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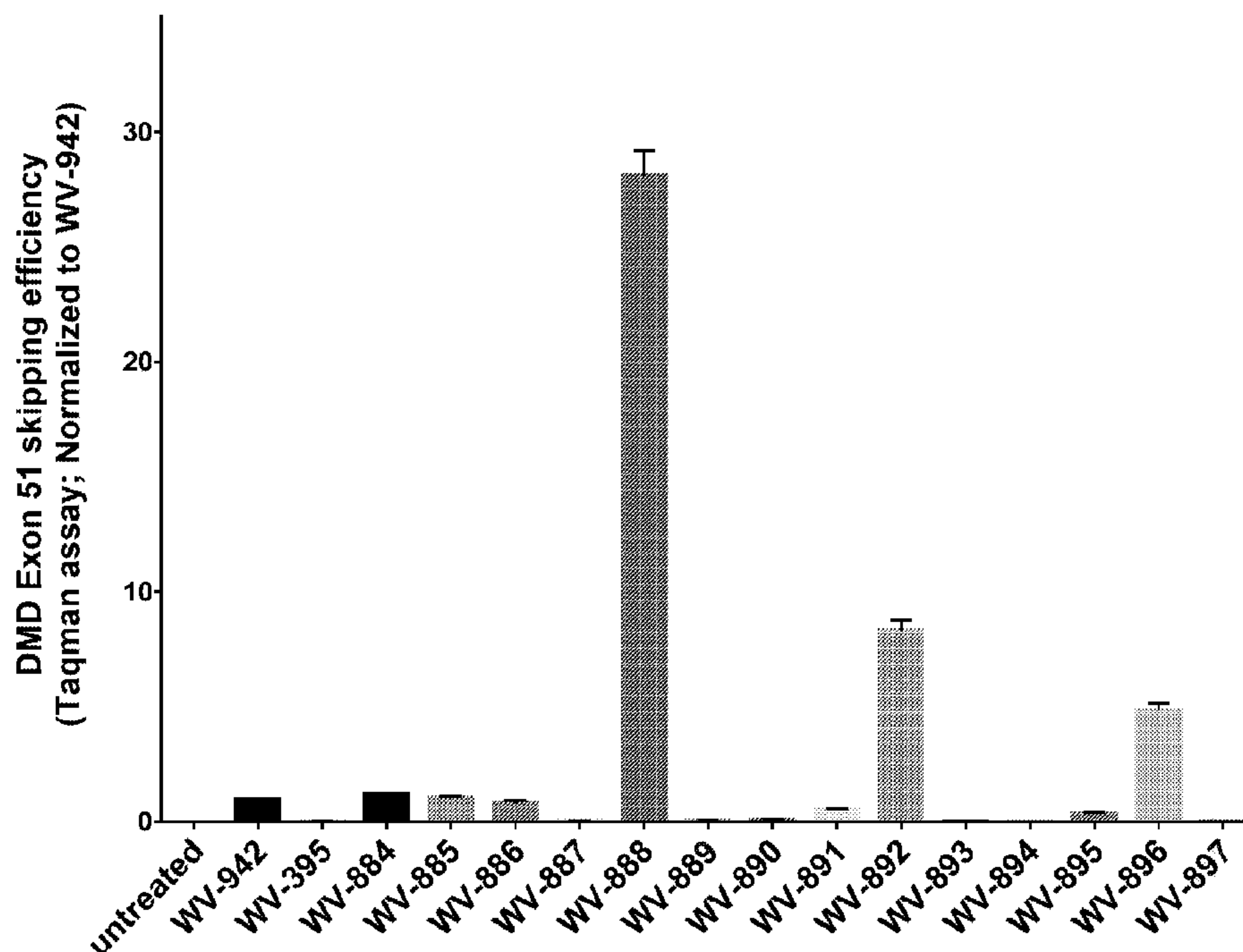
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FIG. 1



(57) Abrégé/Abstract:

Among other things, the present disclosure relates to designed oligonucleotides, compositions, and methods thereof. In some embodiments, provided oligonucleotide compositions provide altered splicing of a transcript. In some embodiments, provided oligonucleotide compositions have low toxicity. In some embodiments, provided oligonucleotide compositions provide improved protein binding profiles. In some embodiments, provided oligonucleotide compositions have improved delivery. In some embodiments, provided oligonucleotide compositions have improved uptake. In some embodiments, the present disclosure provides methods for treatment of diseases using provided oligonucleotide compositions.

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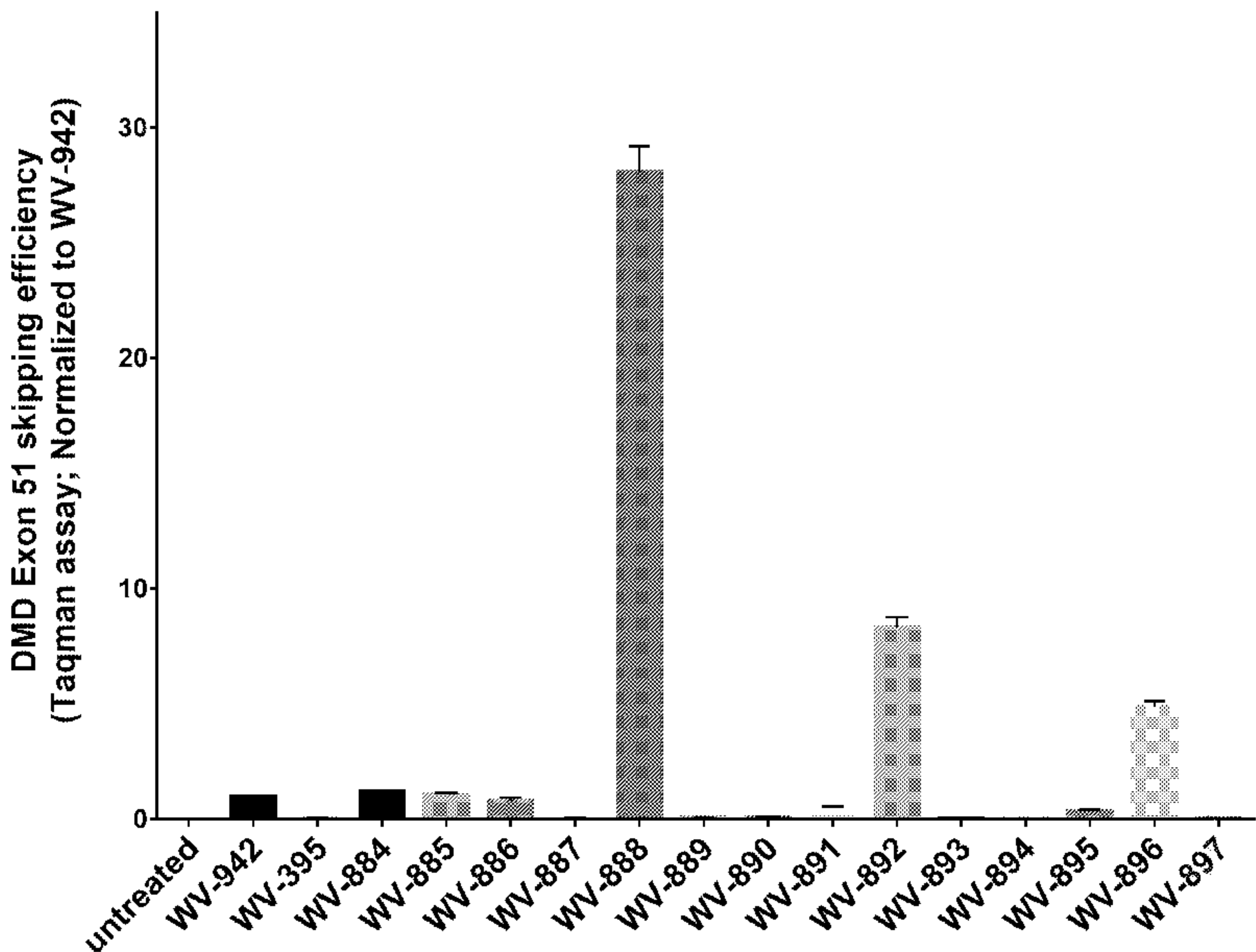
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(54) Title: OLIGONUCLEOTIDE COMPOSITIONS AND METHODS THEREOF

FIG. 1



(57) Abstract: Among other things, the present disclosure relates to designed oligonucleotides, compositions, and methods thereof. In some embodiments, provided oligonucleotide compositions provide altered splicing of a transcript. In some embodiments, provided oligonucleotide compositions have low toxicity. In some embodiments, provided oligonucleotide compositions provide improved protein binding profiles. In some embodiments, provided oligonucleotide compositions have improved delivery. In some embodiments, provided oligonucleotide compositions have improved uptake. In some embodiments, the present disclosure provides methods for treatment of diseases using provided oligonucleotide compositions.

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OLIGONUCLEOTIDE COMPOSITIONS AND METHODS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to United States Provisional Application Nos. 62/239,839, filed October 9, 2015, 62/331,961, filed May 4, 2016, and 62/331,966, 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 oligonucleotide properties, *e.g.*, activities, toxicities, *e.g.*, as may be mediated by protein binding characteristics, stability, splicing-altering capabilities, *etc.* In some embodiments, the present disclosure demonstrates that oligonucleotide compositions comprising oligonucleotides with controlled structural elements, *e.g.*, controlled chemical modification and/or controlled backbone stereochemistry patterns, provide unexpected properties, including but not limited to certain activities, toxicities, *etc.* In some embodiments, the present disclosure demonstrates that oligonucleotide properties, *e.g.*, activities, toxicities, *etc.*, can be modulated by chemical modifications (*e.g.*, modifications of sugars, bases, internucleotidic linkages, *etc.*), chiral structures (*e.g.*, stereochemistry of chiral internucleotidic linkages and patterns thereof, *etc.*), and/or combinations thereof.

[0004] Particularly, in some embodiments, the present disclosure provides compositions

and methods for altering splicing of transcripts. Splicing of a transcript, such as pre-mRNA, is an essential step for the transcript to perform its biological functions in many higher eukaryotes. Defects and/or insufficiency in the splicing process can affect biological functions and/or have pathological consequences. For example, many human genetic diseases are caused by mutations that cause splicing defects, and many diseases are associated with splicing defects that are not attributed to overt mutations. In some embodiments, the present disclosure recognizes that targeting splicing, especially through compositions comprising oligonucleotides having the chemical modifications and/or stereochemistry patterns described in this disclosure, can effectively correct disease-associated aberrant splicing, and/or introduce and/or enhance beneficial splicing that lead to desired products, *e.g.*, mRNA, proteins, *etc.* which can repair, restore, or add new desired biological functions. For example, in some embodiments, inclusion of a mutated exon 51 of DMD can cause a frameshift, premature stop codon and/or deletion of one or more downstream exons. In some embodiments, the present disclosure provides compositions and methods for effectively skipping exon 51 of DMD to restore the reading frame so that a shorter but partially functional dystrophin can be produced. In some embodiments, the present disclosure provides compositions and methods for effectively skipping a mutant exon 51 of DMD to restore the reading frame so that a shorter but partially functional dystrophin can be produced. In some embodiments, provided compositions and methods can alter splicing of a transcript to effectively decrease levels of undesired splicing products. For example, in some embodiments, by skipping one or more exons of a pre-mRNA to produce an mRNA with frameshift and/or premature termination codon, provided compositions and methods effectively knockdown a gene; in some embodiments, such a gene is a mutant gene. A person having ordinary skill in the art appreciates that provided technologies (oligonucleotides, compositions, methods, *etc.*) can also be utilized for exon skipping of other DMD exons, or one or more exons of other transcripts, for example, those described in US 7,534,879 and incorporated herein by reference, in accordance with the present disclosure to treat a disease and/or condition.

[0005] In some embodiments, the present disclosure encompass the recognition that chemical modifications, stereochemistry and combinations thereof can be used to improve properties of oligonucleotide compositions including but not limited to their capabilities to modulate splicing of transcripts. In some embodiments, the present disclosure provides chemical modifications and patterns thereof useful for improving transcript splicing by oligonucleotides.

[0006] Among other things, the present disclosure demonstrates that stereochemistry can be used to modulate transcript splicing by oligonucleotide compositions. In some embodiments, the present disclosure provides combinations of chemical modifications and stereochemistry to improve properties of oligonucleotides, *e.g.*, their capabilities to alter splicing of transcripts. In some embodiments, the present disclosure provides chirally controlled oligonucleotide compositions that, when compared to a reference condition (*e.g.*, absence of the composition, presence of a reference composition (*e.g.*, a stereorandom composition of oligonucleotides having the same base sequence, the same chemical modifications, *etc.*, a chirally controlled oligonucleotide composition of another stereoisomer, *etc.*), and combinations thereof), provide altered splicing that can deliver one or more desired biological effects, for example, increase production of desired proteins, knockdown of a gene by producing mRNA with frameshift mutations and/or premature termination codons, knockdown of a gene expressing a mRNA with a frameshift mutation and/or premature termination codon, *etc.* In some embodiments, compared to a reference condition, provided chirally controlled oligonucleotide compositions are surprisingly effective. In some embodiments, desired biological effects (*e.g.*, as measured by increased levels of desired mRNA, proteins, *etc.*, decreased levels of undesired mRNA, proteins, *etc.*) can be enhanced by more than 5, 10, 15, 20, 25, 30, 40, 50, or 100 fold.

[0007] The present disclosure recognizes challenges of providing low toxicity oligonucleotide compositions and methods thereof. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced toxicity. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced immune responses. In some embodiments, the present disclosure recognizes that various toxicities induced by oligonucleotides are related to complement activation. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced complement activation. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced complement activation via the alternative pathway. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced complement activation via the classical pathway. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced drug-induced vascular injury. In some embodiments, the present disclosure provides oligonucleotide compositions and methods with reduced injection site inflammation. In

some embodiments, reduced toxicity can be evaluated through one or more assays widely known to and practiced by a person having ordinary skill in the art, *e.g.*, evaluation of levels of complete activation product, protein binding, *etc.* as described herein.

[0008] In some embodiments, the present disclosure provides oligonucleotides with enhanced antagonism of hTLR9 activity. In some embodiments, certain diseases, *e.g.*, DMD, are associated with inflammation in, *e.g.*, muscle tissues. In some embodiments, provided technologies (*e.g.*, oligonucleotides, compositions, methods, *etc.*) provides both proteins with enhanced activities (*e.g.*, through exon-skipping of mutant exon 51 (or other exons depending on genotypes) of DMD) and hTLR9 antagonist activities which can be beneficial to one or more conditions and/or diseases associated with inflammation. In some embodiments, provided oligonucleotides and/or compositions thereof provides both exon-skipping capabilities and hTLR9 antagonist activities. In some embodiments, oligonucleotides comprising one or more lipid moieties (*e.g.*, oligonucleotides conjugated with lipids) provide unexpectedly high exon-skipping efficiency and hTLR9 antagonist activities. In some embodiments, a chirally controlled oligonucleotide composition comprising a predetermined level of an oligonucleotide comprising one or more lipid moieties (*e.g.*, oligonucleotides conjugated with lipids) provides unexpectedly high exon-skipping efficiency and hTLR9 antagonist activities

[0009] In some embodiments, the present disclosure demonstrates that oligonucleotide properties, *e.g.*, activities, toxicities, *etc.*, can be modulated through chemical modifications. In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides which have a common base sequence, and comprise one or more modified sugar moieties, one or more natural phosphate linkages, or combinations thereof. In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides which have a common base sequence, comprise one or more modified internucleotidic linkages, and comprise one or more modified sugar moieties, one or more natural phosphate linkages, or combinations thereof. For example, in some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides which have a common base sequence, and comprise one or more modified sugar moieties; in some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides which have a common base sequence, and comprise one or more modified sugar moieties and one or more

natural phosphate linkages; in some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides which have a common base sequence, and comprise one or more modified sugar moieties and one or more modified internucleotidic linkages; in some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides which have a common base sequence, and comprise one or more modified sugar moieties, one or more natural phosphate linkages and one or more modified internucleotidic linkages. In some embodiments, provided oligonucleotide composition comprising a first plurality of oligonucleotides are chirally controlled in that level of the first plurality of oligonucleotides is pre-determined, and oligonucleotides of the first plurality share a common stereochemistry configuration at one or more chiral internucleotidic linkages. For example, in some embodiments, oligonucleotides of the first plurality share a common stereochemistry configuration at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50 or more chiral internucleotidic linkages, each of which is independently *Rp* or *Sp*; in some embodiments, oligonucleotides of the first plurality share a common stereochemistry configuration at each chiral internucleotidic linkages. In some embodiments, a chiral internucleotidic linkage where a predetermined level of oligonucleotides of a composition share a common stereochemistry configuration (independently *Rp* or *Sp*) is referred to as a chirally controlled internucleotidic linkage. In some embodiments, a predetermined level of oligonucleotides of a provided composition, *e.g.*, a first plurality of oligonucleotides of certain example compositions, comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50 or more chiral controlled internucleotidic linkages; in some embodiments, at least 5 internucleotidic linkages are chirally controlled; in some embodiments, at least 10 internucleotidic linkages are chirally controlled; in some embodiments, at least 10 internucleotidic linkages are chirally controlled; in some embodiments, each chiral internucleotidic linkage is chirally controlled. In some embodiments, oligonucleotides of a first plurality have a wing-core-wing structure. In some embodiments, each wing region independently comprises one or more modified phosphate linkages and no natural phosphate linkages, and the core comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, each wing region independently comprises one or more natural phosphate linkages and optionally one or more modified internucleotidic

linkages, and the core comprises one or more modified internucleotidic linkages and optionally one or more natural phosphate linkages. In some embodiments, each wing region independently comprises one or more natural phosphate linkages and one or more modified internucleotidic linkages, and the core comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, each wing region independently comprises one or more natural phosphate linkages and one or more modified internucleotidic linkages, and the core comprises one or more modified internucleotidic linkages and no natural phosphate linkages. In some embodiments, a wing comprises modified sugar moieties. In some embodiments, a modified internucleotidic linkage is phosphorothioate. In some embodiments, a modified internucleotidic linkage is substituted phosphorothioate. In some embodiments, a modified internucleotidic linkage has the structure of formula **I** described in this disclosure. In some embodiments, a modified sugar moiety is 2'-modified. In some embodiments, a 2'-modification is 2'-R¹. In some embodiments, a 2'-modification is 2'-OR¹. In some embodiments, a 2'-modification is 2'-F. As described in more details, provided oligonucleotides may comprise more than one types of sugar modifications; in some embodiments, provided oligonucleotides comprise both 2'-F and 2'-OR¹ modifications. In some embodiments, provided oligonucleotides comprise both 2'-F and 2'-OMe modifications. In some embodiments, provided oligonucleotides comprise both 2'-F and 2'-OMe modifications, and both phosphorothioate and natural phosphate linkages. In some embodiments, each chiral internucleotidic linkage, *e.g.*, phosphorothioate linkage, is chirally controlled. In some embodiments, such provided compositions have lower toxicity. In some embodiments, provided compositions have lower complement activation.

[0010] In some embodiments, the present disclosure provides oligonucleotide compositions with improved protein binding profiles, *e.g.*, lowered harmful protein binding and/or increased beneficial protein binding. In some embodiments, the present disclosure provides methods for improved delivery of oligonucleotide compositions comprising providing oligonucleotide compositions with improved protein binding profile. In some embodiments, the present disclosure demonstrates that protein binding by oligonucleotide compositions can be modulated through chemical modifications, stereochemistry, or combinations thereof. In some embodiments, protein binding by oligonucleotide compositions can be modulated by incorporation of modified internucleotidic linkages. In some embodiments, an increased

percentage of modified internucleotidic linkages provides increased binding of oligonucleotides to certain proteins. In some embodiments, replacement of one or more modified internucleotidic linkages with natural phosphate linkages provides decreased binding to certain proteins. In some embodiments, replacement of one or more natural phosphate linkages with modified internucleotidic linkages provides increased binding to certain proteins. In some embodiments, certain chemical modifications provide increased protein binding to certain proteins. In some embodiments, certain chemical modifications provide decreased protein binding to certain proteins. In some embodiments, different chemical modifications of the same kind provide different protein binding. For example, in some embodiments, 2'-MOE provides decreased protein binding compared to 2'-OMe (at least in certain contexts – *e.g.*, sequence, stereochemistry, *etc.*).

[0011] 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 and/or chemical modifications, they are different chemical entities at least due to their different backbone stereochemistry, and they can have, as demonstrated herein, different properties, *e.g.*, activities, toxicities, distribution *etc.* Among other things, the present disclosure provides chirally controlled compositions that are or contain particular stereoisomers of oligonucleotides of interest; in contrast to chirally uncontrolled compositions, chirally controlled compositions comprise predetermined levels of particular stereoisomers of oligonucleotides. 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, the present disclosure demonstrates that property

improvements (*e.g.*, improved activities, lower toxicities, *etc.*) achieved through inclusion and/or location of particular chiral structures within an oligonucleotide can be comparable to, or even better than those achieved through use of chemical modification, *e.g.*, particular backbone linkages, residue modifications, *etc.* (*e.g.*, through use of certain types of modified phosphates [*e.g.*, phosphorothioate, substituted phosphorothioate, *etc.*], sugar modifications [*e.g.*, 2'-modifications, *etc.*], and/or base modifications [*e.g.*, methylation, *etc.*]). In some embodiments, the present disclosure demonstrates that chirally controlled oligonucleotide compositions of oligonucleotides comprising certain chemical modifications (*e.g.*, 2'-F, 2'-OMe, phosphorothioate internucleotidic linkages, lipid conjugation, *etc.*) demonstrate unexpectedly high exon-skipping efficiency.

[0012] Among other things, the present disclosure demonstrates that stereochemistry can be used to modulate toxicity of oligonucleotide compositions. In some embodiments, the present disclosure provides chirally controlled oligonucleotide compositions that have lower toxicity when compared to a corresponding stereorandom (or chirally uncontrolled) oligonucleotide composition of oligonucleotides sharing the same base sequence and chemical modifications. In some embodiments, chirally controlled oligonucleotide compositions of oligonucleotides comprising more *Rp* chiral internucleotidic linkage have lower toxicity. In some embodiments, chirally controlled oligonucleotide compositions of oligonucleotides having a single *Rp* chiral internucleotidic linkage have increased toxicity compared to other chirally controlled oligonucleotide compositions and/or the corresponding stereorandom oligonucleotide composition of oligonucleotides sharing the same base sequence and chemical modifications. In some embodiments, a single *Rp* chiral internucleotidic linkage is in the middle of a sequence. In some embodiments, chirally controlled oligonucleotide compositions of oligonucleotides which comprise one or more *Rp* chiral internucleotidic linkages at the 5'- and/or the 3'-end provide lower toxicity. In some embodiments, chirally controlled oligonucleotide compositions of oligonucleotides which comprise one or more natural phosphate linkages at the 5'- and/or the 3'-end provide lower toxicity. In some embodiments, a chiral internucleotidic linkage has the structure of formula I. In some embodiments, a chiral internucleotidic linkage is a phosphorothioate linkage. In some embodiments, a chiral internucleotidic linkage is a substituted phosphorothioate linkage.

[0013] Among other things, the present disclosure recognizes that, in some embodiments,

properties (*e.g.*, activities, toxicities, distribution, pharmacokinetics, *etc.*) 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.*, chemical modifications, patterns of modifications such as linkage pattern, nucleoside modification pattern, conjugation to lipids or other moieties, *etc.*) of the oligonucleotide. In some embodiments, the present disclosure recognizes that, in some embodiments, properties (*e.g.*, activities, toxicities, *etc.*) 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.*, chemical modifications, patterns of modifications such as linkage pattern, nucleoside modification pattern, *etc.*) of the oligonucleotide. In some embodiments, the present disclosure recognizes and demonstrates 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.*, activities, toxicities, distribution, pharmacokinetics, *etc.*). In some embodiments, the present disclosure demonstrates that combinations of chemical modifications and stereochemistry can provide unexpected, greatly improved properties (*e.g.*, activities, toxicities, distribution, pharmacokinetics, *etc.*). In some embodiments, chemical combinations, such as modifications of sugars, bases, and/or internucleotidic linkages, are combined with stereochemistry patterns to provide oligonucleotides and compositions thereof with surprisingly enhanced properties including low toxicity, better protein binding profile, *etc.* In some embodiments, a provided oligonucleotide composition comprising a first plurality of oligonucleotides is chirally controlled, and oligonucleotides of the first plurality comprise a combination of 2'-modification of one or more sugar moieties, one or more natural phosphate linkages, and one or more chiral internucleotidic linkages. In some embodiments, a provided oligonucleotide composition comprising a first plurality of oligonucleotides is chirally controlled, and oligonucleotides of the first plurality comprise a combination of 2'-modification of one or more sugar moieties, one or more natural phosphate linkages, one or more chiral internucleotidic linkages, wherein the 5'- and/or the 3'-end internucleotidic linkages are chiral. In some embodiments, both the 5'- and the 3'-end internucleotidic linkages are chiral. In some embodiments, both the 5'- and the 3'-end internucleotidic linkages are chiral and *Sp*. In some embodiments, a provided oligonucleotide

composition comprising a first plurality of oligonucleotides is chirally controlled, and oligonucleotides of the first plurality comprise a combination of 2'-modification of one or more sugar moieties, one or more natural phosphate linkages, one or more chiral internucleotidic 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, a chiral internucleotidic linkage has the structure of formula I. In some embodiments, a chiral internucleotidic linkage is a phosphorothioate linkage. In some embodiments, a chiral internucleotidic linkage is a substituted phosphorothioate linkage.

[0014] In some embodiments, provided oligonucleotides comprise a wing and a core region. In some embodiments, provided oligonucleotides have a wing-core-wing structure, wherein the core region comprises one or more sugar moieties and/or internucleotidic linkages not in the wing regions. In some embodiments, provided oligonucleotides have a wing-core-wing structure, wherein the core region comprises one or more sugar moieties and internucleotidic linkages not in the wing regions. In some embodiments, provided oligonucleotides have a wing-core-wing structure, wherein the core region comprises one or more internucleotidic linkages not in the wing regions. In some embodiments, a core region comprises a modified sugar moiety. In some embodiments, each sugar moiety in a core region is modified. Example sugar modifications are widely known in the art including but not limited to those described in this disclosure. In some embodiments, a core comprises at least one internucleotidic linkage which is chirally controlled (*e.g.*, a phosphorothioate in *Sp* or *Rp* configuration) and at least one internucleotidic linkage which is not chiral (*e.g.*, a phosphodiester or phosphorodithioate). In some embodiments, a core comprises at least one internucleotidic linkage which is chirally controlled phosphorothioate in *Sp* configuration and at least one internucleotidic linkage which is not chiral (*e.g.*, a phosphodiester or phosphorodithioate). In some embodiments, each wing region comprises no modified sugar moieties. In some embodiments, a core region comprises one or more natural phosphate linkages. In some embodiments, each internucleotidic linkage following a core nucleoside is natural phosphate linkage. In some embodiments, a wing comprises at least one internucleotidic linkage which is chirally controlled (*e.g.*, a phosphorothioate in *Sp* or *Rp* configuration) and at least one internucleotidic linkage which is not chiral (*e.g.*, a phosphodiester or phosphorodithioate). In some embodiments, a wing comprises at

least one internucleotidic linkage which is chirally controlled phosphorothioate in *Sp* configuration and at least one internucleotidic linkage which is not chiral (*e.g.*, a phosphodiester or phosphorodithioate). In some embodiments, a wing comprises one or more modified internucleotidic linkages. In some embodiments, each internucleotidic linkage following a core nucleoside is a modified internucleotidic linkage. For example, see WV-1111. In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage which is chirally controlled (*e.g.*, a phosphorothioate in *Sp* or *Rp* configuration) and at least one internucleotidic linkage which is not chiral at the linkage phosphorus (*e.g.*, a phosphodiester or phosphorodithioate). In some embodiments, an oligonucleotide comprises at least one internucleotidic linkage which is chirally controlled phosphorothioate in *Sp* configuration and at least one internucleotidic linkage which is not chiral at the linkage phosphorus (*e.g.*, a phosphodiester or phosphorodithioate).

[0015] In some embodiments, provided oligonucleotides are blockmers. In some embodiments, provided oligonucleotide are altmers. In some embodiments, provided oligonucleotides are altmers comprising alternating blocks. In some embodiments, a blockmer or an altmer can be defined by chemical modifications (including presence or absence), *e.g.*, base modifications, sugar modification, internucleotidic linkage modifications, stereochemistry, *etc.*

[0016] In some embodiments, provided oligonucleotides comprise blocks comprising different internucleotidic linkages. In some embodiments, provided oligonucleotides comprise blocks comprising modified internucleotidic linkages and natural phosphate linkages. In some embodiments, provided oligonucleotides comprise blocks comprising different modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise alternating blocks comprising different internucleotidic linkages. In some embodiments, provided oligonucleotides comprise alternating blocks comprising modified internucleotidic linkages and natural phosphate linkages. In some embodiments, provided oligonucleotides comprise alternating blocks comprising different modified internucleotidic linkages. In some embodiments, a block comprising modified internucleotidic linkages have pattern of backbone chiral centers as described herein. In some embodiments, each block comprising modified internucleotidic linkages has the same pattern of backbone chiral centers. In some embodiments, blocks comprising modified internucleotidic linkages have different patterns of backbone chiral centers. In some embodiments, blocks comprising modified internucleotidic linkages have

different length and/or modifications. In some embodiments, blocks comprising modified internucleotidic linkages have the same length and/or modifications. In some embodiments, blocks comprising modified internucleotidic linkages have the same length. In some embodiments, blocks comprising modified internucleotidic linkages have the same internucleotidic linkages. In some embodiments, provided oligonucleotides comprise a first block at the 5'-end (5'-block), and a second block at the 3'-end (3'-block), each of which independently comprise one or more modified internucleotidic linkages. In some embodiments, each of the 5'- and 3'-blocks independently comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more modified internucleotidic linkages. In some embodiments, a 5'-block comprises 4 or more modified internucleotidic linkages. In some embodiments, a 5'-block comprises 5 or more modified internucleotidic linkages. In some embodiments, a 5'-block comprises 6 or more modified internucleotidic linkages. In some embodiments, a 5'-block comprises 7 or more modified internucleotidic linkages. In some embodiments, a 3'-block comprises 4 or more modified internucleotidic linkages. In some embodiments, a 3'-block comprises 5 or more modified internucleotidic linkages. In some embodiments, a 3'-block comprises 6 or more modified internucleotidic linkages. In some embodiments, a 3'-block comprises 7 or more modified internucleotidic linkages. In some embodiments, each of the 5'- and 3'-blocks independently comprises at least 4 modified internucleotidic linkages. In some embodiments, each of the 5'- and 3'-blocks independently comprises at least 5 modified internucleotidic linkages. In some embodiments, each of the 5'- and 3'-blocks independently comprises at least 6 modified internucleotidic linkages. In some embodiments, each of the 5'- and 3'-blocks independently comprises at least 7 modified internucleotidic linkages. In some embodiments, modified internucleotidic linkages within a block are consecutive. In some embodiments, each linkage of the 5'-block is independently a modified internucleotidic linkage. In some embodiments, each linkage of the 5'-block is independently a phosphorothioate linkage. In some embodiments, each linkage of the 5'-block is independently chirally controlled. In some embodiments, each linkage of the 5'-block is *Sp*. In some embodiments, each linkage of the 3'-block is independently a modified internucleotidic linkage. In some embodiments, each linkage of the 3'-block is independently a phosphorothioate linkage. In some embodiments, each linkage of the 3'-block is independently chirally controlled. In some embodiments, each linkage of the 3'-block is *Sp*.

[0017] In some embodiments, provided oligonucleotides comprise blocks comprising sugar modifications. In some embodiments, provided oligonucleotides comprise one or more blocks comprising one or more 2'-F modifications (2'-F blocks). In some embodiments, provided oligonucleotides comprise blocks comprising consecutive 2'-F modifications. In some embodiments, a block comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more consecutive 2'-F modifications. In some embodiments, a block comprises 4 or more 2'-F modifications. In some embodiments, a block comprises 5 or more 2'-F modifications. In some embodiments, a block comprises 6 or more 2'-F modifications. In some embodiments, a block comprises 7 or more 2'-F modifications. In some embodiments, provided oligonucleotides comprises one or more blocks comprising one or more 2'-OR¹ modifications (2'-OR¹ blocks). In some embodiments, provided oligonucleotides comprise both 2'-F and 2'-OR¹ blocks. In some embodiments, provided oligonucleotides comprise alternating 2'-F and 2'-OR¹ blocks. In some embodiments, provided oligonucleotides comprise a first 2'-F block at the 5'-end, and a second 2'-F block at the 3'-end, each of which independently comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more consecutive 2'-F modifications; in some embodiments, each of which independently comprises 4 or more 2'-F modifications; in some embodiments, each of which independently comprises 5 or more 2'-F modifications; in some embodiments, each of which independently comprises 6 or more 2'-F modifications; in some embodiments, each of which independently comprises 7 or more 2'-F modifications. In some embodiments, provided oligonucleotides comprise a 5'-block wherein each sugar moiety of the 5'-block comprises a 2'-F modification. In some embodiments, provided oligonucleotides comprise a 3'-block wherein each sugar moiety of the 3'-block comprises a 2'-F modification. In some embodiments, such provided oligonucleotides comprise one or more 2'-OR¹ blocks, and optionally one or more 2'-F blocks, between the 5' and 3' 2'-F blocks. In some embodiments, such provided oligonucleotides comprise one or more 2'-OR¹ blocks, and one or more 2'-F blocks, between the 5' and 3' 2'-F blocks (*e.g.*, WV-3407, WV-3408, *etc.*).

[0018] In some embodiments, a block is a stereochemistry block. In some embodiments, a block is an *Rp* block in that each internucleotidic linkage of the block is *Rp*. In some embodiments, a 5'-block is an *Rp* block. In some embodiments, a 3'-block is an *Rp* block. In some embodiments, a block is an *Sp* block in that each internucleotidic linkage of the block is *Sp*. In some embodiments, a 5'-block is an *Sp* block. In some embodiments, a 3'-block is an *Sp*

block. In some embodiments, provided oligonucleotides comprise both *Rp* and *Sp* blocks. In some embodiments, provided oligonucleotides comprise one or more *Rp* but no *Sp* blocks. In some embodiments, provided oligonucleotides comprise one or more *Sp* but no *Rp* blocks. In some embodiments, provided oligonucleotides comprise one or more PO blocks wherein each internucleotidic linkage in a natural phosphate linkage.

[0019] In some embodiments, a 5'-block is an *Sp* block wherein each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block is an *Sp* block wherein each of internucleotidic linkage is a modified internucleotidic linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block is an *Sp* block wherein each of internucleotidic linkage is a phosphorothioate linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 5'-block comprises 4 or more nucleoside units. In some embodiments, a 5'-block comprises 5 or more nucleoside units. In some embodiments, a 5'-block comprises 6 or more nucleoside units. In some embodiments, a 5'-block comprises 7 or more nucleoside units. In some embodiments, a 3'-block is an *Sp* block wherein each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block is an *Sp* block wherein each of internucleotidic linkage is a modified internucleotidic linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block is an *Sp* block wherein each of internucleotidic linkage is a phosphorothioate linkage and each sugar moiety comprises a 2'-F modification. In some embodiments, a 3'-block comprises 4 or more nucleoside units. In some embodiments, a 3'-block comprises 5 or more nucleoside units. In some embodiments, a 3'-block comprises 6 or more nucleoside units. In some embodiments, a 3'-block comprises 7 or more nucleoside units.

[0020] In some embodiments, a type of nucleoside in a region or an oligonucleotide is followed by a specific type of internucleotidic linkage, *e.g.*, natural phosphate linkage, modified internucleotidic linkage, *Rp* chiral internucleotidic linkage, *Sp* chiral internucleotidic linkage, *etc.* In some embodiments, A is followed by *Sp*. In some embodiments, A is followed by *Rp*. In some embodiments, A is followed by natural phosphate linkage (PO). In some embodiments, U is followed by *Sp*. In some embodiments, U is followed by *Rp*. In some embodiments, U is followed by natural phosphate linkage (PO). In some embodiments, C is followed by *Sp*. In some embodiments, C is followed by *Rp*. In some embodiments, C is followed by natural phosphate linkage (PO). In some embodiments, G is followed by *Sp*. In some embodiments, G

is followed by *Rp*. In some embodiments, G is followed by natural phosphate linkage (PO). In some embodiments, C and U are followed by *Sp*. In some embodiments, C and U are followed by *Rp*. In some embodiments, C and U are followed by natural phosphate linkage (PO). In some embodiments, A and G are followed by *Sp*. In some embodiments, A and G are followed by *Rp*. In some embodiments, A and G are followed by natural phosphate linkage (PO). For examples, see WV-1111, WV-1112, WV-XXX1, *etc*.

[0021] In some embodiments, provided oligonucleotides comprise alternating blocks comprising modified sugar moieties and unmodified sugar moieties. In some embodiments, modified sugar moieties comprise 2'-modifications. In some embodiments, provided oligonucleotides comprise alternating 2'-OMe modified sugar moieties and unmodified sugar moieties. For examples, see WV-1112, WV-1113, *etc*.

[0022] In some embodiments, provided oligonucleotides comprise alternating blocks comprising different modified sugar moieties and/or unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise alternating blocks comprising different modified sugar moieties and unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise alternating blocks comprising different modified sugar moieties. In some embodiments, provided oligonucleotides comprise alternating blocks comprising different modified sugar moieties, wherein the modified sugar moieties comprise different 2'-modifications. For example, in some embodiments, provided oligonucleotide comprises alternating blocks comprising 2'-OMe and 2'-F, respectively. For examples, see WV-1712, WV1713, WV-1714, *etc*.

[0023] In some embodiments, a type of nucleoside in a region or an oligonucleotide is modified, optionally with a different modification compared to another type of nucleoside. In some embodiments, a type of nucleoside in a region or an oligonucleotide is modified with a different modification compared to another type of nucleoside. For example, in some embodiments, a pyrimidine nucleoside comprises a 2'-F modification, and a purine nucleoside comprises a 2'-OMe modification. In some other embodiments, a pyrimidine nucleoside comprises a 2'-OMe modification, and a purine nucleoside comprises a 2'-F modification. In some embodiments, G and C has one type of sugar modification, and A and U has another type of sugar modification. In some embodiments, G and C comprises 2'-OMe modification, and A and U comprises 2'-F modification. In some embodiments, G and C comprises 2'-F

modification, and A and U comprises 2'-OMe modification.

[0024] In some embodiments, an internucleotidic linkage following an unmodified sugar moiety is a modified internucleotidic linkage. In some embodiments, an internucleotidic linkage after an unmodified sugar moiety is a phosphorothioate linkage. In some embodiments, each internucleotidic linkage after an unmodified sugar moiety is a modified internucleotidic linkage. In some embodiments, each internucleotidic linkage after an unmodified sugar moiety is a phosphorothioate linkage. In some embodiments, an internucleotidic linkage following a modified sugar moiety is a natural phosphate linkage. In some embodiments, each internucleotidic linkage following a modified sugar moiety is a natural phosphate linkage. For example, see WV-1111, WV1112, *etc.*

[0025] In some embodiments, provided oligonucleotides comprise one or more 2'-F modified sugar moieties whose 3'-internucleotidic linkages are modified internucleotidic linkages. In some embodiments, a modified internucleotidic linkage is phosphorothioate. In some embodiments, a modified internucleotidic linkage is chirally controlled and is *Rp*. In some embodiments, a modified internucleotidic linkage is chirally controlled and is *Sp*. In some embodiments, provided oligonucleotides comprise one or more 2'-OR¹ modified sugar moieties whose 3'-internucleotidic linkages are natural phosphate linkages.

[0026] In some embodiments, a provided pattern of backbone chiral centers comprises repeating (Sp)^m(Rp)ⁿ, (Rp)ⁿ(Sp)^m, (Np)^t(Rp)ⁿ(Sp)^m, or (Sp)^t(Rp)ⁿ(Sp)^m units. In some embodiments, a repeating unit is (Sp)^m(Rp)ⁿ. In some embodiments, a repeating unit is *SpRp*. In some embodiments, a repeating unit is *SpSpRp*. In some embodiments, a repeating unit is *SpRpRp*. In some embodiments, a repeating unit is *RpRpSp*. In some embodiments, a repeating unit is (Rp)ⁿ(Sp)^m. In some embodiments, a repeating unit is (Np)^t(Rp)ⁿ(Sp)^m. In some embodiments, a repeating unit is (Sp)^t(Rp)ⁿ(Sp)^m.

[0027] In some embodiments, a provided pattern of backbone chiral centers comprises (Rp/Sp)-(All *Rp* or All *Sp*)-(Rp/Sp). In some embodiments, a provided pattern of backbone chiral centers comprises (Rp)-(All *Sp*)-(Rp). In some embodiments, a provided pattern of backbone chiral centers comprises (Sp)-(All *Sp*)-(Sp). In some embodiments, a provided pattern of backbone chiral centers comprises (Sp)-(All *Rp*)-(Sp). In some embodiments, a provided pattern of backbone chiral centers comprises (Rp/Sp)-(repeating (Sp)^m(Rp)ⁿ)-(Rp/Sp). In some embodiments, a provided pattern of backbone chiral centers comprises (Rp/Sp)-(repeating

SpSpRp)-(Rp/Sp).

[0028] In some embodiments, a provided pattern of backbone chiral centers is *(Rp/Sp)-(All Rp or All Sp)-(Rp/Sp)*. In some embodiments, a provided pattern of backbone chiral centers is *(Sp)-(All Sp)-(Sp)*. In some embodiments, each chiral internucleotidic linkage is *Sp*. In some embodiments, a provided pattern of backbone chiral centers is *(Rp)-(All Sp)-(Rp)*. In some embodiments, a provided pattern of backbone chiral centers is *(Sp)-(All Rp)-(Sp)*. In some embodiments, a provided pattern of backbone chiral centers is *(Rp/Sp)-(repeating (Sp)_m(Rp)_n)-(Rp/Sp)*. In some embodiments, a provided pattern of backbone chiral centers is *(Rp/Sp)-(repeating SpSpRp)-(Rp/Sp)*.

[0029] In some embodiments, the present disclosure provides oligonucleotide compositions having low toxicity. In some embodiments, the present disclosure provides oligonucleotide compositions having improved protein binding profile. In some embodiments, the present disclosure provides oligonucleotide compositions having improved binding to albumin. In some embodiments, provided compositions have low toxicity and improved binding to certain desired proteins. In some embodiments, provided compositions have low toxicity and improved binding to certain desired proteins. In some embodiments, provided oligonucleotide compositions at the same time provides the same level of, or greatly enhanced, stability and/or activities, *e.g.*, better target-cleavage pattern, better target-cleavage efficiency, better target specificity, *etc.*

[0030] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides which:

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

[0031] In some embodiments, a provided oligonucleotide composition is characterized in that, when it is contacted with the transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[0032] In some embodiments, a reference condition is absence of the composition. In some embodiments, a reference condition is presence of a reference composition. Example reference compositions comprising a reference plurality of oligonucleotides are extensively

described in this disclosure. In some embodiments, oligonucleotides of the reference plurality have a different structural elements (chemical modifications, stereochemistry, *etc.*) compared with oligonucleotides of the first plurality in a provided composition. In some embodiments, a reference composition is a stereorandom preparation of oligonucleotides having the same chemical modifications. In some embodiments, a reference composition is a mixture of stereoisomers while a provided composition is a chirally controlled oligonucleotide composition of one stereoisomer. In some embodiments, oligonucleotides of the reference plurality have the same base sequence as oligonucleotide of the first plurality in a provided composition. In some embodiments, oligonucleotides of the reference plurality have the same chemical modifications as oligonucleotide of the first plurality in a provided composition. In some embodiments, oligonucleotides of the reference plurality have the same sugar modifications as oligonucleotide of the first plurality in a provided composition. In some embodiments, oligonucleotides of the reference plurality have the same base modifications as oligonucleotide of the first plurality in a provided composition. In some embodiments, oligonucleotides of the reference plurality have the same internucleotidic linkage modifications as oligonucleotide of the first plurality in a provided composition. In some embodiments, oligonucleotides of the reference plurality have the same stereochemistry as oligonucleotide of the first plurality in a provided composition but different chemical modifications, *e.g.*, base modification, sugar modification, internucleotidic linkage modifications, *etc.*

[0033] Example splicing systems are widely known in the art. In some embodiments, a splicing system is an *in vivo* or *in vitro* system including components sufficient to achieve splicing of a relevant target transcript. In some embodiments, a splicing system is or comprises a spliceosome (*e.g.*, protein and/or RNA components thereof). In some embodiments, a splicing system is or comprises an organellar membrane (*e.g.*, a nuclear membrane) and/or an organelle (*e.g.*, a nucleus). In some embodiments, a splicing system is or comprises a cell or population thereof. In some embodiments, a splicing system is or comprises a tissue. In some embodiments, a splicing system is or comprises an organism, *e.g.*, an animal, *e.g.*, a mammal such as a mouse, rat, monkey, human, *etc.*

[0034] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides which:

- 1) have a common base sequence complementary to a target sequence in a transcript; and

2) comprise one or more modified sugar moieties and modified internucleotidic linkages, the oligonucleotide composition being characterized in that, when it is contacted with the transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[0035] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides of a particular oligonucleotide type defined by:

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

[0036] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides of a particular oligonucleotide type defined by:

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

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

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

[0037] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising one or more wing regions and a core region, wherein:

- oligonucleotides of the first plurality have the same base sequence; and
- each wing region independently comprises one or more modified internucleotidic

linkages and optionally one or more natural phosphate linkages, and the core region independently comprises one or more modified internucleotidic linkages; or

each wing region independently comprises one or more modified sugar moieties, and the core region comprises one or more un-modified sugar moieties.

[0038] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising one or more wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

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

the core region independently has a length of two or more bases and independently comprises one or more modified internucleotidic linkages.

[0039] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising one or more wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

each wing region independently has a length of two or more bases, and independently comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages; and

the core region independently has a length of two or more bases and independently comprises one or more modified internucleotidic linkages.

[0040] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising two wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

each wing region independently has a length of two or more bases, and independently comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages; and

the core region independently has a length of two or more bases and independently comprises one or more modified internucleotidic linkages.

[0041] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising two wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

each wing region independently has a length of two or more bases, and independently comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages;

the wing region to the 5'-end of the core region comprises at least one modified internucleotidic linkage followed by a natural phosphate linkage in the wing; and

the wing region to the 3'-end of the core region comprises at least one modified internucleotidic linkage preceded by a natural phosphate linkage in the wing;

the core region independently has a length of two or more bases and independently comprises one or more modified internucleotidic linkages.

[0042] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising a wing region and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

the wing region has a length of two or more bases, and comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages;

the wing region is to the 5'-end of the core region and comprises a natural phosphate linkage between the two nucleosides at its 3'-end, or the wing region to the 3'-end of the core region and comprises a natural phosphate linkage between the two nucleosides at its 5'-end; and

the core region independently has a length of two or more bases and independently comprises one or more modified internucleotidic linkages.

[0043] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising two wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

each wing region independently has a length of two or more bases, and independently comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages;

the wing region to the 5'-end of the core region comprises a natural phosphate linkage between the two nucleosides at its 3'-end;

the wing region to the 3'-end of a core region comprises a natural phosphate linkage between the two nucleosides at its 5'-end; and

the core region independently has a length of two or more bases and independently comprises one or more modified internucleotidic linkages.

[0044] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising one or more wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence; and

each wing region independently comprises one or more modified internucleotidic linkages and optionally one or more natural phosphate linkages, and the core region independently comprises one or more modified internucleotidic linkages; and

each wing region independently comprises one or more modified sugar moieties, and the core region comprises one or more un-modified sugar moieties.

[0045] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising one or more wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence; and

each wing region independently comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages, and the core region independently comprises one or more modified internucleotidic linkages; and

each wing region independently comprises one or more modified sugar moieties, and the core region comprises one or more un-modified sugar moieties.

[0046] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides which:

1) have a common base sequence; and

2) comprise one or more wing regions and a core region;

wherein:

each wing region comprises at least one modified sugar moiety; and

each core region comprises at least one un-modified sugar moiety.

[0047] 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.

[0048] 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.

[0049] 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.

[0050] 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.

[0051] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising two wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

each wing region independently has a length of two or more bases, and independently comprises one or more modified internucleotidic linkages and one or more modified sugar moieties;

the core region independently has a length of two or more bases and independently comprises one or more natural phosphate linkages.

[0052] In some embodiments, each wing region of provided oligonucleotides independently comprises 3, 4, 5, 6, 7, 8, 9, 10 or more bases. In some embodiments, each wing region independently comprises 3 or more bases. In some embodiments, each wing region independently comprises 4 or more bases. In some embodiments, each wing region independently comprises 5 or more bases. In some embodiments, each wing region independently comprises 6 or more bases. In some embodiments, each wing region independently comprises 7 or more bases. In some embodiments, each sugar moiety in a wing is modified. In some embodiments, a modification is a 2'-modification. In some embodiments, each modification is a 2'-modification. In some embodiments, a modification is 2'-F. In some embodiments, each modification is 2'-F. In some embodiments, a modification is 2'-OR¹. In some embodiments, each modification is 2'-OR¹. In some embodiments, a modification is 2'-OR¹. In some embodiments, each modification is 2'-OMe. In some embodiments, each modification is 2'-OMe. In some embodiments, each modification is 2'-MOE. In some embodiments, each modification is 2'-MOE. In some embodiments, a modification is an LNA sugar modification. In some embodiments, each modification is an LNA sugar modification. In some embodiments, each internucleotidic linkage in a wing is a chiral internucleotidic linkage. In some embodiments, each internucleotidic linkage in a wing is an Sp chiral internucleotidic

linkage. In some embodiments, a chiral internucleotidic linkage is a phosphorothioate linkage. In some embodiments, a core region comprises one or more natural phosphate linkages and one or more modified internucleotidic linkages. In some embodiments, a core region comprises one or more natural phosphate linkages and one or more chiral internucleotidic linkages. In some embodiments, a core region comprises one or more natural phosphate linkages and one or more *Sp* chiral internucleotidic linkages. In some embodiments, a core region comprises one or more natural phosphate linkages and one or more *Sp* phosphorothioate linkages.

[0053] In some embodiments, level of oligonucleotides, such as level of a plurality of oligonucleotides (*e.g.*, a first plurality of oligonucleotides, a reference plurality of oligonucleotides, *etc.*), in provided compositions is predetermined. For example, as readily appreciated by a person having ordinary skill in the art, provided chirally controlled oligonucleotide compositions of a plurality of oligonucleotides, *e.g.*, a first plurality of oligonucleotides, comprise a predetermined level of such plurality of oligonucleotides.

[0054] In some embodiments, provided oligonucleotides have a base sequence of UCAAGGAAGAUGGCAUUUCU. In some embodiments, provided oligonucleotides have a base sequence comprising UCAAGGAAGAUGGCAUUUCU, and a length of up to 30 bases. In some embodiments, provided oligonucleotides have a base sequence comprising UCAAGGAAGAUGGCAUUUCU, and a length of up to 40 bases. In some embodiments, provided oligonucleotides have a base sequence comprising UCAAGGAAGAUGGCAUUUCU, and a length of up to 50 bases. In some embodiments, provided oligonucleotides have a base sequence comprising at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, and a length of up to 30 bases. In some embodiments, provided oligonucleotides have a base sequence comprising at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, and a length of up to 40 bases. In some embodiments, provided oligonucleotides have a base sequence comprising at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, and a length of up to 50 bases. In some embodiments, provided oligonucleotides have a base sequence comprising a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, and a length of up to 30 bases. In some embodiments, provided oligonucleotides have a base sequence comprising a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, and a length of up to 40 bases. In some embodiments, provided oligonucleotides have a base sequence

comprising a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, and a length of up to 50 bases.

[0055] In some embodiments, a common base sequence of a plurality of oligonucleotides is UCAAGGAAGAUGGCAUUUCU. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 30 bases. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 40 bases. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 50 bases. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 30 bases. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 40 bases. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 50 bases. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 30 bases. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 40 bases. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 50 bases.

[0056] In some embodiments, a common base sequence of a plurality of oligonucleotides is UCAAGGAAGAUGGCAUUUCU, and a common pattern of backbone chiral centers comprises at least one chirally controlled center. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 30 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 40 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center. In some embodiments, a common base sequence comprises

UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 50 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 30 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 40 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 50 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 30 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 40 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 50 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center.

[0057] In some embodiments, a common base sequence is UCAAGGAAGAUGGCAUUUCU, and a common pattern of backbone chiral centers comprises at least one chirally controlled center which is a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 30 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center which is a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 40 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled

center which is a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 50 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center which is a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 30 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center which is a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 40 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center which is a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 50 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center which is a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 30 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center which is a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 40 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center which is a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 50 bases, and a common pattern of backbone chiral centers comprises at least one chirally controlled center which is a phosphorothioate in the *Sp* configuration.

[0058] In some embodiments, a common base sequence is UCAAGGAAGAUGGCAUUUCU, and a common pattern of backbone chiral centers comprises at least three chirally controlled centers. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 30

bases, and a common pattern of backbone chiral centers comprises at least three chirally controlled centers. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 40 bases, and a common pattern of backbone chiral centers comprises at least three chirally controlled centers. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 50 bases, and a common pattern of backbone chiral centers comprises at least three chirally controlled centers. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 30 bases, and a common pattern of backbone chiral centers comprises at least three chirally controlled centers. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 40 bases, and a common pattern of backbone chiral centers comprises at least three chirally controlled centers. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 50 bases, and a common pattern of backbone chiral centers comprises at least three chirally controlled centers. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 30 bases, and a common pattern of backbone chiral centers comprises at least three chirally controlled centers. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 40 bases, and a common pattern of backbone chiral centers comprises at least three chirally controlled centers. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 50 bases, and a common pattern of backbone chiral centers comprises at least three chirally controlled centers.

[0059] In some embodiments, a common base sequence is UCAAGGAAGAUGGCAUUUCU, and a common pattern of backbone chiral centers comprises at least five chirally controlled centers which are each a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises

UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 30 bases, and a common pattern of backbone chiral centers comprises at least five chirally controlled centers which are each a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, and the oligonucleotides have a length of up to 40 bases, and a common pattern of backbone chiral centers comprises at least five chirally controlled centers which are each a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 50 bases, and a common pattern of backbone chiral centers comprises at least five chirally controlled centers which are each a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 30 bases, and a common pattern of backbone chiral centers comprises at least five chirally controlled centers which are each a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 40 bases, and a common pattern of backbone chiral centers comprises at least five chirally controlled centers which are each a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises at least 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 50 bases, and a common pattern of backbone chiral centers comprises at least five chirally controlled centers which are each a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 30 bases, and a common pattern of backbone chiral centers comprises at least five chirally controlled centers which are each a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, the oligonucleotides have a length of up to 40 bases, and a common pattern of backbone chiral centers comprises at least five chirally controlled centers which are each a phosphorothioate in the *Sp* configuration. In some embodiments, a common base sequence comprises a sequence having no more than 5 mismatches from the sequence of bases of UCAAGGAAGAUGGCAUUUCU, the

oligonucleotides have a length of up to 50 bases, and a common pattern of backbone chiral centers comprises at least five chirally controlled centers which are each a phosphorothioate in the *Sp* configuration. In some embodiments, a mismatch is a difference between the base sequence or length when two sequences are maximally aligned and compared. As a non-limiting example, a mismatch is counted if a difference exists between the base at a particular location in one sequence and the base at the corresponding position in another sequence. Thus, a mismatch is counted, for example, if a position in one sequence has a particular base (*e.g.*, A), and the corresponding position on the other sequence has a different base (*e.g.*, G, C or U). A mismatch is also counted, *e.g.*, if a position in one sequence has a base (*e.g.*, A), and the corresponding position on the other sequence has no base (*e.g.*, that position is an abasic nucleotide which comprises a phosphate-sugar backbone but no base) or that position is skipped. A single-stranded nick in either sequence (or in the sense or antisense strand) may not be counted as mismatch, for example, no mismatch would be counted if one sequence comprises the sequence 5'-AG-3', but the other sequence comprises the sequence 5'-AG-3' with a single-stranded nick between the A and the G. A base modification is generally not considered a mismatch, for example, if one sequence comprises a C, and the other sequence comprises a modified C (*e.g.*, with a 2'-modification) at the same position, no mismatch may be counted.

[0060] In some embodiments, a common pattern of backbone chiral centers comprises SSS, SSSS, SSSSS, SSSSSS, SSSSSSS, SOS, SSOSS, SSSOSSS, SSSSOSSSS, SSSSSOSSSSS, SSSSSSOSSSSS, SSSSSSOSSSSSS, SSSSSSOSSSSSSS, SSSSSSOSSSSSSSS, SSSSSSOSSSSSSSSS, SSSSSSOSSSSSSSSS, SOSOSOSOS, SSOSOSOSOSS, SSSOSOSOSOSSS, SSSSOSOSOSOSSS, SSSSSOSOSOSOSSSS, SSSSSSOSOSOSOSSSSS, SOSOSSOOS, SSOSOSSOOSS, SSSOSOSSOOSS, SSSSOSOSSOOSSS, SSSSSOSOSSOOSSSS, SSSSSSOSOSSOOSSSSS, SSSSSSOSSOOSSSSS, SOSOSSOOS, SSOSOSSOOSO, SSSOSOSSOOSOS, SSSSOSOSSOOSOSS, SSSSSOSOSSOOSOSS, SSSSSSOSOSSOOSOSSS, SOSOSSOOSO, SSOSOSSOOSOS, SSSSOSOSSOOSOS, SSSSSOSOSSOOSOS, SSSSSSOSOSSOOSOSSS, SOSOSSOOSOSS, SSSSOSOSSOOSOSS, SSSSSOSOSSOOSOSSS, SSOSOSSOO, SSSOSOSSOOS, SSSSOSOSSOOS, SSSSSOSOSSOOS, SSSSSSOSOSSOOS, OSSSSSSOSOSSOOS, OOSSSSSOSOSSOOS, OOSSSSSSOSOSSOOS, OOSSSSSSOSOSSOOS, OOSSSSSSOSOSSOOS, OOSSSSSSOSOSSOOS, or

[0061] In some embodiments, a common pattern of backbone linkages comprises at least 10 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises at least 11 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises at least 12 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises at least 13 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises at least 14 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises at least 15 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises at least 16 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises at least 17 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises at least 18 modified internucleotidic linkages. In some

embodiments, a common pattern of backbone linkages comprises at least 19 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises no more than 19 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises no more than 18 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises no more than 17 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises no more than 16 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises no more than 15 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises no more than 14 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises 14 to 18 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises 13 to 19 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises 12 to 20 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises 11 to 21 modified internucleotidic linkages. In some embodiments, a common pattern of backbone linkages comprises 0 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 1 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 2 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 3 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 4 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 5 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 6 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 7 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 0 to 7 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 1 to 6 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 2 to 5 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 3 to 4 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 1 to 6 phosphodiester and 13 to 19 modified internucleotidic linkages. In some embodiments, the phosphodiester are optionally contiguous or not contiguous. In some embodiments, the modified internucleotidic linkages are optionally contiguous or not contiguous.

[0062] In some embodiments, a common pattern of backbone linkages comprises at least 10 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises at least 11 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises at least 12 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises at least 13 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises at least 14 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises at least 15 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises at least 16 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises at least 17 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises at least 18 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises at least 19 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises no more than 19 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises no more than 18 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises no more than 17 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises no more than 16 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises no more than 15 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises no more than 14 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises 14 to 18 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises 13 to 19 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises 12 to 20 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises 11 to 21 phosphorothioate linkages. In some embodiments, a common pattern of backbone linkages comprises 0 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 1 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 2 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 3 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 4 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 5 phosphodiester. In some embodiments, a

common pattern of backbone linkages comprises 6 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 7 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 0 to 7 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 1 to 6 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 2 to 5 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 3 to 4 phosphodiester. In some embodiments, a common pattern of backbone linkages comprises 1 to 6 phosphodiester and 13 to 19 phosphorothioate linkages. In some embodiments, the phosphodiester are optionally contiguous or not contiguous. In some embodiments, the phosphorothioate linkages are optionally contiguous or not contiguous.

[0063] In some embodiments, a common pattern of backbone chiral centers comprises at least 5 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 6 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 7 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 8 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 9 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 10 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 11 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 12 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 13 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 14 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 15 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 16 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 17 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 18 internucleotidic linkages in the *Sp*

configuration. In some embodiments, a common pattern of backbone chiral centers comprises at least 19 internucleotidic linkages in the *Sp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 8 internucleotidic linkages in the *Rp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 7 internucleotidic linkages in the *Rp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 6 internucleotidic linkages in the *Rp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 5 internucleotidic linkages in the *Rp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 4 internucleotidic linkages in the *Rp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 3 internucleotidic linkages in the *Rp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 2 internucleotidic linkages in the *Rp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 1 internucleotidic linkages in the *Rp* configuration. In some embodiments, a common pattern of backbone chiral centers comprises no more than 8 internucleotidic linkages which are not chiral (as a non-limiting example, a phosphodiester). In some embodiments, a common pattern of backbone chiral centers comprises no more than 7 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 6 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 5 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 4 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 3 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 2 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises no more than 1 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 10 internucleotidic linkages in the *Sp* configuration, and no more than 8 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 11 internucleotidic linkages in the *Sp* configuration, and no more than 7

internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 12 internucleotidic linkages in the *Sp* configuration, and no more than 6 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 13 internucleotidic linkages in the *Sp* configuration, and no more than 6 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 14 internucleotidic linkages in the *Sp* configuration, and no more than 5 internucleotidic linkages which are not chiral. In some embodiments, a common pattern of backbone chiral centers comprises at least 15 internucleotidic linkages in the *Sp* configuration, and no more than 4 internucleotidic linkages which are not chiral. In some embodiments, the internucleotidic linkages in the *Sp* configuration are optionally contiguous or not contiguous. In some embodiments, the internucleotidic linkages in the *Rp* configuration are optionally contiguous or not contiguous. In some embodiments, the internucleotidic linkages which are not chiral are optionally contiguous or not contiguous.

[0064] A wing and core can be defined by any structural elements. 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. In some embodiments, a wing and core is defined by

pattern of backbone internucleotidic linkages. In some embodiments, a wing comprises a type of internucleotidic linkage, and/or a pattern of internucleotidic linkages, that are not found in a core. In some embodiments, a wing region comprises both a modified internucleotidic linkage and a natural phosphate linkage. In some embodiments, the internucleotidic linkage at the 5'-end of a wing to the 5'-end of the core region is a modified internucleotidic linkage. In some embodiments, the internucleotidic linkage at the 3'-end of a wing to the 3'-end of the core region is a modified internucleotidic linkage. In some embodiments, a modified internucleotidic linkage is a chiral internucleotidic linkage.

[0065] In some embodiments, a wing comprises at least 3 2'-F modifications. In some embodiments, a wing comprises at least 4 2'-F modifications. In some embodiments, a wing comprises at least 5 2'-F modifications. In some embodiments, a wing comprises at least 6 2'-F modifications. In some embodiments, a wing comprises at least 3 consecutive 2'-F modifications. In some embodiments, a wing comprises at least 4 consecutive 2'-F modifications. In some embodiments, a wing comprises at least 5 consecutive 2'-F modifications. In some embodiments, a wing comprises at least 6 consecutive 2'-F modifications. In some embodiments, the internucleotidic linkage of each nucleotide unit having one of the consecutive 2'-F modifications is a modified internucleotidic linkage. In some embodiments, the internucleotidic linkage of each nucleotide unit having one of the consecutive 2'-F modifications is independently an *Sp* chiral internucleotidic linkage. In some embodiments, the internucleotidic linkage of each nucleotide unit having one of the consecutive 2'-F modifications is an *Sp* phosphorothioate linkage. In some embodiments, a core comprising two or more of any of: a 2'-F modification, a 2'-OR¹ modification, or 2'-OH. In some embodiments, a core comprising two or more of any of: a 2'-F modification, a 2'-OMe modification, or 2'-OH. In some embodiments, a core comprises at least 1 2'-OMe modification. In some embodiments, a core comprises at least 2 2'-OMe modifications. In some embodiments, a core comprises at least 3 2'-OMe modifications. In some embodiments, a core comprises at least 2 2'-OMe modifications. In some embodiments, a core comprises at least 4 2'-OMe modifications. In some embodiments, a core comprises at least 1 2'-F modification. In some embodiments, a core comprises at least 2 2'-F modifications. In some embodiments, a core comprises at least 3 2'-F modifications. In some embodiments, a core comprises at least 2 2'-F modifications. In some embodiments, a core comprises at least 4 2'-F modifications. In some embodiments, a core

comprises at least 1 2'-F modification and at least 1 2'-OMe modification. In some embodiments, a core comprises at least 1 2'-F modification and at least 2 2'-OMe modifications. In some embodiments, a core comprises at least 2 2'-F modifications and at least 1 2'-OMe modification. In some embodiments, a core comprises at least 2 2'-F modifications and at least 2 2'-OMe modifications. In some embodiments, the 2'-F modifications in the core and/or wing are contiguous or non-contiguous. In some embodiments, the 2'-OMe modifications in the core and/or wing are contiguous or non-contiguous. In some embodiments, the 2'-OH in the core and/or wing are contiguous or non-contiguous. In some embodiments, a core comprises one or more natural phosphate linkages.

[0066] 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.

[0067] 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 comprises 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, the pattern of backbone chiral centers of a core region 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 core region comprises 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 $m > 2$. In some embodiments, the pattern of backbone chiral centers of a core region is $(Sp)m(Rp)n$, $(Rp)n(Sp)m$, $(Np)t(Rp)n(Sp)m$, or $(Sp)t(Rp)n(Sp)m$, wherein $m > 2$. Among other things, in some embodiments such patterns can provide or enhance controlled cleavage of a target sequence, *e.g.*, an RNA sequence.

[0068] In some embodiments, a wing comprises at least 4 phosphorothioates (phosphorothioate linkages). In some embodiments, a wing comprises at least 5 phosphorothioates. In some embodiments, a wing comprises at least 6 phosphorothioates. In some embodiments, a core comprises at least 2 phosphorothioates. In some embodiments, a core comprises at least 3 phosphorothioates. In some embodiments, a core comprises at least 4 phosphorothioates. In some embodiments, a core comprises at least 5 phosphorothioates. In some embodiments, a core comprises at least 6 phosphorothioates. In some embodiments, a core comprises at least 2 phosphodiester (natural phosphate linkages). In some embodiments, a core comprises at least 3 phosphodiesters. In some embodiments, a core comprises at least 4 phosphodiesters. In some embodiments, a core comprises at least 5 phosphodiesters. In some embodiments, a core comprises at least 6 phosphodiesters. In some embodiments, a core comprises at least 1 phosphodiester and at least 1 phosphorothioate. In some embodiments, a core comprises at least 1 phosphodiester and at least 2 phosphorothioates. In some embodiments, a core comprises at least 2 phosphodiesters and at least 1 phosphorothioates. In some embodiments, a core comprises at least 2 phosphodiesters and at least 2 phosphorothioates. In some embodiments, a core comprises at least 2 phosphodiesters and at least 3 phosphorothioates. In some embodiments, a core comprises at least 3 phosphodiesters and at least 2 phosphorothioates. In some embodiments, a core comprises at least 3 phosphodiesters and at least 3 phosphorothioates. In some embodiments, the phosphodiesters in the core and/or one or both wings are optionally contiguous or not contiguous. In some embodiments, the phosphorothioates in the core and/or one or both wings are optionally contiguous or not contiguous.

[0069] 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.

[0070] 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.

[0071] 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.

[0072] 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 (*Rp/Sp*) of chiral internucleotidic linkages), and the same pattern of backbone phosphorus modifications (*e.g.*, pattern of modifications on the internucleotidic phosphorus atom, such as $-S^-$, and $-L-R^1$ of formula I).

[0073] In some embodiments, the present disclosure provides chirally controlled oligonucleotide compositions of oligonucleotides comprising multiple (*e.g.*, more than 5, 6, 7, 8, 9, or 10) internucleotidic linkages, and particularly for oligonucleotides comprising multiple (*e.g.*, more than 5, 6, 7, 8, 9, or 10) chiral internucleotidic linkages. 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.

[0074] Among other things, the present disclosure provides oligonucleotide compositions and technologies for optimizing properties, *e.g.*, activities, toxicities, *etc.* In some embodiments, the present disclosure provides methods for lowering toxicity of oligonucleotides and compositions thereof. In some embodiments, the present disclosure provides methods for lowering immune response associated with administration of oligonucleotides and compositions thereof (*i.e.*, of administering oligonucleotide compositions so that undesirable immune

responses to oligonucleotides in the compositions are reduced, for example relative to those observed with a reference composition of nucleotides of comparable or identical nucleotide sequence). In some embodiments, the present disclosure provides methods for lowering complement activation associated with administration of oligonucleotides and compositions thereof. In some embodiments, the present disclosure provides methods for improving protein binding profile of oligonucleotides and compositions thereof. In some embodiments, the present disclosure provides methods for increasing binding to certain proteins by oligonucleotides and compositions thereof. In some embodiments, the present disclosure provides methods for increasing binding to certain proteins by oligonucleotides and compositions thereof. In some embodiments, the present disclosure provides methods for enhancing delivery of oligonucleotides and compositions thereof. Among other things, the present disclosure encompasses the recognition that optimal delivery of oligonucleotides to their targets, in some embodiments, involves balance of oligonucleotides binding to certain proteins so that oligonucleotides can be transported to the desired locations, and oligonucleotide release so that oligonucleotides can be properly released from certain proteins to perform their desired functions, for example, hybridization with their targets, cleavage of their targets, inhibition of translation, modulation of transcript processing, *etc.* As exemplified in this disclosure, the present disclosure recognizes, among other things, that improvement of oligonucleotide properties can be achieved through chemical modifications and/or stereochemistry.

[0075] As described herein, provided compositions and methods are capable of altering splicing of transcripts. In some embodiments, provided compositions and methods provide improved splicing patterns of transcripts compared to reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof. An improvement can be an improvement of any desired biological functions. In some embodiments, for example, in DMD, an improvement is production of an mRNA from which a dystrophin protein with improved biological activities is produced. In some other embodiments, for example, an improvement is down-regulation of STAT3, HNRNPH1 and/or KDR to mitigate tumor progression, malignancy, and angiogenesis through forced splicing-induced nonsense-mediated decay (DSD-NMD).

[0076] In some embodiments, the present disclosure provides a method for altering splicing of a target transcript, comprising administering a composition comprising a first

plurality of oligonucleotides, wherein the splicing of the target transcript is altered relative to reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[0077] In some embodiments, the present disclosure provides a method of generating a set of spliced products from a target transcript, the method comprising steps of:

contacting a splicing system containing the target transcript with an oligonucleotide composition comprising a first plurality of oligonucleotides, in an amount, for a time, and under conditions sufficient for a set of spliced products to be generated that is different from a set generated under reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[0078] As widely known in the art, many diseases and/or conditions are associated with transcript splicing. For examples, see Garcia-Blanco, *et al.*, Alternative splicing in disease and therapy, *Nat. Biotechnol.* 2004 May;22(5):535-46; Wang, *et al.*, Splicing in disease: disruption of the splicing code and the decoding machinery, *Nat. Rev. Genet.* 2007 Oct;8(10):749-61; Havens, *et al.*, Targeting RNA splicing for disease therapy, *Wiley Interdiscip. Rev. RNA*. 2013 May-Jun;4(3):247-66. In some embodiments, the present disclosure provides compositions and methods for treating or preventing diseases.

[0079] In some embodiments, the present disclosure provides a method for treating or preventing a disease, comprising administering to a subject an oligonucleotide composition described herein.

[0080] In some embodiments, the present disclosure provides a method for treating or preventing a disease, comprising administering to a subject an oligonucleotide composition comprising a first plurality of oligonucleotides, which:

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

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

[0081] In some embodiments, the present disclosure provides a method for treating or preventing a disease, comprising administering to a subject an oligonucleotide composition

comprising a first plurality of oligonucleotides of a particular oligonucleotide type defined by:

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

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

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

[0082] In some embodiments, a disease is one in which, after administering a provided composition, one or more spliced transcripts repair, restore or introduce a new beneficial function. For example, in DMD, after skipping one or more exons, functions of dystrophin can be restored, or partially restored, through a truncated but (partially) active version. In some embodiments, a disease is one in which, after administering a provided composition, one or more spliced transcripts repair, a gene is effectively knockdown by altering splicing of the gene transcript.

[0083] In some embodiments, a disease is Duchenne muscular dystrophy. In some embodiments, a disease is spinal muscular atrophy. In some embodiments, a disease is cancer.

[0084] In some embodiments, the present disclosure provides a method of treating a disease by administering a composition comprising a first plurality of oligonucleotides sharing a common base sequence comprising a common base sequence, which nucleotide sequence is complementary to a target sequence in the target transcript,

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

[0085] In some embodiments, the present disclosure provides a method of treating a

disease by administering a composition comprising a first plurality of oligonucleotides sharing a common base sequence comprising a common base sequence, which nucleotide sequence is complementary to a target sequence in the target transcript,

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

[0086] In some embodiments, a common sequence comprises a sequence selected from Table A1. In some embodiments, a common sequence is a sequence selected from Table A1.

[0087] In some embodiments, the present disclosure provides a method of administering an oligonucleotide composition comprising a first plurality of oligonucleotides having a common nucleotide sequence, the improvement that comprises:

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

[0088] In some embodiments, the present disclosure provides a method of administering an oligonucleotide composition comprising a first plurality of oligonucleotides having a common nucleotide sequence, the improvement that comprises:

administering an oligonucleotide composition in which each oligonucleotide in the plurality comprises one or more modified sugar moieties and the composition is characterized by reduced toxicity relative to a reference oligonucleotide composition of the same common nucleotide sequence but lacking at least one of the one or more modified sugar moieties.

[0089] In some embodiments, the present disclosure provides a method of administering an oligonucleotide composition comprising a first plurality of oligonucleotides having a common nucleotide sequence, the improvement that comprises:

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

wherein the oligonucleotide composition is characterized by reduced toxicity when tested in at least one assay that is observed with an otherwise comparable reference composition whose

oligonucleotides do not comprise natural phosphate linkages.

[0090] In some embodiments, the present disclosure provides a method of administering an oligonucleotide composition comprising a first plurality of oligonucleotides having a common nucleotide sequence, the improvement that comprises:

administering an oligonucleotide composition in which each oligonucleotide in the plurality comprises one or more modified sugar moieties and the composition is characterized by reduced toxicity relative to a reference oligonucleotide composition of the same common nucleotide sequence but lacking at least one of the one or more modified sugar moieties.

[0091] In some embodiments, the present disclosure provides a method comprising steps of administering to a subject an oligonucleotide composition comprising a first plurality of oligonucleotides each of which has a common base sequence and comprises a modified sugar moiety, wherein the oligonucleotide composition is characterized by reduced toxicity when tested in at least one assay that is observed with an otherwise comparable reference composition that comprises a reference plurality of oligonucleotides which have the same common base sequence but have no modified sugar moieties.

[0092] In some embodiments, the present disclosure provides a method comprising steps of administering to a subject an oligonucleotide composition comprising a first plurality of oligonucleotides each of which has a common base sequence and comprises one or more natural phosphate linkages and one or more modified phosphate linkages, wherein the oligonucleotide composition is characterized by reduced toxicity when tested in at least one assay that is observed with an otherwise comparable reference composition that comprises a reference plurality of oligonucleotides which have the same common base sequence but have no natural phosphate linkages.

[0093] In some embodiments, the present disclosure provides a method comprising steps of administering a chirally controlled oligonucleotide composition to a subject, wherein the chirally controlled oligonucleotide composition is characterized by reduced toxicity when tested in at least one assay that is observed with an otherwise comparable reference composition that includes a different chirally controlled oligonucleotide composition, or a stereorandom oligonucleotide composition, comprising oligonucleotides having the same base sequence.

[0094] In some embodiments, reduced toxicity is or comprises reduced complement activation. In some embodiments, reduced toxicity comprises reduced complement activation.

In some embodiments, reduced toxicity is or comprises reduced complement activation. In some embodiments, reduced toxicity comprises reduced complement activation via the alternative pathway.

[0095] In some embodiments, oligonucleotides can elicit proinflammatory responses. In some embodiments, the present disclosure provides compositions and methods for reducing inflammation. In some embodiments, the present disclosure provides compositions and methods for reducing proinflammatory responses. In some embodiments, the present disclosure provides methods for reducing injection site inflammation using provided compositions. In some embodiments, the present disclosure provides methods for reducing drug-induced vascular injury using provided compositions.

[0096] In some embodiments, the present disclosure provides a method, comprising administering a composition comprising a first plurality of oligonucleotides, which composition displays reduced injection site inflammation as compared with a reference composition comprising a plurality of oligonucleotides, each of which also has the common base sequence but which differs structurally from the oligonucleotides of the first plurality in that:

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

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

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

[0097] In some embodiments, the present disclosure provides a method of administering an oligonucleotide composition comprising a first plurality of oligonucleotides having a common nucleotide sequence, the improvement that comprises:

administering an oligonucleotide comprising a first plurality of oligonucleotides that is characterized by reduced injection site inflammation relative to a reference oligonucleotide composition of the same common nucleotide sequence.

[0098] In some embodiments, the present disclosure provides a method, comprising administering a composition comprising a first plurality of oligonucleotides, which composition displays altered protein binding as compared with a reference composition comprising a plurality of oligonucleotides, each of which also has the common base sequence but which differs

structurally from the oligonucleotides of the first plurality in that:

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

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

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

[0099] In some embodiments, the present disclosure provides a method of administering an oligonucleotide composition comprising a first plurality of oligonucleotides having a common nucleotide sequence, the improvement that comprises:

administering an oligonucleotide composition comprising a first plurality of oligonucleotides that is characterized by altered protein binding relative to a reference oligonucleotide composition of the same common nucleotide sequence.

[00100] In some embodiments, the present disclosure provides a method comprising administering a composition comprising a first plurality of oligonucleotides, which composition displays improved delivery as compared with a reference composition comprising a plurality of oligonucleotides, each of which also has the common base sequence but which differs structurally from the oligonucleotides of the first plurality in that:

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

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

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

[00101] In some embodiments, the present disclosure provides a method of administering an oligonucleotide composition comprising a first plurality of oligonucleotides having a common nucleotide sequence, the improvement that comprises:

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

[00102] In some embodiments, the present disclosure provides a composition comprising

a chirally controlled oligonucleotide composition selected from: WV-887, WV-892, WV-896, WV-1714, WV-2444, WV-2445, WV-2526, WV-2527, WV-2528, WV-2530, WV-2531, WV-2578, WV-2580, WV-2587, WV-3047, WV-3152, WV-3472, WV-3473, WV-3507, WV-3508, WV-3509, WV-3510, WV-3511, WV-3512, WV-3513, WV-3514, WV-3515, WV-3545, and WV-3546. In some embodiments, the present disclosure provides a composition comprising a chirally controlled oligonucleotide composition selected from: WV-887, WV-892, WV-896, WV-1714, WV-2444, WV-2445, WV-2526, WV-2527, WV-2528, and WV-2530. In some embodiments, the present disclosure provides chirally controlled oligonucleotide compositions of WV-887, WV-892, WV-896, WV-1714, WV-2444, WV-2445, WV-2526, WV-2527, WV-2528, WV-2530, WV-2531, WV-2578, WV-2580, WV-2587, WV-3047, WV-3152, WV-3472, WV-3473, WV-3507, WV-3508, WV-3509, WV-3510, WV-3511, WV-3512, WV-3513, WV-3514, WV-3515, WV-3545, or WV-3546. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-887. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-892. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-896. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-1714. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2444. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2445. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2526. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2527. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2528. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2530. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2531. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2578. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2580. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2587. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3047. In some embodiments, the present

disclosure provides a chirally controlled oligonucleotide composition of WV-3152. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3472. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3473. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3507. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3508. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3509. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3510. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3511. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3512. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3513. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3514. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3515. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3545. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3546. As readily appreciated by one skilled in the art, such chirally controlled oligonucleotide compositions comprise predetermined levels of WV-887, WV-892, WV-896, WV-1714, WV-2444, WV-2445, WV-2526, WV-2527, WV-2528, WV-2530, WV-2531, WV-2578, WV-2580, WV-2587, WV-3047, WV-3152, WV-3472, WV-3473, WV-3507, WV-3508, WV-3509, WV-3510, WV-3511, WV-3512, WV-3513, WV-3514, WV-3515, WV-3545, or WV-3546.

[00103] In some embodiments, the present disclosure provides a composition comprising a chirally controlled oligonucleotide composition wherein the sequence of the oligonucleotide comprises or consists of the sequence of an oligonucleotide selected from: WV-887, WV-892, WV-896, WV-1714, WV-2444, WV-2445, WV-2526, WV-2527, WV-2528, WV-2530, WV-2531, WV-2578, WV-2580, WV-2587, WV-3047, WV-3152, WV-3472, WV-3473, WV-3507, WV-3508, WV-3509, WV-3510, WV-3511, WV-3512, WV-3513, WV-3514, WV-3515, WV-3545, and WV-3546. In some embodiments, the present disclosure provides a composition comprising a chirally controlled oligonucleotide composition wherein the sequence of the

oligonucleotide comprises or consists of the sequence of an oligonucleotide selected from: WV-887, WV-892, WV-896, WV-1714, WV-2444, WV-2445, WV-2526, WV-2527, WV-2528, and WV-2530.

[00104] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises the sequence of WV-887. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises the sequence of WV-892. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises the sequence of WV-896. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises the sequence of WV-1714. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises the sequence of WV-2444. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises the sequence of WV-2445. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises the sequence of WV-2526. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises the sequence of WV-2527. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises the sequence of WV-2528. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises the sequence of WV-2530. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-887. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-892. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-896. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of

the sequence of WV-1714. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-2444. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-2445. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-2526. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-2527. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-2528. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-2530. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-2531. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-2578. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-2580. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-2587. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3047. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3152. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3472. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3473. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3507. In some embodiments, the present disclosure provides a chirally

controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3508. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3509. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3510. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3511. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3512. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3513. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3514. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3515. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3545. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide consists of the sequence of WV-3546.

[00105] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-887, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-892, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-896, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-1714, wherein the composition further comprises a lipid. In

some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-2444, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-2445, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-2526, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-2527, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-2528, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-2530, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-2531, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-2578, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-2580, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-2587, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3047, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition,

wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3152, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3472, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3473, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3507, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3508, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3509, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3510, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3511, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3512, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3513, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3514, wherein the composition further comprises a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition, wherein the sequence of the oligonucleotide comprises or consists of the sequence of WV-3515, wherein the

composition further comprises a lipid.

[00106] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-887 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-892 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-896 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-1714 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2444 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2445 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2526 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2527 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2528 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2530 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2531 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2578 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2580 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-2587 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3047 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3152 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3472 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3473 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3507 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3508

conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3509 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3510 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3511 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3512 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3513 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3514 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of WV-3515 conjugated to a lipid. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide selected from any of the Tables. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide selected from any of the Tables, wherein the oligonucleotide is conjugated to a lipid.

[00107] In some embodiments, the oligonucleotide is no more than 25 bases long. In some embodiments, the oligonucleotide is no more than 30 bases long. In some embodiments, the oligonucleotide is no more than 35 bases long. In some embodiments, the oligonucleotide is no more than 40 bases long. In some embodiments, the oligonucleotide is no more than 45 bases long. In some embodiments, the oligonucleotide is no more than 50 bases long. In some embodiments, the oligonucleotide is no more than 55 bases long. In some embodiments, the oligonucleotide is no more than 60 bases long.

[00108] In some embodiments, a lipid is a fatty acid. In some embodiments, an oligonucleotide is conjugated to a fatty acid. In some embodiments, a fatty acid comprises 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more carbon atoms. In some embodiments, a fatty acid comprises 10 or more carbon atoms. In some embodiments, a fatty acid comprises 11 or more carbon atoms. In some embodiments, a fatty acid comprises 12 or more carbon atoms. In some embodiments, a fatty acid comprises 13 or more carbon atoms. In some embodiments, a fatty acid comprises 14 or more carbon atoms. In some embodiments, a fatty acid comprises 15 or more carbon atoms. In some embodiments, a fatty acid comprises 16 or more carbon atoms. In some embodiments, a fatty acid comprises 17 or more carbon atoms.

In some embodiments, a fatty acid comprises 18 or more carbon atoms. In some embodiments, a fatty acid comprises 19 or more carbon atoms. In some embodiments, a fatty acid comprises 20 or more carbon atoms. In some embodiments, a fatty acid comprises 21 or more carbon atoms. In some embodiments, a fatty acid comprises 22 or more carbon atoms. In some embodiments, a fatty acid comprises 23 or more carbon atoms. In some embodiments, a fatty acid comprises 24 or more carbon atoms. In some embodiments, a fatty acid comprises 25 or more carbon atoms. In some embodiments, a fatty acid comprises 26 or more carbon atoms. In some embodiments, a fatty acid comprises 27 or more carbon atoms. In some embodiments, a fatty acid comprises 28 or more carbon atoms. In some embodiments, a fatty acid comprises 29 or more carbon atoms. In some embodiments, a fatty acid comprises 30 or more carbon atoms.

[00109] In some embodiments, a lipid is stearic acid or turbinaric acid. In some embodiments, a lipid is stearic acid acid. In some embodiments, a lipid is turbinaric acid.

[00110] In some embodiments, a lipid comprises an optionally substituted, C₁₀-C₈₀ saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C₁-C₆ alkylene, C₁-C₆ alkenylene, $\text{—C}\equiv\text{C—}$, a C₁-C₆ heteroaliphatic moiety, $\text{—C(R')}_2\text{—}$, —Cy— , —O— , —S— , —S—S— , —N(R')— , —C(O)— , —C(S)— , —C(NR')— , —C(O)N(R')— , —N(R')C(O)N(R')— , —N(R')C(O)— , —N(R')C(O)O— , —OC(O)N(R')— , —S(O)— , $\text{—S(O)}_2\text{—}$, $\text{—S(O)}_2\text{N(R')—}$, $\text{—N(R')S(O)}_2\text{—}$, —SC(O)— , —C(O)S— , —OC(O)— , and —C(O)O— , wherein each variable is independently as defined and described herein.

[00111] In some embodiments, a lipid comprises an optionally substituted C₁₀-C₆₀ saturated or partially unsaturated, aliphatic chain.

[00112] In some embodiments, a lipid comprises an optionally substituted C₁₀-C₆₀ linear, saturated or partially unsaturated, aliphatic chain.

[00113] In some embodiments, a lipid comprises a C₁₀-C₆₀ linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C₁₋₄ aliphatic group.

[00114] In some embodiments, a lipid comprises an optionally substituted, C₁₀-C₆₀ saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C₁-C₆ alkylene, C₁-C₆ alkenylene, $\text{—C}\equiv\text{C—}$, a C₁-C₆ heteroaliphatic moiety, $\text{—C(R')}_2\text{—}$, —Cy— , —O— , —S— , —S—S— , —N(R')— , —C(O)— , —C(S)— , —C(NR')— , —C(O)N(R')— , —N(R')C(O)N(R')— , —N(R')C(O)— , —N(R')C(O)O— , —OC(O)N(R')— , —S(O)— , $\text{—S(O)}_2\text{—}$, $\text{—S(O)}_2\text{N(R')—}$, $\text{—N(R')S(O)}_2\text{—}$, —SC(O)— , —C(O)S— , —OC(O)— , and —C(O)O— , wherein each variable is independently as defined and described herein.

N(R')C(O)- , -N(R')C(O)O- , -OC(O)N(R')- , -S(O)- , $\text{-S(O)}_2\text{-}$, $\text{-S(O)}_2\text{N(R')-}$, $\text{-N(R')S(O)}_2\text{-}$, -SC(O)- , -C(O)S- , -OC(O)- , and -C(O)O- , wherein each variable is independently as defined and described herein.

[00115] In some embodiments, a lipid comprises an optionally substituted $\text{C}_{10}\text{-C}_{60}$ saturated or partially unsaturated, aliphatic chain.

[00116] In some embodiments, a lipid comprises an optionally substituted $\text{C}_{10}\text{-C}_{60}$ linear, saturated or partially unsaturated, aliphatic chain.

[00117] In some embodiments, a lipid comprises a $\text{C}_{10}\text{-C}_{60}$ linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic group.

[00118] In some embodiments, a lipid comprises an optionally substituted, $\text{C}_{10}\text{-C}_{40}$ saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from $\text{C}_1\text{-C}_6$ alkylene, $\text{C}_1\text{-C}_6$ alkenylene, $\text{-C}\equiv\text{C-}$, a $\text{C}_1\text{-C}_6$ heteroaliphatic moiety, $\text{-C(R')}_2\text{-}$, -Cy- , -O- , -S- , -S-S- , -N(R')- , -C(O)- , -C(S)- , -C(NR')- , -C(O)N(R')- , -N(R')C(O)N(R')- , -N(R')C(O)- , -N(R')C(O)O- , -OC(O)N(R')- , -S(O)- , $\text{-S(O)}_2\text{-}$, $\text{-S(O)}_2\text{N(R')-}$, $\text{-N(R')S(O)}_2\text{-}$, -SC(O)- , -C(O)S- , -OC(O)- , and -C(O)O- , wherein each variable is independently as defined and described herein.

[00119] In some embodiments, a lipid comprises an optionally substituted $\text{C}_{10}\text{-C}_{60}$ saturated or partially unsaturated, aliphatic chain.

[00120] In some embodiments, a lipid comprises an optionally substituted $\text{C}_{10}\text{-C}_{60}$ linear, saturated or partially unsaturated, aliphatic chain.

[00121] In some embodiments, a lipid comprises a $\text{C}_{10}\text{-C}_{60}$ linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic group.

[00122] In some embodiments, a lipid comprises an unsubstituted $\text{C}_{10}\text{-C}_{80}$ linear, saturated or partially unsaturated, aliphatic chain.

[00123] In some embodiments, a lipid comprises no more than one optionally substituted $\text{C}_{10}\text{-C}_{60}$ linear, saturated or partially unsaturated, aliphatic chain.

[00124] In some embodiments, a lipid comprises two or more optionally substituted $\text{C}_{10}\text{-C}_{60}$ linear, saturated or partially unsaturated, aliphatic chain.

[00125] In some embodiments, a lipid comprises an unsubstituted $\text{C}_{10}\text{-C}_{60}$ linear, saturated or partially unsaturated, aliphatic chain.

[00126] In some embodiments, a lipid comprises no more than one optionally substituted C₁₀-C₆₀ linear, saturated or partially unsaturated, aliphatic chain.

[00127] In some embodiments, a lipid comprises two or more optionally substituted C₁₀-C₆₀ linear, saturated or partially unsaturated, aliphatic chain.

[00128] In some embodiments, a lipid comprises an unsubstituted C₁₀-C₄₀ linear, saturated or partially unsaturated, aliphatic chain.

[00129] In some embodiments, a lipid comprises no more than one optionally substituted C₁₀-C₆₀ linear, saturated or partially unsaturated, aliphatic chain.

[00130] In some embodiments, a lipid comprises two or more optionally substituted C₁₀-C₆₀ linear, saturated or partially unsaturated, aliphatic chain.

[00131] In some embodiments, a lipid comprises a C₁₀-C₄₀ linear, saturated or partially unsaturated, aliphatic chain.

[00132] In some embodiments, a lipid comprises a C₁₀-C₄₀ linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C₁₋₄ aliphatic group.

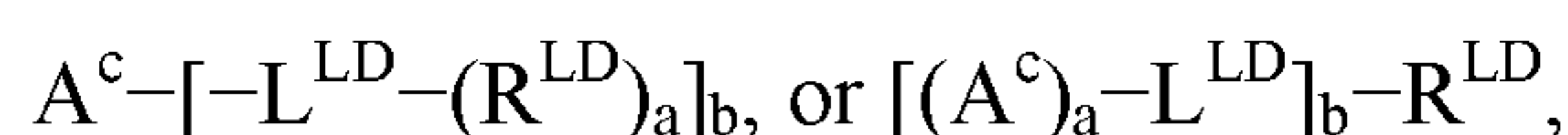
[00133] In some embodiments, a lipid is selected from the group consisting of: lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (DHA or cis-DHA), turbinaric acid and dilinoleyl.

[00134] In some embodiments, a lipid is not conjugated to the oligonucleotide.

[00135] In some embodiments, a lipid is conjugated to the oligonucleotide.

[00136] In some embodiments, a lipid is conjugated to the oligonucleotide with a linker. In some embodiments, a linker has the structure of -L-. In some embodiments, a conjugate has the structure of A^c-[L^{LD}-(R^{LD})_a]_b.

[00137] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a plurality of oligonucleotides having the structure of:



wherein:

A^c is an oligonucleotide chain ([H]_b-A^c is an oligonucleotide);

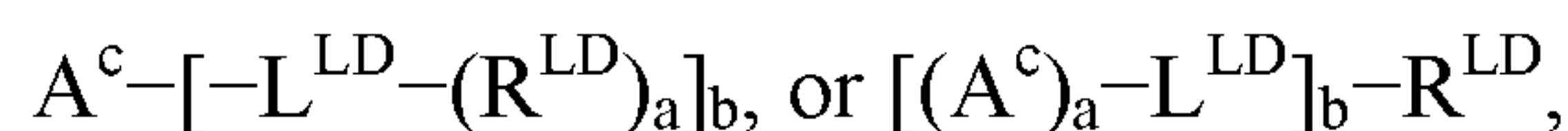
a is 1-1000;

b is 1-1000;

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

each R^{LD} is independently a lipid moiety or a targeting component.

[00138] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a plurality of oligonucleotides having the structure of:



wherein:

A^c is an oligonucleotide chain ($[H]_b-A^c$ is an oligonucleotide);

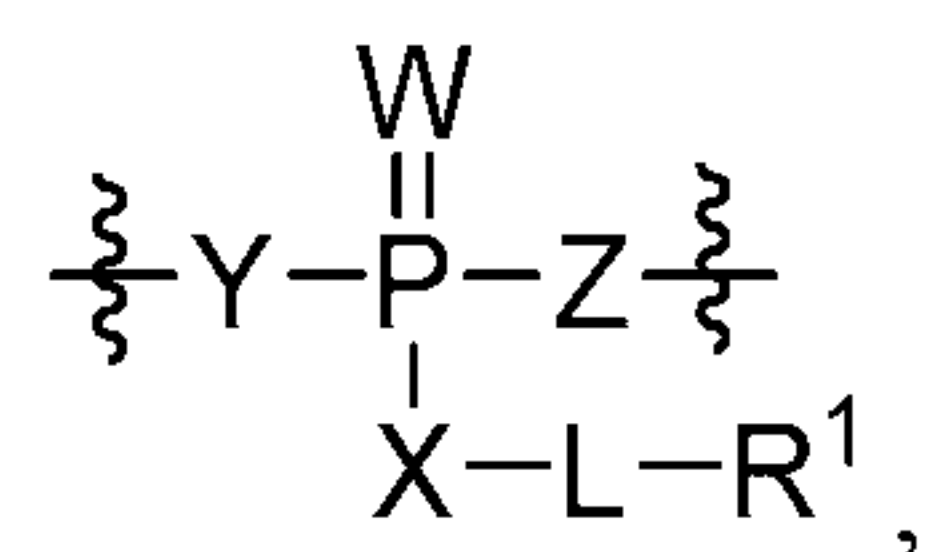
a is 1-1000;

b is 1-1000;

each L^{LD} is independently a covalent bond or an optionally substituted, C_1 - C_{80} saturated or partially unsaturated aliphatic group, wherein one or more methylene units are optionally and independently replaced by T^{LD} or an optionally substituted group selected from C_1 - C_6 alkylene, C_1 - C_6 alkenylene, $-C\equiv C-$, a C_1 - C_6 heteroaliphatic moiety, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, $-SC(O)-$, $-C(O)S-$, $-OC(O)-$, and $-C(O)O-$;

each R^{LD} is independently an optionally substituted, C_1 - 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, $-C\equiv C-$, a C_1 - C_6 heteroaliphatic moiety, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)-$, $-N(R')C(O)O-$, $-OC(O)N(R')-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-N(R')S(O)_2-$, $-SC(O)-$, $-C(O)S-$, $-OC(O)-$, and $-C(O)O-$;

T^{LD} has the structure of:



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, $-C\equiv C-$, a C_1 - C_6 heteroaliphatic moiety, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$,

$-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{N}(\text{R}')-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{S}(\text{O})_2-$, $-\text{SC}(\text{O})-$, $-\text{C}(\text{O})\text{S}-$, $-\text{OC}(\text{O})-$, and $-\text{C}(\text{O})\text{O}-$;

R^1 is halogen, R, or an optionally substituted $\text{C}_1\text{--C}_{50}$ aliphatic wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from $\text{C}_1\text{--C}_6$ alkylene, $\text{C}_1\text{--C}_6$ alkenylene, $-\text{C}\equiv\text{C}-$, a $\text{C}_1\text{--C}_6$ heteroaliphatic moiety, $-\text{C}(\text{R}')_2-$, $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})\text{N}(\text{R}')-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{S}(\text{O})_2-$, $-\text{SC}(\text{O})-$, $-\text{C}(\text{O})\text{S}-$, $-\text{OC}(\text{O})-$, and $-\text{C}(\text{O})\text{O}-$

each R' is independently $-\text{R}$, $-\text{C}(\text{O})\text{R}$, $-\text{CO}_2\text{R}$, or $-\text{SO}_2\text{R}$, or:

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

$-\text{Cy}-$ is an optionally substituted bivalent ring selected from phenylene, carbocyclylene, arylene, heteroarylene, and heterocyclylene; and

each R is independently hydrogen, or an optionally substituted group selected from $\text{C}_1\text{--C}_6$ aliphatic, carbocyclyl, aryl, heteroaryl, and heterocyclyl.

[00139] In some embodiments, $[\text{H}]_b\text{-Ac}$ (wherein b is 1-1000) is an oligonucleotide of any one of the Tables. In some embodiments, $[\text{H}]_b\text{-Ac}$ is an oligonucleotide of Table 2. In some embodiments, $[\text{H}]_b\text{-Ac}$ is an oligonucleotide of Table 3. In some embodiments, $[\text{H}]_b\text{-Ac}$ is an oligonucleotide of Table 4. In some embodiments, $[\text{H}]_b\text{-Ac}$ is an oligonucleotide of Table 4 comprising no lipid moieties.

[00140] In some embodiments, P in T^{LD} is P^* . In some embodiments, a conjugate has the structure of $[(\text{A}^c)_a\text{-L}^{\text{LD}}]_b\text{-R}^{\text{LD}}$. In some embodiments, a conjugate has the structure of $(\text{A}^c)_a\text{-L}^{\text{LD}}\text{-R}^{\text{LD}}$. In some embodiments, a is 1-100. In some embodiments, a is 1-50. In some embodiments, a is 1-40. In some embodiments, a is 1-30. In some embodiments, a is 1-20. In some embodiments, a is 1-15. In some embodiments, a is 1-10. In some embodiments, a is 1-9. In some embodiments, a is 1-8. In some embodiments, a is 1-7. In some embodiments, a is 1-6. In some embodiments, a is 1-5. In some embodiments, a is 1-4. In some embodiments, a is 1-3. In some embodiments, a is 1-2. In some embodiments, a is 1. In some embodiments, a is 2. In some embodiments, a is 3. In some embodiments, a is 4. In some embodiments, a is 5. In some embodiments, a is 6. In some embodiments, a is 7. In some embodiments, a is 8. In some

embodiments, a is 9. In some embodiments, a is 10. In some embodiments, a is more than 10. In some embodiments, b is 1-100. In some embodiments, b is 1-50. In some embodiments, b is 1-40. In some embodiments, b is 1-30. In some embodiments, b is 1-20. In some embodiments, b is 1-15. In some embodiments, b is 1-10. In some embodiments, b is 1-9. In some embodiments, b is 1-8. In some embodiments, b is 1-7. In some embodiments, b is 1-6. In some embodiments, b is 1-5. In some embodiments, b is 1-4. In some embodiments, b is 1-3. In some embodiments, b is 1-2. In some embodiments, b is 1. In some embodiments, b is 2. In some embodiments, b is 3. In some embodiments, b is 4. In some embodiments, b is 5. In some embodiments, b is 6. In some embodiments, b is 7. In some embodiments, b is 8. In some embodiments, b is 9. In some embodiments, b is 10. In some embodiments, b is more than 10. In some embodiments, a conjugate has the structure of $A^c-L^{LD}-R^{LD}$. In some embodiments, A^c is conjugated through one or more of its sugar, base and/or internucleotidic linkage moieties. In some embodiments, A^c is conjugated through its 5'-OH (5'-O-). In some embodiments, A^c is conjugated through its 3'-OH (3'-O-). In some embodiments, before conjugation, $A^c-(H)_b$ (b is an integer of 1-1000 depending on valency of A^c) is an oligonucleotide as described herein, for example, one of those described in any one of the Tables. In some embodiments, L^{LD} is $-L-$. In some embodiments, L^{LD} comprises a phosphorothioate group. In some embodiments, L^{LD} is $-C(O)NH-(CH_2)_6-OP(=O)(S^-)-O-$. In some embodiments, the $-C(O)NH$ end is connected to R^{LD} , and the $-O-$ end is connected to the oligonucleotide, *e.g.*, through 5'- or 3'-end. In some embodiments, R^{LD} is optionally substituted C_{10} , C_{15} , C_{16} , C_{17} , C_{18} , C_{19} , C_{20} , C_{21} , C_{22} , C_{23} , C_{24} , or C_{25} to C_{20} , C_{21} , C_{22} , C_{23} , C_{24} , C_{25} , C_{26} , C_{27} , C_{28} , C_{29} , C_{30} , C_{35} , C_{40} , C_{45} , C_{50} , C_{60} , C_{70} , or C_{80} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{10-80} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{20-80} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{10-70} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{20-70} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{10-60} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{20-60} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{10-50} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{20-50} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{10-40} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{20-40} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{10-30} aliphatic. In some embodiments, R^{LD} is optionally substituted C_{20-30} aliphatic. In some embodiments, R^{LD} is unsubstituted C_{10} , C_{15} , C_{16} , C_{17} , C_{18} , C_{19} , C_{20} , C_{21} , C_{22} ,

C₂₃, C₂₄, or C₂₅ to C₂₀, C₂₁, C₂₂, C₂₃, C₂₄, C₂₅, C₂₆, C₂₇, C₂₈, C₂₉, C₃₀, C₃₅, C₄₀, C₄₅, C₅₀, C₆₀, C₇₀, or C₈₀ aliphatic. In some embodiments, R^{LD} is unsubstituted C₁₀₋₈₀ aliphatic. In some embodiments, R^{LD} is unsubstituted C₂₀₋₈₀ aliphatic. In some embodiments, R^{LD} is unsubstituted C₁₀₋₇₀ aliphatic. In some embodiments, R^{LD} is unsubstituted C₂₀₋₇₀ aliphatic. In some embodiments, R^{LD} is unsubstituted C₁₀₋₆₀ aliphatic. In some embodiments, R^{LD} is unsubstituted C₂₀₋₆₀ aliphatic. In some embodiments, R^{LD} is unsubstituted C₁₀₋₅₀ aliphatic. In some embodiments, R^{LD} is unsubstituted C₂₀₋₅₀ aliphatic. In some embodiments, R^{LD} is unsubstituted C₁₀₋₄₀ aliphatic. In some embodiments, R^{LD} is unsubstituted C₂₀₋₄₀ aliphatic. In some embodiments, R^{LD} is unsubstituted C₁₀₋₃₀ aliphatic. In some embodiments, R^{LD} is unsubstituted C₂₀₋₃₀ aliphatic.

[00141] In some embodiments, a chirally controlled oligonucleotide composition is any one of the preceding compositions, further comprising one or more additional components.

[00142] In some embodiments, conjugation of a lipid to an oligonucleotide improves at least one property of the oligonucleotide. In some embodiments, improved properties include increased activity (*e.g.*, increased ability to induce desirable skipping of a deleterious exon), decreased toxicity, and/or improved distribution to a tissue. In some embodiments, a tissue is muscle tissue. In some embodiments, a tissue is skeletal muscle, gastrocnemius, triceps, heart or diaphragm. In some embodiments, improved properties include reduced hTLR9 agonist activity. In some embodiments, improved properties include hTLR9 antagonist activity. In some embodiments, improved properties include increased hTLR9 antagonist activity.

[00143] In general, properties of oligonucleotide compositions as described herein can be assessed using any appropriate assay. Relative toxicity and/or protein binding properties for different compositions (*e.g.*, stereocontrolled vs non-stereocontrolled, and/or different stereocontrolled compositions) are typically desirably determined in the same assay, in some embodiments substantially simultaneously and in some embodiments with reference to historical results.

[00144] Those of skill in the art will be aware of and/or will readily be able to develop appropriate assays for particular oligonucleotide compositions. The present disclosure provides descriptions of certain particular assays, for example that may be useful in assessing one or more features of oligonucleotide composition behavior *e.g.*, complement activation, injection site inflammation, protein binding, *etc.*

[00145] For example, certain assays that may be useful in the assessment of toxicity and/or protein binding properties of oligonucleotide compositions may include any assay described and/or exemplified herein.

DEFINITIONS

[00146] *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 C₃-C₁₀ 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 C₃-C₆ 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.

[00147] *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.

[00148] *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.

[00149] *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.

[00150] *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.

[00151] *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.

[00152] *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.

[00153] *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.

[00154] *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.

[00155] *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.

[00156] *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.

[00157] *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.

[00158] *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-acetylcysteine), and regulators of cellular trafficking (*e.g.*, Rab1a polypeptide).

[00159] *Heteroaliphatic*: The term “heteroaliphatic” refers to an aliphatic group wherein one or more units selected from C, CH, CH₂, or CH₃ are independently replaced by a heteroatom. In some embodiments, a heteroaliphatic group is heteroalkyl. In some embodiments, a heteroaliphatic group is heteroalkenyl.

[00160] *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(4*H*)-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.

[00161] *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)).

[00162] *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).

[00163] 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.

[00164] *Intraperitoneal:* The phrases “intraperitoneal administration” and “administered intraperitoneally” as used herein have their art-understood meaning referring to administration of a compound or composition into the peritoneum of a subject.

[00165] *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).

[00166] *In vivo:* As used herein, the term “*in vivo*” refers to events that occur within an organism (*e.g.*, animal, plant, and/or microbe).

[00167] *Lower alkyl:* The term “lower alkyl” refers to a C₁₋₄ straight or branched alkyl group. Example lower alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, and tert-butyl.

[00168] *Lower haloalkyl:* The term “lower haloalkyl” refers to a C₁₋₄ straight or branched alkyl group that is substituted with one or more halogen atoms.

[00169] *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.

[00170] 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° ; $-NO_2$; $-CN$; $-N_3$; $-(CH_2)_{0-4}N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$; $-N(R^\circ)C(S)R^\circ$; $-(CH_2)_{0-4}N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)C(S)NR^\circ_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $-N(R^\circ)N(R^\circ)C(O)R^\circ$; $-N(R^\circ)N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)N(R^\circ)C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)R^\circ$; $-C(S)R^\circ$; $-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)SR^\circ$; $-(CH_2)_{0-4}C(O)OSiR^\circ_3$; $-(CH_2)_{0-4}OC(O)R^\circ$; $-OC(O)(CH_2)_{0-4}SR$; $-SC(S)SR^\circ$; $-(CH_2)_{0-4}SC(O)R^\circ$; $-(CH_2)_{0-4}C(O)NR^\circ_2$; $-C(S)NR^\circ_2$; $-C(S)SR^\circ$; $-SC(S)SR^\circ$; $-(CH_2)_{0-4}OC(O)NR^\circ_2$; $-C(O)N(OR^\circ)R^\circ$; $-C(O)C(O)R^\circ$; $-C(O)CH_2C(O)R^\circ$; $-C(NOR^\circ)R^\circ$; $-(CH_2)_{0-4}SSR^\circ$; $-(CH_2)_{0-4}S(O)_2R^\circ$; $-(CH_2)_{0-4}S(O)_2OR^\circ$; $-(CH_2)_{0-4}OS(O)_2R^\circ$; $-S(O)_2NR^\circ_2$; $-(CH_2)_{0-4}S(O)R^\circ$; $-N(R^\circ)S(O)_2NR^\circ_2$; $-N(R^\circ)S(O)_2R^\circ$;

$-\text{N}(\text{OR}^\circ)\text{R}^\circ$; $-\text{C}(\text{NH})\text{NR}^\circ_2$; $-\text{P}(\text{O})_2\text{R}^\circ$; $-\text{P}(\text{O})\text{R}^\circ_2$; $-\text{OP}(\text{O})\text{R}^\circ_2$; $-\text{OP}(\text{O})(\text{OR}^\circ)_2$; $-\text{SiR}^\circ_3$; $-\text{OSiR}^\circ_3$; $-(\text{C}_{1-4} \text{ straight or branched alkylene})\text{O}-\text{N}(\text{R}^\circ)_2$; or $-(\text{C}_{1-4} \text{ straight or branched alkylene})\text{C}(\text{O})\text{O}-\text{N}(\text{R}^\circ)_2$, wherein each R° may be substituted as defined below and is independently hydrogen, C_{1-6} aliphatic, $-\text{CH}_2\text{Ph}$, $-\text{O}(\text{CH}_2)_{0-1}\text{Ph}$, $-\text{CH}_2$ -(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.

[00171] Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $-(\text{CH}_2)_{0-2}\text{R}^\bullet$, $-(\text{haloR}^\bullet)$, $-(\text{CH}_2)_{0-2}\text{OH}$, $-(\text{CH}_2)_{0-2}\text{OR}^\bullet$, $-(\text{CH}_2)_{0-2}\text{CH}(\text{OR}^\bullet)_2$, $-\text{O}(\text{haloR}^\bullet)$, $-\text{CN}$, $-\text{N}_3$, $-(\text{CH}_2)_{0-2}\text{C}(\text{O})\text{R}^\bullet$, $-(\text{CH}_2)_{0-2}\text{C}(\text{O})\text{OH}$, $-(\text{CH}_2)_{0-2}\text{C}(\text{O})\text{OR}^\bullet$, $-(\text{CH}_2)_{0-2}\text{SR}^\bullet$, $-(\text{CH}_2)_{0-2}\text{SH}$, $-(\text{CH}_2)_{0-2}\text{NH}_2$, $-(\text{CH}_2)_{0-2}\text{NHR}^\bullet$, $-(\text{CH}_2)_{0-2}\text{NR}^\bullet_2$, $-\text{NO}_2$, $-\text{SiR}^\bullet_3$, $-\text{OSiR}^\bullet_3$, $-\text{C}(\text{O})\text{SR}^\bullet$, $-(\text{C}_{1-4} \text{ straight or branched alkylene})\text{C}(\text{O})\text{OR}^\bullet$, or $-\text{SSR}^\bullet$ wherein each $\text{R}^\bullet$ is unsubstituted or where preceded by “halo” is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, $-\text{CH}_2\text{Ph}$, $-\text{O}(\text{CH}_2)_{0-1}\text{Ph}$, 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 $=\text{O}$ and $=\text{S}$.

[00172] Suitable divalent substituents on a saturated carbon atom of an “optionally substituted” group include the following: $=\text{O}$, $=\text{S}$, $=\text{NNR}^*_2$, $=\text{NNHC}(\text{O})\text{R}^*$, $=\text{NNHC}(\text{O})\text{OR}^*$, $=\text{NNHS}(\text{O})_2\text{R}^*$, $=\text{NR}^*$, $=\text{NOR}^*$, $-\text{O}(\text{C}(\text{R}^*_2))_{2-3}\text{O}-$, or $-\text{S}(\text{C}(\text{R}^*_2))_{2-3}\text{S}-$, 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: $-\text{O}(\text{CR}^*_2)_{2-3}\text{O}-$, 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.

[00173] Suitable substituents on the aliphatic group of R^* include halogen, $-R^\bullet$, $-(\text{halo}R^\bullet)$, $-\text{OH}$, $-\text{OR}^\bullet$, $-\text{O}(\text{halo}R^\bullet)$, $-\text{CN}$, $-\text{C}(\text{O})\text{OH}$, $-\text{C}(\text{O})\text{OR}^\bullet$, $-\text{NH}_2$, $-\text{NHR}^\bullet$, $-\text{NR}^\bullet_2$, or $-\text{NO}_2$, 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, $-\text{CH}_2\text{Ph}$, $-\text{O}(\text{CH}_2)_{0-1}\text{Ph}$, or a 5-6 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00174] Suitable substituents on a substitutable nitrogen of an “optionally substituted” group include $-R^\dagger$, $-\text{NR}^\dagger_2$, $-\text{C}(\text{O})R^\dagger$, $-\text{C}(\text{O})\text{OR}^\dagger$, $-\text{C}(\text{O})\text{C}(\text{O})R^\dagger$, $-\text{C}(\text{O})\text{CH}_2\text{C}(\text{O})R^\dagger$, $-\text{S}(\text{O})_2R^\dagger$, $-\text{S}(\text{O})_2\text{NR}^\dagger_2$, $-\text{C}(\text{S})\text{NR}^\dagger_2$, $-\text{C}(\text{NH})\text{NR}^\dagger_2$, or $-\text{N}(\text{R}^\dagger)\text{S}(\text{O})_2R^\dagger$; wherein each $R^\dagger$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted $-\text{OPh}$, or an unsubstituted 5-6 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^\dagger$, 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.

[00175] Suitable substituents on the aliphatic group of $R^\dagger$ are independently halogen, $-R^\bullet$, $-(\text{halo}R^\bullet)$, $-\text{OH}$, $-\text{OR}^\bullet$, $-\text{O}(\text{halo}R^\bullet)$, $-\text{CN}$, $-\text{C}(\text{O})\text{OH}$, $-\text{C}(\text{O})\text{OR}^\bullet$, $-\text{NH}_2$, $-\text{NHR}^\bullet$, $-\text{NR}^\bullet_2$, or $-\text{NO}_2$, 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, $-\text{CH}_2\text{Ph}$, $-\text{O}(\text{CH}_2)_{0-1}\text{Ph}$, or a 5-6 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[00176] *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.

[00177] *Parenteral:* The phrases “parenteral administration” and “administered parenterally” as used herein have their art-understood meaning referring to modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, and intrasternal injection and infusion.

[00178] *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.

[00179] *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.

[00180] *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.

[00181] *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.

[00182] *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.

[00183] *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 J. Rautio (Wiley, 2011);
- c) A Textbook of Drug Design and Development, edited by Krogsgaard-Larsen;
- d) Bundgaard, Chapter 5 “Design and Application of Prodrugs”, by H. Bundgaard, p. 113-191 (1991);
- e) Bundgaard, *Advanced Drug Delivery Reviews*, 8:1-38 (1992);
- f) Bundgaard, *et al.*, *Journal of Pharmaceutical Sciences*, 77:285 (1988); and
- g) Kakeya, *et al.*, *Chem. Pharm. Bull.*, 32:692 (1984).

[00184] 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.

[00185] *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 carbamate, 9-fluorenylmethyl carbamate (Fmoc), 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluorenylmethyl carbamate, 2,7-di-*t*-butyl-[9-(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl carbamate (DBD-Tmoc), 4-methoxyphenacyl carbamate

(Phenoc), 2,2,2-trichloroethyl carbamate (Troc), 2-trimethylsilylethyl carbamate (Teoc), 2-phenylethyl carbamate (hZ), 1-(1-adamantyl)-1-methylethyl carbamate (Adpoc), 1,1-dimethyl-2-haloethyl carbamate, 1,1-dimethyl-2,2-dibromoethyl carbamate (DB-*t*-BOC), 1,1-dimethyl-2,2,2-trichloroethyl carbamate (TCBOC), 1-methyl-1-(4-biphenyl)ethyl carbamate (Bpoc), 1-(3,5-di-*t*-butylphenyl)-1-methylethyl carbamate (*t*-Bumeoc), 2-(2'- and 4'-pyridyl)ethyl carbamate (Pyoc), 2-(*N,N*-dicyclohexylcarboxamido)ethyl carbamate, *t*-butyl carbamate (BOC), 1-adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1-isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4-nitrocinnamyl carbamate (Noc), 8-quinolyl carbamate, *N*-hydroxypiperidinyl carbamate, alkylidithio 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*-nitrophenylacetamide, *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-pyroloin-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*-ferrocenylmethylenamine (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 phenacysulfonamide.

[00186] 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.

[00187] Suitable hydroxyl protecting groups include methyl, methoxymethyl (MOM), methylthiomethyl (MTM), *t*-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), *p*-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (*p*-AOM), guaiacolmethyl (GUM), *t*-butoxymethyl, 4-pentenylloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-

benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, *t*-butyl, allyl, *p*-chlorophenyl, *p*-methoxyphenyl, 2,4-dinitrophenyl, benzyl, *p*-methoxybenzyl, 3,4-dimethoxybenzyl, *o*-nitrobenzyl, *p*-nitrobenzyl, *p*-halobenzyl, 2,6-dichlorobenzyl, *p*-cyanobenzyl, *p*-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl *N*-oxido, diphenylmethyl, *p,p'*-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, α -naphthyl diphenylmethyl, *p*-methoxyphenyl diphenylmethyl, di(*p*-methoxyphenyl)phenylmethyl, tri(*p*-methoxyphenyl)methyl, 4-(4'-bromophenacyloxyphenyl)diphenylmethyl, 4,4',4''-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4''-tris(levulinoyloxyphenyl)methyl, 4,4',4''-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4',4''-dimethoxyphenyl)methyl, 1,1-bis(4-methoxyphenyl)-1'-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, benzisothiazolyl *S,S*-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylhexylsilyl, *t*-butyldimethylsilyl (TBDMS), *t*-butyldiphenylsilyl (TBDPS), tribenzylsilyl, tri-*p*-xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), *t*-butylmethoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, *p*-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantate, crotonate, 4-methoxycrotonate, benzoate, *p*-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl)ethyl carbonate (Psec), 2-(triphenylphosphonio)ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate, alkyl allyl carbonate, alkyl *p*-nitrophenyl carbonate, alkyl benzyl carbonate, alkyl *p*-methoxybenzyl carbonate, alkyl 3,4-dimethoxybenzyl carbonate, alkyl *o*-nitrobenzyl carbonate, alkyl *p*-nitrobenzyl carbonate, alkyl *S*-benzyl thiocarbonate, 4-ethoxy-1-naphthyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, *o*-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (*E*)-2-methyl-2-butenate, *o*-

(methoxycarbonyl)benzoate, α -naphthoate, nitrate, alkyl N,N,N',N' -tetramethylphosphorodiamidate, alkyl N -phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-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-ethoxyethylidene ortho ester, 1,2-dimethoxyethylidene ortho ester, α -methoxybenzylidene ortho ester, 1-(N,N -dimethylamino)ethylidene derivative, α -(N,N' -dimethylamino)benzylidene derivative, 2-oxacyclopentylidene ortho ester, di-*t*-butylsilylene group (DTBS), 1,3-(1,1,3,3-tetraisopropylidisiloxanylidene) derivative (TIPDS), tetra-*t*-butoxydisiloxane-1,3-diylidene derivative (TBDS), cyclic carbonates, cyclic boronates, ethyl boronate, and phenyl boronate.

[00188] 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, trifluoroacetyl, pivaloyl, 9-fluorenylmethyl carbonate, mesylate, tosylate, triflate, trityl, monomethoxytrityl (MMTr), 4,4'-dimethoxytrityl, (DMTr) and 4,4',4''-trimethoxytrityl (TMTr), 2-cyanoethyl (CE or Cne), 2-(trimethylsilyl)ethyl (TSE), 2-(2-nitrophenyl)ethyl, 2-(4-cyanophenyl)ethyl 2-(4-nitrophenyl)ethyl (NPE), 2-(4-nitrophenylsulfonyl)ethyl, 3,5-dichlorophenyl, 2,4-dimethylphenyl, 2-nitrophenyl, 4-nitrophenyl, 2,4,6-trimethylphenyl, 2-(2-nitrophenyl)ethyl, butylthiocarbonyl, 4,4',4''-tris(benzoyloxy)trityl, diphenylcarbamoyl, levulinyl, 2-(dibromomethyl)benzoyl (Dbmb), 2-(isopropylthiomethoxymethyl)benzoyl (Ptmt), 9-phenylxanthen-9-yl (pixyl) or 9-(*p*-methoxyphenyl)xanthine-9-yl (MOX). In some embodiments, each of the hydroxyl protecting groups is, independently selected from acetyl, benzyl, *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl and 4,4'-dimethoxytrityl. In some

embodiments, the hydroxyl protecting group is selected from the group consisting of trityl, monomethoxytrityl and 4,4'-dimethoxytrityl group.

[00189] In some embodiments, a phosphorous protecting group is a group attached to the internucleotide phosphorous linkage throughout oligonucleotide synthesis. In some embodiments, the phosphorous protecting group is attached to the sulfur atom of the internucleotide phosphorothioate linkage. In some embodiments, the phosphorous protecting group is attached to the oxygen atom of the internucleotide phosphorothioate linkage. In some embodiments, the phosphorous protecting group is attached to the oxygen atom of the internucleotide phosphate linkage. In some embodiments the phosphorous protecting group is 2-cyanoethyl (CE or Cne), 2-trimethylsilylethyl, 2-nitroethyl, 2-sulfonylethyl, methyl, benzyl, *o*-nitrobenzyl, 2-(*p*-nitrophenyl)ethyl (NPE or Npe), 2-phenylethyl, 3-(*N*-*tert*-butylcarboxamido)-1-propyl, 4-oxopentyl, 4-methylthio-1-butyl, 2-cyano-1,1-dimethylethyl, 4-*N*-methylaminobutyl, 3-(2-pyridyl)-1-propyl, 2-[*N*-methyl-*N*-(2-pyridyl)]aminoethyl, 2-(*N*-formyl,*N*-methyl)aminoethyl, 4-[*N*-methyl-*N*-(2,2,2-trifluoroacetyl)amino]butyl.

[00190] *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.

[00191] *Sample:* A “sample” as used herein is a specific organism or material obtained therefrom. In some embodiments, a sample is a biological sample obtained or derived from a source of interest, as described herein, . In some embodiments, a source of interest comprises an

organism, such as an animal or human. In some embodiments, a biological sample comprises biological tissue or fluid. In some embodiments, a biological sample is or comprises bone marrow; blood; blood cells; ascites; tissue or fine needle biopsy samples; cell-containing body fluids; free floating nucleic acids; sputum; saliva; urine; cerebrospinal fluid, peritoneal fluid; pleural fluid; feces; lymph; gynecological fluids; skin swabs; vaginal swabs; oral swabs; nasal swabs; washings or lavages such as a ductal lavages or bronchoalveolar 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.

[00192] *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.

[00193] *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.

[00194] *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.

[00195] *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.

[00196] *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.

[00197] *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.

[00198] *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.

[00199] *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.

[00200] *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.

[00201] *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.

[00202] *Unsaturated:* The term "unsaturated," as used herein, means that a moiety has one or more units of unsaturation.

[00203] *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.

[00204] *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).

[00205] *Nucleic acid*: The term “nucleic acid” includes any nucleotides, analogs thereof, and polymers thereof. The term “polynucleotide” as used herein refer to a polymeric form of nucleotides of any length, either ribonucleotides (RNA) or deoxyribonucleotides (DNA). These terms refer to the primary structure of the molecules and, thus, include double- and single-stranded DNA, and double- and single-stranded RNA. These terms include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs and modified polynucleotides such as, though not limited to, methylated, protected and/or capped nucleotides or polynucleotides. The terms encompass poly- or oligo-ribonucleotides (RNA) and poly- or oligo-deoxyribonucleotides (DNA); RNA or DNA derived from N-glycosides or C-glycosides of nucleobases and/or modified nucleobases; nucleic acids derived from sugars and/or modified sugars; and nucleic acids derived from phosphate bridges and/or modified phosphorus-atom bridges (also referred to herein as “internucleotide linkages”). The term encompasses nucleic acids containing any combinations of nucleobases, modified nucleobases, sugars, modified sugars, phosphate bridges or modified phosphorus atom bridges. Examples include, and are not limited to, nucleic acids containing ribose moieties, the nucleic acids containing deoxy-ribose moieties, nucleic acids containing both ribose and deoxyribose moieties, nucleic acids containing ribose and modified ribose moieties. The prefix poly- refers to a nucleic acid containing 2 to about 10,000 nucleotide monomer units and wherein the prefix oligo- refers to a nucleic acid containing 2 to about 200 nucleotide monomer units.

[00206] *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.

[00207] *Nucleoside*: The term “nucleoside” refers to a moiety wherein a nucleobase or a modified nucleobase is covalently bound to a sugar or modified sugar.

[00208] *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”).

[00209] *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.

[00210] *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.

[00211] *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.

[00212] *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.

[00213] *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.

[00214] *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.

[00215] *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).

[00216] *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.

[00217] *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).

[00218] *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, 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).

[00219] *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.

[00220] *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.

[00221] *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.

[00222] *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.

[00223] *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.

[00224] *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.

[00225] *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.

[00226] 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).

[00227] *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.

[00228] *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.

[00229] *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.

[00230] *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.

[00231] *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).

[00232] Oligonucleotides can be single-stranded or double-stranded. As used herein, the term “oligonucleotide strand” encompasses a single-stranded oligonucleotide. A single-stranded oligonucleotide can have double-stranded regions and a double-stranded oligonucleotide can have single-stranded regions. Example oligonucleotides include, but are not limited to structural genes, genes including control and termination regions, self-replicating systems such as viral or plasmid DNA, single-stranded and double-stranded siRNAs and other RNA interference reagents (RNAi agents or iRNA agents), shRNA, antisense oligonucleotides, ribozymes, microRNAs, microRNA mimics, supermirs, aptamers, antimirs, antagomirs, UI adaptors, triplex-forming oligonucleotides, G-quadruplex oligonucleotides, RNA activators, immuno-stimulatory oligonucleotides, and decoy oligonucleotides.

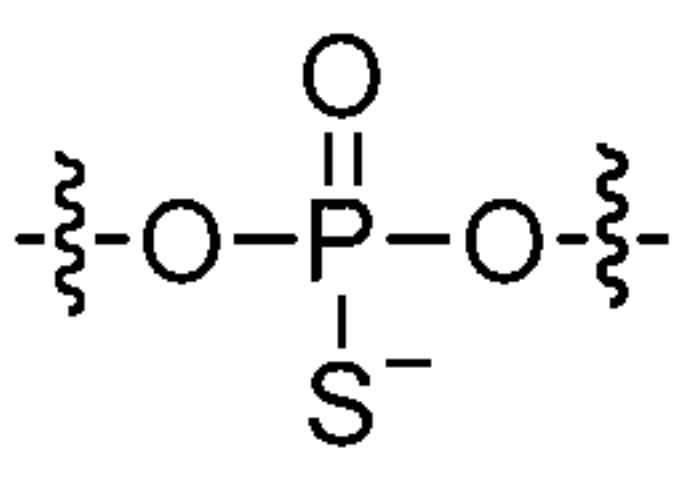
[00233] Double-stranded and single-stranded oligonucleotides that are effective in inducing RNA interference are also referred to as siRNA, RNAi agent, or iRNA agent, herein. In some embodiments, these RNA interference inducing oligonucleotides associate with a cytoplasmic multi-protein complex known 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.

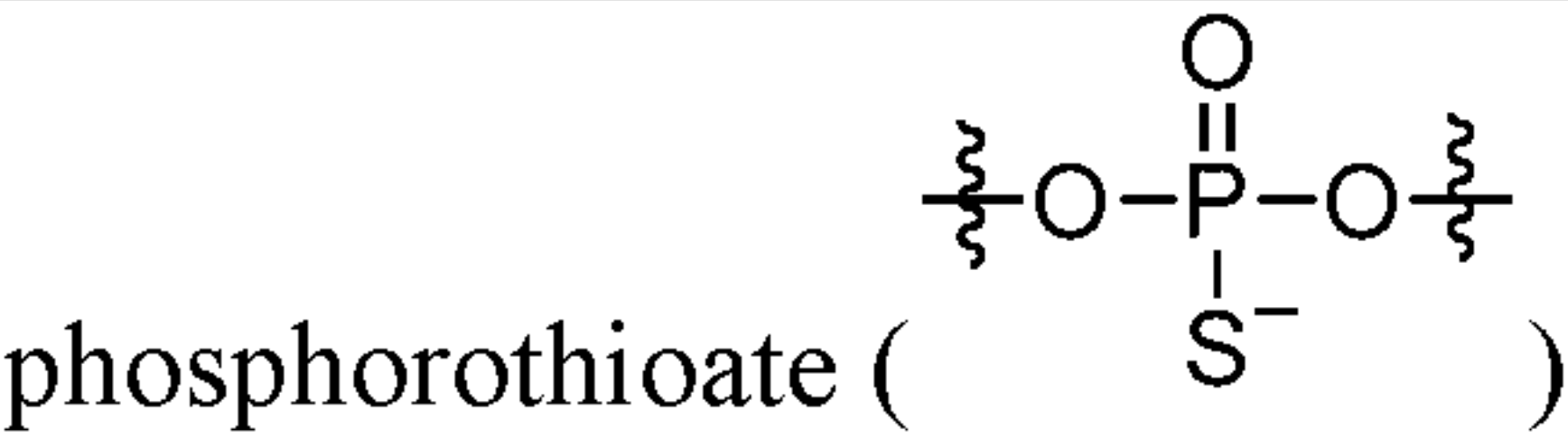
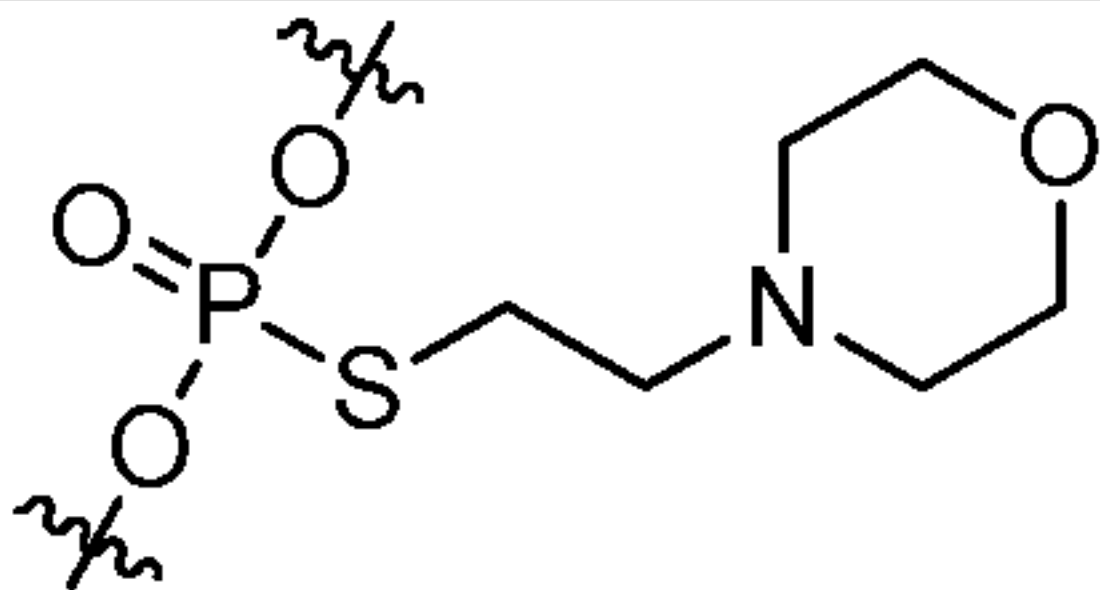
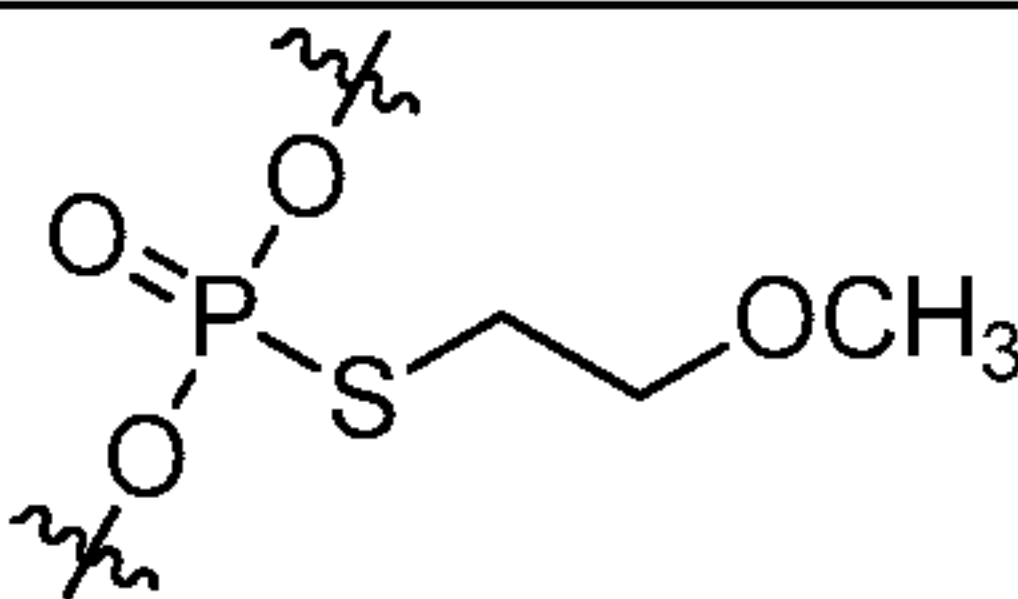
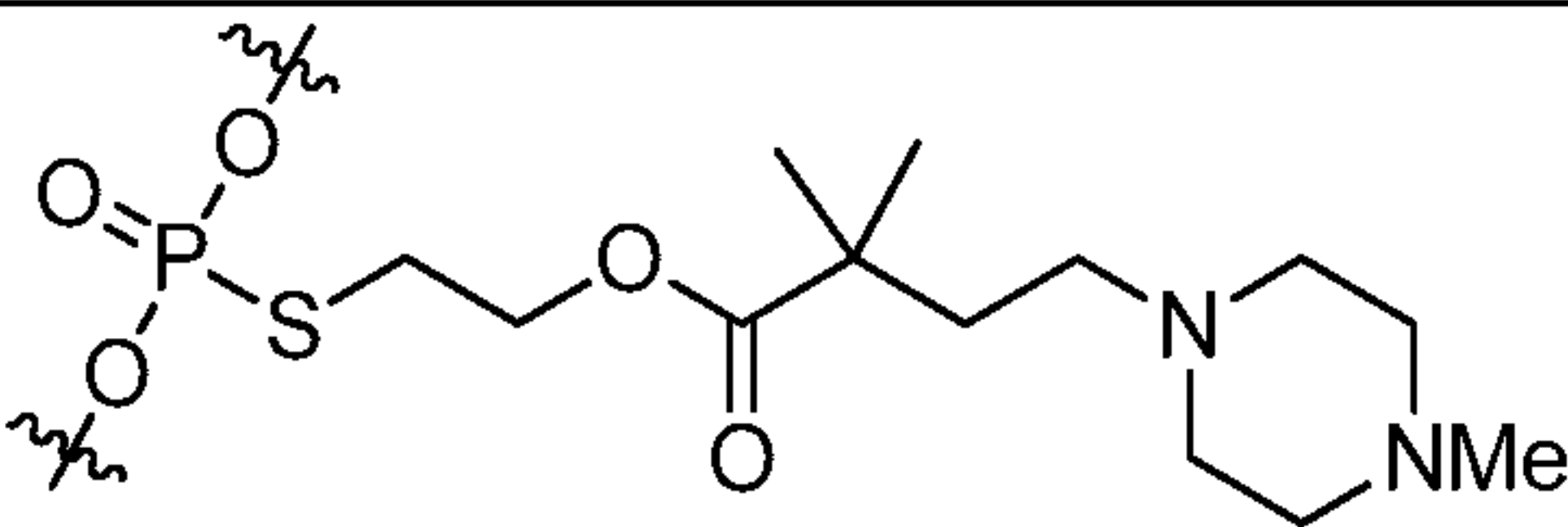
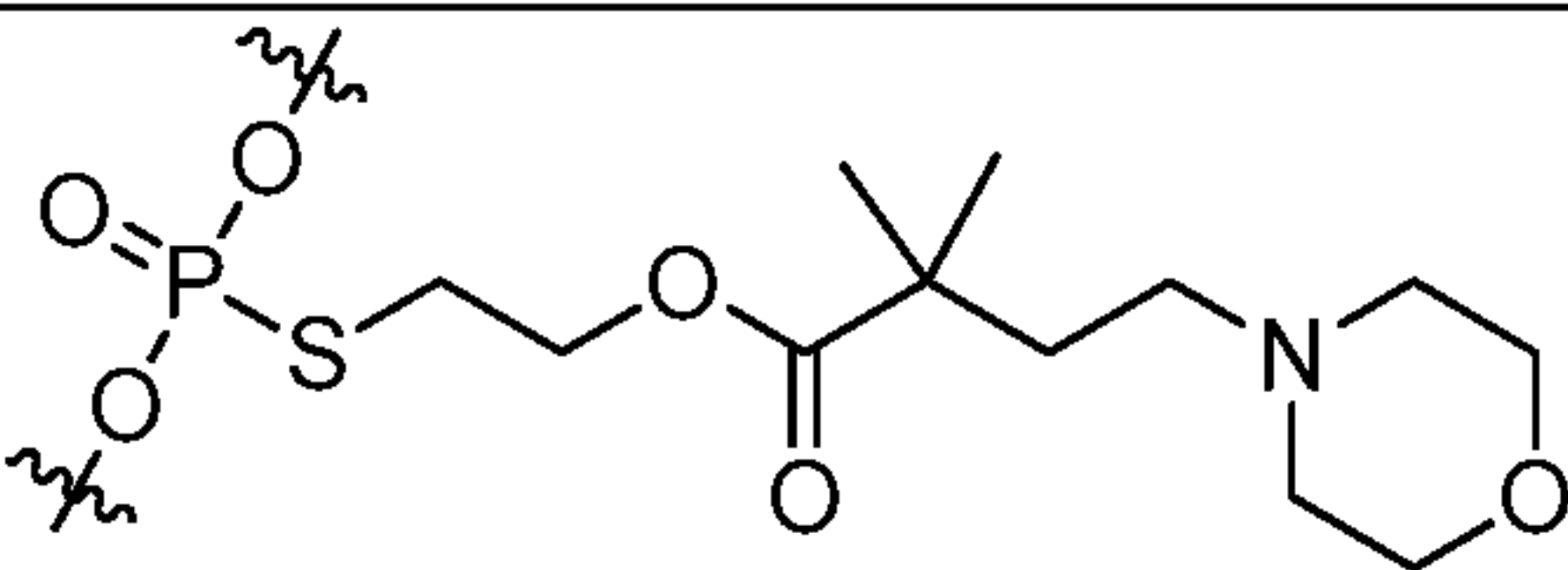
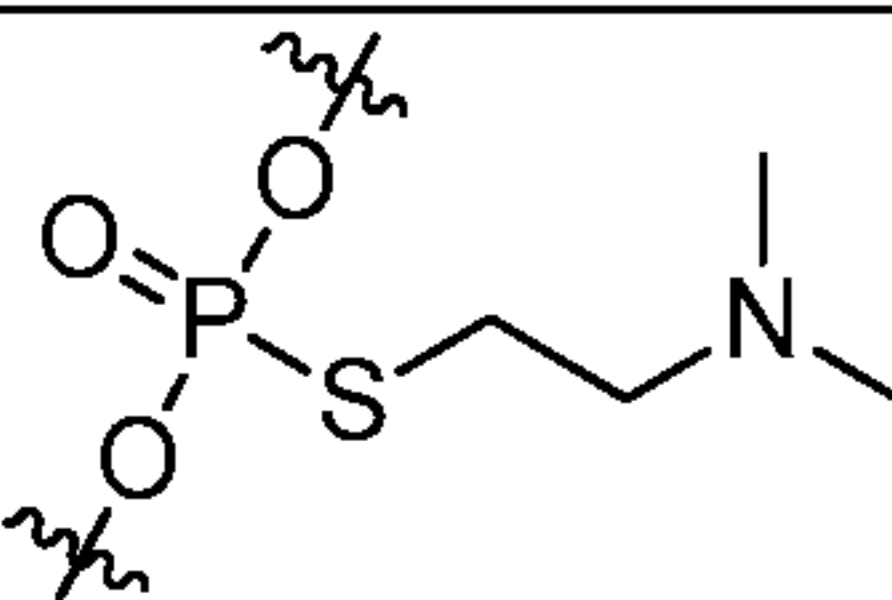
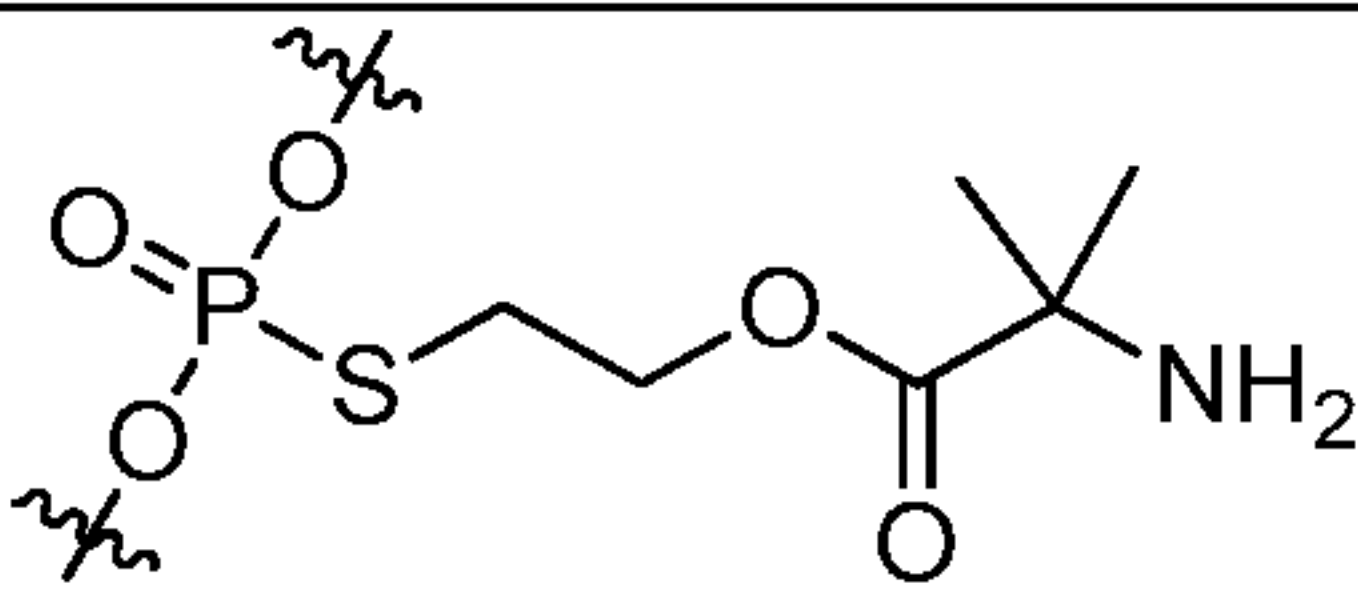
[00234] Oligonucleotides of the present disclosure can be of various lengths. In particular embodiments, oligonucleotides can range from about 2 to about 200 nucleotides in length. In various related embodiments, oligonucleotides, single-stranded, double-stranded, and triple-stranded, can range in length 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.

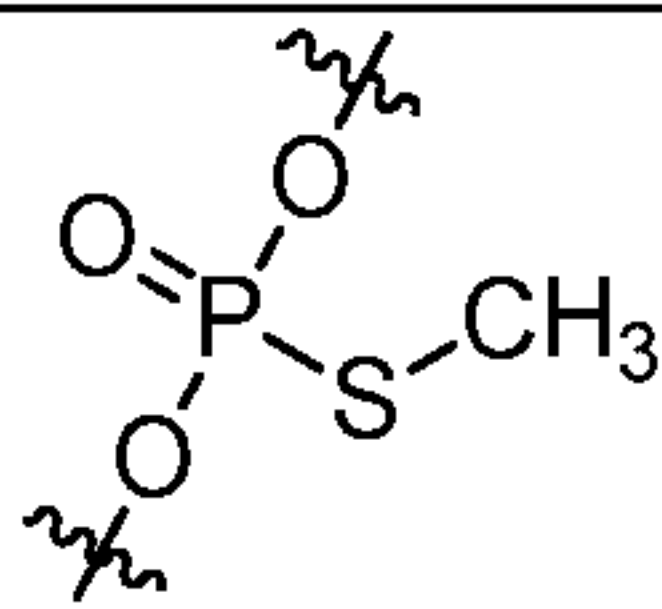
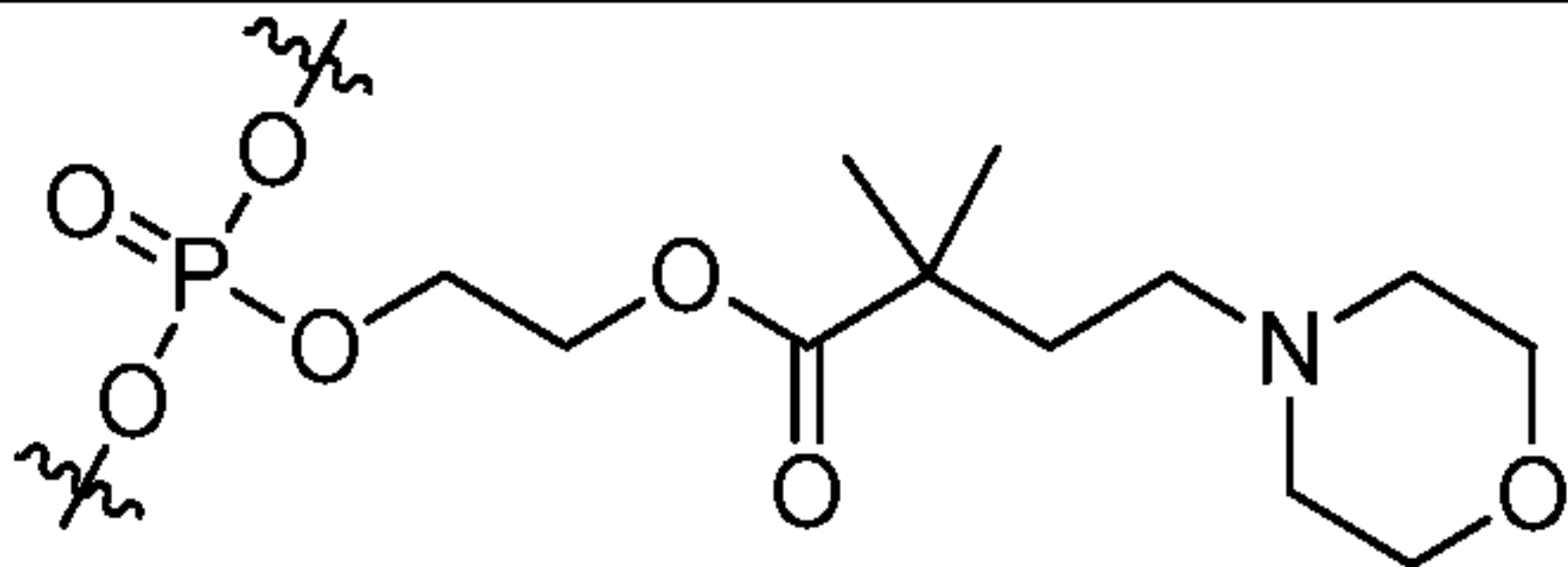
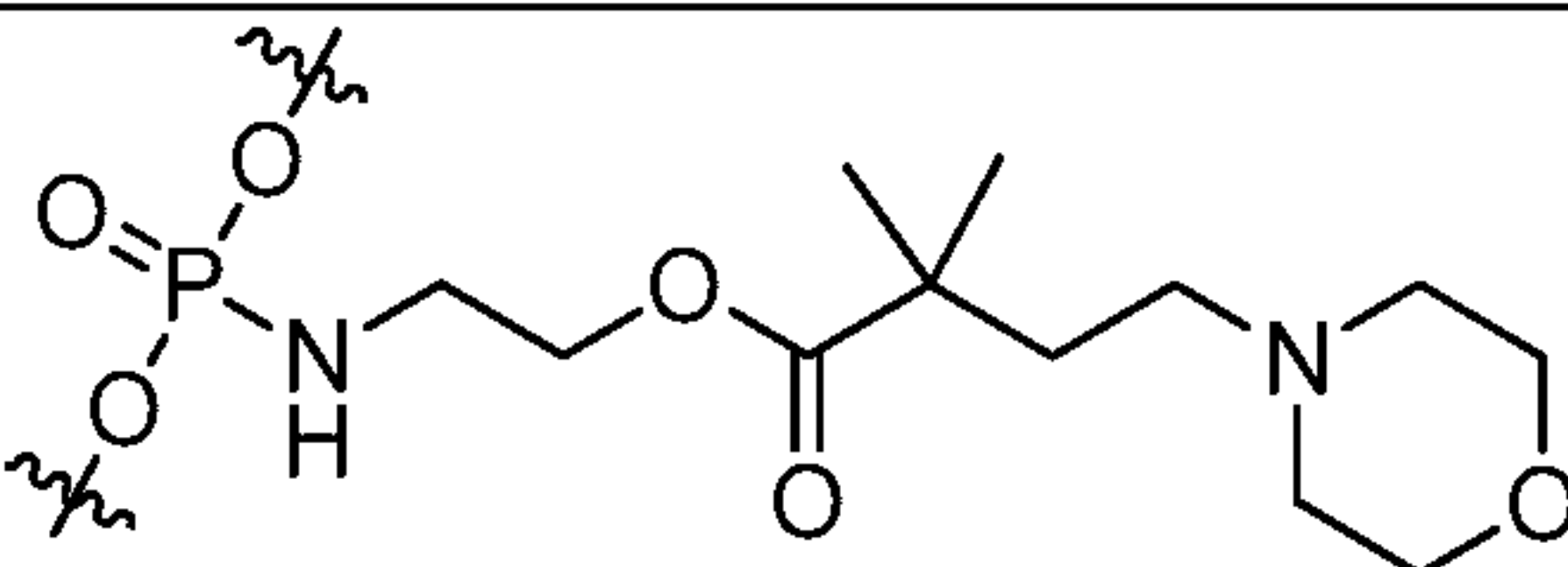
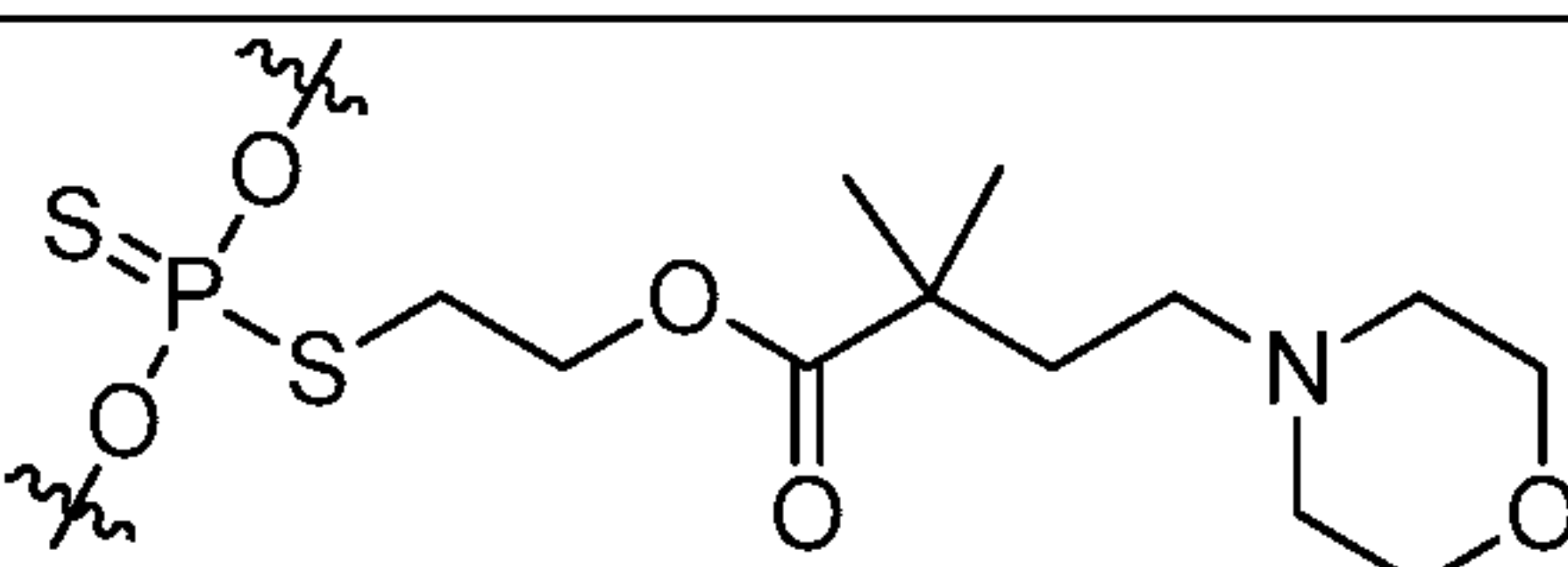
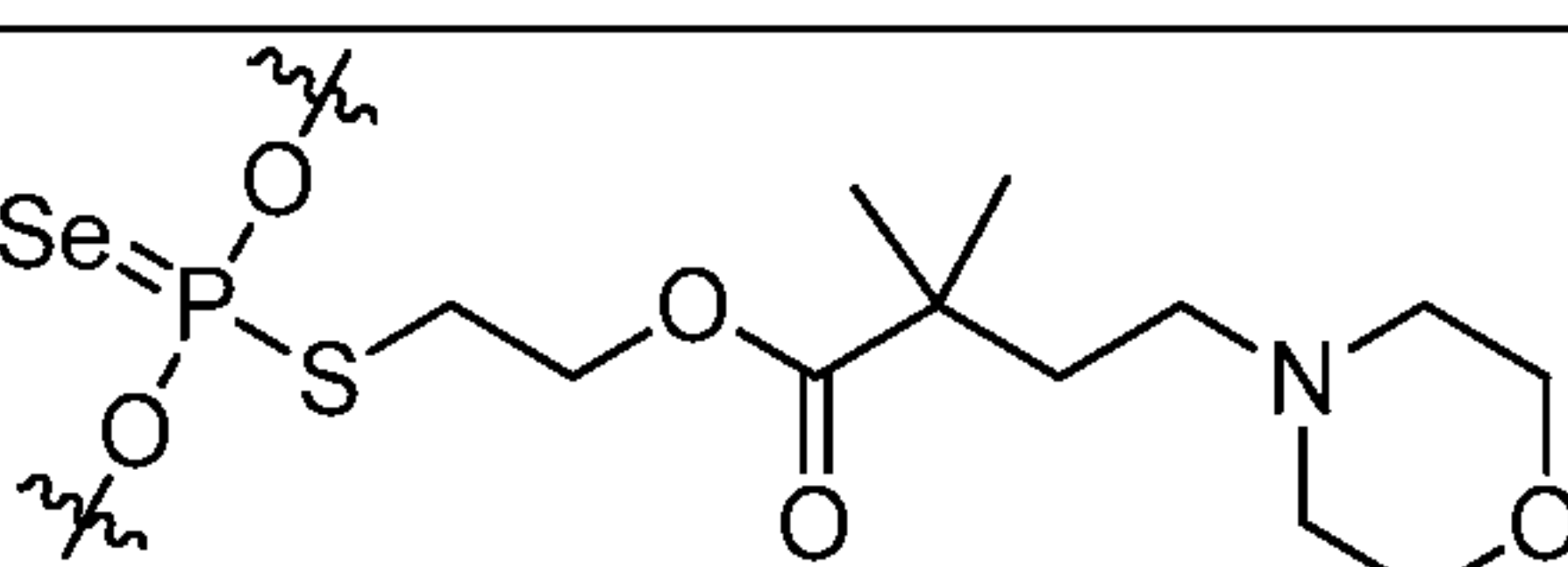
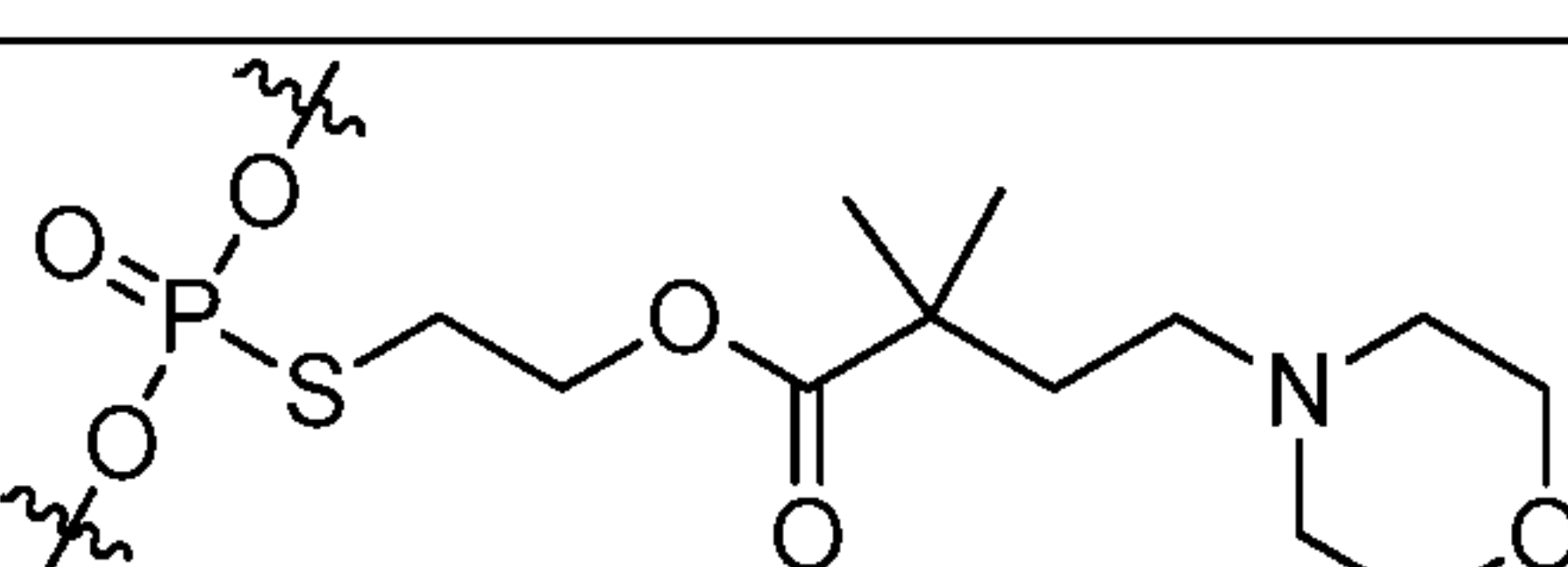
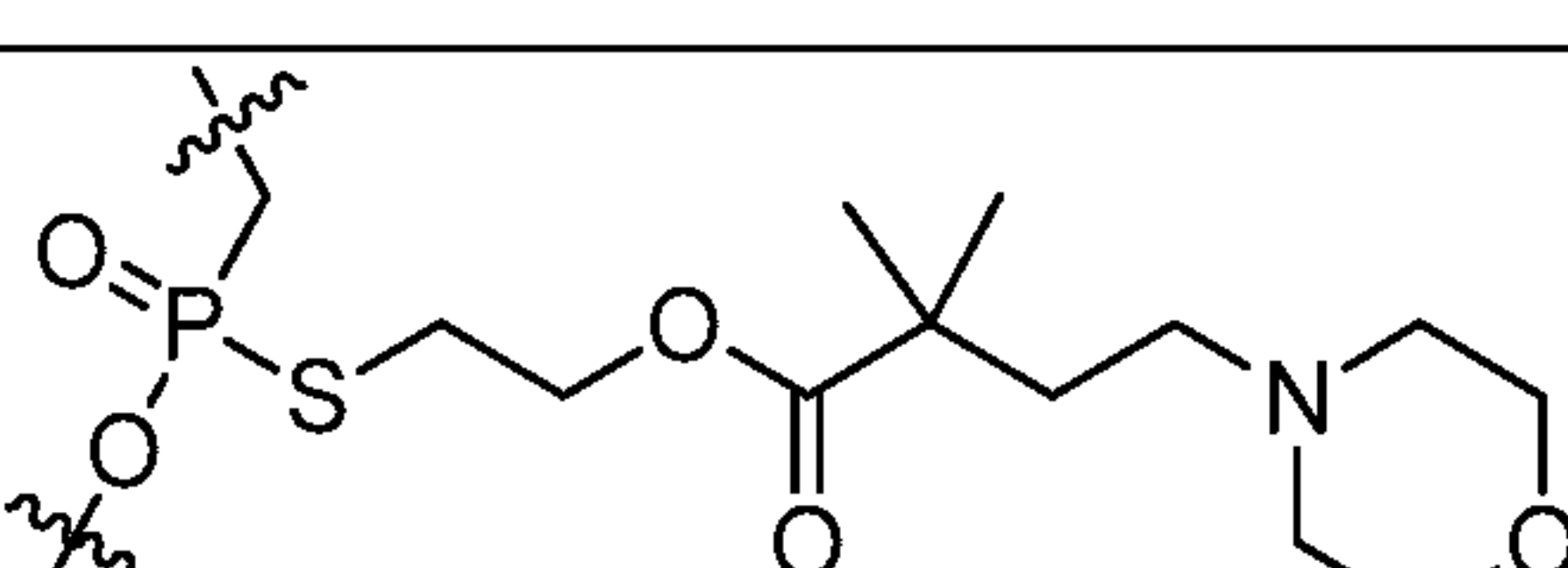
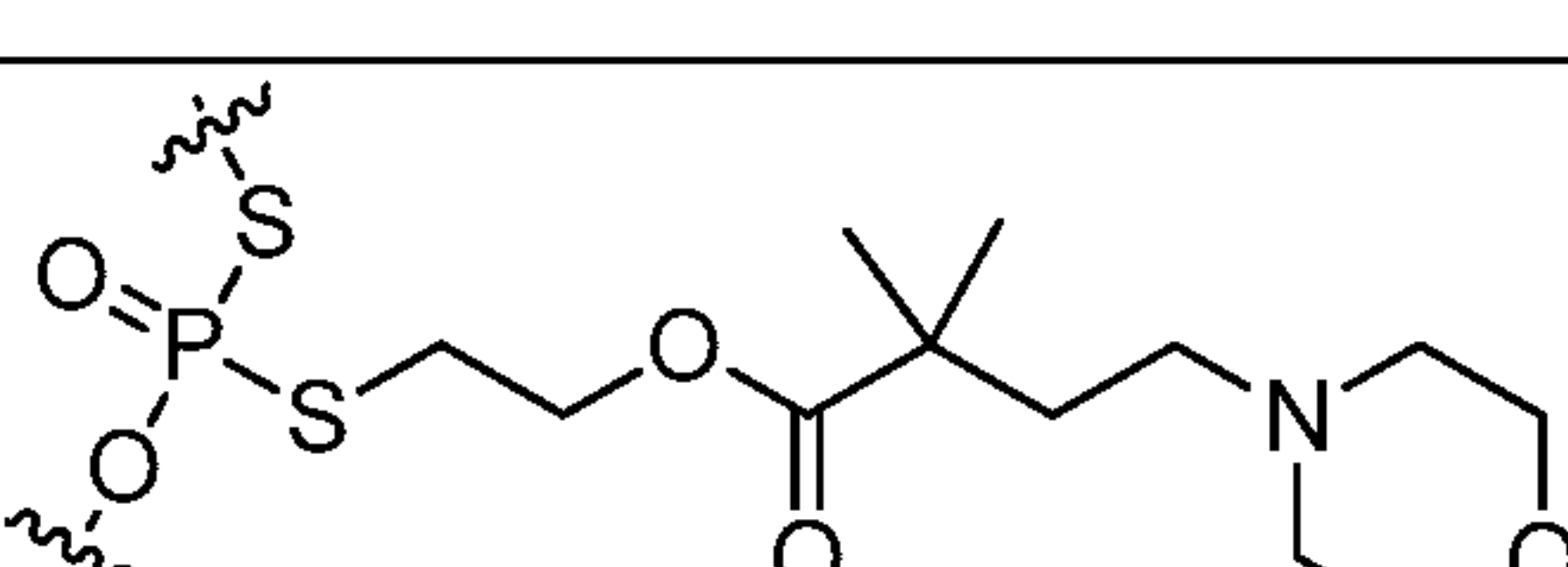
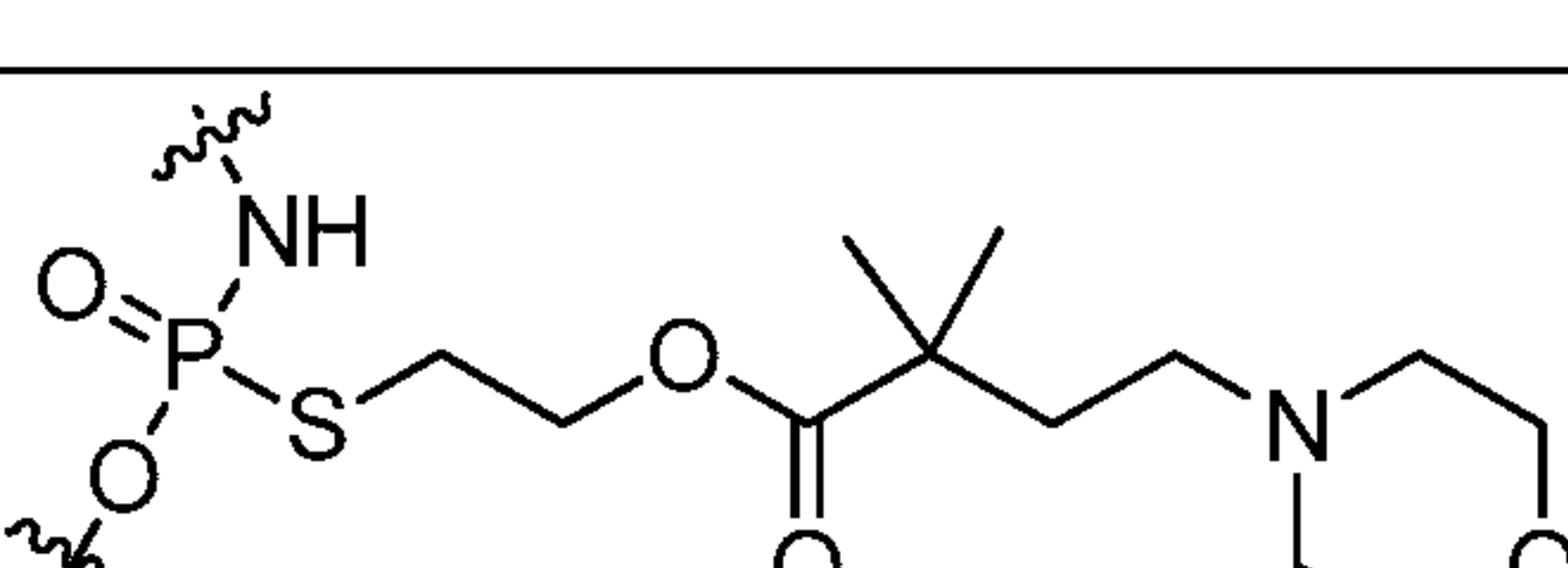
[00235] *Internucleotidic linkage:* As used herein, the phrase “internucleotidic linkage” refers generally to the phosphorus-containing linkage between nucleotide units of an oligonucleotide, and is interchangeable with “inter-sugar linkage” and “phosphorus atom bridge,” as used above and herein. In 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.

[00236] 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 below:

Symbol	Modified Internucleotidic Linkage
s	
s1	
s2	
s3	
s4	
s5	
s6	

s7	
s8	
s9	
s10	
s11	
s12	
s13	
s14	
s15	

s16	
s17	
s18	

[00237] For instance, (Rp, Sp)-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 Rp/Sp 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 (Rp, Sp)-ATsCs1GA, the phosphorus in the “s” linkage between T and C has Rp configuration and the phosphorus in “s1” linkage between C and G has Sp configuration. In some embodiments, “All-(Rp)” or “All-(Sp)” is used to indicate that all chiral linkage phosphorus atoms in oligonucleotide have the same Rp or Sp configuration, respectively. For instance, All-(Rp)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC indicates that all the chiral linkage phosphorus atoms in the oligonucleotide have Rp configuration; All-(Sp)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC indicates that all the chiral linkage phosphorus atoms in the oligonucleotide have Sp configuration.

[00238] *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 (*Rp/Sp*)), and pattern of backbone phosphorus modifications (e.g., pattern of “-XLR¹” groups in formula I). Oligonucleotides of a common designated “type” are structurally identical to one another.

[00239] 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.

[00240] *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.

[00241] *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.

[00242] *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.

[00243] *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.

[00244] *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).

[00245] *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.

[00246] *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.

[00247] *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”.

[00248] In some embodiments, a blockmer is a “stereoblockmer,” *e.g.*, at least two consecutive nucleotide units have the same stereochemistry at the linkage phosphorus. Such at least two consecutive nucleotide units form a “stereoblock.” For instance, (Sp, Sp)-ATsCs1GA is a stereoblockmer because at least two consecutive nucleotide units, the Ts and the Cs1, have the same stereochemistry at the linkage phosphorus (both Sp). In the same oligonucleotide (Sp, Sp)-ATsCs1GA, TsCs1 forms a block, and it is a stereoblock.

[00249] In some embodiments, a blockmer is a “P-modification blockmer,” *e.g.*, at least two consecutive nucleotide units have the same modification at the linkage phosphorus. Such at least two consecutive nucleotide units form a “P-modification block”. For instance, (Rp, Sp)-ATsCsGA is a P-modification blockmer because at least two consecutive nucleotide units, the Ts and the Cs, have the same P-modification (*i.e.*, both are a phosphorothioate diester). In the same oligonucleotide of (Rp, Sp)-ATsCsGA, TsCs forms a block, and it is a P-modification block.

[00250] In some embodiments, a blockmer is a “linkage blockmer,” *e.g.*, at least two consecutive nucleotide units have identical stereochemistry and identical modifications at the linkage phosphorus. At least two consecutive nucleotide units form a “linkage block”. For instance, (Rp, Rp)-ATsCsGA is a linkage blockmer because at least two consecutive nucleotide units, the Ts and the Cs, have the same stereochemistry (both Rp) and P-modification (both phosphorothioate). In the same oligonucleotide of (Rp, Rp)-ATsCsGA, TsCs forms a block, and it is a linkage block.

[00251] 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, (Rp, Rp, Rp, Rp, Rp, Sp, Sp, Sp)–AAsTsCsGsAs1Ts1Cs1Gs1ATCG is a stereoblockmer with respect to the stereoblock AsTsCsGsAs1 (all Rp at linkage phosphorus) or Ts1Cs1Gs1 (all Sp 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 Rp at linkage phosphorus and all s linkage) or Ts1Cs1Gs1 (all Sp at linkage phosphorus and all s1 linkage).

[00252] *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.

[00253] 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, (Rp, Sp, Rp, Sp, Rp, Sp, Rp, Sp, Rp, Sp Rp, Sp, Rp, Sp, Rp, Sp, Rp, Sp, Rp, Sp, Rp)–GsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC.

[00254] 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-(Sp)-CAs1GsT, in which each linkage phosphorus has a different P-modification than the others.

[00255] 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, (Rp, Sp, Rp, Sp, Rp, Sp, Rp, Sp, Rp, Sp Rp, Sp, Rp, Sp, Rp, Sp, Rp, Sp, Rp, Sp, Rp)–GsCs1CsTs1CsAs1GsTs1CsTs1GsCs1TsTs2CsGs3CsAs4CsC.

[00256] *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.

[00257] In some embodiments, a unimer is a “stereounimer,” *e.g.*, all nucleotide units have the same stereochemistry at the linkage phosphorus. For instance, All-(Sp)-CsAs1GsT, in which all the linkages have *Sp* phosphorus.

[00258] 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, (*Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*, *Rp*, *Sp*)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC, in which all the internucleotidic linkages are phosphorothioate diester.

[00259] 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-(*Sp*)-GsCsCsTsCsAsGsTsCsTsGsCsTsTsCsGsCsAsCsC, in which all the internucleotidic linkages are phosphorothioate diester having *Sp* linkage phosphorus.

[00260] *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-(Sp)-CAs1GsT, in which the internucleotidic linkage between C and A is a phosphate diester linkage.

[00261] *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-(Sp)-AsTCs1GAs2TCs3G.

[00262] 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.

[00263] 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

[00264] *Figure 1.* Figure 1 showed relative activity of oligonucleotides inducing exon 51 skipping in human DMD patient derived fibroblasts that bear $\Delta 48-50$ deletion (DMD cells with del48-50). The result was normalized to randomer WV-942. Taqman assay employed specifically detects DMD transcript with exon 51 skipped.

[00265] *Figure 2.* Figure 2 showed relative activity of oligonucleotides inducing exon 51 skipping in human DMD patient derived fibroblasts that bear $\Delta 48-50$ deletion. The result was normalized to untreated control. Taqman assay employed specifically detects DMD transcript with exon 51 skipped.

[00266] *Figures 3A to 3F.* Figures 3A to 3F showed expression of muscle differentiation markers relative to untreated controls in differentiated human DMD fibroblasts with or without oligonucleotide treatments. Taqman assays specific for DMD, DES, MYOD1, MYOG, MYH1 and RYR1 were used.

[00267] *Figure 4.* Example time course of C3a complement activation. Oligonucleotide concentration: 330 $\mu\text{g/mL}$; 37 $^{\circ}\text{C}$.

[00268] *Figure 5.* Example time course of Bb complement activation. Oligonucleotide concentration: 330 $\mu\text{g/mL}$; 37 $^{\circ}\text{C}$.

[00269] *Figure 6.* Example albumin binding.

[00270] *Figures 7A and 7B.* Sequences and the chemistry of various oligonucleotides: WV395 and WV884 to WV897. The suffices .01 and .02 indicate batch numbers. These include stereopure (chirally pure) oligonucleotides or oligonucleotide compositions, including 2'-OMe modifications.

[00271] *Figures 8A and 8B.* Ability of various oligonucleotides to induce skipping of exon 51 of human dystrophin. 8B is a compilation of data, including three or more replicates. Controls: WV-942, WV-1714, and untreated; Concentration: 10 μM ; Duration: 4 days in differentiation medium; treatment was gymnotic (without transfection reagent); Cells: Del 48-50 [Primary human myoblasts from a patient with (dystrophin deletion exon 48–50), DL 589.2 (dystrophin deletion exon 51–55)].

[00272] *Figures 9A and 9B.* Compositions of PS (phosphorothioates) and 2'-F on the

wings of various oligonucleotides, including WV-2095 to WV-2109. WV-2106 to WV-2109 are hemimers.

[00273] *Figures 10A and 10B.* Ability of various oligonucleotides to induce skipping of exon 51 of dystrophin. Figure 10B shows additional data for WV-1714. WV-1683, a negative control in this experiment, targets mouse exon 23.

[00274] *Figures 11A and 11B.* Sequence and chemistry of various oligonucleotides, WV-1108 and WV-2381 to WV-2395. These have PS (phosphorothioates) in the wings and PO (phosphodiester) in the core.

[00275] *Figure 12.* Ability of various oligonucleotides to induce skipping of exon 51 of dystrophin. Controls: WV-942 (Drisapersen, stereorandom) and untreated; Concentration: 10uM; Duration: 4 days in differentiation medium; Cells: Del 48-50; treatment was gymnotic (without transfection reagent).

[00276] *Figures 13A and 13B.* Sequences and chemistry of various oligonucleotides, WV-2366 to WV-2370. These have phosphorothioates in the Sp conformation in the wings and PO (phosphodiester) in the core.

[00277] *Figure 14.* Ability of various oligonucleotides to induce skipping of exon 51 of dystrophin. Controls: WV-942 and untreated; Concentration: 10uM; Duration: 4 days in differentiation medium; Cells: Del 48-50; treatment was gymnotic (without transfection reagent).

[00278] *Figure 15.* Sequences and chemistry of various oligonucleotides, which are 20-mers or 25-mers, including WV-2313 to WV-2320, and WV-2223 to WV-2230.

[00279] *Figure 16.* Location of the sequences of various oligonucleotides, which are 20-mers or 25-mers, including WV-2313 to WV-2320, and WV-2223 to WV-2230, relative to the human (H) and mouse (M) dystrophin sequences.

[00280] *Figure 17.* Ability of various oligonucleotides to induce skipping of exon 51 of dystrophin. Controls: WV-942 and untreated; Concentration: 10uM; Duration: 4 days in differentiation medium; Cells: Del 48-50; treatment was gymnotic (without transfection reagent).

[00281] *Figure 18.* Exon skipping mediated by an active compound, oligonucleotide WV-942, delivered via gymnotic delivery (not conjugated to a lipid), or conjugated to a lipid (listed in Table 5).

[00282] *Figure 19.* In vivo pharmacokinetic (PK) data related to delivery of oligonucleotide WV-942 delivered via gymnotic delivery (not conjugated to a lipid), or

conjugated to a lipid, to gastrocnemius, heart and quadriceps muscle tissues. Tested articles are listed in Table 4. From left to right: WV-942, WV-2588, WV-2581, WV-2582, WV-2584, WV-2585, WV-2586 and WV-2587.

[00283] *Figure 20.* In vivo pharmacokinetic (PK) data related to delivery of WV-942 delivered via gymnotic delivery (not conjugated to a lipid), or conjugated to a lipid, to gastrocnemius, heart and quadriceps and diaphragm muscle tissues. From left to right: WV-942, WV-2588, WV-2581, WV-2582, WV-2584, WV-2585, WV-2586 and WV-2587.

[00284] *Figure 21.* Standard curves for lipid conjugates in different tissues (quadriceps and diaphragm).

[00285] *Figure 22.* Standard curves for lipid conjugates in different tissues (heart and gastrocnemius).

[00286] *Figure 23.* Example structures of lipids and linkers for conjugation to an active compound. Abbreviation: Oligo: an example oligonucleotide.

[00287] *Figure 24.* Figure 24 shows the efficacy of stereopure oligonucleotides with 2'-F wings and either PO or Rp cores, in skipping exon 51 of human dystrophin, compared to WV-942 (Drisapersen). Treatment was 10 μ M, gymnotic treatment.

[00288] *Figure 25.* Figure 25 shows the efficacy of stereopure oligonucleotides in skipping exon 51 of human dystrophin, compared to WV-942. Data for two different doses, 3 μ M and 10 μ M, are presented. On the bottom left are stereorandomers with different patterns of 2'-F and 2'-OMe modifications. On the bottom right are stereopure oligonucleotides.

[00289] *Figure 26.* Figure 26 shows the efficacy of various oligonucleotides; shown are fold-changes compared to WV-942. Data for two different doses, 3 μ M and 10 μ M, are presented.

[00290] *Figures 27A to 27O.* Figures 27A to 27O shows liquid chromatograph and mass spectra data for oligonucleotides: WV887, WV892, WV896, WV1714, WV2444, WV2445, WV2526, WV2527, WV2528, and WV2530. The suffices (01), (02), .01 and .02 indicate batch numbers.

[00291] *Figure 28.* Figure 28 shows example skipping efficiency of certain provided chirally controlled oligonucleotide compositions in skipping exon 51 of human dystrophin, compared to WV-942. Data for three different doses, 1 μ M, 3 μ M and 10 μ M, are presented. Skipping efficiency generally increases with increased concentration. Treatment was gymnotic

(without transfection reagent).

[00292] *Figure 29.* Figure 29 shows example skipping efficiency of certain provided chirally controlled oligonucleotide compositions in skipping exon 51 of human dystrophin, compared to WV-942. Data for different doses from 0.3 μ M to 30 μ M, are presented. Skipping efficiency generally increases with increased concentration. WV-3545 (WV-3473 conjugated to stearic acid by PO and C6 amino linker) and WV-3546 (WV-3473 conjugated to turbinaric acid by PO and C6 amino linker), both containing oligonucleotide-lipid conjugates, demonstrated higher efficiency. Treatment was gymnotic (without transfection reagent). The experiment was done in triplicate, with average data shown.

[00293] *Figure 30.* Figure 30 shows that several example provided oligonucleotides do not have hTLR9 agonist activity under the tested conditions. The experiment was done in triplicate, with average data shown.

[00294] *Figure 31.* Figure 31 shows that various provided oligonucleotides can counteract the hTLR9 agonistic activity of oligonucleotide ODN2006 (and to antagonize hTLR9). As demonstrated, conjugates of lipids (*e.g.*, stearic acid (WV-3545) or turbinaric acid (WV-3546)) and oligonucleotides (*e.g.*, WV-3473 (WV-3545 and WV-3546)) have significantly increased hTLR9 antagonistic activities. The concentration of agonistic oligonucleotide ODN2006 was held constant at 0.3 μ M. Each oligonucleotide was tested at decreasing concentrations of: 5, 2.5, 1.25, 0.6, 0.3, 0.15 and 0.075 μ M (from left to right). Treatment was gymnotic (without transfection reagent). The experiment was done in triplicate, with average data shown.

[00295] *Figure 32.* Figure 32 shows that various oligonucleotides can counteract the hTLR9 agonistic activity of oligonucleotide ODN2006 (and to antagonize hTLR9). As demonstrated, conjugates of lipids (*e.g.*, stearic acid (WV-3545) or turbinaric acid (WV-3546)) and oligonucleotides (*e.g.*, WV-3473 (WV-3545 and WV-3546)) have significantly increased hTLR9 antagonistic activities. neg: negative control (buffer only). ODN2006c: an agonistic control in which the CpG sequence is replaced by GpC. PMO: Eteplirsen. The concentration of agonistic oligonucleotide ODN2006 was held constant at 0.3 μ M. Each oligonucleotide was tested at decreasing concentrations of: 5, 2.5, 1.25, 0.6, 0.3, 0.15 and 0.075 μ M (from left to right). Treatment was gymnotic (without transfection reagent). The experiment was done in triplicate, with average data shown.

[00296] *Figure 33.* Figure 33 shows example skipping efficiency of certain provided

chirally controlled oligonucleotide compositions in skipping exon 51 of human dystrophin, compared to WV-942 and PMO (Eteplirsen). Skipping efficiency generally increases with increased concentration. Data for different doses from 0.1 μM to 10 μM are presented. DMD del48-50 cells were used. Treatment was gymnotic (without transfection reagent).

[00297] *Figure 34.* Figure 34 shows example skipping efficiency of certain provided chirally controlled oligonucleotide compositions in skipping exon 51 of human dystrophin, compared to WV-942 and PMO (Eteplirsen). Skipping efficiency generally increases with increased concentration. Data for three different doses, 1 μM , 3 μM and 10 μM , are presented. DMD del48-50 cells were used. Treatment was gymnotic (without transfection reagent). neg: negative control.

[00298] *Figure 35.* Figure 35 shows example skipping efficiency of certain provided chirally controlled oligonucleotide compositions in skipping exon 51 of human dystrophin. Skipping efficiency generally increases with increased concentration. Data for four different doses, 1 μM , 3 μM , 10 μM and 10 μM are presented. DMD del48-50 cells were used. Treatment was gymnotic (without transfection reagent).

[00299] *Figure 36.* Figure 36 shows example skipping efficiency of certain provided oligonucleotide compositions in skipping exon 51 of human dystrophin, compared to WV-942. Data for a dose of 10 μM are presented. Treatment was gymnotic (without transfection reagent). DMD del48-50 cells were used.

[00300] *Figure 37.* Figure 37 shows example skipping efficiency of certain provided oligonucleotide compositions in skipping exon 51 of human dystrophin, compared to WV-942. Data for two doses, 3 μM (right column) and 10 μM (left column), are presented. Treatment was gymnotic (without transfection reagent). Under tested conditions, lipid conjugation increased skipping efficiency.

[00301] *Figures 38A to 38D.* Figures 38A to 38D show example distribution of oligonucleotides in various muscle tissues: gastrocnemius (Figure 38A); triceps (Figure 38B); heart (Figure 38C); and diaphragm (Figure 38D).

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[00302] Synthetic oligonucleotides provide useful molecular tools in a wide variety of applications. For example, oligonucleotides are useful in therapeutic, diagnostic, research, and

new nanomaterials applications. The use of naturally occurring nucleic acids (*e.g.*, unmodified DNA or RNA) is limited, for example, by their susceptibility to endo- and exo-nucleases. As such, various synthetic counterparts have been developed to circumvent these shortcomings. These include synthetic oligonucleotides that contain chemical modification, *e.g.*, base modifications, sugar modifications, backbone modifications, *etc.*, which, among other things, render these molecules less susceptible to degradation and improve other properties of oligonucleotides. Chemical modifications may also lead to certain undesired effects, such as increased toxicities, *etc.* From a structural point of view, modifications to internucleotide phosphate linkages introduce chirality, and 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).

[00303] 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, toxicities, *e.g.*, as may be mediated by protein binding characteristics, stability, splicing-altering capabilities, *etc.* In some embodiments, oligonucleotide properties can be adjusted by optimizing chemical modifications (modifications of base, sugar, and/or internucleotidic linkage) and/or stereochemistry (pattern of backbone chiral centers).

[00304] 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, provided compositions comprising oligonucleotides having chemical modifications (*e.g.*, base modifications, sugar modification, internucleotidic linkage modifications, *etc.*) have improved properties, such as improved splicing-altering capabilities, lower toxicity, or improved protein binding profile, and/or improved delivery, *etc.* Particularly, in some embodiments, the present

disclosure provides compositions and methods for altering splicing of transcripts. In some embodiments, the present disclosure provides compositions and methods for improving splicing of transcripts. In some embodiments, altered transcript splicing by provided compositions and methods include production of products having desired and/or improved biological functions, and/or knockdown of undesired product by, *e.g.*, modifying splicing products so that undesired biological functions can be suppressed or removed.

[00305] In some embodiments, a transcript is pre-mRNA. In some embodiments, a splicing product is mature RNA. In some embodiments, a splicing product is mRNA. In some embodiments, alteration comprises skipping one or more exons. In some embodiments, splicing of a transcript is improved in that exon skipping increases levels of mRNA and proteins that have improved beneficial activities compared with absence of exon skipping. In some embodiments, an exon causing frameshift is skipped. In some embodiments, an exon comprising an undesired mutation is skipped. In some embodiments, an exon comprising a premature termination codon is skipped. An undesired mutation can be a mutation causing changes in protein sequences; it can also be a silent mutation. In some embodiments, an exon comprising an undesired SNP is skipped.

[00306] In some embodiments, splicing of a transcript is improved in that exon skipping lowers levels of mRNA and proteins that have undesired activities compared with absence of exon skipping. In some embodiments, a target is knocked down through exon skipping which, by skipping one or more exons, causes premature stop codon and/or frameshift mutations.

[00307] Reading frame correction is achieved by skipping one or two exons flanking a deletion, by skipping in-frame exons containing a nonsense mutation, or by skipping duplicated exons.

[00308] In some embodiments, the present disclosure provides compositions and methods for reducing certain undesired repeats, such as CAG repeat (see, *e.g.*, Evers, *et al.*, Targeting several CAG expansion diseases by a single antisense oligonucleotide, PLoS One. 2011;6(9):e24308. doi: 10.1371/journal.pone.0024308; Mulders, *et al.*, Triplet-repeat oligonucleotide-mediated reversal of RNA toxicity in myotonic dystrophy, Proc Natl Acad Sci U S A. 2009 Aug 18;106(33):13915-20; *etc.*) by altering splicing, *e.g.*, exon skipping. Example targets include but are not limited to HTT, ATXN3, DMPK, CNBP, AR, C9ORF72 (target for familial frontotemporal dementia and amyotrophic lateral sclerosis) and those listed below:

Disease	Sequence	Location	Parent of origin of expansion	Repeat number (normal)	Repeat number (pre-mutation)	Repeat number (disease)	Somatic instability
<i>Diseases with coding TNRs</i>							
DRPLA	CAG	<i>ATN1</i> (exon 5)	P	6–35	35–48	49–88	Yes
HD	CAG	<i>HTT</i> (exon 1)	P	6–29	29–37	38–180	Yes
OPMD	GCN	<i>PABPN1</i> (exon 1)	P and M	10	12–17	>11	None found in tissue tested (hypothalamus)
SCA1	CAG	<i>ATXN1</i> (exon 8)	P	6–39	40	41–83	Yes
SCA2	CAG	<i>ATXN2</i> (exon 1)	P	<31	31–32	32–200	Unknown
SCA3 (Machado–Joseph disease)	CAG	<i>ATXN3</i> (exon 8)	P	12–40	41–85	52–86	Unknown
SCA6	CAG	<i>CACNA1A</i> (exon 47)	P	<18	19	20–33	None found
SCA7	CAG	<i>ATXN7</i> (exon 3)	P	4–17	28–33	>36 to >460	Yes
SCA17	CAG	<i>TBP</i> (exon 3)	P > M	25–42	43–48	45–66	Yes
SMBA	CAG	<i>AR</i> (exon 1)	P	13–31	32–39	40	None found
<i>Diseases with non-coding TNRs</i>							
DM1	CTG	<i>DMPK</i> (3' UTR)	M	5–37	37–50	<50	Yes
DM2	CCTG	<i>CNBP</i> (intron 1)	Uncertain	<30	31–74	75–11,000	Yes
FRAX-E	GCC	<i>AFF2</i> (5' UTR)	M	4–39	40–200	>200	Unknown
FRDA	GAA	<i>FXN</i> (intron 1)	Recessive	5–30	31–100	70–1,000	Yes
FXS	CGG	<i>FMR1</i> (5' UTR)	M	6–50	55–200	200–4,000	Yes
HDL2	CTG	<i>JPH3</i> (exon 2A)	M	6–27	29–35	36–57	Unknown
SCA8	CTG	<i>ATXN8OS</i> (3' UTR)	M	15–34	34–89	89–250	Unknown
SCA10	ATTCT	<i>ATXN10</i> (intron 9)	M and P (smaller changes with M)	10–29	29–400	400–4,500	Yes
SCA12	CAG	<i>PPP2R2B</i> (5' UTR)	M and P (more unstable with P)	7–28	28–66	66–78	None found

AFF2, AF4/FMR2 family, member 2; *AR*, androgen receptor; *ATN1*, atrophin 1; *ATXN*, ataxin; *ATXN8OS*, *ATXN8* opposite strand (non-protein coding); *CACNA1A*, calcium channel, voltage-dependent, P/Q type, alpha 1A subunit; *CNBP*, CCHC-type zinc finger nucleic acid binding protein; *DM*, myotonic dystrophy; *DMPK*, dystrophin myotonia-protein kinase; *DRPLA*, dentatorubral-pallidoluysian atrophy; *FMR1*, fragile X mental retardation 1; *FRAX-E*, mental retardation, X-linked, associated with *FRAXE*; *FRDA*, Friedreich's ataxia; *FXN*, frataxin; *FXS*, fragile X syndrome; *FXTAS*, fragile X-associated tremor/ataxia syndrome; *HD*, Huntington's disease; *HDL2*, Huntington's disease-like 2; *HTT*, huntingtin; *JPH3*, junctophilin 3; *M*, maternal; *OPMD*, oculopharyngeal muscular dystrophy; *P*, paternal; *PABPN1*, poly(A) binding protein nuclear 1; *PPP2R2B*, protein phosphatase 2, regulatory subunit B; *SCA*, spinocerebellar ataxia; *SMBA*, spinomuscular bulbar atrophy; *TBP*, TATA-box binding protein; *TNR*, trinucleotide repeat.

[00309] In some embodiments, provided oligonucleotides in provided compositions, *e.g.*, oligonucleotides of a first plurality, comprise base modifications, sugar modifications, and/or internucleotidic linkage modifications. In some embodiments, provided oligonucleotides comprise base modifications and sugar modifications. In some embodiments, provided oligonucleotides comprise base modifications and internucleotidic linkage modifications. In some embodiments, provided oligonucleotides comprise sugar modifications and internucleotidic modifications. In some embodiments, provided compositions comprise base modifications, sugar modifications, and internucleotidic linkage modifications. Example chemical modifications, such as base modifications, sugar modifications, internucleotidic linkage modifications, *etc.* are widely known in the art including but not limited to those described in this disclosure. In some embodiments, a modified base is substituted A, T, C, G or U. In some embodiments, a sugar modification is 2'-modification. In some embodiments, a 2'-modification is 2-F modification. In some embodiments, a 2'-modification is 2'-OR¹. In some embodiments, a 2'-modification is 2'-OR¹, wherein R¹ is optionally substituted alkyl. In some embodiments, a 2'-modification is 2'-OMe. In some embodiments, a 2'-modification is 2'-MOE. In some embodiments, a modified sugar moiety is a bridged bicyclic or polycyclic ring. In some embodiments, a modified sugar moiety is a bridged bicyclic or polycyclic ring having 5-20 ring atoms wherein one or more ring atoms are optionally and independently heteroatoms. Example ring structures are widely known in the art, such as those found in BNA, LNA, *etc.* In some embodiments, provided oligonucleotides comprise both one or more modified internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, oligonucleotides comprising both modified internucleotidic linkage and natural phosphate linkage and compositions thereof provide improved properties, *e.g.*, activities and toxicities, *etc.* In some

embodiments, a modified internucleotidic linkage is a chiral internucleotidic linkage. In some embodiments, a modified internucleotidic linkage is a phosphorothioate linkage. In some embodiments, a modified internucleotidic linkage is a substituted phosphorothioate linkage.

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.*, activities, toxicities, *etc.* 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. In some embodiments, the present disclosure provide an oligonucleotide composition comprising 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. 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. In some embodiments, property 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.*]). Among other things, the present disclosure recognizes that, in some embodiments, properties (*e.g.*, activities, toxicities, *etc.*) 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. As exemplified by various examples in the present disclosure, provided chirally controlled oligonucleotide compositions can demonstrate improved properties, such as lower toxicity, improved protein binding profile, improved delivery, *etc.*

[00310] In some embodiments, provided oligonucleotides contain increased levels of one or more isotopes. In some embodiments, provided oligonucleotides are labeled, *e.g.*, by one or more isotopes of one or more elements, *e.g.*, hydrogen, carbon, nitrogen, *etc.* In some embodiments, provided oligonucleotides in provided compositions, *e.g.*, oligonucleotides of a first plurality, comprise base modifications, sugar modifications, and/or internucleotidic linkage modifications, wherein the oligonucleotides contain an enriched level of deuterium. In some embodiments, provided oligonucleotides are labeled with deuterium (replacing ^1H with ^2H) at one or more positions. In some embodiments, one or more ^1H of an oligonucleotide or any moiety conjugated to the oligonucleotide (*e.g.*, a targeting moiety, lipid, *etc.*) is substituted with ^2H . Such oligonucleotides can be used in any composition or method described herein.

[00311] In some embodiments, oligonucleotide properties can be adjusted by optimizing stereochemistry (pattern of backbone chiral centers) and chemical modifications (modifications of base, sugar, and/or internucleotidic linkage). Among other things, the present disclosure demonstrates that stereochemistry can further improve properties of oligonucleotides comprising chemical modifications. 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 (mG*SmGmCmAmC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SmAmCmUmU*SmC) comprises 2'-OMe modifications, phosphate and phosphorothioate linkages in its 5'- and 3'-

wing regions, and phosphorothioate linkages in its core regions.

[00312] In some embodiments, the present disclosure provides oligonucleotide compositions which, unexpectedly, greatly improve properties of oligonucleotides. In some embodiments, provided oligonucleotide compositions provides surprisingly low toxicity. In some embodiments, provided oligonucleotide compositions provides surprisingly improved protein binding profile. In some embodiments, provided oligonucleotide compositions provides surprisingly enhanced delivery. In some embodiments, certain property improvement, such as lower toxicity, improved protein binding profile, and/or enhanced delivery, *etc.*, are achieved without sacrificing other properties, *e.g.*, activities, specificity, *etc.*. In some embodiments, provided compositions provides lower toxicity, improved protein binding profile, and/or enhanced delivery, and improved activity, stability, and/or specificity (*e.g.*, target-specificity, cleavage site specificity, *etc.*). Example improved activities (*e.g.*, enhanced cleavage rates, increased target-specificity, cleavage site specificity, *etc.*) include but are not limited to those described in WO/2014/012081 and WO/2015/107425.

[00313] 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, a pattern of backbone chiral centers provides surprisingly low toxicity. In some embodiments, a pattern of backbone chiral centers provides surprisingly low immune response. In some embodiments, a pattern of backbone chiral centers provides surprisingly low complement activation. In some embodiments, a pattern of backbone chiral centers provides surprisingly low complement activation via the alternative pathway. In some embodiments, a pattern of backbone chiral centers provides surprisingly improved protein binding profile. In some embodiments, a pattern of backbone chiral centers provides surprisingly increased binding to certain proteins. In some embodiments, a pattern of backbone chiral centers provides surprisingly enhanced delivery. In some embodiments, a pattern of backbone chiral centers comprises or is $(Sp)_m(Rp)_n$, $(Rp)_n(Sp)_m$, $(Rp)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$. In some embodiments, a pattern of backbone chiral centers comprises or is $(Rp)_n(Sp)_m$, $(Rp)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein $m > 2$. In some embodiments, a pattern of backbone chiral centers comprises or is $(Rp)_n(Sp)_m$, $(Rp)_t(Rp)_n(Sp)_m$, or $(Sp)_t(Rp)_n(Sp)_m$, wherein n is 1, $t > 1$, and $m > 2$. In some embodiments, $m > 3$. In some

embodiments, $m > 4$. In some embodiments, a pattern of backbone chiral centers comprises one or more achiral natural phosphate linkages.

[00314] 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, $m > 4$.

[00315] In some embodiments, a pattern of backbone chiral centers comprises or is $(Rp)n(Sp)m$, $(Sp)t(Rp)n$, $(Np)t(Rp)n(Sp)m$, $(Sp)t(Sp)m$ or $(Sp)t(Rp)n(Sp)m$. In some embodiments, a pattern of backbone chiral centers comprises or is $(Rp)n(Sp)m$, $(Sp)t(Rp)n$, $(Np)t(Rp)n(Sp)m$, or $(Sp)t(Rp)n(Sp)m$, and the oligonucleotides comprises one or more 2'-modifications as described herein. In some embodiments, a pattern of backbone chiral centers comprises or is $(Rp)n(Sp)m$, $(Sp)t(Rp)n$, $(Np)t(Rp)n(Sp)m$, or $(Sp)t(Rp)n(Sp)m$, and the oligonucleotides comprises one or more 2'-F modifications as described herein. In some embodiments, a pattern of backbone chiral centers comprises or is $(Rp)n(Sp)m$, $(Sp)t(Rp)n$, $(Np)t(Rp)n(Sp)m$, or $(Sp)t(Rp)n(Sp)m$, and the oligonucleotides comprises one or more 2'-OR modifications as described herein. In some embodiments, a pattern of backbone chiral centers comprises or is $(Rp)n(Sp)m$. In some embodiments, a pattern of backbone chiral centers comprises or is $(Sp)t(Rp)n$. In some embodiments, a pattern of backbone chiral centers comprises or is $(Np)t(Rp)n(Sp)m$. In some embodiments, a pattern of backbone chiral centers comprises or is $(Sp)t(Sp)m$, optionally with n achiral phosphate diester internucleotidic linkages and/or stereorandom (non-chirally controlled) chiral internucleotidic linkages between the section having $(Sp)t$ and the section having $(Sp)m$. In some embodiments, there are n achiral

phosphate diester internucleotidic linkages in between. In some embodiments, there are n stereorandom chiral internucleotidic linkages in between. In some embodiments, a pattern of backbone chiral centers comprises or is $(Sp)t(Rp)n(Sp)m$. In some embodiments, each of t and m is independently greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In some embodiments, each of t and m is independently greater than 1. In some embodiments, each of t and m is independently greater than 2. In some embodiments, each of t and m is independently greater than 2. In some embodiments, each of t and m is independently greater than 2. In some embodiments, each of t and m is independently greater than 3. In some embodiments, each of t and m is independently greater than 4. In some embodiments, each of t and m is independently greater than 5. In some embodiments, each of t and m is independently greater than 6. In some embodiments, each of t and m is independently greater than 7. In some embodiments, each of t and m is independently greater than 8. In some embodiments, each of t and m is independently greater than 9. In some embodiments, each of t and m is independently greater than 10. In some embodiments, each of t and m is independently greater than 11. In some embodiments, each of t and m is independently greater than 12. In some embodiments, each of t and m is independently greater than 13. In some embodiments, each of t and m is independently greater than 14. In some embodiments, each of t and m is independently greater than 15. In some embodiments, t is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In some embodiments, t is greater than 1. In some embodiments, t is greater than 2. In some embodiments, t is greater than 2. In some embodiments, t is greater than 3. In some embodiments, t is greater than 4. In some embodiments, t is greater than 5. In some embodiments, t is greater than 6. In some embodiments, t is greater than 7. In some embodiments, t is greater than 8. In some embodiments, t is greater than 9. In some embodiments, t is greater than 10. In some embodiments, t is greater than 11. In some embodiments, t is greater than 12. In some embodiments, t is greater than 13. In some embodiments, t is greater than 14. In some embodiments, t is greater than 15. In some embodiments, t is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In some embodiments, t is 1. In some embodiments, t is 2. 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, m is greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In some embodiments, m is greater than 1. In some embodiments, m is greater than 2. In some embodiments, m is greater than 2. In some embodiments, m is greater than 3. In some embodiments, m is greater than 4. In some embodiments, m is greater than 5. In some embodiments, m is greater than 6. In some embodiments, m is greater than 7. In some embodiments, m is greater than 8. In some embodiments, m is greater than 9. In some embodiments, m is greater than 10. In some embodiments, m is greater than 11. In some embodiments, m is greater than 12. In some embodiments, m is greater than 13. In some embodiments, m is greater than 14. In some embodiments, m is greater than 15. In some embodiments, m is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In some embodiments, m is 1. In some embodiments, m is 2. 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, $t=m$. In some embodiments, n is greater than 1, 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 1. In some embodiments, n is greater than 2. In some embodiments, n is greater than 2. In some embodiments, n is greater than 3. In some embodiments, n is greater than 4. 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, n is 1, 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 1. In some embodiments, n is 2. 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. In some embodiments, n is 11. In some embodiments, n is 12. In some embodiments, n is 13. In some embodiments, n is 14. In some

[00316] In some embodiments, a common pattern of backbone chiral centers is comprises
 SSS, SSSS, SSSSS, SSSSSS, SSSSSSS, SOS, SSOSS, SSSOSSS, SSSSOSSSS, SSSSSOSSSSSS,
 SSSSSSOSSSSSS, SSSSSSSOSSSSSSSS, SSSSSSSSOSSSSSSSS, SSSSSSSSOSSSSSSSSSS,
 SOSOSOSOS, SSOSOSOSOSS, SSSOSOSOSOSSS, SSSSOSOSOSOSSSS,
 SSSSSOSOSOSOSSSSSS, SSSSSSOSOSOSOSSSSSS, SOSOSSOOS, SSOSOSSOOSS,
 SSSOSOSSOOSSS, SSSSOSOSSOOSSSS, SSSSSOSOSSOOSSSSS, SSSSSSOSSOOSSSSSS,
 SSSSSSOSSOOSSSSSSS, SOSOOSOOS, SSOSOOSOOSS, SSSOSOOSOOSSS,
 SSSSSOSOSOOSO, SSSOSOSSOOSOS, SSSSOSOSSOOSOSS, SSSSSOSOSSOOSOSSS,
 SSSSSSOSSOOSOSSSS, SOSOOSOOSO, SSOSOOSOOSOS, SSSOSOOSOOSOS,
 SSSSOSOSOOSOOS, SSSSSOSOSOOSOOS, SSSSSSOSOSOOSOOS, SSSSSSOOSOOS,
 SSSOSOSSOOS, SSSSOSOSSOOS, SSSSSOSOSSOOSS, SSSSSSOSSOOSSS,
 OSSSSSSOSOSSOOSSS, OOSSSSSSOSOSSOOS, OOSSSSSSOSOSSOOSS, OOSSSSSSOSOSSOOSSS,
 OOSSSSSSOSOSSOOSSSS, OOSSSSSSOSOSSOOSSSSS, OOSSSSSSOSOSSOOSSSSSS, and/or
 OOSSSSSSOSOSSOOSSSSSS, wherein O is a non-chiral internucleotidic linkage and S is an *Sp*
 chiral internucleotidic linkage. In some embodiments, the non-chiral center is a phosphodiester
 linkage. In some embodiments, the chiral center in a *Sp* configuration is a phosphorothioate
 linkage. In some embodiments, a common pattern of backbone chiral centers is selected from:
 SSS, SSSS, SSSSS, SSSSSS, SSSSSSS, SOS, SSOSS, SSSOSSS, SSSSOSSSS, SSSSSOSSSSSS,
 SSSSSSOSSSSSS, SSSSSSSOSSSSSSSS, SSSSSSSSOSSSSSSSS, SSSSSSSSOSSSSSSSSS,
 SOSOSOSOS, SSOSOSOSOSS, SSSOSOSOSOSSS, SSSSOSOSOSOSSSS,
 SSSSSOSOSOSOSSSSSS, SSSSSSOSOSOSOSSSSSS, SOSOSSOOS, SSOSOSSOOSS,
 SSSOSOSSOOSSS, SSSSOSOSSOOSSSS, SSSSSOSOSSOOSSSSS, SSSSSSOSSOOSSSSSS,
 SSSSSSOSSOOSSSSSSS, SOSOOSOOS, SSOSOOSOOSS, SSSOSOOSOOSSS,
 SSSSSOSOSOOSO, SSSOSOSSOOSOS, SSSSOSOSSOOSOSS, SSSSSOSOSSOOSOSSS,
 SSSSSSOSSOOSOSSSS, SOSOOSOOSO, SSOSOOSOOSOS, SSSOSOOSOOSOS,
 SSSSOSOSOOSOOS, SSSSSOSOSOOSOOS, SSSSSSOSOSOOSOOS, SSSSSSOOSOOS,
 SSSOSOSSOOS, SSSSOSOSSOOS, SSSSSOSOSSOOSS, SSSSSSOSSOOSSS,
 OSSSSSSOSOSSOOSSS, OOSSSSSSOSOSSOOS, OOSSSSSSOSOSSOOSS

OOSSSSSSOSOSSOOSSS, OOSSSSSSOSOSSOOSSS, OOSSSSSSOSOSSOOSSS, and OOSSSSSSOSOSSOOSSSSS, wherein O is a non-chiral internucleotidic linkage and S is an *Sp* chiral internucleotidic linkage. In some embodiments, the non-chiral center is a phosphodiester linkage. In some embodiments, the chiral center in a *Sp* configuration is a phosphorothioate linkage.

[00317] In some embodiments, the 5'-end region of provided oligonucleotides, *e.g.*, a 5'-wing, comprises a stereochemistry pattern of S, SS, SSS, SSSS, SSSSS, SSSSSS, or SSSSSS. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSS. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSS. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSSS. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSSSS. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSSSSS. In some embodiments, each S represents an *Sp* phosphorothioate internucleotidic linkage. In some embodiments, the 5'-end region of provided oligonucleotides, *e.g.*, a 5'-wing, comprises a stereochemistry pattern of S, SS, SSS, SSSS, SSSSS, SSSSSS, or SSSSSS, wherein the first S represents the first (the 5'-end) internucleotidic linkage of a provided oligonucleotide. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSS, wherein the first S represents the first internucleotidic linkage. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSS, wherein the first S represents the first internucleotidic linkage. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSSS, wherein the first S represents the first internucleotidic linkage. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSSSS, wherein the first S represents the first internucleotidic linkage. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSSSSS, wherein the first S represents the first internucleotidic linkage. In some embodiments, one or more nucleotidic units comprising an *Sp* internucleotidic linkage in the 5'-end region independently comprise -F. In some embodiments, each nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'-end region independently comprises -F. In some embodiments, one or more nucleotidic units comprising an *Sp* internucleotidic linkage in the 5'-end region independently comprise a sugar modification. In some embodiments, each nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'-end region independently

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

modification is 2'-OR¹. In some embodiments, a 2'-modification is 2'-F. In some embodiments, provided oligonucleotides comprise both a 5'-end region, *e.g.*, a 5'-wing, and a 3'-end region, *e.g.*, a 3'-end wing, as described herein. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SS, wherein one or more nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprise -F. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SS, wherein one or more nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprise a 2'-F sugar modification. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SS, wherein each nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprises -F. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SS, wherein each nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprises a 2'-F sugar modification. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SSS, wherein one or more nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprise -F. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SSS, wherein one or more nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprise a 2'-F sugar modification. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SSS, wherein each nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprises -F. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSS, wherein the first S represents the first

internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SSS, wherein each nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprises a 2'-F sugar modification. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SSSS, wherein one or more nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprise -F. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SSSS, wherein one or more nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprise a 2'-F sugar modification. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SSSS, wherein each nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprises -F. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SSSS, wherein each nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprises a 2'-F sugar modification. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSSS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SSSSS, wherein one or more nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprise -F. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSSS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SSSSS, wherein one or more nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprise a 2'-F sugar modification. In some embodiments, the 5'-end region comprises a stereochemistry pattern of SSSSS, wherein the first S represents the first internucleotidic linkage of a provide oligonucleotide, a the 3'-end region comprises a stereochemistry pattern of SSSSS, wherein each nucleotidic unit comprising an *Sp* internucleotidic linkage in the 5'- or 3'-end region comprises -F. In some embodiments, the 5'-

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

comprises one or more *Rp* internucleotidic linkages. In some embodiments, a middle region comprises one or more *Rp* internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, a middle region comprises one or more *Rp* internucleotidic linkages and one or more *Sp* internucleotidic linkages.

[00318] In some embodiments, provided oligonucleotides comprise one or more nucleotidic units comprising $-F$ in the 5'-end region, *e.g.*, a 5'-wing. In some embodiments, provided oligonucleotides comprise two or more nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise three or more nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise four or more nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise five or more nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise six or more nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise seven or more nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise eight or more nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise nine or more nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise ten or more nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise two or more consecutive nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise three or more consecutive nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise four or more consecutive nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise five or more consecutive nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise six or more consecutive nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise seven or more consecutive nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise eight or more consecutive nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise nine or more consecutive nucleotidic units comprising $-F$ in the 5'-end region. In some embodiments, provided oligonucleotides comprise ten or more

consecutive nucleotidic units comprising -F in the 5'-end region. In some embodiments, provided oligonucleotides comprise two or more consecutive nucleotidic units comprising -F in the 5'-end region, wherein the first nucleotidic unit of the consecutive nucleotidic units is the first nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise three or more consecutive nucleotidic units comprising -F in the 5'-end region, wherein the first nucleotidic unit of the consecutive nucleotidic units is the first nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise four or more consecutive nucleotidic units comprising -F in the 5'-end region, wherein the first nucleotidic unit of the consecutive nucleotidic units is the first nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise five or more consecutive nucleotidic units comprising -F in the 5'-end region, wherein the first nucleotidic unit of the consecutive nucleotidic units is the first nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise six or more consecutive nucleotidic units comprising -F in the 5'-end region, wherein the first nucleotidic unit of the consecutive nucleotidic units is the first nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise seven or more consecutive nucleotidic units comprising -F in the 5'-end region, wherein the first nucleotidic unit of the consecutive nucleotidic units is the first nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise eight or more consecutive nucleotidic units comprising -F in the 5'-end region, wherein the first nucleotidic unit of the consecutive nucleotidic units is the first nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise nine or more consecutive nucleotidic units comprising -F in the 5'-end region, wherein the first nucleotidic unit of the consecutive nucleotidic units is the first nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise ten or more consecutive nucleotidic units comprising -F in the 5'-end region, wherein the first nucleotidic unit of the consecutive nucleotidic units is the first nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise one or more nucleotidic units comprising -F in the 3'-end region, *e.g.*, a 3'-wing. In some embodiments, provided oligonucleotides comprise two or more nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise three or more nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise four or more nucleotidic units comprising -F in the 3'-end

region. In some embodiments, provided oligonucleotides comprise five or more nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise six or more nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise seven or more nucleotidic units comprising - a 3'-wing. In some embodiments, provided oligonucleotides comprise two or more nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise nine or more nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise ten or more nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise two or more consecutive nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise three or more consecutive nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise four or more consecutive nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise five or more consecutive nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise six or more consecutive nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise seven or more consecutive nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise eight or more consecutive nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise nine or more consecutive nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise ten or more consecutive nucleotidic units comprising -F in the 3'-end region. In some embodiments, provided oligonucleotides comprise two or more consecutive nucleotidic units comprising -F in the 3'-end region, wherein the last nucleotidic unit of the consecutive nucleotidic units is the last nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise three or more consecutive nucleotidic units comprising -F in the 3'-end region, wherein the last nucleotidic unit of the consecutive nucleotidic units is the last nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise four or more consecutive nucleotidic units comprising -F in the 3'-end region, wherein the last nucleotidic unit of the consecutive nucleotidic units is the last nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise five or more

consecutive nucleotidic units comprising -F in the 3'-end region, wherein the last nucleotidic unit of the consecutive nucleotidic units is the last nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise six or more consecutive nucleotidic units comprising -F in the 3'-end region, wherein the last nucleotidic unit of the consecutive nucleotidic units is the last nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise seven or more consecutive nucleotidic units comprising -F in the 3'-end region, wherein the last nucleotidic unit of the consecutive nucleotidic units is the last nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise eight or more consecutive nucleotidic units comprising -F in the 3'-end region, wherein the last nucleotidic unit of the consecutive nucleotidic units is the last nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise nine or more consecutive nucleotidic units comprising -F in the 3'-end region, wherein the last nucleotidic unit of the consecutive nucleotidic units is the last nucleotidic unit of the oligonucleotide. In some embodiments, provided oligonucleotides comprise ten or more consecutive nucleotidic units comprising -F in the 3'-end region, wherein the last nucleotidic unit of the consecutive nucleotidic units is the last nucleotidic unit of the oligonucleotide. In some embodiments, a nucleotidic unit comprising -F comprises a sugar moiety comprising -F. In some embodiments, each nucleotidic unit comprising -F comprises a sugar moiety comprising -F. In some embodiments, a nucleotidic unit comprising -F comprises a 2'-F modified sugar moiety. In some embodiments, each nucleotidic unit comprising -F comprises a 2'-F modified sugar moiety. In some embodiments, provided oligonucleotides comprise both a 5'-end and a 3'-end regions as described herein. In some embodiments, provided oligonucleotides comprise one or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise one or more chiral modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise one or more chirally controlled chiral modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise one or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise one or more modified internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, a modified internucleotidic linkage is a phosphorothioate linkage. In some embodiments, each modified internucleotidic linkage is a phosphorothioate linkage.

[00319] In some embodiments, provided oligonucleotides can bind to a transcript, and

change the splicing pattern of the transcript. In some embodiments, provided oligonucleotides provides exon-skipping of an exon, with efficiency greater than a comparable oligonucleotide under one or more suitable conditions, *e.g.*, as described in Figures 35, 36, 37, 38, *etc.* In some embodiments, a provided skipping efficiency is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190% more than, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50 or more fold of, that of a comparable oligonucleotide under one or more suitable conditions, *e.g.*, as described in Figures 35, 36, 37, 38, *etc.*

[00320] In some embodiments, the present disclosure demonstrates that 2'-F modifications, among other things, can improve exon-skipping efficiency. In some embodiments, the present disclosure demonstrates that *Sp* internucleotidic linkages, among other things, at the 5'- and 3'-ends can improve oligonucleotide stability. In some embodiments, the present disclosure demonstrates that, among other things, natural phosphate linkages and/or *Rp* internucleotidic linkages can improve removal of oligonucleotides from a system. As appreciated by a person having ordinary skill in the art, various assays known in the art can be utilized to assess such properties in accordance with the present disclosure.

[00321] In some embodiments, a common base sequence of a plurality of oligonucleotides comprises or consists of the base sequence of UCAAGGAAGAUGGCAUUUCU and the plurality of oligonucleotides has a common pattern of backbone chiral centers comprising SSS, SSSS, SSSSS, SSSSSS, SSSSSSS, SOS, SSOSS, SSSOSSS, SSSSOSSSS, SSSSSOSSSSS, SSSSSSOSSSSS, SSSSSSOSSSSSS, SSSSSSOSSSSSSS, SSSSSSOSSSSSSSS, SSSSSSOSSSSSSSSS, SOSOSOSOS, SSOSOSOSOSS, SSSOSOSOSOSSS, SSSSOSOSOSOSSSS, SSSSSOSOSOSOSSSSS, SSSSSSOSOSOSOSSSSS, SOSOSSOOS, SSOSOSSOOSS, SSSOSOSSOOSSS, SSSSOSOSSOOSSSS, SSSSSOSOSSOOSSSSS, SOSOSSOOS, SSOSOSSOOSO, SSSOSOSSOOSOS, SSSSOSOSSOOSOSS, SSSSSOSOSSOOSOSSS, SSSSSSOSOSSOOSOSSS, SOSOSSOOS, SSOSOSSOOS, SSSOSOSSOOS, SSSSOSOSSOOS, SSSSSOSOSSOOS, OSSSSSSOSOSSOOSSS, OOSSSSSSOSOSSOOS, OOSSSSSSOSOSSOOS,

OOSSSSSSOSOSSOOSSSS, OOSSSSSSOSOSSOOSSSS, OOSSSSSSOSOSSOOSSSSS, and/or OOSSSSSSOSOSSOOSSSSSS, wherein O is a non-chiral internucleotidic linkage and S is an *Sp* internucleotidic linkage. In some embodiments, a plurality of oligonucleotides share a common base sequence comprising or consisting of the base sequence of UCAAGGAAGAUGGCAUUUCU or 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, have a length of 40 bases or less, have a common pattern of backbone chiral centers comprising SSSS, wherein S is a phosphorothioate linkage in an *Sp* configuration, and further comprise one or more of the following: a phosphorothioate in the *Rp* configuration, a phosphodiester, or a phosphorodithioate. In some embodiments, a plurality of oligonucleotides have a common base sequence of an oligonucleotide comprising or consisting of the base sequence of UCAAGGAAGAUGGCAUUUCU or 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, have a length of 40 bases or less, have a common pattern of backbone chiral centers comprising a sequence of SSSS, wherein S is a phosphorothioate in an *Sp* configuration, and further comprise one or more of the following: a phosphorothioate in the *Rp* configuration, a phosphodiester, or a phosphorodithioate. In some embodiments, a plurality of oligonucleotides have a common base sequence of an oligonucleotide comprising or consisting of the base sequence of UCAAGGAAGAUGGCAUUUCU or 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, have a length of 40 bases or less, have a common pattern of backbone chiral centers comprising 4 phosphorothioates in an *Sp* configuration, wherein the 4 phosphorothioates in an *Sp* configuration may be contiguous or non-contiguous, and further comprise one or more of the following: a phosphorothioate in the *Rp* configuration, a phosphodiester, or a phosphorodithioate. In some embodiments, a plurality of oligonucleotides have a common base sequence of an oligonucleotide comprising or consisting of the base sequence of UCAAGGAAGAUGGCAUUUCU or 15 contiguous bases of UCAAGGAAGAUGGCAUUUCU, have a length of 40 bases or less, have a common pattern of backbone chiral centers comprising 5 phosphorothioates in an *Sp* configuration, wherein the 5 phosphorothioates in an *Sp* configuration may be contiguous or non-contiguous, and further comprise 2 or more of the following: a phosphorothioate in the *Rp* configuration, a phosphodiester, or a phosphorodithioate.

[00322] In some embodiments, provided oligonucleotides comprise one or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise one or more

modified sugar moieties. In some embodiments, provided oligonucleotides comprise 2 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 3 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 4 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 5 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 6 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 7 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 8 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 9 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 10 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 11 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 12 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 13 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 14 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 15 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 15 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 16 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 17 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 18 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 19 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 20 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 21 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 22 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 23 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 24 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 25 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 30 or more modified sugar moieties. In some embodiments, a modified sugar moiety comprises a 2'-modification. In some embodiments, a modified sugar moiety comprises a 2'-modification. In some embodiments, a 2'-modification is 2'-OR¹. In some embodiments, a 2'-modification is a 2'-OMe. In some embodiments, a 2'-modification is a 2'-MOE. In some embodiments, a 2'-modification is an LNA sugar modification. In some embodiments, a 2'-modification is 2'-F. In

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

[00323] In some embodiments, 5% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 10% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 15% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 20% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 25% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 30% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 35% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 40% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 45% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 50% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 55% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 60% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 65% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 70% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 75% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 80% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 85% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 90% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 95% or more of the sugar moieties of provided

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

[00324] In some embodiments, provided oligonucleotides comprise one or more 2'-F. In some embodiments, provided oligonucleotides comprise two or more 2'-F. In some embodiments, provided oligonucleotides comprise three or more 2'-F. In some embodiments, provided oligonucleotides comprise four or more 2'-F. In some embodiments, provided oligonucleotides comprise five or more 2'-F. In some embodiments, provided oligonucleotides comprise six or more 2'-F. In some embodiments, provided oligonucleotides comprise seven or more 2'-F. In some embodiments, provided oligonucleotides comprise eight or more 2'-F. In some embodiments, provided oligonucleotides comprise nine or more 2'-F. In some embodiments, provided oligonucleotides comprise ten or more 2'-F. In some embodiments, provided oligonucleotides comprise 11 or more 2'-F. In some embodiments, provided oligonucleotides comprise 12 or more 2'-F. In some embodiments, provided oligonucleotides comprise 13 or more 2'-F. In some embodiments, provided oligonucleotides comprise 14 or more 2'-F. In some embodiments, provided oligonucleotides comprise 15 or more 2'-F. In some embodiments, provided oligonucleotides comprise 16 or more 2'-F. In some embodiments, provided oligonucleotides comprise 17 or more 2'-F. In some embodiments, provided

oligonucleotides comprise 18 or more 2'-F. In some embodiments, provided oligonucleotides comprise 19 or more 2'-F. In some embodiments, provided oligonucleotides comprise 20 or more 2'-F. In some embodiments, provided oligonucleotides comprise 21 or more 2'-F. In some embodiments, provided oligonucleotides comprise 22 or more 2'-F. In some embodiments, provided oligonucleotides comprise 23 or more 2'-F. In some embodiments, provided oligonucleotides comprise 24 or more 2'-F. In some embodiments, provided oligonucleotides comprise 25 or more 2'-F. In some embodiments, provided oligonucleotides comprise 30 or more 2'-F. In some embodiments, provided oligonucleotides comprise 35 or more 2'-F.

[00325] In some embodiments, provided oligonucleotides comprise one 2'-F. In some embodiments, provided oligonucleotides comprise two 2'-F. In some embodiments, provided oligonucleotides comprise three 2'-F. In some embodiments, provided oligonucleotides comprise four 2'-F. In some embodiments, provided oligonucleotides comprise five 2'-F. In some embodiments, provided oligonucleotides comprise six 2'-F. In some embodiments, provided oligonucleotides comprise seven 2'-F. In some embodiments, provided oligonucleotides comprise eight 2'-F. In some embodiments, provided oligonucleotides comprise nine 2'-F. In some embodiments, provided oligonucleotides comprise ten 2'-F. In some embodiments, provided oligonucleotides comprise 11 2'-F. In some embodiments, provided oligonucleotides comprise 12 2'-F. In some embodiments, provided oligonucleotides comprise 13 2'-F. In some embodiments, provided oligonucleotides comprise 14 2'-F. In some embodiments, provided oligonucleotides comprise 15 2'-F. In some embodiments, provided oligonucleotides comprise 16 2'-F. In some embodiments, provided oligonucleotides comprise 17 2'-F. In some embodiments, provided oligonucleotides comprise 18 2'-F. In some embodiments, provided oligonucleotides comprise 19 2'-F. In some embodiments, provided oligonucleotides comprise 20 2'-F. In some embodiments, provided oligonucleotides comprise 21 2'-F. In some embodiments, provided oligonucleotides comprise 22 2'-F. In some embodiments, provided oligonucleotides comprise 23 2'-F. In some embodiments, provided oligonucleotides comprise 24 2'-F. In some embodiments, provided oligonucleotides comprise 25 2'-F. In some embodiments, provided oligonucleotides comprise 30 2'-F. In some embodiments, provided oligonucleotides comprise 35 2'-F.

[00326] In some embodiments, provided oligonucleotides comprise one or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise two or more

consecutive 2'-F. In some embodiments, provided oligonucleotides comprise three or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise four or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise five or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise six or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise seven or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise eight or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise nine or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise ten or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 11 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 12 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 13 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 14 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 15 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 16 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 17 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 18 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 19 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 20 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 21 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 22 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 23 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 24 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 25 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 30 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 35 or more consecutive 2'-F.

[00327] In some embodiments, provided oligonucleotides comprise one consecutive 2'-F. In some embodiments, provided oligonucleotides comprise two consecutive 2'-F. In some embodiments, provided oligonucleotides comprise three consecutive 2'-F. In some embodiments, provided oligonucleotides comprise four consecutive 2'-F. In some embodiments, provided oligonucleotides comprise five consecutive 2'-F. In some embodiments, provided

oligonucleotides comprise six consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise seven consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise eight consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise nine consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise ten consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 11 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 12 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 13 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 14 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 15 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 16 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 17 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 18 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 19 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 20 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 21 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 22 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 23 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 24 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 25 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 30 consecutive 2'-F. In some embodiments, provided
oligonucleotides comprise 35 consecutive 2'-F.

[00328] In some embodiments, a nucleoside comprising a 2'-modification is followed by a modified internucleotidic linkage. In some embodiments, a nucleoside comprising a 2'-modification is preceded by a modified internucleotidic linkage. In some embodiments, a modified internucleotidic linkage is a chiral internucleotidic linkage. In some embodiments, a modified internucleotidic linkage is a phosphorothioate. In some embodiments, a chiral internucleotidic linkage is *Sp*. In some embodiments, a nucleoside comprising a 2'-modification is followed by an *Sp* chiral internucleotidic linkage. In some embodiments, a nucleoside comprising a 2'-F is followed by an *Sp* chiral internucleotidic linkage. In some embodiments, a nucleoside comprising a 2'-modification is preceded by an *Sp* chiral internucleotidic linkage. In

some embodiments, a nucleoside comprising a 2'-F is preceded by an *Sp* chiral internucleotidic linkage. In some embodiments, a chiral internucleotidic linkage is *Rp*. In some embodiments, a nucleoside comprising a 2'-modification is followed by an *Rp* chiral internucleotidic linkage. In some embodiments, a nucleoside comprising a 2'-F is followed by an *Rp* chiral internucleotidic linkage. In some embodiments, a nucleoside comprising a 2'-modification is preceded by an *Rp* chiral internucleotidic linkage. In some embodiments, a nucleoside comprising a 2'-F is preceded by an *Rp* chiral internucleotidic linkage.

[00329] In some embodiments, provided oligonucleotides comprise alternating 2'-F modified sugar moieties and 2'-OR¹ modified sugar moieties. In some embodiments, provided oligonucleotides comprise alternating 2'-F modified sugar moieties and 2'-OMe modified sugar moieties, *e.g.*, [(2'-F)(2'-OMe)]_x, [(2'-OMe)(2'-F)]_x, *etc.*, wherein x is 1-50. In some embodiments, provided oligonucleotides comprise at least two pairs of alternating 2'-F and 2'-OMe modifications. In some embodiments, provided oligonucleotides comprises alternating phosphodiester and phosphorothioate internucleotidic linkages, *e.g.*, [(PO)(PS)]_x, [(PS)(PO)]_x, *etc.*, wherein x is 1-50. In some embodiments, provided oligonucleotides comprise at least two pairs of alternating phosphodiester and phosphorothioate internucleotidic linkages.

[00330] In some embodiments, provided oligonucleotides comprise one or more natural phosphate linkages and one or more modified internucleotidic linkages.

[00331] Provided oligonucleotides can comprise various number of natural phosphate linkages. In some embodiments, provided oligonucleotides comprise no natural phosphate linkages. In some embodiments, provided oligonucleotides comprise one natural phosphate linkage. In some embodiments, provided oligonucleotides comprise 2 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 3 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 4 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 5 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 6 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 7 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 8 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 9 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 10 or more natural phosphate linkages. In some

embodiments, provided oligonucleotides comprise 15 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 20 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 25 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 30 or more natural phosphate linkages.

[00332] In some embodiments, 5% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 10% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 15% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 20% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 25% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 30% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 35% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 40% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 45% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 50% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 55% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 60% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 65% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 70% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 75% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 80% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 85% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 90% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 95% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, each internucleotidic linkage of a wing region is a natural phosphate

linkage.

[00333] In some embodiments, a core region comprises 2 or more modified internucleotidic linkages. In some embodiments, a core region comprises 3 or more modified internucleotidic linkages. In some embodiments, a core region comprises 4 or more modified internucleotidic linkages. In some embodiments, a core region comprises 5 or more modified internucleotidic linkages. In some embodiments, a core region comprises 6 or more modified internucleotidic linkages. In some embodiments, a core region comprises 7 or more modified internucleotidic linkages. In some embodiments, a core region comprises 8 or more modified internucleotidic linkages. In some embodiments, a core region comprises 9 or more modified internucleotidic linkages. In some embodiments, a core region comprises 10 or more modified internucleotidic linkages. In some embodiments, a core region comprises 11 or more modified internucleotidic linkages. In some embodiments, a core region comprises 12 or more modified internucleotidic linkages. In some embodiments, a core region comprises 13 or more modified internucleotidic linkages. In some embodiments, a core region comprises 14 or more modified internucleotidic linkages. In some embodiments, a core region comprises 15 or more modified internucleotidic linkages. In some embodiments, a core region comprises 2 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 3 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 4 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 5 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 6 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 7 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 8 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 9 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 10 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 11 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 12 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 13 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 14 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 15 or consecutive modified internucleotidic linkages. In some embodiments, each

internucleotidic linkage in a core region is independently a modified internucleotidic linkage.

[00334] In some embodiments, 5% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 10% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 15% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 20% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 25% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 30% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 35% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 40% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 45% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 50% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 55% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 60% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 65% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 70% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 75% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 80% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 85% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 90% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 95% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, each internucleotidic linkage of a core region is a modified internucleotidic linkage.

[00335] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence; and

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

[00336] In some embodiments, oligonucleotides of the first plurality comprise one or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise one or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 2 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 3 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 4 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 5 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 6 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 7 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 8 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 9 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 10 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 15 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 20 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 25 or more modified sugar moieties. In some embodiments, provided oligonucleotides comprise 30 or more modified sugar moieties.

[00337] In some embodiments, 5% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 10% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 15% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 20% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 25% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 30% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 35% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 40% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 45% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 50% or more of the sugar moieties of provided

oligonucleotides are modified. In some embodiments, 55% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 60% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 65% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 70% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 75% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 80% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 85% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 90% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, 95% or more of the sugar moieties of provided oligonucleotides are modified. In some embodiments, each sugar moiety of provided oligonucleotides is modified.

[00338] In some embodiments, provided oligonucleotides comprise one or more 2'-F. In some embodiments, provided oligonucleotides comprise two or more 2'-F. In some embodiments, provided oligonucleotides comprise three or more 2'-F. In some embodiments, provided oligonucleotides comprise four or more 2'-F. In some embodiments, provided oligonucleotides comprise five or more 2'-F. In some embodiments, provided oligonucleotides comprise six or more 2'-F. In some embodiments, provided oligonucleotides comprise seven or more 2'-F. In some embodiments, provided oligonucleotides comprise eight or more 2'-F. In some embodiments, provided oligonucleotides comprise nine or more 2'-F. In some embodiments, provided oligonucleotides comprise ten or more 2'-F. In some embodiments, provided oligonucleotides comprise 11 or more 2'-F. In some embodiments, provided oligonucleotides comprise 12 or more 2'-F. In some embodiments, provided oligonucleotides comprise 13 or more 2'-F. In some embodiments, provided oligonucleotides comprise 14 or more 2'-F. In some embodiments, provided oligonucleotides comprise 15 or more 2'-F. In some embodiments, provided oligonucleotides comprise 16 or more 2'-F. In some embodiments, provided oligonucleotides comprise 17 or more 2'-F. In some embodiments, provided oligonucleotides comprise 18 or more 2'-F. In some embodiments, provided oligonucleotides comprise 19 or more 2'-F. In some embodiments, provided oligonucleotides comprise 20 or more 2'-F. In some embodiments, provided oligonucleotides comprise 21 or more 2'-F. In some embodiments, provided oligonucleotides comprise 22 or more 2'-F. In some embodiments,

provided oligonucleotides comprise 23 or more 2'-F. In some embodiments, provided oligonucleotides comprise 24 or more 2'-F. In some embodiments, provided oligonucleotides comprise 25 or more 2'-F. In some embodiments, provided oligonucleotides comprise 30 or more 2'-F. In some embodiments, provided oligonucleotides comprise 35 or more 2'-F.

[00339] In some embodiments, provided oligonucleotides comprise one 2'-F. In some embodiments, provided oligonucleotides comprise two 2'-F. In some embodiments, provided oligonucleotides comprise three 2'-F. In some embodiments, provided oligonucleotides comprise four 2'-F. In some embodiments, provided oligonucleotides comprise five 2'-F. In some embodiments, provided oligonucleotides comprise six 2'-F. In some embodiments, provided oligonucleotides comprise seven 2'-F. In some embodiments, provided oligonucleotides comprise eight 2'-F. In some embodiments, provided oligonucleotides comprise nine 2'-F. In some embodiments, provided oligonucleotides comprise ten 2'-F. In some embodiments, provided oligonucleotides comprise 11 2'-F. In some embodiments, provided oligonucleotides comprise 12 2'-F. In some embodiments, provided oligonucleotides comprise 13 2'-F. In some embodiments, provided oligonucleotides comprise 14 2'-F. In some embodiments, provided oligonucleotides comprise 15 2'-F. In some embodiments, provided oligonucleotides comprise 16 2'-F. In some embodiments, provided oligonucleotides comprise 17 2'-F. In some embodiments, provided oligonucleotides comprise 18 2'-F. In some embodiments, provided oligonucleotides comprise 19 2'-F. In some embodiments, provided oligonucleotides comprise 20 2'-F. In some embodiments, provided oligonucleotides comprise 21 2'-F. In some embodiments, provided oligonucleotides comprise 22 2'-F. In some embodiments, provided oligonucleotides comprise 23 2'-F. In some embodiments, provided oligonucleotides comprise 24 2'-F. In some embodiments, provided oligonucleotides comprise 25 2'-F. In some embodiments, provided oligonucleotides comprise 30 2'-F. In some embodiments, provided oligonucleotides comprise 35 2'-F.

[00340] In some embodiments, provided oligonucleotides comprise one or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise two or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise three or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise four or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise five or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise six or more

consecutive 2'-F. In some embodiments, provided oligonucleotides comprise seven or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise eight or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise nine or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise ten or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 11 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 12 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 13 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 14 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 15 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 16 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 17 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 18 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 19 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 20 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 21 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 22 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 23 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 24 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 25 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 30 or more consecutive 2'-F. In some embodiments, provided oligonucleotides comprise 35 or more consecutive 2'-F.

[00341] In some embodiments, provided oligonucleotides comprise one consecutive 2'-F. In some embodiments, provided oligonucleotides comprise two consecutive 2'-F. In some embodiments, provided oligonucleotides comprise three consecutive 2'-F. In some embodiments, provided oligonucleotides comprise four consecutive 2'-F. In some embodiments, provided oligonucleotides comprise five consecutive 2'-F. In some embodiments, provided oligonucleotides comprise six consecutive 2'-F. In some embodiments, provided oligonucleotides comprise seven consecutive 2'-F. In some embodiments, provided oligonucleotides comprise eight consecutive 2'-F. In some embodiments, provided oligonucleotides comprise nine consecutive 2'-F. In some embodiments, provided

oligonucleotides comprise ten consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 11 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 12 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 13 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 14 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 15 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 16 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 17 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 18 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 19 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 20 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 21 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 22 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 23 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 24 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 25 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 30 consecutive 2'-F. In some embodiments, provided
 oligonucleotides comprise 35 consecutive 2'-F.

[00342] In some embodiments, a nucleoside comprising a 2'-modification is followed by a modified internucleotidic linkage. In some embodiments, a nucleoside comprising a 2'-modification is preceded by a modified internucleotidic linkage. In some embodiments, a modified internucleotidic linkage is a chiral internucleotidic linkage. In some embodiments, a modified internucleotidic linkage is a phosphorothioate. In some embodiments, a chiral internucleotidic linkage is *Sp*. In some embodiments, a nucleoside comprising a 2'-modification is followed by an *Sp* chiral internucleotidic linkage. In some embodiments, a nucleoside comprising a 2'-F is followed by an *Sp* chiral internucleotidic linkage. In some embodiments, a nucleoside comprising a 2'-modification is preceded by an *Sp* chiral internucleotidic linkage. In some embodiments, a nucleoside comprising a 2'-F is preceded by an *Sp* chiral internucleotidic linkage. In some embodiments, a chiral internucleotidic linkage is *Rp*. In some embodiments, a nucleoside comprising a 2'-modification is followed by an *Rp* chiral internucleotidic linkage. In some embodiments, a nucleoside comprising a 2'-F is followed by an *Rp* chiral internucleotidic

linkage. In some embodiments, a nucleoside comprising a 2'-modification is preceded by an *Rp* chiral internucleotidic linkage. In some embodiments, a nucleoside comprising a 2'-F is preceded by an *Rp* chiral internucleotidic linkage.

[00343] In some embodiments, oligonucleotides of the first plurality comprise one or more natural phosphate linkages and one or more modified internucleotidic linkages.

[00344] Provided oligonucleotides can comprise various number of natural phosphate linkages. In some embodiments, provided oligonucleotides comprise no natural phosphate linkages. In some embodiments, provided oligonucleotides comprise one natural phosphate linkage. In some embodiments, provided oligonucleotides comprise 2 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 3 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 4 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 5 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 6 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 7 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 8 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 9 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 10 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 15 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 20 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 25 or more natural phosphate linkages. In some embodiments, provided oligonucleotides comprise 30 or more natural phosphate linkages.

[00345] In some embodiments, 5% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 10% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 15% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 20% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 25% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 30% or more of the internucleotidic linkages of provided

oligonucleotides are natural phosphate linkages. In some embodiments, 35% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 40% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 45% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 50% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 55% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 60% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 65% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 70% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 75% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 80% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 85% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 90% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 95% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages.

[00346] Provided oligonucleotides can comprise various number of modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise one modified internucleotidic linkage. In some embodiments, provided oligonucleotides comprise 2 or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise 3 or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise 4 or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise 5 or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise 6 or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise 7 or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise 8 or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise 9 or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise

10 or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise 15 or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise 20 or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise 25 or more modified internucleotidic linkages. In some embodiments, provided oligonucleotides comprise 30 or more modified internucleotidic linkages.

[00347] In some embodiments, 5% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 10% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 15% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 20% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 25% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 30% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 35% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 40% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 45% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 50% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 55% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 60% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 65% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 70% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 75% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 80% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 85% or more of the internucleotidic linkages of provided oligonucleotides

are modified internucleotidic linkages. In some embodiments, 90% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 95% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, each internucleotidic linkage of provided oligonucleotides is a modified internucleotidic linkage.

[00348] In some embodiments, provided oligonucleotides comprise no more than about 25 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 20 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 15 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 10 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 9 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 8 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 7 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 6 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 5 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 4 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 3 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 2 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 25 unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 20 unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 15 unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 10 unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 5 unmodified sugar moieties.

[00349] In some embodiments, provided oligonucleotides comprise no more than about 95% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 90% unmodified sugar moieties. In some embodiments, provided

oligonucleotides comprise no more than about 85% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 80% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 70% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 60% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 50% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 40% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 30% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 20% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 10% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 5% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 15 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 10 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 9 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 8 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 7 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 6 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 5 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 4 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 3 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 2 consecutive unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 25 unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 20 unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 15 unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 10 unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 5 unmodified sugar

moieties.

[00350] In some embodiments, provided oligonucleotides comprise no more than about 95% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 90% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 85% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 80% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 70% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 60% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 50% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 40% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 30% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 20% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 10% unmodified sugar moieties. In some embodiments, provided oligonucleotides comprise no more than about 5% unmodified sugar moieties. In some embodiments, each sugar moiety of the oligonucleotides of the first plurality is independently modified.

[00351] In some embodiments, provided compositions alter transcript splicing so that an undesired target and/or biological function are suppressed. In some embodiments, in such cases provided composition can also induce cleavage of the transcript after hybridization.

[00352] In some embodiments, provided compositions alter transcript splicing so a desired target and/or biological function is enhanced. In some embodiments, provided compositions, by incorporating chemical modifications, stereochemistry and/or combinations thereof, effectively suppress or prevent cleavage of a target transcript after contact.

[00353] In some embodiments, each oligonucleotide of the first plurality comprises one or more modified sugar moieties and modified internucleotidic linkages. In some embodiments, each oligonucleotide of the first plurality comprises two or more modified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises three or more modified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises four or more modified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises five or more modified sugar moieties. In some embodiments, each

oligonucleotide of the first plurality comprises ten or more modified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises about 15 or more modified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises about 20 or more modified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises about 25 or more modified sugar moieties.

[00354] In some embodiments, about 5% of the sugar moieties in each oligonucleotide of the first plurality are modified sugar moieties. In some embodiments, about 10% or more of the sugar moieties in each oligonucleotide of the first plurality are modified sugar moieties. In some embodiments, about 20% or more of the sugar moieties in each oligonucleotide of the first plurality are modified sugar moieties. In some embodiments, about 30% or more of the sugar moieties in each oligonucleotide of the first plurality are modified sugar moieties. In some embodiments, about 40% or more of the sugar moieties in each oligonucleotide of the first plurality are modified sugar moieties. In some embodiments, about 50% or more of the sugar moieties in each oligonucleotide of the first plurality are modified sugar moieties. In some embodiments, about 60% or more of the sugar moieties in each oligonucleotide of the first plurality are modified sugar moieties. In some embodiments, about 70% or more of the sugar moieties in each oligonucleotide of the first plurality are modified sugar moieties. In some embodiments, about 80% or more of the sugar moieties in each oligonucleotide of the first plurality are modified sugar moieties. In some embodiments, about 85% or more of the sugar moieties in each oligonucleotide of the first plurality are modified sugar moieties. In some embodiments, about 90% or more of the sugar moieties in each oligonucleotide of the first plurality are modified sugar moieties. In some embodiments, about 95% or more of the sugar moieties in each oligonucleotide of the first plurality are modified sugar moieties.

[00355] In some embodiments, each oligonucleotide of the first plurality comprises no more than about 25 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 20 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 15 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 10 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 9 consecutive unmodified sugar moieties. In some embodiments, each

oligonucleotide of the first plurality comprises no more than about 8 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 7 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 6 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 5 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 4 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 3 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 2 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 25 unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 20 unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 15 unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 10 unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 5 unmodified sugar moieties.

[00356] In some embodiments, each oligonucleotide of the first plurality comprises no more than about 95% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 90% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 85% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 80% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 70% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 60% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 50% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 40% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 30% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 20% unmodified sugar

moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 10% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 5% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 15 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 10 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 9 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 8 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 7 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 6 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 5 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 4 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 3 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 2 consecutive unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 25 unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 20 unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 15 unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 10 unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 5 unmodified sugar moieties.

[00357] In some embodiments, each oligonucleotide of the first plurality comprises no more than about 95% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 90% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 85% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 80% unmodified sugar moieties. In some embodiments, each

oligonucleotide of the first plurality comprises no more than about 70% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 60% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 50% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 40% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 30% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 20% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 10% unmodified sugar moieties. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 5% unmodified sugar moieties. In some embodiments, each sugar moiety of the oligonucleotides of the first plurality is independently modified.

[00358] In some embodiments, each oligonucleotide of the first plurality comprises two or more modified internucleotidic linkages. In some embodiments, each oligonucleotide of the first plurality comprises three or more modified internucleotidic linkages. In some embodiments, each oligonucleotide of the first plurality comprises four or more modified internucleotidic linkages. In some embodiments, each oligonucleotide of the first plurality comprises five or more modified internucleotidic linkages. In some embodiments, each oligonucleotide of the first plurality comprises ten or more modified internucleotidic linkages. In some embodiments, each oligonucleotide of the first plurality comprises about 15 or more modified internucleotidic linkages. In some embodiments, each oligonucleotide of the first plurality comprises about 20 or more modified internucleotidic linkages. In some embodiments, each oligonucleotide of the first plurality comprises about 25 or more modified internucleotidic linkages.

[00359] In some embodiments, about 5% of the internucleotidic linkages in each oligonucleotide of the first plurality are modified internucleotidic linkages. In some embodiments, about 10% of the internucleotidic linkages in each oligonucleotide of the first plurality are modified internucleotidic linkages. In some embodiments, about 20% of the internucleotidic linkages in each oligonucleotide of the first plurality are modified internucleotidic linkages. In some embodiments, about 30% of the internucleotidic linkages in each oligonucleotide of the first plurality are modified internucleotidic linkages. In some

embodiments, about 40% of the internucleotidic linkages in each oligonucleotide of the first plurality are modified internucleotidic linkages. In some embodiments, about 50% of the internucleotidic linkages in each oligonucleotide of the first plurality are modified internucleotidic linkages. In some embodiments, about 60% of the internucleotidic linkages in each oligonucleotide of the first plurality are modified internucleotidic linkages. In some embodiments, about 70% of the internucleotidic linkages in each oligonucleotide of the first plurality are modified internucleotidic linkages. In some embodiments, about 80% of the internucleotidic linkages in each oligonucleotide of the first plurality are modified internucleotidic linkages. In some embodiments, about 85% of the internucleotidic linkages in each oligonucleotide of the first plurality are modified internucleotidic linkages. In some embodiments, about 90% of the internucleotidic linkages in each oligonucleotide of the first plurality are modified internucleotidic linkages. In some embodiments, about 95% of the internucleotidic linkages in each oligonucleotide of the first plurality are modified internucleotidic linkages.

[00360] In some embodiments, each oligonucleotide of the first plurality comprises no more than about 25 consecutive natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 20 consecutive natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 15 consecutive natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 10 consecutive natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 9 consecutive natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 8 consecutive natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 7 consecutive natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 6 consecutive natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 5 consecutive natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 4 consecutive natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 3 consecutive natural phosphate linkages. In some embodiments, each

oligonucleotide of the first plurality comprises no more than about 2 consecutive natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 25 natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 20 natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 15 natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 10 natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 5 natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 95% natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 90% natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 85% natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 80% natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 70% natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 60% natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 50% natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 40% natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 30% natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 20% natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 10% natural phosphate linkages. In some embodiments, each oligonucleotide of the first plurality comprises no more than about 5% natural phosphate linkages.

[00361] In some embodiments, oligonucleotides of the first plurality comprise no DNA nucleotide. A DNA nucleotide is a nucleotide in which the sugar moiety is an unmodified DNA sugar moiety, and the internucleotidic linkage is a natural phosphate linkage. In some embodiments, oligonucleotides of the first plurality comprise no more than 2 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 3 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than

4 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 5 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 6 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 7 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 8 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 9 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 10 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 11 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 12 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 13 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 14 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 15 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 20 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 25 DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 30 DNA nucleotides.

[00362] In some embodiments, oligonucleotides of the first plurality comprise no more than 2 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 3 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 4 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 5 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 6 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 7 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 8 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 9 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 10 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 11 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 12 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 13

consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 14 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 15 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 20 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 25 consecutive DNA nucleotides. In some embodiments, oligonucleotides of the first plurality comprise no more than 30 consecutive DNA nucleotides.

[00363] In some embodiments, compared to a reference condition, provided chirally controlled oligonucleotide compositions are surprisingly effective. In some embodiments, desired biological effects (*e.g.*, as measured by increased levels of desired mRNA, proteins, *etc.*, decreased levels of undesired mRNA, proteins, *etc.*) can be enhanced by more than 5, 10, 15, 20, 25, 30, 40, 50, or 100 fold. In some embodiments, a change is measured by increase of a desired mRNA level compared to a reference condition. In some embodiments, a change is measured by decrease of an undesired mRNA level compared to a reference condition. In some embodiments, a reference condition is absence of oligonucleotide treatment. In some embodiments, a reference condition is a stereorandom composition of oligonucleotides having the same base sequence and chemical modifications.

[00364] In some embodiments, a desired biological effect is enhanced by more than 2 fold. In some embodiments, a desired biological effect is enhanced by more than 3 fold. In some embodiments, a desired biological effect is enhanced by more than 4 fold. In some embodiments, a desired biological effect is enhanced by more than 5 fold. In some embodiments, a desired biological effect is enhanced by more than 6 fold. In some embodiments, a desired biological effect is enhanced by more than 7 fold. In some embodiments, a desired biological effect is enhanced by more than 8 fold. In some embodiments, a desired biological effect is enhanced by more than 9 fold. In some embodiments, a desired biological effect is enhanced by more than 10 fold. In some embodiments, a desired biological effect is enhanced by more than 11 fold. In some embodiments, a desired biological effect is enhanced by more than 12 fold. In some embodiments, a desired biological effect is enhanced by more than 13 fold. In some embodiments, a desired biological effect is enhanced by more than 14 fold. In some embodiments, a desired biological effect is enhanced by more than 15 fold. In some

embodiments, a desired biological effect is enhanced by more than 20 fold. In some embodiments, a desired biological effect is enhanced by more than 25 fold. In some embodiments, a desired biological effect is enhanced by more than 30 fold. In some embodiments, a desired biological effect is enhanced by more than 35 fold. In some embodiments, a desired biological effect is enhanced by more than 40 fold. In some embodiments, a desired biological effect is enhanced by more than 45 fold. In some embodiments, a desired biological effect is enhanced by more than 50 fold. In some embodiments, a desired biological effect is enhanced by more than 60 fold. In some embodiments, a desired biological effect is enhanced by more than 70 fold. In some embodiments, a desired biological effect is enhanced by more than 80 fold. In some embodiments, a desired biological effect is enhanced by more than 90 fold. In some embodiments, a desired biological effect is enhanced by more than 100 fold. In some embodiments, a desired biological effect is enhanced by more than 200 fold. In some embodiments, a desired biological effect is enhanced by more than 500 fold.

[00365] In some embodiments, provided oligonucleotides comprise two wing regions and one core regions. In some embodiments, provided oligonucleotides comprises a 5'-wing-core-wing-3' structure. In some embodiments, provided oligonucleotides are of a 5'-wing-core-wing-3' gapmer structure. In some embodiments, the two wing regions are identical. In some embodiments, the two wing regions are different. In some embodiments, the two wing regions are identical in chemical modifications. In some embodiments, the two wing regions are identical in 2'-modifications. In some embodiments, the two wing regions are identical in internucleotidic linkage modifications. In some embodiments, the two regions are identical in patterns of backbone chiral centers. In some embodiments, the two wing regions are identical in pattern of backbone linkages. In some embodiments, the two wing regions are identical in pattern of backbone linkage types. In some embodiments, the two wing regions are identical in pattern of backbone phosphorus modifications.

[00366] In some embodiments, provided oligonucleotides comprise one wing and one core regions. In some embodiments, provided oligonucleotides comprises a 5'-wing-core-3' hemimer structure. In some embodiments, provided oligonucleotides are of a 5'-wing-core-3' hemimer structure. In some embodiments, provided oligonucleotides comprises a 5'-core-wing-3' hemimer structure. In some embodiments, provided oligonucleotides are of a 5'-core-wing-3' hemimer structure.

hemimer structure.

[00367] A wing region can be differentiated from a core region in that a wing region contains a different structure feature than a core region. For example, in some embodiments, a wing region differs from a core region in that they have different sugar modifications, base modifications, internucleotidic linkages, internucleotidic linkage stereochemistry, *etc.* In some embodiments, a wing region differs from a core region in that they have different 2'-modifications of the sugars.

[00368] In some embodiments, an internucleotidic linkage between a wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic linkage between a 5'-wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic linkage between a 3'-wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic linkage between a wing region and a core region is considered part of the core region. In some embodiments, an internucleotidic linkage between a 5'-wing region and a core region is considered part of the core region. In some embodiments, an internucleotidic linkage between a 3'-wing region and a core region is considered part of the core region.

[00369] In some embodiments, an internucleotidic linkage between a wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic linkage between a 5'-wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic linkage between a 3'-wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic linkage between a wing region and a core region is considered part of the core region. In some embodiments, an internucleotidic linkage between a 5'-wing region and a core region is considered part of the core region. In some embodiments, an internucleotidic linkage between a 3'-wing region and a core region is considered part of the core region.

[00370] In some embodiments, a wing region comprises 2 or more nucleosides. In some embodiments, a wing region comprises 3 or more nucleosides. In some embodiments, a wing region comprises 4 or more nucleosides. In some embodiments, a wing region comprises 5 or more nucleosides. In some embodiments, a wing region comprises 6 or more nucleosides. In some embodiments, a wing region comprises 7 or more nucleosides. In some embodiments, a wing region comprises 8 or more nucleosides. In some embodiments, a wing region comprises 9

or more nucleosides. In some embodiments, a wing region comprises 10 or more nucleosides. In some embodiments, a wing region comprises 11 or more nucleosides. In some embodiments, a wing region comprises 12 or more nucleosides. In some embodiments, a wing region comprises 13 or more nucleosides. In some embodiments, a wing region comprises 14 or more nucleosides. In some embodiments, a wing region comprises 15 or more nucleosides.

[00371] In some embodiments, provided oligonucleotides comprise two wing and one core regions. In some embodiments, provided oligonucleotides comprises a 5'-wing-core-wing-3' structure. In some embodiments, provided oligonucleotides are of a 5'-wing-core-wing-3' gapmer structure. In some embodiments, the two wing regions are identical. In some embodiments, the two wing regions are different. In some embodiments, the two wing regions are identical in chemical modifications. In some embodiments, the two wing regions are identical in 2'-modifications. In some embodiments, the two wing regions are identical in internucleotidic linkage modifications. In some embodiments, the two wing regions are identical in patterns of backbone chiral centers. In some embodiments, the two wing regions are identical in pattern of backbone linkages. In some embodiments, the two wing regions are identical in pattern of backbone linkage types. In some embodiments, the two wing regions are identical in pattern of backbone phosphorus modifications.

[00372] In some embodiments, provided oligonucleotides comprise one wing and one core regions. In some embodiments, provided oligonucleotides comprises a 5'-wing-core-3' hemimer structure. In some embodiments, provided oligonucleotides are of a 5'-wing-core-3' hemimer structure. In some embodiments, provided oligonucleotides comprises a 5'-core-wing-3' hemimer structure. In some embodiments, provided oligonucleotides are of a 5'-core-wing-3' hemimer structure.

[00373] A wing region can be differentiated from a core region in that a wing region contains a different structure feature than a core region. For example, in some embodiments, a wing region differs from a core region in that they have different sugar modifications, base modifications, internucleotidic linkages, internucleotidic linkage stereochemistry, *etc.* In some embodiments, a wing region differs from a core region in that they have different 2'-modifications of the sugars.

[00374] In some embodiments, an internucleotidic linkage between a wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic

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

[00375] In some embodiments, an internucleotidic linkage between a wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic linkage between a 5'-wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic linkage between a 3'-wing region and a core region is considered part of the wing region. In some embodiments, an internucleotidic linkage between a wing region and a core region is considered part of the core region. In some embodiments, an internucleotidic linkage between a 5'-wing region and a core region is considered part of the core region. In some embodiments, an internucleotidic linkage between a 3'-wing region and a core region is considered part of the core region.

[00376] In some embodiments, a wing region comprises 2 or more nucleosides. In some embodiments, a wing region comprises 3 or more nucleosides. In some embodiments, a wing region comprises 4 or more nucleosides. In some embodiments, a wing region comprises 5 or more nucleosides. In some embodiments, a wing region comprises 6 or more nucleosides. In some embodiments, a wing region comprises 7 or more nucleosides. In some embodiments, a wing region comprises 8 or more nucleosides. In some embodiments, a wing region comprises 9 or more nucleosides. In some embodiments, a wing region comprises 10 or more nucleosides. In some embodiments, a wing region comprises 11 or more nucleosides. In some embodiments, a wing region comprises 12 or more nucleosides. In some embodiments, a wing region comprises 13 or more nucleosides. In some embodiments, a wing region comprises 14 or more nucleosides. In some embodiments, a wing region comprises 15 or more nucleosides.

[00377] In some embodiments, a wing region comprises 2 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 3 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 4 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 5 or more modified internucleotidic linkages.

internucleotidic linkages. In some embodiments, a wing region comprises 6 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 7 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 8 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 9 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 10 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 11 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 12 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 13 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 14 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 15 or more modified internucleotidic linkages.

[00378] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides which:

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

[00379] In some embodiments, a provided oligonucleotide composition is characterized in that, when it is contacted with the transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof.

[00380] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides of a particular oligonucleotide type defined by:

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

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

the oligonucleotide composition being characterized in that, when it is contacted with the

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

[00381] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising one or more wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence; and
each wing region independently comprises one or more modified internucleotidic linkages and optionally one or more natural phosphate linkages, and the core region independently comprises one or more modified internucleotidic linkages; or

each wing region independently comprises one or more modified sugar moieties, and the core region comprises one or more un-modified sugar moieties.

[00382] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising one or more wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence; and
each wing region independently comprises one or more modified internucleotidic linkages and optionally one or more natural phosphate linkages, and the core region independently comprises one or more modified internucleotidic linkages.

[00383] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising one or more wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence; and
each wing region independently comprises one or more modified sugar moieties, and the core region comprises one or more un-modified sugar moieties.

[00384] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising one or more wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence; and
each wing region independently comprises one or more modified internucleotidic linkages and optionally one or more natural phosphate linkages, and the core region

independently comprises one or more modified internucleotidic linkages; and

each wing region independently comprises one or more modified sugar moieties, and the core region comprises one or more un-modified sugar moieties.

[00385] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising one or more wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence; and

each wing region independently comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages, and the core region independently comprises one or more modified internucleotidic linkages; and

each wing region independently comprises one or more modified sugar moieties, and the core region comprises one or more un-modified sugar moieties.

[00386] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising one or more wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

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

the core region independently has a length of two or more bases and independently comprises one or more modified internucleotidic linkages.

[00387] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising one or more wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

each wing region independently has a length of two or more bases, and independently comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages; and

the core region independently has a length of two or more bases and independently comprises one or more modified internucleotidic linkages.

[00388] In some embodiments, the present disclosure provides an oligonucleotide

composition comprising a first plurality of oligonucleotides comprising two wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

each wing region independently has a length of two or more bases, and independently comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages; and

the core region independently has a length of two or more bases and independently comprises one or more modified internucleotidic linkages.

[00389] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising two wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

each wing region independently has a length of two or more bases, and independently comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages;

the wing region to the 5'-end of the core region comprises at least one modified internucleotidic linkage followed by a natural phosphate linkage in the wing; and

the wing region to the 3'-end of the core region comprises at least one modified internucleotidic linkage preceded by a natural phosphate linkage in the wing;

the core region independently has a length of two or more bases and independently comprises one or more modified internucleotidic linkages.

[00390] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising a wing region and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

the wing region has a length of two or more bases, and comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages;

the wing region is to the 5'-end of the core region and comprises a modified internucleotidic linkage between the two nucleosides at its 3'-end, or the wing region to the 3'-end of the core region and comprises a modified internucleotidic linkage between the two nucleosides at its 5'-end; and

the core region independently has a length of two or more bases and independently comprises one or more modified internucleotidic linkages.

[00391] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides comprising two wing regions and a core region, wherein:

oligonucleotides of the first plurality have the same base sequence;

each wing region independently has a length of two or more bases, and independently comprises one or more modified internucleotidic linkages and one or more natural phosphate linkages;

the wing region to the 5'-end of the core region comprises a modified internucleotidic linkage between the two nucleosides at its 3'-end;

the wing region to the 3'-end of a core region comprises a modified internucleotidic linkage between the two nucleosides at its 5'-end; and

the core region independently has a length of two or more bases and independently comprises one or more modified internucleotidic linkages.

[00392] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more nucleoside units comprising -F.

[00393] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more consecutive nucleoside units comprising -F.

[00394] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more consecutive nucleoside units comprising 2'-F modified sugar moieties..

[00395] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more consecutive nucleoside units comprising 2'-F modified sugar moieties, and 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more modified internucleotidic linkages.

[00396] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more consecutive nucleoside units comprising 2'-F modified sugar moieties, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more modified internucleotidic linkages, and 2, 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages.

[00397] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more consecutive nucleoside units comprising 2'-F modified sugar moieties, and 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more chirally controlled modified internucleotidic linkages.

[00398] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more consecutive nucleoside units comprising 2'-F modified sugar moieties, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more chirally controlled modified internucleotidic linkages, and 2, 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages.

[00399] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a predetermined level of first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise a 5'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising 2'-F modified sugar moieties, and 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more modified internucleotidic linkages, and optionally a 3'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising 2'-F modified sugar moieties.

[00400] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a predetermined level of first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise a 5'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising 2'-F modified sugar moieties, and 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more modified internucleotidic linkages, and a 3'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising 2'-F modified sugar moieties.

[00401] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a predetermined level of first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise a 5'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising 2'-F modified sugar moieties wherein the first nucleoside unit of the consecutive nucleoside units is the first nucleoside unit of the oligonucleotide, and 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more modified internucleotidic linkages, and a 3'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising 2'-F modified sugar moieties wherein the last nucleoside unit of the consecutive nucleoside units is the last nucleoside unit of the oligonucleotide.

[00402] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise 2, 3, 4, 5, 6, 7, 8, 9, 10 or more

consecutive nucleoside units comprising 2'-F modified sugar moieties, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more modified internucleotidic linkages, and 2, 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages.

[00403] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a predetermined level of first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise a 5'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising 2'-F modified sugar moieties, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more modified internucleotidic linkages, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages, and a 3'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising 2'-F modified sugar moieties.

[00404] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a predetermined level of first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise a 5'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising 2'-F modified sugar moieties wherein the first nucleoside unit of the consecutive nucleoside units is the first nucleoside unit of the oligonucleotide, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more modified internucleotidic linkages, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages, and a 3'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising 2'-F modified sugar moieties wherein the last nucleoside unit of the consecutive nucleoside units is the last nucleoside unit of the oligonucleotide.

[00405] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a predetermined level of first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise a 5'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising 2'-F modified sugar moieties wherein the first nucleoside unit of the consecutive nucleoside units is the first nucleoside unit of the

oligonucleotide, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more modified internucleotidic linkages, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages, a 3'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising 2'-F modified sugar moieties wherein the last nucleoside unit of the consecutive nucleoside units is the last nucleoside unit of the oligonucleotide, and 2, 3, 4, 5, 6, 7, 8, 9, 10 or more sugar moieties comprising 2'-OR¹ modifications, wherein R¹ is optionally substituted C₁₋₆ aliphatic.

[00406] In some embodiments, a 5'-end region comprises 2 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 5'-end region comprises 3 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 5'-end region comprises 4 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 5'-end region comprises 5 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 5'-end region comprises 6 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 5'-end region comprises 7 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 5'-end region comprises 8 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 5'-end region comprises 9 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 5'-end region comprises 10 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 3'-end region comprises 2 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 3'-end region comprises 3 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 3'-end region comprises 4 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 3'-end region comprises 5 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 3'-end region comprises 6 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 3'-end region comprises 7 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 3'-end region comprises 8 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 3'-end region comprises 9 or more consecutive nucleoside units comprising 2'-F. In some embodiments, a 3'-end region comprises 10 or more consecutive nucleoside units comprising 2'-F.

[00407] In some embodiments, each of the consecutive nucleoside units is independently preceded and/or followed by a modified internucleotidic linkage. In some embodiments, each of the consecutive nucleoside units is independently preceded and/or followed by a phosphorothioate linkage. In some embodiments, each of the consecutive nucleoside units is

independently preceded and/or followed by a chirally controlled modified internucleotidic linkage. In some embodiments, each of the consecutive nucleoside units is independently preceded and/or followed by a chirally controlled phosphorothioate linkage. In some embodiments, a modified internucleotidic linkage has a structure of formula **I**. In some embodiments, a modified internucleotidic linkage has a structure of formula **I-a**.

[00408] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a predetermined level of first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise a 5'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive *Sp* modified internucleotidic linkages, a 3'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive *Sp* modified internucleotidic linkages, and a middle region between the 5'-end region and the 3'-region comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages.

[00409] In some embodiments, a 5'-end region comprises 2 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 5'-end region comprises 3 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 5'-end region comprises 4 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 5'-end region comprises 5 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 5'-end region comprises 6 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 5'-end region comprises 7 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 5'-end region comprises 8 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 5'-end region comprises 9 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 5'-end region comprises 10 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 3'-end region comprises 2 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 3'-end region comprises 3 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 3'-end region comprises 4 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 3'-end region comprises 5 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 3'-end region comprises 6 or more consecutive *Sp* modified internucleotidic linkages. In some

embodiments, a 3'-end region comprises 7 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 3'-end region comprises 8 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 3'-end region comprises 9 or more consecutive *Sp* modified internucleotidic linkages. In some embodiments, a 3'-end region comprises 10 or more consecutive *Sp* modified internucleotidic linkages.

[00410] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a predetermined level of first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise a 5'-end region comprising 4, 5, 6, 7, 8, 9, 10 or more consecutive *Sp* modified internucleotidic linkages, a 3'-end region comprising 4, 5, 6, 7, 8, 9, 10 or more consecutive *Sp* modified internucleotidic linkages, and a middle region between the 5'-end region and the 3'-region comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages.

[00411] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a predetermined level of first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise a 5'-end region comprising 5, 6, 7, 8, 9, 10 or more consecutive *Sp* modified internucleotidic linkages, a 3'-end region comprising 5, 6, 7, 8, 9, 10 or more consecutive *Sp* modified internucleotidic linkages, and a middle region between the 5'-end region and the 3'-region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages.

[00412] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a predetermined level of first plurality of oligonucleotides, wherein:

oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise a 5'-end region comprising 6, 7, 8, 9, 10 or more consecutive *Sp* modified internucleotidic linkages, a 3'-end region comprising 6, 7, 8, 9, 10 or more consecutive *Sp* modified internucleotidic linkages, and a middle region between the 5'-end region and the 3'-region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate

linkages.

[00413] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a predetermined level of first plurality of oligonucleotides, wherein:

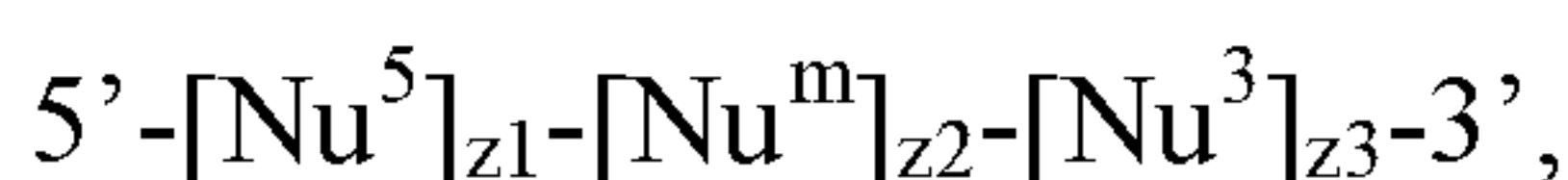
oligonucleotides of the first plurality have the same base sequence;

oligonucleotides of the first plurality comprise a 5'-end region comprising 6, 7, 8, 9, 10 or more consecutive *Sp* modified internucleotidic linkages, a 3'-end region comprising 6, 7, 8, 9, 10 or more consecutive *Sp* modified internucleotidic linkages, and a middle region between the 5'-end region and the 3'-region comprising 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages.

[00414] In some embodiments, a modified internucleotidic linkage has a structure of formula **I**. In some embodiments, a modified internucleotidic linkage has a structure of formula **I-a**.

[00415] As demonstrated in the present disclosure, in some embodiments, a provided oligonucleotide composition is characterized in that, when it is contacted with the transcript in a transcript splicing system, splicing of the transcript is altered relative to that observed under reference conditions selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof. In some embodiments, a desired splicing product is increased 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 fold or more. In some embodiments, a desired splicing reference is absent (*e.g.*, cannot be reliably detected by quantitative PCR) under reference conditions. In some embodiments, as exemplified in the present disclosure, levels of the plurality of oligonucleotides, *e.g.*, a first plurality of oligonucleotides, in provided compositions are pre-determined.

[00416] In some embodiments, a provide oligonucleotide composition comprising a first plurality of oligonucleotides, each of which has the structure of:



wherein:

each Nu^5 is independently a nucleotidic unit, and at least one is Nu^S or Nu^F ;

each Nu^3 is independently a nucleotidic unit, and at least one is Nu^S or Nu^F ;

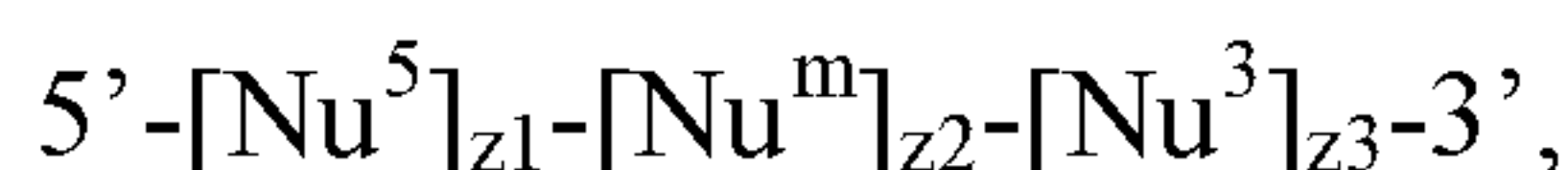
each Nu^{S} is independently a nucleotidic unit comprising a modified internucleotidic linkage;

each Nu^{F} is independently a nucleotidic unit comprises $-\text{F}$;

each of z_1 , z_2 and z_3 is independently 0-100, wherein at least one of z_1 , z_2 and z_3 is not 0; and

each Nu^{m} is independently a nucleotidic unit.

[00417] In some embodiments, a provide oligonucleotide composition comprising a first plurality of oligonucleotides, each of which has the structure of:



wherein:

each Nu^{S} is independently a nucleotidic unit, and at least one is Nu^{S} or Nu^{F} ;

each Nu^{F} is independently a nucleotidic unit, and at least one is Nu^{S} or Nu^{F} ;

each Nu^{S} is independently a nucleotidic unit comprising a modified internucleotidic linkage;

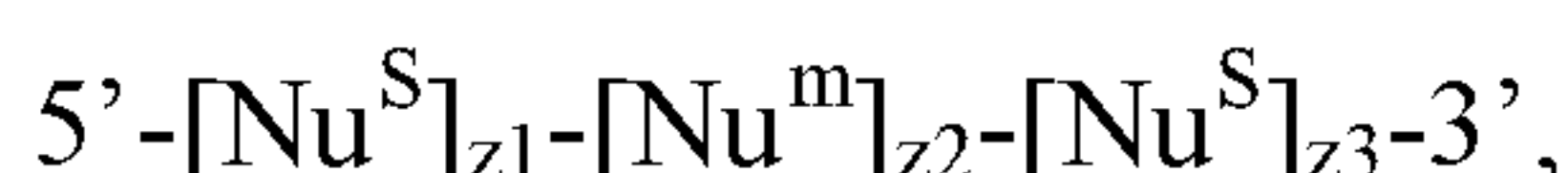
each Nu^{F} is independently a nucleotidic unit comprises $-\text{F}$;

each of z_1 , z_2 and z_3 is independently 0-100, wherein at least one of z_1 , z_2 and z_3 is not 0;

each Nu^{m} is independently a nucleotidic unit; and

the first plurality of oligonucleotides are structurally identical.

[00418] In some embodiments, a provide oligonucleotide composition comprising a first plurality of oligonucleotides, each of which has the structure of:



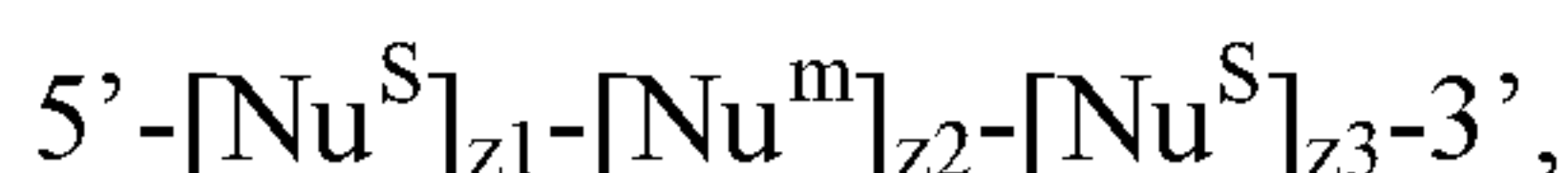
wherein:

each Nu^{S} is independently a nucleotidic unit comprising a modified internucleotidic linkage;

each of z_1 , z_2 and z_3 is independently 0-100, wherein at least one of z_1 , z_2 and z_3 is not 0; and

each Nu^{m} is independently a nucleotidic unit.

[00419] In some embodiments, a provide oligonucleotide composition comprising a first plurality of oligonucleotides, each of which has the structure of:



wherein:

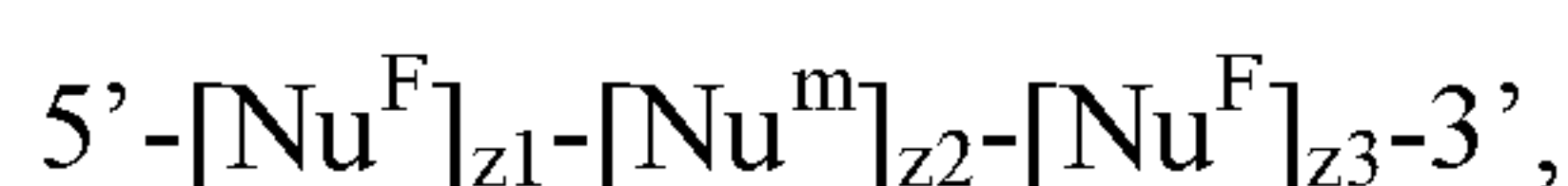
each Nu^{S} is independently a nucleotidic unit comprising a modified internucleotidic linkage;

each of z_1 , z_2 and z_3 is independently 0-100, wherein at least one of z_1 , z_2 and z_3 is not 0;

each Nu^{m} is independently a nucleotidic unit; and

the first plurality of oligonucleotides are structurally identical.

[00420] In some embodiments, a provide oligonucleotide composition comprising a first plurality of oligonucleotides, each of which has the structure of:



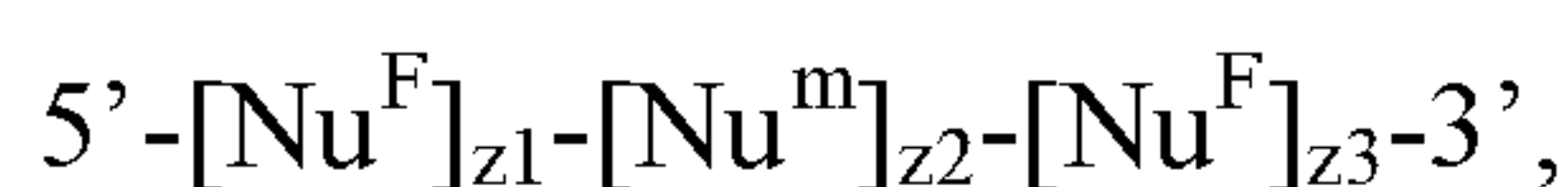
wherein:

each Nu^{F} is independently a nucleotidic unit comprises $-\text{F}$;

each of z_1 , z_2 and z_3 is independently 0-100, wherein at least one of z_1 , z_2 and z_3 is not 0; and

each Nu^{m} is independently a nucleotidic unit.

[00421] In some embodiments, a provide oligonucleotide composition comprising a first plurality of oligonucleotides, each of which has the structure of:



wherein:

each Nu^{F} is independently a nucleotidic unit comprises $-\text{F}$;

each of z_1 , z_2 and z_3 is independently 0-100, wherein at least one of z_1 , z_2 and z_3 is not 0;

each Nu^{m} is independently a nucleotidic unit; and

the first plurality of oligonucleotides are structurally identical.

[00422] In some embodiments, A^{c} is $-5' - [\text{Nu}^{\text{S}}]_{z_1} - [\text{Nu}^{\text{m}}]_{z_2} - [\text{Nu}^{\text{S}}]_{z_3} - 3'$. In some embodiments, A^{c} is $-5' - [\text{Nu}^{\text{S}}]_{z_1} - [\text{Nu}^{\text{m}}]_{z_2} - [\text{Nu}^{\text{S}}]_{z_3} - 3'$. In some embodiments, A^{c} is $-5' - [\text{Nu}^{\text{F}}]_{z_1} - [\text{Nu}^{\text{m}}]_{z_2} - [\text{Nu}^{\text{F}}]_{z_3} - 3'$.

[00423] In some embodiments, at least one Nu^{S} is Nu^{S} . In some embodiments, at least one Nu^{S} is Nu^{S} . In some embodiments, at least one Nu^{S} is Nu^{S} and at least one Nu^{S} is Nu^{S} . In some embodiments, each Nu^{S} and Nu^{S} is independently Nu^{S} . In some embodiments, at least one Nu^{S} is Nu^{F} . In some embodiments, at least one Nu^{S} is Nu^{F} . In some embodiments, at least one Nu^{S} is

Nu^{F} and at least one Nu^3 is Nu^{F} . In some embodiments, each Nu^5 and Nu^3 is independently Nu^{F} .

[00424] In some embodiments, Nu^{S} comprises a modified internucleotidic linkage having the structure of formula **I**. In some embodiments, Nu^{S} comprises a modified internucleotidic linkage having the structure of formula **I-a**. In some embodiments, a modified internucleotidic linkage is a phosphorothioate linkage. In some embodiments, a modified internucleotidic linkage is chirally controlled. In some embodiments, a modified internucleotidic linkage is chirally controlled and *Sp*. In some embodiments, each modified internucleotidic linkage is chirally controlled and *Sp*. In some embodiments, a modified internucleotidic linkage is chirally controlled and *Rp*. In some embodiments, each modified internucleotidic linkage is chirally controlled and *Rp*. In some embodiments, each Nu^{S} comprises a phosphorothioate linkage. In some embodiments, each Nu^{S} comprises a chirally controlled phosphorothioate linkage. In some embodiments, each Nu^{S} comprises a chirally controlled *Sp* phosphorothioate linkage. In some embodiments, each Nu^{S} comprises a chirally controlled *Rp* phosphorothioate linkage. In some embodiments, Nu^{S} comprises $-\text{F}$. In some embodiments, Nu^{S} comprises a sugar moiety comprising $-\text{F}$. In some embodiments, Nu^{S} comprises a 2'- F sugar moiety. In some embodiments, each Nu^{S} comprises a 2'- F sugar moiety. In some embodiments, Nu^{S} comprises a modified sugar moiety. In some embodiments, Nu^{S} comprises a 2'-modified sugar moiety. In some embodiments, Nu^{S} comprises a 2'- R^1 modified sugar moiety. In some embodiments, Nu^{S} comprises a 2'- OR^1 modified sugar moiety. In some embodiments, Nu^{S} comprises a 2'- OR^1 modified sugar moiety, wherein R^1 is optionally substituted C_{1-6} aliphatic. In some embodiments, Nu^{S} comprises a 2'-MOE modified sugar moiety. In some embodiments, Nu^{S} comprises a 2'-OMe modified sugar moiety.

[00425] In some embodiments, Nu^{F} comprises a sugar moiety comprising $-\text{F}$. In some embodiments, Nu^{F} comprises a 2'- F sugar moiety. In some embodiments, each Nu^{F} comprises a 2'- F sugar moiety. In some embodiments, Nu^{F} comprises a modified internucleotidic linkage. In some embodiments, Nu^{F} comprises a modified internucleotidic linkage of formula **I**. In some embodiments, Nu^{S} comprises a modified internucleotidic linkage having the structure of formula **I-a**. In some embodiments, Nu^{F} comprises a chirally controlled modified internucleotidic linkage. In some embodiments, Nu^{F} comprises a chirally controlled, *Sp* modified internucleotidic linkage. In some embodiments, Nu^{F} comprises a phosphorothioate linkage. In some embodiments, Nu^{F} comprises a chirally controlled phosphorothioate linkage. In some

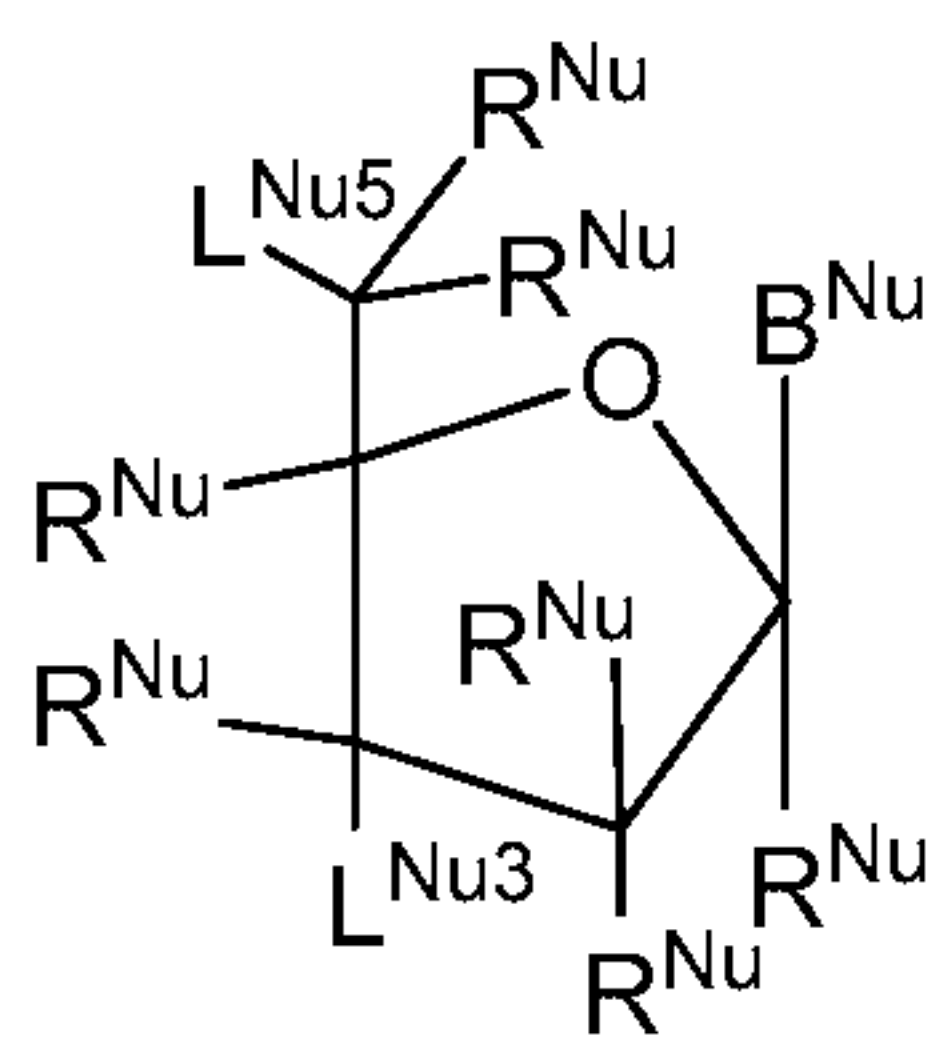
embodiments, Nu^{F} comprises a chirally controlled *Sp* phosphorothioate linkage. In some embodiments, Nu^{F} is a 2'-F phosphorothioate unit. In some embodiments, Nu^{F} is a chirally controlled 2'-F phosphorothioate unit. In some embodiments, Nu^{F} is a chirally controlled *Sp* 2'-F phosphorothioate unit. In some embodiments, each Nu^{F} is a chirally controlled *Sp* 2'-F phosphorothioate unit. In some embodiments, a modified internucleotidic linkage has the structure of formula **I**. In some embodiments, a modified internucleotidic linkage has the structure of formula **I-a**.

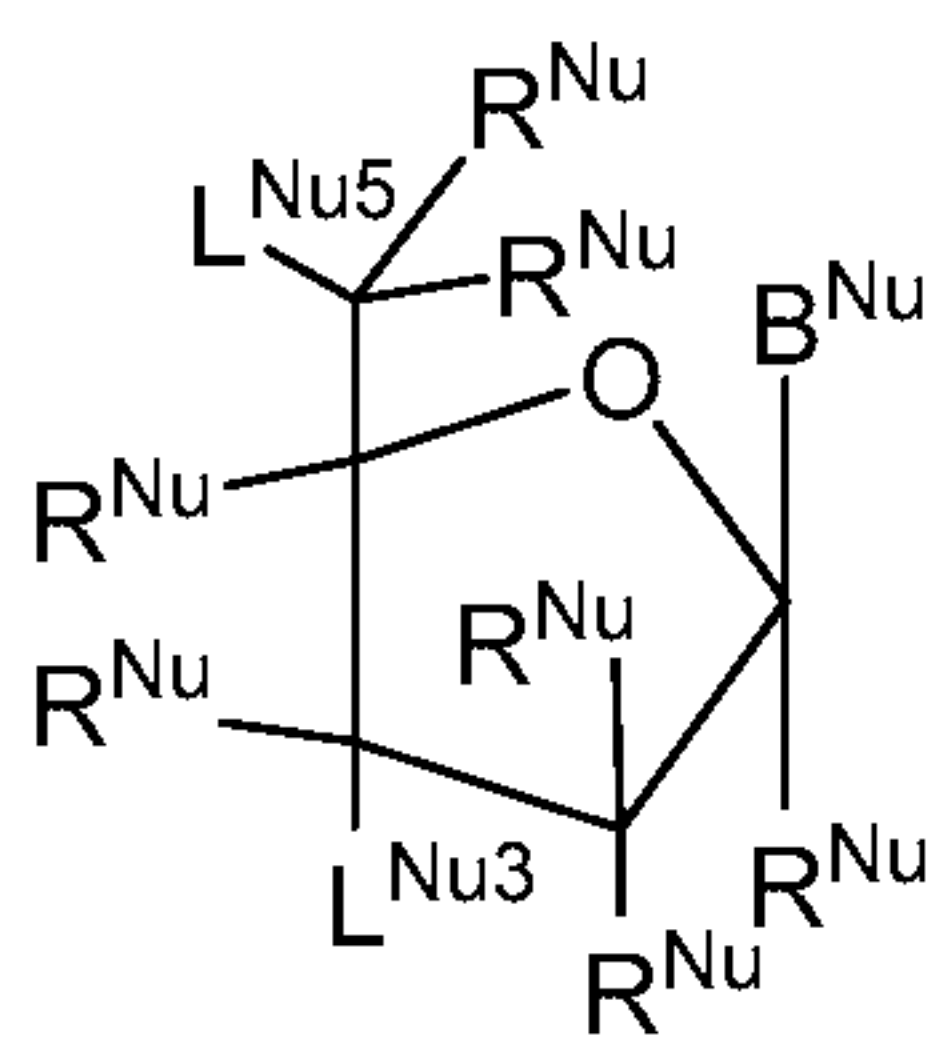
[00426] In some embodiments, Nu^{m} comprises a modified sugar moiety. In some embodiments, Nu^{m} comprises a 2'-modified sugar moiety. In some embodiments, Nu^{m} comprises a 2'-OR¹ modified sugar moiety. In some embodiments, Nu^{m} comprises a 2'-F modified sugar moiety. In some embodiments, at least one Nu^{m} comprises a 2'-OR¹ modified sugar moiety. In some embodiments, at least one Nu^{m} comprises a 2'-OR¹ modified sugar moiety, wherein R¹ is optionally substituted C₁₋₆ alkyl. In some embodiments, at least one Nu^{m} comprises a 2'-F modified sugar moiety. In some embodiments, at least one Nu^{m} comprises a 2'-OR¹ modified sugar moiety, and at least one Nu^{m} comprises a 2'-F modified sugar moiety. In some embodiments, at least one Nu^{m} comprises a 2'-OR¹ modified sugar moiety, and at least one Nu^{m} comprises a 2'-F modified sugar moiety, wherein R¹ is optionally substituted C₁₋₆ aliphatic. In some embodiments, at least one Nu^{m} comprises a 2'-OMe modified sugar moiety, and at least one Nu^{m} comprises a 2'-F modified sugar moiety.

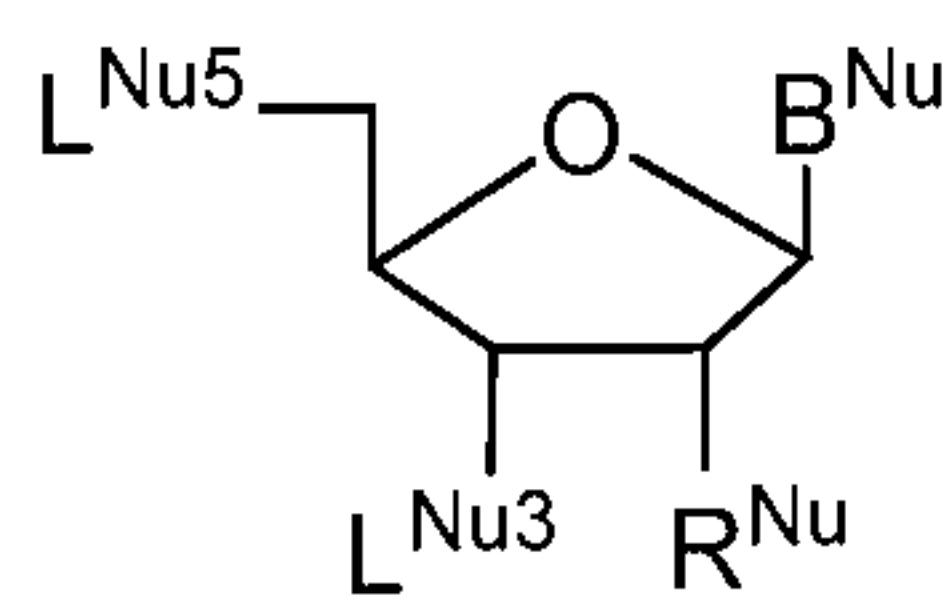
[00427] In some embodiments, Nu^{m} comprises a natural phosphate linkage. In some embodiments, Nu^{m} comprises a modified internucleotidic linkage. In some embodiments, Nu^{m} comprises a phosphorothioate linkage. In some embodiments, Nu^{m} comprises a chirally controlled modified internucleotidic linkage. In some embodiments, Nu^{m} comprises a chirally controlled *Sp* modified internucleotidic linkage. In some embodiments, Nu^{m} comprises a chirally controlled *Rp* modified internucleotidic linkage. In some embodiments, Nu^{m} comprises a chirally controlled phosphorothioate linkage. In some embodiments, Nu^{m} comprises a chirally controlled *Sp* phosphorothioate linkage. In some embodiments, Nu^{m} comprises a chirally controlled *Rp* phosphorothioate linkage. In some embodiments, at least one Nu^{m} comprises a natural phosphate linkage, and at least one Nu^{m} comprises a modified internucleotidic linkage. In some embodiments, at least one Nu^{m} comprises a natural phosphate linkage, and at least one Nu^{m} comprises a chirally controlled modified internucleotidic linkage. In some embodiments, at

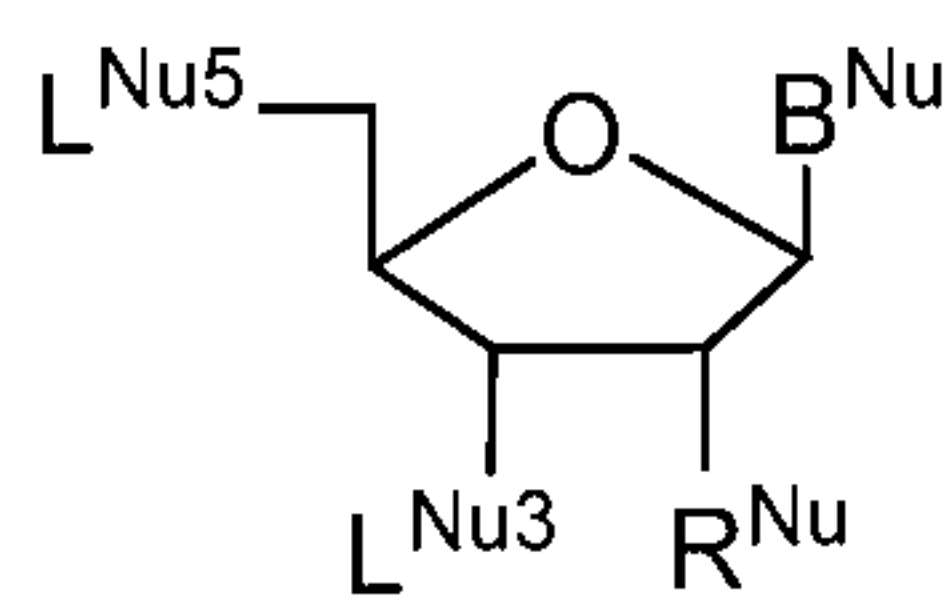
least one Nu^m comprises a natural phosphate linkage, and at least one Nu^m comprises a chirally controlled *Sp* modified internucleotidic linkage. In some embodiments, at least one Nu^m comprises a natural phosphate linkage, and at least one Nu^m comprises a chirally controlled *Rp* modified internucleotidic linkage. In some embodiments, at least one Nu^m comprises a natural phosphate linkage, and at least one Nu^m comprises a phosphorothioate linkage. In some embodiments, at least one Nu^m comprises a natural phosphate linkage, and at least one Nu^m comprises a chirally controlled phosphorothioate linkage. In some embodiments, at least one Nu^m comprises a natural phosphate linkage, and at least one Nu^m comprises a chirally controlled *Sp* phosphorothioate linkage. In some embodiments, at least one Nu^m comprises a natural phosphate linkage, and at least one Nu^m comprises a chirally controlled *Rp* phosphorothioate linkage. In some embodiments, a modified internucleotidic linkage has the structure of formula **I**. In some embodiments, a modified internucleotidic linkage has the structure of formula **I-a**.

[00428] In some embodiments, each of Nu^S, Nu^F and Nu^m independently has the structure



of , wherein B^{Nu} is an optionally substituted nucleobase; each R^{Nu} is independently R¹, R', -L-R¹, or -L-R', wherein each of R¹, R', and L is independently as defined and described; L^{Nu5} is a covalent bond, or if at the 5'-end of an oligonucleotide, R^{Nu}; and L^{Nu3} is a internucleotidic linkage having the structure of formula **I**, or if at the 3'-end of an oligonucleotide, R^{Nu}. In some embodiments, each of Nu^S, Nu^F and Nu^m independently has the



structure of . In some embodiments, B^{Nu} is optionally substituted A, T, C, G and U. In some embodiments, two R^{Nu} groups are taken together with their intervening atoms to form a ring system. In some embodiments, the 2'-R^{Nu} is 2'-OR¹, in some embodiments, 2'-OMe, 2'-MOE, *etc.* In some embodiments, the 2'-R^{Nu} is 2'-F. In some embodiments, L^{Nu5} is a covalent bond. In some embodiments, L^{Nu5} is 5'-OH or protected 5'-OH. In some embodiments, L^{Nu3} has the structure of formula **I**. In some embodiments, L^{Nu3} is 3'-OH or protected 3'-OH.

[00429] In some embodiments, z1 is not 0. In some embodiments, z3 is not 0. In some

embodiments, z_1 and z_3 are not zero. In some embodiments, the sum of z_1 , z_2 and z_3 are 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 50 or more. In some embodiments, the sum of z_1 , z_2 , and z_3 are 15 or more. In some embodiments, the sum of z_1 , z_2 , and z_3 are 16 or more. In some embodiments, the sum of z_1 , z_2 , and z_3 are 17 or more. In some embodiments, the sum of z_1 , z_2 , and z_3 are 18 or more. In some embodiments, the sum of z_1 , z_2 , and z_3 are 19 or more. In some embodiments, the sum of z_1 , z_2 , and z_3 are 20 or more.

[00430] In some embodiments, z_1 is 0. In some embodiments, z_1 is 1. In some embodiments, z_1 is 2. In some embodiments, z_1 is 3. In some embodiments, z_1 is 4. In some embodiments, z_1 is 5. In some embodiments, z_1 is 6. In some embodiments, z_1 is 7. In some embodiments, z_1 is 8. In some embodiments, z_1 is 0. In some embodiments, z_1 is 10. In some embodiments, z_1 is 1 or more. In some embodiments, z_1 is 2 or more. In some embodiments, z_1 is 3 or more. In some embodiments, z_1 is 4 or more. In some embodiments, z_1 is 5 or more. In some embodiments, z_1 is 6 or more. In some embodiments, z_1 is 7 or more. In some embodiments, z_1 is 8 or more. In some embodiments, z_1 is 0 or more. In some embodiments, z_1 is 10 or more.

[00431] In some embodiments, z_2 is 0. In some embodiments, z_2 is 1. In some embodiments, z_2 is 2. In some embodiments, z_2 is 3. In some embodiments, z_2 is 4. In some embodiments, z_2 is 5. In some embodiments, z_2 is 6. In some embodiments, z_2 is 7. In some embodiments, z_2 is 8. In some embodiments, z_2 is 0. In some embodiments, z_2 is 10. In some embodiments, z_2 is 1 or more. In some embodiments, z_2 is 2 or more. In some embodiments, z_2 is 3 or more. In some embodiments, z_2 is 4 or more. In some embodiments, z_2 is 5 or more. In some embodiments, z_2 is 6 or more. In some embodiments, z_2 is 7 or more. In some embodiments, z_2 is 8 or more. In some embodiments, z_2 is 0 or more. In some embodiments, z_2 is 10 or more.

[00432] In some embodiments, z_3 is 0. In some embodiments, z_3 is 1. In some embodiments, z_3 is 2. In some embodiments, z_3 is 3. In some embodiments, z_3 is 4. In some embodiments, z_3 is 5. In some embodiments, z_3 is 6. In some embodiments, z_3 is 7. In some embodiments, z_3 is 8. In some embodiments, z_3 is 0. In some embodiments, z_3 is 10. In some embodiments, z_3 is 1 or more. In some embodiments, z_3 is 2 or more. In some embodiments, z_3 is 3 or more. In some embodiments, z_3 is 4 or more. In some embodiments, z_3 is 5 or more. In some embodiments, z_3 is 6 or more. In some embodiments, z_3 is 7 or more. In some

embodiments, $z3$ is 8 or more. In some embodiments, $z3$ is 0 or more. In some embodiments, $z3$ is 10 or more.

[00433] In some embodiments, each of $z1$ and $z3$ is independently 2 or more. In some embodiments, each of $z1$ and $z3$ is independently 3 or more. In some embodiments, each of $z1$ and $z3$ is independently 4 or more. In some embodiments, each of $z1$ and $z3$ is independently 5 or more. In some embodiments, each of $z1$ and $z3$ is independently 6 or more. In some embodiments, each of $z1$ and $z3$ is independently 7 or more. In some embodiments, each of $z1$ and $z3$ is independently 8 or more. In some embodiments, each of $z1$ and $z3$ is independently 9 or more. In some embodiments, each of $z1$ and $z3$ is independently 10 or more. In some embodiments, $z1$ equals $z3$.

[00434] In some embodiments, $[\text{Nu}^5]_{z1}$ is a 5'-wing. In some embodiments, $[\text{Nu}^S]_{z1}$ is a 5'-wing. In some embodiments, $[\text{Nu}^F]_{z1}$ is a 5'-wing. In some embodiments, $[\text{Nu}^m]_{z2}$ is a core. In some embodiments, $[\text{Nu}^3]_{z3}$ is a 3'-wing. In some embodiments, $[\text{Nu}^S]_{z3}$ is a 3'-wing. In some embodiments, $[\text{Nu}^F]_{z3}$ is a 3'-wing. In some embodiments, $[\text{Nu}^5]_{z1}$ is a 5'-wing, $[\text{Nu}^m]_{z2}$ is a core, and $[\text{Nu}^3]_{z3}$ is a 5'-wing. In some embodiments, $[\text{Nu}^S]_{z1}$ is a 5'-wing, $[\text{Nu}^m]_{z2}$ is a core, and $[\text{Nu}^S]_{z3}$ is a 5'-wing. In some embodiments, $[\text{Nu}^F]_{z1}$ is a 5'-wing, $[\text{Nu}^m]_{z2}$ is a core, and $[\text{Nu}^F]_{z3}$ is a 5'-wing.

[00435] In some embodiments, $[\text{Nu}^5]_{z1}$ is a 5'-end region. In some embodiments, $[\text{Nu}^S]_{z1}$ is a 5'-end region. In some embodiments, $[\text{Nu}^F]_{z1}$ is a 5'-end region. In some embodiments, $[\text{Nu}^m]_{z2}$ is a middle region. In some embodiments, $[\text{Nu}^3]_{z3}$ is a 3'-end region. In some embodiments, $[\text{Nu}^S]_{z3}$ is a 3'-end region. In some embodiments, $[\text{Nu}^F]_{z3}$ is a 3'-end region. In some embodiments, $[\text{Nu}^5]_{z1}$ is a 5'-end region, $[\text{Nu}^m]_{z2}$ is a middle region, and $[\text{Nu}^3]_{z3}$ is a 5'-end region. In some embodiments, $[\text{Nu}^S]_{z1}$ is a 5'-end region, $[\text{Nu}^m]_{z2}$ is a middle region, and $[\text{Nu}^S]_{z3}$ is a 5'-end region. In some embodiments, $[\text{Nu}^F]_{z1}$ is a 5'-end region, $[\text{Nu}^m]_{z2}$ is a middle region, and $[\text{Nu}^F]_{z3}$ is a 5'-end region.

[00436] An example composition is WV-1497 ($\text{mG}^*\text{mGmCmAmC}^*\text{A}^*\text{A}^*\text{G}^*\text{G}^*\text{G}^*\text{C}^*\text{A}^*\text{C}^*\text{A}^*\text{G}^*\text{mAmCmUmU}^*\text{mC}$), wherein the core region is $^*\text{A}^*\text{A}^*\text{G}^*\text{G}^*\text{G}^*\text{C}^*\text{A}^*\text{C}^*\text{A}^*\text{G}^*$, the wing region to the 5'-end of the core region is $\text{mG}^*\text{mGmCmAmC}$, and the wing region to the 3'-end of the core region is $\text{mAmCmUmU}^*\text{mC}$. In some embodiments, a wing region comprises a modified internucleotidic linkage between the two nucleosides at its 3'-end. In some embodiments, a wing region to the 5'-end of a core region

comprises a modified internucleotidic linkage between the two nucleosides at its 3'-end. For example, in WV-1497, mG*mGmCmAmC is a wing to the 5'-end of the core region (*A*A*G*G*G*C*A*C*A*G*), and it comprise a modified internucleotidic linkage between the two nucleosides at its 3'-end (mG*mGmCmAmC). In some embodiments, a wing region comprises a modified internucleotidic linkage between the two nucleosides at its 5'-end. In some embodiments, a wing region to the 3'-end of a core region comprises a modified internucleotidic linkage between the two nucleosides at its 5'-end. For example, in WV-1497, mAmCmUmU*mC is a wing to the 3'-end of the core region (*A*A*G*G*G*C*A*C*A*G*), and it comprise a modified internucleotidic linkage between the two nucleosides at its 5'-end (mAmCmUmU*mC). WV-3507 is an example oligonucleotide comprising 5'-end and 3'-end regions (can also be considered rings for WV-3507) comprising consecutive Sp 2'-F phosphorothioate nucleotidic units, and a middle (can also be considered core region for WV-3507) comprising natural phosphate linkages and 2'-OMe sugar modifications.

[00437] In some embodiments, oligonucleotides of the first plurality have two wing and one core regions. In some embodiments, the two wing regions are identical. In some embodiments, the two wing regions are different.

[00438] In some embodiments, a wing region comprises 2 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 3 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 4 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 5 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 6 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 7 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 8 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 9 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 10 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 11 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 12 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 13 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 14 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 15 or more modified internucleotidic linkages. In some embodiments, a wing region comprises 2 or consecutive

modified internucleotidic linkages. In some embodiments, a chiral internucleotidic linkage or a modified internucleotidic linkage has the structure of formula I. In some embodiments, a chiral internucleotidic linkage or a modified internucleotidic linkage is phosphorothioate. In some embodiments, each chiral internucleotidic linkage or a modified internucleotidic linkage independently has the structure of formula I. In some embodiments, each chiral internucleotidic linkage or a modified internucleotidic linkage is phosphorothioate. In some embodiments, a wing region comprises 3 or consecutive modified internucleotidic linkages. In some embodiments, a wing region comprises 4 or consecutive modified internucleotidic linkages. In some embodiments, a wing region comprises 5 or consecutive modified internucleotidic linkages. In some embodiments, a wing region comprises 6 or consecutive modified internucleotidic linkages. In some embodiments, a wing region comprises 7 or consecutive modified internucleotidic linkages. In some embodiments, a wing region comprises 8 or consecutive modified internucleotidic linkages. In some embodiments, a wing region comprises 9 or consecutive modified internucleotidic linkages. In some embodiments, a wing region comprises 10 or consecutive modified internucleotidic linkages. In some embodiments, a wing region comprises 11 or consecutive modified internucleotidic linkages. In some embodiments, a wing region comprises 12 or consecutive modified internucleotidic linkages. In some embodiments, a wing region comprises 13 or consecutive modified internucleotidic linkages. In some embodiments, a wing region comprises 14 or consecutive modified internucleotidic linkages. In some embodiments, a wing region comprises 15 or consecutive modified internucleotidic linkages. In some embodiments, each internucleotidic linkage in a wing region is independently a modified internucleotidic linkage.

[00439] In some embodiments, 5% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 10% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 15% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 20% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 25% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 30% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In

some embodiments, 35% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 40% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 45% or more of the internucleotidic linkages of a wing region are modified internucleotidic linkages. In some embodiments, 50% or more of the internucleotidic linkages of a wing region are modified internucleotidic linkages. In some embodiments, 55% or more of the internucleotidic linkages of a wing region are modified internucleotidic linkages. In some embodiments, 60% or more of the internucleotidic linkages of a wing region are modified internucleotidic linkages. In some embodiments, 65% or more of the internucleotidic linkages of a wing region are modified internucleotidic linkages. In some embodiments, 70% or more of the internucleotidic linkages of a wing region are modified internucleotidic linkages. In some embodiments, 75% or more of the internucleotidic linkages of a wing region are modified internucleotidic linkages. In some embodiments, 80% or more of the internucleotidic linkages of a wing region are modified internucleotidic linkages. In some embodiments, 85% or more of the internucleotidic linkages of a wing region are modified internucleotidic linkages. In some embodiments, 90% or more of the internucleotidic linkages of a wing region are modified internucleotidic linkages. In some embodiments, 95% or more of the internucleotidic linkages of a wing region are modified internucleotidic linkages. In some embodiments, each internucleotidic linkage of a wing region is a modified internucleotidic linkage.

[00440] In some embodiments, a wing region comprises 2 or more natural phosphate linkages. In some embodiments, a wing region comprises 3 or more natural phosphate linkages. In some embodiments, a wing region comprises 4 or more natural phosphate linkages. In some embodiments, a wing region comprises 5 or more natural phosphate linkages. In some embodiments, a wing region comprises 6 or more natural phosphate linkages. In some embodiments, a wing region comprises 7 or more natural phosphate linkages. In some embodiments, a wing region comprises 8 or more natural phosphate linkages. In some embodiments, a wing region comprises 9 or more natural phosphate linkages. In some embodiments, a wing region comprises 10 or more natural phosphate linkages. In some embodiments, a wing region comprises 11 or more natural phosphate linkages. In some embodiments, a wing region comprises 12 or more natural phosphate linkages. In some embodiments, a wing region comprises 13 or more natural phosphate linkages. In some

embodiments, a wing region comprises 14 or more natural phosphate linkages. In some embodiments, a wing region comprises 15 or more natural phosphate linkages. In some embodiments, a wing region comprises 2 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 3 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 4 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 5 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 6 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 7 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 8 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 9 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 10 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 11 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 12 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 13 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 14 or consecutive natural phosphate linkages. In some embodiments, a wing region comprises 15 or consecutive natural phosphate linkages. In some embodiments, each internucleotidic linkage in a wing region is independently a natural phosphate linkage.

[00441] In some embodiments, 5% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 10% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 15% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 20% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 25% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 30% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 35% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 40% or more of the internucleotidic linkages of provided oligonucleotides are natural phosphate linkages. In some embodiments, 45% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 50% or more of the

internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 55% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 60% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 65% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 70% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 75% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 80% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 85% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 90% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, 95% or more of the internucleotidic linkages of a wing region are natural phosphate linkages. In some embodiments, each internucleotidic linkage of a wing region is a natural phosphate linkage.

[00442] In some embodiments, a core region comprises 2 or more modified internucleotidic linkages. In some embodiments, a core region comprises 3 or more modified internucleotidic linkages. In some embodiments, a core region comprises 4 or more modified internucleotidic linkages. In some embodiments, a core region comprises 5 or more modified internucleotidic linkages. In some embodiments, a core region comprises 6 or more modified internucleotidic linkages. In some embodiments, a core region comprises 7 or more modified internucleotidic linkages. In some embodiments, a core region comprises 8 or more modified internucleotidic linkages. In some embodiments, a core region comprises 9 or more modified internucleotidic linkages. In some embodiments, a core region comprises 10 or more modified internucleotidic linkages. In some embodiments, a core region comprises 11 or more modified internucleotidic linkages. In some embodiments, a core region comprises 12 or more modified internucleotidic linkages. In some embodiments, a core region comprises 13 or more modified internucleotidic linkages. In some embodiments, a core region comprises 14 or more modified internucleotidic linkages. In some embodiments, a core region comprises 15 or more modified internucleotidic linkages. In some embodiments, a core region comprises 2 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 3 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises

4 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 5 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 6 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 7 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 8 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 9 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 10 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 11 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 12 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 13 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 14 or consecutive modified internucleotidic linkages. In some embodiments, a core region comprises 15 or consecutive modified internucleotidic linkages. In some embodiments, each internucleotidic linkage in a core region is independently a modified internucleotidic linkage.

[00443] In some embodiments, 5% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 10% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 15% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 20% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 25% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 30% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 35% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 40% or more of the internucleotidic linkages of provided oligonucleotides are modified internucleotidic linkages. In some embodiments, 45% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 50% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 55% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 60% or more of the internucleotidic linkages of a core region are modified

internucleotidic linkages. In some embodiments, 65% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 70% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 75% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 80% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 85% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 90% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, 95% or more of the internucleotidic linkages of a core region are modified internucleotidic linkages. In some embodiments, each internucleotidic linkage of a core region is a modified internucleotidic linkage.

[00444] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a first plurality of 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.

[00445] 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 linkage 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. For example, for WV-1092, the pattern of backbone linkages is 1 x PS(phosphorothioate)-3 x PO (phosphate)-11 x PS-3 x PO-1 x PS. A pattern of backbone chiral centers of an oligonucleotide can be designated by a combination of linkage phosphorus stereochemistry (*Rp/Sp*) from 5' to 3'.

For example, WV-1092 has a pattern of 1S-3PO (phosphate)-8S-1R-2S-3PO-1S. In some embodiments, all non-chiral linkages (*e.g.*, PO) may be omitted. As exemplified above, locations of non-chiral linkages may be obtained, for example, from pattern of backbone linkages.

[00446] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a first plurality of 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.

[00447] 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 tetrathylthiuram disulfide or (TETD) or 3H-1, 2-benzodithiol-3-one 1, 1-dioxide (BDTD), a well-known process in the art. In some embodiments, substantially racemic preparation of oligonucleotides provides substantially racemic oligonucleotide compositions (or chirally uncontrolled oligonucleotide compositions). In some embodiments, at least one coupling of a nucleotide monomer has a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, 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, each coupling of a nucleotide monomer independently has a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, 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, each internucleotidic linkage independently has a diastereoselectivity lower than about 60:40, 70:30, 80:20, 85:15, 90:10, 91:9, 92:8, 97:3, 98:2, or 99:1. In some embodiments, a diastereoselectivity is lower than about 60:40. In some embodiments, a diastereoselectivity is lower than about 70:30. In some embodiments, a diastereoselectivity is lower than about 80:20. In some embodiments, a diastereoselectivity is lower than about 90:10. In some embodiments, a diastereoselectivity is lower than about 91:9. In some embodiments, 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, each coupling independently has 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. In some embodiments, each internucleotidic linkage independently has a diastereoselectivity lower than about 90:10.

[00448] In some embodiments, a chirally controlled internucleotidic linkage, such as those of oligonucleotides of chirally controlled oligonucleotide compositions, has a diastereoselectivity of 90:10 or more. In some embodiments, each chirally controlled internucleotidic linkage, such as those of oligonucleotides of chirally controlled oligonucleotide compositions, has a diastereoselectivity of 90:10 or more. In some embodiments, the selectivity is 91:9 or more. In some embodiments, the selectivity is 92:8 or more. In some embodiments, the selectivity is 97:3 or more. In some embodiments, the selectivity is 94:6 or more. In some embodiments, the selectivity is 95:5 or more. In some embodiments, the selectivity is 96:4 or more. In some embodiments, the selectivity is 97:3 or more. In some embodiments, the selectivity is 98:2 or more. In some embodiments, the selectivity is 99:1 or more.

[00449] 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*SG*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.*

[00450] In some embodiments, the present disclosure provides chirally controlled (and/or stereochemically pure) oligonucleotide compositions comprising a first plurality of 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.

[00451] In some embodiments, the present disclosure provides chirally controlled oligonucleotide composition of a first plurality 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 a first plurality 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.

[00452] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a first plurality of 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.

[00453] 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.

[00454] 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.

[00455] In some embodiments, a chirally controlled oligonucleotide composition is a substantially pure preparation of an oligonucleotide type in that oligonucleotides in the composition that are not of the oligonucleotide type are impurities from the preparation process of said oligonucleotide type, in some case, after certain purification procedures.

[00456] 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.

[00457] 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.

[00458] 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 a first plurality 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.

[00459] 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.

[00460] 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.

[00461] 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 (*Rp/Sp*) of chiral internucleotidic linkages), and the same pattern of backbone phosphorus modifications (*e.g.*, pattern of modifications on the internucleotidic phosphorus atom, such as $-S^-$, and $-L-R^1$ of formula I).

[00462] 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.

[00463] 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. In some embodiments, stereoselectivity of a chiral internucleotidic linkage in an oligonucleotide may be measured through a model reaction, *e.g.* formation of a dimer under essentially the same or comparable conditions wherein the dimer has the same internucleotidic linkage as the chiral internucleotidic linkage, the 5'-nucleoside of the dimer is the same as the nucleoside to the 5'-end of the chiral internucleotidic linkage, and the 3'-nucleoside of the dimer is the same as the nucleoside to the 3'-end of the chiral internucleotidic linkage (*e.g.*, for fU*SfU*SfC*SfU, through the dimer of fU*SfC). As appreciated by a person having ordinary skill in the art, percentage of oligonucleotides of a particular type having n internucleotidic linkages in a preparation may be calculated as $SE^1 * SE^2 * SE^3 * \dots * SE^n$, wherein SE^1 , SE^2 , SE^3 , ..., SE^n is independently the stereoselectivity of the 1st, 2nd, 3rd, ..., and n^{th} chiral internucleotidic linkage. I

[00464] In some embodiments, in provided compositions, at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 97% or 99% of oligonucleotides that have the base sequence of a particular oligonucleotide type (defined by 1) base sequence; 2) pattern of backbone linkages; 3) pattern of backbone chiral centers; and 4) pattern of backbone phosphorus modifications) are oligonucleotides of the particular

oligonucleotide type. In some embodiments, at least 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 97% or 99% of oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of a particular oligonucleotide type are oligonucleotides of the particular oligonucleotide type. In some embodiments, the percentage is at least 0.5%. In some embodiments, the percentage is at least 1%. In some embodiments, the percentage is at least 2%. In some embodiments, the percentage is at least 3%. In some embodiments, the percentage is at least 4%. In some embodiments, the percentage is at least 5%. In some embodiments, the percentage is at least 6%. In some embodiments, the percentage is at least 7%. In some embodiments, the percentage is at least 8%. In some embodiments, the percentage is at least 9%. In some embodiments, the percentage is at least 10%. In some embodiments, the percentage is at least 20%. In some embodiments, the percentage is at least 30%. In some embodiments, the percentage is at least 40%. In some embodiments, the percentage is at least 50%. In some embodiments, the percentage is at least 60%. In some embodiments, the percentage is at least 70%. In some embodiments, the percentage is at least 75%. In some embodiments, the percentage is at least 80%. In some embodiments, the percentage is at least 81%. In some embodiments, the percentage is at least 82%. In some embodiments, the percentage is at least 83%. In some embodiments, the percentage is at least 84%. In some embodiments, the percentage is at least 85%. In some embodiments, the percentage is at least 86%. In some embodiments, the percentage is at least 87%. In some embodiments, the percentage is at least 88%. In some embodiments, the percentage is at least 89%. In some embodiments, the percentage is at least 90%. In some embodiments, the percentage is at least 91%. In some embodiments, the percentage is at least 92%. In some embodiments, the percentage is at least 93%. In some embodiments, the percentage is at least 94%. In some embodiments, the percentage is at least 95%. In some embodiments, the percentage is at least 96%. In some embodiments, the percentage is at least 97%. In some embodiments, the percentage is at least 98%. In some embodiments, the percentage is at least 99%.

[00465] In some embodiments, oligonucleotides of a particular type in a chirally controlled oligonucleotide composition is enriched at least 5 fold (oligonucleotides of the particular type have a fraction of $5 \cdot (1/2^n)$ of oligonucleotides that have the base sequence, the

pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type, wherein n is the number of chiral internucleotidic linkages; or oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type but are not of the particular oligonucleotide type are no more than $[1-(1/2^n)]/5$ of oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type) compared to a stereorandom preparation of the oligonucleotides (oligonucleotides of the particular type are typically considered to have a fraction of $1/2^n$ of oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type, wherein n is the number of chiral internucleotidic linkages, and oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type but are not of the particular oligonucleotide type are typically considered to have a fraction of $[1-(1/2^n)]$ of oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type). In some embodiments, the enrichment is at least 20 fold. In some embodiments, the enrichment is at least 30 fold. In some embodiments, the enrichment is at least 40 fold. In some embodiments, the enrichment is at least 50 fold. In some embodiments, the enrichment is at least 60 fold. In some embodiments, the enrichment is at least 70 fold. In some embodiments, the enrichment is at least 80 fold. In some embodiments, the enrichment is at least 90 fold. In some embodiments, the enrichment is at least 100 fold. In some embodiments, the enrichment is at least 200 fold. In some embodiments, the enrichment is at least 300 fold. In some embodiments, the enrichment is at least 400 fold. In some embodiments, the enrichment is at least 500 fold. In some embodiments, the enrichment is at least 600 fold. In some embodiments, the enrichment is at least 700 fold. In some embodiments, the enrichment is at least 800 fold. In some embodiments, the enrichment is at least 900 fold. In some embodiments, the enrichment is at least 1,000 fold. In some embodiments, the enrichment is at least 2,000 fold. In some embodiments, the enrichment is at least 4,000 fold. In some embodiments, the enrichment is at least 8,000 fold. In some embodiments, the enrichment is at least 10,000 fold. In some embodiments, the enrichment is at least 20,000 fold. In some embodiments, the enrichment is at least $(1.5)^n$. In some embodiments, the enrichment is at least

(1.6)ⁿ. In some embodiments, the enrichment is at least (1.7)ⁿ. In some embodiments, the enrichment is at least (1.1)ⁿ. In some embodiments, the enrichment is at least (1.8)ⁿ. In some embodiments, the enrichment is at least (1.9)ⁿ. In some embodiments, the enrichment is at least 2ⁿ. In some embodiments, the enrichment is at least 3ⁿ. In some embodiments, the enrichment is at least 4ⁿ. In some embodiments, the enrichment is at least 5ⁿ. In some embodiments, the enrichment is at least 6ⁿ. In some embodiments, the enrichment is at least 7ⁿ. In some embodiments, the enrichment is at least 8ⁿ. In some embodiments, the enrichment is at least 9ⁿ. In some embodiments, the enrichment is at least 10ⁿ. In some embodiments, the enrichment is at least 15ⁿ. In some embodiments, the enrichment is at least 20ⁿ. In some embodiments, the enrichment is at least 25ⁿ. In some embodiments, the enrichment is at least 30ⁿ. In some embodiments, the enrichment is at least 40ⁿ. In some embodiments, the enrichment is at least 50ⁿ. In some embodiments, the enrichment is at least 100ⁿ. In some embodiments, enrichment is measured by increase of the fraction of oligonucleotides of the particular oligonucleotide type in oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type. In some embodiments, an enrichment is measured by decrease of the fraction of oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type but are not of the particular oligonucleotide type in oligonucleotides that have the base sequence, the pattern of backbone linkages, and the pattern of backbone phosphorus modifications of the particular oligonucleotide type.

[00466] 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.

[00467] In some embodiments, the present disclosure provides an oligonucleotide composition comprising a predetermined level of a first plurality 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, wherein the lipids are optionally and independently conjugated to one or more oligonucleotides of the plurality.

[00468] 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 *Sp*/all *Rp*, (*SpRp*) repeats/all *Rp*, *etc.*), backbone phosphorus modification types (*e.g.*, s1/s2, s1/s3, *etc.*), *etc.*

[00469] 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'-OR¹. 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. In some embodiments, a wing and core is defined by internucleotidic linkages, wherein a wing comprises a internucleotidic linkage type (*e.g.*, natural phosphate linkage, a type of modified internucleotidic linkage, *etc.*) that the core region does not have. In some embodiments, a wing and core is defined by internucleotidic linkages, wherein a wing has a pattern of backbone linkage that is different from that of the core.

[00470] 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.

[00471] In some embodiments, provided chirally controlled (and/or stereochemically pure) preparations are antisense oligonucleotides (*e.g.*, chimerasen). 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-microRNAs, antimir, supermir, ribozyme, UI adaptor, RNA activator, RNAi agent, decoy oligonucleotide, triplex forming oligonucleotide, aptamer or adjuvant. In some embodiments, a chirally controlled oligonucleotide composition is of antisense oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of antagomir

oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of microRNA oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of pre-microRNA oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of antimir oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of supermir oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of ribozyme oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of UI adaptor oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of RNA activator oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of RNAi agent oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of decoy oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of triplex forming oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of aptamer oligonucleotides. In some embodiments, a chirally controlled oligonucleotide composition is of adjuvant oligonucleotides.

[00472] 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.

[00473] 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.

[00474] 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 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.

[00475] 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%.

[00476] In some embodiments, such a provided purity can be of one or more chiral internucleotidic linkage is a composition is partially chirally controlled.

[00477] 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 *Sp* conformation. In some embodiments, at least about 10% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Sp* conformation. In some embodiments, at least about 20% chiral phosphorothioate

internucleotidic linkages of a provided oligonucleotide are of the *Sp* conformation. In some embodiments, at least about 30% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Sp* conformation. In some embodiments, at least about 40% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Sp* conformation. In some embodiments, at least about 50% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Sp* conformation. In some embodiments, at least about 60% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Sp* conformation. In some embodiments, at least about 70% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Sp* conformation. In some embodiments, at least about 80% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Sp* conformation. In some embodiments, at least about 90% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Sp* conformation. In some embodiments, at least about 95% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Sp* conformation.

[00478] 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 *Rp* conformation. In some embodiments, at least about 10% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, at least about 20% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, at least about 30% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, at least about 40% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, at least about 50% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, at least about 60% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, at least about 70% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, at least about 80% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, at least about 90% chiral

phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, at least about 95% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation.

[00479] 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 *Rp* conformation. In some embodiments, less than about 10% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, less than about 20% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, less than about 30% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, less than about 40% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, less than about 50% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, less than about 60% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, less than about 70% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, less than about 80% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, less than about 90% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, less than about 95% chiral phosphorothioate internucleotidic linkages of a provided oligonucleotide are of the *Rp* conformation. In some embodiments, a provided oligonucleotide has only one *Rp* chiral phosphorothioate internucleotidic linkages. In some embodiments, a provided oligonucleotide has only one *Rp* chiral phosphorothioate internucleotidic linkages, wherein all internucleotide linkages are chiral phosphorothioate internucleotidic linkages.

[00480] 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 *Rp*.

[00481] 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 modified bases are described above and herein.

[00482] 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.

[00483] 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.

[00484] 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.

[00485] 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.

[00486] 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.

[00487] In some embodiments, provided compositions comprise oligonucleotides containing one or more residues which are modified at the sugar moiety. In some embodiments, provided compositions comprise oligonucleotides containing one or more residues which are modified at the 2' position of the sugar moiety (referred to herein as a "2'-modification"). Examples of such modifications are described 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 compositions comprise oligonucleotides containing one or more residues which are 2'-modified. For example, in some embodiments, provided oligonucleotides contain one or more residues which are 2'-O-methoxyethyl (2'-MOE)-modified residues. In some embodiments, provided compositions comprise oligonucleotides which do not contain any 2'-modifications. In some embodiments, provided compositions are oligonucleotides which do not contain any 2'-MOE residues. That is, in some embodiments, provided oligonucleotides are not MOE-modified. Additional example sugar modifications are described in the present disclosure.

[00488] 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 generally as X-Y-X). In some embodiments, each wing region independently contains one or more residues having a particular modification, which modification is absent from the core "Y" portion. In some embodiments, each wing region 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 region 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 region 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’-OR¹, wherein R¹ is as defined and described in the present disclosure.

[00489] 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.

[00490] 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.

[00491] 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.

[00492] 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.

[00493] In some embodiments, each wing region independently comprises one or more chiral internucleotidic linkages. In some embodiments, each wing region independently comprises one or more natural phosphate linkages. In some embodiments, each wing region independently comprises one or more chiral internucleotidic linkages and one or more natural phosphate linkages. In some embodiments, each wing region independently comprises one or more chiral internucleotidic linkages and two or more natural phosphate linkages. In some embodiments, each wing region independently comprises one or more chiral internucleotidic linkages and two or more natural phosphate linkages, wherein two or more natural phosphate linkages are consecutive.

[00494] In some embodiments, each wing region independently comprises at least one

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

[00495] In some embodiments, each wing region independently comprises one chiral internucleotidic linkage. In some embodiments, each wing region independently comprises two chiral internucleotidic linkages. In some embodiments, each wing region independently comprises three chiral internucleotidic linkages. In some embodiments, each wing region independently comprises four chiral internucleotidic linkages. In some embodiments, each wing region independently comprises five chiral internucleotidic linkages. In some embodiments, each wing region independently comprises six chiral internucleotidic linkages. In some embodiments, each wing region independently comprises seven chiral internucleotidic linkages.

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

[00496] In some embodiments, each wing region independently comprises at least one consecutive natural phosphate linkage. In some embodiments, each wing region independently comprises at least two consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises at least three consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises at least four consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises at least five consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises at least six consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises at least seven consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises at least eight consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises at least nine consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises at least ten consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises at least 11 consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises at least 12 consecutive chiral internucleotidic linkages. In some

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

[00497] In some embodiments, each wing region independently comprises one consecutive natural phosphate linkage. In some embodiments, each wing region independently comprises two consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises three consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises four consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises five consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises six consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises seven consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises eight consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises nine consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises ten consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises 11 consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises 12 consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises 13 consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises 14 consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises 15 consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises 16 consecutive chiral

internucleotidic linkages. In some embodiments, each wing region independently comprises 17 consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises 18 consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises 19 consecutive chiral internucleotidic linkages. In some embodiments, each wing region independently comprises 20 consecutive chiral internucleotidic linkages.

[00498] In some embodiments, each wing region independently comprises at least one natural phosphate linkage. In some embodiments, each wing region independently comprises at least two natural phosphate linkages. In some embodiments, each wing region independently comprises at least three natural phosphate linkages. In some embodiments, each wing region independently comprises at least four natural phosphate linkages. In some embodiments, each wing region independently comprises at least five natural phosphate linkages. In some embodiments, each wing region independently comprises at least six natural phosphate linkages. In some embodiments, each wing region independently comprises at least seven natural phosphate linkages. In some embodiments, each wing region independently comprises at least eight natural phosphate linkages. In some embodiments, each wing region independently comprises at least nine natural phosphate linkages. In some embodiments, each wing region independently comprises at least ten natural phosphate linkages. In some embodiments, each wing region independently comprises at least 11 natural phosphate linkages. In some embodiments, each wing region independently comprises at least 12 natural phosphate linkages. In some embodiments, each wing region independently comprises at least 13 natural phosphate linkages. In some embodiments, each wing region independently comprises at least 14 natural phosphate linkages. In some embodiments, each wing region independently comprises at least 15 natural phosphate linkages. In some embodiments, each wing region independently comprises at least 16 natural phosphate linkages. In some embodiments, each wing region independently comprises at least 17 natural phosphate linkages. In some embodiments, each wing region independently comprises at least 18 natural phosphate linkages. In some embodiments, each wing region independently comprises at least 19 natural phosphate linkages. In some embodiments, each wing region independently comprises at least 20 natural phosphate linkages.

[00499] In some embodiments, each wing region independently comprises one natural

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

[00500] In some embodiments, each wing region independently comprises at least one consecutive natural phosphate linkage. In some embodiments, each wing region independently comprises at least two consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises at least three consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises at least four consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises at least five consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises at least six consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises at least seven consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises at least eight consecutive natural phosphate linkages. In some embodiments, each wing region

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

[00501] In some embodiments, each wing region independently comprises one consecutive natural phosphate linkage. In some embodiments, each wing region independently comprises two consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises three consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises four consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises five consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises six consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises seven consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises eight consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises nine consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises ten consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises 11 consecutive natural phosphate linkages. In some embodiments, each wing region independently comprises 12 consecutive natural phosphate linkages. In some embodiments, each wing region

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

[00502] In some embodiments, a wing is to the 5'-end of a core (5'-end wing). In some embodiments, a wing is to the 3'-end of a core (3'-end wing). For example, in WV-1092 (mG*SmGmCmAmC*SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*SmAmCmUmU*SmC), mG*SmGmCmAmC is a 5'-end wing, *SA*SA*SG*SG*SG*SC*SA*SC*RA*SG*S is a core, and mAmCmUmU*SmC is a 3'-end wing.

[00503] In some embodiments, a 5'-end wing comprises one or more modified internucleotidic linkages and one or more natural phosphate internucleotidic linkages. In some embodiments, a 3'-end wing comprises one or more modified internucleotidic linkages and one or more natural phosphate internucleotidic linkages. In some embodiments, each wing independently comprises one or more modified internucleotidic linkages and one or more natural phosphate internucleotidic linkages. For example, WV-1092 has a 5'-end wing comprises one or more modified internucleotidic linkages and one or more natural phosphate internucleotidic linkages, and a 3'-end wing comprises one or more modified internucleotidic linkages and one or more natural phosphate internucleotidic linkages.

[00504] In some embodiments, a 5'-end wing comprises a modified internucleotidic linkage having one or more natural phosphate linkages connecting two or more nucleosides after (to the 3'-end) the modified internucleotidic linkage in the 5'-end wing. For example, a 5'-end wing mG*SmGmCmAmC comprises a modified internucleotidic linkage (mG*SmG) which has three natural phosphate linkages connecting four nucleosides (mGmCmAmC) after the modified internucleotidic linkage in the 5'-end wing. In some embodiments, a 5'-end wing comprises a modified internucleotidic linkages followed by one or more natural phosphate linkages and/or

one or more modified internucleotidic linkages, which are followed by one or more natural phosphate linkages in the 5'-end wing (for example, mG*SmG and mG*SmC in mG*SmG*SmCmAmC). In some embodiments, a 5'-end wing comprises a modified internucleotidic linkages followed by one or more natural phosphate linkages in the 5'-end wing. In some embodiments, a 5'-end wing comprises a modified internucleotidic linkages followed by one or more consecutive natural phosphate linkages in the 5'-end wing. In some embodiments, a 5'-end wing comprises a natural phosphate linkage between the two nucleosides at its 3'-end. For example, a 5'-end wing mG*SmGmCmAmC has a natural phosphate linkage between the two nucleosides at its 3'-end (mG*SmGmCmAmC).

[00505] In some embodiments, a 3'-end wing comprises a modified internucleotidic linkage having one or more natural phosphate linkages connecting two or more nucleosides before (to the 5'-end) the modified internucleotidic linkage in the 3'-end wing. For example, a 3'-end wing mAmCmUmU*SmC comprises a modified internucleotidic linkage (mU*SmC) which has three natural phosphate linkages connecting four nucleosides (mAmCmUmU) before the modified internucleotidic linkage in the 3'-end wing. In some embodiments, a 3'-end wing comprises a modified internucleotidic linkages preceded by one or more natural phosphate linkages and/or one or more modified internucleotidic linkages, which are preceded by one or more natural phosphate linkages in the 3'-end wing (for example, mU*SmU and mU*SmC in mAmCmU*SmU*SmC). In some embodiments, a 3'-end wing comprises a modified internucleotidic linkages preceded by one or more natural phosphate linkages in the 3'-end wing. In some embodiments, a 3'-end wing comprises a modified internucleotidic linkages preceded by one or more consecutive natural phosphate linkages in the 3'-end wing. In some embodiments, a 3'-end wing comprises a natural phosphate linkage between the two nucleosides at its 5'-end. For example, a 3'-end wing having the structure of mAmCmUmU*SmC has a natural phosphate linkage between the two nucleosides at its 5'-end (mAmCmUmU*SmC).

[00506] In some embodiments, one or more is one. In some embodiments, one or more is two. In some embodiments, one or more is three. In some embodiments, one or more is four. In some embodiments, one or more is five. In some embodiments, one or more is six. In some embodiments, one or more is seven. In some embodiments, one or more is eight. In some embodiments, one or more is nine. In some embodiments, one or more is ten. In some embodiments, one or more is at least one. In some embodiments, one or more is at least two. In

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

[00507] 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 *Rp*. 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 *Sp*. 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 *Rp*. 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 *Sp*.

[00508] 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.

[00509] 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*SA*SA*SG*SG*SG*SC*S**
A*SC*RA*SG*SmAmCmUmU*SmC, the underlined linkages may be considered as part of the core (bolded), its 5'-wing (having 2'-OMe on sugar moieties) has one single *Sp* phosphorothioate linkages at its 5'- end, its 3'-wing (having 2'-OMe on sugar moieties) has one *Sp* phosphorothioate linkage at its 3'-end, and its core has no 2'-modifications on sugar).

[00510] 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.

[00511] 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.

[00512] 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.

[00513] In some embodiments, a sugar moiety without a 2'-modification is a sugar moiety found in a natural DNA nucleoside.

[00514] 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 *Rp*. In some embodiments, the only chiral internucleotidic linkage in the 5'-wing is *Sp*. In some embodiments, the only chiral internucleotidic linkage in the 3'-wing is *Rp*. In some embodiments, the only chiral internucleotidic linkage in the 3'-wing is *Sp*. In some embodiments, the only chiral internucleotidic linkage in both the 5'- and the 3'-wings are *Sp*. In some embodiments, the only chiral internucleotidic linkage in both the 5'- and the 3'-

wings are *Rp*. In some embodiments, the only chiral internucleotidic linkage in the 5'-wing is *Sp*, and the only chiral internucleotidic linkage in the 3'-wing is *Rp*. In some embodiments, the only chiral internucleotidic linkage in the 5'-wing is *Rp*, and the only chiral internucleotidic linkage in the 3'-wing is *Sp*.

[00515] 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.

[00516] 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 *Rp*. In some embodiments, both chiral internucleotidic linkages in the 5'-wing are *Sp*. 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 *Rp*. In some embodiments, chiral internucleotidic linkages in the 5'- and 3'-wings are *Sp*. In some embodiments, chiral internucleotidic linkages in the 5'- and 3'-wings have different stereochemistry.

[00517] In some embodiments, a chiral, modified phosphate linkage is a chiral phosphorothioate linkage, i.e., phosphorothioate internucleotidic linkage. In some embodiments, a wing region 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 wing region are of the *Sp* conformation. In some embodiments, at least about 10% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Sp* conformation. In some embodiments, at least about 20% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Sp* conformation. In some embodiments, at least about 30% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Sp* conformation. In some embodiments, at least about 40% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Sp* conformation. In some embodiments, at least about 50% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Sp* conformation. In some embodiments, at least about 60% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Sp* conformation. In some embodiments, at least about 70% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Sp* conformation. In some embodiments, at least about 80% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Sp* conformation. In some embodiments, at least about 90% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Sp* conformation. In some embodiments, at least about 95% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Sp* conformation.

[00518] In some embodiments, at least about 1 chiral phosphorothioate internucleotidic linkage of a wing region is of the *Sp* conformation. In some embodiments, at least about 2 chiral

phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 3 chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 4 chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 5 chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 6 chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 7 chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 8 chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 9 chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation.

[00519] In some embodiments, at least about 2 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 3 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 4 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 5 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 6 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 7 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 8 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation. In some embodiments, at least about 9 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the Sp conformation.

[00520] In some embodiments, at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 10% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 20% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 30% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at

least about 40% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 50% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 60% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 70% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 80% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 90% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 95% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation.

[00521] In some embodiments, less than about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, less than about 10% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, less than about 20% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, less than about 30% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, less than about 40% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, less than about 50% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, less than about 60% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, less than about 70% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, less than about 80% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, less than about 90% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, less than about 95% chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, a wing region has only one *Rp* chiral phosphorothioate internucleotidic linkages. In some embodiments, a wing region has only one *Rp* chiral phosphorothioate internucleotidic linkages, wherein all internucleotide linkages are chiral

phosphorothioate internucleotidic linkages.

[00522] In some embodiments, at least about 1 chiral phosphorothioate internucleotidic linkage of a wing region is of the *Rp* conformation. In some embodiments, at least about 2 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 3 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 4 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 5 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 6 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 7 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 8 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 9 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation.

[00523] In some embodiments, at least about 2 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 3 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 4 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 5 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 6 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 7 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 8 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 9 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation.

[00524] In some embodiments, a wing comprises one or more modified sugar moieties. In some embodiments, a wing comprises two or more modified sugar moieties. In some embodiments, a wing comprises three or more modified sugar moieties. In some embodiments, a wing comprises four or more modified sugar moieties. In some embodiments, a wing comprises

five or more modified sugar moieties. In some embodiments, a wing comprises six or more modified sugar moieties. In some embodiments, a wing comprises seven or more modified sugar moieties. In some embodiments, a wing comprises eight or more modified sugar moieties. In some embodiments, a wing comprises nine or more modified sugar moieties. In some embodiments, a wing comprises ten or more modified sugar moieties. In some embodiments, a wing comprises 11 or more modified sugar moieties. In some embodiments, a wing comprises 12 or more modified sugar moieties. In some embodiments, a wing comprises 13 or more modified sugar moieties. In some embodiments, a wing comprises 14 or more modified sugar moieties. In some embodiments, a wing comprises 15 or more modified sugar moieties. In some embodiments, a wing comprises 16 or more modified sugar moieties. In some embodiments, a wing comprises 17 or more modified sugar moieties. In some embodiments, a wing comprises 18 or more modified sugar moieties. In some embodiments, a wing comprises 19 or more modified sugar moieties. In some embodiments, a wing comprises 20 or more modified sugar moieties. In some embodiments, a wing comprises 21 or more modified sugar moieties. In some embodiments, a wing comprises 22 or more modified sugar moieties. In some embodiments, a wing comprises 23 or more modified sugar moieties. In some embodiments, a wing comprises 24 or more modified sugar moieties. In some embodiments, a wing comprises 25 or more modified sugar moieties. In some embodiments, a wing comprises 30 or more modified sugar moieties. In some embodiments, a wing comprises 35 or more modified sugar moieties.

[00525] In some embodiments, a wing comprises one or more 2'-modified sugar moieties. In some embodiments, a wing comprises two or more 2'-modified sugar moieties. In some embodiments, a wing comprises three or more 2'-modified sugar moieties. In some embodiments, a wing comprises four or more 2'-modified sugar moieties. In some embodiments, a wing comprises five or more 2'-modified sugar moieties. In some embodiments, a wing comprises six or more 2'-modified sugar moieties. In some embodiments, a wing comprises seven or more 2'-modified sugar moieties. In some embodiments, a wing comprises eight or more 2'-modified sugar moieties. In some embodiments, a wing comprises nine or more 2'-modified sugar moieties. In some embodiments, a wing comprises ten or more 2'-modified sugar moieties. In some embodiments, a wing comprises 11 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 12 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 13 or more 2'-modified sugar moieties. In some embodiments,

a wing comprises 14 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 15 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 16 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 17 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 18 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 19 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 20 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 21 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 22 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 23 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 24 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 25 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 30 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 35 or more 2'-modified sugar moieties.

[00526] In some embodiments, a wing comprises one or more 2'-F. In some embodiments, a wing comprises two or more 2'-F. In some embodiments, a wing comprises three or more 2'-F. In some embodiments, a wing comprises four or more 2'-F. In some embodiments, a wing comprises five or more 2'-F. In some embodiments, a wing comprises six or more 2'-F. In some embodiments, a wing comprises seven or more 2'-F. In some embodiments, a wing comprises eight or more 2'-F. In some embodiments, a wing comprises nine or more 2'-F. In some embodiments, a wing comprises ten or more 2'-F. In some embodiments, a wing comprises 11 or more 2'-F. In some embodiments, a wing comprises 12 or more 2'-F. In some embodiments, a wing comprises 13 or more 2'-F. In some embodiments, a wing comprises 14 or more 2'-F. In some embodiments, a wing comprises 15 or more 2'-F. In some embodiments, a wing comprises 16 or more 2'-F. In some embodiments, a wing comprises 17 or more 2'-F. In some embodiments, a wing comprises 18 or more 2'-F. In some embodiments, a wing comprises 19 or more 2'-F. In some embodiments, a wing comprises 20 or more 2'-F. In some embodiments, a wing comprises 21 or more 2'-F. In some embodiments, a wing comprises 22 or more 2'-F. In some embodiments, a wing comprises 23 or more 2'-F. In some embodiments, a wing comprises 24 or more 2'-F. In some embodiments, a wing comprises 25 or more 2'-F. In some embodiments, a wing comprises 30 or more 2'-F. In some embodiments, a wing comprises 35 or more 2'-F.

[00527] In some embodiments, a wing comprises one 2'-F. In some embodiments, a wing

comprises two 2'-F. In some embodiments, a wing comprises three 2'-F. In some embodiments, a wing comprises four 2'-F. In some embodiments, a wing comprises five 2'-F. In some embodiments, a wing comprises six 2'-F. In some embodiments, a wing comprises seven 2'-F. In some embodiments, a wing comprises eight 2'-F. In some embodiments, a wing comprises nine 2'-F. In some embodiments, a wing comprises ten 2'-F. In some embodiments, a wing comprises 11 2'-F. In some embodiments, a wing comprises 12 2'-F. In some embodiments, a wing comprises 13 2'-F. In some embodiments, a wing comprises 14 2'-F. In some embodiments, a wing comprises 15 2'-F. In some embodiments, a wing comprises 16 2'-F. In some embodiments, a wing comprises 17 2'-F. In some embodiments, a wing comprises 18 2'-F. In some embodiments, a wing comprises 19 2'-F. In some embodiments, a wing comprises 20 2'-F. In some embodiments, a wing comprises 21 2'-F. In some embodiments, a wing comprises 22 2'-F. In some embodiments, a wing comprises 23 2'-F. In some embodiments, a wing comprises 24 2'-F. In some embodiments, a wing comprises 25 2'-F. In some embodiments, a wing comprises 30 2'-F. In some embodiments, a wing comprises 35 2'-F.

[00528] In some embodiments, a wing comprises one or more consecutive 2'-F. In some embodiments, a wing comprises two or more consecutive 2'-F. In some embodiments, a wing comprises three or more consecutive 2'-F. In some embodiments, a wing comprises four or more consecutive 2'-F. In some embodiments, a wing comprises five or more consecutive 2'-F. In some embodiments, a wing comprises six or more consecutive 2'-F. In some embodiments, a wing comprises seven or more consecutive 2'-F. In some embodiments, a wing comprises eight or more consecutive 2'-F. In some embodiments, a wing comprises nine or more consecutive 2'-F. In some embodiments, a wing comprises ten or more consecutive 2'-F. In some embodiments, a wing comprises 11 or more consecutive 2'-F. In some embodiments, a wing comprises 12 or more consecutive 2'-F. In some embodiments, a wing comprises 13 or more consecutive 2'-F. In some embodiments, a wing comprises 14 or more consecutive 2'-F. In some embodiments, a wing comprises 15 or more consecutive 2'-F. In some embodiments, a wing comprises 16 or more consecutive 2'-F. In some embodiments, a wing comprises 17 or more consecutive 2'-F. In some embodiments, a wing comprises 18 or more consecutive 2'-F. In some embodiments, a wing comprises 19 or more consecutive 2'-F. In some embodiments, a wing comprises 20 or more consecutive 2'-F. In some embodiments, a wing comprises 21 or more consecutive 2'-F. In some embodiments, a wing comprises 22 or more consecutive 2'-F.

In some embodiments, a wing comprises 23 or more consecutive 2'-F. In some embodiments, a wing comprises 24 or more consecutive 2'-F. In some embodiments, a wing comprises 25 or more consecutive 2'-F. In some embodiments, a wing comprises 30 or more consecutive 2'-F. In some embodiments, a wing comprises 35 or more consecutive 2'-F.

[00529] In some embodiments, a wing comprises one consecutive 2'-F. In some embodiments, a wing comprises two consecutive 2'-F. In some embodiments, a wing comprises three consecutive 2'-F. In some embodiments, a wing comprises four consecutive 2'-F. In some embodiments, a wing comprises five consecutive 2'-F. In some embodiments, a wing comprises six consecutive 2'-F. In some embodiments, a wing comprises seven consecutive 2'-F. In some embodiments, a wing comprises eight consecutive 2'-F. In some embodiments, a wing comprises nine consecutive 2'-F. In some embodiments, a wing comprises ten consecutive 2'-F. In some embodiments, a wing comprises 11 consecutive 2'-F. In some embodiments, a wing comprises 12 consecutive 2'-F. In some embodiments, a wing comprises 13 consecutive 2'-F. In some embodiments, a wing comprises 14 consecutive 2'-F. In some embodiments, a wing comprises 15 consecutive 2'-F. In some embodiments, a wing comprises 16 consecutive 2'-F. In some embodiments, a wing comprises 17 consecutive 2'-F. In some embodiments, a wing comprises 18 consecutive 2'-F. In some embodiments, a wing comprises 19 consecutive 2'-F. In some embodiments, a wing comprises 20 consecutive 2'-F. In some embodiments, a wing comprises 21 consecutive 2'-F. In some embodiments, a wing comprises 22 consecutive 2'-F. In some embodiments, a wing comprises 23 consecutive 2'-F. In some embodiments, a wing comprises 24 consecutive 2'-F. In some embodiments, a wing comprises 25 consecutive 2'-F. In some embodiments, a wing comprises 30 consecutive 2'-F. In some embodiments, a wing comprises 35 consecutive 2'-F.

[00530] In some embodiments, in vitro studies performed with provided oligonucleotides were performed with gymnotic introduction of the nucleic acids into cells (without transfection reagent). In some embodiments, experimental data has shown that effects of 2'-modifications are not independent of the manner of introduction of oligonucleotides into cells. In some embodiments, for example, some oligonucleotides which were fully 2'-F modified had higher efficacy when transfected (with transfection reagent), particularly at low concentrations.

[00531] 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.

[00532] In some embodiments, a core comprises one or more modified internucleotidic linkages. In some embodiments, a core comprises one or more natural phosphate linkages. In

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

[00533] In some embodiments, a core comprises at least one natural phosphate linkage. In some embodiments, a core comprises at least two natural phosphate linkages. In some embodiments, a core comprises at least three natural phosphate linkages. In some embodiments, a core comprises at least four natural phosphate linkages. In some embodiments, a core comprises at least five natural phosphate linkages. In some embodiments, a core comprises at least six natural phosphate linkages. In some embodiments, a core comprises at least seven natural phosphate linkages. In some embodiments, a core comprises at least eight natural phosphate linkages. In some embodiments, a core comprises at least nine natural phosphate linkages. In some embodiments, a core comprises at least ten natural phosphate linkages. In some embodiments, a core comprises at least two modified internucleotidic linkages. In some embodiments, a core comprises at least three modified internucleotidic linkages. In some embodiments, a core comprises at least four modified internucleotidic linkages. In some embodiments, a core comprises at least five modified internucleotidic linkages. In some embodiments, a core comprises at least six modified internucleotidic linkages. In some embodiments, a core comprises at least seven modified internucleotidic linkages. In some embodiments, a core comprises at least eight modified internucleotidic linkages. In some embodiments, a core comprises at least nine modified internucleotidic linkages. In some embodiments, a core comprises at least ten modified internucleotidic linkages. In some embodiments, a core comprises at least 11 modified internucleotidic linkages. In some embodiments, a core comprises at least 12 modified internucleotidic linkages. In some embodiments, a core comprises at least 13 modified internucleotidic linkages. In some embodiments, a core comprises at least 14 modified internucleotidic linkages. In some embodiments, a core comprises at least 15 modified internucleotidic linkages. In some embodiments, a core comprises at least 16 modified internucleotidic linkages. In some embodiments, a core comprises at least 17 modified internucleotidic linkages. In some embodiments, a core comprises at least 18 modified internucleotidic linkages. In some embodiments, a core comprises at least 19 modified internucleotidic linkages. In some

embodiments, a core comprises at least 20 modified internucleotidic linkages.

[00534] 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 natural phosphate linkages. In some embodiments, each core linkage is a chiral internucleotidic linkage.

[00535] 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.

[00536] 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.

[00537] 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 Np 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.

[00538] In some embodiments, at least 60% of the chiral internucleotidic linkages in the core region are *Sp*. In some embodiments, at least 65% of the chiral internucleotidic linkages in the core region are *Sp*. In some embodiments, at least 66% of the chiral internucleotidic linkages in the core region are *Sp*. In some embodiments, at least 67% of the chiral internucleotidic linkages in the core region are *Sp*. In some embodiments, at least 70% of the chiral internucleotidic linkages in the core region are *Sp*. In some embodiments, at least 75% of the chiral internucleotidic linkages in the core region are *Sp*. In some embodiments, at least 80% of the chiral internucleotidic linkages in the core region are *Sp*. In some embodiments, at least 85% of the chiral internucleotidic linkages in the core region are *Sp*. In some embodiments, at least 90% of the chiral internucleotidic linkages in the core region are *Sp*. In some embodiments, at least 95% of the chiral internucleotidic linkages in the core region are *Sp*.

[00539] In some embodiments, each chiral internucleotidic linkages in the core region is *Sp*.

[00540] In some embodiments, at least 1 core region internucleotidic linkage is *Sp*. In some embodiments, at least 2 core region internucleotidic linkages are *Sp*. In some embodiments, at least 3 core region internucleotidic linkages are *Sp*. In some embodiments, at least 4 core region internucleotidic linkages are *Sp*. In some embodiments, at least 5 core region internucleotidic linkages are *Sp*. In some embodiments, at least 6 core region internucleotidic linkages are *Sp*. In some embodiments, at least 7 core region internucleotidic linkages are *Sp*. In some embodiments, at least 8 core region internucleotidic linkages are *Sp*. In some embodiments, at least 9 core region internucleotidic linkages are *Sp*. In some embodiments, at least 10 core region internucleotidic linkages are *Sp*. In some embodiments, at least 11 core region internucleotidic linkages are *Sp*. In some embodiments, at least 12 core region internucleotidic linkages are *Sp*. In some embodiments, at least 13 core region internucleotidic linkages are *Sp*. In some embodiments, at least 14 core region internucleotidic linkages are *Sp*. In some embodiments, at least 15 core region internucleotidic linkages are *Sp*. In some embodiments, at least 16 core region internucleotidic linkages are *Sp*. In some embodiments, at least 17 core region internucleotidic linkages are *Sp*. In some embodiments, at least 18 core region internucleotidic linkages are *Sp*. In some embodiments, at least 19 core region

internucleotidic linkages are *Sp*. In some embodiments, at least 20 core region internucleotidic linkages are *Sp*. In some embodiments, at least 21 core region internucleotidic linkages are *Sp*. In some embodiments, at least two core region internucleotidic linkages are *Sp*. In some embodiments, the *Sp* internucleotidic linkages are consecutive.

[00541] In some embodiments, at least 60% of the chiral internucleotidic linkages in the core region are *Rp*. In some embodiments, at least 65% of the chiral internucleotidic linkages in the core region are *Rp*. In some embodiments, at least 66% of the chiral internucleotidic linkages in the core region are *Rp*. In some embodiments, at least 67% of the chiral internucleotidic linkages in the core region are *Rp*. In some embodiments, at least 70% of the chiral internucleotidic linkages in the core region are *Rp*. In some embodiments, at least 75% of the chiral internucleotidic linkages in the core region are *Rp*. In some embodiments, at least 80% of the chiral internucleotidic linkages in the core region are *Rp*. In some embodiments, at least 85% of the chiral internucleotidic linkages in the core region are *Rp*. In some emIn some embodiments, each chiral internucleotidic linkages in the core region is *Rp*.

[00542] In some embodiments, at least 1 core region internucleotidic linkage is *Rp*. In some embodiments, at least 2 core region internucleotidic linkages are *Rp*. In some embodiments, at least 3 core region internucleotidic linkages are *Rp*. In some embodiments, at least 4 core region internucleotidic linkages are *Rp*. In some embodiments, at least 5 core region internucleotidic linkages are *Rp*. In some embodiments, at least 6 core region internucleotidic linkages are *Rp*. In some embodiments, at least 7 core region internucleotidic linkages are *Rp*. In some embodiments, at least 8 core region internucleotidic linkages are *Rp*. In some embodiments, at least 9 core region internucleotidic linkages are *Rp*. In some embodiments, at least 10 core region internucleotidic linkages are *Rp*. In some embodiments, at least 11 core region internucleotidic linkages are *Rp*. In some embodiments, at least 12 core region internucleotidic linkages are *Rp*. In some embodiments, at least 13 core region internucleotidic linkages are *Rp*. In some embodiments, at least 14 core region internucleotidic linkages are *Rp*. In some embodiments, at least 15 core region internucleotidic linkages are *Rp*. In some embodiments, at least 16 core region internucleotidic linkages are *Rp*. In some embodiments, at least 17 core region internucleotidic linkages are *Rp*. In some embodiments, at least 18 core region internucleotidic linkages are *Rp*. In some embodiments, at least 19 core region internucleotidic linkages are *Rp*. In some embodiments, at least 20 core region internucleotidic

linkages are *Rp*. In some embodiments, at least 21 core region internucleotidic linkages are *Rp*. In some embodiments, at least two core region internucleotidic linkages are *Rp*. In some embodiments, the *Rp* internucleotidic linkages are consecutive.

[00543] In some embodiments, a core comprises one or more modified sugar moieties. In some embodiments, a core comprises two or more modified sugar moieties. In some embodiments, a core comprises three or more modified sugar moieties. In some embodiments, a core comprises four or more modified sugar moieties. In some embodiments, a core comprises five or more modified sugar moieties. In some embodiments, a core comprises six or more modified sugar moieties. In some embodiments, a core comprises seven or more modified sugar moieties. In some embodiments, a core comprises eight or more modified sugar moieties. In some embodiments, a core comprises nine or more modified sugar moieties. In some embodiments, a core comprises ten or more modified sugar moieties. In some embodiments, a core comprises 11 or more modified sugar moieties. In some embodiments, a core comprises 12 or more modified sugar moieties. In some embodiments, a core comprises 13 or more modified sugar moieties. In some embodiments, a core comprises 14 or more modified sugar moieties. In some embodiments, a core comprises 15 or more modified sugar moieties. In some embodiments, a core comprises 16 or more modified sugar moieties. In some embodiments, a core comprises 17 or more modified sugar moieties. In some embodiments, a core comprises 18 or more modified sugar moieties. In some embodiments, a core comprises 19 or more modified sugar moieties. In some embodiments, a core comprises 20 or more modified sugar moieties. In some embodiments, a core comprises 21 or more modified sugar moieties. In some embodiments, a core comprises 22 or more modified sugar moieties. In some embodiments, a core comprises 23 or more modified sugar moieties. In some embodiments, a core comprises 24 or more modified sugar moieties. In some embodiments, a core comprises 25 or more modified sugar moieties. In some embodiments, a core comprises 30 or more modified sugar moieties. In some embodiments, a core comprises 35 or more modified sugar moieties. In some embodiments, a 2'-modification is 2'-OR¹. In some embodiments, a 2'-modification is 2'-OMe.

[00544] In some embodiments, a core comprises one or more 2'-modified sugar moieties. In some embodiments, a core comprises two or more 2'-modified sugar moieties. In some embodiments, a core comprises three or more 2'-modified sugar moieties. In some embodiments, a core comprises four or more 2'-modified sugar moieties. In some embodiments,

a core comprises five or more 2'-modified sugar moieties. In some embodiments, a core comprises six or more 2'-modified sugar moieties. In some embodiments, a core comprises seven or more 2'-modified sugar moieties. In some embodiments, a core comprises eight or more 2'-modified sugar moieties. In some embodiments, a core comprises nine or more 2'-modified sugar moieties. In some embodiments, a core comprises ten or more 2'-modified sugar moieties. In some embodiments, a core comprises 11 or more 2'-modified sugar moieties. In some embodiments, a core comprises 12 or more 2'-modified sugar moieties. In some embodiments, a core comprises 13 or more 2'-modified sugar moieties. In some embodiments, a core comprises 14 or more 2'-modified sugar moieties. In some embodiments, a core comprises 15 or more 2'-modified sugar moieties. In some embodiments, a core comprises 16 or more 2'-modified sugar moieties. In some embodiments, a core comprises 17 or more 2'-modified sugar moieties. In some embodiments, a core comprises 18 or more 2'-modified sugar moieties. In some embodiments, a core comprises 19 or more 2'-modified sugar moieties. In some embodiments, a core comprises 20 or more 2'-modified sugar moieties. In some embodiments, a core comprises 21 or more 2'-modified sugar moieties. In some embodiments, a core comprises 22 or more 2'-modified sugar moieties. In some embodiments, a core comprises 23 or more 2'-modified sugar moieties. In some embodiments, a core comprises 24 or more 2'-modified sugar moieties. In some embodiments, a core comprises 25 or more 2'-modified sugar moieties. In some embodiments, a core comprises 30 or more 2'-modified sugar moieties. In some embodiments, a core comprises 35 or more 2'-modified sugar moieties. In some embodiments, a 2'-modification is 2'-OR¹. In some embodiments, a 2'-modification is 2'-OMe.

[00545] In some embodiments, at least about 1 chiral phosphorothioate internucleotidic linkage of a wing region is of the *Rp* conformation. In some embodiments, at least about 2 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 3 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 4 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 5 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 6 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 7 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation.

In some embodiments, at least about 8 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 9 chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation.

[00546] In some embodiments, at least about 2 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 3 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 4 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 5 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 6 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 7 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 8 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation. In some embodiments, at least about 9 consecutive chiral phosphorothioate internucleotidic linkages of a wing region are of the *Rp* conformation.

[00547] In some embodiments, a wing comprises one or more modified sugar moieties. In some embodiments, a wing comprises two or more modified sugar moieties. In some embodiments, a wing comprises three or more modified sugar moieties. In some embodiments, a wing comprises four or more modified sugar moieties. In some embodiments, a wing comprises five or more modified sugar moieties. In some embodiments, a wing comprises six or more modified sugar moieties. In some embodiments, a wing comprises seven or more modified sugar moieties. In some embodiments, a wing comprises eight or more modified sugar moieties. In some embodiments, a wing comprises nine or more modified sugar moieties. In some embodiments, a wing comprises ten or more modified sugar moieties. In some embodiments, a wing comprises 11 or more modified sugar moieties. In some embodiments, a wing comprises 12 or more modified sugar moieties. In some embodiments, a wing comprises 13 or more modified sugar moieties. In some embodiments, a wing comprises 14 or more modified sugar moieties. In some embodiments, a wing comprises 15 or more modified sugar moieties. In some embodiments, a wing comprises 16 or more modified sugar moieties. In some embodiments, a wing comprises 17 or more modified sugar moieties. In some embodiments, a wing comprises

18 or more modified sugar moieties. In some embodiments, a wing comprises 19 or more modified sugar moieties. In some embodiments, a wing comprises 20 or more modified sugar moieties. In some embodiments, a wing comprises 21 or more modified sugar moieties. In some embodiments, a wing comprises 22 or more modified sugar moieties. In some embodiments, a wing comprises 23 or more modified sugar moieties. In some embodiments, a wing comprises 24 or more modified sugar moieties. In some embodiments, a wing comprises 25 or more modified sugar moieties. In some embodiments, a wing comprises 30 or more modified sugar moieties. In some embodiments, a wing comprises 35 or more modified sugar moieties.

[00548] In some embodiments, a wing comprises one or more 2'-modified sugar moieties. In some embodiments, a wing comprises two or more 2'-modified sugar moieties. In some embodiments, a wing comprises three or more 2'-modified sugar moieties. In some embodiments, a wing comprises four or more 2'-modified sugar moieties. In some embodiments, a wing comprises five or more 2'-modified sugar moieties. In some embodiments, a wing comprises six or more 2'-modified sugar moieties. In some embodiments, a wing comprises seven or more 2'-modified sugar moieties. In some embodiments, a wing comprises eight or more 2'-modified sugar moieties. In some embodiments, a wing comprises nine or more 2'-modified sugar moieties. In some embodiments, a wing comprises ten or more 2'-modified sugar moieties. In some embodiments, a wing comprises 11 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 12 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 13 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 14 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 15 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 16 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 17 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 18 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 19 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 20 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 21 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 22 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 23 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 24 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 25 or more 2'-modified sugar moieties. In some embodiments, a wing comprises 30 or more 2'-modified sugar

moieties. In some embodiments, a wing comprises 35 or more 2'-modified sugar moieties.

[00549] In some embodiments, a wing comprises one or more 2'-F. In some embodiments, a wing comprises two or more 2'-F. In some embodiments, a wing comprises three or more 2'-F. In some embodiments, a wing comprises four or more 2'-F. In some embodiments, a wing comprises five or more 2'-F. In some embodiments, a wing comprises six or more 2'-F. In some embodiments, a wing comprises seven or more 2'-F. In some embodiments, a wing comprises eight or more 2'-F. In some embodiments, a wing comprises nine or more 2'-F. In some embodiments, a wing comprises ten or more 2'-F. In some embodiments, a wing comprises 11 or more 2'-F. In some embodiments, a wing comprises 12 or more 2'-F. In some embodiments, a wing comprises 13 or more 2'-F. In some embodiments, a wing comprises 14 or more 2'-F. In some embodiments, a wing comprises 15 or more 2'-F. In some embodiments, a wing comprises 16 or more 2'-F. In some embodiments, a wing comprises 17 or more 2'-F. In some embodiments, a wing comprises 18 or more 2'-F. In some embodiments, a wing comprises 19 or more 2'-F. In some embodiments, a wing comprises 20 or more 2'-F. In some embodiments, a wing comprises 21 or more 2'-F. In some embodiments, a wing comprises 22 or more 2'-F. In some embodiments, a wing comprises 23 or more 2'-F. In some embodiments, a wing comprises 24 or more 2'-F. In some embodiments, a wing comprises 25 or more 2'-F. In some embodiments, a wing comprises 30 or more 2'-F. In some embodiments, a wing comprises 35 or more 2'-F.

[00550] In some embodiments, a wing comprises one 2'-F. In some embodiments, a wing comprises two 2'-F. In some embodiments, a wing comprises three 2'-F. In some embodiments, a wing comprises four 2'-F. In some embodiments, a wing comprises five 2'-F. In some embodiments, a wing comprises six 2'-F. In some embodiments, a wing comprises seven 2'-F. In some embodiments, a wing comprises eight 2'-F. In some embodiments, a wing comprises nine 2'-F. In some embodiments, a wing comprises ten 2'-F. In some embodiments, a wing comprises 11 2'-F. In some embodiments, a wing comprises 12 2'-F. In some embodiments, a wing comprises 13 2'-F. In some embodiments, a wing comprises 14 2'-F. In some embodiments, a wing comprises 15 2'-F. In some embodiments, a wing comprises 16 2'-F. In some embodiments, a wing comprises 17 2'-F. In some embodiments, a wing comprises 18 2'-F. In some embodiments, a wing comprises 19 2'-F. In some embodiments, a wing comprises 20 2'-F. In some embodiments, a wing comprises 21 2'-F. In some embodiments, a wing

comprises 22 2'-F. In some embodiments, a wing comprises 23 2'-F. In some embodiments, a wing comprises 24 2'-F. In some embodiments, a wing comprises 25 2'-F. In some embodiments, a wing comprises 30 2'-F. In some embodiments, a wing comprises 35 2'-F.

[00551] In some embodiments, a wing comprises one or more consecutive 2'-F. In some embodiments, a wing comprises two or more consecutive 2'-F. In some embodiments, a wing comprises three or more consecutive 2'-F. In some embodiments, a wing comprises four or more consecutive 2'-F. In some embodiments, a wing comprises five or more consecutive 2'-F. In some embodiments, a wing comprises six or more consecutive 2'-F. In some embodiments, a wing comprises seven or more consecutive 2'-F. In some embodiments, a wing comprises eight or more consecutive 2'-F. In some embodiments, a wing comprises nine or more consecutive 2'-F. In some embodiments, a wing comprises ten or more consecutive 2'-F. In some embodiments, a wing comprises 11 or more consecutive 2'-F. In some embodiments, a wing comprises 12 or more consecutive 2'-F. In some embodiments, a wing comprises 13 or more consecutive 2'-F. In some embodiments, a wing comprises 14 or more consecutive 2'-F. In some embodiments, a wing comprises 15 or more consecutive 2'-F. In some embodiments, a wing comprises 16 or more consecutive 2'-F. In some embodiments, a wing comprises 17 or more consecutive 2'-F. In some embodiments, a wing comprises 18 or more consecutive 2'-F. In some embodiments, a wing comprises 19 or more consecutive 2'-F. In some embodiments, a wing comprises 20 or more consecutive 2'-F. In some embodiments, a wing comprises 21 or more consecutive 2'-F. In some embodiments, a wing comprises 22 or more consecutive 2'-F. In some embodiments, a wing comprises 23 or more consecutive 2'-F. In some embodiments, a wing comprises 24 or more consecutive 2'-F. In some embodiments, a wing comprises 25 or more consecutive 2'-F. In some embodiments, a wing comprises 30 or more consecutive 2'-F. In some embodiments, a wing comprises 35 or more consecutive 2'-F.

[00552] In some embodiments, a wing comprises one consecutive 2'-F. In some embodiments, a wing comprises two consecutive 2'-F. In some embodiments, a wing comprises three consecutive 2'-F. In some embodiments, a wing comprises four consecutive 2'-F. In some embodiments, a wing comprises five consecutive 2'-F. In some embodiments, a wing comprises six consecutive 2'-F. In some embodiments, a wing comprises seven consecutive 2'-F. In some embodiments, a wing comprises eight consecutive 2'-F. In some embodiments, a wing comprises nine consecutive 2'-F. In some embodiments, a wing comprises ten consecutive 2'-F.

In some embodiments, a wing comprises 11 consecutive 2'-F. In some embodiments, a wing comprises 12 consecutive 2'-F. In some embodiments, a wing comprises 13 consecutive 2'-F. In some embodiments, a wing comprises 14 consecutive 2'-F. In some embodiments, a wing comprises 15 consecutive 2'-F. In some embodiments, a wing comprises 16 consecutive 2'-F. In some embodiments, a wing comprises 17 consecutive 2'-F. In some embodiments, a wing comprises 18 consecutive 2'-F. In some embodiments, a wing comprises 19 consecutive 2'-F. In some embodiments, a wing comprises 20 consecutive 2'-F. In some embodiments, a wing comprises 21 consecutive 2'-F. In some embodiments, a wing comprises 22 consecutive 2'-F. In some embodiments, a wing comprises 23 consecutive 2'-F. In some embodiments, a wing comprises 24 consecutive 2'-F. In some embodiments, a wing comprises 25 consecutive 2'-F. In some embodiments, a wing comprises 30 consecutive 2'-F. In some embodiments, a wing comprises 35 consecutive 2'-F.

[00553] 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.

[00554] 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.*

[00555] 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 *Sp* conformation.

[00556] 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.

[00557] In some embodiments, a core region of a wing-core-wing motif optionally contains chiral, modified phosphate internucleotidic linkages. In some embodiments, a core region of a wing-core-wing motif optionally contains chiral phosphorothioate internucleotidic linkages. In some embodiments, a core region of a wing-core-wing motif comprises a repeating pattern of internucleotidic linkage stereochemistry. In some embodiments, a core region of a wing-core-wing motif has a repeating pattern of internucleotidic linkage stereochemistry. In some embodiments, a 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, a 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, a 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, a 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, a 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, a 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, a 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, a 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, a 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, a 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, a 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 $RpRpSp$ and $SpSpRp$. In some embodiments, a core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a repeating $RpRpSp$. In some embodiments, a core region of a wing-core-wing motif has repeating pattern of internucleotidic linkage stereochemistry, wherein the repeating pattern is a repeating $SpSpRp$.

[00558] 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 $(Sp)_mRp$. 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 $(Sp)_2Rp(Sp)_2$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers in the core region 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 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 $(Sp)_2Rp$.

[00559] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers 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 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 $(Sp)mRp$. 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 $(Sp)_2Rp(Sp)_2$. In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition of an oligonucleotide type whose pattern of backbone chiral centers comprises $(Rp)_2Rp(Sp)_2$. 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 $(Sp)_2Rp$.

[00560] 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.

[00561] 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 $(Sp)_2(Rp)$.

[00562] 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-

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CLAIMS

1. An oligonucleotide composition, comprising a first plurality of oligonucleotides of a particular oligonucleotide type defined by:

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

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

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

2. The composition of claim 1, wherein the reference condition is absence of the composition, and the pattern of backbone chiral centers comprises at least two chirally controlled centers independently selected from *Rp* and *Sp*.

3. The composition of claim 2, wherein the pattern of backbone linkages comprises one or more backbone linkages selected from phosphodiester, phosphorothioate and phosphodithioate linkages.

4. The composition of claim 3, wherein oligonucleotides of the particular oligonucleotide type each further comprise one or more sugar modifications.

5. The composition of claim 4, wherein the sugar modifications comprise one or more modifications selected from: 2'-O-methyl, 2'-MOE, 2'-F, morpholino and bicyclic sugar moieties.

6. The composition of claim 5, wherein the sugar modifications comprise one or more 2'-F sugar moieties.

7. The composition of claim 6, wherein oligonucleotides of the particular oligonucleotide type each comprise:

- 1) a 5'-end region comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleoside units comprising a 2'-F modified sugar moiety;
- 2) a 3'-end region comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleoside units

comprising a 2'-F modified sugar moiety; and

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

8. The composition of claim 7, wherein the 5'-end region comprises 1 or more nucleoside units not comprising a 2'-F modified sugar moiety; the 3'-end region comprises 1 or more nucleoside units not comprising a 2'-F modified sugar moiety; and/or the middle region comprises 1 or more nucleotidic units not comprising a phosphodiester linkage.

9. The composition of claim 7, wherein the first of the 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleoside units comprising a 2'-F modified sugar moiety and a modified internucleotidic linkage of the 5'-end is the first, second, third, fourth or fifth nucleoside unit of the oligonucleotide from the 5'-end, and the last of the 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleoside units comprising a 2'-F modified sugar moiety and a modified internucleotidic linkage of the 3'-end is the last, second last, third last, fourth last, or fifth last nucleoside unit of the oligonucleotide.

10. The composition of claim 9, wherein the 5'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising a 2'-F modified sugar moiety, and the 3'-end region comprising 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising a 2'-F modified sugar moiety.

11. The composition of claim 10, wherein each nucleotidic unit comprising a nucleoside unit of the 2, 3, 4, 5, 6, 7, 8, 9, 10 or more consecutive nucleoside units comprising a 2'-F modified sugar moiety of the 5'-end or the 3'-end region and an internucleotidic linkage comprises a modified internucleotidic linkage, and the middle region comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotidic units, each of which independently comprises a phosphodiester linkage and 2'-OR¹ modified sugar moiety, wherein R¹ is optionally substituted C₁₋₆ alkyl.

12. The composition of claim 2, wherein the base sequence comprises a sequence having no more than 5 mismatches from a 20 base long portion of the dystrophin gene or its complement, the length is no more than 50 bases, the pattern of backbone chiral centers comprises at least three chirally controlled centers independently of *R*_p or *S*_p, and the oligonucleotides of the particular oligonucleotide type are capable of mediating the skipping of an exon of the dystrophin gene.

13. The composition of claim 12, wherein the oligonucleotides of the particular oligonucleotide type are capable of mediating the skipping of exon 51 of the dystrophin gene.

14. The composition of claim 4, wherein the base sequence comprises a sequence having no more than 5 mismatches from the sequence of UCAAGGAAGAUGGCAUUUCU.

15. The composition of claim 4, wherein the base sequence comprises the sequence of bases of UCAAGGAAGAUGGCAUUUCU.

16. The composition of claim 4, wherein the base sequence consists of the sequence of bases of UCAAGGAAGAUGGCAUUUCU.

17. The composition of claim 1, wherein oligonucleotides of the particular oligonucleotide type are:

WV-2530:

fU*SfC*SfA*SfA*SfG*SfG*SfA*SfA*SmGmAmUmG*SfG*SfC*SfA*SfU*SfU*SfU*SfC*SfU;

WV-2531:

fU*SfC*SfA*SfA*SfG*SfG*SfA*SfA*SfG*SmAmU*SfG*SfG*SfC*SfA*SfU*SfU*SfU*SfC*SfU;

WV-3152:

fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGfA*SmUfG*SmGfC*SfA*SfU*SfU*SfU*SfC*SfU

WV-3472:

fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGfA*SfU*SmGmGfC*SfA*SfU*SfU*SfU*SfC*SfU;

WV-3473:

fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGmA*SfU*SmGmGfC*SfA*SfU*SfU*SfU*SfC*SfU;

WV-3507:

fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGmAfU*SmGmGfC*SfA*SfU*SfU*SfU*SfC*SfU;

WV-3508:

fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGfA*SfU*SmGmGfC*SfAfU*SfU*SfU*SfC*SfU;

WV-3509:

fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGmA*SfU*SmGmGfC*SfAfU*SfU*SfU*SfC*SfU;

WV-3510:

fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGfAfU*SmGmGfC*SmA*SfU*SfU*SfU*SfC*SfU;

WV-3511:

fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGmAfU*SmGmGfC*SmA*SfU*SfU*SfU*SfC*SfU;

WV-3512:

fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGfAfU*SmGmGfC*SmAfU*SfU*SfU*SfC*SfU;

WV-3513:

fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGmAfU*SmGmGfC*SmAfU*SfU*SfU*SfC*SfU;

WV-3514:

fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGfAfU*SmGmGfC*SfAfU*SfU*SfU*SfC*SfU;

WV-3515:

fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGmAfU*SmGmGfC*SfAfU*SfU*SfU*SfC*SfU;

WV-3545:

Mod015L001fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGmA*SfU*SmGmGfC*SfA*SfU*SfU*
SfU*SfC*SfU; or

WV-3546:

Mod020L001fU*SfC*SfA*SfA*SfG*SfG*SmAfA*SmGmA*SfU*SmGmGfC*SfA*SfU*SfU*
SfU*SfC*SfU,

wherein:

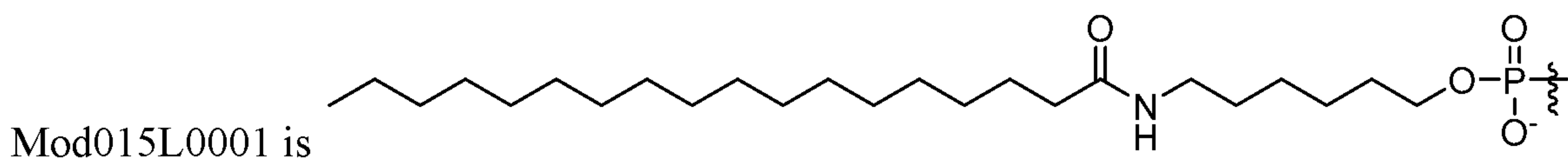
f is 2'-F modification;

m is 2'-OME-;

* is phosphorothioate;

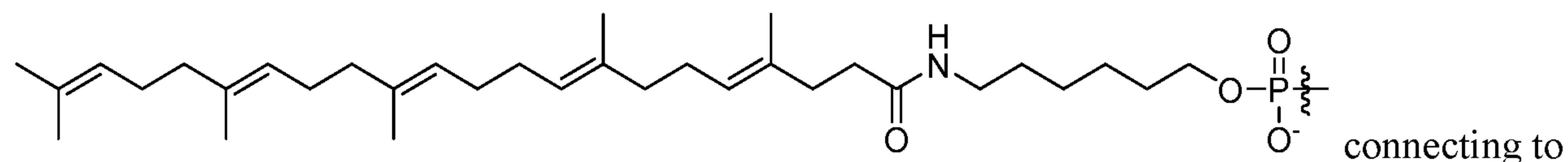
*S is Phosphorothioate in *Sp* conformation;

*R is Phosphorothioate in *Sp* conformation;



connecting to 5'-end -O- of an oligonucleotide chain; and

Mod020L0001 is



5'-end -O- of an oligonucleotide chain.

18. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-2530.
19. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-2531.
20. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3152.
21. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3472.
22. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3473.
23. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3507.
24. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3508.
25. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3509.
26. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3510.
27. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3511.
28. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3512.
29. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3513.
30. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3514.
31. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3515.
32. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3545.

33. The composition of claim 17, wherein oligonucleotides of the particular oligonucleotide type are oligonucleotides of WV-3546.

34. The composition of any one of claim 1-32, wherein at least 10% of the oligonucleotides that have the base sequence of the particular oligonucleotide type are oligonucleotides of the particular oligonucleotide type.

35. The composition of any one of claim 1-32, wherein at least 10% of the oligonucleotides that have the base sequence, pattern of backbone linkages, and pattern of backbone phosphorus modifications of the particular oligonucleotide type are oligonucleotides of the particular oligonucleotide type.

36. A method for altering splicing of a target transcript, comprising administering an oligonucleotide composition comprising a first plurality of oligonucleotides of a particular oligonucleotide type defined by:

- 1) base sequence;
- 2) pattern of backbone linkages;
- 3) pattern of backbone chiral centers; and

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

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

37. The method of claim 36, wherein the splicing of the target transcript is altered relative to absence of the composition.

38. The method of claim 37, wherein the target transcript is pre-mRNA of dystrophin, and wherein the alteration is that one or more exon is skipped at an increased level relative to absence of the composition.

39. The method of claim 38, wherein exon 51 of dystrophin is skipped at an increased level relative to absence of the composition.

40. A composition, oligonucleotide, method, or compound of any one of embodiments 1-1223.
41. The method of claim 36, wherein the oligonucleotide composition is a composition of any one of claims 1-35 or 40.
42. A method for treating Duchenne muscular dystrophy, comprising administering to a subject susceptible thereto or suffering therefrom a composition of any one of claims 1-35 or 40.

FIG. 1

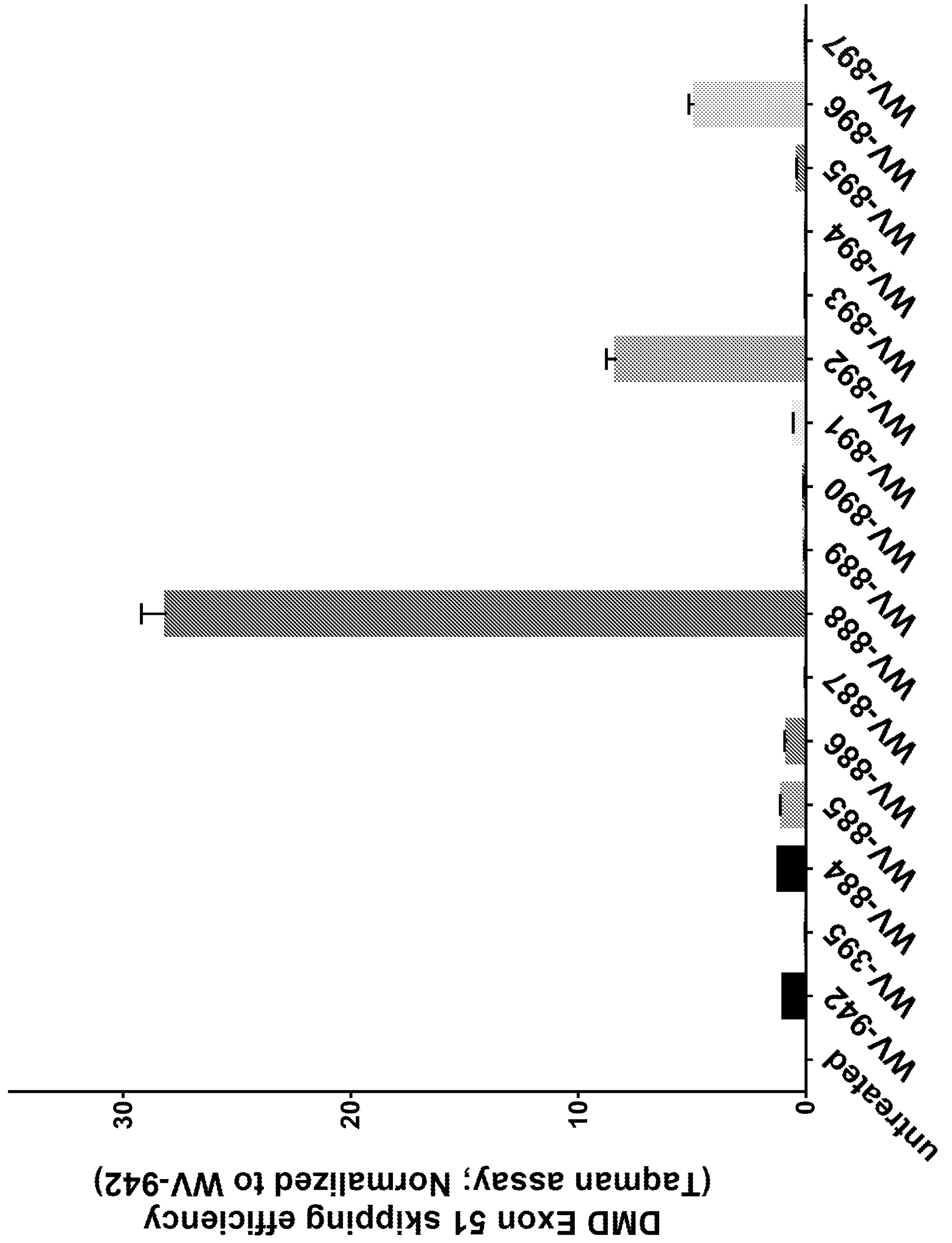


FIG. 2

DMD Exon 51 skipping efficiency
(Taqman assay; Normalized to untreated control)

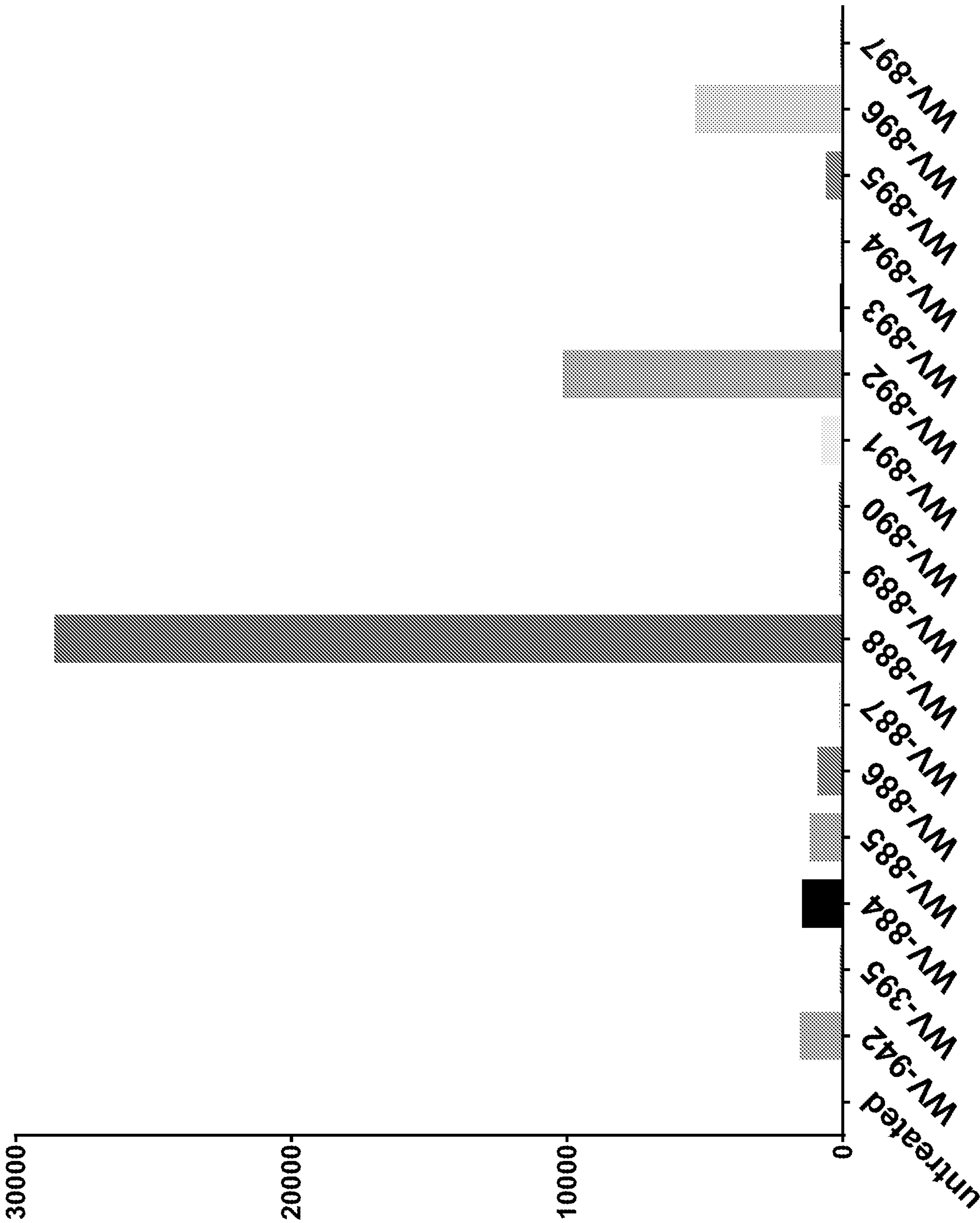
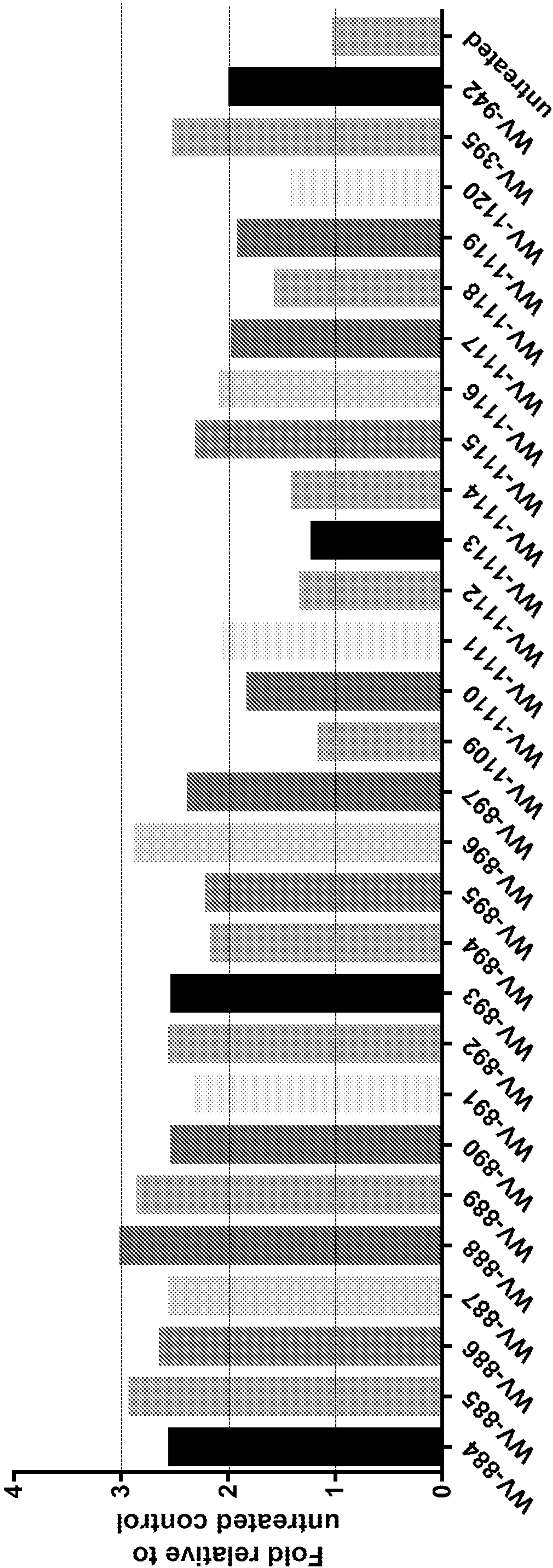


FIG. 3A

DMD
(normalized to GAPDH)



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FIG. 3B

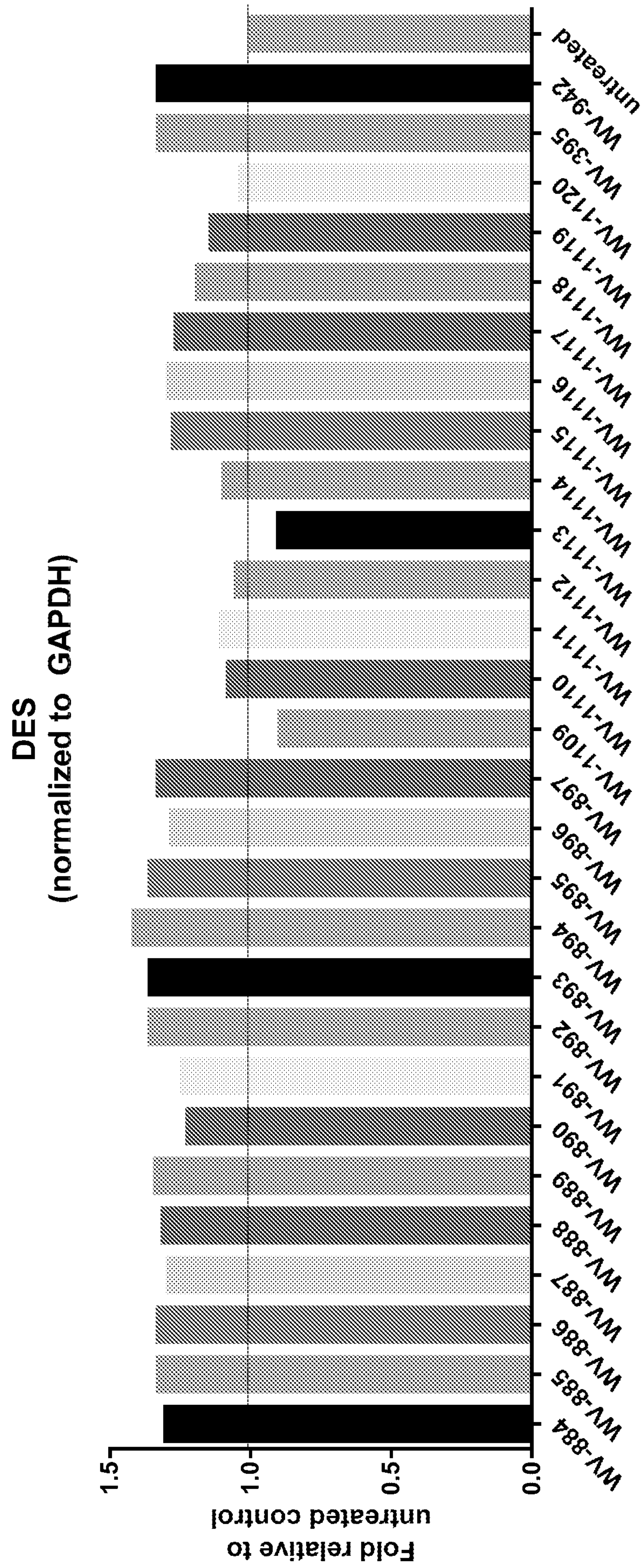
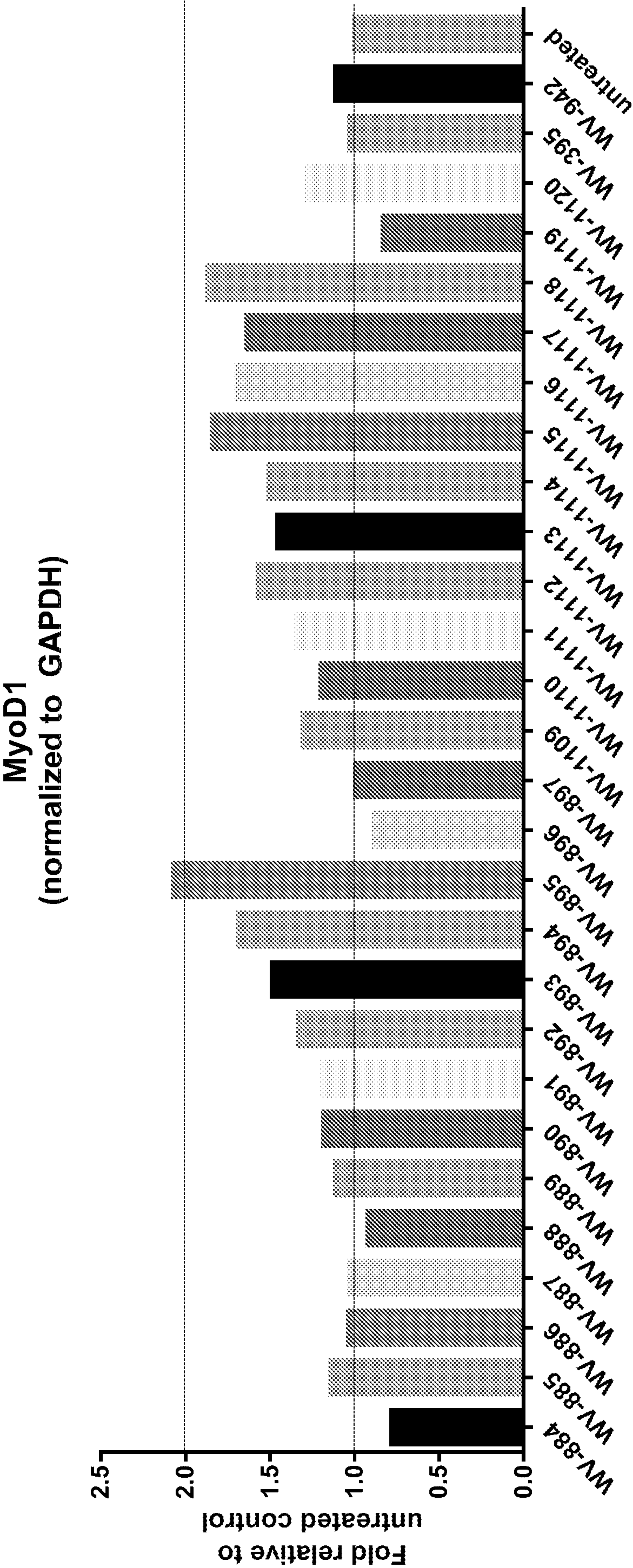


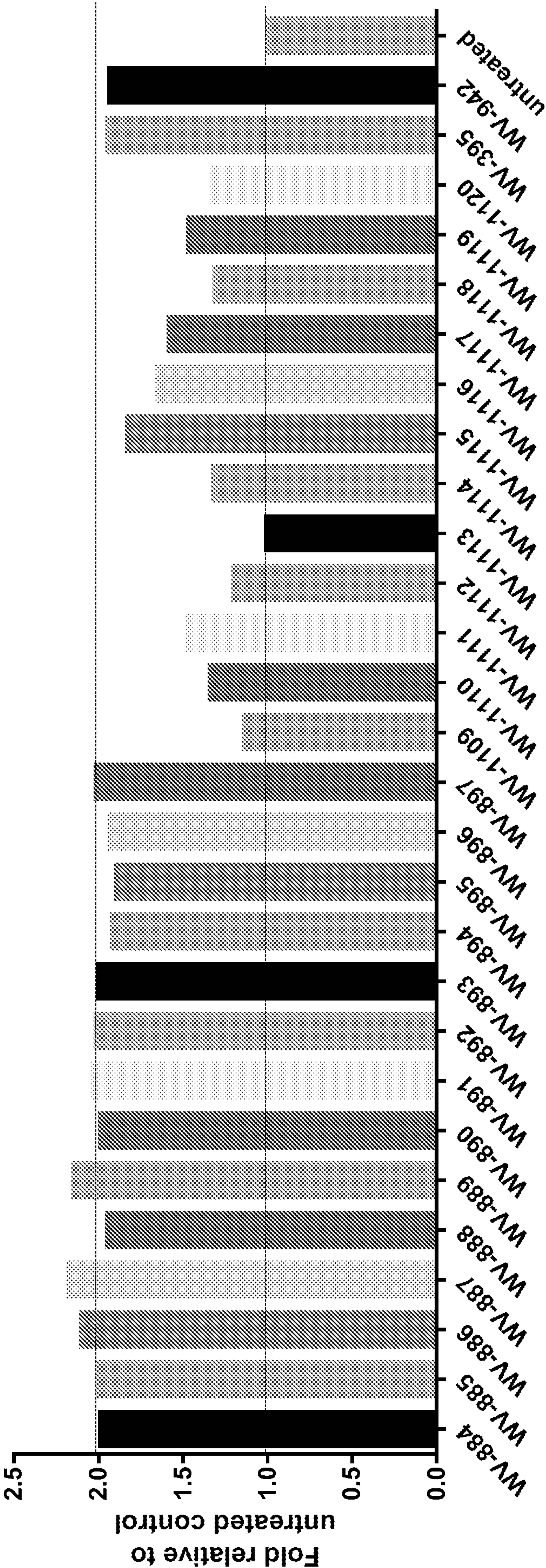
FIG. 3C



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FIG. 3D

MYOG
(normalized to GAPDH)



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MYH1
(normalized to GAPDH)

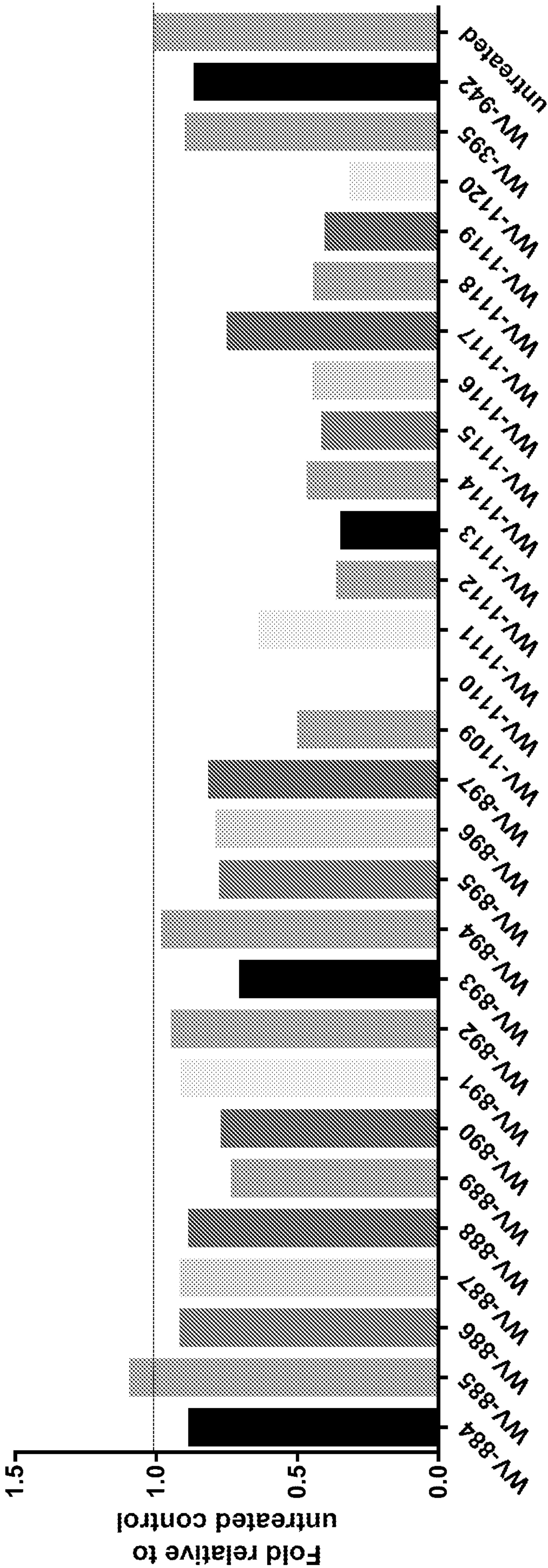


FIG. 3E

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FIG. 3F

RYR1
(normalized to GAPDH)

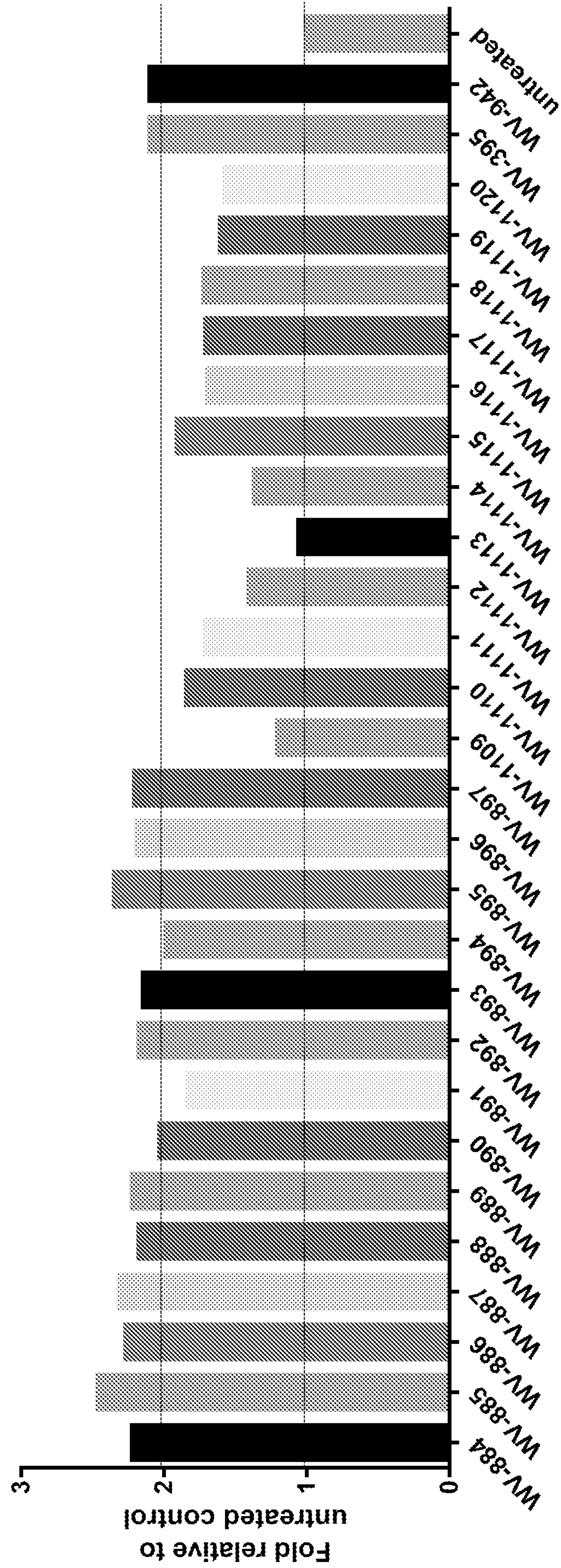
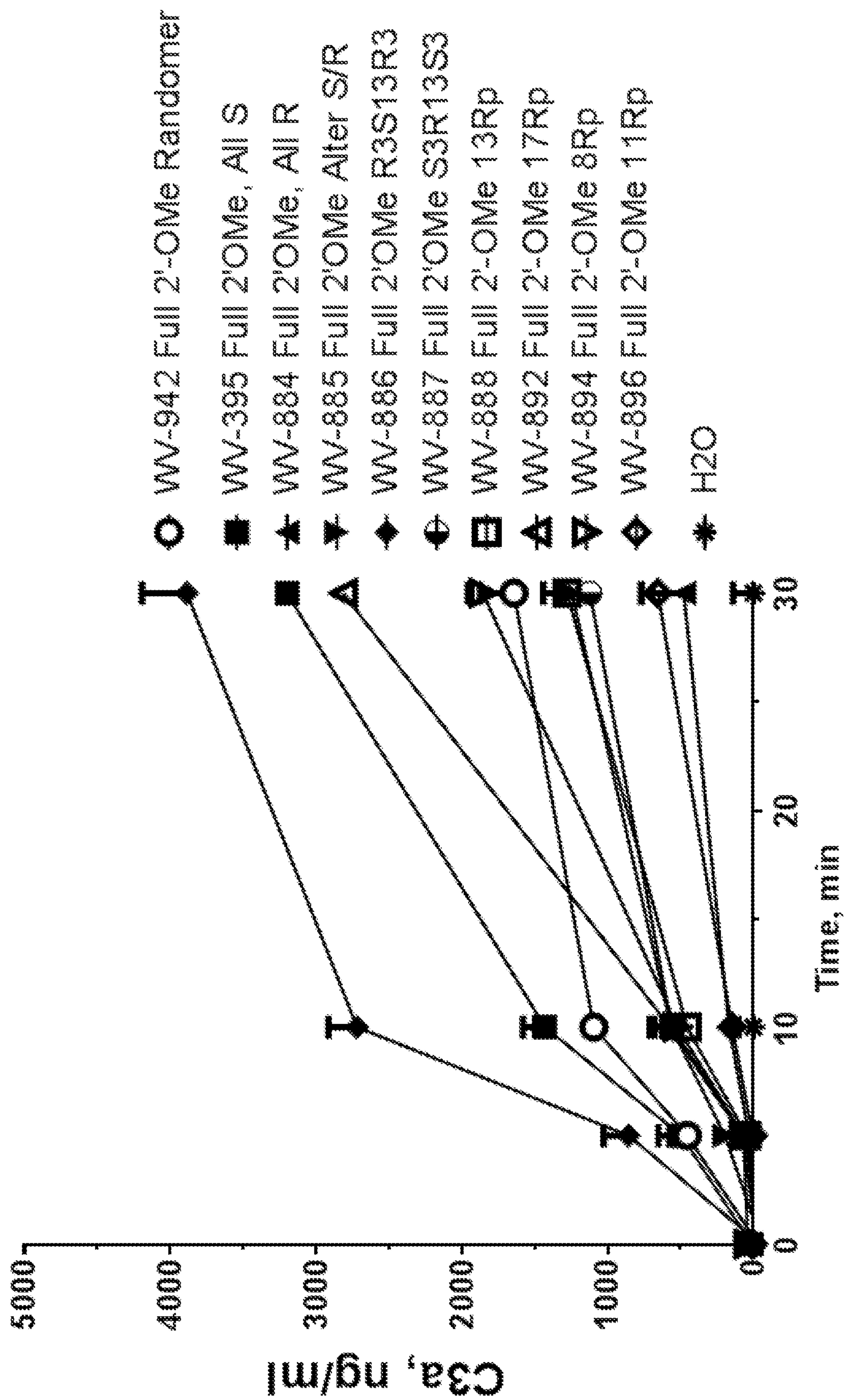


FIG. 4



5
6
7
8

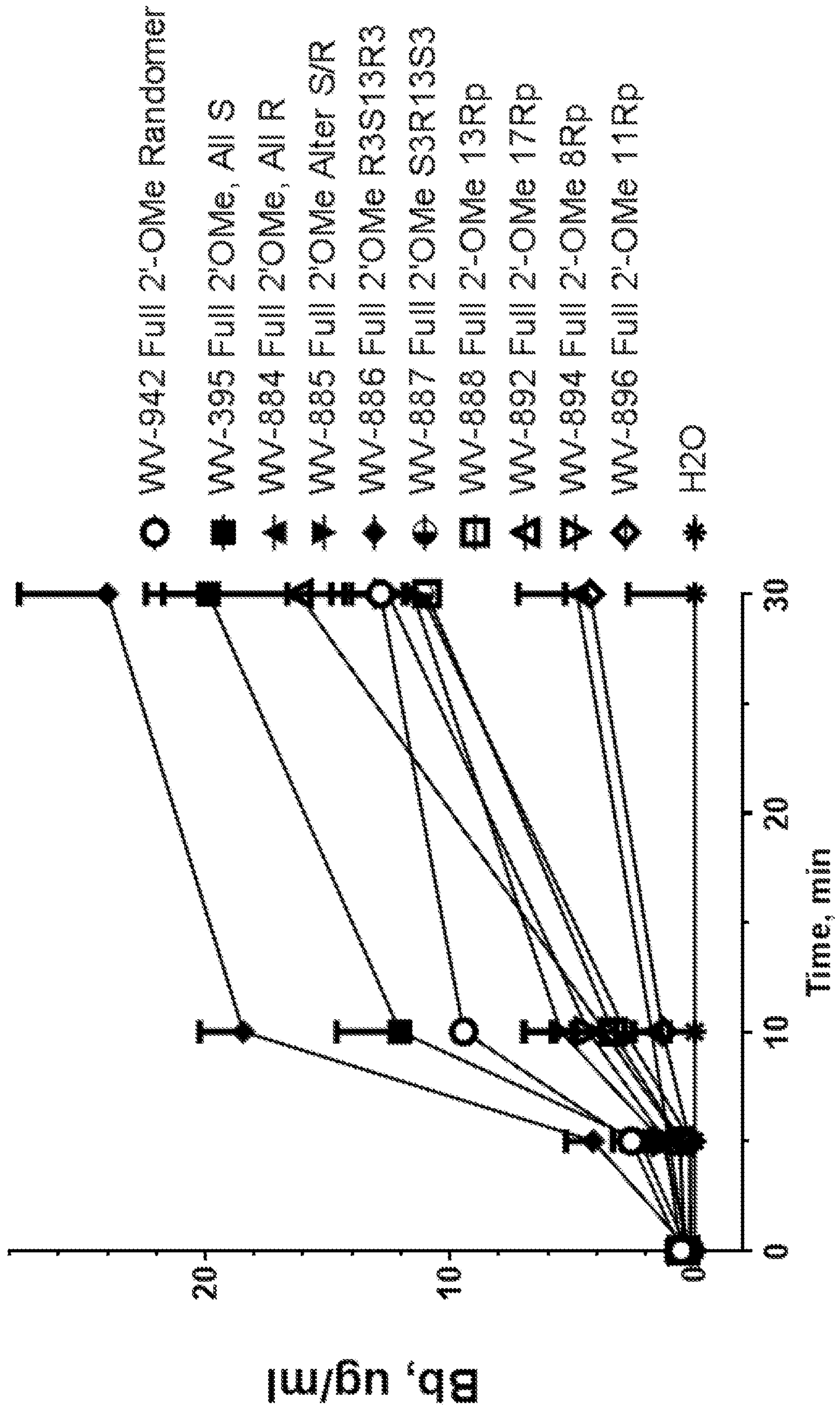


FIG. 6

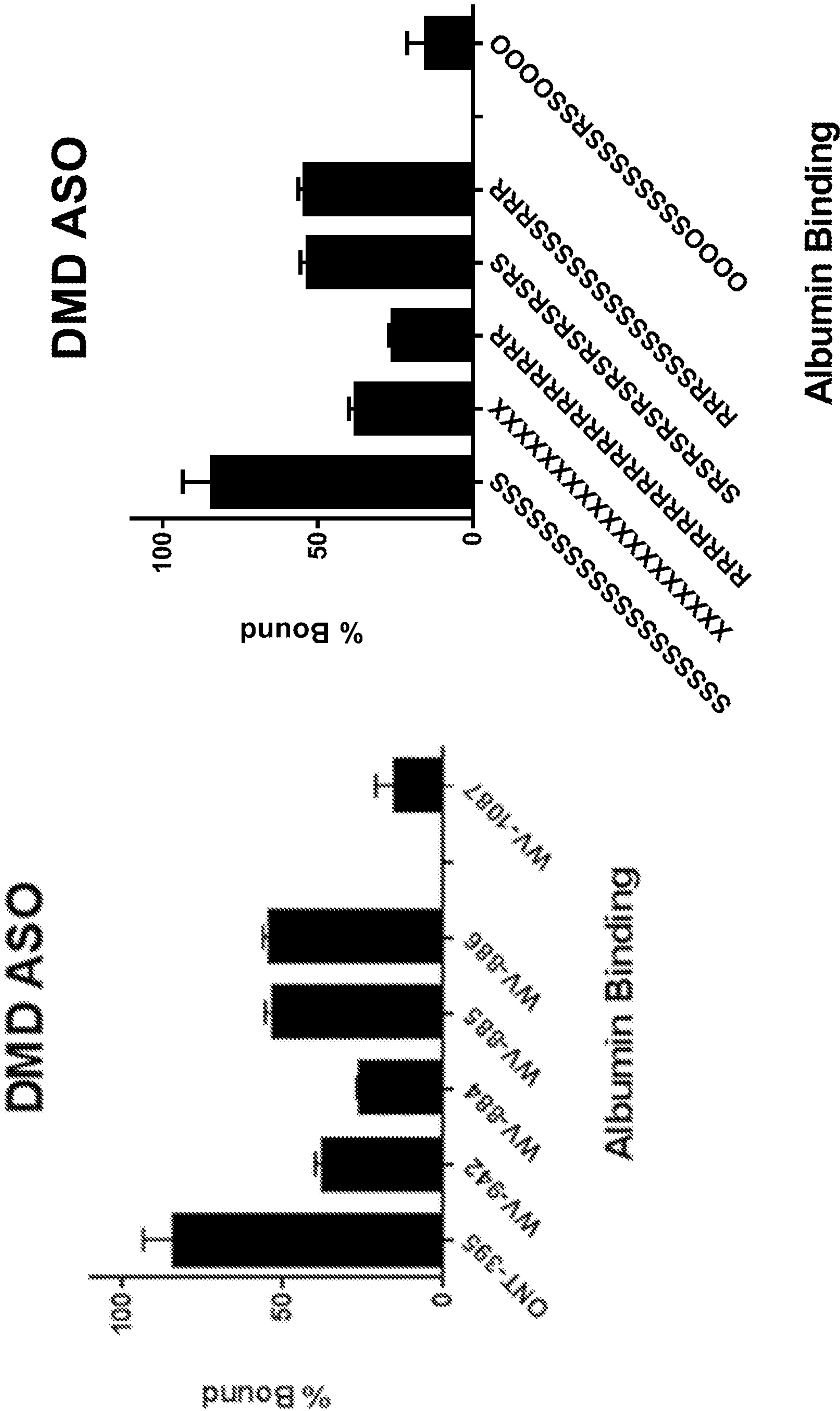


FIG. 6 (Continued)

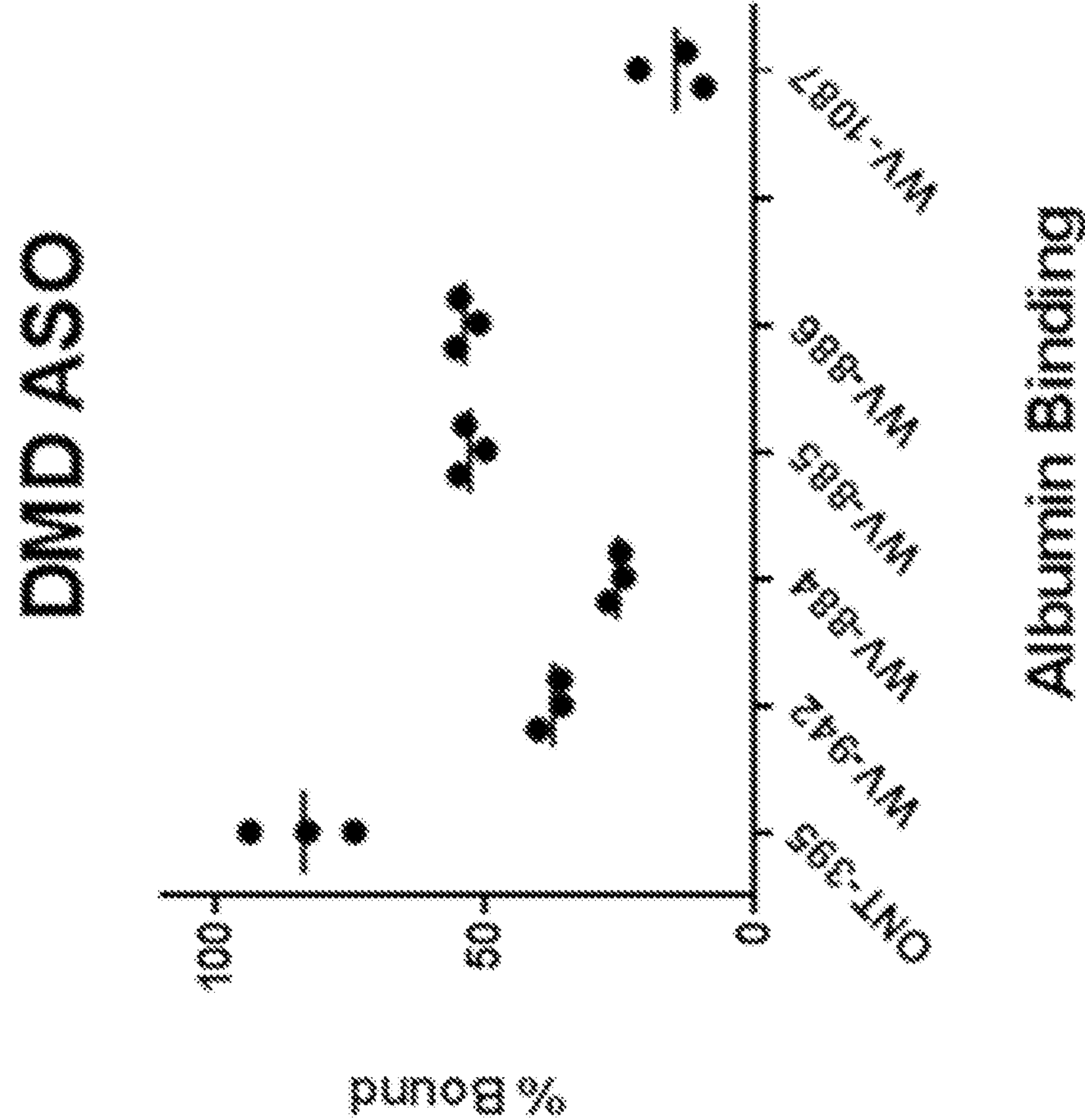


FIG. 7A

WV395.02	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV884.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV885.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV886.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV887.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV888.02	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV889.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV890.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV891.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV892.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV893.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV894.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV895.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV896.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU
WV897.01	mU*SmC*SmA*SmA*SmG*SmG*SmA*SmU*SmG*SmC*SmA*SmU*SmU*SmC*SmU

FIG. 7B

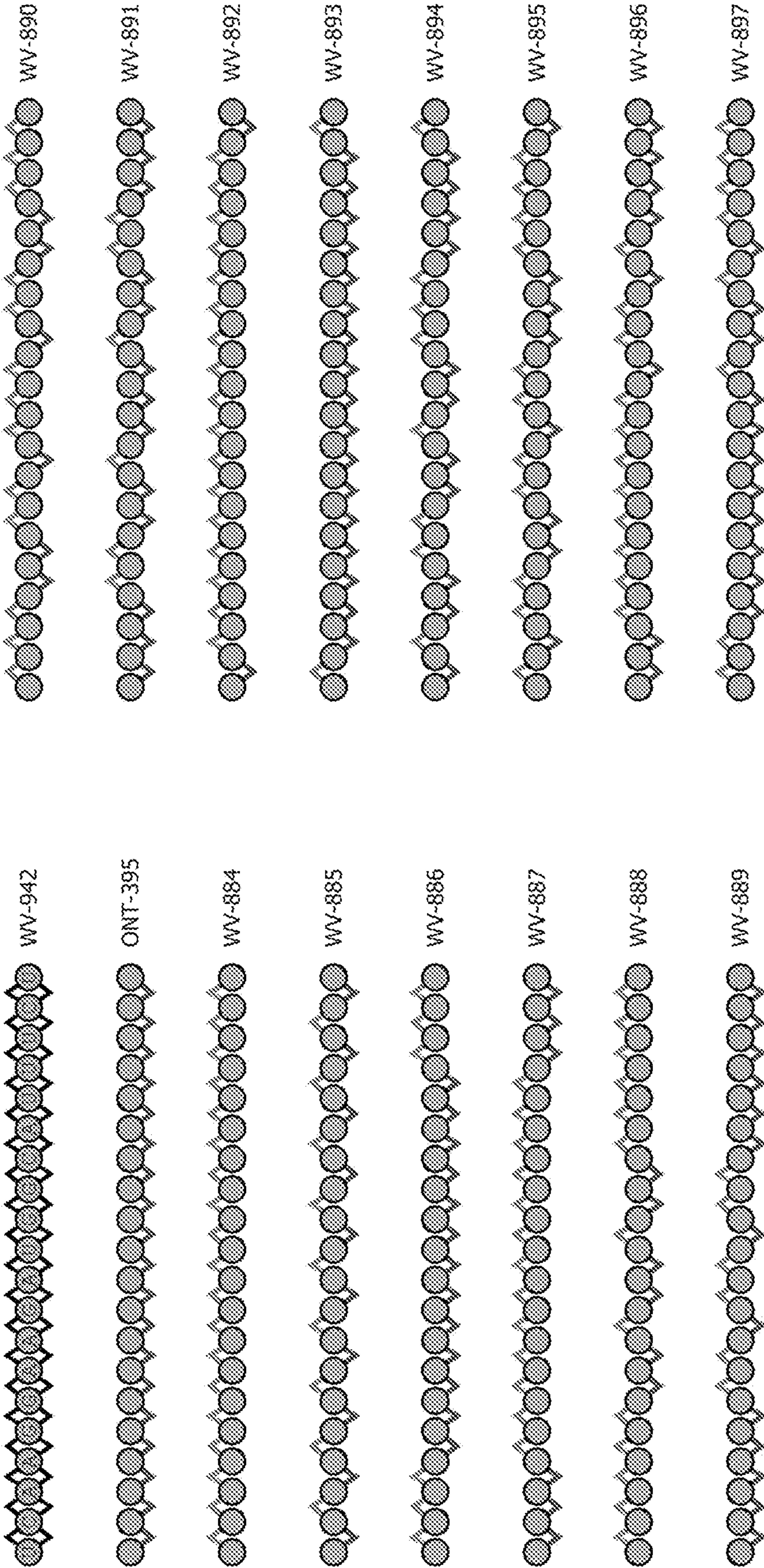


FIG. 8A

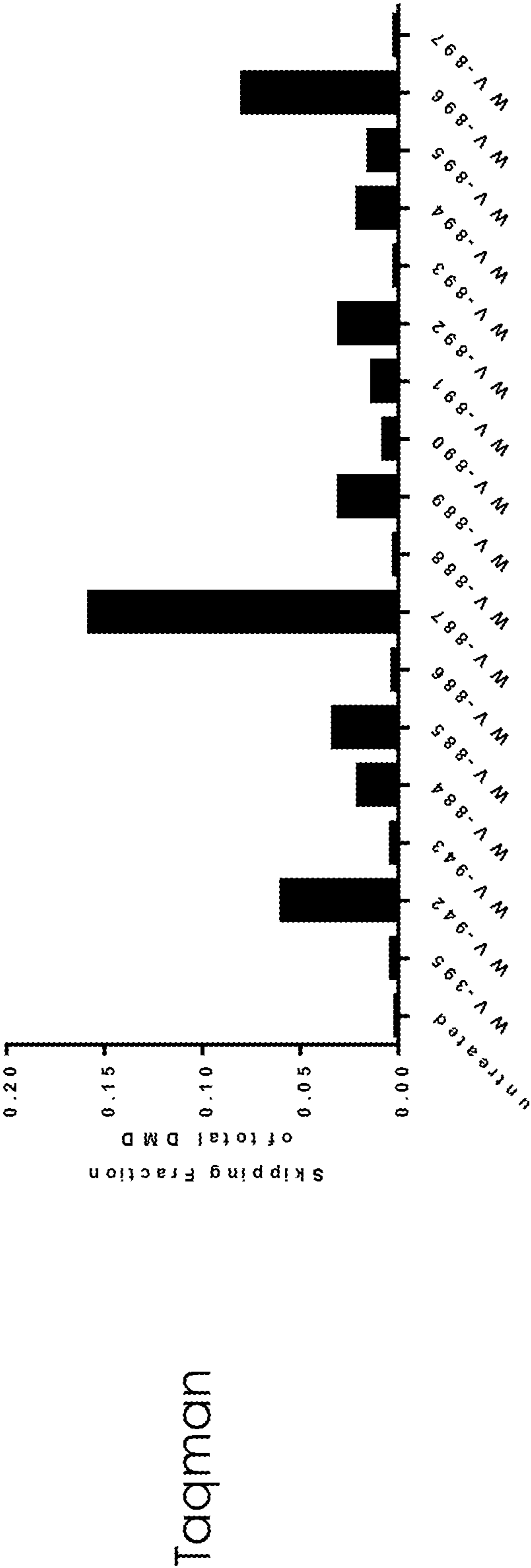


FIG. 8B

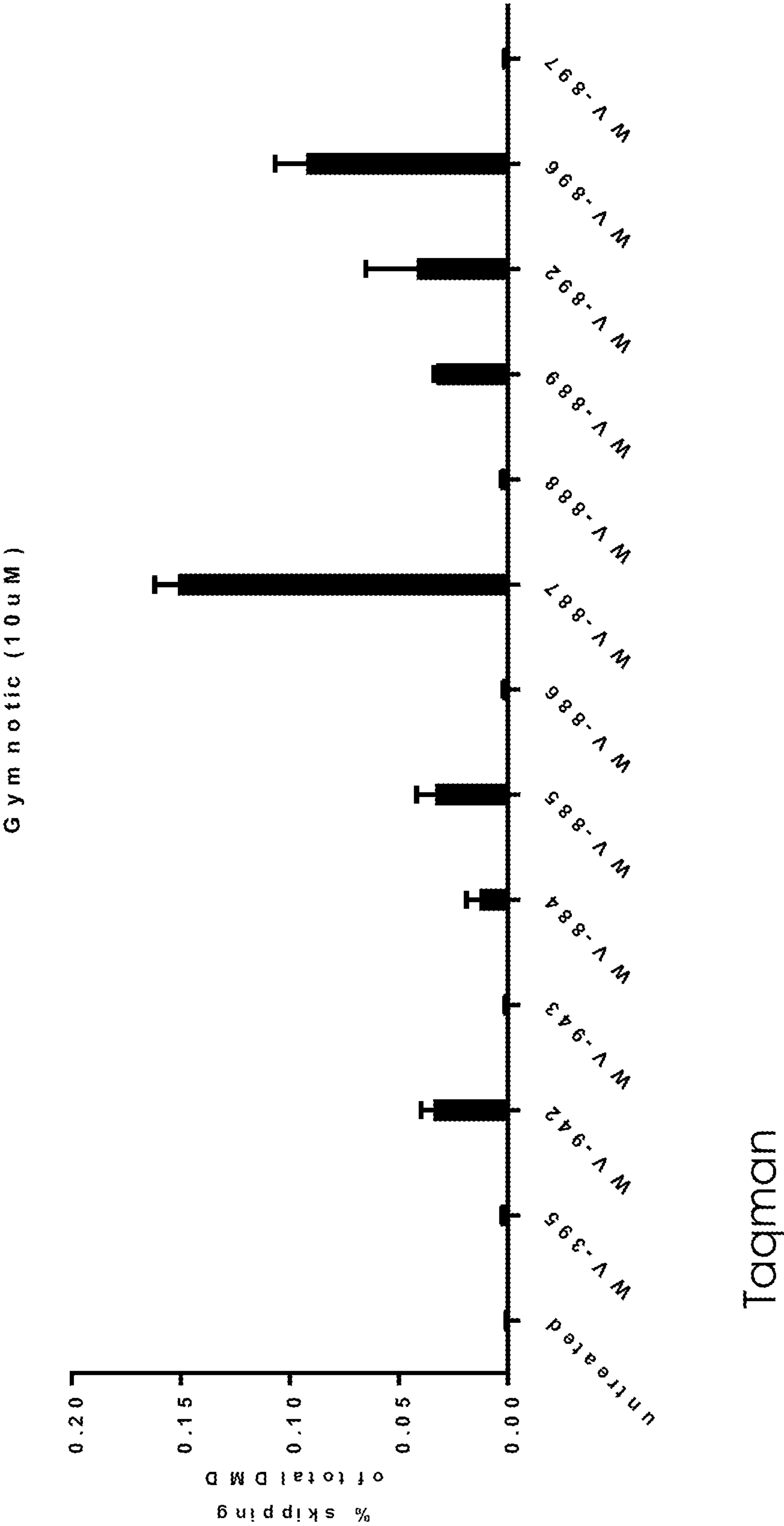


FIG. 9A



Different # of
2'F

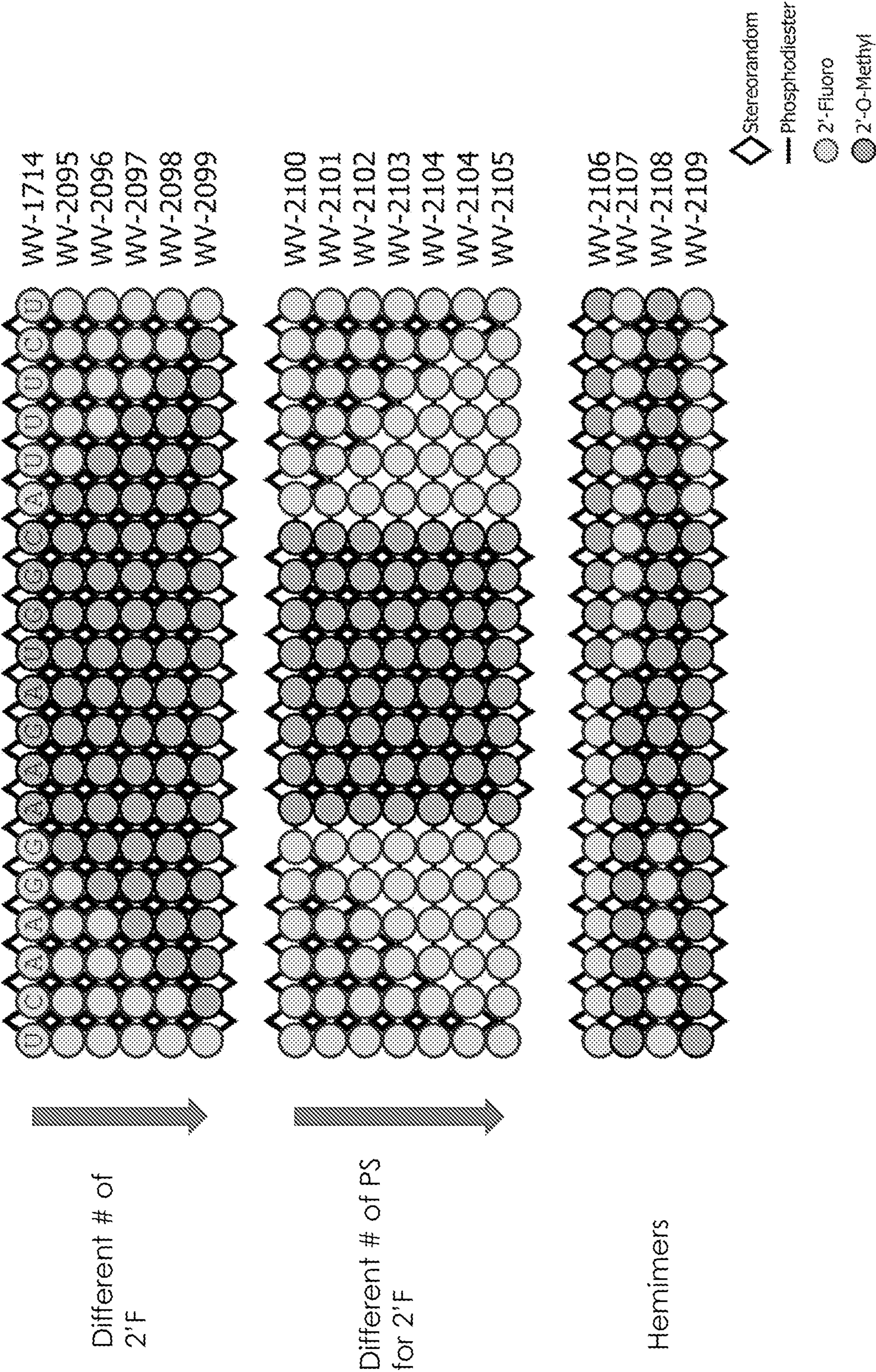
WV-2095	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*fU*fU*fU*fc*fU
WV-2096	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*mU*fU*fU*fc*fU
WV-2097	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*mU*fU*fU*fc*fU
WV-2098	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*mU*fU*fU*fc*fU
WV-2099	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*mU*fU*fU*fc*fU
WV-2100	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*fU*fU*fU*fc*fU
WV-2101	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*fU*fU*fU*fc*fU
WV-2102	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*fU*fU*fU*fc*fU
WV-2103	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*fU*fU*fU*fc*fU
WV-2104	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*fU*fU*fU*fc*fU
WV-2105	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*fU*fU*fU*fc*fU
WV-2106	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*mU*fU*fU*fU*fc*fU
WV-2107	mU*mC*mA*mA*fg*mG*mA*mA*mG*mA*fU*fg*fg*fc*fa*fU*fU*fU*fc*fU
WV-2108	fU*fc*fa*fa*fg*mG*mA*mA*mG*mA*mU*mG*mC*mA*mU*fU*fU*fU*fc*fU
WV-2109	mU*mC*mA*mA*fg*mG*mA*mA*mG*mA*fU*fg*fg*fc*fa*fU*fU*fU*fc*fU

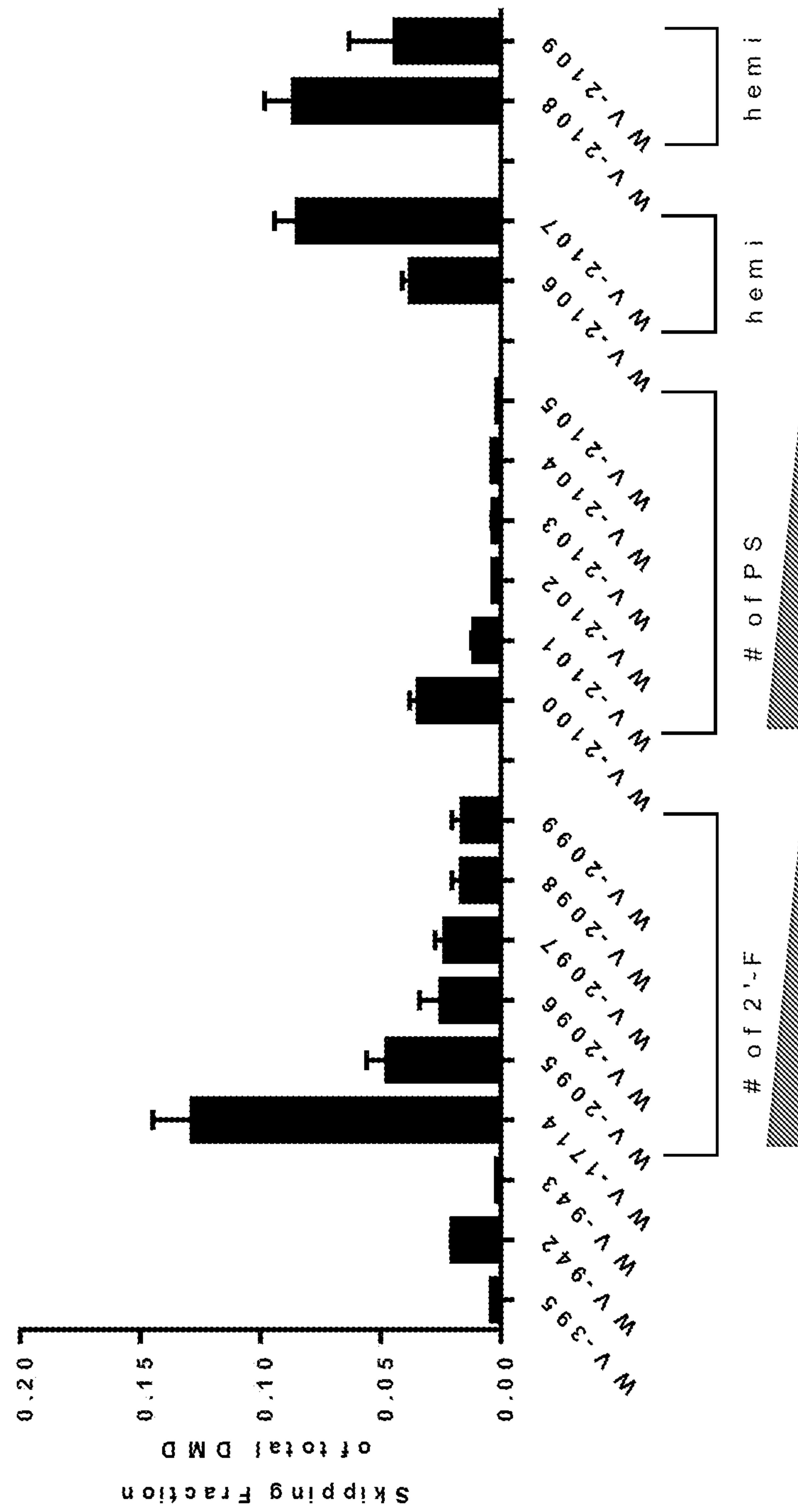


Different # of PS
for 2'F

Hemimers

FIG. 9B





Taqman

FIG. 10A

FIG. 10B

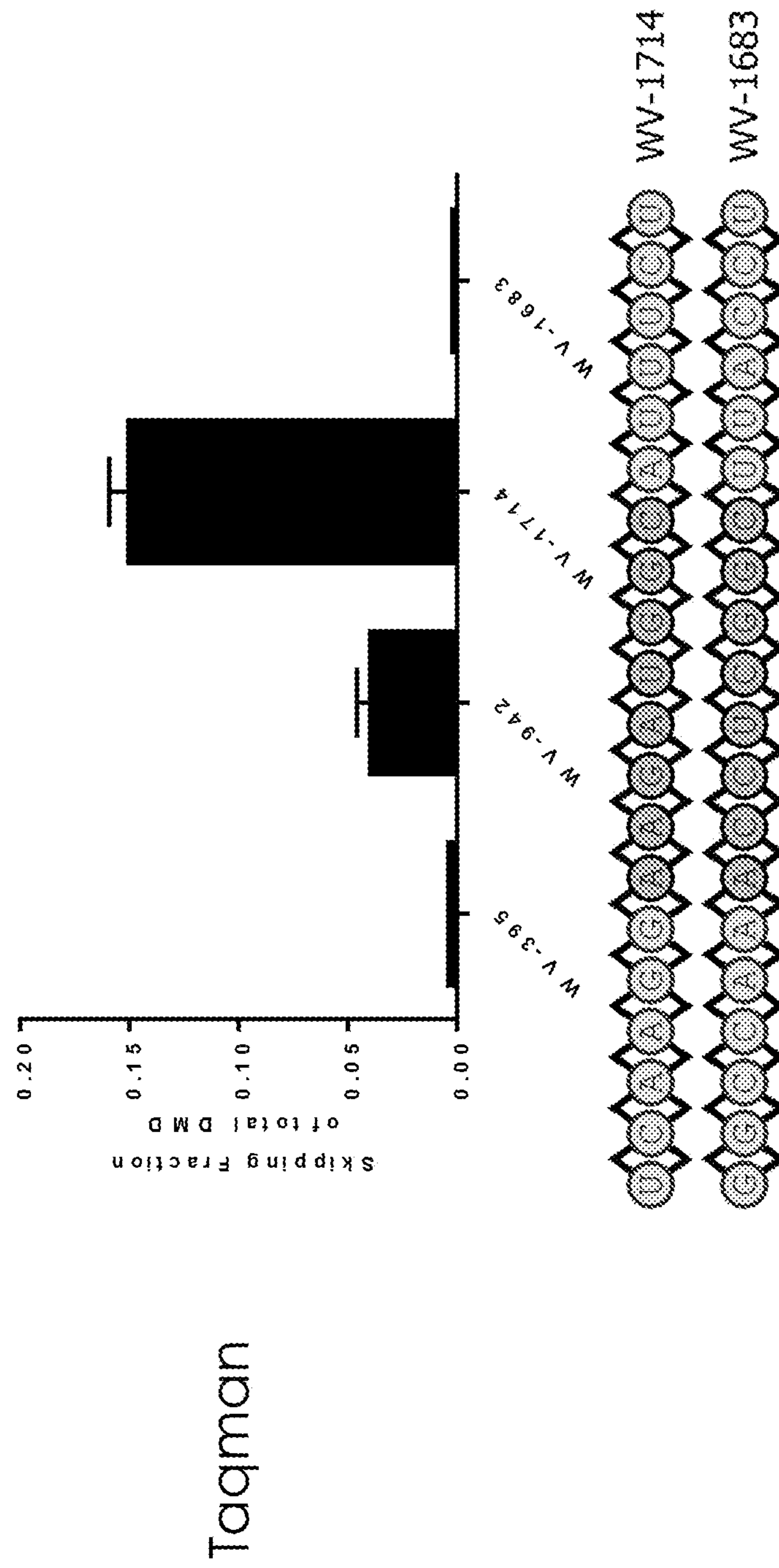


FIG. 11A

WV-1108	mUmCmAmAmGmGmAmAmGmAmUmGmGmCmAmUmUmUmCmU
WV-2381	mU*mCmAmAmGmGmAmAmGmAmUmGmGmCmAmUmUmUmC*mU
WV-2382	mU*mC*mAmAmGmGmAmAmGmAmUmGmGmCmAmUmUmU*mC*mU
WV-2383	mU*mC*mA*mAmGmGmAmAmGmAmUmGmGmCmAmUmU*mU*mC*mU
WV-2384	mU*mC*mA*mA*mGmGmAmAmGmAmUmGmGmCmAmU*mU*mC*mU
WV-2385	mU*mC*mA*mA*mG*mGmAmAmGmAmUmGmGmCmA*mU*mU*mC*mU

FIG. 11B

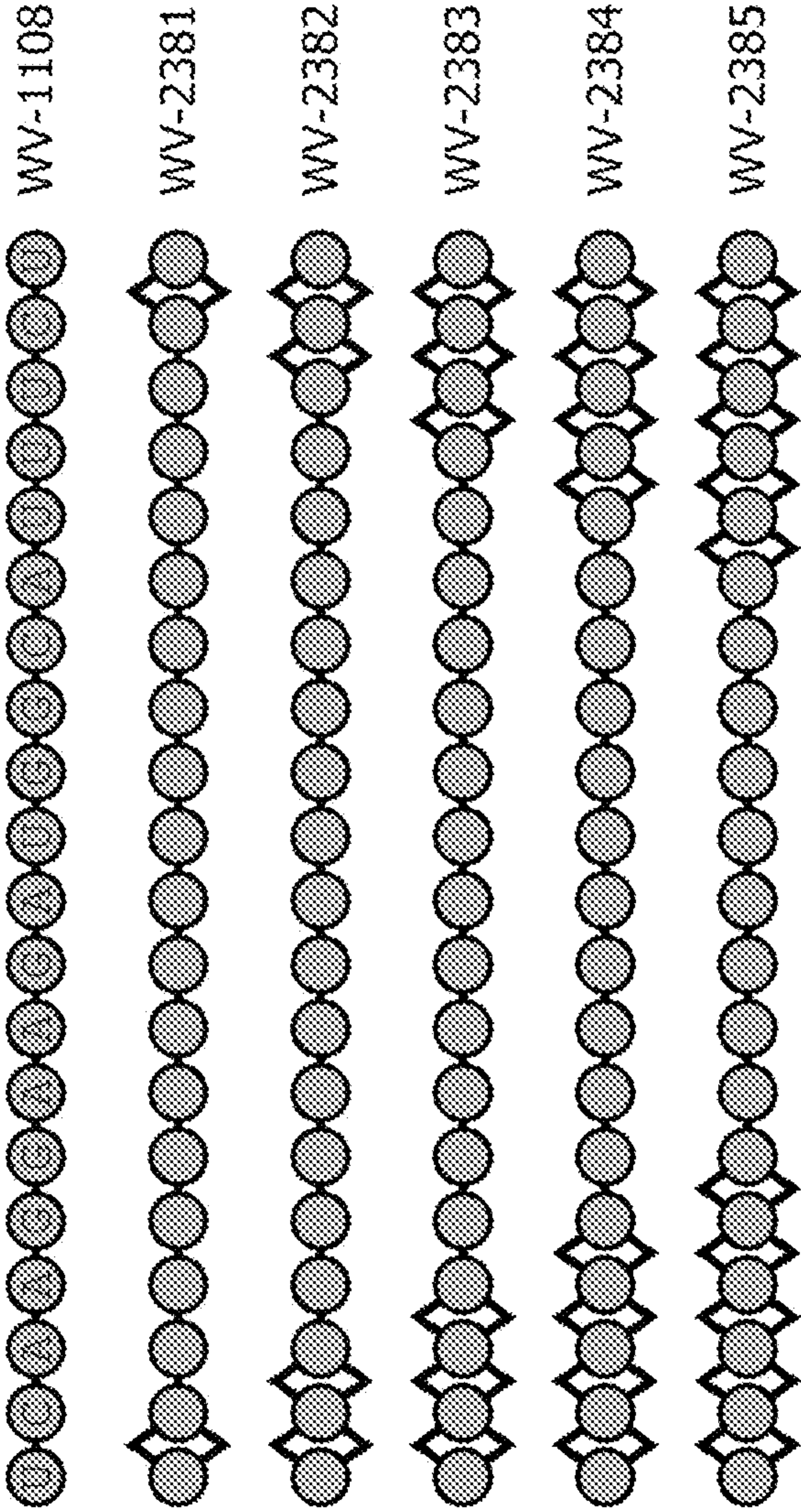
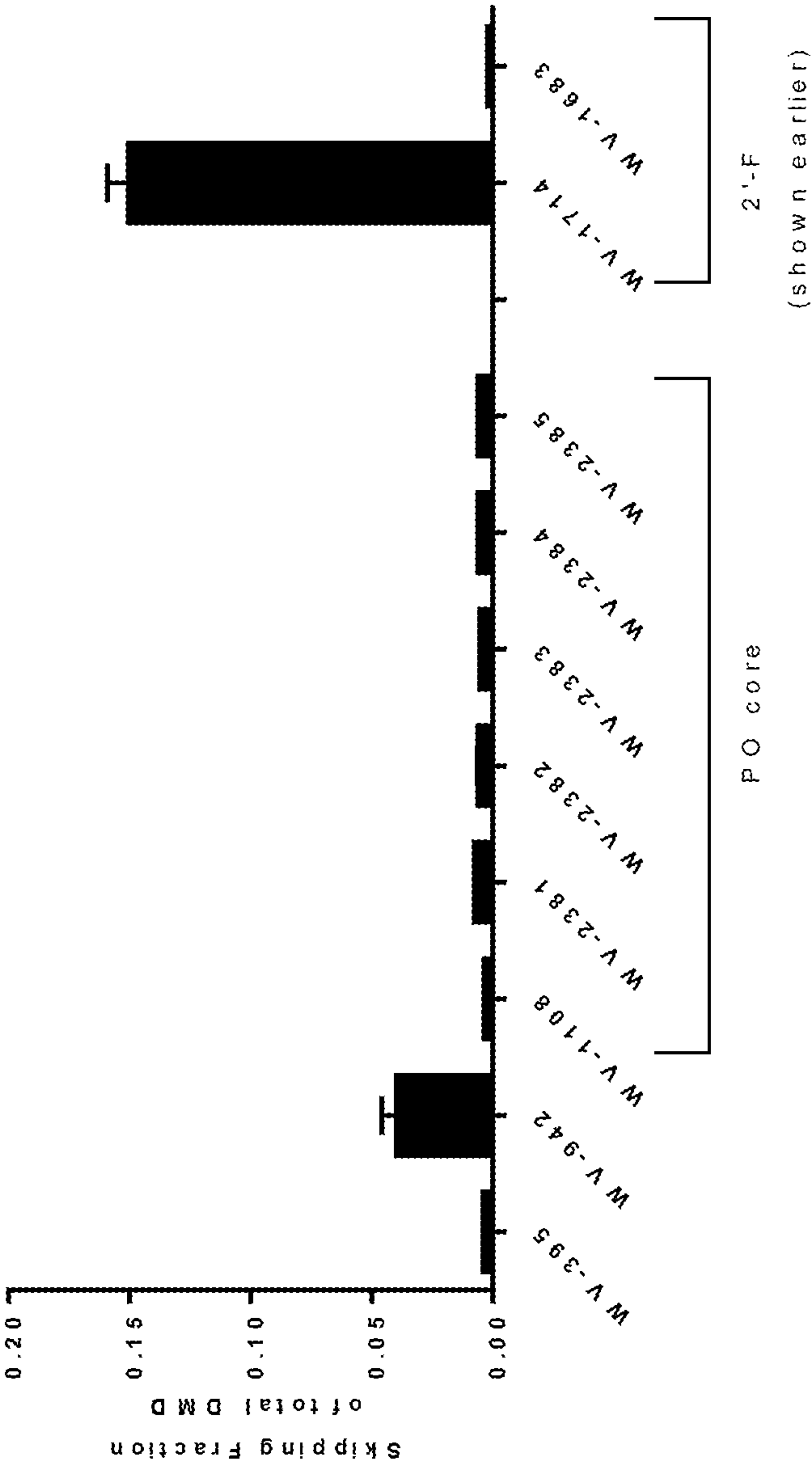


FIG. 12



Taqman

FIG. 13A

WV-2366	mU*SmCmAmGmGmAmAmGmAmUmGmGmCmAmUmUmUmC*SmU
WV-2367	mU*SmC*SmAmAmGmGmAmAmGmAmUmGmGmCmAmUmUmU*SmC*SmU
WV-2368	mU*SmC*SmA*SmAmGmGmAmAmGmAmUmGmGmCmAmUmU*SmU*SmC*SmU
WV-2369	mU*SmC*SmA*SmA*SmGmGmAmAmGmAmUmGmGmCmAmU*SmU*SmC*SmU
WV-2370	mU*SmC*SmA*SmA*SmG*SmGmAmAmGmAmUmGmGmCmA*SmU*SmU*SmC*SmU

FIG. 13B

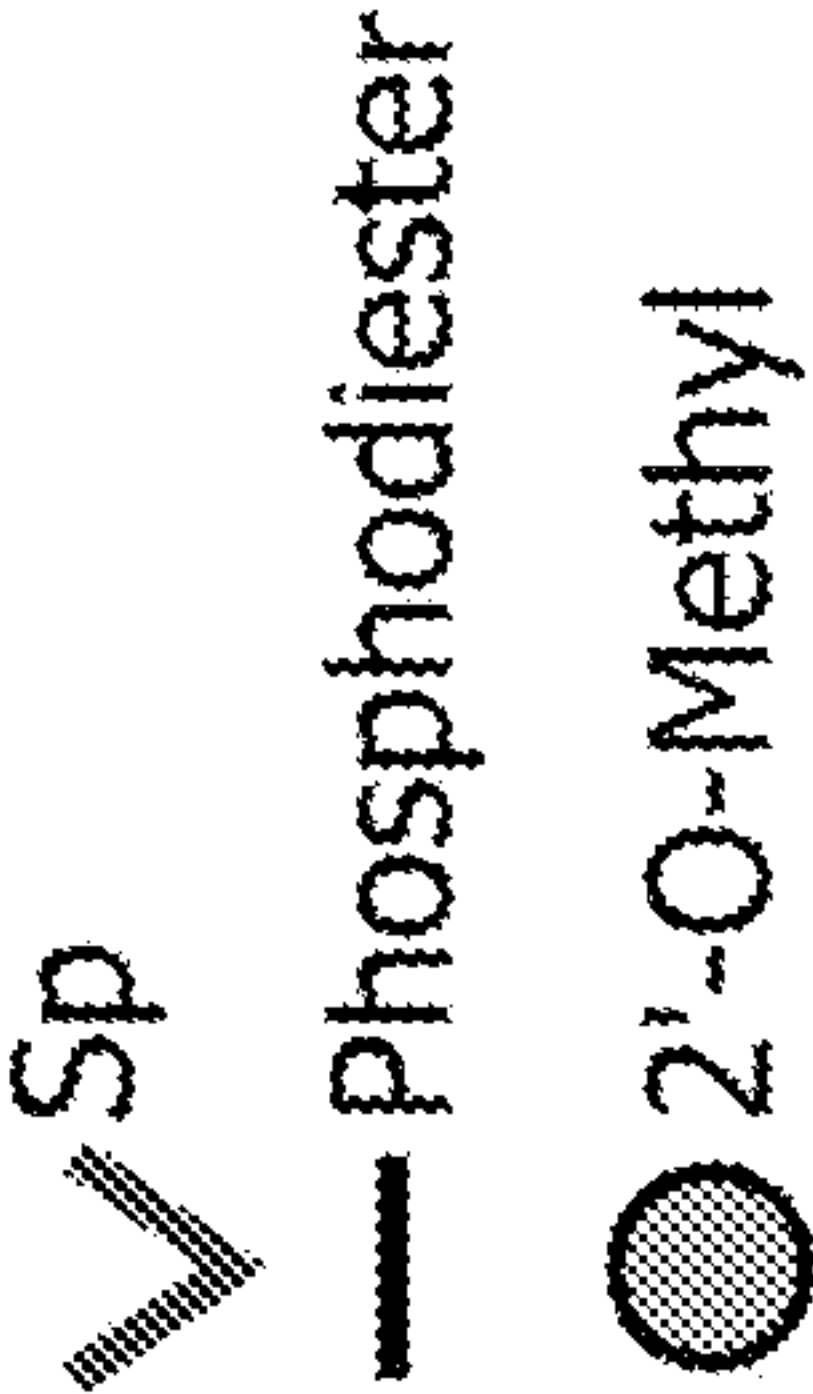
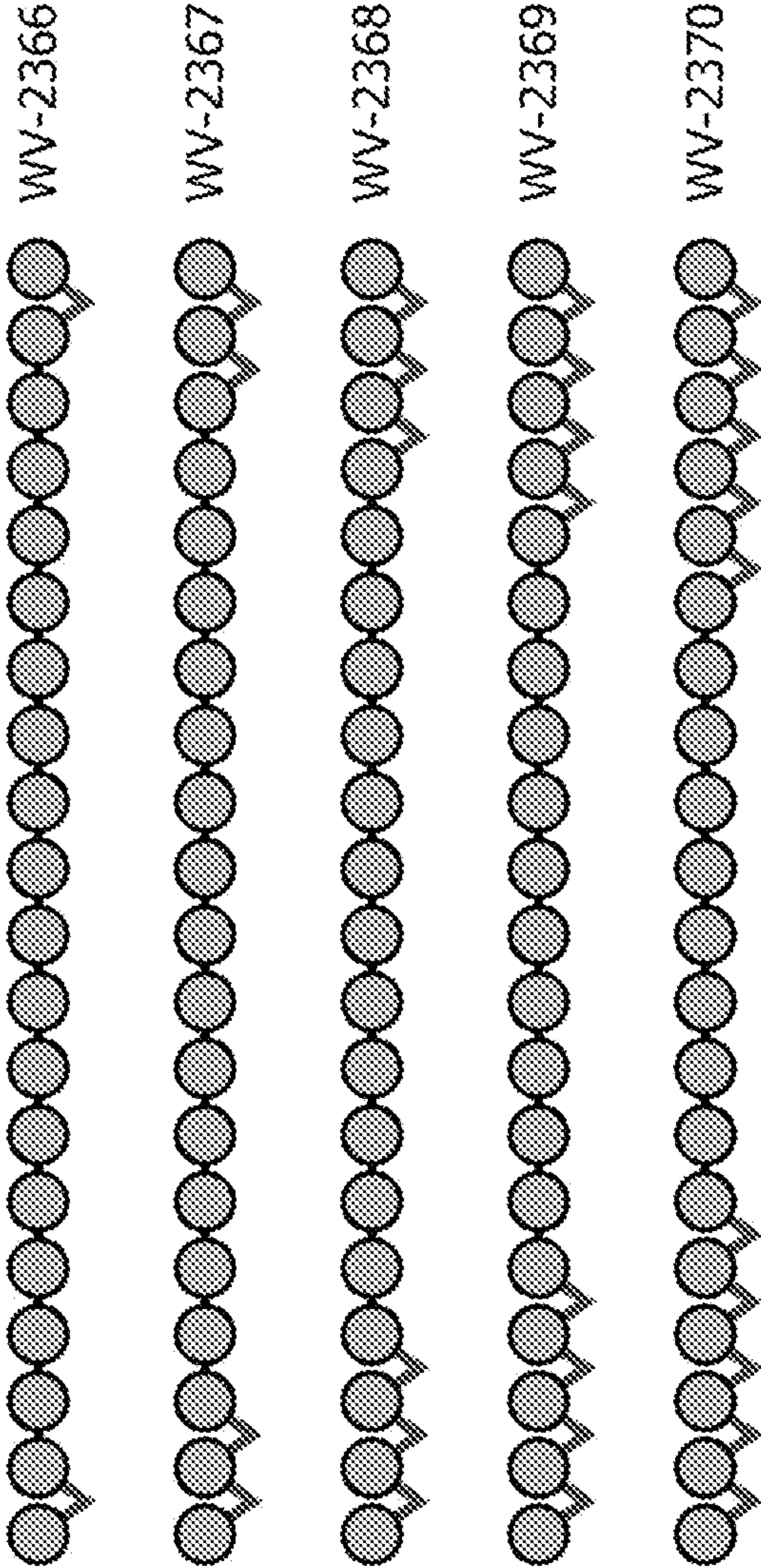


FIG. 14

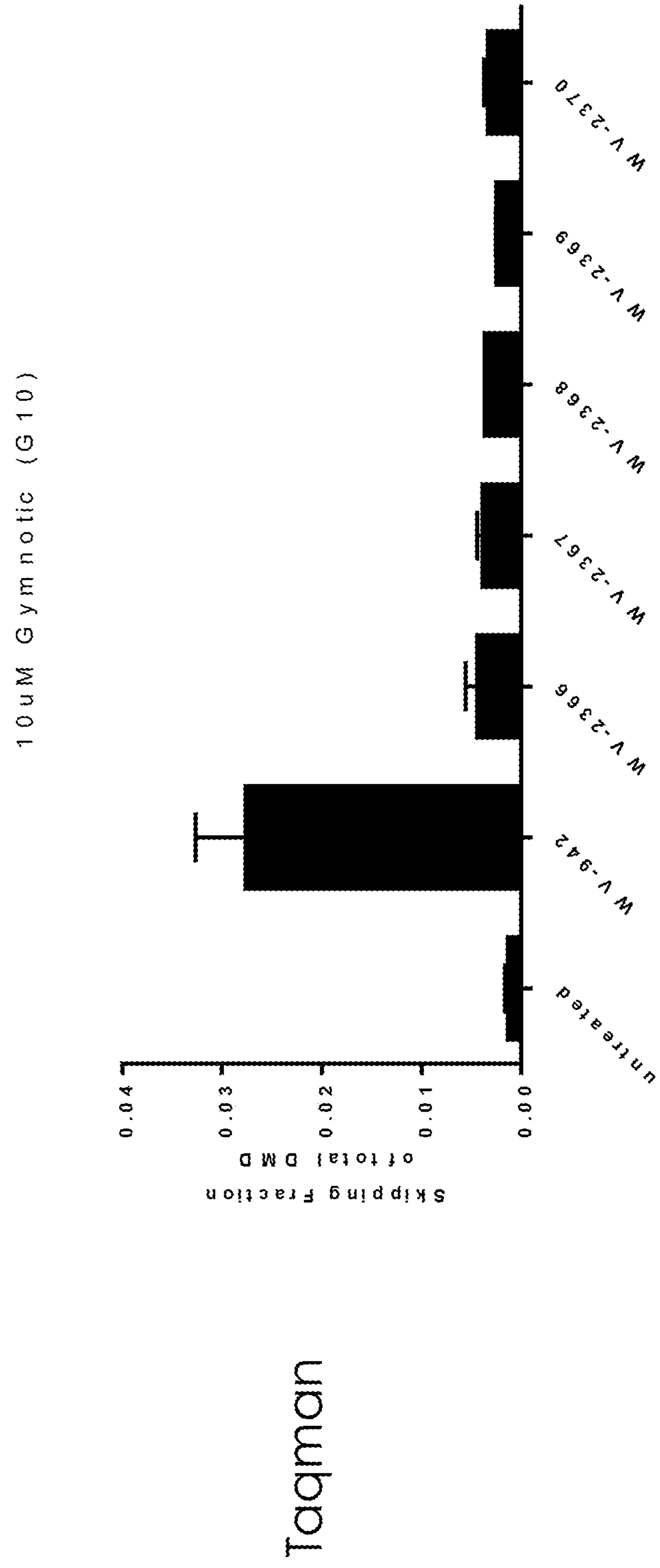


FIG. 15

WV-2223	mC*mA*mA* mG*mG*mA* mA*mG*mA* mU*mG*mG* mC*mA*mU* mU*mU*mC* mU*mA*mG* mU*mU*mU* mG
WV-2224	mC*mA*mU* mC*mA*mA* mG*mG*mA* mA*mG*mA* mU*mG*mG* mC*mA*mU* mU*mU*mC* mU*mA*mG* mU
WV-2225	mA*mC*mA* mU*mC*mA* mA*mG*mG* mA*mA*mG* mA*mU*mG* mG*mC*mA* mU*mU*mU* mC*mU*mA* mG
WV-2226	mA*mA*mC* mA*mU*mC* mA*mA*mG* mG*mA*mA* mG*mG*mC* mA*mU*mU* mU*mC*mU* mA
WV-2227	mC*mA*mA* mC*mA*mU* mC*mA*mA* mG*mG*mA* mA*mG*mA* mU*mG*mG* mC*mA*mU* mU*mU*mC* mU
WV-2228	mC*mU*mC* mC*mA*mA* mC*mA*mU* mC*mA*mA* mG*mG*mA* mA*mG*mG* mU*mG*mG* mC*mA*mU* mU
WV-2229	mA*mC*mC* mU*mC*mC* mA*mA*mC* mA*mU*mC* mA*mA*mG* mG*mA*mA* mG*mA*mU* mG*mG*mC* mA
WV-2230	mG*mU*mA* mC*mC*mU* mC*mC*mA* mA*mC*mA* mU*mC*mA* mA*mG*mG* mA*mA*mG* mA*mU*mG* mG
WV-2313	mA*mU*mU* mU*mC*mU* mA*mG*mU* mU*mU*mG* mG*mA*mG* mA*mU*mG* mG*mC
WV-2314	mU*mG*mG* mC*mA*mU* mU*mU*mC* mU*mA*mG* mU*mU*mU* mG*mG*mA* mG*mA
WV-2315	mG*mA*mU* mG*mG*mC* mA*mU*mU* mU*mC*mU* mA*mG*mU* mU*mU*mG* mG*mA
WV-2316	mA*mG*mA* mU*mG*mG* mC*mA*mU* mU*mU*mC* mU*mA*mG* mU*mU*mU* mG*mG
WV-2317	mA*mA*mG* mA*mU*mG* mG*mC*mA* mU*mU*mU* mC*mU*mA* mG*mU*mU* mU*mG
WV-2318	mA*mG*mG* mA*mA*mG* mA*mU*mG* mG*mC*mA* mU*mU*mU* mC*mU*mA* mG*mU
WV-2319	mA*mA*mG* mG*mA*mA* mG*mA*mU* mG*mG*mC* mA*mU*mU* mU*mC*mU* mA*mG
WV-2320	mC*mA*mA* mG*mG*mA* mA*mG*mA* mU*mG*mG* mC*mA*mU* mU*mU*mC* mU*mA

FIG. 16

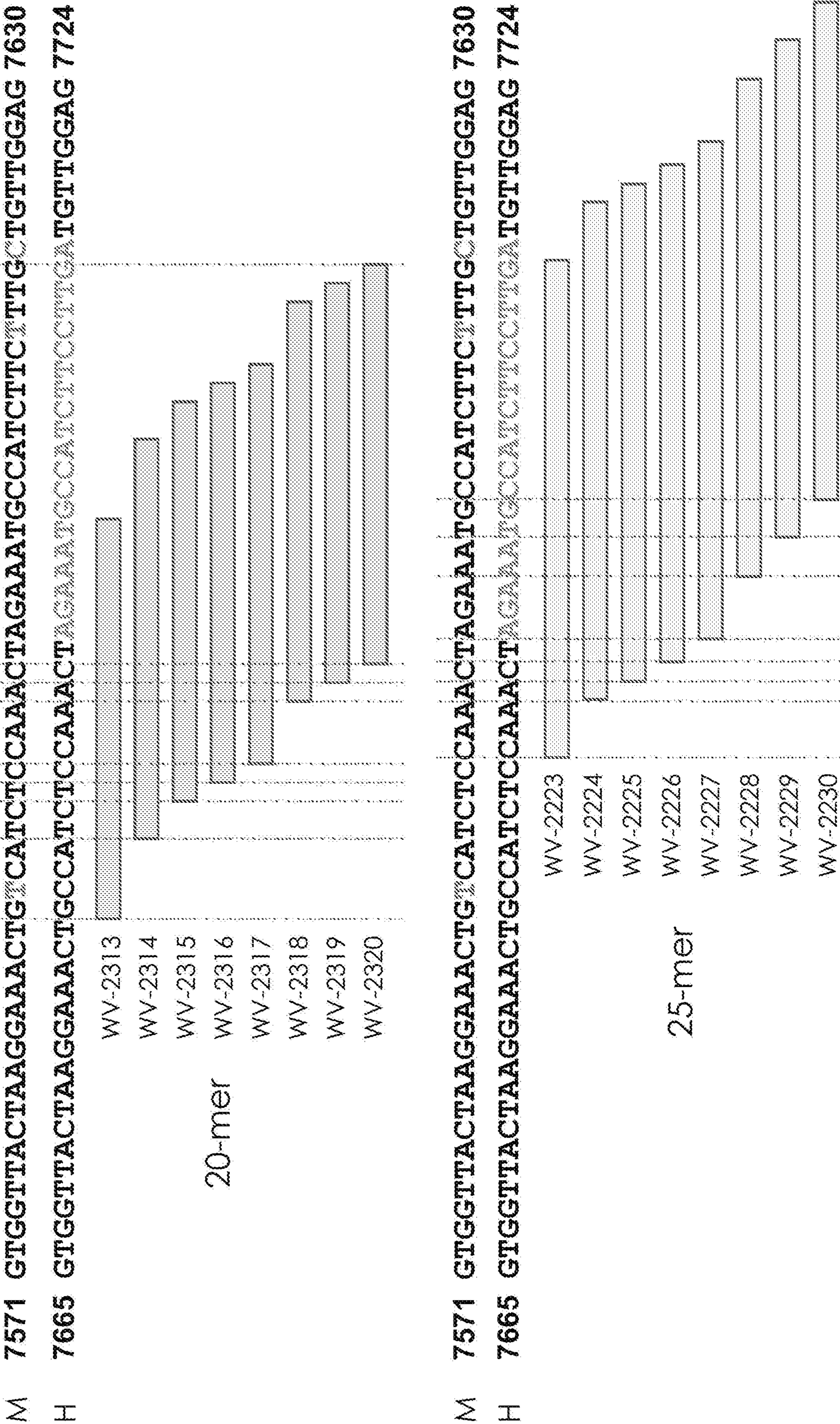


FIG. 17

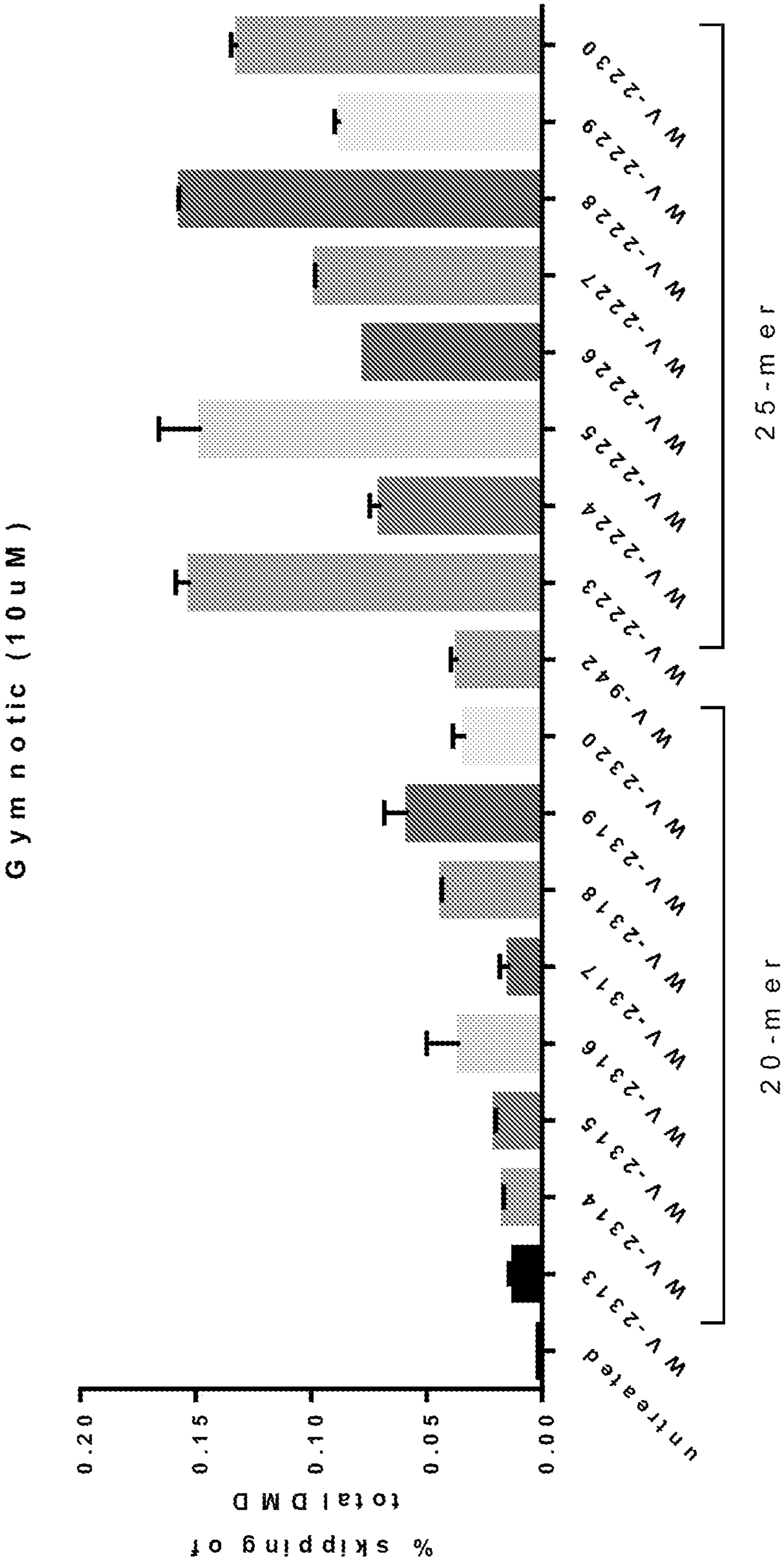


FIG. 18

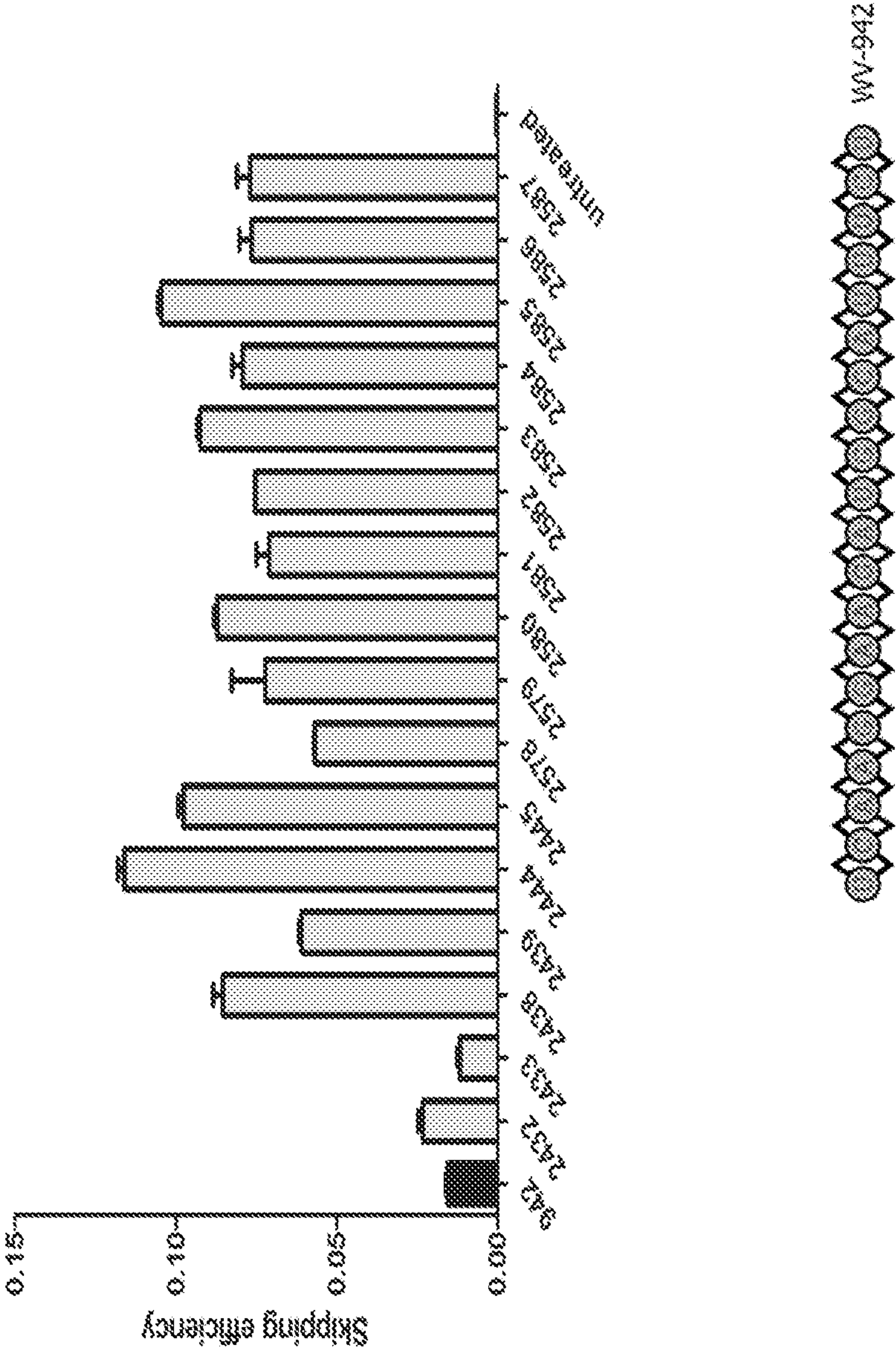
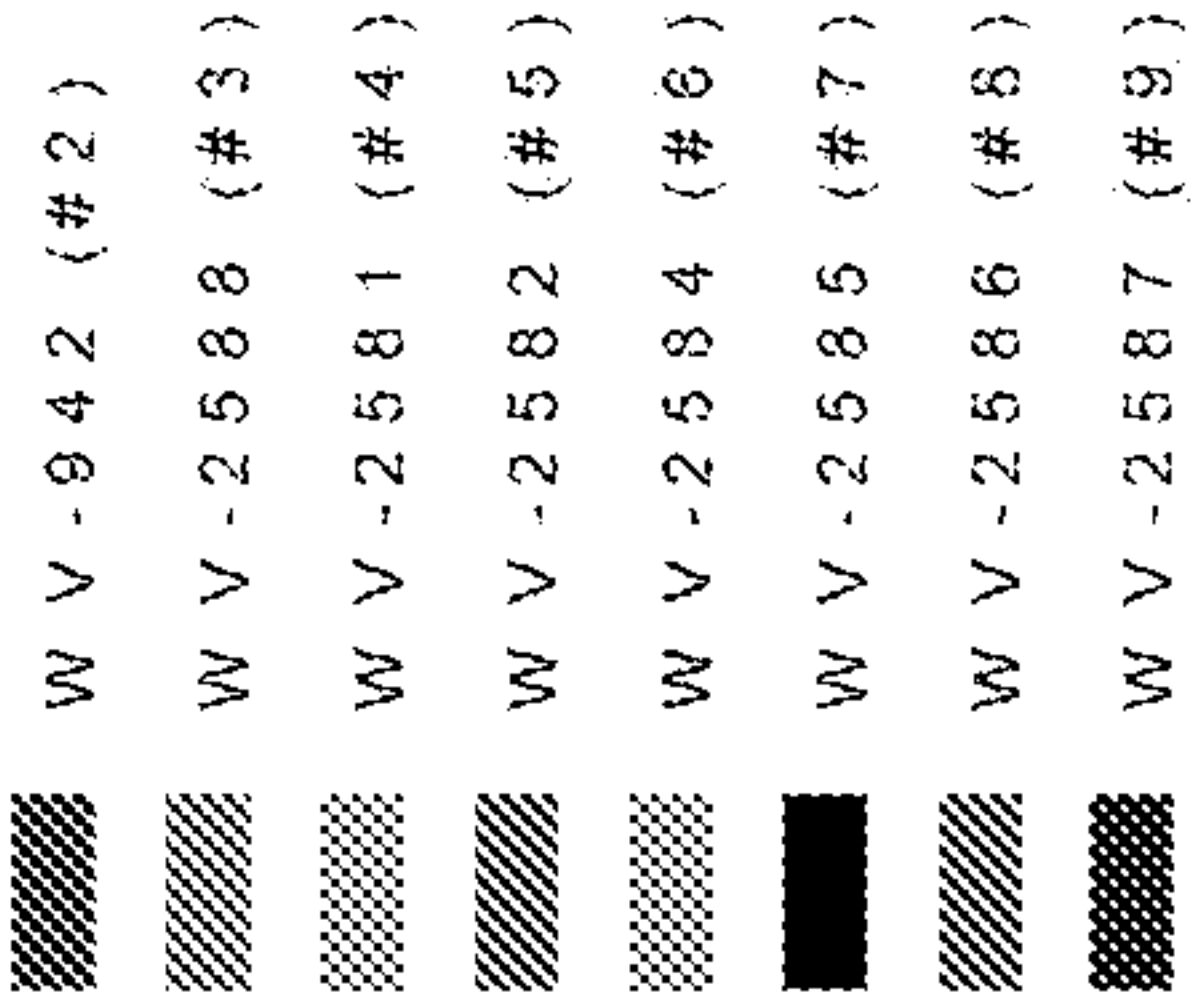


FIG. 19

Oligo (WaVe #)	Conjugated Acid	Carbon	double bond
WV-2578	Lauric acid	12	0
WV-2579	Myristic Acid	14	0
WV-2580	Palmitic acid	16	0
WV-2581	Stearic acid	18	0
WV-2582	Oleic acid	18	1
WV-2583	Linoleic acid	18	2
WV-2584	α -Linolenic acid	18	3
WV-2585	g-Linolenic acid	18	3
WV-2586	cis-DHA	22	6
WV-2587	Turbinaric acid	27	5
WV-2588	Dilinoleyl	37	4



PK data (DMD-03)

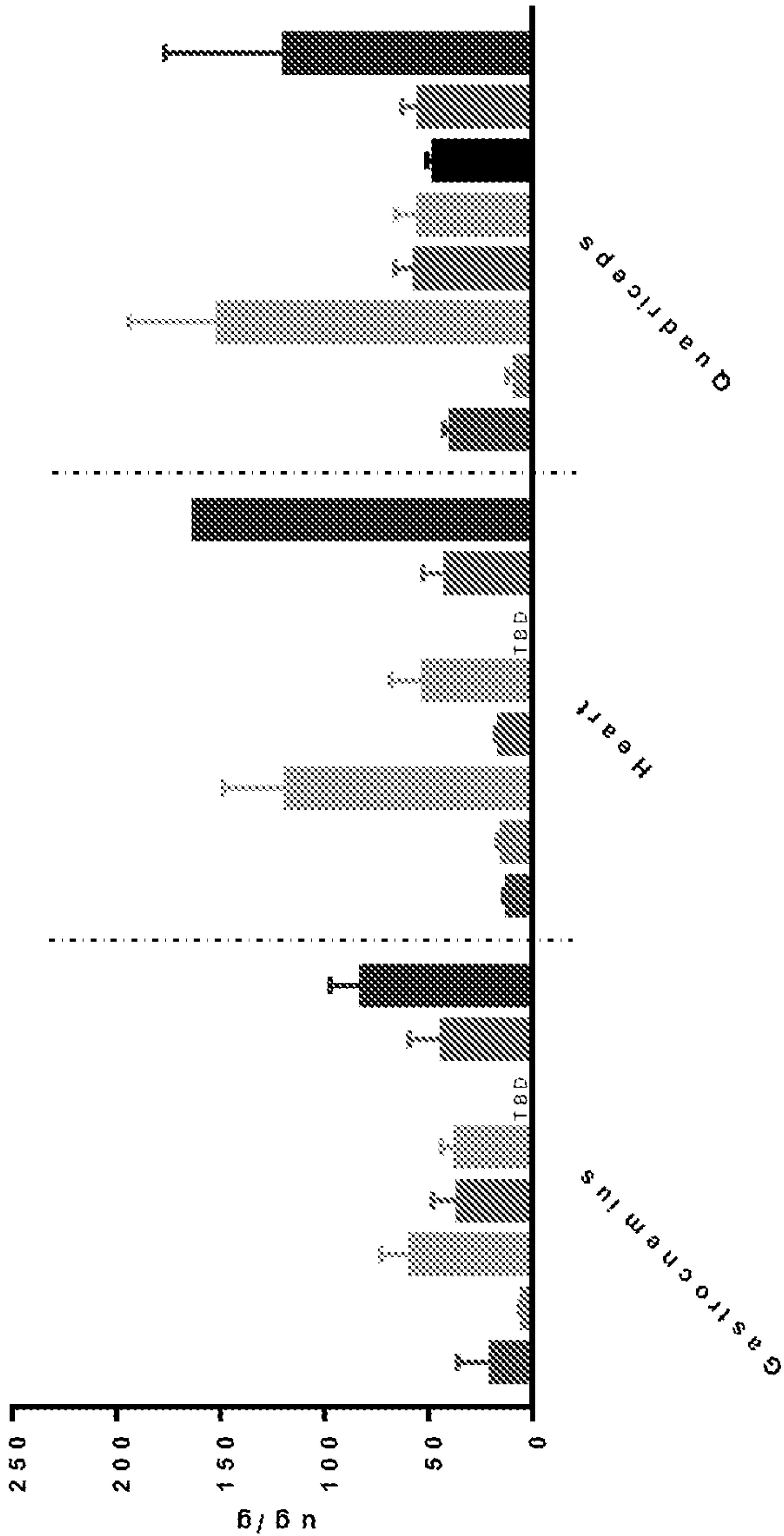
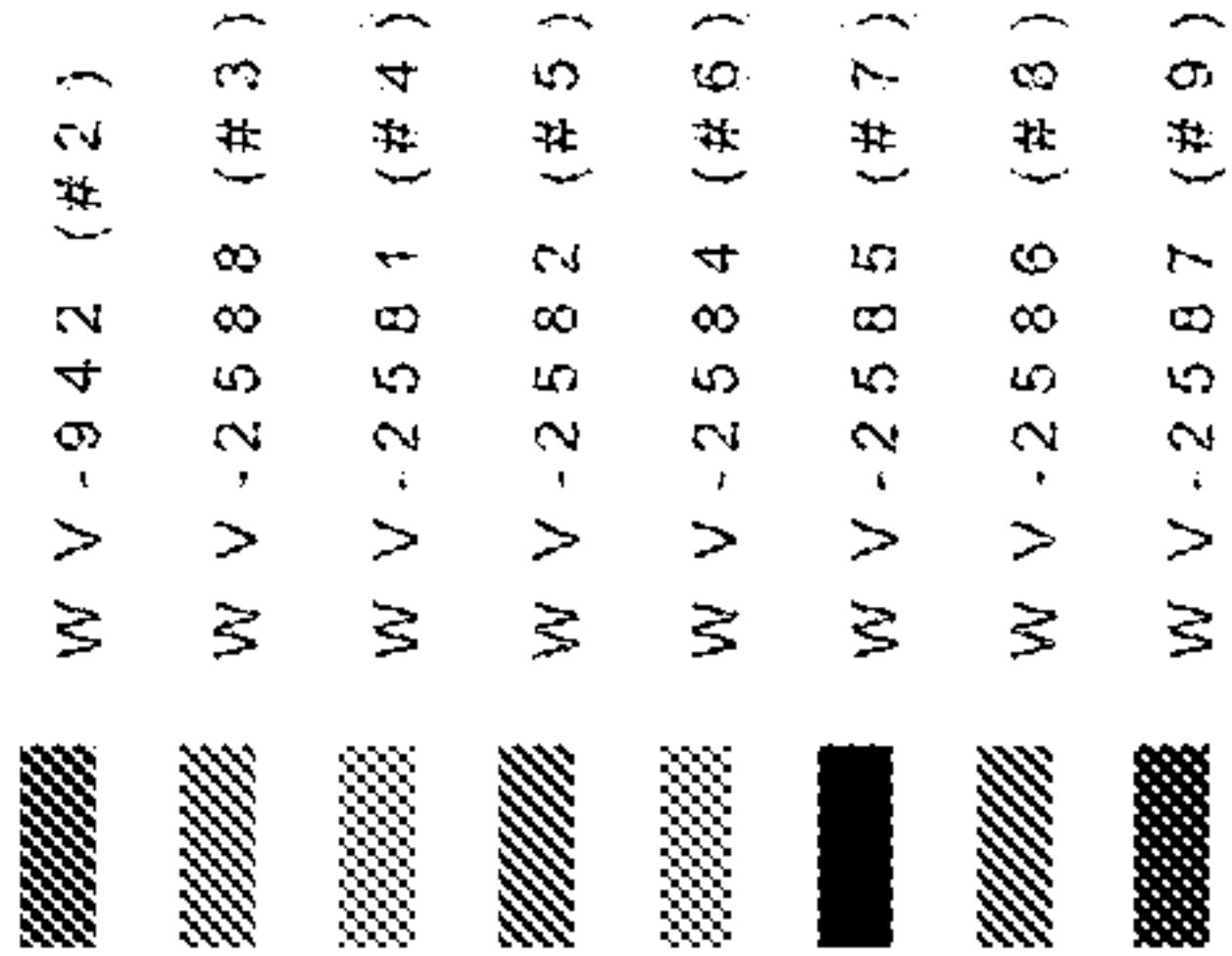


FIG. 20

Oligo (WaVe #)	Conjugated Acid	Carbon	double bond
WV-2578	Lauric acid	12	0
WV-2579	Myristic Acid	14	0
WV-2580	Palmitic acid	16	0
WV-2581	Stearic acid	18	0
WV-2582	Oleic acid	18	1
WV-2583	Linoleic acid	18	2
WV-2584	α -Linolenic acid	18	3
WV-2585	g-Linolenic acid	18	3
WV-2586	cis-DHA	22	6
WV-2587	Turbinaric acid	27	5
WV-2588	Dilinoleyl	37	4



P K data (D M D -03)

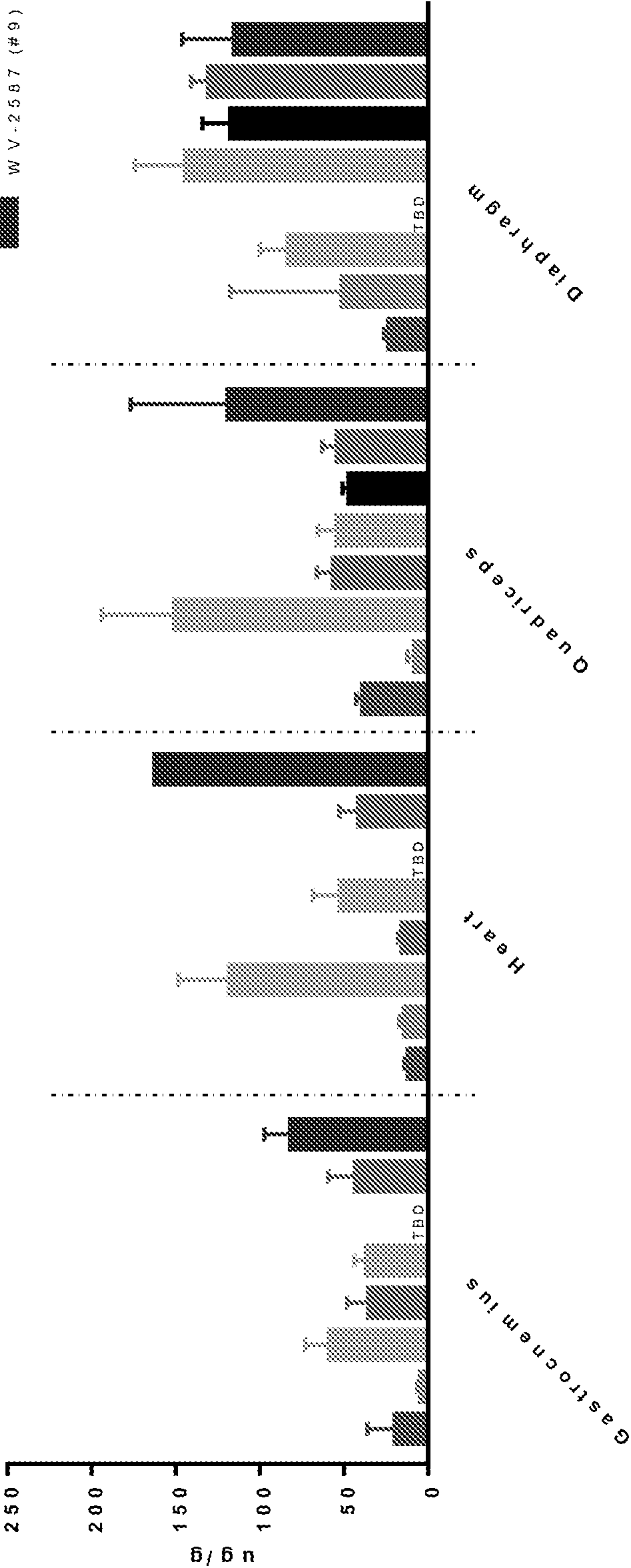
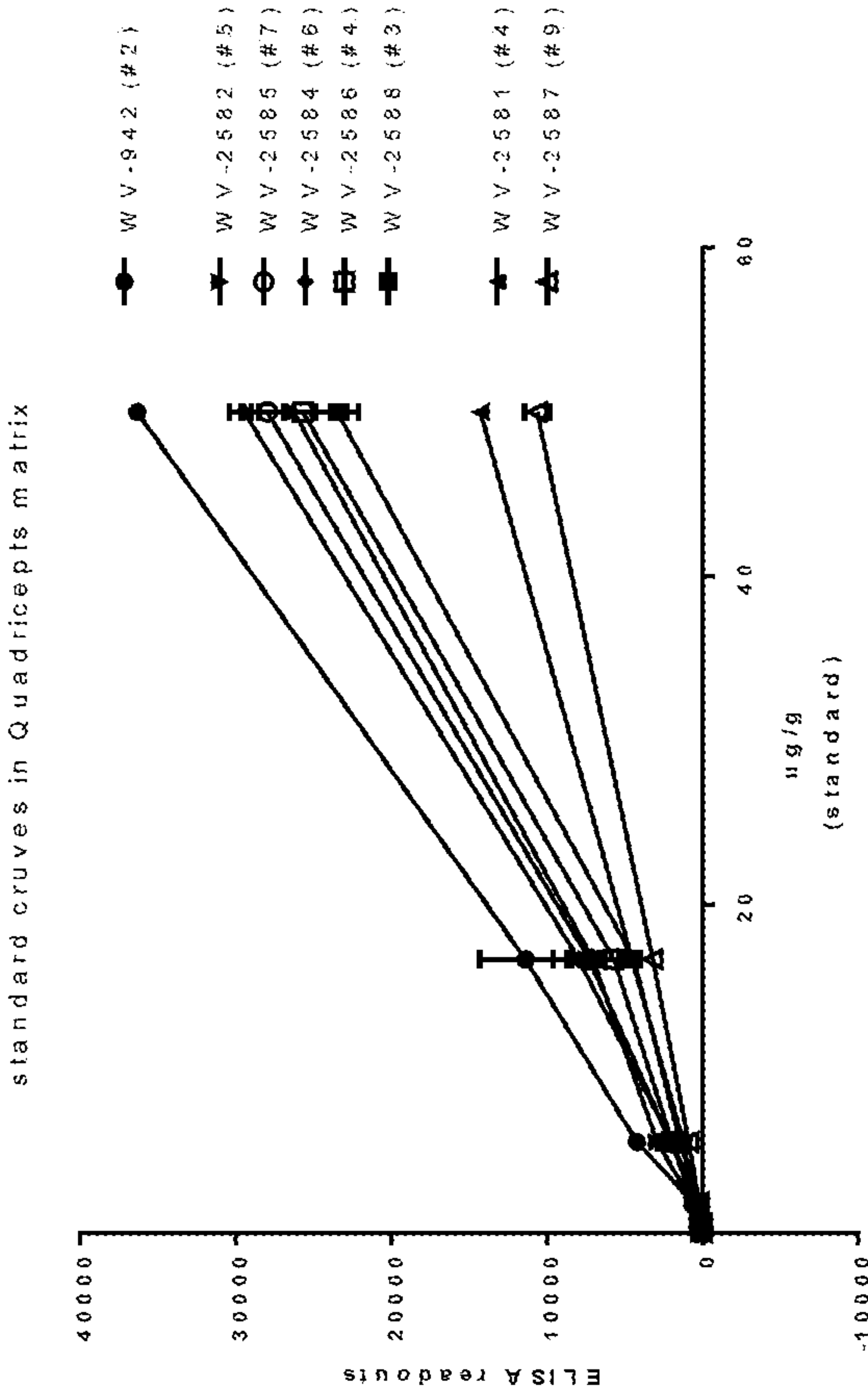


FIG. 21

Quadriceps



Diaphragm

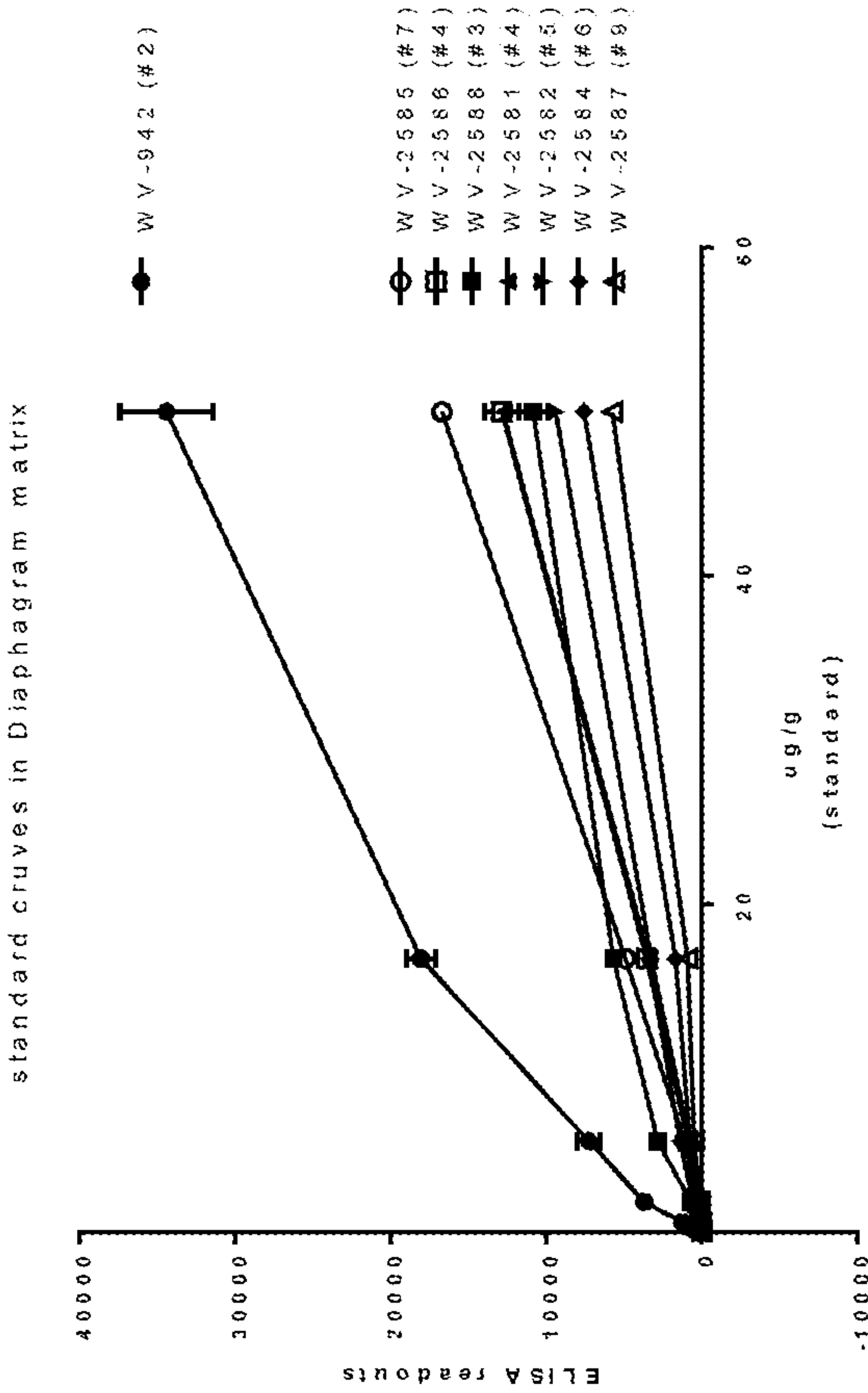
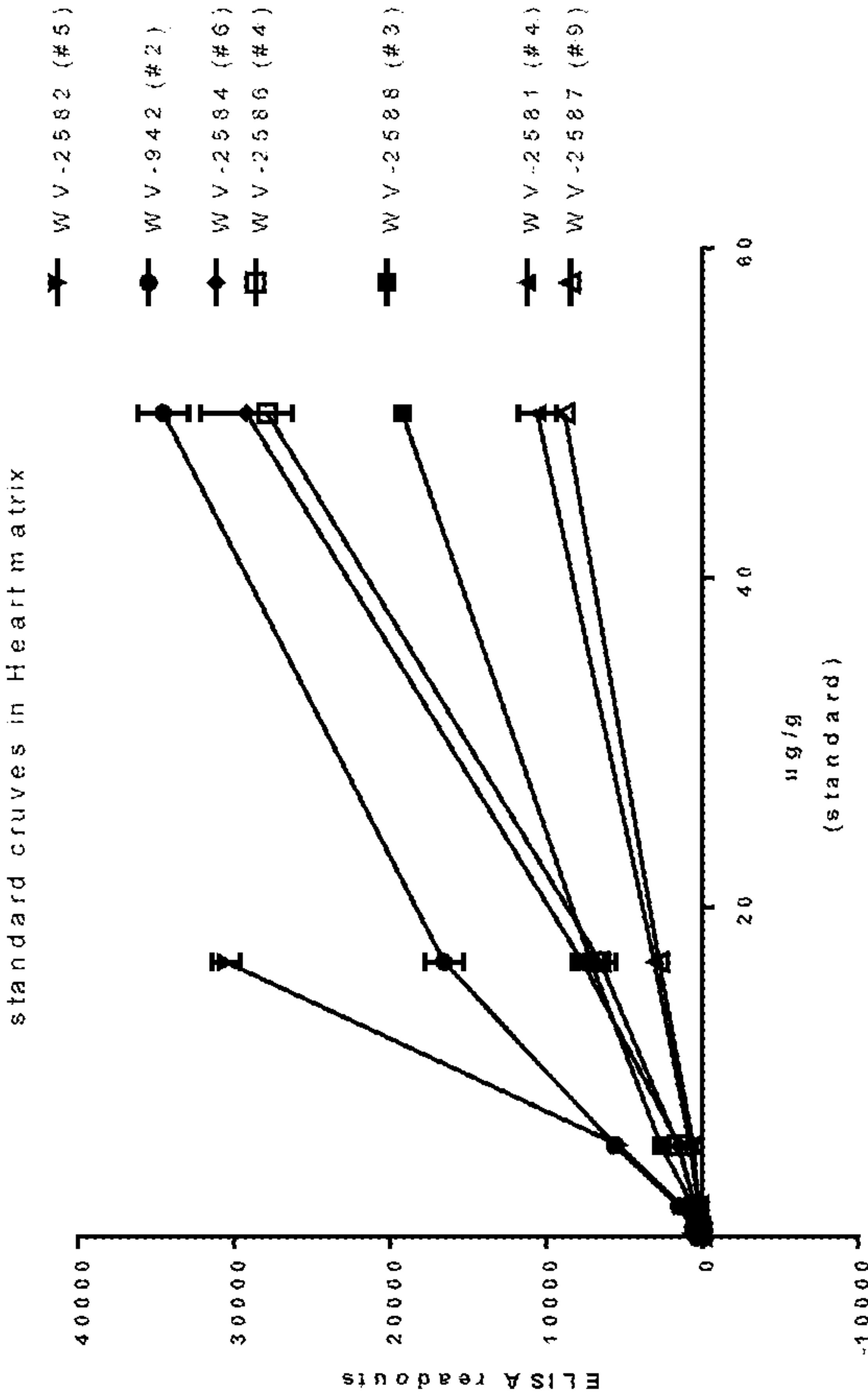
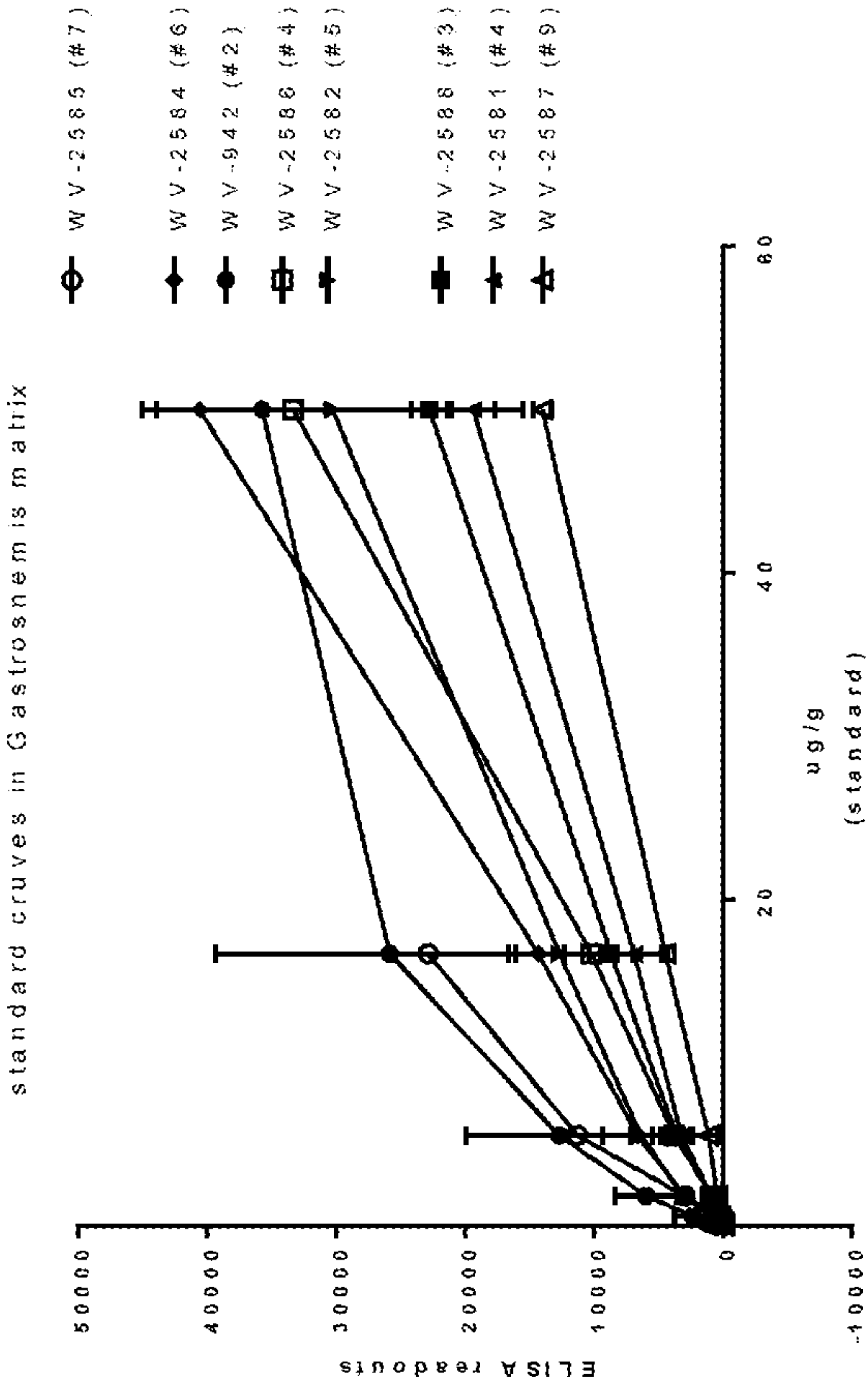


FIG. 22



Heart



Gastrocnemius

FIG. 23

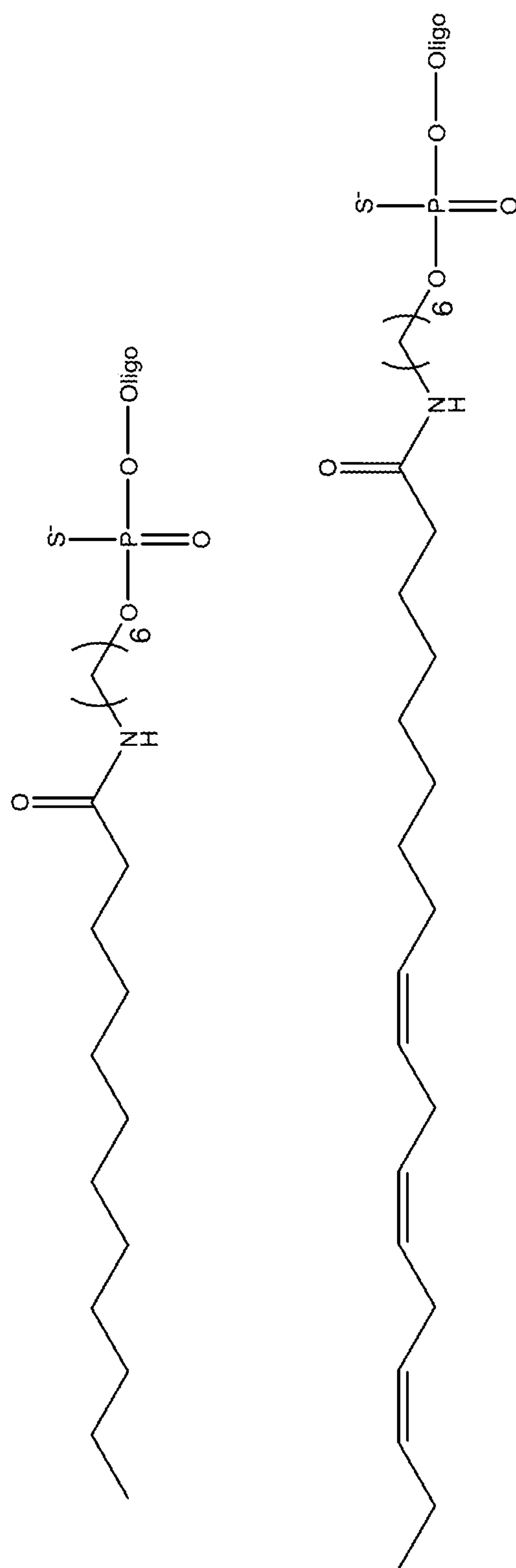


FIG. 24

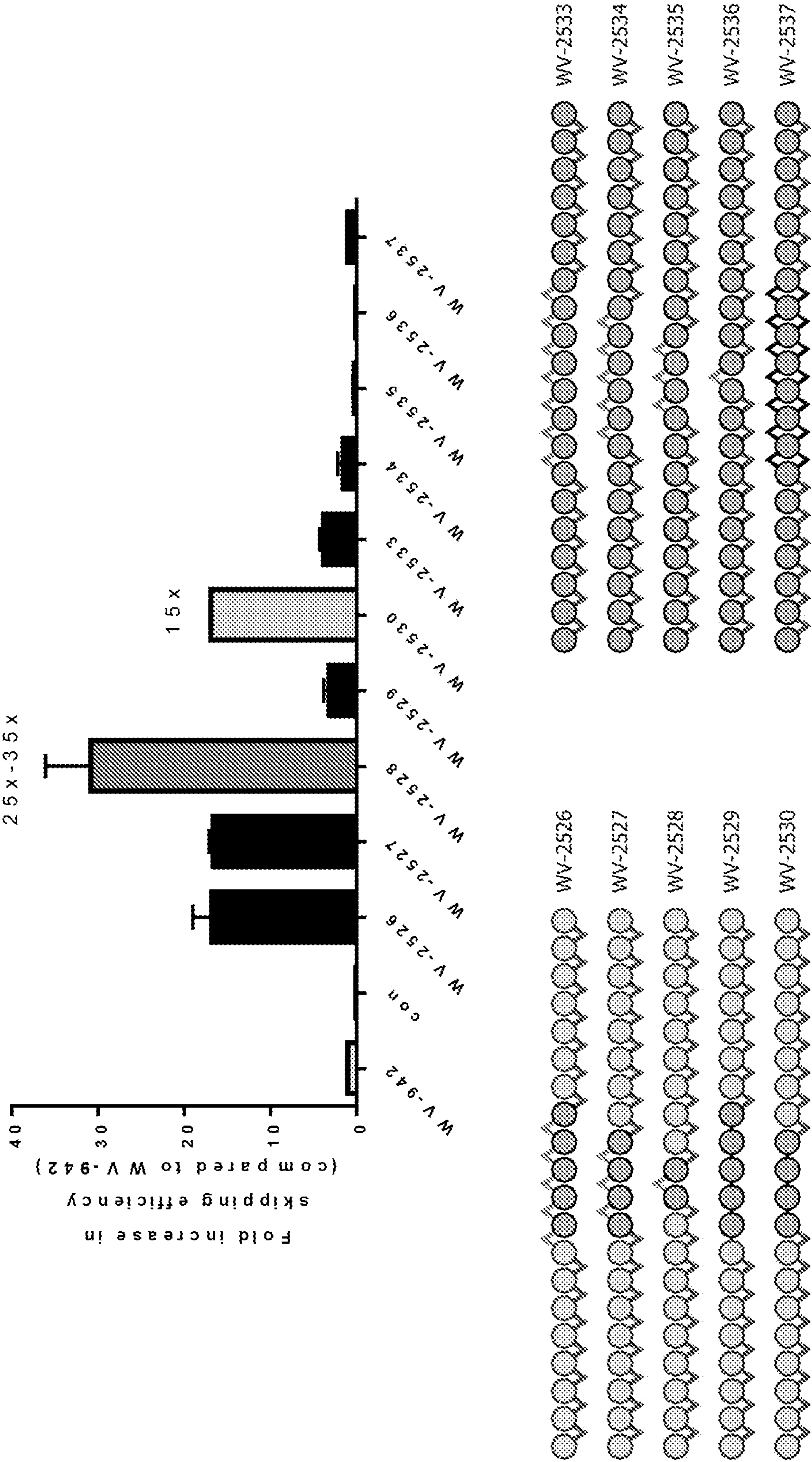


FIG. 25

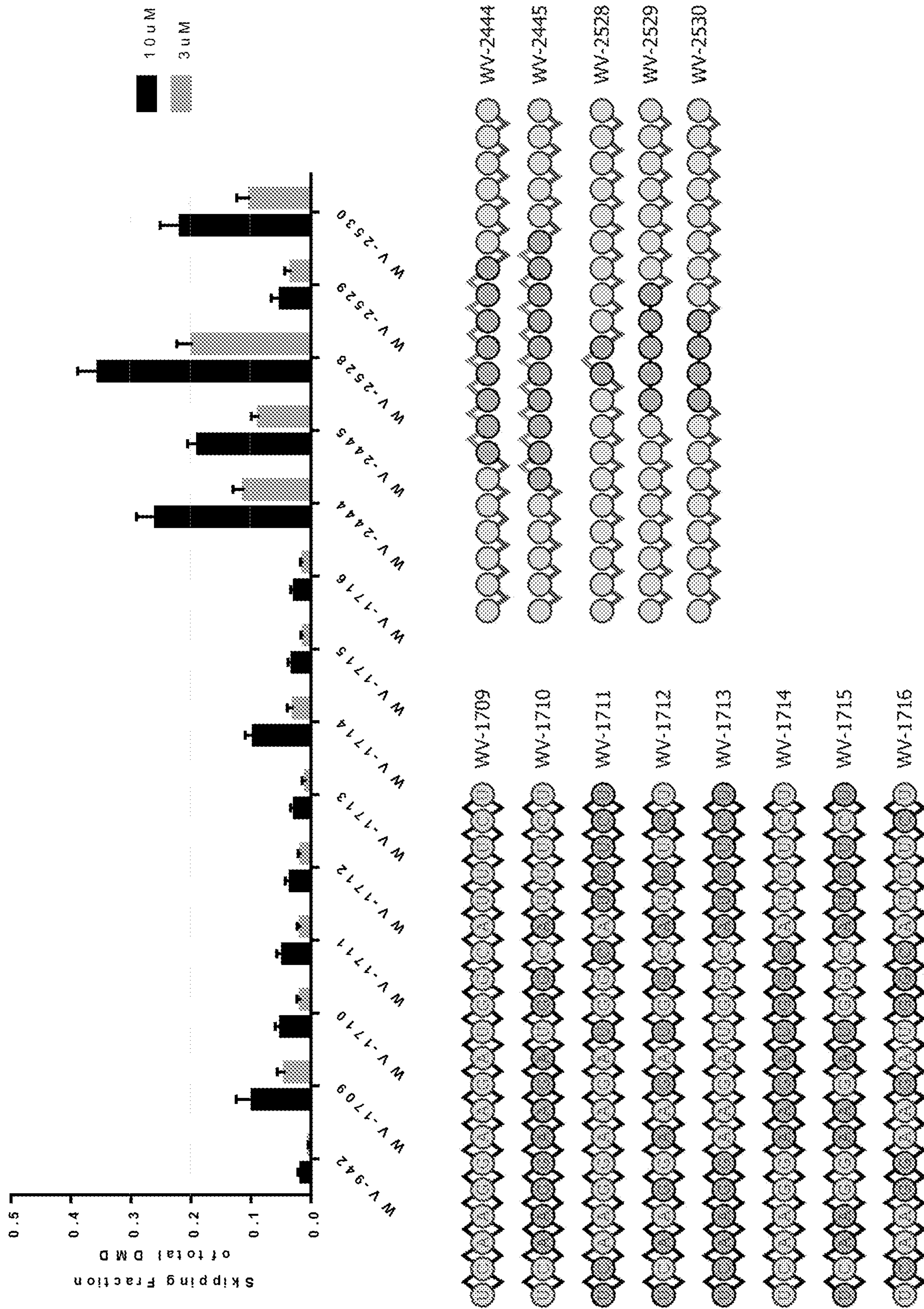


FIG. 26

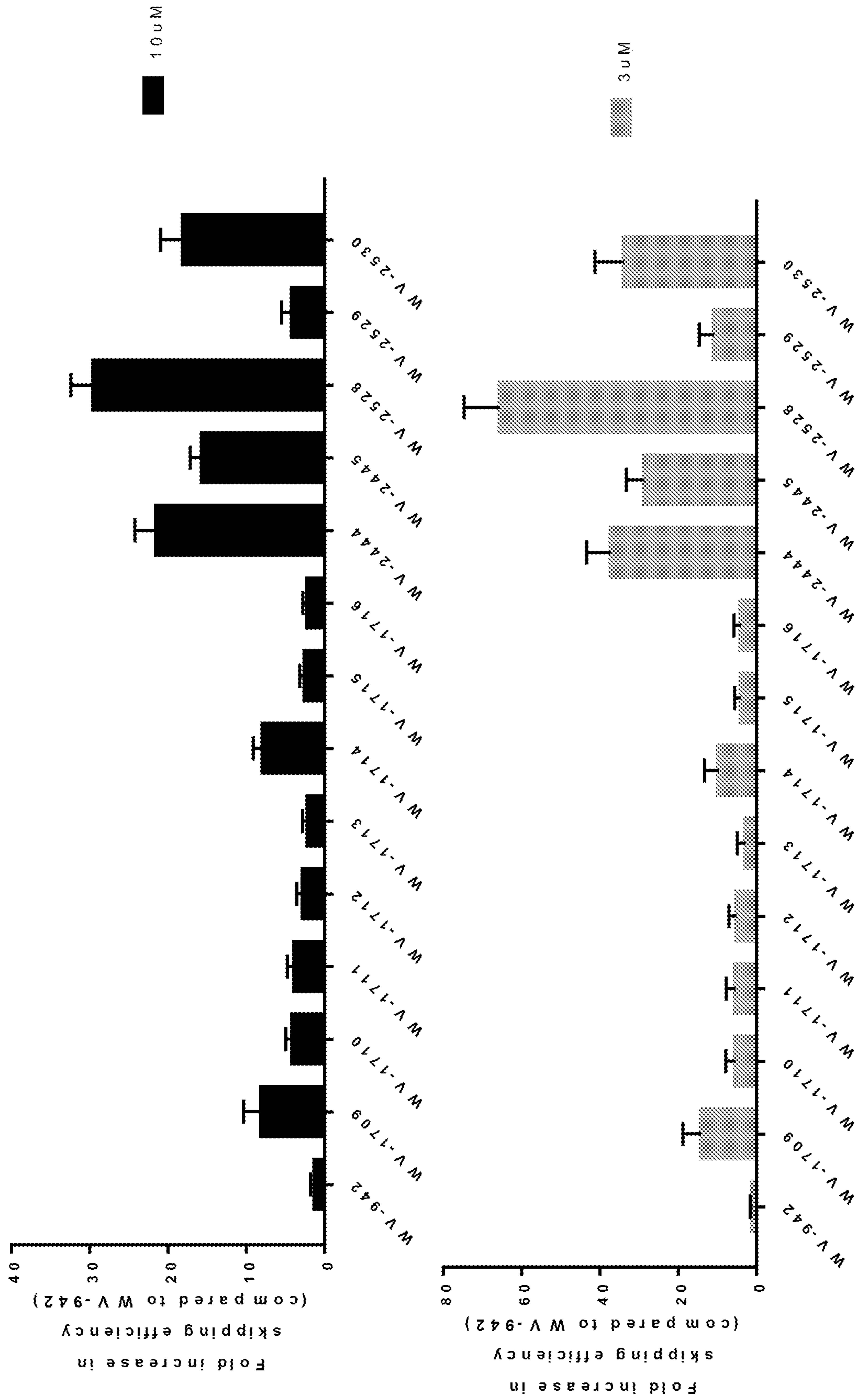
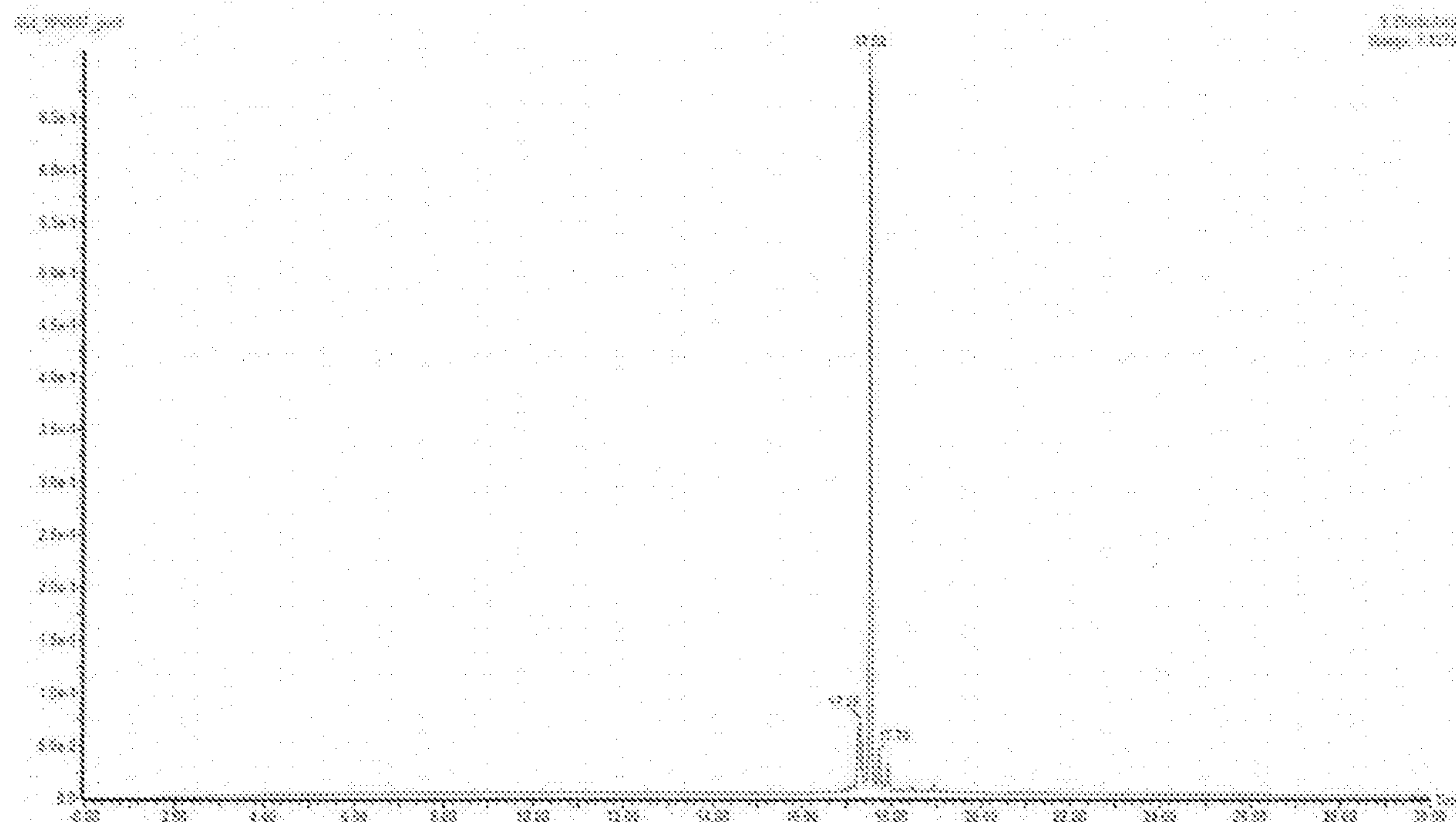


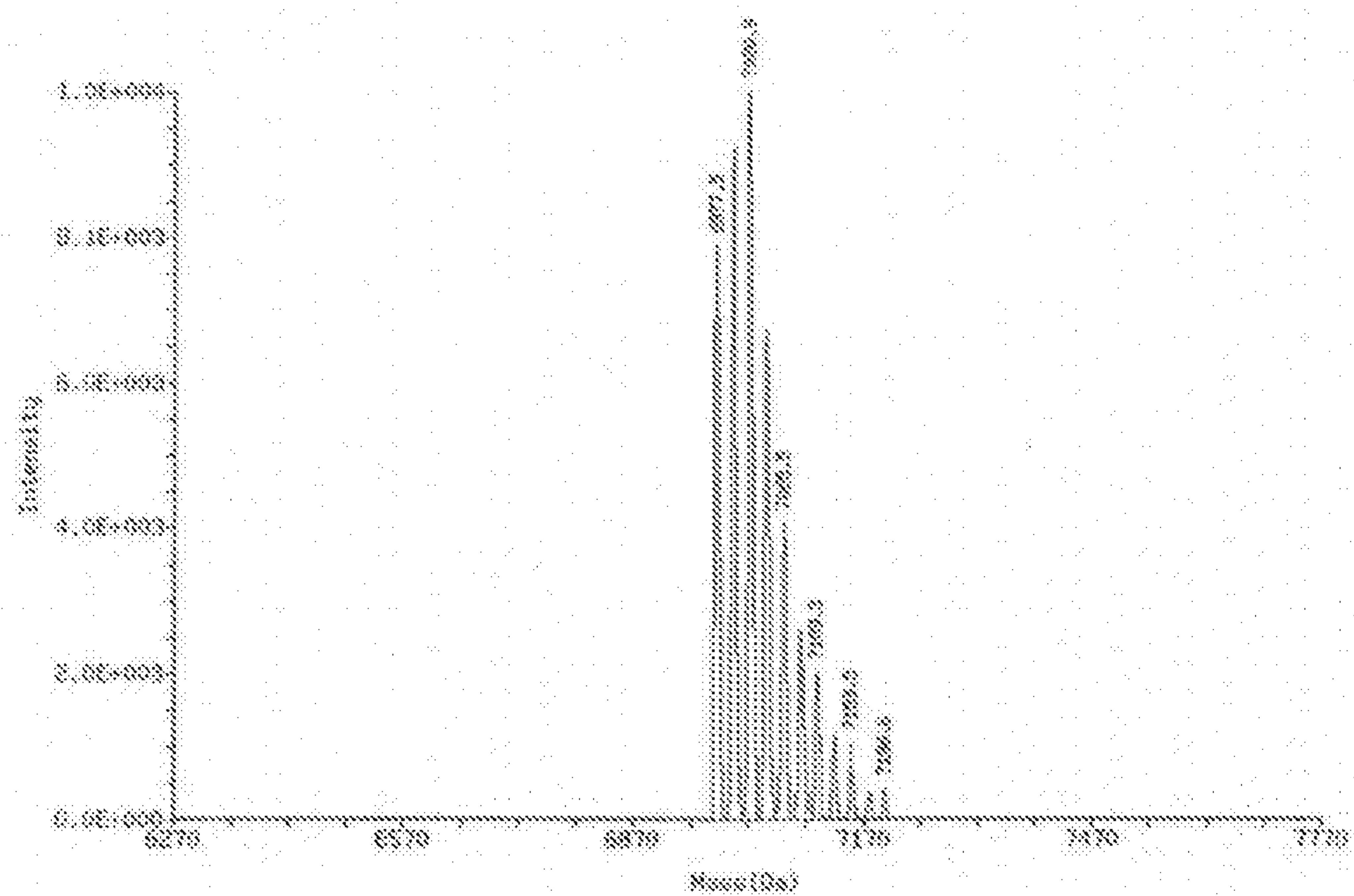
FIG. 27A.

WV887.02

UPLC(RP) analysis



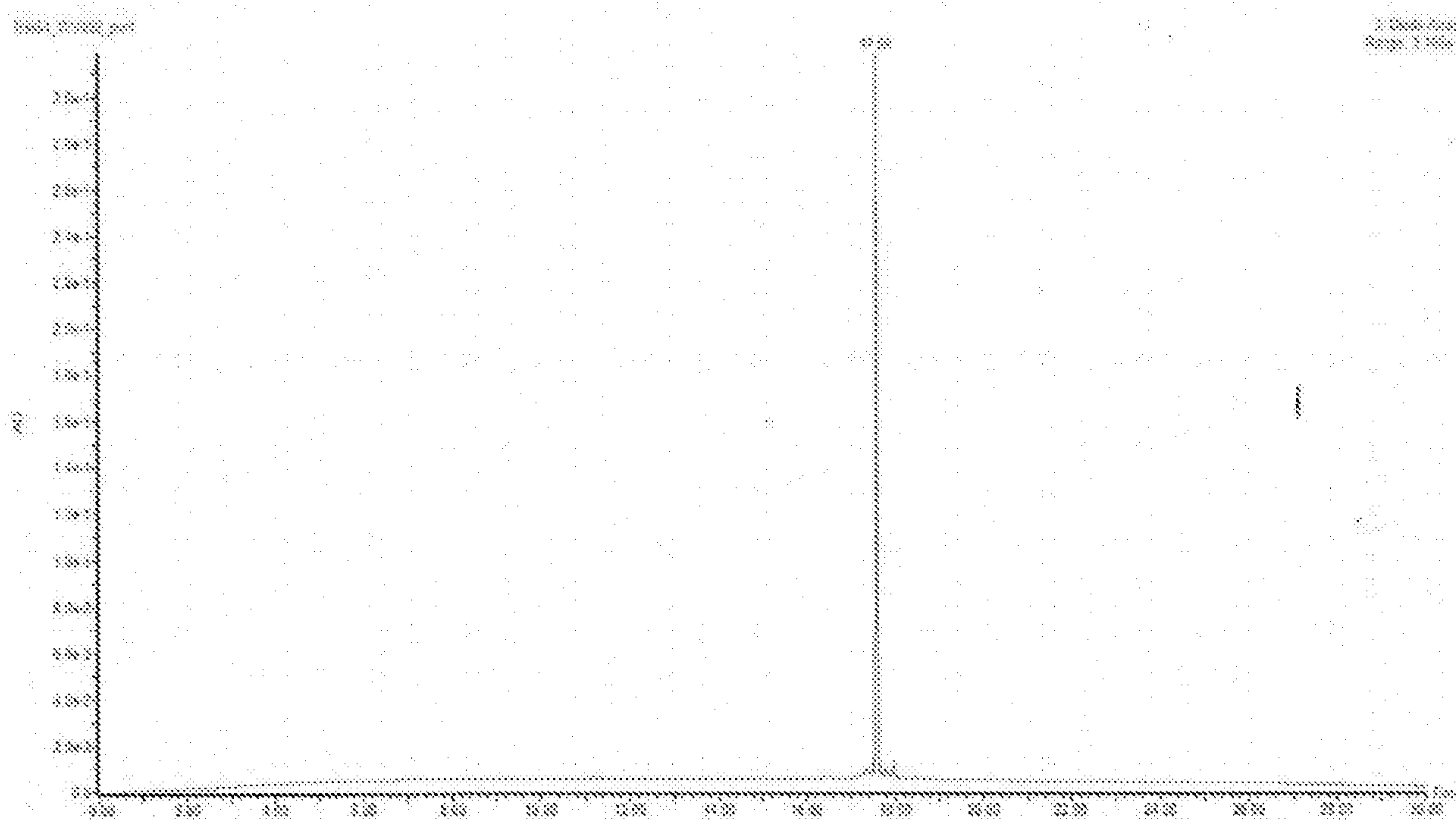
UPLC-MS analysis [MW] calcd 8977.81, found 8977.5



WV887.02

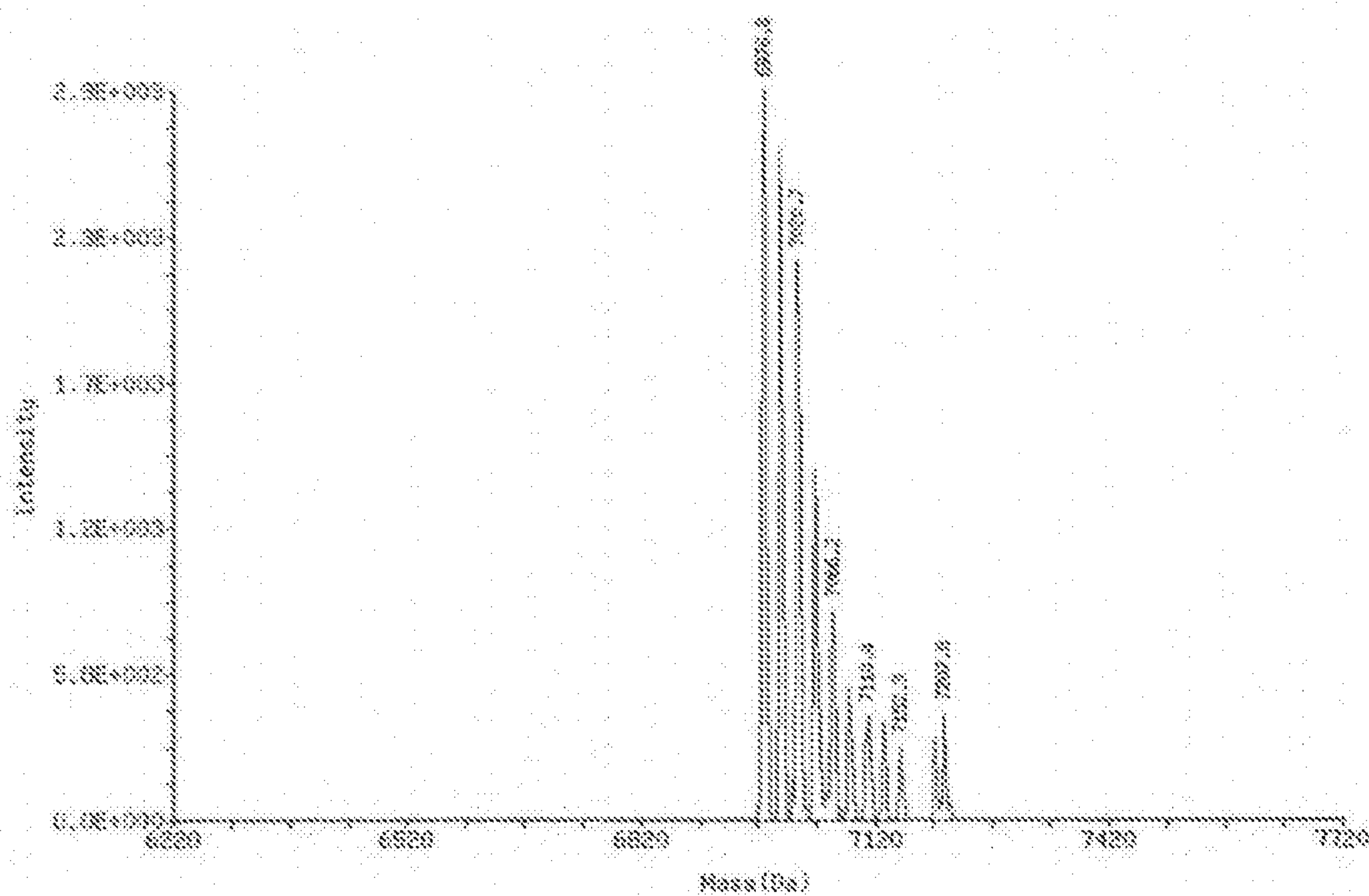
FIG. 27B.

UPLC(RP) analysis



WV892.02

UPLC-MS analysis [MW]: calcd 6977.61, found 6976.8



WV892.02

FIG. 27C.

UPLC(RP) Analysis

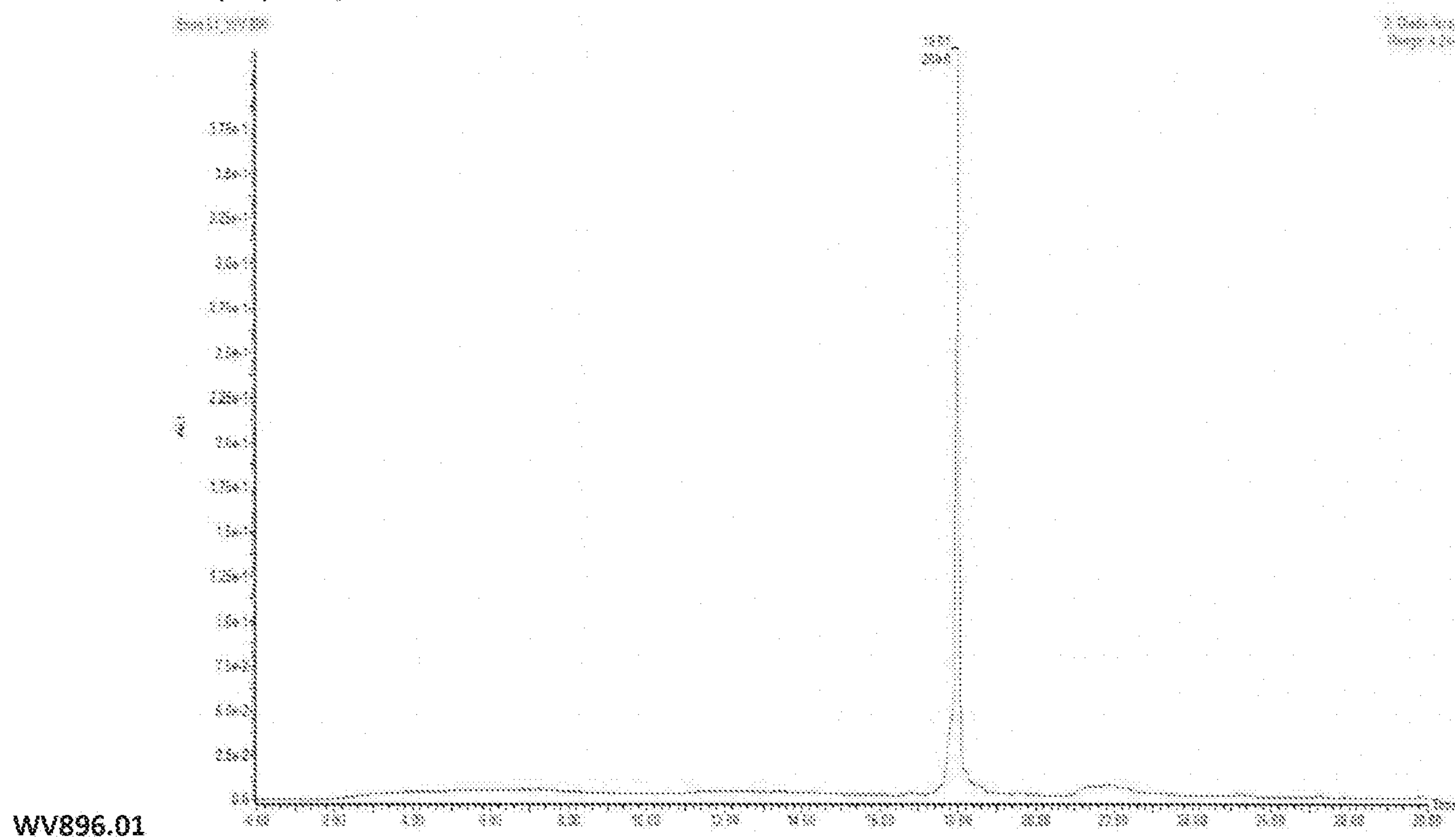
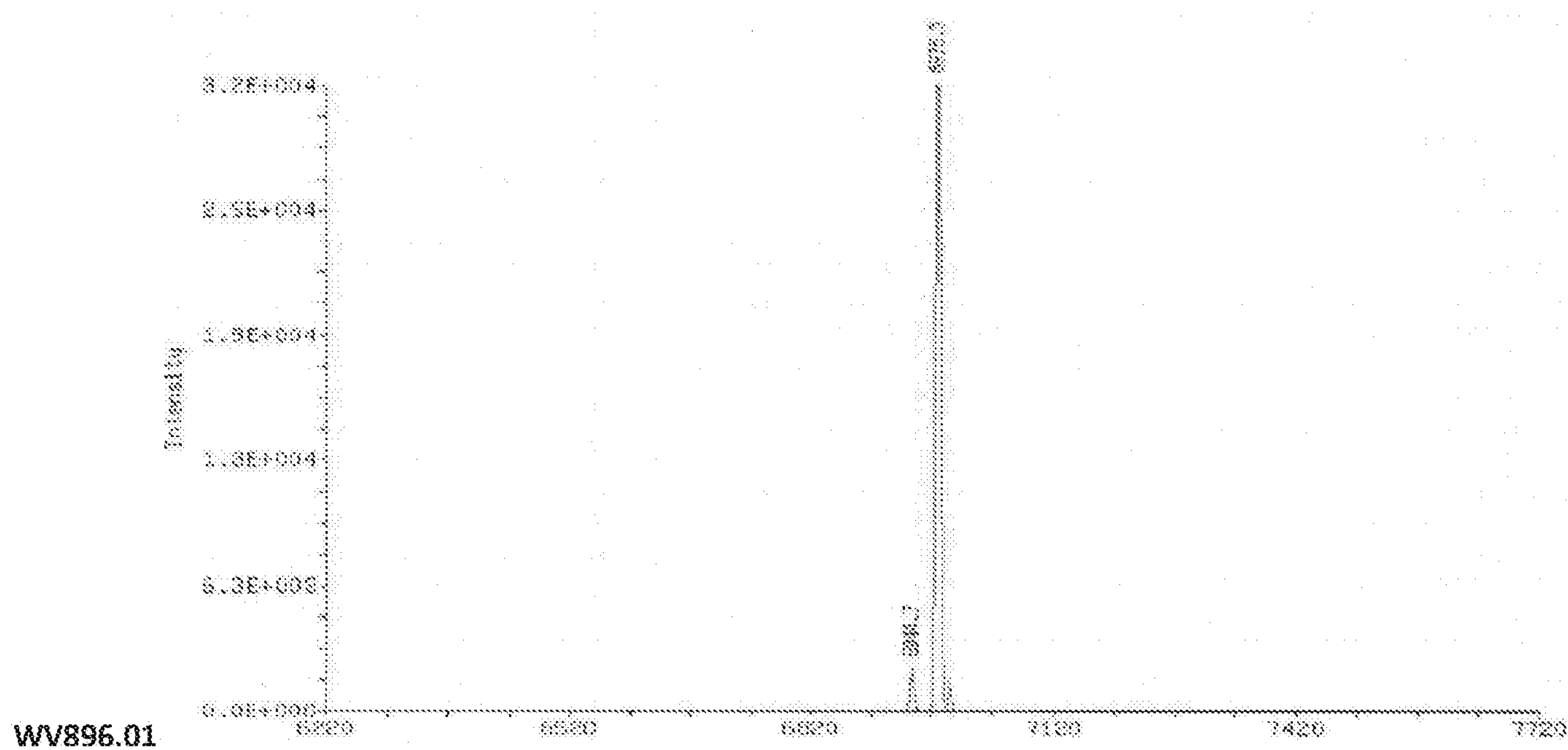
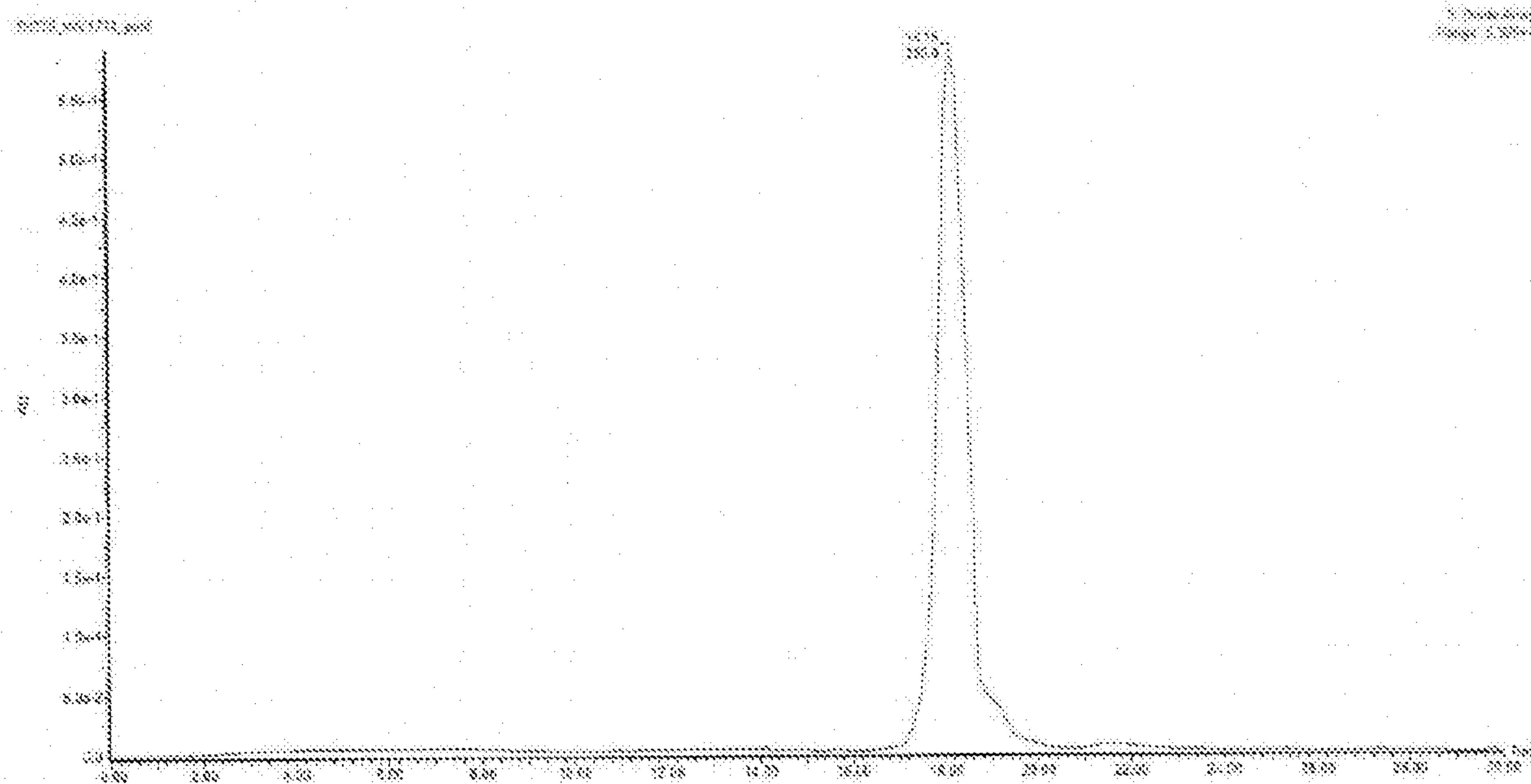
UPLC-MS Analysis (M-H)⁺: calcd 6976.61, found 6976.5

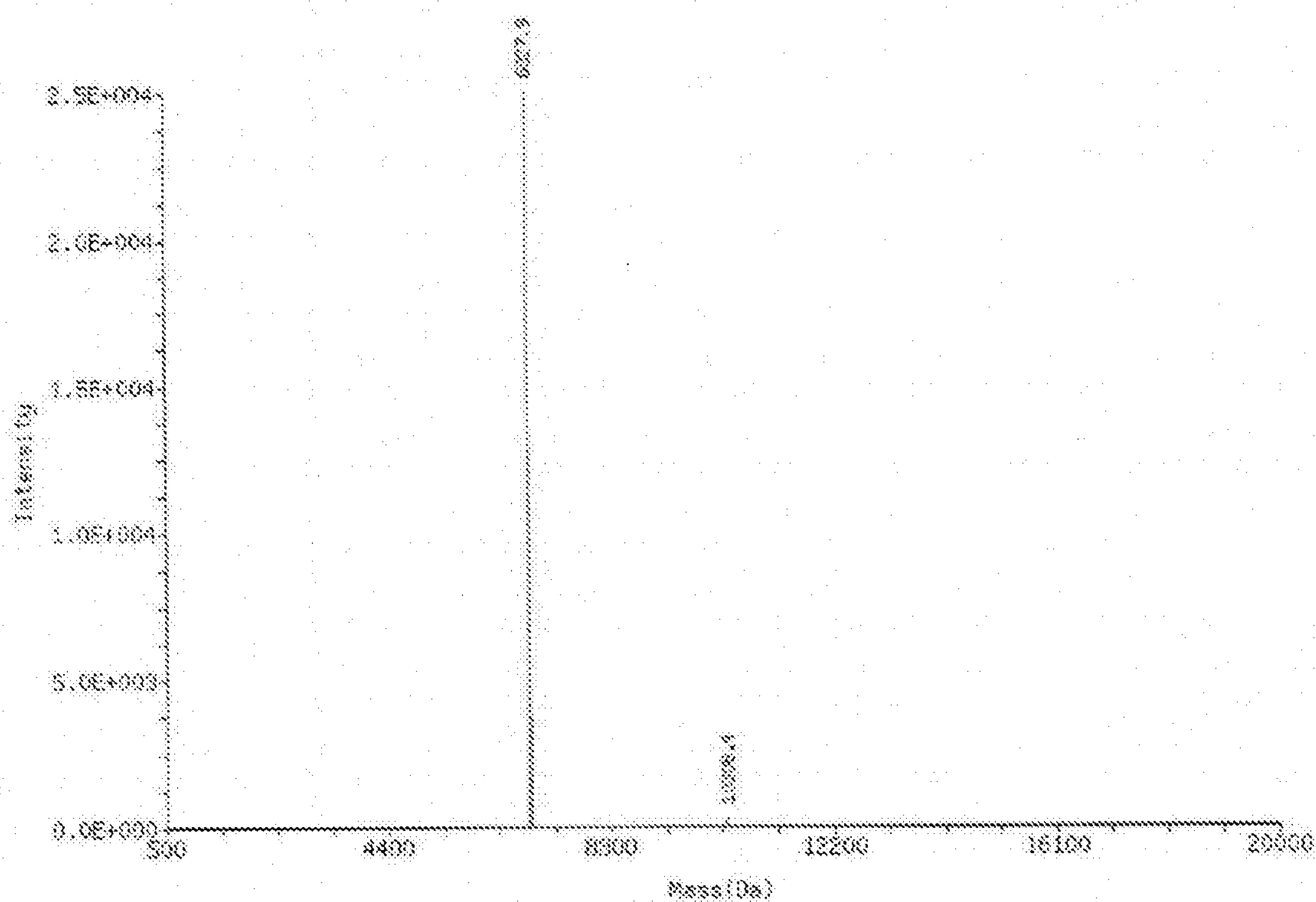
FIG. 27D.

UPLC(RP) analysis



WV1714.01

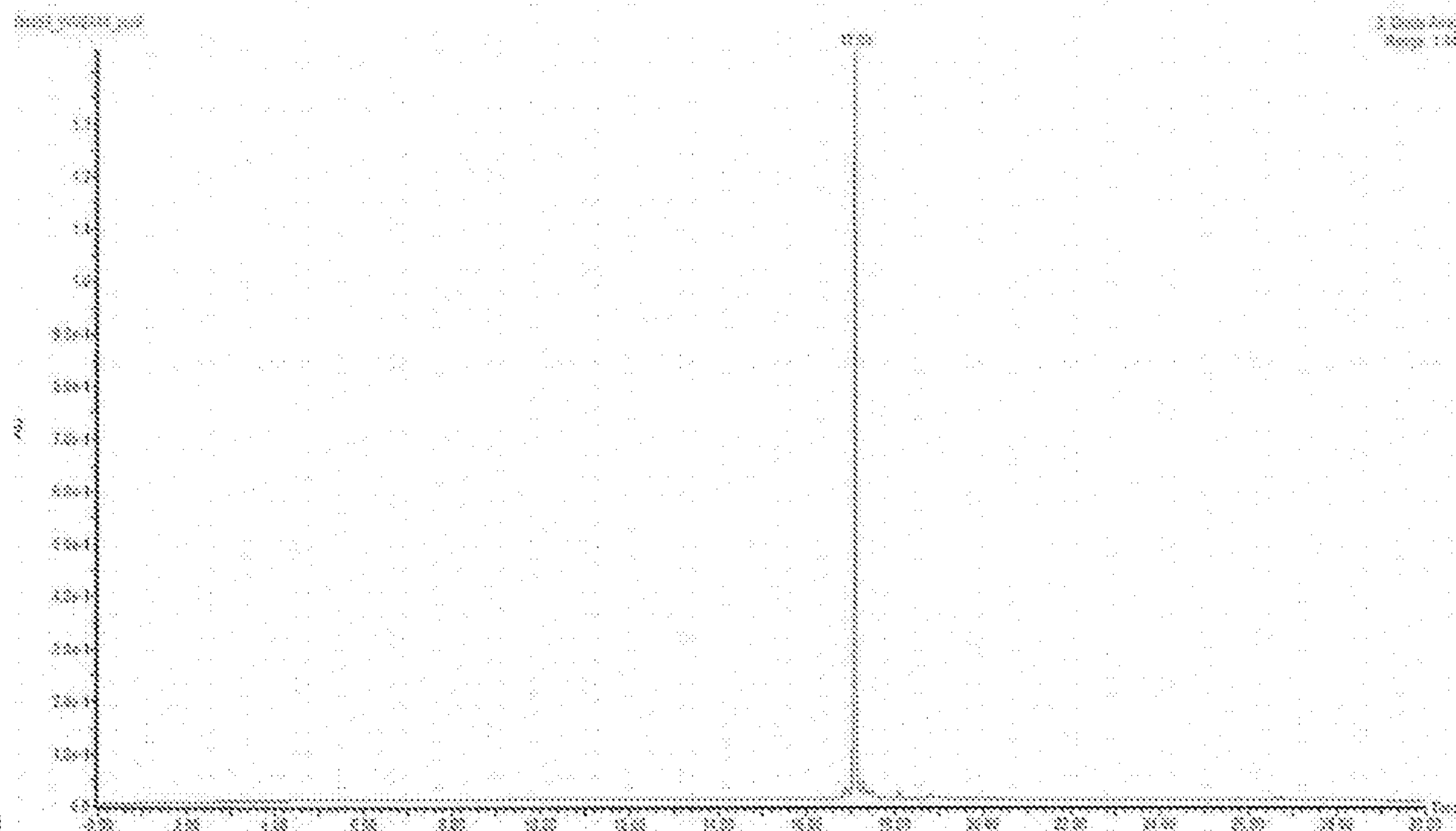
UPLC-MS analysis [MW]: calcd 6833.19, found 6827.9



WV1714.01

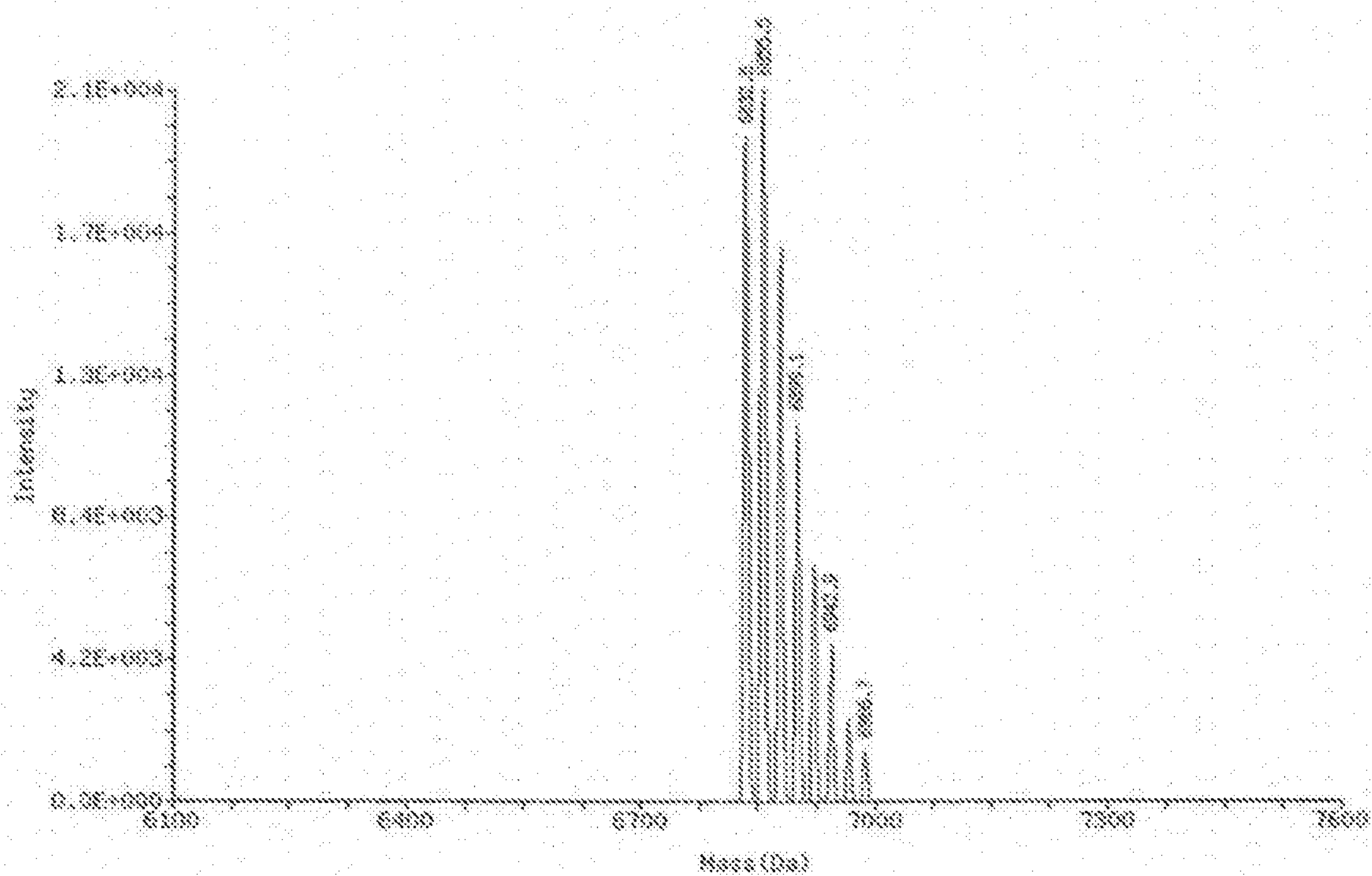
FIG. 27E.

UPLC(RP) analysis



WV2444.01

UPLC-MS analysis [MW]: calcd 6833.18, found 6832.8



WV2444.01

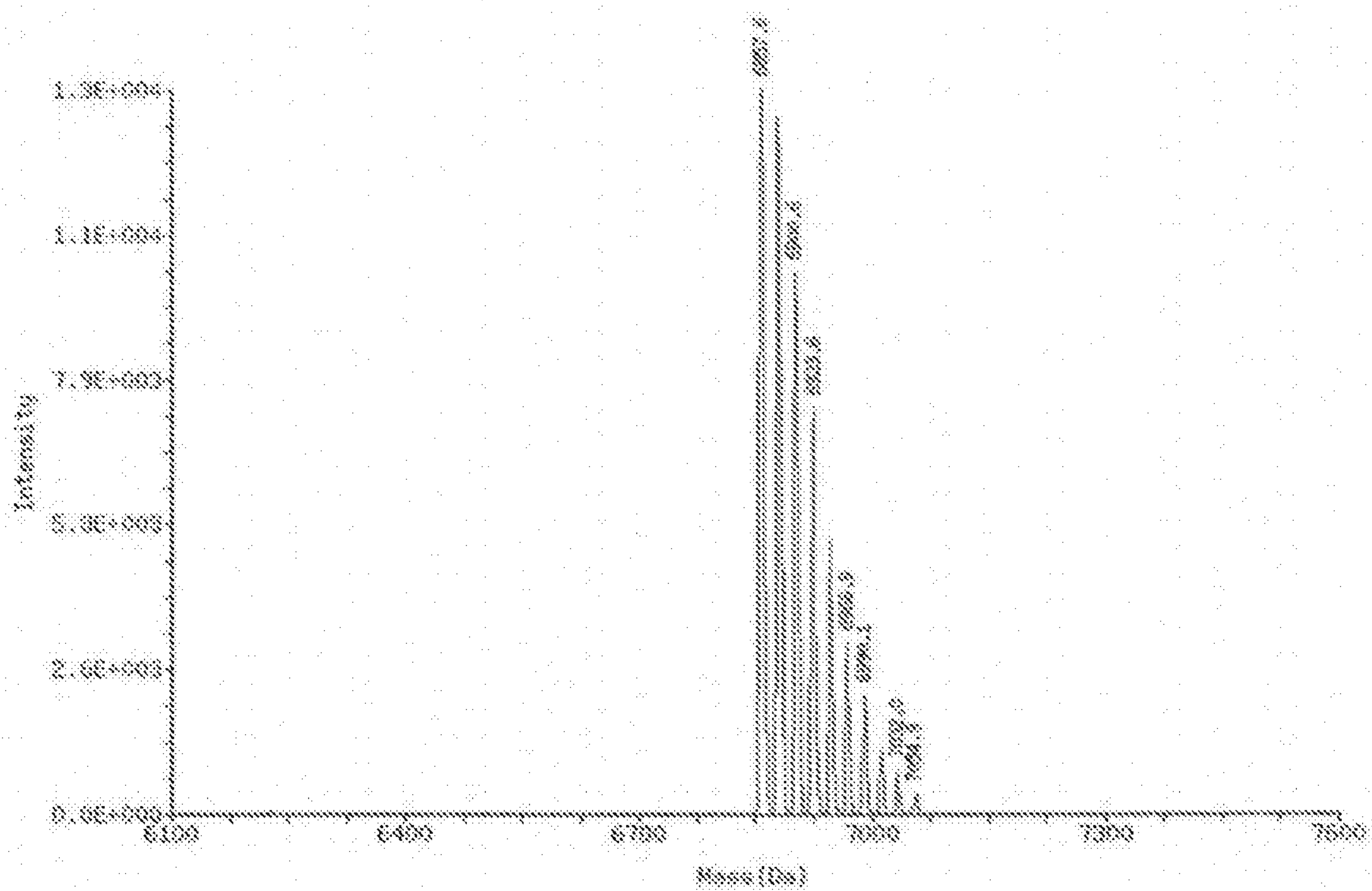
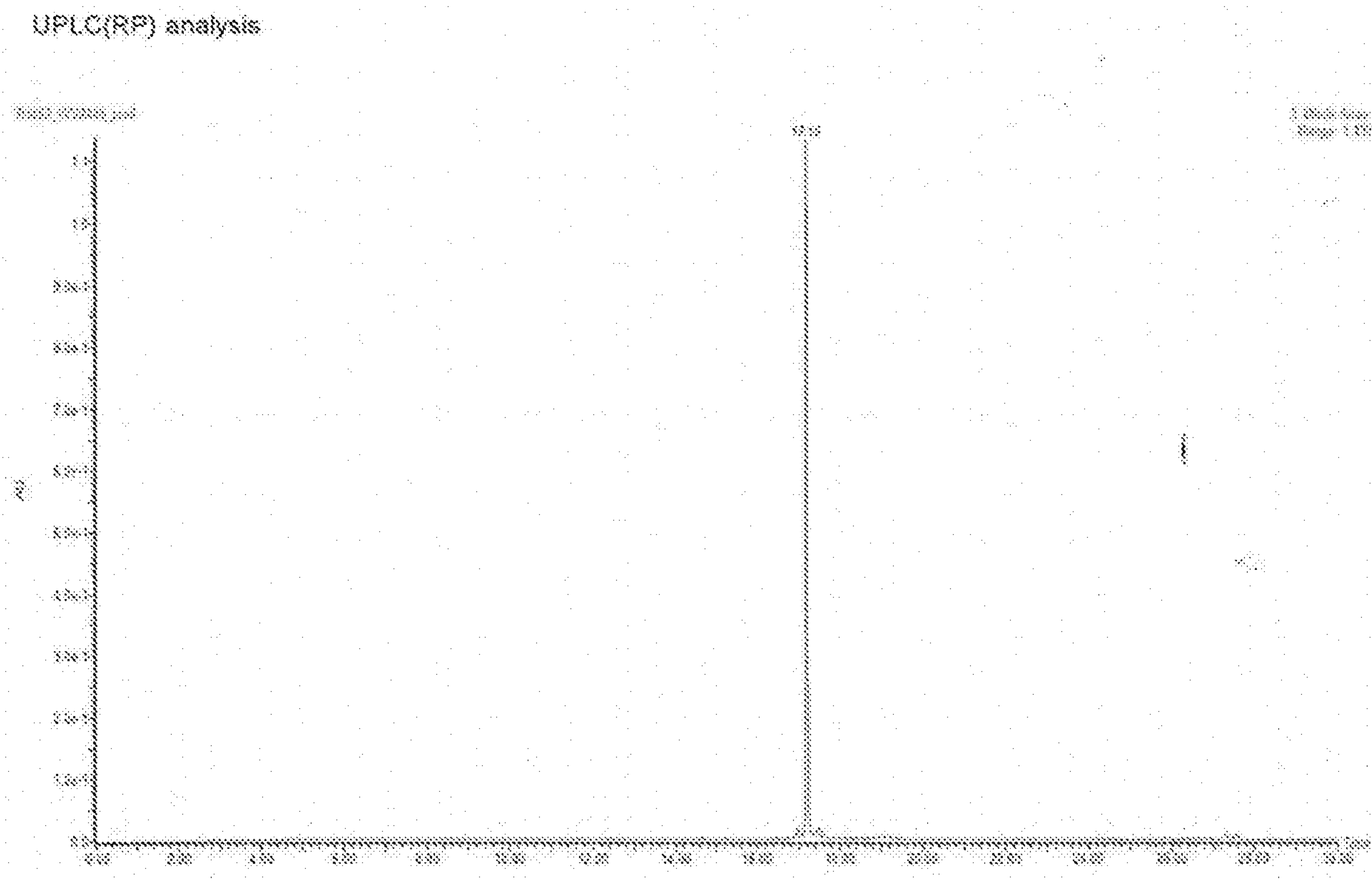
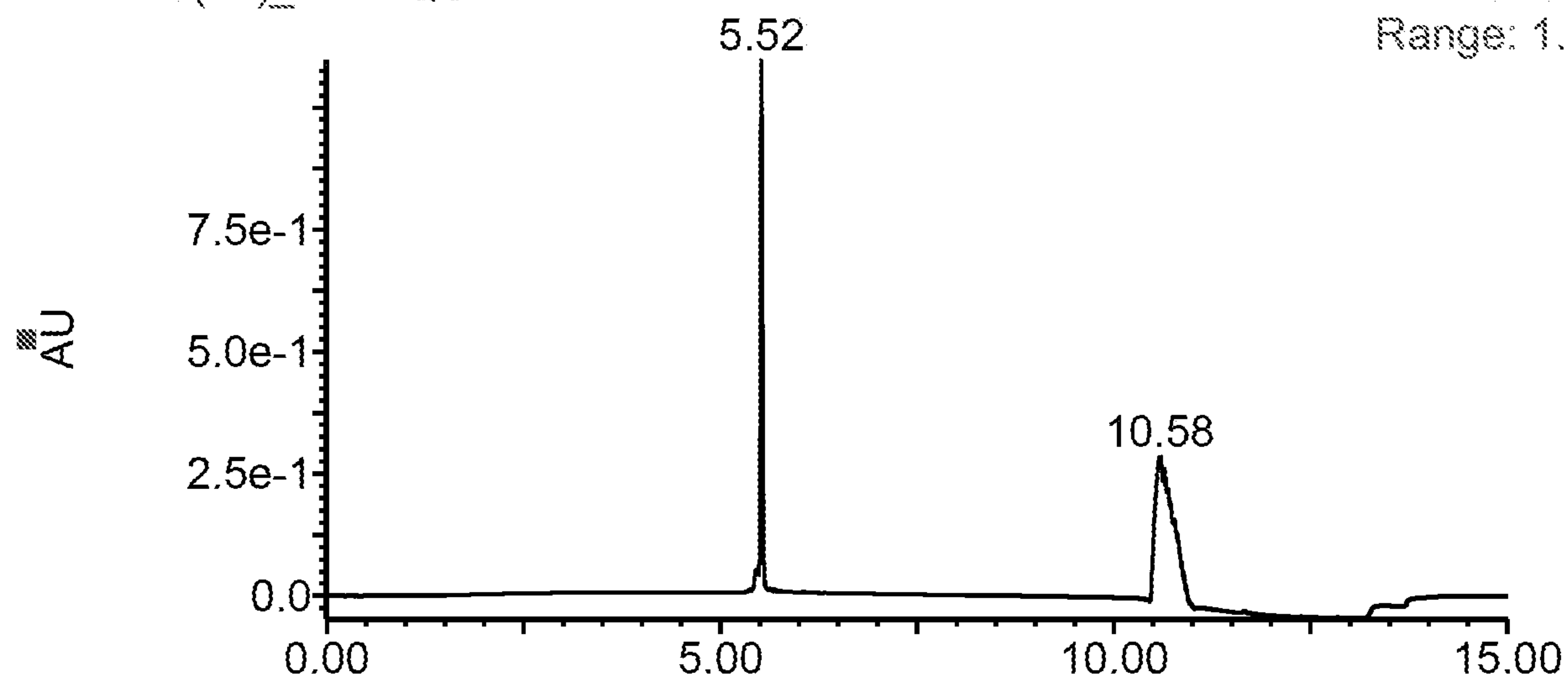
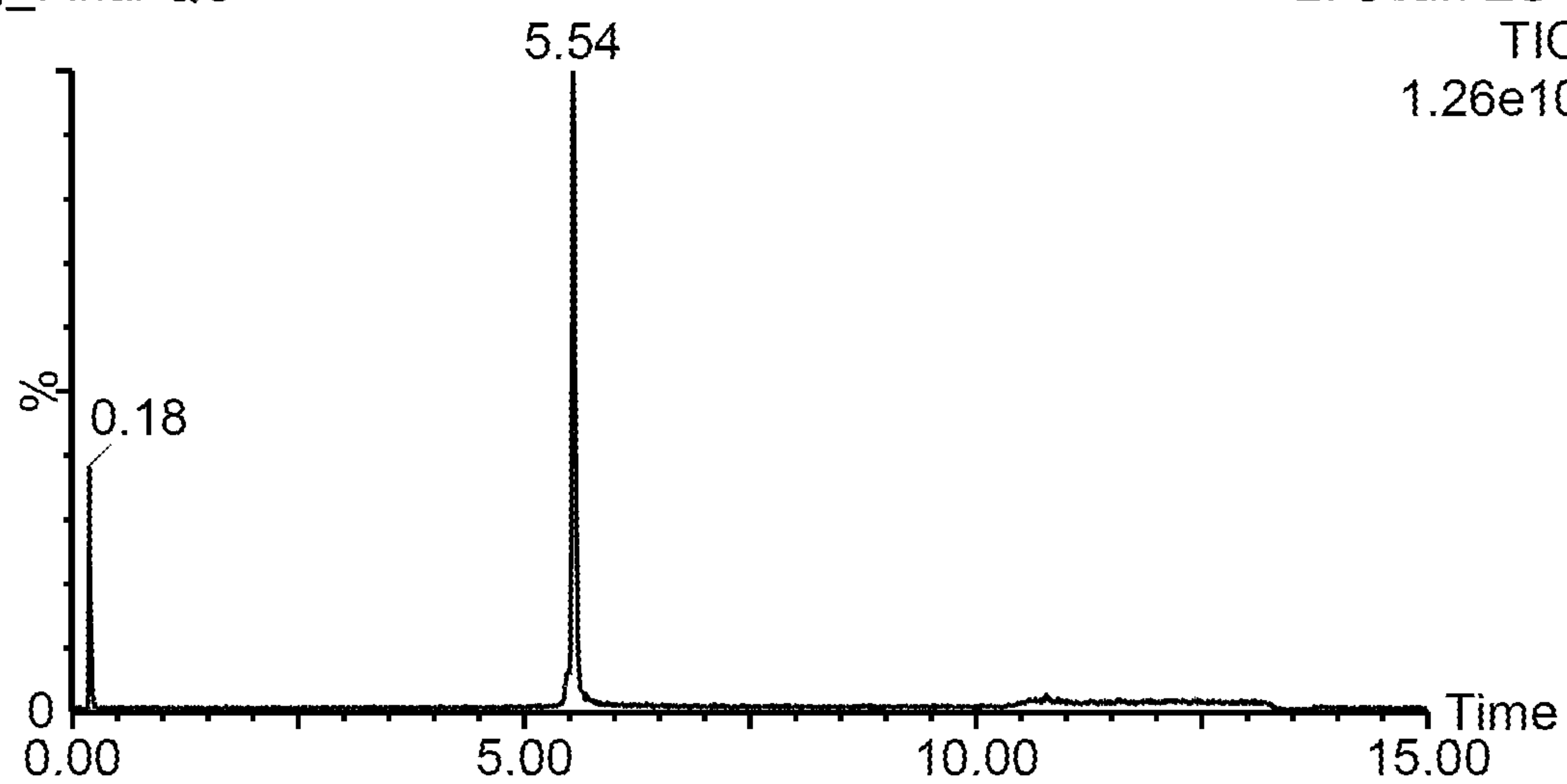


FIG. 27G.

WV2526(01)_Final QC

3: Diode Array
Range: 1.142

WV2526(01)_Final QC

2: Scan ES-
TIC
1.26e10

WV2526.01

FIG. 27H.

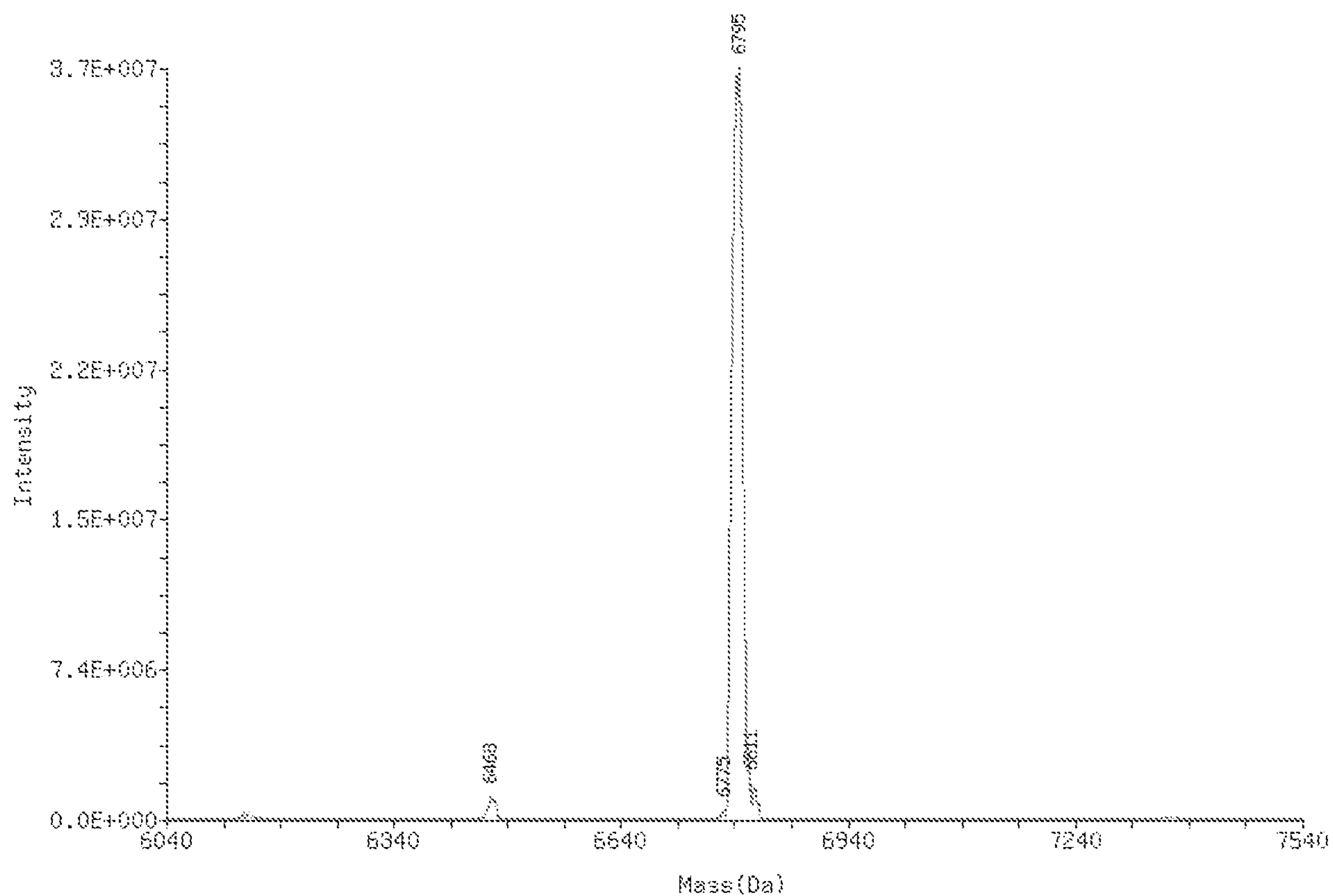
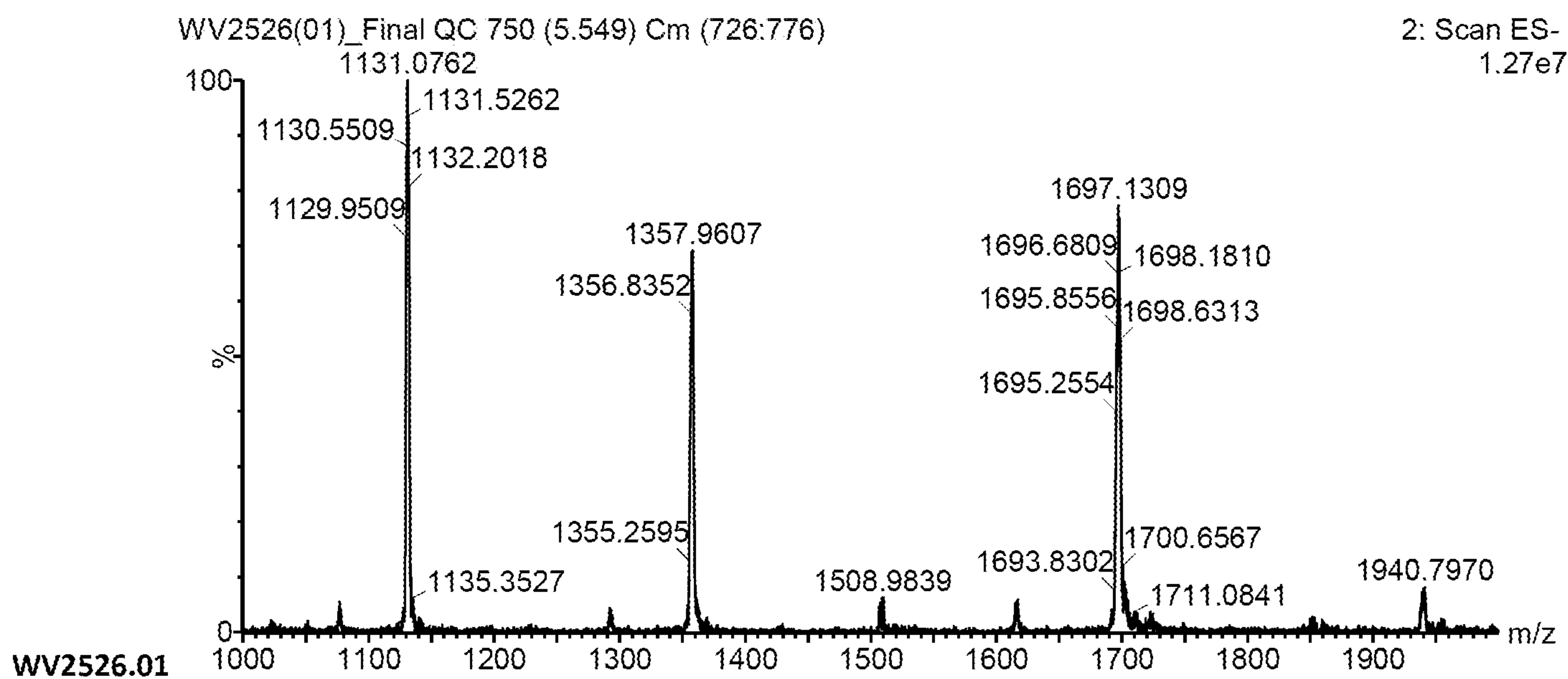
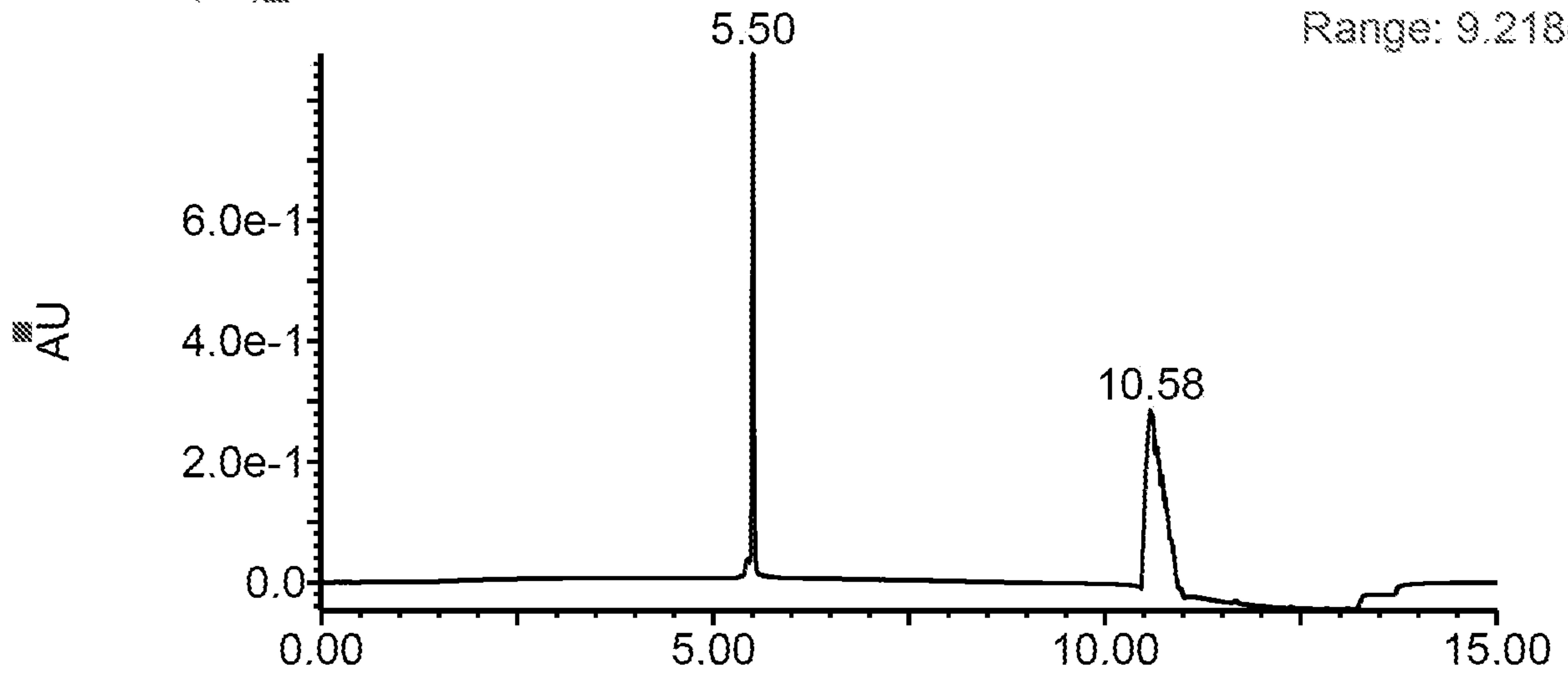
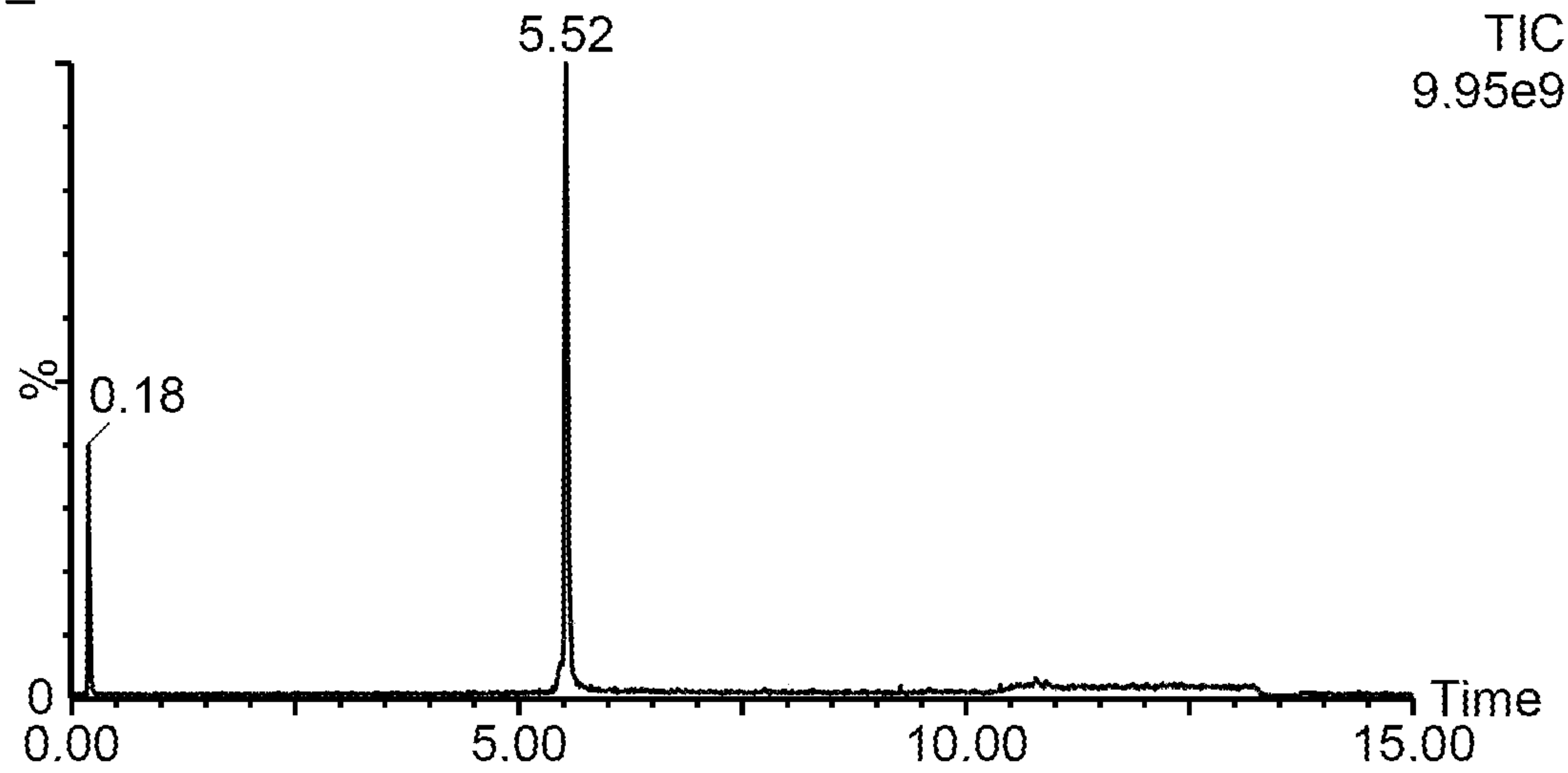


FIG. 27I.

WV2527(01)_Final QC

3: Diode Array
Range: 9.218e-1

WV2527(01)_Final QC

2: Scan ES-
TIC
9.95e9

WV2527.01

FIG. 27J.

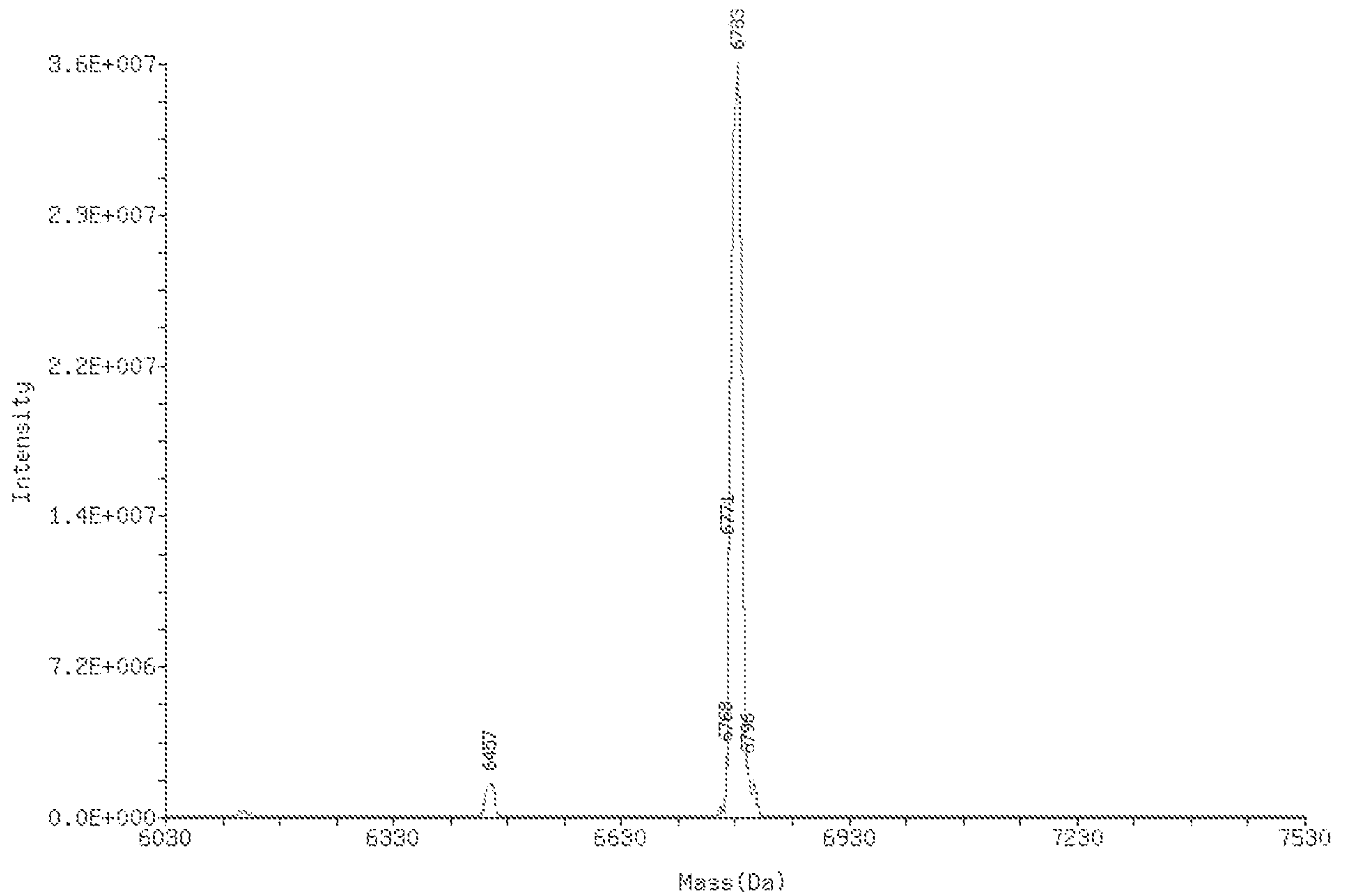
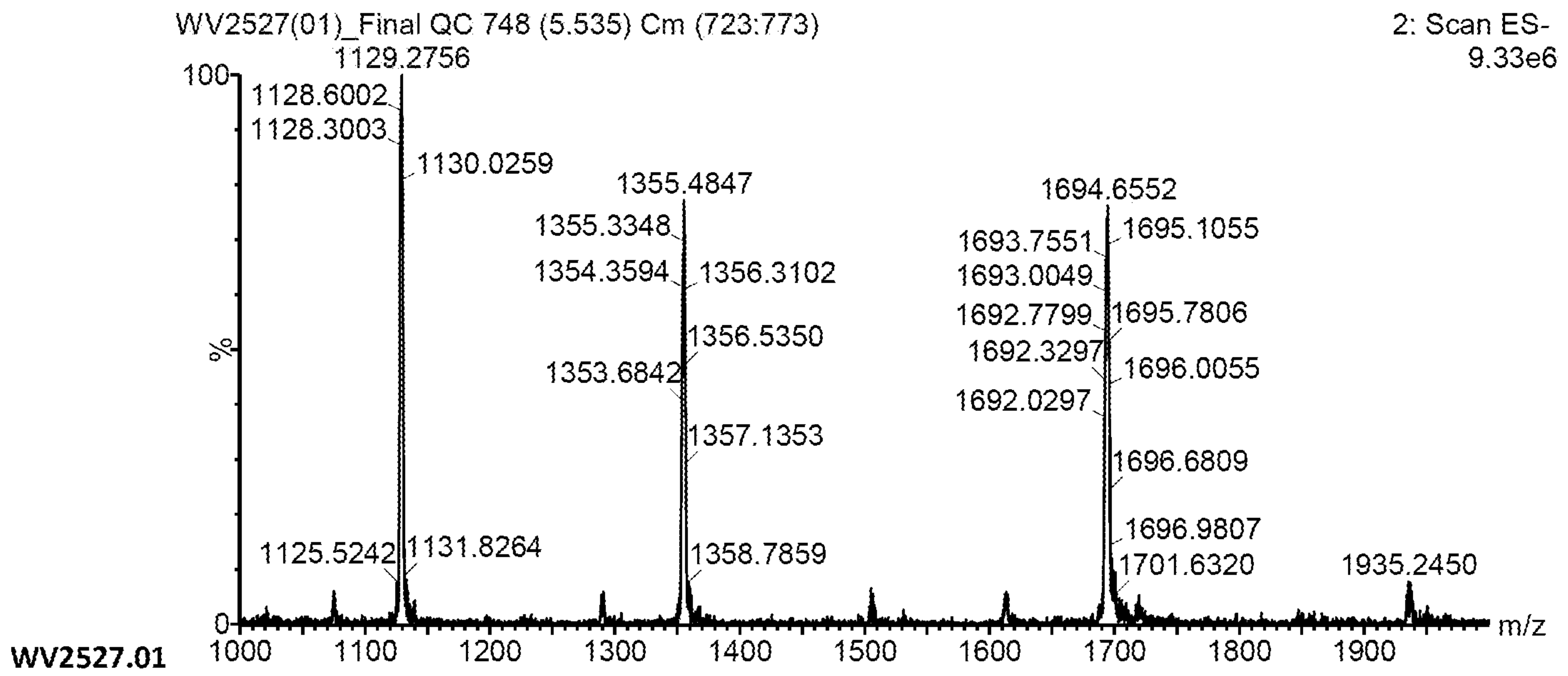
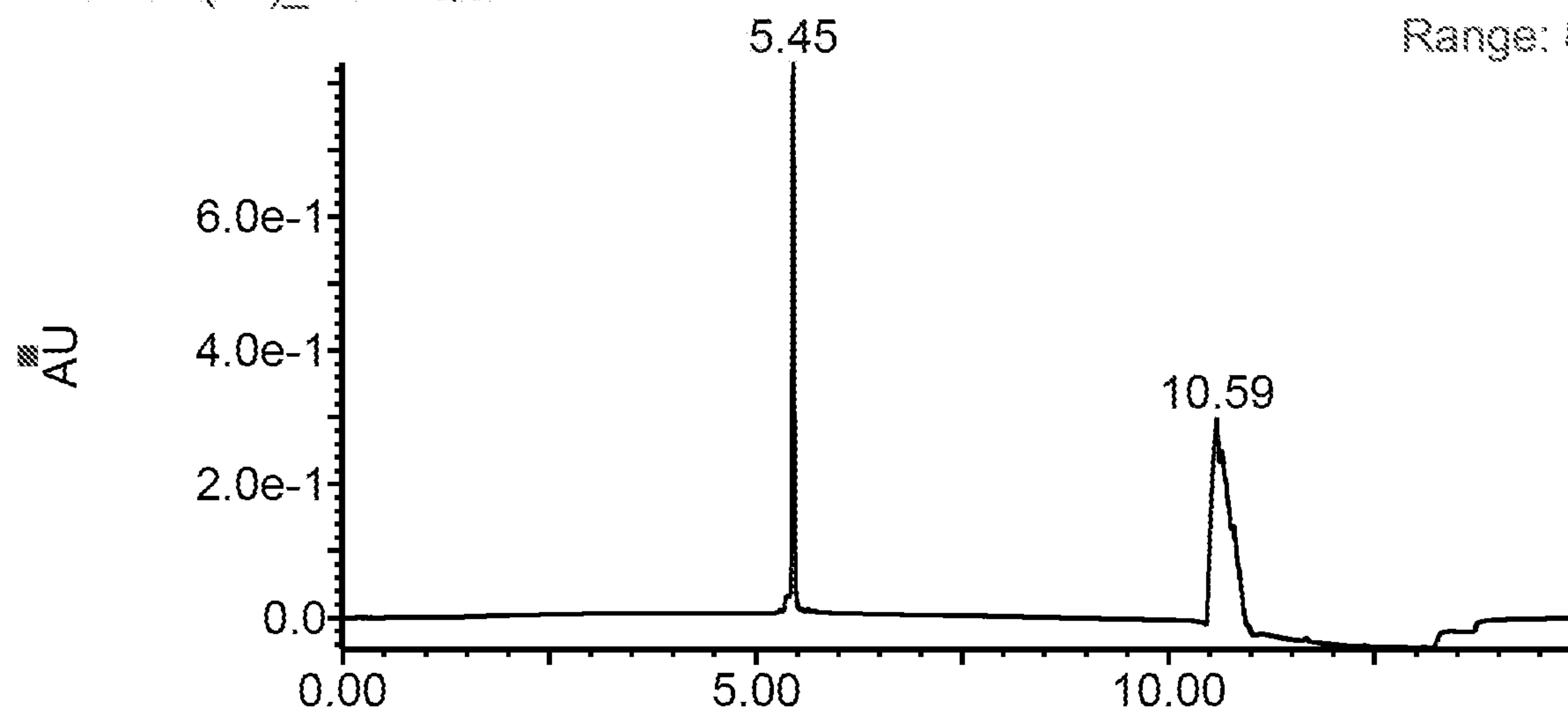
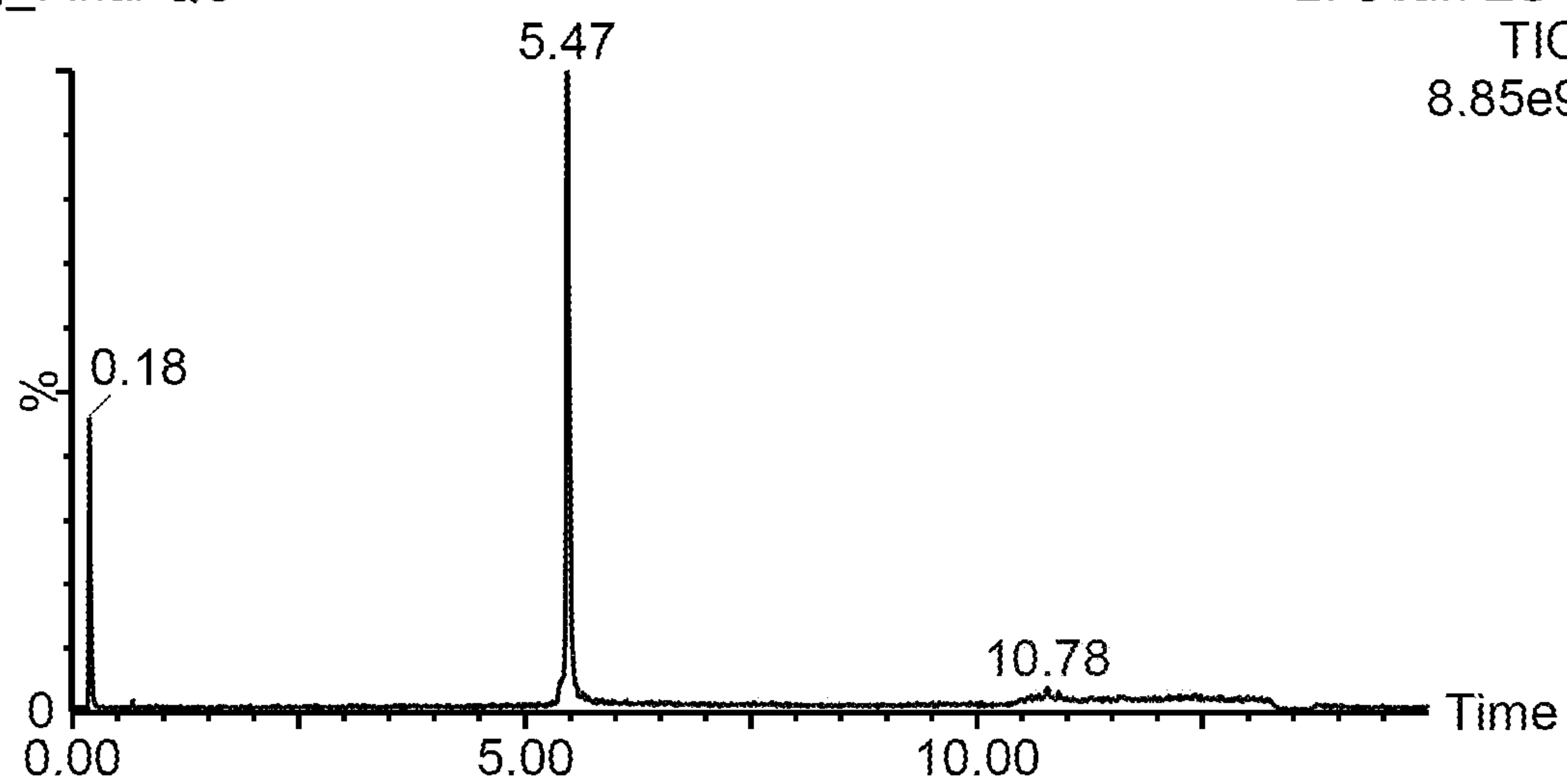


FIG. 27K.

WV2528(01)_Final QC

3: Diode Array
Range: 8.75e-1

WV2528(01)_Final QC

2: Scan ES-
TIC
8.85e9

WV2528.01

FIG. 27L.

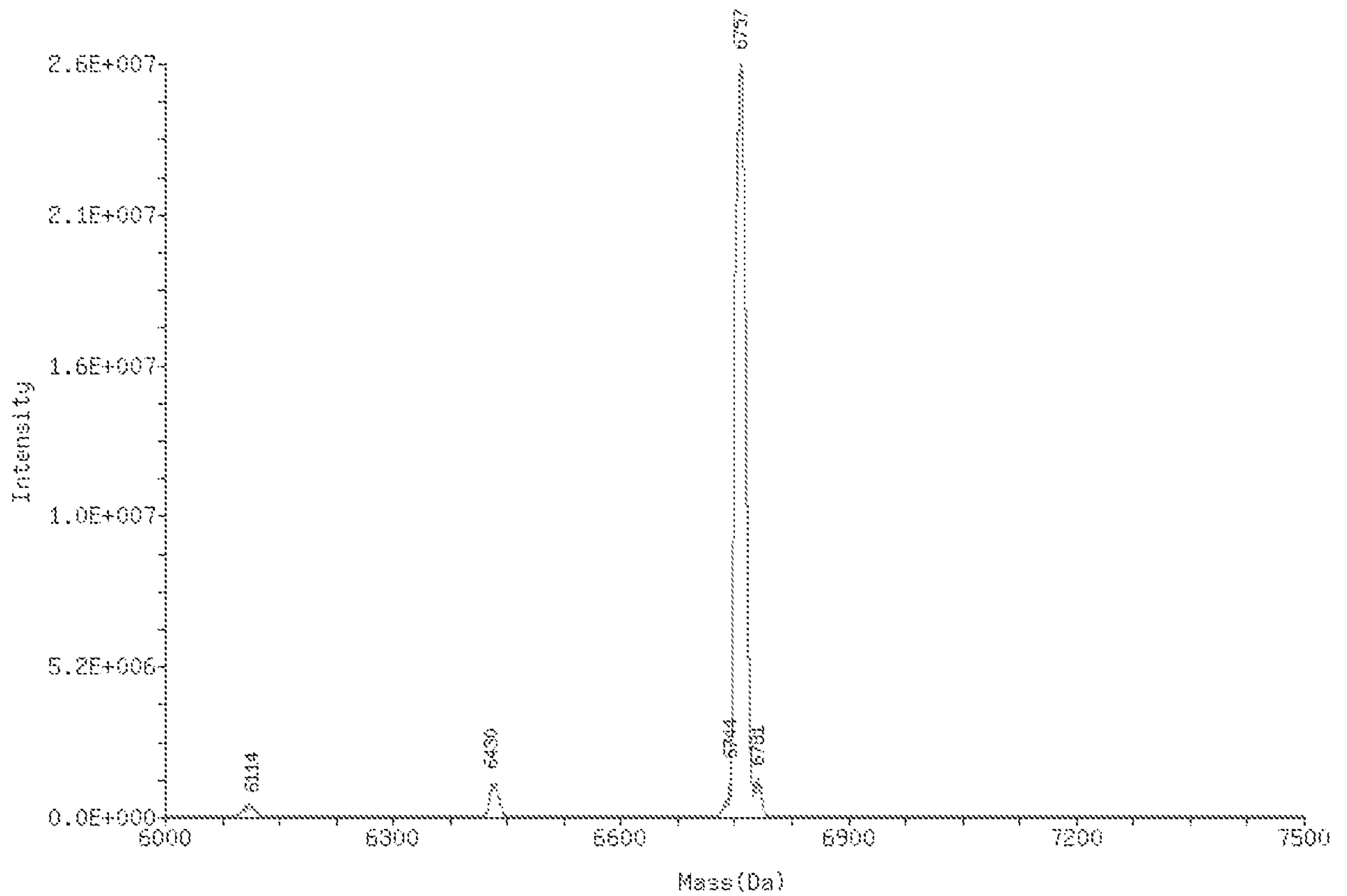
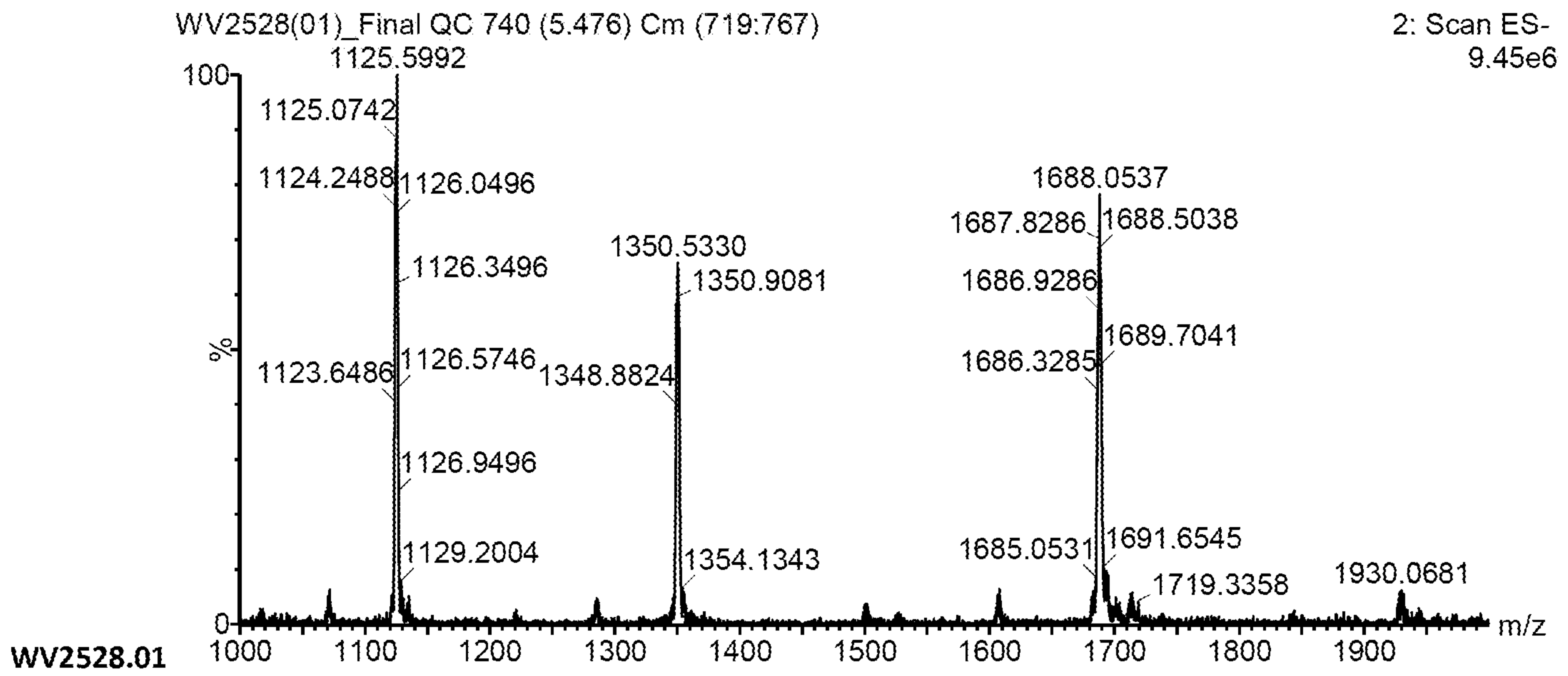
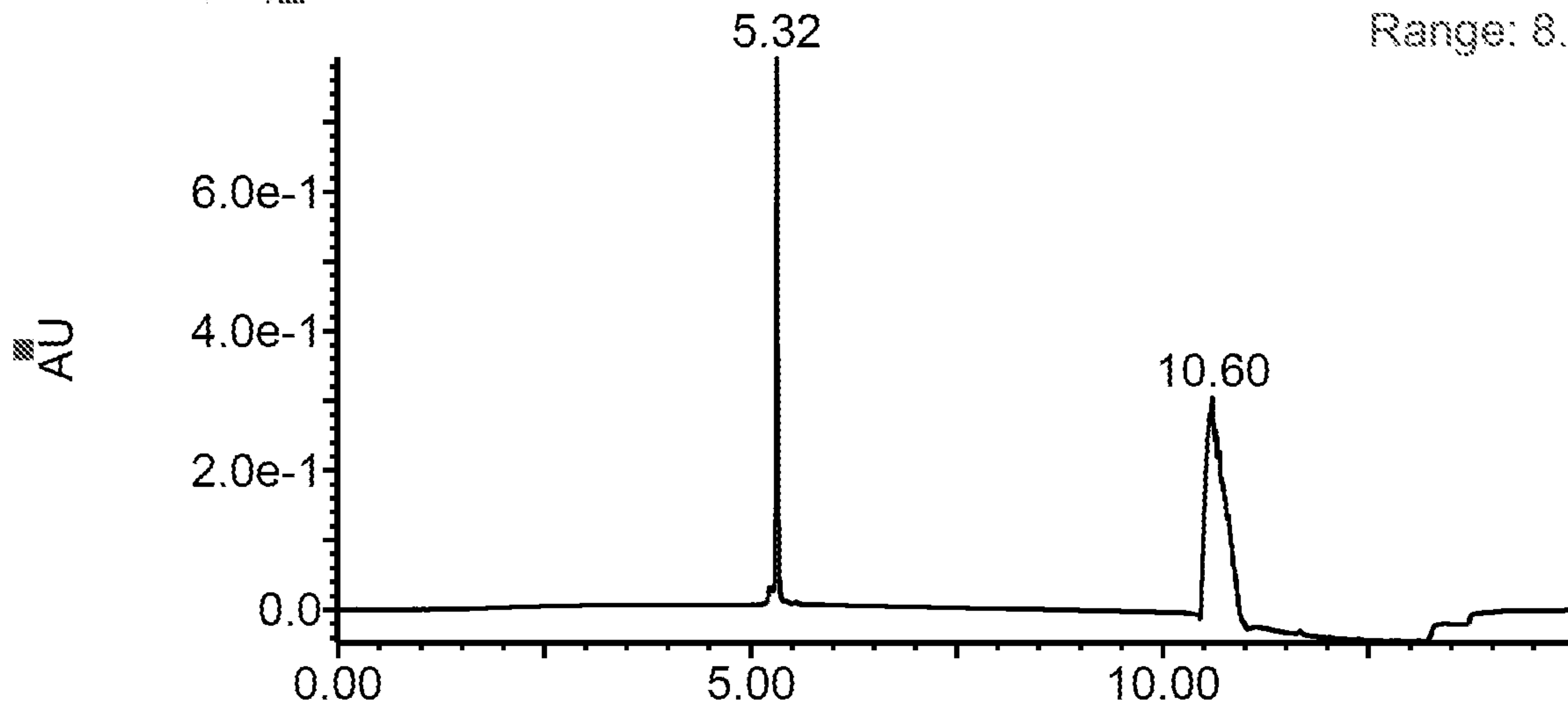
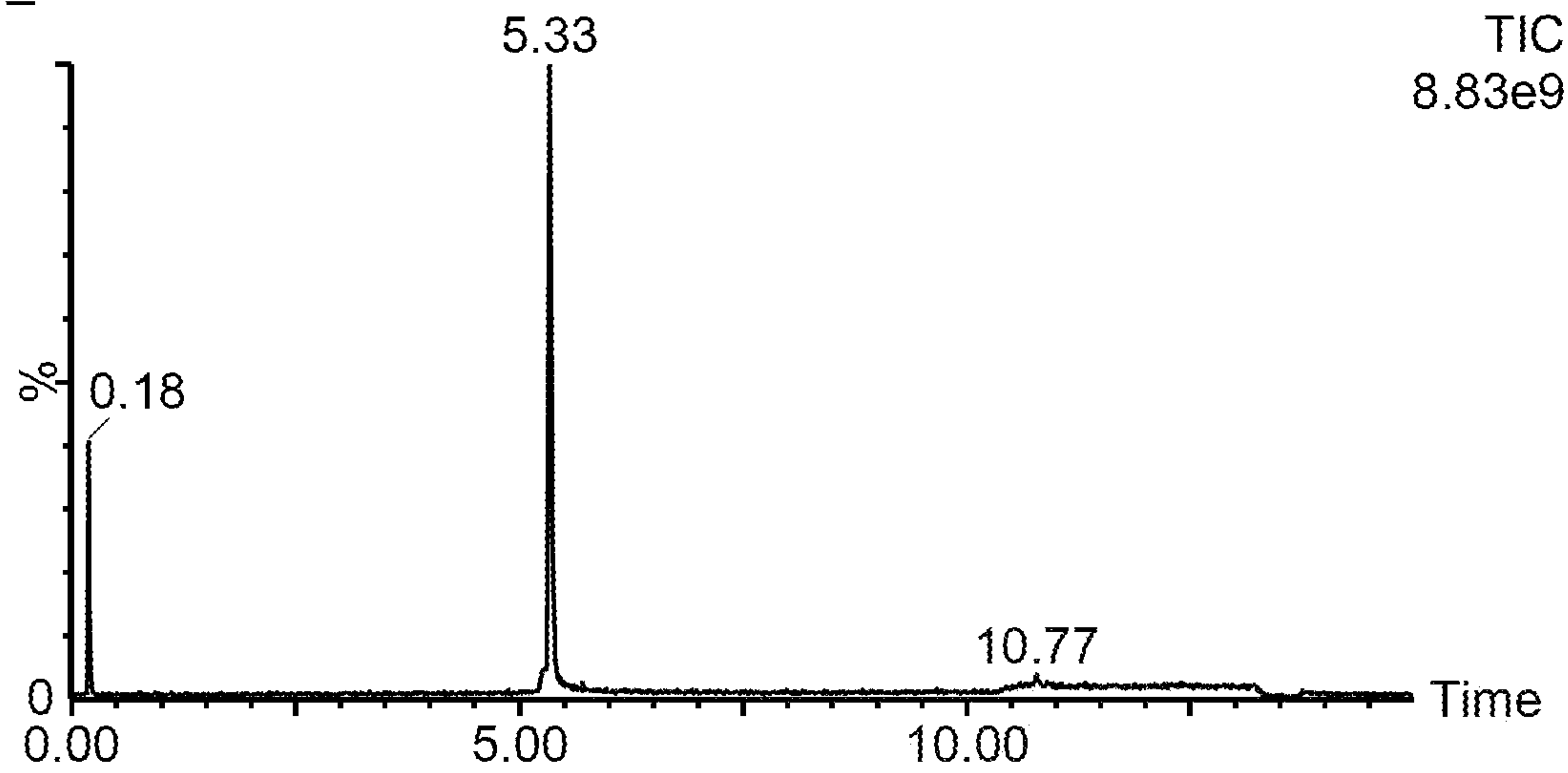


FIG. 27M.

WV2530(01)_Final QC

3: Diode Array
Range: 8.373e-1

WV2530(01)_Final QC

2: Scan ES-
TIC
8.83e9

WV2530.01

FIG. 27N.

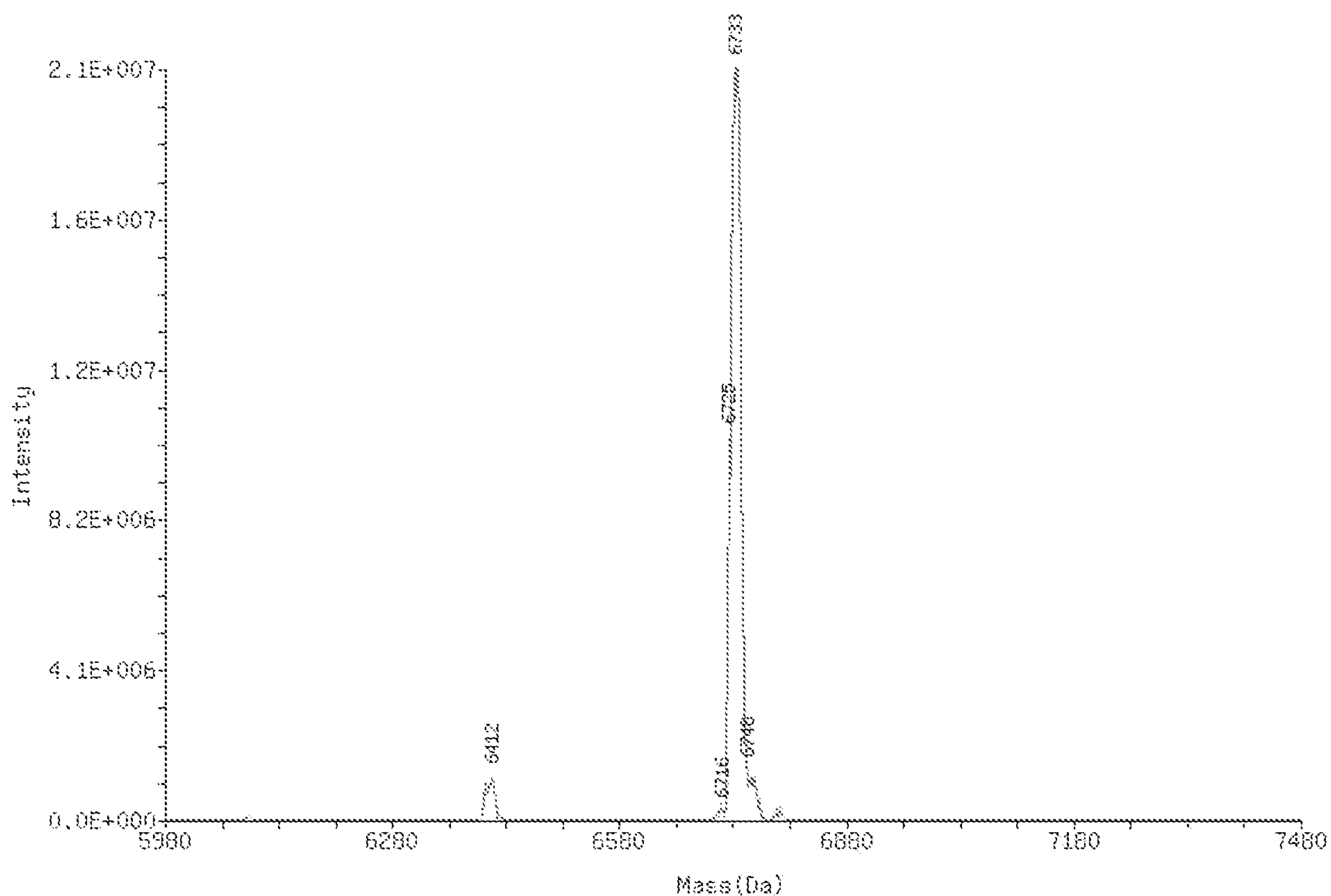
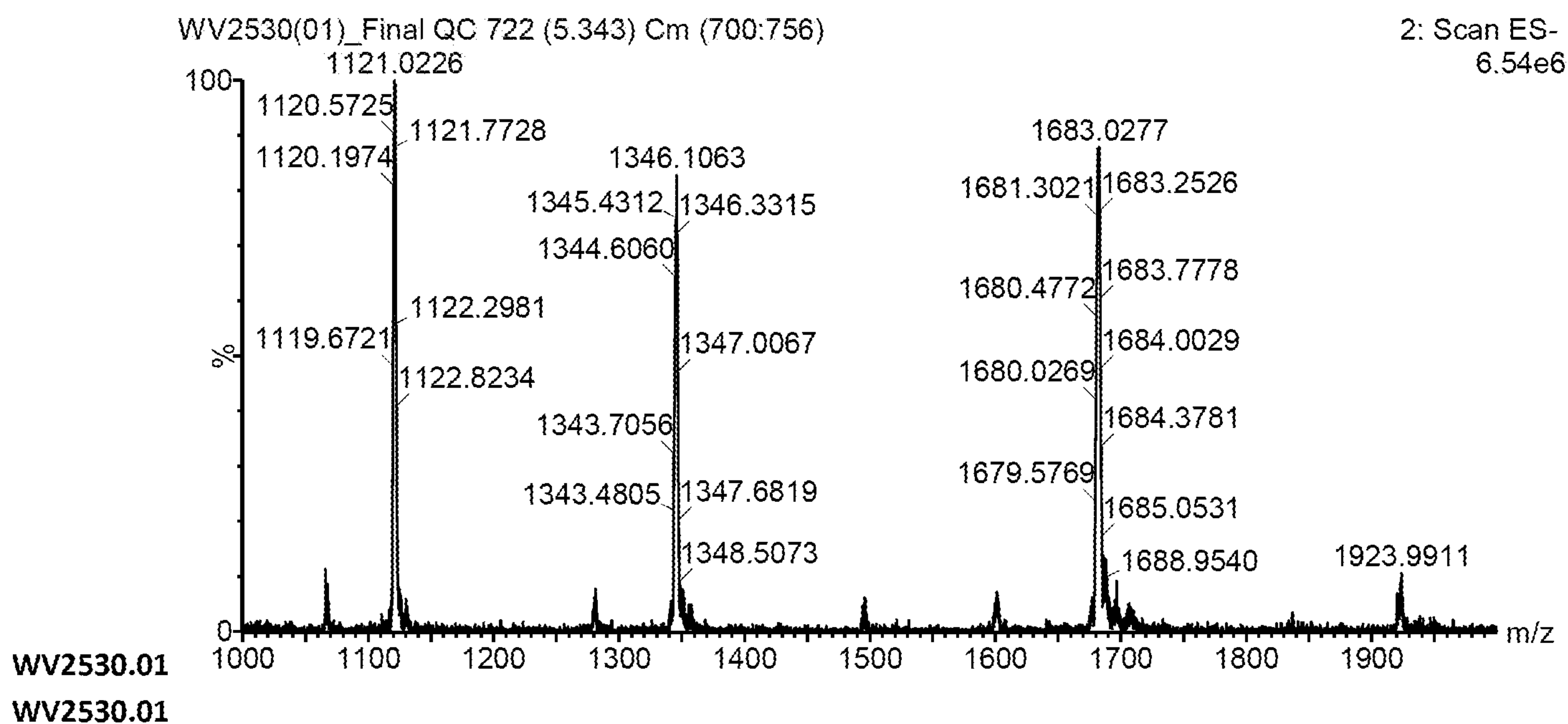
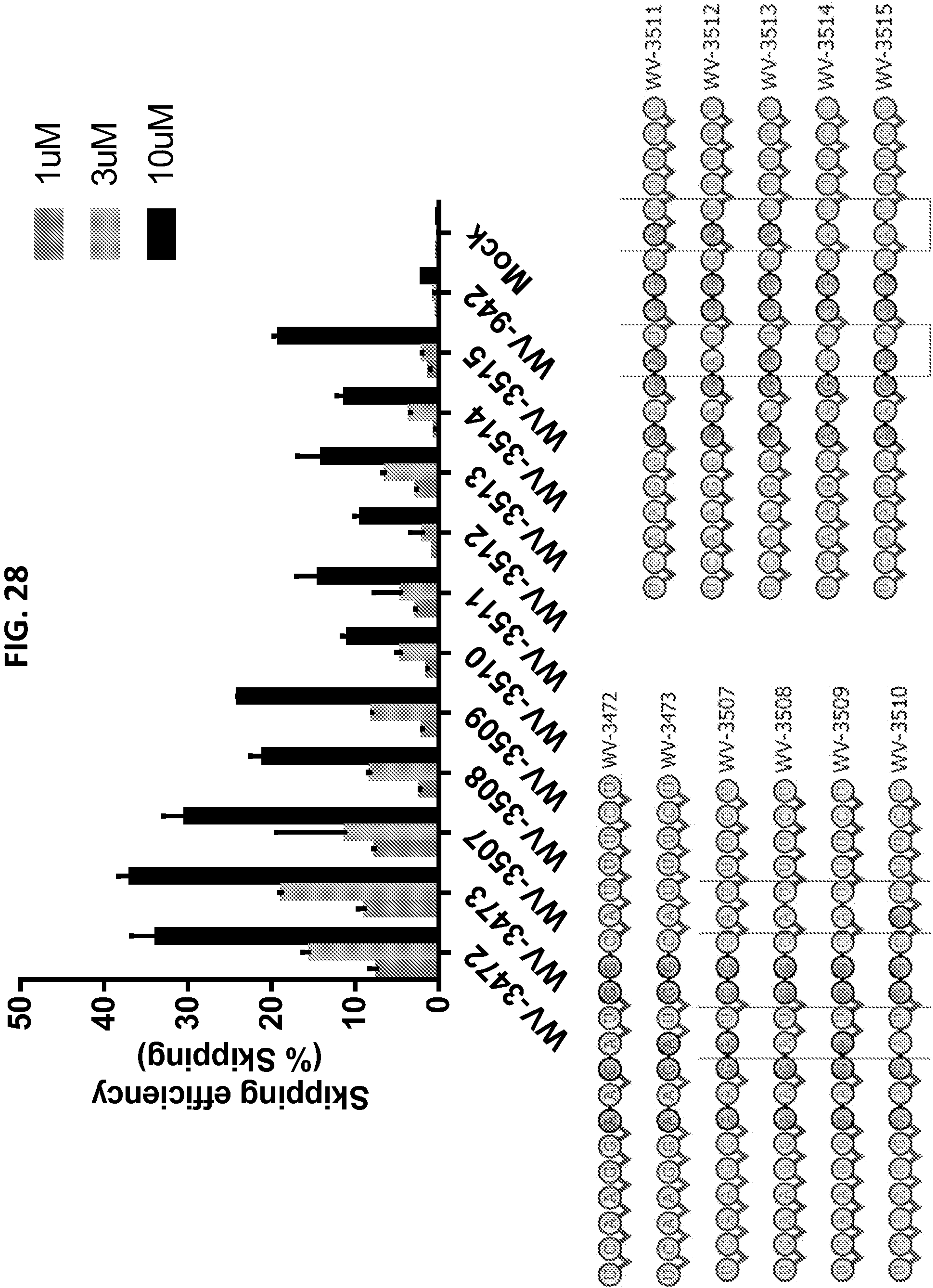


FIG. 270.

WV#	Calculated Mass (Da)	Found Mass (Da)
WV887.02	6977.61544	6977.5
WV892.02	6977.61544	6976.8
WV896.01	6977.61544	6976.5
WV1714.01	6833.18920	6827.9
WV2444.01	6833.18920	6832.8
WV2445.01	6857.26024	6857.6
WV2526.01	6797.08264	6795
WV2527.01	6785.04712	6783
WV2528.01	6760.97608	6757
WV2530.01	6736.85032	6733



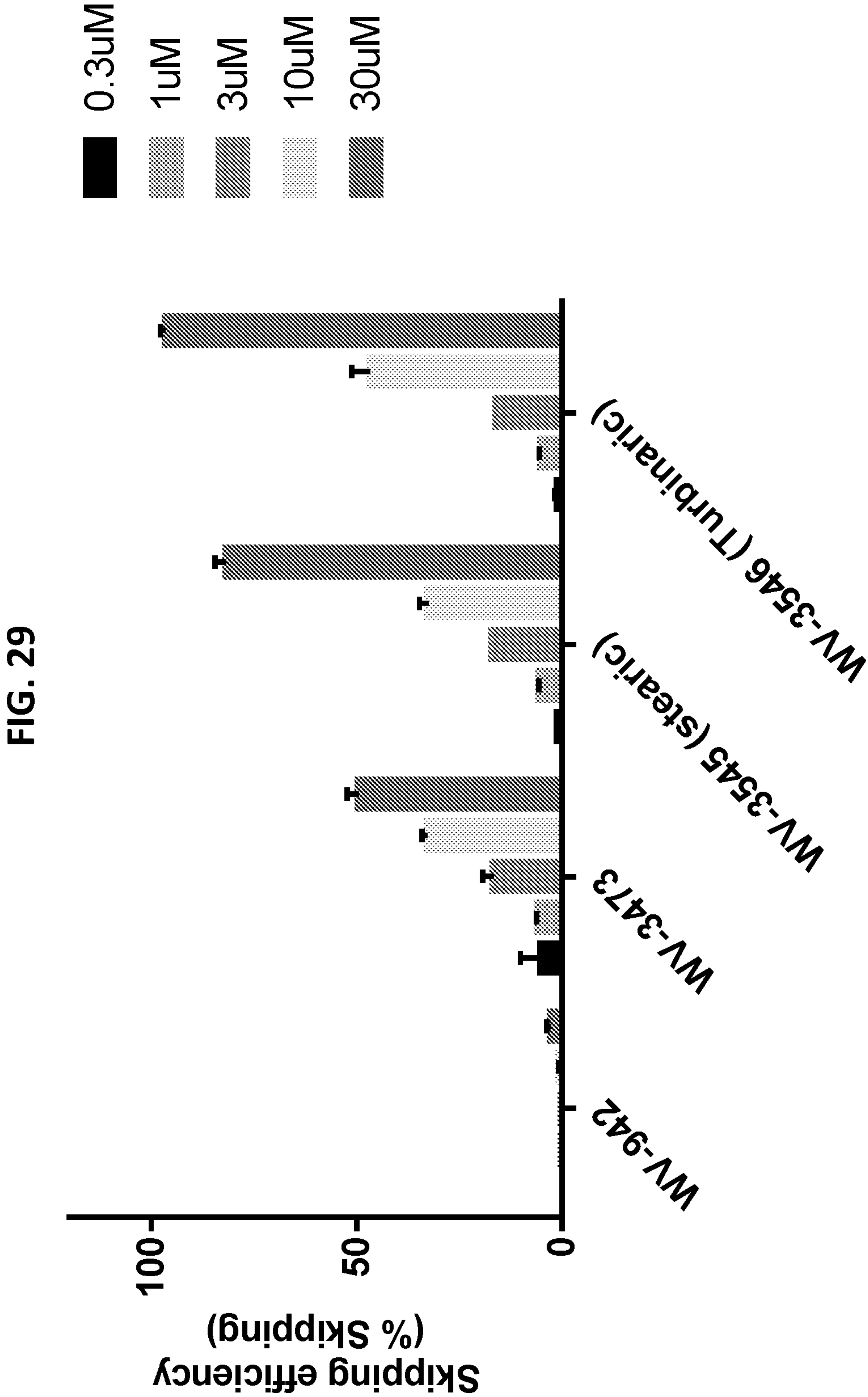


FIG. 30

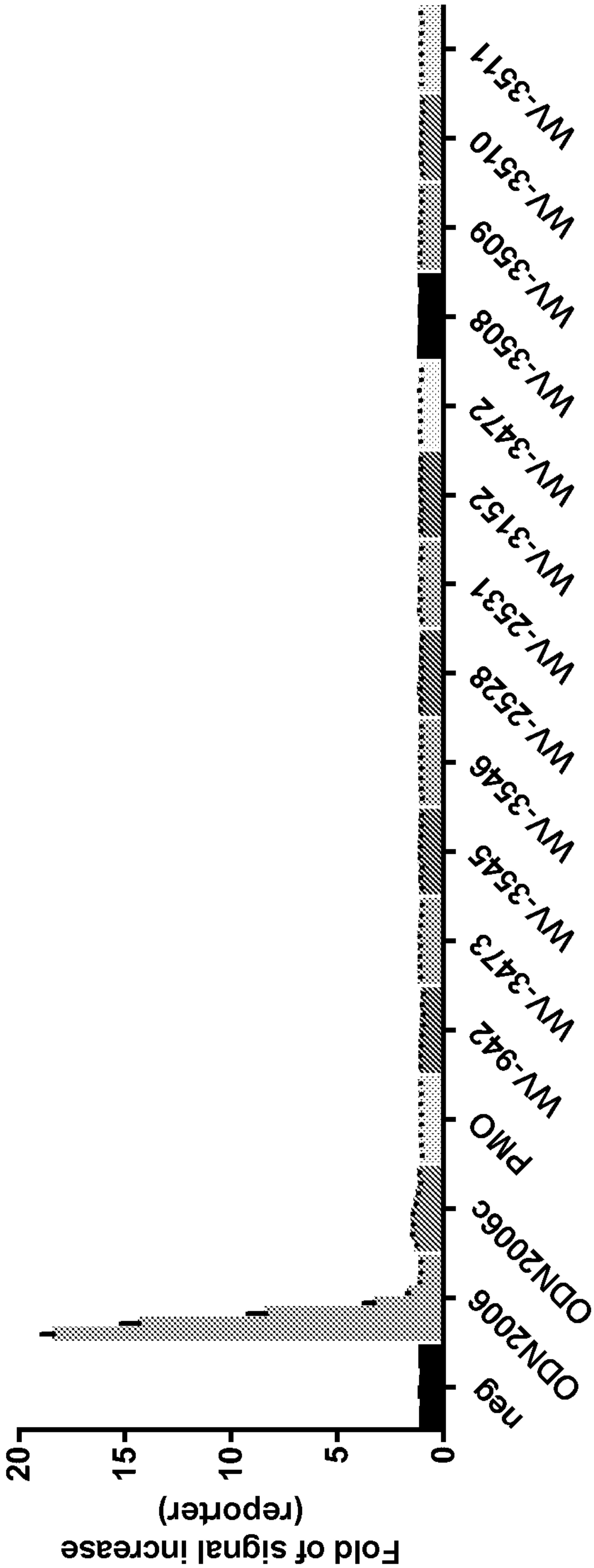


FIG. 31

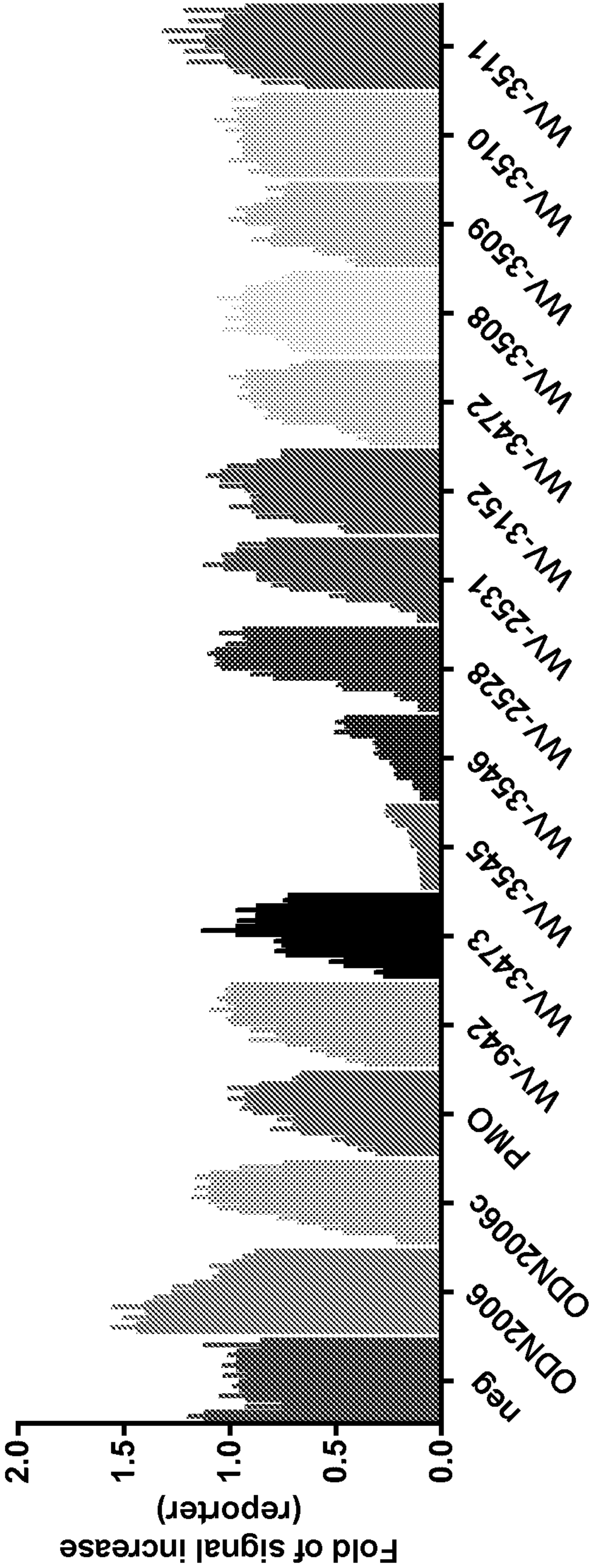
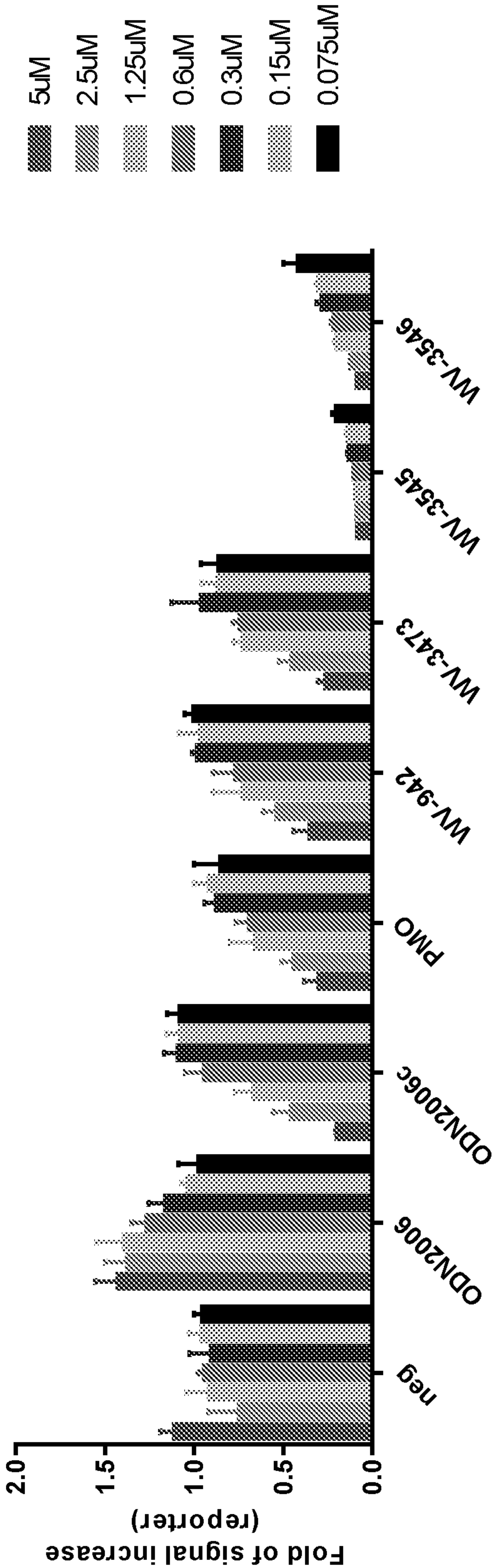


FIG. 32

TLR9 antagonist activity (in the presence of 0.3uM ODN2006)



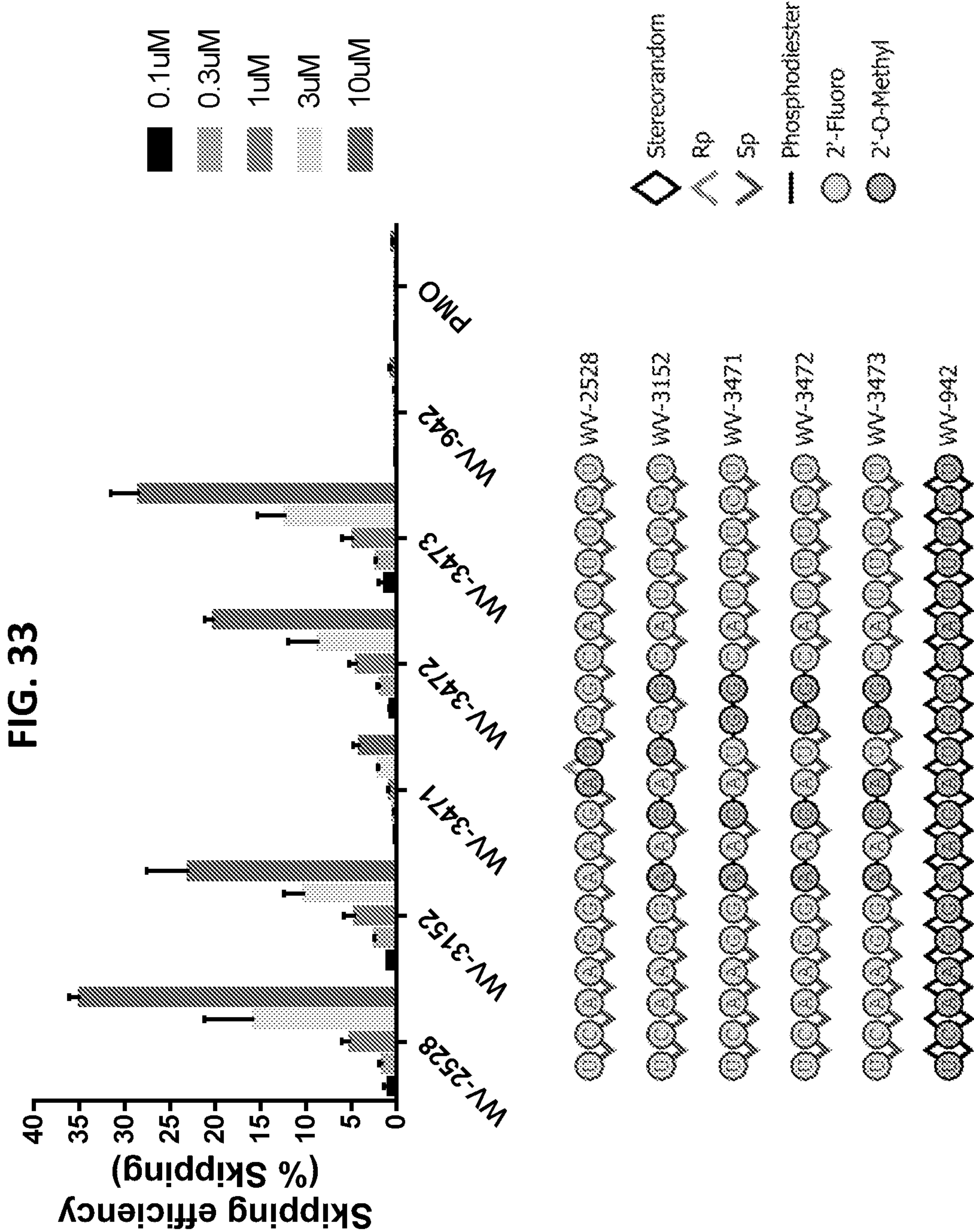


FIG. 34

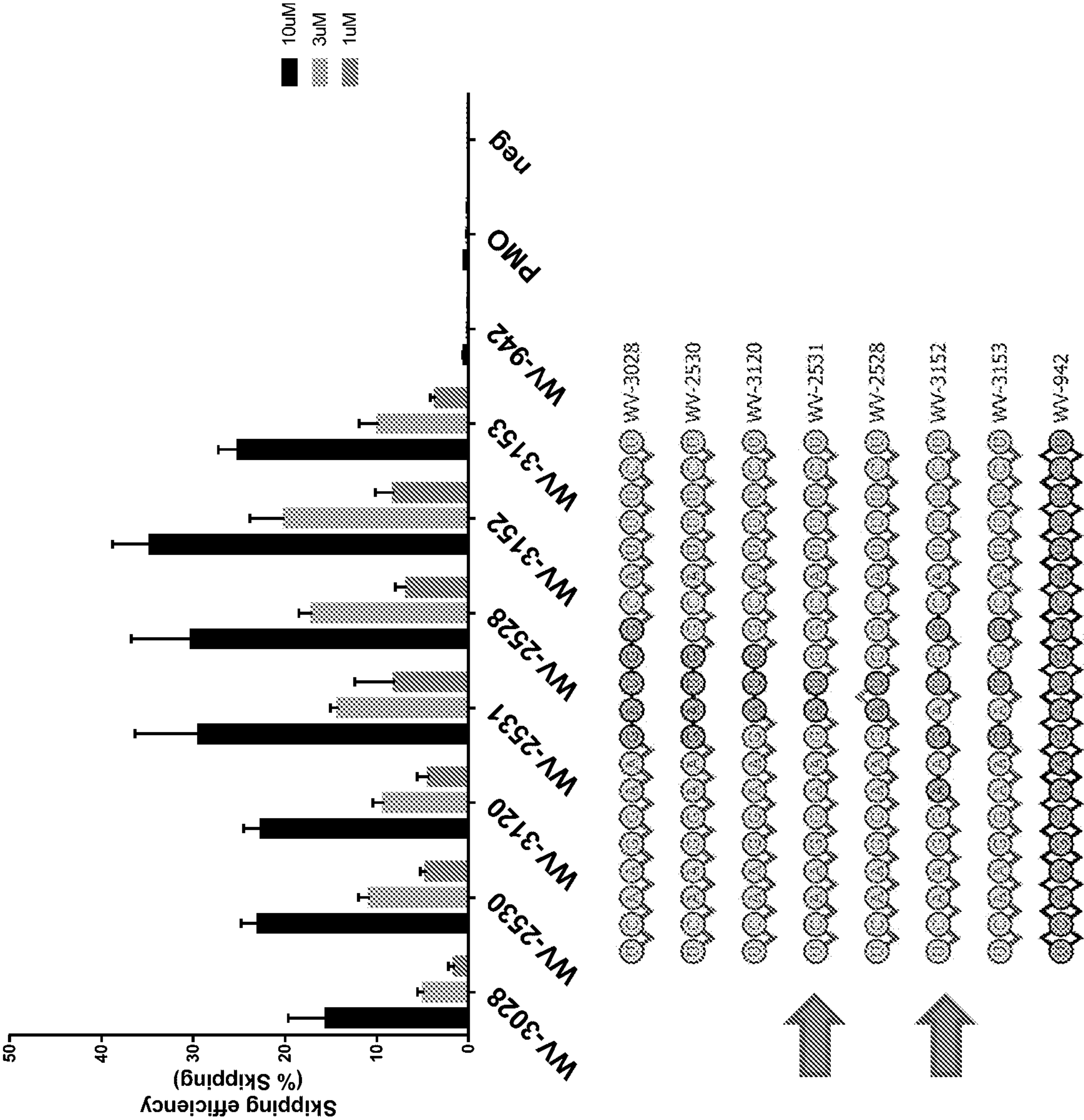


FIG. 35

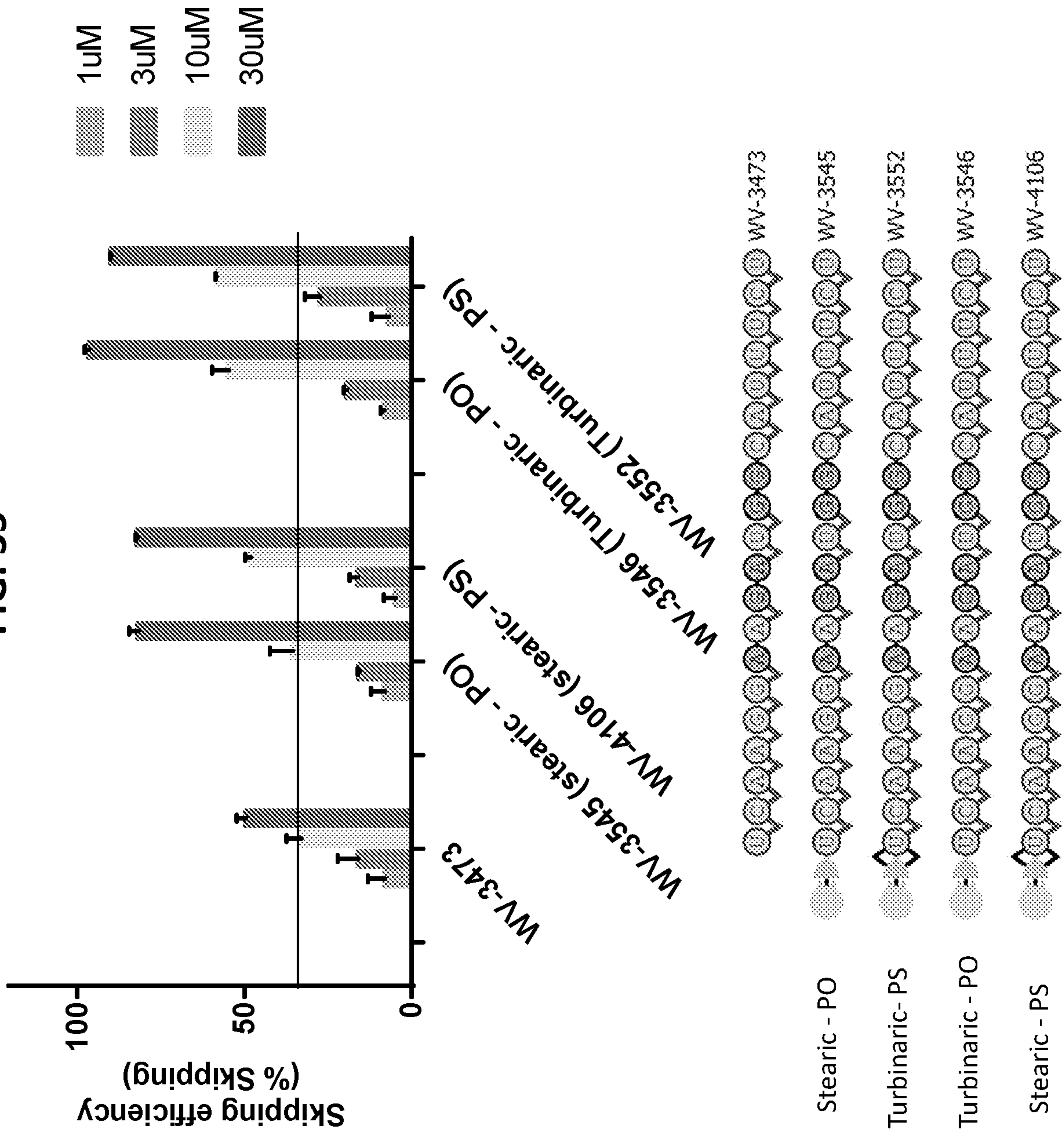
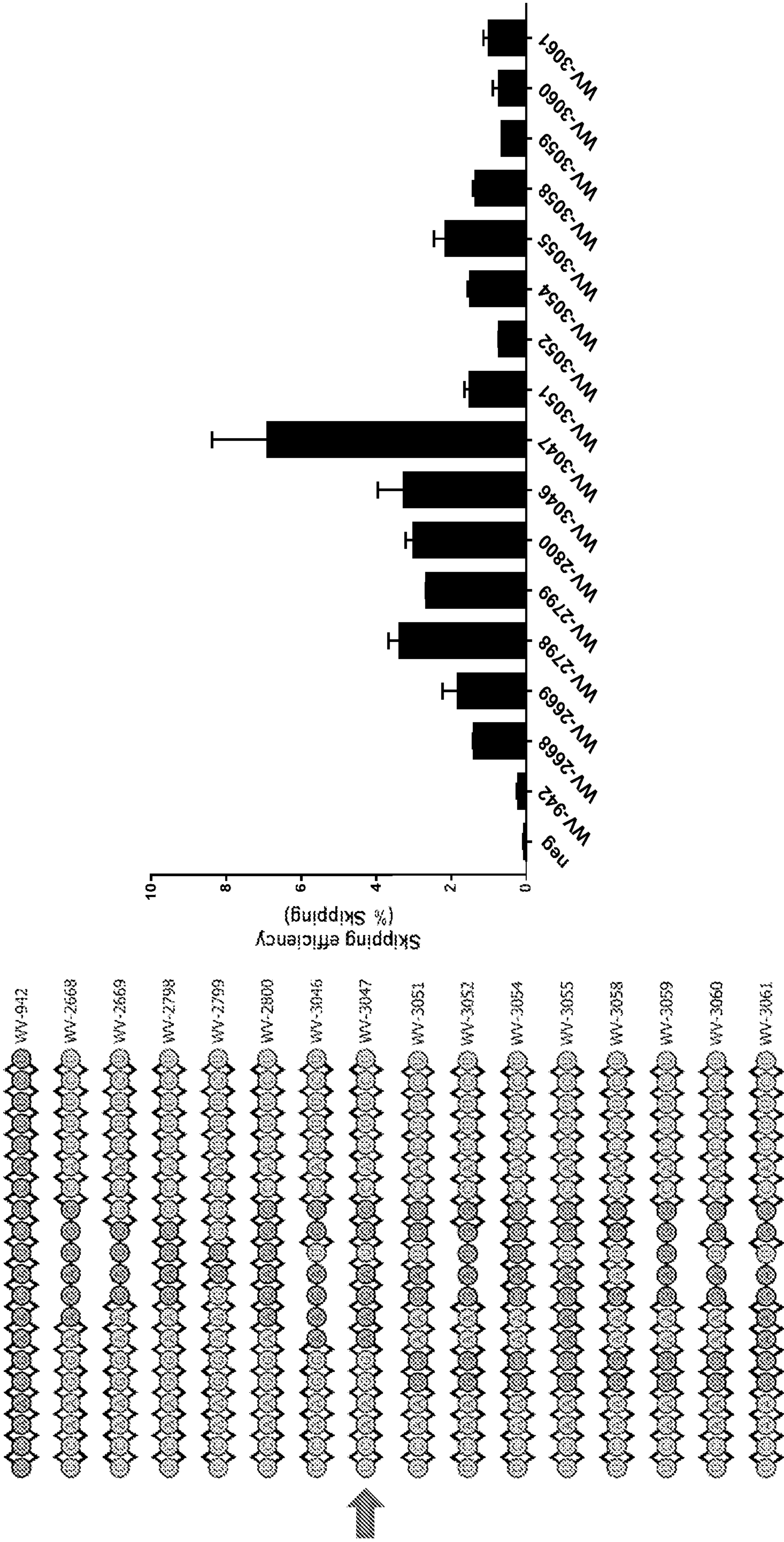


FIG. 36



10uM Gymnotic experiment

FIG. 37

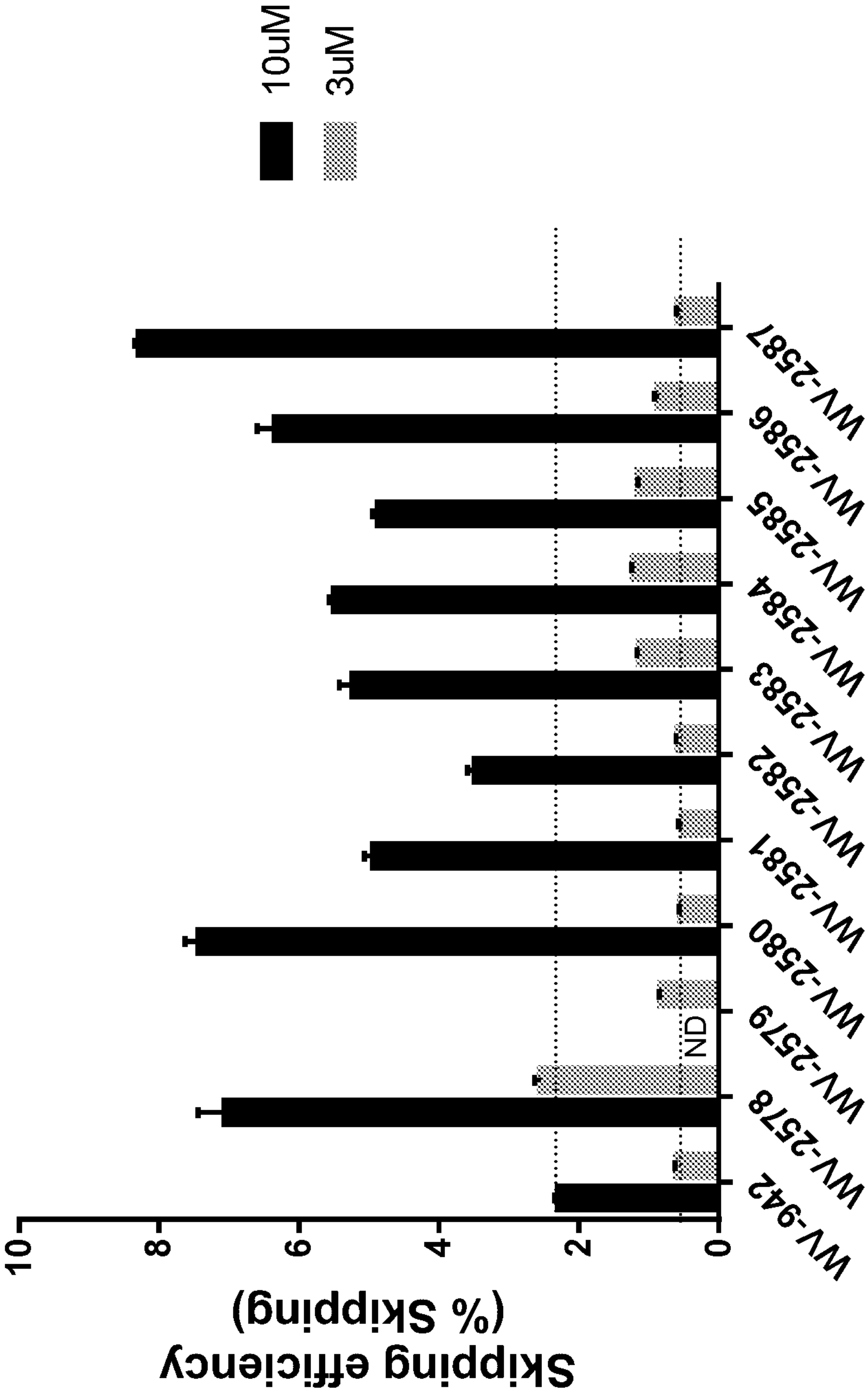


FIG. 38B

Distribution of oligonucleotide in Triceps

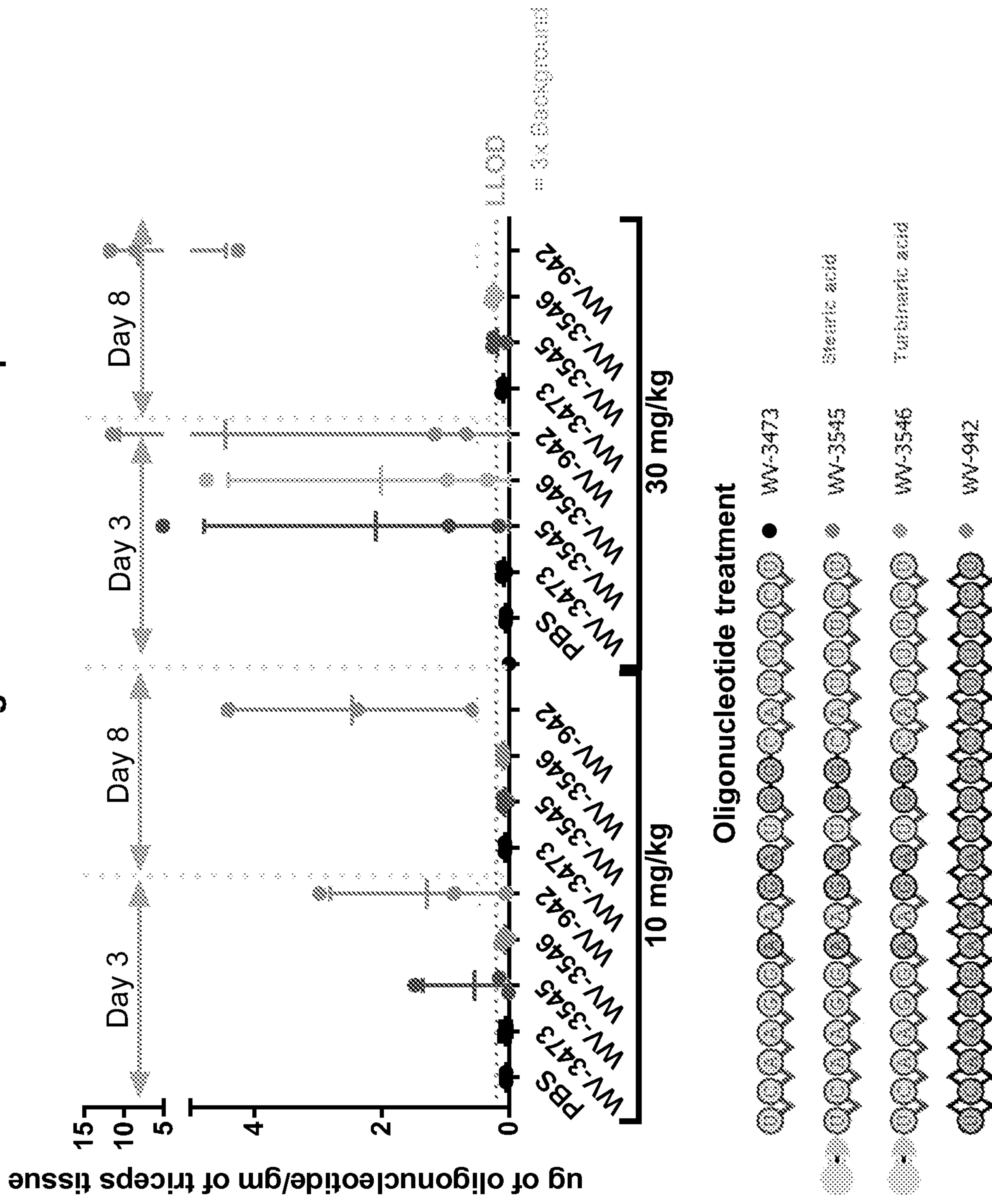


FIG. 38C
Distribution of oligonucleotide in Heart

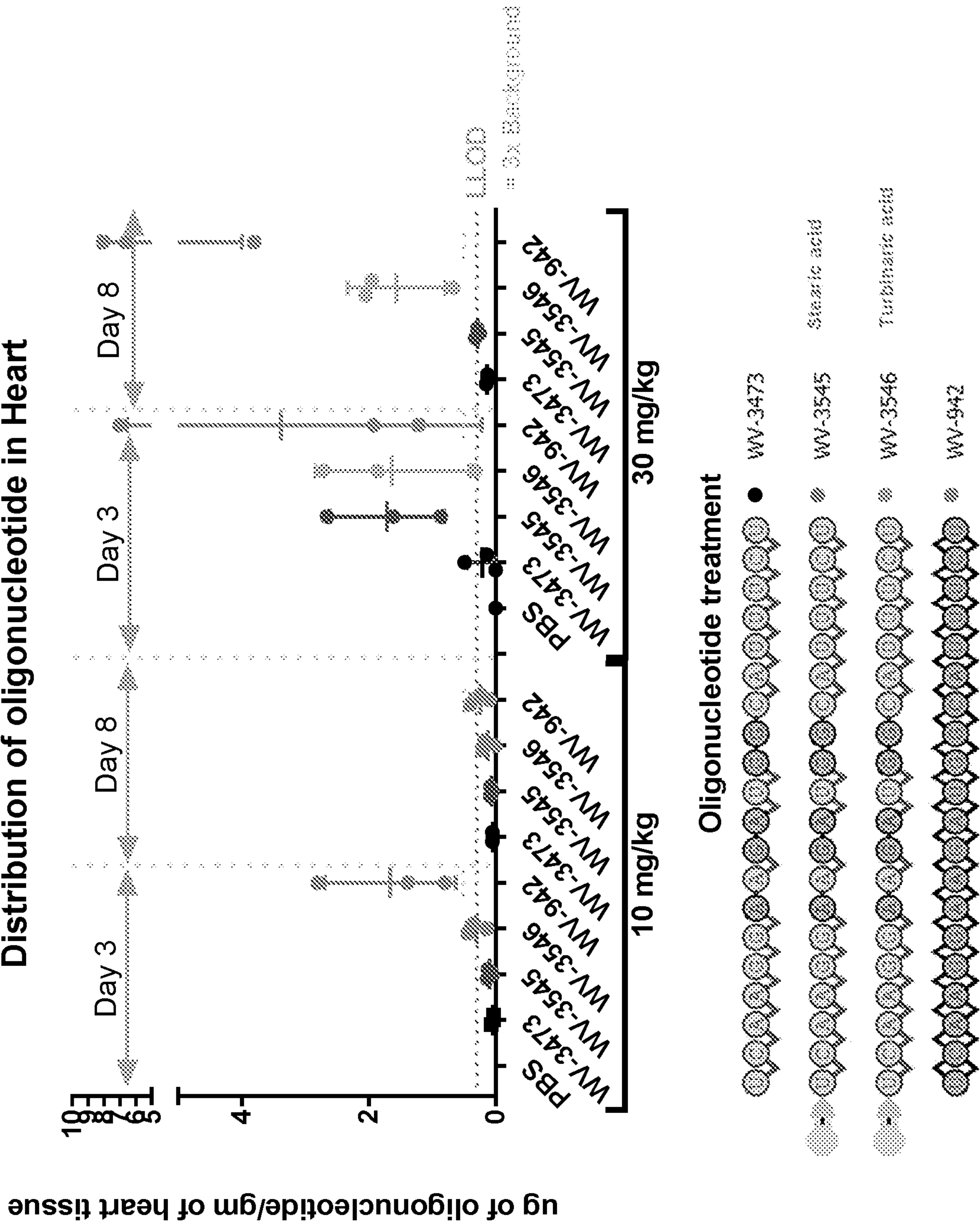


FIG. 38D
Distribution of oligonucleotide in Diaphragm

