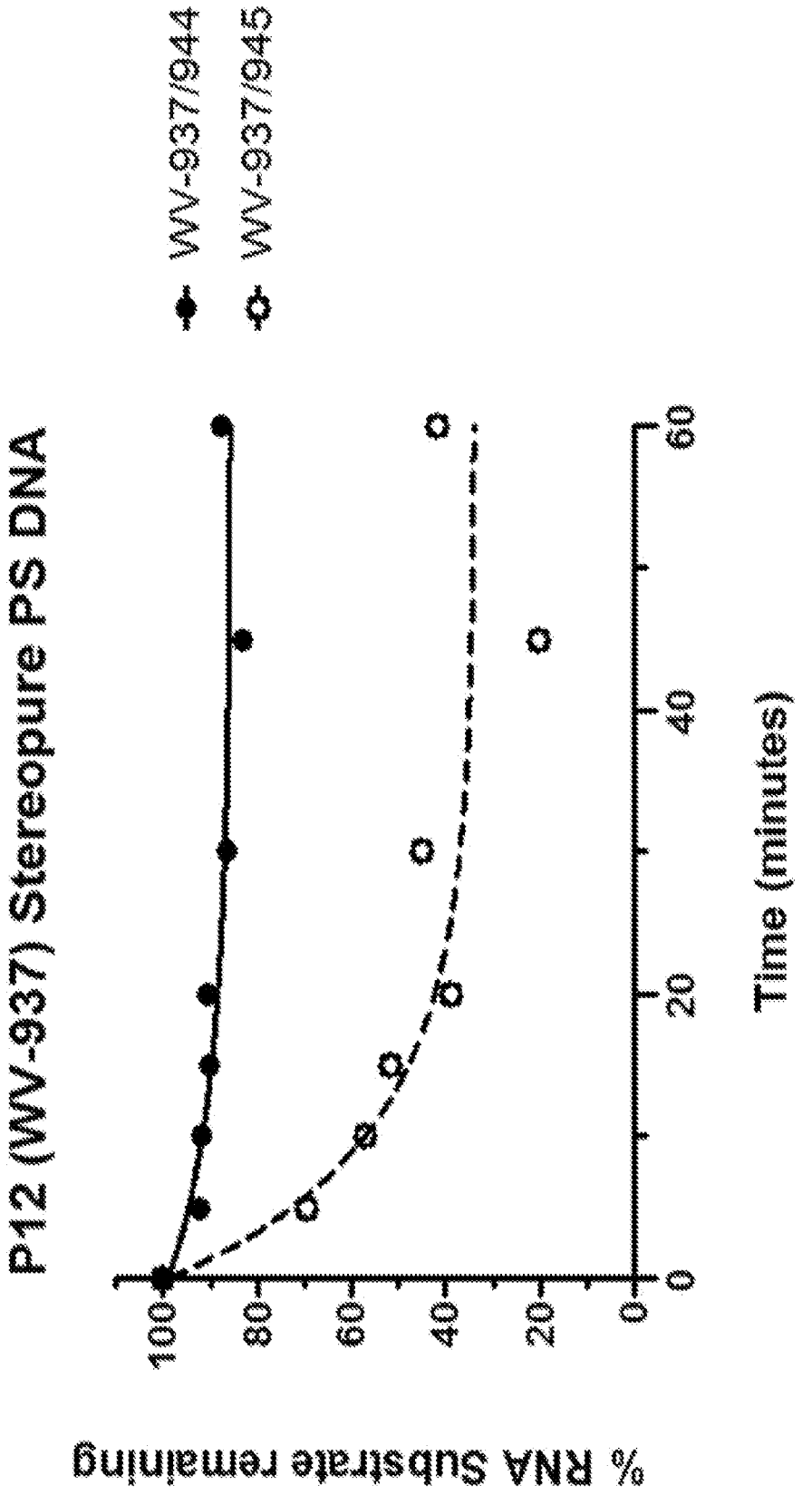
(PANEL J)
FIG. 32 (CONTINUED)



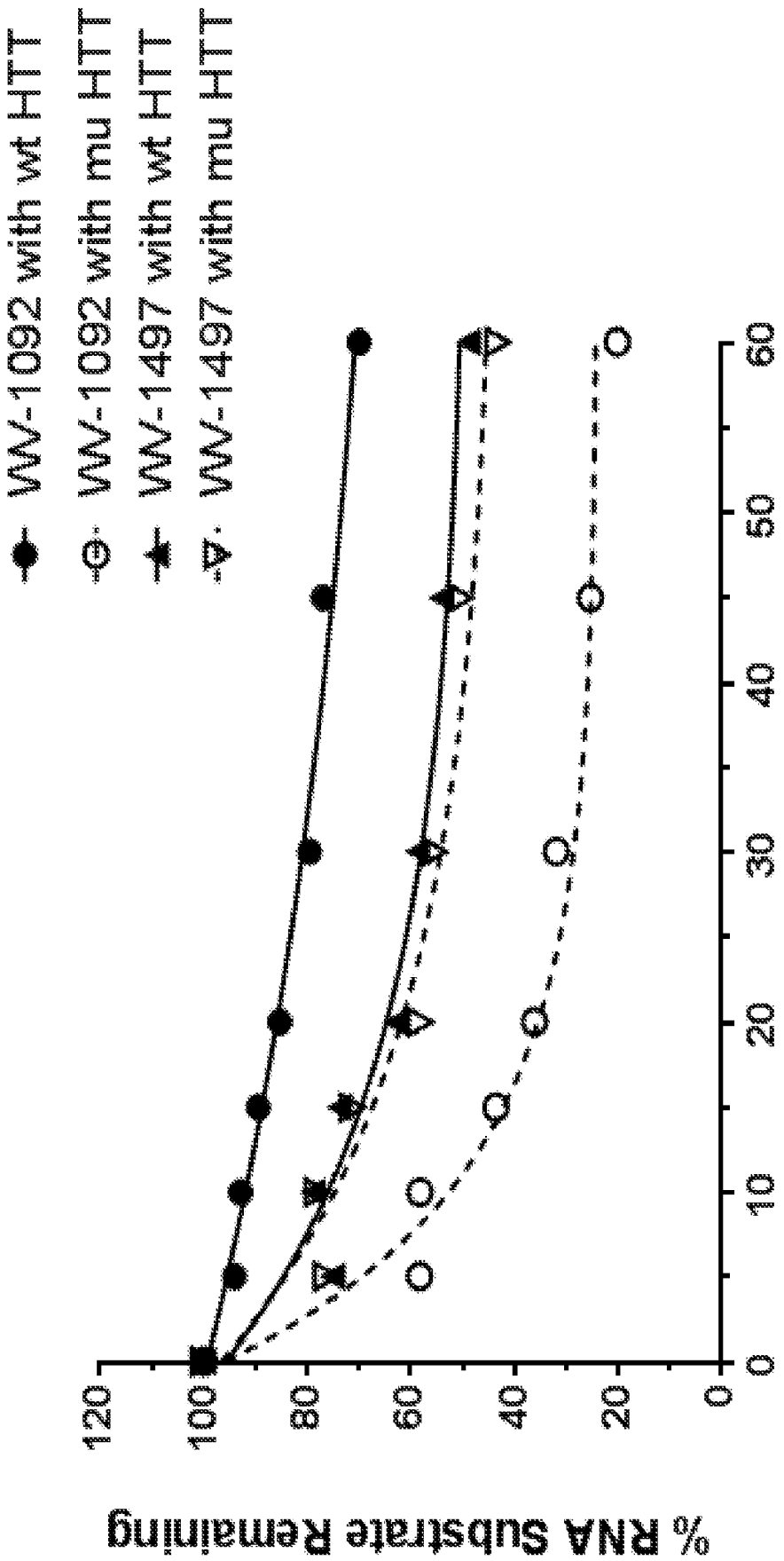
(PANEL K)
FIG. 32 (CONTINUED)



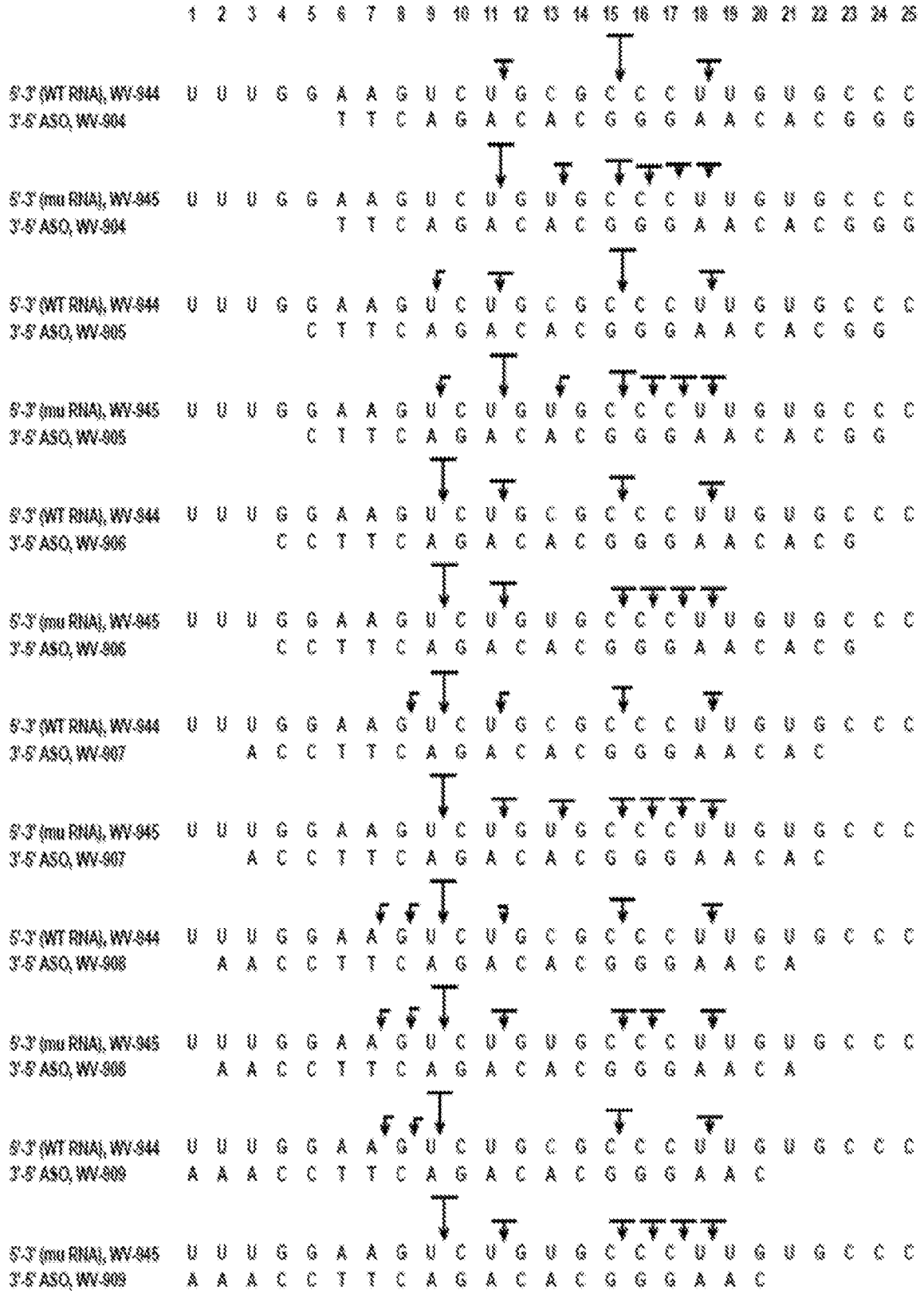
(PANEL L)
FIG. 32 (CONTINUED)



(PANEL M)
FIG. 32 (CONTINUED)

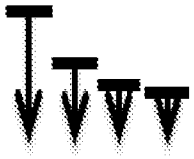


(PANEL N)
FIG. 32 (CONTINUED)

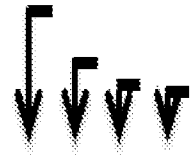


(PANEL A)

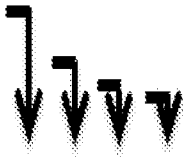
FIG. 33



Ranked cleavage site when both 5' P and 3' OH fragments were identified



Ranked cleavage site when only 3' OH fragments were identified



Ranked cleavage site when only 5' P fragments were identified



The highest amount of metabolite represents the relative major cleavage site



This size arrow represents 50-99% of the metabolite relative to the major metabolite



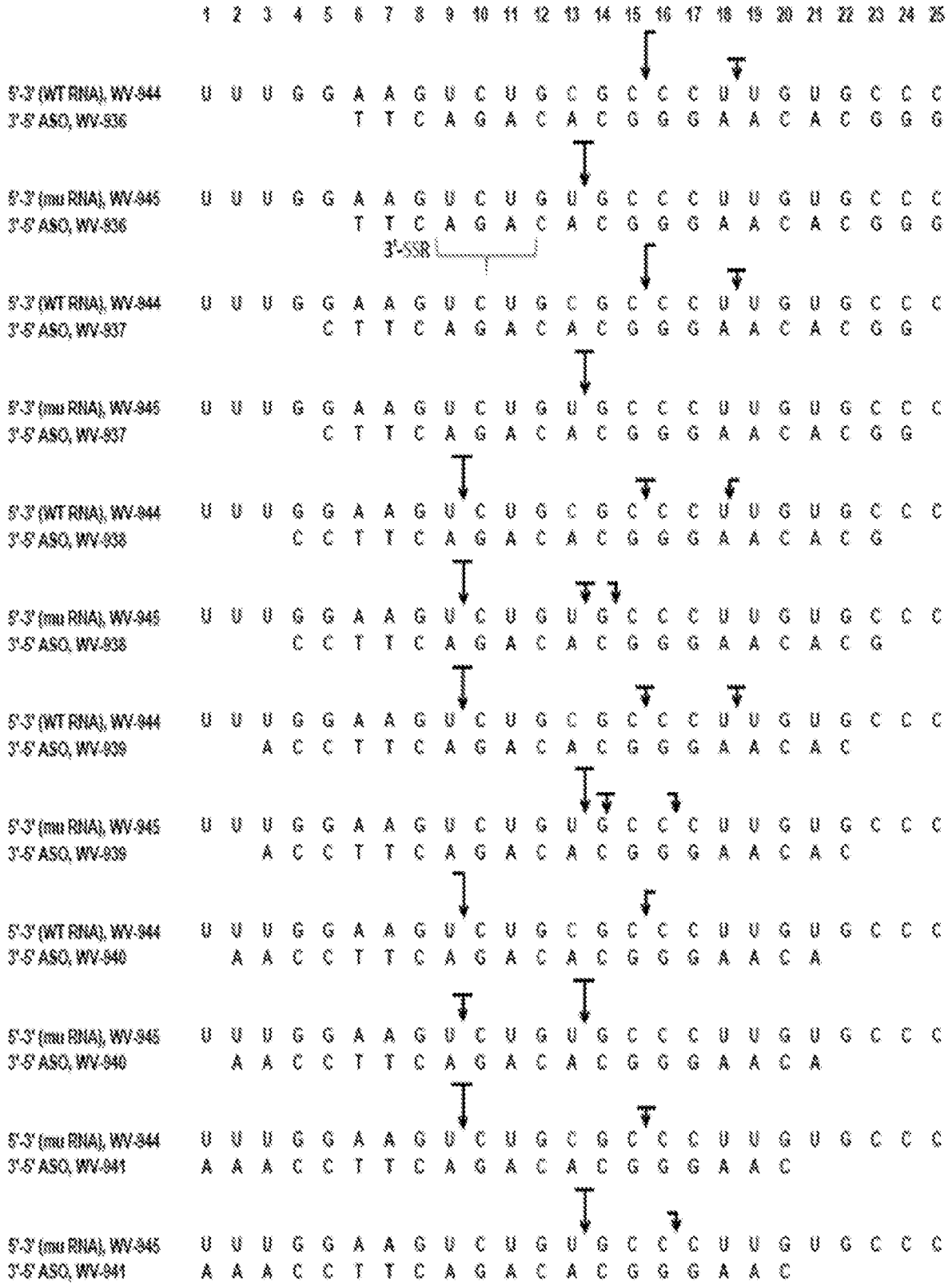
This size arrow represents 10-49% of the metabolite relative to the major arrow



This size arrow represents <10% of the metabolite relative to the major arrow

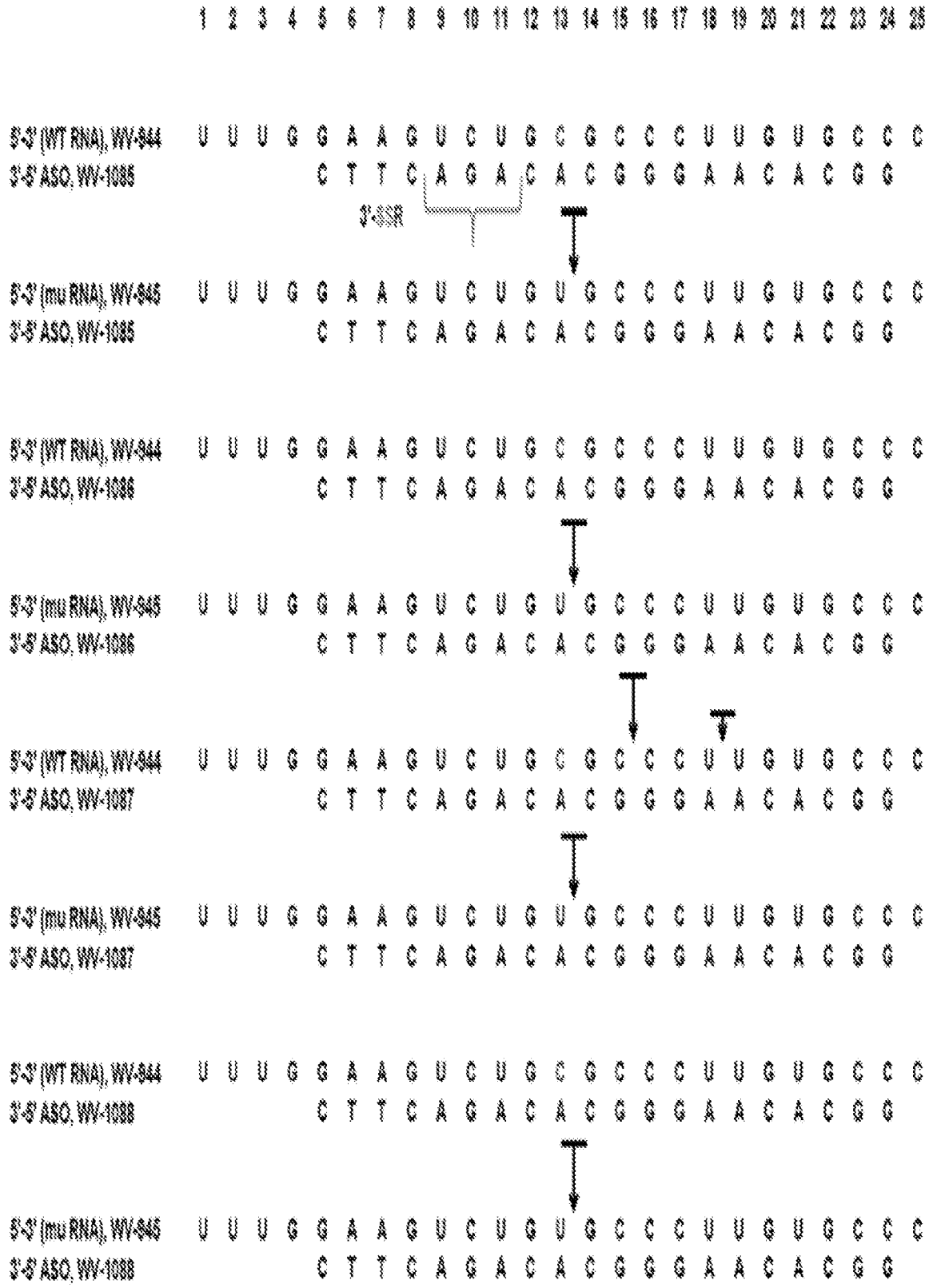
(PANEL B)

FIG. 33 (CONTINUED)



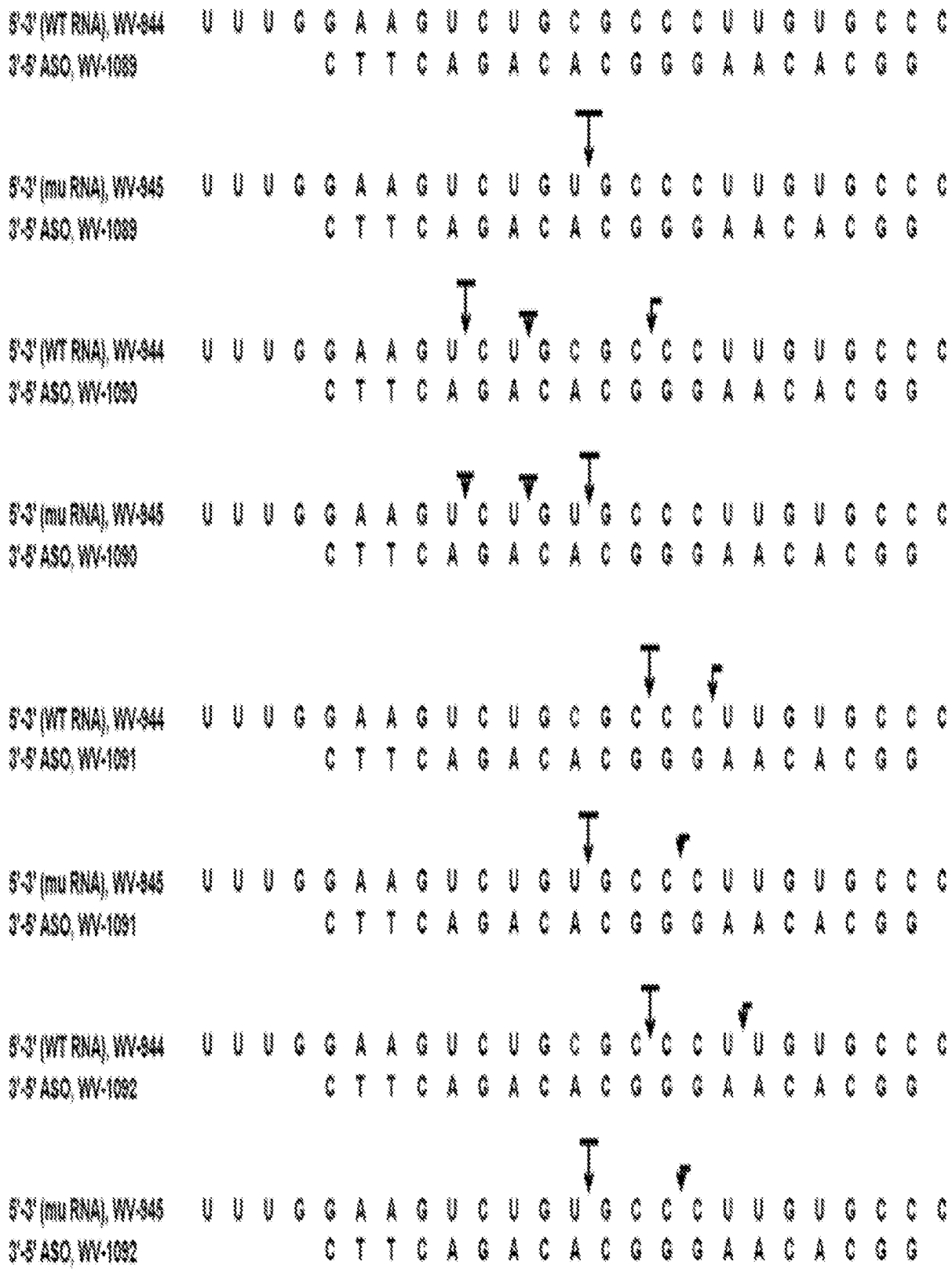
(PANEL A)

FIG. 34



(PANEL B)

FIG. 34 (CONTINUED)



(PANEL C)

FIG. 34 (CONTINUED)

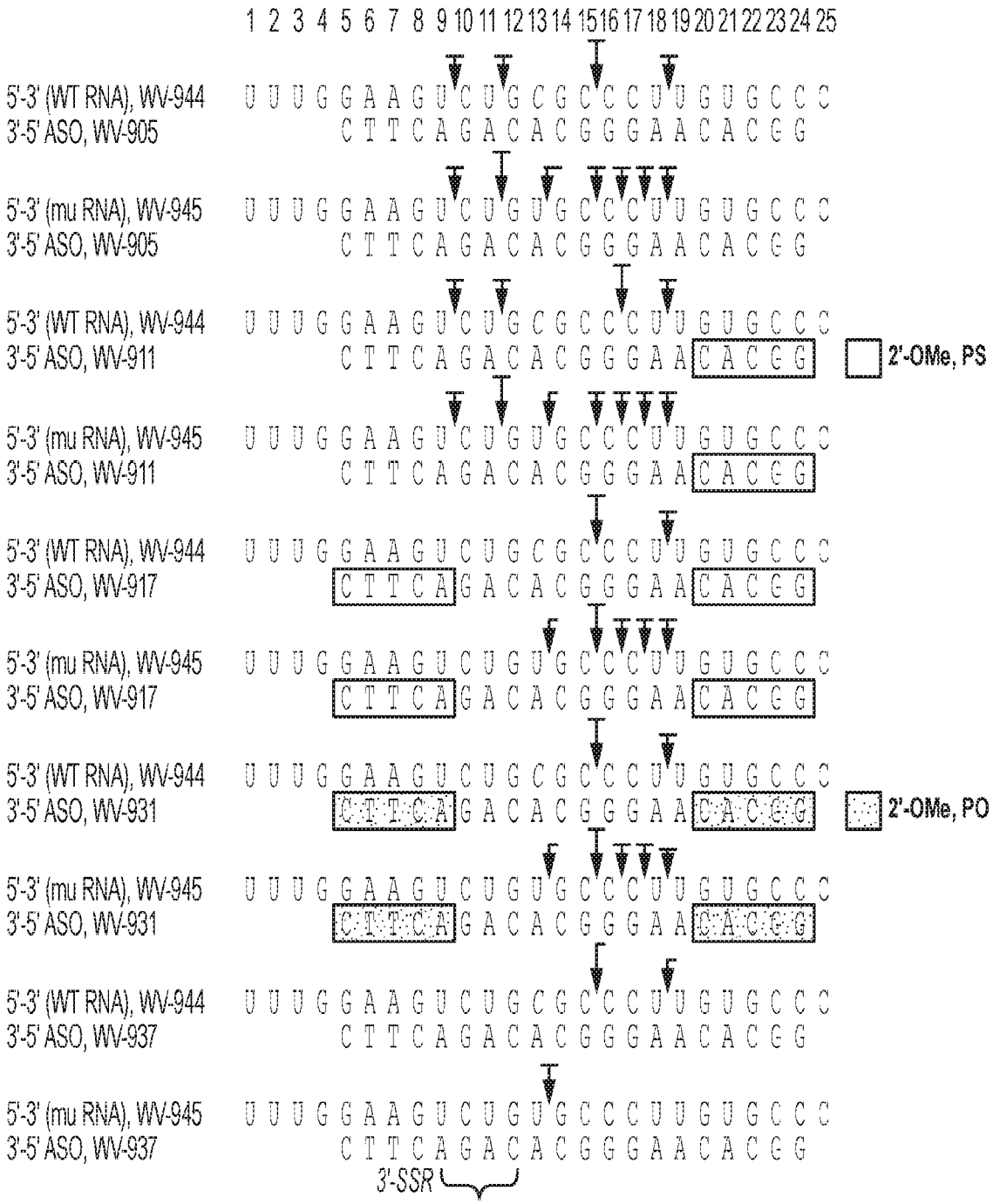

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
5'-3' (WT RNA), WV-944
3'-5' ASO, WV-905
5'-3' (mu RNA), WV-945
3'-5' ASO, WV-905
5'-3' (WT RNA), WV-944
3'-5' ASO, WV-911
5'-3' (mu RNA), WV-945
3'-5' ASO, WV-911
5'-3' (WT RNA), WV-944
3'-5' ASO, WV-917
5'-3' (mu RNA), WV-945
3'-5' ASO, WV-917
5'-3' (WT RNA), WV-944
3'-5' ASO, WV-931
5'-3' (mu RNA), WV-945
3'-5' ASO, WV-931
5'-3' (WT RNA), WV-944
3'-5' ASO, WV-937
5'-3' (mu RNA), WV-945
3'-5' ASO, WV-937
3'-SSR
2'-OMe, PS
2'-OMe, PO

FIG. 35

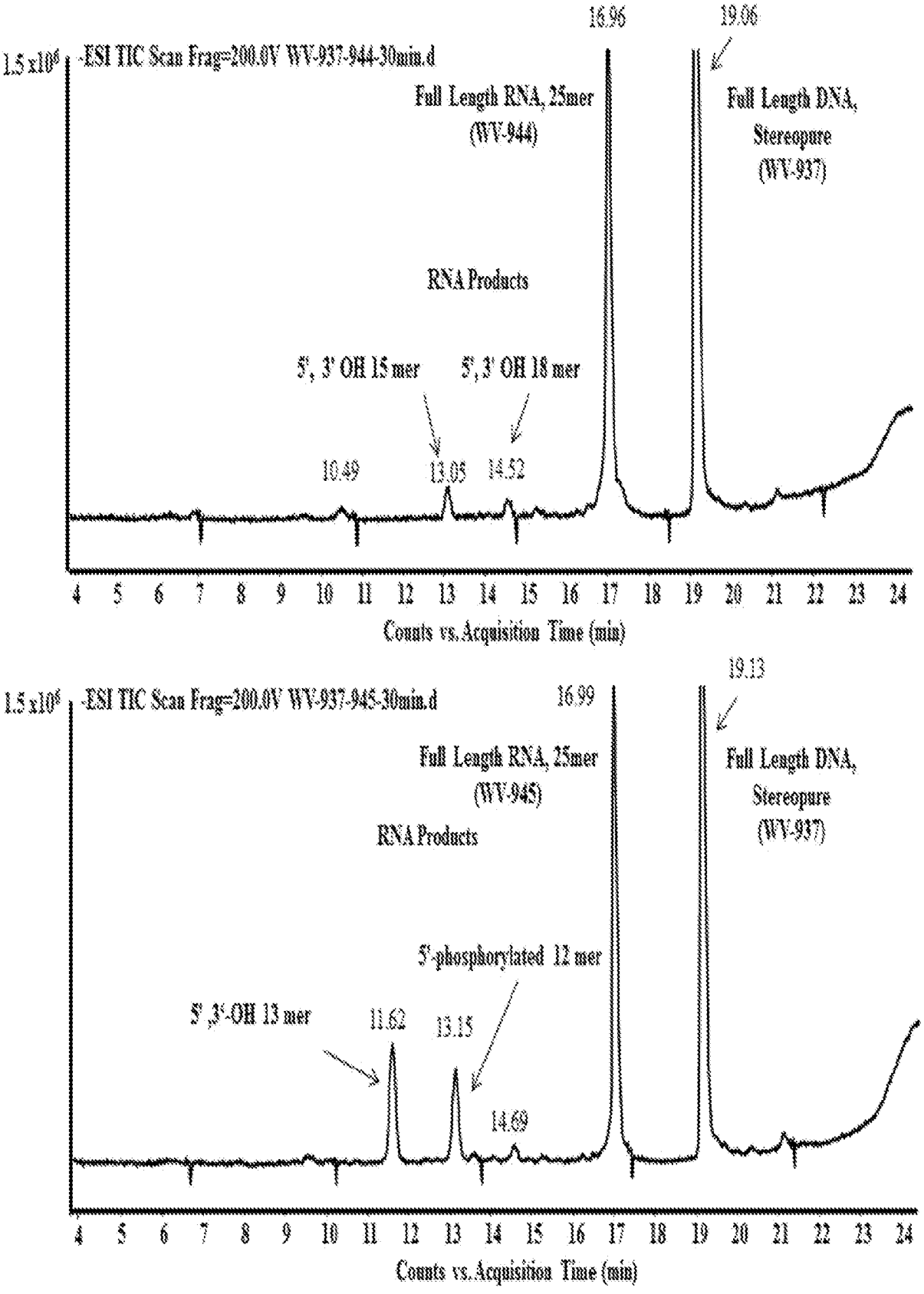

1.5 x10^6
-ESI TIC Scan Frag=200.0V WV-937-944-30min.d
16.96
19.06
Full Length RNA, 25mer
(WV-944)
Full Length DNA,
Stereopure
(WV-937)
RNA Products
5', 3' OH 15 mer
5', 3' OH 18 mer
10.49
13.05
14.52
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Counts vs. Acquisition Time (min)
1.5 x10^6
-ESI TIC Scan Frag=200.0V WV-937-945-30min.d
16.99
19.13
Full Length RNA, 25mer
(WV-945)
RNA Products
Full Length DNA,
Stereopure
(WV-937)
5'-phosphorylated 12 mer
5', 3'-OH 13 mer
11.62
13.15
14.69
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Counts vs. Acquisition Time (min)

FIG. 36

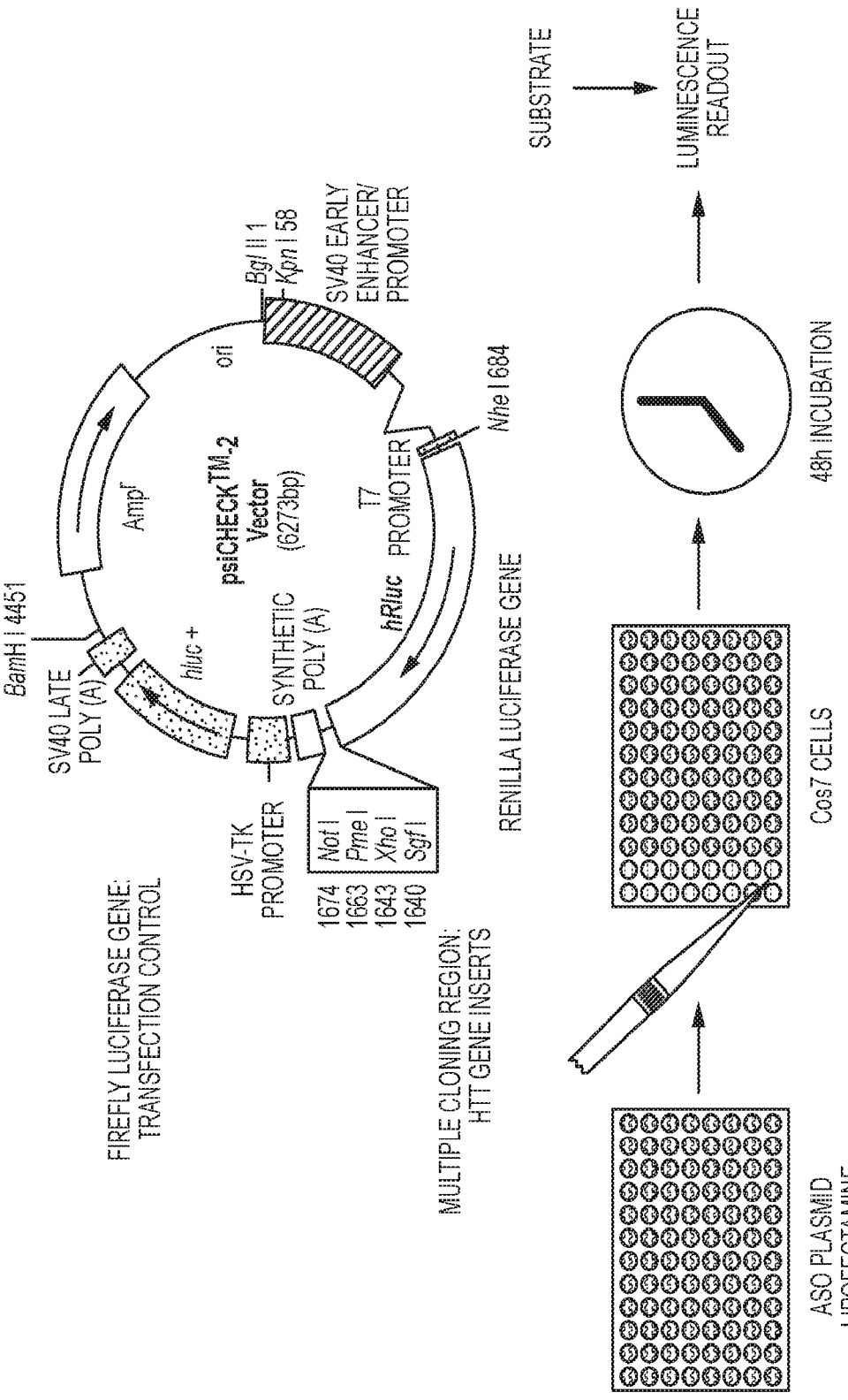

SUBSTRATE
LUMINESCENCE READOUT
48h INCUBATION
Cos7 CELLS
ASO PLASMID LIPOFECTAMINE
psiCHECK™-2 Vector (6273bp)
Amp^r
ori
Bgl II 1
Kpn I 58
SV40 EARLY ENHANCER/ PROMOTER
Nhe I 684
T7 PROMOTER
hRluc
RENILLA LUCIFERASE GENE
BamH I 4451
SV40 LATE POLY (A)
hluc +
SYNTHETIC POLY (A)
HSV-TK PROMOTER
Not I
Pme I
Xho I
Sgf I
1674
1663
1643
1640
MULTIPLE CLONING REGION: HTT GENE INSERTS
FIREFLY LUCIFERASE GENE: TRANSFECTION CONTROL

FIG. 37

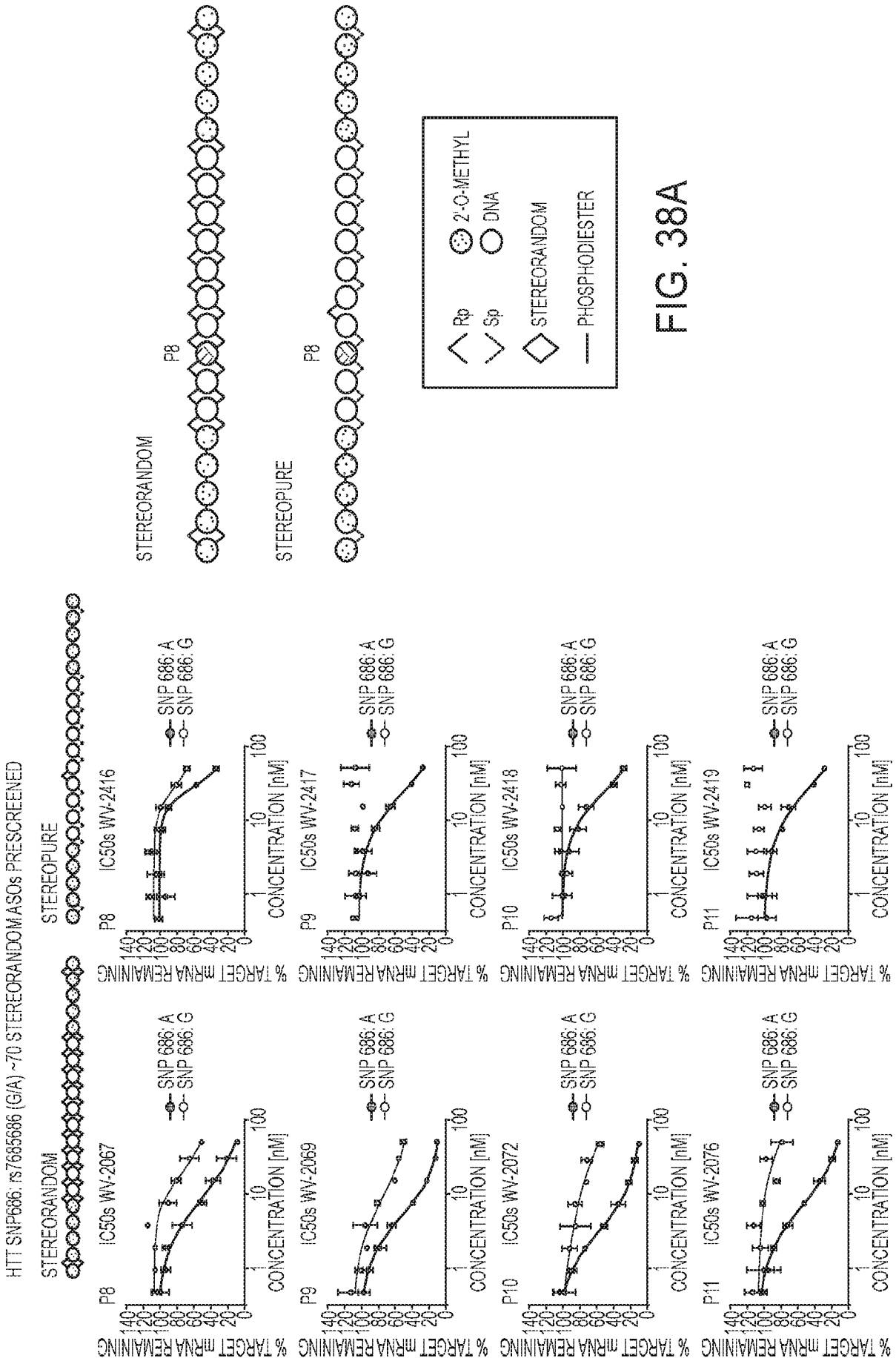
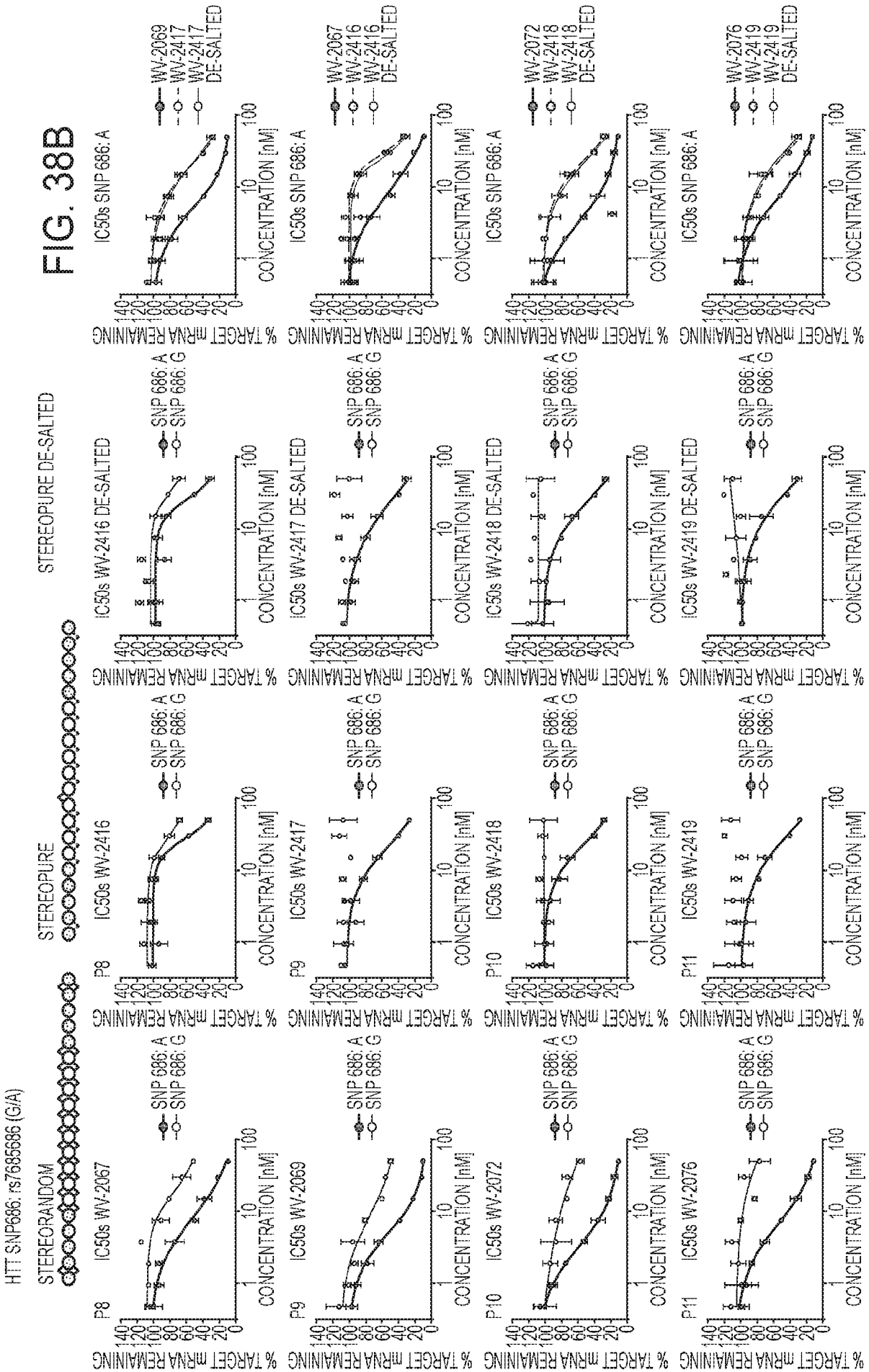

HTT SNP686: rs7685686 (G/A) ~70 STEREORANDOM ASOs PRESCREENED
STEREORANDOM
STEREOPURE
P8 IC50s WV-2067
P9 IC50s WV-2069
P10 IC50s WV-2072
P11 IC50s WV-2076
P8 IC50s WV-2416
P9 IC50s WV-2417
P10 IC50s WV-2418
P11 IC50s WV-2419
SNP 686: A
SNP 686: G
% TARGET mRNA REMAINING
CONCENTRATION [nM]
STEREORANDOM
STEREOPURE
P8
Rp
Sp
2'-O-METHYL
DNA
STEREORANDOM
PHOSPHODIESTER

FIG. 38A

FIG. 38B


HTT SNP686: rs7685686 (G/A)
STEREORANDOM
STEREOPURE
STEREOPURE DE-SALTED
P8
IC50s WV-2067
IC50s WV-2416
IC50s WV-2416 DE-SALTED
P9
IC50s WV-2069
IC50s WV-2417
IC50s WV-2417 DE-SALTED
P10
IC50s WV-2072
IC50s WV-2418
IC50s WV-2418 DE-SALTED
P11
IC50s WV-2076
IC50s WV-2419
IC50s WV-2419 DE-SALTED
SNP 686: A
SNP 686: G
WV-2069
WV-2417
WV-2417 DE-SALTED
WV-2067
WV-2416
WV-2416 DE-SALTED
WV-2072
WV-2418
WV-2418 DE-SALTED
WV-2076
WV-2419
WV-2419 DE-SALTED
% TARGET mRNA REMAINING
CONCENTRATION [nM]

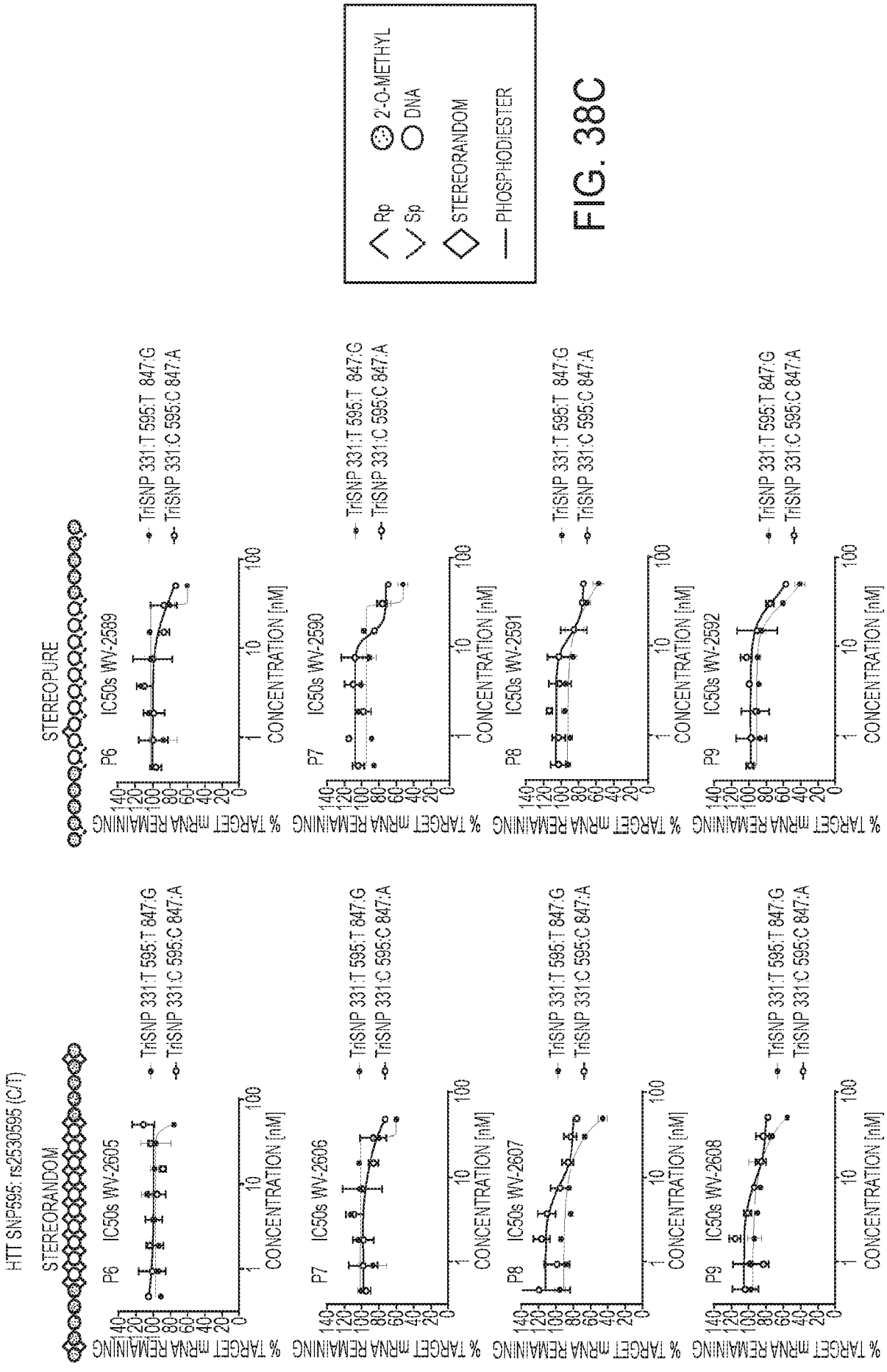

HTT SNP595: rs2530595 (C/T)
STEREORANDOM
STEREOPURE
P6 IC50s WV-2605
P7 IC50s WV-2606
P8 IC50s WV-2607
P9 IC50s WV-2608
P6 IC50s WV-2589
P7 IC50s WV-2590
P8 IC50s WV-2591
P9 IC50s WV-2592
TriSNP 331:T 595:T 847:G
TriSNP 331:C 595:C 847:A
% TARGET mRNA REMAINING
CONCENTRATION [nM]
Rp
Sp
STEREORANDOM
PHOSPHODIESTER
2'-O-METHYL
DNA

FIG. 38C

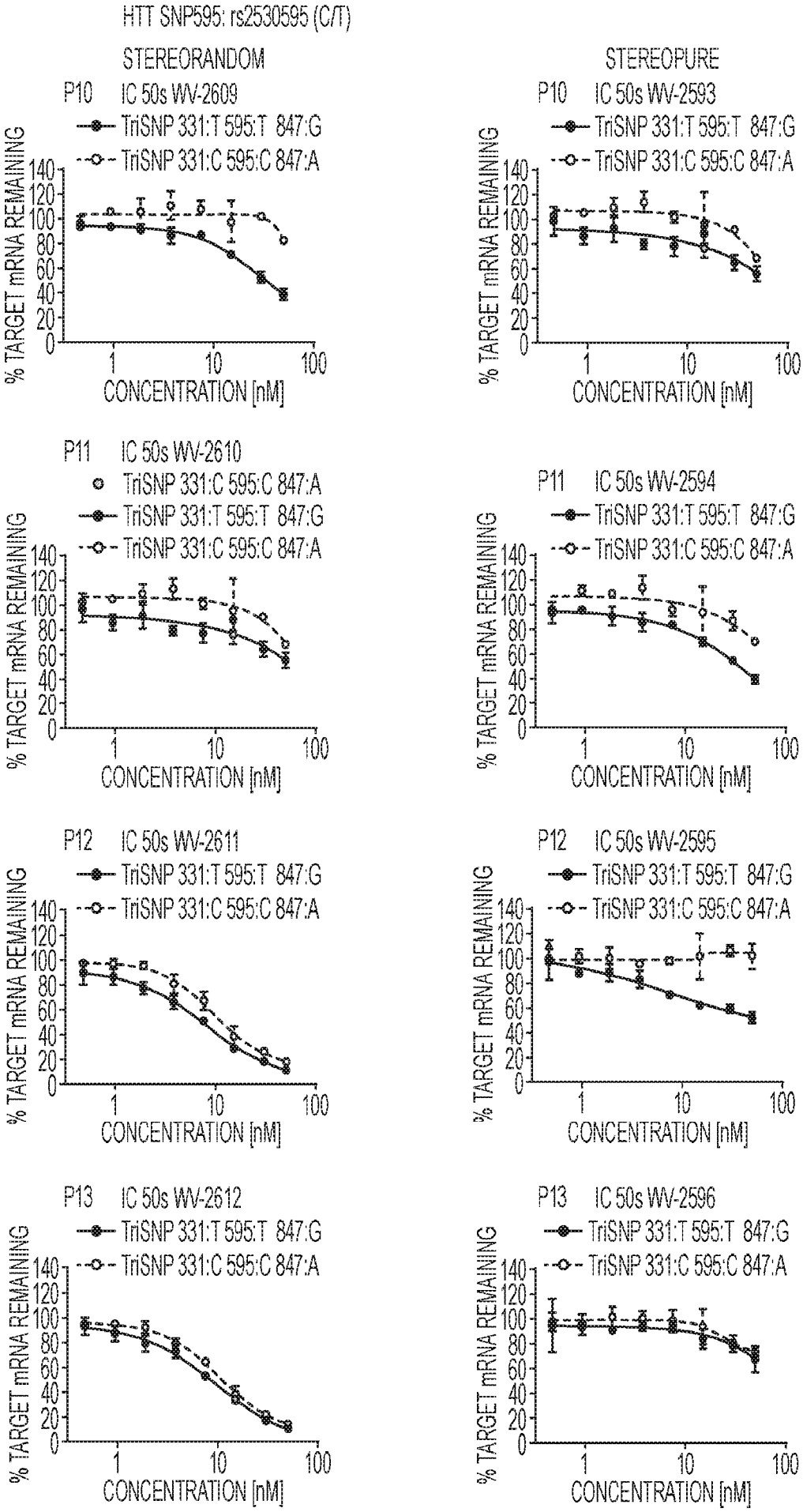

HTT SNP595: rs2530595 (C/T)
STEREORANDOM
STEREOPURE
P10 IC 50s WV-2609
P10 IC 50s WV-2593
P11 IC 50s WV-2610
P11 IC 50s WV-2594
P12 IC 50s WV-2611
P12 IC 50s WV-2595
P13 IC 50s WV-2612
P13 IC 50s WV-2596
TriSNP 331:T 595:T 847:G
TriSNP 331:C 595:C 847:A
% TARGET mRNA REMAINING
CONCENTRATION [nM]

FIG. 38D

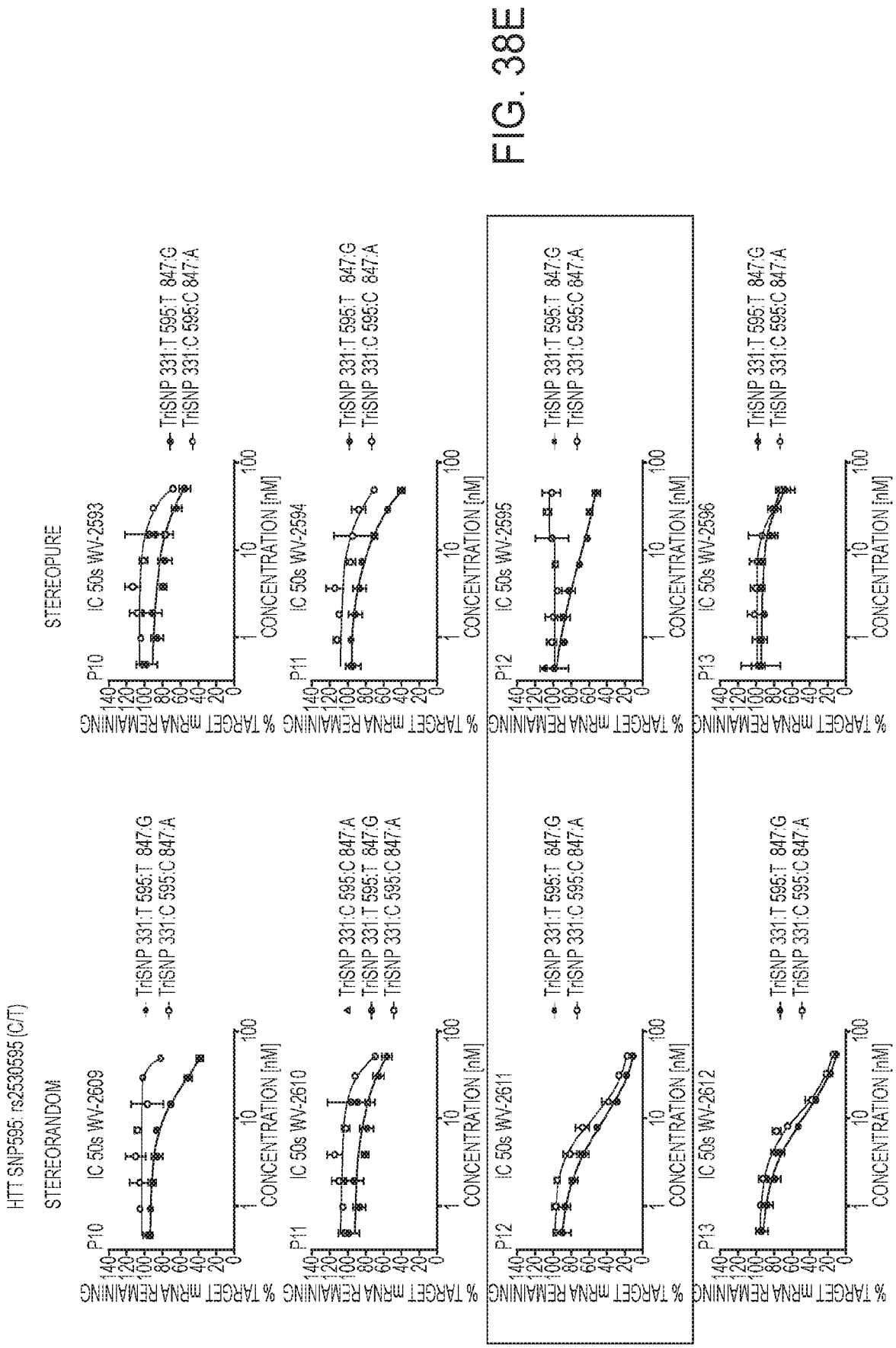

HTT SNP595: rs2530595 (C/T)
STEREORANDOM
STEREOPURE
P10
IC 50s WV-2609
P11
IC 50s WV-2610
P12
IC 50s WV-2611
P13
IC 50s WV-2612
P10
IC 50s WV-2593
P11
IC 50s WV-2594
P12
IC 50s WV-2595
P13
IC 50s WV-2596
% TARGET mRNA REMAINING
CONCENTRATION [nM]
TriSNP 331:T 595:T 847:G
TriSNP 331:C 595:C 847:A
TriSNP 331:C 595:C 847:A
TriSNP 331:T 595:T 847:G
TriSNP 331:C 595:C 847:A

FIG. 38E

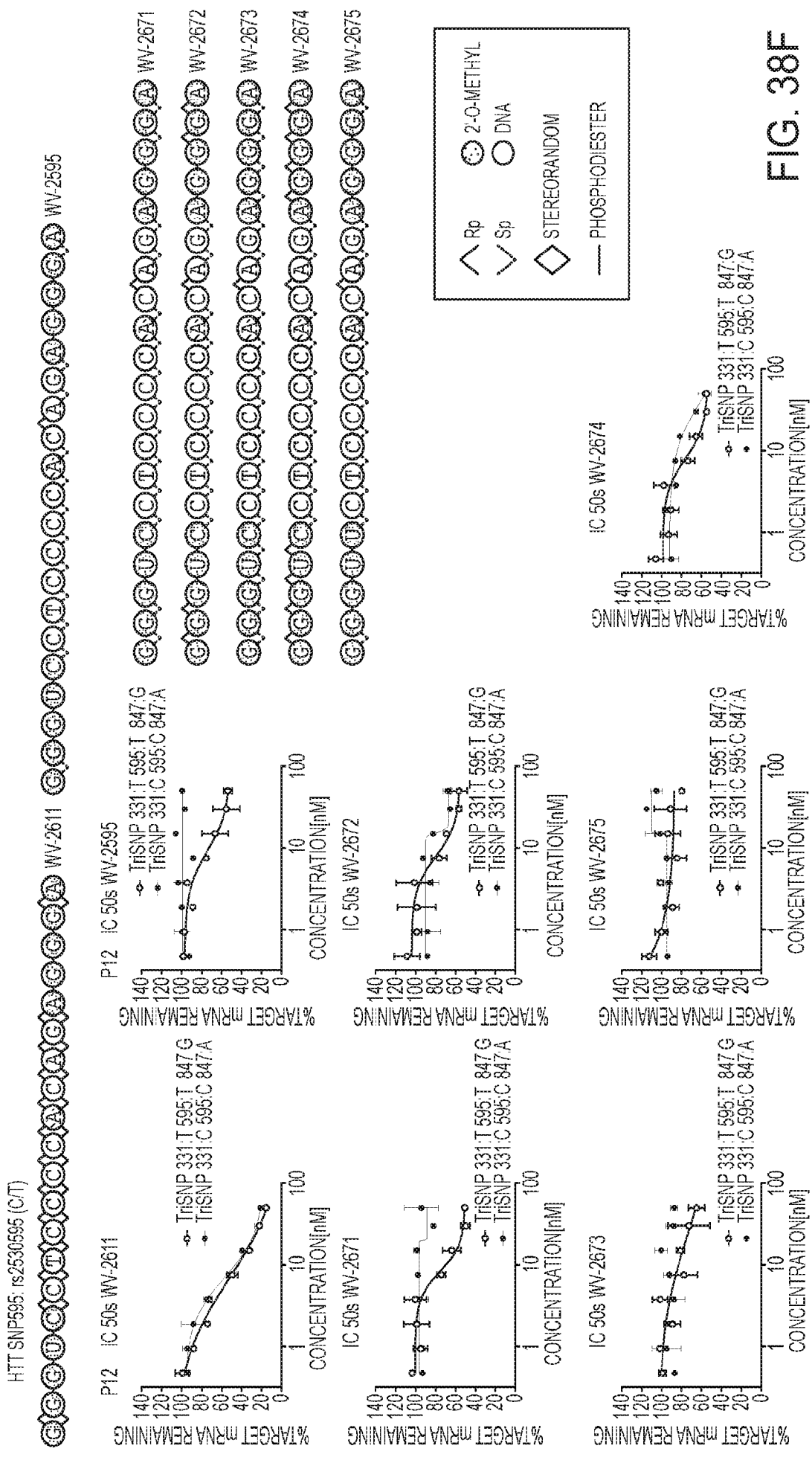

HTT SNP595: rs2530595 (C/T)
WV-2611
WV-2595
WV-2671
WV-2672
WV-2673
WV-2674
WV-2675
Rp
Sp
STEREORANDOM
2-O-METHYL
DNA
PHOSPHODIESTER
P12 IC 50s WV-2611
P12 IC 50s WV-2595
IC 50s WV-2671
IC 50s WV-2672
IC 50s WV-2673
IC 50s WV-2674
IC 50s WV-2675
TriSNP 331:T 595:T 847:G
TriSNP 331:C 595:C 847:A
%TARGET mRNA REMAINING
CONCENTRATION[nM]

FIG. 38F

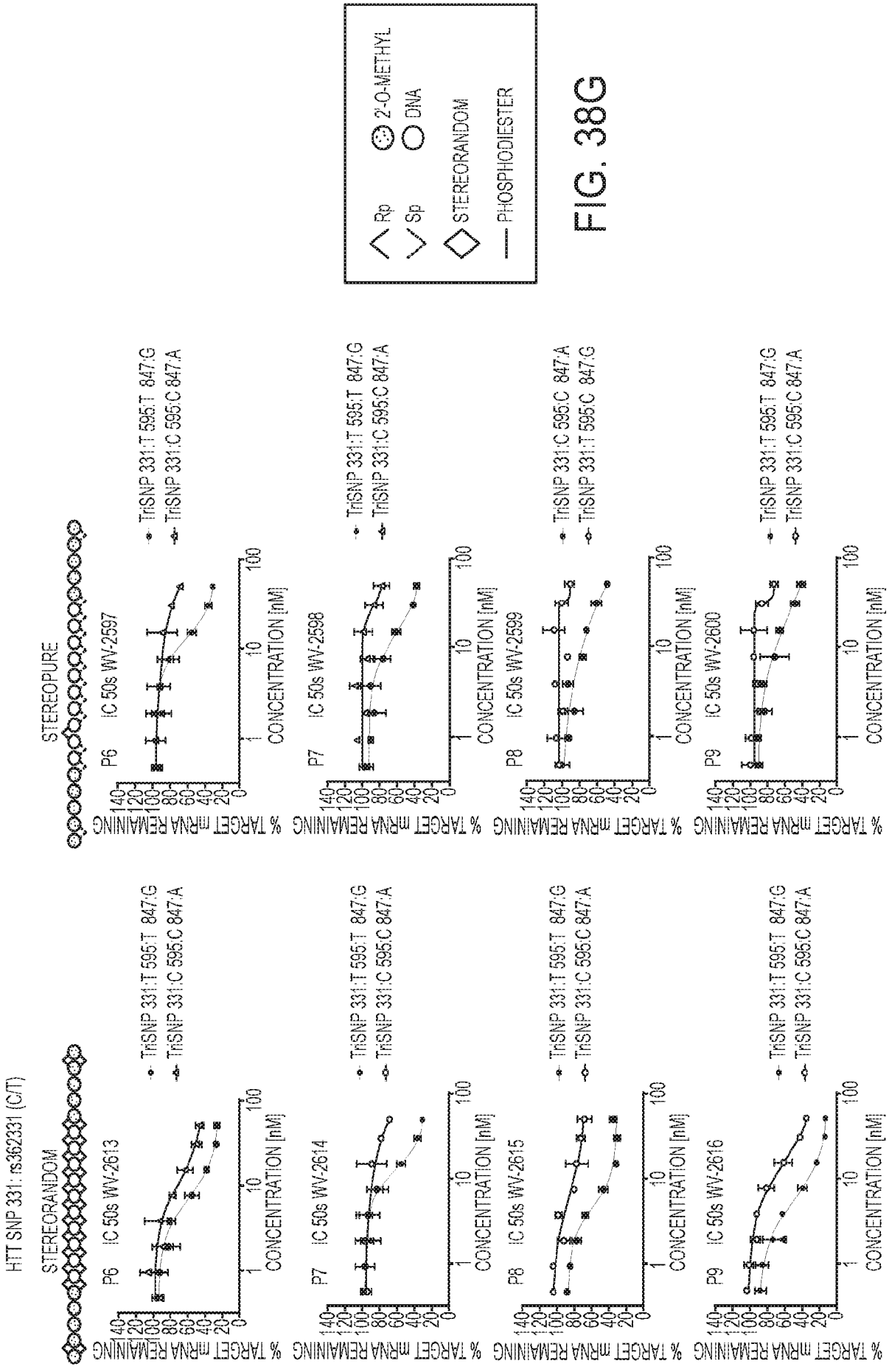

HTT SNP 331: rs362331 (C/T)
STEREORANDOM
STEREOPURE
P6 IC 50s WV-2613
P7 IC 50s WV-2614
P8 IC 50s WV-2615
P9 IC 50s WV-2616
P6 IC 50s WV-2597
P7 IC 50s WV-2598
P8 IC 50s WV-2599
P9 IC 50s WV-2600
TrISNP 331:T 595:T 847:G
TrISNP 331:C 595:C 847:A
% TARGET mRNA REMAINING
CONCENTRATION [nM]
Rp
Sp
STEREORANDOM
PHOSPHODIESTER
2'-O-METHYL
DNA

FIG. 38G

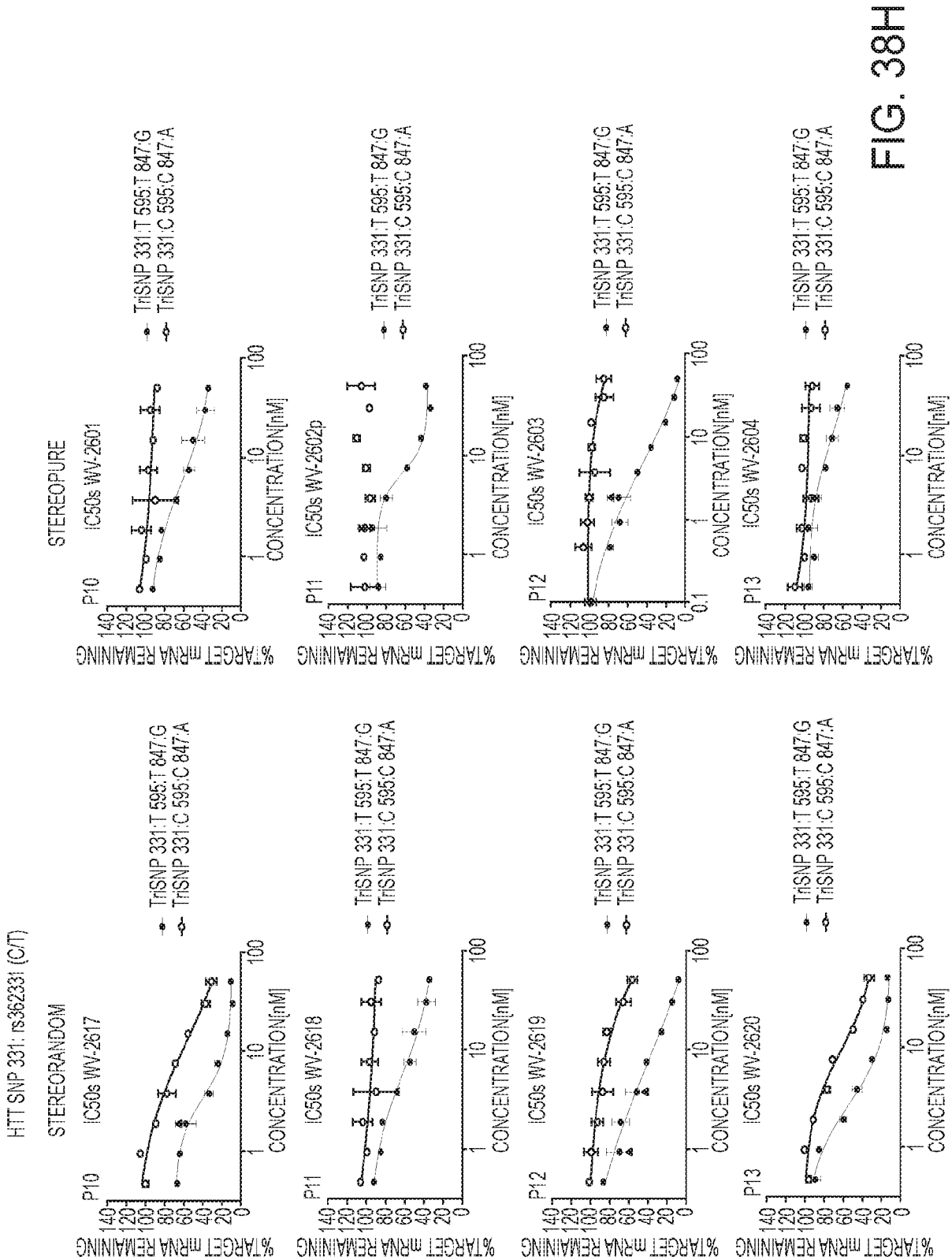

HTT SNP 331: rs362331 (C/T)
STEREORANDOM
STEREOPURE
P10
IC50s WV-2617
IC50s WV-2601
P11
IC50s WV-2618
IC50s WV-2602p
P12
IC50s WV-2619
IC50s WV-2603
P13
IC50s WV-2620
IC50s WV-2604
TriSNP 331:T 595:T 847:G
TriSNP 331:C 595:C 847:A
%TARGET mRNA REMAINING
CONCENTRATION[nM]

FIG. 38H

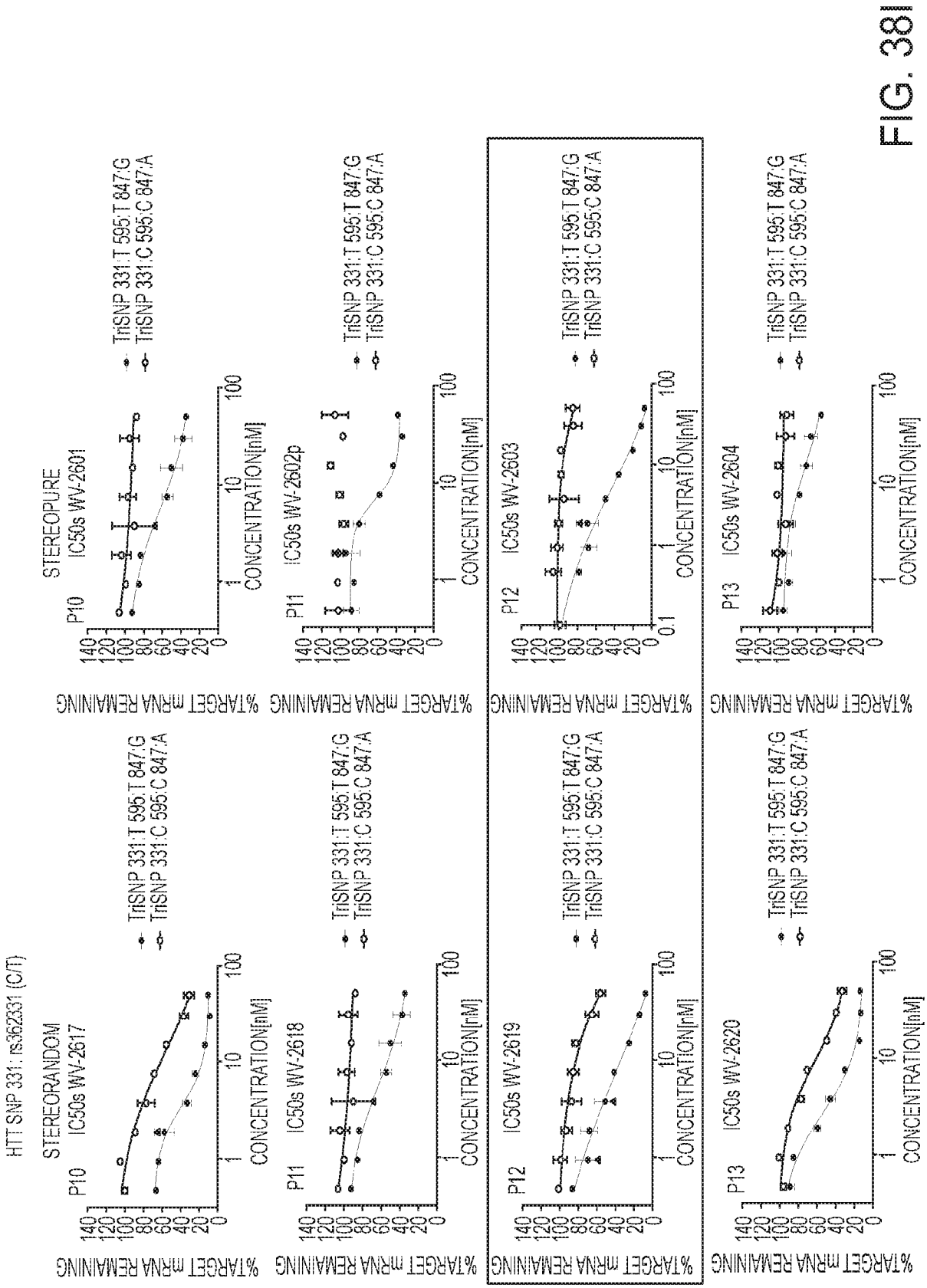

HTT SNP 331: rs362331 (C/T)
STEREORANDOM
P10 IC50s WV-2617
P11 IC50s WV-2618
P12 IC50s WV-2619
P13 IC50s WV-2620
STEREOPURE
P10 IC50s WV-2601
P11 IC50s WV-2602p
P12 IC50s WV-2603
P13 IC50s WV-2604
TriSNP 331:T 595:T 847:G
TriSNP 331:C 595:C 847:A
%TARGET mRNA REMAINING
CONCENTRATION[nM]

FIG. 38I

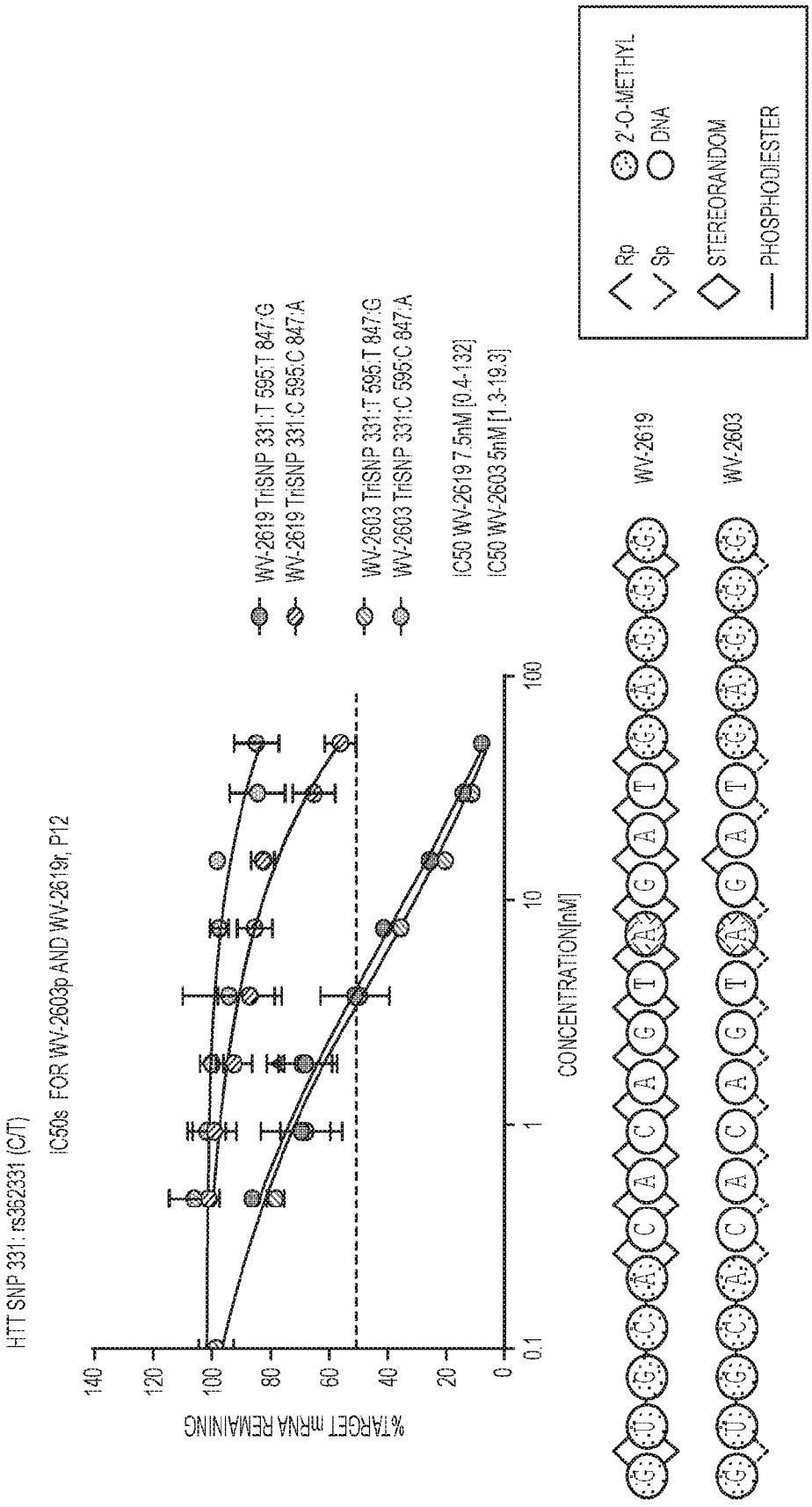

HTT SNP 331: rs362331 (C/T)
IC50s FOR WV-2603p AND WV-2619r, P12
%TARGET mRNA REMAINING
CONCENTRATION[nM]
WV-2619 TriSNP 331:T 595:T 847:G
WV-2619 TriSNP 331:C 595:C 847:A
WV-2603 TriSNP 331:T 595:T 847:G
WV-2603 TriSNP 331:C 595:C 847:A
IC50 WV-2619 7.5nM [0.4-132]
IC50 WV-2603 5nM [1.3-19.3]
Rp
Sp
2'-O-METHYL
DNA
STEREORANDOM
PHOSPHODIESTER
WV-2619
WV-2603

FIG. 39A

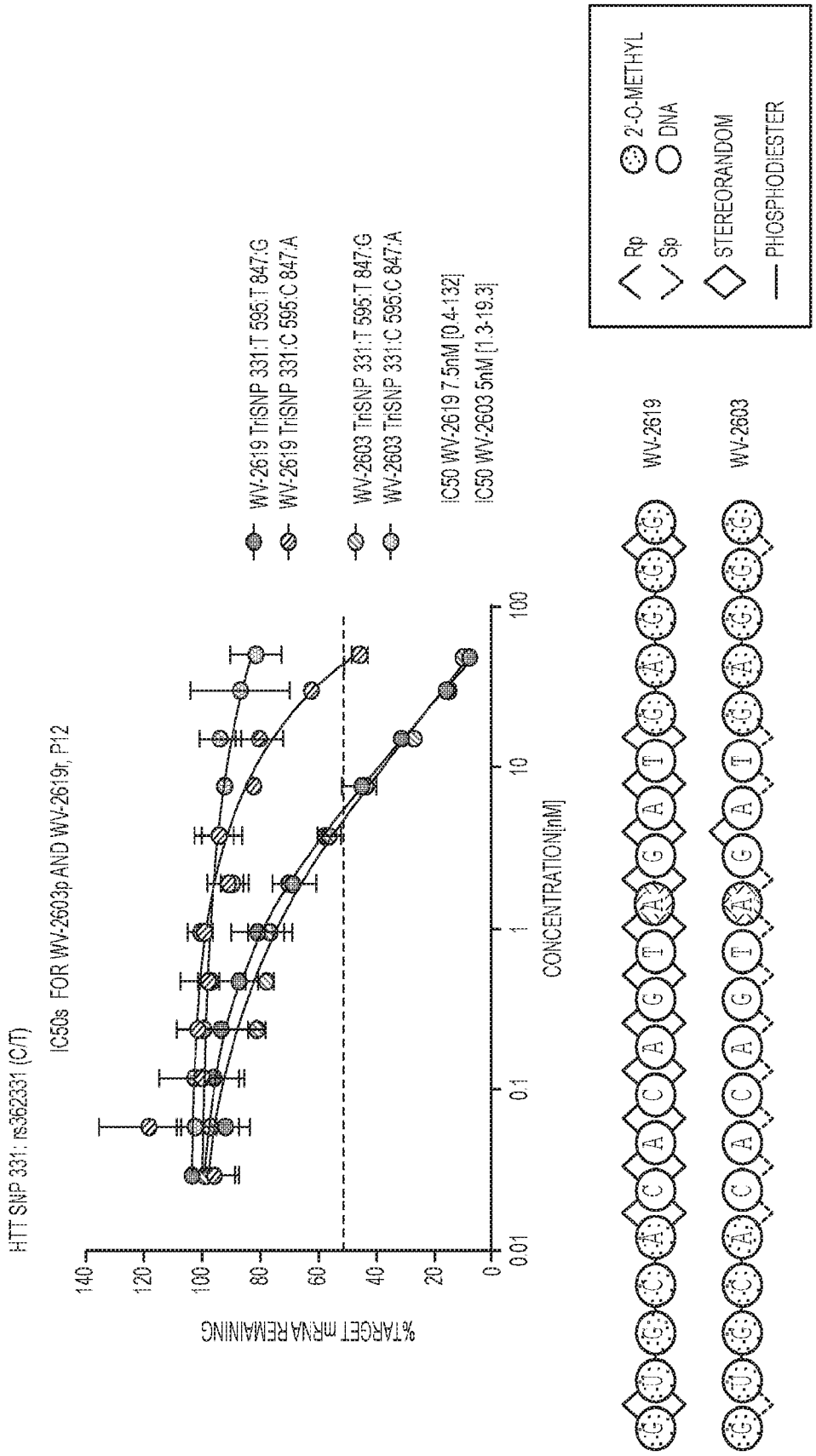

HTT SNP 331: rs362331 (C/T)
IC50s FOR WV-2603p AND WV-2619r, P12
%TARGET mRNA REMAINING
CONCENTRATION[nM]
WV-2619 TriSNP 331:T 595:T 847:G
WV-2619 TriSNP 331:C 595:C 847:A
WV-2603 TriSNP 331:T 595:T 847:G
WV-2603 TriSNP 331:C 595:C 847:A
IC50 WV-2619 7.5nM [0.4-132]
IC50 WV-2603 5nM [1.3-19.3]
Rp
Sp
STEREORANDOM
2'-O-METHYL
DNA
PHOSPHODIESTER
WV-2619
WV-2603

FIG. 39B

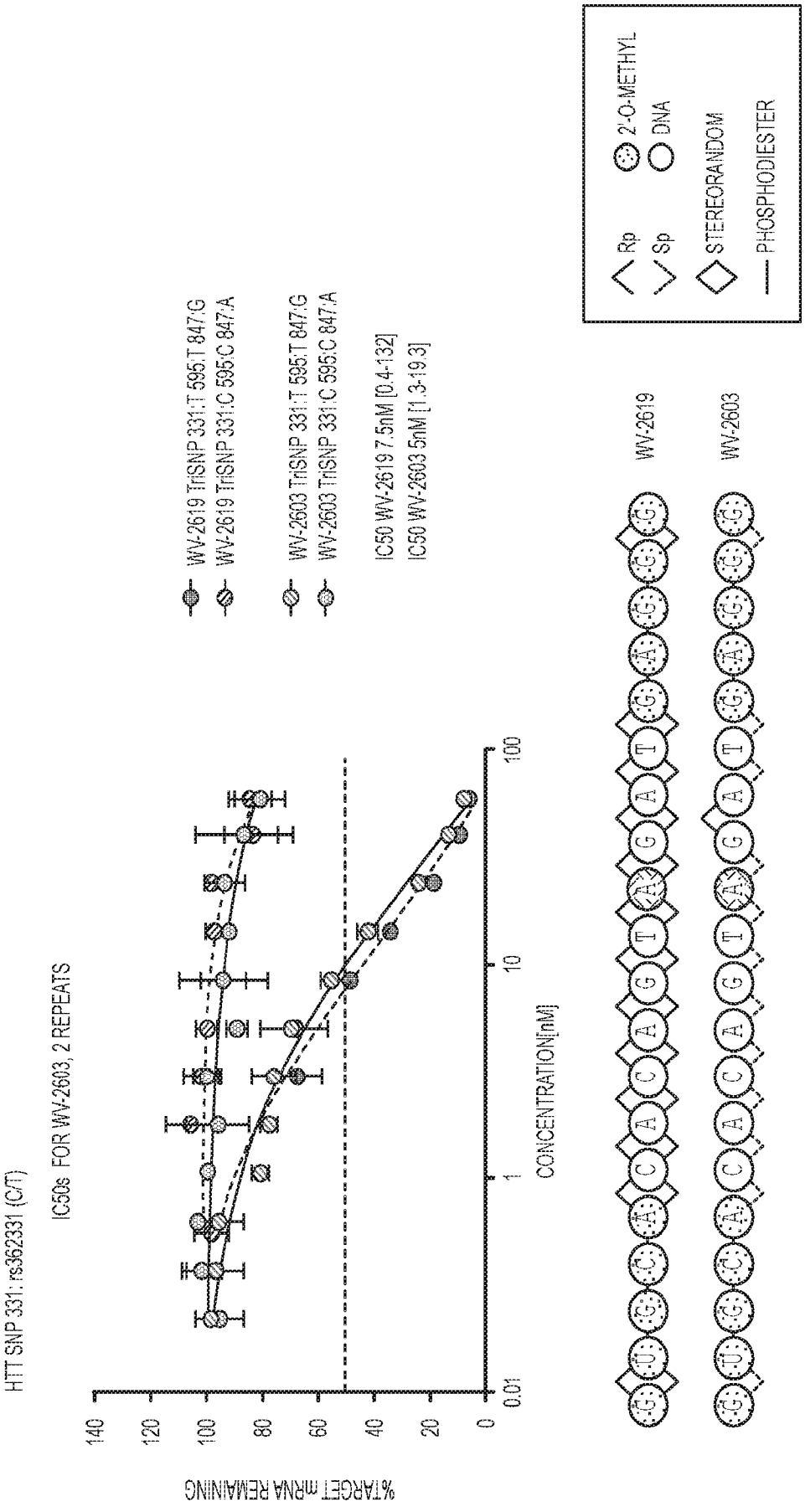

HTT SNP 331: rs362331 (C/T)
IC50s FOR WV-2603, 2 REPEATS
%TARGET mRNA REMAINING
CONCENTRATION[nM]
WV-2619 TriSNP 331:T 595:T 847:G
WV-2619 TriSNP 331:C 595:C 847:A
WV-2603 TriSNP 331:T 595:T 847:G
WV-2603 TriSNP 331:C 595:C 847:A
IC50 WV-2619 7.5nM [0.4-132]
IC50 WV-2603 5nM [1.3-19.3]
Rp
Sp
2'-O-METHYL
DNA
STEREORANDOM
PHOSPHODIESTER
WV-2619
WV-2603

FIG. 39C

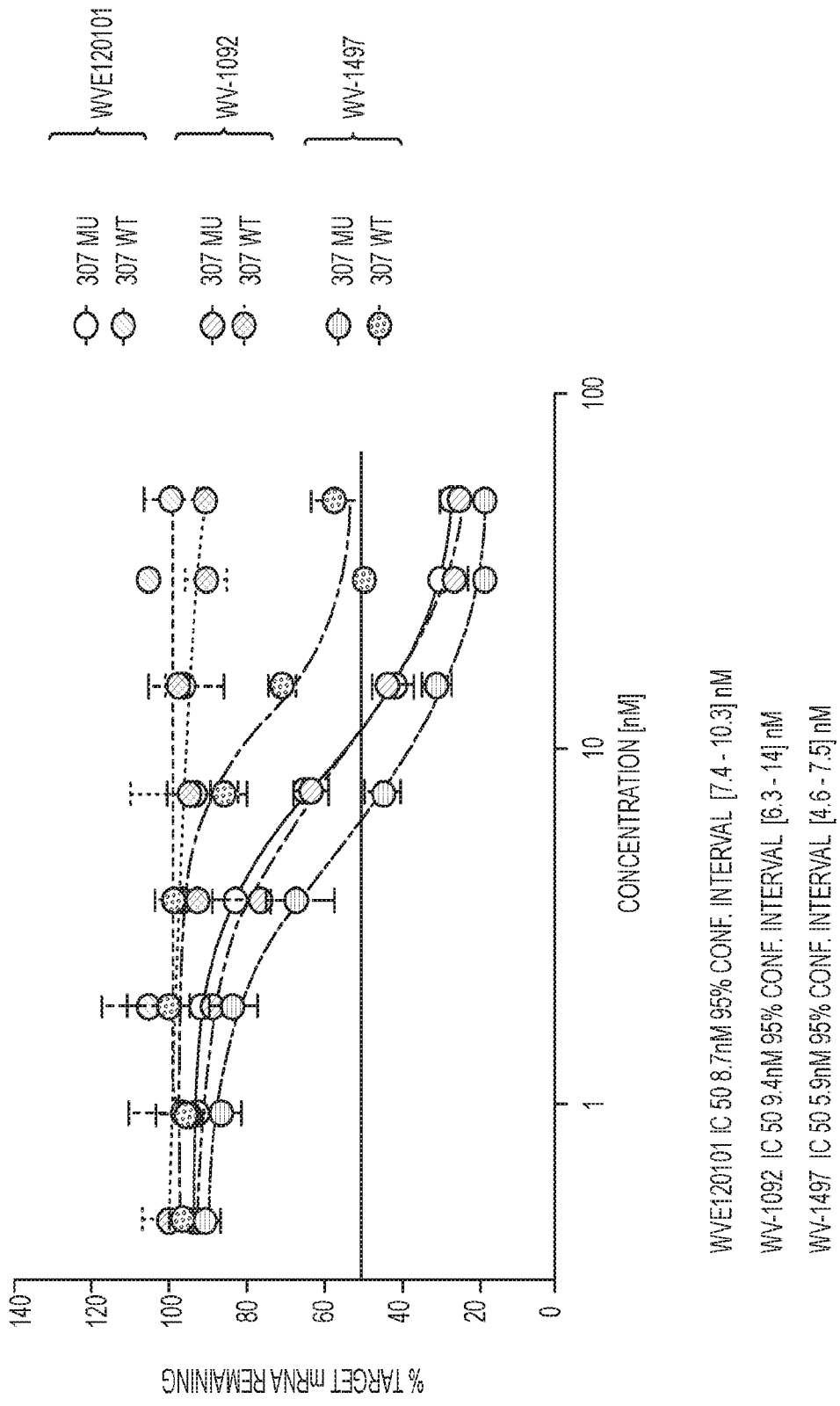

WVE120101
WV-1092
WV-1497
307 MU
307 WT
307 MU
307 WT
307 MU
307 WT
% TARGET mRNA REMAINING
140
120
100
80
60
40
20
0
1
10
100
CONCENTRATION [nM]
WVE120101 IC 50 8.7nM 95% CONF. INTERVAL [7.4 - 10.3] nM
WV-1092 IC 50 9.4nM 95% CONF. INTERVAL [6.3 - 14] nM
WV-1497 IC 50 5.9nM 95% CONF. INTERVAL [4.6 - 7.5] nM

FIG. 39D

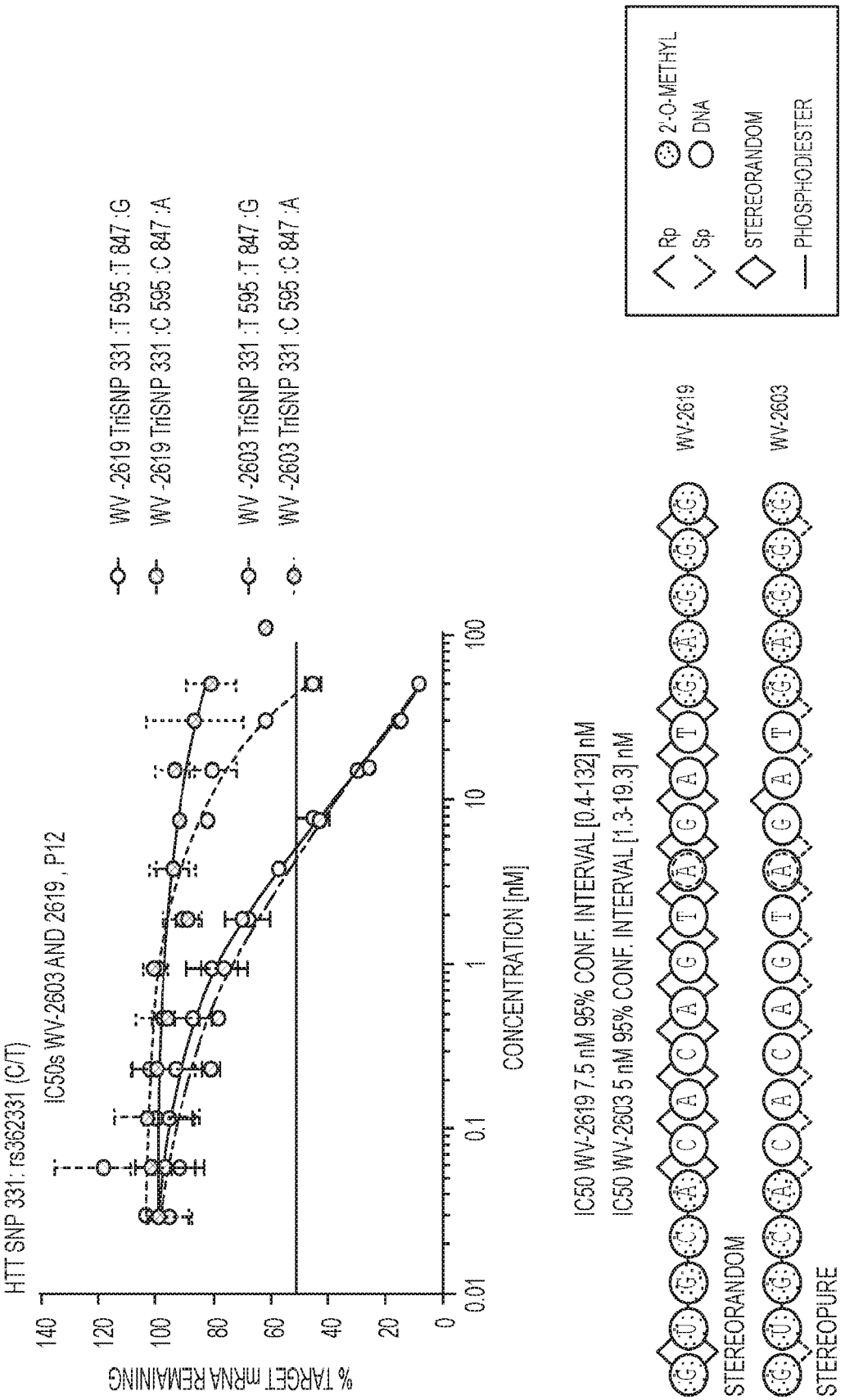

HTT SNP 331: rs362331 (C/T)
IC50s WV-2603 AND 2619 , P12
WV -2619 TriSNP 331 :T 595 :T 847 :G
WV -2619 TriSNP 331 :C 595 :C 847 :A
WV -2603 TriSNP 331 :T 595 :T 847 :G
WV -2603 TriSNP 331 :C 595 :C 847 :A
% TARGET mRNA REMAINING
CONCENTRATION [nM]
IC50 WV-2619 7.5 nM 95% CONF. INTERVAL [0.4-132] nM
IC50 WV-2603 5 nM 95% CONF. INTERVAL [1.3-19.3] nM
STEREORANDOM
STEREOPURE
WV-2619
WV-2603
Rp
Sp
2'-O-METHYL
DNA
STEREORANDOM
PHOSPHODIESTER

FIG. 39E

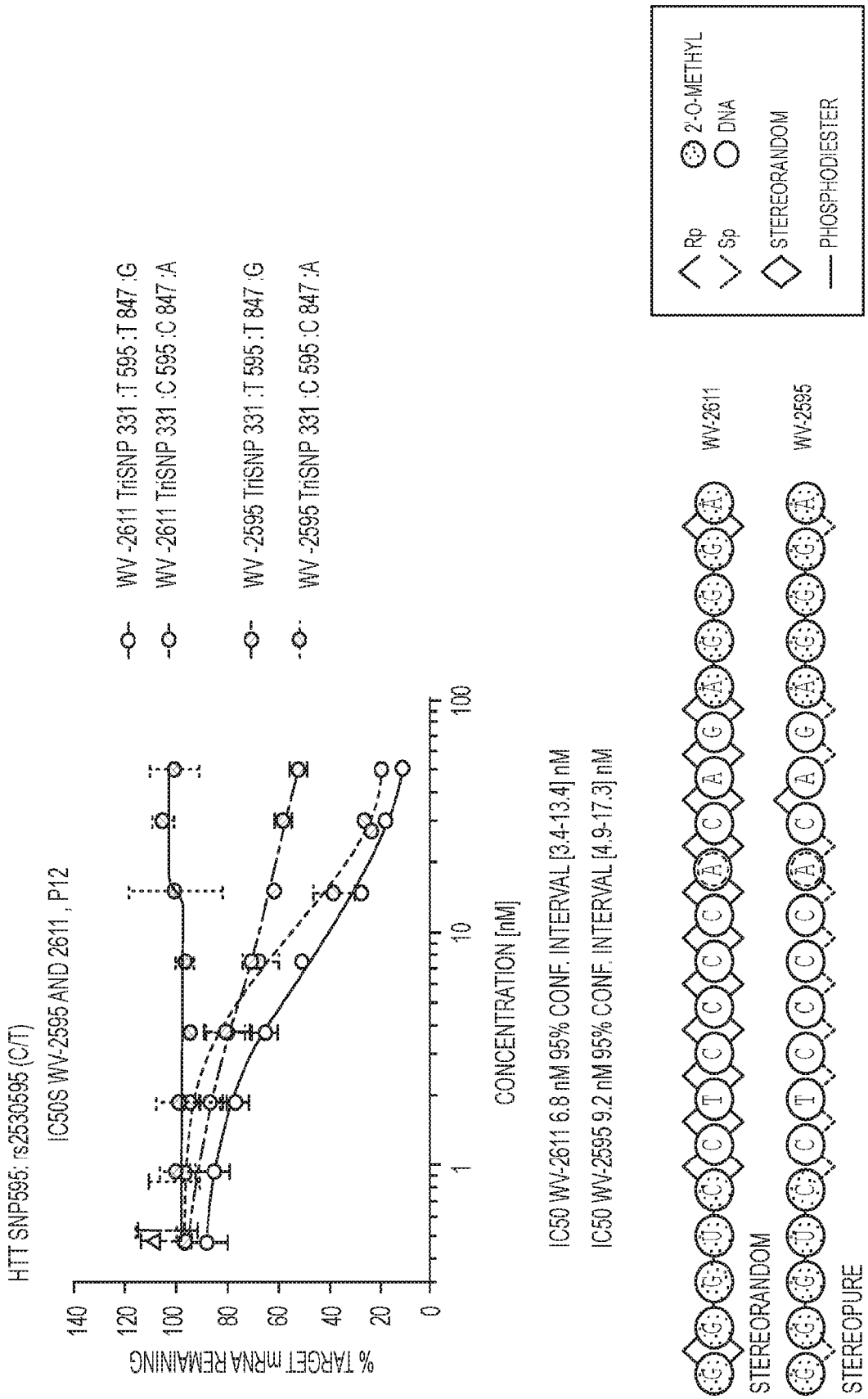

HTT SNP595: rs2530595 (C/T)
IC50S WV-2595 AND 2611 , P12
% TARGET mRNA REMAINING
CONCENTRATION [nM]
WV -2611 TriSNP 331 :T 595 :T 847 :G
WV -2611 TriSNP 331 :C 595 :C 847 :A
WV -2595 TriSNP 331 :T 595 :T 847 :G
WV -2595 TriSNP 331 :C 595 :C 847 :A
IC50 WV-2611 6.8 nM 95% CONF. INTERVAL [3.4-13.4] nM
IC50 WV-2595 9.2 nM 95% CONF. INTERVAL [4.9-17.3] nM
Rp
Sp
2'-O-METHYL
DNA
STEREORANDOM
PHOSPHODIESTER
WV-2611
STEREORANDOM
WV-2595
STEREOPURE

FIG. 39F

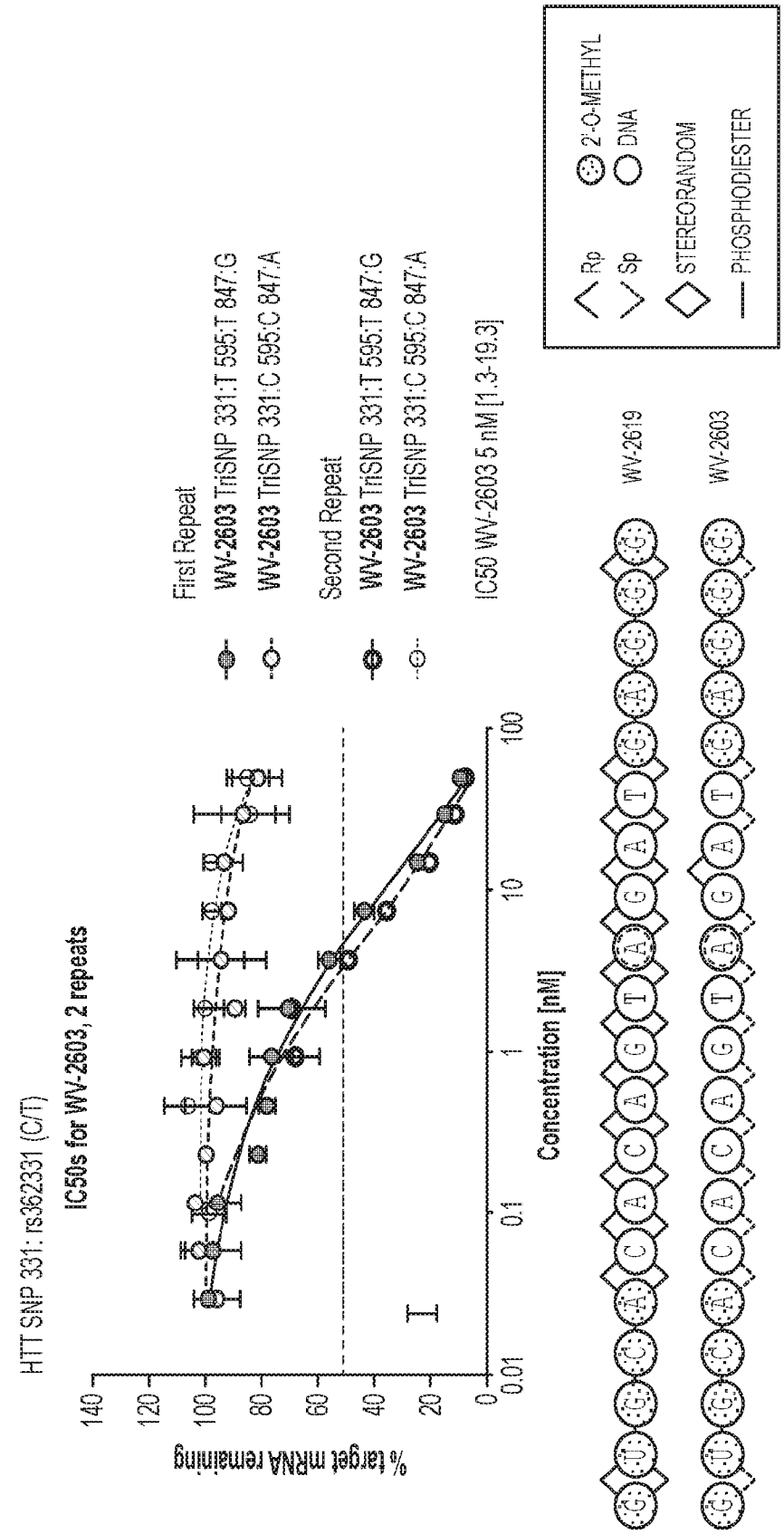

HTT SNP 331: rs362331 (C/T)
IC50s for WV-2603, 2 repeats
% target mRNA remaining
Concentration [nM]
First Repeat
WV-2603 TriSNP 331:T 595:T 847:G
WV-2603 TriSNP 331:C 595:C 847:A
Second Repeat
WV-2603 TriSNP 331:T 595:T 847:G
WV-2603 TriSNP 331:C 595:C 847:A
IC50 WV-2603 5 nM [1.3-19.3]
Rp
Sp
2'-O-METHYL
DNA
STEREORANDOM
PHOSPHODIESTER
WV-2619
WV-2603

FIG. 39G

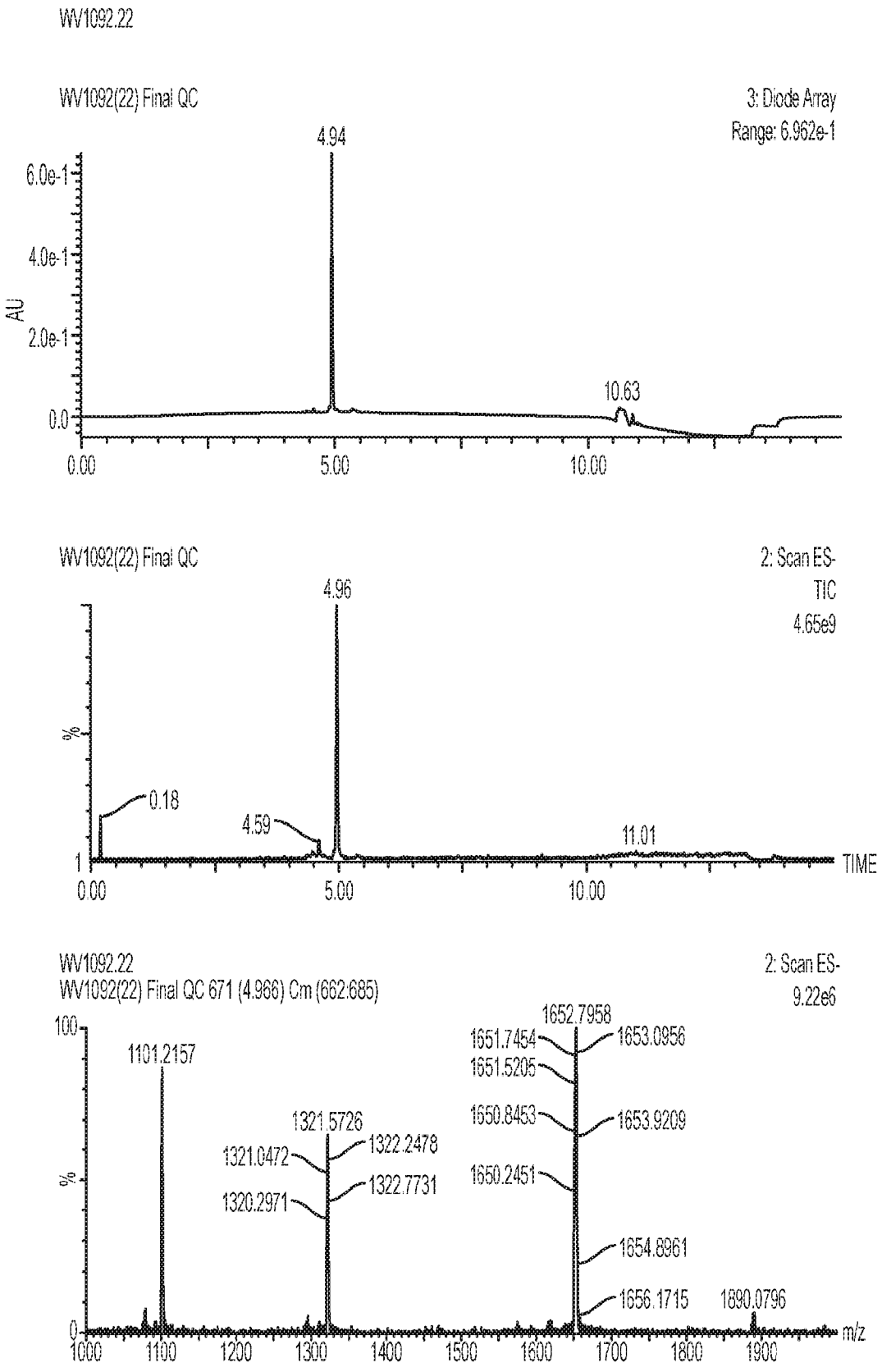

WV1092.22
WV1092(22) Final QC
3: Diode Array
Range: 6.962e-1
4.94
6.0e-1
4.0e-1
2.0e-1
0.0
AU
10.63
0.00
5.00
10.00
WV1092(22) Final QC
2: Scan ES-
TIC
4.65e9
4.96
%
0.18
4.59
11.01
1
TIME
0.00
5.00
10.00
WV1092.22
WV1092(22) Final QC 671 (4.966) Cm (662:685)
2: Scan ES-
9.22e6
1652.7958
100
1101.2157
1651.7454
1653.0956
1651.5205
1321.5726
1650.8453
1653.9209
1322.2478
1321.0472
1650.2451
1322.7731
1320.2971
%
1654.8961
1656.1715
1890.0796
0
m/z
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900

FIG. 40A

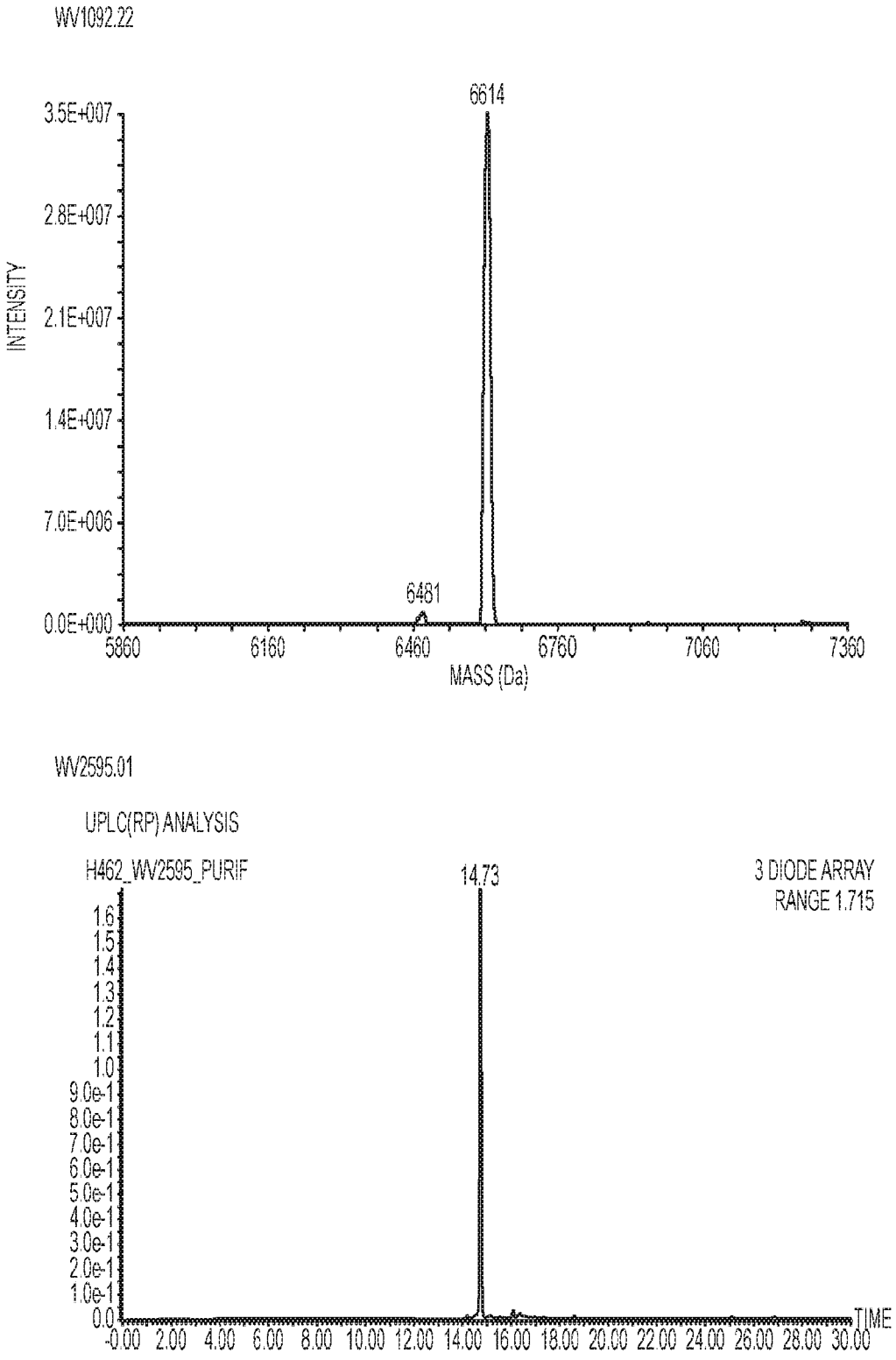
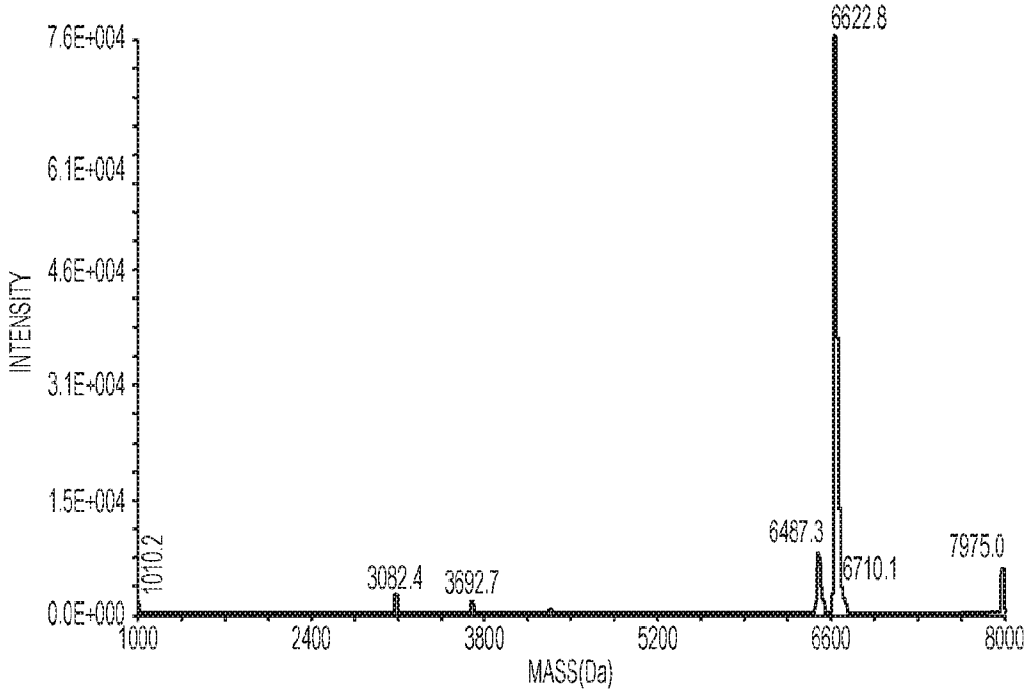

WV1092.22
6614
3.5E+007
2.8E+007
2.1E+007
1.4E+007
7.0E+006
0.0E+000
INTENSITY
6481
5860
6160
6460
6760
7060
7360
MASS (Da)
WV2595.01
UPLC(RP) ANALYSIS
H462_WV2595_PURIF
14.73
3 DIODE ARRAY
RANGE 1.715
1.6
1.5
1.4
1.3
1.2
1.1
1.0
9.0e-1
8.0e-1
7.0e-1
6.0e-1
5.0e-1
4.0e-1
3.0e-1
2.0e-1
1.0e-1
0.0
-0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00
TIME

FIG. 40B

WV2595.01

UPLC-MS ANALYSIS [MW]: CALCD 6623.04, FOUND 6622.8



WV2603.01

UPLC(RP) ANALYSIS

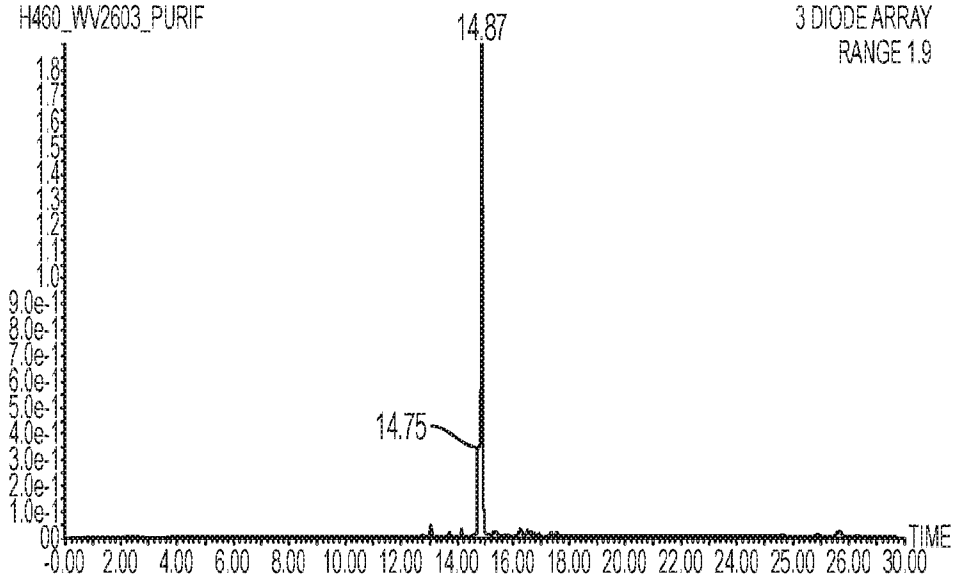
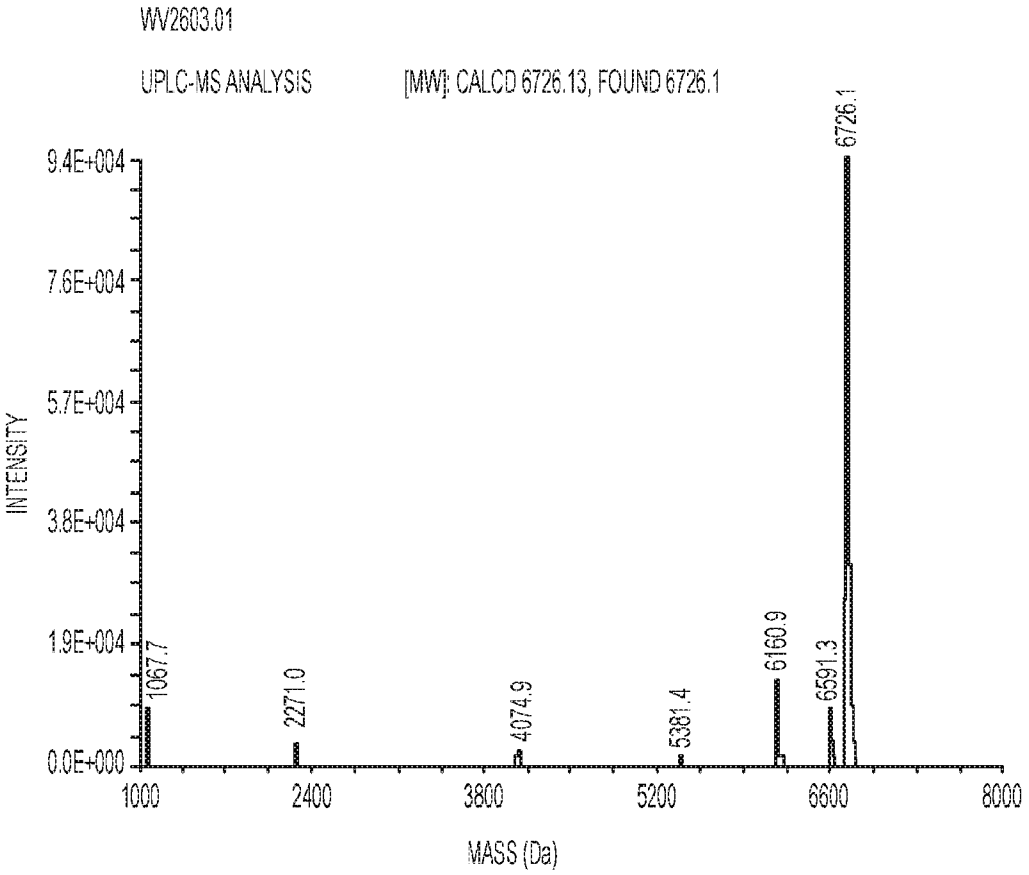
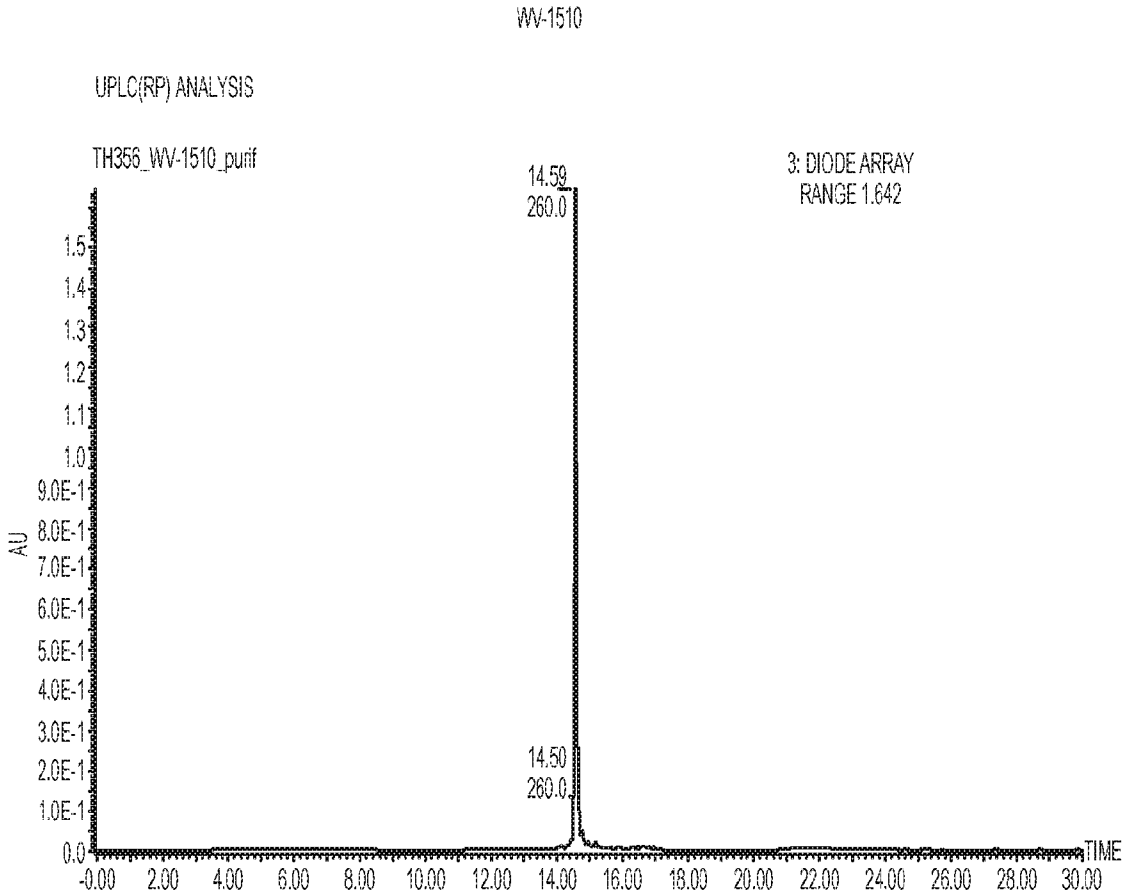


FIG. 40C



WV#	CALCULATED MASS (Da)	FOUND MASS (Da)
WV1092.22	6617.04710	6614
WV2595.01	6623.04838	6622.8
WV2603.01	6726.13322	6726.1

FIG. 40D


WV-1510
UPLC(RP) ANALYSIS
TH356_WV-1510_purif
3: DIODE ARRAY
RANGE 1.642
14.59
260.0
14.50
260.0
AU
TIME
1.5
1.4
1.3
1.2
1.1
1.0
9.0E-1
8.0E-1
7.0E-1
6.0E-1
5.0E-1
4.0E-1
3.0E-1
2.0E-1
1.0E-1
0.0
-0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00

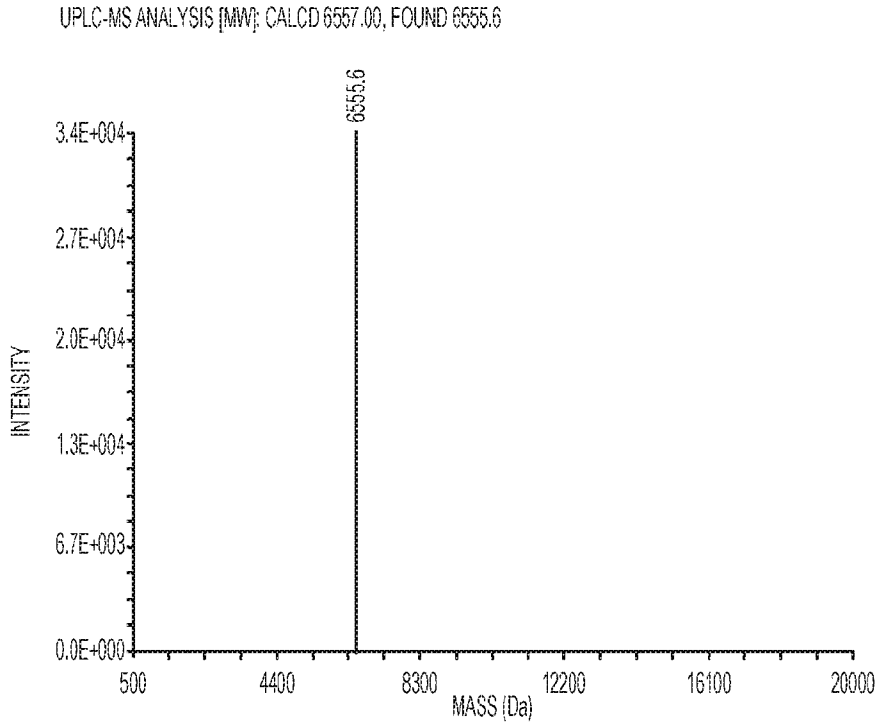
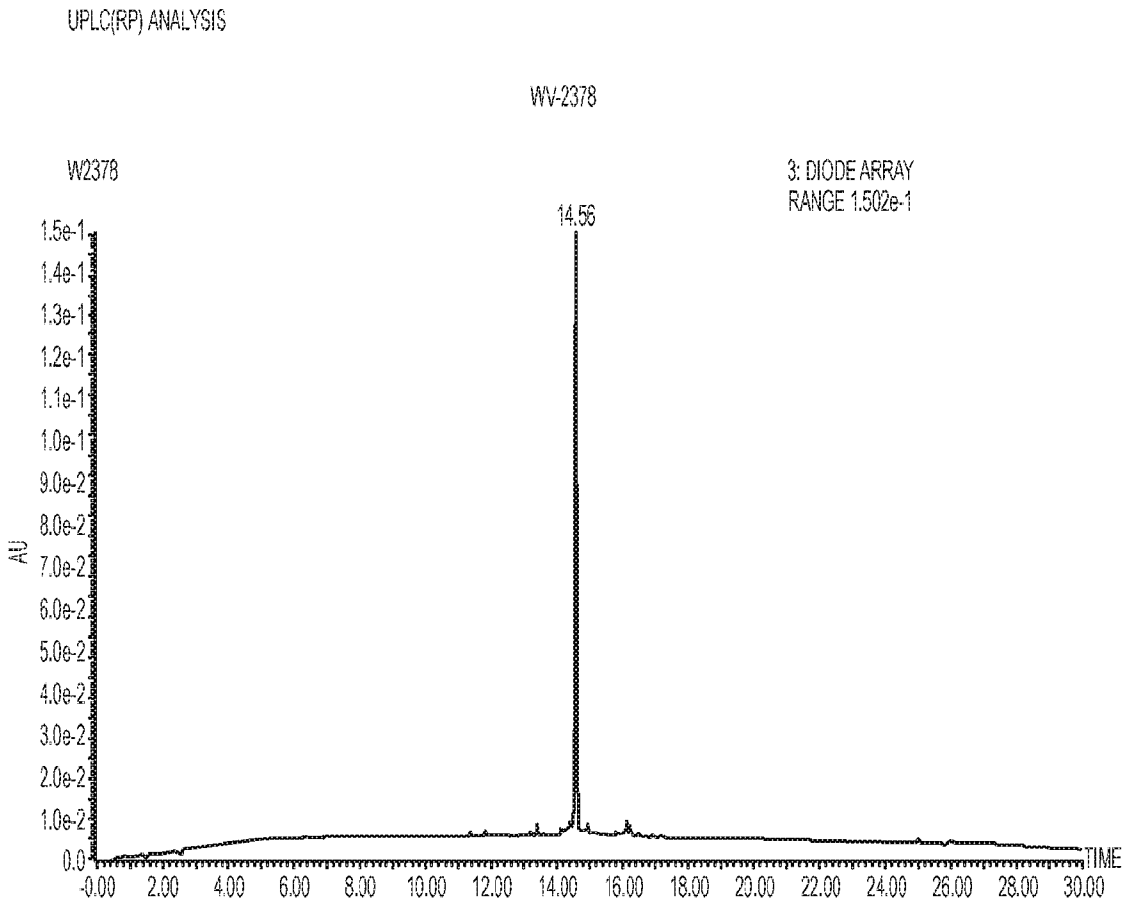

UPLC-MS ANALYSIS [MW]: CALCD 6557.00, FOUND 6555.6
6555.6
INTENSITY
3.4E+004
2.7E+004
2.0E+004
1.3E+004
6.7E+003
0.0E+000
500
4400
8300
12200
16100
20000
MASS (Da)

FIG. 41



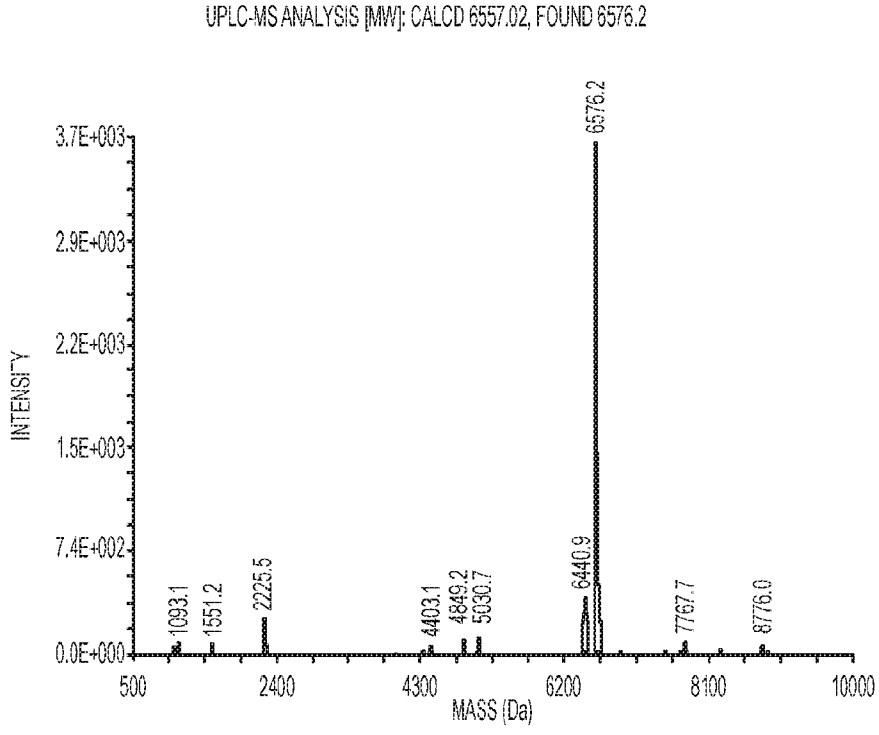


FIG. 41
CONTINUED

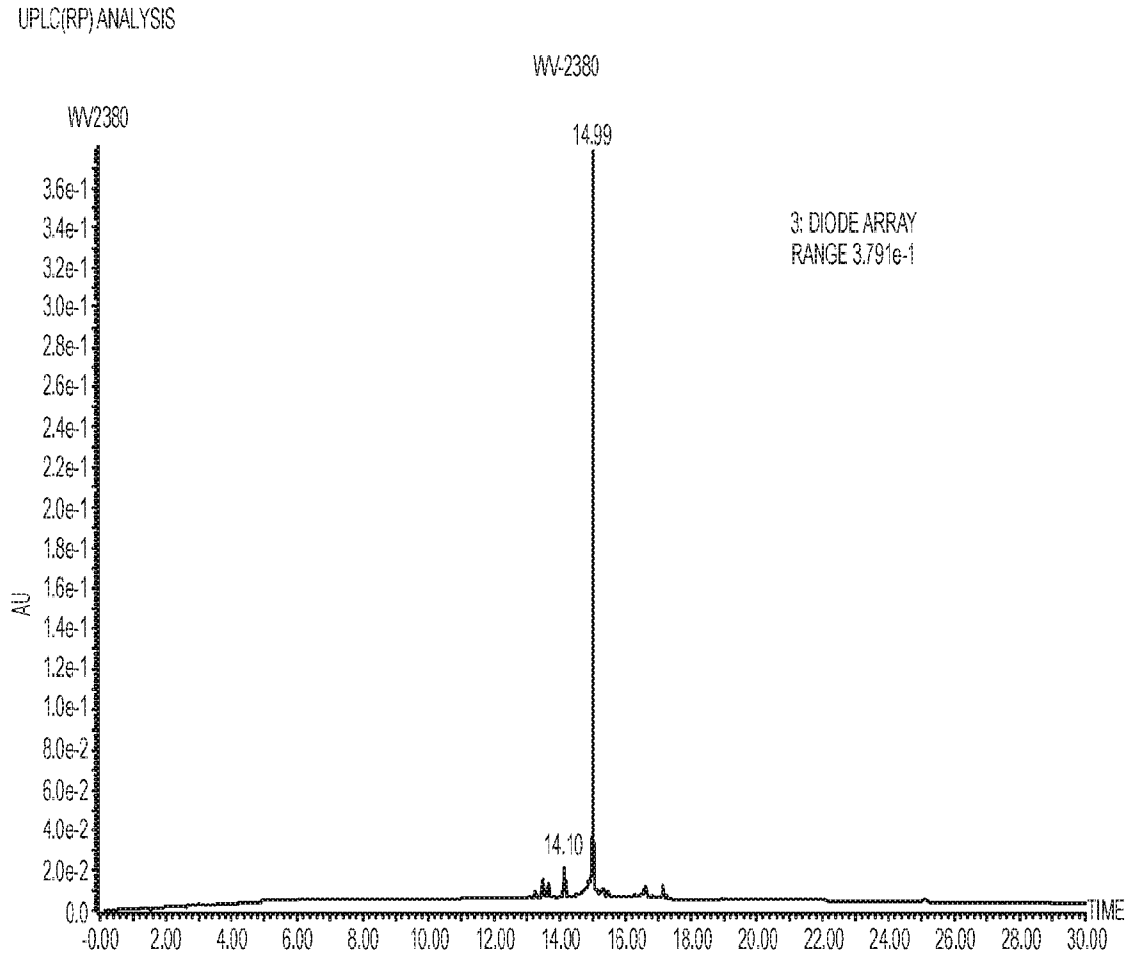


FIG. 41
CONTINUED

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/43542

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/43542

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 40
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-28, 32(in part), 33-39, 41(in part), directed to a composition comprising a chirally controlled oligonucleotide targeting a mutant Huntingtin gene and a method of treating Huntington's disease. The chirally controlled oligonucleotide composition will be searched to the extent that the oligonucleotide targets to Huntingtin gene SNP rs362307 and encompasses the first named chirally controlled oligonucleotide in Table N1A, WV-936 [see instant application, pg 294, para [00757], Table N1A; pg 589, Table 8]. It is believed that claims 1-25, 32(in part), 33-36, 38-39, 41(in part) encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass rs362307 and WV-936. Additional Huntingtin gene SNPs and chirally controlled oligonucleotides will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected SNPs and oligonucleotides.

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-25, 32/1, 33-36, 38-39, 41 limited to rs362307 and WV-936

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/43542

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 48/00, C12N 15/113 (2016.01)
CPC - C12N 15/113, C12N 2310/11
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8)- A61K 48/00, C12N 15/113 (2016.01)
CPC- C12N 15/113, C12N 2310/11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC- C12N 2310/313, C12N 2310/341, C12N 2310/345 (keyword search, terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (USPT, PGPB, EPAB, JPAB), Google Patents/Scholar
Search Terms Used: Chiral oligonucleotide, phosphorothioate, phosphate, chirally pure, huntington, huntingtin, antisense

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2014/012081 A2 (ONTORII, INC.) 16 January 2014 (16.01.2014) para [0097], [00101], [00105], [00200], [00217], [00425], [00426]	2, 21/2, 41 --- 1, 3-20, 21/1, 22-25, 32/1, 33-36, 38-39
Y	US 2015/0051389 A1 (Seth et al.) 19 February 2015 (19.02.2015) (hereinafter 'Seth') para [0039], [0045], [0064], [0410], [0423], [0483], [0592], [0654], [0829], [0830], Table 57, SEQ ID NO: 11	1, 3-20, 21/1, 22-25, 32/1, 33-36, 38-39
Y	US 2014/0256578 A1 (The University of British Columbia) 11 September 2014 (11.09.2014) abstract, Table 5, SEQ ID NO: 335	25, 34-36
Y	US 2014/0221395 A1 (DHANOA) 07 August 2014 (07.08.2014) abstract, claim 12	38

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search	Date of mailing of the international search report
14 December, 2016	28 DEC 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/43542

Continuation of:

Box NO III. Observations where unity of invention is lacking

Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be rs7685686 and ONT-451 (Table N4A) (Claims 1-25, 32(in part), 33-36, 38-39, 41(in part).

Group II: Claims 29-31, 32(in part), 41(in part) directed to a method for controlled cleavage of a nucleic acid polymer.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I+ includes the special technical feature of a composition comprising a chirally controlled oligonucleotide targeting a mutant Huntingtin gene, not required by Group II.

Group II includes the special technical feature of a method for controlled cleavage of a nucleic acid polymer, not required by Group I+.

The technical feature of each of the inventions listed as Group I+ is the specific Huntingtin SNPs and chirally controlled oligonucleotides, recited therein. Each invention requires a specific chirally controlled oligonucleotide targeting to a Huntingtin SNP, not required by any of the other inventions.

Common Technical Features

The inventions of Groups I+ and II share the technical feature of a chirally controlled oligonucleotide comprising oligonucleotides of a articular oligonucleotide type characterized by:
1) a common base sequence and length;
2) a common pattern of backbone linkages; and
3) a common pattern of backbone chiral centers;
which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length, for oligonucleotides of the particular oligonucleotide type.
The inventions of Groups I+ share the technical feature of claim 2.

However, these shared technical features do not represent a contribution over prior art in view of WO 2014/012081 A2 to ONTORII, INC. (hereinafter 'Ontorii'). Ontorii teaches (instant claim 2) a chirally controlled oligonucleotide composition comprising oligonucleotides defined by having:
1) a common base sequence and length;
2) a common pattern of backbone linkages; and
3) a common pattern of backbone chiral centers (para [00426], Oligo103, 5R-9S-5R, (Rp, Rp, Rp, Rp, Rp, Sp, Sp, Sp, Sp, Sp Sp, Sp, Sp, Sp, Rp, Rp, Rp, Rp,Rp)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]; [00217], the present invention provides chirally controlled compositions comprising a plurality of oligonucleotides of the same type, wherein each type is defined by: 1) base sequence; 2) pattern of backbone linkages; 3) pattern of backbone chiral centers; and 4) pattern of backbone P-modifications.), which composition is a substantially pure preparation of a single oligonucleotide (para [00105], Chirally pure: as used herein, the phrase "chirally pure" is used to describe a chirally controlled oligonucleotide composition in which all of the oligonucleotides exist in a single diastereomeric form with respect to the linkage phosphorus) in that a predetermined level of the oligonucleotides in the composition have the common base sequence and length, the common pattern of backbone linkages, and the common pattern of backbone chiral centers (para [00426], [00217]).

The inventions of Groups I+ share the technical feature of claim 1 and Huntingtin SNPs. However, these shared technical features do not represent a contribution over prior art in view of US 2015/0051389 A1 to Seth et al. (hereinafter 'Seth') and Ontorii, as applied above. Seth teaches a chirally pure oligonucleotide composition (para [0423], In certain embodiments, internucleoside linkages having a chiral atom can be prepared as a racemic mixture, or as separate enantiomers. Representative chiral linkages include, but are not limited to, alkylphosphonates and phosphorothioates.) comprising oligonucleotides of a particular oligonucleotide type characterized by:

- Please see extra sheet for continuation -

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/43542

Continuation of:

Box NO III. Observations where unity of invention is lacking

1) a common base sequence and length;
2) a common pattern of backbone linkages; and
3) a common backbone chiral centers (para [0830], The modified oligonucleotides were created with a 3-9-3 motif and are described in Table 57. The internucleoside linkages throughout each gapmer are phosphorothioate (P=S) linkages. For example, ISIS NO. 539560 T.sub.eA.sub.kA.sub.kATTG.sup.pUCATCA.sub.kC.sub.kC.sub.e, SEQ ID NO: 11). Note, since the internucleoside linkages throughout each gapmer are phosphorothioate, this, ISIS NO. 539560 has a common pattern of backbone linkages; and a common backbone chiral centers.) which composition is chirally enriched, relative to a substantially racemic preparation of oligonucleotides having the same base sequence and length (para [0423]), for oligonucleotides of the particular oligonucleotide type, wherein the oligonucleotides target a mutant Huntingtin gene (para 0829], These modified oligonucleotides were designed by introducing various chemical modifications in the central gap region and were tested for their ability to selectively inhibit mutant (mut) HTT mRNA expression levels targeting rs7685686 while leaving the expression of the wild-type (wt) intact.), and the length is from about 10 to about 50 nucleotides, wherein the backbone linkages comprise at least one phosphorothioate (Table 57, ISIS NO. 539560). Seth does not specifically teach a a common backbone chiral centers, and wherein the pattern of backbone chiral centers comprises at least one Rp chiral center and at least one Sp chiral center. Ontorii teaches said chirally controlled oligonucleotide (para [00426], Oligo103, 5R-9S-5R, (Rp, Rp, Rp, Rp, Rp, Sp, Sp, Sp, Sp, Sp Sp, Sp, Sp, Sp, Rp, Rp, Rp, Rp,Rp)-d[GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC]; [00217], the present invention provides chirally controlled compositions comprising a plurality of oligonucleotides of the same type, wherein each type is defined by: 1) base sequence; 2) pattern of backbone linkages; 3) pattern of backbone chiral centers; and 4) pattern of backbone P-modifications.). Ontorii further teaches that said chirally controlled oligonucleotides exhibit "chirally controlled oligonucleotides that are structurally optimized to exhibit certain desirable characteristics, such as, e.g., increased stability and improved efficacy for in vitro and/or in vivo applications." (para [00200]). Accordingly, one of ordinary skill in the art would have modified the chirally pure oligonucleotides targeting Huntingtin SNPs of Set"h to a chirally controlled oligonucleotides of Ontorii, to increase the in vivo half-life of oligonucleotides.

As said technical features were known in the art at the time of the invention, these cannot be considered special technical feature that would otherwise unify the groups.

Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

摘要
本公开内容尤其涉及具有选定设计的手性控制的寡核苷酸、手性控制的寡核苷酸组合物、及其制备和使用方法。在一些实施方案中，提供的手性控制的寡核苷酸组合物提供了与参照寡核苷酸组合物不同的核酸聚合物切割样式。在一些实施方案中，提供的手性控制的寡核苷酸组合物在核酸聚合物的互补序列中提供了单一位点切割。在一些实施方案中，手性控制的寡核苷酸组合物具有本文中所描述的任何碱基序列和/或碱基修饰、糖修饰、骨架修饰和/或立体化学的样式 或者这些要素的组合。