DOUBLE STRANDED OLIGONUCLEOTIDE COMPOSITIONS AND METHODS RELATING THERETO

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application Serial No. 63/029,060, filed May 22, 2021, the contents of which are incorporated by reference in their entirety.

BACKGROUND

Gene-targeting oligonucleotides are useful in various applications, *e.g.*, therapeutic, diagnostic, research and nanomaterials applications. The use of naturally-occurring nucleic acids (*e.g.*, unmodified DNA or RNA) in such applications can be limited by, for example, 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 modifications, e.g., base modifications, sugar modifications, backbone modifications. There remains, however, a need in the art for double-stranded (ds) oligonucleotides with improved properties for use in connection with the above-described applications.

SUMMARY

The present disclosure is directed, in part, to the recognition that controlling structural elements of the oligonucleotides of a double-stranded (ds) oligonucleotide can have a significant impact on the ds oligonucleotide's properties and/or activity. In certain embodiments, such structural elements include one or more of: (1) chemical modifications (*e.g.*, modifications of a sugar, base and/or internucleotidic linkage) and patterns thereof; and (2) alterations in stereochemistry (*e.g.*, stereochemistry of a backbone chiral internucleotidic linkage) and patterns thereof One or more of such structural elements can, in certain embodiments, be independently present in one or both oligonucleotides of a ds oligonucleotide. In certain embodiments, the properties and/or activities impacted by such structural elements include, but are not limited to, participation in, direction of a decrease in expression, activity or level of a gene or a gene product thereof, mediated, for example, by RNA interference (RNAi interference), RNase H-mediated knockdown, steric hindrance of translation, etc.

In certain embodiments, the present disclosure demonstrates that compositions comprising ds oligonucleotides (e.g., dsRNAi oligonucleotides, also referred to as dsRNAi agents) with controlled structural elements provide unexpected properties and/or activities.

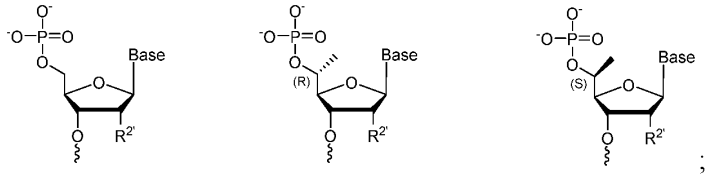
In certain embodiments, the present disclosure encompasses the recognition that stereochemistry, e.g., stereochemistry of backbone chiral centers, can unexpectedly maintain or improve properties of ds oligonucleotides. For example, but not by way of limitation, the instant disclosure relates, in part, to ds oligonucleotides comprising one or more of: (1) a guide strand comprising backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream, i.e., in the 5' direction, (N-2) nucleotide; (2) a guide strand comprising backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream, i.e., in the 3' direction, (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide; (3) a guide strand comprising one or more backbone phosphorothioate chiral centers upstream, i.e., in the 5' direction, relative to backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, where the upstream backbone phosphorothioate chiral centers are in Rp or Sp configuration; (4) a guide strand comprising one or more backbone phosphorothioate chiral centers in Rp or Sp configuration between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide, as well as between one or both of: (a) the +3 nucleotide and the +4 nucleotide; and (b) the +5 nucleotide and the +6 nucleotide; and (5) a passenger strand in combination with one or more of the aforementioned guide strands, comprising one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the present disclosure encompasses the recognition that stereochemistry, e.g., stereochemistry of chiral centers at the 5' terminal modification of guide strands, can unexpectedly maintain or improve properties of ds oligonucleotides wherein the guide strand of the ds oligonucleotide also comprises a phosphorothioate chiral center in Rp or Sp configuration. For example, but not by way of limitation, the instant disclosure relates, in part, to ds oligonucleotides comprising a guide stranding comprising a

phosphorothioate chiral center in Rp or Sp configuration and a 5' terminal modification selected from:

In certain embodiments, the present disclosure encompasses the recognition that stereochemistry, e.g., stereochemistry of chiral centers at a 5' terminal modification of guide strands, can unexpectedly maintain or improve properties of ds oligonucleotides wherein the guide strand of the ds oligonucleotide also comprises a phosphorothioate chiral center in Rp or Sp configuration. For example, but not by way of limitation, the instant disclosure relates, in part, to ds oligonucleotides comprising a guide stranding comprising a phosphorothioate chiral center in Rp or Sp configuration and a 5' terminal modification selected from:

(a) 5' PO modifications, such as, but not limited to:



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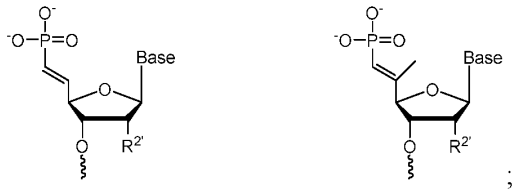
(b) 5' VP modifications, such as, but not limited to:



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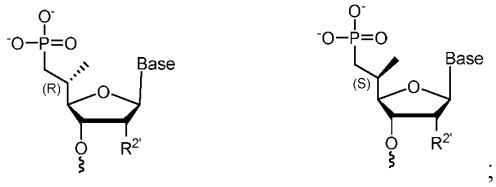
(c) 5' MeP modifications, such as, but not limited to:



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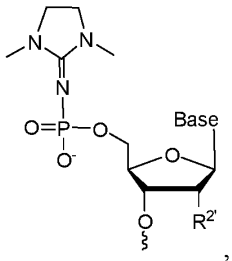
(d) 5' PN and 5' Trizole-P modifications, such as, but not limited to:



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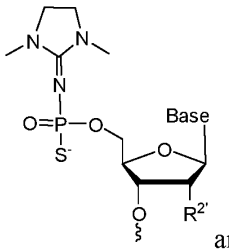
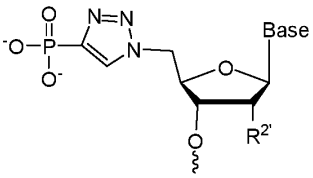
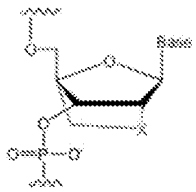
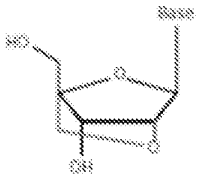


and


;

Wherein Base is selected from A, C, G, T, U, abasic and modified nucleobases;

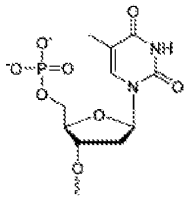
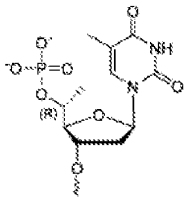
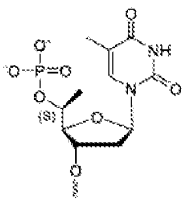
$R^{2'}$ is selected from H, OH, O-alkyl, F, MOE, locked nucleic acid (LNA) bridges and bridged nucleic acid (BNA) bridges to the 4' C, such as, but not limited to:

, and .

In certain other embodiments, the present disclosure encompasses the recognition that stereochemistry, e.g., stereochemistry of chiral centers at the 5' terminal nucleotide of guide strands, can unexpectedly maintain or improve properties of ds oligonucleotides wherein the guide strand of the ds oligonucleotide also comprises a phosphorothioate chiral center in Rp or Sp configuration. For example, but not by way of limitation, the instant disclosure relates, in part, to ds oligonucleotides comprising a guide stranding comprising a phosphorothioate chiral center in Rp or Sp configuration and a 5' terminal nucleotide selected from:

(a) 5' PO nucleotides, such as, but not limited to: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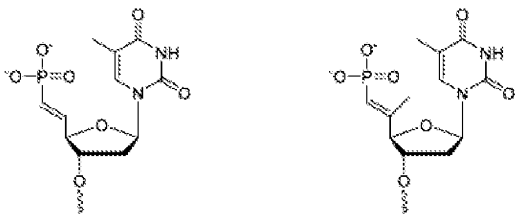
(b) 5' VP nucleotides, such as, but not limited to: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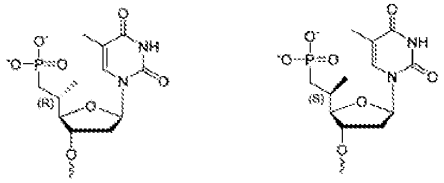
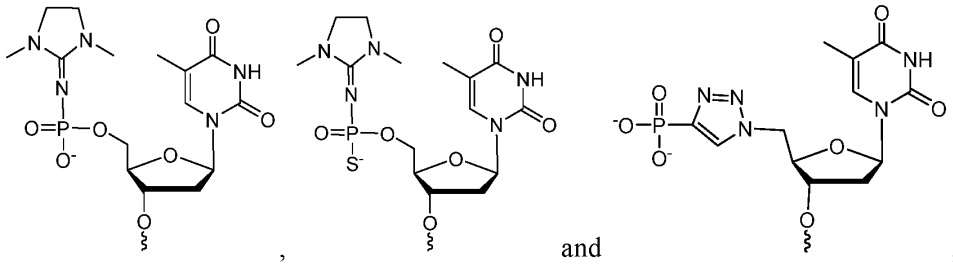
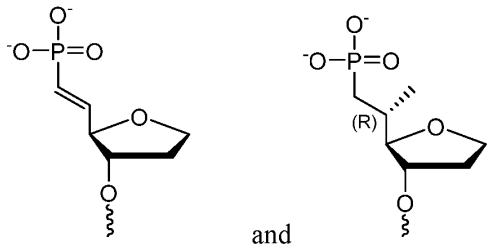
(c) 5' MeP nucleotides, such as, but not limited to:


;

(d) 5' PN and 5' Trizole-P nucleotides, such as, but not limited to:


and ;

(e) 5' abasic VP and 5' abasic MeP nucleotides, such as, but not limited to:


and .

In certain embodiments, the present disclosure encompasses the recognition that non-naturally-occurring internucleotidic linkages, e.g., neutral internucleotidic linkages, can, in certain embodiments, unexpectedly maintain or improve properties of ds oligonucleotides. For example, the present disclosure demonstrates that, in certain embodiments, modified internucleotidic linkages can be introduced into ds oligonucleotide without significantly decreasing the activity of the ds oligonucleotide. For example, but not by way of limitation, the instant disclosure relates, in part, to ds oligonucleotides comprising one or more of: (1) a guide strand where one or both of the 5' and 3' terminal dinucleotides

are ***not*** linked by non-negatively charged internucleotidic linkages, i.e., the guide strand comprises one more non-negatively charged internucleotidic linkages downstream, i.e., in the 3' direction, relative to the linkage between the 5' terminal dinucleotide and/or upstream, i.e., in the 5' direction, relative to the linkage between the 3' terminal dinucleotide; (2) a guide strand where one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, where N is the 3' terminal nucleotide; (3) a guide strand where a non-negatively charged internucleotidic linkage occurs between the third (+3) and fourth (+4) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and/or between the tenth (+10) and eleventh (+11) nucleotides, relative to the 5' terminal nucleotide; (4) a passenger strand where one or more non-negatively charged internucleotidic linkage occurs upstream, i.e., in the 5' direction, relative to the central nucleotide of the passenger strand; and (5) Passenger strand where one or more non-negatively charged internucleotidic linkage occurs downstream, i.e., in the 3' direction, relative to the central nucleotide of the passenger strand.

In certain embodiments, the present disclosure encompasses the recognition that non-naturally occurring internucleotidic linkages, e.g., neutral internucleotidic linkages, can, in certain embodiments, be used to link one or more molecules to the double-stranded oligonucleotides described herein. In certain embodiments, such linked molecules can facilitate targeting and/or delivery of the double-stranded oligonucleotide. For example, but not limitation, such linked molecules an include lipophilic molecules. In certain embodiments, the linked molecule is a molecule comprising one or more GalNAc moieties. In certain embodiments, the the linked molecule is a receptor. In certain embodiments, the linked molecule is a receptor ligand.

In certain embodiments, the present disclosure provides technologies for incorporating various additional chemical moieties into ds oligonucleotides. In certain embodiments, the present disclosure provides, for example, reagents and methods for introducing additional chemical moieties through nucleobases (*e.g.*, by covalent linkage, optionally via a linker, to a site on a nucleobase).

In certain embodiments, the present disclosure provides technologies, e.g., ds oligonucleotide compositions and methods thereof, that achieve allele-specific suppression, wherein transcripts from one allele of a particular target gene is selectively knocked down relative to at least one other allele of the same gene.

Among other things, the present disclosure provides structural elements, technologies and/or features that can be incorporated into ds oligonucleotides and can impart or tune one or more properties thereof (*e.g.*, relative to an otherwise identical ds oligonucleotide lacking the relevant technology or feature). In certain embodiments, the present disclosure documents that one or more provided technologies and/or features can usefully be incorporated into ds oligonucleotides of various sequences.

In certain embodiments, the present disclosure demonstrates that certain provided structural elements, technologies and/or features are particularly useful for ds oligonucleotides that participate in and/or direct RNAi mechanisms (*e.g.*, RNAi agents). Regardless, however, the teachings of the present disclosure are not limited to ds oligonucleotides that participate in or operate via any particular mechanism. In certain embodiments, the present disclosure pertains to any ds oligonucleotide, useful for any purpose, which operates through any mechanism, and which comprises any sequence, structure or format (or portion thereof) described herein. In certain embodiments, the present disclosure provides a ds oligonucleotide, useful for any purpose, which operates through any mechanism, and which comprises any sequence, structure or format (or portion thereof) described herein, including, but not limited to, (1) a guide strand comprising backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide; (2) a guide strand comprising backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide; (3) a guide strand comprising one or more backbone phosphorothioate chiral centers upstream, i.e., in the 5' direction, relative to backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, where the upstream backbone phosphorothioate chiral centers are in Rp or Sp configuration; (4) a guide strand comprising one or more backbone phosphorothioate chiral centers in Rp or Sp configuration between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide, as well as between one or both of: (a) the +3 nucleotide and the +4 nucleotide; and (b) the +5 nucleotide and the +6 nucleotide, and (5) a passenger strand in combination with one or more of the aforementioned guide strands, comprising one or more backbone chiral centers

in Rp or Sp configuration. In certain embodiments, the present disclosure provides a ds oligonucleotide, useful for any purpose, which operates through any mechanism, and which comprises any sequence, structure or format (or portion thereof) described herein, including, but not limited to, (1) a guide strand where one or both of the 5' and 3' terminal dinucleotides are ***not*** linked by non-negatively charged internucleotidic linkages, i.e., the guide strand comprises one more non-negatively charged internucleotidic linkages downstream, i.e., in the 3' direction, relative to the linkage between the 5' terminal dinucleotide and/or upstream, i.e., in the 5' direction, relative to the linkage between the 3' terminal dinucleotide; (2) a guide strand where one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, where N is the 3' terminal nucleotide; (3) a guide strand where a non-negatively charged internucleotidic linkage occurs between the third (+3) and fourth (+4) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and/or between the tenth (+10) and eleventh (+11) nucleotides, relative to the 5' terminal nucleotide; (4) a passenger strand where one or more non-negatively charged internucleotidic linkage occurs upstream, i.e., in the 5' direction, relative to the central nucleotide of the passenger strand; and (5) Passenger strand where one or more non-negatively charged internucleotidic linkage occurs downstream, i.e., in the 3' direction, relative to the central nucleotide of the passenger strand.

In certain embodiments, the present disclosure provides a ds oligonucleotide, useful for any purpose, which operates through any mechanism, and which comprises any sequence, structure or format (or portion thereof) described herein, including, but not limited to: (1) a guide strand comprising backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide; (2) a guide strand comprising backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide; (3) a guide strand comprising one or more backbone phosphorothioate chiral centers upstream, i.e., in the 5' direction, relative to backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, where the upstream backbone phosphorothioate chiral centers are in Rp or Sp configuration; (4) a guide strand comprising

one or more backbone phosphorothioate chiral centers in Rp or Sp configuration between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide, as well as between one or both of: (a) the +3 nucleotide and the +4 nucleotide; and (b) the +5 nucleotide and the +6 nucleotide, and (5) a passenger strand in combination with one or more of the aforementioned guide strands, comprising one or more backbone chiral centers in Rp or Sp configuration, and where the the present disclosure provides a ds oligonucleotide, useful for any purpose, and which also comprises any sequence, structure or format (or portion thereof) described herein, including, but not limited to: (1) a guide strand where one or both of the 5' and 3' terminal dinucleotides are ***not*** linked by non-negatively charged internucleotidic linkages, i.e., the guide strand comprises one more non-negatively charged internucleotidic linkages downstream, i.e., in the 3' direction, relative to the linkage between the 5' terminal dinucleotide and/or upstream, i.e., in the 5' direction, relative to the linkage between the 3' terminal dinucleotide; (2) a guide strand where one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, where N is the 3' terminal nucleotide; (3) a guide strand where a non-negatively charged internucleotidic linkage occurs between the third (+3) and fourth (+4) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and/or between the tenth (+10) and eleventh (+11) nucleotides, relative to the 5' terminal nucleotide; (4) a passenger strand where one or more non-negatively charged internucleotidic linkage occurs upstream, i.e., in the 5' direction, relative to the central nucleotide of the passenger strand; and (5) Passenger strand where one or more non-negatively charged internucleotidic linkage occurs downstream, i.e., in the 3' direction, relative to the central nucleotide of the passenger strand. In certain embodiments, the provided ds oligonucleotides may participate in (*e.g.*, direct) RNAi mechanisms. In certain embodiments, provided ds oligonucleotides may participate in RNase H (ribonuclease H) mechanisms. In certain embodiments, provided ds oligonucleotides may act as translational inhibitors (*e.g.*, may provide steric blocks of translation).

In certain embodiments, the guide strand comprises backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the

immediately upstream (N-2) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49.

In certain embodiments, the guide strand comprises backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49.

In certain embodiments, the guide strand comprises one or more backbone phosphorothioate chiral centers in Rp or Sp configuration upstream of backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49.

In certain embodiments, the guide strand comprises one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49.

In certain embodiments, the guide strand comprises backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises one or more backbone phosphorothioate chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide, and the passenger strand comprises one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises one or more backbone phosphorothioate chiral centers in Rp or Sp configuration upstream of backbone chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprisies one or more backbone phosphorothioate chiral centers in Rp or Sp configuration between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the (+2) nucleotide and the immediately downstream (+3) nucleotide, as well as between one or both of: (a) the (+3) nucleotide and the (+4) nucleotide; and (b) the (+5) nucleotide and the (+6) nucleotide.

In certain embodiments, the guide strand comprises one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, and the passenger strand comprises one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49 and one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49 and one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises one or more backbone phosphorothioate chiral centers in Rp or Sp configuration upstream of backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49 and one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, and the passenger strand

comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49 and one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, provided ds oligonucleotides may participate in exon skipping mechanisms. In certain embodiments, provided ds oligonucleotides may be aptamers. In certain embodiments, provided ds oligonucleotides may bind to and inhibit the function of a protein, small molecule, nucleic acid or cell. In certain embodiments, provided ds oligonucleotides may participate in forming a triplex helix with a double-stranded nucleic acid in the cell. In certain embodiments, provided ds oligonucleotides may bind to genomic (*e.g.*, chromosomal) nucleic acid. In certain embodiments, provided ds oligonucleotides may bind to genomic (*e.g.*, chromosomal) nucleic acid, thus preventing or decreasing expression of the nucleic acid (*e.g.*, by preventing or decreasing transcription, transcriptional enhancement, modification, etc.). In certain embodiments, provided ds oligonucleotides may bind to DNA quadruplexes. In certain embodiments, provided ds oligonucleotides may be immunomodulatory. In certain embodiments, provided ds oligonucleotides may be immunostimulatory. In certain embodiments, provided oligonucleotides may be immunostimulatory and may comprise a CpG sequence. In certain embodiments, provided ds oligonucleotides may be immunostimulatory and may comprise a CpG sequence and may be useful as an adjuvant. In certain embodiments, provided ds oligonucleotides may be immunostimulatory and may comprise a CpG sequence and may be useful as an adjuvant in treating a disease (*e.g.*, an infectious disease or cancer). In certain embodiments, provided ds oligonucleotides may be therapeutic. In certain embodiments, provided ds oligonucleotides may be non-therapeutic. In certain embodiments, provided ds oligonucleotides may be therapeutic or non-therapeutic. In certain embodiments, provided ds oligonucleotides are useful in therapeutic, diagnostic, research and/or nanomaterials applications. In certain embodiments, provided ds oligonucleotides may be useful for experimental purposes. In certain embodiments, provided ds oligonucleotides may be useful for experimental purposes, *e.g.*, as a probe, in a microarray, etc. In certain embodiments, provided ds oligonucleotides may participate in more than one biological mechanism; in certain such embodiments, for example, provided ds oligonucleotides may participate in both RNAi and RNase H mechanisms.

In certain embodiments, provided ds oligonucleotides are directed to a target (*e.g.*, a target sequence, a target RNA, a target mRNA, a target pre-mRNA, a target gene, etc.). A target gene is a gene with respect to which expression and/or activity of one or more gene products (*e.g.*, RNA and/or protein products) are intended to be altered. In certain

embodiments, a target gene is intended to be inhibited. Thus, when a ds oligonucleotide as described herein acts on a particular target gene, presence and/or activity of one or more gene products of that gene are altered when the ds oligonucleotide is present as compared with when it is absent.

In certain embodiments, a target is a specific allele with respect to which expression and/or activity of one or more products (*e.g.*, RNA and/or protein products) are intended to be altered. In certain embodiments, a target allele is one whose presence and/or expression is associated (*e.g.*, correlated) with presence, incidence, and/or severity, of one or more diseases and/or conditions. Alternatively or additionally, in certain embodiments, a target allele is one for which alteration of level and/or activity of one or more gene products correlates with improvement (*e.g.*, delay of onset, reduction of severity, responsiveness to other therapy, etc) in one or more aspects of a disease and/or condition.

In certain embodiments, e.g., where presence and/or activity of a particular allele (a disease-associated allele) is associated (*e.g.*, correlated) with presence, incidence and/or severity of one or more disorders, diseases and/or conditions, a different allele of the same gene exists and is not so associated, or is associated to a lesser extent (*e.g.*, shows less significant, or statistically insignificant correlation), ds oligonucleotides and methods thereof as described herein may preferentially or specifically target the associated allele relative to the one or more less-associated/unassociated allele(s), thus mediating allele-specific suppression.

In certain embodiments, a target sequence is a sequence to which an oligonucleotide as described herein binds. In certain embodiments, a target sequence is identical to, or is an exact complement of, a sequence of a provided oligonucleotide, or of consecutive residues therein (*e.g.*, a provided oligonucleotide includes a target-binding sequence that is identical to, or an exact complement of, a target sequence). In certain embodiments, a target-binding sequence is an exact complement of a target sequence of a transcript (*e.g.*, pre-mRNA, mRNA, *etc.*). A target-binding sequence/target sequence can be of various lengths to provided oligonucleotides with desired activities and/or properties. In certain embodiments, a target binding sequence/target sequence comprises 5-50 (*e.g.*, 10-40, 15-30, 15-25, 16-25, 17-25, 18-25, 19-25, 20-25, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more) bases. In certain embodiments, a small number of differences/mismatches is tolerated between (a relevant portion of) an oligonucleotide and its target sequence, including but not limited to the 5' and/or 3'-end regions of the target and/or oligonucleotide sequence. In certain embodiments, a target sequence is present

within a target gene. In certain embodiments, a target sequence is present within a transcript (*e.g.*, an mRNA and/or a pre-mRNA) produced from a target gene.

In certain embodiments, a target sequence includes one or more allelic sites (*i.e.*, positions within a target gene at which allelic variation occurs). In certain embodiments, an allelic site is a mutation. In certain embodiments, an allelic site is a SNP. In some such embodiments, a provided oligonucleotide binds to one allele preferentially or specifically relative to one or more other alleles. In certain embodiments, a provided oligonucleotide binds preferentially to a disease-associated allele. For example, in certain embodiments, an oligonucleotide (or a target-binding sequence portion thereof) provided herein has a sequence that is, fully or at least in part, identical to, or an exact complement of a particular allelic version of a target sequence.

In certain embodiments, an oligonucleotide (or a target-binding sequence portion thereof) provided herein has a sequence that is identical to, or an exact complement of a target sequence comprising an allelic site, or an allelic site, of a disease-associated allele. In certain embodiments, an oligonucleotide provided herein has a target binding sequence that is an exact complement of a target sequence comprising an allelic site of a transcript of an allele (in certain embodiments, a disease-associated allele), wherein the allelic site is a mutation. In certain embodiments, an oligonucleotide provided herein has a target binding sequence that is an exact complement of a target sequence comprising an allelic site of a transcript of an allele (in certain embodiments, a disease-associated allele), wherein the allelic site is a SNP. In certain embodiments, a sequence is any sequence disclosed herein.

Unless otherwise noted, all sequences (including, but not limited to base sequences and patterns of chemistry, modification, and/or stereochemistry) are presented in 5' to 3' order, with the 5' terminal nucleotide identified as the "+1" position and the 3' terminal nucleotide identified either by the number of nucleotides of the full sequence or by "N", with the penultimate nucleotide identified, e.g., as "N-1", and so on.

In certain embodiments, the present disclosure provides compositions and methods related to an oligonucleotide which is specific to a target and which has any format, structural element or base sequence of any oligonucleotide disclosed herein.

In certain embodiments, the present disclosure provides compositions and methods related to an oligonucleotide which is specific to a target and which has or comprises the base sequence of any oligonucleotide disclosed herein, or a region of at least 15 contiguous nucleotides of the base sequence of any oligonucleotide disclosed herein,

wherein the first nucleotide of the base sequence or the first nucleotide of the at least 15 contiguous nucleotides can be optionally replaced by T or DNA T.

In certain embodiments, the present disclosure provides compositions and methods for RNA interference directed by a RNAi agent (also referred to as a RNAi oligonucleotides). In certain embodiments, oligonucleotides of such compositions can have a format, structural element or base sequence of an oligonucleotide disclosed herein.

In certain embodiments, the present disclosure provides compositions and methods for RNase H-mediated knockdown of a target gene RNA directed by an oligonucleotide (*e.g.*, an antisense oligonucleotide).

Provided oligonucleotides and oligonucleotide compositions can have any format, structural element or base sequence of any oligonucleotide disclosed herein. In certain embodiments, a structural element is a 5'-end structure, 5'-end region, 5'-nucleotide, seed region, post-seed region, 3'-end region, 3'-terminal dinucleotide, 3'-end cap, or any portion of any of these structures, GC content, long GC stretch, and/or any modification, chemistry, stereochemistry, pattern of modification, chemistry or stereochemistry, or a chemical moiety (*e.g.*, including but not limited to, a targeting moiety, a lipid moiety, a GalNAc moiety, a carbohydrate moiety, etc.), any component, or any combination of any of the above.

In certain embodiments, the present disclosure provides compositions and methods of use of an oligonucleotide.

In certain embodiments, the present disclosure provides compositions and methods of use of an oligonucleotide which can direct both RNA interference and RNase H-mediated knockdown of a target gene RNA. In certain embodiments, oligonucleotides of such compositions can have a format, structural element or base sequence of an oligonucleotide disclosed herein.

In certain embodiments, an oligonucleotide directing a particular event or activity participates in the particular event or activity, e.g., a decrease in the expression, level or activity of a target gene or a gene product thereof. In certain embodiments, an oligonucleotide is deemed to "direct" a particular event or activity when presence of the oligonucleotide in a system in which the event or activity can occur correlates with increased detectable incidence, frequency, intensity and/or level of the event or activity.

In certain embodiments, a provided oligonucleotide comprises any one or more structural elements of an oligonucleotide as described herein, *e.g.*, a base sequence (or a portion thereof of at least 15 contiguous bases); a pattern of internucleotidic linkages (or

a portion thereof of at least 5 contiguous internucleotidic linkage); a pattern of stereochemistry of internucleotidic linkages (or a portion thereof of at least 5 contiguous internucleotidic linkages); a 5'-end structure; a 5'-end region; a first region; a second region; and a 3'-end region (which can be a 3'-terminal dinucleotide and/or a 3'-end cap); and an optional additional chemical moiety; and, in certain embodiments, at least one structural element comprises a chirally controlled chiral center. In certain embodiments, a 3'-terminal dinucleotide can comprise two total nucleotides. In certain embodiments, an oligonucleotide further comprises a chemical moiety selected from, as non-limiting examples, a targeting moiety, a carbohydrate moiety, a GalNAc moiety, a lipid moiety, and any other chemical moiety described herein or known in the art. In certain embodiments, a moiety that binds APGR is a moiety of GalNAc, or a variant, derivative or modified version thereof, as described herein and/or known in the art. In certain embodiments, an oligonucleotide is a RNAi agent. In certain embodiments, a first region is a seed region. In certain embodiments, a second region is a post-seed region.

In certain embodiments, a provided oligonucleotide comprises any one or more structural elements of a RNAi agent as described herein, *e.g.*, a 5'-end structure; a 5'-end region; a seed region; a post-seed region (the region between the seed region and the 3'-end region); and a 3'-end region (which can be a 3'-terminal dinucleotide and/or a 3'-end cap); and an optional additional chemical moiety; and, in certain embodiments, at least one structural element comprises a chirally controlled chiral center. In certain embodiments, a 3'-terminal dinucleotide can comprise two total nucleotides. In certain embodiments, an oligonucleotide further comprises a chemical moiety selected from, as non-limiting examples, a targeting moiety, a carbohydrate moiety, a GalNAc moiety, and a lipid moiety. In certain embodiments, a moiety that binds APGR is any GalNAc, or variant, derivative or modification thereof, as described herein or known in the art.

In certain embodiments, a provided oligonucleotide comprises any one or more structural elements of an oligonucleotide as described herein, *e.g.*, a 5'-end structure, a 5'-end region, a first region, a second region, a 3'-end region, and an optional additional chemical moiety, wherein at least one structural element comprises a chirally controlled chiral center. In certain embodiments, the oligonucleotide comprises a span of at least 5 total nucleotides without 2'-modifications. In certain embodiments, the oligonucleotide further comprises an additional chemical moiety selected from, as non-limiting examples, a targeting moiety, a carbohydrate moiety, a GalNAc moiety, and a lipid moiety. In certain embodiments, a provided oligonucleotide is capable of directing RNA interference. In

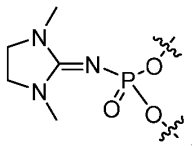
certain embodiments, a provided oligonucleotide is capable of directing RNase H-mediated knockdown. In certain embodiments, a provided oligonucleotide is capable of directing both RNA interference and RNase H-mediated knockdown. In certain embodiments, a first region is a seed region. In certain embodiments, a second region is a post-seed region.

In certain embodiments, a provided oligonucleotide comprises any one or more structural elements of a RNAi agent, *e.g.*, a 5'-end structure, a 5'-end region, a seed region, a post-seed region, and a 3'-end region and an optional additional chemical moiety, wherein at least one structural element comprises a chirally controlled chiral center; and, in certain embodiments, the oligonucleotide is also capable of directing RNase H-mediated knockdown of a target gene RNA. In certain embodiments, the oligonucleotide comprises a span of at least 5 total 2'-deoxy nucleotides. In certain embodiments, the oligonucleotide further comprises a chemical moiety selected from, as non-limiting examples, a targeting moiety, a carbohydrate moiety, a GalNAc moiety, and a lipid moiety, and any other additional chemical moiety described herein.

In certain embodiments, the present disclosure demonstrates that oligonucleotide properties can be modulated through chemical modifications. In certain 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 internucleotidic linkage, sugar, and/or base modifications. In certain embodiments, the present disclosure provides an oligonucleotide composition capable of directing RNA interference and comprising a first plurality of oligonucleotides which have a common base sequence and comprise one or more internucleotidic linkage, and/or one or more sugar, and/or one or more base modifications. In certain embodiments, an oligonucleotide or oligonucleotide composition is also capable of directing RNase H-mediated knockdown of a target gene RNA. In certain embodiments, the present disclosure demonstrates that oligonucleotide properties, *e.g.*, activities, toxicities, *etc.*, can be modulated through chemical modifications of sugars, nucleobases, and/or internucleotidic linkages. In certain embodiments, the present disclosure provides an oligonucleotide composition comprising a plurality of oligonucleotides which have a common base sequence, and comprise one or more modified internucleotidic linkages (or "non-natural internucleotidic linkages", linkages that can be utilized in place of a natural phosphate internucleotidic linkage (−OP(O)(OH)O−, which may exist as a salt form (−OP(O)(O^-)O−) at a physiological pH) found in natural DNA and RNA), one or more modified sugar moieties, and/or one or more natural phosphate linkages. In certain embodiments, provided oligonucleotides may

comprise two or more types of modified internucleotidic linkages. In certain embodiments, a provided oligonucleotide comprises a non-negatively charged internucleotidic linkage. In certain embodiments, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage. In certain embodiments, a neutral internucleotidic linkage comprises a cyclic guanidine moiety. Such moieties an optionally substituted. In certain embodiments, a provided oligonucleotide comprises a neutral internucleotidic linkage and another internucleotidic linkage which is not a neutral backbone. In certain embodiments, a provided oligonucleotide comprises a neutral internucleotidic linkage and a phosphorothioate internucleotidic linkage. In certain embodiments, provided oligonucleotide compositions comprising a plurality of oligonucleotides are chirally controlled and level of the plurality of oligonucleotides in the composition is controlled or pre-determined, and oligonucleotides of the plurality share a common stereochemistry configuration at one or more chiral internucleotidic linkages. For example, in certain embodiments, oligonucleotides of a plurality share a common stereochemistry configuration at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50 or more chiral internucleotidic linkages, each of which is independently *R*p or *S*p; in certain embodiments, oligonucleotides of a plurality share a common stereochemistry configuration at each chiral internucleotidic linkages. In certain embodiments, a chiral internucleotidic linkage where a controlled level of oligonucleotides of a composition share a common stereochemistry configuration (independently in the *R*p or *S*p configuration) is referred to as a chirally controlled internucleotidic linkage. In certain embodiments, a modified internucleotidic linkage is a non-negatively charged (neutral or cationic) internucleotidic linkage in that at a pH, (e.g., human physiological pH (~ 7.4), pH of a delivery site (e.g., an organelle, cell, tissue, organ, organism, etc.), etc.), it largely (*e.g.*, at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, etc.; in certain embodiments, at least 30%; in certain embodiments, at least 40%; in certain embodiments, at least 50%; in certain embodiments, at least 60%; in certain embodiments, at least 70%; in certain embodiments, at least 80%; in certain embodiments, at least 90%; in certain embodiments, at least 99%; etc.;) exists as a neutral or cationic form (as compared to an anionic form (e.g., $-O-P(O)(O^-)-O-$ (the anionic form of natural phosphate linkage), $-O-P(O)(S^-)-O-$ (the anionic form of phosphorothioate linkage), etc.)), respectively. In certain embodiments, a modified internucleotidic linkage is a neutral internucleotidic linkage in that at a pH, it largely exists as a neutral form. In certain embodiments, a modified internucleotidic linkage is a cationic internucleotidic linkage in that at a pH, it largely exists

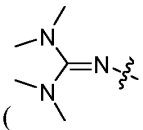
as a cationic form. In certain embodiments, a pH is human physiological pH (~ 7.4). In certain embodiments, a modified internucleotidic linkage is a neutral internucleotidic linkage in that at pH 7.4 in a water solution, at least 90% of the internucleotidic linkage exists as its neutral form. In certain embodiments, a modified internucleotidic linkage is a neutral internucleotidic linkage in that in a water solution of the oligonucleotide, at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the internucleotidic linkage exists in its neutral form. In certain embodiments, the percentage is at least 90%. In certain embodiments, the percentage is at least 95%. In certain embodiments, the percentage is at least 99%. In certain embodiments, a non-negatively charged internucleotidic linkage, e.g., a neutral internucleotidic linkage, when in its neutral form has no moiety with a pKa that is less than 8, 9, 10, 11. 12, 13, or 14. In certain embodiments, pKa of an internucleotidic linkage in the present disclosure can be represented by pKa of CH_3–the internucleotidic linkage–CH_3 (i.e., replacing the two nucleoside units connected by the internucleotidic linkage with two $-CH_3$ groups). Without wishing to be bound by any particular theory, in at least some cases, a neutral internucleotidic linkage in an oligonucleotide can provide improved properties and/or activities, e.g., improved delivery, improved resistance to exonucleases and endonucleases, improved cellular uptake, improved endosomal escape and/or improved nuclear uptake, etc., compared to a comparable nucleic acid which does not comprises a neutral internucleotidic linkage.

In certain embodiments, a non-negatively charged internucleotidic linkage has the structure of e.g., of formula **I-n-1**, **I-n-2**, **I-n-3**, **II**, **II-a-1**, **II-a-2, II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, as described in US 9394333, US 9744183, US 9605019, US 9598458, US 9982257, US 10160969, US 10479995, US 2020/0056173, US 2018/0216107, US 2019/0127733, US 10450568, US 2019/0077817, US 2019/0249173, US 2019/0375774, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612 etc. In certain embodiments, a non-negatively charged internucleotidic linkage comprises a cyclic guanidine moiety. In certain embodiments, a modified internucleotidic linkage comprising a cyclic guanidine moiety has the structure of: . In certain embodiments, a neutral internucleotidic linkage comprising a cyclic guanidine moiety is chirally controlled. In certain embodiments, the

present disclosure pertains to a composition comprising an oligonucleotide comprising at least one neutral internucleotidic linkage and at least one phosphorothioate internucleotidic linkage.

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

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

In certain embodiments, the present disclosure pertains to a composition comprising an oligonucleotide comprising at least one neutral internucleotidic linkage of a neutral internucleotidic linkage comprising a Tmg group (), and at least one phosphorothioate.

In certain embodiments, each internucleotidic linkage in an oligonucleotide is independently selected from a natural phosphate linkage, a phosphorothioate linkage, and a non-negatively charged internucleotidic linkage (e.g., n001, n003, n004, n006, n008, n009, n013, n020, n021, n025, n026, n029, n031, n037, n046, n047, n048, n054, or n055). In some embodiments, each internucleotidic linkage in an oligonucleotide is independently selected from a natural phosphate linkage, a phosphorothioate linkage, and a neutral internucleotidic linkage (e.g., n001, n003, n004, n006, n008, n009, n013 n020, n021, n025, n026, n029, n031, n037, n046, n047, n048, n054, or n055).

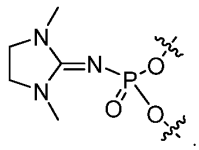
In certain embodiments, the present disclosure pertains to a composition comprising an oligonucleotide comprising at least one neutral internucleotidic linkage of a neutral internucleotidic linkage comprising a Tmg group, and at least one phosphorothioate, wherein the phosphorothioate is a chirally controlled internucleotidic linkage in the Sp configuration.

In certain embodiments, the present disclosure pertains to a composition comprising an oligonucleotide comprising at least one neutral internucleotidic linkage selected from a neutral internucleotidic linkage of a neutral internucleotidic linkage

comprising a Tmg group, and at least one phosphorothioate, wherein the phosphorothioate is a chirally controlled internucleotidic linkage in the Rp configuration.

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

In certain embodiments, a chirally controlled neutral internucleotidic linkage sis neutral at physiological pH, chirally controlled, stable in vivo, hydrophobic, and may increase endosomal escape.

In certain embodiments, provided oligonucleotides comprise one or more regions, e.g., a block, wing, core, 5'-end, 3'-end, middle, seed, post-seed region, etc. In certain embodiments, a region (e.g., a block, wing, core, 5'-end, 3'-end, middle region, etc.) comprises a non-negatively charged internucleotidic linkage, e.g., of formula **I-n-1**, **I-n-2**, **I-n-3**, **II**, **II-a-1**, **II-a-2, II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, etc as described in US 9394333, US 9744183, US 9605019, US 9598458, US 9982257, US 10160969, US 10479995, US 2020/0056173, US 2018/0216107, US 2019/0127733, US 10450568, US 2019/0077817, US 2019/0249173, US 2019/0375774, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612. In certain embodiments, a region comprises a neutral internucleotidic linkage. In certain embodiments, a region comprises an internucleotidic linkage which comprises a cyclic guanidine guanidine. In certain embodiments, a region comprises an internucleotidic linkage which comprises a cyclic guanidine moiety. In certain embodiments, a region comprises an internucleotidic linkage having the structure of . In certain embodiments, such internucleotidic linkages are chirally controlled.

In certain embodiments, a nucleotide is a natural nucleotide. In certain embodiments, a nucleotide is a modified nucleotide. In certain embodiments, a nucleotide

is a nucleotide analog. In certain embodiments, a base is a modified base. In certain embodiments, a base is protected nucleobase, such as a protected nucleobase used in oligonucleotide synthesis. In certain embodiments, a base is a base analog. In certain embodiments, a sugar is a modified sugar. In certain embodiments, a sugar is a sugar analog. In certain embodiments, an internucleotidic linkage is a modified internucleotidic linkage. In certain embodiments, a nucleotide comprises a base, a sugar, and an internucleotidic linkage, wherein each of the base, the sugar, and the internucleotidic linkage is independently and optionally naturally-occurring or non-naturally occurring. In certain embodiments, a nucleoside comprises a base and a sugar, wherein each of the base and the sugar is independently and optionally naturally-occurring or non-naturally occurring. Non-limiting examples of nucleotides include DNA (2'-deoxy) and RNA (2'-OH) nucleotides; and those which comprise one or more modifications at the base, sugar and/or internucleotidic linkage. Non-limiting examples of sugars include ribose and deoxyribose; and ribose and deoxyribose with 2'-modifications, including but not limited to 2'-F, LNA, 2'-OMe, and 2'-MOE modifications. In certain embodiments, an internucleotidic linkage is a moiety which does not a comprise a phosphorus but serves to link two natural or non-natural sugars.

In certain embodiments, a composition comprises a multimer of two or more of any: oligonucleotides of a first plurality and/or oligonucleotides of a second plurality, wherein the oligonucleotides of the first and second plurality can independently direct knockdown of the same or different targets independently via RNA interference and/or RNase H-mediated knockdown.

In certain embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides which share:

1) a common base sequence;

2) a common pattern of backbone linkages;

3) common stereochemistry independently at at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, or 50 chiral internucleotidic linkages ("chirally controlled internucleotidic linkages"); which composition is chirally controlled in that level of the first plurality of oligonucleotides in the composition is predetermined.

In certain embodiments, an oligonucleotide composition comprising a plurality of oligonucleotides (e.g., a first plurality of oligonucleotides) is chirally controlled in that oligonucleotides of the plurality share a common stereochemistry independently at

one or more chiral internucleotidic linkages. In certain embodiments, oligonucleotides of the 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 *R*p or *S*p In certain embodiments, oligonucleotides of the plurality share a common stereochemistry configuration at each chiral internucleotidic linkages. In certain embodiments, a chiral internucleotidic linkage where a predetermined level of oligonucleotides of a composition share a common stereochemistry configuration (independently *R*p or *S*p) is referred to as a chirally controlled internucleotidic linkage.

In certain 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 chirally controlled internucleotidic linkages.

In certain embodiments, at least 5 internucleotidic linkages are chirally controlled; in certain embodiments, at least 10 internucleotidic linkages are chirally controlled; in certain embodiments, at least 15 internucleotidic linkages are chirally controlled; in certain embodiments, each chiral internucleotidic linkage is chirally controlled.

In certain embodiments, 1%-100% of chiral internucleotidic linkages are chirally controlled. In certain embodiments, at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of chiral internucleotidic linkages are chirally controlled.

In certain embodiments, the present disclosure provides an oligonucleotide composition comprising a first plurality of oligonucleotides which share:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) a common pattern of backbone chiral centers, which composition is a substantially pure preparation of 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. In certain embodiments, the common pattern of backbone chiral centers comprises at least one internucleotidic linkage comprising a chirally controlled chiral center. In certain embodiments, a predetermined level of oligonucleotides is at least 1%, 5%, 10%, 15%, 20%,

25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides in a provided composition. In certain embodiments, a predetermined level of oligonucleotides is at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides in a provided composition that are of or comprise a common base sequence. In certain embodiments, all oligonucleotides in a provided composition that are of or comprise a common base sequence are at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides in the composition. In certain embodiments, a predetermined level of oligonucleotides is at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides in a provided composition that are of or comprise a common base sequence, base modification, sugar modification and/or modified internucleotidic linkage. In certain embodiments, all oligonucleotides in a provided composition that are of or comprise a common base sequence, base modification, sugar modification and/or modified internucleotidic linkage are at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides in the composition. In certain embodiments, a predetermined level of oligonucleotides is at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides in a provided composition that are of or comprise a common base sequence, pattern of base modification, pattern of sugar modification, and/or pattern of modified internucleotidic linkage. In certain embodiments, all oligonucleotides in a provided composition that are of or comprise a common base sequence, pattern of base modification, pattern of sugar modification, and/or pattern of modified internucleotidic linkage are at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides in the composition. In certain embodiments, a predetermined level of oligonucleotides is at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides in a provided composition that share a common base sequence, a common pattern of base modification, a common pattern of sugar modification, and/or a common pattern of modified internucleotidic linkages. In

certain embodiments, all oligonucleotides in a provided composition that share a common base sequence, a common pattern of base modification, a common pattern of sugar modification, and/or a common pattern of modified internucleotidic linkages are at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides in the composition. In certain embodiments, a predetermined level is 1-100%. In certain embodiments, a predetermined level is at least 1%. In certain embodiments, a predetermined level is at least 5%. In certain embodiments, a predetermined level is at least 10%. In certain embodiments, a predetermined level is at least 20%. In certain embodiments, a predetermined level is at least 30%. In certain embodiments, a predetermined level is at least 40%. In certain embodiments, a predetermined level is at least 50%. In certain embodiments, a predetermined level is at least 60%. In certain embodiments, a predetermined level is at least 10%. In certain embodiments, a predetermined level is at least 70%. In certain embodiments, a predetermined level is at least 80%. In certain embodiments, a predetermined level is at least 90%. In certain embodiments, a predetermined level is at least 5*(1/2g), wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, a predetermined level is at least 10*(1/2g), wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, a predetermined level is at least 100*(1/2g), wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, a predetermined level is at least (0.80)g, wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, a predetermined level is at least (0.80)g, wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, a predetermined level is at least (0.80)g, wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, a predetermined level is at least (0.85)g, wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, a predetermined level is at least (0.90)g, wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, a predetermined level is at least (0.95)g, wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, a predetermined level is at least (0.96)g, wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, a predetermined level is at least (0.97)g, wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, a predetermined level is at least (0.98)g, wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, a predetermined

level is at least (0.99)g, wherein g is the number of chirally controlled internucleotidic linkages. In certain embodiments, to determine level of oligonucleotides having g chirally controlled internucleotidic linkages in a composition, product of diastereopurity of each of the g chirally controlled internucleotidic linkages: (diastereopurity of chirally controlled internucleotidic linkage 1) * (diastereopurity of chirally controlled internucleotidic linkage 2) * … * (diastereopurity of chirally controlled internucleotidic linkage g) is utilized as the level, wherein diastereopurity of each chirally controlled internucleotidic linkage is independently represented by diastereopurity of a dimer comprising the same internucleotidic linkage and nucleosides flanking the internucleotidic linkage and prepared under comparable methods as the oligonucleotides (e.g., comparable or preferably identical oligonucleotide preparation cycles, including comparable or preferably identical reagents and reaction conditions).In certain embodiments, levels of oligonucleotides and/or diastereopurity can be determined by analytical methods, e.g., chromatographic, spectrometric, spectroscopic methods or any combinations thereof. 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 (or stereochemistry) 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., sensitivity to nucleases, activities, distribution, etc. In certain 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. In certain embodiments, the present disclosure demonstrates that improvements in properties and activities achieved through control of stereochemistry within an oligonucleotide can be comparable to, or even better than those achieved through use of chemical modification.

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 (or stereochemistry) 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.*, sensitivity to nucleases, activities, distribution, etc. In certain 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. In certain embodiments, the present disclosure demonstrates that improvements in properties and activities achieved through control of stereochemistry within an oligonucleotide can be comparable to, or even better than those achieved through use of chemical modification.

I. DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

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

Definitions

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

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

Unless otherwise specified, description of oligonucleotides and elements thereof (e.g., base sequence, sugar modifications, internucleotidic linkages, linkage

phosphorus stereochemistry, patterns thereof, etc.) is from 5' to 3', with the 5' terminal nucleotide identified as the "+1" position and the 3' terminal nucleotide identified either by the number of nucleotides of the full sequence or by "N", with the penultimate nucleotide identified, e.g., as "N-1", and so on. As those skilled in the art will appreciate, in certain embodiments, oligonucleotides may be provided and/or utilized as salt forms, particularly pharmaceutically acceptable salt forms, e.g., sodium salts. As those skilled in the art will also appreciate, in certain embodiments, individual oligonucleotides within a composition may be considered to be of the same constitution and/or structure even though, within such composition (e.g., a liquid composition), particular such oligonucleotides might be in different salt form(s) (and may be dissolved and the oligonucleotide chain may exist as an anion form when, e.g., in a liquid composition) at a particular moment in time. For example, those skilled in the art will appreciate that, at a given pH, individual internucleotidic linkages along an oligonucleotide chain may be in an acid (H) form, or in one of a plurality of possible salt forms (e.g., a sodium salt, or a salt of a different cation, depending on which ions might be present in the preparation or composition), and will understand that, so long as their acid forms (e.g., replacing all cations, if any, with H^+) are of the same constitution and/or structure, such individual oligonucleotides may properly be considered to be of the same constitution and/or structure.

Aliphatic: As used herein, "aliphatic" means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation (but not aromatic), or a substituted or unsubstituted monocyclic, bicyclic, or polycyclic hydrocarbon ring that is completely saturated or that contains one or more units of unsaturation (but not aromatic), or combinations thereof. In certain embodiments, aliphatic groups contain 1-50 aliphatic carbon atoms. In certain embodiments, aliphatic groups contain 1-20 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-10 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-9 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-8 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-7 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-6 aliphatic carbon atoms. In still other embodiments, aliphatic groups contain 1-5 aliphatic carbon atoms, and in yet other embodiments, aliphatic groups contain 1, 2, 3, or 4 aliphatic carbon atoms. Suitable aliphatic groups include, but are not limited to, linear or branched, substituted or unsubstituted alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

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

Alkyl: As used herein, the term "alkyl" is given its ordinary meaning in the art and may include saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In certain embodiments, alkyl has 1-100 carbon atoms. In certain embodiments, a straight chain or branched chain alkyl has about 1-20 carbon atoms in its backbone (e.g., C_1-C_{20} for straight chain, C_2-C_{20} for branched chain), and alternatively, about 1-10. In certain embodiments, cycloalkyl rings have from about 3-10 carbon atoms in their ring structure where such rings are monocyclic, bicyclic, or polycyclic, and alternatively about 5, 6 or 7 carbons in the ring structure. In certain embodiments, an alkyl group may be a lower alkyl group, wherein a lower alkyl group comprises 1-4 carbon atoms (e.g., C_1-C_4 for straight chain lower alkyls).

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

Analog: The term "analog" includes any chemical moiety which differs structurally from a reference chemical moiety or class of moieties, but which is capable of performing at least one function of such a reference chemical moiety or class of moieties. As non-limiting examples, a nucleotide analog differs structurally from a nucleotide but performs at least one function of a nucleotide; a nucleobase analog differs structurally from a nucleobase but performs at least one function of a nucleobase; etc.

Animal: As used herein, the term "animal" refers to any member of the animal kingdom. In certain embodiments, "animal" refers to humans, at any stage of development. In certain 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 certain embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish and/or worms. In certain embodiments, an animal may be a transgenic animal, a genetically-engineered animal and/or a clone.

Aryl: The term "aryl", as used herein, used alone or as part of a larger moiety as in "aralkyl," "aralkoxy," or "aryloxyalkyl," refers to monocyclic, bicyclic or polycyclic ring systems having a total of five to thirty ring members, wherein at least one ring in the system is aromatic. In certain embodiments, an aryl group is a monocyclic, bicyclic or polycyclic ring system having a total of five to fourteen ring members, wherein at least one

ring in the system is aromatic, and wherein each ring in the system contains 3 to 7 ring members. In certain embodiments, each monocyclic ring unit is aromatic. In certain embodiments, an aryl group is a biaryl group. The term "aryl" may be used interchangeably with the term "aryl ring." In certain embodiments of the present disclosure, "aryl" refers to an aromatic ring system which includes, but is not limited to, phenyl, biphenyl, naphthyl, binaphthyl, anthracyl and the like, which may bear one or more substituents. Also included within the scope of the term "aryl," as it is used herein, is a group in which an aromatic ring is fused to one or more non–aromatic rings, such as indanyl, phthalimidyl, naphthimidyl, phenanthridinyl, or tetrahydronaphthyl, and the like.

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

Chirally controlled oligonucleotide composition: The terms "chirally controlled oligonucleotide composition", "chirally controlled nucleic acid composition", and the like, as used herein, refers to a composition that comprises a plurality of oligonucleotides (or nucleic acids) which share a common base sequence, wherein the plurality of oligonucleotides (or nucleic acids) share the same linkage phosphorus stereochemistry at one or more chiral internucleotidic linkages (chirally controlled or stereodefined internucleotidic linkages, whose chiral linkage phosphorus is Rp or Sp in the composition ("stereodefined"), not a random Rp and Sp mixture as non-chirally controlled internucleotidic linkages). In certain embodiments, a chirally controlled oligonucleotide composition comprises a plurality of oligonucleotides (or nucleic acids) that share: 1) a common base sequence, 2) a common pattern of backbone linkages, and 3) a common

pattern of backbone phosphorus modifications, wherein the plurality of oligonucleotides (or nucleic acids) share the same linkage phosphorus stereochemistry at one or more chiral internucleotidic linkages (chirally controlled or stereodefined internucleotidic linkages, whose chiral linkage phosphorus is Rp or Sp in the composition ("stereodefined"), not a random Rp and Sp mixture as non-chirally controlled internucleotidic linkages). Level of the plurality of oligonucleotides (or nucleic acids) in a chirally controlled oligonucleotide composition is pre-determined/controlled or enriched (e.g., through chirally controlled oligonucleotide preparation to stereoselectively form one or more chiral internucleotidic linkages) compared to a random level in a non-chirally controlled oligonucleotide composition. In certain embodiments, about 1%-100%, (e.g., about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80-100%, 90-100%, 95-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all oligonucleotides in a chirally controlled oligonucleotide composition are oligonucleotides of the plurality. In certain embodiments, about 1%-100%, (e.g., about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80-100%, 90-100%, 95-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all oligonucleotides in a chirally controlled oligonucleotide composition that share the common base sequence, the common pattern of backbone linkages, and the common pattern of backbone phosphorus modifications are oligonucleotides of the plurality. In certain embodiments, a level is about 1%-100%, (e.g., about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80-100%, 90-100%, 95-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all oligonucleotides in a composition, or of all oligonucleotides in a composition that share a common base sequence (e.g., of a plurality of oligonucleotide or an oligonucleotide type), or of all oligonucleotides in a composition that share a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone phosphorus modifications, or of all oligonucleotides in a composition that share a common base sequence, a common patter

of base modifications, a common pattern of sugar modifications, a common pattern of internucleotidic linkage types, and/or a common pattern of internucleotidic linkage modifications. In certain embodiments, the plurality of oligonucleotides share the same stereochemistry at about 1-50 (e.g., about 1-10, 1-20, 5-10, 5-20, 10-15, 10-20, 10-25, 10-30, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) chiral internucleotidic linkages. In certain embodiments, the plurality of oligonucleotides share the same stereochemistry at about 1%-100% (e.g., about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80-100%, 90-100%, 95-100%, 50%-90%, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%, or at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%) of chiral internucleotidic linkages. In certain embodiments, oligonucleotides (or nucleic acids) of a plurality share the same pattern of sugar and/or nucleobase modifications, in any. In certain embodiments, oligonucleotides (or nucleic acids) of a plurality are various forms of the same oligonucleotide (e.g., acid and/or various salts of the same oligonucleotide). In certain embodiments, oligonucleotides (or nucleic acids) of a plurality are of the same constitution. In certain embodiments, level of the oligonucleotides (or nucleic acids) of the plurality is about 1%-100%, (e.g., about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80-100%, 90-100%, 95-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all oligonucleotides (or nucleic acids) in a composition that share the same constitution as the oligonucleotides (or nucleic acids) of the plurality. In certain embodiments, each chiral internucleotidic linkage is a chiral controlled internucleotidic linkage, and the composition is a completely chirally controlled oligonucleotide composition. In certain embodiments, oligonucleotides (or nucleic acids) of a plurality are structurally identical. In certain embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%, typically at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%. In certain embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 95%. In certain embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 96%. In certain embodiments, a chirally controlled

internucleotidic linkage has a diastereopurity of at least 97%. In certain embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 98%. In certain embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 99%. In certain embodiments, a percentage of a level is or is at least $(DS)^{nc}$, wherein DS is a diastereopurity as described in the present disclosure (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% or more) and nc is the number of chirally controlled internucleotidic linkages as described in the present disclosure (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more). In certain embodiments, a percentage of a level is or is at least $(DS)^{nc}$, wherein DS is 95%-100%. For example, when DS is 99% and nc is 10, the percentage is or is at least 90% ($(99\%)^{10} \approx 0.90 = 90\%$). In certain embodiments, level of a plurality of oligonucleotides in a composition is represented as the product of the diastereopurity of each chirally controlled internucleotidic linkage in the oligonucleotides. In certain embodiments, diastereopurity of an internucleotidic linkage connecting two nucleosides in an oligonucleotide (or nucleic acid) is represented by the diastereopurity of an internucleotidic linkage of a dimer connecting the same two nucleosides, wherein the dimer is prepared using comparable conditions, in some instances, identical synthetic cycle conditions (e.g., for the linkage between Nx and Ny in an oligonucleotide ….NxNy….., the dimer is NxNy). In certain embodiments, not all chiral internucleotidic linkages are chiral controlled internucleotidic linkages, and the composition is a partially chirally controlled oligonucleotide composition. In certain embodiments, a non-chirally controlled internucleotidic linkage has a diastereopurity of less than about 80%, 75%, 70%, 65%, 60%, 55%, or of about 50%, as typically observed in stereorandom oligonucleotide compositions (e.g., as appreciated by those skilled in the art, from traditional oligonucleotide synthesis, e.g., the phosphoramidite method). In certain embodiments, oligonucleotides (or nucleic acids) of a plurality are of the same type. In certain embodiments, a chirally controlled oligonucleotide composition comprises non-random or controlled levels of individual oligonucleotide or nucleic acids types. For instance, in certain embodiments a chirally controlled oligonucleotide composition comprises one and no more than one oligonucleotide type. In certain embodiments, a chirally controlled oligonucleotide composition comprises more than one oligonucleotide type. In certain embodiments, a chirally controlled oligonucleotide composition comprises multiple oligonucleotide types. In certain embodiments, a chirally controlled oligonucleotide composition is a composition of oligonucleotides of an oligonucleotide type, which composition comprises a non-random

or controlled level of a plurality of oligonucleotides of the oligonucleotide type.

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 certain 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.

Cycloaliphatic: The term "cycloaliphatic," "carbocycle," "carbocyclyl," "carbocyclic radical," and "carbocyclic ring," are used interchangeably, and as used herein, refer to saturated or partially unsaturated, but non-aromatic, cyclic aliphatic monocyclic, bicyclic, or polycyclic ring systems, as described herein, having, unless otherwise specified, from 3 to 30 ring members. Cycloaliphatic groups include, without limitation, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, cycloheptenyl, cyclooctyl, cyclooctenyl, norbornyl, adamantyl, and cyclooctadienyl. In certain embodiments, a cycloaliphatic group has 3–6 carbons. In certain embodiments, a cycloaliphatic group is saturated and is cycloalkyl. The term "cycloaliphatic" may also include aliphatic rings that are fused to one or more aromatic or nonaromatic rings, such as decahydronaphthyl or tetrahydronaphthyl. In certain embodiments, a cycloaliphatic group is bicyclic. In certain embodiments, a cycloaliphatic group is tricyclic. In certain embodiments, a cycloaliphatic group is polycyclic. In certain embodiments, "cycloaliphatic" refers to C_3-C_6 monocyclic hydrocarbon, or C_8-C_{10} bicyclic or polycyclic hydrocarbon, that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule, or a C_9-C_{16} polycyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule.

Heteroaliphatic: The term "heteroaliphatic", as used herein, is given its ordinary meaning in the art and refers to aliphatic groups as described herein in which one or more carbon atoms are independently replaced with one or more heteroatoms (e.g., oxygen, nitrogen, sulfur, silicon, phosphorus, and the like). In certain embodiments, one or

more units selected from C, CH, CH_2, and CH_3 are independently replaced by one or more heteroatoms (including oxidized and/or substituted forms thereof). In certain embodiments, a heteroaliphatic group is heteroalkyl. In certain embodiments, a heteroaliphatic group is heteroalkenyl.

Heteroalkyl: The term "heteroalkyl", as used herein, is given its ordinary meaning in the art and refers to alkyl groups as described herein in which one or more carbon atoms are independently replaced with one or more heteroatoms (e.g., oxygen, nitrogen, sulfur, silicon, phosphorus, and the like). Examples of heteroalkyl groups include, but are not limited to, alkoxy, poly(ethylene glycol)-, alkyl-substituted amino, tetrahydrofuranyl, piperidinyl, morpholinyl, etc.

Heteroaryl: The terms "heteroaryl" and "heteroar–", as used herein, used alone or as part of a larger moiety, e.g., "heteroaralkyl," or "heteroaralkoxy," refer to monocyclic, bicyclic or polycyclic ring systems having a total of five to thirty ring members, wherein at least one ring in the system is aromatic and at least one aromatic ring atom is a heteroatom. In certain embodiments, a heteroaryl group is a group having 5 to 10 ring atoms (i.e., monocyclic, bicyclic or polycyclic), in certain embodiments 5, 6, 9, or 10 ring atoms. In certain embodiments, each monocyclic ring unit is aromatic. In certain embodiments, a heteroaryl group has 6, 10, or 14 π electrons shared in a cyclic array; and having, in addition to carbon atoms, from one to five heteroatoms. Heteroaryl groups include, without limitation, thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, indolizinyl, purinyl, naphthyridinyl, and pteridinyl. In certain embodiments, a heteroaryl is a heterobiaryl group, such as bipyridyl and the like. The terms "heteroaryl" and "heteroar–", as used herein, also include groups in which a heteroaromatic ring is fused to one or more aryl, cycloaliphatic, or heterocyclyl rings, where the radical or point of attachment is on the heteroaromatic ring. Non-limiting examples include indolyl, isoindolyl, benzothienyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4H–quinolizinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, and pyrido[2,3–b]–1,4–oxazin–3(4H)–one. A heteroaryl group may be monocyclic, bicyclic or polycyclic. The term "heteroaryl" may be used interchangeably with the terms "heteroaryl ring," "heteroaryl group," or "heteroaromatic," any of which terms include rings that are optionally substituted. The term "heteroaralkyl" refers to an alkyl group substituted by a heteroaryl group, wherein the alkyl

and heteroaryl portions independently are optionally substituted.

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

Heterocycle: As used herein, the terms “heterocycle,” “heterocyclyl,” “heterocyclic radical,” and “heterocyclic ring", as used herein, are used interchangeably and refer to a monocyclic, bicyclic or polycyclic ring moiety (e.g., 3-30 membered) that is saturated or partially unsaturated and has one or more heteroatom ring atoms. In certain embodiments, a heterocyclyl group is a stable 5– to 7–membered monocyclic or 7– to 10–membered bicyclic heterocyclic moiety that is either saturated or partially unsaturated, and having, in addition to carbon atoms, one or more, preferably one to four, heteroatoms, as defined above. When used in reference to a ring atom of a heterocycle, the term "nitrogen" includes substituted nitrogen. As an example, in a saturated or partially unsaturated ring having 0–3 heteroatoms selected from oxygen, sulfur and nitrogen, the nitrogen may be N (as in 3,4–dihydro–2H–pyrrolyl), NH (as in pyrrolidinyl), or $^{+}$NR (as in N–substituted pyrrolidinyl). A heterocyclic ring can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted. Examples of such saturated or partially unsaturated heterocyclic radicals include, without limitation, tetrahydrofuranyl, tetrahydrothienyl, pyrrolidinyl, piperidinyl, pyrrolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, dioxanyl, dioxolanyl, diazepinyl, oxazepinyl, thiazepinyl, morpholinyl, and quinuclidinyl. The terms “heterocycle,” “heterocyclyl,” “heterocyclyl ring,” “heterocyclic group,” “heterocyclic moiety,” and “heterocyclic radical,” are used interchangeably herein, and also include groups in which a heterocyclyl ring is fused to one or more aryl, heteroaryl, or cycloaliphatic rings, such as indolinyl, 3H–indolyl, chromanyl, phenanthridinyl, or tetrahydroquinolinyl. A heterocyclyl group may be monocyclic, bicyclic or polycyclic. The term “heterocyclylalkyl” refers to an alkyl group substituted by a heterocyclyl, wherein the alkyl and heterocyclyl portions independently are optionally substituted.

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

Internucleotidic linkage: As used herein, the phrase "internucleotidic linkage" refers generally to a linkage linking nucleoside units of an oligonucleotide or a nucleic acid. In certain embodiments, an internucleotidic linkage is a phosphodiester linkage, as extensively found in naturally occurring DNA and RNA molecules (natural phosphate linkage (−OP(=O)(OH)O−), which as appreciated by those skilled in the art may exist as a salt form). In certain embodiments, an internucleotidic linkage is a modified internucleotidic linkage (not a natural phosphate linkage). In certain embodiments, an

internucleotidic linkage is a "modified internucleotidic linkage" wherein at least one oxygen atom or −OH of a phosphodiester linkage is replaced by a different organic or inorganic moiety. In certain embodiments, such an organic or inorganic moiety is selected from =S, =Se, =NR', –SR', –SeR', $–N(R')_2$, $B(R')_3$, –S–, –Se–, and –N(R')–, wherein each R' is independently as defined and described in the present disclosure. In certain embodiments, an internucleotidic linkage is a phosphotriester linkage, phosphorothioate linkage (or phosphorothioate diester linkage, −OP(=O)(SH)O−, which as appreciated by those skilled in the art may exist as a salt form), or phosphorothioate triester linkage. In certain embodiments, a modified internucleotidic linkage is a phosphorothioate linkage. In certain embodiments, an internucleotidic linkage is one of, e.g., PNA (peptide nucleic acid) or PMO (phosphorodiamidate Morpholino oligomer) linkage. In certain embodiments, a modified internucleotidic linkage is a non-negatively charged internucleotidic linkage. In certain embodiments, a modified internucleotidic linkage is a neutral internucleotidic linkage (e.g., n001 in certain provided oligonucleotides). It is understood by a person of ordinary skill in the art that an internucleotidic linkage may exist as an anion or cation at a given pH due to the existence of acid or base moieties in the linkage. In certain embodiments, a modified internucleotidic linkages is a modified internucleotidic linkages designated as s, s1, s2, s3, s4, s5, s6, s7, s8, s9, s10, s11, s12, s13, s14, s15, s16, s17 and s18 as described in WO 2017/210647.

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).

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

Linkage phosphorus: as defined herein, the phrase "linkage phosphorus" is used to indicate that the particular phosphorus atom being referred to is the phosphorus atom present in the internucleotidic linkage, which phosphorus atom corresponds to the phosphorus atom of a phosphodiester internucleotidic linkage as occurs in naturally occurring DNA and RNA. In certain 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 certain embodiments, a linkage phosphorus atom is chiral (e.g., as in phosphorothioate internucleotidic linkages). In certain embodiments, a linkage phosphorus atom is achiral (e.g., as in natural phosphate linkages).

Modified nucleobase: The terms "modified nucleobase", "modified base" and the like refer to a chemical moiety which is chemically distinct from a nucleobase, but which is capable of performing at least one function of a nucleobase. In certain embodiments, a modified nucleobase is a nucleobase which comprises a modification. In certain embodiments, a modified nucleobase is capable of at least one function of a nucleobase, e.g., forming a moiety in a polymer capable of base-pairing to a nucleic acid comprising an at least complementary sequence of bases. In certain embodiments, a modified nucleobase is substituted A, T, C, G, or U, or a substituted tautomer of A, T, C, G, or U. In certain embodiments, a modified nucleobase in the context of oligonucleotides refer to a nucleobase that is not A, T, C, G or U.

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

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

Modified sugar: The term "modified sugar" refers to a moiety that can replace a sugar. A modified sugar mimics the spatial arrangement, electronic properties, or some other physicochemical property of a sugar. In certain embodiments, as described in the present disclosure, a modified sugar is substituted ribose or deoxyribose. In certain embodiments, a modified sugar comprises a 2'-modification. Examples of useful 2'-modification are widely utilized in the art and described herein. In certain embodiments, a 2'-modification is 2'-F. In certain embodiments, a 2'-modification is 2'-OR, wherein R is

optionally substituted C_{1-10} aliphatic. In certain embodiments, a 2'-modification is 2'-OMe. In certain embodiments, a 2'-modification is 2'-MOE. In certain embodiments, a modified sugar is a bicyclic sugar (e.g., a sugar used in LNA, BNA, etc.). In certain embodiments, in the context of oligonucleotides, a modified sugar is a sugar that is not ribose or deoxyribose as typically found in natural RNA or DNA.

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

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 certain embodiments, a naturally-occurring nucleobases are modified adenine, guanine, uracil, cytosine, or thymine. In certain embodiments, a naturally-occurring nucleobases are methylated adenine, guanine, uracil, cytosine, or thymine. In certain embodiments, a nucleobase comprises a heteroaryl ring wherein a ring atom is nitrogen, and when in a nucleoside, the nitrogen is bonded to a sugar moiety. In certain embodiments, a

nucleobase comprises a heterocyclic ring wherein a ring atom is nitrogen, and when in a nucleoside, the nitrogen is bonded to a sugar moiety. In certain embodiments, a nucleobase is a "modified nucleobase," a nucleobase other than adenine (A), guanine (G), uracil (U), cytosine (C), and thymine (T). In certain embodiments, a modified nucleobase is substituted A, T, C, G or U. In certain embodiments, a modified nucleobase is a substituted tautomer of A, T, C, G, or U. In certain embodiments, a modified nucleobase is methylated adenine, guanine, uracil, cytosine, or thymine. In certain embodiments, a modified nucleobase mimics the spatial arrangement, electronic properties, or some other physicochemical property of the nucleobase and retains the property of hydrogen-bonding that binds one nucleic acid strand to another in a sequence specific manner. In certain embodiments, a modified nucleobase can pair with all of the five naturally occurring bases (uracil, thymine, adenine, cytosine, or guanine) without substantially affecting the melting behavior, recognition by intracellular enzymes or activity of the oligonucleotide duplex. As used herein, the term "nucleobase" also encompasses structural analogs used in lieu of natural or naturally-occurring nucleotides, such as modified nucleobases and nucleobase analogs. In certain embodiments, a nucleobase is optionally substituted A, T, C, G, or U, or an optionally substituted tautomer of A, T, C, G, or U. In certain embodiments, a "nucleobase" refers to a nucleobase unit in an oligonucleotide or a nucleic acid (e.g., A, T, C, G or U as in an oligonucleotide or a nucleic acid).

Nucleoside: The term "nucleoside" refers to a moiety wherein a nucleobase or a modified nucleobase is covalently bound to a sugar or a modified sugar. In certain embodiments, a nucleoside is a natural nucleoside, e.g., adenosine, deoxyadenosine, guanosine, deoxyguanosine, thymidine, uridine, cytidine, or deoxycytidine. In certain embodiments, a nucleoside is a modified nucleoside, e.g., a substituted natural nucleoside selected from adenosine, deoxyadenosine, guanosine, deoxyguanosine, thymidine, uridine, cytidine, and deoxycytidine. In certain embodiments, a nucleoside is a modified nucleoside, e.g., a substituted tautomer of a natural nucleoside selected from adenosine, deoxyadenosine, guanosine, deoxyguanosine, thymidine, uridine, cytidine, and deoxycytidine. In certain embodiments, a "nucleoside" refers to a nucleoside unit in an oligonucleotide or a nucleic acid.

Nucleotide: The term "nucleotide" as used herein refers to a monomeric unit of a polynucleotide that consists of a nucleobase, a sugar, and one or more internucleotidic linkages (e.g., phosphate linkages in natural DNA and RNA). The naturally occurring bases [guanine, (G), adenine, (A), cytosine, (C), thymine, (T), and uracil (U)] are derivatives of

purine or pyrimidine, though it should be understood that naturally and non-naturally occurring base analogs are also included. The naturally occurring sugar is the pentose (five-carbon sugar) deoxyribose (which forms DNA) or ribose (which forms RNA), though it should be understood that naturally and non-naturally occurring sugar analogs are also included. Nucleotides are linked via internucleotidic linkages to form nucleic acids, or polynucleotides. Many internucleotidic linkages are known in the art (such as, though not limited to, phosphate, phosphorothioates, boranophosphates and the like). Artificial nucleic acids include PNAs (peptide nucleic acids), phosphotriesters, phosphorothionates, H-phosphonates, phosphoramidates, boranophosphates, methylphosphonates, phosphonoacetates, thiophosphonoacetates and other variants of the phosphate backbone of native nucleic acids, such as those described herein. In certain embodiments, a natural nucleotide comprises a naturally occurring base, sugar and internucleotidic linkage. As used herein, the term “nucleotide” also encompasses structural analogs used in lieu of natural or naturally-occurring nucleotides, such as modified nucleotides and nucleotide analogs. In certain embodiments, a “nucleotide” refers to a nucleotide unit in an oligonucleotide or a nucleic acid.

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

Oligonucleotides can be single-stranded or double-stranded. A single-stranded oligonucleotide can have double-stranded regions (formed by two portions of the single-stranded oligonucleotide) and a double-stranded oligonucleotide, which comprises two oligonucleotide chains, can have single-stranded regions for example, at regions where the two oligonucleotide chains are not complementary to each other. Example oligonucleotides include, but are not limited to structural genes, genes including control and termination regions, self-replicating systems such as viral or plasmid DNA, single-stranded and double-stranded RNAi agents and other RNA interference reagents (RNAi agents or iRNA agents), shRNA, antisense oligonucleotides, ribozymes, microRNAs, microRNA mimics, supermirs, aptamers, antimirs, antagomirs, Ul adaptors, triplex-forming oligonucleotides, G-quadruplex oligonucleotides, RNA activators, immuno-stimulatory oligonucleotides, and decoy oligonucleotides.

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

stranded, or triple-stranded, can range in length from about 4 to about 10 nucleosides, from about 10 to about 50 nucleosides, from about 20 to about 50 nucleosides, from about 15 to about 30 nucleosides, from about 20 to about 30 nucleosides in length. In certain embodiments, the oligonucleotide is from about 9 to about 39 nucleosides in length. In certain embodiments, the oligonucleotide is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleosides in length. In certain embodiments, the oligonucleotide is at least 4 nucleosides in length. In certain embodiments, the oligonucleotide is at least 5 nucleosides in length. In certain embodiments, the oligonucleotide is at least 6 nucleosides in length. In certain embodiments, the oligonucleotide is at least 7 nucleosides in length. In certain embodiments, the oligonucleotide is at least 8 nucleosides in length. In certain embodiments, the oligonucleotide is at least 9 nucleosides in length. In certain embodiments, the oligonucleotide is at least 10 nucleosides in length. In certain embodiments, the oligonucleotide is at least 11 nucleosides in length. In certain embodiments, the oligonucleotide is at least 12 nucleosides in length. In certain embodiments, the oligonucleotide is at least 15 nucleosides in length. In certain embodiments, the oligonucleotide is at least 15 nucleosides in length. In certain embodiments, the oligonucleotide is at least 16 nucleosides in length. In certain embodiments, the oligonucleotide is at least 17 nucleosides in length. In certain embodiments, the oligonucleotide is at least 18 nucleosides in length. In certain embodiments, the oligonucleotide is at least 19 nucleosides in length. In certain embodiments, the oligonucleotide is at least 20 nucleosides in length. In certain embodiments, the oligonucleotide is at least 25 nucleosides in length. In certain embodiments, the oligonucleotide is at least 30 nucleosides in length. In certain embodiments, each nucleoside counted in an oligonucleotide length independently comprises a nucleobase comprising a ring having at least one nitrogen ring atom. In certain embodiments, each nucleoside counted in an oligonucleotide length independently comprises A, T, C, G, or U, or optionally substituted A, T, C, G, or U, or an optionally substituted tautomer of A, T, C, G or U.

Oligonucleotide type: As used herein, the phrase “oligonucleotide type” is used to define an oligonucleotide that has a particular base sequence, pattern of backbone linkages (i.e., pattern of internucleotidic linkage types, for example, phosphate, phosphorothioate, phosphorothioate triester, etc.), pattern of backbone chiral centers (i.e., pattern of linkage phosphorus stereochemistry (Rp/Sp)), and pattern of backbone phosphorus modifications. In certain embodiments, oligonucleotides of a common

designated "type" are structurally identical to one another.

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 certain embodiments, an oligonucleotide strand is designed and/or selected in advance to have a particular combination of stereocenters at the linkage phosphorus. In certain embodiments, an oligonucleotide strand is designed and/or determined to have a particular combination of modifications at the linkage phosphorus. In certain embodiments, an oligonucleotide strand is designed and/or selected to have a particular combination of bases. In certain embodiments, an oligonucleotide strand is designed and/or selected to have a particular combination of one or more of the above structural characteristics. In certain embodiments, the present disclosure provides compositions comprising or consisting of a plurality of oligonucleotide molecules (e.g., chirally controlled oligonucleotide compositions). In certain embodiments, all such molecules are of the same type (i.e., are structurally identical to one another). In certain embodiments, however, provided compositions comprise a plurality of oligonucleotides of different types, typically in pre-determined relative amounts.

Optionally Substituted: As described herein, compounds, e.g., oligonucleotides, of the disclosure may contain optionally substituted and/or 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. In certain embodiments, an optionally substituted group is unsubstituted. 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. Certain substituents are described below.

Suitable monovalent substituents on a substitutable atom, e.g., a suitable

carbon atom, are independently halogen; –$(CH_2)_{0–4}R°$; –$(CH_2)_{0–4}OR°$; –$O(CH_2)_{0-4}R°$, –O–$(CH_2)_{0–4}C(O)OR°$; –$(CH_2)_{0–4}CH(OR°)_2$; –$(CH_2)_{0–4}Ph$, which may be substituted with R°; –$(CH_2)_{0–4}O(CH_2)_{0–1}Ph$ which may be substituted with R°; –CH=CHPh, which may be substituted with R°; –$(CH_2)_{0–4}O(CH_2)_{0–1}$-pyridyl which may be substituted with R°; –NO_2; –CN; –N_3; -$(CH_2)_{0–4}N(R°)_2$; –$(CH_2)_{0–4}N(R°)C(O)R°$; –$N(R°)C(S)R°$; –$(CH_2)_{0–4}N(R°)C(O)NR°_2$; –$N(R°)C(S)NR°_2$; –$(CH_2)_{0–4}N(R°)C(O)OR°$; –$N(R°)N(R°)C(O)R°$; –$N(R°)N(R°)C(O)NR°_2$; –$N(R°)N(R°)C(O)OR°$; –$(CH_2)_{0–4}C(O)R°$; –$C(S)R°$; –$(CH_2)_{0–4}C(O)OR°$; –$(CH_2)_{0–4}C(O)SR°$; -$(CH_2)_{0–4}C(O)OSiR°_3$; –$(CH_2)_{0–4}OC(O)R°$; –$OC(O)(CH_2)_{0–4}SR°$, –$SC(S)SR°$; –$(CH_2)_{0–4}SC(O)R°$; –$(CH_2)_{0–4}C(O)NR°_2$; –$C(S)NR°_2$; –$C(S)SR°$; -$(CH_2)_{0–4}OC(O)NR°_2$; -$C(O)N(OR°)R°$; –$C(O)C(O)R°$; –$C(O)CH_2C(O)R°$; –$C(NOR°)R°$; -$(CH_2)_{0–4}SSR°$; –$(CH_2)_{0–4}S(O)_2R°$; –$(CH_2)_{0–4}S(O)_2OR°$; –$(CH_2)_{0–4}OS(O)_2R°$; –$S(O)_2NR°_2$; -$(CH_2)_{0–4}S(O)R°$; –$N(R°)S(O)_2NR°_2$; –$N(R°)S(O)_2R°$; –$N(OR°)R°$; –$C(NH)NR°_2$; –$Si(R°)_3$; –$OSi(R°)_3$; –$B(R°)_2$; –$OB(R°)_2$; –$OB(OR°)_2$; –$P(R°)_2$; –$P(OR°)_2$; –$P(R°)(OR°)$; –$OP(R°)_2$; –$OP(OR°)_2$; –$OP(R°)(OR°)$; –$P(O)(R°)_2$; –$P(O)(OR°)_2$; –$OP(O)(R°)_2$; –$OP(O)(OR°)_2$; –$OP(O)(OR°)(SR°)$; –$SP(O)(R°)_2$; –$SP(O)(OR°)_2$; –$N(R°)P(O)(R°)_2$; –$N(R°)P(O)(OR°)_2$; –$P(R°)_2[B(R°)_3]$; –$P(OR°)_2[B(R°)_3]$; –$OP(R°)_2[B(R°)_3]$; –$OP(OR°)_2[B(R°)_3]$; –($C_{1–4}$ straight or branched alkylene)O–$N(R°)_2$; or –($C_{1–4}$ straight or branched alkylene)C(O)O–$N(R°)_2$, wherein each R° may be substituted as defined herein and is independently hydrogen, $C_{1–20}$ aliphatic, $C_{1–20}$ heteroaliphatic having 1–5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, –CH_2–(C_{6-14} aryl), –$O(CH_2)_{0–1}$(C_{6-14} aryl), –CH_2-(5-14 membered heteroaryl ring), a 5–20 membered, monocyclic, bicyclic, or polycyclic, saturated, partially unsaturated or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 5–20 membered, monocyclic, bicyclic, or polycyclic, saturated, partially unsaturated or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, which may be substituted as defined below.

Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, –$(CH_2)_{0–2}R^•$, –(halo$R^•$), –$(CH_2)_{0–2}OH$, –$(CH_2)_{0–2}OR^•$, –$(CH_2)_{0–2}CH(OR^•)_2$; –O(halo$R^•$), –CN, –N_3, –$(CH_2)_{0–2}C(O)R^•$, –$(CH_2)_{0–2}C(O)OH$, –$(CH_2)_{0–2}C(O)OR^•$, –

$(CH_2)_{0-2}SR^{\bullet}$, $-(CH_2)_{0-2}SH$, $-(CH_2)_{0-2}NH_2$, $-(CH_2)_{0-2}NHR^{\bullet}$, $-(CH_2)_{0-2}NR^{\bullet}_2$, $-NO_2$, $-SiR^{\bullet}_3$, $-OSiR^{\bullet}_3$, -C(O)$SR^{\bullet}$, $-(C_{1-4}$ straight or branched alkylene)C(O)$OR^{\bullet}$, or $-SSR^{\bullet}$ wherein each $R^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, and a 5–6–membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, and sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

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

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

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

unsaturated, or aryl mono– or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, and sulfur.

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

P-modification: as used herein, the term "P-modification" refers to any modification at the linkage phosphorus other than a stereochemical modification. In certain embodiments, a P-modification comprises addition, substitution, or removal of a pendant moiety covalently attached to a linkage phosphorus.

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.

Pharmaceutical composition: As used herein, the term "pharmaceutical composition" refers to an active agent, formulated together with one or more pharmaceutically acceptable carriers. In certain embodiments, an active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In certain 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.

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.

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.

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 certain 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 certain embodiments, pharmaceutically acceptable salts include,

but are not limited to, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. In certain embodiments, a provided compound comprises one or more acidic groups, e.g., an oligonucleotide, and a pharmaceutically acceptable salt is an alkali, alkaline earth metal, or ammonium (e.g., an ammonium salt of $N(R)_3$, wherein each R is independently defined and described in the present disclosure) salt. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. In certain embodiments, a pharmaceutically acceptable salt is a sodium salt. In certain embodiments, a pharmaceutically acceptable salt is a potassium salt. In certain embodiments, a pharmaceutically acceptable salt is a calcium salt. In certain embodiments, pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl having from 1 to 6 carbon atoms, sulfonate and aryl sulfonate. In certain embodiments, a provided compound comprises more than one acid groups, for example, an oligonucleotide may comprise two or more acidic groups (e.g., in natural phosphate linkages and/or modified internucleotidic linkages). In certain embodiments, a pharmaceutically acceptable salt, or generally a salt, of such a compound comprises two or more cations, which can be the same or different. In certain embodiments, in a pharmaceutically acceptable salt (or generally, a salt), all ionizable hydrogen (e.g., in an aqueous solution with a pKa no more than about 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2; in certain embodiments, no more than about 7; in certain embodiments, no more than about 6; in certain embodiments, no more than about 5; in certain embodiments, no more than about 4; in certain embodiments, no more than about 3) in the acidic groups are replaced with cations. In certain embodiments, each phosphorothioate and phosphate group independently exists in its salt form (e.g., if sodium salt, −O−P(O)(SNa)−O− and −O−P(O)(ONa)−O−, respectively). In certain embodiments, each phosphorothioate and phosphate internucleotidic linkage independently exists in its salt form (e.g., if sodium salt, −O−P(O)(SNa)−O− and −O−P(O)(ONa)−O−, respectively). In certain embodiments, a

pharmaceutically acceptable salt is a sodium salt of an oligonucleotide. In certain embodiments, a pharmaceutically acceptable salt is a sodium salt of an oligonucleotide, wherein each acidic phosphate and modified phosphate group (e.g., phosphorothioate, phosphate, etc.), if any, exists as a salt form (all sodium salt).

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

Protecting group: The term "protecting group," as used herein, is well known in the art and includes those described in detail in Protecting Groups in Organic Synthesis, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of which is incorporated herein by reference. Also included are those protecting groups specially adapted for nucleoside and nucleotide chemistry described in Current Protocols in Nucleic Acid Chemistry, edited by Serge L. Beaucage et al. 06/2012, the entirety of Chapter 2 is incorporated herein by reference. Suitable amino–protecting groups include methyl carbamate, ethyl carbamante, 9–fluorenylmethyl carbamate (Fmoc), 9–(2–sulfo)fluorenylmethyl carbamate, 9–(2,7–dibromo)fluoroenylmethyl carbamate, 2,7–di–t–butyl–[9–(10,10–dioxo–10,10,10,10–tetrahydrothioxanthyl)]methyl carbamate (DBD–Tmoc), 4–methoxyphenacyl carbamate (Phenoc), 2,2,2–trichloroethyl carbamate (Troc), 2–trimethylsilylethyl carbamate (Teoc), 2–phenylethyl carbamate (hZ), 1–(1–adamantyl)–1–methylethyl carbamate (Adpoc), 1,1–dimethyl–2–haloethyl carbamate, 1,1–dimethyl–2,2–

dibromoethyl carbamate (DB–t–BOC), 1,1–dimethyl–2,2,2–trichloroethyl carbamate (TCBOC), 1–methyl–1–(4–biphenylyl)ethyl carbamate (Bpoc), 1–(3,5–di–t–butylphenyl)–1–methylethyl carbamate (t–Bumeoc), 2–(2'– and 4'–pyridyl)ethyl carbamate (Pyoc), 2–(N,N–dicyclohexylcarboxamido)ethyl carbamate, t–butyl carbamate (BOC), 1–adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1–isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4–nitrocinnamyl carbamate (Noc), 8–quinolyl carbamate, N–hydroxypiperidinyl carbamate, alkyldithio carbamate, benzyl carbamate (Cbz), p–methoxybenzyl carbamate (Moz), p–nitobenzyl carbamate, p–bromobenzyl carbamate, p–chlorobenzyl carbamate, 2,4–dichlorobenzyl carbamate, 4–methylsulfinylbenzyl carbamate (Msz), 9–anthrylmethyl carbamate, diphenylmethyl carbamate, 2–methylthioethyl carbamate, 2–methylsulfonylethyl carbamate, 2–(p–toluenesulfonyl)ethyl carbamate, [2–(1,3–dithianyl)]methyl carbamate (Dmoc), 4–methylthiophenyl carbamate (Mtpc), 2,4–dimethylthiophenyl carbamate (Bmpc), 2–phosphonioethyl carbamate (Peoc), 2–triphenylphosphonioisopropyl carbamate (Ppoc), 1,1–dimethyl–2–cyanoethyl carbamate, m–chloro–p–acyloxybenzyl carbamate, p–(dihydroxyboryl)benzyl carbamate, 5–benzisoxazolylmethyl carbamate, 2–(trifluoromethyl)–6–chromonylmethyl carbamate (Tcroc), m–nitrophenyl carbamate, 3,5–dimethoxybenzyl carbamate, o–nitrobenzyl carbamate, 3,4–dimethoxy–6–nitrobenzyl carbamate, phenyl(o–nitrophenyl)methyl carbamate, phenothiazinyl–(10)–carbonyl derivative, N'–p–toluenesulfonylaminocarbonyl derivative, N'–phenylaminothiocarbonyl derivative, t–amyl carbamate, S–benzyl thiocarbamate, p–cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, p–decyloxybenzyl carbamate, 2,2–dimethoxycarbonylvinyl carbamate, o–(N,N–dimethylcarboxamido)benzyl carbamate, 1,1–dimethyl–3–(N,N–dimethylcarboxamido)propyl carbamate, 1,1–dimethylpropynyl carbamate, di(2–pyridyl)methyl carbamate, 2–furanylmethyl carbamate, 2–iodoethyl carbamate, isoborynl carbamate, isobutyl carbamate, isonicotinyl carbamate, p–(p'–methoxyphenylazo)benzyl carbamate, 1–methylcyclobutyl carbamate, 1–methylcyclohexyl carbamate, 1–methyl–1–cyclopropylmethyl carbamate, 1–methyl–1–(3,5–dimethoxyphenyl)ethyl carbamate, 1–methyl–1–(p–phenylazophenyl)ethyl carbamate, 1–methyl–1–phenylethyl carbamate, 1–methyl–1–(4–pyridyl)ethyl carbamate, phenyl carbamate, p–(phenylazo)benzyl carbamate, 2,4,6–tri–t–butylphenyl carbamate, 4–(trimethylammonium)benzyl carbamate, 2,4,6–trimethylbenzyl carbamate, formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3–phenylpropanamide, picolinamide, 3–

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

dimethoxy–4–methylbenzenesulfonamide (iMds), 2,2,5,7,8–pentamethylchroman–6–sulfonamide (Pmc), methanesulfonamide (Ms), β–trimethylsilylethanesulfonamide (SES), 9–anthracenesulfonamide, 4–(4',8'–dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzylsulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamide.

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.

Suitable hydroxyl protecting groups include methyl, methoxylmethyl (MOM), methylthiomethyl (MTM), t–butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), p–methoxybenzyloxymethyl (PMBM), (4–methoxyphenoxy)methyl (p–AOM), guaiacolmethyl (GUM), t–butoxymethyl, 4–pentenyloxymethyl (POM), siloxymethyl, 2–methoxyethoxymethyl (MEM), 2,2,2–trichloroethoxymethyl, bis(2–chloroethoxy)methyl, 2–(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3–bromotetrahydropyranyl, tetrahydrothiopyranyl, 1–methoxycyclohexyl, 4–methoxytetrahydropyranyl (MTHP), 4–methoxytetrahydrothiopyranyl, 4–methoxytetrahydrothiopyranyl S,S–dioxide, 1–[(2–chloro–4–methyl)phenyl]–4–methoxypiperidin–4–yl (CTMP), 1,4–dioxan–2–yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a–octahydro–7,8,8–trimethyl–4,7–methanobenzofuran–2–yl, 1–ethoxyethyl, 1–(2–chloroethoxy)ethyl, 1–methyl–1–methoxyethyl, 1–methyl–1–benzyloxyethyl, 1–methyl–1–benzyloxy–2–fluoroethyl, 2,2,2–trichloroethyl, 2–trimethylsilylethyl, 2–(phenylselenyl)ethyl, t–butyl, allyl, p–chlorophenyl, p–methoxyphenyl, 2,4–dinitrophenyl, benzyl, p–methoxybenzyl, 3,4–dimethoxybenzyl, o–nitrobenzyl, p–nitrobenzyl, p–halobenzyl, 2,6–dichlorobenzyl, p–cyanobenzyl, p–phenylbenzyl, 2–picolyl, 4–picolyl, 3–methyl–2–picolyl N–oxido, diphenylmethyl, p,p'–dinitrobenzhydryl, 5–dibenzosuberyl, triphenylmethyl, α–naphthyldiphenylmethyl, p–methoxyphenyldiphenylmethyl, di(p–methoxyphenyl)phenylmethyl, tri(p–

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

benzylidene acetal, p–methoxybenzylidene acetal, 2,4–dimethoxybenzylidene ketal, 3,4–dimethoxybenzylidene acetal, 2–nitrobenzylidene acetal, methoxymethylene acetal, ethoxymethylene acetal, dimethoxymethylene ortho ester, 1–methoxyethylidene ortho ester, 1–ethoxyethylidine ortho ester, 1,2–dimethoxyethylidene ortho ester, α–methoxybenzylidene ortho ester, 1–(N,N–dimethylamino)ethylidene derivative, α–(N,N'–dimethylamino)benzylidene derivative, 2–oxacyclopentylidene ortho ester, di–t–butylsilylene group (DTBS), 1,3–(1,1,3,3–tetraisopropyldisiloxanylidene) derivative (TIPDS), tetra–t–butoxydisiloxane–1,3–diylidene derivative (TBDS), cyclic carbonates, cyclic boronates, ethyl boronate, and phenyl boronate.

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

trimethylsilylethyl, 2-nitroethyl, 2-sulfonylethyl, methyl, benzyl, o-nitrobenzyl, 2-(p-nitrophenyl)ethyl (NPE or Npe), 2-phenylethyl, 3-(N-tert-butylcarboxamido)-1-propyl, 4-oxopentyl, 4-methylthio-l-butyl, 2-cyano-1,1-dimethylethyl, 4-N-methylaminobutyl, 3-(2-pyridyl)-1-propyl, 2-[N-methyl-N-(2-pyridyl)]aminoethyl, 2-(N-formyl,N-methyl)aminoethyl, or 4-[N-methyl-N-(2,2,2-trifluoroacetyl)amino]butyl.

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

Substantially: As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. A base sequence which is substantially identical or complementary to a second sequence is not fully identical or complementary to the second sequence, but is mostly or nearly identical or complementary to the second sequence. In certain embodiments, an oligonucleotide with a substantially complementary sequence to another oligonucleotide or nucleic acid forms duplex with the oligonucleotide or nucleic acid in a similar fashion as an oligonucleotide with a fully complementary sequence. In addition, one of ordinary skill in the biological and/or chemical arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and/or chemical phenomena.

Sugar: The term "sugar" refers to a monosaccharide or polysaccharide in closed and/or open form. In certain embodiments, sugars are monosaccharides. In certain embodiments, sugars are polysaccharides. Sugars include, but are not limited to, ribose, deoxyribose, pentofuranose, pentopyranose, and hexopyranose moieties. As used herein, the term "sugar" also encompasses structural analogs used in lieu of conventional sugar molecules, such as glycol, polymer of which forms the backbone of the nucleic acid analog, glycol nucleic acid ("GNA"), etc. As used herein, the term "sugar" also encompasses structural analogs used in lieu of natural or naturally-occurring nucleotides, such as modified sugars and nucleotide sugars. In certain embodiments, a sugar is a RNA or DNA

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

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

Therapeutic agent: As used herein, the term "therapeutic agent" in general refers to any agent that elicits a desired effect (e.g., a desired biological, clinical, or pharmacological effect) when administered to a subject. In certain embodiments, an agent, e.g., a dsRNAi agent, is considered to be a therapeutic agent if it demonstrates a statistically significant effect across an appropriate population. In certain embodiments, an appropriate population is a population of subjects suffering from and/or susceptible to a disease, disorder or condition. In certain embodiments, an appropriate population is a population of model organisms. In certain embodiments, an appropriate population may be defined by one or more criterion such as age group, gender, genetic background, preexisting clinical conditions, prior exposure to therapy. In certain embodiments, a therapeutic agent is a substance that alleviates, ameliorates, relieves, inhibits, prevents, delays onset of, reduces severity of, and/or reduces incidence of one or more hepaticsymptoms or features of a disease, disorder, and/or condition in a subject when administered to the subject in an effective amount. In certain embodiments, a "therapeutic agent" is an agent that has been

or is required to be approved by a government agency before it can be marketed for administration to humans. In certain embodiments, a "therapeutic agent" is an agent for which a medical prescription is required for administration to humans. In certain embodiments, a therapeutic agent is a provided compound, e.g., a provided oligonucleotide.

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 certain 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 certain embodiments, a therapeutically effective amount is administered in a single dose; in certain embodiments, multiple unit doses are required to deliver a therapeutically effective amount.

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 certain 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.

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

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).

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

1. Description of Certain Embodiments

Oligonucleotides are useful tools for a wide variety of applications. For example, RNAi oligonucleotides are useful in therapeutic, diagnostic, and research applications, including the treatment of a variety of conditions, disorders, and diseases. 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 and/or to further improve various properties and activities. These include synthetic oligonucleotides that contain chemical modifications, e.g., base modifications, sugar modifications, backbone modifications, etc., which, among other things, render these molecules less susceptible to degradation and improve other properties and/or activities. From a structural point of view, modifications to internucleotidic linkages can introduce chirality and/or alter charge, and certain properties may be affected by configurations of linkage phosphorus atoms of oligonucleotides. For example, binding affinity, sequence specific binding to complementary RNA, stability against nucleases, cleavage of target nucleic acids, delivery, pharmacokinetics, etc., can be affected by, inter alia, chirality and/or charge of backbone linkage atoms.

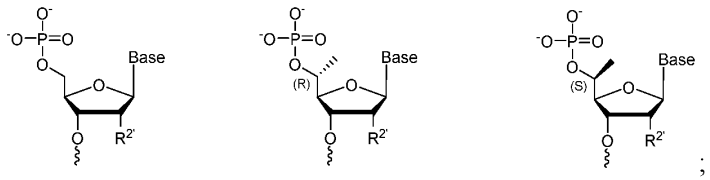
In certain embodiments, the present disclosure demonstrates that compositions comprising ds oligonucleotides (e.g., dsRNAi oligonucleotides, also referred to as dsRNAi agents) with controlled structural elements provide unexpected properties and/or activities.

In certain embodiments, the present disclosure encompasses the recognition that stereochemistry, e.g., stereochemistry of backbone chiral centers, can unexpectedly maintain or improve properties of ds oligonucleotides. In contrast to many prior observations that some structural elements that increase stability can also lower activity, for example, RNA interference, the present disclosure demonstrates that control of stereochemistry can, surprisingly, maintain increase stability while not significantly decreasing activity. For example, but not by way of limitation, the instant disclosure relates, in part, to ds oligonucleotides comprising one or more of: (1) a guide strand comprising backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1)

nucleotide and the immediately upstream (N-2) nucleotide; (2) a guide strand comprising backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide; (3) a guide strand comprising one or more backbone phosphorothioate chiral centers upstream, i.e., in the 5' direction, relative to backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, where the upstream backbone phosphorothioate chiral centers are in Rp or Sp configuration; 4) a guide strand comprising one or more backbone phosphorothioate chiral centers in Rp or Sp configuration between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the (+2) nucleotide and the immediately downstream (+3) nucleotide, as well as between one or both of: (a) the (+3) nucleotide and the (+4) nucleotide; and (b) the (+5) nucleotide and the (+6) nucleotide, and (5) a passenger strand in combination with one or more of the aforementioned guide strands, comprising one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the present disclosure encompasses the recognition that stereochemistry, e.g., stereochemistry of chiral centers at a 5' terminal modification of guide strands, can unexpectedly maintain or improve properties of ds oligonucleotides wherein the guide strand of the ds oligonucleotide also comprises a phosphorothioate chiral center in Rp or Sp configuration. For example, but not by way of limitation, the instant disclosure relates, in part, to ds oligonucleotides comprising a guide stranding comprising a phosphorothioate chiral center in Rp or Sp configuration and a 5' terminal modification selected from:

(a) 5' PO modifications, such as, but not limited to:



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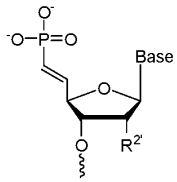
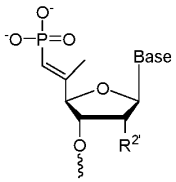
(b) 5' VP modifications, such as, but not limited to: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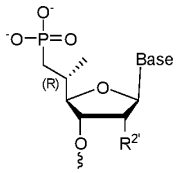
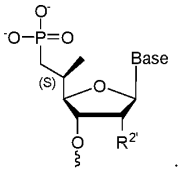
(c) 5' MeP modifications, such as, but not limited to: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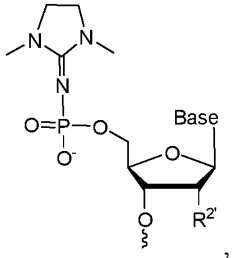
(d) 5' PN and 5' Trizole-P modifications, such as, but not limited to: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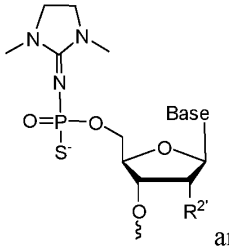
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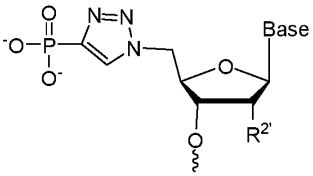
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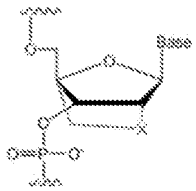
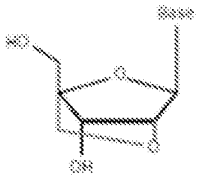
Wherein Base is selected from A, C, G, T, U, abasic and modified nucleobases;

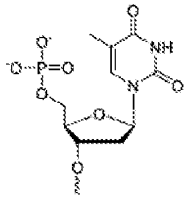
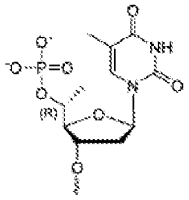
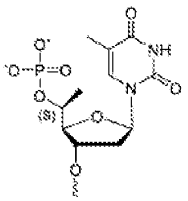
$R^{2'}$ is selected from H, OH, O-alkyl, F, MOE, locked nucleic acid (LNA) bridges and bridged nucleic acid (BNA) bridges to the 4' C, such as, but not limited to:



, and .

In certain other embodiments, the present disclosure encompasses the recognition that stereochemistry, e.g., stereochemistry of chiral centers at the 5' terminal nucleotide of guide strands, can unexpectedly maintain or improve properties of ds oligonucleotides wherein the guide strand of the ds oligonucleotide also comprises a phosphorothioate chiral center in Rp or Sp configuration. For example, but not by way of

limitation, the instant disclosure relates, in part, to ds oligonucleotides comprising a guide stranding comprising a phosphorothioate chiral center in Rp or Sp configuration and a 5' terminal nucleotide selected from:

(a) 5' PO nucleotides, such as, but not limited to: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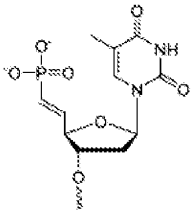
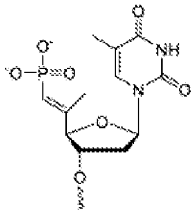
(b) 5' VP nucleotides, such as, but not limited to: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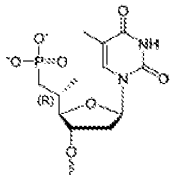
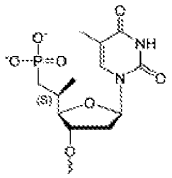
(c) 5' MeP nucleotides, such as, but not limited to: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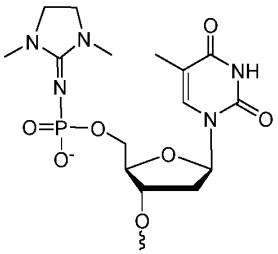
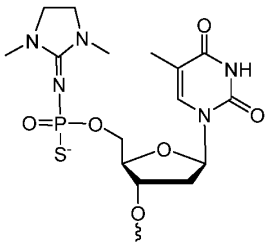
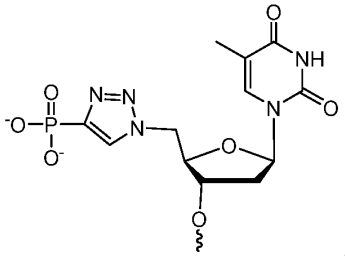
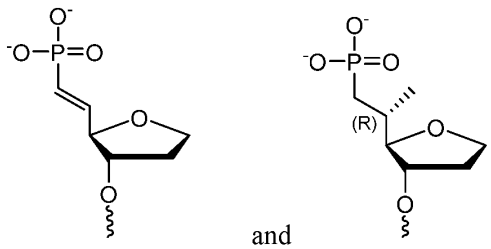
(d) 5' PN and 5' Trizole-P nucleotides, such as, but not limited to:


,

and

;

(e) 5' abasic VP and 5' abasic MeP nucleotides, such as, but not limited to:



and .

In certain embodiments, the present disclosure encompasses the recognition that non-naturally-occurring internucleotidic linkages, e.g., neutral internucleotidic linkages, can unexpectedly maintain or improve properties of ds oligonucleotides. For example, the present disclosure demonstrates that modified internucleotidic linkages can be introduced into ds oligonucleotide without significantly decreasing the activity of the ds oligonucleotide. For example, but not by way of limitation, the instant disclosure relates, in part, to ds oligonucleotides comprising one or more of: (1) a guide strand where one or both of the 5' and 3' terminal dinucleotides are ***not*** linked by non-negatively charged internucleotidic linkages, i.e., the guide strand comprises one more non-negatively charged internucleotidic linkages downstream, i.e., in the 3' direction, relative to the linkage between the 5' terminal dinucleotide and/or upstream, i.e., in the 5' direction, relative to the linkage between the 3' terminal dinucleotide; (2) a guide strand where one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, where N is the 3' terminal nucleotide; (3) a guide strand where a non-negatively charged internucleotidic linkage occurs between the third (+3) and fourth (+4) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and/or between the tenth (+10) and eleventh (+11) nucleotides, relative to the 5' terminal nucleotide; (4) a passenger strand where one or more non-negatively charged internucleotidic linkage occurs upstream, i.e., in the 5' direction, relative to the central nucleotide of the passenger strand; and (5) Passenger strand where one or more non-negatively charged internucleotidic linkage occurs downstream, i.e., in the 3' direction, relative to the central nucleotide of the passenger strand.

In certain embodiments, the present disclosure encompasses the recognition that non-naturally occurring internucleotidic linkages, e.g., neutral internucleotidic linkages, can, in certain embodiments, be used to link one or more molecules to the double-stranded oligonucleotides described herein. In certain embodiments, such linked molecules can facilitate targeting and/or delivery of the double-stranded oligonucleotide. For example, but

not limitation, such linked molecules an include lipophilic molecules. In certain embodiments, the linked molecule is a molecule comprising one or more GalNac moieties. In certain embodiments, the the linked molecule is a receptor. In certain embodiments, the linked molecule is a receptor ligand.

In certain embodiments, the present disclosure provides technologies (*e.g.*, compounds, methods, etc.) for improving oligonucleotide stability while maintaining or increasing activity, including compositions of improved-stability oligonucleotides.

In certain embodiments, the present disclosure provides technologies for incorporating various additional chemical moieties into ds oligonucleotides. In certain embodiments, the present disclosure provides, for example, reagents and methods for introducing additional chemical moieties through nucleobases (*e.g.*, by covalent linkage, optionally via a linker, to a site on a nucleobase).

In certain embodiments, the present disclosure provides technologies, e.g., ds oligonucleotide compositions and methods thereof, that achieve allele-specific suppression, wherein transcripts from one allele of a particular target gene is selectively knocked down relative to at least one other allele of the same gene.

Among other things, the present disclosure provides structural elements, technologies and/or features that can be incorporated into ds oligonucleotides and can impart or tune one or more properties thereof (*e.g.*, relative to an otherwise identical ds oligonucleotide lacking the relevant technology or feature). In certain embodiments, the present disclosure documents that one or more provided technologies and/or features can usefully be incorporated into ds oligonucleotides of various sequences.

In certain embodiments, the present disclosure demonstrates that certain provided structural elements, technologies and/or features are particularly useful for ds oligonucleotides that participate in and/or direct RNAi mechanisms (*e.g.*, RNAi agents). Regardless, however, the teachings of the present disclosure are not limited to ds oligonucleotides that participate in or operate via any particular mechanism. In certain embodiments, the present disclosure pertains to any ds oligonucleotide, useful for any purpose, which operates through any mechanism, and which comprises any sequence, structure or format (or portion thereof) described herein. In certain embodiments, the present disclosure provides a ds oligonucleotide, useful for any purpose, which operates through any mechanism, and which comprises any sequence, structure or format (or portion thereof) described herein, including, but not limited to, (1) a guide strand comprising backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal

nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide; (2) a guide strand comprising backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide; (3) a guide strand comprising one or more backbone phosphorothioate chiral centers upstream, i.e., in the 5' direction, relative to backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, where the upstream backbone phosphorothioate chiral centers are in Rp or Sp configuration; 4) a guide strand comprising one or more backbone phosphorothioate chiral centers in Rp or Sp configuration between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the (+2) nucleotide and the immediately downstream (+3) nucleotide, as well as between one or both of: (a) the (+3) nucleotide and the (+4) nucleotide; and (b) the (+5) nucleotide and the (+6) nucleotide, and (5) a passenger strand in combination with one or more of the aforementioned guide strands, comprising one or more backbone chiral centers in Rp or Sp configuration. In certain embodiments, the present disclosure provides a ds oligonucleotide, useful for any purpose, which operates through any mechanism, and which comprises any sequence, structure or format (or portion thereof) described herein, including, but not limited to, (1) a guide strand where one or both of the 5' and 3' terminal dinucleotides are ***not*** linked by non-negatively charged internucleotidic linkages, i.e., the guide strand comprises one more non-negatively charged internucleotidic linkages downstream, i.e., in the 3' direction, relative to the linkage between the 5' terminal dinucleotide and/or upstream, i.e., in the 5' direction, relative to the linkage between the 3' terminal dinucleotide; (2) a guide strand where one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, where N is the 3' terminal nucleotide; (3) a guide strand where a non-negatively charged internucleotidic linkage occurs between the third (+3) and fourth (+4) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and/or between the tenth (+10) and eleventh (+11) nucleotides, relative to the 5' terminal nucleotide; (4) a passenger strand where one or more non-negatively charged internucleotidic linkage occurs upstream, i.e., in the 5' direction, relative to the central nucleotide of the passenger strand; and (5) Passenger strand where one or more non-

negatively charged internucleotidic linkage occurs downstream, i.e., in the 3' direction, relative to the central nucleotide of the passenger strand.

In certain embodiments, the present disclosure provides a ds oligonucleotide, useful for any purpose, which operates through any mechanism, and which comprises any sequence, structure or format (or portion thereof) described herein, including, but not limited to: (1) a guide strand comprising backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide; (2) a guide strand comprising backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide; (3) a guide strand comprising one or more backbone phosphorothioate chiral centers upstream, i.e., in the 5' direction, relative to backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, where the upstream backbone phosphorothioate chiral centers are in Rp or Sp configuration; 4) a guide strand comprising one or more backbone phosphorothioate chiral centers in Rp or Sp configuration between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the (+2) nucleotide and the immediately downstream (+3) nucleotide, as well as between one or both of: (a) the (+3) nucleotide and the (+4) nucleotide; and (b) the (+5) nucleotide and the (+6) nucleotide, and (5) a passenger strand in combination with one or more of the aforementioned guide strands, comprising one or more backbone chiral centers in Rp or Sp configuration, and where the the present disclosure provides a ds oligonucleotide, useful for any purpose, and which also comprises any sequence, structure or format (or portion thereof) described herein, including, but not limited to: (1) a guide strand where one or both of the 5' and 3' terminal dinucleotides are ***not*** linked by non-negatively charged internucleotidic linkages, i.e., the guide strand comprises one more non-negatively charged internucleotidic linkages downstream, i.e., in the 3' direction, relative to the linkage between the 5' terminal dinucleotide and/or upstream, i.e., in the 5' direction, relative to the linkage between the 3' terminal dinucleotide; (2) a guide strand where one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, where N is the 3' terminal

nucleotide; (3) a guide strand where a non-negatively charged internucleotidic linkage occurs between the third (+3) and fourth (+4) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and/or between the tenth (+10) and eleventh (+11) nucleotides, relative to the 5' terminal nucleotide; (4) a passenger strand where one or more non-negatively charged internucleotidic linkage occurs upstream, i.e., in the 5' direction, relative to the central nucleotide of the passenger strand; and (5) Passenger strand where one or more non-negatively charged internucleotidic linkage occurs downstream, i.e., in the 3' direction, relative to the central nucleotide of the passenger strand. In certain embodiments, the provided ds oligonucleotides may participate in (*e.g.*, direct) RNAi mechanisms. In certain embodiments, provided ds oligonucleotides may participate in RNase H (ribonuclease H) mechanisms. In certain embodiments, provided ds oligonucleotides may act as translational inhibitors (*e.g.*, may provide steric blocks of translation).

In certain embodiments, the guide strand comprises backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49.

In certain embodiments, the guide strand comprises backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49.

In certain embodiments, the guide strand comprises one or more backbone phosphorothioate chiral centers in Rp or Sp configuration upstream of backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49.

In certain embodiments, the guide strand comprises one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49.

In certain embodiments, the guide strand comprises backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide, and the passenger strand comprises one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises one or more backbone phosphorothioate chiral centers in Rp or Sp configuration upstream of backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises one or more backbone phosphorothioate chiral centers in Rp or Sp configuration between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the (+2) nucleotide and the immediately downstream (+3) nucleotide, as well as between one or both of: (a) the (+3) nucleotide and the (+4) nucleotide; and (b) the (+5) nucleotide and the (+6) nucleotide.

In certain embodiments, the guide strand comprises one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, and the passenger strand comprises one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49 and one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49and one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises one or more backbone phosphorothioate chiral centers in Rp or Sp configuration upstream of backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49 and one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, the guide strand comprises one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49 and one or more backbone chiral centers in Rp or Sp configuration.

In certain embodiments, a RNAi oligonucleotide comprises a sequence that is completely or substantially identical to or is completely or substantially complementary to 10 or more (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) contiguous bases of a target genomic sequence or a transcript therefrom (e.g., mRNA (e.g., pre-mRNA, mRNA after splicing, etc.)). In certain embodiments, a RNAi oligonucleotide comprises a sequence that is completely complementary to 10 or more (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) contiguous bases of a target transcript. In certain embodiments, the number of contiguous bases is about 15-20. In certain embodiments, the number of contiguous bases is about 20. In certain embodiments, an RNAi oligonucleotide that can hybridize with a target transcript (e.g., pre-mRNA, RNA, etc.) and can reduce the level of the target transcript and/or a protein encoded by the target transcript.

In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide as disclosed herein, e.g., in Table 1A, Table 1B, Table 1C or Table 1D. In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide having a base sequence disclosed herein, e.g., in Table 1B, or a portion thereof comprising at least 10 (e.g.,

10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) contiguous bases, wherein the RNAi oligonucleotide is stereorandom or not chirally controlled, and wherein each T can be independently substituted with U and vice versa.

In certain embodiments, internucleotidic linkages of an oligonucleotide comprise or consist of 1-5, 1-10, 1-15, 1-20, 1-25, 1-30, 1-40, 1-50, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more chirally controlled internucleotidic linkages. In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide composition wherein the dsRNAi oligonucleotides comprise at least one chirally controlled internucleotidic linkage. In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide composition wherein the dsRNAi oligonucleotides are stereorandom or not chirally controlled. In certain embodiments, in a dsRNAi oligonucleotide, at least one internucleotidic linkage is stereorandom and at least one internucleotidic linkage is chirally controlled.

In certain embodiments, internucleotidic linkages of an oligonucleotide comprise or consist of one or more neutrally charged internucleotidic linkages.

1.1 Double Stranded Oligonucleotides

In certain embodiments, the present disclosure provides oligonucleotides of various designs, which may comprise various nucleobases and patterns thereof, sugars and patterns thereof, internucleotidic linkages and patterns thereof, and/or additional chemical moieties and patterns thereof as described in the present disclosure. In certain embodiments, provided dsRNAi oligonucleotides can direct a decrease in the expression, level and/or activity of a gene and/or one or more of its products (e.g., transcripts, mRNA, proteins, etc.). In certain embodiments, provided dsRNAi oligonucleotides can direct a decrease in the expression, level and/or activity of a gene and/or one or more of its products in a cell of a subject or patient. In certain embodiments, a cell normally expresses or produces a protein. In certain embodiments, provided dsRNAi oligonucleotides can direct a decrease in the expression, level and/or activity of a target gene or a gene product and has a base sequence which consists of, comprises, or comprises a portion (e.g., a span of 1-5, 1-10, 1-15, 1-20, 1-25, 1-30, 1-40, 1-50, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more contiguous bases) of the base sequence of a dsRNAi oligonucleotide disclosed herein, wherein each T can be independently substituted with U and vice versa, and the ds oligonucleotide comprises at least one non-naturally-occurring modification of a base, sugar and/or internucleotidic linkage.

In certain embodiments, dsRNAi oligonucleotides can direct a decrease in the expression, level and/or activity of a target gene, e.g., a target gene, or a product thereof. In certain embodiments, provided ds oligonucleotides can direct a decrease in the expression and/or level of a target gene or its gene product. In certain embodiments, provided ds oligonucleotides can direct a decrease in levels of target products. In certain embodiments, provided ds oligonucleotide can reduce levels of transcripts of target genes. In certain embodiments, provided ds oligonucleotide can reduce levels of mRNA of target genes. In certain embodiments, provided ds oligonucleotide can reduce levels of proteins encoded by target genes. In certain embodiments, provided ds oligonucleotides can direct a decrease in the expression and/or level of a target gene or its gene product via RNA interference. In certain embodiments, provided ds oligonucleotides can direct a decrease in the expression and/or level of a target gene or its gene product via a biochemical mechanism which does not involve RNA interference or RISC (including, but not limited to, RNaseH-mediated knockdown or steric hindrance of gene expression). In certain embodiments, provided ds oligonucleotides can direct a decrease in the expression and/or level of a target gene or its gene product via RNA interference and/or RNase H-mediated knockdown. In certain embodiments, provided ds oligonucleotides can direct a decrease in the expression and/or level of a target gene or its gene product by sterically blocking translation after binding to a target gene mRNA, and/or by altering or interfering with mRNA splicing and/or exon inclusion or exclusion. In certain embodiments, provided ds oligonucleotides comprise one or more structural elements described herein or known in the art in accordance with the present disclosure, e.g., base sequences; modifications; stereochemistry; patterns of internucleotidic linkages; GC contents; long GC stretches; patterns of backbone linkages; patterns of backbone chiral centers; patterns of backbone phosphorus modifications; additional chemical moieties, including but not limited to, one or more targeting moieties, lipid moieties, and/or carbohydrate moieties, etc.; seed regions; post-seed regions; 5'-end structures; 5'-end regions; 5' nucleotide moieties; 3'-end regions; 3'-terminal dinucleotides; 3'-end caps; etc. In certain embodiments, a seed region of an oligonucleotide is or comprises the second to eighth, second to seventh, second to sixth, third to eighth, third to seventh, third to seven, or fourth to eighth or fourth to seventh nucleotides, counting from the 5' end; and the post-seed region of the oligonucleotide is the region immediately 3' to the seed region, and interposed between the seed region and the 3' end region. In certain embodiments, a provided composition comprises a ds oligonucleotide. In certain embodiments, a provided composition comprises one or more lipid moieties, one or more

carbohydrate moieties (unless otherwise specified, other than sugar moieties of nucleoside units that form oligonucleotide chain with internucleotidic linkages), and/or one or more targeting components. In certain embodiments, ds RNAi oligonucleotides can direct a decrease in the expression, level and/or activity of a target gene or a product thereof by sterically blocking translation after binding to a target gene mRNA, and/or by altering or interfering with mRNA splicing. Regardless, however, the present disclosure is not limited to any particular mechanism. In certain embodiments, the present disclosure provides ds oligonucleotides, compositions, methods, etc., capable of operating via double-stranded RNA interference, single-stranded RNA interference, RNase H-mediated knock- down, steric hindrance of translation, or a combination of two or more such mechanisms.

In certain embodiments, a dsRNAi oligonucleotide comprises a structural element or a portion thereof described herein, e.g., in Table 1A or Table 1B or Table 1C or Table 1D. In certain embodiments, a dsRNAi oligonucleotide comprises a base sequence (or a portion thereof) described herein, wherein each T can be independently substituted with U and vice versa, a chemical modification or a pattern of chemical modifications (or a portion thereof), and/or a format or a portion thereof described herein. In certain embodiments, a dsRNAi oligonucleotide has a base sequence which comprises the base sequence (or a portion thereof) wherein each T can be independently substituted with U, pattern of chemical modifications (or a portion thereof), and/or a format of an oligonucleotide disclosed herein, e.g., in Table 1A or 1B, or Table 1C or Table 1D, or otherwise disclosed herein. In certain embodiments, such ds oligonucleotides, e.g., dsRNAi oligonucleotides reduce expression, level and/or activity of a gene, e.g., a gene, or a gene product thereof.

Among other things, dsRNAi oligonucleotides may hybridize to their target nucleic acids (e.g., pre- mRNA, mature mRNA, etc.). For example, in certain embodiments, a dsRNAi oligonucleotide can hybridize to a nucleic acid derived from a DNA strand (either strand of the gene). In certain embodiments, a dsRNAi oligonucleotide can hybridize to a transcript. In certain embodiments, a dsRNAi oligonucleotide can hybridize to a target nucleic acid in any stage of RNA processing, including but not limited to a pre-mRNA or a mature mRNA. In certain embodiments, a dsRNAi oligonucleotide can hybridize to any element of a target nucleic acid or its complement, including but not limited to: a promoter region, an enhancer region, a transcriptional stop region, a translational start signal, a translation stop signal, a coding region, a non-coding region, an exon, an intron, an intron/exon or exon/intron junction, the 5' UTR, or the 3' UTR. In certain embodiments,

dsRNAi oligonucleotides can hybridize to their targets with no more than 2 mismatches. In certain embodiments, dsRNAi oligonucleotides can hybridize to their targets with no more than one mismatch. In certain embodiments, dsRNAi oligonucleotides can hybridize to their targets with no mismatches (e.g., when all C-G and/or A-T/U base paring).

In certain embodiments, a ds oligonucleotide can hybridize to two or more variants of transcripts. In certain embodiments, a dsRNAi oligonucleotide can hybridize to two or more or all variants of a transcript. In certain embodiments, a dsRNAi oligonucleotide can hybridize to two or more or all variants of a transcript derived from the sense strand.

In certain embodiments, a target of a dsRNAi oligonucleotide is a RNA which is not a mRNA.

In certain embodiments, ds oligonucleotides, e.g., dsRNAi oligonucleotides, contain increased levels of one or more isotopes. In certain embodiments, ds oligonucleotides, e.g., dsRNAi oligonucleotides, are labeled, e.g., by one or more isotopes of one or more elements, e.g., hydrogen, carbon, nitrogen, etc. In certain embodiments, ds oligonucleotides, e.g., dsRNAi oligonucleotides, in provided compositions, e.g., ds oligonucleotides of a plurality of a composition, comprise base modifications, sugar modifications, and/or internucleotidic linkage modifications, wherein the ds oligonucleotides contain an enriched level of deuterium. In certain embodiments, oligonucleotides, e.g., RNAi oligonucleotides, are labeled with deuterium (replacing $-^{1}H$ with $-^{2}H$) at one or more positions. In certain embodiments, one or more ^{1}H of a ds oligonucleotide chain or any moiety conjugated to the ds oligonucleotide chain (e.g., a targeting moiety, etc.) is substituted with ^{2}H. Such ds oligonucleotides can be used in compositions and methods described herein.

In certain embodiments, the present disclosure provides a ds oligonucleotide composition comprising a plurality of ds oligonucleotides which:

1) have a common base sequence complementary to a target sequence (e.g., a target sequence) in a transcript; and

2) comprise one or more modified sugar moieties and/or modified internucleotidic linkages.

In certain embodiments, dsRNAi oligonucleotides having a common base sequence may have the same pattern of nucleoside modifications, e.g., sugar modifications, base modifications, etc. In certain embodiments, a pattern of nucleoside modifications may be represented by a combination of locations and modifications. In certain embodiments, a

pattern of backbone linkages comprises locations and types (e.g., phosphate, phosphorothioate, substituted phosphorothioate, etc.) of each internucleotidic linkage.

In certain embodiments, ds oligonucleotides of a plurality, e.g., in provided compositions, are of the same ds oligonucleotide type. In certain embodiments, ds oligonucleotides of an ds oligonucleotide type have a common pattern of sugar modifications. In certain embodiments, ds oligonucleotides of a ds oligonucleotide type have a common pattern of base modifications. In certain embodiments, ds oligonucleotides of a ds oligonucleotide type have a common pattern of nucleoside modifications. In certain embodiments, ds oligonucleotides of a ds oligonucleotide type have the same constitution. In certain embodiments, ds oligonucleotides of a ds oligonucleotide type are identical. In certain embodiments, ds oligonucleotides of a plurality are identical. In certain embodiments, ds oligonucleotides of a plurality share the same constitution.

In certain embodiments, as exemplified herein, dsRNAi oligonucleotides are chiral controlled, comprising one or more chirally controlled internucleotidic linkages. In certain embodiments, ds RNAi oligonucleotides are stereochemically pure. In certain embodiments, dsRNAi oligonucleotides are substantially separated from other stereoisomers.

In certain embodiments, RNAi oligonucleotides comprise one or more modified nucleobases, one or more modified sugars, and/or one or more modified internucleotidic linkages.

In certain embodiments, dsRNAi oligonucleotides comprise one or more modified sugars. In certain embodiments, ds oligonucleotides of the present disclosure comprise one or more modified nucleobases. Various modifications can be introduced to a sugar and/or nucleobase in accordance with the present disclosure. For example, in certain embodiments, a modification is a modification described in US 9006198. In certain embodiments, a modification is a modification described in US 9394333, US 9744183, US 9605019, US 9598458, US 9982257, US 10160969, US 10479995, US 2020/0056173, US 2018/0216107, US 2019/0127733, US 10450568, US 2019/0077817, US 2019/0249173, US 2019/0375774, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the sugar, base, and internucleotidic linkage modifications of each of which are independently incorporated herein by reference.

As used in the present disclosure, in certain embodiments, “one or more” is 1-200, 1-150, 1-100, 1- 90, 1-80, 1-70, 1-60, 1-50, 1-40, 1-30, or 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. In certain embodiments, "one or more" is one. In certain embodiments, "one or more" is two. In certain embodiments, "one or more" is three. In certain embodiments, "one or more" is four. In certain embodiments, "one or more" is five. In certain embodiments, "one or more" is six. In certain embodiments, "one or more" is seven. In certain embodiments, "one or more" is eight. In certain embodiments, "one or more" is nine. In certain embodiments, "one or more" is ten. In certain embodiments, "one or more" is at least one. In certain embodiments, "one or more" is at least two. In certain embodiments, "one or more" is at least three. In certain embodiments, "one or more" is at least four. In certain embodiments, "one or more" is at least five. In certain embodiments, "one or more" is at least six. In certain embodiments, "one or more" is at least seven. In certain embodiments, "one or more" is at least eight. In certain embodiments, "one or more" is at least nine. In certain embodiments, "one or more" is at least ten.

As used in the present disclosure, in certain embodiments, "at least one" is 1-200, 1-150, 1-100, 1- 90, 1-80, 1-70, 1-60, 1-50, 1-40, 1-30, or 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. In certain embodiments, "at least one" is one. In certain embodiments, "at least one" is two. In certain embodiments, "at least one" is three. In certain embodiments, "at least one" is four. In certain embodiments, "at least one" is five. In certain embodiments, "at least one" is six. In certain embodiments, "at least one" is seven. In certain embodiments, "at least one" is eight. In certain embodiments, "at least one" is nine. In certain embodiments, "at least one" is ten.

In certain embodiments, a dsRNAi oligonucleotide is or comprises a dsRNAi oligonucleotide described in Table 1A or 1B or Table 1C or Table 1D.

As demonstrated in the present disclosure, in certain embodiments, a provided ds oligonucleotide (e.g., a dsRNAi oligonucleotide) is characterized in that, when it is contacted with the transcript in a knockdown system, knockdown of its target (e.g., a transcript for a target oligonucleotide).

In certain embodiments, ds oligonucleotides are provided as salt forms. In certain embodiments, ds oligonucleotides are provided as salts comprising negatively-charged internucleotidic linkages (e.g., phosphorothioate internucleotidic linkages, natural phosphate linkages, etc.) existing as their salt forms. In certain embodiments, ds oligonucleotides are provided as pharmaceutically acceptable salts. In certain embodiments, ds oligonucleotides are provided as metal salts. In certain embodiments, ds oligonucleotides are provided as sodium salts. In certain embodiments, ds oligonucleotides are provided as

metal salts, e.g., sodium salts, wherein each negatively-charged internucleotidic linkage is independently in a salt form (e.g., for sodium salts, −O−P(O)(SNa)−O− for a phosphorothioate internucleotidic linkage, −O−P(O)(ONa)−O− for a natural phosphate linkage, etc.).

1.2 Regions of Double Stranded Oligonucleotides

1.2.1 Base Sequences

In certain embodiments, a dsRNAi oligonucleotide comprises a base sequence described herein or a portion (e.g., a span of 5-50, 5-40, 5-30, 5-20, or 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 20 or at least 10, at least 15, contiguous nucleobases) thereof with 0-5 (e.g., 0, 1, 2, 3, 4 or 5) mismatches, wherein each T can be independently substituted with U and vice versa. In certain embodiments, a dsRNAi oligonucleotide comprises a base sequence described herein, or a portion thereof, wherein a portion is a span of at least 10 contiguous nucleobases, or a span of at least 15 contiguous nucleobases with 1-5 mismatches. In certain embodiments, dsRNAi oligonucleotides comprise a base sequence described herein, or a portion thereof, wherein a portion is a span of at least 10 contiguous nucleobases, or a span of at least 10 contiguous nucleobases with 1-5 mismatches, wherein each T can be independently substituted with U and vice versa. In certain embodiments, base sequences of ds oligonucleotides comprise or consists of 10-50 (e.g., about or at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45; in certain embodiments, at least 15; in certain embodiments, at least 16; in certain embodiments, at least 17; in certain embodiments, at least 18; in certain embodiments, at least 19; in certain embodiments, at least 20; in certain embodiments, at least 21; in certain embodiments, at least 22; in certain embodiments, at least 23; in certain embodiments, at least 24; in certain embodiments, at least 25) contiguous bases of a base sequence that is identical to or complementary to a base sequence of a gene or a transcript (e.g., mRNA) thereof.

Base sequences of the guide strand of dsRNAi oligonucleotides, as appreciated by those skilled in the art, typically have sufficient length and complementarity to their targets, e.g., RNA transcripts (e.g., pre-mRNA, mature mRNA, etc.) to mediate target-specific knockdown. In certain embodiments, the base sequence of a dsRNAi oligonucleotide guide strand has a sufficient length and identity to a transcript target to mediate target-specific knockdown. In certain embodiments, the dsRNAi oligonucleotide guide strand is complementary to a portion of a transcript (a transcript target sequence). In certain embodiments, the base sequence of a dsRNAi oligonucleotide has 90% or more

identity with the base sequence of a ds oligonucleotide disclosed in a Table 1A or 1B, or Table 1C or Table 1D, wherein each T can be independently substituted with U and vice versa. In certain embodiments, the base sequence of a dsRNAi oligonucleotide has 95% or more identity with the base sequence of an oligonucleotide disclosed in Table 1A or 1B, or Table 1C or Table 1D, wherein each T can be independently substituted with U and vice versa. In certain embodiments, the base sequence of a dsRNAi oligonucleotide comprises a continuous span of 15 or more bases of an oligonucleotide disclosed in Table 1A or 1B, or Table 1C or Table 1D, wherein each T can be independently substituted with U and vice versa, except that one or more bases within the span are abasic (e.g., a nucleobase is absent from a nucleotide). In certain embodiments, the base sequence of a dsRNAi oligonucleotide comprises a continuous span of 19 or more bases of a dsRNAi oligonucleotide disclosed herein, except that one or more bases within the span are abasic (e.g., a nucleobase is absent from a nucleotide). In certain embodiments, the base sequence of a dsRNAi oligonucleotide comprises a continuous span of 19 or more bases of a ds oligonucleotide disclosed herein, wherein each T can be independently substituted with U and vice versa, except for a difference in the 1 or 2 bases at the 5' end and/or 3' end of the base sequences.

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

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

In certain embodiments, the present disclosure pertains to a ds oligonucleotide having a base sequence which is at least 90% identical to the base sequence of any ds oligonucleotide disclosed herein, wherein each T may be independently replaced with U and vice versa.

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

In certain embodiments, a base sequence of a ds oligonucleotide is, comprises, or comprises 10-20, e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 contiguous

bases of the base sequence of any ds oligonucleotide described herein, wherein each T may be independently replaced with U and vice versa.

In certain embodiments, a dsRNAi oligonucleotide is selected from Table 1A or 1B or Table 1C or Table 1D.

In certain embodiments, a dsRNAi oligonucleotide target two or more or all alleles (if multiple alleles exist in a relevant system). In certain embodiments, a ds oligonucleotide reduces expressions, levels and/or activities of both wild-type allele and mutant allele, and/or transcripts and/or products thereof.

In certain embodiments, base sequences of provided ds oligonucleotides are fully complementary to both human and a non-human primate (NHP) target sequences. In certain embodiments, such sequences can be particularly useful as they can be readily assessed in both human and non-human primates.

In certain embodiments, a dsRNAi oligonucleotide comprises a base sequence or portion thereof described in Table 1A or 1B, or Table 1C or Table 1D, wherein each T may be independently replaced with U and vice versa, and/or a sugar, nucleobase, and/or internucleotidic linkage modification and/or a pattern thereof described in Table 1A or 1B, or Table 1C or Table 1D, and/or an additional chemical moiety (in addition to an oligonucleotide chain, e.g., a target moiety, a lipid moiety, a carbohydrate moiety, etc.) described in Table 1A or 1B or Table 1C or Table 1D.

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

functions (e.g., knockdown of target nucleic acids. Typically when determining complementarity, A and T (or U) are complementary nucleobases and C and G are complementary nucleobases.

In certain embodiments, a "portion" (e.g., of a base sequence or a pattern of modifications) is at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 monomeric units long (e.g., for a base sequence, at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 bases long). In certain embodiments, a "portion" of a base sequence is at least 5 bases long. In certain embodiments, a "portion" of a base sequence is at least 10 bases long. In certain embodiments, a "portion" of a base sequence is at least 15 bases long. In certain embodiments, a "portion" of a base sequence is at least 16, 17, 18, 19 or 20 bases long. In certain embodiments, a "portion" of a base sequence is at least 20 bases long. In certain embodiments, a portion of a base sequence is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or more contiguous (consecutive) bases. In certain embodiments, a portion of a base sequence is 15 or more contiguous (consecutive) bases. In certain embodiments, a portion of a base sequence is 16, 17, 18, 19 or 20 or more contiguous (consecutive) bases. In certain embodiments, a portion of a base sequence is 20 or more contiguous (consecutive) bases.

In certain embodiments, a portion is a span of at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 total nucleotides. In certain embodiments, a portion is a span of at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 total nucleotides with 0-3 mismatches. In certain embodiments, a portion is a span of at least 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 total nucleotides with 0-3 mismatches, wherein a span with 0 mismatches is complementary and a span with 1 or more mismatches is a non-limiting example of substantial complementarity. In certain embodiments, a base comprises a portion characteristic of a nucleic acid (e.g., a gene) in that the portion is identical or complementary to a portion of the nucleic acid or a transcript thereof, and is not identical or complementary to a portion of any other nucleic acid (e.g., a gene) or a transcript thereof in the same genome. In certain embodiments, a portion is characteristic of human dsRNAi.

In certain embodiments, a provided oligonucleotide, e.g., a dsRNAi oligonucleotide, has a length of no more than about 49, 45, 40, 30, 35, 25, or 23 total nucleotides as described herein. In certain embodiments, wherein the sequence recited herein starts with a U or T at the 5'-end, the U can be deleted and/or replaced by another base.

In certain embodiments, ds oligonucleotides, e.g., dsRNAi oligonucleotides are stereorandom. In certain embodiments, RNAi oligonucleotides are chirally controlled.

In certain embodiments, a ds RNAi oligonucleotide is chirally pure (or "stereopure", "stereochemically pure"), wherein the ds oligonucleotide exists as a single stereoisomeric form (in many cases a single diastereoisomeric (or "diastereomeric") form as multiple chiral centers may exist in a ds oligonucleotide, e.g., at linkage phosphorus, sugar carbon, etc.). As appreciated by those skilled in the art, a chirally pure ds oligonucleotide is separated from its other stereoisomeric forms (to the extent that some impurities may exist as chemical and biological processes, selectivities and/or purifications etc. rarely, if ever, go to absolute completeness). In a chirally pure ds oligonucleotide, each chiral center is independently defined with respect to its configuration (for a chirally pure ds oligonucleotide, each internucleotidic linkage is independently stereodefined or chirally controlled). In contrast to chirally controlled and chirally pure ds oligonucleotides which comprise stereodefined linkage phosphorus, racemic (or "stereorandom", "non- chirally controlled") ds oligonucleotides comprising chiral linkage phosphorus, e.g., from traditional phosphoramidite oligonucleotide synthesis without stereochemical control during coupling steps in combination with traditional sulfurization (creating stereorandom phosphorothioate internucleotidic linkages), refer to a random mixture of various stereoisomers (typically diastereoisomers (or "diastereomers") as there are multiple chiral centers in a ds oligonucleotide; *e.g.*, from traditional ds oligonucleotide preparation using reagents containing no chiral elements other than those in nucleosides and linkage phosphorus). For example, for A*A*A wherein * is a phosphorothioate internucleotidic linkage (which comprises a chiral linkage phosphorus), a racemic oligonucleotide preparation includes four diastereomers [$2^2 = 4$, considering the two chiral linkage phosphorus, each of which can exist in either of two configurations (*S*p or *R*p)]: A *S A *S A, A *S A *R A, A *R A *S A, and A *R A *R A, wherein *S represents a *S*p phosphorothioate internucleotidic linkage and *R represents a *R*p phosphorothioate internucleotidic linkage. For a chirally pure oligonucleotide, e.g., A *S A *S A, it exists in a single stereoisomeric form and it is separated from the other stereoisomers (e.g., the diastereomers A *S A *R A, A *R A *S A, and A *R A *R A).

In certain embodiments, dsRNAi oligonucleotides comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more stereorandom internucleotidic linkages (mixture of *R*p and *S*p linkage phosphorus at the internucleotidic linkage, e.g., from traditional non-chirally controlled oligonucleotide synthesis). In certain embodiments, dsRNAi oligonucleotides comprise one or more (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more) chirally controlled internucleotidic linkages (*R*p

or *S*p linkage phosphorus at the internucleotidic linkage, e.g., from chirally controlled oligonucleotide synthesis).

In certain embodiments, an internucleotidic linkage is a phosphorothioate internucleotidic linkage. In certain embodiments, an internucleotidic linkage is a stereorandom phosphorothioate internucleotidic linkage. In certain embodiments, an internucleotidic linkage is a chirally controlled phosphorothioate internucleotidic linkage.

Among other things, the present disclosure provides technologies for preparing chirally controlled (in certain embodiments, stereochemically pure) ds oligonucleotides. In certain embodiments, ds oligonucleotides are stereochemically pure. In certain embodiments, ds oligonucleotides of the present disclosure are about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80-100%, 90-100%, 95-100%, 50%-90%, or about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%, or at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%, pure. In certain embodiments, internucleotidic linkages of ds oligonucleotides comprise or consist of one or more (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more) chiral internucleotidic linkages, each of which independently has a diastereopurity of at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%, typically at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%. In certain embodiments, ds oligonucleotides of the present disclosure, e.g., dsRNAi oligonucleotides, have a diastereopurity of $(DS)^{CIL}$, wherein DS is a diastereopurity as described in the present disclosure (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% or more) and CIL is the number of chirally controlled internucleotidic linkages (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more). In certain embodiments, DS is 95%-100%. In certain embodiments, each internucleotidic linkage is independently chirally controlled, and CIL is the number of chirally controlled internucleotidic linkages.

As examples, certain dsRNAi oligonucleotides comprising certain example base sequences, nucleobase modifications and patterns thereof, sugar modifications and patterns thereof, internucleotidic linkages and patterns thereof, linkage phosphorus stereochemistry and patterns thereof, linkers, and/or additional chemical moieties are presented in Table 1A and Table 1B, or Table 1C or Table 1D, below. Among other things,

ds oligonucleotides, e.g., those in Table 1A, may be utilized to target a transcript, e.g., to reduce the level of a transcript and/or a product thereof.

Table 1A. Example Oligonucleotides/Compositions that target Factor VII.

ID	Description	Naked Sequence	Stereochemistry/linkage
WV-34349	mU*fU*mAfAmGfAmCfUmUfGmAmGmAfUmGfAmU fCmCfUmG*mG*mC	UUAAGACUUGAGAUGA UCCUGGC	XXOOOOOOOOOOOOO OOOOOXX
WV-34350	fC*mA*fGmGfAmUfCmAfUfCfUmCfAmAfGmUfCmU fUmAfAL001Mod001	CAGGAUCAUCUCAAGU CUUAA	XXOOOOOOOOOOOOO OOOOOO
WV-34351	mU*SfU*SmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*SmG*SmC	UUAAGACUUGAGAUGA UCCUGGC	SSOOOOOOOOOOOOO OOOOSS
WV-34352	mU*RfU*SmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*SmG*SmC	UUAAGACUUGAGAUGA UCCUGGC	RSOOOOOOOOOOOOO OOOOSS
WV-34353	mU*SfU*RmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*SmG*SmC	UUAAGACUUGAGAUGA UCCUGGC	SROOOOOOOOOOOOO OOOOSS
WV-34354	mU*RfU*RmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*SmG*SmC	UUAAGACUUGAGAUGA UCCUGGC	RROOOOOOOOOOOOO OOOOSS
WV-34355	mU*SfU*SmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*RmG*SmC	UUAAGACUUGAGAUGA UCCUGGC	SSOOOOOOOOOOOOO OOOORS
WV-34356	mU*RfU*SmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*RmG*SmC	UUAAGACUUGAGAUGA UCCUGGC	RSOOOOOOOOOOOOO OOOORS
WV-34357	mU*SfU*RmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*RmG*SmC	UUAAGACUUGAGAUGA UCCUGGC	SROOOOOOOOOOOOO OOOORS
WV-34358	mU*RfU*RmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*RmG*SmC	UUAAGACUUGAGAUGA UCCUGGC	RROOOOOOOOOOOOO OOOORS
WV-34359	mU*SfU*SmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*SmG*RmC	UUAAGACUUGAGAUGA UCCUGGC	SSOOOOOOOOOOOOO OOOOSR
WV-34360	mU*RfU*SmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*SmG*RmC	UUAAGACUUGAGAUGA UCCUGGC	RSOOOOOOOOOOOOO OOOOSR
WV-34361	mU*SfU*RmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*SmG*RmC	UUAAGACUUGAGAUGA UCCUGGC	SROOOOOOOOOOOOO OOOOSR
WV-34362	mU*RfU*RmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*SmG*RmC	UUAAGACUUGAGAUGA UCCUGGC	RROOOOOOOOOOOOO OOOOSR
WV-34363	mU*SfU*SmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*RmG*RmC	UUAAGACUUGAGAUGA UCCUGGC	SSOOOOOOOOOOOOO OOOORR

WV-34364	mU*RfU*SmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*RmG*RmC	UUAAGACUUGAGAUGA UCCUGGC	RSOOOOOOOOOOOOO OOOORR
WV-34365	mU*SfU*RmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*RmG*RmC	UUAAGACUUGAGAUGA UCCUGGC	SROOOOOOOOOOOOO OOOORR
WV-34366	mU*RfU*RmAfAmGfAmCfUmUfGmAmGmAfUmGfA mUfCmCfUmG*RmG*RmC	UUAAGACUUGAGAUGA UCCUGGC	RROOOOOOOOOOOO OOOORR
WV-34367	fC*SmA*SfGmGfAmUfUfCmAfUfCfUmCfAmAfGmUfC mUfUmAfAL001Mod001	CAGGAUCAUCUCAAGU CUUAA	SSOOOOOOOOOOOOO OOOOO
WV-34368	fC*RmA*SfGmGfAmUfUfCmAfUfCfUmCfAmAfGmUfC mUfUmAfAL001Mod001	CAGGAUCAUCUCAAGU CUUAA	RSOOOOOOOOOOOOO OOOOO
WV-34369	fC*SmA*RfGmGfAmUfUfCmAfUfCfUmCfAmAfGmUfC mUfUmAfAL001Mod001	CAGGAUCAUCUCAAGU CUUAA	SROOOOOOOOOOOOO OOOOO
WV-34370	fC*RmA*RfGmGfAmUfUfCmAfUfCfUmCfAmAfGmUfC mUfUmAfAL001Mod001	CAGGAUCAUCUCAAGU CUUAA	RROOOOOOOOOOOO OOOOO

Table 1B, Example oligonucleotides and/or compositions that target mTTR.

ID	Description	Naked Sequence	Stereochemistry/linkage
WV-20167	mU*fU*mAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXOOOOOOOOOOOOO OOOOOXX
WV-20169	mU*SfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSOOOOOOOOOOOOO OOOOSS
WV-20170	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOO OOOOSS
WV-20171	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOO OOOOSS
WV-20172	mU*RfU*RmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RROOOOOOOOOOOO OOOOSS
WV-20173	mU*SfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*RmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSOOOOOOOOOOOOO OOOORS
WV-20174	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*RmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOO OOOORS
WV-20175	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*RmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOO OOOORS

WV-20176	mU*RfU*RmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmU*RmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RROOOOOOOOOOOOO OOOOORS
WV-20177	mU*SfU*SmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmU*SmU*RmU	UUAUAGAGCAAGAACA CUGUUUU	SSOOOOOOOOOOOOO OOOOSR
WV-20178	mU*RfU*SmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmU*SmU*RmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOOOSR
WV-20179	mU*SfU*RmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmU*SmU*RmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OOOOOSR
WV-20180	mU*RfU*RmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmU*SmU*RmU	UUAUAGAGCAAGAACA CUGUUUU	RROOOOOOOOOOOOO OOOOOSR
WV-20181	mU*SfU*SmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmU*RmU*RmU	UUAUAGAGCAAGAACA CUGUUUU	SSOOOOOOOOOOOOO OOOORR
WV-20182	mU*RfU*SmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmU*RmU*RmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOORR
WV-20183	mU*SfU*RmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmU*RmU*RmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OOOORR
WV-20184	mU*RfU*RmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmU*RmU*RmU	UUAUAGAGCAAGAACA CUGUUUU	RROOOOOOOOOOOOO OOOORR
WV-28310	fA*mA*fCmAfGmUfGmUfUfCfUmUfGmCfUmCfUmA fUmAfAL001Mod001	AACAGUGUUCUUGCUC UAUAA	XXOOOOOOOOOOOOO OOOOOO
WV-28311	fA*SmA*SfCmAfGmUfGmUfUfCfUmUfGmCfUmCfU mAfUmAfAL001Mod001	AACAGUGUUCUUGCUC UAUAA	SSOOOOOOOOOOOOO OOOOO
WV-28312	fA*RmA*SfCmAfGmUfGmUfUfCfUmUfGmCfUmCfU mAfUmAfAL001Mod001	AACAGUGUUCUUGCUC UAUAA	RSOOOOOOOOOOOOO OOOOOO
WV-28313	fA*SmA*RfCmAfGmUfGmUfUfCfUmUfGmCfUmCfU mAfUmAfAL001Mod001	AACAGUGUUCUUGCUC UAUAA	SROOOOOOOOOOOOO OOOOOO
WV-28314	fA*RmA*RfCmAfGmUfGmUfUfCfUmUfGmCfUmCfU mAfUmAfAL001Mod001	AACAGUGUUCUUGCUC UAUAA	RROOOOOOOOOOOOO OOOOOO
WV-36831	mUn001fUn001mAfUmAfGmAfGmCfCfAmAmGmAfAm CfAmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	nXnXOOOOOOOOOOOO OOOOOOXX
WV-36832	mU*fU*mAfUmAfGmAfGmCfCfAmAmGmAfAmCfAmC fUmGfUmUn001mUn001mU	UUAUAGAGCAAGAACA CUGUUUU	XXOOOOOOOOOOOOO OOOOOnXnX
WV-36833	mUn001fU*mAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmU*mUn001mU	UUAUAGAGCAAGAACA CUGUUUU	nXXOOOOOOOOOOOO OOOOOOXnX

WV-36834	mUn001fUmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmUmUn001mU	UUAUAGAGCAAGAACA CUGUUUU	nXOOOOOOOOOOOO OOOOOOOnX
WV-36835	mUn001fUn001mAfUmAfGmAfGmCfAmAmGmAfAm CfAmCfUmGfUmUn001mUn001mU	UUAUAGAGCAAGAACA CUGUUUU	nXnXOOOOOOOOOOO OOOOOOnXnX
WV-36836	mUn001fU*mAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	nXXOOOOOOOOOOO OOOOOOXX
WV-36837	mU*fUn001mAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XnXOOOOOOOOOOO OOOOOOXX
WV-36838	mU*fU*mAn001fUmAfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXnXOOOOOOOOOO OOOOOOXX
WV-36839	mU*fU*mAfUn001mAfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXOnXOOOOOOOOO OOOOOOXX
WV-36840	mU*fU*mAfUmAn001fGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XX0OnXOOOOOOOO OOOOOOXX
WV-36841	mU*fU*mAfUmAfGn001mAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XX0OOnXOOOOOOO OOOOOOXX
WV-36842	mU*fU*mAfUmAfGmAn001fGmCfAmAmGmAfAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XX0OOOnXOOOOOO OOOOOOXX
WV-36843	mU*fU*mAfUmAfGmAfGn001mCfAmAmGmAfAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XX0OOOOnXOOOOO OOOOOOXX
WV-36844	mU*fU*mAfUmAfGmAfGmCn001fAmAmGmAfAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XX0OOOOOnXOOOO OOOOOOXX
WV-36845	mU*fU*mAfUmAfGmAfGmCfAn001mAmGmAfAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XX0OOOOOOnXOOOO OOOOOOXX
WV-36846	mU*fU*mAfUmAfGmAfGmCfAmAn001mGmAfAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XX0OOOOOOOnXOOO OOOOOOXX
WV-36847	mU*fU*mAfUmAfGmAfGmCfAmAmGn001mAfAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XX0OOOOOOOOnXOO OOOOOOXX
WV-36848	mU*fU*mAfUmAfGmAfGmCfAmAmGmAn001fAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XX0OOOOOOOOOnXO OOOOOOXX
WV-36849	mU*fU*mAfUmAfGmAfGmCfAmAmGmAfAn001mCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XX0OOOOOOOOOOnX OOOOOOXX
WV-36850	mU*fU*mAfUmAfGmAfGmCfAmAmGmAfAmCn001f AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XX0OOOOOOOOOOOn XOOOOOXX

WV-36851	mU*fU*mAfUmAfGmAfGmCfAmAmGmAfAmCfAn00 1mCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXOOOOOOOOOOOOO nXOOOOXX
WV-36852	mU*fU*mAfUmAfGmAfGmCfAmAmGmAfAmCfAmC n001fUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXOOOOOOOOOOOOO OnXOOOXX
WV-36853	mU*fU*mAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUn001mGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXOOOOOOOOOOOOO OOnXOOOXX
WV-36854	mU*fU*mAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGn001fUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXOOOOOOOOOOOOO OOOnXOXX
WV-36855	mU*fU*mAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGfUn001mU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXOOOOOOOOOOOOO OOOOnXXX
WV-36856	mU*fU*mAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGfUmUn001mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXOOOOOOOOOOOOO OOOOOnXX
WV-36857	mU*fU*mAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGfUmU*mUn001mU	UUAUAGAGCAAGAACA CUGUUUU	XXOOOOOOOOOOOOO OOOOOXnX
WV-36858	mAmAfCmAfGmUfGmUfUfCfUmUfGmCfUmCfUmAf UmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOOO OOOOOXX
WV-36859	mAmAfCmAfGmUfGmUfUfCfUmUfGmCfUmCfUmAf UmAfAn001mUn001mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOOO OOOOOnXnX
WV-36860	Mod001L001mAmAfCmAfGmUfGmUfUfCfUmUfGmC fUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOOO OOOOOOXX
WV-36861	Mod001L001mAmAfCmAfGmUfGmUfUfCfUmUfGmC fUmCfUmAfUmAfAn001mUn001mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOOO OOOOOOnXnX
WV-37856	mU*RfU*SmAn001fUmAfGmAfGn001mCfAmAmGmA fAmCfAmCfUmGfUn001mU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnXOOOOnXOOOOOO OOOOOnXSS
WV-37857	mU*RfU*SmAn001fUmAfGmAn001fGmCfAmAmGmA fAmCfAmCfUmGfUn001mU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnXOOOnXOOOOOO OOOOOnXSS
WV-37874	mU*RfU*SmAn001RfUmAfGmAfGn001RmCfAmAmG mAfAmCfAmCfUmGfUn001RmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOnROOOOO OOOOOnRSS
WV-37875	mU*RfU*SmAn001RfUmAfGmAn001RfGmCfAmAmG mAfAmCfAmCfUmGfUn001RmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOnROOOOOO OOOOOnRSS
WV-37892	mU*RfU*SmAn001SfUmAfGmAfGn001SmCfAmAmG mAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOnSOOOOO OOOOOnSSS
WV-37893	mU*RfU*SmAn001SfUmAfGmAn001SfGmCfAmAmG mAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOnSOOOOOO OOOOOnSSS

WV-38058	Mod001L001mAn001mAfCmAfGmUfUfCfUmUf GmCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OnXOOOOOOOOOOO OOOOOOOXX
WV-38059	Mod001L001mAmAn001fCmAfGmUfGmUfUfCfUmUf GmCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOnXOOOOOOOOOO OOOOOOOXX
WV-38060	Mod001L001mAmAfCn001mAfGmUfGmUfUfCfUmUf GmCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOnXOOOOOOOOO OOOOOOOXX
WV-38061	Mod001L001mAmAfCmAn001fGmUfGmUfUfCfUmUf GmCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOnXOOOOOOOO OOOOOOOXX
WV-38062	Mod001L001mAmAfCmAfGn001mUfGmUfUfCfUmUf GmCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOnXOOOOOOO OOOOOOOXX
WV-38063	Mod001L001mAmAfCmAfGmUn001fGmUfUfCfUmUf GmCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOnXOOOOOO OOOOOOOXX
WV-38064	Mod001L001mAmAfCmAfGmUfGn001mUfUfCfUmUf GmCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOnXOOOOO OOOOOOOXX
WV-38065	Mod001L001mAmAfCmAfGmUfGmUn001fUfCfUmUf GmCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOnXOOOO OOOOOOOXX
WV-38066	Mod001L001mAmAfCmAfGmUfGmUfUn001fCfUmUf GmCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOnXOOO OOOOOOOXX
WV-38067	Mod001L001mAmAfCmAfGmUfGmUfUfCn001fUmUf GmCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOnXOO OOOOOOOXX
WV-38068	Mod001L001mAmAfCmAfGmUfGmUfUfCfUn001mUf GmCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOnXO OOOOOOOXX
WV-38069	Mod001L001mAmAfCmAfGmUfGmUfUfCfUmUn001f GmCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOnX OOOOOOOXX
WV-38070	Mod001L001mAmAfCmAfGmUfGmUfUfCfUmUfGn00 1mCfUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOnX OOOOOOOXX
WV-38071	Mod001L001mAmAfCmAfGmUfGmUfUfCfUmUfGmC n001fUmCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOn XOOOOOOOXX
WV-38072	Mod001L001mAmAfCmAfGmUfGmUfUfCfUmUfGmC fUn001mCfUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOOO nXOOOOOXX
WV-38073	Mod001L001mAmAfCmAfGmUfGmUfUfCfUmUfGmC fUmCn001fUmAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOOO OnXOOOOXX
WV-38074	Mod001L001mAmAfCmAfGmUfGmUfUfCfUmUfGmC fUmCfUn001mAfUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOOO OOnXOOOXX

WV-38075	Mod001L001mAmAfCmAfGmUfUfCfUmUfGmC fUmCfUmAfUn001fUmAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOOO OOOnXOOXX
WV-38076	Mod001L001mAmAfCmAfGmUfUfCfUmUfGmC fUmCfUmAfUn001mAfA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOOO OOOOnXOXX
WV-38077	Mod001L001mAmAfCmAfGmUfUfCfUmUfGmC fUmCfUmAfUmAn001fA*mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOOO OOOOOnXXX
WV-38078	Mod001L001mAmAfCmAfGmUfUfCfUmUfGmC fUmCfUmAfUmAfAn001mU*mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOOO OOOOOOnXX
WV-38079	Mod001L001mAmAfCmAfGmUfUfCfUmUfGmC fUmCfUmAfUmAfA*mUn001mU	AACAGUGUUCUUGCUC UAUAAUU	OOOOOOOOOOOOOOO OOOOOOXnX
WV-38703	mU*RfU*SmAn001fUmAfGmAfGmCfAn001mAmGmA fAmCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnXOOOOOOnXOOOO OOOOOOSS
WV-38704	mU*RfU*SmAn001fUmAfGmAfGmCfAn001mAmGmA fAmCfAmCfUmGn001fUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnXOOOOOOnXOOOO OOOOnXOSS
WV-38705	mU*RfU*SmAn001RfUmAfGmAfGmCfAn001RmAmG mAfAmCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOOOnROOOO OOOOOOSS
WV-38706	mU*RfU*SmAn001RfUmAfGmAfGmCfAn001RmAmG mAfAmCfAmCfUmGn001RfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOOOnROOOO OOOOnROSS
WV-38707	mU*RfU*SmAn001SfUmAfGmAfGmCfAn001SmAmG mAfAmCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOnSOOOO OOOOOOSS
WV-38708	mU*RfU*SmAn001SfUmAfGmAfGmCfAn001SmAmG mAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOnSOOOO OOOOnSOSS

Table 1C, Example oligonucleotides and/or compositions that target mTTR.

ID	Description	Naked Sequence	Stereochemistry/linkage
WV-39029	Mod001L001mAmAfCmAfGmUfGmUfUfCfUmUfGmC fUmCfUmAfUmAfA	AACAGUGUUCUUGCUC UAUAA	OOOOOOOOOOOOOOO OOOOOO
WV-39058	L001mAmAfCmAfGmUfGmUfUfCfUmUfGmCfUmCfU mAfUmAfA	AACAGUGUUCUUGCUC UAUAA	OOOOOOOOOOOOOOO OOOOOO
WV-39295	mU*5MSfU*mAfUmAfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXOOOOOOOOOOOOO OOOOOXX

WV-39296	mU*5MRfU*mAfUmAfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXOOOOOOOOOOOOO OOOOOXX
WV-39297	mU*S5MSfU*SmAfUmAfGmAfGmCfAmAmGmAfAm CfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSOOOOOOOOOOOOO OOOOSS
WV-39298	mU*S5MRfU*SmAfUmAfGmAfGmCfAmAmGmAfAm CfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSOOOOOOOOOOOOO OOOOSS
WV-39299	mU*R5MSfU*SmAfUmAfGmAfGmCfAmAmGmAfAm CfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOOSS
WV-39300	mU*R5MRfU*SmAfUmAfGmAfGmCfAmAmGmAfAm CfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOOSS
WV-40362	Mod001L001mA*mAfCmAfGmUfGmUfUfCfUmUfGm CfUmCfUmAfUmA*fA	AACAGUGUUCUUGCUC UAUAA	OXOOOOOOOOOOOOO OOOOOX
WV-40363	Mod001L001mA*SmAfCmAfGmUfGmUfUfCfUmUfG mCfUmCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOOOOOOOOO OOOOOS
WV-40364	Mod001L001mA*SmAfCmAfGmUfGmUfUfCfUmUfG mCfUmCfUmAfUmA*RfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOOOOOOOOO OOOOOR
WV-40365	Mod001L001mA*RmAfCmAfGmUfGmUfUfCfUmUfG mCfUmCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OROOOOOOOOOOOOO OOOOOS
WV-40366	Mod001L001mA*RmAfCmAfGmUfGmUfUfCfUmUfG mCfUmCfUmAfUmA*RfA	AACAGUGUUCUUGCUC UAUAA	OROOOOOOOOOOOOO OOOOOR
WV-40367	L001mA*mAfCmAfGmUfGmUfUfCfUmUfGmCfUmCf UmAfUmA*fA	AACAGUGUUCUUGCUC UAUAA	OXOOOOOOOOOOOOO OOOOOX
WV-40368	L001mA*SmAfCmAfGmUfGmUfUfCfUmUfGmCfUmC fUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOOOOOOOOO OOOOOS
WV-40369	L001mA*SmAfCmAfGmUfGmUfUfCfUmUfGmCfUmC fUmAfUmA*RfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOOOOOOOOO OOOOOR
WV-40370	L001mA*RmAfCmAfGmUfGmUfUfCfUmUfGmCfUm CfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OROOOOOOOOOOOOO OOOOOS
WV-40371	L001mA*RmAfCmAfGmUfGmUfUfCfUmUfGmCfUm CfUmAfUmA*RfA	AACAGUGUUCUUGCUC UAUAA	OROOOOOOOOOOOOO OOOOOR
WV-40400	fU*fU*mAfUmAfGmAfGmCfAmAmGmAfAmCfAmCf UmG*mU*mU	UUAUAGAGCAAGAACA CUGUU	XXOOOOOOOOOOOOO OOOXX
WV-40401	fCmAfGmUfGmUfUfCfUmUfGmCfUmCfUmAfUmAfA	CAGUGUUCUUGCUCUA UAA	OOOOOOOOOOOOOOO OOO

WV-40539	mU*RfU*SmAn001SfUmAfGmAfGn001RmCfAmAmG mAfAmCfAmCfUmGfUn001RmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOnROOOOO OOOOOnRSS
WV-40540	mU*RfU*SmAn001RfUmAfGmAfGn001SmCfAmAmG mAfAmCfAmCfUmGfUn001RmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOnSOOOOO OOOOOnRSS
WV-40541	mU*RfU*SmAn001RfUmAfGmAfGn001RmCfAmAmG mAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOnROOOOO OOOOOnSSS
WV-40542	mU*RfU*SmAn001SfUmAfGmAfGn001SmCfAmAmG mAfAmCfAmCfUmGfUn001RmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOnSOOOOO OOOOOnRSS
WV-40543	mU*RfU*SmAn001RfUmAfGmAfGn001SmCfAmAmG mAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOnSOOOOO OOOOOnSSS
WV-40544	mU*RfU*SmAn001SfUmAfGmAfGn001RmCfAmAmG mAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOnROOOOO OOOOOnSSS
WV-40545	mU*RfU*SmAn001SfUmAfGmAn001RfGmCfAmAmG mAfAmCfAmCfUmGfUn001RmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOnROOOOOO OOOOOnRSS
WV-40546	mU*RfU*SmAn001RfUmAfGmAn001SfGmCfAmAmG mAfAmCfAmCfUmGfUn001RmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOnSOOOOOO OOOOOnRSS
WV-40547	mU*RfU*SmAn001RfUmAfGmAn001RfGmCfAmAmG mAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOnROOOOOO OOOOOnSSS
WV-40548	mU*RfU*SmAn001SfUmAfGmAn001SfGmCfAmAmG mAfAmCfAmCfUmGfUn001RmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOnSOOOOOO OOOOOnRSS
WV-40549	mU*RfU*SmAn001RfUmAfGmAn001SfGmCfAmAmG mAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOnSOOOOOO OOOOOnSSS
WV-40550	mU*RfU*SmAn001SfUmAfGmAn001RfGmCfAmAmG mAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOnROOOOOO OOOOOnSSS
WV-40551	mU*SfU*SmAn001RfUmAfGmAfGmCfAn001RmAmG mAfAmCfAmCfUmGn001RfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSnROOOOOOnROOOO OOOOnROSS
WV-40552	mU*RfU*SmAn001SfUmAfGmAfGmCfAn001RmAmG mAfAmCfAmCfUmGn001RfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOnROOOO OOOOnROSS
WV-40553	mU*RfU*SmAn001RfUmAfGmAfGmCfAn001RmAmG mAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOOOnROOOO OOOOnSOSS
WV-40554	mU*SfU*SmAn001SfUmAfGmAfGmCfAn001RmAmG mAfAmCfAmCfUmGn001RfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSnSOOOOOOnROOOO OOOOnROSS
WV-40555	mU*RfU*SmAn001RfUmAfGmAfGmCfAn001SmAmG mAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOOOnSOOOO OOOOnSOSS

WV-40556	mU*RfU*SmAn001SfUmAfGmAfGmCfAn001RmAmG mAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOnROOOO OOOOnSOSS
WV-40796	mU*RfU*SmAn001RfUmAfGmAfGmCfAn001SmAmG mAfAmCfAmCfUmGn001RfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOOOnSOOOO OOOOnROSS
WV-40797	mU*RfU*SmAn001SfUmAfGmAfGmCfAn001SmAmG mAfAmCfAmCfUmGn001RfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOnSOOOO OOOOnROSS
WV-40797	mU*RfU*SmAn001SfUmAfGmAfGmCfAn001SmAmG mAfAmCfAmCfUmGn001RfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOnSOOOO OOOOnROSS
WV-40836	mU*RfU*SmAn001SfUmAfGmAn001SfGmCfAn001Sm AmGmAfAmCfAmCfUn001SmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOnSOOnSOOOO OOOnSOOSS
WV-40837	mU*RfU*SmAn001SfUmAfGmAfGn001SmCfAn001Sm AmGmAfAmCfAmCfUn001SmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOnSOnSOOOO OOOnSOOSS
WV-40838	mU*RfU*SmAn001SfUmAfGmAn001SfGmCfAn001Sm AmGmAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOnSOOnSOOOO OOOOnSOSS
WV-40839	mU*RfU*SmAn001SfUmAfGmAfGn001SmCfAn001Sm AmGmAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOnSOnSOOOO OOOOnSOSS
WV-37896	mU*RfU*SmAn001SfUmAfGmAn001SfGmCfAn001Sm AmGmAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOnSOOnSOOOO OOOOOnSSS
WV-37900	mU*RfU*SmAn001SfUmAfGmAfGn001SmCfAn001Sm AmGmAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOnSOnSOOOO OOOOOnSSS
WV-40840	mU*RfU*SmAn001SfUmAfGmAn001SfGmCfAn001Sm AmGmAfAmCfAmCfAn001SmCfUn001SmGfUmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOnSOOnSOOOO OnSOnSOOSS
WV-40841	mU*RfU*SmAn001SfUmAfGmAfGn001SmCfAn001Sm AmGmAfAmCfAmCfAn001SmCfUn001SmGfUmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOnSOnSOOOO OnSOnSOOSS
WV-40842	mU*RfU*SmAn001SfUmAfGmAn001SfGmCfAn001Sm AmGmAfAmCfAmCfAn001SmCfUmGn001SfUmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOnSOOnSOOOO OnSOOnSOSS
WV-40843	mU*RfU*SmAn001SfUmAfGmAfGn001SmCfAn001Sm AmGmAfAmCfAmCfAn001SmCfUmGn001SfUmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOnSOnSOOOO OnSOOnSOSS
WV-40844	mU*RfU*SmAn001SfUmAfGmAn001SfGmCfAn001Sm AmGmAfAmCfAmCfAn001SmCfUmGfUn001SmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOnSOOnSOOOO OnSOOOnSSS

WV-40845	mU*RfU*SmAn001SfUmAfGmAfGn001SmCfAn001Sm AmGmAfAmCfAn001SmCfUmGfUn001SmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOnSOnSOOOO OnSOOOnSSS
WV-40846	mU*SfU*RmAn001SfUmAfGmAn001SfGmCfAn001Sm AmGmAfAmCfAmCfUn001SmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOnSOOnSOOOO OOOnSOOSS
WV-40847	mU*SfU*RmAn001SfUmAfGmAfGn001SmCfAn001Sm AmGmAfAmCfAmCfUn001SmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOnSOnSOOOO OOOnSOOSS
WV-40848	mU*SfU*RmAn001SfUmAfGmAn001SfGmCfAn001Sm AmGmAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOnSOOnSOOOO OOOOnSOSS
WV-40849	mU*SfU*RmAn001SfUmAfGmAfGn001SmCfAn001Sm AmGmAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOnSOnSOOOO OOOOnSOSS
WV-40850	mU*SfU*RmAn001SfUmAfGmAn001SfGmCfAn001Sm AmGmAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOnSOOnSOOOO OOOOOnSSS
WV-40851	mU*SfU*RmAn001SfUmAfGmAfGn001SmCfAn001Sm AmGmAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOnSOnSOOOO OOOOOnSSS
WV-40852	mU*SfU*RmAn001SfUmAfGmAn001SfGmCfAn001Sm AmGmAfAmCfAn001SmCfUn001SmGfUmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOnSOOnSOOOO OnSOnSOOSS
WV-40853	mU*SfU*RmAn001SfUmAfGmAfGn001SmCfAn001Sm AmGmAfAmCfAn001SmCfUn001SmGfUmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOnSOnSOOOO OnSOnSOOSS
WV-40854	mU*SfU*RmAn001SfUmAfGmAn001SfGmCfAn001Sm AmGmAfAmCfAn001SmCfUmGn001SfUmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOnSOOnSOOOO OnSOOnSOSS
WV-40855	mU*SfU*RmAn001SfUmAfGmAfGn001SmCfAn001Sm AmGmAfAmCfAn001SmCfUmGn001SfUmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOnSOnSOOOO OnSOOnSOSS
WV-40856	mU*SfU*RmAn001SfUmAfGmAn001SfGmCfAn001Sm AmGmAfAmCfAn001SmCfUmGfUn001SmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOnSOOnSOOOO OnSOOOnSSS
WV-40857	mU*SfU*RmAn001SfUmAfGmAfGn001SmCfAn001Sm AmGmAfAmCfAn001SmCfUmGfUn001SmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOnSOnSOOOO OnSOOOnSSS
WV-40858	mU*SfUmAn001SfUmAfGmAn001SfGmCfAn001SmA mGmAfAmCfAmCfUn001SmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOnSOOnSOOOO OOOnSOOSS

WV-40859	mU*SfUmAn001SfUmAfGmAfGn001SmCfAn001SmA mGmAfAmCfAmCfUn001SmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOOnSOnSOOOO OOOnSOOSS
WV-40860	mU*SfUmAn001SfUmAfGmAn001SfGmCfAn001SmA mGmAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOnSOOnSOOOO OOOOnSOSS
WV-40861	mU*SfUmAn001SfUmAfGmAfGn001SmCfAn001SmA mGmAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOOnSOnSOOOO OOOOnSOSS
WV-40862	mU*SfUmAn001SfUmAfGmAn001SfGmCfAn001SmA mGmAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOnSOOnSOOOO OOOOOnSSS
WV-40863	mU*SfUmAn001SfUmAfGmAfGn001SmCfAn001SmA mGmAfAmCfAmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOOnSOnSOOOO OOOOOnSSS
WV-40864	mU*SfUmAn001SfUmAfGmAn001SfGmCfAn001SmA mGmAfAmCfAn001SmCfUn001SmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOnSOOnSOOOO OnSOnSOOSS
WV-40865	mU*SfUmAn001SfUmAfGmAfGn001SmCfAn001SmA mGmAfAmCfAn001SmCfUn001SmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOOnSOnSOOOO OnSOnSOOSS
WV-40866	mU*SfUmAn001SfUmAfGmAn001SfGmCfAn001SmA mGmAfAmCfAn001SmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOnSOOnSOOOO OnSOOnSOSS
WV-40867	mU*SfUmAn001SfUmAfGmAfGn001SmCfAn001SmA mGmAfAmCfAn001SmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOOnSOnSOOOO OnSOOnSOSS
WV-40868	mU*SfUmAn001SfUmAfGmAn001SfGmCfAn001SmA mGmAfAmCfAn001SmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOnSOOnSOOOO OnSOOOnSSS
WV-40869	mU*SfUmAn001SfUmAfGmAfGn001SmCfAn001SmA mGmAfAmCfAn001SmCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOOnSOnSOOOO OnSOOOnSSS
WV-40870	Mod001L001mA*SmAfCmAfGmUfGn001mUfUfCfCfUm UfGmCfUn001mCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOnXOOOOOOO nXOOOOS
WV-40871	Mod001L001mA*SmAfCmAfGmUfGn001RmUfUfCfU mUfGmCfUn001RmCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOnROOOOOOO nROOOOS
WV-40872	Mod001L001mA*SmAfCmAfGmUfGn001SmUfUfCfU mUfGmCfUn001SmCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOnSOOOOOOO nSOOOOS
WV-40873	Mod001L001mA*SmAfCmAfGmUfGn001mUfUfCfUm UfGmCfUn001mCfUn001mAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOnXOOOOOOO nXOnXOOOS
WV-40874	Mod001L001mA*SmAfCmAfGmUfGn001RmUfUfCfU mUfGmCfUn001RmCfUn001RmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOnROOOOOOO nROnROOS
WV-40875	Mod001L001mA*SmAfCmAfGmUfGn001SmUfUfCfU mUfGmCfUn001SmCfUn001SmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOnSOOOOOOO nSOnSOOS

WV-40876	Mod001L001mA*SmAfCmAn001fGmUfGn001mUfUfC fUmUfGmCfUn001mCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOnXOOnXOOOOOO OnXOOOOS
WV-40877	Mod001L001mA*SmAfCmAn001RfGmUfGn001RmUf UfCfUmUfGmCfUn001RmCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOnROOnROOOOOO OnROOOOS
WV-40878	Mod001L001mA*SmAfCmAn001SfGmUfGn001SmUfU fCfUmUfGmCfUn001SmCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOnSOOnSOOOOOO OnSOOOOS
WV-40879	Mod001L001mA*SmAfCmAfGmUfGn001mUfUfCfUm UfGmCfUn001mCfUmAfUn001mA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOnXOOOOOOO nXOOOnXS
WV-40880	Mod001L001mA*SmAfCmAfGmUfGn001RmUfUfCfU mUfGmCfUn001RmCfUmAfUn001RmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOnROOOOOOO nROOOnRS
WV-40881	Mod001L001mA*SmAfCmAfGmUfGn001SmUfUfCfU mUfGmCfUn001SmCfUmAfUn001SmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOnSOOOOOOO nSOOOnSS
WV-40882	Mod001L001mAnf001mAfCmAfGmUfGn001mUfUfCfU mUfGmCfUn001mCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OnXOOOOOnXOOOOOO OnXOOOOS
WV-40883	Mod001L001mAn001RmAfCmAfGmUfGn001RmUfUf CfUmUfGmCfUn001RmCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OnROOOOOnROOOOOO OnROOOOS
WV-40884	Mod001L001mAn001SmAfCmAfGmUfGn001SmUfUfC fUmUfGmCfUn001SmCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OnSOOOOOnSOOOOOO OnSOOOOS
WV-40885	Mod001L001mA*SmAfCmAfGmUfGn001mUfUfCfUm UfGmCfUn001mCfUmAfUmAn001fA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOnXOOOOOOO nXOOOOnX
WV-40886	Mod001L001mA*SmAfCmAfGmUfGn001RmUfUfCfU mUfGmCfUn001RmCfUmAfUmAn001RfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOnROOOOOOO nROOOOnR
WV-40887	Mod001L001mA*SmAfCmAfGmUfGn001SmUfUfCfU mUfGmCfUn001SmCfUmAfUmAn001SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOnSOOOOOOO nSOOOOnS
WV-40888	Mod001L001mAn001mAfCmAfGmUfGn001mUfUfCfU mUfGmCfUn001mCfUmAfUmAn001fA	AACAGUGUUCUUGCUC UAUAA	OnXOOOOOnXOOOOOO OnXOOOOnX
WV-40889	Mod001L001mAn001RmAfCmAfGmUfGn001RmUfUf CfUmUfGmCfUn001RmCfUmAfUmAn001RfA	AACAGUGUUCUUGCUC UAUAA	OnROOOOOnROOOOOO OnROOOOnR
WV-40890	Mod001L001mAn001SmAfCmAfGmUfGn001SmUfUfC fUmUfGmCfUn001SmCfUmAfUmAn001SfA	AACAGUGUUCUUGCUC UAUAA	OnSOOOOOnSOOOOOO OnSOOOOnS
WV-41318	mU*fU*mA*fU*mA*fG*mA*fG*mCfAmAmGmAfAm CfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	XXXXXXXXOOOOOOO OOOOOSS
WV-40877	Mod001L001mA*SmAfCmAn001RfGmUfGn001RmUf UfCfUmUfGmCfUn001RmCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOnROOnROOOOOO OnROOOOS

WV-41389	mA*SmAfCmAn001RfGmUfGn001RmUfUfCfUmUfGmCfUn001RmCfUmAfUmA*SfA	AACAGUGUUCUUGCUCUAUAA	SOOnROOnROOOOOOnR0OOOS
WV-41390	L025mA*SmAfCmAn001RfGmUfGn001RmUfUfCfUmUfGmCfUn001RmCfUmAfUmA*SfA	AACAGUGUUCUUGCUCUAUAA	OSOOnROOnROOOOOOOnROOOOS
WV-41391	L025L025mA*SmAfCmAn001RfGmUfGn001RmUfUfCfUmUfGmCfUn001RmCfUmAfUmA*SfA	AACAGUGUUCUUGCUCUAUAA	OOSOOnROOnROOOOOOnR0OOOS
WV-41392	L025L025L025mA*SmAfCmAn001RfGmUfGn001RmUfUfCfUmUfGmCfUn001RmCfUmAfUmA*SfA	AACAGUGUUCUUGCUCUAUAA	OOOSOOnROOnROOOOOOnR0OOOS
WV-40889	Mod001L001mAn001RmAfCmAfGmUfGn001RmUfUfCfUmUfGmCfUn001RmCfUmAfUmAn001RfA	AACAGUGUUCUUGCUCUAUAA	OnROOOOOnROOOOOOnR0OOOnR
WV-41393	mAn001RmAfCmAfGmUfGn001RmUfUfCfUmUfGmCfUn001RmCfUmAfUmAn001RfA	AACAGUGUUCUUGCUCUAUAA	nROOOOOnROOOOOOnR0OOOnR
WV-41394	L025mAn001RmAfCmAfGmUfGn001RmUfUfCfUmUfGmCfUn001RmCfUmAfUmAn001RfA	AACAGUGUUCUUGCUCUAUAA	OnROOOOOnROOOOOOnR0OOOnR
WV-41395	L025L025mAn001RmAfCmAfGmUfGn001RmUfUfCfUmUfGmCfUn001RmCfUmAfUmAn001RfA	AACAGUGUUCUUGCUCUAUAA	OOnROOOOOnROOOOOOnR0OOOnR
WV-41396	L025L025L025mAn001RmAfCmAfGmUfGn001RmUfUfCfUmUfGmCfUn001RmCfUmAfUmAn001RfA	AACAGUGUUCUUGCUCUAUAA	OOOnROOOOOnROOOOOOnR0OOOnR
WV-41397	mA*SmAfCmAfGmUfGmUfUfCfUmUfGmCfUmCfUmAfUmA*SfA	AACAGUGUUCUUGCUCUAUAA	SOOOOOOOOOOOOOOOOOOOS
WV-41398	L025mA*SmAfCmAfGmUfGmUfUfCfUmUfGmCfUmCfUmAfUmA*SfA	AACAGUGUUCUUGCUCUAUAA	OSOOOOOOOOOOOOOOOOOOOS
WV-41399	L025L025mA*SmAfCmAfGmUfGmUfUfCfUmUfGmCfUmCfUmAfUmA*SfA	AACAGUGUUCUUGCUCUAUAA	OOSOOOOOOOOOOOOOOOOOOS
WV-41400	L025L025L025mA*SmAfCmAfGmUfGmUfUfCfUmUfGmCfUmCfUmAfUmA*SfA	AACAGUGUUCUUGCUCUAUAA	OOOSOOOOOOOOOOOOOOOOOS
WV-41825	mU*fU*mAmUmAfGmAfGfCmAmAmGmAfAmCfAmCmUmGmUmU*mU*mU	UUAUAGAGCAAGAACACUGUUUU	XXOOOOOOOOOOOOOOOOOOOO
WV-41826	mU*fU*mAmUmAfGmAmGmCmAmAmGmAfAmCfAmCnUmGmUmU*mU*mU	UUAUAGAGCAAGAACACUGUUUU	XXOOOOOOOOOOOOOOOOOOOO
WV-41827	mA*mA*mCmAmGmUfGmUfUfCfUmUmGmCmUmCmUmAmUmAmAL001Mod001	AACAGUGUUCUUGCUCUAUAA	XXOOOOOOOOOOOOOOOOOOOO
WV-41828	Mod001L001mA*mAmCmAmGmUfGmUfUfCfUmUmGmCmUmCmUmAmUmA*mA	AACAGUGUUCUUGCUCUAUAA	OXOOOOOOOOOOOOOOOOOOOX

WV-41829	mA*mA*mCmAmGmUfGmUfUfCfUmUmGmCmUmC mUmAmUmAmAL001	AACAGUGUUCUUGCUC UAUAA	XXOOOOOOOOOOOOO OOOOO
WV-41830	L001mA*mAmCmAmGmUfGmUfUfCfUmUmGmCmU mCmUmAmUmA*mA	AACAGUGUUCUUGCUC UAUAA	OXOOOOOOOOOOOOO OOOOX
WV-41841	mU*fU*mA*fUmA*fGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	XXXOXOOOOOOOOOO OOOOSS
WV-41842	mU*RfU*RmA*RfUmA*RfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RRROROOOOOOOOOO OOOOSS
WV-41843	mU*SfU*RmA*RfUmA*RfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRROROOOOOOOOOO OOOOSS
WV-41844	mU*RfU*SmA*RfUmA*RfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSROROOOOOOOOOO OOOOSS
WV-41845	mU*SfU*SmA*RfUmA*RfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSROROOOOOOOOOO OOOOSS
WV-41846	mU*RfU*RmA*SfUmA*RfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RRSOROOOOOOOOOO OOOOSS
WV-41847	mU*SfU*RmA*SfUmA*RfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRSOROOOOOOOOOO OOOOSS
WV-41848	mU*RfU*SmA*SfUmA*RfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSSOROOOOOOOOOO OOOOSS
WV-41849	mU*SfU*SmA*SfUmA*RfGmAfGmCfAmAmGmAfAm CfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSSOROOOOOOOOOO OOOOSS
WV-41850	mU*RfU*RmA*RfUmA*SfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RRRSOOOOOOOOOO OOOOSS
WV-41851	mU*SfU*RmA*RfUmA*SfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRRSOOOOOOOOOO OOOOSS
WV-41852	mU*RfU*SmA*RfUmA*SfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSRSOOOOOOOOOO OOOOSS
WV-41853	mU*SfU*SmA*RfUmA*SfGmAfGmCfAmAmGmAfAm CfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSRSOOOOOOOOOO OOOOSS
WV-41854	mU*RfU*RmA*SfUmA*SfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RRSOSOOOOOOOOO OOOOSS
WV-41855	mU*SfU*RmA*SfUmA*SfGmAfGmCfAmAmGmAfAm CfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRSOSOOOOOOOOO OOOOSS

WV-41856	mU*RfU*SmA*SfUmA*SfGmAfGmCfAmAmGmAfAm CfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSSOSOOOOOOOOOO OOOOSS
WV-41857	mU*SfU*SmA*SfUmA*SfGmAfGmCfAmAmGmAfAm CfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSSOSOOOOOOOOOO OOOOSS
WV-41858	mUfU*mA*fUmA*fGmAfGmCfAmAmGmAfAmCfAm CfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	OXXOXOOOOOOOOOO OOOOOSS
WV-41859	mUfU*RmA*RfUmA*RfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	ORRORROOOOOOOOO OOOOOSS
WV-41860	mUfU*SmA*RfUmA*RfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	OSRORROOOOOOOOO OOOOOSS
WV-41861	mUfU*RmA*SfUmA*RfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	ORSORROOOOOOOOO OOOOOSS
WV-41862	mUfU*SmA*SfUmA*RfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	OSSORROOOOOOOOO OOOOSS
WV-41863	mUfU*RmA*RfUmA*SfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	ORROSOOOOOOOOO OOOOOSS
WV-41864	mUfU*SmA*RfUmA*SfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	OSROSOOOOOOOOO OOOOSS
WV-41865	mUfU*RmA*SfUmA*SfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	ORSOSOOOOOOOOO OOOOSS
WV-41866	mUfU*SmA*SfUmA*SfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	OSSOSOOOOOOOOO OOOOSS
WV-41867	mU*fUmA*fUmA*fGmAfGmCfAmAmGmAfAmCfAm CfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	XOXOXOOOOOOOOO OOOOOSS
WV-41868	mU*RfUmA*RfUmA*RfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RORORROOOOOOOOO OOOOOSS
WV-41869	mU*SfUmA*RfUmA*RfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SORORROOOOOOOOO OOOOOSS
WV-41870	mU*RfUmA*SfUmA*RfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	ROSORROOOOOOOOO OOOOOSS
WV-41871	mU*SfUmA*SfUmA*RfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOSORROOOOOOOOO OOOOSS
WV-41872	mU*RfUmA*RfUmA*SfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RORSOSOOOOOOOOO OOOOOSS

WV-41873	mU*SfUmA*RfUmA*SfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SORSSOOOOOOOOOO OOOOSS
WV-41874	mU*RfUmA*SfUmA*SfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	ROSOSOOOOOOOOOO OOOOSS
WV-41875	mU*SfUmA*SfUmA*SfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOSOSOOOOOOOOOO OOOOSS
WV-41876	mU*fU*mAfUmA*fGmAfGmCfAmAmGmAfAmCfAm CfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	XXOOXOOOOOOOOOO OOOOOSS
WV-41877	mU*RfU*RmAfUmA*RfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RROORSOOOOOOOOO OOOOOSS
WV-41878	mU*SfU*RmAfUmA*RfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROORSOOOOOOOOO OOOOOSS
WV-41879	mU*RfU*SmAfUmA*RfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOORSOOOOOOOOO OOOOOSS
WV-41880	mU*SfU*SmAfUmA*RfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSOORSOOOOOOOOO OOOOSS
WV-41881	mU*RfU*RmAfUmA*SfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RROOSSOOOOOOOOO OOOOOSS
WV-41882	mU*SfU*RmAfUmA*SfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOSSOOOOOOOOO OOOOSS
WV-41883	mU*RfU*SmAfUmA*SfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOSSOOOOOOOOO OOOOSS
WV-41884	mU*SfU*SmAfUmA*SfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSOOSSOOOOOOOOO OOOOSS
WV-41885	mU*fU*mA*fUmAfGmAfGmCfAmAmGmAfAmCfAm CfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	XXXOOOOOOOOOOOO OOOOOSS
WV-41886	mU*RfU*RmA*RfUmAfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RRROOOOOOOOOOO OOOOOSS
WV-41887	mU*SfU*RmA*RfUmAfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRROOOOOOOOOOO OOOOOSS
WV-41888	mU*RfU*SmA*RfUmAfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSROOOOOOOOOOO OOOOOSS
WV-41889	mU*SfU*SmA*RfUmAfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSROOOOOOOOOOO OOOOOSS

WV-41890	mU*RfU*RmA*SfUmAfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RRSOOOOOOOOOOO OOOOOSS
WV-41891	mU*SfU*RmA*SfUmAfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRSOOOOOOOOOOO OOOOOSS
WV-41892	mU*RfU*SmA*SfUmAfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSSOOOOOOOOOOO OOOOOSS
WV-41893	mU*SfU*SmA*SfUmAfGmAfGmCfAmAmGmAfAmCf AmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SSSOOOOOOOOOOO OOOOOSS
WV-41894	mUn001RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	nRSOOOOOOOOOOO OOOOOSS
WV-41895	mU*RfUn001RmAfUmAfGmAfGmCfAmAmGmAfAm CfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RnROOOOOOOOOOO OOOOOSS
WV-41896	mU*RfU*SmAn001RfUmAfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOOOOOOO OOOOOSS
WV-41897	mU*RfU*SmAfUn001RmAfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOnROOOOOOOOO OOOOOSS
WV-41898	mU*RfU*SmAfUmAn001RfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOnROOOOOOOO OOOOOSS
WV-41899	mU*RfU*SmAfUmAfGn001RmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOnROOOOOOO OOOOOSS
WV-41900	mU*RfU*SmAfUmAfGmAn001RfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOnROOOOOO OOOOOSS
WV-41901	mU*RfU*SmAfUmAfGmAfGn001RmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOnROOOOO OOOOOSS
WV-41902	mU*RfU*SmAfUmAfGmAfGmCn001RfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOnROOOO OOOOOSS
WV-41903	mU*RfU*SmAfUmAfGmAfGmCfAn001RmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOnROOO OOOOOSS
WV-41904	mU*RfU*SmAfUmAfGmAfGmCfAmAn001RmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOnRO OOOOOSS
WV-41905	mU*RfU*SmAfUmAfGmAfGmCfAmAmGn001RmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOnR OOOOOSS
WV-41906	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAn001RfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOnR OOOOOSS

WV-41907	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAn001R mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOnRO OOOOOSS
WV-41908	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCn00 1RfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOnR OOOOOSS
WV-41909	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA n001RmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOn ROOOOSS
WV-41910	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCn001RfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OnROOOSS
WV-41911	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUn001RmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOnROOSS
WV-41912	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGn001RfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOnROSS
WV-41913	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUn001RmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOOnRSS
WV-41914	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmUn001RmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOOOnRS
WV-41915	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*SmUn001RmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOOOSnR
WV-41916	mUn001SfU*SmAfUmAfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	nSSOOOOOOOOOOOOO OOOOOSS
WV-41917	mU*RfUn001SmAfUmAfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RnSOOOOOOOOOOOOO OOOOOSS
WV-41918	mU*RfU*SmAn001SfUmAfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOOOOOO OOOOOSS
WV-41919	mU*RfU*SmAfUn001SmAfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOnSOOOOOOOOOO OOOOOSS
WV-41920	mU*RfU*SmAfUmAn001SfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOnSOOOOOOOOO OOOOOSS
WV-41921	mU*RfU*SmAfUmAfGn001SmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOnSOOOOOOOO OOOOOSS
WV-41922	mU*RfU*SmAfUmAfGmAn001SfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOnSOOOOOOO OOOOOSS
WV-41923	mU*RfU*SmAfUmAfGmAfGn001SmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOnSOOOOOOO OOOOOSS

WV-41924	mU*RfU*SmAfUmAfGmAfGmCn001SfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOnSOOOOOO OOOOOSS
WV-41925	mU*RfU*SmAfUmAfGmAfGmCfAn001SmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOnSOOOOO OOOOOSS
WV-41926	mU*RfU*SmAfUmAfGmAfGmCfAmAn001SmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOnSOOOO OOOOOSS
WV-41927	mU*RfU*SmAfUmAfGmAfGmCfAmAmGn001SmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOnSOOO OOOOOSS
WV-41928	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAn001SfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOnSOO OOOOOSS
WV-41929	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAn001S mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOnSO OOOOOSS
WV-41930	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCn00 1SfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOnS OOOOOSS
WV-41931	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA n001SmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOOn SOOOOSS
WV-41932	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCn001SfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OnSOOOSS
WV-41933	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUn001SmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOnSOOSS
WV-41934	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOnSOSS
WV-41935	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOOnSSS
WV-41936	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmUn001SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOOOnSS
WV-41937	mU*RfU*SmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*SmUn001SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOOOSnS
WV-41938	mUn001RfU*RmAfUmAfGmAfGmCfAmAmGmAfAm CfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	nRROOOOOOOOOOOOO OOOOOSS
WV-41939	mU*SfUn001RmAfUmAfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SnROOOOOOOOOOOO OOOOOSS
WV-41940	mU*SfU*RmAn001RfUmAfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnROOOOOOOOOOOO OOOOOSS

WV-41941	mU*SfU*RmAfUn001RmAfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROnROOOOOOOOOO OOOOOSS
WV-41942	mU*SfU*RmAfUmAn001RfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOnROOOOOOOOO OOOOOSS
WV-41943	mU*SfU*RmAfUmAfGn001RmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOnROOOOOOOO OOOOOSS
WV-41944	mU*SfU*RmAfUmAfGmAn001RfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOnROOOOOOO OOOOOSS
WV-41945	mU*SfU*RmAfUmAfGmAfGn001RmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOnROOOOOO OOOOOSS
WV-41946	mU*SfU*RmAfUmAfGmAfGmCn001RfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOnROOOOO OOOOOSS
WV-41947	mU*SfU*RmAfUmAfGmAfGmCfAn001RmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOnROOOO OOOOOSS
WV-41948	mU*SfU*RmAfUmAfGmAfGmCfAmAn001RmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOnROOO OOOOOSS
WV-41949	mU*SfU*RmAfUmAfGmAfGmCfAmAmGn001RmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOnROO OOOOOSS
WV-41950	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAn001RfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOnRO OOOOOSS
WV-41951	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAn001R mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOnRO OOOOOSS
WV-41952	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAmCn00 1RfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOnR OOOOOSS
WV-41953	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAmCfA n001RmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOn ROOOOSS
WV-41954	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCn001RfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOO OnROOOSS
WV-41955	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUn001RmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOO OOnROOSS
WV-41956	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGn001RfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOO OOOnROSS
WV-41957	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUn001RmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOO OOOOnRSS

WV-41958	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmUn001RmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOO OOOOOnRS
WV-41959	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*SmUn001RmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOO OOOOOSnR
WV-41960	mUn001SfU*RmAfUmAfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	nSROOOOOOOOOOOO OOOOOSS
WV-41961	mU*SfUn001SmAfUmAfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SnSOOOOOOOOOOOO OOOOOSS
WV-41962	mU*SfU*RmAn001SfUmAfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOOOOOO OOOOOSS
WV-41963	mU*SfU*RmAfUn001SmAfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROnSOOOOOOOOOO OOOOOSS
WV-41964	mU*SfU*RmAfUmAn001SfGmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOnSOOOOOOOOO OOOOOSS
WV-41965	mU*SfU*RmAfUmAfGn001SmAfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOnSOOOOOOOO OOOOOSS
WV-41966	mU*SfU*RmAfUmAfGmAn001SfGmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOnSOOOOOOO OOOOOSS
WV-41967	mU*SfU*RmAfUmAfGmAfGn001SmCfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOnSOOOOOO OOOOOSS
WV-41968	mU*SfU*RmAfUmAfGmAfGmCn001SfAmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOnSOOOOO OOOOOSS
WV-41969	mU*SfU*RmAfUmAfGmAfGmCfAn001SmAmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOnSOOOO OOOOOSS
WV-41970	mU*SfU*RmAfUmAfGmAfGmCfAmAn001SmGmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOnSOOO OOOOOSS
WV-41971	mU*SfU*RmAfUmAfGmAfGmCfAmAmGn001SmAfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOnSOO OOOOOSS
WV-41972	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAn001SfA mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOnSO OOOOOSS
WV-41973	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAn001S mCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOnS OOOOOSS
WV-41974	mU*SfU*RmAfUmAfGmAfGmCfAmAmGmAfAmCn00 1SfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOnS OOOOOSS

WV-41975	mU*SfU*RmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA n001SmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOn SOOOOSS
WV-41976	mU*SfU*RmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCn001SfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OnSOOOSS
WV-41977	mU*SfU*RmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUn001SmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OOnSOOSS
WV-41978	mU*SfU*RmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OOOnSOSS
WV-41979	mU*SfU*RmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OOOOnSSS
WV-41980	mU*SfU*RmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmUn001SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OOOOOnSS
WV-41981	mU*SfU*RmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmU*SmUn001SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OOOOOSnS
WV-41982	mUn001RfUmAfUmAfGmAfGmCfCfAmAmGmAfAmCfA mCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	nROOOOOOOOOOOOO OOOOOOSS
WV-41939	mU*SfUn001RmAfUmAfGmAfGmCfCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SnROOOOOOOOOOOOO OOOOOSS
WV-41983	mU*SfUmAn001RfUmAfGmAfGmCfCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnROOOOOOOOOOOO OOOOOSS
WV-41984	mU*SfUmAfUn001RmAfGmAfGmCfCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOnROOOOOOOOOOO OOOOOSS
WV-41985	mU*SfUmAfUmAn001RfGmAfGmCfCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOnROOOOOOOOOO OOOOOSS
WV-41986	mU*SfUmAfUmAfGn001RmAfGmCfCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOnROOOOOOOOO OOOOOSS
WV-41987	mU*SfUmAfUmAfGmAn001RfGmCfCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOnROOOOOOOO OOOOOSS
WV-41988	mU*SfUmAfUmAfGmAfGn001RmCfCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOnROOOOOOO OOOOOSS
WV-41989	mU*SfUmAfUmAfGmAfGmCn001RfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOnROOOOOO OOOOOSS
WV-41990	mU*SfUmAfUmAfGmAfGmCfCfAn001RmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOnROOOOO OOOOOSS

WV-41991	mU*SfUmAfUmAfGmAfGmCfAmAn001RmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOnROOOO OOOOOSS
WV-41992	mU*SfUmAfUmAfGmAfGmCfAmAmGn001RmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOnROOO OOOOOSS
WV-41993	mU*SfUmAfUmAfGmAfGmCfAmAmGmAn001RfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOnROO OOOOOSS
WV-41994	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAn001RmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOnRO OOOOOSS
WV-41995	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCn001R fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOnR OOOOOSS
WV-41996	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAn00 1RmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOn ROOOOSS
WV-41997	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAmC n001RfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOO OnROOOSS
WV-41998	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUn001RmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOO OOnROOSS
WV-41999	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGn001RfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOO OOOnROSS
WV-42000	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGfUn001RmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOO OOOOnRSS
WV-42001	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGfUmUn001RmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOO OOOOOnRS
WV-42002	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGfUmU*SmUn001RmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOO OOOOOSnR
WV-42003	mUn001SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfA mCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	nSOOOOOOOOO OOOOOSS
WV-42004	mU*SfUmAn001SfUmAfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOnSOOOOOOOO OOOOOSS
WV-42005	mU*SfUmAfUn001SmAfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOnSOOOOOOO OOOOOSS
WV-42006	mU*SfUmAfUmAn001SfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOnSOOOOOO OOOOOSS
WV-42007	mU*SfUmAfUmAfGn001SmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOnSOOOOO OOOOOSS

WV-42008	mU*SfUmAfUmAfGmAn001SfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOnSOOOOOOO OOOOOSS
WV-42009	mU*SfUmAfUmAfGmAfGn001SmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOnSOOOOOO OOOOOSS
WV-42010	mU*SfUmAfUmAfGmAfGmCn001SfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOnSOOOOO OOOOOSS
WV-42011	mU*SfUmAfUmAfGmAfGmCfAn001SmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOnSOOOO OOOOOSS
WV-42012	mU*SfUmAfUmAfGmAfGmCfAmAn001SmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOnSOOO OOOOOSS
WV-42013	mU*SfUmAfUmAfGmAfGmCfAmAmGn001SmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOOnSOO OOOOOSS
WV-42014	mU*SfUmAfUmAfGmAfGmCfAmAmGmAn001SfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOOnSOO OOOOOSS
WV-42015	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAn001SmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOOOnSO OOOOOSS
WV-42016	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCn001S fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOOOOnS OOOOOSS
WV-42017	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAn00 1SmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOOOOOn SOOOOSS
WV-42018	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAmC n001SfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOOOOOO OnSOOOSS
WV-42019	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUn001SmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOOOOOO OOnSOOSS
WV-42020	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOOOOOO OOOnSOSS
WV-42021	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGfUn001SmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOOOOOO OOOOnSSS
WV-42022	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGfUmUn001SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOOOOOO OOOOOnSS
WV-42023	mU*SfUmAfUmAfGmAfGmCfAmAmGmAfAmCfAmC fUmGfUmU*SmUn001SmU	UUAUAGAGCAAGAACA CUGUUUU	SOOOOOOOOOOOOOO OOOOOSnS
WV-42078	mU*RfU*SmAn001SmUmAfGmAmGmCmAn001SmA mGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOnSOOOO OOOOnSOSS

WV-42079	mU*SfU*RmAn001SmUmAfGmAmGmCmAn001SmA mGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-42080	Mod001L001mA*SmAmCmAmGmUfGmUfUfCfUmUm GmCmUmCmUmAmUmA*SmA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOOOOOOOOO OOOOOS
WV-42081	L001mA*SmAmCmAmGmUfGmUfUfCfUmUmGmCm UmCmUmAmUmA*SmA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOOOOOOOOO OOOOOS
WV-41939	mU*SfUn001RmAfUmAfGmAfGmCfAmAmGmAfAmC fAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SnROOOOOOOOOOOOO OOOOOSS
WV-42940	Mod155L001mA*SmAfCmAfGmUfGmUfUfCfUmUfG mCfUmCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOOOOOOOOO OOOOOS
WV-42941	Mod155L001mA*SmAmCmAmGmUfGmUfUfCfUmUm GmCmUmCmUmAmUmA*SmA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOOOOOOOOO OOOOOS
WV-42904	mU*fU*mAmUmAfGmAmGmCmAmAmGmA*fA*mC *fA*mC*mU*mG*mU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXOOOOOOOOOOXXX XXXXXXX
WV-42905	mU*RfU*SmAn001SmUmAfGmAmGmCmAn001SmA mGmA*SfA*SmC*SfA*SmC*SmU*SmGn001SmU*Sm U*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOnSOOSSS SSSnSSSS	
WV-42906	mU*SfU*RmAn001SmUmAfGmAmGmCmAn001SmA mGmA*SfA*SmC*SfA*SmC*SmU*SmGn001SmU*Sm U*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOSSS SSSnSSSS	
WV-43256	mU*SfU*RmAn001SfUmAfGmAfGmCfAn001SmAmG mAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS	
WV-43257	mU*SfU*RmAn001SfUmAfGmAfGmCfAn001SmAfGm AfAmCfAmCfUmGn001SfUmU*SfU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS	
WV-43258	mU*SfU*RmAn001SfU*SmAfG*SmAfG*SmCfAn001S mAfG*SmAfA*SmCfA*SmCfU*SmGn001SfU*SmU*Sf U*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSSOSOSOSOnSOSOSOS OSnSSSS	
WV-43259	mU*SfU*RmAn001SfUmAfGmAfGmCfAn001SmAfGm A*SfA*SmC*SfA*SmC*SfU*SmGn001SfU*SmU*SfU* SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOSSS SSSnSSSS	
WV-43260	mU*SfU*RmAn001SfU*SmAfG*SmAfG*SmCfAn001S mAfG*SmA*SfA*SmC*SfA*SmC*SfU*SmGn001SfU* SmU*SfU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSSOSOSOnSOSSSSSS SnSSSS	
WV-43261	mU*SfU*RfAn001SfUfAfGfAfGmCfAn001SmAfGmAf AmCfAmCfUmGn001SfUmU*SfU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS	

WV-43262	mU*SfU*RfAn001SfU*SfAfG*SfAfG*SmCfAn001SmAfG*SmAfA*SmCfA*SmCfU*SmGn001SfU*SmU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSSOSOSOnSOSOSOSOSnSSSS
WV-43263	mU*SfU*RfAn001SfUfAfGfAfGmCfAn001SmAfGmA*SfA*SmC*SfA*SmC*SfU*SmGn001SfU*SmU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSOOOOOOnSOOSSSSSSnSSSS
WV-43264	mU*SfU*RfAn001SfU*SfAfG*SfAfG*SmCfAn001SmAfG*SmA*SfA*SmC*SfA*SmC*SfU*SmGn001SfU*SmU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSSOSOSOnSOSSSSSSSnSSSS
WV-43265	mU*SfU*RmAn001SfUmAfGmAfGmCfAn001SmAfGmAfAmCfAfCfUfGn001SfUfU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSOOOOOOnSOOOOOOnSOSS
WV-43266	mU*SfU*RmAn001SfU*SmAfG*SmAfG*SmCfAn001SmAfG*SmAfA*SmCfA*SfCfU*SfGn001SfU*SfU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSSOSOSOnSOSOSOSOSnSSSS
WV-43267	mU*SfU*RmAn001SfUmAfGmAfGmCfAn001SmAfGmA*SfA*SmC*SfA*SfC*SfU*SfGn001SfU*SfU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSOOOOOOnSOOSSSSSSnSSSS
WV-43268	mU*SfU*RmAn001SfU*SmAfG*SmAfG*SmCfAn001SmAfG*SmA*SfA*SmC*SfA*SfC*SfU*SfGn001SfU*SfU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSSOSOSOnSOSSSSSSnSSSS
WV-43269	mU*SfU*RfAn001SfUfAfGfAfGmCfAn001SmAfGmAfAmCfAfCfUfGn001SfUfU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSOOOOOOnSOOOOOOnSOSS
WV-43270	mU*SfU*RfAn001SfU*SfAfG*SfAfG*SmCfAn001SmAfG*SmAfA*SmCfA*SfCfU*SfGn001SfU*SfU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSSOSOSOnSOSOSOSOSnSSSS
WV-43271	mU*SfU*RfAn001SfUfAfGfAfGmCfAn001SmAfGmA*SfA*SmC*SfA*SfC*SfU*SfGn001SfU*SfU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSOOOOOOnSOOSSSSSSnSSSS
WV-43272	mU*SfU*RfAn001SfU*SfAfG*SfAfG*SmCfAn001SmAfG*SmA*SfA*SmC*SfA*SfC*SfU*SfGn001SfU*SfU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSSOSOSOnSOSSSSSSSnSSSS
WV-43273	mU*SfU*RfAn001SfUfAfGfAfGfCfAn001SfAfGfAfAfCfAfCfUfGn001SfUfU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSOOOOOOnSOOOOOOnSOSS
WV-43274	mU*SfU*RfAn001SfU*SfAfG*SfAfG*SfCfAn001SfAfG*SfAfA*SfCfA*SfCfU*SfGn001SfU*SfU*SfU*SmU	UUAUAGAGCAAGAACACUGUUUU	SRnSSOSOSOnSOSOSOSOSnSSSS

WV-43275	mU*SfU*RfAn001SfUfAfGfAfGfCfAn001SfAfGfA*Sf A*SfC*SfA*SfC*SfU*SfGn001SfU*SfU*SfU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOSSS SSSnSSSSS
WV-43276	mU*SfU*RfAn001SfU*SfAfG*SfAfG*SfCfAn001SfAf G*SfA*SfA*SfC*SfA*SfC*SfU*SfGn001SfU*SfU*SfU *SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSSOSOSOnSOSSSSSS SnSSSS
WV-43277	mU*SfU*RmAn001SfUmAfGmAfGmCfAn001SmAfGm AfAmCfAmCfUmGn001SfUmU*STeo*STeo	UUAUAGAGCAAGAACA CUGUUTT	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43278	mU*SfU*RmAn001SfU*SmAfG*SmAfG*SmCfAn001S mAfG*SmAfA*SmCfA*SmCfU*SmGn001SfU*SmU*S Teo*STeo	UUAUAGAGCAAGAACA CUGUUTT	SRnSSOSOSOnSOSOSOS OSnSSSS
WV-43279	mU*SfU*RmAn001SfUmAfGmAfGmCfAn001SmAfGm A*SfA*SmC*SfA*SmC*SfU*SmGn001SfU*SmU*STeo *STeo	UUAUAGAGCAAGAACA CUGUUTT	SRnSOOOOOOnSOOSSS SSSnSSSS
WV-43280	mU*SfU*RmAn001SfU*SmAfG*SmAfG*SmCfAn001S mAfG*SmA*SfA*SmC*SfA*SmC*SfU*SmGn001SfU* SmU*STeo*STeo	UUAUAGAGCAAGAACA CUGUUTT	SRnSSOSOSOnSOSSSSSS SnSSSS
WV-40363	Mod001L001mA*SmAfCmAfGmUfGmUfUfCfUmUfG mCfUmCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOOOOOOOO OOOOOS
WV-43281	Mod001L001mA*SmAfC*SmAfG*SmUfGmUfUfCfUm UfG*SmCfU*SmCfU*SmAfU*SmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOSOSOOOOOOOSOS OSOSS
WV-43282	Mod001L001mA*SmAfC*SmAfG*SmUfG*SmUfU*SfC *SfU*SmUfG*SmCfU*SmAfU*SmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOSOSOSOSSSOSOSO SOSS
WV-43283	Mod001L001mA*SmAfC*SmAfG*SmUfG*SmUfU*SfC *SfUmUfG*SmCfU*SmCfU*SmAfU*SmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOSOSOSOSSOOSOSO SOSS
WV-43987	mU*SfU*RfAn001SmUmAfGmAmGmCfAn001SmAm GmAfAmCfAmCmUfGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43988	mU*SfU*RmAn001SfUmAfGmAmGmCmAn001SfAm GmAfAmCfAmCmUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43989	mU*SfU*RmAn001SfUmAfGmAmGmCfAn001SmAm GmAfAmCfAmCmUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43990	mU*SfU*RfAn001SfUmAfGmAmGmCfAn001SfAmGm AfAmCfAmCmUfGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43991	mU*SfU*RmAn001SmUmAfGmAfGmCmAn001SmAm GmAfAmCfAmCfUmGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS

WV-43992	mU*SfU*RfAn001SmUmAfGmAfGmCfAn001SmAmG mAfAmCfAmCfUfGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43993	mU*SfU*RmAn001SfUmAfGmAfGmCmAn001SfAmG mAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43256	mU*SfU*RmAn001SfUmAfGmAfGmCfAn001SmAmG mAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43994	mU*SfU*RfAn001SfUmAfGmAfGmCfAn001SfAmGm AfAmCfAmCfUfGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43773	mU*SfU*RmAn001SmUmAfGmAfGfCmAn001SmAm GmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43995	mU*SfU*RfAn001SmUmAfGmAfGfCfAn001SmAmGm AfAmCfAmCmUfGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43996	mU*SfU*RmAn001SfUmAfGmAfGfCmAn001SfAmGm AfAmCfAmCmUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43997	mU*SfU*RmAn001SfUmAfGmAfGfCfAn001SmAmGm AfAmCfAmCmUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43998	mU*SfU*RfAn001SfUmAfGmAfGfCfAn001SfAmGmA fAmCfAmCmUfGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43768	Mod001L001fA*mAfCmAfGmUfGmUfUfCfUmUfGmC fUmCfUmAfUmA*fA	AACAGUGUUCUUGCUC UAUAA	OXOOOOOOOOOOOOO OOOOOX
WV-43769	Mod001L001fA*SmAfCmAfGmUfGmUfUfCfUmUfGm CfUmCfUmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOOOOOOOOO OOOOOS
WV-43770	mU*RfU*SmAmUmAfGmAfGfCmAmAmGmAfAmCfA mCmUmGmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOOOSS
WV-43771	mU*SfU*RmAmUmAfGmAfGfCmAmAmGmAfAmCfA mCmUmGmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OOOOOSS
WV-43772	mU*RfU*SmAn001SmUmAfGmAfGfCmAn001SmAm GmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOnSOOOO OOOOnSOSS
WV-43774	mU*RfU*SmAmUmAfGmAmGmCmAmAmGmAfAmC fAmCmUmGmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOOO OOOOOSS
WV-43775	mU*SfU*RmAmUmAfGmAmGmCmAmAmGmAfAmC fAmCmUmGmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OOOOOSS
WV-43999	L001fA*mAfCmAfGmUfGmUfUfCfUmUfGmCfUmCfU mAfUmA*fA	AACAGUGUUCUUGCUC UAUAA	OXOOOOOOOOOOOOO OOOOOX

WV-44000	L001fA*SmAfCmAfGmUfGmUfUfCfUmUfGmCfUmCf UmAfUmA*SfA	AACAGUGUUCUUGCUC UAUAA	OSOOOOOOOOOOOO OOOOOS
WV-44150	mU*RfU*SAsm01n001SfUmAfGmAfGmCAsm01n001S mAmGmAfAmCfAmCfUGsm01n001SfUmU*SmU*Sm U	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOnSOOOO OOOOnSOSS
WV-44151	mU*RfU*SAsm01n001RfUmAfGmAfGmCAsm01n001 RmAmGmAfAmCfAmCfUGsm01n001RfUmU*SmU*S mU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOOOnROOOO OOOOnROSS
WV-44166	mU*RfU*SAsm01n013fUmAfGmAfGmCAsm01n013m AmGmAfAmCfAmCfUGsm01n013fUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOO OOOOOSS
WV-44353	mU*RfU*SAsm01n001RfUmAfGmAfGmCfAmAmGm AfAmCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOOOOOOOO OOOOOSS
WV-44354	mU*RfU*SAsm01n001SfUmAfGmAfGmCfAmAmGmA fAmCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOOOOOO OOOOOSS
WV-44355	mU*RfU*SAsm01n013fUmAfGmAfGmCfAmAmGmAf AmCfAmCfUmGfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOOOOOO OOOOOSS
WV-44144	mU*RfU*SAsm01n001SfUmAfGmAfGmCfAn001SmA mGmAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnSOOOOOOnSOOOO OOOOnSOSS
WV-44145	mU*RfU*SAsm01n001RfUmAfGmAfGmCfAn001SmA mGmAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSnROOOOOOnSOOOO OOOOnSOSS
WV-44167	mU*RfU*SAsm01n013mUmAfGmAmGmCmAn001Sm AmGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOnSOOOOO OOOnSOSS
WV-44463	mU*RfU*SAsm01n013fUmAfGmAmGmCfAn001SmA mGmAfAmCfAmCmUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOnSOOOOO OOOnSOSS
WV-44462	mU*RfU*SAsm01n013fUmAfGmAmGmCmAn001SmA mGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOnSOOOOO OOOnSOSS
WV-44163	mU*RfU*SAsm01n013fUmAfGmAfGmCfAn001SmAm GmAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	RSOOOOOOOnSOOOOO OOOnSOSS
WV-43988	mU*SfU*RmAn001SfUmAfGmAmGmCmAn001SfAm GmAfAmCfAmCmUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43989	mU*SfU*RmAn001SfUmAfGmAmGmCfAn001SmAm GmAfAmCfAmCmUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43994	mU*SfU*RfAn001SfUmAfGmAfGmCfAn001SfAmGm AfAmCfAmCfUfGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS

WV-43996	mU*SfU*RmAn001SfUmAfGmAfGfCmAn001SfAmGm AfAmCfAmCmUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-43256	mU*SfU*RmAn001SfUmAfGmAfGmCfAn001SmAmG mAfAmCfAmCfUmGn001SfUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44433	fU*SfU*RmAn001SmUmAfGmAmGmCmAn001SmAm GmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44434	T*SfU*RmAn001SmUmAfGmAmGmCmAn001SmAm GmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	TUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44435	POT*SfU*RmAn001SmUmAfGmAmGmCmAn001SmA mGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	TUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44436	PO5MSdT*SfU*RmAn001SmUmAfGmAmGmCmAn00 1SmAmGmAfAmCfAmCmUmGn001SmUmU*SmU*S mU	TUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44437	PO5MRdT*SfU*RmAn001SmUmAfGmAmGmCmAn00 1SmAmGmAfAmCfAmCmUmGn001SmUmU*SmU*S mU	TUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44438	VPT*SfU*RmAn001SmUmAfGmAmGmCmAn001SmA mGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	TUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44439	5mvpdT*SfU*RmAn001SmUmAfGmAmGmCmAn001S mAmGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	TUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44440	5mspdT*SfU*RmAn001SmUmAfGmAmGmCmAn001S mAmGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	TUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44441	5mrpdT*SfU*RmAn001SmUmAfGmAmGmCmAn001S mAmGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	TUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44442	PNT*SfU*RmAn001SmUmAfGmAmGmCmAn001SmA mGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	TUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44443	SPNT*SfU*RmAn001SmUmAfGmAmGmCmAn001Sm AmGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	TUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44444	L026*SfU*RmAn001SmUmAfGmAmGmCmAn001Sm AmGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	UAUAGAGCAAGAACAC UGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44445	L027*SfU*RmAn001SmUmAfGmAmGmCmAn001Sm AmGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	UAUAGAGCAAGAACAC UGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS
WV-44446	5ptzdT*SfU*RmAn001SmUmAfGmAmGmCmAn001S mAmGmAfAmCfAmCmUmGn001SmUmU*SmU*SmU	TUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOnSOSS

WV-44447	mU*fU*mAn001mUmAfGmAmGmCmAn001mAmGm AfAmCfAmCmUmGn001mUmU*mU*mU	UUAUAGAGCAAGAACA CUGUUUU	XXnXOOOOOOnXOOOO OOOOnXOXX
WV-44448	mU*SfU*RmAmUmAfGmAmGmCmAn001SmAmGmA fAmCfAmCmUmGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOnSOOOOO OOOnSOSS
WV-44449	mU*SfU*RmAn001SmUmAfGmAmGmCmAmAmGmA fAmCfAmCmUmGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOOOOOOO OOOnSOSS
WV-44450	mU*SfU*RmAn001SmUmAfGmAmGmCmAn001SmA mGmAfAmCfAmCmUmGmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOOOSS
WV-44451	mU*SfU*RmAmUmAfGmAmGmCmAmAmGmAfAmC fAmCmUmGn001SmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OOOnSOSS
WV-44452	mU*SfU*RmAmUmAfGmAmGmCmAn001SmAmGmA fAmCfAmCmUmGmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOnSOOOOO OOOOOSS
WV-44453	mU*SfU*RmAn001SmUmAfGmAmGmCmAmAmGmA fAmCfAmCmUmGmUmU*SmU*SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOOOOOOO OOOOOSS
WV-44454	mU*SfU*RmAn001SmUmAfGmAmGmCmAn001SmA mGmAfAmCfAmCmUmGmUmU*SmUn001SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOOOSnS
WV-44455	mU*SfU*RmAn001SmUmAfGmAmGmCmAmAmGmA fAmCfAmCmUmGmUmU*SmUn001SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOOOOOOO OOOOOSnS
WV-44456	mU*SfU*RmAmUmAfGmAmGmCmAn001SmAmGmA fAmCfAmCmUmGmUmU*SmUn001SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOnSOOOOO OOOOOSnS
WV-44457	mU*SfU*RmAmUmAfGmAmGmCmAmAmGmAfAmC fAmCmUmGmUmU*SmUn001SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OOOOOSnS
WV-44458	mU*SfU*RmAn001SmUmAfGmAmGmCmAn001SmA mGmAfAmCfAmCmUmGmUmUmUn001SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOnSOOOO OOOOOOOnS
WV-44459	mU*SfU*RmAn001SmUmAfGmAmGmCmAmAmGmA fAmCfAmCmUmGmUmUmUn001SmU	UUAUAGAGCAAGAACA CUGUUUU	SRnSOOOOOOOOOOOO OOOOOOnS
WV-44460	mU*SfU*RmAmUmAfGmAmGmCmAn001SmAmGmA fAmCfAmCmUmGmUmUmUn001SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOnSOOOOO OOOOOOnS
WV-44461	mU*SfU*RmAmUmAfGmAmGmCmAmAmGmAfAmC fAmCmUmGmUmUmUn001SmU	UUAUAGAGCAAGAACA CUGUUUU	SROOOOOOOOOOOOO OOOOOOnS

Table 1D, Example oligonucleotides and/or compositions that target PTEN.

ID	Description	Naked Sequence	Stereochemistry/linkage

WV-40398	fU*fA*mUfAmGfUmUfUmCfAmAmAmCfAmUfCmAf UmC*mU*mU	UAUAGUUUCAAACAUC AUCUU	XXOOOOOOOOOOOOO OOOXX
WV-40399	mGmAfUmGfAmUfGfUfUmUfGmAfAmAfCmUfAmUf A	GAUGAUGUUUGAAACU AUA	OOOOOOOOOOOOOOO OOO
WV-40984	fU*RfA*SmUfAmGfUmUfUmCfAmAmAmCfAmUfCm AfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSOOOOOOOOOOOOO OOOSS
WV-40985	fU*RfA*SmUn001SfAmGfUmUn001SfUmCfAmAmAm CfAmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOnSOOOOOOO OnSOOSS
WV-40986	fU*RfA*SmUn001SfAmGfUmUn001SfUmCfAmAmAm CfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOnSOOOOOOO OOOnSSS
WV-40987	fU*RfA*SmUn001SfAmGfUmUfUn001SmCfAmAmAm CfAmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOOnSOOOOOO OnSOOSS
WV-40988	fU*RfA*SmUn001SfAmGfUmUfUn001SmCfAmAmAm CfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOOnSOOOOOO OOOnSSS
WV-40989	fU*RfA*SmUn001SfAmGfUmUfUmCfAn001SmAmAm CfAmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOOOOnSOOOO OnSOOSS
WV-40990	fU*RfA*SmUn001SfAmGfUmUfUmCfAn001SmAmAm CfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOOOOnSOOOO OOOnSSS
WV-40991	fU*RfA*SmUn001SfAmGfUmUn001SfUmCfAn001Sm AmAmCfAmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOnSOOnSOOOO OnSOOSS
WV-40992	fU*RfA*SmUn001SfAmGfUmUn001SfUmCfAn001Sm AmAmCfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOnSOOnSOOOO OOOnSSS
WV-40993	fU*RfA*SmUn001SfAmGfUmUfUn001SmCfAn001Sm AmAmCfAmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOOnSOnSOOOO OnSOOSS
WV-40994	fU*RfA*SmUn001SfAmGfUmUfUn001SmCfAn001Sm AmAmCfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOOnSOnSOOOO OOOnSSS
WV-40995	fU*RfA*SmUn001SfAmGfUmUn001SfUmCfAn001Sm AmAmCfAmUfCn001SmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOnSOOnSOOOO OnSOnSSS
WV-40996	fU*RfA*SmUn001SfAmGfUmUfUn001SmCfAn001Sm AmAmCfAmUfCn001SmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOOnSOnSOOOO OnSOnSSS
WV-40997	fU*SfA*RmUfAmGfUmUfUmCfAmAmAmCfAmUfCm AfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SROOOOOOOOOOOOO OOOSS
WV-40998	fU*SfA*RmUn001SfAmGfUmUn001SfUmCfAmAmAm CfAmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOnSOOOOOOO OnSOOSS

WV-40999	fU*SfA*RmUn001SfAmGfUmUn001SfUmCfAmAmAm CfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOnSOOOOOO OOOnSSS
WV-41000	fU*SfA*RmUn001SfAmGfUmUfUn001SmCfAmAmAm CfAmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOOnSOOOOO OnSOOSS
WV-41001	fU*SfA*RmUn001SfAmGfUmUfUn001SmCfAmAmAm CfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOOnSOOOOO OOOnSSS
WV-41002	fU*SfA*RmUn001SfAmGfUmUfUmCfAn001SmAmAm CfAmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOOOOnSOOOO OnSOOSS
WV-41003	fU*SfA*RmUn001SfAmGfUmUfUmCfAn001SmAmAm CfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOOOOnSOOOO OOOnSSS
WV-41004	fU*SfA*RmUn001SfAmGfUmUn001SfUmCfAn001Sm AmAmCfAmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOnSOOnSOOOO OnSOOSS
WV-41005	fU*SfA*RmUn001SfAmGfUmUn001SfUmCfAn001Sm AmAmCfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOnSOOnSOOOO OOOnSSS
WV-41006	fU*SfA*RmUn001SfAmGfUmUfUn001SmCfAn001Sm AmAmCfAmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOOnSOnSOOOO OnSOOSS
WV-41007	fU*SfA*RmUn001SfAmGfUmUfUn001SmCfAn001Sm AmAmCfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOOnSOnSOOOO OOOnSSS
WV-41008	fU*SfA*RmUn001SfAmGfUmUn001SfUmCfAn001Sm AmAmCfAmUfCn001SmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOnSOOnSOOOO OnSOnSSS
WV-41009	fU*SfA*RmUn001SfAmGfUmUfUn001SmCfAn001Sm AmAmCfAmUfCn001SmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOOnSOnSOOOO OnSOnSSS
WV-41010	fU*SfAmUfAmGfUmUfUmCfAmAmAmCfAmUfCmAf UmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SOOOOOOOOOOOOO OOOSS
WV-41011	fU*SfAmUn001SfAmGfUmUn001SfUmCfAmAmAmCf AmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SOnSOOOnSOOOOOO OnSOOSS
WV-41012	fU*SfAmUn001SfAmGfUmUn001SfUmCfAmAmAmCf AmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SOnSOOOnSOOOOOO OOOnSSS
WV-41013	fU*SfAmUn001SfAmGfUmUfUn001SmCfAmAmAmCf AmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SOnSOOOOnSOOOOO OnSOOSS
WV-41014	fU*SfAmUn001SfAmGfUmUfUn001SmCfAmAmAmCf AmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SOnSOOOOnSOOOOO OOOnSSS
WV-41015	fU*SfAmUn001SfAmGfUmUfUmCfAn001SmAmAmCf AmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SOnSOOOOOOnSOOOO OnSOOSS

WV-41016	fU*SfAmUn001SfAmGfUmUfUmCfAn001SmAmAmCfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SOnSOOOOOnSOOOOOOnSSS
WV-41017	fU*SfAmUn001SfAmGfUmUn001SfUmCfAn001SmAmAmCfCfAmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SOnSOOOnSOOnSOOOOOnSSS
WV-41018	fU*SfAmUn001SfAmGfUmUn001SfUmCfAn001SmAmAmCfCfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SOnSOOOnSOOnSOOOOOnSSS
WV-41019	fU*SfAmUn001SfAmGfUmUfUn001SmCfAn001SmAmAmCfCfAmUfCn001SmAfUmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SOnSOOOOnSOnSOnSOOOOnSSS
WV-41020	fU*SfAmUn001SfAmGfUmUfUn001SmCfAn001SmAmAmCfCfAmUfCmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SOnSOOOOnSOnSOnSOOOOnSSS
WV-41021	fU*SfAmUn001SfAmGfUmUn001SfUmCfAn001SmAmAmCfCfAmUfCn001SmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SOnSOOOnSOOnSOnSOOOOnSSS
WV-41022	fU*SfAmUn001SfAmGfUmUfUn001SmCfAn001SmAmAmCfCfAmUfCn001SmAfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SOnSOOOOnSOnSOnSOOOOnSSS
WV-41023	mG*SmAfUmGfAmUfGfUfUmUfGmAfAmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOOOOOOOOOOOOnXOOOS
WV-41024	mG*SmAfUmGfAn001mUfGfUfUmUfGmAfAn001mAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnXOOOOOOOnXOOOOS
WV-41025	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn001RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-41026	mG*SmAfUmGfAn001SmUfGfUfUmUfGmAfAn001SmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnSOOOOOOOnSOOOOS
WV-41027	mGn001mAfUmGfAn001mUfGfUfUmUfGmAfAn001mAfCmUfAmUn001fA	GAUGAUGUUUGAAACUAUA	nXOOOnXOOOOOOOnXOOOOnX
WV-41028	mGn001RmAfUmGfAn001RmUfGfUfUmUfGmAfAn001RmAfCmUfAmUn001RfA	GAUGAUGUUUGAAACUAUA	nROOOnROOOOOOOnROOOOnR
WV-41029	mGn001SmAfUmGfAn001SmUfGfUfUmUfGmAfAn001SmAfCmUfAmUn001SfA	GAUGAUGUUUGAAACUAUA	nSOOOnSOOOOOOOnSOOOOnS
WV-42309	fU*RfA*SmUn001SfAmGfUmUn001SfUmCfAmAmAmCfSfA*SmU*SfC*SmA*SfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	RSnSOOOnSOOOOOOSSSSSnSSS
WV-42310	fU*SfA*RmUn001SfAmGfUmUn001SfUmCfAmAmAmCfSfA*SmU*SfC*SmA*SfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SRnSOOOnSOOOOOOSSSSSnSSS
WV-42311	fU*RfA*SmUn001SfAmGfUmUn001SfUmCfAmAmAmCfRfA*RmU*RfC*RmA*RfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	RSnSOOOnSOOOOOORRRRRnSSS

WV-42312	fU*SfA*RmUn001SfAmGfUmUn001SfUmCfAmAmAm C*RfA*RmU*RfC*RmA*RfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOnSOOOOORRR RRnSSS
WV-42323	fU*RfA*SmUn001SfAmGfUmUn001SfUmCfAmAmAm CfAmUfCmAfUn009SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOnSOOOOOOO OOOnSSS
WV-42324	fU*SfA*RmUn001SfAmGfUmUn001SfUmCfAmAmAm CfAmUfCmAfUn009SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOnSOOOOOOO OOOnSSS
WV-42325	fU*RfA*SmUn001SfAmGfUmUn001SfUmCfAmAmAm C*SfA*SmU*SfC*SmA*SfUn009SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOnSOOOOOSSS SSnSSS
WV-42326	fU*SfA*RmUn001SfAmGfUmUn001SfUmCfAmAmAm C*SfA*SmU*SfC*SmA*SfUn009SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	SRnSOOOnSOOOOOSSS SSnSSS
WV-42643	L023n001L009n001L009n001mG*SmAfUmGfAmUfGf UfUmUfGmAfAmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	nXnXnXSOOOOOOOOO OOOOOOOS
WV-42644	L023n009mG*SmAfUmGfAmUfGfUfUmUfGmAfAmAf CmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	nXSOOOOOOOOOOOOO OOOS
WV-42645	L023n033mG*SmAfUmGfAmUfGfUfUmUfGmAfAmAf CmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	nXSOOOOOOOOOOOOO OOOS
WV-42646	L023n009L009n009mG*SmAfUmGfAmUfGfUfUmUfG mAfAmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	nXnXSOOOOOOOOOOO OOOOOS
WV-42647	L023n033L009n033mG*SmAfUmGfAmUfGfUfUmUfG mAfAmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	nXnXSOOOOOOOOOOO OOOOOS
WV-42648	L023n009L009n009L009n009mG*SmAfUmGfAmUfGf UfUmUfGmAfAmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	nXnXnXSOOOOOOOOO OOOOOOOS
WV-42649	L023n033L009n033L009n033mG*SmAfUmGfAmUfGf UfUmUfGmAfAmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	nXnXnXSOOOOOOOOO OOOOOOOS
WV-42650	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn009R mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	SOOOnROOOOOOOnRO OOOS
WV-42651	mG*SmAfUmGfAn009RmUfGfUfUmUfGmAfAn001R mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	SOOOnROOOOOOOnRO OOOS
WV-42652	mG*SmAfUmGfAn009RmUfGfUfUmUfGmAfAn009R mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	SOOOnROOOOOOOnRO OOOS
WV-42653	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn033R mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	SOOOnROOOOOOOnRO OOOS
WV-42654	mG*SmAfUmGfAn033RmUfGfUfUmUfGmAfAn001R mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	SOOOnROOOOOOOnRO OOOS

WV-42655	mG*SmAfUmGfAn033RmUfGfUfUmUfGmAfAn033R mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	SOOOnROOOOOOOnRO OOOS
WV-42944	Mod077L001mG*SmAfUmGfAmUfGfUfUmUfUmUfGmAfA mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOOOOOOOOOOO OOOS
WV-42945	Mod102L001mG*SmAfUmGfAmUfGfUfUmUfUmUfGmAfA mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOOOOOOOOOOO OOOS
WV-42946	Mod071L001mG*SmAfUmGfAmUfGfUfUmUfUmUfGmAfA mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOOOOOOOOOOO OOOS
WV-42947	Mod105L001mG*SmAfUmGfAmUfGfUfUmUfUmUfGmAfA mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOOOOOOOOOOO OOOS
WV-42948	Mod029L001mG*SmAfUmGfAmUfGfUfUmUfUmUfGmAfA mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOOOOOOOOOOO OOOS
WV-42949	Mod020L001mG*SmAfUmGfAmUfGfUfUmUfUmUfGmAfA mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOOOOOOOOOOO OOOS
WV-42950	Mod015L001mG*SmAfUmGfAmUfGfUfUmUfUmUfGmAfA mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOOOOOOOOOOO OOOS
WV-42951	Mod077L001mG*SmAfUmGfAn001RmUfGfUfUmUfUmUfG mAfAn001RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOnROOOOOOOnR OOOOS
WV-42952	Mod102L001mG*SmAfUmGfAn001RmUfGfUfUmUfUmUfG mAfAn001RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOnROOOOOOOnR OOOOS
WV-42953	Mod071L001mG*SmAfUmGfAn001RmUfGfUfUmUfUmUfG mAfAn001RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOnROOOOOOOnR OOOOS
WV-42954	Mod105L001mG*SmAfUmGfAn001RmUfGfUfUmUfUmUfG mAfAn001RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOnROOOOOOOnR OOOOS
WV-42955	Mod029L001mG*SmAfUmGfAn001RmUfGfUfUmUfUmUfG mAfAn001RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOnROOOOOOOnR OOOOS
WV-42956	Mod020L001mG*SmAfUmGfAn001RmUfGfUfUmUfUmUfG mAfAn001RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOnROOOOOOOnR OOOOS
WV-42957	Mod015L001mG*SmAfUmGfAn001RmUfGfUfUmUfUmUfG mAfAn001RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOnROOOOOOOnR OOOOS
WV-42942	L001mG*SmAfUmGfAmUfGfUfUmUfUmUfGmAfAmAfCm UfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOOOOOOOOOOO OOOS
WV-42943	L001mG*SmAfUmGfAn001RmUfGfUfUmUfUmUfGmAfAn0 01RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	OSOOOnROOOOOOOnR OOOOS

WV-43223	fU*SfA*RmUn001SfAmGfUmUn001SfUmCfA*SmA*SmA*SmC*SfA*SmU*SfC*SmA*SfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SRnSOOOnSOOSSSSSSSSnSSS
WV-43224	fU*SfA*RmUn001SfAmGfUmUn001SfU*SmC*SfA*SmA*SmA*SmC*SfA*SmU*SfC*SmA*SfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SRnSOOOnSSSSSSSSSSSSnSSS
WV-43225	fU*SfA*RmUn001SfA*SmG*SfU*SmUn001SfU*SmC*SfA*SmA*SmA*SmC*SfA*SmU*SfC*SmA*SfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SRnSSSSnSSSSSSSSSSSSnSSS
WV-43226	fU*SfA*RmUn001SfA*SmGfU*SmUn001SfU*SmCfA*SmAmA*SmC*SfA*SmU*SfC*SmA*SfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SRnSSOSnSSOSOSSSSSSSnSSS
WV-43227	fU*SfA*RmUn001SfAmG*SfUmUn001SfUmC*SfAmA*SmAmC*SfA*SmU*SfC*SmA*SfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SRnSOSOnSOSOSOSSSSnSSS
WV-43228	fU*SfA*RmUn001SfA*SmGfU*SmUn001SfU*SmCfA*SmA*SmA*SmC*SfA*SmU*SfC*SmA*SfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SRnSSOSnSSOSSSSSSSSSnSSS
WV-43229	fU*SfA*RmUn001SfAmG*SfUmUn001SfUmC*SfA*SmA*SmA*SmC*SfA*SmU*SfC*SmA*SfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SRnSOSOnSOSSSSSSSSSnSSS
WV-43230	fU*SfA*RmUn001SfA*SmGfU*SmUn001SfU*SmC*SfA*SmA*SmA*SmC*SfA*SmU*SfC*SmA*SfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SRnSSOSnSSSSSSSSSSSSnSSS
WV-43231	fU*SfA*RmUn001SfAmG*SfUmUn001SfU*SmC*SfA*SmA*SmA*SmC*SfA*SmU*SfC*SmA*SfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUCAUCUU	SRnSOSOnSSSSSSSSSSSSnSSS
WV-41025	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn001RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-43232	mG*SmA*SfU*SmG*SfAn001RmUfGfUfUmUfGmAfAn001RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SSSSnROOOOOOOnROOOOS
WV-43233	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn001RmA*SfC*SmU*SfA*SmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnRSSSSS
WV-43234	mG*SmA*SfU*SmG*SfAn001RmUfGfUfUmUfGmAfAn001RmA*SfC*SmU*SfA*SmU*SfA	GAUGAUGUUUGAAACUAUA	SSSSnROOOOOOOnRSSSSS

WV-43235	mG*SmA*SfU*SmG*SfAn001RmU*SfG*SfU*SfUmUfGmAfAn001RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SSSSnRSSSOOOOnROOOOS
WV-43236	mG*SmAfUmGfAn001RmUfGfUfUmU*SfG*SmA*SfAn001RmA*SfC*SmU*SfA*SmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOSSSSnRSSSSS
WV-43237	mG*SmA*SfU*SmG*SfAn001RmU*SfG*SfU*SfUmU*SfG*SmA*SfAn001RmA*SfC*SmU*SfA*SmU*SfA	GAUGAUGUUUGAAACUAUA	SSSSnRSSSOSSSSnRSSSSS
WV-43238	mG*SmA*SfU*SmG*SfAn001RmU*SfG*SfU*SfU*SmU*SfG*SmA*SfAn001RmA*SfC*SmU*SfA*SmU*SfA	GAUGAUGUUUGAAACUAUA	SSSSnRSSSSSSSnRSSSSS
WV-44330	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn029RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-44331	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn031RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-44332	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn037RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-44333	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn046RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-44334	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn047RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-44335	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn054RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-44336	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn055RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-44337	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn006RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-44338	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn020RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-44339	mG*SmAfUmGfAn001RmUfGfUfUmUfGmAfAn021RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-44371	mG*SmAfUmGfAn029RmUfGfUfUmUfGmAfAn029RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-44372	mG*SmAfUmGfAn031RmUfGfUfUmUfGmAfAn031RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS
WV-44373	mG*SmAfUmGfAn037RmUfGfUfUmUfGmAfAn037RmAfCmUfAmU*SfA	GAUGAUGUUUGAAACUAUA	SOOOnROOOOOOOnROOOOS

WV-44374	mG*SmAfUmGfAn046RmUfGfUfUmUfGmAfAn046R mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	SOOOnROOOOOOOnRO OOOS
WV-44375	mG*SmAfUmGfAn047RmUfGfUfUmUfGmAfAn047R mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	SOOOnROOOOOOOnRO OOOS
WV-44376	mG*SmAfUmGfAn020RmUfGfUfUmUfGmAfAn020R mAfCmUfAmU*SfA	GAUGAUGUUUGAAACU AUA	SOOOnROOOOOOOnRO OOOS
WV-42309	fU*RfA*SmUn001SfAmGfUmUn001SfUmCfAmAmAm C*SfA*SmU*SfC*SmA*SfUn001SmC*SmU*SmU	UAUAGUUUCAAACAUC AUCUU	RSnSOOOnSOOOOOSSS SSnSSS

Notes:

Description, Base Sequence and Stereochemistry/Linkage, due to their length, may be divided into multiple lines in Tables 1A-1D. Unless otherwise specified, all oligonucleotides in Table 1A-1D are single-stranded. As appreciated by those skilled in the art, nucleoside units are unmodified and contain unmodified nucleobases and 2'-deoxy sugars unless otherwise indicated (e.g., with r, m, etc.); linkages, unless otherwise indicated, are natural phosphate linkages; and acidic/basic groups may independently exist in their salt forms. If a sugar is not specified, the sugar is a natural DNA sugar; and if an internucleotidic linkage is not specified, the internucleotidic linkage is a natural phosphate linkage. Moieties and modifications:

m: 2'-OMe;

f: 2'-F;

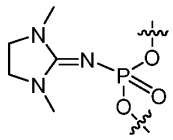
O, PO: phosphodiester (phosphate). It can a linkage or be an end group (or a component thereof), e.g., a linkage between a linker and an oligonucleotide chain, an internucleotidic linkage (a natural phosphate linkage), etc. Phosphodiesters are typically indicated with "O" in the Stereochemistry/Linkage column and are typically not marked in the Description column (if it is an end group, e.g., a 5'-end group, it is indicated in the Description and typically not in Stereochemistry/Linkage); if no linkage is indicated in the Description column, it is typically a phosphodiester unless otherwise indicated. Note that a phosphate linkage between a linker (e.g., L001) and an oligonucleotide chain may not be marked in the Description column, but may be indicated with "O" in the Stereochemistry/Linkage column;

*, PS: Phosphorothioate. It can be an end group (if it is an end group, e.g., a 5'-end group, it is indicated in the Description and typically not in Stereochemistry/Linkage), or a linkage, e.g., a linkage between linker (e.g., L001) and an oligonucleotide chain, an internucleotidic linkage (a phosphorothioate internucleotidic linkage), etc.;

R, *R*p: Phosphorothioate in the *R*p configuration. Note that * R in Description indicates a single phosphorothioate linkage in the *R*p configuration;

S, *S*p: Phosphorothioate in the *S*p configuration. Note that * S in Description indicates a single phosphorothioate linkage in the *S*p configuration;

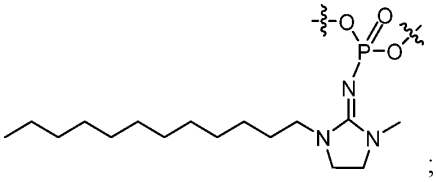
X: stereorandom phosphorothioate;

n001:  ;

nX: stereorandom n001;

nR or n001R: n001 in Rp configuration;

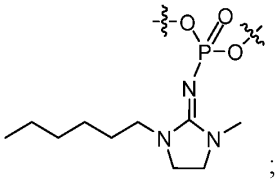
nS or n001S: n001 in Sp configuration;

n009:  ;

nX: stereorandom n009;

nR or n009R: n009 in Rp configuration;

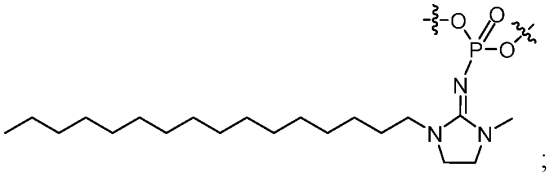
nS or n009S: n009 in Sp configuration;

n031:  ;

nX: stereorandom n031;

nR or n031R: n031 in Rp configuration;

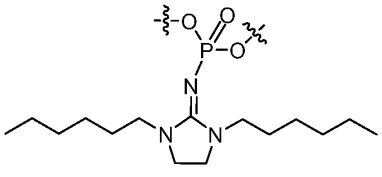
nS or n031S: n031 in Sp configuration;

n033:  ;

nX: stereorandom n033;

nR or n033R: n033in Rp configuration;

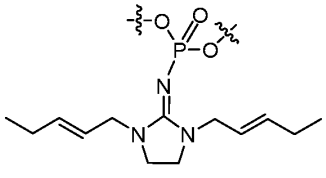
nS or n033S: n033 in Sp configuration;

n037:  ;

nX: stereorandom n037;

nR or n037R: n037in Rp configuration;

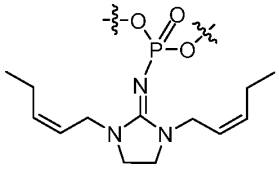
nS or n037S: n037 in Sp configuration;

n046:  ;

nX: stereorandom n046;

nR or n046R: n046in Rp configuration;

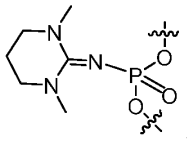
nS or n046S: n046 in Sp configuration;

n047:  ;

nX: stereorandom n047;

nR or n047R: n047in Rp configuration;

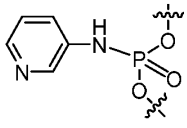
nS or n047S: n047 in Sp configuration;

n025:  ;

nX: stereorandom n025;

nR or n025R: n025 in Rp configuration;

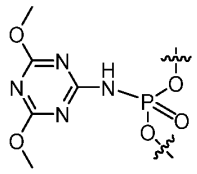
nS or n025S: n025 in Sp configuration;

n054:  ;

nX: stereorandom n054;

nR or n054R: n054 in Rp configuration;

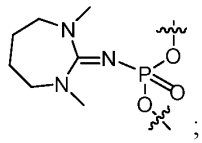
nS or n054S: n054 in Sp configuration;

n055:  ;

nX: stereorandom n055;

nR or n055R: n055 in Rp configuration;

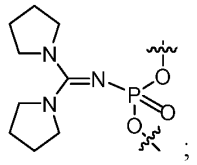
nS or n055S: n055 in Sp configuration;

n026:  ;

nX: stereorandom n001;

nR or n026R: n026 in Rp configuration;

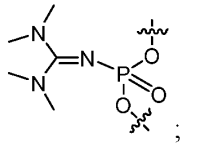
nS or n026S: n026 in Sp configuration;

n004:  ;

nX: stereorandom n004;

nR or n004R: n004 in Rp configuration;

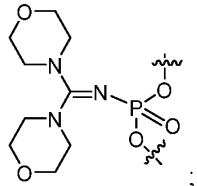
nS or n004S: n004 in Sp configuration;

n003:  ;

nX: stereorandom n003;

nR or n003R: n003 in Rp configuration;

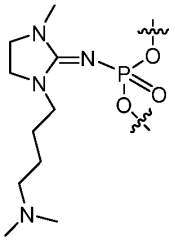
nS or n003S: n003 in Sp configuration;

n008:  ;

nX: stereorandom n008;

nR or n008R: n008 in Rp configuration;

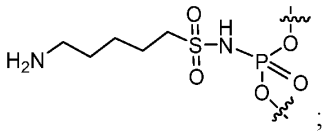
nS or n008S: n008 in Sp configuration;

n029:  ;

nX: stereorandom n029;

nR or n029R: n029 in Rp configuration;

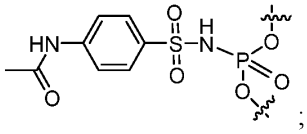
nS or n029S: n029 in Sp configuration;

n021:  ;

nX: stereorandom n021;

nR or n021R: n021 in Rp configuration;

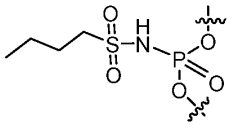
nS or n021S: n021 in Sp configuration;

n006:  ;

nX: stereorandom n006;

nR or n006R: n006 in Rp configuration;

nS or n006S: n006 in Sp configuration;

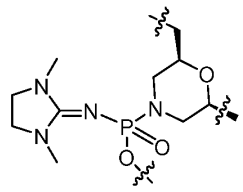
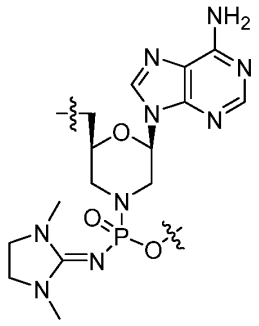
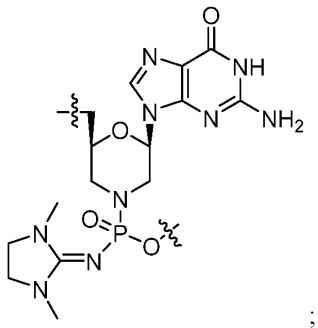
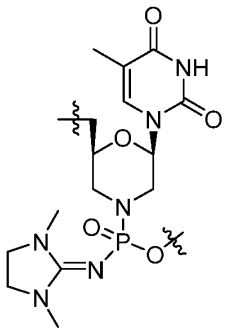
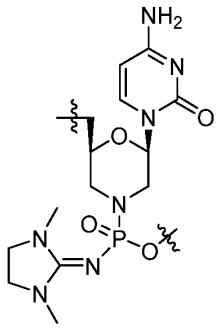
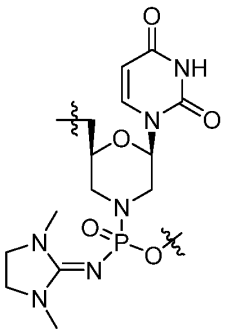
n020:  ;

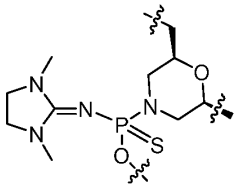
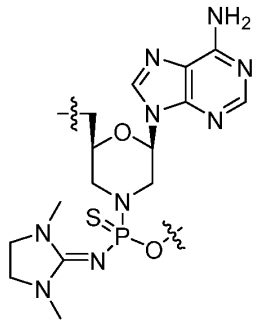
nX: stereorandom n020;

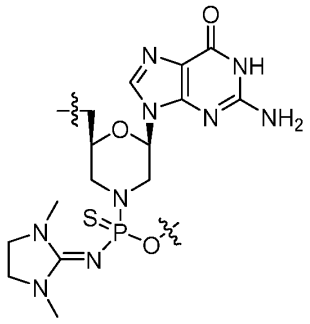
nR or n020R: n020 in Rp configuration;

nS or n020S: n020 in Sp configuration;

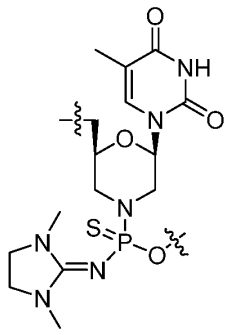
X: stereorandom phosphorothioate;

sm01n001:  (e.g., Asm01n001:  ; Gsm01n001:  ; Tsm01n001:  ; Csm01n001:   Usm01n001:);

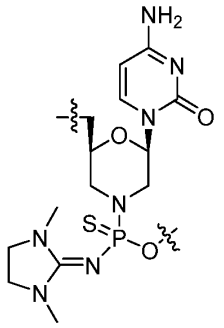
5 sm01*n001:  (e.g., Asm01*n001:  ; Gsm01*n001:



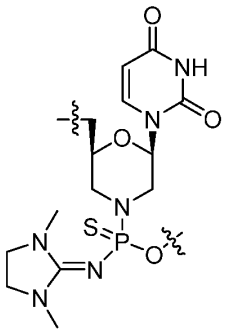
; Tsm01*n001:



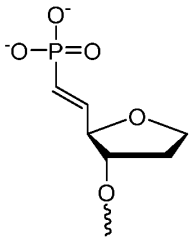
; Csm01*n001:



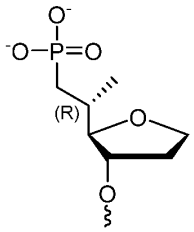
Usm01*n001:



);

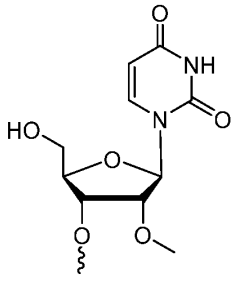


L026 ;

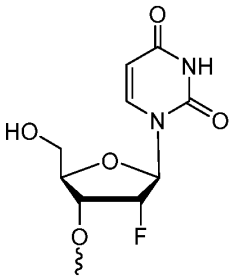


L027 ;

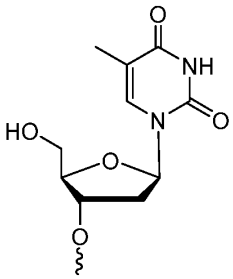
mU



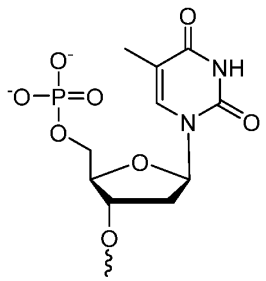
;



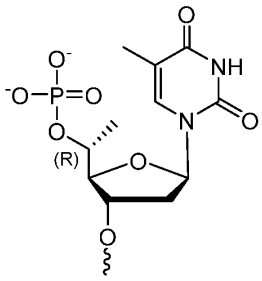
fU ;

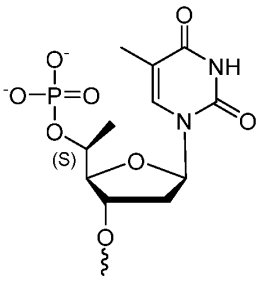


dT ; POdT or PO4-dT

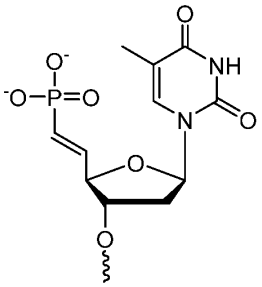


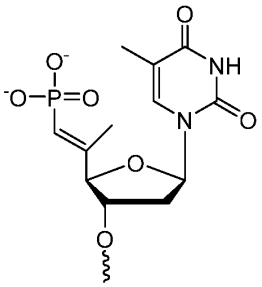
;



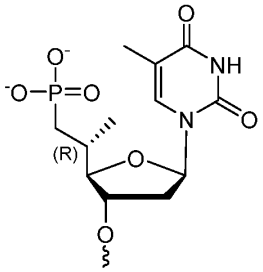
PO5MRdT ; 

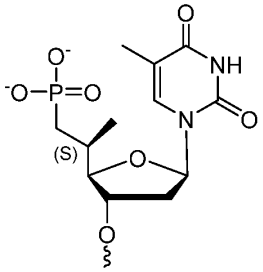
PO5MSdT ;



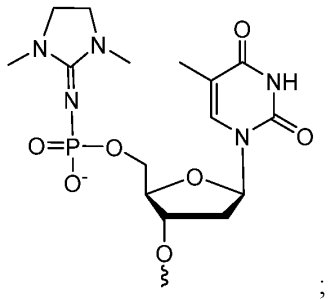
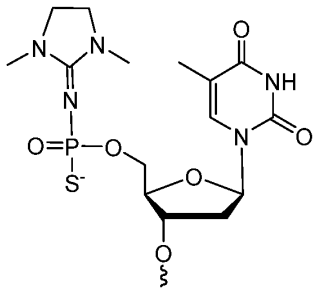
VPdT ; 

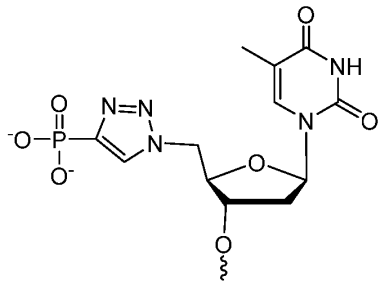
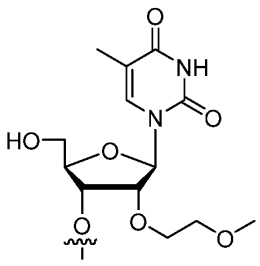
5mvpdT ;

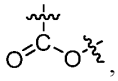


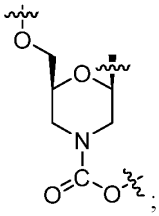
5mrpdT ; 

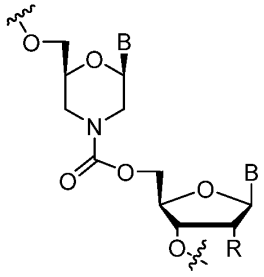
5mspdT ;

PNdT  ; SPNdT  ;

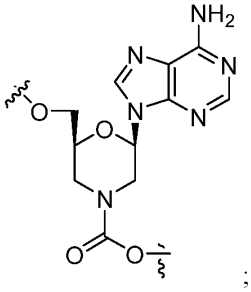
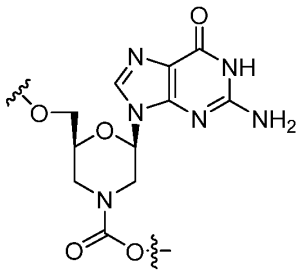
5ptzdT  ; Teo  ;

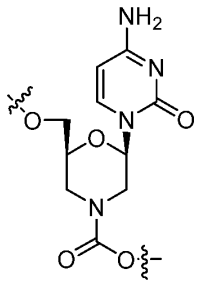
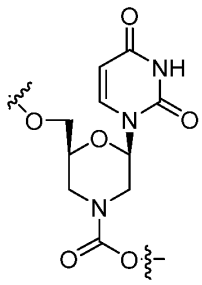
n013: , wherein −C(O)− is bonded to nitrogen;

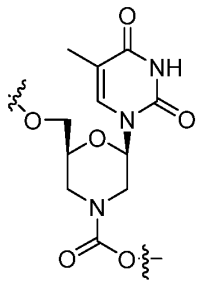
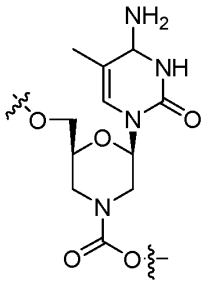
sm01n013: ;



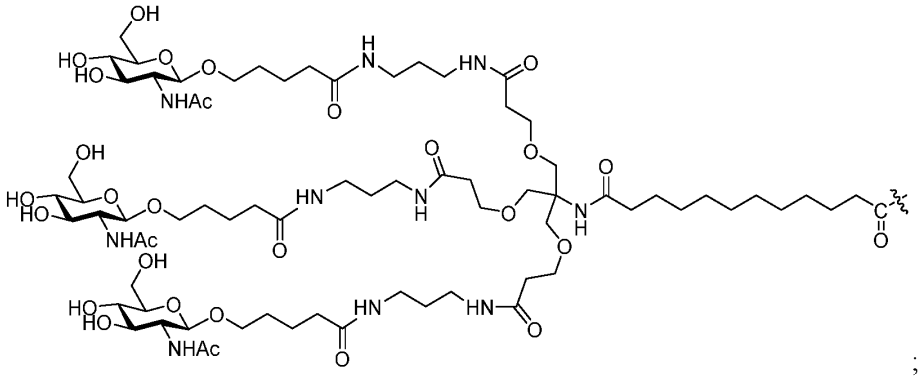
i.e. morpholine carbamate internucleotidic linkage (sm01n013)

; Asm01n013  ; Gsm01n013:  ;

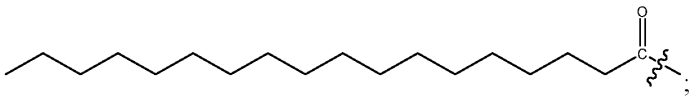
Csm01n013:  ; Usm01n013:  ; Tsm01n013:

 ; m5Csm01n013:  ;

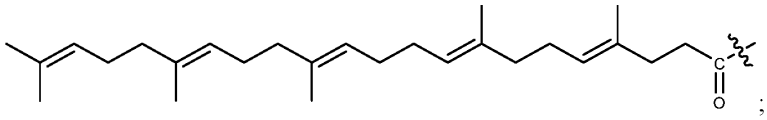
Mod001:

 ;

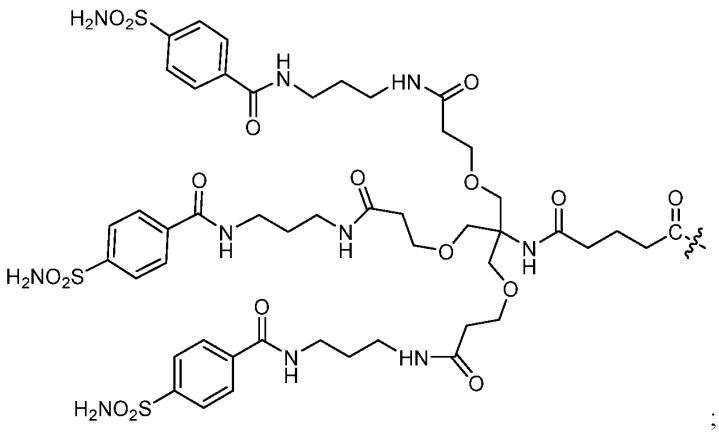
Mod015:

 ;

Mod020:

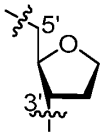
 ;

Mod029:

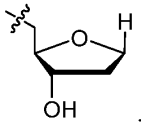


;

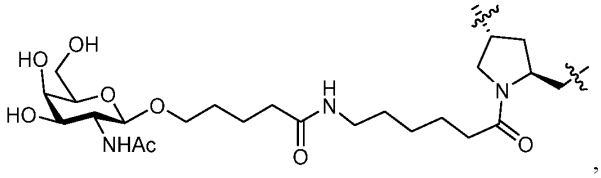
L001 : −NH−$(CH_2)_6$− linker (C6 linker, C6 amine linker or C6 amino linker), connected to Mod (e.g., Mod001) through −NH−, and, in the case of, for example, WV-38061, the 5'-end of the oligonucleotide chain through a phosphate linkage (O or PO). For example, in WV-38061, L001 is connected to Mod001 through –NH− (forming an amide group –C(O)−NH−), and is connected to the oligonucleotide chain through a phosphate linkage (O).

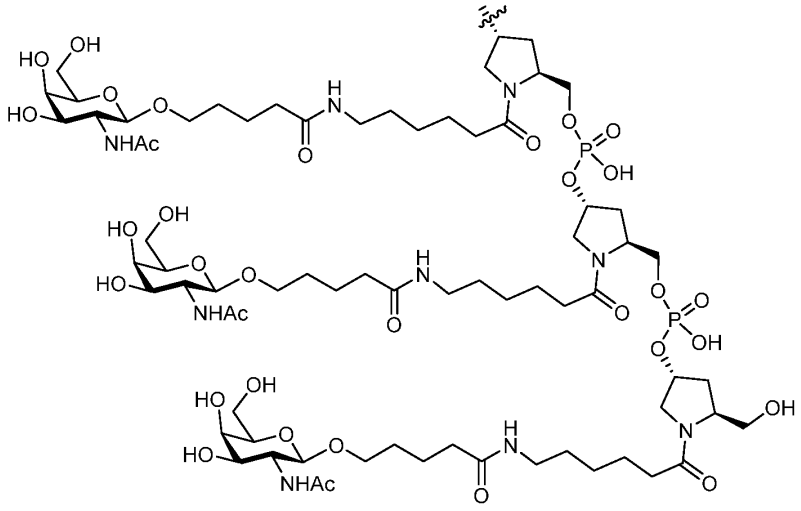
L010: . In some embodiments, when L010 is present in the middle of an oligonucleotide, it is bonded to internucleotidic linkages as other sugars (e.g., DNA sugars), e.g., its 5'-carbon is connected to another unit (e.g., 3' of a sugar) and its 3'-carbon is connected to another unit (e.g., a 5'-carbon of a carbon) independently, e.g., via a linkage (e.g., a phosphate linkage (O or PO) or a phosphorothioate linkage (can be either not chirally controlled or chirally controlled (*S*p or *R*p)));

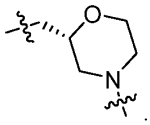
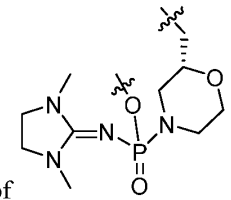
L012:−$CH_2CH_2OCH_2CH_2OCH_2CH_2$−. When L012 is present in the middle of an oligonucleotide, each of its two ends is independently bonded to an internucleotidic linkage (e.g., a phosphate linkage (O or PO) or a phosphorothioate linkage (can be either not chirally controlled or chirally controlled (*S*p or *R*p)));

L022: , wherein L022 is connected to the rest of a molecule through a phosphate unless indicated otherwise;

L023: HO−$(CH_2)_6$−, wherein CH_2 is connected to the rest of a molecule through a phosphate unless indicated otherwise. For example, in WV-42644 (wherein the **O** in OnRnRnRnRSSSSSSSSSSSSSSSSSSnRSSSSSnRSSnR indicates a phosphate linkage connecting L023 to the rest of the molecule);

L025:  , wherein the $-CH_2-$ connection site is utilized as a C5 connection site of a sugar (e.g., a DNA sugar) and is connected to another unit (e.g., 3' of a sugar), and the connection site on the ring is utilized as a C3 connection site and is connected to another unit (e.g., a 5'-carbon of a carbon), each of which is independently, e.g., via a linkage (e.g., a phosphate linkage (O or PO) or a phosphorothioate linkage (can be either not chirally controlled or chirally controlled (*S*p or *R*p))). When L025 is at a5'-end without any modifications, its $-CH_2-$ connection site is bonded to $-OH$. For example, L025L025L025− in various oligonucleotides has the structure of

 (may exist as various salt forms) and is connected to 5'-carbon of an oligonucleotide chain via a linkage as indicated (e.g., a phosphate linkage (O or PO) or a phosphorothioate linkage (can be either not chirally controlled or chirally controlled (*S*p or *R*p)));

L016:  . wherein L016 is connected to the rest of a molecule through a phosphate unless indicated otherwise; L016 is utilized with n001 to form L016n001, which has the structure of  .

1.2.2 Double Stranded Oligonucleotide Lengths

As appreciated by those skilled in the art, ds oligonucleotides can be of various lengths to provide desired properties and/or activities for various uses. Many technologies for assessing, selecting and/or optimizing ds oligonucleotide length are available in the art and can be utilized in accordance with the present disclosure. As demonstrated herein, in certain embodiments, dsRNAi oligonucleotides are of suitable lengths to hybridize with their targets and reduce levels of their targets and/or an encoded product thereof. In certain embodiments, a ds oligonucleotide is long enough to recognize a target nucleic acid (e.g., a target mRNA). In certain embodiments, a ds oligonucleotide is sufficiently long to distinguish between a target nucleic acid and other nucleic acids (e.g., a nucleic acid having a base sequence which is not a target sequence) to reduce off-target effects. In certain embodiments, a dsRNAi oligonucleotide is sufficiently short to reduce complexity of manufacture or production and to reduce cost of products.

In certain embodiments, the base sequence of a ds oligonucleotide is about 10-500 nucleobases in length. In certain embodiments, a base sequence is about 10-500 nucleobases in length. In certain embodiments, a base sequence is about 10-50 nucleobases in length. In certain embodiments, a base sequence is about 15-50 nucleobases in length. In certain embodiments, a base sequence is from about 15 to about 30 nucleobases in length. In certain embodiments, a base sequence is from about 10 to about 25 nucleobases in length. In certain embodiments, a base sequence is from about 15 to about 22 nucleobases in length. In certain embodiments, a base sequence is about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobases in length. In certain embodiments, a base sequence is about 18 nucleobases in length. In certain embodiments, a base sequence is about 19 nucleobases in length. In certain embodiments, a base sequence is about 20 nucleobases in length. In certain embodiments, a base sequence is about 21 nucleobases in length. In certain embodiments, a base sequence is about 22 nucleobases in length. In certain embodiments, a base sequence is about 23 nucleobases in length. In certain embodiments, a base sequence is about 24 nucleobases in length. In certain embodiments, a base sequence is about 25 nucleobases in length. In certain embodiments, each nucleobase is optionally substituted A, T, C, G, U, or an optionally substituted tautomer of A, T, C, G, or U.

2.2.3. Internucleotidic Linkages

In certain embodiments, ds oligonucleotides comprise base modifications, sugar modifications, and/or internucleotidic linkage modifications. Various internucleotidic

linkages can be utilized in accordance with the present disclosure to link units comprising nucleobases, e.g., nucleosides. In certain embodiments, provided ds oligonucleotides comprise both one or more modified internucleotidic linkages and one or more natural phosphate linkages. As widely known by those skilled in the art, natural phosphate linkages are widely found in natural DNA and RNA molecules; they have the structure of −OP(O)(OH)O−, connect sugars in the nucleosides in DNA and RNA, and may be in various salt forms, for example, at physiological pH (about 7.4), natural phosphate linkages are predominantly exist in salt forms with the anion being $-OP(O)(O^-)O-$. A modified internucleotidic linkage, or a non-natural phosphate linkage, is an internucleotidic linkage that is not natural phosphate linkage or a salt form thereof. Modified internucleotidic linkages, depending on their structures, may also be in their salt forms. For example, as appreciated by those skilled in the art, phosphorothioate internucleotidic linkages which have the structure of −OP(O)(SH)O− may be in various salt forms, e.g., at physiological pH (about 7.4) with the anion being $-OP(O)(S^-)O-$.

In certain embodiments, a ds oligonucleotide comprises an internucleotidic linkage which is a modified internucleotidic linkage, e.g., phosphorothioate, phosphorodithioate, methylphosphonate, phosphoroamidate, thiophosphate, 3'-thiophosphate, or 5'-thiophosphate.

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

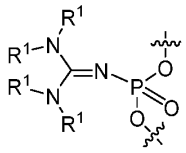
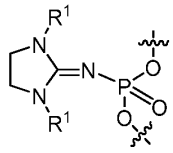
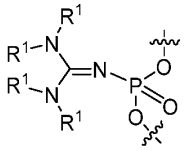
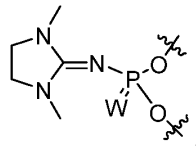
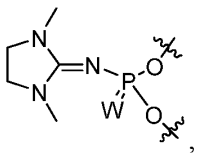
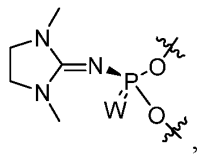
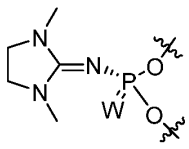
In certain embodiments, an internucleotidic linkage comprises a P-modification, wherein a P-modification is a modification at a linkage phosphorus. In certain

embodiments, a modified internucleotidic linkage is a moiety which does not comprise a phosphorus but serves to link two sugars or two moieties that each independently comprises a nucleobase, e.g., as in peptide nucleic acid (PNA).

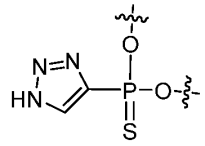
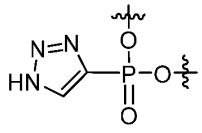
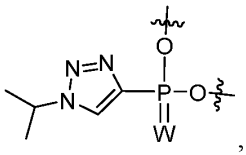
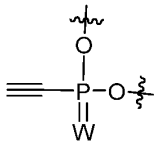
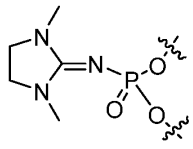
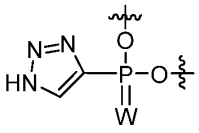
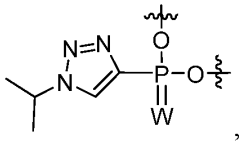
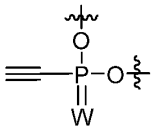
In certain embodiments, a ds oligonucleotide comprises a modified internucleotidic linkage, e.g., those having the structure of Formula **I**, **I-a**, **I-b**, or **I-c** and described herein and/or in: WO 2018/022473, WO 2018/098264, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO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

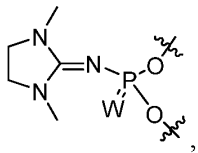
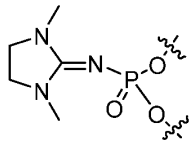
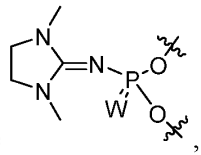
In certain embodiments, a modified internucleotidic linkage is a non-negatively charged internucleotidic linkage. In certain embodiments, provided ds oligonucleotides comprise one or more non-negatively charged internucleotidic linkages. In certain embodiments, a non-negatively charged internucleotidic linkage is a positively charged internucleotidic linkage. In certain embodiments, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage. In certain embodiments, the present disclosure provides ds oligonucleotides comprising one or more neutral internucleotidic linkages. In certain embodiments, a non-negatively charged internucleotidic linkage has the structure of Formula **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, etc., or a salt form thereof, as described herein and/or in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the non-negatively charged internucleotidic linkages (e.g., those of Formula **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, etc., or a suitable salt form thereof) of each of which are independently incorporated herein by reference.

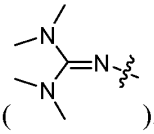
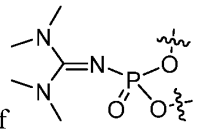
In certain embodiments, a non-negatively charged internucleotidic linkage can improve the delivery and/or activities (e.g., adenosine editing activity).

In certain embodiments, a modified internucleotidic linkage (e.g., a non-negatively charged internucleotidic linkage) comprises optionally substituted triazolyl. In certain embodiments, a modified internucleotidic linkage (e.g., a non-negatively charged internucleotidic linkage) comprises optionally substituted alkynyl. In certain embodiments, a modified internucleotidic linkage comprises a triazole or alkyne moiety. In certain embodiments, a triazole moiety, e.g., a triazolyl group, is optionally substituted. In certain embodiments, a triazole moiety, e.g., a triazolyl group) is substituted. In certain embodiments, a triazole moiety is unsubstituted. In certain embodiments, a modified internucleotidic linkage comprises an optionally substituted cyclic guanidine moiety. In certain embodiments, a modified internucleotidic linkage has the structure of  and is optionally chirally controlled, wherein R^1 is −L−R', wherein L is L^B as described herein, and R' is as described herein. In certain embodiments, each R^1 is independently R'. In certain embodiments, each R' is independently R. In certain embodiments, two R^1 are R and are taken together to form a ring as described herein. In certain embodiments, two R^1 on two different nitrogen atoms are R and are taken together to form a ring as described herein. In certain embodiments, R^1 is independently optionally substituted C_{1-6} aliphatic as described herein. In certain embodiments, R^1 is methyl. In certain embodiments, two R' on the same nitrogen atom are R and are taken together to form a ring as described herein. In certain embodiments, a modified internucleotidic linkage has the structure of  and is optionally chirally controlled. In certain embodiments,  is . In certain embodiments, a modified internucleotidic linkage comprises an optionally substituted cyclic guanidine moiety and has the structure of: , , or , wherein W is

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

In certain embodiments, a non-negatively charged internucleotidic linkage or a neutral internucleotidic linkage is an internucleotidic linkage comprising a triazole moiety. In some embodiments, an internucleotidic linkage comprising a triazole moiety (e.g., an optionally substituted triazolyl group) has the structure of . In some embodiments, an internucleotidic linkage comprising a triazole moiety has the structure of . In some embodiments, an internucleotidic linkage comprising a triazole moiety has the formula of , where W is O or S. In some embodiments, an internucleotidic linkage comprising an alkyne moiety (e.g., an optionally substituted alkynyl group) has the formula of , wherein W is O or S. In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, a neutral internucleotidic linkage, comprises a cyclic guanidine moiety. In some embodiments, an internucleotidic linkage comprising a cyclic guanidine moiety has the structure of . In some embodiments, a non-negatively charged internucleotidic linkage, or a neutral internucleotidic linkage, is or comprising a structure selected from , , , or

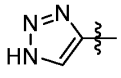
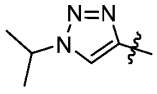
, wherein W is O or S. In certain embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, a neutral internucleotidic linkage, comprises a cyclic guanidine moiety. In certain embodiments, an internucleotidic linkage comprising a cyclic guanidine moiety has the structure of . In certain embodiments, a non-negatively charged internucleotidic linkage, or a neutral internucleotidic linkage, is or comprising a structure , wherein W is O or S.

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

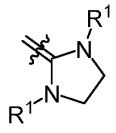
In certain embodiments, a non-negatively charged internucleotidic linkage has the structure of Formula **I**, **I-a**, **I-b**, **I-c**, **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, etc., or a salt form thereof. In certain embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 3-20 membered heterocyclyl or heteroaryl group having 1-10 heteroatoms. In certain embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 3-20 membered heterocyclyl or heteroaryl group having 1-10 heteroatoms, wherein at least one heteroatom is nitrogen. In certain embodiments, such a heterocyclyl or heteroaryl group is of a 5-membered ring. In certain embodiments, such a heterocyclyl or heteroaryl group is of a 6-membered ring.

In certain embodiments, a non-negatively charged internucleotidic linkage

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

In certain embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heterocyclyl group having 1-10 heteroatoms. In certain embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heterocyclyl group having 1-10 heteroatoms, wherein at least one heteroatom is nitrogen. In certain embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-6 membered heterocyclyl group having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In certain embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-membered heterocyclyl group having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In certain embodiments, at least two heteroatoms are nitrogen. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted triazolyl group. In some embodiments, a non-negatively charged internucleotidic linkage comprises an unsubstituted triazolyl group, e.g., . In some embodiments, a non-negatively charged internucleotidic linkage comprises a substituted triazolyl group, e.g., .

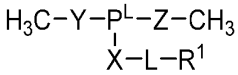
In certain embodiments, a heterocyclyl group is directly bonded to a linkage phosphorus. In certain embodiments, a heterocyclyl group is bonded to a linkage phosphorus through a linker, e.g., =N− when the heterocyclyl group is part of a guanidine moiety who directed bonded to a linkage phosphorus through its =N−. In certain embodiments, a non-negatively charged internucleotidic linkage comprises an optionally

substituted  group. In certain embodiments, a non-negatively charged internucleotidic linkage comprises an substituted  group. In certain embodiments, a non-negatively charged internucleotidic linkage comprises a  group, wherein each R^1 is independently −L−R. In certain embodiments, each R^1 is independently optionally substituted C_{1-6} alkyl. In certain embodiments, each R^1 is independently methyl.

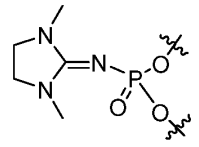
In certain embodiments, a modified internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, comprises a triazole or alkyne moiety, each of which is optionally substituted. In certain embodiments, a modified internucleotidic linkage comprises a triazole moiety. In certain embodiments,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

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

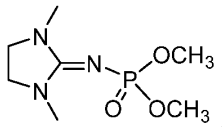
at least one non-negatively charged internucleotidic linkage. In certain embodiments, ds oligonucleotides comprise one or more, e.g., 1-50, 1-40, 1-30, 1-20, 1-15, 1-10, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more non-negatively charged internucleotidic linkages. In certain embodiments, a non-negatively charged internucleotidic linkage is not negatively charged in that at a given pH in an aqueous solution less than 50%, 40%, 40%, 30%, 20%, 10%, 5%, or 1% of the internucleotidic linkage exists in a negatively charged salt form. In certain embodiments, a pH is about pH 7.4. In certain embodiments, a pH is about 4-9. In certain embodiments, the percentage is less than 10%. In certain embodiments, the percentage is less than 5%. In certain embodiments, the percentage is less than 1%. In certain embodiments, an internucleotidic linkage is a non-negatively charged internucleotidic linkage in that the neutral form of the internucleotidic linkage has no pKa that is no more than about 1, 2, 3, 4, 5, 6, or 7 in water. In certain embodiments, no pKa is 7 or less. In certain embodiments, no pKa is 6 or less. In certain embodiments, no pKa is 5 or less. In certain embodiments, no pKa is 4 or less. In certain embodiments, no pKa is 3 or less. In certain embodiments, no pKa is 2 or less. In certain embodiments, no pKa is 1 or less. In certain embodiments, pKa of the neutral form of an internucleotidic linkage can be represented by pKa of the neutral form of a compound having the structure of CH_3–the internucleotidic linkage–CH_3. For example, pKa of the neutral form of an internucleotidic linkage having the structure of Formula I may be represented by the pKa of the neutral form of a compound having the structure of



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



can be represented by pKa

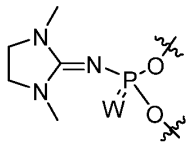
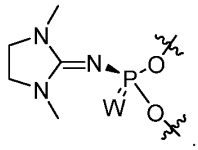
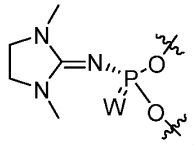
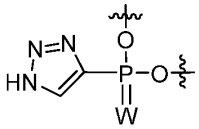


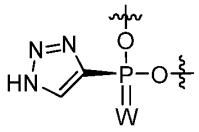
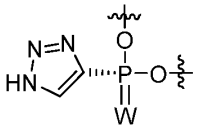
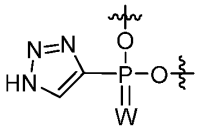
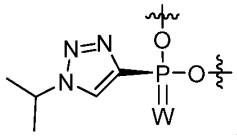
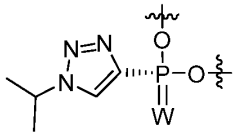
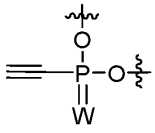
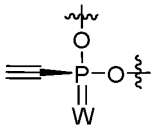
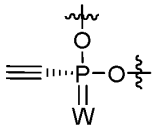
. In certain embodiments, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage. In certain embodiments, a non-negatively charged internucleotidic linkage is a positively-charged internucleotidic linkage. In certain embodiments, a non-negatively charged internucleotidic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

internucleotidic linkage comprises a triazole moiety. In certain embodiments, a non-negatively charged internucleotidic linkage comprises an alkynyl moiety.

In certain embodiments, a neutral or non-negatively charged internucleotidic linkage has the structure of any neutral or non-negatively charged internucleotidic linkage described in any of: US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612,2607, WO2019032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, each neutral or non-negatively charged internucleotidic linkage of each of which is hereby incorporated by reference.

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

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

has the structure of . In some embodiments, a non-negatively charged internucleotidic linkage has the structure of . In some embodiments, a non-negatively charged internucleotidic linkage has the structure of . In some embodiments, a non-negatively charged internucleotidic linkage has the structure of . In some embodiments, a non-negatively charged internucleotidic linkage has the structure of . In some embodiments, a non-negatively charged internucleotidic linkage has the structure of . In some embodiments, a non-negatively charged internucleotidic linkage has the structure of . In some embodiments, a non-negatively charged internucleotidic linkage has the structure of . In some embodiments, W is O. In some embodiments, W is S. In some embodiments, a neutral internucleotidic linkage is a non-negatively charged internucleotidic linkage described above.

In certain embodiments, provided ds oligonucleotides comprise 1 or more internucleotidic linkages of Formula **I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1,** or **II-d-2**, which are described in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO

2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612,2607, WO2019032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the Formula **I, I-a, I-b, I-c, I-n-1, I-n-2, I-n-3, I-n-4, II, II-a-1, II-a-2, II-b-1, II-b-2, II-c-1, II-c-2, II-d-1,** or **II-d-2**, or salt forms thereof, each of which are independently incorporated herein by reference.

In certain embodiments, a ds oligonucleotide comprises a neutral internucleotidic linkage and a chirally controlled internucleotidic linkage. In certain embodiments, a ds oligonucleotide comprises a neutral internucleotidic linkage and a chirally controlled internucleotidic linkage which is not the neutral internucleotidic linkage. In certain embodiments, a ds oligonucleotide comprises a neutral internucleotidic linkage and a chirally controlled phosphorothioate internucleotidic linkage. In certain embodiments, the present disclosure provides a ds oligonucleotide comprising one or more non-negatively charged internucleotidic linkages and one or more phosphorothioate internucleotidic linkages, wherein each phosphorothioate internucleotidic linkage in the oligonucleotide is independently a chirally controlled internucleotidic linkage. In certain embodiments, the present disclosure provides a ds oligonucleotide comprising one or more neutral internucleotidic linkages and one or more phosphorothioate internucleotidic linkage, wherein each phosphorothioate internucleotidic linkage in the ds oligonucleotide is independently a chirally controlled internucleotidic linkage. In certain embodiments, a ds oligonucleotide comprises at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more chirally controlled phosphorothioate internucleotidic linkages. In certain embodiments, non-negatively charged internucleotidic linkage is chirally controlled. In certain embodiments, non-negatively charged internucleotidic linkage is not chirally controlled. In certain embodiments, a neutral internucleotidic linkage is chirally controlled. In certain embodiments, a neutral internucleotidic linkage is not chirally controlled.

Without wishing to be bound by any particular theory, the present disclosure notes that a neutral internucleotidic linkage can be more hydrophobic than a phosphorothioate internucleotidic linkage (PS), which can be more hydrophobic than a natural phosphate linkage (PO). Typically, unlike a PS or PO, a neutral internucleotidic linkage bears less charge. Without wishing to be bound by any particular theory, the present

disclosure notes that incorporation of one or more neutral internucleotidic linkages into a ds oligonucleotide may increase the ds oligonucleotides' ability to be taken up by a cell and/or to escape from endosomes. Without wishing to be bound by any particular theory, the present disclosure notes that incorporation of one or more neutral internucleotidic linkages can be utilized to modulate melting temperature of duplexes formed between a ds oligonucleotide and its target nucleic acid.

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

As appreciated by those skilled in the art, internucleotidic linkages such as natural phosphate linkages and those of Formula **I**, **I-a**, **I-b**, **I-c**, **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, or salt forms thereof typically connect two nucleosides (which can either be natural or modified) as described in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO2019032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the Formula **I**, **I-a**, **I-b**, **I-c**, **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, or salt forms thereof, each of which are independently incorporated herein by reference. A typical connection, as in natural DNA and RNA, is that an internucleotidic linkage forms bonds with two sugars (which can be either unmodified or modified as described herein). In many embodiments, as exemplified herein an internucleotidic linkage forms bonds through its oxygen atoms or heteroatoms (e.g., Y and Z in various formulae) with one optionally modified ribose or deoxyribose at its 5' carbon, and the other optionally modified ribose or deoxyribose at its 3' carbon. In certain embodiments, each nucleoside units connected by an internucleotidic linkage independently comprises a nucleobase which is independently an optionally substituted A, T, C, G, or U, or a substituted tautomer of A, T, C, G or U, or a nucleobase comprising an optionally substituted heterocyclyl and/or a heteroaryl ring having at least one nitrogen atom.

In some embodiments, a linkage has the structure of or comprises

−Y−P^L(−X−R^L)−Z−, or a salt form thereof, wherein:

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

W is O, N(–L^L–R^L), S or Se;

P^N is P=N−C(–L^L–R')(=L^N–R') or P=N–L^L–R^L;

L^N is =N−L^{L1}–, =CH−L^{L1}− wherein CH is optionally substituted, or =N^+(R')(Q^-)−L^{L1}–;

Q^- is an anion;

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

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

each of L^{L1} and L^L is independently L;

−Cy^{IL}− is −Cy−;

each L is independently a covalent bond, or a bivalent, optionally substituted, linear or branched group selected from a C_{1-30} aliphatic group and a C_{1-30} heteroaliphatic group having 1-10 heteroatoms, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_{1-6} alkylene, C_{1-6} alkenylene, —C≡C—, a bivalent C_1–C_6 heteroaliphatic group having 1-5 heteroatoms, −C(R')$_2$−, −Cy−, −O−, −S−, −S−S−, −N(R')−, −C(O)−, −C(S)−, −C(NR')−, −C(NR')N(R')−, −N(R')C(NR')N(R')−, −C(O)N(R')−, −N(R')C(O)N(R')−, −N(R')C(O)O−, −S(O)−, −S(O)$_2$−, −S(O)$_2$N(R')−, −C(O)S−, −C(O)O−, −P(O)(OR')−, −P(O)(SR')−, −P(O)(R')−, −P(O)(NR')−, −P(S)(OR')−, −P(S)(SR')−, −P(S)(R')−, −P(S)(NR')−, −P(R')−, −P(OR')−, −P(SR')−, −P(NR')−, −P(OR')[B(R')$_3$]−, −OP(O)(OR')O−, −OP(O)(SR')O−, −OP(O)(R')O−, −OP(O)(NR')O−, −OP(OR')O−, −OP(SR')O−, −OP(NR')O−, −OP(R')O−, −OP(OR')[B(R')$_3$]O−, and −[C(R')$_2$C(R')$_2$O]n−, wherein n is 1-50, and one or more nitrogen or carbon atoms are optionally and independently replaced with Cy^L;

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

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

heteroatoms;

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

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

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

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

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

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

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

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

internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, P of such an internucleotidic linkage is bonded to N of a sugar.

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

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

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

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

W is O or S;

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

each R' is independently $-R$, $-C(O)R$, $-C(O)N(R)_2$, $-C(O)OR$, or $-S(O)_2R$;

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

membered heteroaryl having 1-10 heteroatoms, and 3-30 membered heterocyclyl having 1-10 heteroatoms, or

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

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

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

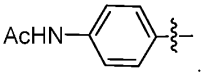
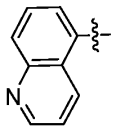
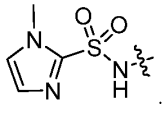
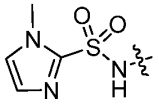
In some embodiments, W is O. In some embodiments, an internucleotidic linkage has the structure of −P(=O)(−N=C(R")$_2$)−, −P(=O)(−N(R')SO$_2$R")−, −P(=O)(−N(R')C(O)R")−, −P(=O)(−N(R")$_2$)−, −P(=O)(−N(R')P(O)(R")$_2$)−, −OP(=O)(−N=C(R")$_2$)O−, −OP(=O)(−N(R')SO$_2$R")O−, −OP(=O)(−N(R')C(O)R")O−, −OP(=O)(−N(R")$_2$)O−,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

In some embodiments, W is S. In some embodiments, an internucleotidic

linkage has the structure of −P(=S)(−N=C(R”)$_2$)−, −P(=S)(−N(R’)SO$_2$R”)−, −P(=S)(−N(R’)C(O)R”)−, −P(=S)(−N(R”)$_2$)−, −P(=S)(−N(R’)P(O)(R”)$_2$)−, −OP(=S)(−N=C(R”)$_2$)O−, −OP(=S)(−N(R’)SO$_2$R”)O−, −OP(=S)(−N(R’)C(O)R”)O−, −OP(=S)(−N(R”)$_2$)O−, −OP(=S)(−N(R’)P(O)(R”)$_2$)O−, −P(=S)(−N=C(R”)$_2$)O−, −P(=S)(−N(R’)SO$_2$R”)O−, −P(=S)(−N(R’)C(O)R”)O−, −P(=S)(−N(R”)$_2$)O−, or −P(=S)(−N(R’)P(O)(R”)$_2$)O−, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of −P(=S)(−N=C(R”)$_2$)− −P(=S)(−N(R”)$_2$)−, −OP(=S)(−N=C(R”)$_2$)−O−, −OP(=S)(−N(R”)$_2$)−O−, −P(=S)(−N=C(R”)$_2$)−O− or −P(=S)(−N(R”)$_2$)−O− or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of −OP(=S)(−N=C(R”)$_2$)−O− or −OP(=S)(−N(R”)$_2$)−O−, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of −OP(=S)(−N=C(R”)$_2$)−O−, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of −OP(=S)(−N(R”)$_2$)−O−, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of −OP(=S)(−N(R’)SO$_2$R”)O−, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of −OP(=S)(−N(R’)C(O)R”)O−, or a salt form thereof. In some embodiments, an internucleotidic linkage has the structure of −OP(=S)(−N(R’)P(O)(R”)$_2$)O−, or a salt form thereof. In some embodiments, a internucleotidic linkage is *n001.

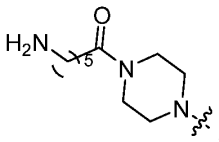
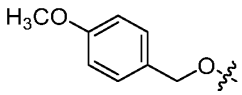
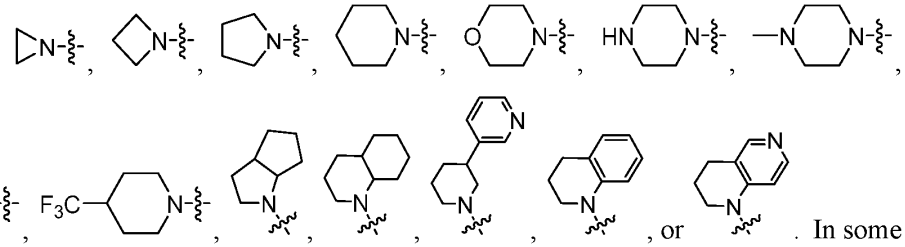
In some embodiments, an internucleotidic linkage has the structure of −P(=O)(−N(R’)SO$_2$R”)−, wherein R” is as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=S)(−N(R’)SO$_2$R”)−, wherein R” is as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=O)(−N(R’)SO$_2$R”)O−, wherein R” is as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=S)(−N(R’)SO$_2$R”)O−, wherein R” is as described herein. In some embodiments, an internucleotidic linkage has the structure of −OP(=O)(−N(R’)SO$_2$R”)O−, wherein R” is as described herein. In some embodiments, an internucleotidic linkage has the structure of −OP(=S)(−N(R’)SO$_2$R”)O−, wherein R” is as described herein. In some embodiments, R’, e.g., of −N(R’)−, is hydrogen or optionally substituted C$_{1-6}$ aliphatic. In some embodiments, R’ is C$_{1-6}$ alkyl. In some embodiments, R’ is hydrogen. In some embodiments, R”, e.g., in −SO$_2$R”, is R’ as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=O)(−NHSO$_2$R”)−, wherein R” is as described herein. In some embodiments, an internucleotidic linkage has

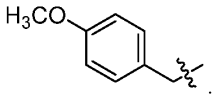
the structure of −P(=S)(−NHSO$_2$R")−, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=O)(−NHSO$_2$R")O−, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=S)(−NHSO$_2$R")O−, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of −OP(=O)(−NHSO$_2$R")O−, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of −OP(=S)(−NHSO$_2$R")O−, wherein R" is as described herein. In some embodiments, −X−R^L is −N(R')SO$_2$R^L, wherein each of R' and R^L is independently as described herein. In some embodiments, R^L is R". In some embodiments, R^L is R'. In some embodiments, −X−R^L is −N(R')SO$_2$R", wherein R' is as described herein. In some embodiments, −X−R^L is −N(R')SO$_2$R', wherein R' is as described herein. In some embodiments, −X−R^L is −NHSO$_2$R', wherein R' is as described herein. In some embodiments, R' is R as described herein. In some embodiments, R' is optionally substituted C$_{1-6}$ aliphatic. In some embodiments, R' is optionally substituted C$_{1-6}$ alkyl. In some embodiments, R' is optionally substituted phenyl. In some embodiments, R' is optionally substituted heteroaryl. In some embodiments, R", e.g., in −SO$_2$R", is R. In some embodiments, R is an optionally substituted group selected from C$_{1-6}$ aliphatic, aryl, heterocyclyl, and heteroaryl. In some embodiments, R is optionally substituted C$_{1-6}$ aliphatic. In some embodiments, R is optionally substituted C$_{1-6}$ alkyl. In some embodiments, R is optionally substituted C$_{1-6}$ alkenyl. In some embodiments, R is optionally substituted C$_{1-6}$ alkynyl. In some embodiments, R is optionally substituted methyl. In some embodiments, −X−R^L is −NHSO$_2$CH$_3$. In some embodiments, R is −CF$_3$. In some embodiments, R is methyl. In some embodiments, R is optionally substituted ethyl. In some embodiments, R is ethyl. In some embodiments, R is −CH$_2$CHF$_2$. In some embodiments, R is −CH$_2$CH$_2$OCH$_3$. In some embodiments, R is optionally substituted propyl. In some embodiments, R is optionally substituted butyl. In some embodiments, R is n-butyl. In some embodiments, R is −(CH$_2$)$_6$NH$_2$. In some embodiments, R is an optionally substituted linear C$_{2-20}$ aliphatic. In some embodiments, R is optionally substituted linear C$_{2-20}$ alkyl. In some embodiments, R is linear C$_{2-20}$ alkyl. In some embodiments, R is optionally substituted C$_1$, C$_2$, C$_3$, C$_4$, C$_5$, C$_6$, C$_7$, C$_8$, C$_9$, C$_{10}$, C$_{11}$, C$_{12}$, C$_{13}$, C$_{14}$, C$_{15}$, C$_{16}$, C$_{17}$, C$_{18}$, C$_{19}$, or C$_{20}$ aliphatic. In some embodiments, R is optionally substituted C$_1$, C$_2$, C$_3$, C$_4$, C$_5$, C$_6$, C$_7$, C$_8$, C$_9$, C$_{10}$, C$_{11}$, C$_{12}$, C$_{13}$, C$_{14}$, C$_{15}$, C$_{16}$, C$_{17}$, C$_{18}$, C$_{19}$, or C$_{20}$ alkyl. In some embodiments, R is optionally substituted linear C$_1$, C$_2$, C$_3$, C$_4$, C$_5$, C$_6$,

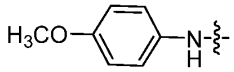
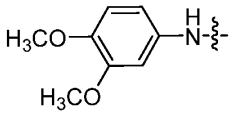
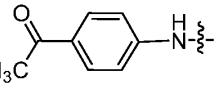
C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is linear C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is phenyl. In some embodiments, R is p-methylphenyl. In some embodiments, R is 4-dimethylaminophenyl. In some embodiments, R is 3-pyridinyl. In some embodiments, R is . In some embodiments, R is . In some embodiments, R is benzyl. In some embodiments, R is optionally substituted heteroaryl. In some embodiments, R is optionally substituted 1,3-diazolyl. In some embodiments, R is optionally substituted 2-(1,3)-diazolyl. In some embodiments, R is optionally substituted 1-methyl-2-(1,3)-diazolyl. In some embodiments, R is isopropyl. In some embodiments, R" is $-N(R')_2$. In some embodiments, R" is $-N(CH_3)_2$. In some embodiments, R", e.g., in $-SO_2R$", is $-OR'$, wherein R' is as described herein. In some embodiments, R' is R as described herein. In some embodiments, R" is $-OCH_3$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2R)O-$, wherein R is as described herein. In some embodiments, R is optionally substituted linear alkyl as described herein. In some embodiments, R is linear alkyl as described herein. In some embodiments, a linkage is $-OP(=O)(-NHSO_2CH_3)O-$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2CH_2CH_3)O-$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2CH_2CH_2OCH_3)O-$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2CH_2Ph)O-$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2CH_2CHF_2)O-$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2(4\text{-methylphenyl}))O-$. In some embodiments, $-X-R^L$ is . In some embodiments, a linkage is $-OP(=O)(-X-R^L)O-$, wherein $-X-R^L$ is . In some embodiments, a linkage is $-OP(=O)(-NHSO_2CH(CH_3)_2)O-$. In some embodiments, a linkage is $-OP(=O)(-NHSO_2N(CH_3)_2)O-$.

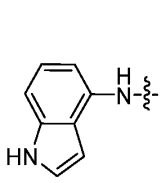
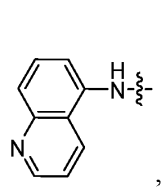
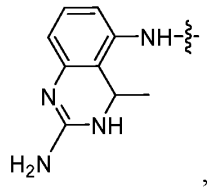
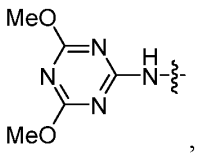
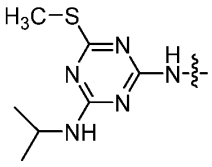
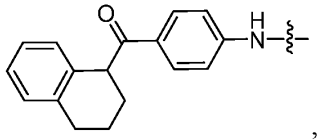
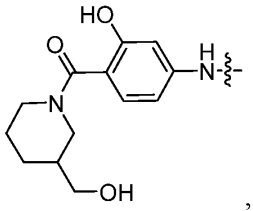
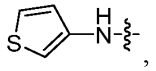
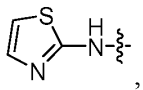
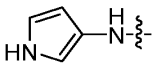
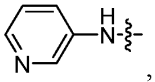
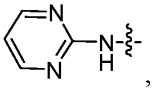
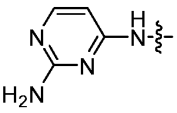
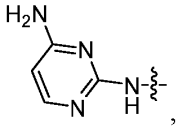
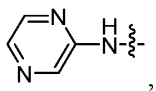
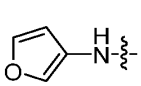
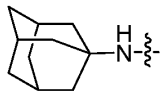
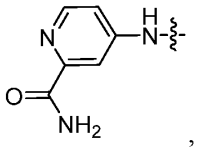
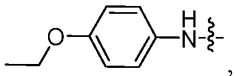
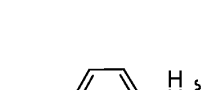
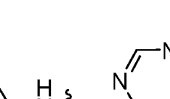
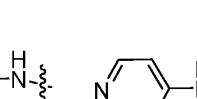
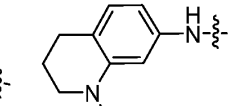
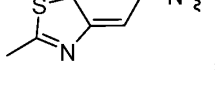
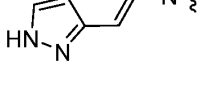
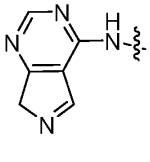
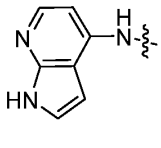
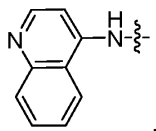
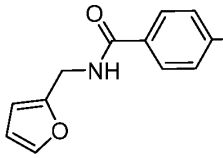
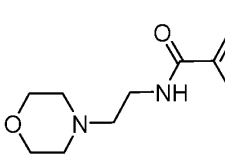
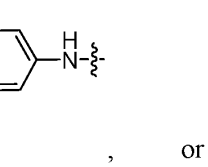
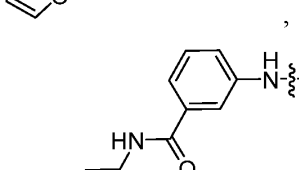
In some embodiments, an internucleotidic linkage has the structure of $-P(=O)(-N(R')C(O)R")-$, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=S)(-N(R')C(O)R")-$, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of

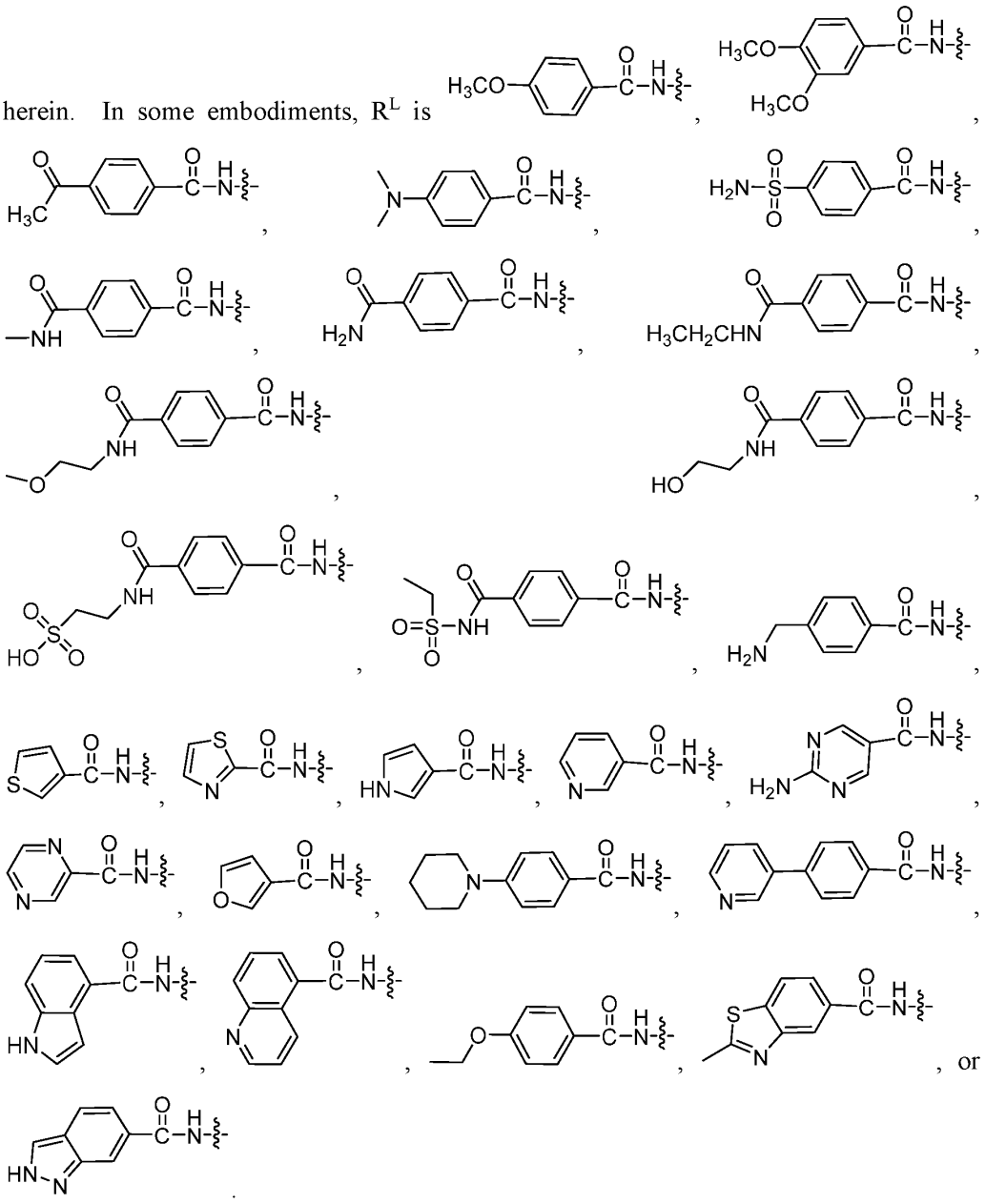
−P(=O)(−N(R')C(O)R")O−, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=S)(−N(R')C(O)R")O−, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of −OP(=O)(−N(R')C(O)R")O−, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of −OP(=S)(−N(R')C(O)R")O−, wherein R" is as described herein. In some embodiments, R', e.g., of −N(R')−, is hydrogen or optionally substituted C_{1-6} aliphatic. In some embodiments, R' is C_{1-6} alkyl. In some embodiments, R' is hydrogen. In some embodiments, R", e.g., in −C(O)R", is R' as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=O)(−NHC(O)R")−, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=S)(−NHC(O)R")−, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=O)(−NHC(O)R")O−, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=S)(−NHC(O)R")O−, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of −OP(=O)(−NHC(O)R")O−, wherein R" is as described herein. In some embodiments, an internucleotidic linkage has the structure of −OP(=S)(−NHC(O)R")O−, wherein R" is as described herein. In some embodiments, $-X-R^L$ is $-N(R')COR^L$, wherein R^L is as described herein. In some embodiments, $-X-R^L$ is −N(R')COR", wherein R" is as described herein. In some embodiments, $-X-R^L$ is −N(R')COR', wherein R' is as described herein. In some embodiments, $-X-R^L$ is −NHCOR', wherein R' is as described herein. In some embodiments, R' is R as described herein. In some embodiments, R' is optionally substituted C_{1-6} aliphatic. In some embodiments, R' is optionally substituted C_{1-6} alkyl. In some embodiments, R' is optionally substituted phenyl. In some embodiments, R' is optionally substituted heteroaryl. In some embodiments, R", e.g., in −C(O)R", is R. In some embodiments, R is an optionally substituted group selected from C_{1-6} aliphatic, aryl, heterocyclyl, and heteroaryl. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted C_{1-6} alkyl. In some embodiments, R is optionally substituted C_{1-6} alkenyl. In some embodiments, R is optionally substituted C_{1-6} alkynyl. In some embodiments, R is methyl. In some embodiments, $-X-R^L$ is $-NHC(O)CH_3$. In some embodiments, R is optionally substituted methyl. In some embodiments, R is $-CF_3$. In some embodiments, R is optionally substituted ethyl. In some embodiments, R is ethyl. In some embodiments, R is $-CH_2CHF_2$.

In some embodiments, R is $-CH_2CH_2OCH_3$. In some embodiments, R is optionally substituted C_{1-20} (e.g., C_{1-6}, C_{2-6}, C_{3-6}, C_{1-10}, C_{2-10}, C_{3-10}, C_{2-20}, C_{3-20}, C_{10-20}, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, etc.) aliphatic. In some embodiments, R is optionally substituted C_{1-20} (e.g., C_{1-6}, C_{2-6}, C_{3-6}, C_{1-10}, C_{2-10}, C_{3-10}, C_{2-20}, C_{3-20}, C_{10-20}, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, etc.) alkyl. In some embodiments, R is an optionally substituted linear C_{2-20} aliphatic. In some embodiments, R is optionally substituted linear C_{2-20} alkyl. In some embodiments, R is linear C_{2-20} alkyl. In some embodiments, R is optionally substituted C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} aliphatic. In some embodiments, R is optionally substituted C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is optionally substituted linear C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is linear C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is optionally substituted aryl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is p-methylphenyl. In some embodiments, R is benzyl. In some embodiments, R is optionally substituted heteroaryl. In some embodiments, R is optionally substituted 1,3-diazolyl. In some embodiments, R is optionally substituted 2-(1,3)-diazolyl. In some embodiments, R is optionally substituted 1-methyl-2-(1,3)-diazolyl. In some embodiments, R^L is $-(CH_2)_5NH_2$. In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R" is $-N(R')_2$. In some embodiments, R" is $-N(CH_3)_2$. In some embodiments, $-X-R^L$ is $-N(R')CON(R^L)_2$, wherein each of R' and R^L is independently as described herein. In some embodiments, $-X-R^L$ is $-NHCON(R^L)_2$, wherein R^L is as described herein. In some embodiments, two R' or two R^L are taken together with the nitrogen atom to which they are attached to form a ring as described herein, e.g., optionally substituted , or . In some embodiments, R", e.g., in $-C(O)R$", is $-OR'$, wherein R' is as described herein. In some

embodiments, R' is R as described herein. In some embodiments, is optionally substituted C_{1-6} aliphatic. In some embodiments, is optionally substituted C_{1-6} alkyl. In some embodiments, R" is $-OCH_3$. In some embodiments, $-X-R^L$ is $-N(R')C(O)OR^L$, wherein each of R' and R^L is independently as described herein. In some embodiments, R is . In some embodiments, $-X-R^L$ is $-NHC(O)OCH_3$. In some embodiments, $-X-R^L$ is $-NHC(O)N(CH_3)_2$. In some embodiments, a linkage is $-OP(O)(NHC(O)CH_3)O-$. In some embodiments, a linkage is $-OP(O)(NHC(O)OCH_3)O-$. In some embodiments, a linkage is $-OP(O)(NHC(O)(p\text{-methylphenyl}))O-$. In some embodiments, a linkage is $-OP(O)(NHC(O)N(CH_3)_2)O-$. In some embodiments, $-X-R^L$ is $-N(R')R^L$, wherein each of R' and R^L is independently as described herein. In some embodiments, $-X-R^L$ is $-N(R')R^L$, wherein each of R' and R^L is independently not hydrogen. In some embodiments, $-X-R^L$ is $-NHR^L$, wherein R^L is as described herein. In some embodiments, R^L is not hydrogen. In some embodiments, R^L is optionally substituted aryl or heteroaryl. In some embodiments, R^L is optionally substituted aryl. In some embodiments, R^L is optionally substituted phenyl. In some embodiments, $-X-R^L$ is $-N(R')_2$, wherein each R' is independently as described herein. In some embodiments, $-X-R^L$ is $-NHR'$, wherein R' is as described herein. In some embodiments, $-X-R^L$ is $-NHR$, wherein R is as described herein. In some embodiments, $-X-R^L$ is R^L, wherein R^L is as described herein. In some embodiments, R^L is $-N(R')_2$, wherein each R' is independently as described herein. In some embodiments, R^L is $-NHR'$, wherein R' is as described herein. In some embodiments, R^L is $-NHR$, wherein R is as described herein. In some embodiments, R^L is $-N(R')_2$, wherein each R' is independently as described herein. In some embodiments, none of R' in $-N(R')_2$ is hydrogen. In some embodiments, R^L is $-N(R')_2$, wherein each R' is independently C_{1-6} aliphatic. In some embodiments, R^L is $-L-R'$, wherein each of L and R' is independently as described herein. In some embodiments, R^L is $-L-R$, wherein each of L and R is independently as described herein. In some embodiments, R^L is $-N(R')-Cy-N(R')-R'$. In some embodiments, R^L is $-N(R')-Cy-C(O)-R'$. In some embodiments, R^L is $-N(R')-Cy-O-R'$. In some embodiments, R^L is $-N(R')-Cy-SO_2-R'$. In some embodiments, R^L is $-N(R')-Cy-SO_2-N(R')_2$. In some embodiments, R^L is $-N(R')-Cy-C(O)-N(R')_2$. In some embodiments, R^L is $-N(R')-Cy-OP(O)(R")_2$. In some embodiments, $-Cy-$ is an optionally substituted bivalent aryl group. In some embodiments, $-Cy-$ is optionally substituted

phenylene. In some embodiments, −Cy− is optionally substituted 1,4-phenylene. In some embodiments, −Cy− is 1,4-phenylene. In some embodiments, R^L is $-N(CH_3)_2$. In some embodiments, R^L is $-N(i\text{-}Pr)_2$. In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is 

embodiments, R^L is , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , , or . In some embodiments, $-X-R^L$ is $-N(R')-C(O)-Cy-R^L$. In some embodiments, $-X-R^L$ is R^L. In some embodiments, R^L is $-N(R')-C(O)-Cy-O-R'$. In some embodiments, R^L is $-N(R')-C(O)-Cy-R'$. In some embodiments, R^L is $-N(R')-C(O)-Cy-C(O)-R'$. In some embodiments, R^L is $-N(R')-C(O)-Cy-N(R')_2$. In some embodiments, R^L is $-N(R')-C(O)-Cy-SO_2-N(R')_2$.

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

As described herein, in some embodiments, one or more methylene units of L, or a variable which comprises or is L, are independently replaced with $-O-$, $-N(R')-$, $-C(O)-$, $-C(O)N(R')-$, $-SO_2-$, $-SO_2N(R')-$, or $-Cy-$. In some embodiments, a methylene unit is replaced with $-Cy-$. In some embodiments, $-Cy-$ is an optionally substituted bivalent aryl group. In some embodiments, $-Cy-$ is optionally substituted phenylene. In

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

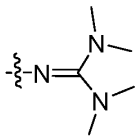
In some embodiments, an internucleotidic linkage has the structure of −P(=O)(−N(R')P(O)(R")$_2$)−, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=S)(−N(R')P(O)(R")$_2$)−, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=O)(−N(R')P(O)(R")$_2$)O−, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=S)(−N(R')P(O)(R")$_2$)O−, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of −OP(=O)(−N(R')P(O)(R")$_2$)O−, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of −OP(=S)(−N(R')P(O)(R")$_2$)O−, wherein each R" is independently as described herein. In some embodiments, R', e.g., of −N(R')−, is hydrogen or optionally substituted C_{1-6} aliphatic. In some embodiments, R' is C_{1-6} alkyl. In some embodiments, R' is hydrogen. In some embodiments, R", e.g., in −P(O)(R")$_2$, is R' as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=O)(−NHP(O)(R")$_2$)−, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=S)(−NHP(O)(R")$_2$)−, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of −P(=O)(−NHP(O)(R")$_2$)O−, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of

−P(=S)(−NHP(O)(R")$_2$)O−, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of −OP(=O)(−NHP(O)(R")$_2$)O−, wherein each R" is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of −OP(=S)(−NHP(O)(R")$_2$)O−, wherein each R" is independently as described herein. In some embodiments, an occurrence of R", e.g., in −P(O)(R")$_2$, is R. In some embodiments, R is an optionally substituted group selected from C_{1-6} aliphatic, aryl, heterocyclyl, and heteroaryl. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted C_{1-6} alkyl. In some embodiments, R is optionally substituted C_{1-6} alkenyl. In some embodiments, R is optionally substituted C_{1-6} alkynyl. In some embodiments, R is methyl. In some embodiments, R is optionally substituted methyl. In some embodiments, R is $-CF_3$. In some embodiments, R is optionally substituted ethyl. In some embodiments, R is ethyl. In some embodiments, R is $-CH_2CHF_2$. In some embodiments, R is $-CH_2CH_2OCH_3$. In some embodiments, R is optionally substituted C_{1-20} (e.g., C_{1-6}, C_{2-6}, C_{3-6}, C_{1-10}, C_{2-10}, C_{3-10}, C_{2-20}, C_{3-20}, C_{10-20}, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, etc.) aliphatic. In some embodiments, R is optionally substituted C_{1-20} (e.g., C_{1-6}, C_{2-6}, C_{3-6}, C_{1-10}, C_{2-10}, C_{3-10}, C_{2-20}, C_{3-20}, C_{10-20}, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, etc.) alkyl. In some embodiments, R is an optionally substituted linear C_{2-20} aliphatic. In some embodiments, R is optionally substituted linear C_{2-20} alkyl. In some embodiments, R is linear C_{2-20} alkyl. In some embodiments, R is isopropyl. In some embodiments, R is optionally substituted C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} aliphatic. In some embodiments, R is optionally substituted C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is optionally substituted linear C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, R is linear C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, or C_{20} alkyl. In some embodiments, each R" is independently R as described herein, for example, in some embodiments, each R" is methyl. In some embodiments, R" is optionally substituted aryl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is p-methylphenyl. In some embodiments, R is benzyl. In some embodiments, R is optionally substituted heteroaryl. In some embodiments, R is optionally substituted 1,3-diazolyl. In some embodiments, R is optionally substituted 2-(1,3)-diazolyl. In some embodiments, R is optionally substituted 1-methyl-2-(1,3)-diazolyl. In some embodiments, an occurrence of

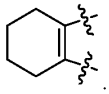
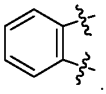
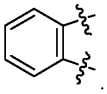
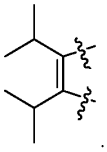
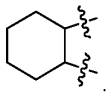
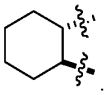
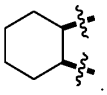
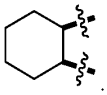
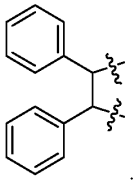
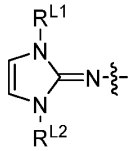
R" is $-N(R')_2$. In some embodiments, R" is $-N(CH_3)_2$. In some embodiments, an occurrence of R", e.g., in $-P(O)(R")_2$, is $-OR'$, wherein R' is as described herein. In some embodiments, R' is R as described herein. In some embodiments, is optionally substituted C_{1-6} aliphatic. In some embodiments, is optionally substituted C_{1-6} alkyl. In some embodiments, R" is $-OCH_3$. In some embodiments, each R" is $-OR'$ as described herein. In some embodiments, each R" is $-OCH_3$. In some embodiments, each R" is $-OH$. In some embodiments, a linkage is $-OP(O)(NHP(O)(OH)_2)O-$. In some embodiments, a linkage is $-OP(O)(NHP(O)(OCH_3)_2)O-$. In some embodiments, a linkage is $-OP(O)(NHP(O)(CH_3)_2)O-$.

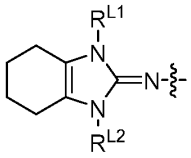
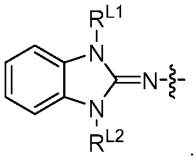
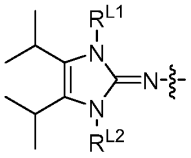
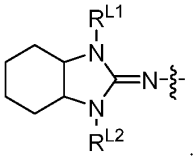
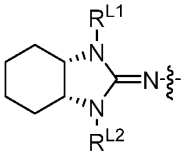
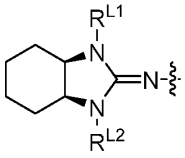
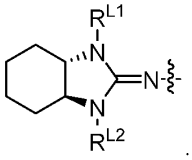
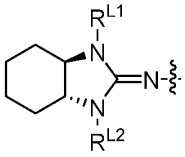
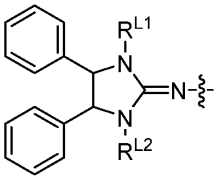
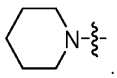
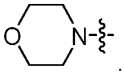
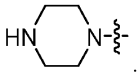
In some embodiments, $-N(R")_2$ is $-N(R')_2$. In some embodiments, $-N(R")_2$ is $-NHR$. In some embodiments, $-N(R")_2$ is $-NHC(O)R$. In some embodiments, $-N(R")_2$ is $-NHC(O)OR$. In some embodiments, $-N(R")_2$ is $-NHS(O)_2R$.

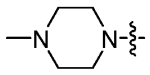
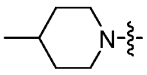
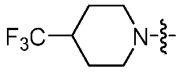
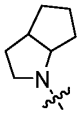
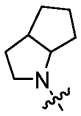
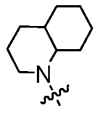
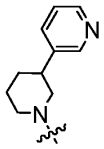
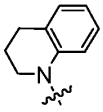
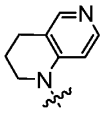
In some embodiments, an internucleotidic linkage is a phosphoryl guanidine internucleotidic linkage. In some embodiments, an internucleotidic linkage comprises $-X-R^L$ as described herein. In some embodiments, $-X-R^L$ is $-N=C(-L^L-R^L)_2$. In some embodiments, $-X-R^L$ is $-N=C[N(R^L)_2]_2$. In some embodiments, $-X-R^L$ is $-N=C[NR'R^L]_2$. In some embodiments, $-X-R^L$ is $-N=C[N(R')_2]_2$. In some embodiments, $-X-R^L$ is $-N=C[N(R^L)_2](CHR^{L1}R^{L2})$, wherein each of R^{L1} and R^{L2} is independently as described herein. In some embodiments, $-X-R^L$ is $-N=C(NR'R^L)(CHR^{L1}R^{L2})$, wherein each of R^{L1} and R^{L2} is independently as described herein. In some embodiments, $-X-R^L$ is $-N=C(NR'R^L)(CR'R^{L1}R^{L2})$, wherein each of R^{L1} and R^{L2} is independently as described herein. In some embodiments, $-X-R^L$ is $-N=C[N(R')_2](CHR'R^{L2})$. In some embodiments, $-X-R^L$ is $-N=C[N(R^L)_2](R^L)$. In some embodiments, $-X-R^L$ is $-N=C(NR'R^L)(R^L)$. In some embodiments, $-X-R^L$ is $-N=C(NR'R^L)(R')$. In some embodiments, $-X-R^L$ is $-N=C[N(R')_2](R')$. In some embodiments, $-X-R^L$ is $-N=C(NR'R^{L1})(NR'R^{L2})$, wherein each R^{L1} and R^{L2} is independently R^L, and each R' and R^L is independently as described herein. In some embodiments, $-X-R^L$ is $-N=C(NR'R^{L1})(NR'R^{L2})$, wherein variable is independently as described herein. In some embodiments, $-X-R^L$ is $-N=C(NR'R^{L1})(CHR'R^{L2})$, wherein variable is independently as described herein. In some embodiments, $-X-R^L$ is $-N=C(NR'R^{L1})(R')$, wherein variable is independently as described herein. In some embodiments, each R' is independently R. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is methyl.



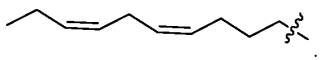
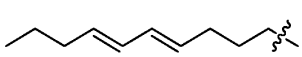
In some embodiments, $-X-R^L$ is . In some embodiments, two groups selected from R', R^L, R^{L1}, R^{L2}, etc. (in some embodiments, on the same atom (e.g., $-N(R')_2$, or $-NR'R^L$, or $-N(R^L)^2$, wherein R' and R^L can independently be R as described herein), etc.), or on different atoms (e.g., the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

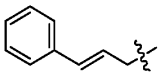
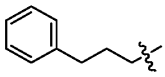
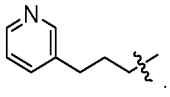
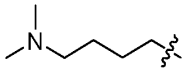
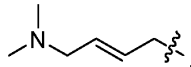
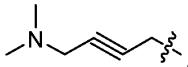
heteroaliphatic chain is unsaturated. In some embodiments, a chain is optionally substituted $-(CH_2)-$. In some embodiments, a chain is optionally substituted $-(CH_2)_2-$. In some embodiments, a chain is optionally substituted $-(CH_2)-$. In some embodiments, a chain is optionally substituted $-(CH_2)_2-$.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

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

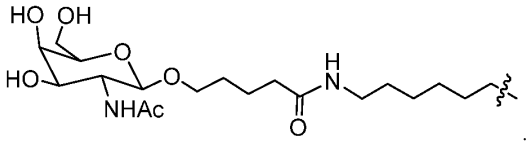
. In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted . In some embodiments, a ring is optionally substituted .

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

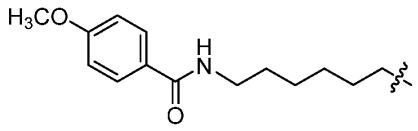
In some embodiments, R^{L} is optionally substituted C_{1-30} aliphatic. In some embodiments, R^{L} is optionally substituted C_{1-30} alkyl. In some embodiments, R^{L} is linear. In some embodiments, R^{L} is optionally substituted linear C_{1-30} alkyl. In some embodiments, R^{L} is optionally substituted C_{1-6} alkyl. In some embodiments, R^{L} is methyl. In some embodiments, R^{L} is ethyl. In some embodiments, R^{L} is n-propyl. In some embodiments, R^{L} is isopropyl. In some embodiments, R^{L} is n-butyl. In some embodiments, R^{L} is tert-butyl. In some embodiments, R^{L} is (E)−CH_2−CH=CH−CH_2−CH_3. In some embodiments, R^{L} is (Z)−CH_2−CH=CH−CH_2−CH_3. In some embodiments, R^{L} is . In some embodiments, R^{L} is . In some embodiments, R^{L} is $CH_3(CH_2)_2C{\equiv}CC{\equiv}C(CH_2)_3$−. In some embodiments, R^{L} is $CH_3(CH_2)_5C{\equiv}C$−. In some embodiments, R^{L} optionally substituted aryl. In some embodiments, R^{L} is optionally substituted phenyl. In some embodiments, R^{L} is phenyl

substituted with one or more halogen. In some embodiments, R^L is phenyl optionally substituted with halogen, −N(R'), or −N(R')C(O)R'. In some embodiments, R^L is phenyl optionally substituted with −Cl, −Br, −F, $-N(Me)_2$, or $-NHCOCH_3$. In some embodiments, R^L is $-L^L-R'$, wherein L^L is an optionally substituted C_{1-20} saturated, partially unsaturated or unsaturated hydrocarbon chain. In some embodiments, such a hydrocarbon chain is linear. In some embodiments, such a hydrocarbon chain is unsubstituted. In some embodiments, L^L is (E)$-CH_2-CH=CH-$. In some embodiments, L^L is $-CH_2-C\equiv C-CH_2-$. In some embodiments, L^L is $-(CH_2)_3-$. In some embodiments, L^L is $-(CH_2)_4-$. In some embodiments, L^L is $-(CH_2)_n-$, wherein n is 1-30 (e.g., 1-20, 5-30, 6-30, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, etc.). In some embodiments, R' is optionally substituted aryl as described herein. In some embodiments, R' is optionally substituted phenyl. In some embodiments, R' is phenyl. In some embodiments, R' is optionally substituted heteroaryl as described herein. In some embodiments, R' is 2'-pyridinyl. In some embodiments, R' is 3'-pyridinyl. In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is $-L^L-N(R')_2$, wherein each variable is independently as described herein. In some embodiments, each R' is independently C_{1-6} aliphatic as described herein. In some embodiments, $-N(R')_2$ is $-N(CH_3)_2$. In some embodiments, $-N(R')_2$ is $-NH_2$. In some embodiments, R^L is $-(CH_2)_n-N(R')_2$, wherein n is 1-30 (e.g., 1-20, 5-30, 6-30, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, etc.). In some embodiments, R^L is $-(CH_2CH_2O)_n-CH_2CH_2-N(R')_2$, wherein n is 1-30 (e.g., 1-20, 5-30, 6-30, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, etc.). In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is . In some embodiments, R^L is $-(CH_2)_n-NH_2$. In some embodiments, R^L is $-(CH_2CH_2O)_n-CH_2CH_2-NH_2$. In some embodiments, R^L is $-(CH_2CH_2O)_n-CH_2CH_2-R'$, wherein n is 1-30 (e.g., 1-20, 5-30, 6-30, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, etc.). In some embodiments, R^L is

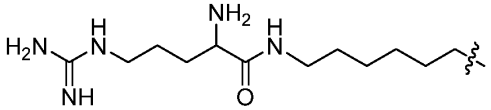
$-(CH_2CH_2O)_n-CH_2CH_2CH_3$, wherein n is 1-30 (e.g., 1-20, 5-30, 6-30, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, etc.). In some embodiments, R^L is $-(CH_2CH_2O)_n-CH_2CH_2OH$, wherein n is 1-30 (e.g., 1-20, 5-30, 6-30, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, etc.). In some embodiments, R^L is or comprises a carbohydrate moiety, e.g., GalNAc. In some embodiments, R^L is $-L^L-$GalNAc. In some embodiments, R^L is



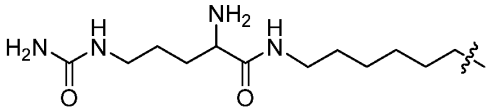
. In some embodiments, one or more methylene units of L^L are independently replaced with $-Cy-$ (e.g., optionally substituted 1,4-phenylene, a 3-30 membered bivalent optionally substituted monocyclic, bicyclic, or polycyclic cycloaliphatic ring, etc.), $-O-$, $-N(R')-$ (e.g., $-NH$), $-C(O)-$, $-C(O)N(R')-$ (e.g., $-C(O)NH-$), $-C(NR')-$ (e.g., $-C(NH)-$), $-N(R')C(O)(N(R')-$ (e.g., $-NHC(O)NH-$), $-N(R')C(NR')(N(R')-$ (e.g., $-NHC(NH)NH-$), $-(CH_2CH_2O)_n-$, etc. For example, in some embodiments, R^L is



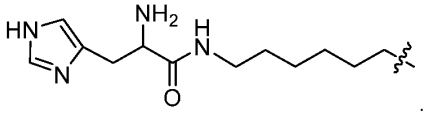
. In some embodiments, R^L is



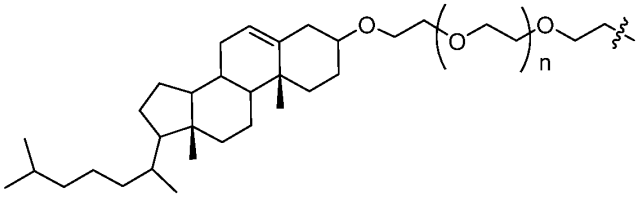
. In some embodiments, R^L is



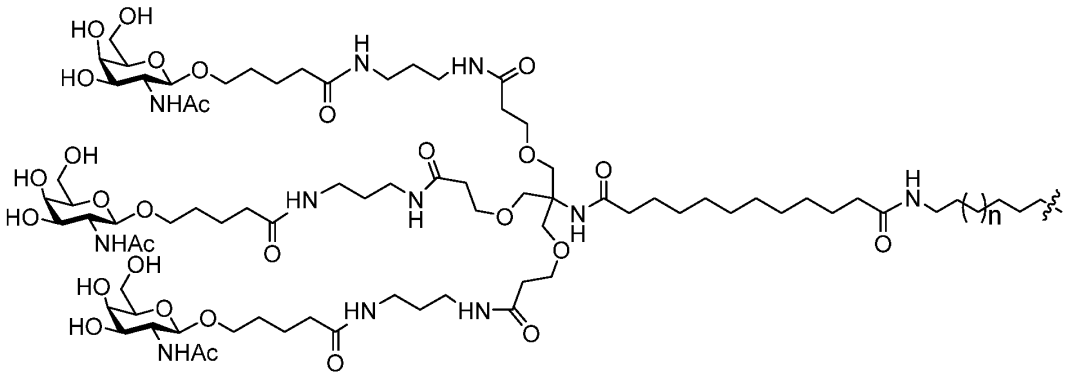
. In some embodiments, R^L is

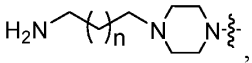
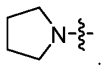
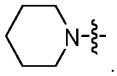
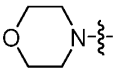
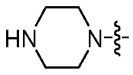
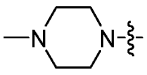
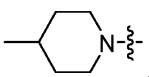
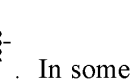


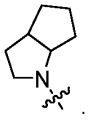
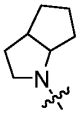
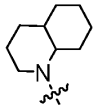
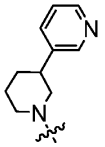
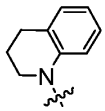
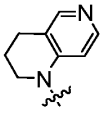
. In some embodiments, R^L is

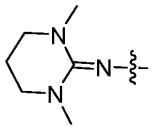
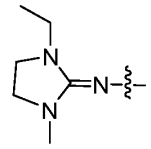
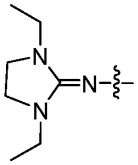
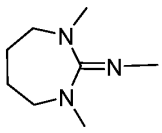
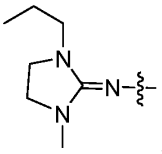
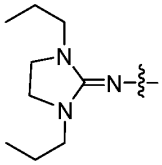
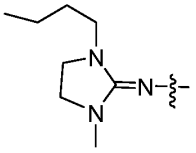
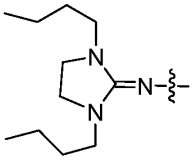
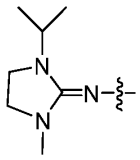
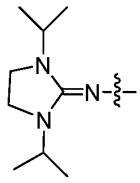


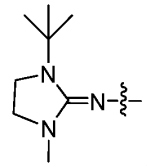
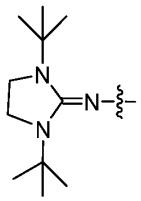
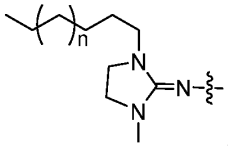
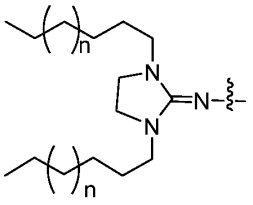
wherein n is 0-20. In some embodiments, R^L is or comprises one or more additional chemical moieties (e.g., carbohydrate moieties, GalNAc moieties, etc.) optionally substituted connected through a linker (which can be bivalent or polyvalent). For example, in some embodiments, R^L is

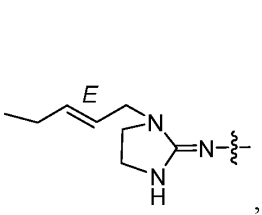
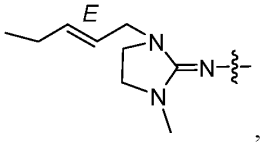
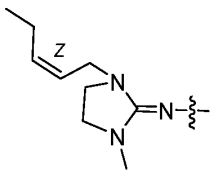
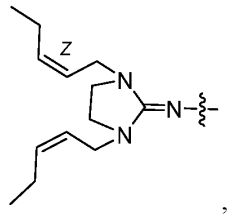


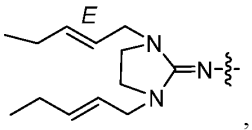
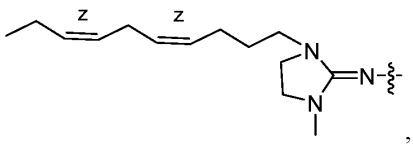
, wherein n is 0-20. In some embodiments, R^L is , wherein n is 0-20. In some embodiments, R^L is R' as described herein. As described herein, many variable can independently be R'. In some embodiments, R' is R as described herein. As described herein, various variables can independently be R. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted C_{1-6} alkyl. In some embodiments, R is methyl. In some embodiments, R is optionally substituted cycloaliphatic. In some embodiments, R is optionally substituted cycloalkyl. In some embodiments, R is optionally substituted aryl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is optionally substituted heteroaryl. In some embodiments, R is optionally substituted heterocyclyl. In some embodiments, R is optionally substituted C_{1-20} heterocyclyl having 1-5 heteroatoms, e.g., one of which is nitrogen. In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted . In some embodiments, R is optionally substituted

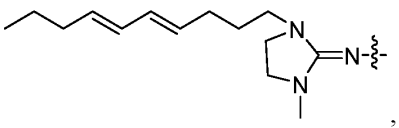
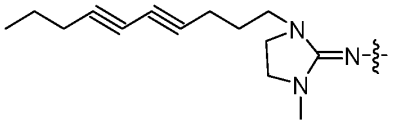

. In some embodiments, R is optionally substituted  In some embodiments, R is optionally substituted  . In some embodiments, R is optionally substituted  . In some embodiments, R is optionally substituted  . In some embodiments, R is optionally substituted  .

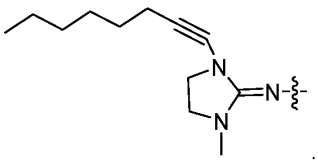
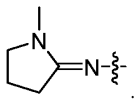
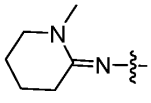
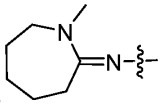
[01] In some embodiments, $-X-R^L$ is  . In some embodiments, $-X-R^L$ is  . In some embodiments, $-X-R^L$ is  . In some embodiments, $-X-R^L$ is  . In some embodiments, $-X-R^L$ is  . In some embodiments, $-X-R^L$ is  . In some embodiments, $-X-R^L$ is  . In some embodiments, $-X-R^L$ is  . In some embodiments, $-X-R^L$ is  . In some embodiments, $-X-R^L$ is  . In some embodiments, $-X-R^L$

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

, , , ,

, ,

,  and

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

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

In some embodiments, R" or R^L is or comprises an additional chemical

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

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

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

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

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

In some embodiments, $-X-R^L$ is −SH. In some embodiments, $-X-R^L$ is −OH.

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

In some embodiments, a non-negatively charged internucleotidic linkage has the structure of $-OP(=O)(-N=C((N(R')_2)_2-O-$. In some embodiments, a R' group of one $N(R')_2$ is R, a R' group of the other $N(R')_2$ is R, and the two R groups are taken together

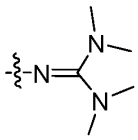
with their intervening atoms to form an optionally substituted ring, e.g., a 5-membered ring as in n001. In some embodiments, each R' is independently R, wherein each R is independently optionally substituted C_{1-6} aliphatic.

In some embodiments, $-X-R^L$ is $-N=C(-L^L-R')_2$. In some embodiments, $-X-R^L$ is $-N=C(-L^{L1}-L^{L2}-L^{L3}-R')_2$, wherein each L^{L1}, L^{L2} and L^{L3} is independently L", wherein each L" is independently a covalent bond, or a bivalent, optionally substituted, linear or branched group selected from a C_{1-10} aliphatic group and a C_{1-10} heteroaliphatic group having 1-5 heteroatoms, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_{1-6} alkylene, C_{1-6} alkenylene, —C≡C—, a bivalent C_1–C_6 heteroaliphatic group having 1-5 heteroatoms, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, $-C(O)O-$, $-P(O)(OR')-$, $-P(O)(SR')-$, $-P(O)(R')-$, $-P(O)(NR')-$, $-P(S)(OR')-$, $-P(S)(SR')-$, $-P(S)(R')-$, $-P(S)(NR')-$, $-P(R')-$, $-P(OR')-$, $-P(SR')-$, $-P(NR')-$, $-P(OR')[B(R')_3]-$, $-OP(O)(OR')O-$, $-OP(O)(SR')O-$, $-OP(O)(R')O-$, $-OP(O)(NR')O-$, $-OP(OR')O-$, $-OP(SR')O-$, $-OP(NR')O-$, $-OP(R')O-$, or $-OP(OR')[B(R')_3]O-$, and one or more nitrogen or carbon atoms are optionally and independently replaced with Cy^L. In some embodiments, L^{L2} is $-Cy-$. In some embodiments, L^{L1} is a covalent bond. In some embodiments, L^{L3} is a covalent bond. In some embodiments, $-X-R^L$ is $-N=C(-L^{L1}-Cy-L^{L3}-R')_2$. In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is .

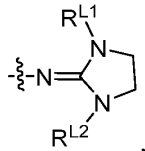
In some embodiments, as utilized in the present disclosure, L is covalent bond. In some embodiments, L is a bivalent, optionally substituted, linear or branched group selected from a C_{1-30} aliphatic group and a C_{1-30} heteroaliphatic group having 1-10 heteroatoms, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from C_{1-6} alkylene, C_{1-6} alkenylene, —C≡C—, a bivalent C_1–C_6 heteroaliphatic group having 1-5 heteroatoms, $-C(R')_2-$, $-Cy-$, $-O-$, $-S-$, $-S-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-C(O)S-$, $-C(O)O-$, $-P(O)(OR')-$, $-P(O)(SR')-$, $-P(O)(R')-$, $-P(O)(NR')-$, $-P(S)(OR')-$, $-P(S)(SR')-$, $-P(S)(R')-$, $-P(S)(NR')-$, $-P(R')-$, $-P(OR')-$, $-P(SR')-$, $-P(NR')-$, $-P(OR')[B(R')_3]-$, $-OP(O)(OR')O-$, $-OP(O)(SR')O-$, $-OP(O)(R')O-$, $-OP(O)(NR')O-$,

−OP(OR')O−, −OP(SR')O−, −OP(NR')O−, −OP(R')O−, or −OP(OR')[B(R')$_3$]O−, and one or more nitrogen or carbon atoms are optionally and independently replaced with Cy^L. In some embodiments, L is a bivalent, optionally substituted, linear or branched group selected from a C_{1-30} aliphatic group and a C_{1-30} heteroaliphatic group having 1-10 heteroatoms, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from —C≡C—, −C(R')$_2$−, −Cy−, −O−, −S−, −S−S−, −N(R')−, −C(O)−, −C(S)−, −C(NR')−, −C(O)N(R')−, −N(R')C(O)N(R')−, −N(R')C(O)O−, −S(O)−, −S(O)$_2$−, −S(O)$_2$N(R')−, −C(O)S−, −C(O)O−, −P(O)(OR')−, −P(O)(SR')−, −P(O)(R')−, −P(O)(NR')−, −P(S)(OR')−, −P(S)(SR')−, −P(S)(R')−, −P(S)(NR')−, −P(R')−, −P(OR')−, −P(SR')−, −P(NR')−, −P(OR')[B(R')$_3$]−, −OP(O)(OR')O−, −OP(O)(SR')O−, −OP(O)(R')O−, −OP(O)(NR')O−, −OP(OR')O−, −OP(SR')O−, −OP(NR')O−, −OP(R')O−, or −OP(OR')[B(R')$_3$]O−, and one or more nitrogen or carbon atoms are optionally and independently replaced with Cy^L. In some embodiments, L is a bivalent, optionally substituted, linear or branched group selected from a C_{1-10} aliphatic group and a C_{1-10} heteroaliphatic group having 1-10 heteroatoms, wherein one or more methylene units are optionally and independently replaced by an optionally substituted group selected from —C≡C—, −C(R')$_2$−, −Cy−, −O−, −S−, −S−S−, −N(R')−, −C(O)−, −C(S)−, −C(NR')−, −C(O)N(R')−, −N(R')C(O)N(R')−, −N(R')C(O)O−, −S(O)−, −S(O)$_2$−, −S(O)$_2$N(R')−, −C(O)S−, −C(O)O−, −P(O)(OR')−, −P(O)(SR')−, −P(O)(R')−, −P(O)(NR')−, −P(S)(OR')−, −P(S)(SR')−, −P(S)(R')−, −P(S)(NR')−, −P(R')−, −P(OR')−, −P(SR')−, −P(NR')−, −P(OR')[B(R')$_3$]−, −OP(O)(OR')O−, −OP(O)(SR')O−, −OP(O)(R')O−, −OP(O)(NR')O−, −OP(OR')O−, −OP(SR')O−, −OP(NR')O−, −OP(R')O−, or −OP(OR')[B(R')$_3$]O−, and one or more nitrogen or carbon atoms are optionally and independently replaced with Cy^L. In some embodiments, one or more methylene units are optionally and independently replaced by an optionally substituted group selected from —C≡C—, −C(R')$_2$−, −Cy−, −O−, −S−, −S−S−, −N(R')−, −C(O)−, −C(S)−, −C(NR')−, −C(O)N(R')−, −N(R')C(O)N(R')−, −N(R')C(O)O−, −S(O)−, −S(O)$_2$−, −S(O)$_2$N(R')−, −C(O)S−, or −C(O)O−.

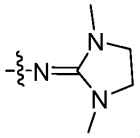
In some embodiments, an internucleotidic linkage is a phosphoryl guanidine internucleotidic linkage. In some embodiments, $-X-R^L$ is $-N=C[N(R')_2]_2$. In some embodiments, each R' is independently R. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is methyl. In some embodiments,



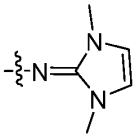
$-X-R^L$ is . In some embodiments, one R' on a nitrogen atom is taken with a R' on the other nitrogen to form a ring as described herein.



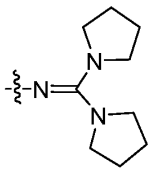
In some embodiments, $-X-R^L$ is , wherein R^1 and R^2 are



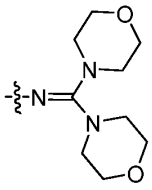
independently R'. In some embodiments, $-X-R^L$ is . In some embodiments,



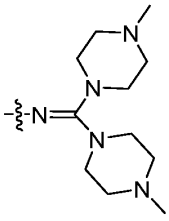
$-X-R^L$ is . In some embodiments, two R' on the same nitrogen are taken



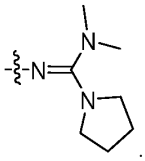
together to form a ring as described herein. In some embodiments, $-X-R^L$ is



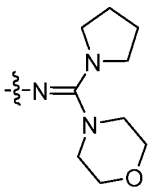
. In some embodiments, $-X-R^L$ is . In some embodiments, $-X-R^L$ is



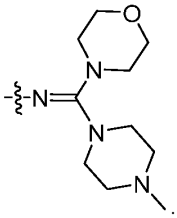
. In some embodiments, $-X-R^L$ is



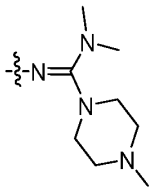
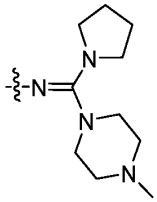
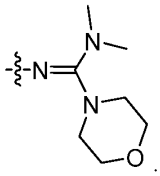
. In some embodiments, $-X-R^L$ is



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



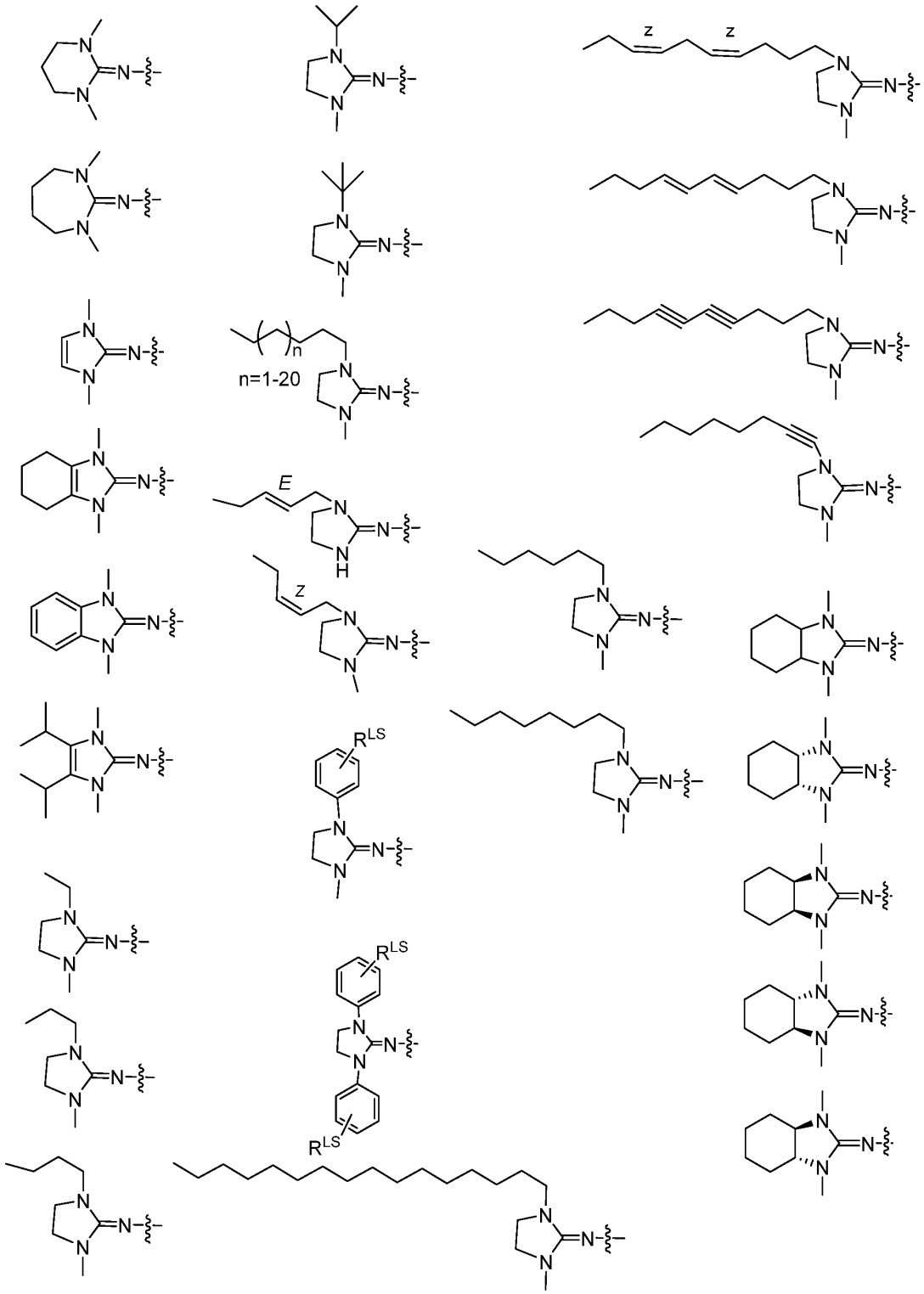
. In some

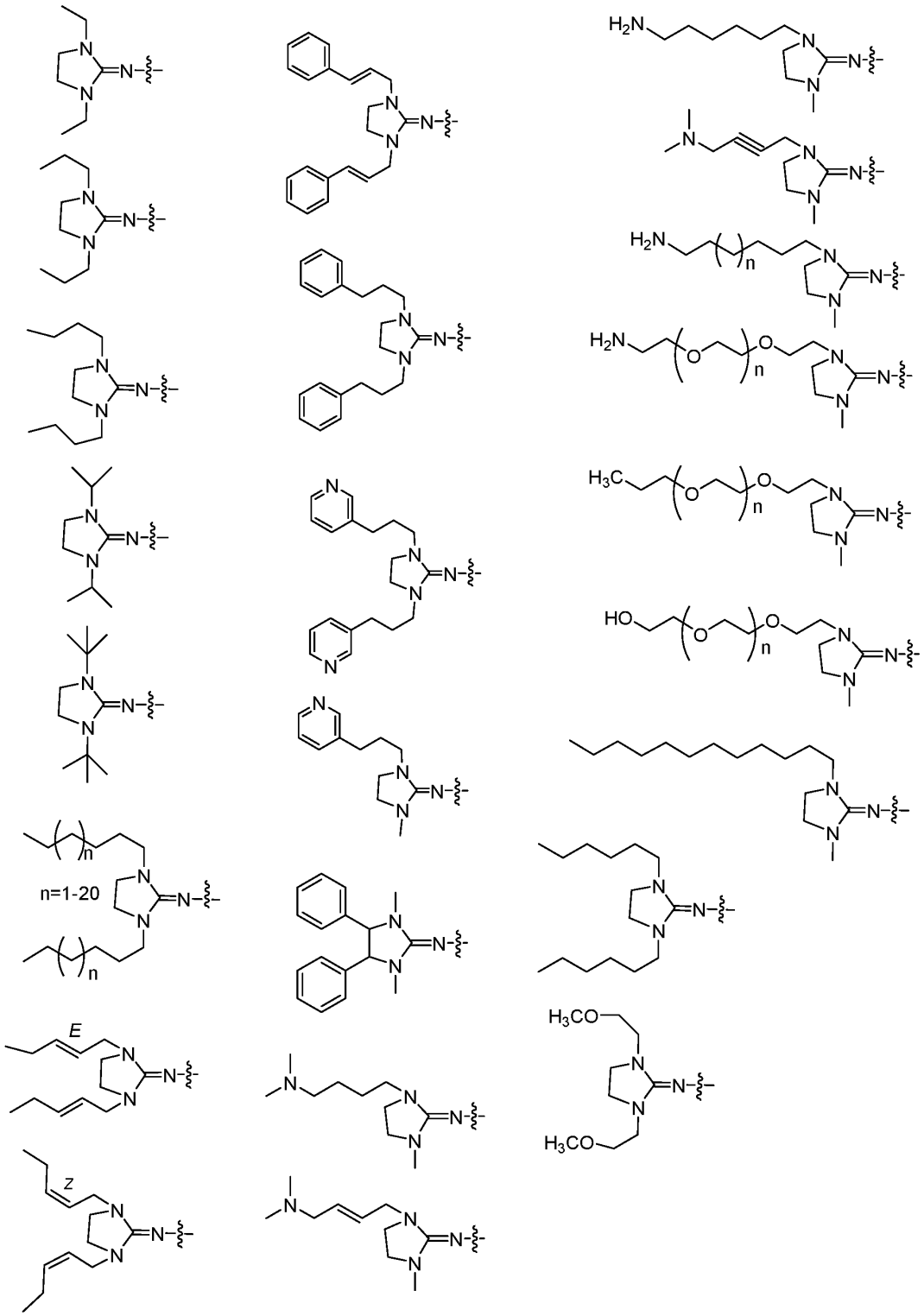
embodiments, −X−R^L is . In some embodiments, −X−R^L is . In some embodiments, −X−R^L is .

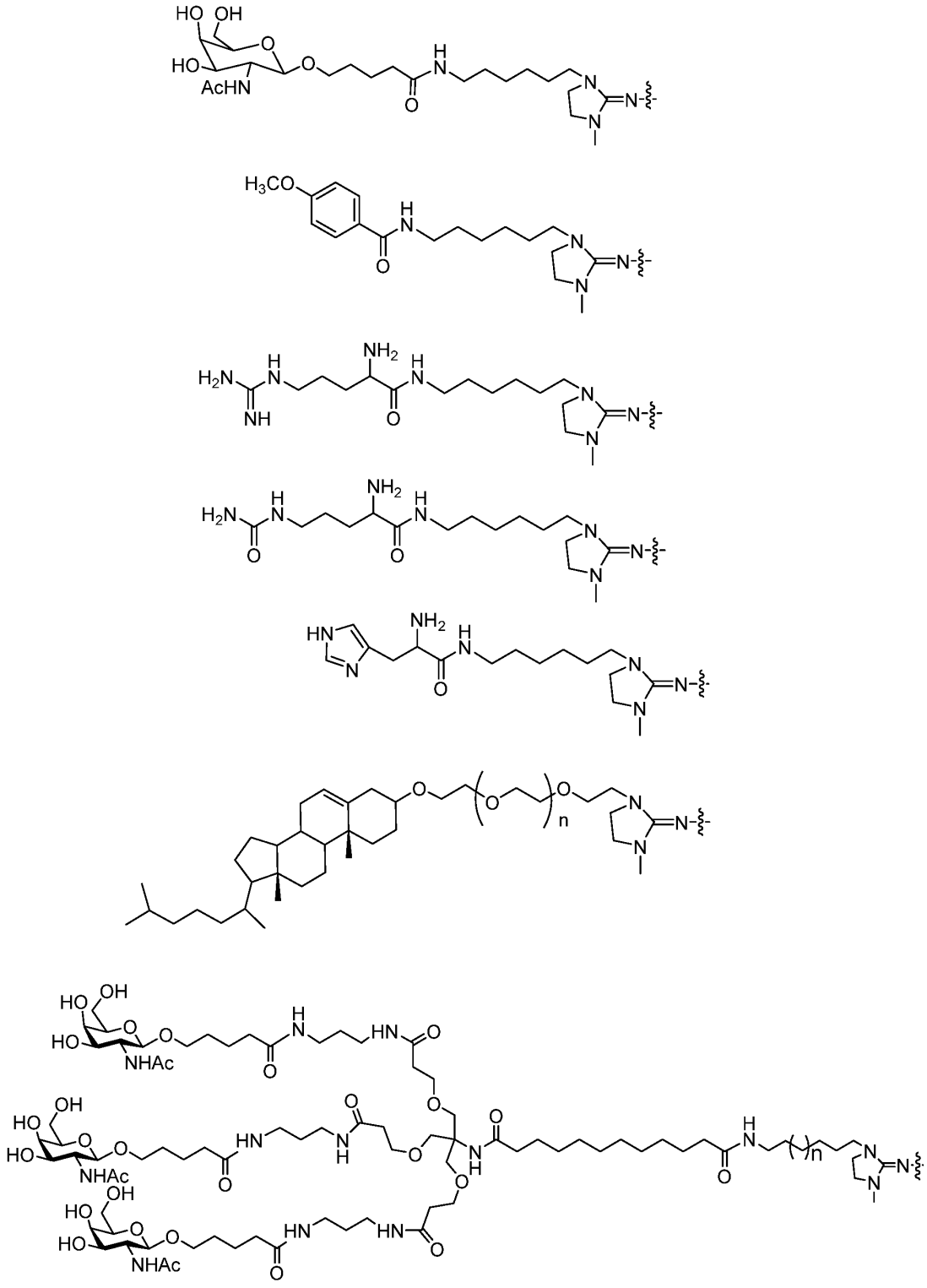
In some embodiments, −X−R^L is R as described herein. In some embodiments, R is not hydrogen. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted C_{1-6} alkyl. In some embodiments, R is methyl.

In some embodiments, −X−R^L is selected from Tables below. In some embodiments, X is as described herein. In some embodiments, R^L is as described herein. In some embodiments, a linkage has the structure of −Y−P^L(−X−R^L)−Z−, wherein −X−R^L is selected from Tables below, and each other variable is independently as described herein. In some embodiments, a linkage has the structure of or comprises −P(O)(−X−R^L)−, wherein −X−R^L is selected from Tables below. In some embodiments, a linkage has the structure of or comprises −P(S)(−X−R^L)−, wherein −X−R^L is selected from Tables below. In some embodiments, a linkage has the structure of or comprises −P(−X−R^L)−, wherein −X−R^L is selected from Tables below. In some embodiments, a linkage has the structure of or comprises −O−P(O)(−X−R^L)−O−, wherein −X−R^L is selected from Tables below. In some embodiments, a linkage has the structure of or comprises −O−P(S)(−X−R^L)−O−, wherein −X−R^L is selected from Tables below. In some embodiments, a linkage has the structure of or comprises −O−P(−X−R^L)−O−, wherein −X−R^L is selected from Tables below. In some embodiments, a linkage has the structure of −O−P(O)(−X−R^L)−O−, wherein −X−R^L is selected from Tables below. In some embodiments, a linkage has the structure of −O−P(S)(−X−R^L)−O−, wherein −X−R^L is selected from Tables below. In some embodiments, a linkage has the structure of −O−P(−X−R^L)−O−, wherein −X−R^L is selected from Tables below. In some embodiments, the Tables below, n is 0-20 or as described herein.

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


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


n=1-20
E
Z



, wherein each R^{LS} is independently R^{s}. In some embodiments, each R^{LS} is independently −Cl, −Br, −F, $-N(Me)_2$, or $-NHCOCH_3$.

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

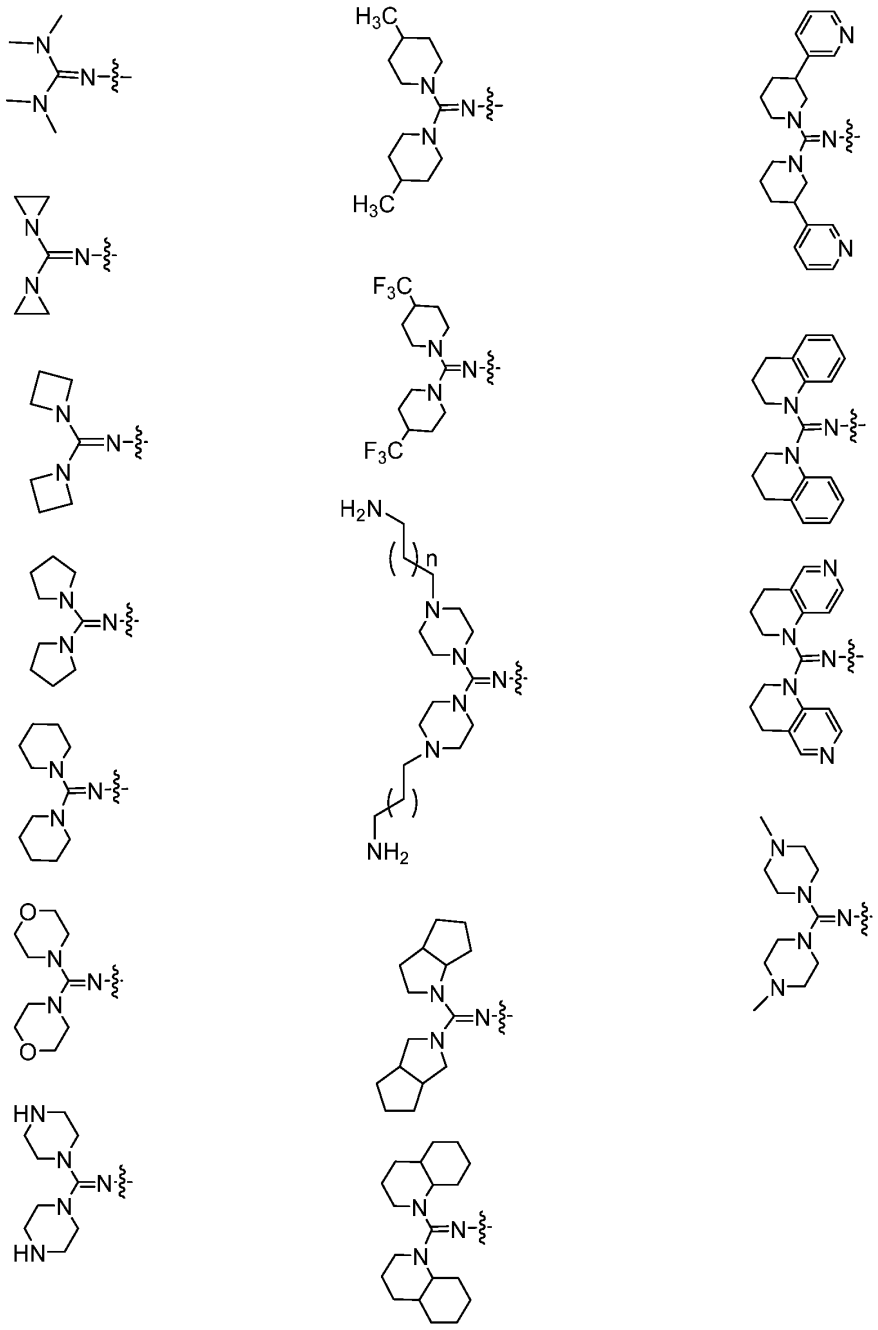


Table L-3. Certain useful moieties bonded to linkage phosphorus (e.g., −X−R^L).

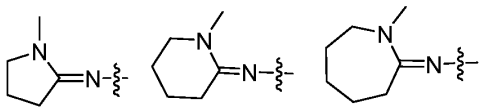


Table L-4. Certain useful moieties bonded to linkage phosphorus (e.g., −X−R^L).

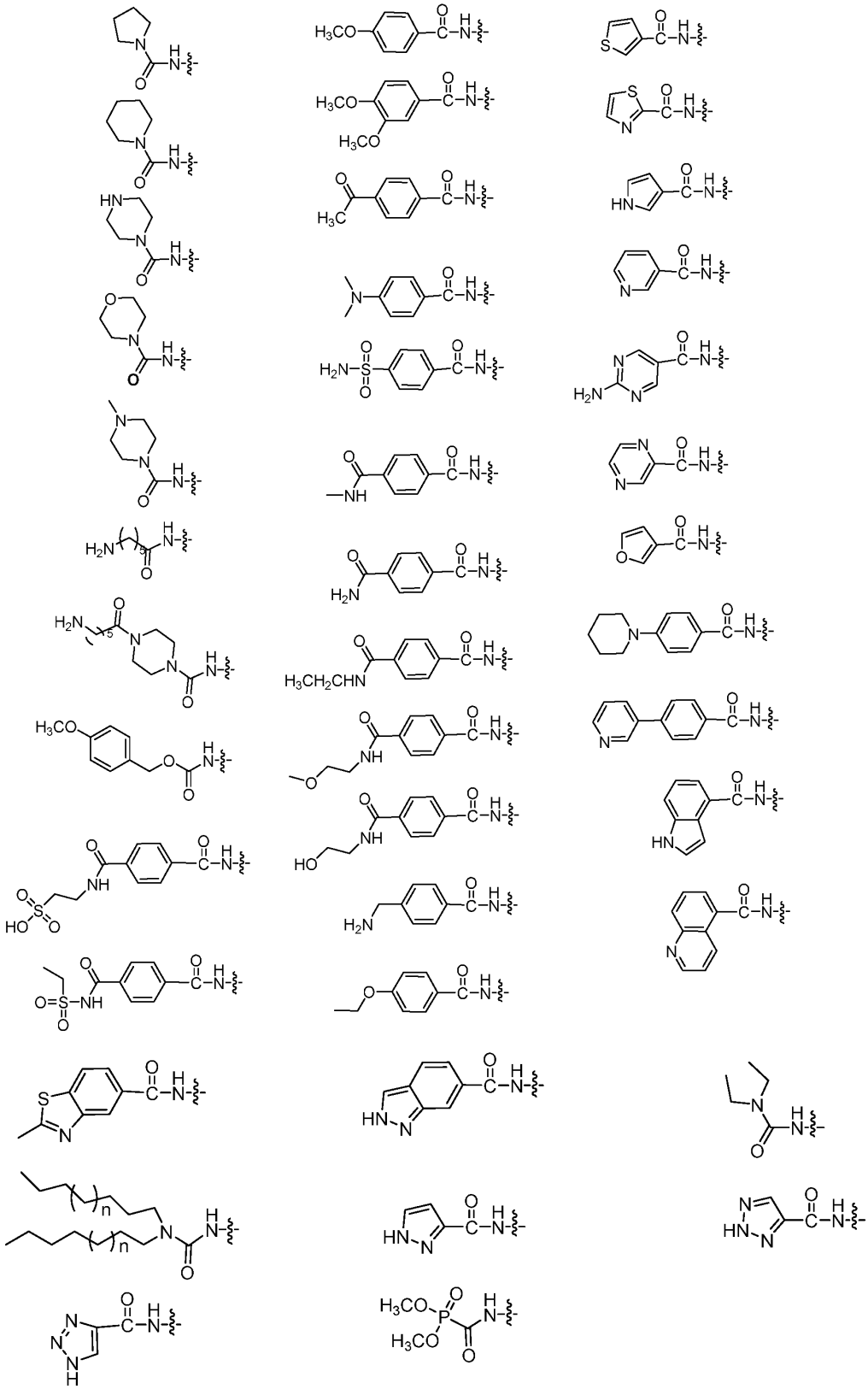
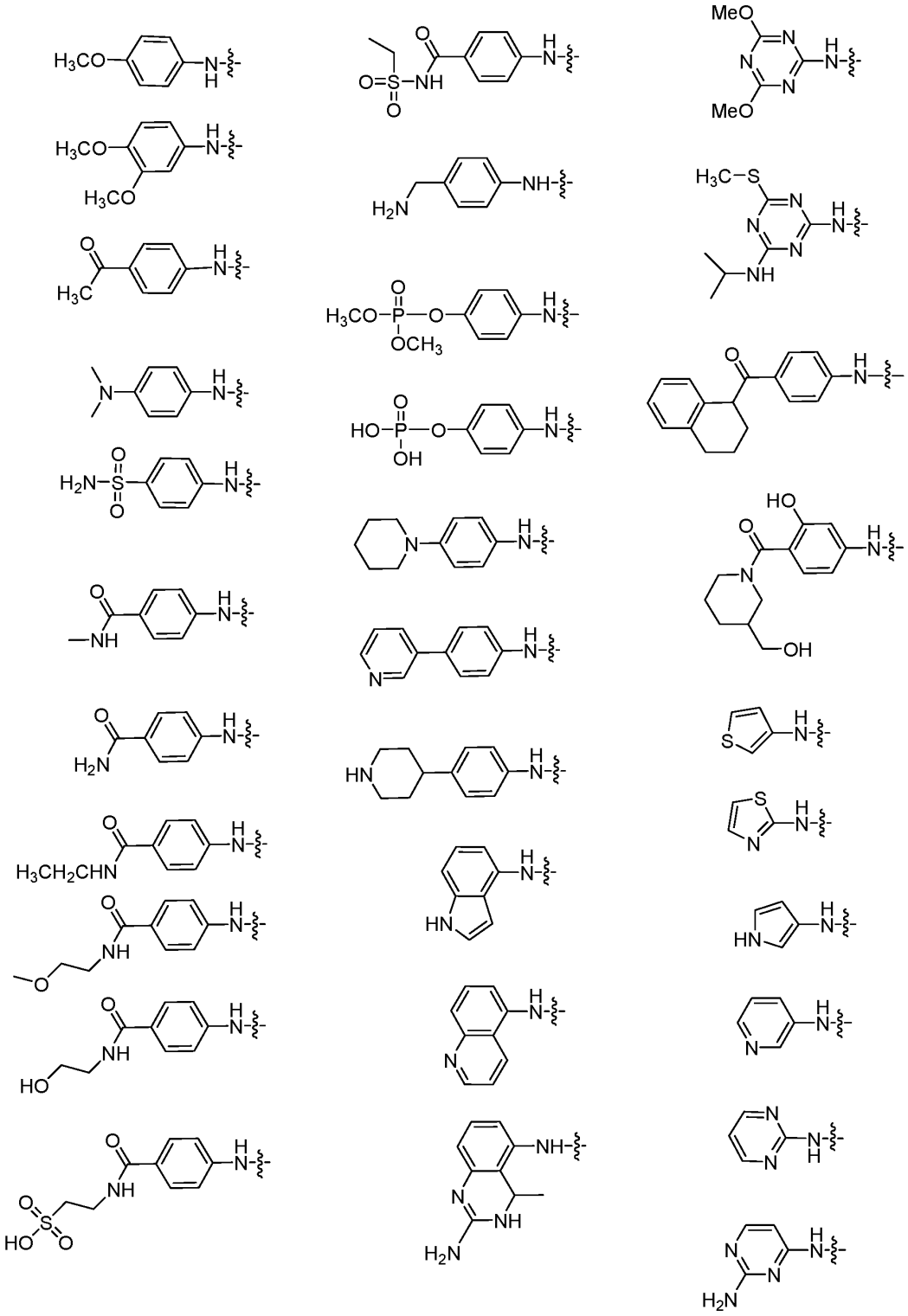


Table L-5. Certain useful moieties bonded to linkage phosphorus (e.g., −X−R^L).



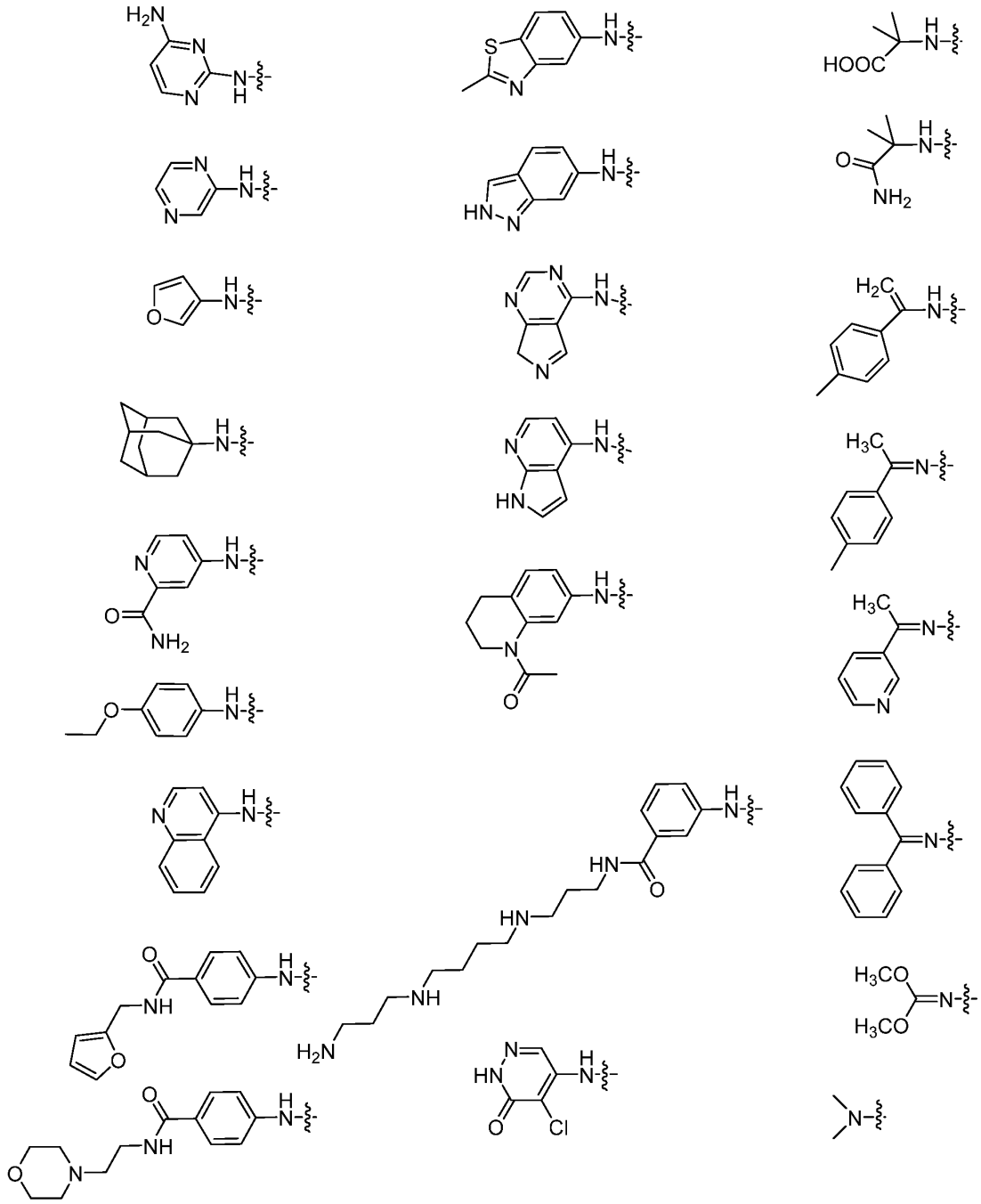
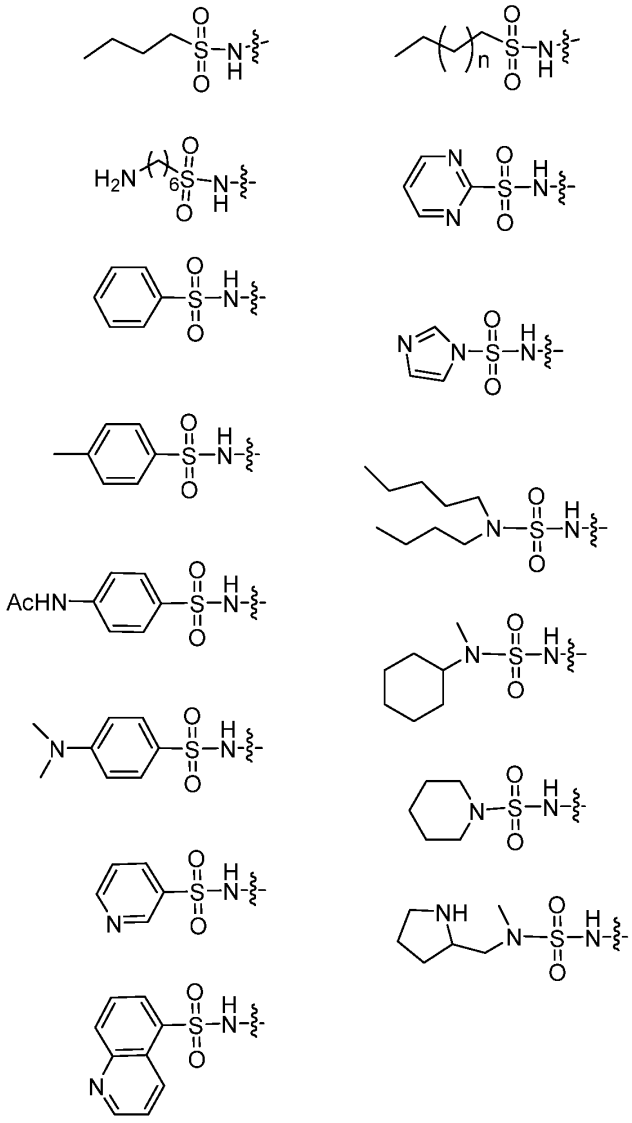
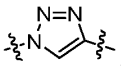
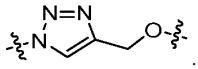


Table L-6. Certain useful moieties bonded to linkage phosphorus (e.g., $-X-R^L$).



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

ring. In some embodiments, Cy^{IL} is . In some embodiments, a linkage is .

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

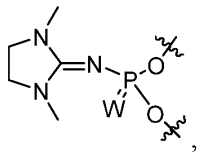
In some embodiments, R' is R. In some embodiments, R' is H. In some embodiments, R' is $-C(O)R$. In some embodiments, R' is $-C(O)OR$. In some embodiments, R' is $-S(O)_2R$.

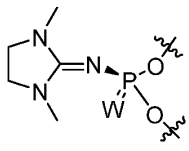
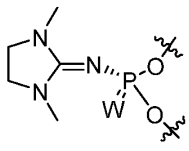
In some embodiments, R" is $-NHR'$. In some embodiments, $-N(R')_2$ is $-NHR'$.

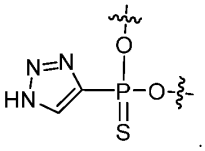
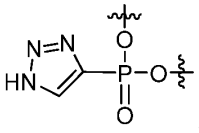
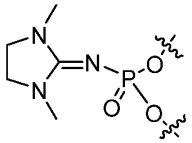
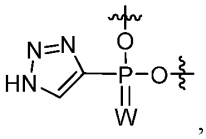
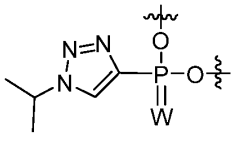
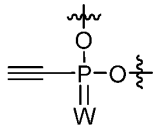
As described herein, some embodiments, R is H. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted C_{1-6} alkyl. In some embodiments, R is methyl. In some embodiments, R is substituted methyl. In some embodiments, R is ethyl. In some embodiments,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

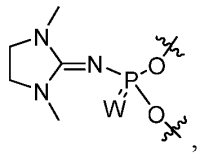
In some embodiments, as described herein, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage.

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

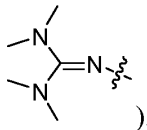
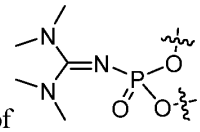
guanidine moiety and an internucleotidic linkage has the structure of: ,

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

In some embodiments, a non-negatively charged internucleotidic linkage or a neutral internucleotidic linkage is an internucleotidic linkage comprising a triazole moiety. In some embodiments, a non-negatively charged internucleotidic linkage or a non-negatively charged internucleotidic linkage comprises an optionally substituted triazolyl group. In some embodiments, an internucleotidic linkage comprising a triazole moiety (e.g., an optionally substituted triazolyl group) has the structure of . In some embodiments, an internucleotidic linkage comprising a triazole moiety has the structure of . In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, a neutral internucleotidic linkage, comprises a cyclic guanidine moiety. In some embodiments, an internucleotidic linkage comprising a cyclic guanidine moiety has the structure of . In some embodiments, a non-negatively charged internucleotidic linkage, or a neutral internucleotidic linkage, is or comprising a structure selected from , , ,

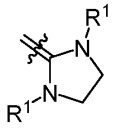

, or , wherein W is O or S.

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


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

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

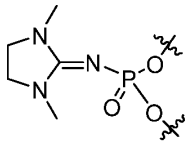
In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heteroaryl group having 1-10 heteroatoms. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heteroaryl group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

group having 1-10 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-6 membered heterocyclyl group having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-membered heterocyclyl group having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, at least two heteroatoms are nitrogen. In some embodiments, a heterocyclyl group is directly bonded to a linkage phosphorus. In some embodiments, a heterocyclyl group is bonded to a linkage phosphorus through a linker, e.g., =N− when the heterocyclyl group is part of a guanidine moiety who directed bonded to a linkage phosphorus through its =N−. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted  group. In some embodiments, a non-negatively charged internucleotidic linkage comprises an substituted  group. In some embodiments, a non-negatively charged internucleotidic linkage comprises a  group. In some embodiments, each R^1 is independently optionally substituted C_{1-6} alkyl. In some embodiments, each R^1 is independently methyl.

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

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

some embodiments, an internucleotidic linkage is or comprises −C(O)−O− which is part of a urea moiety.

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

In many embodiments, as demonstrated extensively, oligonucleotides of the present disclosure comprise two or more different internucleotidic linkages. In some embodiments, an oligonucleotide comprises a phosphorothioate internucleotidic linkage and a non-negatively charged internucleotidic linkage. In some embodiments, an oligonucleotide comprises a phosphorothioate internucleotidic linkage, a non-negatively charged internucleotidic linkage, and a natural phosphate linkage. In some embodiments, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, a non-negatively charged internucleotidic linkage is n001, n003, n004, n006, n008 or n009, n013, n020, n021, n025, n026, n029, n031, n037, n046, n047, n048, n054, or n055). In some embodiments, a non-negatively charged internucleotidic linkage is n001. In some embodiments, each phosphorothioate internucleotidic linkage is independently chirally controlled. In some embodiments, each chiral modified internucleotidic linkage is independently chirally controlled. In some embodiments, one or more non-negatively charged internucleotidic linkage are not chirally controlled.

A typical connection, as in natural DNA and RNA, is that an internucleotidic

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

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

In some embodiments, an oligonucleotide comprises a modified internucleotidic linkage (e.g., a modified internucleotidic linkage having the structure of Formula **I**, **I-a**, **I-b**, or **I-c**, **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, etc., or a salt form thereof) as described in US 9394333, US 9744183, US 9605019, US 9598458, US 9982257, US 10160969, US 10479995, US 2020/0056173, US 2018/0216107, US 2019/0127733, US 10450568, US 2019/0077817, US 2019/0249173, US 2019/0375774, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612 the internucleotidic linkages (e.g., those of Formula **I**, **I-a**, **I-b**, or **I-c**, **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, etc.,) of each of which are independently incorporated herein by reference. In some embodiments, a modified internucleotidic linkage is a non-negatively charged internucleotidic linkage. In some embodiments, provided oligonucleotides comprise one or more non-negatively charged internucleotidic linkages. In some embodiments, a non-negatively charged internucleotidic linkage is a positively charged internucleotidic linkage. In some embodiments, a non-negatively charged internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, the present disclosure provides oligonucleotides comprising one or more neutral internucleotidic linkages. In some embodiments, a non-negatively charged internucleotidic linkage or a neutral internucleotidic linkage (e.g., one of Formula **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, etc.) is as described in US 9394333, US 9744183, US 9605019, US 9598458, US 9982257, US 10160969, US 10479995, US 2020/0056173, US 2018/0216107, US 2019/0127733, US 10450568, US

2019/0077817, US 2019/0249173, US 2019/0375774, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612. In some embodiments, a non-negatively charged internucleotidic linkage or neutral internucleotidic linkage is one of Formula **I-n-1**, **I-n-2**, **I-n-3**, **I-n-4**, **II**, **II-a-1**, **II-a-2**, **II-b-1**, **II-b-2**, **II-c-1**, **II-c-2**, **II-d-1**, **II-d-2**, etc. as described in WO 2018/223056, WO 2019/032607, WO 2019/075357, WO 2019/032607, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, such internucleotidic linkages of each of which are independently incorporated herein by reference.

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

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

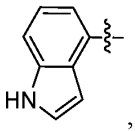
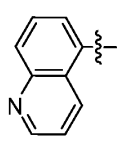
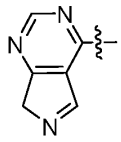
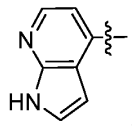
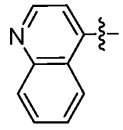
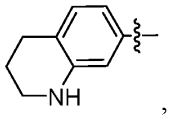
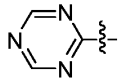
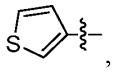
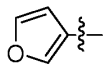
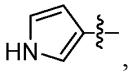
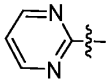
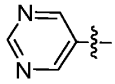
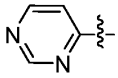
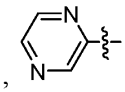
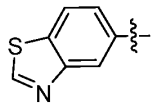
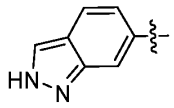
In some embodiments, R is optionally substituted C_{1-30} (e.g., 1, 2, 3, 4, 5, 6,

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

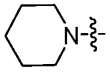
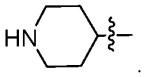
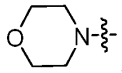
In some embodiments, R is optionally substituted C_{6-30} aryl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is C_{6-14} aryl. In some embodiments, R is optionally substituted bicyclic aryl. In some embodiments,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

In some embodiments, R is optionally substituted 5-30 (5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) membered heteroaryl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-20 membered heteroaryl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-10 membered heteroaryl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-5 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-4 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-3 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having one heteroatom. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-5 heteroatoms. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-4 heteroatoms. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-3 heteroatoms. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 6-membered heteroaryl having one

heteroatom. In some embodiments, R is optionally substituted monocyclic heteroaryl. In some embodiments, R is optionally substituted bicyclic heteroaryl. In some embodiments, R is optionally substituted polycyclic heteroaryl. In some embodiments, a heteroatom is nitrogen.

In some embodiments, R is optionally substituted 2-pyridinyl. In some embodiments, R is optionally substituted 3-pyridinyl. In some embodiments, R is optionally substituted 4-pyridinyl. In some embodiments, R is optionally substituted , , , , , , , , , , , , , , , , , , , , or .

In some embodiments, R is optionally substituted 3-30 (3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) membered heterocyclyl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 3-membered heterocyclyl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 4-membered heterocyclyl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 5-20 membered heterocyclyl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-10 membered heterocyclyl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-5 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-4 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-3 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having one heteroatom. In some embodiments, R is optionally substituted 6-membered heterocyclyl having 1-5 heteroatoms. In some embodiments, R is optionally substituted 6-membered heterocyclyl having 1-4 heteroatoms. In some embodiments, R is optionally substituted 6-membered heterocyclyl

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

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

Various variables may comprises an optionally substituted ring, or can be taken together with their intervening atom(s) to form a ring. In some embodiments, a ring is 3-30 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) membered. In some embodiments, a ring is 3-20 membered. In some embodiments, a ring is 3-15 membered. In some embodiments, a ring is 3-10 membered. In some embodiments, a ring is 3-8 membered. In some embodiments, a ring is 3-7 membered. In some embodiments, a ring is 3-6 membered. In some embodiments, a ring is 4-20 membered. In some embodiments, a ring is 5-20 membered. In some embodiments, a ring is monocyclic. In some embodiments, a ring is bicyclic. In some embodiments, a ring is polycyclic. In some embodiments, each monocyclic ring or each monocyclic ring unit in bicyclic or polycyclic rings is independently saturated, partially saturated or aromatic. In some embodiments, each monocyclic ring or each monocyclic ring unit in bicyclic or polycyclic rings is independently 3-10 membered and has 0-5 heteroatoms.

In some embodiments, each heteroatom is independently selected oxygen,

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

As appreciated by those skilled in the art, many other types of internucleotidic linkages may be utilized in accordance with the present disclosure, for example, those described in U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,177,195; 5,023,243; 5,034,506; 5,166,315; 5,185,444; 5,188,897; 5,214,134; 5,216,141; 5,235,033; 5,264,423; 5,264,564; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,938; 5,405,939; 5,434,257; 5,453,496; 5,455,233; 5,466,677; 5,466,677; 5,470,967; 5,476,925; 5,489,677; 5,519,126; 5,536,821; 5,541,307; 5,541,316; 5,550,111; 5,561,225; 5,563,253; 5,571,799; 5,587,361; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,625,050; 5,633,360; 5,64,562; 5,663,312; 5,677,437; 5,677,439; 6,160,109; 6,239,265; 6,028,188; 6,124,445; 6,169,170; 6,172,209; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; or RE39464. In certain embodiments, a modified internucleotidic linkage is one described in US 9982257, US 20170037399, US 20180216108, WO 2017192664, WO 2017015575, WO2017062862, WO 2018067973, WO 2017160741, WO 2017192679, WO 2017210647, WO 2018098264, PCT/US18/35687, PCT/US18/38835, or PCT/US18/51398, the nucleobases, sugars, internucleotidic linkages, chiral auxiliaries/reagents, and technologies for oligonucleotide synthesis (reagents, conditions, cycles, etc.) of each of which is independently incorporated herein by reference.

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

In certain embodiments, a ds oligonucleotide comprises one or more nucleotides that independently comprise a phosphorus modification prone to "autorelease" under certain conditions. That is, under certain conditions, a particular phosphorus modification is designed such that it self-cleaves from the ds oligonucleotide to provide,

e.g., a natural phosphate linkage. In certain embodiments, such a phosphorus modification has a structure of –O–L–R^1, wherein L is L^B as described herein, and R^1 is R' as described herein. In certain embodiments, a phosphorus modification has a structure of –S–L–R^1, wherein each L and R^1 is independently as described in the present disclosure. Certain examples of such phosphorus modification groups can be found in US 9982257. In certain embodiments, an autorelease group comprises a morpholino group. In certain embodiments, an autorelease group is characterized by the ability to deliver an agent to the internucleotidic phosphorus linker, which agent facilitates further modification of the phosphorus atom such as, e.g., desulfurization. In certain embodiments, the agent is water and the further modification is hydrolysis to form a natural phosphate linkage.

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

Ds Oligonucleotides can comprise various number of natural phosphate linkages. In certain embodiments, 5% or more of the internucleotidic linkages of provided ds oligonucleotides are natural phosphate linkages. In certain embodiments, 10% or more of the internucleotidic linkages of provided ds oligonucleotides are natural phosphate linkages. In certain embodiments, 15% or more of the internucleotidic linkages of provided ds oligonucleotides are natural phosphate linkages. In certain embodiments, 20% or more of the internucleotidic linkages of provided ds oligonucleotides are natural phosphate linkages. In certain embodiments, 25% or more of the internucleotidic linkages of provided ds oligonucleotides are natural phosphate linkages. In certain embodiments, 30% or more of the internucleotidic linkages of provided ds oligonucleotides are natural phosphate linkages. In certain embodiments, 35% or more of the internucleotidic linkages of provided ds oligonucleotides are natural phosphate linkages. In certain embodiments, 40% or more

of the internucleotidic linkages of provided ds oligonucleotides are natural phosphate linkages. In certain embodiments, provided ds oligonucleotides comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages. In certain embodiments, provided ds oligonucleotides comprises 4, 5, 6, 7, 8, 9, 10 or more natural phosphate linkages. In certain embodiments, the number of natural phosphate linkages is 2. In certain embodiments, the number of natural phosphate linkages is 3. In certain embodiments, the number of natural phosphate linkages is 4. In certain embodiments, the number of natural phosphate linkages is 5. In certain embodiments, the number of natural phosphate linkages is 6. In certain embodiments, the number of natural phosphate linkages is 7. In certain embodiments, the number of natural phosphate linkages is 8. In certain embodiments, some or all of the natural phosphate linkages are consecutive.

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

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

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

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

In certain embodiments, one or more chiral internucleotidic linkages are

chirally controlled and one or more chiral internucleotidic linkages are not chirally controlled. In certain embodiments, each phosphorothioate internucleotidic linkage is independently chirally controlled, and one or more non-negatively charged internucleotidic linkage are not chirally controlled. In certain embodiments, each phosphorothioate internucleotidic linkage is independently chirally controlled, and each non-negatively charged internucleotidic linkage is not chirally controlled. In certain embodiments, the internucleotidic linkage between the first two nucleosides of a ds oligonucleotide is a non-negatively charged internucleotidic linkage. In certain embodiments, the internucleotidic linkage between the last two nucleosides are each independently a non-negatively charged internucleotidic linkage. In certain embodiments, both are independently non-negatively charged internucleotidic linkages. In certain embodiments, each non-negatively charged internucleotidic linkage is independently neutral internucleotidic linkage. In certain embodiments, each non-negatively charged internucleotidic linkage is independently n001.

In certain embodiments, a controlled level of ds oligonucleotides in a composition are desired ds oligonucleotides. In certain embodiments, of all ds oligonucleotides in a composition that share a common base sequence (e.g., a desired sequence for a purpose), or of all ds oligonucleotides in a composition, level of desired ds oligonucleotides (which may exist in various forms (e.g., salt forms) and typically differ only at non-chirally controlled internucleotidic linkages (various forms of the same stereoisomer can be considered the same for this purpose)) is about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80-100%, 90-100%, 95-100%, 50%-90%, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%, or at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%. In certain embodiments, a level is at least about 50%. In certain embodiments, a level is at least about 60%. In certain embodiments, a level is at least about 70%. In certain embodiments, a level is at least about 75%. In certain embodiments, a level is at least about 80%. In certain embodiments, a level is at least about 85%. In certain embodiments, a level is at least about 90%. In certain embodiments, a level is or is at least $(DS)^{nc}$, wherein DS is about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% and nc is the number of chirally controlled internucleotidic linkages as described in the present disclosure (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more). In certain embodiments,

a level is or is at least $(DS)^{nc}$, wherein DS is 95%-100%.

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

2.3. Double Stranded Oligonucleotide Compositions

Among other things, the present disclosure provides various ds oligonucleotide compositions. In certain embodiments, the present disclosure provides ds oligonucleotide compositions of ds oligonucleotides described herein. In certain embodiments, a ds oligonucleotide composition, e.g., a dsRNAi oligonucleotide composition, comprises a plurality of a ds oligonucleotide described in the present disclosure. In certain embodiments, a ds oligonucleotide composition, e.g., a dsRNAi oligonucleotide composition, is chirally controlled. In certain embodiments, a ds oligonucleotide composition, e.g., a dsRNAi oligonucleotide composition, is not chirally controlled (stereorandom).

Linkage phosphorus of natural phosphate linkages is achiral. Linkage phosphorus of many modified internucleotidic linkages, e.g., phosphorothioate internucleotidic linkages, are chiral. In certain embodiments, during preparation of ds oligonucleotide compositions (e.g., in traditional phosphoramidite ds oligonucleotide synthesis), configurations of chiral linkage phosphorus are not purposefully designed or controlled, creating non-chirally controlled (stereorandom) ds oligonucleotide compositions (substantially racemic preparations) which are complex, random mixtures of various stereoisomers (diastereoisomers) - for ds oligonucleotides with n chiral internucleotidic linkages (linkage phosphorus being chiral), typically 2^n stereoisomers (e.g., when n is 10, 2^{10} =1,032; when n is 20, 2^{20} = 1,048,576). These stereoisomers have the same constitution, but differ with respect to the pattern of stereochemistry of their linkage phosphorus.

In certain embodiments, stereorandom ds oligonucleotide compositions have sufficient properties and/or activities for certain purposes and/or applications. In certain

embodiments, stereorandom ds oligonucleotide compositions can be cheaper, easier and/or simpler to produce than chirally controlled ds oligonucleotide compositions. However, stereoisomers within stereorandom compositions may have different properties, activities, and/or toxicities, resulting in inconsistent therapeutic effects and/or unintended side effects by stereorandom compositions, particularly compared to certain chirally controlled ds oligonucleotide compositions of ds oligonucleotides of the same constitution.

2.3.1. Chirally Controlled Double Stranded Oligonucleotide Compositions

In certain embodiments, the present disclosure encompasses technologies for designing and preparing chirally controlled ds oligonucleotide compositions. In certain embodiments, a chirally controlled ds oligonucleotide composition comprises a controlled/pre-determined (not random as in stereorandom compositions) level of a plurality of ds oligonucleotides, wherein the ds oligonucleotides share the same linkage phosphorus stereochemistry at one or more chiral internucleotidic linkages (chirally controlled internucleotidic linkages). In certain embodiments, ds oligonucleotides of a plurality share the same pattern of backbone chiral centers (stereochemistry of linkage phosphorus). In certain embodiments, a pattern of backbone chiral centers is as described in the present disclosure. In certain embodiments, ds oligonucleotides of a plurality share a common constitution. In certain embodiments, they are structurally identical.

For example, in certain embodiments, the present disclosure provides a ds oligonucleotide composition comprising a plurality of ds oligonucleotides, wherein ds oligonucleotides of the plurality share:

1) a common base sequence, and

2) the same linkage phosphorus stereochemistry independently at one or more (e.g., about 1-50, 1-40, 1-30, 1-25, 1-20, 1-15, 1-10, 5-50, 5-40, 5-30, 5-25, 5-20, 5-15, 5-10, 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 or more) chiral internucleotidic linkages ("chirally controlled internucleotidic linkages"); wherein level of ds oligonucleotides of the plurality in the composition is non-random (e.g., controlled/pre- determined as described herein).

In certain embodiments, the present disclosure provides a ds oligonucleotide composition comprising a plurality of ds oligonucleotides, wherein ds oligonucleotides of the plurality share:

1) a common base sequence, and

2) the same linkage phosphorus stereochemistry independently at one or more (e.g.,

about 1-50, 1-40, 1-30, 1-25, 1-20, 1-15, 1-10, 5-50, 5-40, 5-30, 5-25, 5-20, 5-15, 5-10, 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 or more) chiral internucleotidic linkages ("chirally controlled internucleotidic linkages"); wherein the composition is enriched relative to a substantially racemic preparation of ds oligonucleotides sharing the common base sequence for oligonucleotides of the plurality.

In certain embodiments, the present disclosure provides a ds oligonucleotide composition comprising a plurality of ds oligonucleotides, wherein ds oligonucleotides of the plurality share:

1) a common base sequence, and

2) the same linkage phosphorus stereochemistry independently at one or more (e.g., about 1-50, 1-40, 1-30, 1-25, 1-20, 1-15, 1-10, 5-50, 5-40, 5-30, 5-25, 5-20, 5-15, 5-10, 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 or more) chiral internucleotidic linkages ("chirally controlled internucleotidic linkages"); wherein about 1%-100%, (e.g., about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80-100%, 90-100%, 95-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all ds oligonucleotides in the composition that share the common base sequence are ds oligonucleotides of the plurality.

In certain embodiments, the percentage/level of the ds oligonucleotides of a plurality is or is at least $(DS)^{nc}$, wherein DS is 90%-100%, and nc is the number of chirally controlled internucleotidic linkages. In certain embodiments, nc is 5, 6, 7, 8, 9, 10 or more. In certain embodiments, a percentage/level is at least 10%.

In certain embodiments, a percentage/level is at least 20%. In certain embodiments, a percentage/level is at least 30%. In certain embodiments, a percentage/level is at least 40%. In certain embodiments, a percentage/level is at least 50%. In certain embodiments, a percentage/level is at least 60%. In certain embodiments, a percentage/level is at least 65%. In certain embodiments, a percentage/level is at least 70%. In certain embodiments, a percentage/level is at least 75%. In certain embodiments, a percentage/level is at least 80%. In certain embodiments, a percentage/level is at least 85%. In certain embodiments, a percentage/level is at least 90%. In certain embodiments, a percentage/level is at least 95%.

In certain embodiments, ds oligonucleotides of a plurality share a common pattern of backbone linkages. In certain embodiments, each ds oligonucleotide of a plurality independently has an internucleotidic linkage of a particular constitution (e.g., −O−P(O)(SH)−O−) or a salt form thereof (e.g., −O−P(O)(SNa)−O−) independently at each internucleotidic linkage site. In certain embodiments, internucleotidic linkages at each internucleotidic linkage site are of the same form. In certain embodiments, internucleotidic linkages at each internucleotidic linkage site are of different forms.

In certain embodiments, ds oligonucleotides of a plurality share a common constitution. In certain embodiments, ds oligonucleotides of a plurality are of the same form of a common constitution. In certain embodiments, ds oligonucleotides of a plurality are of two or more forms of a common constitution. In certain embodiments, ds oligonucleotides of a plurality are each independently of a particularly oligonucleotide or a pharmaceutically acceptable salt thereof, or of a ds oligonucleotide having the same constitution as the particularly ds oligonucleotide or a pharmaceutically acceptable salt thereof. In certain embodiments, about 1%- 100%, (e.g., about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80-100%, 90-100%, 95-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all ds oligonucleotides in the composition that share a common constitution are ds oligonucleotides of the plurality. In certain embodiments, a percentage of a level is or is at least $(DS)^{nc}$, wherein DS is 90%-100%, and nc is the number of chirally controlled internucleotidic linkages. In certain embodiments, nc is 5, 6, 7, 8, 9, 10 or more. In certain embodiments, a level is at least 10%. In certain embodiments, a level is at least 20%. In certain embodiments, a level is at least 30%. In certain embodiments, a level is at least 40%. In certain embodiments, a level is at least 50%. In certain embodiments, a level is at least 60%. In certain embodiments, a level is at least 65%. In certain embodiments, a level is at least 70%. In certain embodiments, a level is at least 75%. In certain embodiments, a level is at least 80%. In certain embodiments, a level is at least 85%. In certain embodiments, a level is at least 90%. In certain embodiments, a level is at least 95%.

In certain embodiments, each phosphorothioate internucleotidic linkage is independently a chirally controlled internucleotidic linkage.

In certain embodiments, the present disclosure provides a chirally controlled ds oligonucleotide composition comprising a plurality of ds oligonucleotides of a particular ds oligonucleotide type characterized by:

a) a common base sequence;

b) a common pattern of backbone linkages;

c) a common pattern of backbone chiral centers; wherein the composition is enriched, relative to a substantially racemic preparation of ds oligonucleotides having the same common base sequence, for ds oligonucleotides of the particular oligonucleotide type.

In certain embodiments, the present disclosure provides a chirally controlled ds oligonucleotide composition comprising a plurality of ds oligonucleotides of a particular ds oligonucleotide type characterized by:

a) a common base sequence;

b) a common pattern of backbone linkages;

c) a common pattern of backbone chiral centers; wherein ds oligonucleotides of the plurality comprise at least one internucleotidic linkage comprising a common linkage phosphorus in the *S*p configuration; wherein the composition is enriched, relative to a substantially racemic preparation of d oligonucleotides having the same common base sequence, for ds oligonucleotides of the particular ds oligonucleotide type.

Common patterns of backbone chiral centers, as appreciated by those skilled in the art, comprise at least one *R*p or at least one *S*p. Certain patterns of backbone chiral centers are illustrated in, e.g., Table 1A and 1B or Table 1C or Table 1D.

In certain embodiments, a chirally controlled ds oligonucleotide composition is enriched, relative to a substantially racemic preparation of ds oligonucleotides share the same common base sequence and a common pattern of backbone linkages, for ds oligonucleotides of the particular ds oligonucleotide type.

In certain embodiments, ds oligonucleotides of a plurality, e.g., a particular ds oligonucleotide type, have a common pattern of backbone phosphorus modifications and a common pattern of nucleoside modifications. In certain embodiments, ds oligonucleotides of a plurality have a common pattern of sugar modifications. In certain embodiments, ds oligonucleotides of a plurality have a common pattern of base modifications. In certain embodiments, ds oligonucleotides of a plurality have a common pattern of nucleoside modifications. In certain embodiments, ds oligonucleotides of a plurality have the same constitution. In certain embodiments, ds oligonucleotides of a plurality are identical. In

certain embodiments, ds oligonucleotides of a plurality are of the same ds oligonucleotide (as those skilled in the art will appreciate, such ds oligonucleotides may each independently exist in one of the various forms of the ds oligonucleotide, and may be the same, or different forms of the ds oligonucleotide). In certain embodiments, ds oligonucleotides of a plurality are each independently of the same ds oligonucleotide or a pharmaceutically acceptable salt thereof.

In certain embodiments, the present disclosure provides chirally controlled ds oligonucleotide compositions, e.g., of many oligonucleotides in Table 1A or 1B or Table 1C or Table 1D, whose "stereochemistry/linkage" contain S and/or R. In certain embodiments, ds oligonucleotides of a plurality are each independently a particular ds oligonucleotide in Table 1 whose "stereochemistry/linkage" contains S and/or R, optionally in various forms. In certain embodiments, ds oligonucleotides of a plurality are each independently a particular ds oligonucleotide in Table 1A or 1B or 1C or 1D, whose "stereochemistry/linkage" contains S and/or R, or a pharmaceutically acceptable salt thereof.

In certain embodiments, level of a plurality of ds oligonucleotides in a composition can be determined as the product of the diastereopurity of each chirally controlled internucleotidic linkage in the ds oligonucleotides. In certain embodiments, diastereopurity of an internucleotidic linkage connecting two nucleosides in a ds oligonucleotide (or nucleic acid) is represented by the diastereopurity of an internucleotidic linkage of a dimer connecting the same two nucleosides, wherein the dimer is prepared using comparable conditions, in some instances, identical synthetic cycle conditions.

In certain embodiments, all chiral internucleotidic linkages are independently chiral controlled, and the composition is a completely chirally controlled ds oligonucleotide composition. In certain embodiments, not all chiral internucleotidic linkages are chiral controlled internucleotidic linkages, and the composition is a partially chirally controlled ds oligonucleotide composition.

Ds Oligonucleotides may comprise or consist of various patterns of backbone chiral centers (patterns of stereochemistry of chiral linkage phosphorus). Certain useful patterns of backbone chiral centers are described in the present disclosure. In certain embodiments, a plurality of ds oligonucleotides share a common pattern of backbone chiral centers, which is or comprises a pattern described in the present disclosure (e.g., as in "Stereochemistry and Patterns of Backbone Chiral Centers", a pattern of backbone chiral

centers of a chirally controlled ds oligonucleotide in Table 1A or 1B, or Table 1C or Table 1D, etc.).

In certain embodiments, a chirally controlled ds oligonucleotide composition is chirally pure (or stereopure, stereochemically pure) ds oligonucleotide composition, wherein the ds oligonucleotide composition comprises a plurality of ds oligonucleotides, wherein the ds oligonucleotides are independently of the same stereoisomer (including that each chiral element of the ds oligonucleotides, including each chiral linkage phosphorus, is independently defined (stereodefined)). A chirally pure (or stereopure, stereochemically pure) ds oligonucleotide composition of a ds oligonucleotide stereoisomer does not contain other stereoisomers (as appreciated by those skilled in the art, one or more unintended stereoisomers may exist as impurities from, e.g., preparation, storage, etc.).

2.3.2 Stereochemistry and Patterns of Backbone Chiral Centers

In contrast to natural phosphate linkages, linkage phosphorus of chiral modified internucleotidic linkages, e.g., phosphorothioate internucleotidic linkages, are chiral. Among other things, the present disclosure provides technologies (e.g., oligonucleotides, compositions, methods, etc.) comprising control of stereochemistry of chiral linkage phosphorus in chiral internucleotidic linkages. In certain embodiments, as demonstrated herein, control of stereochemistry can provide improved properties and/or activities, including desired stability, reduced toxicity, improved reduction of target nucleic acids, etc. In certain embodiments, the present disclosure provides useful patterns of backbone chiral centers for oligonucleotides and/or regions thereof, which pattern is a combination of stereochemistry of each chiral linkage phosphorus (*R*p or *S*p) of chiral linkage phosphorus, indication of each achiral linkage phosphorus (Op, if any), etc. from 5' to 3'. In certain embodiments, patterns of backbone chiral centers can control cleavage patterns of target nucleic acids when they are contacted with provided ds oligonucleotides or compositions thereof in a cleavage system (e.g., in vitro assay, cells, tissues, organs, organisms, subjects, etc.). In certain embodiments, patterns of backbone chiral centers improve cleavage efficiency and/or selectivity of target nucleic acids when they are contacted with provided ds oligonucleotides or compositions thereof in a cleavage system.

In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof comprises or is any (Np)n(Op)m, wherein Np is *R*p or *S*p, Op represents a linkage phosphorus being achiral (e.g., as for the linkage phosphorus of natural phosphate linkages), and each of n and m is independently as defined and described

in the present disclosure. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof comprises or is (*S*p)n(Op)m, wherein each variable is independently as defined and described in the present disclosure. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof comprises or is (*R*p)n(Op)m, wherein each variable is independently as defined and described in the present disclosure. In certain embodiments, n is 1. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof comprises or is (*S*p)(Op)m, wherein m is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In certain embodiments, a pattern of backbone chiral centers of an oligonucleotide or a region thereof comprises or is (*R*p)(Op)m, wherein m is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In certain embodiments, the pattern of backbone chiral centers of a 5'-wing is or comprises (Np)n(Op)m. In certain embodiments, the pattern of backbone chiral centers of a 5'-wing is or comprises (*S*p)n(Op)m. In certain embodiments, the pattern of backbone chiral centers of a 5'-wing is or comprises (*R*p)n(Op)m. In certain embodiments, the pattern of backbone chiral centers of a 5'-wing is or comprises (*S*p)(Op)m. In certain embodiments, the pattern of backbone chiral centers of a 5'-wing is or comprises (*R*p)(Op)m. In certain embodiments, the pattern of backbone chiral centers of a 5'-wing is (*S*p)(Op)m. In certain embodiments, the pattern of backbone chiral centers of a 5'-wing is (*R*p)(Op)m. In certain embodiments, the pattern of backbone chiral centers of a 5'-wing is (*S*p)(Op)m, wherein *S*p is the linkage phosphorus configuration of the first internucleotidic linkage of the oligonucleotide from the 5'-end. In certain embodiments, the pattern of backbone chiral centers of a 5'-wing is (*R*p)(Op)m, wherein *R*p is the linkage phosphorus configuration of the first internucleotidic linkage of the oligonucleotide from the 5'- end. In certain embodiments, as described in the present disclosure, m is 2; in certain embodiments, m is 3; in certain embodiments, m is 4; in certain embodiments, m is 5; in certain embodiments, m is 6.

In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof comprises or is (Op)m(Np)n, wherein Np is *R*p or *S*p, Op represents a linkage phosphorus being achiral (e.g., as for the linkage phosphorus of natural phosphate linkages), and each of n and m is independently as defined and described in the present disclosure. In certain embodiments, a pattern of backbone chiral centers of an oligonucleotide or a region thereof comprises or is (Op)m(*S*p)n, wherein each variable is independently as defined and described in the present disclosure. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof comprises or

is (Op)m(*R*p)n, wherein each variable is independently as defined and described in the present disclosure. In certain embodiments, n is 1. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof comprises or is (Op)m(*S*p), wherein m is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In certain embodiments, a pattern of backbone chiral centers of an oligonucleotide or a region thereof comprises or is (Op)m(*R*p), wherein m is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In certain embodiments, the pattern of backbone chiral centers of a 3'-wing is or comprises (Op)m(Np)n. In certain embodiments, the pattern of backbone chiral centers of a 3'-wing is or comprises (Op)m(*S*p)n. In certain embodiments, the pattern of backbone chiral centers of a 3'-wing is or comprises (Op)m(*R*p)n. In certain embodiments, the pattern of backbone chiral centers of a 3'-wing is or comprises (Op)m(*S*p). In certain embodiments, the pattern of backbone chiral centers of a 3'-wing is or comprises (Op)m(*R*p). In certain embodiments, the pattern of backbone chiral centers of a 3'-wing is (Op)m(*S*p). In certain embodiments, the pattern of backbone chiral centers of a 3'-wing is (Op)m(*R*p). In certain embodiments, the pattern of backbone chiral centers of a 3'-wing is (Op)m(*S*p), wherein *S*p is the linkage phosphorus configuration of the last internucleotidic linkage of the ds oligonucleotide from the 5'-end. In certain embodiments, the pattern of backbone chiral centers of a 3'-wing is (Op)m(*R*p), wherein *R*p is the linkage phosphorus configuration of the last internucleotidic linkage of the oligonucleotide from the 5'- end. In certain embodiments, as described in the present disclosure, m is 2; in certain embodiments, m is 3; in certain embodiments, m is 4; in certain embodiments, m is 5; in certain embodiments, m is 6.

In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof (e.g., a core) comprises or is (*S*p)m(*R*p/Op)n or (*R*p/Op)n(*S*p)m, wherein each variable is independently as described in the present disclosure. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof (e.g., a core) comprises or is (*S*p)m(*R*p)n or (*R*p)n(*S*p)m, wherein each variable is independently as described in the present disclosure. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof (e.g., a core) comprises or is (*S*p)m(Op)n or (Op)n(*S*p)m, wherein each variable is independently as described in the present disclosure. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof (e.g., a core) comprises or is (Np)t[(*R*p/Op)n(*S*p)m]y or [(*R*p/Op)n(*S*p)m]y(Np)t, wherein y is 1-50, and each other variable is independently as described in the present disclosure. In certain embodiments, a

pattern of backbone chiral centers of a ds oligonucleotide or a region thereof (e.g., a core) comprises or is (Np)t[(*R*p)n(*S*p)m]y or [(*R*p)n(*S*p)m]y(Np)t, wherein each variable is independently as described in the present disclosure. In certain embodiments, a pattern of backbone chiral centers of a a ds n oligonucleotide or a region thereof (e.g., a core) comprises or is [(*R*p/Op)n(*S*p)m]y(*R*p)k, [(*R*p/Op)n(*S*p)m]y, (*S*p)t[(*R*p/Op)n(*S*p)m]y, (*S*p)t[(*R*p/Op)n(*S*p)m]y(*R*p)k, wherein k is 1-50, and each other variable is independently as described in the present disclosure. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof (e.g., a core) comprises or is [(Op)n(*S*p)m]y(*R*p)k, [(Op)n(*S*p)m]y, (*S*p)t[(Op)n(*S*p)m]y, (*S*p)t[(Op)n(*S*p)m]y(*R*p)k, wherein each variable is independently as described in the present disclosure. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region thereof (e.g., a core) comprises or is [(*R*p)n(*S*p)m]y(*R*p)k, [(*R*p)n(*S*p)m]y, (*S*p)t[(*R*p)n(*S*p)m]y, (*S*p)t[(*R*p)n(*S*p)m]y(*R*p)k, wherein each variable is independently as described in the present disclosure. In certain embodiments, an oligonucleotide comprises a core region. In certain embodiments, an oligonucleotide comprises a core region, wherein each sugar in the core region does not contain a 2'-OR^1, wherein R^1 is as described in the present disclosure. In certain embodiments, a ds oligonucleotide comprises a core region, wherein each sugar in the core region is independently a natural DNA sugar. In certain embodiments, the pattern of backbone chiral centers of the core comprises or is (*R*p)(*S*p)m. In certain embodiments, the pattern of backbone chiral centers of the core comprises or is (Op)(*S*p)m. In certain embodiments, the pattern of backbone chiral centers of the core comprises or is (Np)t[(*R*p/Op)n(*S*p)m]y or [(*R*p/Op)n(*S*p)m]y(Np)t. In certain embodiments, the pattern of backbone chiral centers of the core comprises or is (Np)t[(*R*p/Op)n(*S*p)m]y or [(*R*p/Op)n(*S*p)m]y(Np)t. In certain embodiments, the pattern of backbone chiral centers of the core comprises or is (Np)t[(*R*p)n(*S*p)m]y or [(*R*p)n(*S*p)m]y(Np)t. In certain embodiments, the pattern of backbone chiral centers of a core comprises or is [(*R*p/Op)n(*S*p)m]y(*R*p)k, [(*R*p/Op)n(*S*p)m]y, (*S*p)t[(*R*p/Op)n(*S*p)m]y, (*S*p)t[(*R*p/Op)n(*S*p)m]y(*R*p)k. In certain embodiments, a pattern of backbone chiral centers of a core comprises or is [(Op)n(*S*p)m]y(*R*p)k, [(Op)n(*S*p)m]y, (*S*p)t[(Op)n(*S*p)m]y, (*S*p)t[(Op)n(*S*p)m]y(*R*p)k. In certain embodiments, a pattern of backbone chiral centers of a core comprises or is [(*R*p)n(*S*p)m]y(*R*p)k, [(*R*p)n(*S*p)m]y, (*S*p)t[(*R*p)n(*S*p)m]y, or (*S*p)t[(*R*p)n(*S*p)m]y(*R*p)k. In certain embodiments, a pattern of backbone chiral centers of a core comprises [(*R*p)n(*S*p)m]y(*R*p)k. In certain embodiments, a pattern of backbone chiral

centers of a core comprises [(*R*p)n(*S*p)m]y(*R*p). In certain embodiments, a pattern of backbone chiral centers of a core comprises [(*R*p)n(*S*p)m]y. In certain embodiments, a pattern of backbone chiral centers of a core comprises (*S*p)t[(*R*p)n(*S*p)m]y. In certain embodiments, a pattern of backbone chiral centers of a core comprises (*S*p)t[(*R*p)n(*S*p)m]y(*R*p)k. In certain embodiments, a pattern of backbone chiral centers of a core comprises (*S*p)t[(*R*p)n(*S*p)m]y(*R*p). In certain embodiments, a pattern of backbone chiral centers of a core is [(*R*p)n(*S*p)m]y(*R*p)k. In certain embodiments, a pattern of backbone chiral centers of a core is [(*R*p)n(*S*p)m]y(*R*p). In certain embodiments, a pattern of backbone chiral centers of a core is [(*R*p)n(*S*p)m]y. In certain embodiments, a pattern of backbone chiral centers of a core is (*S*p)t[(*R*p)n(*S*p)m]y. In certain embodiments, a pattern of backbone chiral centers of a core is (*S*p)t[(*R*p)n(*S*p)m]y(*R*p)k. In certain embodiments, a pattern of backbone chiral centers of a core is (*S*p)t[(*R*p)n(*S*p)m]y(*R*p). In certain embodiments, each n is 1. In certain embodiments, each t is 1. In certain embodiments, t is 2, 3, 4, 5, 6, 7, 8, 9, or 10. In certain embodiments, each of t and n is 1. In certain embodiments, each m is 2 or more. In certain embodiments, k is 1. In certain embodiments, k is 2-10.

In certain embodiments, a pattern of backbone chiral centers comprises or is (*S*p)m(*R*p)n, (*R*p)n(*S*p)m, (Np)t(*R*p)n(*S*p)m, (*S*p)t(*R*p)n(*S*p)m, (Np)t[(*R*p)n(*S*p)m]2, (*S*p)t[(*R*p)n(*S*p)m]2, (Np)t(Op)n(*S*p)m, (*S*p)t(Op)n(*S*p)m, (Np)t[(Op)n(*S*p)m]2, or (*S*p)t[(Op)n(*S*p)m]2. In certain embodiments, a pattern is (Np)t(Op/*R*p)n(*S*p)m(Op/*R*p)n(*S*p)m. In certain embodiments, a pattern is (Np)t(Op/*R*p)n(*S*p)1- 5(Op/*R*p)n(*S*p)m. In certain embodiments, a pattern is (Np)t(Op/*R*p)n(*S*p)2-5(Op/*R*p)n(*S*p)m. In certain embodiments, a pattern is (Np)t(Op/*R*p)n(*S*p)2(Op/*R*p)n(*S*p)m. In certain embodiments, a pattern is (Np)t(Op/*R*p)n(*S*p)3(Op/*R*p)n(*S*p)m. In certain embodiments, a pattern is (Np)t(Op/*R*p)n(*S*p)4(Op/*R*p)n(*S*p)m. In certain embodiments, a pattern is (Np)t(Op/*R*p)n(*S*p)5(Op/*R*p)n(*S*p)m.

In certain embodiments, Np is *S*p. In certain embodiments, (Op/*R*p) is Op. In certain embodiments, (Op/*R*p) is *R*p. In certain embodiments, Np is *S*p and (Op/*R*p) is *R*p. In certain embodiments, Np is *S*p and (Op/*R*p) is Op. In certain embodiments, Np is *S*p and at least one (Op/*R*p) is *R*p, and at least one (Op/*R*p) is Op. In certain embodiments, a pattern of backbone chiral centers comprises or is (*R*p)n(*S*p)m, (Np)t(*R*p)n(*S*p)m, or (*S*p)t(*R*p)n(*S*p)m, wherein m > 2. In certain embodiments, a pattern of backbone chiral

centers comprises or is (*R*p)n(*S*p)m, (Np)t(*R*p)n(*S*p)m, or (*S*p)t(*R*p)n(*S*p)m, wherein n is 1, at least one t >1, and at least one m > 2.

In certain embodiments, oligonucleotides comprising core regions whose patterns of backbone chiral centers starting with *R*p can provide high activities and/or improved properties. In certain embodiments, oligonucleotides comprising core regions whose patterns of backbone chiral centers ending with *R*p can provide high activities and/or improved properties. In certain embodiments, oligonucleotides comprising core regions whose patterns of backbone chiral centers starting with *R*p provide high activities (e.g., target cleavage) without significantly impacting its properties, e.g., stability. In certain embodiments, oligonucleotides comprising core regions whose patterns of backbone chiral centers ending with *R*p provide high activities (e.g., target cleavage) without significantly impacting its properties, e.g., stability. In certain embodiments, patterns of backbone chiral centers start with *R*p and end with *S*p. In certain embodiments, patterns of backbone chiral centers start with *R*p and end with *R*p. In certain embodiments, patterns of backbone chiral centers start with *S*p and end with *R*p.

In certain embodiments, a pattern of backbone chiral centers of a RNAi oligonucleotide or a region thereof (e.g., a core) comprises or is (Op)[(*R*p/Op)n(*S*p)m]y(*R*p)k(Op), (Op)[(*R*p/Op)n(*S*p)m]y(Op), (Op)(*S*p)t[(*R*p/Op)n(*S*p)m]y(Op), or (Op)(*S*p)t[(*R*p/Op)n(*S*p)m]y(*R*p)k(Op), wherein k is 1-50, and each other variable is independently as described in the present disclosure. In certain embodiments, a pattern of backbone chiral centers of a RNAi oligonucleotide comprises or is (Op)[(*R*p/Op)n(*S*p)m]y(*R*p)k(Op), (Op)[(*R*p/Op)n(*S*p)m]y(Op), (Op)(*S*p)t[(*R*p/Op)n(*S*p)m]y(Op), or (Op)(*S*p)t[(*R*p/Op)n(*S*p)m]y(*R*p)k(Op), wherein each of f, g, h and j is independently 1-50, and each other variable is independently as described in the present disclosure, and the oligonucleotide comprises a core region whose pattern of backbone chiral centers comprises or is [(*R*p/Op)n(*S*p)m]y(*R*p)k, [(*R*p/Op)n(*S*p)m]y, (*S*p)t[(*R*p/Op)n(*S*p)m]y, or (*S*p)t[(*R*p/Op)n(*S*p)m]y(*R*p)k as described in the present disclosure. In certain embodiments, a pattern of backbone chiral centers is or comprises (Op)[(*R*p/Op)n(*S*p)m]y(*R*p)k(Op). In certain embodiments, a pattern of backbone chiral centers is or comprises (Op)[(Rp/Op)n(Sp)m]y(Rp)(Op). In certain embodiments, a pattern of backbone chiral centers is or comprises (Op)[(*R*p/Op)n(*S*p)m]y(Op). In certain embodiments, a pattern of backbone chiral centers is or comprises (Op)(*S*p)t[(*R*p/Op)n(*S*p)m]y(Op). In certain embodiments, a pattern of backbone chiral

centers is or comprises (Op)(*S*p)t[(*R*p/Op)n(*S*p)m]y(*R*p)k(Op). In certain embodiments, a pattern of backbone chiral centers is or comprises (Op)(*S*p)t[(*R*p/Op)n(*S*p)m]y(*R*p)(Op). In certain embodiments, a pattern of backbone chiral centers is or comprises (Op)[(Rp)n(Sp)m]y(Rp)k(Op). In certain embodiments, a pattern of backbone chiral centers is or comprises (Op)[(*R*p)n(*S*p)m]y(*R*p)(Op). In certain embodiments, a pattern of backbone chiral centers is or comprises (Op)[(*R*p)n(*S*p)m]y(Op). In certain embodiments, a pattern of backbone chiral centers is or comprises (Op)(*S*p)t[(*R*p)n(*S*p)m]y(Op). In certain embodiments, a pattern of backbone chiral centers is or comprises (Op)(*S*p)t[(*R*p)n(*S*p)m]y(*R*p)k(Op). In certain embodiments, a pattern of backbone chiral centers is or comprises (Op)(*S*p)t[(*R*p)n(*S*p)m]y(*R*p)(Op). In certain embodiments, each n is 1. In certain embodiments, k is 1. In certain embodiments, k is 2-10.

In certain embodiments, a pattern of backbone chiral centers of a RNAi oligonucleotide or a region thereof (e.g., a core) comprises or is (Np)f(Op)g[(*R*p/Op)n(*S*p)m]y(*R*p)k(Op)h(Np)j, (Np)f(Op)g[(*R*p/Op)n(*S*p)m]y(Op)h(Np)j, (Np)f(Op)g(*S*p)t[(*R*p/Op)n(*S*p)m]y(Op)h(Np)j, or (Np)f(Op)g(*S*p)t[(*R*p/Op)n(*S*p)m]y(*R*p)k(Op)h(Np)j, wherein each of f, g, h and j is independently 1-50, and each other variable is independently as described in the present disclosure.

In certain embodiments, a pattern of backbone chiral centers of a RNAi oligonucleotide comprises or is (Np)f(Op)g[(*R*p/Op)n(*S*p)m]y(*R*p)k(Op)h(Np)j, (Np)f(Op)g[(*R*p/Op)n(*S*p)m]y(Op)h(Np)j, (Np)f(Op)g(*S*p)t[(*R*p/Op)n(*S*p)m]y(Op)h(Np)j, or (Np)f(Op)g(*S*p)t[(*R*p/Op)n(*S*p)m]y(*R*p)k(Op)h(Np)j, and the oligonucleotide comprises a core region whose pattern of backbone chiral centers comprises or is [(*R*p/Op)n(*S*p)m]y(*R*p)k, [(*R*p/Op)n(*S*p)m]y, (*S*p)t[(*R*p/Op)n(*S*p)m]y, or (*S*p)t[(*R*p/Op)n(*S*p)m]y(*R*p)k as described in the present disclosure.

In certain embodiments, a pattern of backbone chiral centers of a RNAi oligonucleotide is (Np)f(Op)g[(*R*p/Op)n(*S*p)m]y(*R*p)k(Op)h(Np)j, (Np)f(Op)g[(*R*p/Op)n(*S*p)m]y(Op)h(Np)j, (Np)f(Op)g(*S*p)t[(*R*p/Op)n(*S*p)m]y(Op)h(Np)j, or (Np)f(Op)g(Sp)t[(Rp/Op)n(Sp)m]y(Rp)k(Op)h(Np)j, and the oligonucleotide comprises a core region whose pattern of backbone chiral centers comprises or is [(Rp/Op)n(Sp)m]y(Rp)k, [(Rp/Op)n(Sp)m]y, (Sp)t[(Rp/Op)n(Sp)m]y, or (Sp)t[(Rp/Op)n(Sp)m]y(Rp)k as described in the present disclosure. In certain

embodiments, a pattern of backbone chiral centers is or comprises (Np)f(Op)g[(Rp/Op)n(Sp)m]y(Rp)k(Op)h(Np)j.

In certain embodiments, a pattern of backbone chiral centers is or comprises (Np)f(Op)g[(Rp/Op)n(Sp)m]y(Rp)(Op)h(Np)j. In certain embodiments, a pattern of backbone chiral centers is or comprises (Np)f(Op)g[(Rp/Op)n(Sp)m]y(Op)h(Np)j. In certain embodiments, a pattern of backbone chiral centers is or comprises (Np)f(Op)g(Sp)t[(Rp/Op)n(Sp)m]y(Op)h(Np)j. In certain embodiments, a pattern of backbone chiral centers is or comprises (Np)f(Op)g(Sp)t[(Rp/Op)n(Sp)m]y(Rp)k(Op)h(Np)j. In certain embodiments, a pattern of backbone chiral centers is or comprises (Np)f(Op)g(Sp)t[(Rp/Op)n(Sp)m]y(Rp)(Op)h(Np)j.

In certain embodiments, a pattern of backbone chiral centers is or comprises (Np)f(Op)g[(Rp)n(Sp)m]y(Rp)k(Op)h(Np)j. In certain embodiments, a pattern of backbone chiral centers is or comprises (Np)f(Op)g[(Rp)n(Sp)m]y(Rp)(Op)h(Np)j. In certain embodiments, a pattern of backbone chiral centers is or comprises (Np)f(Op)g[(Rp)n(Sp)m]y(Op)h(Np)j. In certain embodiments, a pattern of backbone chiral centers is or comprises (Np)f(Op)g(Sp)t[(Rp)n(Sp)m]y(Op)h(Np)j.

In certain embodiments, a pattern of backbone chiral centers is or comprises (Np)f(Op)g(Sp)t[(Rp)n(Sp)m]y(Rp)k(Op)h(Np)j. In certain embodiments, a pattern of backbone chiral centers is or comprises (Np)f(Op)g(Sp)t[(Rp)n(Sp)m]y(Rp)(Op)h(Np)j.

In certain embodiments, at least one Np is Sp. In certain embodiments, at least one Np is Rp. In certain embodiments, the 5' most Np is Sp. In certain embodiments, the 3' most Np is Sp. In certain embodiments, each Np is Sp. In certain embodiments, (Np)f(Op)g[(Rp/Op)n(Sp)m]y(Rp)k(Op)h(Np)j is (Sp)(Op)g[(Rp)n(Sp)m]y(Rp)k(Op)h(Sp).

In certain embodiments, (Np)f(Op)g[(Rp/Op)n(Sp)m]y(Rp)k(Op)h(Np)j is (Sp)(Op)g[(Rp)n(Sp)m]y(Rp)(Op)h(Sp). In certain embodiments, a pattern of backbone chiral center of a ds oligonucleotide is or comprises (Sp)(Op)g[(Rp)n(Sp)m]y(Rp)(Op)h(Sp). In certain embodiments, a pattern of backbone chiral center of a ds oligonucleotide is (Sp)(Op)g[(Rp)n(Sp)m]y(Rp)(Op)h(Sp). In certain embodiments, (Np)f(Op)g[(Rp/Op)n(Sp)m]y(Op)h(Np)j is (Sp)(Op)g[(Rp)n(Sp)m]y(Op)h(Sp). In certain embodiments, a pattern of backbone chiral center of a ds oligonucleotide is or comprises (Sp)(Op)g[(Rp)n(Sp)m]y(Op)h(Sp). In

certain embodiments, a pattern of backbone chiral center of a ds oligonucleotide is (Sp)(Op)g[(Rp)n(Sp)m]y(Op)h(Sp).

In certain embodiments, (Np)f(Op)g(Sp)t[(Rp/Op)n(Sp)m]y(Op)h(Np)j is (Sp)(Op)g(Sp)t[(Rp)n(Sp)m]y(Op)h(Sp). In certain embodiments, a pattern of backbone chiral center of a ds oligonucleotide is or comprises (Sp)(Op)g(Sp)t[(Rp)n(Sp)m]y(Op)h(Sp). In certain embodiments, a pattern of backbone chiral center of a ds oligonucleotide is (Sp)(Op)g(Sp)t[(Rp)n(Sp)m]y(Op)h(Sp). In certain embodiments, (Np)f(Op)g(Sp)t[(Rp/Op)n(Sp)m]y(Rp)k(Op)h(Np)j is (Sp)(Op)g(Sp)t[(Rp)n(Sp)m]y(Rp)k(Op)h(Sp).

In certain embodiments, (Np)f(Op)g(Sp)t[(Rp/Op)n(Sp)m]y(Rp)k(Op)h(Np)j is (Sp)(Op)g(Sp)t[(Rp)n(Sp)m]y(Rp)(Op)h(Sp). In certain embodiments, a pattern of backbone chiral center of a ds oligonucleotide is or comprises (Sp)(Op)g(Sp)t[(Rp)n(Sp)m]y(Rp)(Op)h(Sp). In certain embodiments, a pattern of backbone chiral center of a ds oligonucleotide is (Sp)(Op)g(Sp)t[(Rp)n(Sp)m]y(Rp)(Op)h(Sp). In certain embodiments, each n is 1. In certain embodiments, f is 1. In certain embodiments, g is 1. In certain embodiments, g is greater than 1. In certain embodiments, g is 2. In certain embodiments, g is 3. In certain embodiments, g is 4. In certain embodiments, g is 5. In certain embodiments, g is 6. In certain embodiments, g is 7. In certain embodiments, g is 8. In certain embodiments, g is 9. In certain embodiments, g is 10. In certain embodiments, h is 1. In certain embodiments, h is greater than 1. In certain embodiments, h is 2. In certain embodiments, h is 3. In certain embodiments, h is 4. In certain embodiments, h is 5. In certain embodiments, h is 6. In certain embodiments, h is 7. In certain embodiments, h is 8. In certain embodiments, h is 9. In certain embodiments, h is 10. In certain embodiments, j is 1. In certain embodiments, k is 1. In certain embodiments, k is 2-10.

In certain embodiments, a pattern of backbone chiral centers of a RNAi oligonucleotide or a region thereof (e.g., a core) comprises or is [(Rp/Op)n(Sp)m]y, (Sp)t[(Rp/Op)n(Sp)m]y, (Sp)t[(Rp/Op)n(Sp)m]yRp, [(Rp/Op)n(Sp)m]y(Rp)k, (Sp)t[(Rp/Op)n(Sp)m]y(Rp)k, (Sp)t[(Rp/Op)n(Sp)m]y(Rp)k(Op)h, (Sp)t[(Rp/Op)n(Sp)m]y(Rp)k(Op)h(Np)j, wherein each variable is independently as described in the present disclosure.

In certain embodiments, in a provided pattern of backbone chiral centers, at least one (*R*p/Op) is *R*p. In certain embodiments, at least one (*R*p/Op) is Op. In certain embodiments, each (*R*p/Op) is *R*p. In certain embodiments, each (*R*p/Op) is Op. In certain embodiments, at least one of [(*R*p)n(*S*p)m]y or [(*R*p/Op)n(*S*p)m]y of a pattern is *R*p*S*p. In certain embodiments, at least one of [(*R*p)n(*S*p)m]y or [(*R*p/Op)n(*S*p)m]y of a pattern is or comprises *R*p*S*p*S*p. In certain embodiments, at least one of [(*R*p)n(*S*p)m]y or [(*R*p/Op)n(*S*p)m]y in a pattern is *R*p*S*p, and at least one of [(*R*p)n(*S*p)m]y or [(*R*p/Op)n(*S*p)m]y in a pattern is or comprises *R*p*S*p*S*p. For example, in certain embodiments, [(*R*p)n(*S*p)m]y in a pattern is (*R*p*S*p)[(*R*p)n(*S*p)m]$_{(y-1)}$; in certain embodiments, [(*R*p)n(*S*p)m]y in a pattern is (*R*p*S*p)[*R*p*S*p*S*p(*S*p)$_{(m-2)}$][(*R*p)n(*S*p)m]$_{(y-2)}$. In certain embodiments, (*S*p)t[(*R*p)n(*S*p)m]y(*R*p) is (*S*p)t(*R*p*S*p)[(*R*p)n(*S*p)m]$_{(y-1)}$(*R*p). In certain embodiments, (*S*p)t[(*R*p)n(*S*p)m]y(*R*p) is (*S*p)t(*R*p*S*p)[*R*p*S*p*S*p(*S*p)$_{(m-2)}$][(*R*p)n(*S*p)m]$_{(y-2)}$(*R*p). In certain embodiments, each [(Rp/Op)n(Sp)m] is independently [Rp(Sp)m]. In certain embodiments, the first Sp of (Sp)t represents linkage phosphorus stereochemistry of the first internucleotidic linkage of a ds oligonucleotide from 5' to 3'. In certain embodiments, the first Sp of (Sp)t represents linkage phosphorus stereochemistry of the first internucleotidic linkage of a region from 5' to 3', e.g., a core. In certain embodiments, the last Np of (Np)j represents linkage phosphorus stereochemistry of the last internucleotidic linkage of the oligonucleotide from 5' to 3'. In certain embodiments, the last Np is Sp.

In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region (*e.g.*, of a 5'-wing) is or comprises *S*p(Op)$_3$. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region (*e.g.*, of a 5'-wing) is or comprises *R*p(Op)$_3$. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region (*e.g.*, of a 3'-wing) is or comprises (Op)$_3$*S*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region (*e.g.*, of a 3'-wing) is or comprises (Op)$_3$*R*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region (*e.g.*, of a core) is or comprises *R*p(*S*p)$_4$*R*p(*S*p)$_4$*R*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region (*e.g.*, of a core) is or comprises (*S*p)$_5$*R*p(*S*p)$_4$*R*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region (*e.g.*, of a core) is or comprises (*S*p)$_5$*R*p(*S*p)$_5$. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide or a region (*e.g.*, of a core) is or comprises

*R*p(*S*p)$_4$*R*p(*S*p)$_5$. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide is or comprises *N*p(Op)$_3$*R*p(*S*p)$_4$*R*p(*S*p)$_4$*R*p(Op)$_3$*N*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide is or comprises *N*p(Op)$_3$(*S*p)$_5$*R*p(*S*p)$_4$*R*p(Op)$_3$*N*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide is or comprises *N*p(Op)$_3$(*S*p)$_5$*R*p(*S*p)$_5$(Op)$_3$*N*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide is or comprises *N*p(Op)$_3$*R*p(*S*p)$_4$*R*p(*S*p)$_5$(Op)$_3$*N*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide is or comprises *S*p(Op)$_3$*R*p(*S*p)$_4$*R*p(*S*p)$_4$*R*p(Op)$_3$*S*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide is or comprises *S*p(Op)$_3$(*S*p)$_5$*R*p(*S*p)$_4$*R*p(Op)$_3$*S*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide is or comprises *S*p(Op)$_3$(*S*p)$_5$*R*p(*S*p)$_5$(Op)$_3$*S*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide is or comprises *S*p(Op)$_3$*R*p(*S*p)$_4$*R*p(*S*p)$_5$(Op)$_3$*S*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide is or comprises *R*p(Op)$_3$*R*p(*S*p)$_4$*R*p(*S*p)$_4$*R*p(Op)$_3$*R*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide is or comprises *R*p(Op)$_3$(*S*p)$_5$*R*p(*S*p)$_4$*R*p(Op)$_3$*R*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide is or comprises *R*p(Op)$_3$(*S*p)$_5$*R*p(*S*p)$_5$(Op)$_3$*R*p. In certain embodiments, a pattern of backbone chiral centers of a ds oligonucleotide is or comprises *R*p(Op)$_3$*R*p(*S*p)$_4$*R*p(*S*p)$_5$(Op)$_3$*R*p.

In certain embodiments, each of m, y, t, n, k, f, g, h, and j is independently 1-25.

In certain embodiments, m is 1-25. In certain embodiments, m is 1-20. In certain embodiments, m is 1-15. In certain embodiments, m is 1-10. In certain embodiments, m is 1-5. In certain embodiments, m is 2-20. In certain embodiments, m is 2-15. In certain embodiments, m is 2-10. In certain embodiments, m is 2-5. In certain embodiments, m is 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. In certain embodiments, in a pattern of backbone chiral centers each m is independently 2 or more. In certain embodiments, each m is independently 2, 3, 4, 5, 6, 7, 8, 9, or 10. In certain embodiments, each m is independently 2-3, 2-5, 2-6, or 2-10. In certain embodiments, m is 2. In certain embodiments, m is 3. In certain embodiments, m is 4. In certain embodiments, m is 5. In certain embodiments, m is 6. In certain embodiments, m is 7. In certain embodiments, m is 8. In certain embodiments, m is 9. In certain embodiments, m is 10. In certain embodiments, where there are two or more

occurrences of m, they can be the same or different, and each of them is independently as described in the present disclosure.

In certain embodiments, y is 1-25. In certain embodiments, y is 1-20. In certain embodiments, y is 1- 15. In certain embodiments, y is 1-10. In certain embodiments, y is 1-5. In certain embodiments, y is 2-20. In certain embodiments, y is 2-15. In certain embodiments, y is 2-10. In certain embodiments, y is 2-5. In certain embodiments, y is 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. In certain embodiments, y is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In certain embodiments, y is 1. In certain embodiments, y is 2. In certain embodiments, y is 3. In certain embodiments, y is 4. In certain embodiments, y is 5. In certain embodiments, y is 6. In certain embodiments, y is 7. In certain embodiments, y is 8. In certain embodiments, y is 9. In certain embodiments, y is 10.

In certain embodiments, t is 1-25. In certain embodiments, t is 1-20. In certain embodiments, t is 1-15. In certain embodiments, t is 1-10. In certain embodiments, t is 1-5. In certain embodiments, t is 2-20. In certain embodiments, t is 2-15. In certain embodiments, t is 2-10. In certain embodiments, t is 2-5. In certain embodiments, t is 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. In certain embodiments, each t is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In certain embodiments, t is 2 or more. In certain embodiments, t is 1. In certain embodiments, t is 2. In certain embodiments, t is 3. In certain embodiments, t is 4. In certain embodiments, t is 5. In certain embodiments, t is 6. In certain embodiments, t is 7. In certain embodiments, t is 8. In certain embodiments, t is 9. In certain embodiments, t is 10. In certain embodiments, where there are two or more occurrences of t, they can be the same or different, and each of them is independently as described in the present disclosure.

In certain embodiments, n is 1-25. In certain embodiments, n is 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. In certain embodiments, n is 1. In certain embodiments, n is 2. In certain embodiments, n is 3. In certain embodiments, n is 4. In certain embodiments, n is 5. In certain embodiments, n is 6. In certain embodiments, n is 7. In certain embodiments, n is 8. In certain embodiments, n is 9. In certain embodiments, n is 10. In certain embodiments, where there are two or more occurrences of n, they can be the same or different, and each of them is independently as described in the present disclosure. In certain embodiments, in a pattern of backbone chiral centers, at least one occurrence of n is 1; in some cases, each n is 1.

In certain embodiments, k is 1-25. In certain embodiments, k is 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. In certain embodiments, k is 1. In certain embodiments, k is 2. In certain embodiments, k is 3. In certain embodiments, k is 4. In certain embodiments, k is 5. In certain embodiments, k is 6. In certain embodiments, k is 7. In certain embodiments, k is 8. In certain embodiments, k is 9. In certain embodiments, k is 10.

In certain embodiments, f is 1-25. In certain embodiments, f is 1-20. In certain embodiments, f is 1-10. In certain embodiments, f is 1-5. In certain embodiments, f is 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. In certain embodiments, f is 1. In certain embodiments, f is 2. In certain embodiments, f is 3. In certain embodiments, f is 4. In certain embodiments, f is 5. In certain embodiments, f is 6. In certain embodiments, f is 7. In certain embodiments, f is 8. In certain embodiments, f is 9. In certain embodiments, f is 10.

In certain embodiments, g is 1-25. In certain embodiments, g is 1-20. In certain embodiments, g is 1-9. In certain embodiments, g is 1-5. In certain embodiments, g is 2-10. In certain embodiments, g is 2-5. In certain embodiments, g is 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. In certain embodiments, g is 1. In certain embodiments, g is 2. In certain embodiments, g is 3. In certain embodiments, g is 4. In certain embodiments, g is 5. In certain embodiments, g is 6. In certain embodiments, g is 7. In certain embodiments, g is 8. In certain embodiments, g is 9. In certain embodiments, g is 10.

In certain embodiments, h is 1-25. In certain embodiments, h is 1-10. In certain embodiments, h is 1-5. In certain embodiments, h is 2-10. In certain embodiments, h is 2-5. In certain embodiments, h is 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. In certain embodiments, h is 1. In certain embodiments, h is 2. In certain embodiments, h is 3. In certain embodiments, h is 4. In certain embodiments, h is 5. In certain embodiments, h is 6. In certain embodiments, h is 7. In certain embodiments, h is 8. In certain embodiments, h is 9. In certain embodiments, h is 10.

In certain embodiments, j is 1-25. In certain embodiments, j is 1-10. In certain embodiments, j is 1-5. In certain embodiments, j is 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. In certain embodiments, j is 1. In certain embodiments, j is 2. In certain embodiments, j is 3. In certain embodiments, j is 4. In certain embodiments, j is 5. In certain embodiments, j is 6. In certain embodiments, j is

7. In certain embodiments, j is 8. In certain embodiments, j is 9. In certain embodiments, j is 10.

In certain embodiments, at least one n is 1, and at least one m is no less than 2. In certain embodiments, at least one n is 1, at least one t is no less than 2, and at least one m is no less than 3. In certain embodiments, each n is 1. In certain embodiments, t is 1. In certain embodiments, at least one $t > 1$. In certain embodiments, at least one $t > 2$. In certain embodiments, at least one $t > 3$. In certain embodiments, at least one $t > 4$. In certain embodiments, at least one $m > 1$. In certain embodiments, at least one $m > 2$. In certain embodiments, at least one $m > 3$. In certain embodiments, at least one $m > 4$. In certain embodiments, a pattern of backbone chiral centers comprises one or more achiral natural phosphate linkages. In certain embodiments, the sum of m, t, and n (or m and n if no t is in a pattern) is no less than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In certain embodiments, the sum is 5. In certain embodiments, the sum is 6. In certain embodiments, the sum is 7. In certain embodiments, the sum is 8. In certain embodiments, the sum is 9. In certain embodiments, the sum is 10. In certain embodiments, the sum is 11. In certain embodiments, the sum is 12. In certain embodiments, the sum is 13. In certain embodiments, the sum is 14. In certain embodiments, the sum is 15.

In certain embodiments, a number of linkage phosphorus in chirally controlled internucleotidic linkages are *S*p. In certain embodiments, at least 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of chirally controlled internucleotidic linkages have *S*p linkage phosphorus. In certain embodiments, at least 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of all chiral internucleotidic linkages are chirally controlled internucleotidic linkages having *S*p linkage phosphorus. In certain embodiments, at least 10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of all internucleotidic linkages are chirally controlled internucleotidic linkages having *S*p linkage phosphorus. In certain embodiments, the percentage is at least 20%. In certain embodiments, the percentage is at least 30%. In certain embodiments, the percentage is at least 40%. In certain embodiments, the percentage is at least 50%. In certain embodiments, the percentage is at least 60%.

In certain embodiments, the percentage is at least 65%. In certain embodiments, the percentage is at least 70%. In certain embodiments, the percentage is at least 75%. In certain embodiments, the percentage is at least 80%. In certain embodiments,

the percentage is at least 90%. In certain embodiments, the percentage is at least 95%. In certain embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 internucleotidic linkages are chirally controlled internucleotidic linkages having *Sp* linkage phosphorus. In certain embodiments, at least 5 internucleotidic linkages are chirally controlled internucleotidic linkages having *Sp* linkage phosphorus. In certain embodiments, at least 6 internucleotidic linkages are chirally controlled internucleotidic linkages having *Sp* linkage phosphorus. In certain embodiments, at least 7 internucleotidic linkages are chirally controlled internucleotidic linkages having *Sp* linkage phosphorus. In certain embodiments, at least 8 internucleotidic linkages are chirally controlled internucleotidic linkages having *Sp* linkage phosphorus. In certain embodiments, at least 9 internucleotidic linkages are chirally controlled internucleotidic linkages having *Sp* linkage phosphorus. In certain embodiments, at least 10 internucleotidic linkages are chirally controlled internucleotidic linkages having *Sp* linkage phosphorus. In certain embodiments, at least 11 internucleotidic linkages are chirally controlled internucleotidic linkages having *Sp* linkage phosphorus. In certain embodiments, at least 12 internucleotidic linkages are chirally controlled internucleotidic linkages having *Sp* linkage phosphorus. In certain embodiments, at least 13 internucleotidic linkages are chirally controlled internucleotidic linkages having *Sp* linkage phosphorus. In certain embodiments, at least 14 internucleotidic linkages are chirally controlled internucleotidic linkages having *Sp* linkage phosphorus. In certain embodiments, at least 15 internucleotidic linkages are chirally controlled internucleotidic linkages having *Sp* linkage phosphorus. In certain embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 internucleotidic linkages are chirally controlled internucleotidic linkages having *Rp* linkage phosphorus. In certain embodiments, no more than 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 internucleotidic linkages are chirally controlled internucleotidic linkages having *Rp* linkage phosphorus. In certain embodiments, one and no more than one internucleotidic linkage in a ds oligonucleotide is a chirally controlled internucleotidic linkage having *Rp* linkage phosphorus. In certain embodiments, 2 and no more than 2 internucleotidic linkages in a ds oligonucleotide are chirally controlled internucleotidic linkages having *Rp* linkage phosphorus. In certain embodiments, 3 and no more than 3 internucleotidic linkages in a ds oligonucleotide are chirally controlled internucleotidic linkages having *Rp* linkage phosphorus. In certain embodiments, 4 and no more than 4 internucleotidic linkages in a ds oligonucleotide are

chirally controlled internucleotidic linkages having *R*p linkage phosphorus. In certain embodiments, 5 and no more than 5 internucleotidic linkages in a ds oligonucleotide are chirally controlled internucleotidic linkages having *R*p linkage phosphorus.

In certain embodiments, all, essentially all or most of the internucleotidic linkages in a ds oligonucleotide are in the *S*p configuration (e.g., about 50%-100%, 55%-100%, 60%-100%, 65%-100%, 70%-100%, 75%-100%, 80%-100%, 85%-100%, 90%-100%, 55%-95%, 60%-95%, 65%-95%, or about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99% or more of all chirally controlled internucleotidic linkages, or of all chiral internucleotidic linkages, or of all internucleotidic linkages in the oligonucleotide) except for one or a minority of internucleotidic linkages (e.g., 1, 2, 3, 4, or 5, and/or less than 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% of all chirally controlled internucleotidic linkages, or of all chiral internucleotidic linkages, or of all internucleotidic linkages in the oligonucleotide) being in the *R*p configuration. In certain embodiments, all, essentially all or most of the internucleotidic linkages in a core are in the *S*p configuration (e.g., about 50%-100%, 55%-100%, 60%-100%, 65%-100%, 70%-100%, 75%-100%, 80%-100%, 85%-100%, 90%-100%, 55%-95%, 60%-95%, 65%-95%, or about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99% or more of all chirally controlled internucleotidic linkages, or of all chiral internucleotidic linkages, or of all internucleotidic linkages, in the core) except for one or a minority of internucleotidic linkages (e.g., 1, 2, 3, 4, or 5, and/or less than 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% of all chirally controlled internucleotidic linkages, or of all chiral internucleotidic linkages, or of all internucleotidic linkages, in the core) being in the *R*p configuration. In certain embodiments, all, essentially all or most of the internucleotidic linkages in the core are a phosphorothioate in the *S*p configuration (e.g., about 50%-100%, 55%-100%, 60%-100%, 65%-100%, 70%-100%, 75%-100%, 80%-100%, 85%-100%, 90%-100%, 55%-95%, 60%-95%, 65%-95%, or about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 99% or more of all chirally controlled internucleotidic linkages, or of all chiral internucleotidic linkages, or of all internucleotidic linkages, in the core) except for one or a minority of internucleotidic linkages (e.g., 1, 2, 3, 4, or 5, and/or less than 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% of all chirally controlled internucleotidic linkages, or of all chiral internucleotidic linkages, or of all internucleotidic linkages, in the core) being a phosphorothioate in the *R*p configuration. In certain embodiments, each internucleotidic linkage in the core is a phosphorothioate in the *S*p configuration except for one

phosphorothioate in the *R*p configuration. In certain embodiments, each internucleotidic linkage in the core is a phosphorothioate in the *S*p configuration except for one phosphorothioate in the *R*p configuration.

In certain embodiments, a ds oligonucleotide comprises one or more *R*p internucleotidic linkages. In certain embodiments, a ds oligonucleotide comprises one and no more than one *R*p internucleotidic linkages. In certain embodiments, a ds oligonucleotide comprises two or more *R*p internucleotidic linkages. In certain embodiments, a ds oligonucleotide comprises three or more *R*p internucleotidic linkages. In certain embodiments, a ds oligonucleotide comprises four or more *R*p internucleotidic linkages. In certain embodiments, a ds oligonucleotide comprises five or more *R*p internucleotidic linkages. In certain embodiments, about 5%-50% of all chirally controlled internucleotidic linkages in a ds oligonucleotide are *R*p. In certain embodiments, about 5%- 40% of all chirally controlled internucleotidic linkages in a ds oligonucleotide are *R*p. In certain embodiments, about 10%-40% of all chirally controlled internucleotidic linkages in a ds oligonucleotide are *R*p. In certain embodiments, about 15%-40% of all chirally controlled internucleotidic linkages in a ds oligonucleotide are *R*p. In certain embodiments, about 20%-40% of all chirally controlled internucleotidic linkages in a ds oligonucleotide are *R*p. In certain embodiments, about 25%-40% of all chirally controlled internucleotidic linkages in a ds oligonucleotide are *R*p. In certain embodiments, about 30%-40% of all chirally controlled internucleotidic linkages in a ds oligonucleotide are *R*p. In certain embodiments, about 35%-40% of all chirally controlled internucleotidic linkages in a ds oligonucleotide are *R*p.

In certain embodiments, instead of an *R*p internucleotidic linkage, a natural phosphate linkage may be similarly utilized, optionally with a modification, e.g., a sugar modification (e.g., a 5'-modification such as R^{5s} as described herein). In certain embodiments, a modification improves stability of a natural phosphate linkage.

In certain embodiments, the present disclosure provides a ds oligonucleotide having a pattern of backbone chiral centers as described herein. In certain embodiments, oligonucleotides in a chirally controlled ds oligonucleotide composition share a common pattern of backbone chiral centers as described herein.

In certain embodiments, at least about 25% of the internucleotidic linkages of a dsRNAi oligonucleotide are chirally controlled and have *S*p linkage phosphorus. In certain embodiments, at least about 30% of the internucleotidic linkages of a ds

oligonucleotide are chirally controlled and have *Sp* linkage phosphorus. In certain embodiments, at least about 40% of the internucleotidic linkages of a provided ds oligonucleotide are chirally controlled and have *Sp* linkage phosphorus. In certain embodiments, at least about 50% of the internucleotidic linkages of a provided ds oligonucleotide are chirally controlled and have *Sp* linkage phosphorus. In certain embodiments, at least about 60% of the internucleotidic linkages of a provided ds oligonucleotide are chirally controlled and have *Sp* linkage phosphorus. In certain embodiments, at least about 65% of the internucleotidic linkages of a provided ds oligonucleotide are chirally controlled and have *Sp* linkage phosphorus. In certain embodiments, at least about 70% of the internucleotidic linkages of a provided ds oligonucleotide are chirally controlled and have *Sp* linkage phosphorus. In certain embodiments, at least about 75% of the internucleotidic linkages of a provided ds oligonucleotide are chirally controlled and have *Sp* linkage phosphorus. In certain embodiments, at least about 80% of the internucleotidic linkages of a provided ds oligonucleotide are chirally controlled and have *Sp* linkage phosphorus. In certain embodiments, at least about 85% of the internucleotidic linkages of a provided ds oligonucleotide are chirally controlled and have *Sp* linkage phosphorus. In certain embodiments, at least about 90% of the internucleotidic linkages of a provided ds oligonucleotide are chirally controlled and have *Sp* linkage phosphorus. In certain embodiments, at least about 95% of the internucleotidic linkages of a provided ds oligonucleotide are chirally controlled and have *Sp* linkage phosphorus.

In certain embodiments, the present disclosure provides chirally controlled ds oligonucleotide compositions, e.g., chirally controlled dsRNAi oligonucleotide compositions, wherein the composition comprises a non-random or controlled level of a plurality of oligonucleotides, wherein oligonucleotides of the plurality share a common base sequence, and share the same configuration of linkage phosphorus independently at 1-50, 1-40, 1-30, 1-25, 1-20, 1-15, 1-10, 5-50, 5-40, 5-30, 5-25, 5-20, 5-15, 5-10, 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 or more chiral internucleotidic linkages.

In certain embodiments, dsRNAi oligonucleotides comprise 2-30 chirally controlled internucleotidic linkages. In certain embodiments, provided ds oligonucleotide compositions comprise 5-30 chirally controlled internucleotidic linkages. In certain

embodiments, provided ds oligonucleotide compositions comprise 10-30 chirally controlled internucleotidic linkages.

In certain embodiments, a percentage is about 5%-100%. In certain embodiments, a percentage is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 965, 96%, 98%, or 99%. In certain embodiments, a percentage is about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 965, 96%, 98%, or 99%.

In certain embodiments, a pattern of backbone chiral centers in a dsRNAi oligonucleotide comprises a pattern of i^o-i^s-i^o-i^s-i^o, i^o-i^s-i^s-i^s-i^o, i^o-i^s-i^s-i^s-i^o-i^s, i^s-i^o-i^s-i^o, i^s-i^o-i^s-i^o, i^s-i^o-i^s-i^o-i^s, i^s-i^o-i^s-i^o-i^s-i^o, i^s-i^o-i^s-i^o-i^s-i^o-i^s, i^s-i^o-i^s-i^s-i^s-i^o, i^s-i^s-i^o-i^s-i^s-i^s-i^o-i^s-i^s, i^s-i^s-i^s-i^o-i^s-i^o-i^s-i^s-i^s, i^s-i^s-i^s-i^s-i^o-i^s-i^o-i^s-i^s-i^s-i^s, i^s-i^s-i^s-i^s-i^s, i^s-i^s-i^s-i^s-i^s-i^s, i^s-i^s-i^s- i^s-i^s-i^s-i^s, i^s-i^s-i^s-i^s-i^s-i^s-i^s, i^s-i^s-i^s-i^s-i^s-i^s-i^s-i^s, or i^r-i^r-i^r, wherein i^s represents an internucleotidic linkage in the *S*p configuration; i^o represents an achiral internucleotidic linkage; and i^r represents an internucleotidic linkage in the *R*p configuration.

In certain embodiments, an internucleotidic linkage in the *S*p configuration (having a *S*p linkage phosphorus) is a phosphorothioate internucleotidic linkage. In certain embodiments, an achiral internucleotidic linkage is a natural phosphate linkage. In certain embodiments, an internucleotidic linkage in the *R*p configuration (having a *R*p linkage phosphorus) is a phosphorothioate internucleotidic linkage. In certain embodiments, each internucleotidic linkage in the *S*p configuration is a phosphorothioate internucleotidic linkage. In certain embodiments, each achiral internucleotidic linkage is a natural phosphate linkage. In certain embodiments, each internucleotidic linkage in the *R*p configuration is a phosphorothioate internucleotidic linkage. In certain embodiments, each internucleotidic linkage in the *S*p configuration is a phosphorothioate internucleotidic linkage, each achiral internucleotidic linkage is a natural phosphate linkage, and each internucleotidic linkage in the *R*p configuration is a phosphorothioate internucleotidic linkage.

In certain embodiments, dsRNAi oligonucleotides in chirally controlled oligonucleotide compositions each comprise different types of internucleotidic linkages. In certain embodiments, dsRNAi oligonucleotides comprise at least one natural phosphate linkage and at least one modified internucleotidic linkage. In certain embodiments, dsRNAi oligonucleotides comprise at least one natural phosphate linkage and at least two modified internucleotidic linkages. In certain embodiments, dsRNAi oligonucleotides comprise at least one natural phosphate linkage and at least three modified internucleotidic linkages. In

certain embodiments, dsRNAi oligonucleotides comprise at least one natural phosphate linkage and at least four modified internucleotidic linkages. In certain embodiments, dsRNAi oligonucleotides comprise at least one natural phosphate linkage and at least five modified internucleotidic linkages. In certain embodiments, dsRNAi oligonucleotides comprise at least one natural phosphate linkage and 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 modified internucleotidic linkages. In certain embodiments, a modified internucleotidic linkage is a phosphorothioate internucleotidic linkage. In certain embodiments, each modified internucleotidic linkage is a phosphorothioate internucleotidic linkage. In certain embodiments, a modified internucleotidic linkage is a phosphorothioate triester internucleotidic linkage. In certain embodiments, each modified internucleotidic linkage is a phosphorothioate triester internucleotidic linkage. In certain embodiments, RNAi oligonucleotides comprise at least one natural phosphate linkage and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 consecutive modified internucleotidic linkages. In certain embodiments, RNAi oligonucleotides comprise at least one natural phosphate linkage and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 consecutive phosphorothioate internucleotidic linkages. In certain embodiments, dsRNAi oligonucleotides comprise at least one natural phosphate linkage and at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 consecutive phosphorothioate triester internucleotidic linkages.

In certain embodiments, oligonucleotides in a chirally controlled ds oligonucleotide composition each comprise at least two internucleotidic linkages that have different stereochemistry and/or different P- modifications relative to one another. In certain embodiments, at least two internucleotidic linkages have different stereochemistry relative to one another, and the ds oligonucleotides each comprise a pattern of backbone chiral centers comprising alternating linkage phosphorus stereochemistry.

In certain embodiments, a linkage comprises a chiral auxiliary, which, for example, is used to control the stereoselectivity of a reaction, e.g., a coupling reaction in a ds oligonucleotide synthesis cycle. In certain embodiments, a phosphorothioate triester linkage does not comprise a chiral auxiliary. In certain embodiments, a phosphorothioate triester linkage is intentionally maintained until and/or during the administration of the oligonucleotide composition to a subject.

In certain embodiments, purity, particularly stereochemical purity, and particularly diastereomeric purity of many ds oligonucleotides and compositions thereof wherein all other chiral centers in the ds oligonucleotides but the chiral linkage phosphorus centers have been stereodefined (e.g., carbon chiral centers in the sugars, which are defined in, *e.g.*, phosphoramidites for ds oligonucleotide synthesis), can be controlled by stereoselectivity (as appreciated by those skilled in this art, diastereoselectivity in many cases of ds oligonucleotide synthesis wherein the ds oligonucleotide comprise more than one chiral centers) at chiral linkage phosphorus in coupling steps when forming chiral internucleotidic linkages. In certain embodiments, a coupling step has a stereoselectivity (diastereoselectivity when there are other chiral centers) of 60% at the linkage phosphorus. After such a coupling step, the new internucleotidic linkage formed may be referred to have a 60% stereochemical purity (for ds oligonucleotides, typically diastereomeric purity in view of the existence of other chiral centers). In certain embodiments, each coupling step independently has a stereoselectivity of at least 60%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 70%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 80%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 85%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 90%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 91%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 92%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 93%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 94%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 95%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 96%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 97%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 98%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 99%. In certain embodiments, each coupling step independently has a stereoselectivity of at least 99.5%. In certain embodiments, each coupling step independently has a stereoselectivity of virtually 100%. In certain embodiments, a coupling step has a stereoselectivity of virtually 100% in that each detectable product from the coupling step analyzed by an analytical method (e.g., NMR, HPLC, etc.) has the intended stereoselectivity. In certain embodiments,

a chirally controlled internucleotidic linkage is typically formed with a stereoselectivity of at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99.5% or virtually 100% (in certain embodiments, at least 90%; in certain embodiments, at least 95%; in certain embodiments, at least 96%; in certain embodiments, at least 97%; in certain embodiments, at least 98%; in certain embodiments, at least 99%). In certain embodiments, a chirally controlled internucleotidic linkage has a stereochemical purity (typically diastereomeric purity for oligonucleotides with multiple chiral centers) of at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99.5% or virtually 100% (in certain embodiments, at least 90%; in certain embodiments, at least 95%; in certain embodiments, at least 96%; in certain embodiments, at least 97%; in certain embodiments, at least 98%; in certain embodiments, at least 99%) at its chiral linkage phosphorus. In certain embodiments, each chirally controlled internucleotidic linkage independently has a stereochemical purity (typically diastereomeric purity for oligonucleotides with multiple chiral centers) of at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99.5% or virtually 100% (in certain embodiments, at least 90%; in certain embodiments, at least 95%; in certain embodiments, at least 96%; in certain embodiments, at least 97%; in certain embodiments, at least 98%; in certain embodiments, at least 99%) at its chiral linkage phosphorus. In certain embodiments, a non-chirally controlled internucleotidic linkage is typically formed with a stereoselectivity of less than 60%, 70%, 80%, 85%, or 90% (in certain embodiments, less than 60%; in certain embodiments, less than 70%; in certain embodiments, less than 80%; in certain embodiments, less than 85%; in certain embodiments, less than 90%). In certain embodiments, each non-chirally controlled internucleotidic linkage is independently formed with a stereoselectivity of less than 60%, 70%, 80%, 85%, or 90% (in certain embodiments, less than 60%; in certain embodiments, less than 70%; in certain embodiments, less than 80%; in certain embodiments, less than 85%; in certain embodiments, less than 90%). In certain embodiments, a non-chirally controlled internucleotidic linkage has a stereochemical purity (typically diastereomeric purity for oligonucleotides with multiple chiral centers) of less than 60%, 70%, 80%, 85%, or 90% (in certain embodiments, less than 60%; in certain embodiments, less than 70%; in certain embodiments, less than 80%; in certain embodiments, less than 85%; in certain embodiments, less than 90%) at its chiral linkage phosphorus. In certain embodiments, each non-chirally controlled internucleotidic linkage independently has a stereochemical purity (typically diastereomeric purity for oligonucleotides with multiple chiral centers) of less than 60%, 70%, 80%, 85%, or 90% (in

certain embodiments, less than 60%; in certain embodiments, less than 70%; in certain embodiments, less than 80%; in certain embodiments, less than 85%; in certain embodiments, less than 90%) at its chiral linkage phosphorus.

In certain embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 couplings of a monomer (as appreciated by those skilled in the art in certain embodiments a phosphoramidite for oligonucleotide synthesis) independently have a stereoselectivity less than about 60%, 70%, 80%, 85%, or 90% [for oligonucleotide synthesis, typically diastereoselectivity with respect to formed linkage phosphorus chiral center(s)]. In certain embodiments, at least one coupling has a stereoselectivity less than about 60%, 70%, 80%, 85%, or 90%. In certain embodiments, at least two couplings independently have a stereoselectivity less than about 60%, 70%, 80%, 85%, or 90%. In certain embodiments, at least three couplings independently have a stereoselectivity less than about 60%, 70%, 80%, 85%, or 90%. In certain embodiments, at least four couplings independently have a stereoselectivity less than about 60%, 70%, 80%, 85%, or 90%. In certain embodiments, at least five couplings independently have a stereoselectivity less than about 60%, 70%, 80%, 85%, or 90%. In certain embodiments, each coupling independently has a stereoselectivity less than about 60%, 70%, 80%, 85%, or 90%. In certain embodiments, each non-chirally controlled internucleotidic linkage is independently formed with a stereoselectivity less than about 60%, 70%, 80%, 85%, or 90%. In certain embodiments, a stereoselectivity is less than about 60%. In certain embodiments, a stereoselectivity is less than about 70%. In certain embodiments, a stereoselectivity is less than about 80%. In certain embodiments, a stereoselectivity is less than about 90%. In certain embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 couplings independently have a stereoselectivity less than about 90%. In certain embodiments, at least one coupling has a stereoselectivity less than about 90%. In certain embodiments, at least two couplings have a stereoselectivity less than about 90%. In certain embodiments, at least three couplings have a stereoselectivity less than about 90%. In certain embodiments, at least four couplings have a stereoselectivity less than about 90%. In certain embodiments, at least five couplings have a stereoselectivity less than about 90%. In certain embodiments, each coupling independently has a stereoselectivity less than about 90%. In certain embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 couplings independently have a stereoselectivity less than about 85%. In certain embodiments, each coupling independently has a stereoselectivity less than about

85%. In certain embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 couplings independently have a stereoselectivity less than about 80%. In certain embodiments, each coupling independently has a stereoselectivity less than about 80%. In certain embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 couplings independently have a stereoselectivity less than about 70%. In certain embodiments, each coupling independently has a stereoselectivity less than about 70%.

In certain embodiments, ds oligonucleotides and compositions of the present disclosure have high purity. In certain embodiments, ds oligonucleotides and compositions of the present disclosure have high stereochemical purity. In certain embodiments, a stereochemical purity, e.g., diastereomeric purity, is about 60%-100%. In certain embodiments, a diastereomeric purity, is about 60%-100%. In certain embodiments, the percentage is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 93%, 95%, 96%, 97%, 98%, or 99%. In certain embodiments, the percentage is at least 80%, 85%, 90%, 91%, 92%, 93%, 93%, 95%, 96%, 97%, 98%, or 99%. In certain embodiments, the percentage is at least 90%, 91%, 92%, 93%, 93%, 95%, 96%, 97%, 98%, or 99%. In certain embodiments, a diastereomeric purity is at least 60%. In certain embodiments, a diastereomeric purity is at least 70%. In certain embodiments, a diastereomeric purity is at least 80%. In certain embodiments, a diastereomeric purity is at least 85%. In certain embodiments, a diastereomeric purity is at least 90%. In certain embodiments, a diastereomeric purity is at least 91%. In certain embodiments, a diastereomeric purity is at least 92%. In certain embodiments, a diastereomeric purity is at least 93%. In certain embodiments, a diastereomeric purity is at least 94%. In certain embodiments, a diastereomeric purity is at least 95%. In certain embodiments, a diastereomeric purity is at least 96%. In certain embodiments, a diastereomeric purity is at least 97%. In certain embodiments, a diastereomeric purity is at least 98%. In certain embodiments, a diastereomeric purity is at least 99%. In certain embodiments, a diastereomeric purity is at least 99.5%.

In certain embodiments, compounds of the present disclosure (e.g., oligonucleotides, chiral auxiliaries, etc.) comprise multiple chiral elements (e.g., multiple carbon and/or phosphorus (e.g., linkage phosphorus of chiral internucleotidic linkages) chiral centers). In certain embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or more chiral elements of a provided compound (e.g., a ds oligonucleotide) each independently have a

diastereomeric purity as described herein. In certain embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or more chiral carbon centers of a provided compound each independently have a diastereomeric purity as described herein. In certain embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or more chiral phosphorus centers of a provided compound each independently have a diastereomeric purity as described herein. In certain embodiments, each chiral element independently has a diastereomeric purity as described herein. In certain embodiments, each chiral center independently has a diastereomeric purity as described herein. In certain embodiments, each chiral carbon center independently has a diastereomeric purity as described herein. In certain embodiments, each chiral phosphorus center independently has a diastereomeric purity as described herein. In certain embodiments, each chiral phosphorus center independently has a diastereomeric purity of at least 90%, 91%, 92%, 93%, 93%, 95%, 96%, 97%, 98%, or 99% or more.

As understood by a person having ordinary skill in the art, in certain embodiments, diastereoselectivity of a coupling or diastereomeric purity of a chiral linkage phosphorus center can be assessed through the diastereoselectivity of a dimer formation or diastereomeric purity of a dimer prepared under the same or comparable conditions, wherein the dimer has the same 5'- and 3'-nucleosides and internucleotidic linkage.

Various technologies can be utilized for identifying or confirming stereochemistry of chiral elements (e.g., configuration of chiral linkage phosphorus) and/or patterns of backbone chiral centers, and/or for assessing stereoselectivity (e.g., diastereoselectivity of couple steps in oligonucleotide synthesis) and/or stereochemical purity (e.g., diastereomeric purity of internucleotidic linkages, compounds (e.g., oligonucleotides), etc.). Example technologies include NMR [e.g., 1D (one-dimensional) and/or 2D (two- dimensional) ^{1}H-^{31}P HETCOR (heteronuclear correlation spectroscopy)], HPLC, RP-HPLC, mass spectrometry, LC-MS, and cleavage of internucleotidic linkages by stereospecific nucleases, etc., which may be utilized individually or in combination. Example useful nucleases include benzonase, micrococcal nuclease, and svPDE (snake venom phosphodiesterase), which are specific for certain internucleotidic linkages with *R*p linkage phosphorus (e.g., a *R*p phosphorothioate linkage); and nuclease P1, mung bean nuclease, and nuclease S1, which are specific for internucleotidic linkages with *S*p linkage phosphorus (e.g., a *S*p phosphorothioate linkage). Without wishing to be bound by any particular theory, the present disclosure notes that, in at least some cases, cleavage of oligonucleotides by a particular nuclease may be impacted by structural elements, e.g.,

chemical modifications (e.g., 2'-modifications of a sugars), base sequences, or stereochemical contexts. For example, it is observed that in some cases, benzonase and micrococcal nuclease, which are specific for internucleotidic linkages with *R*p linkage phosphorus, were unable to cleave an isolated *R*p phosphorothioate internucleotidic linkage flanked by *S*p phosphorothioate internucleotidic linkages.

In certain embodiments, ds oligonucleotides sharing a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone chiral centers share a common pattern of backbone phosphorus modifications and a common pattern of base modifications. In certain embodiments, sd oligonucleotide compositions sharing a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone chiral centers share a common pattern of backbone phosphorus modifications and a common pattern of nucleoside modifications. In certain embodiments, ds oligonucleotides share a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone chiral centers have identical structures.

In certain embodiments, the present disclosure provides a ds oligonucleotide composition comprising a plurality of oligonucleotides capable of directing RNAi knockdown, wherein ds oligonucleotides of the plurality are of a particular ds oligonucleotide type, which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of ds oligonucleotides having the same base sequence, for ds oligonucleotides of the particular ds oligonucleotide type.

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

In certain embodiments, the present disclosure provides dsRNAi oligonucleotide compositions comprising a plurality of oligonucleotides. In certain embodiments, the present disclosure provides chirally controlled oligonucleotide compositions of dsRNAi oligonucleotides. In certain embodiments, the present disclosure

provides a dsRNAi oligonucleotide whose base sequence is or is complementary to a dsRNAi sequence disclosed herein or a portion thereof (e.g., various bases sequences in Table 1A or 1B, or Table 1C or Table 1D, wherein each T may be independently replaced with U and vice versa). In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide whose base sequence comprises a base sequence that is or is complementary to a dsRNAi sequence disclosed herein or a portion thereof (e.g., various bases sequences in Table 1A or 1B or Table 1C or Table 1D). In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide whose base sequence comprises 15 contiguous bases of a base sequence that is or is complementary to a dsRNAi sequence disclosed herein or a portion thereof (e.g., various bases sequences in Table 1A or 1B, or Table 1C or Table 1D, wherein each T may be independently replaced with U and vice versa). In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide which has a base sequence comprising 15 contiguous bases with 0-3 mismatches of a base sequence that is or is complementary to a dsRNAi sequence disclosed herein or a portion thereof (e.g., various bases sequences in Table 1A or 1B, or Table 1C or Table 1D, wherein each T may be independently replaced with U and vice versa). In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide composition wherein the dsRNAi oligonucleotides comprise at least one chiral internucleotidic linkage which is not chirally controlled. In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide comprising a non-chirally controlled chiral internucleotidic linkage, wherein the base sequence of the dsRNAi oligonucleotide comprises a base sequence that is or is complementary to a dsRNAi sequence disclosed herein or a portion thereof (e.g., various bases sequences in Table 1A or 1B, or Table 1C or Table 1D, wherein each T may be independently replaced with U and vice versa). In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide composition comprising a non-chirally controlled chiral internucleotidic linkage, wherein the base sequence of the dsRNAi oligonucleotide is a base sequence that is or is complementary to a dsRNAi sequence disclosed herein or a portion thereof (e.g., various bases sequences in Table 1A or 1B, or Table 1C or Table 1D, wherein each T may be independently replaced with U and vice versa). In certain embodiments, the present disclosure provides a RNAi oligonucleotide comprising a non-chirally controlled chiral internucleotidic linkage, wherein the base sequence of the dsRNAi oligonucleotide comprises 15 contiguous bases of a base sequence that is or is complementary to a dsRNAi sequence disclosed herein or a portion thereof (e.g.,

various bases sequences in Table 1A or 1B, or Table 1C or Table 1D, wherein each T may be independently replaced with U and vice versa). In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide comprising a non-chirally controlled chiral internucleotidic linkage, wherein the base sequence of the dsRNAi oligonucleotides comprises 15 contiguous bases with 0-3 mismatches of a base sequence that is or is complementary to a RNAi sequence disclosed herein or a portion thereof (e.g., various bases sequences in Table 1A or 1B, or Table 1C or Table 1D, wherein each T may be independently replaced with U and vice versa). In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide comprising a chirally controlled chiral internucleotidic linkage, wherein the base sequence of the dsRNAi oligonucleotide comprises a base sequence that is or is complementary to a dsRNAi sequence disclosed herein or a portion thereof (e.g., various bases sequences in Table 1A or 1B, or 1C or 1D, wherein each T may be independently replaced with U and vice versa). In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide composition comprising a chirally controlled chiral internucleotidic linkage, wherein the base sequence of the RNAi oligonucleotide is a base sequence that is or is complementary to a dsRNAi sequence disclosed herein or a portion thereof (e.g., various bases sequences in Table 1A or 1B, or Table 1C or Table 1D, wherein each T may be independently replaced with U and vice versa). In certain embodiments, the present disclosure provides a dsRNAi oligonucleotide comprising a chirally controlled chiral internucleotidic linkage, wherein the base sequence of the dsRNAi oligonucleotide comprises 15 contiguous bases of a base sequence that is or is complementary to a dsRNAi sequence disclosed herein or a portion thereof (e.g., various bases sequences in Table 1A or 1B, or Table 1C or Table 1D, wherein each T may be independently replaced with U and vice versa). In certain embodiments, the present disclosure provides a RNAi oligonucleotide comprising a chirally controlled chiral internucleotidic linkage, wherein the base sequence of the RNAi oligonucleotides comprises 15 contiguous bases with 0-3 mismatches of a base sequence that is or is complementary to a dsRNAi sequence disclosed herein or a portion thereof (e.g., various bases sequences in Table 1A or 1B, or Table 1C or Table 1D, wherein each T may be independently replaced with U and vice versa).

In certain embodiments, ds oligonucleotides of the same ds oligonucleotide type have a common pattern of backbone phosphorus modifications and a common pattern of nucleoside modifications. In certain embodiments, ds oligonucleotides of the same ds

doligonucleotide type have a common pattern of sugar modifications. In certain embodiments, ds oligonucleotides of the same ds oligonucleotide type have a common pattern of base modifications. In certain embodiments, ds oligonucleotides of the same ds oligonucleotide type have a common pattern of nucleoside modifications. In certain embodiments, ds oligonucleotides of the same ds oligonucleotide type have the same constitution. In certain embodiments, ds oligonucleotides of the same ds oligonucleotide type are identical. In certain embodiments, ds oligonucleotides of the same ds oligonucleotide type are of the same ds oligonucleotide (as those skilled in the art will appreciate, such ds oligonucleotides may each independently exist in one of the various forms of the ds oligonucleotide, and may be the same, or different forms of the ds oligonucleotide). In certain embodiments, ds oligonucleotides of the same ds oligonucleotide type are each independently of the same ds oligonucleotide or a pharmaceutically acceptable salt thereof.

[02] In certain embodiments, a plurality of ds oligonucleotides or ds oligonucleotides of a particular ds oligonucleotide type in a provided ds oligonucleotide composition are sdRNAi oligonucleotides. In certain embodiments, the present disclosure provides a chirally controlled dsRNAi oligonucleotide composition comprising a plurality of dsRNAi oligonucleotides, wherein the ds oligonucleotides share:

1) a common base sequence;

2) a common pattern of backbone linkages; and

3) the same linkage phosphorus stereochemistry at one or more chiral internucleotidic linkages (chirally controlled internucleotidic linkages), wherein the composition is enriched, relative to a substantially racemic preparation of oligonucleotides sharing the common base sequence and pattern of backbone linkages, for oligonucleotides of the plurality.

In certain embodiments, as used herein, “one or more” or “at least one” is 1-50, 1-40, 1-30, 1-25, 1- 20, 1-15, 1-10, 5-50, 5-40, 5-30, 5-25, 5-20, 5-15, 5-10, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more.

In certain embodiments, a ds oligonucleotide type is further defined by: 4) additional chemical moiety, if any.

In certain embodiments, the percentage is at least about 10%. In certain embodiments, the percentage is at least about 20%. In certain embodiments, the percentage is at least about 30%. In certain embodiments, the percentage is at least about 40%. In

certain embodiments, the percentage is at least about 50%. In certain embodiments, the percentage is at least about 60%. In certain embodiments, the percentage is at least about 70%. In certain embodiments, the percentage is at least about 75%. In certain embodiments, the percentage is at least about 80%. In certain embodiments, the percentage is at least about 85%. In certain embodiments, the percentage is at least about 90%. In certain embodiments, the percentage is at least about 91%. In certain embodiments, the percentage is at least about 92%. In certain embodiments, the percentage is at least about 93%. In certain embodiments, the percentage is at least about 94%. In certain embodiments, the percentage is at least about 95%. In certain embodiments, the percentage is at least about 96%. In certain embodiments, the percentage is at least about 97%. In certain embodiments, the percentage is at least about 98%. In certain embodiments, the percentage is at least about 99%. In certain embodiments, the percentage is or is greater than $(DS)^{nc}$, wherein DS and nc are each independently as described in the present disclosure.

In certain embodiments, a plurality of ds oligonucleotides, e.g., dsRNAi oligonucleotides, share the same constitution. In certain embodiments, a plurality of oligonucleotides, e.g., dsRNAi oligonucleotides, are identical (the same stereoisomer). In certain embodiments, a chirally controlled ds oligonucleotide composition, e.g., a chirally controlled dsRNAi oligonucleotide composition, is a stereopure ds oligonucleotide composition wherein ds oligonucleotides of the plurality are identical (the same stereoisomer), and the composition does not contain any other stereoisomers. Those skilled in the art will appreciate that one or more other stereoisomers may exist as impurities as processes, selectivities, purifications, etc. may not achieve completeness.

In certain embodiments, a provided composition is characterized in that when it is contacted with a target nucleic acid (e.g., a transcript (e.g., pre-mRNA, mature mRNA, other types of RNA, etc. that hybridizes with oligonucleotides of the composition)), levels of the target nucleic acid and/or a product encoded thereby is reduced compared to that observed under a reference condition. In certain embodiments, a reference condition is selected from the group consisting of absence of the composition, presence of a reference composition, and combinations thereof. In certain embodiments, a reference condition is absence of the composition. In certain embodiments, a reference condition is presence of a reference composition. In certain embodiments, a reference composition is a composition whose oligonucleotides do not hybridize with the target nucleic acid. In certain embodiments, a reference composition is a composition whose oligonucleotides do not

comprise a sequence that is sufficiently complementary to the target nucleic acid. In certain embodiments, a provided composition is a chirally controlled oligonucleotide composition and a reference composition is a non- chirally controlled oligonucleotide composition which is otherwise identical but is not chirally controlled (e.g., a racemic preparation of oligonucleotides of the same constitution as oligonucleotides of a plurality in the chirally controlled oligonucleotide composition).

In certain embodiments, the present disclosure provides a chirally controlled dsRNAi oligonucleotide composition comprising a plurality of dsRNAi oligonucleotides capable of directing RNAi knockdown, wherein the oligonucleotides share:

1) a common base sequence,

2) a common pattern of backbone linkages, and

3) the same linkage phosphorus stereochemistry at one or more (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 1- 15, 1-10, 5-50, 5-40, 5-30, 5-25, 5-20, 5-15, 5-10, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) chiral internucleotidic linkages (chirally controlled internucleotidic linkages),

wherein the composition is enriched, relative to a substantially racemic preparation of oligonucleotides sharing the common base sequence and pattern of backbone linkages, for oligonucleotides of the plurality, the ds oligonucleotide composition being characterized in that, when it is contacted with a transcript in a dsRNAi knockdown system, knockdown of the transcript is improved 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.

As noted above and understood in the art, in certain embodiments, the base sequence of a ds 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 ds oligonucleotide and/or to the hybridization character (i.e., the ability to hybridize with particular complementary residues) of such residues.

As demonstrated herein, ds oligonucleotide structural elements (e.g., patterns of sugar modifications, backbone linkages, backbone chiral centers, backbone phosphorus modifications, etc.) and combinations thereof can provide surprisingly improved properties and/or bioactivities.

In certain embodiments, ds oligonucleotide compositions are capable of reducing the expression, level and/or activity of a target gene or a gene product thereof. In certain embodiments, ds oligonucleotide compositions are capable of reducing in the expression, level and/or activity of a target gene or a gene product thereof by sterically blocking translation after annealing to a target gene mRNA, by cleaving mRNA (pre-mRNA or mature mRNA), and/or by altering or interfering with mRNA splicing. In certain embodiments, provided dsRNAi oligonucleotide compositions are capable of reducing the expression, level and/or activity of a target gene or a gene product thereof. In certain embodiments, provided dsRNAi oligonucleotide compositions are capable of reducing in the expression, level and/or activity of a target gene or a gene product thereof by sterically blocking translation after annealing to a target gene mRNA, by cleaving target mRNA (pre-mRNA or mature mRNA), and/or by altering or interfering with mRNA splicing.

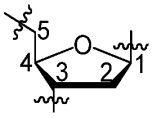
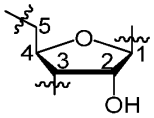
In certain embodiments, a ds oligonucleotide composition, e.g., a dsdRNAi oligonucleotide composition, is a substantially pure preparation of a single ds oligonucleotide stereoisomer, e.g., a dsRNAi oligonucleotide stereoisomer, in that oligonucleotides in the composition that are not of the oligonucleotide stereoisomer are impurities from the preparation process of said ds oligonucleotide stereoisomer, in some case, after certain purification procedures.

In certain embodiments, the present disclosure provides ds oligonucleotides and oligonucleotide compositions that are chirally controlled, and in certain embodiments, stereopure. For instance, in certain embodiments, a provided composition contains non-random or controlled levels of one or more individual oligonucleotide types as described herein. In certain embodiments, oligonucleotides of the same oligonucleotide type are identical.

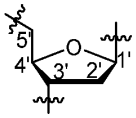
3. Sugars

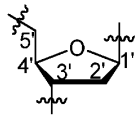
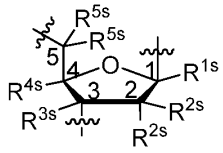
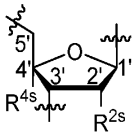
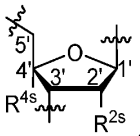
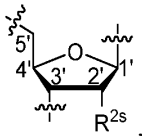
Various sugars, including modified sugars, can be utilized in accordance with the present disclosure. In certain embodiments, the present disclosure provides sugar modifications and patterns thereof optionally in combination with other structural elements (e.g., internucleotidic linkage modifications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

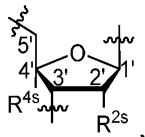
The most common naturally occurring nucleosides comprise ribose sugars (e.g., in RNA) or deoxyribose sugars (e.g., in DNA) linked to the nucleobases adenosine

(A), cytosine (C), guanine (G), thymine (T) or uracil (U). In certain embodiments, a sugar, e.g., various sugars in many oligonucleotides in Table 1 (unless otherwise notes), is a natural DNA sugar (in DNA nucleic acids or oligonucleotides, having the structure of , wherein a nucleobase is attached to the 1' position, and the 3' and 5' positions are connected to internucleotidic linkages (as appreciated by those skilled in the art, if at the 5'-end of a ds oligonucleotide, the 5' position may be connected to a 5'-end group (e.g., −OH), and if at the 3'-end of a ds oligonucleotide, the 3' position may be connected to a 3'-end group (e.g., −OH). In certain embodiments, a sugar is a natural RNA sugar (in RNA nucleic acids or oligonucleotides, having the structure of , wherein a nucleobase is attached to the 1' position, and the 3' and 5' positions are connected to internucleotidic linkages (as appreciated by those skilled in the art, if at the 5'-end of a ds oligonucleotide, the 5' position may be connected to a 5'-end group (e.g., −OH), and if at the 3'-end of a ds oligonucleotide, the 3' position may be connected to a 3'-end group (e.g., −OH). In certain embodiments, a sugar is a modified sugar in that it is not a natural DNA sugar or a natural RNA sugar. Among other things, modified sugars may provide improved stability. In certain embodiments, modified sugars can be utilized to alter and/or optimize one or more hybridization characteristics. In certain embodiments, modified sugars can be utilized to alter and/or optimize target recognition. In certain embodiments, modified sugars can be utilized to optimize Tm. In certain embodiments, modified sugars can be utilized to improve oligonucleotide activities.

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

In certain embodiments, a sugar is an optionally substituted natural DNA or RNA sugar. In certain embodiments, a sugar is optionally substituted . In certain embodiments, the 2' position is optionally substituted. In certain embodiments, a

sugar is . In certain embodiments, a sugar has the structure of  or , wherein each of R^{1s}, R^{2s}, R^{3s}, R^{4s}, and R^{5s} is independently −H, a suitable substituent or suitable sugar modification (e.g., those described in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO2019/032612, WO 2019/055951, and/or WO 2019/075357, the substituents, sugar modifications, descriptions of R^{1s}, R^{2s}, R^{3s}, R^{4s}, and R^{5s}, and modified sugars of each of which are independently incorporated herein by reference). In certain embodiments, a sugar has the structure of . In certain embodiments, R^{4s} is −H. In certain embodiments, a sugar has the structure of , wherein R^{2s} is −H, halogen, or −OR, wherein R is optionally substituted C_{1-6} aliphatic. In certain embodiments, R^{2s} is −H. In certain embodiments, R^{2s} is −F. In certain embodiments, R^{2s} is −OMe. In certain embodiments, R^{2s} is $-OCH_2CH_2OMe$.

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

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

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

In certain embodiments, a sugar modification is a 2'-modification. Commonly used 2'-modifications include but are not limited to 2'−OR, wherein R is optionally substituted C_{1-6} aliphatic. In certain embodiments, a modification is 2'−OR, wherein R is optionally substituted C_{1-6} alkyl. In certain embodiments, a modification is 2'−OMe. In certain embodiments, a modification is 2'-MOE. In certain embodiments, a 2'-modification is S-cEt. In certain embodiments, a modified sugar is an LNA sugar. In certain embodiments, a 2'-modification is −F.

In certain embodiments, a sugar modification replaces a sugar moiety with another cyclic or acyclic moiety. Examples of such moieties are widely known in the art, including but not limited to those used in morpholino (optionally with its phosphorodiamidate linkage), glycol nucleic acids, etc.

In certain embodiments, one or more of the sugars of an ATXN3 oligonucleotide are modified. In certain embodiments, each sugar of a ds oligonucleotide is independently modified. In certain embodiments, a modified sugar comprises a 2'-modification. In certain embodiments, each modified sugar independently comprises a 2'-modification. In certain embodiments, a 2'-modification is 2'-OR, wherein R is optionally substituted C_{1-6} aliphatic. In certain embodiments, a 2'-modification is a 2'-OMe. In certain embodiments, a 2'-modification is a 2'-MOE. In certain embodiments, a 2'-modification is an LNA sugar modification. In certain embodiments, a 2'-modification is 2'-F. In certain embodiments, each sugar modification is independently a 2'-modification. In certain embodiments, each sugar modification is independently 2'-OR. In certain embodiments, each sugar modification is independently 2'-OR, wherein R is optionally substituted C_{1-6} alkyl. In certain embodiments, each sugar modification is 2'-OMe. In certain embodiments, each sugar modification is 2'-MOE. In certain embodiments, each sugar modification is independently 2'-OMe or 2'-MOE. In certain embodiments, each sugar modification is independently 2'-OMe, 2'-MOE, or a LNA sugar.

As those skilled in the art will appreciate, modifications of sugars, nucleobases, internucleotidic linkages, etc. can and are often utilized in combination in oligonucleotides, e.g., see various oligonucleotides in Table 1. For example, a combination of sugar modification and nucleobase modification is 2'-F (sugar) 5-methyl (nucleobase) modified nucleosides. In certain embodiments, a combination is replacement of a ribosyl ring oxygen atom with S and substitution at the 2'-position.

In certain embodiments, a sugar is one described in US 9394333, US 9744183, US 9605019, US 9598458, US 9982257, US 10160969, US 10479995, US 2020/0056173, US 2018/0216107, US 2019/0127733, US 10450568, US 2019/0077817, US 2019/0249173, US 2019/0375774, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the sugars of each of which is incorporated herein by reference.

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

4. Nucleobases

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

In certain embodiments, a ds oligonucleotide comprises one or more A, T, C, G or U. In certain embodiments, a ds oligonucleotide comprises one or more optionally substituted A, T, C, G or U. In certain embodiments, a ds oligonucleotide comprises one or more 5-methylcytidine, 5-hydroxymethylcytidine, 5- formylcytosine, or 5-carboxylcytosine. In certain embodiments, a ds oligonucleotide comprises one or more 5-methylcytidine. In certain embodiments, each nucleobase in a ds oligonucleotide is selected

from the group consisting of optionally substituted A, T, C, G and U, and optionally substituted tautomers of A, T, C, G and U.

In certain embodiments, each nucleobase in a ds oligonucleotide is optionally protected A, T, C, G and U. In certain embodiments, each nucleobase in a ds oligonucleotide is optionally substituted A, T, C, G or U. In certain embodiments, each nucleobase in a ds oligonucleotide is selected from the group consisting of A, T, C, G, U, and 5mC.

In certain embodiments, a nucleobase is optionally substituted 2AP or DAP. In certain embodiments, a nucleobase is optionally substituted 2AP. In certain embodiments, a nucleobase is optionally substituted DAP. In certain embodiments, a nucleobase is 2AP. In certain embodiments, a nucleobase is DAP.

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

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

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

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

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

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

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

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

In certain embodiments, a modified nucleobase is a modified nucleobase known in the art, e.g., WO2017/210647. In certain embodiments, modified nucleobases are expanded-size nucleobases in which one or more aryl and/or heteroaryl rings, such as phenyl rings, have been added.

In certain embodiments, a modified nucleobase is selected from 5-substituted pyrimidines, 6- azapyrimidines, alkyl or alkynyl substituted pyrimidines, alkyl substituted purines, and N-2, N-6 and O-6 substituted purines. In certain embodiments, modified nucleobases are selected from 2-aminopropyladenine, 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-N-methylguanine, 6-N- methyladenine, 2-propyladenine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl ($-C\equiv C-CH_3$) uracil, 5-propynylcytosine, 6-azouracil, 6-azocytosine, 6-azothymine, 5-ribosyluracil (pseudouracil), 4- thiouracil, 8- halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl, 8-aza and other 8-substituted purines, 5-halo, particularly 5- bromo, 5-trifluoromethyl, 5-halouracil, and 5-halocytosine, 7-methylguanine, 7-methyladenine, 2-F-adenine, 2-aminoadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, 3-deazaadenine, 6-N- benzoyladenine, 2-N-isobutyrylguanine, 4-N-benzoylcytosine, 4-N-benzoyluracil, 5-methyl 4-N-

benzoylcytosine, 5-methyl 4-N- benzoyluracil, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. In certain embodiments, modified nucleobases are tricyclic pyrimidines, such as l,3-diazaphenoxazine-2- one, l,3-diazaphenothiazine-2-one or 9-(2-aminoethoxy)-l,3-diazaphenoxazine-2- one (G-clamp). In certain embodiments, modified nucleobases are those in which the purine or pyrimidine base is replaced with other heterocycles, for example, 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine or 2- pyridone.

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

In certain embodiments, nucleosides that can be utilized in provided technologies comprise modified nucleobases and/or modified sugars, e.g., 4-acetylcytidine; 5-(carboxyhydroxylmethyl)uridine; 2'-O- methylcytidine; 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyluridine; dihydrouridine; 2'-O-methylpseudouridine; beta,D-galactosylqueosine; 2'-O-methylguanosine; N^6-isopentenyladenosine; 1-methyladenosine; 1-methylpseudouridine; 1-methylguanosine; l-methylinosine; 2,2- dimethylguanosine; 2-methyladenosine; 2-methylguanosine; N^7-methylguanosine; 3-methyl-cytidine; 5- methylcytidine; 5-hydroxymethylcytidine; 5-formylcytosine; 5-carboxylcytosine; N^6-methyladenosine; 7- methylguanosine; 5-methylaminoethyluridine; 5-methoxyaminomethyl-2-thiouridine; beta,D-mannosylqueosine; 5-methoxycarbonylmethyluridine; 5-methoxyuridine; 2-methylthio-N^6- isopentenyladenosine; N-((9-beta,D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine; N-((9- beta,D-ribofuranosylpurine-6-yl)-N-methylcarbamoyl)threonine; uridine-5-oxyacetic acid methylester; uridine-5-oxyacetic acid (v); pseudouridine; queosine; 2-thiocytidine; 5-methyl-2-thiouridine; 2-thiouridine; 4-thiouridine; 5-methyluridine; 2'-O-methyl-5-methyluridine; and 2'-O-methyluridine. In certain embodiments, a nucleobase, e.g., a modified nucleobase comprises one or more biomolecule binding moieties such as e.g., antibodies, antibody fragments, biotin, avidin, streptavidin, receptor ligands, or chelating moieties. In other embodiments, a nucleobase is 5-bromouracil, 5 iodouracil, or 2,6- diaminopurine. In certain embodiments, a nucleobase

comprises substitution with a fluorescent or biomolecule binding moiety. In certain embodiments, a substituent is a fluorescent moiety.

In certain embodiments, a substituent is biotin or avidin.

In certain embodiments, a nucleobase is one described in US 9394333, US 9744183, US 9605019, US 9598458, US 9982257, US 10160969, US 10479995, US 2020/0056173, US 2018/0216107, US 2019/0127733, US 10450568, US 2019/0077817, US 2019/0249173, US 2019/0375774, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the nucleobases of each of which is incorporated herein by reference.

5. Additional Chemical Moieties

In certain embodiments, a ds oligonucleotide comprises one or more additional chemical moieties. Various additional chemical moieties, e.g., targeting moieties, carbohydrate moieties, lipid moieties, etc. are known in the art and can be utilized in accordance with the present disclosure to modulate properties and/or activities of provided oligonucleotides, e.g., stability, half-life, activities, delivery, pharmacodynamics properties, pharmacokinetic properties, etc. In certain embodiments, certain additional chemical moieties facilitate delivery of oligonucleotides to desired cells, tissues and/or organs, including but not limited the cells of the central nervous system. In certain embodiments, certain additional chemical moieties facilitate internalization of oligonucleotides. In certain embodiments, certain additional chemical moieties increase oligonucleotide stability. In certain embodiments, the present disclosure provides technologies for incorporating various additional chemical moieties into oligonucleotides.

In certain embodiments, a ds oligonucleotide comprises an additional chemical moiety demonstrates increased delivery to and/or activity in a tissue compared to a reference oligonucleotide, e.g., a reference oligonucleotide which does not have the additional chemical moiety but is otherwise identical.

In certain embodiments, non-limiting examples of additional chemical moieties include carbohydrate moieties, targeting moieties, etc., which, when incorporated into oligonucleotides, can improve one or more properties. In certain embodiments, an additional chemical moiety is selected from: glucose, GluNAc (N-acetyl amine glucosamine) and anisamide moieties. In certain embodiments, a provided ds oligonucleotide can comprise two or more additional chemical moieties, wherein the

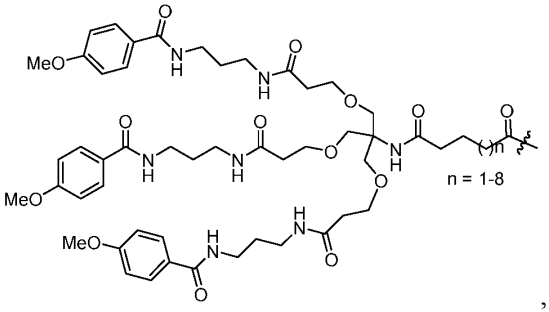
additional chemical moieties are identical or non-identical, or are of the same category (e.g., carbohydrate moiety, sugar moiety, targeting moiety, etc.) or not of the same category.

In certain embodiments, an additional chemical moiety is a targeting moiety. In certain embodiments, an additional chemical moiety is or comprises a carbohydrate moiety. In certain embodiments, an additional chemical moiety is or comprises a lipid moiety. In certain embodiments, an additional chemical moiety is or comprises a ligand moiety for, e.g., cell receptors such as a sigma receptor, an asialoglycoprotein receptor, etc. In certain embodiments, a ligand moiety is or comprises an anisamide moiety, which may be a ligand moiety for a sigma receptor. In certain embodiments, a ligand moiety is or comprises a GalNAc moiety, which may be a ligand moiety for an asialoglycoprotein receptor. In certain embodiments, an additional chemical moiety facilitates delivery to liver.

In certain embodiments, a provided ds oligonucleotide can comprise one or more linkers and additional chemical moieties (e.g., targeting moieties), and/or can be chirally controlled or not chirally controlled, and/or have a bases sequence and/or one or more modifications and/or formats as described herein.

Various linkers, carbohydrate moieties and targeting moieties, including many known in the art, can be utilized in accordance with the present disclosure. In certain embodiments, a carbohydrate moiety is a targeting moiety. In certain embodiments, a targeting moiety is a carbohydrate moiety.

In certain embodiments, a provided ds oligonucleotide comprises an additional chemical moiety suitable for delivery, e.g., glucose, GluNAc (N-acetyl amine glucosamine), anisamide, or a structure selected from:



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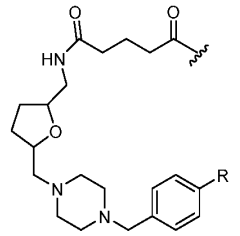
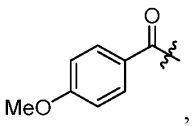


R = F, OMe, OH, NHAc, $NHCOCF_3$,



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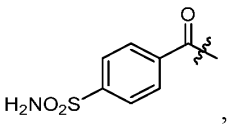

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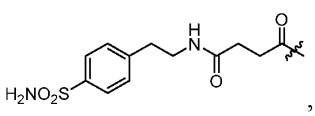

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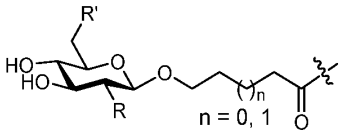

R = NHAc, R' = OH; R = NHCOC6H4OMe(p-anisoyl), R'=OH;
R = NHAc, R' = NHCOC6H4OMe(p-anisoyl);
R = OH, R' = NHCOC6H4OMe(p-anisoyl)

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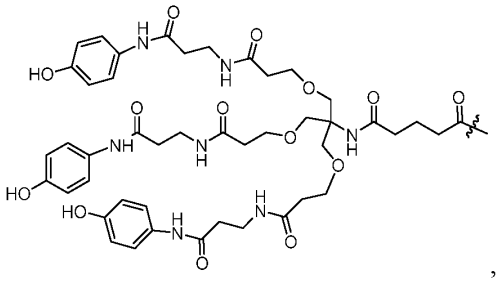

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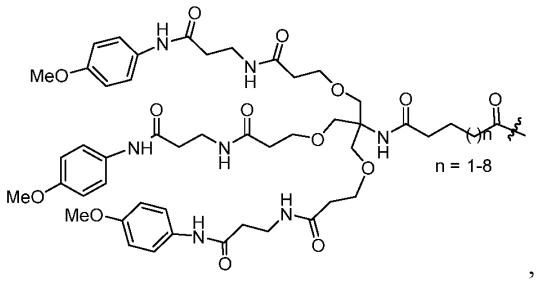

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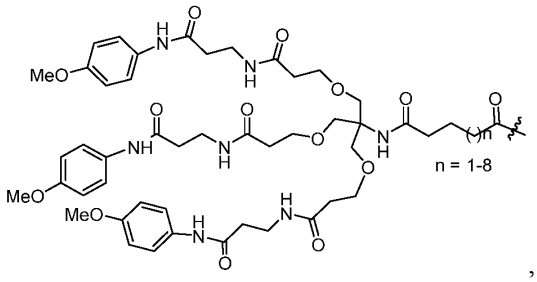

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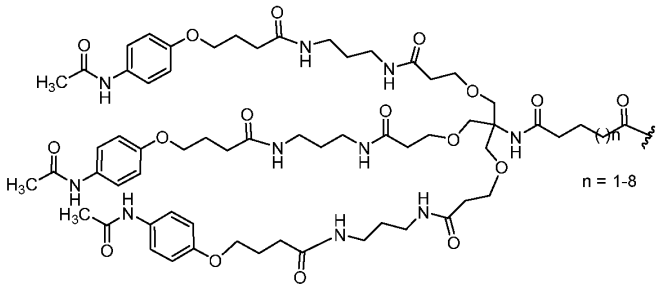


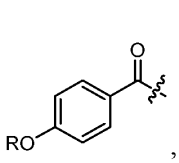
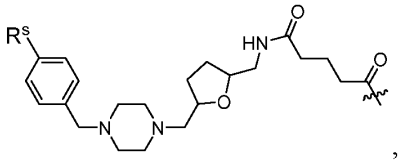
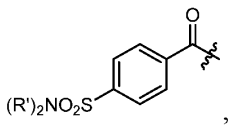
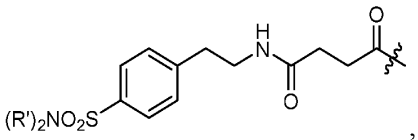
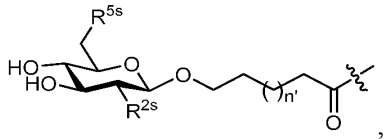
. In certain embodiments, n is 1. In certain embodiments, n is 2. In certain embodiments, n is 3. In certain embodiments, n is 4. In certain embodiments, n is 5. In certain embodiments, n is 6. In certain embodiments, n is 7. In certain embodiments, n is 8.

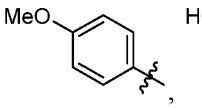
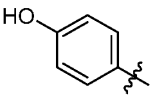
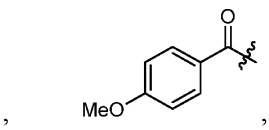
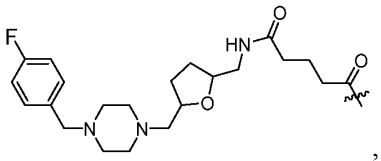
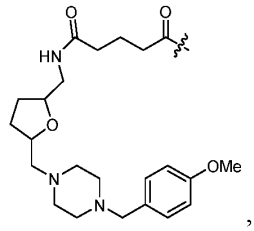
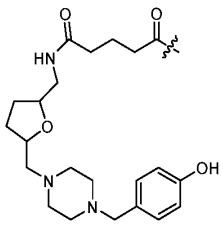
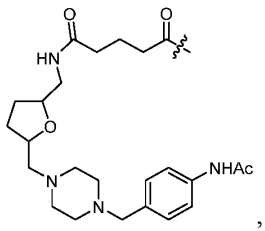
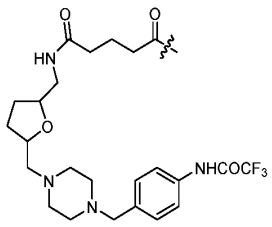
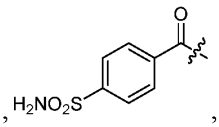
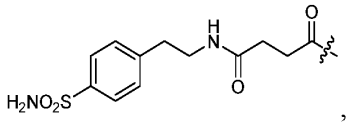
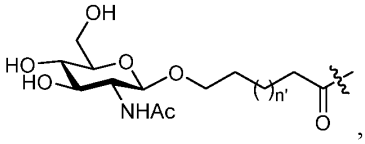
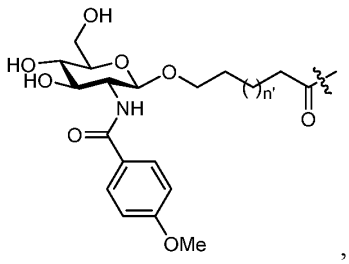
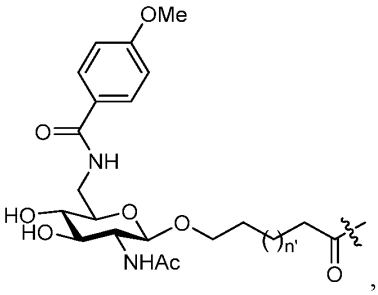
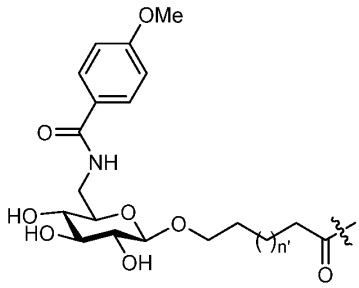
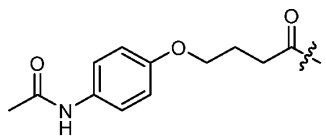
In certain embodiments, additional chemical moieties are any of ones described in the Examples, including examples of various additional chemical moieties incorporated into various ds oligonucleotides.

In certain embodiments, an additional chemical moiety conjugated to a ds oligonucleotide is capable of targeting the ds oligonucleotide to a cell in the central nervous system.

In certain embodiments, an additional chemical moiety comprises or is a cell receptor ligand. In certain embodiments, an additional chemical moiety comprises or is a protein binder, e.g., one binds to a cell surface protein. Such moieties among other things can be useful for targeted delivery of ds oligonucleotides to cells expressing the corresponding receptors or proteins. In certain embodiments, an additional chemical moiety of a provided ds oligonucleotide comprises anisamide or a derivative or an analog thereof and is capable of targeting the ds oligonucleotide to a cell expressing a particular receptor, such as the sigma 1 receptor.

In certain embodiments, a provided ds oligonucleotide is formulated for administration to a body cell and/or tissue expressing its target. In certain embodiments, an additional chemical moiety conjugated to a ds oligonucleotide is capable of targeting the oligonucleotide to a cell.

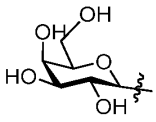
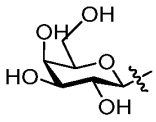
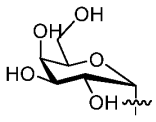
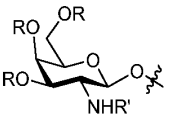
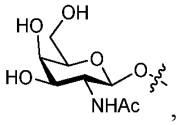
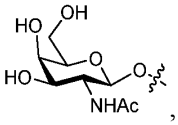
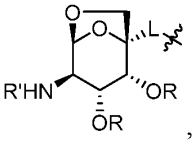
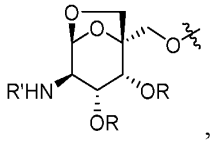
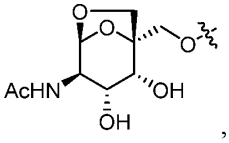
In certain embodiments, an additional chemical moiety is selected from optionally substituted phenyl, , , , , and , wherein n' is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, and each other variable is as described in the present disclosure. In certain embodiments, R^s is F. In certain embodiments, R^s is OMe. In certain embodiments, R^s is OH. In certain embodiments, R^s is NHAc. In certain embodiments, R^s is $NHCOCF_3$. In certain embodiments, R' is H. In certain embodiments, R is H. In certain embodiments, R^{2s} is NHAc, and R^{5s} is OH. In

certain embodiments, R^{2s} is p-anisoyl, and R^{5s} is OH. In certain embodiments, R^{2s} is NHAc and R^{5s} is p-anisoyl. In certain embodiments, R^{2s} is OH, and R^{5s} is p-anisoyl. In certain embodiments, an additional chemical moiety is selected from , , , , , , , , , , , , , , and . In certain embodiments, n' is 1. In certain embodiments, n' is 0. In certain embodiments, n" is 1. In certain embodiments, n" is 2.

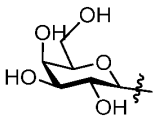
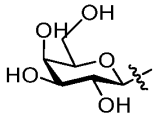
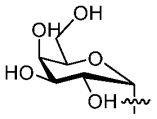
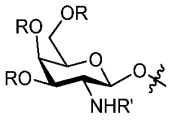
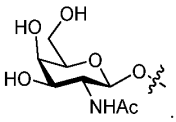
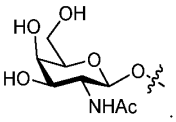
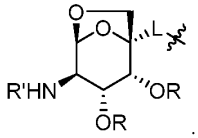
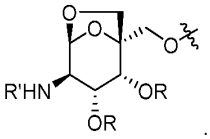
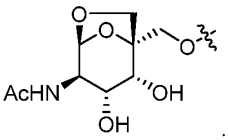
In certain embodiments, an additional chemical moiety is or comprises an

asialoglycoprotein receptor (ASGPR) ligand.

Without wishing to be bound by any particular theory, the present disclosure notes that ASGPR1 has also been reported to be expressed in the hippocampus region and/or cerebellum Purkinje cell layer of the mouse. http://mouse.brain-map.org/experiment/show/2048

Various other ASGPR ligands are known in the art and can be utilized in accordance with the present disclosure. In certain embodiments, an ASGPR ligand is a carbohydrate. In certain embodiments, an ASGPR ligand is GalNac or a derivative or an analog thereof. In certain embodiments, an ASGPR ligand is one described in Sanhueza et al. J. Am. Chem. Soc., 2017, 139 (9), pp 3528–3536. In certain embodiments, an ASGPR ligand is one described in Mamidyala et al. J. Am. Chem. Soc., 2012, 134, pp 1978−1981. In certain embodiments, an ASGPR ligand is one described in US 20160207953. In certain embodiments, an ASGPR ligand is a substituted-6,8-dioxabicyclo[3.2.1]octane-2,3-diol derivative disclosed in, e.g., US 20160207953. In certain embodiments, an ASGPR ligand is one described in, e.g., US 20150329555. In certain embodiments, an ASGPR ligand is a substituted-6,8-dioxabicyclo[3.2.1]octane-2,3-diol derivative disclosed e.g., in US 20150329555. In certain embodiments, an ASGPR ligand is one described in US 8877917, US 20160376585, US 10086081, or US 8106022. ASGPR ligands described in these documents are incorporated herein by reference. Those skilled in the art will appreciate that various technologies are known in the art, including those described in these documents, for assessing binding of a chemical moiety to ASGPR and can be utilized in accordance with the present disclosure. In certain embodiments, a provided ds oligonucleotide is conjugated to an ASGPR ligand. In certain embodiments, a provided ds oligonucleotide comprises an ASGPR ligand. In certain embodiments, an additional chemical moiety comprises an ASGPR ligand is , , , , , , , , or , wherein each variable is independently as described in the present

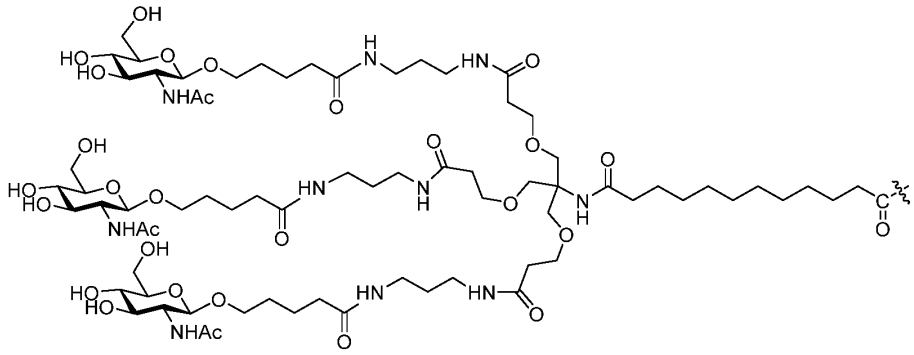
disclosure. In certain embodiments, R is −H. In certain embodiments, R' is −C(O)R.

In certain embodiments, an additional chemical moiety is or comprises . In certain embodiments, an additional chemical moiety is or comprises . In certain embodiments, an additional chemical moiety is or comprises . In certain embodiments, an additional chemical moiety is or comprises . In certain embodiments, an additional chemical moiety is or comprises optionally substituted . In certain embodiments, an additional chemical moiety is or comprises . In certain embodiments, an additional chemical moiety is or comprises . In certain embodiments, an additional chemical moiety is or comprises . In certain embodiments, an additional chemical moiety is or comprises . In certain embodiments, an additional chemical moiety is or comprises .

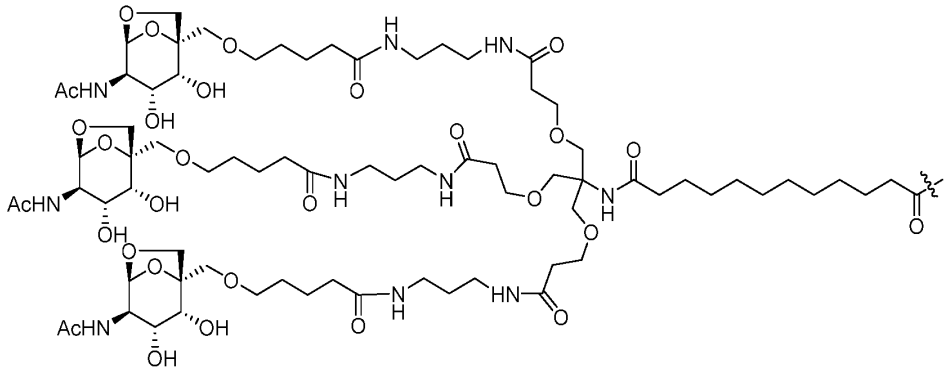
In certain embodiments, an additional chemical moiety comprises one or more moieties that can bind to, e.g., oligonucleotide target cells. For example, in certain embodiments, an additional chemistry moiety comprises one or more protein ligand

moieties, e.g., in certain embodiments, an additional chemical moiety comprises multiple moieties, each of which independently is an ASGPR ligand. In certain embodiments, as in Mod 001, Mod083, Mod071, Mod153, and Mod155, an additional chemical moiety comprises three such ligands.

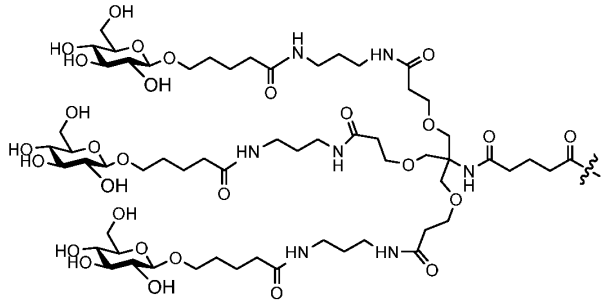
Mod001:



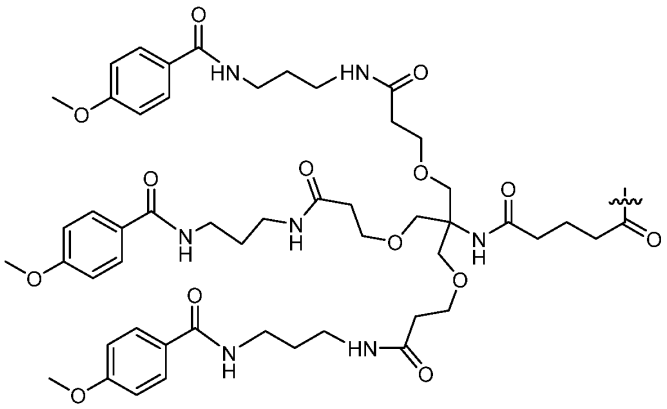
Mod083:



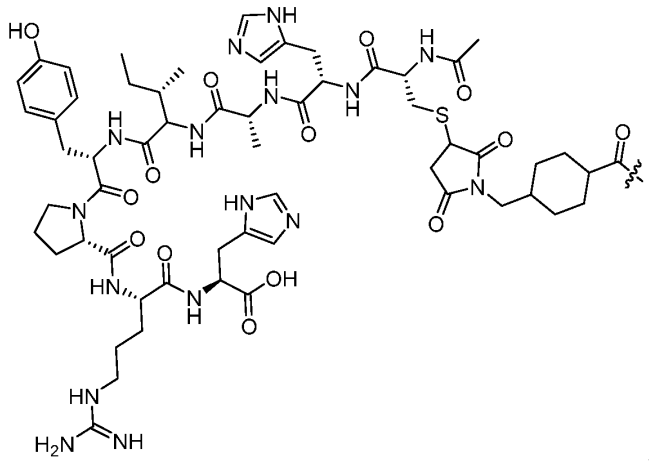
Mod071



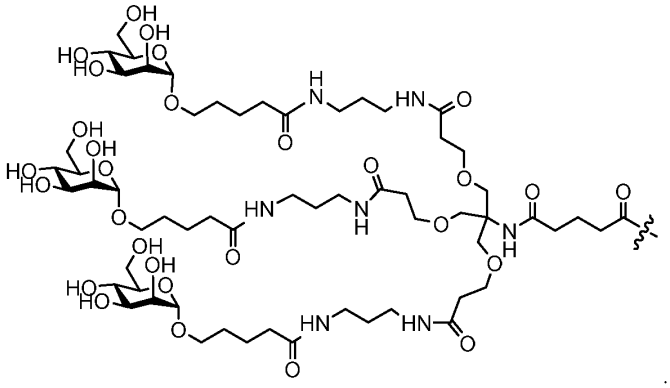
Mod077



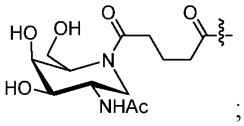
Mod102:



Mod105:

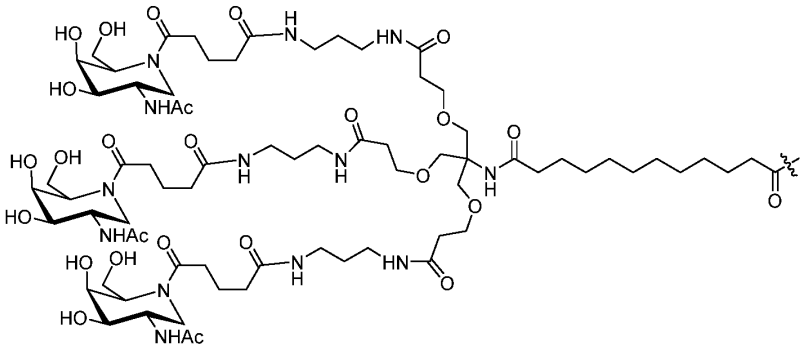


Mod152 (in certain embodiments, −C(O)− connects to −NH− of a linker such as Mod153):

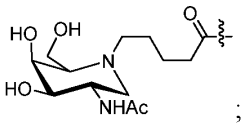


;

Mod153

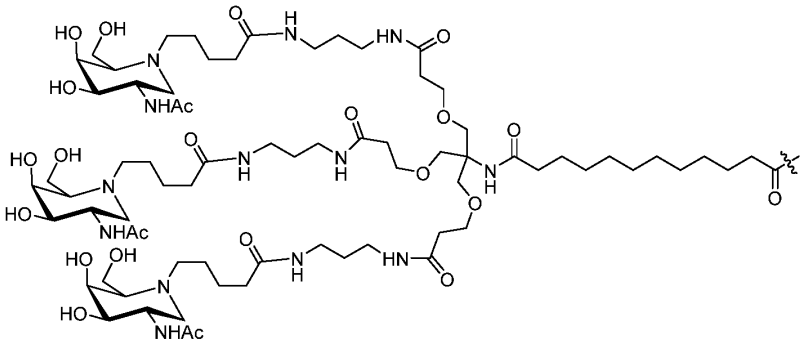


Mod154 (in certain embodiments, −C(O)− connects to −NH− of a linker such as Mod155):



;

Mod155



.

In some embodiments, an oligonucleotide comprises OBJ, wherein each variable is independently as described herein. In some embodiments, each −OR' is −OAc, and −N(R')$_2$ is −NHAc. In some embodiments, an oligonucleotide comprises OBJ. In some embodiments, each R' is −H. In some embodiments, each −OR' is −OH, and each −N(R')$_2$ is −NHC(O)R. In some embodiments, each −OR' is −OH, and each −N(R')$_2$ is −NHAc. In some embodiments, an oligonucleotide comprises OBJ (L025). In some embodiments, the −CH_2− connection site is utilized as a C5 connection site in a sugar. In some embodiments,

the connection site on the ring is utilized as a C3 connection site in a sugar. Such moieties may be introduced utilizing, e.g., phosphoramidites such as [OBJ], e.g., [OBJ] (those skilled in the art appreciate that one or more other groups, such as protection groups for −OH, $-NH_2-$, $-N(i\text{-}Pr)_2$, $-OCH_2CH_2CN$, etc., may be alternatively utilized, and protection groups can be removed under various suitable conditions, sometimes during oligonucleotide de-protection and/or cleavage steps). In some embodiments, an oligonucleotide comprises 2, 3 or more (e.g., 3 and no more than 3) [OBJ]. In some embodiments, an oligonucleotide comprises 2, 3 or more (e.g., 3 and no more than 3) [OBJ]. In some embodiments, copies of such moieties are linked by internucleotidic linkages, e.g., natural phosphate linkages, as described herein. In some embodiments, when at a 5'-end, a $-CH_2-$ connection site is bonded to −OH. In some embodiments, an oligonucleotide comprises [OBJ]. In some embodiments, an oligonucleotide comprises [OBJ]. In some embodiments, each −OR' is −OAc, and $-N(R')_2$ is −NHAc. In some embodiments, an oligonucleotide comprises [OBJ]. Among other things, [OBJ] may be utilized to introduce [OBJ] with comparable and/or better activities and/or properties. In some embodiments, it provides improved preparation efficiency and/or lower cost for the same number of [OBJ] (e.g., when compared to Mod001).

In certain embodiments, an additional chemical moiety is a Mod group described herein, e.g., in Table 1.

In certain embodiments, an additional chemical moiety is Mod001. In certain embodiments, an additional chemical moiety is Mod083. In certain embodiments, an additional chemical moiety, e.g., a Mod group, is directly conjugated (e.g., without a linker) to the remainder of the ds oligonucleotide. In certain embodiments, an additional chemical moiety is conjugated via a linker to the remainder of the ds oligonucleotide. In certain embodiments, additional chemical moieties, e.g., Mod groups, may be directly connected, and/or via a linker, to nucleobases, sugars and/or internucleotidic linkages of ds oligonucleotides. In certain embodiments, Mod groups are connected, either directly or via a linker, to sugars. In certain embodiments, Mod groups are connected, either directly or via a linker, to 5'-end sugars. In certain embodiments, Mod groups are connected, either directly or via a linker, to 5'-end sugars via 5' carbon. For examples, see various ds oligonucleotides in Table 1A and 1B or Table 1C or Table 1D. In certain embodiments, Mod groups are connected, either directly or via a linker, to 3'-end sugars. In certain embodiments, Mod groups are connected, either directly or via a linker, to 3'-end sugars via 3' carbon. In certain embodiments, Mod groups are connected, either directly or via a linker,

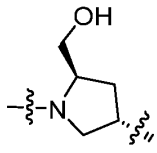
to nucleobases. In certain embodiments, Mod groups are connected, either directly or via a linker, to internucleotidic linkages. In certain embodiments, provided oligonucleotides comprise Mod001 connected to 5'-end of oligonucleotide chains through L001.

As appreciated by those skilled in the art, an additional chemical moiety may be connected to a ds oligonucleotide chain at various locations, e.g., 5'-end, 3'-end, or a location in the middle (e.g., on a sugar, a base, an internucleotidic linkage, etc.). In certain embodiments, it is connected at a 5'-end. In certain embodiments, it is connected at a 3'-end. In certain embodiments, it is connected at a nucleotide in the middle.

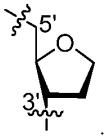
Certain additional chemical moieties (e.g., lipid moieties, targeting moieties, carbohydrate moieties), including but not limited to Mod012, Mod039, Mod062, Mod085, Mod086, and Mod094, and various linkers for connecting additional chemical moieties to ds oligonucleotide chains, including but not limited to L001, L003, L004, L008, L009, and L010, are described in WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO2019032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the additional chemical moieties and linkers of each of which are independently incorporated herein by reference, and can be utilized in accordance with the present disclosure. In certain embodiments, an additional chemical moiety is digoxigenin or biotin or a derivative thereof.

In certain embodiments, a ds oligonucleotide comprises a linker, e.g., L001 L004, L008, and/or an additional chemical moiety, e.g., Mod012, Mod039, Mod062, Mod085, Mod086, or Mod094. In certain embodiments, a linker, *e.g.*, L001, L003, L004, L008, L009, L110, *etc.* is linked to a Mod, *e.g.*, Mod012, Mod039, Mod062, Mod085, Mod086, Mod094, Mod152, Mod153, Mod154, Mod155 *etc*. L001: $-NH-(CH_2)_6-$ linker (also known as a C6 linker, C6 amine linker or C6 amino linker), connected to Mod, if any, through $-NH-$, and the 5'-end or 3'-end of the ds oligonucleotide chain through either a phosphate linkage ($-O-P(O)(OH)-O-$, which may exist as a salt form, and may be indicated as O or PO) or a phosphorothioate linkage ($-O-P(O)(SH)-O-$, which may exist as a salt form, and may be indicated as * if the phosphorothioate is not chirally controlled; or *S, S, or *Sp*, if the phosphorothioate is chirally controlled and has an *Sp* configuration, or *R, R, or *Rp*, if the phosphorothioate is chirally controlled and has an *Rp* configuration) as indicated at the $-CH_2-$ connecting site. If no Mod is present, L001 is connected to $-H$

through −NH−;

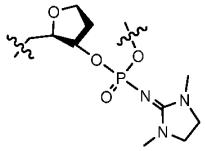
L003:  linker. In certain embodiments, it is connected to Mod, if any (if no Mod, −H), through its amino group, and the 5'-end or 3'-end of an oligonucleotide chain e.g., via a linkage (e.g., a phosphate linkage (O or PO) or a phosphorothioate linkage (can be either not chirally controlled or chirally controlled (*Sp* or *Rp*))); L004: linker having the structure of $-NH(CH_2)_4CH(CH_2OH)CH_2-$, wherein −NH− is connected to Mod (through −C(O)−) or −H, and the $-CH_2-$ connecting site is connected to an oligonucleotide chain (e.g., at the 3'-end) through a linkage, e.g., phosphodiester (−O−P(O)(OH)−O−, which may exist as a salt form, and may be indicated as O or PO), phosphorothioate (−O−P(O)(SH)−O−, which may exist as a salt form, and may be indicated as * if the phosphorothioate is not chirally controlled; or *S, S, or *Sp*, if the phosphorothioate is chirally controlled and has an *Sp* configuration, or *R, R, or *Rp*, if the phosphorothioate is chirally controlled and has an *Rp* configuration), or phosphorodithioate (−O−P(S)(SH)−O−, which may exist as a salt form, and may be indicated as PS2 or : or D) linkage. For example, an asterisk immediately preceding a L004 (e.g., *L004) indicates that the linkage is a phosphorothioate linkage, and the absence of an asterisk immediately preceding L004 indicates that the linkage is a phosphodiester linkage. For example, in an oligonucleotide which terminates in ...mAL004, the linker L004 is connected (via the $-CH_2-$ site) through a phosphodiester linkage to the 3' position of the 3'-terminal sugar (which is 2'-OMe modified and connected to the nucleobase A), and the L004 linker is connected via −NH− to −H. Similarly, in one or more oligonucleotides, the L004 linker is connected (via the $-CH_2-$ site) through the phosphodiester linkage to the 3' position of the 3'-terminal sugar, and the L004 is connected via −NH− to, e.g., Mod012, Mod085, Mod086, etc.; L008: linker having the structure of $-C(O)-(CH_2)_9-$, wherein −C(O)− is connected to Mod (through −NH−) or −OH (if no Mod indicated), and the $-CH_2-$ connecting site is connected to an oligonucleotide chain (e.g., at the 5'-end) through a linkage, e.g., phosphodiester (−O−P(O)(OH)−O−, which may exist as a salt form, and may be indicated as O or PO), phosphorothioate (−O−P(O)(SH)−O−, which may exist as a salt form, and may be indicated as * if the phosphorothioate is not chirally controlled; or *S, S, or *Sp*, if the phosphorothioate is chirally controlled and has an *Sp* configuration, or *R, R, or *Rp*, if the phosphorothioate is chirally controlled and has an *Rp* configuration), or phosphorodithioate

(−O−P(S)(SH)−O−, which may exist as a salt form, and may be indicated as PS2 or : or D) linkage. For example, in an example oligonucleotide which has the sequence of 5'-L008mN * mN * mN * mN * N * N * N * N * N * N * N * N * N * N * mN * mN * mN * mN-3', and which has a Stereochemistry/Linkage of OXXXXXXXXX XXXXXXXX, wherein N is a base, wherein O is a natural phosphate internucleotidic linkage, and wherein X is a stereorandom phosphorothioate, L008 is connected to −OH through −C(O)−, and the 5'-end of an oligonucleotide chain through a phosphate linkage (indicated as "O" in "Stereochemistry/Linkage"); in another example oligonucleotide, which has the sequence of 5'-Mod062L008mN * mN * mN * mN * N * N * N * N * N * N * N * N * N * N * mN * mN * mN * mN-3', and which has a Stereochemistry/Linkage of OXXXXXXXXX XXXXXXXX, wherein N is a base, L008 is connected to Mod062 through −C(O)−, and the 5'-end of an oligonucleotide chain through a phosphate linkage (indicated as "O" in "Stereochemistry/Linkage");

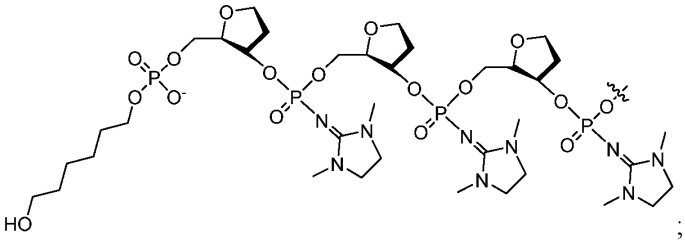
L009: $-CH_2CH_2CH_2-$. In certain embodiments, when L009 is present at the 5'-end of an oligonucleotide without a Mod, one end of L009 is connected to −OH and the other end connected to a 5'-carbon of the oligonucleotide chain e.g., via a linkage (e.g., a phosphate linkage (O or PO) or a phosphorothioate linkage (can be either not chirally controlled or chirally controlled (*S*p or *Rp*))); L010: 

. In certain embodiments, when L010 is present at the 5'-end of an oligonucleotide without a Mod, the 5'-carbon of L010 is connected to −OH and the 3'-carbon connected to a 5'-carbon of the oligonucleotide chain e.g., via a linkage (e.g., a phosphate linkage (O or PO) or a phosphorothioate linkage (can be either not chirally controlled or chirally controlled (*S*p or *Rp*))); Mod012 (in certain embodiments, −C(O)− connects to −NH− of a linker such as L001, L004, L008, *etc.*):

L010 is utilized with n001R to form L010n001R, which has the structure of

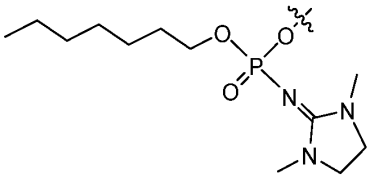


and wherein the configuration of linkage phosphorus is *R*p. In some embodiments, multiple L010n001R may be utilized. For example, L023L010n001RL010n001RL010n001R, which has the following structure (which is bonded to the 5'-carbon at the 5'-end of the oligonucleotide chain, and each linkage phosphorus is independently *R*p):



;

L023 is utilized with n001 to form L023n001, which has the structure of



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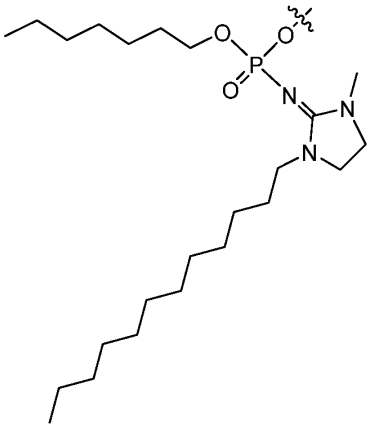
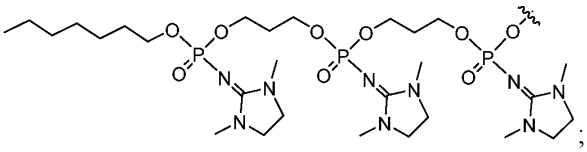
L023 is utilized with n009 to form L023n009, as in WV-42644 which has the structure of



;

In some embodiments, L023n001L009n001L009n001 may be utilized. For example, L023n001L009n001L009n001 as in WV-42643



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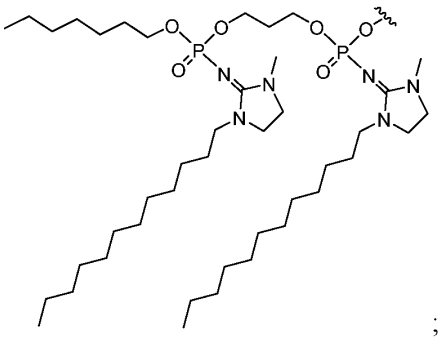
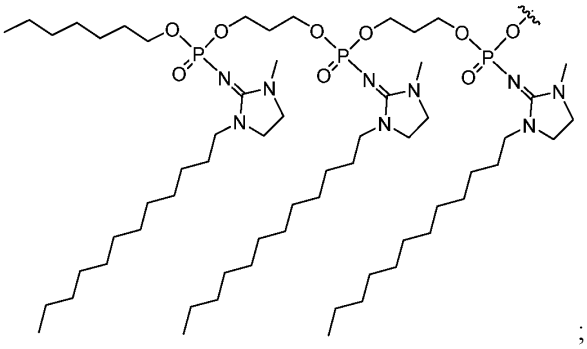
In some embodiments, L023n009L009n009 may be utilized. For example, as in WV-42646



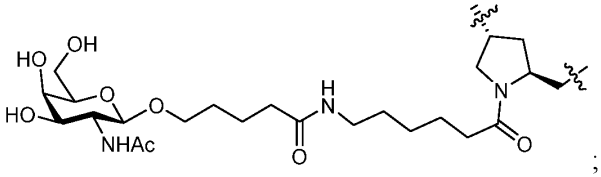
;

In some embodiments, L023n009L009n009L009n009 may be utilized. For example, as in WV-42648

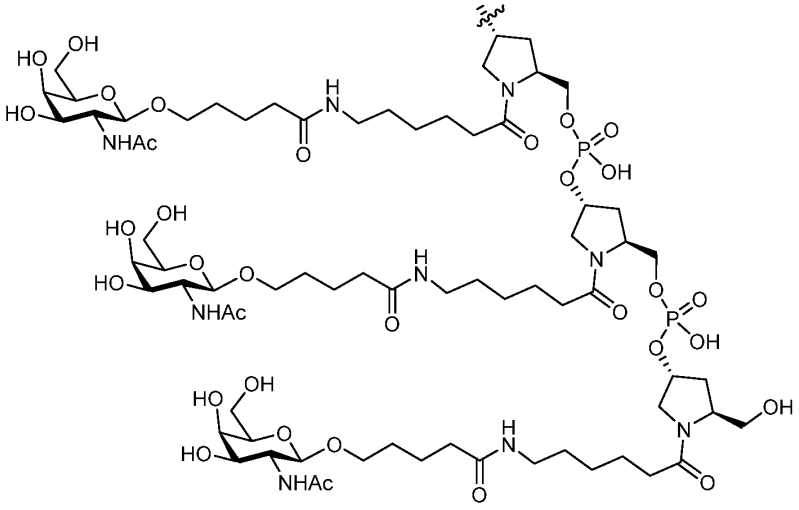


;

In some embodiments L025 may be utilized; as in WV-41390,

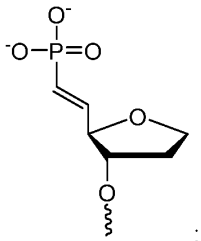


; wherein the $-CH_2-$ connection site is utilized as a C5 connection site of a sugar (e.g., a DNA sugar) and is connected to another unit (e.g., 3' of a sugar), and the connection site on the ring is utilized as a C3 connection site and is connected to another unit (e.g., a 5'-carbon of a carbon), each of which is independently, e.g., via a linkage (e.g., a phosphate linkage (O or PO) or a phosphorothioate linkage (can be either not chirally controlled or chirally controlled (*S*p or *R*p))). When L025 is at a5'-end without any modifications, its $-CH_2-$ connection site is bonded to $-OH$. For example, L025L025L025− in various oligonucleotides has the structure of



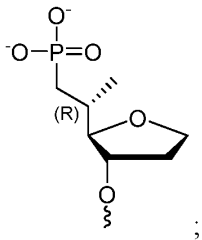
(may exist as various salt forms) and is connected to 5'-carbon of an oligonucleotide chain via a linkage as indicated (e.g., a phosphate linkage (O or PO) or a phosphorothioate linkage (can be either not chirally controlled or chirally controlled (*S*p or *R*p)));

In some embodiments L026 may be utilized; as in WV-44444,



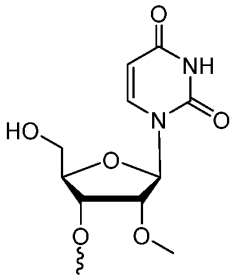
;

In some embodiments L027 may be utilized; as in WV-44445,



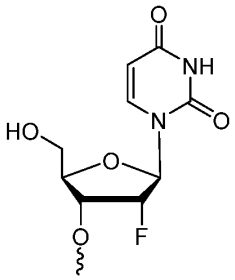
;

In some embodiments mU may be utilized; as in WV-42079,



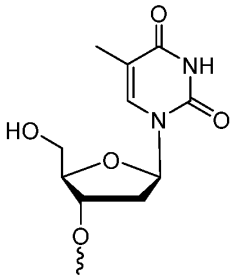
;

In some embodiments fU may be utilized; as in WV-44433,



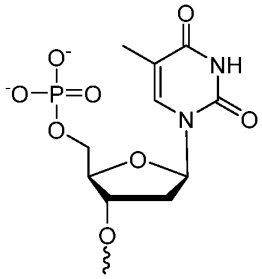
;

In some embodiments dT may be utilized; as in WV-44434,



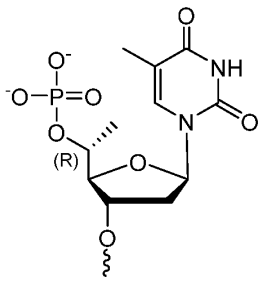
5 ;

In some embodiments POdT or PO4-dTmay be utilized; as in WV-44435,



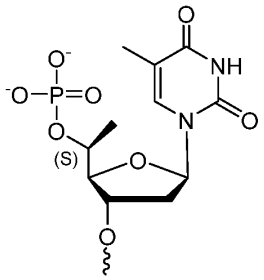
;

In some embodiments PO5MRdT may be utilized; as in WV-44436,



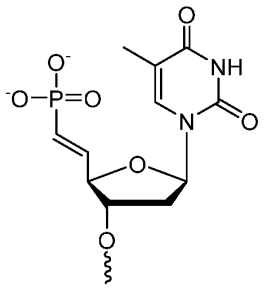
;

In some embodiments PO5MSdT may be utilized; as in WV-44437,



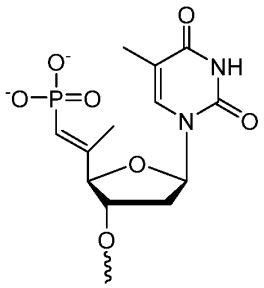
;

In some embodiments VPdT may be utilized; as in WV-44438,



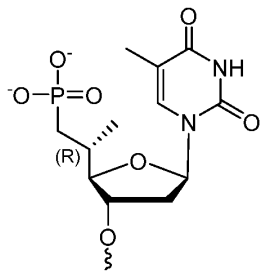
;

In some embodiments 5mvpdT may be utilized; as in WV-44439,



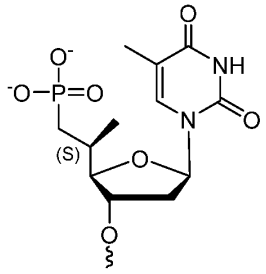
;

In some embodiments 5mrpdT may be utilized; as in WV-44440,



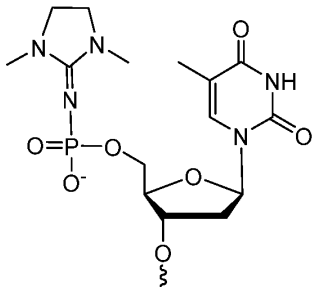
;

In some embodiments 5mspdT may be utilized; as in WV-44441,



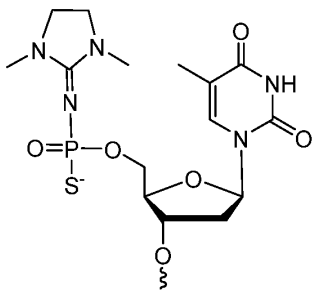
;

In some embodiments PNdT may be utilized; as in WV-44442,



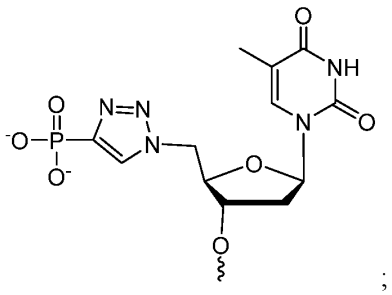
;

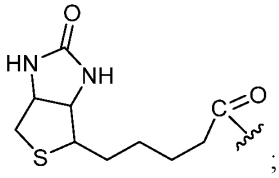
In some embodiments SPNdT may be utilized; as in WV-44443,

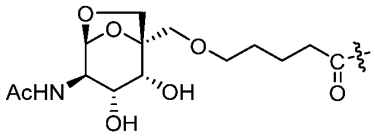


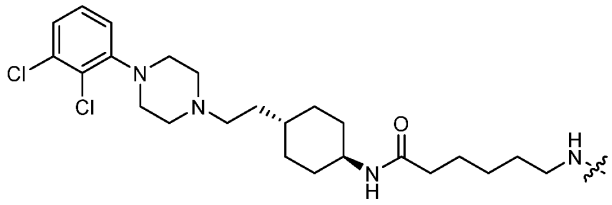
;

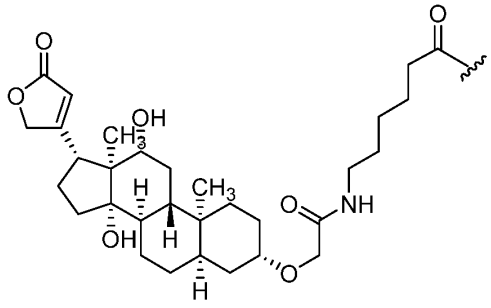
In some embodiments 5ptzdT may be utilized; as in WV-44446,

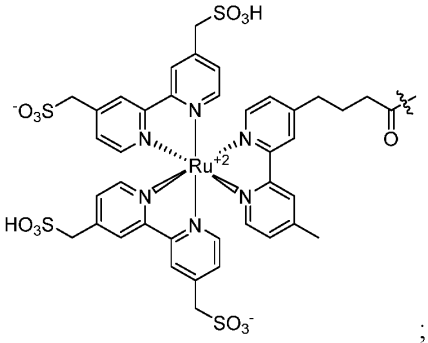

;


; Mod039 (in certain embodiments, −C(O)− connects to −NH− of a linker such as L001, L003, L004, L008, L009, L110, *etc.*):

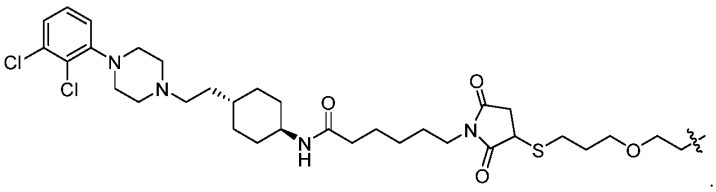

; Mod062 (in certain embodiments, −C(O)− connects to −NH− of a linker such as L001, L003, L004, L008, L009, L110, *etc.*):


; Mod085 (in certain embodiments, −C(O)− connects to −NH− of a linker such as L001, L003, L004, L008, L009, L110, *etc.*):


; Mod086 (in certain embodiments, −C(O)− connects to −NH− of a linker such as L001, L003, L004, L008, L009, L110, *etc.*):



; Mod094 (in certain embodiments, connects to an internucleotidic linkage, or to the 5'-end or 3'-end of an oligonucleotide via a linkage, *e.g.*, a phosphate linkage, a phosphorothioate linkage (which is optionally chirally controlled), *etc.*. For example, in an example oligonucleotide which has the sequence of 5'-mN * mN * mN * mN * N * N * N * N * N * N * N * N * N * N * mN * mN * mN * mNMod094-3', and which has a Stereochemistry/Linkage of XXXXX XXXXX XXXXX XXO, wherein N is a base, Mod094 is connected to the 3'-end of the oligonucleotide chain (3'-carbon of the 3'-end sugar) through a phosphate group (which is not shown below and which may exist as a salt form; and which is indicated as "O" in "Stereochemistry/Linkage" (...XXXX**O**))):



.

In certain embodiments, an additional chemical moiety is one described in WO 2012/030683. In certain embodiments, a provided ds oligonucleotide comprise a chemical structure (e.g., a linker, lipid, solubilizing group, and/or targeting ligand) described in WO 2012/030683.

In certain embodiments, a provided ds oligonucleotide comprises an additional chemical moiety and/or a modification (e.g., of nucleobase, sugar, internucleotidic linkage, etc.) described in: U.S. Pat. Nos. 5,688,941; 6,294,664; 6,320,017; 6,576,752; 5,258,506; 5,591,584; 4,958,013; 5,082,830; 5,118,802; 5,138,045; 6,783,931; 5,254,469; 5,414,077; 5,486,603; 5,112,963; 5,599,928; 6,900,297; 5,214,136; 5,109,124; 5,512,439; 4,667,025; 5,525,465; 5,514,785; 5,565,552; 5,541,313; 5,545,730; 4,835,263; 4,876,335; 5,578,717; 5,580,731; 5,451,463; 5,510,475; 4,904,582; 5,082,830; 4,762,779; 4,789,737; 4,824,941; 4,828,979; 5,595,726; 5,214,136; 5,245,022; 5,317,098; 5,371,241; 5,391,723; 4,948,882; 5,218,105; 5,112,963; 5,567,810; 5,574,142; 5,578,718; 5,608,046;

4,587,044; 4,605,735; 5,585,481; 5,292,873; 5,552,538; 5,512,667; 5,597,696; 5,599,923; 7,037,646; 5,587,371; 5,416,203; 5,262,536; 5,272,250; or 8,106,022.

In certain embodiments, an additional chemical moiety, e.g., a Mod, is connected via a linker. Various linkers are available in the art and may be utilized in accordance with the present disclosure, for example, those utilized for conjugation of various moieties with proteins (e.g., with antibodies to form antibody-drug conjugates), nucleic acids, etc. Certain useful linkers are described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/223056, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the linker moieties of each which are independently incorporated herein by reference. In certain embodiments, a linker is, as non-limiting examples, L001, L004, L009 or L010. In certain embodiments, an oligonucleotide comprises a linker, but not an additional chemical moiety other than the linker. In certain embodiments, a ds oligonucleotide comprises a linker, but not an additional chemical moiety other than the linker, wherein the linker is L001, L004, L009, or L010.

As demonstrated herein, provided technologies can provide high levels of activities and/or desired properties, in certain embodiments, without utilizing particular structural elements (e.g., modifications, linkage configurations and/or patterns, etc.) reported to be desired and/or necessary (e.g., those reported in WO 2019/219581), though certain such structural elements may be incorporated into ds oligonucleotides in combination with various other structural elements in accordance with the present disclosure. For example, in certain embodiments, ds oligonucleotides of the present disclosure have fewer nucleosides 3' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine), contain one or more phosphorothioate internucleotidic linkages at one or more positions where a phosphorothioate internucleotidic linkage was reportedly not favored or not allowed, contain one or more Sp phosphorothioate internucleotidic linkages at one or more positions where a Sp phosphorothioate internucleotidic linkage was reportedly not favored or not allowed, contain one or more Rp phosphorothioate internucleotidic linkages at one or more positions where a Rp phosphorothioate internucleotidic linkage was reportedly not favored or not allowed, and/or contain different modifications (e.g., internucleotidic linkage modifications, sugar modifications, etc.) and/or

stereochemistry at one or more locations compared to those reportedly favorable or required for certain oligonucleotide properties and/or activities (e.g., presence of 2'-MOE, absence of phosphorothioate linkages at certain positions, absence of *S*p phosphorothioate linkages at certain positions, and/or absence of *R*p phosphorothioate linkages at certain positions were reportedly favorable or required for certain oligonucleotide properties and/or activities; as demonstrated herein, provided technologies can provide desired properties and/or high activities without utilizing 2'-MOE, without avoiding phosphorothioate linkages at one or more such certain positions, without avoiding *S*p phosphorothioate linkages at one or more such certain positions, and/or without avoiding *R*p phosphorothioate linkages at one or more such certain positions). Additionally or alternatively, provided ds oligonucleotides incorporates structural elements that were not previously recognized such as utilization of certain modifications (e.g., base modifications, sugar modifications (e.g., 2'-F), linkage modifications (e.g., non-negatively charged internucleotidic linkages), additional moieties, etc.) and levels, patterns, and combinations thereof.

For example, in certain embodiments, as described herein, provided d oligonucleotides contain no more than 5, 6, 7, 8, 9, 10, 11 or 12 nucleosides 3' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine).

Alternatively or additionally, as described herein (e.g., illustrated in certain Examples), for structural elements 3' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine), in certain embodiments, about 50%-100% (e.g., about or at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%) of internucleotidic linkages 3' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine) are each independently a modified internucleotidic linkage, which is optionally chirally controlled. In certain embodiments, no more than 1, 2, or 3 internucleotidic linkages 3' to a nucleoside opposite to a target nucleoside are natural phosphate linkages. In certain embodiments, no such internucleotidic linkage is natural phosphate linkages. In certain embodiments, no more than 1 such internucleotidic linkage is natural phosphate linkages. In certain embodiments, no more than 2 such internucleotidic linkages are natural phosphate linkages. In certain embodiments, no more than 3 such internucleotidic linkages are natural phosphate linkages. In certain embodiments, each modified internucleotidic linkage is independently a phosphorothioate or a non-negatively charged internucleotidic linkage (e.g., n001). In certain embodiments, each phosphorothioate internucleotidic linkage is chirally controlled. In certain embodiments, no more than 1, 2, or 3 internucleotidic linkages 3' to a nucleoside

opposite to a target nucleoside are *R*p phosphorothioate internucleotidic linkage.

Alternatively or additionally, as described herein (e.g., illustrated in certain Examples), in certain embodiments, about 50%-100% (e.g., about or at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%) of internucleotidic linkages 5' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine) are each independently a modified internucleotidic linkage, which is optionally chirally controlled. In certain embodiments, no or no more than 1, 2, or 3 internucleotidic linkages 5' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine) are not modified internucleotidic linkages. In certain embodiments, no or no more than 1, 2, or 3 internucleotidic linkages 5' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine) are not phosphorothioate internucleotidic linkages. In certain embodiments, no or no more than 1, 2, or 3 internucleotidic linkages 5' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine) are not *S*p phosphorothioate internucleotidic linkages. In certain embodiments, no more than 1, 2, or 3 internucleotidic linkages 5' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine) are natural phosphate linkages. In certain embodiments, no such internucleotidic linkage is natural phosphate linkages. In certain embodiments, no more than 1 such internucleotidic linkage is natural phosphate linkages. In certain embodiments, no more than 2 such internucleotidic linkages are natural phosphate linkages. In certain embodiments, no more than 3 such internucleotidic linkages are natural phosphate linkages. In certain embodiments, each modified internucleotidic linkage is independently a phosphorothioate or a non-negatively charged internucleotidic linkage (e.g., n001). In certain embodiments, there are no 2, 3, or 4 consecutive internucleotidic linkages 5' to a nucleoside opposite to a target nucleoside, each of which is not a phosphorothioate internucleotidic linkage. In certain embodiments, there are no 2, 3, or 4 consecutive internucleotidic linkages 5' to a nucleoside opposite to a target nucleoside, each of which is chirally controlled and is not a *S*p phosphorothioate internucleotidic linkage. In certain embodiments, no or no more than 1, 2, 3, 4, or 5 internucleotidic linkages 5' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine) are *R*p phosphorothioate internucleotidic linkage. In certain embodiments, at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, or 32, or about 50%-100% (e.g., about or at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%) of internucleotidic linkages 5' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine) are each independently

chirally controlled and a *Sp* internucleotidic linkage. In certain embodiments, at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, or 32, or about 50%-100% (e.g., about or at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%) of phosphorothioate internucleotidic linkages 5' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine) are each independently chirally controlled and are *Sp*. In certain embodiments, each phosphorothioate internucleotidic linkages 5' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine) is chirally controlled. In certain embodiments, each phosphorothioate internucleotidic linkages 5' to a nucleoside opposite to a target nucleoside (e.g., a target adenosine) is *Sp*.

6. Production of Oligonucleotides and Compositions

Various methods can be utilized for production of ds oligonucleotides and compositions and can be utilized in accordance with the present disclosure. For example, traditional phosphoramidite chemistry can be utilized to prepare stereorandom oligonucleotides and compositions, and certain reagents and chirally controlled technologies can be utilized to prepare chirally controlled oligonucleotide compositions, e.g., as described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/223056, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the reagents and methods of each of which is incorporated herein by reference.

In certain embodiments, chirally controlled/stereoselective preparation of ds oligonucleotides and compositions thereof comprise utilization of a chiral auxiliary, e.g., as part of monomeric phosphoramidites. Examples of such chiral auxiliary reagents and phosphoramidites are described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/223056, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the chiral auxiliary reagents and phosphoramidites of each of which are independently incorporated herein by reference. In certain embodiments, a chiral auxiliary is a chiral auxiliary described in any of: WO 2018/022473, WO 2018/098264, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO

2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the chiral auxiliaries of each of which are independently incorporated herein by reference.

In certain embodiments, chirally controlled preparation technologies, including oligonucleotide synthesis cycles, reagents and conditions are described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, and/WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO2019032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the oligonucleotide synthesis methods, cycles, reagents and conditions of each of which are independently incorporated herein by reference.

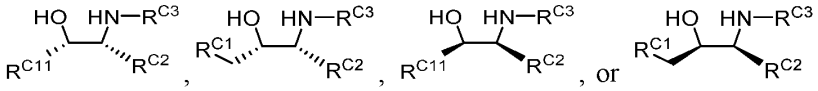
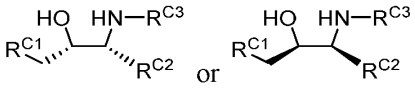
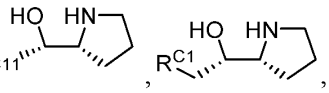
Once synthesized, provided ds oligonucleotides and compositions are typically further purified. Suitable purification technologies are widely known and practiced by those skilled in the art, including but not limited to those described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US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

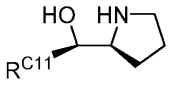
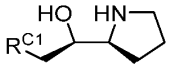
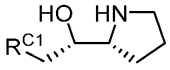
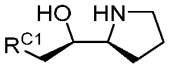
In certain embodiments, a cycle comprises or consists of coupling, capping, modification and deblocking. In certain embodiments, a cycle comprises or consists of coupling, capping, modification, capping and deblocking. These steps are typically performed in the order they are listed, but in certain embodiments, as appreciated by those skilled in the art, the order of certain steps, e.g., capping and modification, may be altered. If desired, one or more steps may be repeated to improve conversion, yield and/or purity as those skilled in the art often perform in syntheses. For example, in certain embodiments, coupling may be repeated; in certain embodiments, modification (e.g., oxidation to install =O, sulfurization to install =S, etc.) may be repeated; in certain embodiments, coupling is repeated after modification which can convert a P(III) linkage to a P(V) linkage which can

be more stable under certain circumstances, and coupling is routinely followed by modification to convert newly formed P(III) linkages to P(V) linkages. In certain embodiments, when steps are repeated, different conditions may be employed (e.g., concentration, temperature, reagent, time, etc.).

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

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

In certain embodiments, a useful chiral auxiliary has the structure of , or a salt thereof, wherein R^{C11} is $-L^{C1}-R^{C1}$, L^{C1} is optionally substituted $--CH_2-$. R^{C1} is R, $-Si(R)_3$, $-SO_2R$ or an electron-withdrawing group, and R^{C2} and R^{C3} are taken together with their intervening atoms to form an optionally substituted 3-10 membered saturated ring having, in addition to the nitrogen atom, 0-2 heteroatoms. In certain embodiments, a useful chiral auxiliary has the structure of  or , wherein R^{C1} is R, $-Si(R)_3$ or $-SO_2R$, and R^{C2} and R^{C3} are taken together with their intervening atoms to form an optionally substituted 3-7 membered saturated ring having, in addition to the nitrogen atom, 0-2 heteroatoms. is a formed ring is an optionally substituted 5-membered ring. In certain embodiments, a useful chiral auxiliary has the structure of ,

 or , or a salt thereof. In certain embodiments, a useful chiral auxiliary has the structure of  or . In certain embodiments, a useful chiral auxiliary is a DPSE chiral auxiliary. In certain embodiments, purity or stereochemical purity of a chiral auxiliary is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%. In certain embodiments, it is at least 85%. In certain embodiments, it is at least 90%. In certain embodiments, it is at least 95%. In certain embodiments, it is at least 96%. In certain embodiments, it is at least 97%. In certain embodiments, it is at least 98%. In certain embodiments, it is at least 99%.

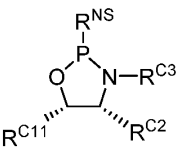
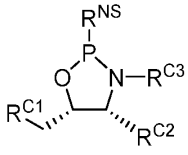
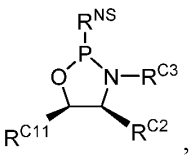
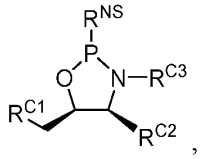
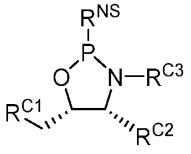
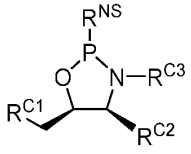
In certain embodiments, L^{C1} is $-CH_2-$. In certain embodiments, L^{C1} is substituted $-CH_2-$. In certain embodiments, L^{C1} is monosubstituted $-CH_2-$.

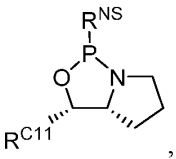
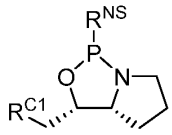
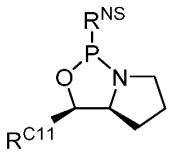
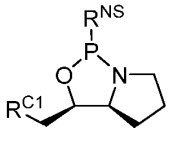
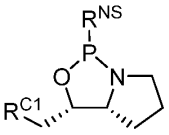
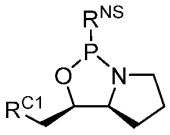
In certain embodiments, R^{C1} is R. In certain embodiments, R^{C1} is optionally substituted phenyl. In certain embodiments, R^{C1} is $-SiR_3$. In certain embodiments, R^{C1} is $-SiPh_2Me$. In certain embodiments, R^{C1} is $-SO_2R$. In certain embodiments, R is not hydrogen. In certain embodiments, R is optionally substituted phenyl. In certain embodiments, R is phenyl. In certain embodiments, R is optionally substituted C_{1-6} alphatic. In certain embodiments, R is C_{1-6} alkyl. In certain embodiments, R is methyl. In certain embodiments, R is t-butyl.

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

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

In certain embodiments, the present disclosure provides useful reagents for preparation of ds oligonucleotides and compositions thereof. In certain embodiments, phosphoramidites comprise nucleosides, nucleobases and sugars as described herein. In

certain embodiments, nucleobases and sugars are properly protected for oligonucleotide synthesis as those skilled in the art will appreciate. In certain embodiments, a phosphoramidite has the structure of R^{NS}–P(OR)N$(R)_2$, wherein R^{NS} is a optionally protected nucleoside moiety. In certain embodiments, a phosphoramidite has the structure of R^{NS}–P(OCH_2CH_2CN)N(i-Pr)$_2$. In certain embodiments, a phosphoramidite comprises a nucleobase which is or comprises Ring BA, wherein Ring BA has the structure of BA-I, BA-I-a, BA-I-b, BA-II, BA-II-a, BA-II-b, BA-III, BA-III-a, BA-III-b, BA-IV, BA-IV-a, BA-IV-b, BA-V, BA-V-a, BA-V-b, or BA-VI, or a tautomer of Ring BA, wherein the nucleobase is optionally substituted or protected. In certain embodiments, a phosphoramidite comprises a chiral auxiliary moiety, wherein the phosphorus is bonded to an oxygen and a nitrogen atom of the chiral auxiliary moiety. In certain embodiments, a phosphoramidite has the structure of , , , , or a salt thereof, wherein R^{NS} is a protected nucleoside moiety (e.g., 5'-OH and/or nucleobases suitably protected for oligonucleotide synthesis), and each other variable is independently as described herein. In certain embodiments, a phosphoramidite has the structure of  or , wherein R^{NS} is a protected nucleoside moiety (e.g., 5'-OH and/or nucleobases suitably protected for oligonucleotide synthesis), R^{C1} is R, $-Si(R)_3$ or $-SO_2R$, and R^{C2} and R^{C3} are taken together with their intervening atoms to form an optionally substituted 3-7 membered saturated ring having, in addition to the nitrogen atom, 0-2 heteroatoms, wherein the coupling forms an internucleotidic linkage. In certain embodiments, 5'-OH of R^{NS} is protected. In certain embodiments, 5'-OH of R^{NS} is protected as $-$ODMTr. In certain embodiments, R^{NS} is bonded to phosphorus through its 3'-O-. In certain embodiments, a formed ring by R^{C2} and R^{C3} is an optionally substituted 5-membered ring. In certain embodiments, a

phosphoramidite has the structure of , ,  or , or a salt thereof. In certain embodiments, a phosphoramidite has the structure of  or .

In certain embodiments, purity or stereochemical purity of a phosphoramidite is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99%. In certain embodiments, it is at least 85%. In certain embodiments, it is at least 90%. In certain embodiments, it is at least 95%.

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

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

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

W is O, S, or W^N;

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

Q^- is an anion;

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

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

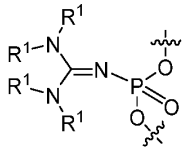
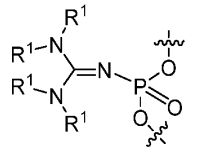
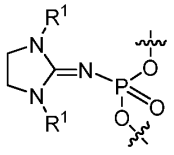
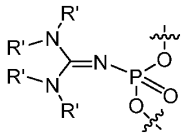
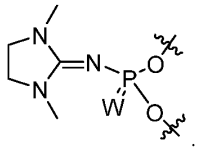
O^3 is an oxygen bonded to a 3'-carbon of a sugar.

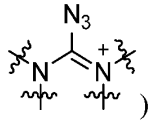
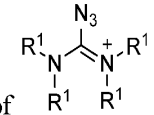
In certain embodiments, a modified internucleotidic linkage is optionally chirally controlled. In certain embodiments, a modified internucleotidic linkage is optionally chirally controlled.

In certain embodiments, a provided methods comprising removing R^{CA} from such a modified internucleotidic linkages. In certain embodiments, after removal, bonding to R^{CA} is replaced with −OH. In certain embodiments, after removal, bonding to R^{CA} is

replaced with =O, and bonding to W^N is replaced with $-N=C(N(R^1)_2)_2$.

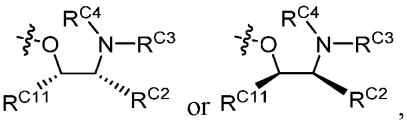
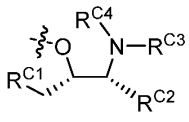
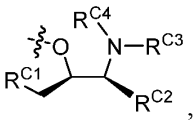
In certain embodiments, P^L is P=S, and when R^{CA} is removed, such an internucleotidic linkage is converted into a phosphorothioate internucleotidic linkage.

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

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

As appreciated by those skilled in the art, Q^- can be various suitable anion

present in a system (e.g., in oligonucleotide synthesis), and may vary during oligonucleotide preparation processes depending on cycles, process stages, reagents, solvents, etc. In certain embodiments, Q^- is PF_6^-.

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

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

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

8. Characterization and Assessment

In certain embodiments, properties and/or activities of dsRNAi oligonucleotides and compositions thereof can be characterized and/or assessed using various technologies available to those skilled in the art, e.g., biochemical assays, cell based assays, animal models, clinical trials, etc.

In certain embodiments, a method of identifying and/or characterizing an oligonucleotide composition, e.g., a dsRNAi oligonucleotide composition, comprises steps of: providing at least one composition comprising a plurality of oligonucleotides; and assessing delivery relative to a reference composition.

In certain embodiments, the present disclosure provides a method of identifying and/or characterizing a ds oligonucleotide composition, e.g., a dsRNAi oligonucleotide composition, comprises steps of: providing at least one composition comprising a plurality of ds oligonucleotides; and assessing cellular uptake relative to a reference composition.

In certain embodiments, the present disclosure provides a method of identifying and/or characterizing a ds oligonucleotide composition, e.g., a dsRNAi oligonucleotide composition, comprises steps of: providing at least one composition comprising a plurality of ds oligonucleotides; and assessing reduction of transcripts of a target gene and/or a product encoded thereby relative to a reference composition.

In certain embodiments, the present disclosure provides a method of identifying and/or characterizing a ds oligonucleotide composition, e.g., a dsRNAi oligonucleotide composition, comprises steps of: providing at least one composition comprising a plurality of ds oligonucleotides; and assessing reduction of tau levels, its aggregation and/or spreading relative to a reference composition.

In certain embodiments, properties and/or activities of ds oligonucleotides, e.g., dsRNAi oligonucleotides, and compositions thereof are compared to reference ds oligonucleotides and compositions thereof, respectively.

In certain embodiments, a reference ds oligonucleotide composition is a stereorandom ds oligonucleotide composition. In certain embodiments, a reference ds oligonucleotide composition is a stereorandom composition of ds oligonucleotides of which all internucleotidic linkages are phosphorothioate. In certain embodiments, a reference ds oligonucleotide composition is a ds DNA oligonucleotide composition with all phosphate linkages. In certain embodiments, a reference ds oligonucleotide composition is otherwise identical to a provided chirally controlled ds oligonucleotide composition except that it is not chirally controlled. In certain embodiments, a reference ds oligonucleotide composition is otherwise identical to a provided chirally controlled oligonucleotide composition except that it has a different pattern of stereochemistry. In certain embodiments, a reference ds oligonucleotide composition is similar to a provided ds oligonucleotide composition except that it has a different modification of one or more sugar, base, and/or internucleotidic linkage, or pattern of modifications. In certain embodiments, a ds oligonucleotide composition is stereorandom and a reference ds oligonucleotide composition is also stereorandom, but they differ in regard to sugar and/or base modification(s) or patterns thereof.

In certain embodiments, a reference composition is a composition of ds oligonucleotides having the same base sequence and the same chemical modifications. In certain embodiments, a reference composition is a composition of ds oligonucleotides having the same base sequence and the same pattern of chemical modifications. In certain

embodiments, a reference composition is a non-chirally controlled (or stereorandom) composition of ds oligonucleotides having the same base sequence and chemical modifications. In certain embodiments, a reference composition is a non-chirally controlled (or stereorandom) composition of ds oligonucleotides of the same constitution but is otherwise identical to a provided chirally controlled ds oligonucleotide composition.

In certain embodiments, a reference ds oligonucleotide composition is of ds oligonucleotides having a different base sequence. In certain embodiments, a reference ds oligonucleotide composition is of ds oligonucleotides that do not target RNAi (e.g., as negative control for certain assays).

In certain embodiments, a reference composition is a composition of ds oligonucleotides having the same base sequence but different chemical modifications, including but not limited to chemical modifications described herein. In certain embodiments, a reference composition is a composition of ds oligonucleotides having the same base sequence but different patterns of internucleotidic linkages and/or stereochemistry of internucleotidic linkages and/or chemical modifications.

Various methods are known in the art for detection of gene products, the expression, level and/or activity of which may be altered after introduction or administration of a provided ds oligonucleotide. For example, transcripts and their knockdown can be detected and quantified with qPCR, and protein levels can be determined via Western blot.

In certain embodiments, assessment of efficacy of ds oligonucleotides can be performed in biochemical assays or in vitro in cells. In certain embodiments, dsRNAi oligonucleotides can be introduced to cells via various methods available to those skilled in the art, e.g., gymnotic delivery, transfection, lipofection, etc.

In certain embodiments, the efficacy of a putative dsRNAi oligonucleotide can be tested in vitro.

In certain embodiments, the efficacy of a putative dsRNAi oligonucleotide can be tested in vitro using any known method of testing the expression, level and/or activity of a gene or gene product thereof.

In certain embodiments, dsRNAi soluble aggregates can be observed by immunoblotting.

In certain embodiments, a dsRNAi oligonucleotide is tested in a cell or animal model of a disease.

In certain embodiments, an animal model administered a dsRNAi oligonucleotide can be evaluated for safety and/or efficacy.

In certain embodiments, the effect(s) of administration of a ds oligonucleotide to an animal can be evaluated, including any effects on behavior, inflammation, and toxicity. In certain embodiments, following dosing, animals can be observed for signs of toxicity including trouble grooming, lack of food consumption, and any other signs of lethargy. In certain embodiments, in a mouse model, following administration of a dsRNAi oligonucleotide, the animals can be monitored for timing of onset of a rear paw clasping phenotype.

In certain embodiments, following administration of a dsRNAi oligonucleotide to an animal, the animal can be sacrificed and analysis of tissues or cells can be performed to determine changes in RNAi activity, or other biochemical or other changes. In certain embodiments, following necropsy, liver, heart, lung, kidney, and spleen can be collected, fixed, and processed for histopathological evaluation (standard light microscopic examination of hematoxylin and eosin-stained tissue slides).

In certain embodiments, following administration of a dsRNAi oligonucleotide to an animal, behavioral changes can be monitored or assessed. In certain embodiments, such an assessment can be performed using a technique described in the scientific literature.

Various effects of testing in animals described herein can also be monitored in human subjects or patients following administration of a dsRNAi oligonucleotide.

In addition, the efficacy of a dsRNAi oligonucleotide in a human subject can be measured by evaluating, after administration of the oligonucleotide, any of various parameters known in the art, including but not limited to a reduction in a symptom, or a decrease in the rate of worsening or onset of a symptom of a disease.

In certain embodiments, following human treatment with a ds oligonucleotide, or contacting a cell or tissue in vitro with an oligonucleotide, cells and/or tissues are collected for analysis.

In certain embodiments, in various cells and/or tissues, target nucleic acid levels can be quantitated by methods available in the art, many of which can be accomplished with commercially available kits and materials. Such methods include, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), quantitative real-time PCR, etc. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA.

Probes and primers are designed to hybridize to a nucleic acid to be detected. Methods for designing real-time PCR probes and primers are well known and widely practiced in the art. For example, to detect and quantify RNAi RNA, an example method comprises isolation of total RNA (e.g., including mRNA) from a cell or animal treated with an oligonucleotide or a composition and subjecting the RNA to reverse transcription and/or quantitative real-time PCR, for example, as described herein, or in: Moon et al. 2012 Cell Metab. 15: 240-246.

In certain embodiments, protein levels can be evaluated or quantitated in various methods known in the art, e.g., enzyme-linked immunosorbent assay (ELISA), Western blot analysis (immunoblotting), immunocytochemistry, fluorescence-activated cell sorting (FACS), immuno-histochemistry, immunoprecipitation, protein activity assays (for example, caspase activity assays), and quantitative protein assays. Antibodies useful for the detection of mouse, rat, monkey, and human proteins are commercially available or can be generated if needed. For example, various RNAi antibodies have been reported.

Various technologies are available and/or known in the art for detecting levels of ds oligonucleotides or other nucleic acids. Such technologies are useful for detecting dsRNAi oligonucleotides when administered to assess, e.g., delivery, cell uptake, stability, distribution, etc.

In certain embodiments, selection criteria are used to evaluate the data resulting from various assays and to select particularly desirable ds oligonucleotides, e.g., desirable dsRNAi oligonucleotides, with certain properties and activities. In certain embodiments, selection criteria include an IC_{50} of less than about 10 nM, less than about 5 nM or less than about 1 nM. In certain embodiments, selection criteria for a stability assay include at least 50% stability [at least 50% of an oligonucleotide is still remaining and/or detectable] at Day 1. In certain embodiments, selection criteria for a stability assay include at least 50% stability at Day 2. In certain embodiments, selection criteria for a stability assay include at least 50% stability at Day 3. In certain embodiments, selection criteria for a stability assay include at least 50% stability at Day 4. In certain embodiments, selection criteria for a stability assay include at least 50% stability at Day 5. In certain embodiments, selection criteria for a stability assay include at least 80% [at least 80% of the oligonucleotide remains] at Day 5.

In certain embodiments, efficacy of a dsRNAi oligonucleotide is assessed directly or indirectly by monitoring, measuring or detecting a change in a condition, disorder or disease or a biological pathway.

In certain embodiments, efficacy of a dsRNAi oligonucleotide is assessed directly or indirectly by monitoring, measuring or detecting a change in a response to be affected by knockdown.

In certain embodiments, a provided ds oligonucleotide (e.g., a dsRNAi oligonucleotide) can by analyzed by a sequence analysis to determine what other genes (e.g., genes which are not a target gene) have a sequence which is complementary to the base sequence of the provided ds oligonucleotide (e.g., the dsRNAi oligonucleotide) or which have 0, 1, 2 or more mismatches from the base sequence of the provided ds oligonucleotide (e.g., the dsRNAi oligonucleotide). Knockdown, if any, by the ds oligonucleotide of these potential off-targets can be determined to evaluate potential off-target effects of a ds oligonucleotide (e.g., a dsRNAi oligonucleotide). In certain embodiments, an off-target effect is also termed an unintended effect and/or related to hybridization to a bystander (non-target) sequence or gene.

In certain embodiments, a dsRNAi oligonucleotide which has been evaluated and tested for its ability to provide a particular biological effect (e.g., reduction of level, expression and/or activity of a target gene or a gene product thereof) can be used to treat, ameliorate and/or prevent a condition, disorder or disease.

9. Biologically active oligonucleotides

In certain embodiments, the present disclosure encompasses ds oligonucleotides which capable of acting as dsRNAi agents.

In certain embodiments, provided compositions include one or more oligonucleotides fully or partially complementary to a strand of: structural genes, genes control and/or termination regions, and/or self-replicating systems such as viral or plasmid DNA. In certain embodiments, provided compositions include one or more oligonucleotides that are or act as RNAi agents or other RNA interference reagents (RNAi agents or iRNA agents), shRNA, antisense oligonucleotides, self-cleaving RNAs, ribozymes, fragment thereof and/or variants thereof (such as Peptidyl transferase 23S rRNA, RNase P, Group I and Group II introns, GIR1 branching ribozymes, Leadzyme, Hairpin ribozymes, Hammerhead ribozymes, HDV ribozymes, Mammalian CPEB3 ribozyme, VS ribozymes, glmS ribozymes, CoTC ribozyme, etc.), microRNAs, microRNA mimics, supermirs, aptamers, antimirs, antagomirs, Ul adaptors, triplex-forming oligonucleotides, RNA activators, long non-coding RNAs, short non-coding RNAs (*e.g.*, piRNAs), immunomodulatory oligonucleotides (such as immunostimulatory oligonucleotides,

immunoinhibitory oligonucleotides), GNA, LNA, ENA, PNA, TNA, morpholinos, G-quadruplex (RNA and DNA), antiviral oligonucleotides, and decoy oligonucleotides.

In certain embodiments, provided compositions include one or more hybrid (*e.g.*, chimeric) oligonucleotides. In the context of the present disclosure, the term "hybrid" broadly refers to mixed structural elements of oligonucleotides. Hybrid oligonucleotides may refer to, for example, (1) an oligonucleotide molecule having mixed classes of nucleotides, *e.g.*, part DNA and part RNA within the single molecule (*e.g.*, DNA-RNA); (2) complementary pairs of nucleic acids of different classes, such that DNA:RNA base pairing occurs either intramolecularly or intermolecularly; or both; (3) an oligonucleotide with two or more kinds of the backbone or internucleotide linkages.

In certain embodiments, provided compositions include one or more oligonucleotide that comprises more than one classes of nucleic acid residues within a single molecule. For example, in any of the embodiments described herein, an oligonucleotide may comprise a DNA portion and an RNA portion. In certain embodiments, an oligonucleotide may comprise a unmodified portion and modified portion.

Provided ds oligonucleotide compositions can include oligonucleotides containing any of a variety of modifications, for example as described herein. In certain embodiments, particular modifications are selected, for example, in light of intended use. In certain embodiments, it is desirable to modify one or both strands of a double-stranded oligonucleotide (or a double-stranded portion of a single-stranded oligonucleotide). In certain embodiments, the two strands (or portions) include different modifications. In certain embodiments, the two strands include the same modifications. One of skill in the art will appreciate that the degree and type of modifications enabled by methods of the present disclosure allow for numerous permutations of modifications to be made. Examples of such modifications are described herein and are not meant to be limiting.

The phrase "antisense strand" or "guide strand" as used herein, refers to an oligonucleotide that is substantially or 100% complementary to a target sequence of interest. The phrase "antisense strand" or "guide strand" includes the antisense region of both oligonucleotides that are formed from two separate strands, as well as unimolecular oligonucleotides that are capable of forming hairpin or dumbbell type structures. In reference to a double-stranded RNAi agent such as a siRNA, the antisense strand is the strand preferentially incorporated into RISC, and which targets RISC-mediated knockdown of a RNA target. In reference to a double-stranded RNAi agent, the terms "antisense strand"

and "guide strand" are used interchangeably herein; and the terms "sense strand" or "passenger strand" are used interchangeably herein in reference to the strand which is not the antisense strand.

The phrase "sense strand" refers to an oligonucleotide that has the same nucleoside sequence, in whole or in part, as a target sequence such as a messenger RNA or a sequence of DNA.

By "target sequence" is meant any nucleic acid sequence whose expression or activity is to be modulated. The target nucleic acid can be DNA or RNA, such as endogenous DNA or RNA, viral DNA or viral RNA, or other RNA encoded by a gene, virus, bacteria, fungus, mammal, or plant. In certain embodiments, a target sequence is associated with a disease or disorder. In reference to RNA interference and RNase H-mediated knockdown, a target sequence is generally a RNA target sequence.

By "specifically hybridizable" and "complementary" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non- traditional types. In reference to the nucleic molecules of the present disclosure, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, *e.g.*, RNAi activity. Determination of binding free energies for nucleic acid molecules is well known in the art (see, *e.g.*, Turner et al, 1987, CSH Symp. Quant. Biol. LIT pp. 123-133; Frier et al., 1986, Proc. Nat. Acad. Sci. USA83:9373-9377; Turner et al., 1987, J. Am. Chem. Soc. 109:3783-3785)

A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (*e.g.*, Watson-Crick base pairing) with a second nucleic acid sequence (*e.g.*, 5, 6, 7, 8, 9,10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" or 100% complementarity means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. Less than perfect complementarity refers to the situation in which some, but not all, nucleoside units of two strands can hydrogen bond with each other. "Substantial complementarity" refers to polynucleotide strands exhibiting 90% or greater complementarity, excluding regions of the polynucleotide strands, such as overhangs, that are selected so as to be noncomplementary. Specific binding requires a sufficient degree of complementarity to avoid non-specific binding of the oligomeric compound to non-target

sequences under conditions in which specific binding is desired, *e.g.*, under physiological conditions in the case of *in vivo* assays or therapeutic treatment, or in the case of *in vitro* assays, under conditions in which the assays are performed. In certain embodiments, non-target sequences differ from corresponding target sequences by at least 5 nucleotides.

When used as therapeutics, a provided ds oligonucleotide is administered as a pharmaceutical composition. In certain embodiments, the pharmaceutical composition comprises a therapeutically effective amount of a provided oligonucleotide comprising, or a pharmaceutically acceptable salt thereof, and at least one pharmaceutically acceptable inactive ingredient selected from pharmaceutically acceptable diluents, pharmaceutically acceptable excipients, and pharmaceutically acceptable carriers. In certain embodiments, the pharmaceutical composition is formulated for intravenous injection, oral administration, buccal administration, inhalation, nasal administration, topical administration, ophthalmic administration or otic administration. In further embodiments, the pharmaceutical composition is a tablet, a pill, a capsule, a liquid, an inhalant, a nasal spray solution, a suppository, a suspension, a gel, a colloid, a dispersion, a suspension, a solution, an emulsion, an ointment, a lotion, an eye drop or an ear drop.

10. Administration of Oligonucleotides and Compositions

Many delivery methods, regimen, etc. can be utilized in accordance with the present disclosure for administering provided ds oligonucleotides and compositions thereof (typically pharmaceutical compositions for therapeutic purposes), including various technologies known in the art.

In certain embodiments, a ds oligonucleotide composition, e.g., a dsRNAi oligonucleotide composition, is administered at a dose and/or frequency lower than that of an otherwise comparable reference ds oligonucleotide composition and has comparable or improved effects. In certain embodiments, a chirally controlled ds oligonucleotide composition is administered at a dose and/or frequency lower than that of a comparable, otherwise identical stereorandom reference ds oligonucleotide composition and with comparable or improved effects, e.g., in improving the knockdown of the target transcript.

In certain embodiments, the present disclosure recognizes that properties and activities, e.g., knockdown activity, stability, toxicity, etc. of ds oligonucleotides and compositions thereof can be modulated and optimized by chemical modifications and/or stereochemistry. In certain embodiments, the present disclosure provides methods for optimizing ds oligonucleotide properties and/or activities through chemical modifications

and/or stereochemistry. In certain embodiments, the present disclosure provides ds oligonucleotides and compositions thereof with improved properties and/or activities. Without wishing to be bound by any theory, due to, e.g., their better activity, stability, delivery, distribution, toxicity, pharmacokinetic, pharmacodynamics and/or efficacy profiles, Applicant notes that provided ds oligonucleotides and compositions thereof in certain embodiments can be administered at lower dosage and/or reduced frequency to achieve comparable or better efficacy, and in certain embodiments can be administered at higher dosage and/or increased frequency to provide enhanced effects.

In certain embodiments, the present disclosure provides, in a method of administering a ds oligonucleotide composition comprising a plurality of ds oligonucleotides sharing a common base sequence, the improvement comprising administering a ds oligonucleotide comprising a plurality of ds oligonucleotides that is characterized by improved delivery relative to a reference ds oligonucleotide composition of the same common base sequence.

In certain embodiments, provided ds oligonucleotides, compositions and methods provide improved delivery. In certain embodiments, provided ds oligonucleotides, compositions and methods provide improved cytoplasmatic delivery. In certain embodiments, improved delivery is to a population of cells. In certain embodiments, improved delivery is to a tissue. In certain embodiments, improved delivery is to an organ. In certain embodiments, improved delivery is to an organism, e.g., a patient or subject. Example structural elements (e.g., chemical modifications, stereochemistry, combinations thereof, etc.), oligonucleotides, compositions and methods that provide improved delivery are extensively described in the present disclosure.

Various dosing regimens can be utilized to administer ds oligonucleotides and compositions of the present disclosure. In certain embodiments, multiple unit doses are administered, separated by periods of time. In certain embodiments, a given composition has a recommended dosing regimen, which may involve one or more doses. In certain 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 certain embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In certain embodiments, all doses within a dosing regimen are of the same unit dose amount. In certain embodiments, different doses within a dosing regimen are of different amounts. In certain 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 certain embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second (or subsequent) dose amount that is the same as or different from the first dose (or another prior dose) amount. In certain embodiments, a chirally controlled ds oligonucleotide composition is administered according to a dosing regimen that differs from that utilized for a non-chirally controlled (e.g., stereorandom) ds oligonucleotide composition of the same sequence, and/or of a different chirally controlled ds oligonucleotide composition of the same sequence. In certain embodiments, a chirally controlled ds oligonucleotide composition is administered according to a dosing regimen that is reduced as compared with that of a chirally uncontrolled (e.g., stereorandom) ds oligonucleotide composition of the same sequence in that it achieves a lower level of total exposure over a given unit of time, involves one or more lower unit doses, and/or includes a smaller number of doses over a given unit of time. In certain embodiments, a chirally uncontrolled ds oligonucleotide is administered according to a dosing regimen that extends for a longer period of time than does that of a chirally uncontrolled (e.g., stereorandom) ds oligonucleotide composition of the same sequence. Without wishing to be limited by theory, Applicant notes that in certain embodiments, the shorter dosing regimen, and/or longer time periods between doses, may be due to the improved stability, bioavailability, and/or efficacy of a chirally controlled ds oligonucleotide composition. In certain embodiments, with their improved delivery (and other properties), provided compositions can be administered in lower dosages and/or with lower frequency to achieve biological effects, for example, clinical efficacy.

11. Pharmaceutical Compositions

When used as therapeutics, a provided ds oligonucleotide, e.g., a dsRNAi oligonucleotide, or ds oligonucleotide composition thereof is typically administered as a pharmaceutical composition. In certain embodiments, the present disclosure provides pharmaceutical compositions comprising a provided compound, e.g., a ds oligonucleotide, or a pharmaceutically acceptable salt thereof, and a pharmaceutical carrier. In certain embodiments, for therapeutic and clinical purposes, ds oligonucleotides of the present disclosure are provided as pharmaceutical compositions. As appreciated by those skilled in the art, ds oligonucleotides of the present disclosure can be provided in their acid, base or salt forms. In certain embodiments, ds oligonucleotides can be in acid forms, e.g., for natural phosphate linkages, in the form of −OP(O)(OH)O−; for phosphorothioate

internucleotidic linkages, in the form of −OP(O)(SH)O−; etc. In certain embodiments, dsRNAi oligonucleotides can be in salt forms, e.g., for natural phosphate linkages, in the form of −OP(O)(ONa)O− in sodium salts; for phosphorothioate internucleotidic linkages, in the form of −OP(O)(SNa)O− in sodium salts; etc. Unless otherwise noted, ds oligonucleotides of the present disclosure can exist in acid, base and/or salt forms.

In certain embodiments, a pharmaceutical composition is a liquid composition. In certain embodiments, a pharmaceutical composition is provided by dissolving a solid ds oligonucleotide composition, or diluting a concentrated ds oligonucleotide composition, using a suitable solvent, e.g., water or a pharmaceutically acceptable buffer. In certain embodiments, liquid compositions comprise anionic forms of provided ds oligonucleotides and one or more cations. In certain embodiments, liquid compositions have pH values in the weak acidic, about neutral, or basic range. In certain embodiments, pH of a liquid composition is about a physiological pH, e.g., about 7.4.

In certain embodiments, a provided ds oligonucleotide is formulated for administration to and/or contact with a body cell and/or tissue expressing its target. For example, in certain embodiments, a provided dsRNAi oligonucleotide is formulated for administration to a body cell and/or tissue. In certain embodiments such a body cell and/or tissue is selected from the group consisting of: immune cells, blood cells, cardiac cells, lung cells, muscle cells, optic cells, liver cells, kidney cells, brain cells, cells of the central nervous system, and cells of the peripheral nervous system. In certain embodiments, such a body cell and/or tissue are a neuron or a cell and/or tissue of the liver. In certain embodiments, broad distribution of ds oligonucleotides and compositions may be achieved with intraparenchymal administration, intrathecal administration, or intracerebroventricular administration. In certain embodiments, the pharmaceutical composition is formulated for intravenous injection, oral administration, buccal administration, inhalation, nasal administration, topical administration, ophthalmic administration or optic administration. In certain embodiments, the pharmaceutical composition is a tablet, a pill, a capsule, a liquid, an inhalant, a nasal spray solution, a suppository, a suspension, a gel, a colloid, a dispersion, a suspension, a solution, an emulsion, an ointment, a lotion, an eye drop or an ear drop.

In certain embodiments, the present disclosure provides a pharmaceutical composition comprising chirally controlled ds oligonucleotide or composition thereof, in admixture with a pharmaceutically acceptable inactive ingredient (e.g., a pharmaceutically acceptable excipient, a pharmaceutically acceptable carrier, etc.). One of skill in the art will

recognize that the pharmaceutical compositions include pharmaceutically acceptable salts of provided ds oligonucleotide or compositions. In certain embodiments, a pharmaceutical composition is a chirally controlled ds oligonucleotide composition. In certain embodiments, a pharmaceutical composition is a stereopure ds oligonucleotide composition.

In certain embodiments, the present disclosure provides salts of ds oligonucleotides and pharmaceutical compositions thereof. In certain embodiments, a salt is a pharmaceutically acceptable salt. In certain embodiments, a pharmaceutical composition comprises a ds oligonucleotide, optionally in its salt form, and a sodium salt. In certain embodiments, a pharmaceutical composition comprises a ds oligonucleotide, optionally in its salt form, and sodium chloride. In certain embodiments, each hydrogen ion of a ds oligonucleotide that may be donated to a base (e.g., under conditions of an aqueous solution, a pharmaceutical composition, etc.) is replaced by a non-H^+ cation. For example, in certain embodiments, a pharmaceutically acceptable salt of a ds oligonucleotide is an all-metal ion salt, wherein each hydrogen ion (for example, of −OH, −SH, etc.) of each internucleotidic linkage (e.g., a natural phosphate linkage, a phosphorothioate internucleotidic linkage, etc.) is replaced by a metal ion. Various suitable metal salts for pharmaceutical compositions are widely known in the art and can be utilized in accordance with the present disclosure. In certain embodiments, a pharmaceutically acceptable salt is a sodium salt. In certain embodiments, a pharmaceutically acceptable salt is magnesium salt. In certain embodiments, a pharmaceutically acceptable salt is a calcium salt. In certain embodiments, a pharmaceutically acceptable salt is a potassium salt. In certain embodiments, a pharmaceutically acceptable salt is an ammonium salt (cation $N(R)_4^+$). In certain embodiments, a pharmaceutically acceptable salt comprises one and no more than one types of cation. In certain embodiments, a pharmaceutically acceptable salt comprises two or more types of cation. In certain embodiments, a cation is Li^+, Na^+, K^+, Mg^{2+} or Ca^{2+}. In certain embodiments, a pharmaceutically acceptable salt is an all-sodium salt. In certain embodiments, a pharmaceutically acceptable salt is an all-sodium salt, wherein each internucleotidic linkage which is a natural phosphate linkage (acid form −O−P(O)(OH)−O−), if any, exists as its sodium salt form (−O−P(O)(ONa)−O−), and each internucleotidic linkage which is a phosphorothioate internucleotidic linkage (acid form −O−P(O)(SH)−O−), if any, exists as its sodium salt form (−O−P(O)(SNa)−O−).

Various technologies for delivering nucleic acids and/or oligonucleotides are known in the art can be utilized in accordance with the present disclosure. For example, a

variety of supramolecular nanocarriers can be used to deliver nucleic acids. Example nanocarriers include, but are not limited to liposomes, cationic polymer complexes and various polymeric compounds. Complexation of nucleic acids with various polycations is another approach for intracellular delivery; this includes use of PEGylated polycations, polyethyleneamine (PEI) complexes, cationic block co-polymers, and dendrimers. Several cationic nanocarriers, including PEI and polyamidoamine dendrimers help to release contents from endosomes. Other approaches include use of polymeric nanoparticles, microspheres, liposomes, dendrimers, biodegradable polymers, conjugates, prodrugs, inorganic colloids such as sulfur or iron, antibodies, implants, biodegradable implants, biodegradable microspheres, osmotically controlled implants, lipid nanoparticles, emulsions, oily solutions, aqueous solutions, biodegradable polymers, poly(lactide-coglycolic acid), poly(lactic acid), liquid depot, polymer micelles, quantum dots and lipoplexes. In certain embodiments, a ds oligonucleotide is conjugated to another molecule.

In therapeutic and/or diagnostic applications, compounds, e.g., ds oligonucleotides, of the disclosure can be formulated for a variety of modes of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remington, The Science and Practice of Pharmacy (20th ed. 2000).

Pharmaceutically acceptable salts for basic moieties are generally well known to those of ordinary skill in the art, and may include, e.g., acetate, benzenesulfonate, besylate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, carnsylate, carbonate, citrate, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, mucate, napsylate, nitrate, pamoate (embonate), pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, or teoclate. Other pharmaceutically acceptable salts may be found in, for example, Remington, The Science and Practice of Pharmacy (20th ed. 2000). Preferred pharmaceutically acceptable salts include, for example, acetate, benzoate, bromide, carbonate, citrate, gluconate, hydrobromide, hydrochloride, maleate, mesylate, napsylate, pamoate (embonate), phosphate, salicylate, succinate, sulfate, or tartrate.

In certain embodiments, dsRNAi oligonucleotides are formulated in pharmaceutical compositions described in WO 2005/060697, WO 2011/076807 or WO 2014/136086.

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

For injection, provided agents, e.g., oligonucleotides may be formulated and diluted in aqueous solutions, such as in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulations. Such penetrants are generally known in the art and can be utilized in accordance with the present disclosure.

Use of pharmaceutically acceptable carriers to formulate compounds, e.g., provided ds oligonucleotides, for the practice of the disclosure into dosages suitable for various mods of administration is well known in the art. With proper choice of carrier and suitable manufacturing practice, compositions of the present disclosure, e.g., those formulated as solutions, may be administered via various routes, e.g., parenterally, such as by intravenous injection.

In certain embodiments, a composition comprising a dsRNAi oligonucleotide further comprises any or all of: calcium chloride dihydrate, magnesium chloride hexahydrate, potassium chloride, sodium chloride, sodium phosphate dibasic anhydrous, sodium phosphate, monobasic dihydrate, and/or water for Injection. In certain embodiments, a composition further comprises any or all of: calcium chloride dihydrate (0.21 mg) USP, magnesium chloride hexahydrate (0.16 mg) USP, potassium chloride (0.22

mg) USP, sodium chloride (8.77 mg) USP, sodium phosphate dibasic anhydrous (0.10 mg) USP, sodium phosphate monobasic dihydrate (0.05 mg) USP, and Water for Injection USP.

In certain embodiments, a composition comprising a ds oligonucleotide further comprises any or all of: cholesterol, (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate(DLin- MC3-DMA), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), alpha-(3'-{[1,2-di(myristyloxy)propanoxy]carbonylamino}propyl)-omega-methoxy, polyoxyethylene(PEG2000-C-DMG), potassium phosphate monobasic anhydrous NF, sodium chloride, sodium phosphate dibasic heptahydrate, and Water for Injection. In certain embodiments, the pH of a composition comprising a RNAi oligonucleotide is ~7.0. In certain embodiments, a composition comprising an oligonucleotide further comprises any or all of: 6.2 mg cholesterol USP, 13.0 mg (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate(DLin- MC3-DMA), 3.3 mg 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1.6 mg α-(3'-{[1,2- di(myristyloxy)propanoxy] carbonylamino}propyl)-ω-methoxy, polyoxyethylene(PEG2000-C-DMG), 0.2 mg potassium phosphate monobasic anhydrous NF, 8.8 mg sodium chloride USP, 2.3 mg sodium phosphate dibasic heptahydrate USP, and Water for Injection USP, in an approximately 1 mL total volume.

Provided compounds, e.g., ds oligonucleotides, can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. In certain embodiments, such carriers enable provided oligonucleotides to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for, e.g., oral ingestion by a subject (e.g., patient) to be treated.

For nasal or inhalation delivery, provided compounds, e.g., ds oligonucleotides, may be formulated by methods known to those of skill in the art, and may include, e.g., examples of solubilizing, diluting, or dispersing substances such as saline, preservatives, such as benzyl alcohol, absorption promoters, and fluorocarbons.

In certain embodiments, methods of specifically localizing provided compounds, e.g., ds oligonucleotides, such as by bolus injection, may decrease median effective concentration (EC50) by a factor of 20, 25, 30, 35, 40, 45 or 50. In certain embodiments, a targeted tissue is brain tissue. In certain embodiments, a targeted tissue is striatal tissue. In certain embodiments, decreasing EC50 is desirable because it reduces the dose required to achieve a pharmacological result in a patient in need thereof.

In certain embodiments, a provided ds oligonucleotide is delivered by injection or infusion once every month, every two months, every 90 days, every 3 months, every 6 months, twice a year or once a year.

Pharmaceutical compositions suitable for use in the present disclosure include compositions wherein the active ingredients, e.g., ds oligonucleotides, are contained in effective amounts to achieve their intended purposes. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

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

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

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

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin, and a plasticizer, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients, e.g., ds oligonucleotides, in admixture with fillers such as lactose, binders such as starches, and/or

lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, active compounds, e.g., ds oligonucleotides, may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols (PEGs). In addition, stabilizers may be added.

In certain embodiments, a provided composition comprises a lipid. In certain embodiments, a lipid is conjugated to an active compound, e.g., an oligonucleotide. In certain embodiments, a lipid is not conjugated to an active compound. In certain embodiments, a lipid comprises a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain. In certain embodiments, a lipid comprises a C_{10}-C_{40} linear, saturated or partially unsaturated, aliphatic chain, optionally substituted with one or more C_{1-4} aliphatic group. In certain embodiments, the 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 (cis-DHA), turbinaric acid and dilinoleyl alcohol. In certain embodiments, an active compound is a provided oligonucleotide. In certain embodiments, a composition comprises a lipid and an an active compound, and further comprises another component which is another lipid or a targeting compound or moiety. In certain embodiments, a lipid is an amino lipid; an amphipathic lipid; an anionic lipid; an apolipoprotein; a cationic lipid; a low molecular weight cationic lipid; a cationic lipid such as CLinDMA and DLinDMA; an ionizable cationic lipid; a cloaking component; a helper lipid; a lipopeptide; a neutral lipid; a neutral zwitterionic lipid; a hydrophobic small molecule; a hydrophobic vitamin; a PEG-lipid; an uncharged lipid modified with one or more hydrophilic polymers; phospholipid; a phospholipid such as 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; a stealth lipid; a sterol; a cholesterol; a targeting lipid; or another lipid described herein or reported in the art suitable for pharmaceutical uses. In certain embodiments, a composition comprises a lipid and a portion of another lipid capable of mediating at least one function of another lipid. In certain embodiments, a targeting compound or moiety is capable of targeting a compound (e.g., a ds oligonucleotide) to a particular cell or tissue or subset of cells or tissues. In certain embodiments, a targeting moiety is designed to take advantage of cell- or tissue-specific expression of particular targets, receptors, proteins, or another subcellular component. In certain embodiments, a targeting moiety is a ligand (e.g., a small molecule, antibody, peptide, protein, carbohydrate, aptamer, etc.) that targets a composition to a cell or tissue, and/or binds to a target, receptor, protein, or another subcellular component.

Certain example lipids for delivery of an active compound, e.g., a ds oligonucleotide, allow (e.g., do not prevent or interfere with) the function of an active compound. In certain embodiments, a lipid is lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid or dilinoleyl alcohol.

As described in the present disclosure, lipid conjugation, such as conjugation with fatty acids, may improve one or more properties of ds oligonucleotides.

In certain embodiments, a composition for delivery of an active compound, e.g., a ds oligonucleotide, is capable of targeting an active compound to particular cells or tissues as desired. In certain embodiments, a composition for delivery of an active compound is capable of targeting an active compound to a muscle cell or tissue. In certain embodiments, the present disclosure provides compositions and methods related to delivery of active compounds, wherein the compositions comprise an active compound and a lipid. In various embodiments to a hepatic cell or tissue, a lipid is selected from lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, gamma-linolenic acid, docosahexaenoic acid (cis-DHA), turbinaric acid and dilinoleyl alcohol.

In certain embodiments, a dsRNAi oligonucleotide is delivered to the central nervous or hepetic system, or a cell or tissue or portion thereof, via a delivery method or composition designed for delivery of nucleic acids to the central nervous or hepetic system, or a cell or tissue or portion thereof.

In certain embodiments, a dsRNAi oligonucleotide is delivered via a composition comprising any one or more of, or a method of delivery involving the use of any one or more of: transferrin receptor-targeted nanoparticle; cationic liposome-based delivery strategy; cationic liposome; polymeric nanoparticle; viral carrier; retrovirus; adeno-associated virus; stable nucleic acid lipid particle; polymer; cell-penetrating peptide; lipid; dendrimer; neutral lipid; cholesterol; lipid-like molecule; fusogenic lipid; hydrophilic molecule; polyethylene glycol (PEG) or a derivative thereof; shielding lipid; PEGylated lipid; PEG-C-DMSO; PEG-C- DMSA; DSPC; ionizable lipid; a guanidinium-based cholesterol derivative; ion-coated nanoparticle; metal-ion coated nanoparticle; manganese ion-coated nanoparticle; angubindin-1; nanogel; incorporation of the dsRNAi into a branched nucleic acid structure; and/or incorporation of the dsRNAi into a branched nucleic acid structure comprising 2, 3, 4 or more oligonucleotides.

In certain embodiments, a composition comprising a ds oligonucleotide is lyophilized. In certain embodiments, a composition comprising a ds oligonucleotide is lyophilized, and the lyophilized ds oligonucleotide is in a vial. In certain embodiments, the vial is back filled with nitrogen. In certain embodiments, the lyophilized ds oligonucleotide composition is reconstituted prior to administration. In certain embodiments, the lyophilized ds oligonucleotide composition is reconstituted with a sodium chloride solution prior to administration. In certain embodiments, the lyophilized ds oligonucleotide composition is reconstituted with a 0.9% sodium chloride solution prior to administration. In certain embodiments, reconstitution occurs at the clinical site for administration. In certain embodiments, in a lyophilized composition, a ds oligonucleotide composition is chirally controlled or comprises at least one chirally controlled internucleotidic linkage and/or the ds oligonucleotide targets.

II. EXEMPLIFICATION

Various technologies can be utilized to assess properties and/or activities of provided oligonucleotides and compositions thereof. Some such technologies are described in this Example. Those skilled in the art appreciate that many other technologies can be readily utilized. As demonstrated herein, provided oligonucleotides and compositions, among other things, can be highly active, e.g., in reducing levels of their target nucleic acids.

Certain examples of provided technologies (compounds (oligonucleotides, reagents, etc.), compositions, methods (methods of preparation, use, assessment, etc.), etc.) were presented herein.

EXAMPLE 1. Oligonucleotide Synthesis

Various technologies for preparing oligonucleotides and oligonucleotide compositions (both stereorandom and chirally controlled) are known and can be utilized in accordance with the present disclosure, including, for example, those in US 9394333, US 9744183, US 9605019, US 9598458, US 9982257, US 10160969, US 10479995, US 2020/0056173, US 2018/0216107, US 2019/0127733, US 10450568, US 2019/0077817, US 2019/0249173, US 2019/0375774, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, and/or WO 2019/032612, the methods and reagents of each of which are incorporated herein by reference. Stereorandom and chirally controlled

guide strand sequences were prepared utilizing the synthetic procedures as exemplified in above mentioned disclosures. Respective passenger strands were designed to have covalently linked GalNAc moiety as delivery vehicle at either end of sequences. Oligonucleotides with 5'-GalNAc modifications were synthesized by coupling C6-amino modifier linker at the 5'-end of sequence. Oligonucleotides with 3'-GalNAc moiety as delivery vehicle were synthesized by utilizing 3'-C6 amino modified support. The single strand was cleaved from CPG by using deprotection condition as exemplified in earlier disclosures. The resulting amino group containing crude oligonucleotide was purified by ion exchange chromatography on AKTA pure system using a sodium chloride gradient. Desired product was desalted and further used for conjugation with GalNAc acid. After conjugation reaction was found to be complete the material was further purified by ion exchange chromatography and desalted to achieve desired material. For introduction of PN linkages in guide and passenger strands, specific PN coupling cycles were introduced at desired positions in oligonucleotide sequence utilizing the conditions as exemplified in WO2019/200185.

In certain embodiments, oligonucleotides were prepared using suitable chiral auxiliaries, e.g., DPSE and PSM chiral auxiliaries. Various oligonucleotides, e.g., those in **Table 1A-1D**, and compositions thereof, were prepared in accordance with the present disclosure.

Various technologies can be utilized to assess properties and/or activities of provided oligonucleotides and compositions thereof. Some such technologies are described in this Example. Those skilled in the art appreciate that many other technologies can be readily utilized. As demonstrated herein, provided oligonucleotides and compositions, among other things, can be highly active, e.g., in reducing levels of their target nucleic acids.

EXAMPLE 2. Provided Oligonucleotides and Compositions Can Effectively Knockdown mouse Transthyretin (mTTR) and mouse Factor VII (mF7) in vitro.

Various siRNAs for mouse TTR or Factor VII were designed and constructed. A number of siRNAs were tested *in vitro* in mouse primary hepatocytes at one or a range of concentrations. Some siRNA were also tested in mice (e.g., C57BL6 wild type mice).

Example protocol for *in vitro* determination of siRNA activity: For determination of siRNAs activity, siRNAs at specific concentration were gymnotically

delivered to mouse primary hepatocytes plated at 96-well plates, with 10,000 cells/well. Following 48 hours treatment, total RNA was extracted using SV96 Total RNA Isolation kit (Promega). cDNA production from RNA samples were performed using High-Capacity cDNA Reverse Transcription kit (Thermo Fisher) following manufacturer's instructions and qPCR analysis performed in CFX System using iQ Multiplex Powermix (Bio-Rad). For mouse TTR mRNA, the following qPCR assay were utilized: IDT Taqman qPCR assay ID Mm.PT.58.11922308.

For mouse Factor VII mRNA, the following qPCR assay were utilized: Thermofisher Taqman qPCR assay ID Mm00487332_m1. Mouse HPRT was used as normalizer (Forward 5'CAAACTTTGCTTTCCCTGGTT3', Reverse 5'TGGCCTGTATCCAACACTTC3', Probe 5'/5HEX/ACCAGCAAG/Zen/CTTGCAACCTTAACC/3IABkFQ/3'. mRNA knockdown levels were calculated as %mRNA remaining relative to mock treatment.

Table 2 shows % mouse TTR mRNA remaining (at 500 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 2

		500 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/mHPRT)-2	Mean
WV-20167	WV-28310	40.38	32.27	36.32
WV-20169	WV-28311	42.91	34.94	38.93
WV-20170	WV-28311	28.64	34.78	31.71
WV-20171	WV-28311	25.11	30.15	27.63
WV-20172	WV-28311	27.00	30.52	28.76
WV-20173	WV-28311	54.68	60.68	57.68
WV-20174	WV-28311	36.47	38.24	37.36
WV-20175	WV-28311	46.82	44.90	45.86
WV-20176	WV-28311	41.86	40.67	41.27
WV-20177	WV-28311	50.98	61.96	56.47
WV-20178	WV-28311	28.99	34.79	31.89
WV-20179	WV-28311	28.88	37.07	32.98
WV-20180	WV-28311	N.D.	11.30	11.30

WV-20181	WV-28311	73.38	72.53	72.95
WV-20182	WV-28311	47.02	51.02	49.02
WV-20183	WV-28311	72.55	60.64	66.59
WV-20184	WV-28311	58.51	57.05	57.78
WV-20169	WV-28312	49.49	39.57	44.53
WV-20170	WV-28312	46.39	37.53	41.96
WV-20171	WV-28312	29.17	24.20	26.68
WV-20172	WV-28312	37.87	31.88	34.87
WV-20173	WV-28312	58.20	58.73	58.46
WV-20174	WV-28312	36.89	41.81	39.35
WV-20175	WV-28312	37.90	45.09	41.49
WV-20176	WV-28312	30.45	35.39	32.92
WV-20177	WV-28312	46.75	58.99	52.87
WV-20178	WV-28312	31.14	41.09	36.11
WV-20179	WV-28312	42.46	50.67	46.57
WV-20180	WV-28312	40.20	42.08	41.14
WV-20181	WV-28312	93.65	98.19	95.92
WV-20182	WV-28312	76.10	71.81	73.96
WV-20183	WV-28312	97.06	88.93	92.99
WV-20184	WV-28312	71.15	70.67	70.91
WV-20169	WV-28313	40.02	49.99	45.01
WV-20170	WV-28313	32.03	35.16	33.59
WV-20171	WV-28313	15.42	22.23	18.82
WV-20172	WV-28313	23.95	25.93	24.94
WV-20173	WV-28313	41.75	55.68	48.71
WV-20174	WV-28313	25.86	36.69	31.28
WV-20175	WV-28313	25.59	46.11	35.85
WV-20176	WV-28313	23.82	34.25	29.03
WV-20177	WV-28313	42.40	57.10	49.75
WV-20178	WV-28313	30.77	39.98	35.37
WV-20179	WV-28313	37.23	51.45	44.34
WV-20180	WV-28313	26.15	41.49	33.82

WV-20181	WV-28313	81.35	99.41	90.38
WV-20182	WV-28313	43.29	60.42	51.85
WV-20183	WV-28313	64.22	N.D.	64.22
WV-20184	WV-28313	52.24	N.D.	52.24
WV-20169	WV-28314	28.92	N.D.	28.92
WV-20170	WV-28314	25.36	N.D.	25.36
WV-20171	WV-28314	18.05	N.D.	18.05
WV-20172	WV-28314	20.50	N.D.	20.50
WV-20173	WV-28314	55.87	N.D.	55.87
WV-20174	WV-28314	32.63	N.D.	32.63
WV-20175	WV-28314	33.26	N.D.	33.26
WV-20176	WV-28314	28.50	N.D.	28.50
WV-20177	WV-28314	46.98	N.D.	46.98
WV-20178	WV-28314	24.87	N.D.	24.87
WV-20179	WV-28314	28.78	N.D.	28.78
WV-20180	WV-28314	24.43	33.41	28.92
WV-20181	WV-28314	80.17	72.26	76.22
WV-20182	WV-28314	36.55	52.66	44.60
WV-20183	WV-28314	69.35	86.28	77.82
WV-20184	WV-28314	46.26	68.01	57.14

Table 3 shows % mouse F7 mRNA remaining (at 150 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 3.

		150 pM		
Guide	Passenger	%remaining mRNA (mF7/mHPRT)-1	%remaining mRNA (mF7/mHPRT)-2	Mean
WV-34349	WV-34350	65.14	48.88	57.01
WV-34351	WV-34367	43.79	39.64	41.71
WV-34352	WV-34367	50.89	46.81	48.85
WV-34353	WV-34367	47.90	47.11	47.50
WV-34354	WV-34367	68.79	82.39	75.59
WV-34355	WV-34367	43.54	69.78	56.66

WV-34356	WV-34367	51.26	68.28	59.77
WV-34357	WV-34367	41.81	49.34	45.57
WV-34358	WV-34367	69.27	66.21	67.74
WV-34359	WV-34367	49.76	58.58	54.17
WV-34360	WV-34367	45.56	64.72	55.14
WV-34361	WV-34367	45.11	50.85	47.98
WV-34362	WV-34367	102.08	124.08	113.08
WV-34363	WV-34367	60.25	85.27	72.76
WV-34364	WV-34367	75.67	101.06	88.37
WV-34365	WV-34367	69.15	63.76	66.45
WV-34366	WV-34367	60.09	46.53	53.31
WV-34351	WV-34368	46.28	48.91	47.60
WV-34352	WV-34368	45.04	53.96	49.50
WV-34353	WV-34368	38.31	61.02	49.67
WV-34354	WV-34368	66.32	94.62	80.47
WV-34355	WV-34368	60.68	88.88	74.78
WV-34356	WV-34368	58.13	79.17	68.65
WV-34357	WV-34368	35.65	48.21	41.93
WV-34358	WV-34368	62.61	68.59	65.60
WV-34359	WV-34368	49.50	53.20	51.35
WV-34360	WV-34368	58.62	67.11	62.87
WV-34361	WV-34368	43.13	82.32	62.72
WV-34362	WV-34368	89.64	133.38	111.51
WV-34363	WV-34368	62.37	100.69	81.53
WV-34364	WV-34368	88.26	104.36	96.31
WV-34365	WV-34368	68.99	69.42	69.21
WV-34366	WV-34368	54.89	52.90	53.90
WV-34351	WV-34369	39.32	44.58	41.95
WV-34352	WV-34369	49.55	52.60	51.08
WV-34353	WV-34369	38.87	59.47	49.17
WV-34354	WV-34369	73.25	96.87	85.06
WV-34355	WV-34369	37.51	80.37	58.94

WV-34356	WV-34369	47.19	68.48	57.83
WV-34357	WV-34369	41.36	45.90	43.63
WV-34358	WV-34369	68.31	54.30	61.31
WV-34359	WV-34369	41.06	40.42	40.74
WV-34360	WV-34369	58.44	57.76	58.10
WV-34361	WV-34369	40.72	61.73	51.22
WV-34362	WV-34369	79.25	100.36	89.80
WV-34363	WV-34369	53.21	78.07	65.64
WV-34364	WV-34369	93.71	114.65	104.18
WV-34365	WV-34369	79.94	73.00	76.47
WV-34366	WV-34369	63.95	56.42	60.19
WV-34351	WV-34370	32.90	38.12	35.51
WV-34352	WV-34370	40.51	43.71	42.11
WV-34353	WV-34370	29.93	48.13	39.03
WV-34354	WV-34370	76.90	94.72	85.81
WV-34355	WV-34370	52.12	74.11	63.11
WV-34356	WV-34370	69.57	77.23	73.40
WV-34357	WV-34370	41.00	38.47	39.74
WV-34358	WV-34370	57.30	62.49	59.89
WV-34359	WV-34370	42.60	36.37	39.48
WV-34360	WV-34370	52.17	62.64	57.40
WV-34361	WV-34370	39.45	43.27	41.36
WV-34362	WV-34370	81.50	86.32	83.91
WV-34363	WV-34370	60.45	56.48	58.47
WV-34364	WV-34370	89.18	95.62	92.40
WV-34365	WV-34370	58.91	50.69	54.80
WV-34366	WV-34370	41.60	39.37	40.48

Table 4 shows % mouse TTR mRNA remaining (at 500, 150 and 50 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 4.

		500 pM			150 pM			50 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-20167	WV-28310	22.22	6.59	14.40	29.48	21.94	25.71	58.12	47.68	52.90
WV-36831	WV-28310	109.82	114.13	111.98	119.25	122.80	121.02	114.53	130.99	122.76
WV-36832	WV-28310	57.98	38.94	48.46	101.03	80.94	90.98	110.08	99.86	104.97
WV-36833	WV-28310	101.36	N.D.	101.36	108.87	N.D.	108.87	110.39	N.D.	110.39
WV-36834	WV-28310	96.91	111.96	104.44	119.42	112.86	116.14	117.68	120.44	119.06
WV-36835	WV-28310	104.97	94.56	99.76	133.50	128.15	130.82	120.56	115.19	117.88

WV-36836	WV-28310	96.51	85.30	90.90	135.00	105.41	120.20	114.44	123.74	119.09
WV-36837	WV-28310	46.94	35.51	41.22	74.49	82.60	78.55	97.29	103.88	100.59
WV-36838	WV-28310	20.95	5.35	13.15	40.83	9.24	25.03	66.52	28.78	47.65
WV-36839	WV-28310	34.07	14.35	24.21	71.33	22.97	47.15	84.87	55.24	70.05
WV-36840	WV-28310	31.35	10.35	20.85	59.08	22.62	40.85	86.17	48.16	67.17
WV-36841	WV-28310	30.04	N.D.	30.04	61.36	N.D.	61.36	74.19	N.D.	74.19
WV-36842	WV-28310	20.16	6.77	13.46	48.02	14.57	31.29	75.48	44.70	60.09
WV-36843	WV-28310	24.49	12.21	18.35	59.84	21.53	40.68	81.30	49.09	65.19
WV-36844	WV-28310	21.68	8.69	15.19	47.21	17.13	32.17	84.38	45.38	64.88
WV-36845	WV-28310	11.20	6.55	8.88	33.74	11.95	22.84	68.59	35.65	52.12
WV-	WV-	15.27	18.44	16.85	37.87	48.53	43.20	67.42	70.96	69.19

36846	28310									
WV-36847	WV-28310	15.04	17.97	16.51	42.44	48.18	45.31	65.69	73.10	69.39
WV-36848	WV-28310	N.D.	22.54	22.54	N.D.	47.52	47.52	N.D.	80.26	80.26
WV-36849	WV-28310	19.70	23.05	21.38	53.22	62.29	57.75	28.14	33.45	30.80
WV-36850	WV-28310	17.11	19.76	18.44	44.30	53.61	48.96	70.96	80.27	75.62
WV-36851	WV-28310	12.92	13.91	13.42	33.72	40.17	36.94	64.14	60.46	62.30
WV-36852	WV-28310	15.56	12.71	14.13	44.60	40.38	42.49	73.03	70.20	71.62
WV-36853	WV-28310	13.76	10.35	12.06	35.83	36.91	36.37	72.69	60.84	66.76
WV-36854	WV-28310	6.78	18.91	12.85	22.57	41.53	32.05	51.62	74.70	63.16
WV-36855	WV-28310	9.52	27.47	18.49	29.51	66.44	47.98	75.55	85.49	80.52
WV-36856	WV-28310	N.D.	27.95	27.95	N.D.	61.28	61.28	N.D.	96.91	96.91

WV-36857	WV-28310	31.39	53.30	42.34	72.19	84.15	78.17	105.17	110.88	108.02

Table 5 shows % mouse TTR mRNA remaining (at 500, 150 and 50 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 5.

		500 pM			150 pM			50 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-20167	WV-28310	10.08	7.51	8.79	24.67	20.11	22.39	33.37	48.14	40.75
WV-20167	WV-36860	8.06	11.00	9.53	25.33	19.35	22.34	48.14	46.24	47.19
WV-20167	WV-36861	8.80	8.69	8.74	31.28	25.15	28.21	62.43	38.86	50.64
WV-36838	WV-28310	15.92	9.65	12.79	32.43	21.28	26.85	47.04	52.61	49.82

WV-20167	WV-38058	12.26	9.79	11.03	42.11	31.88	37.00	76.07	45.21	60.64
WV-20167	WV-38059	11.34	11.38	11.36	34.62	37.11	35.87	70.58	49.21	59.89
WV-20167	WV-38060	15.35	14.04	14.69	41.68	43.36	42.52	76.82	60.03	68.43
WV-20167	WV-38061	15.03	15.46	15.25	39.02	44.24	41.63	69.35	57.06	63.21
WV-20167	WV-38062	20.83	19.31	20.07	29.25	43.66	36.45	77.17	68.49	72.83
WV-20167	WV-38063	14.46	18.71	16.59	28.23	42.18	35.20	61.75	60.94	61.35
WV-20167	WV-38064	14.59	9.34	11.96	27.66	22.07	24.86	53.07	47.44	50.26
WV-20167	WV-38065	25.98	12.58	19.28	46.64	33.51	40.08	65.63	52.02	58.82
WV-20167	WV-38066	22.61	17.76	20.19	50.34	41.48	45.91	67.89	49.85	58.87
WV-20167	WV-38067	37.19	30.41	33.80	48.85	62.55	55.70	68.47	64.60	66.53
WV-	WV-	67.15	66.08	66.61	92.36	85.59	88.98	121.38	88.36	104.87

20167	38068									
WV-20167	WV-38069	25.04	22.34	23.69	52.22	47.48	49.85	58.04	76.38	67.21
WV-20167	WV-38070	22.07	25.88	23.97	41.44	47.80	44.62	70.23	54.36	62.29
WV-20167	WV-38071	17.64	22.17	19.90	27.46	40.44	33.95	41.89	61.33	51.61
WV-20167	WV-38073	13.26	9.01	11.14	26.72	25.57	26.14	35.87	37.96	36.91
WV-20167	WV-38073	16.83	13.56	15.20	33.66	21.23	27.44	53.40	50.83	52.11
WV-20167	WV-38074	17.63	12.14	14.88	39.00	25.29	32.14	48.86	41.36	45.11
WV-20167	WV-38075	17.77	16.60	17.18	48.45	36.40	42.43	67.81	43.58	55.69
WV-20167	WV-38076	23.07	20.46	21.77	39.69	37.45	38.57	56.79	40.72	48.75
WV-20167	WV-38077	25.51	16.02	20.77	57.81	45.60	51.70	72.33	50.94	61.63
WV-20167	WV-38078	23.73	20.69	22.21	43.45	43.57	43.51	66.42	45.57	55.99

WV-20167	WV-38079	17.77	20.17	18.97	30.38	39.19	34.78	66.15	44.95	55.55

EXAMPLE 3. Provided Oligonucleotides and Compositions Are Active *in vivo*

In vivo determination of mouse TTR siRNA activity: All animal procedures were performed under IACUC guidelines at Biomere (Worcester, MA). Male 8-10 weeks of age C57BL/6 mice were dose at 2 or 6 mg/kg at desired oligonucleotide concentration on Day 1 by subcutaneous administration to the interscapular area. For interim blood collection, whole blood was collected by tail snip into serum separator tubes, and processed serum samples were kept at -70°C. Animals were euthanized on Day 8 by CO_2 asphyxiation followed by thoracotomy and terminal blood collection. Blood samples were collected by cardiac puncture into serum separator tubes, and processed serum samples were kept at -70°C. After cardiac perfusion with saline, liver samples were harvested and flash-frozen in dry ice. Mouse TTR protein concentration in the serum was assessed using the Mouse Prealbumin ELISA kit (Novus Biologicals or Crystal Chem) and following manufacturer's instructions.

Table 6 shows % mouse TTR protein remaining relative to PBS control. N = 5. N.D.: Not determined.

Table 6.

		2 mg/kg					
	PBS	WV-20167/28310		WV-20170/28314		WV-20183/28311	
animal No.	%remaining of mTTR protein	animal No.	%remaining of mTTR protein	animal No.	%remaining of mTTR protein	animal No.	%remaining of mTTR protein
1	86.15	6	30.68	11	21.79	16	55.76
2	119.24	7	25.31	12	24.02	17	55.04
3	96.87	8	34.07	13	18.46	18	54.99
4	101.21	9	39.21	14	20.92	19	64.25
5	96.54	10	26.47	15	20.56	20	72.68
Mean	100	Mean	31.15	Mean	21.15	Mean	60.54
6 mg/kg							
WV-20167/28310		WV-20170/28314		WV-20183/28311			
animal No.	%remaining of mTTR protein	animal No.	%remaining of mTTR protein	animal No.	%remaining of mTTR protein		
21	8.95	26	3.83	31	28.8		
22	5.7	27	4.33	32	34.6		
23	5.95	28	4.89	33	36.53		
24	5.97	29	3.94	34	39.44		

25	13.24	30	3.23	35	45.6		
Mean	7.96	Mean	4.04	Mean	36.99		

EXAMPLE 4. Provided Oligonucleotides and Compositions Are Active *in vivo* with longer duration

In vivo determination of mouse TTR siRNA activity: All animal procedures were performed under IACUC guidelines at Biomere (Worcester, MA). Male 8-10 weeks of age C57BL/6 mice were dose at 6 mg/kg at desired oligonucleotide concentration on Day 1 by subcutaneous administration to the interscapular area. Blood samples were collected on Day 8, 15, 22, 29, 36 and 43 by tail snip into serum separator tubes, and processed serum samples were kept at -70ºC. Mouse TTR protein concentration in the serum was assessed using the Mouse Prealbumin ELISA kit (Novus Biologicals) and following manufacturer's instructions.

Table 7. shows % mouse TTR protein remaining relative to PBS control. N = 5. N.D.: Not determined.

Table 7.

	PBS					
Day	animal1	animal2	animal3	animal4	animal5	Mean
1	135.65	112.62	77.03	76.9	97.79	100
8	117.21	115.99	76.18	74.14	116.47	100
15	119.9	108.19	59.91	83.16	128.84	100
22	109.62	101.21	80.95	87.12	121.1	100
29	142.01	87.52	75.09	79.65	115.73	100
36	115.25	115.35	105.23	71.22	92.96	100
43	106.86	113.19	116.54	68.79	94.62	100
	WV-20167/28310					
Day	animal6	animal7	animal8	animal9	animal10	Mean
1	120.7	106.43	124	121.65	119.73	118.5
8	10.26	14.68	8	12.66	9.6	11.04
15	33.38	43.97	35.94	36.62	30.37	36.06
22	67.29	83.77	86.46	89.26	63.58	78.07
29	97.59	112.79	36.38	111.62	90.79	89.83
36	96.33	107.97	101.59	117.86	108.02	106.35
43	88.85	110.11	119.21	132.27	104.66	111.02
	WV-20170/28314					
Day	animal11	animal12	animal13	animal14	animal15	Mean
1	111.59	109.64	122.69	138.83	81.47	112.84

8	N.D.	N.D.	3.36	N.D.	N.D.	3.36
15	13.28	14.84	20.28	13.69	16.64	15.75
22	37.05	46.55	66.57	33.01	31.41	42.92
29	70.68	75.3	82.14	41.87	114.55	76.91
36	88.77	79.44	85.91	66.93	N.D.	80.26
43	102.89	79.63	116.54	77.16	96.35	94.51

EXAMPLE 5. Provided Oligonucleotides and Compositions Can Effectively Knockdown mouse Transthyretin (mTTR) in vitro.

Various siRNAs for mouse TTR were designed and constructed. A number of siRNAs were tested *in vitro* in mouse primary hepatocytes at one or a range of concentrations. Some siRNAs were also tested in mice (e.g., C57BL6 wild type mice).

Example protocol for *in vitro* determination of siRNA activity: For determination of siRNAs activity, siRNAs at specific concentration were gymnotically delivered to mouse primary hepatocytes plated at 96-well plates, with 10,000 cells/well. Following 48 hours treatment, total RNA was extracted using SV96 Total RNA Isolation kit (Promega). cDNA production from RNA samples were performed using High-Capacity cDNA Reverse Transcription kit (Thermo Fisher) following manufacturer's instructions and qPCR analysis performed in CFX System using iQ Multiplex Powermix (Bio-Rad). For mouse TTR mRNA, the following qPCR assay were utilized: IDT Taqman qPCR assay ID Mm.PT.58.11922308. Mouse HPRT was used as normalizer (Forward 5'CAAACTTTGCTTTCCCTGGTT3', Reverse 5'TGGCCTGTATCCAACACTTC3', Probe 5'/5HEX/ACCAGCAAG/Zen/CTTGCAACCTTAACC/3IABkFQ/3'. mRNA knockdown levels were calculated as %mRNA remaining relative to mock treatment.

Table 8 shows % mouse TTR mRNA remaining (at 1000, 300 and 100 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 8.

		1000 pM			300 pM			100 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-20167	WV-40362	7.37	12.03	9.70	29.25	35.63	32.44	44.31	47.88	46.10
WV-20167	WV-40363	7.90	5.93	6.92	24.75	29.60	27.17	59.68	46.05	52.86
WV-20167	WV-40364	14.31	15.09	14.70	42.71	42.74	42.72	64.31	43.31	53.81
WV-20167	WV-40365	5.81	13.65	9.73	22.52	42.09	32.30	51.92	51.07	51.50
WV-20167	WV-40366	5.36	24.05	14.70	28.88	39.09	33.98	61.57	56.27	58.92
WV-20169	WV-40362	6.86	10.74	8.80	29.83	35.36	32.60	38.68	42.89	40.79
WV-20169	WV-40363	7.23	6.47	6.85	23.79	35.98	29.89	51.94	51.92	51.93
WV-20169	WV-40364	12.44	20.37	16.40	41.75	51.88	46.81	64.96	46.36	55.66
WV-20169	WV-40365	6.41	12.50	9.45	27.36	26.53	26.94	55.04	52.87	53.95
WV-20169	WV-40366	5.38	24.63	15.00	31.54	23.17	27.35	61.01	55.49	58.25
WV-20170	WV-40362	5.90	4.71	5.31	21.43	27.32	24.38	53.12	40.86	46.99
WV-20170	WV-40363	6.90	10.54	8.72	21.32	29.74	25.53	45.66	31.99	38.83
WV-20170	WV-40364	6.69	12.18	9.44	25.73	26.77	26.25	44.94	49.94	47.44
WV-20170	WV-40365	3.00	13.34	8.17	26.71	28.11	27.41	55.72	44.74	50.23

WV-20170	WV-40366	4.46	17.53	10.99	20.93	18.36	19.65	53.39	53.46	53.43
WV-20171	WV-40362	4.88	4.73	4.80	25.57	26.77	26.17	43.46	29.41	36.44
WV-20171	WV-40363	3.78	8.83	6.30	14.28	19.73	17.00	30.23	30.75	30.49
WV-20171	WV-40364	3.1	12.341	7.75	28.42	26.21	27.32	47.00	38.35	42.68
WV-20171	WV-40365	2.13	8.55	5.34	19.51	17.13	18.32	54.40	40.88	47.64
WV-20171	WV-40366	3.26	9.37	6.31	23.77	16.06	19.91	N.D.	41.10	41.10
WV-20172	WV-40362	4.81	6.33	5.57	26.55	47.86	37.21	44.67	48.51	46.59
WV-20172	WV-40363	2.21	8.71	5.46	24.50	15.80	20.15	37.06	35.63	36.35
WV-20172	WV-40364	2.82	10.5	6.66	18.34	21.90	20.12	54.86	62.99	58.92
WV-20172	WV-40365	2.75	8.14	5.45	21.56	12.57	17.07	43.04	35.57	39.30
WV-20172	WV-40366	3.43	7.93	5.68	29.41	20.77	25.09	43.06	40.82	41.94
WV-20173	WV-40362	8.09	19.75	13.92	57.44	25.78	41.61	79.01	74.59	76.80
WV-20173	WV-40363	4.65	15.43	10.04	30.91	32.20	31.56	61.93	54.63	58.28
WV-20173	WV-40364	14.58	32.45	23.51	62.63	53.15	57.89	84.18	76.66	80.42
WV-20173	WV-40365	4.72	N.D.	4.72	35.18	34.82	35.00	46.65	55.65	51.15
WV-20173	WV-40366	20.70	14.31	17.51	49.17	52.41	50.79	65.83	62.61	64.22
WV-20174	WV-40362	5.26	6.93	6.10	23.32	27.10	25.21	45.67	52.46	49.07
WV-20174	WV-40363	3.15	8.51	5.83	31.44	19.72	25.58	52.76	41.46	47.11
WV-20174	WV-40364	4.58	10.33	7.45	28.20	25.36	26.78	47.45	44.91	46.18
WV-20174	WV-40365	6.78	6.34	6.56	24.75	27.82	26.28	44.37	40.62	42.50
WV-20174	WV-40366	8.18	7.51	7.85	21.31	31.51	26.41	57.27	43.01	50.14
WV-20175	WV-40362	5.59	8.76	7.18	39.70	24.40	32.05	57.70	53.15	55.42

WV-20175	WV-40363	2.99	7.69	5.34	26.20	18.62	22.41	38.56	N.D.	38.56
WV-20175	WV-40364	12.09	14.45	13.27	47.72	49.23	48.48	57.02	58.36	57.69
WV-20175	WV-40365	6.81	6.23	6.52	28.22	29.90	29.06	58.91	44.24	51.58
WV-20175	WV-40366	17.21	21.39	19.30	48.81	42.27	45.54	65.46	42.50	53.98
WV-20176	WV-40362	4.54	5.89	5.21	21.80	20.41	21.11	40.18	31.97	36.07
WV-20176	WV-40363	5.60	8.32	6.96	26.17	26.17	26.17	38.71	41.56	40.13
WV-20176	WV-40364	7.85	6.72	7.28	25.34	37.89	31.62	62.22	52.50	57.36
WV-20176	WV-40365	6.15	11.28	8.71	27.66	31.41	29.54	53.06	28.81	40.94
WV-20176	WV-40366	6.28	16.62	11.44	26.05	63.25	44.65	48.94	53.11	51.02
WV-20177	WV-40362	12.35	18.22	15.28	49.76	55.57	52.67	61.16	66.35	63.75
WV-20177	WV-40363	9.83	10.05	9.94	35.66	44.99	40.33	72.85	63.27	68.06
WV-20177	WV-40364	35.24	42.42	38.83	95.13	81.27	88.20	87.67	64.74	76.20
WV-20177	WV-40365	7.77	20.49	14.13	37.26	27.12	32.19	62.42	60.16	61.29
WV-20177	WV-40366	18.87	51.23	35.05	59.02	21.57	40.29	72.05	72.96	72.50
WV-20178	WV-40362	6.70	8.67	7.69	26.32	31.37	28.84	50.68	55.46	53.07
WV-20178	WV-40363	6.52	12.27	9.39	34.41	30.05	32.23	47.08	36.72	41.90
WV-20178	WV-40364	6.97	16.00	11.48	33.08	52.60	42.84	54.91	53.81	54.36
WV-20178	WV-40365	4.41	15.90	10.16	26.30	32.79	29.55	49.41	44.61	47.013
WV-20178	WV-40366	5.44	N.D.	5.44	24.82	22.77	23.79	60.54	56.40	58.47
WV-20179	WV-40362	7.48	10.52	9.00	37.37	44.66	41.01	65.98	38.84	52.41
WV-20179	WV-40363	4.95	17.23	11.09	27.22	24.07	25.65	N.D.	48.08	48.08
WV-20179	WV-40364	10.16	31.42	20.79	61.13	28.58	44.86	76.64	68.51	72.58

WV-20179	WV-40365	3.38	N.D.	3.38	23.94	23.91	23.93	61.94	53.93	57.94
WV-20179	WV-40366	9.46	22.61	16.04	46.20	40.67	43.44	67.37	65.53	66.45
WV-20180	WV-40362	3.75	8.55	6.15	21.80	87.72	54.76	42.55	40.27	41.41
WV-20180	WV-40363	3.60	13.09	8.35	30.21	29.17	29.69	46.53	45.61	46.07
WV-20180	WV-40364	3.96	15.43	9.69	35.83	22.43	29.13	61.90	58.09	59.99
WV-20180	WV-40365	3.19	7.94	5.56	22.57	13.92	18.24	48.30	40.49	44.40
WV-20180	WV-40366	3.80	10.58	7.19	32.93	34.51	33.72	43.35	50.01	46.68
WV-20181	WV-40362	34.30	67.43	50.87	94.09	21.17	57.63	97.73	98.17	97.95
WV-20181	WV-40363	27.05	63.63	45.34	72.72	73.71	73.21	98.34	100.33	99.34
WV-20181	WV-40364	54.22	74.30	64.26	109.17	87.17	98.17	94.63	97.06	95.84
WV-20181	WV-40365	28.47	N.D.	28.47	73.41	70.01	71.71	90.12	98.63	94.38
WV-20181	WV-40366	63.14	44.48	53.81	91.57	87.11	89.34	106.33	108.89	107.61
WV-20182	WV-40362	7.41	17.25	12.33	34.45	31.90	33.17	67.80	66.10	66.95
WV-20182	WV-40363	6.14	14.28	10.21	42.49	N.D.	42.49	74.54	67.57	71.05
WV-20182	WV-40364	6.39	14.26	10.32	38.53	33.63	36.08	55.37	56.34	55.85
WV-20182	WV-40365	12.69	10.51	11.60	32.98	40.19	36.59	56.20	54.92	55.56
WV-20182	WV-40366	12.46	12.29	12.37	32.75	38.69	35.72	60.97	50.31	55.64
WV-20183	WV-40362	25.21	37.76	31.48	72.43	61.40	66.91	83.05	88.69	85.87
WV-20183	WV-40363	17.73	26.69	22.21	62.85	49.44	56.15	78.66	75.83	77.24
WV-20183	WV-40364	40.84	47.17	44.00	78.77	86.93	82.85	84.29	96.39	90.34
WV-20183	WV-40365	27.55	22.20	24.88	52.51	66.16	59.33	94.82	81.84	88.33
WV-20183	WV-40366	52.38	69.83	61.11	75.88	82.44	79.16	98.08	67.02	82.55

WV-20184	WV-40362	10.09	13.38	11.73	35.80	32.74	34.27	61.83	51.98	56.91
WV-20184	WV-40363	7.73	8.86	8.30	31.68	40.66	36.17	52.06	55.34	53.70
WV-20184	WV-40364	16.73	13.37	15.05	36.47	50.37	43.42	71.54	68.46	70.00
WV-20184	WV-40365	9.49	10.73	10.11	42.21	N.D.	42.21	63.87	45.73	54.80
WV-20184	WV-40366	8.19	23.03	15.61	37.39	34.04	35.72	62.51	66.27	64.39

Table 9 shows % IC50 of knocking down mouse TTR mRNA in mouse primary hepatocyte

Table 9

Guide	Passenger	IC50 (pM)	95% CI
WV-39295	WV-40363	33.72	25.41 to 44.79
WV-39296	WV-40363	28.64	23.72 to 34.58
WV-20167	WV-40363	16.62	13.35 to 20.67
WV-39297	WV-40363	425.2	163.0 to 1232
WV-39298	WV-40363	27.80	17.98 to 42.99
WV-20169	WV-40363	11.29	7.58 to 16.74
WV-39299	WV-40363	12.61	7.81 to 20.42
WV-39300	WV-40363	21.50	15.52 to 29.79
WV-20170	WV-40363	12.14	9.59 to 15.36

Table 10 shows % mouse TTR mRNA remaining (at 1500, 500 and 150 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 10.

		1500 pM			500 pM			150 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-20167	WV-28310	3.95	3.95	3.95	16.45	18.80	17.63	58.43	54.97	56.70
WV-20170	WV-28314	1.91	2.22	2.07	6.07	5.39	5.73	35.83	31.25	33.54
WV-38082	WV-28314	5.69	4.75	5.22	16.55	14.85	15.70	65.38	54.54	59.96
WV-38083	WV-28314	3.87	3.09	3.48	13.70	17.40	15.55	63.65	47.59	55.62
WV-38084	WV-28314	6.57	4.57	5.57	20.56	20.07	20.32	70.25	55.57	62.91
WV-38085	WV-28314	3.60	2.74	3.17	12.69	8.46	10.57	53.93	39.07	46.50
WV-38086	WV-28314	3.24	2.27	2.76	17.34	8.15	12.75	49.15	40.93	45.04
WV-38087	WV-28314	1.89	0.86	1.38	6.67	3.98	5.32	25.94	17.79	21.87
WV-38088	WV-28314	2.29	1.86	2.08	9.67	4.44	7.05	36.35	23.50	29.92
WV-38089	WV-28314	1.75	1.75	1.75	N.D.	4.82	4.82	55.75	24.67	40.21
WV-38090	WV-28314	4.35	4.77	4.56	16.40	13.58	14.99	58.83	48.66	53.74
WV-38091	WV-28314	3.96	3.53	3.74	17.65	15.23	16.44	55.09	47.10	51.10
WV-38092	WV-28314	4.94	3.28	4.11	13.17	11.28	12.22	53.21	43.48	48.34
WV-38093	WV-28314	3.85	3.14	3.50	15.40	12.17	13.78	51.71	39.78	45.75
WV-38094	WV-28314	3.40	2.72	3.06	13.40	9.34	11.37	40.87	34.08	37.47

WV-38095	WV-28314	2.94	2.29	2.61	17.64	10.81	14.23	51.66	34.35	43.00
WV-38096	WV-28314	3.63	3.01	3.32	12.13	8.10	10.11	54.01	33.26	43.64
WV-38097	WV-28314	3.13	2.07	2.60	30.86	9.96	20.41	62.62	32.88	47.75
WV-38098	WV-28314	3.21	3.59	3.40	13.02	11.91	12.47	51.93	39.34	45.63
WV-38099	WV-28314	3.63	3.27	3.45	15.62	13.69	14.65	54.14	50.27	52.20
WV-38100	WV-28314	4.12	3.40	3.76	12.06	14.09	13.08	56.83	47.30	52.06
WV-38101	WV-28314	4.50	4.57	4.54	16.52	13.32	14.92	56.39	50.58	53.48
WV-38102	WV-28314	5.33	3.42	4.38	18.15	10.30	14.22	47.72	47.80	47.76
WV-38103	WV-28314	4.67	2.98	3.82	20.99	12.71	16.85	54.94	40.61	47.77
WV-38104	WV-28314	3.89	3.53	3.71	17.26	9.52	13.39	54.91	39.61	47.26
WV-38105	WV-28314	3.16	1.95	2.55	N.D.	8.64	8.64	54.50	27.77	41.13
WV-38106	WV-28314	4.34	4.03	4.19	15.63	15.33	15.48	62.14	47.23	54.69
WV-38107	WV-28314	2.22	1.83	2.03	8.48	8.65	8.56	44.25	31.27	37.76
WV-38108	WV-28314	4.64	3.53	4.08	14.78	13.13	13.95	60.80	41.51	51.16
WV-38109	WV-28314	3.25	2.96	3.11	13.23	11.55	12.39	53.71	44.86	49.28
WV-38110	WV-28314	5.56	4.11	4.84	18.41	9.33	13.87	51.87	44.41	48.14
WV-38111	WV-28314	3.99	3.18	3.59	17.15	10.19	13.67	52.16	38.23	45.20
WV-38112	WV-28314	3.82	3.31	3.57	13.62	9.63	11.62	52.34	35.13	43.74
WV-38113	WV-28314	3.73	2.43	3.08	23.73	9.66	16.69	47.05	42.22	44.64
WV-38114	WV-28314	4.51	3.81	4.16	14.16	18.70	16.43	61.69	63.97	62.83
WV-38115	WV-28314	3.13	2.76	2.94	13.96	13.88	13.92	47.65	41.83	44.74
WV-38116	WV-28314	4.63	2.88	3.76	10.39	11.82	11.11	50.04	43.95	46.99
WV-38117	WV-28314	3.14	2.76	2.95	9.44	10.25	9.84	47.09	40.57	43.83
WV-38118	WV-28314	5.04	3.51	4.27	14.72	7.73	11.23	49.19	42.76	45.97
WV-38119	WV-28314	2.63	2.20	2.41	15.54	11.01	13.27	45.33	31.55	38.44
WV-38120	WV-28314	5.02	3.21	4.11	13.31	8.80	11.05	48.51	37.27	42.89

WV-38121	WV-28314	2.60	2.28	2.44	18.27	8.05	13.16	47.10	31.86	39.48
WV-38122	WV-28314	4.14	4.81	4.48	11.45	16.14	13.80	50.63	51.86	51.24
WV-38123	WV-28314	3.79	3.26	3.52	15.64	15.43	15.54	55.79	48.60	52.19
WV-38124	WV-28314	5.25	3.95	4.60	13.32	12.48	12.90	56.94	52.72	54.83
WV-38125	WV-28314	3.62	3.43	3.53	16.42	15.46	15.94	53.97	49.01	51.49
WV-38126	WV-28314	5.30	3.65	4.47	12.21	8.29	10.25	50.01	43.21	46.61
WV-38127	WV-28314	2.04	1.46	1.75	9.47	5.67	7.57	37.00	19.09	28.04
WV-38128	WV-28314	3.97	3.42	3.70	10.60	8.87	9.73	43.75	26.64	35.19
WV-38129	WV-28314	2.35	1.36	1.85	10.69	5.45	8.07	35.88	24.05	29.96
WV-38130	WV-28314	4.64	5.20	4.92	10.76	16.56	13.66	66.33	59.81	63.07
WV-38131	WV-28314	3.55	3.21	3.38	11.19	12.45	11.82	53.37	44.39	48.88
WV-38132	WV-28314	4.01	4.24	4.13	9.46	9.69	9.57	48.70	44.22	46.46
WV-38133	WV-28314	3.32	2.83	3.07	11.60	13.72	12.66	51.97	48.79	50.38
WV-38134	WV-28314	5.04	2.90	3.97	16.70	9.51	13.11	49.46	43.88	46.67
WV-38135	WV-28314	2.93	2.90	2.91	17.14	12.61	14.88	48.48	40.59	44.53
WV-38136	WV-28314	4.28	3.31	3.80	9.80	7.35	8.58	48.77	31.64	40.21
WV-38137	WV-28314	3.01	1.84	2.42	12.94	5.68	9.31	42.95	24.14	33.55
WV-38138	WV-28314	3.66	4.40	4.03	9.00	11.25	10.13	56.14	51.22	53.68
WV-38139	WV-28314	2.86	3.05	2.95	10.59	10.68	10.64	49.76	46.66	48.21
WV-38140	WV-28314	3.64	3.66	3.65	10.49	10.04	10.27	50.79	49.06	49.93
WV-38141	WV-28314	2.80	2.46	2.63	14.16	14.84	14.50	53.33	46.05	49.69
WV-38142	WV-28314	4.19	3.45	3.82	13.65	8.27	10.96	49.46	43.94	46.70
WV-38143	WV-28314	3.46	2.57	3.01	16.07	13.16	14.61	50.83	38.52	44.68
WV-38144	WV-28314	4.31	3.43	3.87	12.68	8.17	10.42	50.41	28.88	39.65
WV-38145	WV-28314	2.33	1.89	2.11	13.52	4.59	9.06	40.04	22.05	31.05
WV-38146	WV-28314	3.34	3.98	3.66	9.09	9.36	9.22	49.18	47.55	48.37

Table 11 shows % mouse TTR mRNA remaining (at 300 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 11.

		300 pM		
Guide	Passenger	%remaining mRNA (mF7/mHPRT)-1	%remaining mRNA (mF7/mHPRT)-2	Mean
WV-20167	WV-36860	4.96	7.55	6.25
WV-20170	WV-36860	4.66	4.30	4.48
WV-36980	WV-36860	10.45	12.15	11.30
WV-36981	WV-36860	11.70	17.11	14.40
WV-36982	WV-36860	15.92	18.13	17.03
WV-36983	WV-36860	13.71	15.55	14.63
WV-36984	WV-36860	11.85	18.24	15.04
WV-36985	WV-36860	12.44	14.93	13.69
WV-36986	WV-36860	18.07	18.17	18.12
WV-36987	WV-36860	17.20	18.34	17.77
WV-36988	WV-36860	10.55	14.15	12.35
WV-36989	WV-36860	14.72	20.00	17.36
WV-36990	WV-36860	22.40	28.32	25.36
WV-36991	WV-36860	16.07	21.66	18.87
WV-36992	WV-36860	N.D.	25.82	25.82
WV-36993	WV-36860	19.83	20.94	20.39
WV-36994	WV-36860	22.87	29.77	26.32
WV-36995	WV-36860	12.82	19.02	15.92
WV-36996	WV-36860	7.65	10.26	8.95
WV-36997	WV-36860	11.30	15.70	13.50
WV-36998	WV-36860	20.41	24.08	22.25
WV-36999	WV-36860	10.21	14.35	12.28
WV-37000	WV-36860	10.37	22.91	16.64
WV-37001	WV-36860	14.19	13.64	13.91
WV-37002	WV-36860	15.51	18.74	17.12

WV-37003	WV-36860	14.41	16.53	15.47
WV-37004	WV-36860	13.00	17.81	15.40
WV-37005	WV-36860	18.85	24.90	21.88
WV-37006	WV-36860	30.86	37.82	34.34
WV-37007	WV-36860	14.46	28.59	21.52
WV-37008	WV-36860	19.63	33.41	26.52
WV-37009	WV-36860	21.78	24.35	23.07
WV-37010	WV-36860	28.91	32.02	30.46
WV-37011	WV-36860	14.68	18.32	16.50
WV-37012	WV-36860	9.58	13.44	11.51
WV-37013	WV-36860	12.23	15.55	13.89
WV-37014	WV-36860	13.76	19.67	16.71
WV-37015	WV-36860	8.07	11.92	9.99
WV-37016	WV-36860	9.08	16.83	12.96
WV-37017	WV-36860	9.89	13.94	11.91
WV-37018	WV-36860	13.06	15.27	14.16
WV-37019	WV-36860	17.71	21.43	19.57
WV-37020	WV-36860	11.88	16.12	14.00
WV-37021	WV-36860	17.48	21.88	19.68
WV-37022	WV-36860	27.35	32.90	30.12
WV-37023	WV-36860	14.85	27.62	21.24
WV-37024	WV-36860	20.58	28.68	24.63
WV-37025	WV-36860	22.78	22.02	22.40
WV-37026	WV-36860	23.29	27.62	25.46
WV-37027	WV-36860	11.40	14.66	13.03
WV-37028	WV-36860	9.12	12.89	11.01
WV-37029	WV-36860	14.10	15.81	14.95
WV-37030	WV-36860	18.51	23.80	21.15
WV-37031	WV-36860	12.70	12.94	12.82
WV-37032	WV-36860	12.11	21.10	16.61
WV-37033	WV-36860	19.41	16.91	18.16
WV-37034	WV-36860	20.27	19.93	20.10
WV-37035	WV-36860	29.52	30.52	30.02

WV-37036	WV-36860	20.19	25.96	23.08
WV-37037	WV-36860	25.20	33.74	29.47
WV-37038	WV-36860	37.69	52.95	45.32
WV-37039	WV-36860	26.60	32.58	29.59
WV-37040	WV-36860	26.40	45.42	35.91
WV-37041	WV-36860	39.05	31.95	35.50
WV-37042	WV-36860	39.92	35.74	37.83
WV-37043	WV-36860	16.06	20.28	18.17
WV-37044	WV-36860	8.92	11.41	10.17
WV-37045	WV-36860	13.40	14.47	13.94
WV-37046	WV-36860	14.78	20.31	17.54
WV-37047	WV-36860	11.98	18.60	15.29
WV-37048	WV-36860	10.16	18.77	14.46
WV-37049	WV-36860	15.14	14.47	14.80
WV-37050	WV-36860	14.01	15.40	14.71
WV-37051	WV-36860	17.92	19.96	18.94
WV-37052	WV-36860	11.95	16.30	14.13
WV-37053	WV-36860	20.31	22.86	21.58
WV-37054	WV-36860	22.15	31.45	26.80
WV-37055	WV-36860	19.01	21.43	20.22
WV-37056	WV-36860	17.25	29.98	23.61
WV-37057	WV-36860	20.18	22.55	21.36
WV-37058	WV-36860	21.88	22.30	22.09
WV-37059	WV-36860	22.52	20.27	21.40
WV-37060	WV-36860	7.08	9.99	8.53
WV-37061	WV-36860	17.59	18.37	17.98
WV-37062	WV-36860	17.97	25.49	21.73
WV-37063	WV-36860	13.41	15.27	14.34
WV-37064	WV-36860	11.58	20.70	16.14
WV-37065	WV-36860	22.02	19.12	20.57
WV-37066	WV-36860	24.22	26.13	25.17
WV-37067	WV-36860	29.39	26.95	28.17
WV-37068	WV-36860	19.04	18.36	18.70

WV-37069	WV-36860	29.43	28.57	29.00
WV-37070	WV-36860	35.17	47.16	41.17
WV-37071	WV-36860	18.85	19.74	19.29
WV-37072	WV-36860	29.00	19.56	24.28
WV-37073	WV-36860	32.87	28.97	30.92
WV-37074	WV-36860	34.42	33.66	34.04
WV-37075	WV-36860	18.54	17.28	17.91
WV-37076	WV-36860	18.08	18.80	18.44
WV-37077	WV-36860	20.97	19.73	20.35
WV-37078	WV-36860	17.84	16.48	17.16
WV-37079	WV-36860	16.73	11.51	14.12
WV-37080	WV-36860	17.05	14.78	15.91
WV-37081	WV-36860	16.89	16.25	16.57
WV-37082	WV-36860	15.86	16.90	16.38
WV-37083	WV-36860	32.12	30.15	31.14
WV-37084	WV-36860	29.69	30.52	30.10
WV-37085	WV-36860	43.72	42.64	43.18
WV-37086	WV-36860	37.71	40.91	39.31
WV-37087	WV-36860	37.78	28.62	33.20
WV-37088	WV-36860	42.60	40.64	41.62
WV-37089	WV-36860	47.89	38.09	42.99
WV-37090	WV-36860	31.94	35.73	33.84
WV-37091	WV-36860	20.27	17.99	19.13
WV-37092	WV-36860	16.69	13.96	15.32
WV-37093	WV-36860	27.10	24.23	25.67
WV-37094	WV-36860	18.15	24.11	21.13
WV-37095	WV-36860	23.06	16.50	19.78
WV-37096	WV-36860	21.05	20.91	20.98
WV-37097	WV-36860	28.37	29.09	28.73
WV-37098	WV-36860	22.55	19.14	20.85
WV-37099	WV-36860	35.95	38.93	37.44
WV-37100	WV-36860	48.24	43.85	46.05
WV-37101	WV-36860	62.03	52.51	57.27

WV-37102	WV-36860	63.45	54.72	59.08
WV-37103	WV-36860	50.88	43.76	47.32
WV-37104	WV-36860	53.22	55.81	54.51
WV-37105	WV-36860	62.45	59.58	61.02
WV-37106	WV-36860	55.27	51.08	53.17
WV-37107	WV-36860	28.10	21.88	24.99
WV-37108	WV-36860	27.57	22.02	24.80
WV-37109	WV-36860	35.51	29.47	32.49
WV-37110	WV-36860	20.08	21.07	20.57
WV-37111	WV-36860	45.48	30.09	37.79
WV-37112	WV-36860	26.92	23.32	25.12
WV-37113	WV-36860	42.08	36.71	39.39
WV-37114	WV-36860	27.89	20.92	24.40
WV-37115	WV-36860	45.99	40.94	43.46
WV-37116	WV-36860	52.11	39.68	45.90
WV-37117	WV-36860	59.72	58.26	58.99
WV-37118	WV-36860	59.87	52.26	56.07
WV-37119	WV-36860	66.99	43.20	55.09
WV-37120	WV-36860	53.22	38.95	46.08
WV-37121	WV-36860	77.16	68.69	72.92
WV-37122	WV-36860	62.07	52.14	57.11
WV-37123	WV-36860	29.03	27.82	28.43
WV-37124	WV-36860	31.49	26.84	29.17
WV-37125	WV-36860	33.05	29.99	31.52
WV-37126	WV-36860	29.24	27.84	28.54
WV-37127	WV-36860	26.69	24.59	25.64
WV-37128	WV-36860	24.17	21.53	22.85
WV-37129	WV-36860	32.63	29.59	31.11
WV-37130	WV-36860	34.42	22.15	28.28
WV-37131	WV-36860	36.55	32.52	34.53
WV-37132	WV-36860	41.37	35.96	38.67
WV-37133	WV-36860	61.38	54.90	58.14
WV-37134	WV-36860	59.51	49.42	54.46

WV-37135	WV-36860	51.57	43.19	47.38
WV-37136	WV-36860	52.03	45.12	48.58
WV-37137	WV-36860	64.51	65.73	65.12
WV-37138	WV-36860	66.68	60.05	63.36
WV-37139	WV-36860	59.06	61.02	60.04
WV-37140	WV-36860	54.71	51.79	53.25
WV-37141	WV-36860	61.43	63.45	62.44
WV-37142	WV-36860	53.31	44.70	49.00
WV-37143	WV-36860	55.33	52.49	53.91
WV-37144	WV-36860	54.11	48.60	51.35
WV-37145	WV-36860	65.86	72.58	69.22
WV-37146	WV-36860	59.99	45.20	52.59
WV-37147	WV-36860	48.91	58.42	53.67
WV-37148	WV-36860	76.15	78.10	77.12
WV-37149	WV-36860	63.99	66.80	65.39
WV-37150	WV-36860	77.66	68.63	73.15
WV-37151	WV-36860	52.66	48.07	50.37
WV-37152	WV-36860	82.36	68.59	75.48
WV-37153	WV-36860	69.32	72.20	70.76
WV-37154	WV-36860	83.46	66.38	74.92
WV-37155	WV-36860	51.65	57.70	54.67
WV-37156	WV-36860	46.34	59.01	52.68
WV-37157	WV-36860	49.27	56.39	52.83
WV-37158	WV-36860	57.36	52.83	55.10
WV-37159	WV-36860	49.17	57.70	53.44
WV-37160	WV-36860	50.97	56.57	53.77
WV-37161	WV-36860	45.80	52.23	49.01
WV-37162	WV-36860	47.43	55.55	51.49
WV-37163	WV-36860	58.36	59.39	58.88
WV-37164	WV-36860	69.41	74.03	71.72
WV-37165	WV-36860	58.38	63.70	61.04
WV-37166	WV-36860	77.37	71.77	74.57
WV-37167	WV-36860	55.82	62.63	59.22

WV-37168	WV-36860	82.45	74.57	78.51
WV-37169	WV-36860	69.31	61.24	65.27
WV-37170	WV-36860	62.78	63.48	63.13
WV-37171	WV-36860	59.69	62.51	61.10
WV-37172	WV-36860	48.90	52.06	50.48
WV-37173	WV-36860	53.34	53.26	53.30
WV-37174	WV-36860	41.51	43.72	42.62
WV-37175	WV-36860	59.39	56.94	58.17
WV-37176	WV-36860	48.80	42.83	45.81
WV-37177	WV-36860	41.85	49.79	45.82
WV-37178	WV-36860	43.61	53.46	48.53
WV-37179	WV-36860	72.22	70.68	71.45
WV-37180	WV-36860	55.66	60.85	58.26
WV-37181	WV-36860	77.03	66.20	71.62
WV-37182	WV-36860	67.07	68.51	67.79
WV-37183	WV-36860	74.15	64.31	69.23
WV-37184	WV-36860	60.93	56.36	58.64
WV-37185	WV-36860	61.54	71.20	66.37
WV-37186	WV-36860	56.82	63.59	60.20
WV-37187	WV-36860	58.65	59.88	59.27
WV-37188	WV-36860	40.87	50.23	45.55
WV-37189	WV-36860	60.76	61.28	61.02
WV-37190	WV-36860	52.86	55.59	54.22
WV-37191	WV-36860	51.29	54.07	52.68
WV-37192	WV-36860	44.53	51.97	48.25
WV-37193	WV-36860	43.44	55.82	49.63
WV-37194	WV-36860	47.94	52.99	50.46
WV-37195	WV-36860	78.18	74.89	76.54
WV-37196	WV-36860	63.23	65.80	64.52
WV-37197	WV-36860	75.99	80.42	78.21
WV-37198	WV-36860	78.13	84.00	81.07
WV-37199	WV-36860	73.88	74.96	74.42
WV-37200	WV-36860	66.51	71.03	68.77

WV-37201	WV-36860	57.03	77.37	67.20
WV-37202	WV-36860	63.86	74.89	69.37
WV-37203	WV-36860	70.26	80.10	75.18
WV-37204	WV-36860	66.38	68.55	67.46
WV-37205	WV-36860	78.93	71.67	75.30
WV-37206	WV-36860	77.74	77.71	77.73
WV-37207	WV-36860	81.80	88.59	85.19
WV-37208	WV-36860	73.80	69.00	71.40
WV-37209	WV-36860	63.50	79.72	71.61
WV-37210	WV-36860	68.59	72.71	70.65
WV-37211	WV-36860	87.57	95.25	91.41
WV-37212	WV-36860	79.02	88.59	83.81
WV-37213	WV-36860	79.76	94.58	87.17
WV-37214	WV-36860	N.D.	79.85	79.85
WV-37215	WV-36860	84.42	89.24	86.83
WV-37216	WV-36860	80.77	85.09	82.93
WV-37217	WV-36860	69.35	87.78	78.57
WV-37218	WV-36860	75.86	82.70	79.28
WV-37219	WV-36860	75.97	80.08	78.03
WV-37220	WV-36860	65.12	77.99	71.56
WV-37221	WV-36860	75.99	88.34	82.17
WV-37222	WV-36860	74.35	74.70	74.52
WV-37223	WV-36860	77.78	88.76	83.27
WV-37224	WV-36860	68.06	78.57	73.32
WV-37225	WV-36860	64.01	84.68	74.35
WV-37226	WV-36860	70.70	74.04	72.37
WV-37227	WV-36860	92.04	89.72	90.88
WV-37228	WV-36860	79.82	88.77	84.30
WV-37229	WV-36860	83.68	97.66	90.67
WV-37230	WV-36860	89.52	82.54	86.03
WV-37231	WV-36860	88.86	96.62	92.74
WV-37232	WV-36860	78.97	95.50	87.24
WV-37233	WV-36860	67.62	89.80	78.71

WV-37234	WV-36860	85.88	88.72	87.30
WV-37235	WV-36860	20.97	22.92	21.95
WV-37236	WV-36860	17.96	22.66	20.31

Table 12 shows % mouse TTR mRNA remaining (at 1000, 300 and 100 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 12.

		1000 pM			300 pM			100 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-41841	WV-40363	12.24	14.12	13.18	16.82	18.55	17.68	44.46	42.52	43.49
WV-41842	WV-40363	18.98	12.60	15.79	21.86	17.00	19.43	40.96	35.70	38.33
WV-41843	WV-40363	12.84	7.75	10.29	20.74	18.31	19.52	47.24	31.88	39.56
WV-41844	WV-40363	12.20	13.86	13.03	20.25	26.75	23.50	47.97	33.65	40.81
WV-41845	WV-40363	19.45	13.29	16.37	42.67	37.50	40.08	65.63	39.03	52.33
WV-41846	WV-40363	12.57	9.18	10.87	33.29	22.90	28.10	72.75	39.24	55.99
WV-41847	WV-40363	30.22	N.D.	30.22	71.44	67.23	69.34	120.84	78.65	99.74
WV-41848	WV-40363	13.18	2.31	7.74	21.38	33.83	27.61	76.24	N.D.	76.24
WV-41849	WV-40363	71.01	80.33	75.67	69.02	75.82	72.42	112.70	83.84	98.27
WV-41850	WV-40363	19.56	11.43	15.49	17.14	24.03	20.58	55.26	40.33	47.80
WV-41851	WV-40363	13.88	9.05	11.47	19.36	31.71	25.53	53.90	39.04	46.47
WV-41852	WV-40363	10.47	12.24	11.35	19.22	24.45	21.83	60.37	31.67	46.02
WV-41853	WV-40363	16.53	17.51	17.02	32.86	37.24	35.05	72.33	37.79	55.06
WV-41854	WV-40363	9.86	10.35	10.10	25.67	27.52	26.59	75.44	38.48	56.96
WV-41855	WV-40363	36.06	35.07	35.56	81.81	62.91	72.36	124.09	77.32	100.70

WO 2021/234459 PCT/IB2021/000351

WV-41856	WV-40363	16.31	14.25	15.28	23.56	40.28	31.92	96.48	69.35	82.91
WV-41857	WV-40363	81.80	58.71	70.25	76.98	77.04	77.01	107.24	89.99	98.61
WV-41858	WV-40363	25.97	12.41	19.19	28.29	24.37	26.33	88.48	63.60	76.04
WV-41859	WV-40363	21.09	7.52	14.31	25.63	26.67	26.15	71.09	59.36	65.23
WV-41860	WV-40363	19.97	29.21	24.59	39.57	30.80	35.18	84.73	61.76	73.25
WV-41861	WV-40363	19.47	22.74	21.11	48.92	30.60	39.76	76.88	54.99	65.93
WV-41862	WV-40363	21.84	19.74	20.79	49.72	38.08	43.90	91.69	65.27	78.48
WV-41863	WV-40363	14.69	13.82	14.25	40.60	33.91	37.25	82.85	52.50	67.67
WV-41864	WV-40363	18.77	16.52	17.64	27.95	37.75	32.85	76.72	70.37	73.55
WV-41865	WV-40363	20.79	11.65	16.22	26.39	29.96	28.18	72.78	46.96	59.87
WV-41866	WV-40363	37.68	24.28	30.98	48.84	42.00	45.42	90.90	74.84	82.87
WV-41867	WV-40363	9.44	4.14	6.79	13.26	13.52	13.39	47.73	29.71	38.72
WV-41868	WV-40363	9.34	14.66	12.00	20.57	14.51	17.54	53.96	31.43	42.70
WV-41869	WV-40363	8.18	10.25	9.21	20.08	15.84	17.96	45.84	34.16	40.00
WV-41870	WV-40363	9.41	10.03	9.72	21.64	23.59	22.62	62.04	47.29	54.67
WV-41871	WV-40363	37.89	35.38	36.64	64.95	67.41	66.18	100.49	80.89	90.69
WV-41872	WV-40363	5.05	4.52	4.79	8.33	14.77	11.55	33.71	34.14	33.93
WV-41873	WV-40363	9.31	5.11	7.21	11.80	14.00	12.90	53.31	22.90	38.11
WV-41874	WV-40363	14.20	9.23	11.71	18.51	20.10	19.30	76.48	41.45	58.96
WV-41875	WV-40363	67.73	35.01	51.37	74.00	66.34	70.17	114.20	85.75	99.97
WV-41876	WV-40363	10.48	13.58	12.03	13.85	17.20	15.53	54.18	35.37	44.77
WV-41877	WV-40363	5.97	11.06	8.52	15.38	18.40	16.89	48.50	28.16	38.33
WV-41878	WV-40363	5.85	5.52	5.68	7.43	14.41	10.92	47.23	34.12	40.68
WV-41879	WV-40363	5.63	6.74	6.18	9.62	21.27	15.44	46.87	32.91	39.89

WV-41880	WV-40363	10.29	8.44	9.37	15.31	28.63	21.97	51.46	47.33	49.40
WV-41881	WV-40363	9.92	4.17	7.05	10.33	14.46	12.39	43.85	21.59	32.72
WV-41882	WV-40363	13.65	5.75	9.70	10.88	15.83	13.35	55.85	36.53	46.19
WV-41883	WV-40363	9.07	3.28	6.17	8.94	13.46	11.20	45.02	33.10	39.06
WV-41884	WV-40363	10.71	19.29	15.00	28.36	19.07	23.72	52.92	40.91	46.91
WV-41885	WV-40363	N.D.	12.96	12.96	23.88	13.45	18.66	41.25	29.12	35.18
WV-41886	WV-40363	4.17	5.94	5.06	12.97	11.50	12.23	39.75	31.68	35.71
WV-41887	WV-40363	4.80	3.90	4.35	10.94	12.86	11.90	29.11	31.18	30.15
WV-41888	WV-40363	6.46	5.25	5.85	10.21	20.24	15.23	40.51	30.90	35.71
WV-41889	WV-40363	13.27	4.90	9.09	13.00	15.52	14.26	46.61	28.85	37.73
WV-41890	WV-40363	15.63	6.01	10.82	12.18	19.22	15.70	52.87	42.09	47.48
WV-41891	WV-40363	36.52	13.82	25.17	34.15	45.99	40.07	82.93	69.08	76.00
WV-41892	WV-40363	11.43	15.85	13.64	11.28	20.93	16.11	55.21	48.83	52.02
WV-41893	WV-40363	48.93	57.98	53.45	60.30	73.86	67.08	105.27	90.58	97.93

EXAMPLE 6. Provided Oligonucleotides and Compositions Can Effectively Knockdown mouse Transthyretin (mTTR) in vitro.

Various siRNAs for mouse TTR were designed and constructed. A number of siRNAs were tested *in vitro* in mouse primary hepatocytes at one or a range of concentrations. Some siRNAs were also tested in mice (e.g., C57BL6 wild type mice).

Example protocol for *in vitro* determination of siRNA activity: For determination of siRNAs activity, siRNAs at specific concentration were gymnotically delivered to mouse primary hepatocytes plated at 96-well plates, with 10,000 cells/well. Following 48 hours treatment, total RNA was extracted using SV96 Total RNA Isolation kit (Promega). cDNA production from RNA samples were performed using High-Capacity cDNA Reverse Transcription kit (Thermo Fisher) following manufacturer's instructions and qPCR analysis performed in CFX System using iQ Multiplex Powermix (Bio-Rad). For mouse TTR mRNA, the following qPCR assay were utilized: IDT Taqman qPCR assay ID Mm.PT.58.11922308. Mouse HPRT was used as normalizer (Forward 5'CAAACTTTGCTTTCCCTGGTT3', Reverse 5'TGGCCTGTATCCAACACTTC3', Probe 5'/5HEX/ACCAGCAAG/Zen/CTTGCAACCTTAACC/3IABkFQ/3'. mRNA knockdown levels were calculated as %mRNA remaining relative to mock treatment.

Table 13 shows % mouse TTR mRNA remaining (at 1500, 500 and 150 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 13.

		1500 pM			500 pM			150 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-36838	WV-28310	4.78	6.16	5.47	6.76	10.19	8.47	18.80	26.37	22.59
WV-20167	WV-28310	6.44	5.87	6.15	5.41	6.55	5.98	18.95	28.62	23.79
WV-38670	WV-28310	34.75	53.82	44.29	74.53	85.75	80.14	78.70	87.42	83.06
WV-38671	WV-28310	13.34	22.07	17.70	26.51	32.91	29.71	53.34	54.79	54.07
WV-38672	WV-28310	8.76	12.61	10.69	18.32	23.18	20.75	38.99	44.87	41.93
WV-38673	WV-28310	7.53	11.99	9.76	13.38	14.73	14.05	29.86	33.98	31.92
WV-38674	WV-28310	7.11	12.59	9.85	11.88	15.23	13.55	33.65	35.09	34.37
WV-38675	WV-28310	6.56	10.15	8.35	10.16	14.26	12.21	22.57	26.20	24.38
WV-38676	WV-28310	8.05	8.49	8.27	11.99	13.55	12.77	29.09	33.58	31.34
WV-38677	WV-28310	7.61	8.70	8.15	12.52	15.12	13.82	30.43	35.58	33.00
WV-38678	WV-28310	5.65	6.34	5.99	8.72	11.26	9.99	22.45	31.68	27.06
WV-38679	WV-28310	6.96	9.08	8.02	8.45	9.88	9.16	20.26	27.50	23.88
WV-38680	WV-28310	6.60	7.87	7.24	8.05	11.79	9.92	22.73	29.50	26.11
WV-38681	WV-28310	7.05	9.18	8.12	8.64	14.03	11.34	21.39	34.24	27.81
WV-38682	WV-28310	7.73	8.77	8.25	9.64	10.63	10.13	27.06	33.41	30.23
WV-38683	WV-28310	8.63	10.90	9.76	11.67	13.18	12.42	26.30	34.73	30.51

WV-38684	WV-28310	6.37	8.52	7.44	7.45	9.74	8.59	23.87	25.98	24.92
WV-38685	WV-28310	5.93	7.11	6.52	9.21	11.13	10.17	17.97	29.56	23.76
WV-38686	WV-28310	4.76	6.12	5.44	7.98	12.38	10.18	27.64	31.31	29.47
WV-38687	WV-28310	5.96	8.38	7.17	6.85	8.19	7.52	17.15	24.69	20.92
WV-38688	WV-28310	6.60	6.82	6.71	7.41	9.84	8.63	19.52	27.74	23.63
WV-38689	WV-28310	7.92	10.35	9.14	9.66	14.63	12.15	24.21	31.35	27.78
WV-38690	WV-28310	9.44	10.95	10.19	13.44	20.90	17.17	44.40	53.75	49.08
WV-36838	WV-36860	9.64	9.47	9.55	11.40	18.35	14.87	27.23	43.32	35.27
WV-38670	WV-36860	47.13	57.26	52.20	56.95	65.93	61.44	74.12	84.89	79.50
WV-38671	WV-36860	21.08	24.23	22.66	41.42	48.49	44.95	56.63	73.49	65.06
WV-38672	WV-36860	8.33	10.90	9.61	19.81	28.09	23.95	53.76	67.37	60.56
WV-38673	WV-36860	9.28	12.76	11.02	15.29	26.40	20.84	36.73	48.22	42.47
WV-38674	WV-36860	9.72	12.07	10.90	18.45	26.19	22.32	46.77	63.83	55.30
WV-38675	WV-36860	8.85	11.04	9.94	14.39	18.05	16.22	31.57	43.68	37.62
WV-38676	WV-36860	9.38	11.90	10.64	15.10	21.39	18.24	43.86	56.07	49.97
WV-38677	WV-36860	11.74	14.89	13.32	19.99	26.72	23.36	38.90	45.24	42.07
WV-38678	WV-36860	6.09	8.01	7.05	8.66	12.09	10.37	29.67	29.78	29.72
WV-38679	WV-36860	5.90	6.93	6.41	9.93	12.17	11.05	28.08	34.17	31.13
WV-38680	WV-36860	5.07	6.71	5.89	9.21	11.39	10.30	29.31	38.52	33.92
WV-38681	WV-36860	7.15	8.47	7.81	9.34	12.97	11.16	27.08	36.99	32.03
WV-38682	WV-36860	7.74	9.77	8.76	10.21	12.23	11.22	33.92	40.78	37.35
WV-38683	WV-36860	9.32	11.36	10.34	13.67	16.91	15.29	32.30	43.92	38.11
WV-38684	WV-36860	6.96	8.92	7.94	9.34	11.10	10.22	31.40	38.88	35.14
WV-38685	WV-36860	8.19	9.65	8.92	17.01	17.75	17.38	29.17	38.02	33.59

WV-38686	WV-36860	6.82	9.13	7.98	10.45	12.98	11.71	26.63	37.42	32.02
WV-38687	WV-36860	6.47	7.10	6.79	12.29	13.65	12.97	26.65	40.32	33.49
WV-38688	WV-36860	7.11	8.03	7.57	12.57	16.32	14.44	33.72	48.26	40.99
WV-38689	WV-36860	8.93	9.52	9.22	19.21	18.30	18.76	43.50	53.09	48.30
WV-38690	WV-36860	29.40	32.54	30.97	53.13	47.10	50.11	73.07	74.04	73.56

Table 14 shows % mouse TTR mRNA remaining (at 1500, 500 and 150 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 14.

		1500 pM			500 pM			150 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-20167	WV-28310	2.14	2.13	2.13	3.84	3.75	3.79	10.75	11.50	11.13
WV-20167	WV-36860	1.95	2.04	1.99	4.32	3.94	4.13	9.85	9.88	9.87
WV-20170	WV-28310	1.93	1.87	1.90	3.05	2.75	2.90	6.20	5.96	6.08
WV-20170	WV-36860	1.87	2.29	2.08	3.77	3.23	3.50	7.90	7.79	7.84
WV-37856	WV-28310	3.61	2.81	3.21	7.75	4.83	6.29	20.74	19.52	20.13
WV-37857	WV-28310	3.18	2.81	3.00	7.54	6.24	6.89	26.43	23.14	24.78
WV-37874	WV-28310	71.32	71.58	71.45	91.66	92.47	92.06	82.83	82.81	82.82
WV-37875	WV-28310	79.08	75.27	77.18	97.35	93.96	95.66	97.76	97.54	97.65
WV-37892	WV-28310	2.57	2.64	2.60	6.08	5.78	5.93	16.86	16.78	16.82

WV-37893	WV-28310	1.99	1.88	1.94	3.54	3.68	3.61	10.16	10.57	10.37
WV-38703	WV-28310	2.67	2.81	2.74	6.37	6.14	6.25	19.77	22.14	20.96
WV-38704	WV-28310	3.44	3.44	3.44	6.94	6.78	6.86	24.82	22.99	23.91
WV-38705	WV-28310	40.98	26.10	33.54	83.19	57.86	70.52	85.33	89.22	87.27
WV-38706	WV-28310	36.47	33.27	34.87	79.02	61.53	70.27	96.22	98.60	97.41
WV-38707	WV-28310	1.65	1.64	1.65	2.71	2.79	2.75	11.77	12.04	11.90
WV-38708	WV-28310	1.92	1.94	1.93	3.98	3.79	3.89	8.28	8.07	8.17
WV-37856	WV-36860	4.11	4.28	4.20	11.06	10.94	11.00	26.16	25.89	26.02
WV-37857	WV-36860	3.54	3.50	3.52	12.29	12.61	12.45	24.47	23.07	23.77
WV-37874	WV-36860	68.59	69.82	69.21	81.35	86.08	83.71	80.88	80.02	80.45
WV-37875	WV-36860	87.42	66.61	77.01	104.70	75.74	90.22	77.59	99.62	88.60
WV-37892	WV-36860	2.53	2.67	2.60	6.69	5.90	6.29	18.31	18.42	18.36
WV-37893	WV-36860	2.22	2.58	2.40	4.47	4.68	4.58	8.79	9.06	8.93
WV-38703	WV-36860	2.96	3.05	3.00	7.73	7.75	7.74	15.13	16.86	15.99
WV-38704	WV-36860	3.61	3.74	3.68	8.83	8.59	8.71	23.69	22.57	23.13
WV-38705	WV-36860	48.37	48.88	48.62	80.89	80.85	80.87	104.36	103.44	103.90
WV-38706	WV-36860	42.63	47.69	45.16	70.80	68.41	69.61	88.46	86.25	87.36
WV-38707	WV-36860	1.96	2.06	2.01	3.30	3.60	3.45	8.20	8.52	8.36
WV-38708	WV-36860	2.19	1.75	1.97	4.20	3.94	4.07	20.65	9.82	15.23

Table 15 shows % mouse TTR mRNA remaining (at 300 and 100 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 15.

		300 pM			100 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-37856	WV-40362	19.14	19.60	19.37	32.79	60.24	46.52
WV-37874	WV-40362	72.88	68.84	70.86	68.25	92.19	80.22
WV-37892	WV-40362	1.97	2.65	2.31	4.39	11.53	7.96
WV-40539	WV-40362	2.15	3.18	2.66	5.15	13.06	9.10
WV-40540	WV-40362	35.81	39.94	37.87	51.71	95.45	73.58
WV-40541	WV-40362	8.39	9.49	8.94	15.67	33.26	24.47
WV-40542	WV-40362	40.68	47.35	44.01	47.46	74.58	61.02
WV-40543	WV-40362	84.06	N.D.	84.06	71.83	88.15	79.99
WV-40544	WV-40362	24.13	23.19	23.66	42.33	61.56	51.94
WV-37857	WV-40362	6.01	N.D.	6.01	12.62	30.78	21.70
WV-37875	WV-40362	66.29	58.23	62.26	74.22	84.39	79.31
WV-37893	WV-40362	2.81	3.01	2.91	6.54	11.65	9.09
WV-40545	WV-40362	2.05	2.27	2.16	7.00	10.86	8.93
WV-40546	WV-40362	5.74	6.09	5.91	15.96	26.23	21.10
WV-40547	WV-40362	86.05	83.11	84.58	75.97	85.26	80.62
WV-40548	WV-40362	68.26	69.91	69.08	68.25	79.56	73.91
WV-40549	WV-40362	33.88	38.55	36.21	53.52	61.76	57.64
WV-40550	WV-40362	4.31	5.17	4.74	9.66	16.74	13.20
WV-38704	WV-40362	1.92	2.23	2.07	5.50	7.01	6.26
WV-38706	WV-40362	56.29	76.07	66.18	77.92	92.39	85.15
WV-38708	WV-40362	2.07	1.96	2.01	5.58	8.45	7.01
WV-40552	WV-40362	0.53	0.52	0.52	0.67	0.99	0.83
WV-40796	WV-40362	30.68	36.77	33.72	45.85	35.79	40.82
WV-40553	WV-40362	67.09	67.40	67.25	N.D.	67.84	67.84
WV-40797	WV-40362	2.34	2.58	2.46	4.68	9.72	7.20

WV-40555	WV-40362	20.65	27.89	24.27	40.66	51.56	46.11
WV-40556	WV-40362	1.15	1.11	1.13	1.83	3.10	2.46
WV-37856	WV-40363	8.63	13.12	10.87	26.15	41.69	33.92
WV-37874	WV-40363	62.50	61.86	62.18	77.87	88.17	83.02
WV-37892	WV-40363	0.51	0.65	0.58	0.76	0.92	0.84
WV-40539	WV-40363	11.29	N.D.	11.29	31.65	30.95	31.30
WV-40540	WV-40363	63.43	83.64	73.53	78.70	75.62	77.16
WV-40541	WV-40363	36.81	40.62	38.72	66.34	66.57	66.46
WV-40542	WV-40363	2.60	2.82	2.71	5.34	8.50	6.92
WV-40543	WV-40363	11.69	14.44	13.06	33.12	40.22	36.67
WV-40544	WV-40363	35.04	40.17	37.60	54.56	67.07	60.81
WV-37857	WV-40363	6.17	8.24	7.20	23.45	28.25	25.85
WV-37875	WV-40363	27.16	35.49	31.32	55.62	58.21	56.92
WV-37893	WV-40363	5.31	N.D.	5.31	22.77	20.81	21.79
WV-40545	WV-40363	27.39	21.95	24.67	37.26	40.02	38.64
WV-40546	WV-40363	32.94	42.86	37.90	55.36	58.87	57.12
WV-40547	WV-40363	53.68	57.83	55.76	68.83	75.06	71.95
WV-40548	WV-40363	2.28	N.D.	2.28	3.44	6.18	4.81
WV-40549	WV-40363	53.24	89.24	71.24	77.22	81.69	79.46
WV-40550	WV-40363	6.14	10.46	8.30	21.02	32.11	26.57
WV-38704	WV-40363	0.64	0.75	0.70	1.06	1.31	1.19
WV-38706	WV-40363	28.57	62.83	45.70	74.13	58.06	66.09
WV-38708	WV-40363	18.91	24.61	21.76	49.00	49.33	49.16
WV-40552	WV-40363	1.29	1.74	1.52	2.35	5.16	3.75
WV-40796	WV-40363	32.41	35.36	33.88	48.21	64.51	56.36
WV-40553	WV-40363	36.59	36.14	36.37	53.39	72.68	63.04
WV-40797	WV-40363	101.33	98.62	99.98	92.36	95.55	93.96
WV-40555	WV-40363	46.02	60.72	53.37	74.42	91.30	82.86
WV-40556	WV-40363	0.83	0.79	0.81	1.03	1.64	1.33
WV-37856	WV-40364	7.47	10.71	9.09	33.82	29.38	31.60
WV-37874	WV-40364	75.54	89.39	82.47	92.77	81.22	86.99
WV-37892	WV-40364	3.77	4.22	3.99	10.56	20.53	15.54
WV-40539	WV-40364	7.69	7.94	7.82	15.08	33.77	24.42
WV-40540	WV-40364	71.89	60.91	66.40	85.07	79.92	82.50
WV-40541	WV-40364	103.05	119.53	111.29	106.03	97.25	101.64
WV-40542	WV-40364	55.01	67.12	61.07	81.80	89.07	85.43

WV-40543	WV-40364	2.88	3.97	3.43	17.35	22.71	20.03
WV-40544	WV-40364	7.56	12.97	10.27	N.D.	33.60	33.60
WV-37857	WV-40364	62.03	79.96	70.99	93.37	81.41	87.39
WV-37875	WV-40364	83.79	80.06	81.92	88.03	85.21	86.62
WV-37893	WV-40364	1.57	2.51	2.04	2.96	6.08	4.52
WV-40545	WV-40364	2.31	2.84	2.58	6.72	8.68	7.70
WV-40546	WV-40364	64.11	106.42	85.27	105.26	87.19	96.22
WV-40547	WV-40364	79.40	98.68	89.04	102.73	91.19	96.96
WV-40548	WV-40364	0.65	0.58	0.61	0.99	1.18	1.09
WV-40549	WV-40364	6.59	40.61	23.60	76.37	34.85	55.61
WV-40550	WV-40364	43.88	68.23	56.06	91.61	73.77	82.69
WV-38704	WV-40364	5.59	6.29	5.94	15.37	30.74	23.05
WV-38706	WV-40364	62.63	63.30	62.96	70.47	85.78	78.13
WV-38708	WV-40364	2.25	1.96	2.10	5.02	9.31	7.16
WV-40552	WV-40364	76.38	113.04	94.71	103.23	91.86	97.55
WV-40796	WV-40364	79.89	104.99	92.44	104.37	98.77	101.57
WV-40553	WV-40364	2.34	2.10	2.22	14.27	22.75	18.51
WV-40797	WV-40364	5.22	10.08	7.65	35.07	35.64	35.35
WV-40555	WV-40364	82.01	84.64	83.32	99.13	87.24	93.18
WV-40556	WV-40364	7.86	8.03	7.95	15.48	29.11	22.29
WV-37856	WV-40365	16.52	23.35	19.94	32.42	66.57	49.50
WV-37874	WV-40365	90.68	76.33	83.51	120.32	94.07	107.19
WV-37892	WV-40365	19.60	12.76	16.18	37.97	35.48	36.73
WV-40539	WV-40365	22.08	13.19	17.64	46.36	48.14	47.25
WV-40540	WV-40365	62.11	52.76	57.44	93.14	103.12	98.13
WV-40541	WV-40365	90.98	74.62	82.80	117.81	111.99	114.90
WV-40542	WV-40365	16.76	10.63	13.70	46.16	43.11	44.63
WV-40543	WV-40365	65.91	42.83	54.37	70.88	94.77	82.83
WV-40544	WV-40365	21.28	21.60	21.44	32.26	45.94	39.10
WV-37857	WV-40365	23.70	13.84	18.77	38.09	43.53	40.81
WV-37875	WV-40365	96.57	76.55	86.56	97.98	93.03	95.51
WV-37893	WV-40365	10.40	5.58	7.99	12.32	23.03	17.68
WV-40545	WV-40365	9.58	5.29	7.44	18.20	28.85	23.52
WV-40546	WV-40365	83.14	74.39	78.76	102.03	104.63	103.33
WV-40547	WV-40365	91.56	56.75	74.16	101.91	113.91	107.91
WV-40548	WV-40365	9.25	4.84	7.04	15.40	26.43	20.91

WV-40549	WV-40365	58.03	53.80	55.91	78.30	82.72	80.51
WV-40550	WV-40365	13.47	6.91	10.19	14.37	28.25	21.31
WV-38704	WV-40365	18.84	19.10	18.97	31.88	40.27	36.07
WV-38706	WV-40365	71.63	59.55	65.59	74.87	100.88	87.87
WV-38708	WV-40365	9.18	5.43	7.31	16.02	28.91	22.47
WV-40552	WV-40365	10.13	7.76	8.94	16.45	24.26	20.36
WV-40796	WV-40365	63.00	52.31	57.66	82.62	84.45	83.54
WV-40553	WV-40365	68.22	51.92	60.07	66.41	100.39	83.40
WV-40797	WV-40365	6.97	N.D.	6.97	13.05	24.16	18.61
WV-40555	WV-40365	46.23	39.73	42.98	54.14	71.82	62.98
WV-40556	WV-40365	9.78	7.84	8.81	16.64	27.05	21.85
WV-37856	WV-40366	21.25	18.03	19.64	34.52	41.08	37.80
WV-37874	WV-40366	80.85	77.50	79.18	99.10	125.34	112.22
WV-37892	WV-40366	23.50	17.67	20.58	31.86	47.88	39.87
WV-40539	WV-40366	26.56	18.72	22.64	42.76	55.59	49.17
WV-40540	WV-40366	96.75	88.81	92.78	93.69	118.06	105.88
WV-40541	WV-40366	79.18	88.92	84.05	105.73	122.69	114.21
WV-40542	WV-40366	14.45	8.95	11.70	19.94	26.73	23.34
WV-40543	WV-40366	60.46	59.64	60.05	79.07	83.34	81.20
WV-40544	WV-40366	34.78	19.97	27.37	39.90	52.21	46.05
WV-37857	WV-40366	N.D.	16.31	16.31	33.17	51.40	42.28
WV-37875	WV-40366	94.86	90.47	92.67	100.02	109.77	104.89
WV-37893	WV-40366	11.74	8.38	10.06	24.86	38.09	31.48
WV-40545	WV-40366	13.08	8.57	10.82	21.10	31.30	26.20
WV-40546	WV-40366	75.98	N.D.	75.98	85.77	106.67	96.22
WV-40547	WV-40366	104.64	83.88	94.26	93.09	112.45	102.77
WV-40548	WV-40366	9.16	10.31	9.73	14.63	23.28	18.96
WV-40549	WV-40366	88.66	77.61	83.14	85.27	89.09	87.18
WV-40550	WV-40366	15.63	9.26	12.45	26.09	38.01	32.05
WV-38704	WV-40366	21.83	16.41	19.12	32.25	50.52	41.39
WV-38706	WV-40366	74.20	60.70	67.45	97.14	102.29	99.71
WV-38708	WV-40366	11.72	8.26	9.99	20.30	27.84	24.07
WV-40552	WV-40366	7.08	6.10	6.59	11.33	19.38	15.36
WV-40796	WV-40366	85.16	64.19	74.67	88.56	81.93	85.24
WV-40553	WV-40366	77.45	56.47	66.96	82.29	82.12	82.20
WV-40797	WV-40366	N.D.	4.79	4.79	16.93	21.88	19.41

WV-40555	WV-40366	63.12	53.63	58.38	97.04	85.53	91.29
WV-40556	WV-40366	14.77	9.47	12.12	21.70	37.37	29.54

Table 16 shows % mouse TTR mRNA remaining (at 1000, 300 and 100 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 16.

		1000 pM			300 pM			100 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-20170	WV-40363	7.35	7.04	7.20	17.96	12.47	15.22	29.23	28.46	28.85
WV-40836	WV-40363	21.09	12.56	16.83	36.04	28.19	32.12	48.25	69.21	58.73
WV-40837	WV-40363	30.63	23.56	27.09	50.90	54.67	52.79	73.91	72.12	73.01
WV-40838	WV-40363	12.53	14.29	13.41	35.87	33.01	34.44	43.45	59.25	51.35
WV-40839	WV-40363	19.08	27.61	23.35	56.17	60.08	58.13	78.08	141.60	109.84
WV-37896	WV-40363	11.72	25.05	18.38	40.55	41.32	40.94	48.61	50.45	49.53
WV-37900	WV-40363	22.19	29.23	25.71	60.59	47.83	54.21	64.53	65.04	64.79
WV-40840	WV-40363	18.35	22.20	20.28	53.05	43.69	48.37	56.56	60.28	58.42
WV-40841	WV-40363	28.30	38.83	33.56	61.39	62.49	61.94	69.26	84.16	76.71
WV-40842	WV-40363	14.73	13.80	14.27	39.62	29.16	34.39	52.53	70.35	61.44
WV-40843	WV-40363	33.41	24.59	29.00	47.32	54.22	50.77	68.43	84.06	76.25
WV-40844	WV-40363	15.31	14.37	14.84	40.00	33.21	36.60	43.83	60.76	52.29
WV-40845	WV-40363	27.56	37.55	32.55	59.80	66.87	63.33	82.84	100.84	91.84
WV-20171	WV-40363	4.80	4.67	4.73	13.25	10.00	11.62	22.26	23.65	22.95
WV-40846	WV-40363	9.52	12.50	11.01	29.56	26.08	27.82	37.91	44.65	41.28

WO 2021/234459 PCT/IB2021/000351

WV-40847	WV-40363	13.62	21.75	17.69	48.17	41.13	44.65	52.65	62.28	57.46
WV-40848	WV-40363	12.19	11.20	11.69	30.64	24.77	27.71	36.07	40.54	38.30
WV-40849	WV-40363	21.25	20.96	21.10	44.05	39.29	41.67	50.67	64.82	57.75
WV-40850	WV-40363	8.59	8.78	8.68	27.39	21.37	24.38	40.64	67.47	54.05
WV-40851	WV-40363	12.02	13.89	12.96	39.28	35.95	37.61	55.45	77.18	66.32
WV-40852	WV-40363	13.11	18.39	15.75	37.88	40.57	39.22	51.19	64.76	57.97
WV-40853	WV-40363	23.52	35.34	29.43	57.76	60.55	59.16	71.01	101.47	86.24
WV-40854	WV-40363	9.45	16.28	12.87	31.74	34.37	33.06	46.58	40.28	43.43
WV-40855	WV-40363	20.60	27.24	23.92	56.22	46.95	51.58	59.79	75.58	67.68
WV-40856	WV-40363	9.51	11.54	10.52	112.77	57.30	85.04	45.55	43.19	44.37
WV-40857	WV-40363	15.19	23.76	19.47	46.61	47.86	47.23	58.28	63.09	60.68
WV-38107	WV-40363	6.76	7.35	7.05	19.46	16.27	17.87	27.23	32.01	29.62
WV-40858	WV-40363	12.03	12.11	12.07	29.90	22.57	26.24	46.14	78.54	62.34
WV-40859	WV-40363	25.61	22.43	24.02	50.73	42.01	46.37	66.99	88.59	77.79
WV-40860	WV-40363	14.43	12.00	13.22	32.79	29.92	31.36	40.75	51.12	45.93
WV-40861	WV-40363	20.60	26.22	23.41	45.16	57.88	51.52	64.89	74.57	69.73
WV-40862	WV-40363	12.97	13.90	13.44	31.29	30.70	30.99	43.89	56.15	50.02
WV-40863	WV-40363	20.98	24.20	22.59	58.79	40.48	49.64	61.06	65.22	63.14
WV-40864	WV-40363	17.32	23.31	20.32	55.25	45.84	50.54	54.47	63.20	58.83
WV-40865	WV-40363	31.57	34.07	32.82	68.07	57.26	62.67	72.99	78.61	75.80
WV-40866	WV-40363	14.91	10.52	12.72	35.98	24.83	30.40	44.31	76.93	60.62
WV-40867	WV-40363	29.62	29.97	29.79	59.59	47.23	53.41	66.93	87.50	77.22
WV-40868	WV-40363	16.63	13.57	15.10	30.32	24.59	27.46	45.41	50.56	47.98
WV-40869	WV-40363	32.09	38.31	35.20	54.16	52.59	53.37	78.45	108.82	93.64

Table 17 shows % mouse TTR mRNA remaining (at 300, 100 and 30 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 17.

		300 pM			100 pM			30 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-20170	WV-40870	26.39	22.52	24.45	45.59	38.59	42.09	58.85	53.11	55.98
WV-20170	WV-40871	24.43	17.14	20.79	51.95	45.72	48.83	53.10	54.58	53.84
WV-20170	WV-40872	22.62	22.12	22.37	36.53	35.49	36.01	53.60	55.33	54.46
WV-20170	WV-40873	24.03	23.16	23.59	50.98	48.60	49.79	59.90	65.20	62.55
WV-20170	WV-40874	32.54	28.04	30.29	52.97	44.22	48.59	60.30	69.09	64.69
WV-20170	WV-40875	28.94	27.72	28.33	49.27	50.96	50.11	49.13	60.95	55.04
WV-20170	WV-40876	26.66	24.00	25.33	40.23	N.D.	40.23	61.39	61.86	61.63
WV-20170	WV-40877	31.64	28.58	30.11	48.11	50.61	49.36	68.73	70.03	69.38
WV-20170	WV-40878	27.10	23.04	25.07	41.49	34.10	37.79	50.58	71.44	61.01
WV-20170	WV-40879	32.38	23.76	28.07	44.54	45.75	45.14	53.65	59.37	56.51
WV-20170	WV-40881	29.44	27.88	28.66	54.71	49.40	52.05	69.42	62.19	65.80
WV-20170	WV-40882	29.90	31.56	30.73	49.26	55.60	52.43	59.38	61.94	60.66
WV-20170	WV-40883	25.35	24.16	24.76	38.69	35.87	37.28	46.48	58.87	52.67
WV-20170	WV-40884	33.34	26.13	29.74	52.03	46.60	49.31	55.94	63.50	59.72
WV-20170	WV-40885	30.07	24.64	27.36	40.22	50.83	45.52	57.31	65.49	61.40

WO 2021/234459 PCT/IB2021/000351

WV-20170	WV-40886	25.17	21.76	23.46	45.56	44.08	44.82	65.38	71.08	68.23
WV-20170	WV-40887	27.37	19.82	23.60	47.66	40.68	44.17	45.25	62.30	53.77
WV-20170	WV-40888	37.63	24.41	31.02	57.86	49.41	53.64	58.08	64.88	61.48
WV-20170	WV-40889	29.88	28.40	29.14	49.79	47.27	48.53	61.22	71.96	66.59
WV-20170	WV-40890	33.04	31.30	32.17	54.45	60.93	57.69	59.05	68.45	63.75
WV-38708	WV-40870	27.72	25.82	26.77	52.69	49.46	51.08	56.26	65.04	60.65
WV-38708	WV-40871	27.11	23.67	25.39	52.20	50.09	51.15	50.51	64.68	57.60
WV-38708	WV-40872	17.21	23.51	20.36	46.31	46.74	46.53	57.88	62.44	60.16
WV-38708	WV-40873	25.95	17.58	21.77	38.56	37.08	37.82	50.12	55.70	52.91
WV-38708	WV-40874	34.60	32.26	33.43	61.76	56.40	59.08	58.01	63.67	60.84
WV-38708	WV-40875	16.49	16.66	16.57	33.44	33.80	33.62	43.79	48.04	45.92
WV-38708	WV-40876	19.13	18.07	18.60	38.39	40.97	39.68	40.86	N.D.	40.86
WV-38708	WV-40877	34.00	30.82	32.41	50.47	52.29	51.38	68.70	62.10	65.40
WV-38708	WV-40878	34.75	34.91	34.83	74.04	63.62	68.83	64.16	65.95	65.06
WV-38708	WV-40879	23.53	23.94	23.73	44.51	45.01	44.76	52.67	62.15	57.41
WV-38708	WV-40881	27.85	31.21	29.53	53.91	58.06	55.98	68.98	68.21	68.59
WV-38708	WV-40882	16.07	12.82	14.44	43.32	34.24	38.78	38.97	40.79	39.88
WV-38708	WV-40883	39.77	33.01	36.39	55.31	49.16	52.23	61.55	63.98	62.76
WV-38708	WV-40884	30.66	29.18	29.92	61.02	60.10	60.56	63.95	70.95	67.45
WV-38708	WV-40885	37.17	34.25	35.71	54.21	60.26	57.24	77.94	71.99	74.97
WV-38708	WV-40886	42.56	33.47	38.01	60.24	69.73	64.99	61.29	85.68	73.48
WV-38708	WV-40887	36.08	29.91	33.00	66.12	60.82	63.47	73.05	71.85	72.45
WV-38708	WV-40888	25.62	23.85	24.73	62.50	60.49	61.49	57.08	62.64	59.86
WV-38708	WV-40889	42.87	42.31	42.59	70.29	60.52	65.40	77.53	72.19	74.86
WV-38708	WV-40890	35.77	35.81	35.79	85.18	63.51	74.35	58.53	69.72	64.13

WV-20170	WV-40363	21.07	16.24	18.66	36.61	36.01	36.31	41.13	51.98	46.56
WV-38708	WV-40363	32.33	24.96	28.64	46.84	40.47	43.65	54.54	65.25	59.90
WV-20167	WV-40362	25.67	19.69	22.68	52.39	57.14	54.76	50.77	68.74	59.76
WV-38704	WV-40362	51.23	50.40	50.81	70.91	70.07	70.49	76.11	79.19	77.65

Table 18 shows % mouse TTR mRNA remaining (at 500 and 150 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 18.

		500 pM			150 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-41894	WV-40363	12.15	9.57	10.86	44.47	51.04	47.76
WV-41895	WV-40363	60.06	67.53	63.79	61.67	80.92	71.29
WV-41896	WV-40363	70.93	81.76	76.35	86.66	104.01	95.33
WV-41897	WV-40363	43.99	45.52	44.75	42.77	62.09	52.43
WV-41898	WV-40363	16.05	18.99	17.52	49.79	78.78	64.29
WV-41899	WV-40363	28.89	41.37	35.13	34.69	39.68	37.18
WV-41900	WV-40363	5.59	6.07	5.83	11.39	8.40	9.89
WV-41901	WV-40363	N.D.	9.79	9.79	9.58	10.78	10.18
WV-41902	WV-40363	2.89	4.30	3.59	4.43	7.17	5.80
WV-41903	WV-40363	2.64	3.03	2.83	6.84	6.09	6.47
WV-41904	WV-40363	2.22	2.10	2.16	4.54	4.15	4.34
WV-41905	WV-40363	2.32	2.19	2.26	3.76	4.34	4.05
WV-41906	WV-40363	1.70	1.71	1.71	5.40	4.98	5.19
WV-41907	WV-40363	2.90	2.55	2.72	5.62	6.69	6.15
WV-41908	WV-40363	3.32	2.62	2.97	4.13	3.32	3.73
WV-41909	WV-40363	3.28	3.73	3.51	4.93	4.46	4.70
WV-41910	WV-40363	2.80	4.64	3.72	3.54	4.98	4.26
WV-41911	WV-40363	2.52	2.48	2.50	5.01	6.61	5.81
WV-41912	WV-40363	2.43	2.70	2.56	4.18	5.33	4.75
WV-41913	WV-40363	2.40	3.36	2.88	4.07	5.41	4.74
WV-41914	WV-40363	2.19	2.34	2.26	3.62	5.08	4.35
WV-41915	WV-40363	20.08	24.28	22.18	12.31	14.58	13.45
WV-41916	WV-40363	26.00	33.95	29.97	101.38	81.86	91.62
WV-41917	WV-40363	4.88	5.62	5.25	6.47	5.78	6.12
WV-41918	WV-40363	2.98	4.26	3.62	2.97	3.12	3.05
WV-41919	WV-40363	3.69	5.58	4.63	5.54	5.54	5.54

WV-41920	WV-40363	3.15	3.78	3.46	5.18	6.29	5.73
WV-41921	WV-40363	4.73	7.42	6.08	7.49	9.81	8.65
WV-41922	WV-40363	2.30	2.59	2.44	3.75	3.19	3.47
WV-41923	WV-40363	3.73	5.08	4.40	6.32	5.71	6.02
WV-41924	WV-40363	3.96	5.50	4.73	8.17	10.12	9.14
WV-41925	WV-40363	2.64	4.05	3.34	4.83	6.00	5.42
WV-41926	WV-40363	4.25	5.09	4.67	2.97	3.97	3.47
WV-41927	WV-40363	2.57	3.78	3.17	2.83	3.93	3.38
WV-41928	WV-40363	2.60	2.38	2.49	3.21	4.41	3.81
WV-41929	WV-40363	2.78	2.54	2.66	4.48	5.08	4.78
WV-41930	WV-40363	1.83	3.93	2.88	16.15	26.00	21.07
WV-41931	WV-40363	4.25	2.83	3.54	4.10	4.49	4.29
WV-41932	WV-40363	2.97	4.98	3.98	2.96	3.49	3.22
WV-41933	WV-40363	4.83	5.49	5.16	5.68	5.24	5.46
WV-41934	WV-40363	5.41	7.24	6.32	3.18	4.97	4.07
WV-41935	WV-40363	4.26	4.47	4.36	2.94	3.86	3.40
WV-41936	WV-40363	4.04	4.72	4.38	3.41	5.16	4.28
WV-41937	WV-40363	22.43	22.50	22.47	7.82	9.35	8.58
WV-20170	WV-40363	2.73	2.31	2.52	11.66	19.99	15.83
WV-41938	WV-40363	27.09	37.76	32.43	75.33	84.05	79.69
WV-41939	WV-40363	86.04	90.31	88.17	94.64	107.26	100.95
WV-41940	WV-40363	111.64	108.10	109.87	102.19	102.88	102.53
WV-41941	WV-40363	76.88	85.72	81.30	71.71	77.15	74.43
WV-41942	WV-40363	56.56	63.75	60.15	103.53	105.50	104.51
WV-41943	WV-40363	70.45	79.76	75.10	72.87	66.87	69.87
WV-41944	WV-40363	16.32	20.09	18.21	14.44	16.87	15.65
WV-41945	WV-40363	19.68	20.03	19.86	24.19	25.21	24.70
WV-41946	WV-40363	14.42	19.72	17.07	27.36	40.07	33.71
WV-41947	WV-40363	10.57	14.29	12.43	22.58	29.17	25.87
WV-41948	WV-40363	11.59	15.29	13.44	22.87	23.83	23.35
WV-41949	WV-40363	10.85	12.65	11.75	14.97	25.27	20.12
WV-41950	WV-40363	12.40	16.09	14.25	21.19	30.19	25.69
WV-41951	WV-40363	13.11	17.41	15.26	22.75	30.72	26.74
WV-41952	WV-40363	11.45	15.94	13.70	10.10	12.13	11.12
WV-41953	WV-40363	10.59	16.95	13.77	12.48	14.97	13.73
WV-41954	WV-40363	10.32	13.96	12.14	19.48	24.92	22.20

WV-41955	WV-40363	8.92	11.51	10.21	23.68	28.39	26.03
WV-41956	WV-40363	9.81	12.06	10.93	22.42	23.75	23.09
WV-41957	WV-40363	9.50	15.64	12.57	19.57	16.88	18.23
WV-41958	WV-40363	14.08	22.07	18.07	22.14	25.84	23.99
WV-41959	WV-40363	58.55	59.68	59.12	46.12	41.69	43.91
WV-41960	WV-40363	55.65	65.66	60.65	62.63	70.24	66.43
WV-41961	WV-40363	13.49	17.60	15.55	12.09	14.06	13.07
WV-41962	WV-40363	7.06	8.32	7.69	17.54	24.01	20.77
WV-41963	WV-40363	11.74	14.15	12.95	22.83	32.42	27.62
WV-41964	WV-40363	16.30	19.92	18.11	20.34	30.64	25.49
WV-41965	WV-40363	22.30	27.14	24.72	30.50	33.28	31.89
WV-41966	WV-40363	9.95	15.82	12.88	20.30	23.16	21.73
WV-41967	WV-40363	13.56	19.47	16.51	21.89	31.48	26.69
WV-41968	WV-40363	16.73	23.13	19.93	18.56	23.89	21.22
WV-41969	WV-40363	7.65	11.54	9.59	11.09	13.36	12.23
WV-41970	WV-40363	10.24	7.35	8.79	16.93	28.54	22.73
WV-41971	WV-40363	8.59	10.87	9.73	13.98	26.41	20.19
WV-41972	WV-40363	10.05	12.53	11.29	18.62	23.66	21.14
WV-41973	WV-40363	8.62	12.05	10.33	15.28	17.75	16.51
WV-41974	WV-40363	11.82	20.17	16.00	21.19	21.48	21.33
WV-41975	WV-40363	10.49	15.48	12.98	16.91	22.66	19.78
WV-41976	WV-40363	8.68	20.46	14.57	15.64	23.37	19.51
WV-41977	WV-40363	9.57	22.41	15.99	15.65	14.73	15.19
WV-41978	WV-40363	13.32	17.37	15.34	18.48	30.22	24.35
WV-41979	WV-40363	13.53	17.93	15.73	19.53	32.25	25.89
WV-41980	WV-40363	8.92	16.45	12.69	20.99	30.79	25.89
WV-41981	WV-40363	43.01	47.35	45.18	34.24	48.80	41.52
WV-20171	WV-40363	9.94	10.84	10.39	12.13	17.95	15.04
WV-41982	WV-40363	137.03	136.65	136.84	56.16	68.38	62.27
WV-41939	WV-40363	90.32	117.89	104.11	32.26	51.97	42.12
WV-41983	WV-40363	129.71	114.72	122.22	75.99	73.93	74.96
WV-41984	WV-40363	78.18	88.65	83.42	51.48	54.12	52.80
WV-41985	WV-40363	100.80	99.58	100.19	64.13	62.14	63.14
WV-41986	WV-40363	68.50	79.10	73.80	47.25	45.52	46.39
WV-41987	WV-40363	14.74	16.44	15.59	7.56	7.72	7.64
WV-41988	WV-40363	27.40	38.77	33.08	16.69	19.28	17.98

WV-41989	WV-40363	52.06	63.28	57.67	13.23	13.10	13.16
WV-41990	WV-40363	39.26	48.12	43.69	9.63	11.93	10.78
WV-41991	WV-40363	18.98	26.81	22.89	6.95	7.27	7.11
WV-41992	WV-40363	12.23	22.60	17.42	7.86	8.91	8.38
WV-41993	WV-40363	15.12	17.06	16.09	8.57	11.88	10.22
WV-41994	WV-40363	11.02	15.08	13.05	8.35	7.88	8.11
WV-41995	WV-40363	18.98	20.28	19.63	10.63	9.71	10.17
WV-41996	WV-40363	12.99	17.58	15.28	6.51	7.30	6.90
WV-41997	WV-40363	46.26	56.39	51.32	10.48	11.84	11.16
WV-41998	WV-40363	53.98	56.69	55.34	15.65	16.09	15.87
WV-41999	WV-40363	25.22	28.49	26.86	8.35	8.61	8.48
WV-42000	WV-40363	17.98	17.33	17.65	9.44	10.71	10.08
WV-42001	WV-40363	15.45	19.03	17.24	9.14	8.30	8.72
WV-42002	WV-40363	83.51	80.91	82.21	44.30	47.84	46.07
WV-42003	WV-40363	100.25	90.39	95.32	65.55	61.39	63.47
WV-41961	WV-40363	15.42	17.38	16.40	8.16	7.37	7.76
WV-42004	WV-40363	59.55	66.48	63.01	20.07	24.78	22.42
WV-42005	WV-40363	114.07	121.81	117.94	45.87	41.93	43.90
WV-42006	WV-40363	53.02	67.33	60.18	19.00	17.62	18.31
WV-42007	WV-40363	12.40	12.69	12.55	6.52	4.94	5.73
WV-42008	WV-40363	17.99	18.29	18.14	9.20	10.95	10.08
WV-42009	WV-40363	23.79	23.43	23.61	15.03	12.62	13.82
WV-42010	WV-40363	17.32	18.32	17.82	9.96	8.72	9.34
WV-42011	WV-40363	12.53	13.79	13.16	8.33	6.70	7.52
WV-42012	WV-40363	31.07	35.66	33.36	3.03	3.77	3.40
WV-42013	WV-40363	54.79	53.54	54.17	7.17	8.23	7.70
WV-42014	WV-40363	23.47	39.85	31.66	5.27	5.89	5.58
WV-42015	WV-40363	16.09	22.38	19.23	10.91	11.90	11.41
WV-42016	WV-40363	10.60	14.33	12.47	13.69	13.63	13.66
WV-42017	WV-40363	15.37	23.57	19.47	14.87	14.16	14.52
WV-42018	WV-40363	12.90	18.53	15.72	16.00	16.53	16.27
WV-42019	WV-40363	17.06	27.24	22.15	22.35	24.23	23.29
WV-42020	WV-40363	42.13	52.57	47.35	7.82	8.08	7.95
WV-42021	WV-40363	52.13	57.33	54.73	12.44	13.94	13.19
WV-42022	WV-40363	101.26	122.82	112.04	47.56	57.90	52.73
WV-42023	WV-40363	13.69	18.82	16.25	12.87	9.95	11.41

WV-31807	WV-40363	10.98	16.48	13.73	9.14	8.09	8.62

Table 19 shows % mouse TTR mRNA remaining (at 500, 125 and 31 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 19.

		500 pM			125 pM			31 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-20167	WV-43768	4.03	3.55	3.79	14.88	18.00	16.44	33.08	44.15	38.62
WV-20170	WV-43769	3.33	3.92	3.63	15.05	19.90	17.48	46.89	43.93	45.41
WV-20171	WV-43769	2.34	2.90	2.62	10.09	12.76	11.42	32.50	36.14	34.32
WV-38708	WV-43769	2.88	4.37	3.62	14.77	22.91	18.84	45.11	53.07	49.09
WV-43256	WV-43769	4.11	4.61	4.36	16.31	24.85	20.58	43.61	53.08	48.34
WV-41825	WV-41828	3.15	4.71	3.93	15.41	20.74	18.07	44.16	52.39	48.27
WV-43770	WV-42080	1.34	2.11	1.72	5.93	11.25	8.59	24.44	32.93	28.68
WV-43771	WV-42080	1.38	2.27	1.83	5.87	9.06	7.46	19.99	31.20	25.60
WV-43772	WV-42080	2.10	2.44	2.27	11.29	9.76	10.52	35.17	35.67	35.42
WV-43773	WV-42080	7.68	7.11	7.40	22.29	22.91	22.60	54.76	47.86	51.31
WV-41826	WV-41828	3.61	4.90	4.26	15.66	19.64	17.65	46.23	43.39	44.81
WV-43774	WV-42080	1.59	3.24	2.41	7.52	11.37	9.45	29.94	39.62	34.78
WV-43775	WV-42080	1.76	2.52	2.14	7.37	8.59	7.98	23.53	36.17	29.85
WV-42078	WV-42080	2.28	3.97	3.13	9.02	16.95	12.99	39.73	52.74	46.24
WV-42079	WV-42080	4.69	7.16	5.93	16.22	20.62	18.42	35.94	44.44	40.19

WO 2021/234459 PCT/IB2021/000351

Table 20 shows % mouse TTR mRNA remaining (at 200 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 20.

		200 pM		
Guide	Passenger	%remaining mRNA (mF7/mHPRT)-1	%remaining mRNA (mF7/mHPRT)-2	Mean
WV-38708	WV-40363	6.44	6.95	6.69
WV-43256	WV-40363	13.06	18.60	15.83
WV-43257	WV-40363	7.69	5.90	6.80
WV-43258	WV-40363	15.21	15.12	15.17
WV-43259	WV-40363	10.73	11.38	11.05
WV-43260	WV-40363	42.99	37.27	40.13
WV-43261	WV-40363	17.93	20.41	19.17
WV-43262	WV-40363	20.17	8.09	14.13
WV-43263	WV-40363	24.34	23.55	23.94
WV-43264	WV-40363	33.66	28.56	31.11
WV-43265	WV-40363	15.56	13.92	14.74
WV-43266	WV-40363	28.89	30.84	29.87
WV-43267	WV-40363	14.38	16.58	15.48
WV-43268	WV-40363	39.44	38.15	38.79
WV-43269	WV-40363	49.12	48.65	48.88
WV-43270	WV-40363	31.51	23.15	27.33
WV-43271	WV-40363	33.89	34.08	33.99
WV-43272	WV-40363	56.74	47.45	52.09
WV-43273	WV-40363	70.13	57.57	63.85
WV-43274	WV-40363	49.67	45.44	47.56
WV-43275	WV-40363	68.37	61.26	64.82
WV-43276	WV-40363	68.37	63.82	66.09
WV-43277	WV-40363	68.33	65.82	67.07
WV-43278	WV-40363	73.49	59.33	66.41
WV-43279	WV-40363	56.09	49.51	52.80
WV-43280	WV-40363	89.62	87.28	88.45
WV-38708	WV-43281	25.36	26.56	25.96
WV-43256	WV-43281	19.85	16.95	18.40
WV-43257	WV-43281	23.93	22.69	23.31

WV-43258	WV-43281	53.95	44.29	49.12
WV-43259	WV-43281	28.39	32.96	30.67
WV-43260	WV-43281	82.39	51.48	66.93
WV-43261	WV-43281	28.72	26.38	27.55
WV-43262	WV-43281	41.97	39.01	40.49
WV-43263	WV-43281	53.41	51.61	52.51
WV-43264	WV-43281	44.38	50.39	47.38
WV-43265	WV-43281	36.33	33.54	34.93
WV-43266	WV-43281	59.68	59.75	59.72
WV-43267	WV-43281	33.56	35.37	34.47
WV-43268	WV-43281	62.17	54.54	58.36
WV-43269	WV-43281	51.66	56.42	54.04
WV-43270	WV-43281	55.92	53.77	54.85
WV-43271	WV-43281	57.34	56.94	57.14
WV-43272	WV-43281	74.73	69.66	72.19
WV-43273	WV-43281	91.70	73.80	82.75
WV-43274	WV-43281	72.14	69.11	70.63
WV-43275	WV-43281	80.95	98.20	89.57
WV-43276	WV-43281	79.52	67.01	73.27
WV-43277	WV-43281	68.33	76.65	72.49
WV-43278	WV-43281	102.23	94.33	98.28
WV-43279	WV-43281	85.34	78.50	81.92
WV-43280	WV-43281	94.42	98.23	96.32
WV-38708	WV-43282	67.25	47.17	57.21
WV-43256	WV-43282	80.18	45.92	63.05
WV-43257	WV-43282	78.48	53.13	65.81
WV-43258	WV-43282	96.26	76.28	86.27
WV-43259	WV-43282	68.05	55.62	61.83
WV-43260	WV-43282	116.61	85.12	100.87
WV-43261	WV-43282	93.29	70.02	81.66
WV-43262	WV-43282	85.72	N.D.	85.72
WV-43263	WV-43282	84.77	57.28	71.03
WV-43264	WV-43282	83.76	60.99	72.38
WV-43265	WV-43282	85.64	51.63	68.64
WV-43266	WV-43282	107.49	72.69	90.09
WV-43267	WV-43282	71.17	61.39	66.28

WV-43268	WV-43282	107.42	86.48	96.95
WV-43269	WV-43282	86.41	76.52	81.46
WV-43270	WV-43282	85.38	84.95	85.16
WV-43271	WV-43282	68.86	56.55	62.70
WV-43272	WV-43282	89.31	62.63	75.97
WV-43273	WV-43282	89.73	69.26	79.49
WV-43274	WV-43282	95.38	82.62	89.00
WV-43275	WV-43282	94.26	73.01	83.63
WV-43276	WV-43282	104.28	77.85	91.06
WV-43277	WV-43282	103.76	84.21	93.98
WV-43278	WV-43282	84.96	105.06	95.01
WV-43279	WV-43282	88.33	75.68	82.00
WV-43280	WV-43282	99.88	87.58	93.73
WV-38708	WV-43283	13.42	3.63	8.53
WV-43256	WV-43283	32.95	13.42	23.18
WV-43257	WV-43283	28.45	13.39	20.92
WV-43258	WV-43283	93.60	54.73	74.17
WV-43259	WV-43283	38.74	22.70	30.72
WV-43260	WV-43283	83.80	74.33	79.07
WV-43261	WV-43283	30.08	12.38	21.23
WV-43262	WV-43283	43.40	24.88	34.14
WV-43263	WV-43283	49.54	24.79	37.16
WV-43264	WV-43283	73.00	57.37	65.19
WV-43265	WV-43283	35.70	21.34	28.52
WV-43266	WV-43283	90.65	67.64	79.14
WV-43267	WV-43283	43.91	34.17	39.04
WV-43268	WV-43283	82.18	76.22	79.20
WV-43269	WV-43283	57.24	29.12	43.18
WV-43270	WV-43283	60.93	24.38	42.65
WV-43271	WV-43283	57.07	33.65	45.36
WV-43272	WV-43283	90.41	74.02	82.21
WV-43273	WV-43283	82.27	55.10	68.69
WV-43274	WV-43283	93.24	70.07	81.66
WV-43275	WV-43283	80.11	67.69	73.90
WV-43276	WV-43283	80.56	85.74	83.15
WV-43277	WV-43283	67.58	57.35	62.46

WV-43278	WV-43283	90.30	74.76	82.53
WV-43279	WV-43283	88.07	64.96	76.52
WV-43280	WV-43283	108.49	102.18	105.33

Table 21 shows % mouse TTR mRNA remaining (at 500, 125 and 31 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 21.

		500 pM			125 pM			31 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-42079	WV-42080	2.93	3.64	3.29	10.84	21.29	16.06	32.30	40.01	36.16
WV-43987	WV-42080	4.84	10.35	7.59	30.68	31.57	31.12	66.64	60.53	63.59
WV-43988	WV-42080	2.93	4.89	3.91	16.30	17.19	16.74	45.70	40.57	43.14
WV-43989	WV-42080	4.39	7.83	6.11	23.04	23.65	23.34	60.20	54.36	57.28
WV-43990	WV-42080	4.59	5.75	5.17	22.90	22.03	22.47	51.05	50.60	50.82
WV-43991	WV-42080	6.63	9.64	8.14	23.36	31.46	27.41	66.91	62.75	64.83
WV-43992	WV-42080	11.06	9.84	10.45	31.51	28.20	29.85	65.24	56.62	60.93
WV-43993	WV-42080	4.09	5.66	4.88	16.15	18.08	17.11	45.42	68.98	57.20
WV-43256	WV-42080	7.23	8.28	7.76	25.74	27.70	26.72	54.26	61.48	57.87
WV-43994	WV-42080	5.19	N.D.	5.19	19.79	19.94	19.86	43.10	39.64	41.37
WV-43773	WV-42080	9.14	7.83	8.49	21.96	26.24	24.10	43.55	49.39	46.47
WV-43995	WV-42080	7.15	7.27	7.21	27.11	25.76	26.44	51.18	64.90	58.04
WV-43996	WV-42080	5.39	6.20	5.80	24.95	20.45	22.70	47.37	45.00	46.18
WV-43997	WV-42080	6.90	9.86	8.38	24.28	31.71	28.00	56.75	59.04	57.90
WV-43998	WV-42080	11.13	12.06	11.59	22.02	25.61	23.81	36.85	46.31	41.58

Table 22 shows % mouse TTR mRNA remaining (at 300, 100 and 30 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 22.

		300 pM			100 pM			30 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-42079	WV-42080	31.65	21.04	26.35	47.14	31.61	39.38	74.20	64.86	69.53
WV-44454	WV-42080	73.88	65.91	69.89	90.99	81.23	86.11	92.84	88.19	90.51
WV-44455	WV-42080	55.03	46.31	50.67	71.33	62.85	67.09	87.81	95.62	91.72
WV-44456	WV-42080	96.65	76.09	86.37	94.65	94.87	94.76	136.75	86.60	111.68
WV-44457	WV-42080	78.48	58.81	68.64	88.37	71.21	79.79	111.33	99.04	105.19
WV-44458	WV-42080	89.72	81.79	85.75	100.21	97.15	98.68	129.88	89.02	109.45
WV-44459	WV-42080	77.99	78.91	78.45	97.49	99.55	98.52	113.94	121.14	117.54
WV-44460	WV-42080	101.83	102.23	102.03	124.56	97.10	110.83	84.12	72.88	78.50
WV-44461	WV-42080	84.32	91.90	88.11	100.37	88.32	94.35	86.67	105.75	96.21

Table 23 shows % mouse TTR mRNA remaining (at 300 and 100 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 23.

		300 pM			100 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-41826	WV-41828	22.58	20.88	21.73	67.61	53.81	60.71
WV-43775	WV-42080	10.31	10.88	10.59	42.45	34.78	38.61
WV-43774	WV-42080	10.24	12.44	11.34	40.84	43.10	41.97
WV-45141	WV-42080	12.02	12.19	12.11	47.21	31.18	39.19
WV-45142	WV-42080	16.57	17.19	16.88	46.39	41.80	44.09
WV-42079	WV-42080	19.20	15.77	17.48	55.89	47.83	51.86
WV-42078	WV-42080	22.35	17.94	20.15	53.96	48.32	51.14
WV-45149	WV-42080	27.40	21.58	24.49	46.75	41.65	44.20
WV-45156	WV-42080	27.97	29.48	28.72	61.19	55.30	58.25
WV-44448	WV-42080	27.68	28.02	27.85	57.69	58.55	58.12
WV-45143	WV-42080	26.23	23.82	25.02	55.76	60.24	58.00
WV-45150	WV-42080	27.69	25.14	26.41	58.40	49.24	53.82
WV-45157	WV-42080	46.70	44.42	45.56	71.59	86.66	79.13
WV-44449	WV-42080	17.98	17.43	17.70	42.91	37.02	39.97
WV-45144	WV-42080	22.54	15.81	19.17	51.94	36.45	44.19

WV-45151	WV-42080	16.99	17.37	17.18	44.43	41.63	43.03
WV-45158	WV-42080	21.86	24.49	23.18	49.40	40.59	44.99
WV-44450	WV-42080	17.02	14.31	15.67	49.00	43.14	46.07
WV-45145	WV-42080	20.17	14.43	17.30	55.71	39.80	47.75
WV-45152	WV-42080	17.20	15.47	16.34	51.18	53.79	52.48
WV-45159	WV-42080	16.92	16.12	16.52	61.15	55.71	58.43
WV-44451	WV-42080	31.28	21.74	26.51	75.71	52.55	64.13
WV-45146	WV-42080	28.22	17.12	22.67	45.15	40.25	42.70
WV-45153	WV-42080	21.24	21.05	21.14	52.74	46.81	49.78
WV-45160	WV-42080	35.76	31.61	33.68	89.05	75.58	82.32
WV-44452	WV-42080	12.26	11.98	12.12	57.45	38.54	48.00
WV-45147	WV-42080	10.41	12.29	11.35	31.96	35.53	33.74
WV-45154	WV-42080	9.40	11.32	10.36	44.76	42.97	43.86
WV-45161	WV-42080	18.93	15.93	17.43	49.15	49.86	49.50
WV-44453	WV-42080	9.88	10.59	10.23	38.62	28.15	33.39
WV-45148	WV-42080	12.19	17.25	14.72	40.06	30.75	35.41
WV-45155	WV-42080	11.36	12.40	11.88	37.41	33.19	35.30
WV-45162	WV-42080	18.64	16.76	17.70	N.D.	40.05	40.05

Table 24 shows % mouse TTR mRNA remaining (at 300, 100 and 30 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 24.

		300 pM			100 pM			30 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-44447	WV-41828	74.61	59.39	67.00	96.54	105.15	100.85	129.43	129.30	129.36
WV-42079	WV-42080	4.68	4.23	4.45	16.69	10.73	13.71	44.52	26.62	35.57
WV-44433	WV-42080	11.32	7.51	9.42	35.27	21.12	28.20	65.71	56.94	61.33
WV-44434	WV-42080	14.87	12.30	13.58	42.12	30.49	36.31	87.93	71.34	79.64
WV-44435	WV-42080	18.26	12.88	15.57	39.55	32.96	36.26	83.92	77.31	80.62
WV-44436	WV-42080	28.93	21.30	25.11	63.96	53.80	58.88	118.86	96.85	107.85
WV-44437	WV-42080	28.07	22.75	25.41	56.45	41.16	48.81	90.25	64.93	77.59
WV-44438	WV-42080	9.55	7.88	8.71	27.96	23.96	25.96	62.76	37.54	50.15
WV-44439	WV-42080	10.32	6.84	8.58	25.67	22.25	23.96	53.10	26.34	39.72
WV-44444	WV-42080	9.56	6.49	8.02	20.36	13.00	16.68	49.91	32.61	41.26
WV-44440	WV-42080	9.27	6.49	7.88	28.50	17.29	22.89	65.84	23.48	44.66
WV-44441	WV-42080	8.47	5.55	7.01	23.88	13.06	18.47	67.84	28.89	48.36
WV-44445	WV-42080	14.87	9.01	11.94	33.96	27.14	30.55	61.72	32.80	47.26
WV-44442	WV-42080	17.21	12.74	14.97	37.41	25.56	31.48	96.68	39.89	68.28
WV-44446	WV-42080	14.36	10.42	12.39	31.51	30.02	30.76	50.94	39.63	45.28

Table 25 shows % mouse TTR mRNA remaining (at 300 and 100 pM siRNA treatment) relative to mouse HPRT control. N = 2. N.D.: Not determined.

Table 25.

		300 pM			100 pM		
Guide	Passenger	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-20167	WV-43769	40.66	29.46	35.06	55.67	64.69	60.18
WV-20170	WV-43769	41.53	22.31	31.92	64.17	54.96	59.56
WV-41918	WV-43769	43.65	20.15	31.90	61.52	43.31	52.42
WV-44353	WV-43769	86.03	88.28	87.16	88.92	94.23	91.57
WV-44354	WV-43769	77.09	65.39	71.24	80.35	74.83	77.59
WV-44355	WV-43769	76.32	76.02	76.17	83.00	82.86	82.93
WV-38708	WV-43769	34.30	22.03	28.16	51.66	46.20	48.93
WV-44144	WV-43769	75.12	59.00	67.06	89.02	88.87	88.95
WV-44145	WV-43769	85.19	77.53	81.36	93.56	94.43	93.99
WV-44163	WV-43768	75.30	63.52	69.41	111.99	82.91	97.45

Table 26 shows % IC50 of knocking down mouse TTR mRNA in mouse primary hepatocyte

Table 26

Guide	Passenger	IC50 (pM)	95% CI
WV-38708	WV-40363	8.22	6.47 to 10.43
WV-38708	WV-42940	18.29	12.89 to 25.98
WV-42078	WV-42080	5.62	4.82 to 6.56
WV-42078	WV-42941	15.44	9.68 to 24.57

EXAMPLE 7. Provided Oligonucleotides and Compositions Are Active *in vivo*

In vivo determination of mouse TTR siRNA activity: All animal procedures were performed under IACUC guidelines. To evaluate the potency and liver exposure of provided oligonucleotides and compositions, male 8-10 weeks of age C57BL/6 mice were dose at 0.6, 2 or 6 mg/kg at desired oligonucleotide concentration on Day 1 by subcutaneous administration. Animals were euthanized on Day 8 by CO_2 asphyxiation followed by thoracotomy and terminal blood collection. After cardiac perfusion with PBS, liver samples were harvested and flash-frozen in dry ice. Liver total RNA was extracted using SV96 Total RNA Isolation kit (Promega), after tissue lysis with TRIzol and bromochloropropane. cDNA production from RNA samples were performed using High-Capacity cDNA Reverse Transcription kit (Thermo Fisher) following manufacturer's instructions and qPCR analysis performed in CFX System using iQ Multiplex Powermix (Bio-Rad). For mouse TTR mRNA, the following qPCR assay were utilized: IDT Taqman qPCR assay ID Mm.PT.58.11922308. Oligonucleotide accumulation in liver was determined by hybrid ELISA.

To evaluate the durability of provided oligonucleotides and compositions, male 8-10 weeks of age C57BL/6 mice were dose at 2 or 6 mg/kg at desired oligonucleotide

concentration on Day 1 by subcutaneous administration. On Day 1 (pre-dose) and then weekly, whole blood was collected via submandibular bleeding into serum separator tubes, and processed serum samples were kept at -70ºC. Mouse TTR protein concentration in the serum was assessed using the Mouse Prealbumin ELISA kit (Crystal Chem) and following manufacturer's instructions.

Table 27 shows % mouse TTR mRNA remaining relative to PBS control. N = 5. N.D.: Not determined.

Table 27.

		WV-20167/WV-36860					
	PBS	0.6 mg/kg		2 mg/kg		6 mg/kg	
animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA
1	126.89	6	81.73	11	38.85	16	35.76
2	86.61	7	70.20	12	42.98	17	17.77
3	95.66	8	75.48	13	40.83	18	20.10
4	93.00	9	65.90	14	48.03	19	11.25
5	97.84	10	77.26	15	42.39	20	10.30
Mean	100	Mean	74.11	Mean	42.62	Mean	19.03
WV-20170/WV-36807						WV-38708/WV-36807	
0.6 mg/kg		2 mg/kg		6 mg/kg		0.6 mg/kg	
animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA
21	59.04	26	27.62	31	8.38	36	74.11
22	64.79	27	24.76	32	9.90	37	51.80
23	57.41	28	39.43	33	4.61	38	75.30
24	63.78	29	33.50	34	6.79	39	58.13
25	61.99	30	28.70	35	14.13	40	75.99
Mean	61.40	Mean	30.80	Mean	8.76	Mean	67.07
WV-38708/WV-36807							
2 mg/kg		6 mg/kg					
animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA				
41	14.89	46	3.01				
42	12.19	47	5.27				
43	19.18	48	3.76				
44	23.48	49	2.98				
45	17.54	50	3.94				
Mean	17.46	Mean	3.79				

Table 28. shows the accumulation of antisence strand in liver tissue. N = 5. N.D.: Not determined.

Table 28.

		WV-20167/WV-36860					
	PBS	0.6 mg/kg		2 mg/kg		6 mg/kg	
animal No.	antisense strand (µg/g of tissue)	animal No.	antisense strand (µg/g of tissue)	animal No.	% antisense strand (µg/g of tissue)	animal No.	antisense strand (µg/g of tissue)
1	0.000	6	0.024	11	0.031	16	0.055
2	0.000	7	0.048	12	0.084	17	0.121
3	0.000	8	0.032	13	0.069	18	0.113
4	0.000	9	0.025	14	0.049	19	0.101
5	0.000	10	0.026	15	0.042	20	0.131
Mean	0.000	Mean	0.031	Mean	0.055	Mean	0.104
WV-20170/WV-36807						WV-38708/WV-36807	
0.6 mg/kg		2 mg/kg		6 mg/kg		0.6 mg/kg	
animal No.	antisense strand (µg/g of tissue)	animal No.	antisense strand (µg/g of tissue)	animal No.	antisense strand (µg/g of tissue)	animal No.	antisense strand (µg/g of tissue)
21	0.040	26	0.066	31	0.057	36	0.017
22	0.024	27	0.032	32	0.062	37	0.015
23	0.006	28	0.015	33	0.110	38	0.011
24	0.000	29	0.019	34	0.072	39	0.004
25	0.000	30	0.022	35	0.103	40	0.010
Mean	0.014	Mean	0.031	Mean	0.081	Mean	0.012
WV-38708/WV-36807							
2 mg/kg		6 mg/kg					
animal No.	antisense strand (µg/g of tissue)	animal No.	antisense strand (µg/g of tissue)				
41	0.063	46	0.298				
42	0.088	47	0.365				
43	0.065	48	0.196				
44	0.092	49	0.229				
45	0.050	50	0.322				
Mean	0.072	Mean	0.282				

Table 29. shows % mouse TTR protein remaining relative to PBS control. N = 5. N.D.: Not determined.

Table 29.

	PBS					
Day	animal1	animal2	animal3	animal4	animal5	Mean
1	91	114	104	91	100	100
8	103	120	98	91	87	100
15	91	100	112	103	93	100
22	94	107	91	102	106	100
29	93	101	87	108	111	100
36	87	102	95	112	104	100
43	98	95	95	101	112	100
	WV-20167/WV-36860, 2 mg/kg					
Day	animal6	animal7	animal8	animal9	animal10	Mean
1	97	105	72	80	90	89
8	54	48	39	51	44	47
15	68	70	62	62	63	65
22	90	96	80	92	92	90
29	85	116	82	98	91	94
36	115	120	108	88	122	110
43	102	115	99	99	94	102
	WV-20167/WV-36860, 6 mg/kg					
Day	animal11	animal12	animal13	animal14	animal15	Mean
1	92	102	109	119	92	103
8	18	18	19	18	16	18
15	33	38	39	30	34	35
22	57	78	72	55	66	66
29	72	80	85	71	81	78
36	90	119	104	96	100	102
43	94	98	115	105	106	104
	WV-20170/WV-36807, 2 mg/kg					
Day	animal16	animal17	animal18	animal19	animal20	Mean
1	65	83	95	83	103	86
8	36	37	43	41	34	38
15	53	47	49	60	55	53
22	67	76	71	84	76	75
29	78	85	85	103	69	84
36	90	93	93	96	93	93
43	88	93	94	117	79	94
	WV-20170/WV-36807, 6 mg/kg					
Day	animal21	animal22	animal23	animal24	animal25	Mean
1	108	129	100	105	107	110
8	9	11	4	8	15	10
15	17	19	9	19	29	19

22	39	40	18	39	58	39
29	67	69	41	56	79	62
36	90	94	61	84	110	88
43	108	114	80	90	107	100
	WV-38708/WV-36807, 2 mg/kg					
Day	animal26	animal27	animal28	animal29	animal30	Mean
1	120	102	117	99	141	116
8	32	31	21	22	30	27
15	46	40	38	36	44	41
22	77	79	69	65	70	72
29	85	69	82	77	87	80
36	100	95	92	98	103	98
43	106	101	91	113	119	106
	WV-38708/WV-36807, 6 mg/kg					
	animal31	animal32	animal33	animal34	animal35	Mean
Day	102	103	107	105	117	107
1	7	4	4	5	7	6
8	13	9	7	11	12	10
15	27	20	19	27	30	25
22	48	38	40	52	63	48
29	77	66	66	72	83	73
36	82	89	77	115	94	91
43	102	103	107	105	117	107

EXAMPLE 8. Provided Oligonucleotides and Compositions Are Active *in vivo*

In vivo determination of mouse TTR siRNA activity: All animal procedures were performed under IACUC guidelines. To evaluate the potency and liver exposure of provided oligonucleotides and compositions, male 8-10 weeks of age C57BL/6 mice were dose at 0.6, 2 or 6 mg/kg at desired oligonucleotide concentration on Day 1 by subcutaneous administration. Animals were euthanized on Day 8 by CO_2 asphyxiation followed by thoracotomy and terminal blood collection. After cardiac perfusion with PBS, liver samples were harvested and flash-frozen in dry ice. Liver total RNA was extracted using SV96 Total RNA Isolation kit (Promega), after tissue lysis with TRIzol and bromochloropropane. cDNA production from RNA samples were performed using High-Capacity cDNA Reverse Transcription kit (Thermo Fisher) following manufacturer's instructions and qPCR analysis performed in CFX System using iQ Multiplex Powermix (Bio-Rad). For mouse TTR mRNA, the following qPCR assay were utilized: IDT Taqman qPCR assay ID Mm.PT.58.11922308. Oligonucleotide accumulation in liver was determined by hybrid ELISA.

To evaluate the durability of provided oligonucleotides and compositions, male 8-10 weeks of age C57BL/6 mice were dose at 2 or 6 mg/kg at desired oligonucleotide concentration on Day 1 by subcutaneous administration. On Day 1 (pre-dose) and then weekly, whole blood was collected via submandibular bleeding into serum separator tubes, and processed serum samples were kept at -70ºC. Mouse TTR protein concentration in the serum was assessed using the Mouse Prealbumin ELISA kit (Crystal Chem) and following manufacturer's instructions.

Table 30 shows % mouse TTR mRNA remaining relative to PBS control. N = 5. N.D.: Not determined.

Table 30.

		WV-41826/WV-41828					
	PBS	0.6 mg/kg		2 mg/kg		6 mg/kg	
animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA
1	97.15	6	32.47	11	4.20	16	0.79
2	99.64	7	21.18	12	5.27	17	1.12
3	104.22	8	26.82	13	4.30	18	0.46
4	101.70	9	31.64	14	1.34	19	1.08
5	97.29	10	34.12	15	1.72	20	0.72
Mean	100.00	Mean	29.25	Mean	3.36	Mean	0.83
WV-42078/WV-42080						WV-42079/WV-42080	
0.6 mg/kg		2 mg/kg		6 mg/kg		0.6 mg/kg	
animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA
21	25.18	26	4.08	31	0.70	36	17.31
22	28.20	27	3.27	32	0.96	37	17.10
23	17.05	28	4.45	33	0.78	38	35.99
24	10.34	29	4.04	34	0.48	39	11.06
25	41.64	30	4.54	35	0.60	40	19.05
Mean	24.48	Mean	4.08	Mean	0.71	Mean	20.10
WV-42079/WV-42080							
2 mg/kg		6 mg/kg					
animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA				
41	3.64	46	0.99				
42	4.47	47	0.60				
43	3.67	48	0.79				
44	5.35	49	2.29				

45	6.34	50	0.69				
Mean	4.69	Mean	1.07				

Table 31. shows the accumulation of antisence strand in liver tissue. N = 5.

N.D.: Not determined.

Table 31.

		WV-41826/WV-41828					
	PBS	0.6 mg/kg		2 mg/kg		6 mg/kg	
animal No.	antisense strand (µg/g of tissue)	animal No.	antisense strand (µg/g of tissue)	animal No.	% antisense strand (µg/g of tissue)	animal No.	antisense strand (µg/g of tissue)
1	0.000	6	0.076	11	0.182	16	0.360
2	0.000	7	0.111	12	0.223	17	0.472
3	0.000	8	0.078	13	0.228	18	0.335
4	0.000	9	0.107	14	0.191	19	0.429
5	0.000	10	0.098	15	0.203	20	0.438
Mean	0.000	Mean	0.094	Mean	0.206	Mean	0.407
WV-42078/WV-42080						WV-42079/WV-42080	
0.6 mg/kg		2 mg/kg		6 mg/kg		0.6 mg/kg	
animal No.	antisense strand (µg/g of tissue)	animal No.	antisense strand (µg/g of tissue)	animal No.	antisense strand (µg/g of tissue)	animal No.	antisense strand (µg/g of tissue)
21	1.820	26	12.395	31	61.452	36	1.682
22	2.338	27	13.538	32	55.954	37	1.812
23	2.160	28	14.009	33	45.669	38	2.348
24	2.080	29	14.480	34	44.396	39	1.631
25	2.287	30	10.596	35	57.765	40	1.589
Mean	2.137	Mean	13.003	Mean	53.047	Mean	1.812
WV-42079/WV-42080							
2 mg/kg		6 mg/kg					
animal No.	antisense strand (µg/g of tissue)	animal No.	antisense strand (µg/g of tissue)				
41	11.650	46	52.453				
42	9.906	47	48.244				
43	9.640	48	56.305				
44	9.509	49	51.949				
45	9.667	50	48.769				
Mean	10.074	Mean	51.544				

Table 32. shows % mouse TTR protein remaining relative to PBS control. N = 5. N.D.: Not determined.

Table 32.

	PBS					
Day	animal1	animal2	animal3	animal4	animal5	Mean
1	91	108	101	119	80	100
8	93	125	82	115	84	100
15	84	119	94	114	89	100
22	91	107	99	114	89	100
29	109	93	95	115	88	100
36	100	112	78	116	93	100
43	95	99	91	116	99	100
50	99	101	77	119	104	100
57	94	115	86	105	100	100
64	84	116	85	126	89	100
	WV-41826/WV-41828, 0.6 mg/kg					
Day	animal6	animal7	animal8	animal9	animal10	Mean
1	133	99	120	62	74	97
8	22	5	2	17	15	12
15	23	9	6	29	20	17
22	33	23	14	42	34	29
29	57	49	38	67	57	53
36	77	76	66	76	82	75
43	116	98	97	95	108	103
	WV-41826/WV-41828, 2 mg/kg					
Day	animal11	animal12	animal13	animal14	animal15	Mean
1	85	86	101	124	127	105
8	4	1	4	3	4	3
15	5	3	6	5	6	5
22	8	5	9	6	7	7
29	17	14	18	13	15	15
36	28	31	33	20	27	28
43	57	51	37	55	47	49
50	76	82	71	49	62	68
57	80	89	83	55	73	76
64	113	98	90	65	98	93
	WV-42078/WV-42080, 0.6 mg/kg					
Day	animal16	animal17	animal18	animal19	animal20	Mean
1	135	91	106	103	123	112
8	16	13	23	11	18	16
15	23	15	29	16	24	21
22	31	21	34	21	31	28

29	49	38	47	40	44	44
36	63	47	65	48	60	57
43	93	65	79	84	85	81
	WV-42078/WV-42080, 2 mg/kg					
Day	animal21	animal22	animal23	animal24	animal25	Mean
1	103	112	118	143	93	114
8	9	4	3	3	4	4
15	13	5	4	5	4	6
22	17	5	5	6	6	8
29	28	11	10	10	10	14
36	38	14	14	14	14	19
43	52	33	28	28	29	34
50	55	45	37	37	44	43
57	68	58	56	52	65	60
64	83	98	85	86	85	87
	WV-42079/WV-42080, 0.6 mg/kg					
Day	animal26	animal27	animal28	animal29	animal30	Mean
1	93	128	88	114	123	109
8	2	21	19	16	20	16
15	6	24	29	19	20	19
22	15	36	39	26	35	30
29	45	61	50	49	55	52
36	64	79	58	79	70	70
43	104	110	82	110	109	103
	WV-42079/WV-42080, 2 mg/kg					
Day	animal31	animal32	animal33	animal34	animal35	Mean
1	N.D.	114	62	111	146	108
8	3	3	8	4	2	4
15	4	4	12	5	4	6
22	6	7	18	7	6	9
29	14	16	37	14	15	19
36	24	27	46	21	25	29
43	46	51	53	81	75	61
50	66	63	65	75	68	68
57	89	88	77	88	91	87
64	114	99	88	123	156	116

EXAMPLE 9. Provided Oligonucleotides and Compositions Are Active *in vivo*

In vivo determination of mouse TTR siRNA activity: All animal procedures were performed under IACUC guidelines at Alpha Preclinical (North Grafton, MA). To evaluate the durability of provided oligonucleotides and compositions, male 8-10 weeks of age C57BL/6 mice were dose at 0.5 or 1.5 mg/kg at desired oligonucleotide concentration on Day 1 by subcutaneous administration. On Day 1 (pre-dose) and then weekly, whole

blood was collected by tail snip into serum separator tubes, and processed serum samples were kept at -70°C. Mouse TTR protein concentration in the serum was assessed using the Mouse Prealbumin ELISA kit (Crystal Chem) and following manufacturer's instructions.

Table 33. shows % mouse TTR protein remaining relative to PBS control. N = 5. N.D.: Not determined.

Table 33.

	PBS					
Day	animal1	animal2	animal3	animal4	animal5	Mean
1	100	100	110	105	86	100
8	99	101	112	95	93	100
15	87	105	106	111	91	100
22	97	103	113	100	86	100
29	91	118	101	103	87	100
36	76	122	112	116	75	100
43	124	62	116	115	83	100
	WV-42078/WV-42080, 0.5 mg/kg					
Day	animal6	animal7	animal8	animal9	animal10	Mean
1	115	120	107	90	97	106
8	33	30	32	37	37	34
15	34	27	29	39	32	32
22	53	43	46	48	51	48
29	62	53	55	47	75	59
36	86	57	80	55	87	73
43	136	79	75	60	101	90
	WV-42078/WV-40363, 0.5 mg/kg					
Day	animal11	animal12	animal13	animal14	animal15	Mean
1	113	120	105	102	112	110
8	44	42	41	37	51	43
15	48	54	50	48	49	50
22	67	80	82	67	70	73
29	98	91	96	89	90	93
36	123	103	108	93	91	104
43	96	114	84	97	100	98
	WV-38708/WV-42080, 0.5 mg/kg					
Day	animal16	animal17	animal18	animal19	animal20	Mean
1	114	81	100	124	102	104
8	40	29	34	36	36	35
15	46	37	39	41	41	41
22	72	66	60	64	74	67
29	74	67	63	60	83	69
36	101	90	90	125	135	108
43	80	84	90	77	131	92
	WV-20167/WV-36860, 1.5 mg/kg					

Day	animal21	animal22	animal23	animal24	animal25	Mean
1	95	94	119	118	111	108
8	79	69	52	54	37	58
15	93	73	71	75	53	73
22	99	81	91	113	85	94
29	106	108	95	107	90	101
36	81	94	136	92	129	106
43	85	104	176	144	120	126
	WV-41826/WV-41828, 1.5 mg/kg					
Day	animal26	animal27	animal28	animal29	animal30	Mean
1	93	89	104	91	120	99
8	5	5	N.D.	4	10	6
15	9	10	3	12	11	9
22	18	20	6	20	20	17
29	28	32	N.D.	35	45	35
36	36	51	23	35	42	37
43	41	64	37	68	57	53
	WV-42078/WV-42080, 1.5 mg/kg					
Day	animal31	animal32	animal33	animal34	animal35	Mean
1	124	109	119	113	118	117
8	1	3	6	5	2	4
15	6	5	7	8	8	7
22	10	9	10	12	10	10
29	16	16	21	23	17	18
36	22	20	26	43	20	26
43	45	39	49	65	37	47
	WV-42078/WV-40363, 1.5 mg/kg					
Day	animal36	animal37	animal38	animal39	animal40	Mean
1	97	106	82	85	96	93
8	15	12	14	17	5	13
15	17	17	23	23	9	18
22	31	35	36	43	15	32
29	39	59	39	55	29	44
36	80	85	52	91	39	69
43	76	101	88	102	58	85
	WV-38708/WV-42080, 1.5 mg/kg					
Day	animal41	animal42	animal43	animal44	animal45	Mean
1	88	98	96	109	75	93
8	14	9	10	7	14	11
15	20	12	12	10	22	15
22	35	24	23	21	33	27
29	43	38	28	30	39	36
36	65	65	53	50	54	57
43	59	74	58	57	58	61
	WV-38708/WV-40363, 1.5 mg/kg					
Day	animal46	animal47	animal48	animal49	animal50	Mean

1	104	101	108	103	91	102
8	11	14	12	10	11	12
15	17	17	14	16	16	16
22	33	34	26	30	34	31
29	60	38	36	52	51	47
36	73	58	66	60	83	68
43	101	129	76	132	92	106

EXAMPLE 10. Provided Oligonucleotides and Compositions Are Active *in vivo*

In vivo determination of mouse TTR siRNA activity: All animal procedures were performed under IACUC guidelines at Alpha Preclinical (North Grafton, MA). To evaluate the durability of provided oligonucleotides and compositions, male 8-10 weeks of age C57BL/6 mice were dose at 0.5 or 1.5 mg/kg at desired oligonucleotide concentration on Day 1 by subcutaneous administration. On Day 1 (pre-dose) and then weekly, whole blood was collected by tail snip into serum separator tubes, and processed serum samples were kept at -70°C. Mouse TTR protein concentration in the serum was assessed using the Mouse Prealbumin ELISA kit (Novus Biologicals or Crystal Chem) and following manufacturer's instructions

Table 34. shows % mouse TTR protein remaining relative to PBS control. N = 5. N.D.: Not determined.

Table 34.

	PBS					
Day	animal1	animal2	animal3	animal4	animal5	Mean
1	87	101	102	108	102	100
8	102	110	96	103	90	100
15	95	113	95	108	90	100
22	108	104	99	101	87	100
29	78	118	100	105	98	100
36	85	113	101	110	91	100
43	89	117	98	108	88	100
	WV-41826/WV-41828, 1.5 mg/kg					
Day	animal6	animal7	animal8	animal9	animal10	Mean
1	104	96	84	75	106	93
8	8	2	10	8	10	8
15	9	7	10	9	10	9
22	13	11	14	15	14	13
29	18	16	20	22	19	19
36	33	33	30	29	36	32
43	50	44	45	46	46	46

	WV-42904/WV-41828, 1.5 mg/kg					
Day	animal11	animal12	animal13	animal14	animal15	Mean
1	82	88	87	93	91	88
8	10	8	N.D.	13	16	12
15	13	11	11	16	16	13
22	22	20	25	18	25	22
29	37	31	N.D.	29	19	29
36	53	53	55	40	57	52
43	64	62	72	55	72	65
	WV-42905/WV-42080, 1.5 mg/kg					
Day	animal16	animal17	animal18	animal19	animal20	Mean
1	106	107	N.D.	107	93	103
8	6	13	15	12	12	11
15	4	11	15	11	13	11
22	4	17	20	15	18	15
29	N.D.	29	23	30	26	27
36	N.D.	30	47	39	44	40
43	N.D.	35	65	52	58	52
	WV-42906/WV-42080, 1.5 mg/kg					
Day	animal21	animal22	animal23	animal24	animal25	Mean
1	108	107	105	108	102	106
8	11	9	5	10	11	9
15	10	10	11	12	12	11
22	15	18	14	17	18	16
29	25	35	24	24	23	26
36	35	50	36	40	35	39
43	48	63	56	60	46	55
	WV-42905/WV-40363, 1.5 mg/kg					
Day	animal26	animal27	animal28	animal29	animal30	Mean
1	99	111	105	107	84	101
8	20	21	20	24	21	21
15	21	20	21	22	23	21
22	40	33	33	35	34	35
29	57	54	49	53	56	54
36	65	70	67	63	66	66
43	79	81	81	83	93	83
	WV-42906/WV-40363, 1.5 mg/kg					
Day	animal31	animal32	animal33	animal34	animal35	Mean
1	125	113	110	105	119	114
8	19	16	20	21	21	19
15	21	21	22	19	23	21
22	34	33	44	36	36	36
29	53	57	41	53	63	54
36	68	73	70	69	84	73
43	86	98	88	96	89	91

EXAMPLE 11. Provided Oligonucleotides and Compositions Are Active *in vivo*

In vivo determination of mouse TTR siRNA activity: All animal procedures were performed under IACUC guidelines at Alpha Preclinical (North Grafton, MA). To evaluate the potency and liver exposure of provided oligonucleotides and compositions, male 8-10 weeks of age C57BL/6 mice were dose at 0.5 or 1.5 mg/kg at desired oligonucleotide concentration on Day 1 by subcutaneous administration to the interscapular area. Animals were euthanized on Day 8. After cardiac perfusion with saline, liver samples were harvested and flash-frozen in dry ice. Liver total RNA was extracted using SV96 Total RNA Isolation kit (Promega), after tissue lysis with TRIzol and bromochloropropane. cDNA production from RNA samples were performed using High-Capacity cDNA Reverse Transcription kit (Thermo Fisher) following manufacturer's instructions and qPCR analysis performed in CFX System using iQ Multiplex Powermix (Bio-Rad). For mouse TTR mRNA, the following qPCR assay were utilized: IDT Taqman qPCR assay ID Mm.PT.58.11922308.

To evaluate the durability of provided oligonucleotides and compositions, male 8-10 weeks of age C57BL/6 mice were dose at 1.5 mg/kg at desired oligonucleotide concentration on Day 1 by subcutaneous administration to the interscapular area. On Day 1 (pre-dose) and then weekly, whole blood was collected by tail snip into serum separator tubes, and processed serum samples were kept at -70ºC. Mouse TTR protein concentration in the serum was assessed using the Mouse Prealbumin ELISA kit (Novus Biologicals or Crystal Chem) and following manufacturer's instructions.

Table 35 shows % mouse TTR mRNA remaining relative to PBS control. N = 5. N.D.: Not determined.

Table 35.

		WV-42078/WV-42080				WV-42078/WV-42941	
	PBS	0.5 mg/kg		1.5 mg/kg		0.5 mg/kg	
animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA	animal No.	%remaining of mTTR mRNA
1	91.74	6	45.07	11	16.07	16	15.61
2	73.63	7	24.87	12	8.96	17	34.14
3	120.91	8	17.46	13	4.70	18	32.83
4	106.99	9	41.95	14	6.72	19	14.46
5	106.74	10	22.71	15	4.45	20	17.12
Mean	100.00	Mean	30.41	Mean	8.18	Mean	22.83

WV-42078/WV-42941							
1.5 mg/kg							
animal No.	%remaining of mTTR mRNA						
21	4.23						
22	4.49						
23	6.29						
24	4.87						
25	4.94						
Mean	4.96						

Table 36. shows % mouse TTR protein remaining relative to PBS control. N = 5. N.D.: Not determined.

Table 36.

	PBS					
Day	animal1	animal2	animal3	animal4	animal5	Mean
1	102	106	121	77	94	100
8	104	95	111	87	104	100
15	105	99	106	92	98	100
22	107	111	93	90	99	100
29	119	107	104	72	98	100
36	106	81	94	96	122	100
43	109	97	94	99	102	100
	WV-42078/WV-42080, 1.5 mg/kg					
Day	animal6	animal7	animal8	animal9	animal10	Mean
1	96	80	69	117	105	93
8	3	5	3	6	5	4
15	3	10	5	6	7	6
22	7	13	10	10	10	10
29	11	13	17	16	11	14
36	24	30	34	33	20	28
43	29	49	71	52	63	53
	WV-42078/WV-42941, 1.5 mg/kg					
Day	animal11	animal12	animal13	animal14	animal15	Mean
1	98	93	95	69	90	89
8	10	7	7	15	4	9
15	16	14	16	27	13	17
22	23	21	26	37	19	25
29	49	41	40	66	45	48
36	56	47	69	79	60	62
43	75	67	86	69	104	80

EXAMPLE 12. Provided Oligonucleotides and Compositions Can Effectively Knockdown Phosphatase and tensin homolog (PTEN) in vitro.

Various siRNAs for PTEN were designed and constructed. A number of siRNAs were tested *in vitro* in iCell Neurons and mouse H2K cell line at one or a range of concentrations.

Example protocol for *in vitro* determination of siRNA activity: For determination of siRNAs activity, siRNAs at specific concentrations were gymnotically delivered to iCell Neurons plated into 96-well plates at 400,000 cells/mL for 6 days. Next, total RNA was extracted using the SV96 Total RNA Isolation Kit (Promega). cDNA was generated from RNA samples using the High-Capacity Reverse Transcription kit (Thermo Fisher) according to the manufacturer's instructions and qPCR analysis was performed using the CFX System with iQ Multiplex Powermix (Bio-Rad). For the mouse H2K cell assay, cells were predifferentiated for 4 days, and then treated with siRNA for 4 additional days prior to RNA extraction. For human PTEN mRNA, the following qPCR assay was utilized (Thermo Fisher Hs02621230_s1). Human SRSF9 was used as a normalizer (Forward 5' TGGAATATGCCCTGCGTAAA 3', Reverse 5' TGGTGCTTCTCTCAGGATAAAC, Probe 5'/5HEX/TG GAT GAC A/Zen/C CAA ATT CCG CTC TCA/3IABkFQ/3'. For mouse PTEN mRNA, the following qPCR assay was utilized (Thermo Fisher Mm00477208_m1). Mouse HPRT was used as a normalizer. mRNA knockdown levels were calculated as the %mRNA remaining relative to mock treatment.

Table 37 shows % human PTEN mRNA remaining (at 15 and 5μM siRNA treatment) relative to human SRSF9 control, determined in iCell Neuron. N = 2. N.D.: Not determined.

Table 37.

-	-	15 μM			5 μM		
Guide	Passenger	%remaining mRNA (PTEN/ SRSF9)-1	%remaining mRNA (PTEN/ SRSF9)-2	Mean	%remaining mRNA (PTEN/ SRSF9)-1	%remaining mRNA (PTEN/ SRSF9)-2	Mean
WV-40986	WV-41023	37.3	32.1	34.7	49.3	36.9	43.1

WV-40986	WV-41024	49.7	37.6	43.7	55.3	58.2	56.8
WV-40986	WV-41025	37.2	27.7	32.5	44.5	49.2	46.9
WV-40986	WV-41026	37.8	26.1	32.0	57.9	53.8	55.9
WV-40986	WV-41027	35.6	33.2	34.4	63.2	65	64.1
WV-40986	WV-41028	52.7	48.6	50.7	74.3	91.8	83.1
WV-40986	WV-41029	58.5	51.6	55.1	115.4	69.6	92.5
WV-40990	WV-41023	55	34.2	44.6	102.4	107.7	105.1
WV-40990	WV-41024	34.6	34.8	34.7	112.6	134.8	123.7
WV-40990	WV-41025	34.3	32	33.2	130.9	145.3	138.1
WV-40990	WV-41026	25.6	24.4	25.0	117.9	120.7	119.3
WV-40990	WV-41027	31	33.9	32.5	105.1	79.6	92.4
WV-40990	WV-41028	38.3	40.2	39.3	99.8	93.3	96.6
WV-	WV-41029	44.8	39.1	42.0	75.5	ND	75.5

40990							
WV-40398	WV-40399	80.6	82.8	81.7	123.7	119.8	121.8

Table 38 shows % human PTEN mRNA remaining (at 15 and 5μM siRNA treatment) relative to human SRSF9 control, determined in iCell Neuron. N = 2. N.D.: Not determined.

Table 38.

		15 μM			5 μM		
Guide	Passenger	%remaining mRNA (PTEN/ SRSF9)-1	%remaining mRNA (PTEN/ SRSF9)-2	Mean	%remaining mRNA (PTEN/ SRSF9)-1	%remaining mRNA (PTEN/ SRSF9)-2	Mean
WV-42309	WV-41025	17.8	21.7	19.75	28.2	28.7	28.5
WV-42310	WV-41025	23.7	11.8	17.75	42	39.5	40.8
WV-42311	WV-41025	23.6	24.3	23.95	39.8	44.7	42.3
WV-42312	WV-41025	34	33.2	33.6	49	57.7	53.4
WV-42323	WV-41025	19.5	18	18.75	29.9	33.4	31.7
WV-42324	WV-41025	23.2	24.9	24.05	39.6	42.8	41.2
WV-42325	WV-41025	21.3	17.6	19.45	29.6	36.2	32.9
WV-42326	WV-41025	33.1	21.5	27.3	36.5	38.5	37.5
WV-40986	WV-41025	36.6	35	35.8	62.9	62.3	62.6
WV-40999	WV-41025	34.9	54.7	44.8	72.5	77.3	74.9

Table 39 shows % human PTEN mRNA remaining (at 15 and 5μM siRNA treatment) relative to human SRSF9 control, determined in iCell Neuron. N = 2. N.D.: Not determined.

Table 39.

		15 μM			5 μM		
Guide	Passenger	%remaining mRNA (PTEN/ SRSF9)-1	%remaining mRNA (PTEN/ SRSF9)-2	Mean	%remaining mRNA (PTEN/ SRSF9)-1	%remaining mRNA (PTEN/ SRSF9)-2	Mean
WV-42309	WV-41023	35.2	31.2	33.2	40.9	63.9	52.4
WV-42309	WV-40399	30.0	35.3	32.7	81.2	ND	81.2
WV-42309	WV-42643	43.1	42.8	43.0	43.4	67.3	55.4
WV-42309	WV-42644	36.8	26.4	31.6	46.9	53.4	50.2
WV-42309	WV-42645	38.0	43.2	40.6	ND	50.5	50.5
WV-42309	WV-42646	42.8	47.4	45.1	60.0	62.6	61.3
WV-42309	WV-42647	22.3	34.5	28.4	43.0	51.0	47.0
WV-42309	WV-42648	19.9	24.5	22.2	44.8	46.8	45.8
WV-42309	WV-42649	17.4	22.2	19.8	56.4	ND	56.4
WV-42309	WV-41025	30.0	35.7	32.9	68.5	ND	68.5
WV-42309	WV-42650	30.5	19.5	25.0	24.4	ND	24.4
WV-	WV-42651	33.8	49.0	41.4	33.6	ND	33.6

42309							
WV-42309	WV-42652	27.1	15.2	21.2	24.6	ND	24.6
WV-42309	WV-42653	25.7	15.9	20.8	27.8	ND	27.8
WV-42309	WV-42654	25.8	29.4	27.6	39.5	ND	39.5
WV-42309	WV-42655	22.8	21.3	22.1	30.4	ND	30.4
WV-40398	WV-40399	69.1	79.8	74.5	96.1	ND	96.1

Table 40 shows % human PTEN mRNA remaining (at 5μM siRNA treatment) relative to human SRSF9 control, determined in iCell Neuron. N = 2. N.D.: Not determined.

Table 40.

		5 μM		
Guide	Passenger	%remaining mRNA (PTEN/ SRSF9)-1	%remaining mRNA (PTEN/ SRSF9)-2	Mean
WV-42310	WV-41025	55.2	49.7	52.5
WV-43223	WV-41025	66.3	38.0	52.2
WV-43224	WV-41025	56.9	45.0	51.0
WV-43225	WV-41025	74.4	60.6	67.5
WV-43226	WV-41025	79.4	55.1	67.3
WV-43227	WV-41025	61.4	32.6	47.0
WV-43228	WV-41025	64.1	43.2	53.7
WV-43229	WV-41025	52.6	41.1	46.9
WV-43230	WV-41025	56.1	44.5	50.3
WV-42310	WV-43232	53.1	55.5	54.3
WV-43223	WV-43232	45.7	50.9	48.3
WV-43224	WV-43232	51.0	51.2	51.1
WV-43225	WV-43232	70.6	71.9	71.3
WV-43226	WV-43232	55.2	54.7	55.0
WV-43227	WV-43232	45.2	66.5	55.9
WV-43228	WV-43232	54.6	67.0	60.8
WV-43229	WV-43232	33.3	40.0	36.7
WV-43230	WV-43232	50.2	66.5	58.4
WV-42310	WV-43233	49.0	30.7	39.9
WV-	WV-43233	44.7	29.0	36.9

43223				
WV-43224	WV-43233	46.5	37.1	41.8
WV-43225	WV-43233	68.2	48.1	58.2
WV-43226	WV-43233	58.1	40.1	49.1
WV-43227	WV-43233	42.4	35.0	38.7
WV-43228	WV-43233	51.9	46.6	49.3
WV-43229	WV-43233	35.0	32.8	33.9
WV-43230	WV-43233	46.0	50.9	48.5
WV-42310	WV-43234	39.9	31.7	35.8
WV-43223	WV-43234	31.0	31.3	31.2
WV-43224	WV-43234	41.4	34.9	38.2
WV-43225	WV-43234	60.4	47.2	53.8
WV-43226	WV-43234	50.0	36.1	43.1
WV-43227	WV-43234	30.0	30.8	30.4
WV-43228	WV-43234	36.6	38.8	37.7
WV-43229	WV-43234	28.5	26.2	27.4
WV-43230	WV-43234	44.4	32.5	38.5
WV-42310	WV-43235	57.2	33.6	45.4
WV-43223	WV-43235	ND	32.7	32.7
WV-43224	WV-43235	48.8	27.6	38.2
WV-43225	WV-43235	52.8	38.2	45.5
WV-43226	WV-43235	55.7	37.2	46.5
WV-43227	WV-43235	39.5	30.3	34.9
WV-43228	WV-43235	52.5	40.0	46.3
WV-43229	WV-43235	33.6	27.1	30.4
WV-43230	WV-43235	52.0	43.6	47.8
WV-42310	WV-43236	ND	31.9	31.9

WV-43223	WV-43236	ND	25.7	25.7
WV-43224	WV-43236	ND	29.0	29.0
WV-43225	WV-43236	ND	35.1	35.1
WV-43226	WV-43236	ND	34.1	34.1
WV-43227	WV-43236	ND	28.7	28.7
WV-43228	WV-43236	ND	40.1	40.1
WV-43229	WV-43236	ND	24.0	24.0
WV-43230	WV-43236	ND	37.8	37.8
WV-42310	WV-43237	28.0	26.7	27.4
WV-43223	WV-43237	28.6	26.9	27.8
WV-43224	WV-43237	33.7	28.5	31.1
WV-43225	WV-43237	45.7	37.4	41.6
WV-43226	WV-43237	26.9	32.3	29.6
WV-43227	WV-43237	24.8	28.4	26.6
WV-43228	WV-43237	35.6	45.9	40.8
WV-43229	WV-43237	23.5	29.2	26.4
WV-43230	WV-43237	38.2	41.6	39.9
WV-42310	WV-43238	35.4	31.5	33.5
WV-43223	WV-43238	29.8	29.0	29.4
WV-43224	WV-43238	35.5	34.0	34.8
WV-43225	WV-43238	62.3	54.0	58.2
WV-43226	WV-43238	36.9	38.7	37.8
WV-43227	WV-43238	27.3	30.9	29.1
WV-43228	WV-43238	39.0	49.8	44.4
WV-43229	WV-43238	28.4	34.0	31.2
WV-43230	WV-43238	46.0	46.3	46.2

Table 41 shows % mouse PTEN mRNA remaining (at 15 and 5 μM siRNA treatment) relative to human SFRS control, determined in iCell neuron cell line. N = 2. N.D.: Not determined.

Table 41.

		15 uM			5 pM		
Guide	Passenger	%remaining mRNA (mPTEN/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-42309	WV-41025	51.0	44.8	47.9	63.5	69.2	66.4
WV-42309	WV-44331	44.2	N.D.	44.2	59.5	83.6	71.6
WV-42309	WV-44332	36.7	23.6	30.2	56.6	70.8	63.7
WV-42309	WV-44333	30.9	31.7	31.3	58.2	60.7	59.5
WV-42309	WV-44334	43.2	35.6	39.4	73.4	53.3	63.4
WV-42309	WV-44337	45.9	39.7	42.8	48.3	40.1	44.2
WV-42309	WV-44338	50.1	52.7	51.4	62.4	58.6	60.5
WV-42309	WV-44372	45.4	N.D.	45.4	55.8	57.5	56.7
WV-42309	WV-44373	31.8	26.8	29.3	123.0	68.1	95.6
WV-42309	WV-44374	41.4	28.4	34.9	170.5	109.9	140.2
WV-42309	WV-44375	37.6	27.2	32.4	185.1	179.9	182.5
WV-42309	WV-44376	36.9	42.7	39.8	162.8	172.6	167.7
WV-40398	WV-40399	109.8	113.3	111.6	64.2	63.1	63.7

Table 42 shows % mouse PTEN mRNA remaining (at 10, 3, and 1 μM siRNA treatment) relative to mouse HPRT control, determined in H2K cell line. N = 2. N.D.: Not determined.

Table 42.

		10 uM			3 pM			1 pM		
Guide	Passenger	%remaining mRNA (mPTEN/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-40986	WV-41025	44.1	47.6	45.9	55.5	59.9	57.7	69.6	59.5	64.6
WV-40999	WV-41025	49.0	50.0	49.5	60.3	62.4	61.3	81.4	77.5	79.5
WV-42309	WV-41025	36.7	34.4	35.5	54.0	50.1	52.1	52.5	54.3	53.4
WV-42310	WV-41025	41.9	48.5	45.2	58.8	59.3	59.1	66.1	67.7	66.9
WV-42311	WV-41025	40.5	44.4	42.5	56.2	60.2	58.2	67.4	66.2	66.8
WV-42312	WV-41025	45.6	52.5	49.0	58.2	60.5	59.4	68.5	71.3	69.9
WV-42323	WV-41025	33.4	40.3	36.9	52.8	53.0	52.9	65.8	67.8	66.8
WV-42324	WV-41025	37.5	43.2	40.4	53.8	55.5	54.7	59.8	66.7	63.3
WV-42325	WV-41025	24.0	28.5	26.2	43.9	46.2	45.0	62.3	65.1	63.7
WV-42326	WV-41025	30.4	32.1	31.3	55.2	52.1	53.6	65.2	68.8	67.0

Table 43 shows % mouse PTEN mRNA remaining (at 15 and 5 μM siRNA treatment) relative to mouse HPRT control, determined in H2K cell line. N = 2. N.D.: Not determined.

Table 43

		15 uM			5 pM		
Guide	Passenger	%remaining mRNA (mPTEN/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-40398	WV-40399	62.9	62.7	62.8	86.1	78.2	82.2
WV-42309	WV-41023	48.9	48.6	48.8	68.2	70.2	69.2
WV-42309	WV-42643	50.7	49.0	49.9	67.2	63.5	65.4
WV-42309	WV-42644	34.7	35.2	35.0	53.8	59.2	56.5
WV-42309	WV-42645	27.5	31.2	29.4	55.1	57.4	56.3
WV-42309	WV-42646	23.5	26.9	25.2	67.6	67.3	67.5
WV-42309	WV-42647	17.6	19.7	18.7	55.2	55.6	55.4
WV-42309	WV-42648	25.8	29.8	27.8	57.9	54.9	56.4
WV-42309	WV-42649	23.7	24.2	24.0	60.9	58.3	59.6
WV-42309	WV-40399	50.5	49.6	50.1	68.0	69.6	68.8
WV-42309	WV-41025	49.3	44.9	47.1	73.9	71.4	72.7
WV-42309	WV-42650	37.2	37.0	37.1	59.1	57.5	58.3
WV-42309	WV-42651	34.6	32.3	33.5	53.4	48.5	51.0

WV-42309	WV-42652	26.5	25.2	25.9	45.2	44.8	45.0
WV-42309	WV-42653	37.3	37.9	37.6	64.8	60.6	62.7
WV-42309	WV-42654	22.1	22.4	22.3	47.5	45.8	46.7
WV-42309	WV-42655	32.3	37.8	35.1	73.7	67.3	70.5

Table 44 shows % mouse PTEN mRNA remaining (at 15 and 5 μM siRNA treatment) relative to mouse HPRT control, determined in H2K cell line. N = 2. N.D.: Not determined.

Table 44.

		15 uM			5 pM		
Guide	Passenger	%remaining mRNA (mPTEN/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-42310	WV-41025	35.5	42.1	38.8	58.8	40.5	49.6
WV-43223	WV-41025	31.3	36.3	33.8	57.7	42.2	50.0
WV-43224	WV-41025	36.6	39.3	37.9	57.5	41.7	49.6
WV-43225	WV-41025	51.7	49.2	50.4	73.2	59.8	66.5
WV-43226	WV-41025	53.6	47.2	50.4	71.2	62.3	66.8
WV-43227	WV-41025	48.2	50.6	49.4	64.1	56.8	60.5
WV-43228	WV-41025	55.7	55.5	55.6	69.2	68.1	68.6
WV-43229	WV-41025	39.6	34.4	37.0	55.2	52.3	53.7

WV-43230	WV-41025	47.3	43.0	45.2	59.9	56.8	58.4
WV-42310	WV-43232	27.5	30.4	29.0	66.2	48.4	57.3
WV-43223	WV-43232	25.6	28.4	27.0	57.5	43.1	50.3
WV-43224	WV-43232	31.0	32.8	31.9	55.8	42.4	49.1
WV-43225	WV-43232	47.7	47.9	47.8	77.3	57.4	67.3
WV-43226	WV-43232	43.6	43.5	43.6	73.9	52.4	63.1
WV-43227	WV-43232	36.2	38.1	37.1	64.0	50.9	57.4
WV-43228	WV-43232	50.0	47.7	48.9	75.2	58.6	66.9
WV-43229	WV-43232	30.8	29.5	30.2	56.8	46.4	51.6
WV-43230	WV-43232	42.2	45.4	43.8	69.3	53.7	61.5
WV-42310	WV-43233	31.8	38.2	35.0	58.4	46.9	52.7
WV-43223	WV-43233	22.1	27.3	24.7	44.7	40.1	42.4
WV-43224	WV-43233	29.0	33.6	31.3	52.5	42.9	47.7
WV-43225	WV-43233	44.0	41.8	42.9	66.2	60.0	63.1
WV-43226	WV-43233	43.0	42.7	42.8	64.8	54.5	59.6
WV-43227	WV-43233	34.9	38.4	36.7	58.0	49.9	54.0
WV-43228	WV-43233	51.0	48.5	49.8	70.5	53.8	62.1
WV-43229	WV-43233	26.1	29.9	28.0	48.9	40.0	44.5
WV-43230	WV-43233	39.1	39.4	39.3	65.9	51.7	58.8
WV-42310	WV-43234	26.7	31.8	29.2	54.5	47.1	50.8
WV-43223	WV-43234	26.0	28.3	27.1	44.5	39.5	42.0
WV-43224	WV-43234	32.5	35.7	34.1	53.3	46.1	49.7

WV-43225	WV-43234	48.3	52.9	50.6	80.4	62.5	71.5
WV-43226	WV-43234	44.1	46.1	45.1	62.5	50.2	56.4
WV-43227	WV-43234	35.4	36.5	35.9	59.1	47.5	53.3
WV-43228	WV-43234	48.7	54.7	51.7	75.1	62.7	68.9
WV-43229	WV-43234	26.2	39.0	32.6	52.6	45.9	49.3
WV-43230	WV-43234	41.7	55.9	48.8	58.8	58.9	58.8
WV-42310	WV-43235	27.0	35.0	31.0	58.7	44.9	51.8
WV-43223	WV-43235	24.6	29.1	26.8	50.1	40.3	45.2
WV-43224	WV-43235	31.8	34.2	33.0	52.2	44.4	48.3
WV-43225	WV-43235	47.9	48.6	48.2	75.0	56.1	65.6
WV-43226	WV-43235	44.0	45.0	44.5	74.6	51.3	63.0
WV-43227	WV-43235	29.2	36.5	32.9	64.0	42.7	53.4
WV-43228	WV-43235	44.3	48.5	46.4	70.6	59.0	64.8
WV-43229	WV-43235	26.4	34.0	30.2	48.8	41.9	45.4
WV-43230	WV-43235	44.1	45.0	44.5	61.5	60.7	61.1
WV-42310	WV-43236	26.9	31.8	29.4	53.0	45.1	49.0
WV-43223	WV-43236	25.2	26.6	25.9	46.4	36.2	41.3
WV-43224	WV-43236	31.5	30.5	31.0	54.5	46.3	50.4
WV-43225	WV-43236	45.4	47.7	46.5	72.3	54.9	63.6
WV-43226	WV-43236	36.0	41.0	38.5	56.4	52.7	54.5
WV-43227	WV-43236	29.1	30.0	29.5	54.0	48.9	51.4
WV-43228	WV-43236	47.2	46.2	46.7	76.7	56.7	66.7

WV-43229	WV-43236	24.1	28.5	26.3	51.4	35.8	43.6
WV-43230	WV-43236	40.8	41.3	41.0	58.2	59.8	59.0
WV-42310	WV-43237	28.3	34.3	31.3	52.3	50.4	51.4
WV-43223	WV-43237	26.0	29.1	27.5	48.0	42.6	45.3
WV-43224	WV-43237	32.5	33.4	32.9	52.4	54.7	53.6
WV-43225	WV-43237	39.9	41.9	40.9	62.2	57.7	59.9
WV-43226	WV-43237	40.2	47.0	43.6	75.8	66.3	71.1
WV-43227	WV-43237	35.3	40.5	37.9	54.1	48.5	51.3
WV-43228	WV-43237	45.3	48.2	46.8	71.6	69.1	70.4
WV-43229	WV-43237	26.0	31.2	28.6	46.3	46.3	46.3
WV-43230	WV-43237	32.9	33.4	33.2	66.2	57.8	62.0
WV-42310	WV-43238	28.9	33.7	31.3	88.2	51.0	69.6
WV-43223	WV-43238	27.3	27.5	27.4	47.8	48.8	48.3
WV-43224	WV-43238	27.5	32.5	30.0	55.2	65.4	60.3
WV-43225	WV-43238	41.3	46.0	43.6	67.0	77.8	72.4
WV-43226	WV-43238	31.9	38.6	35.2	61.7	64.4	63.1
WV-43227	WV-43238	30.8	33.8	32.3	57.6	57.0	57.3
WV-43228	WV-43238	47.0	48.4	47.7	75.3	86.0	80.6
WV-43229	WV-43238	23.3	27.9	25.6	47.5	44.8	46.1
WV-43230	WV-43238	46.1	43.2	44.7	53.2	71.7	62.4

Table 45 shows % mouse PTEN mRNA remaining (at 10, 3, and 1 μM siRNA treatment) relative to mouse HPRT control, determined in H2K cell line. N = 2. N.D.: Not determined.

Table 45.

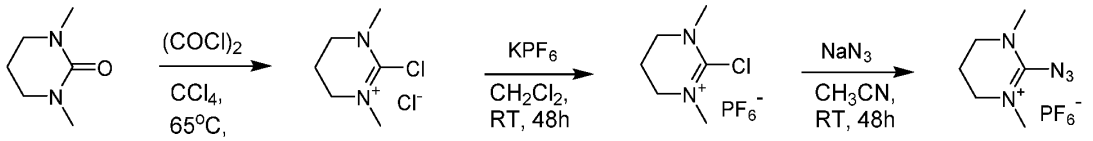
		10 uM			3 pM			1 pM		
Guide	Passenger	%remaining mRNA (mPTEN/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean	%remaining mRNA (mTTR/ mHPRT)-1	%remaining mRNA (mTTR/ mHPRT)-2	Mean
WV-42309	WV-41025	48.5	37.0	42.8	65.9	55.2	60.6	69.4	58.4	63.9
WV-42309	WV-44331	35.1	43.2	39.2	49.3	51.4	50.4	55.3	58.5	56.9
WV-42309	WV-44332	40.3	46.8	43.6	58.0	58.4	58.2	66.9	66.2	66.5
WV-42309	WV-44333	58.4	44.2	51.3	63.0	71.9	67.4	67.5	81.0	74.3
WV-42309	WV-44334	48.3	42.0	45.1	62.1	71.4	66.7	77.9	82.9	80.4
WV-42309	WV-44337	58.0	58.9	58.5	69.7	79.4	74.6	80.1	89.1	84.6
WV-42309	WV-44338	53.8	40.9	47.3	60.9	53.7	57.3	67.2	68.0	67.6
WV-42309	WV-44372	41.9	48.9	45.4	52.8	58.3	55.5	66.2	61.6	63.9
WV-42309	WV-44373	48.5	56.0	52.2	58.8	64.4	61.6	76.0	75.7	75.8
WV-42309	WV-44374	66.1	52.3	59.2	76.1	72.7	74.4	81.0	84.3	82.7
WV-42309	WV-44375	51.8	61.2	56.5	74.4	81.0	77.7	76.5	87.9	82.2
WV-42309	WV-44376	46.2	53.6	49.9	57.8	62.4	60.1	78.0	76.6	77.3

WV-40398	WV-40399		58.6		70.8	64.7	62.3	68.5	65.4	63.7	72.4	68.0

400

EXAMPLE 13. **Synthesis and Experimental Procedures for all PN variations Azides:**

Synthesis of 2-azido-(1,3-dimethyl-3,4,5,6-tetrahydropyrimidinium) hexafluorophosphate (1d). PN code: n025



Synthesis of 2-chloro-1,3-dimethyl-3,4,5,6-tetrahydropyrimidinium chloride (1b).

To 1,3-dimethyltetrahydropyrimidin-2(1H)-one, **1a** (25.0 g, 0.195 mol, 1.0 equiv) in dry two neck round bottom flask (1 liter) under argon atmosphere was added anhydrous carbon tetrachloride (375 mL). To the reaction mixture was added freshly distilled oxalyl chloride (25.0 mL, 0.292 mol, 1.5 equiv) using additional funnel over a period of 20 min. Then reaction mixture was heated to 65 °C for 48 hrs. After completion of reaction (TLC – 5% CH_3OH:CH_2Cl_2; TLC charring – Phosphomolybdic acid), reaction mixture was cooled to room temperature and was added diethyl ether (300 mL). The reaction mixture was stirred at room temperature for 5 min. The obtained reaction mixture was filtered, and precipitate was washed with diethyl ether (3 x 500 mL). Compound was dried on high vacuum to give 2-chloro-1,3-dimethyl-3,4,5,6-tetrahydropyrimidinium chloride **1b** as a brown solid (31g, 87% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm 3.97 (t, 4H, J = 5.8 Hz), 3.51 (s, 6H), 2.37-2.31 (m, 2H).

MS: m/z calcd for $C_6H_{12}Cl_2N_2$ ([M-Cl]$^+$), 147.06; found 146.95.

Synthesis of 2-chloro-1,3-dimethyl-3,4,5,6-tetrahydropyrimidinium hexafluorophosphate (1c).

To 2-chloro-1,3-dimethyl-3,4,5,6-tetrahydropyrimidinium chloride, **1b** (31.0 g, 0.169 mol, 1.0 equiv), in a dry round bottomed flask (1 liter) under argon atmosphere was added CH_2Cl_2 (310 mL). To the solution was added KPF_6 (31.16 g, 0.169 mol, 1.0 equiv) in a portion wise over a period of 10 min. The reaction mixture was stirred at room temperature for 1.5 h. After completion of reaction (TLC – 5% CH_3OH:CH_2Cl_2; TLC charring –

Phosphomolybdic acid), the reaction mixture was filter through celite and filter cake was washed with CH_2Cl_2 (150 mL). The filtrate was concentrated to dryness under reduced pressure to obtain crude product. The crude product was dissolved in CH_2Cl_2 (25 mL). Compound was precipitate by dropwise addition of diethyl ether. After complete precipitation, solvent was decanted to get product. Obtained was dried under vacuum to give 2-chloro-1,3-dimethyl-3,4,5,6-tetrahydropyrimidinium hexafluorophosphate (**1c**), as white solid (45.0 g, 91% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 3.84 (s, 4H), 3.47 (s, 6H), 2.30 (s, 2H).

^{19}F NMR (500 MHz, $CDCl_3$): δ in ppm = -73.02 and -74.54.

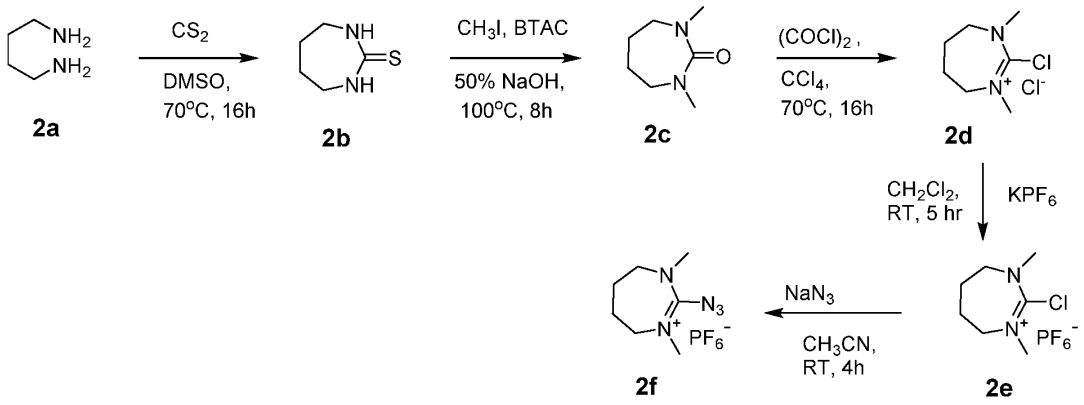
Synthesis of 2-azido-(1,3-dimethyl-3,4,5,6-tetrahydropyrimidinium) hexafluorophosphate (1d).

To 2-chloro-1,3-dimethyl-3,4,5,6-tetrahydropyrimidinium hexafluorophosphate (**1c**), (45.0 g, 0.154 mol, 1.0 equiv) in a dry round bottomed flask (1 liter) was added anhydrous acetonitrile (450 mL) under argon atmosphere. To the solution was added sodium azide (14.99 g, 0.231 mol, 1.5 equiv) in a portion wise over the period of 10 min. The reaction mixture stirred at room temperature for 8 hrs. After completion of reaction (TLC – 5% CH_3OH:CH_2Cl_2; TLC charring – ninhydrin), reaction mixture was filtered through a pad of celite and washed with CH_3CN (30 mL). The obtained filtrate was dried under reduced pressure to get crude product. The crude compound was dissolved in CH_3CN (150 mL). Product was precipitate by dropwise addition of diethyl ether: hexane mixture. After complete precipitation, the solvent was decanted and solid was dried under vacuum. The above precipitation procedure repeats two more times to get pure 2-azido-(1,3-dimethyl-3,4,5,6-tetrahydropyrimidinium) hexafluorophosphate **1d** as white solid (26g, 57% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.59 (t, 4H, J = 6.0 Hz), 3.33 (s, 6H), 2.26-2.20 (m, 2H).

^{19}F NMR (400 MHz, $CDCl_3$): δ in ppm = -72.99 and -74.88. MS: m/z calcd for $C_6H_{12}F_6N_5P$ ($[M-PF_6]^+$), 154.11; found 154.29. IR (KBr pellet): N_3 (2184 cm^{-1})

Synthesis of 2-azido-(1,3-dimethyl-4,5,6,7-tetrahydro-1H-1,3-diazepinium) hexafluoro phosphate (2f). PN code: n026



[OBJ]

Synthesis of 1,3-diazepane-2-thione (2b)

To butane-1,4-diamine **2a** (50.0 g, 0.567 mol, 1.0 equiv) in a dry round bottomed flask (1 liter) under argon atmosphere was added DMSO (500 mL). Solution was cooled 0 °C by using ice bath and carbon disulphide (41.2 mL, 0.682 mol, 1.2 equiv) was added by using addition funnel. Then, reaction mixture was heated 70 °C for 16 hrs. After completion of reaction (TLC – 5% CH_3OH:CH_2Cl_2), the reaction mixture was cooled to room temperature. The precipitated solid was filtered off and dried under high vacuum to get 32.0 g of product. To obtained filtrate was diluted with water (1.0 liter) and organic layer was extract with CH_2Cl_2 (3 x 1000 mL). The combined the organic layer dried on sodium sulphate and evaporated under reduced pressure to get crude product. This crude product was dissolved in minimum volume of CH_2Cl_2 then precipitated by dropwise addition of hexane. The precipitate was filtered and dried under high vacuum to give3-diazepane-2-thione **2b** (18.0 g), as a white solid (50g, 68% yield).

^1H NMR (400 MHz, $CDCl_3$): δ in ppm = 6.69 (s, 2H), 3.28-3.24 (m, 4H), 1.77-1.74 (m, 4H).

Synthesis of 1,3-dimethyl-1,3-diazepan-2-one (2c).

To 1,3-diazepane-2-thione **2b** (21.0 g, 0.161 mol, 1.0 equiv) in a dry single neck round bottomed flask (250 mL) under argon atmosphere was added CH_2Cl_2 (100 mL) and solution was cooled by using ice bath. To solution was added benzyltrimethylammonium chloride (BTAC, 1.49 g, 0.008 mol, 2 mol%) followed by dropwise addition of methyl iodide (65.0 mL, 1.044 mol, 6.5 equiv) and 50% aq. NaOH solution (58.68 mL) respectively. The reaction mixture was heated to 100 °C for 8 hrs. After completion of reaction (TLC – 5%

CH_3OH:CH_2Cl_2), the reaction mixture was cooled to room temperature. Organic layer was extracted with chloroform (3 x 1000 mL). Combined organic layer was dried over sodium sulphate and solvent was removed under reduced pressure to get crude product. The compound was purified via silica gel (100-200 mesh) column chromatography, the product was eluted with 30-80% Ethyl acetate in hexanes to give 1,3-dimethyl-1,3-diazepan-2-one, **2c** as a light-yellow oil (9.00 g, 39% yield).

^1H NMR (500 MHz, $CDCl_3$): δ in ppm = 3.13-3.11 (m, 4H), 2.84 (s, 6H), 1.68-1.65 (m, 4H).

Synthesis of 2-chloro-1,3-dimethyl-4,5,6,7-tetrahydro-1H-1,3-diazepinium chloride (2d).

To 1,3-dimethyl-1,3-diazepan-2-one **2c,** (25.0 g, 0.176 mol, 1.0 equiv) in dry two neck round bottomed flask (1 liter) under argon atmosphere was added anhydrous carbon tetrachloride (250 mL). To the solution was added freshly distilled oxalyl chloride (22.6 mL, 0.264 mol, 1.5 equiv) using addition funnel over a period of 20 min. The reaction mixture was heated to 70 °C for 16 hrs. After completion of reaction (TLC – 10% CH_3OH:CH_2Cl_2), reaction mixture was cooled to room temperature, then diluted with of diethyl ether (500 mL) and stirred for 5 min. The precipitate was collected after filtration and washed with diethyl ether (2 x 500 mL). Obtained crude product was dissolved in minimum amount of solvent and precipitated by addition of 50% ethyl acetate and hexanes. Compound was collected via filtration and dried under vacuum to give 2-chloro-1,3-dimethyl-4,5,6,7-tetrahydro-1H-1,3-diazepinium chloride, **2d** as a white solid (30.0 g). The crude compound was directly used for next reaction without any further purification.

Synthesis of 2-chloro-1,3-dimethyl-4,5,6,7-tetrahydro-1H-1,3-diazepinium hexafluoro phosphate (2e).

To 2-chloro-1,3-dimethyl-4,5,6,7-tetrahydro-1H-1,3-diazepinium chloride, **2d** (30.0g, 0.152 mol, 1.0 equiv) in dry round bottomed flak (1 liter) under argon atmosphere was added CH_2Cl_2 (300 mL). To the solution was added KPF_6 (42.02 g, 0.228 mol, 1.5 equiv) in a portion wise over a period of 10 min. The reaction mixture was stirred at room temperature for 4.5 hrs. After completion of reaction (TLC – 10% CH_3OH:CH_2Cl_2), the reaction mixture

was filter through celite and filter cake was washed with CH_2Cl_2 (150 mL), the filtrate was concentrated to dryness. The crude compound was dissolved in CH_2Cl_2 and washed with water (2 x 500 mL). The organic layer dried over sodium sulphate and solvent was removed under reduced pressure to give 2-chloro-1,3-dimethyl-4,5,6,7-tetrahydro-1H-1,3-diazepinium hexafluoro phosphate, **2e** as white solid (25.0 g, 54% yield).

^1H NMR (500 MHz, $CDCl_3$): δ in ppm = 3.90 (t, 4H, *J* = 5.9 Hz), 3.38 (s, 6H), 2.09-2.07 (m, 4H).

^{19}F NMR (500 MHz, $CDCl_3$): δ in ppm = -72.66 and -74.16.

Synthesis of 2-azido-(1,3-dimethyl-4,5,6,7-tetrahydro-1H-1,3-diazepinium) hexafluoro phosphate (2f).

To 2-chloro-1,3-dimethyl-4,5,6,7-tetrahydro-1H-1,3-diazepinium hexafluoro phosphate, **2e** (25.0 g, 0.081 mol, 1.0 equiv) in a round bottomed flask (1 liter) under argon atmosphere was added anhydrous CH_3CN (250 mL). To the solution was added sodium azide (7.95 g, 0.122 mol, 1.5 equiv) in a portion wise over a period of 10 min. The reaction mixture was stirred at room temperature for 4 hrs. After completion of reaction (TLC – 10% CH_3OH:CH_2Cl_2; TLC charring – ninhydrin), reaction mixture was filtered through a pad of celite and washed with CH_3CN (30 mL). The organic layer was evaporated under reduced pressure to give crude product. The crude product was dissolved in CH_3CN (50 mL) and product was precipitated by adding diethyl ether at -78°C. The solvent was removed and the solid obtained was dried under vacuum. The above precipitation procedure was repeated two times to give 2-azido-(1,3-dimethyl-4,5,6,7-tetrahydro-1H-1,3-diazepinium) hexafluoro phosphate **2f,** as pale-yellow solid g (21.0g, 82% yield).

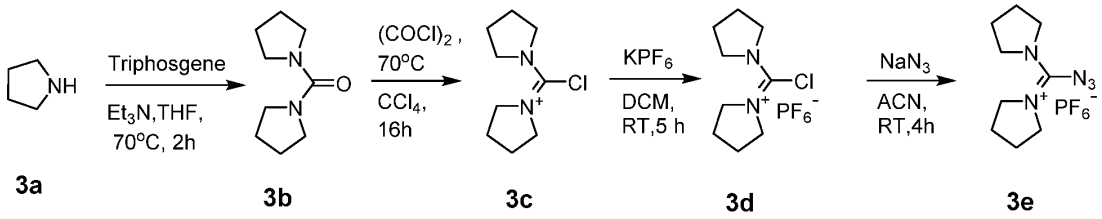
^1H NMR (500 MHz, $CDCl_3$): δ in ppm = 3.63 (t, 4H, *J* = 5.5 Hz), 3.51 (d, 4H, *J* = 25.5 Hz), 3.25 (s, 6H), 3.15 (s, 6H), 2.02-1.96 (m, 4H), 1.89 (s, 4H).

^{19}F NMR (500 MHz, $CDCl_3$): δ in ppm = -72.15, -72.56, -73.67 and -74.08.

MS: m/z calcd for $C_{67}H_{14}F_6N_5P$ ($[M\text{-}PF_6]^+$), 168.22; found 168.15. IR (KBr pellet): N_3 (2162 cm^{-1})

Synthesis of 1-azido(pyrrolidin-1-yl)methylene)pyrrolidinium)hexafluorophosphate, (3e)

PN code:n004



3a 3b 3c 3d 3e

Synthesis of di(pyrrolidin-1-yl)methanone (3b).

To pyrrolidine **3a** (117 mL, 1.424 mol, 1.0 equiv) in a dry three neck round bottom flask (3 liters) was added in anhydrous THF (1380 mL). To the solution was added triethylamine (212 mL, 1.521 mol, 1.1 equiv) and reaction mixture was cooled to 0 °C by using ice bath. To reaction mixture was added, a solution of triphosgene (70.0 g, 0.236 mol, 0.16 equiv, in 224 mL THF) dropwise using dropping funnel over a period of 30 min. The resulting precipitated mixture was heated at 70 °C for 2 hrs. Then reaction mixture was cooled to room temperature and stirred for another 2 hrs. TLC showed the reaction was complete (TLC - 5% CH_3OH:CH_2Cl_2; TLC charring – $KMnO_4$). Then reaction mixture was filtered through Buckner funnel and Whatman filter paper. Obtained cake was washed with THF (250 mL). Filtrate was collected and solvent was removed under reduced pressure to give di(pyrrolidin-1-yl)methanone, **3b** as a brown color liquid (124.0 g, 52% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 3.37 (t, 8H, J = 6.9 Hz), 1.81-1.84 (m, 8H).

MS: m/z calcd for $C_9H_{16}N_2O$ ($[M+H]^+$), 169.24; found 169.11.

Synthesis of 1-(chloro(pyrrolidin-1-yl)methylene)pyrrolidinium chloride (3c)

To di(pyrrolidin-1-yl)methanone **3b** (124 g, 0.737 mol, 1.0 equiv) in a dry three-neck round bottom flask (3 liter) was added dry CH_2Cl_2 (1340 mL) under argon atmosphere at room temperature. To the solution was added a solution of oxalyl chloride (63.2 mL, 0.737 mol, 1.0 equiv) in dry CH_2Cl_2 (520 mL) dropwise using dropping funnel at room temperature for over a period of 40 min. Then reaction mixture was heated to 60 °C for 5 hrs. TLC showed the reaction was complete (TLC - 5% CH_3OH:CH_2Cl_2; TLC charring – $KMnO_4$). Then the solvent was evaporated to dryness to get 1-(chloro(pyrrolidin-1-yl)methylene)pyrrolidinium chloride **3c** as a brown color liquid (160.0 g). The crude material was directly used for next step.

Synthesis of 1-(chloro(pyrrolidin-1-yl)methylene)pyrrolidinium hexafluorophosphate (3d)

To 1-(chloro(pyrrolidin-1-yl)methylene) pyrrolidinium chloride **3c** (160 g, 0.717 mol, 1.0 equiv) in a dry round bottom flask (2 liter) was added water (1525 mL) at room temperature. To solution, was added a saturated solution of KPF_6 (158.9 g, 0.863 mol, 1.2 equiv in 326 mL water) dropwise using dropping funnel over a period of 20 min. While adding some of product was precipitated out. Stirring was continue for another 10 min at room temperature. Then reaction mixture was filtered through Buckner funnel using Whatman filter paper. The solid was washed with water (1500 mL) and dried on high vacuum to get crude product. The crude product was dissolved in acetone (110 mL) and precipitated by dropwise addition of diethyl ether (1000 mL). The above precipitation method was repeated one more time to give 1-(chloro(pyrrolidin-1-yl) methylene)pyrrolidinium hexafluorophosphate, **3d** as a cream color solid. (142.1g, 60% yield).

^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 3.92 (t, 8H, J = 6.2 Hz), 2.10 (t, 8H, J = 6.5 Hz).

Synthesis of 1-(azido(pyrrolidin-1-yl)methylene)pyrrolidinium hexafluorophosphate (3e)

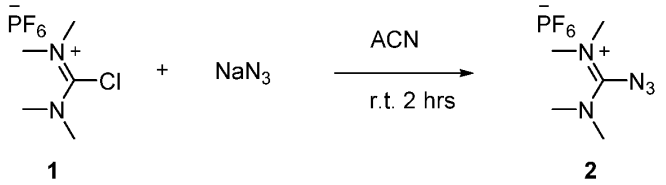
To 1-(chloro(pyrrolidin-1-yl)methylene)pyrrolidinium hexafluorophosphate, **3d** (71.0 g, 0.213 mol, 1.0 equiv) in a dry round bottomed flask (500 mL) was aziotroped with acetonitrile (3 x 100 mL) while maintaining the bath temperature 28°C. The compound was dried on high vacuum pump for 1 hrs. To the flask was added anhydrous CH_3CN (213 mL) under argon atmosphere. To the solution was added sodium azide (3.58 g, 0.055 mol) and stir for 3 hrs at 30 °C. After completion of reaction (TLC – 5% CH_3OH:CH_2Cl_2; TLC charring – ninhydrin), reaction mixture was filtered through a pad of celite and washed with CH_3CN (50 mL). The organic layer was removed under reduced pressure to get crude product. The solid obtained was dissolved in CH_3CN (60 mL) and precipitate by adding dropwise diethyl ether (850 mL). The above precipitation method was repeated one more time to geve **3e** as a white color solid (65.1 g, 89% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.77 (t, 8H, J = 6.5 Hz), 2.03-2.06 (m, 8H).

^{19}F NMR (400 MHz, $CDCl_3$): δ in ppm = -73.36 and -75.26. MS: m/z calcd for $C_9H_{16}N_5PF_6$ ($[M-PF_6]^+$), 194.26; found 194.16. IR (KBr pellet): N_3 (2153 cm^{-1})

Synthesis of *N*-(azido(dimethylamino)methylene)-*N*-methylmethanaminium

hexafluorophosphate, (2). PN code: n003



1 2

OBJ

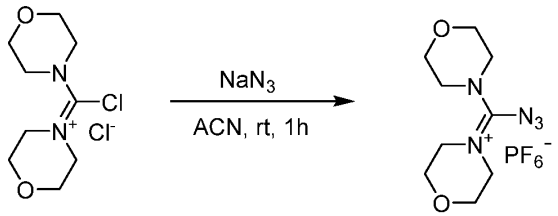
To commercially available *N*-(chloro(dimethylamino)methylene)-*N*-methylmethanaminium hexafluorophosphate(V) (**1**) (35.0 g, 124.7 mmol, 1.0 equiv) in a round bottom flask was added acetonitrile (100 mL). To the solution was added sodium azide (12.2 g, 187.1 mmol, 1.5 equiv). The mixture was stirred at room temperature for 1.5 hrs. After completion of reaction the reaction mixture was filtered through celite pad. The cake was washed with acetonitrile (3 x 40 mL). The filtrate was collected, and solvent was removed under reduced pressure to get crude product. The residue was dissolved in acetone (15 mL), then toluene was added to precipitate out product to give *N*-(azido(dimethylamino)methylene)-*N*-methylmethanaminium hexafluorophosphate, (**2**) as while solid (35.4g, 99 % yield).

^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 3.12(s, 12H).

^{19}F NMR (400 MHz, Acetonitrile-d_3): δ in ppm = -69.57 and -70.83

Synthesis of 4-(azido(morpholino)methylene)morpholinium hexafluorophosphate (4b).

PN code: n008



4a 4b

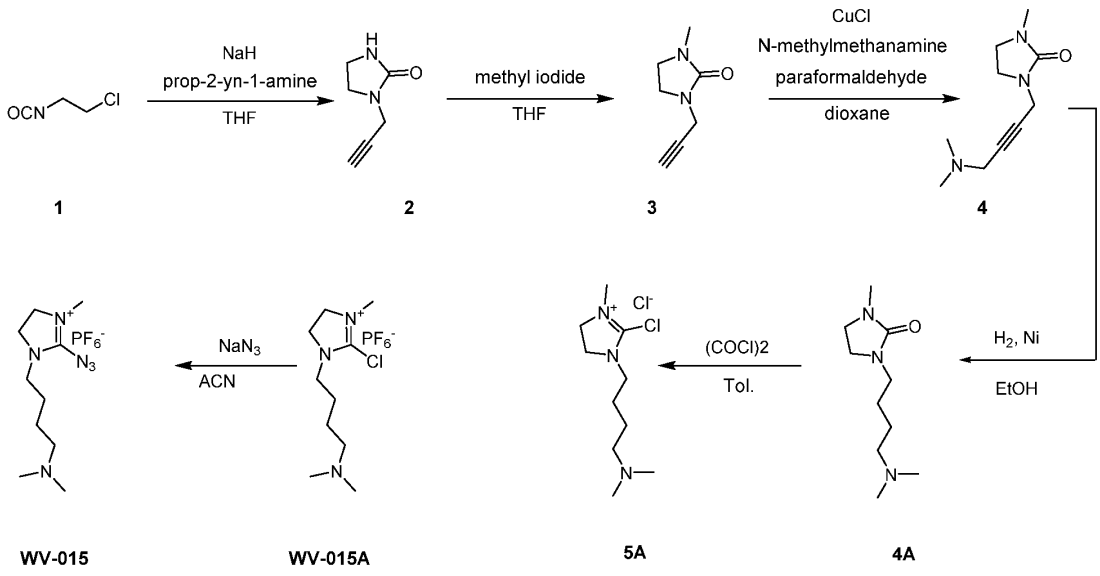
To commercially available 4-[chloro(morpholinium-4-ylidene)methyl]morpholine chloride **4a** (41.2g, 0.115mole, 1.0 equiv) in a round bottomed flask was added acetonitrile (115 mL).

To the solution was added sodium azide (11.2g, 0.172mole, 1.5 equiv). The mixture was stirred at room temperature for 1hrs. After completion of reaction the reaction mixture was filtered through celite pad. The cake was washed with acetonitrile (3 x 40 mL). The filtrate was collected, and solvent was removed under reduced pressure to get crude product. The residue was dissolved in 1:1 toluene: acetone (160 mL) and left it in freezer overnight for formation of crystallization. The compound was collected by filtration and dried under vacuum to 4-(azido(morpholino)methylene)morpholinium hexafluorophosphate, **4b**,(27g, 64% yield).

^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 3.86 – 3.71 (m, 4H), 3.65 – 3.58 (m, 2H), 2.34 (br.s, 8H).

^{19}F NMR (400 MHz, Acetonitrile-d_3): δ in ppm = -71.98 and -73.80

Synthesis of 2-chloro-1-(4-(dimethylamino)butyl)-3-methyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(WV-015A), PN code: n029



1-(prop-2-yn-1-yl)imidazolidin-2-one (2).

1-chloro-2-isocyanatoethane **1** (100 g, 947.66 mmol) was added at 0 °C to a stirred solution of prop-2-yn-1-amine (propargyl amine, 57.42 g, 1.04 mol, 1.0 equiv) in THF (1000 mL).

The solution was warmed to 20°C and NaH (39.80 g, 995.05 mmol, 60% purity, 0.99 equiv) was added, the mixture was stirred for 3 hr. TLC indicated prop-2-yn-1-amine was consumed completely and one new spot formed. The reaction was quenched with acetic acid (50.0 mL), the THF was removed under reduced pressure, and the residue was diluted with water 400 mL and extracted with ethyl acetate 900 mL (300 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by crystallization from ethyl acetate/hexane to give 1-(prop-2-yn-1-yl)imidazolidin-2-one (**2**) as a white solid (89 g, 75.65% yield).

1-methyl-3-(prop-2-yn-1-yl)imidazolidin-2-one (3)

To a solution of 1-(prop-2-yn-1-yl)imidazolidin-2-one (**2**) (89 g, 716.93 mmol, 1.0 equiv) in THF (900 mL) was added NaH (57.35 g, 1.43 mol, 60% purity, 2.0 equiv) at 0 °C, 15 min later MeI (122.11 g, 860.32 mmol,) was added. The mixture was stirred at 0-20 °C for 2 hr. TLC indicated compound 2 was consumed completely and one new spot formed. The reaction mixture was quenched by addition H_2O 500 mL, and then extracted with EtOAc 1500 mL (500 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 0/1) to give 1-methyl-3-(prop-2-yn-1-yl)imidazolidin-2-one (**3**) as a yellow oil (99 g, crude).
TLC (Petroleum ether: Ethyl acetate = 0:1), Rf = 0.6

1-(4-(dimethylamino)but-2-yn-1-yl)-3-methylimidazolidin-2-one (4).

To a solution of 1-methyl-3-(prop-2-yn-1-yl)imidazolidin-2-one (3) (99 g, 716.53 mmol, 1.0 equiv) in dioxane (1000 mL) was added CuCl (92.22 g, 931.48 mmol, 1.3 equiv), PARAFORMALDEHYDE (20 g, 2.53 mmol) and *N*-methylmethanamine (84.80 g, 752.35 mmol, 40% purity, 1.05 equiv). The mixture was stirred at 55 °C for 6 hr. LCMS showed the desired mass was detected. 500 g Na_2CO_3 was added to the reaction mixture then stirred for 1 hr, filtered the mixture and the filtrate was concentrated under reduced pressure. The residue was purified by RP-MPLC (DAC-150 Agela C18, 450ml/min, 5-25% 40min; 25-25% 40min) to give a crude mixture. The crude was purified by column chromatography (SiO_2, Ethyl acetate /Methanol=1/0 to 5/1) to give 1-(4-(dimethylamino)but-2-yn-1-yl)-3-

methylimidazolidin-2-one (**4**) as a yellow oil (50 g, 35.74% yield). LCMS (M+H+): 196.2 TLC (Ethyl acetate: Methanol = 5:1), Rf = 0.4

1-(4-(dimethylamino)butyl)-3-methylimidazolidin-2-one (4A).

A mixture of 1-(4-(dimethylamino)but-2-yn-1-yl)-3-methylimidazolidin-2-one (**4**) (30 g, 153.64 mmol, 1.0 equiv), Ni (10 g) in EtOH (500 mL) was degassed and purged with H_2 for 3 times, and then the mixture was stirred at 80 °C for 12 hr under H_2 atmosphere (15psi). LCMS showed compound 4 was consumed completely and one main peak with desired mass was detected. The mixture was filtered through celite pad and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, Dichloromethane: Methanol = 1/0 to 0/1) to give 1-(4-(dimethylamino)butyl)-3-methylimidazolidin-2-one (**4A**) as a yellow oil (30 g, crude).
LCMS (M+H+): 200.3. TLC (DCM: MeOH = 5:1, Rf = 0.2)

2-chloro-1-(4-(dimethylamino)butyl)-3-methyl-4,5-dihydro-1H-imidazol-3-ium chloride (5A).

To a solution of 1-(4-(dimethylamino)butyl)-3-methylimidazolidin-2-one (4A) (15 g, 75.27 mmol, 1.0 equiv) in toluene (50 mL) was added $(COCl)_2$ (191.06 g, 1.51 mol), the mixture was stirred at 65 °C for 12 hr. LCMS showed the desired mass was detected. The reaction mixture was concentrated under reduced pressure to remove solvent. The crude product was purified by re-crystallization from ACN 100 mL at 15 °C to give 2-chloro-1-(4-(dimethylamino)butyl)-3-methyl-4,5-dihydro-1H-imidazol-3-ium chloride (**5A**). as a brown solid (10 g, 52.27% yield,) . LCMS (M+H+): 218.3

2-chloro-1-(4-(dimethylamino)butyl)-3-methyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(WV-015A)

To a solution of 2-chloro-1-(4-(dimethylamino)butyl)-3-methyl-4,5-dihydro-1H-imidazol-3-ium chloride (**5A**) (9.75 g, 38.36 mmol, 1.0 equiv) in DCM (50 mL) and H_2O (30 mL) was added potassium;hexafluorophosphate (7.06 g, 38.36 mmol, 1.0 equiv) at 15 °C. The reaction mixture was stirred at 15 °C for 1 h. A large number of solids are precipitated form

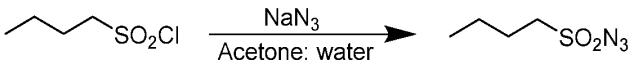
the reaction mixture. The reaction mixture was filtered, and the filter cake was washed with DCM (30mL x 2), concentrated under reduced pressure to get 10 g crude. The crude was added to 200 mL H_2O, filtered, the filter cake was desired compound 2-chloro-1-(4-(dimethylamino)butyl)-3-methyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate (**WV-015A**) (8.2 g, 58.75% yield).

2-azido-1-(4-(dimethylamino)butyl)-3-methyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate (WV-015A)

To a solution of 2-chloro-1-(4-(dimethylamino)butyl)-3-methyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate (**WV-015A**) (5.5 g, 15.1 mmol, 1.0 equiv) in dry round bottom flask (500 mL) was added dry acetonitrile (300 mL) and cooled to 0°C. To the solution was added sodium azide (1.18 g, 18.2 mmol, 1.2 equiv) and stirred for 2 hours. TLC showed completion of reaction. The reaction mixture was filtered through celite pad. Filtrate was evaporated under reduced pressure to get crude compound.

MS (ESI) 371.31 $(M+1)^+$

Synthesis of butane-1-sulfonyl azide (WLS-05). PN Code: n020



WLS-05

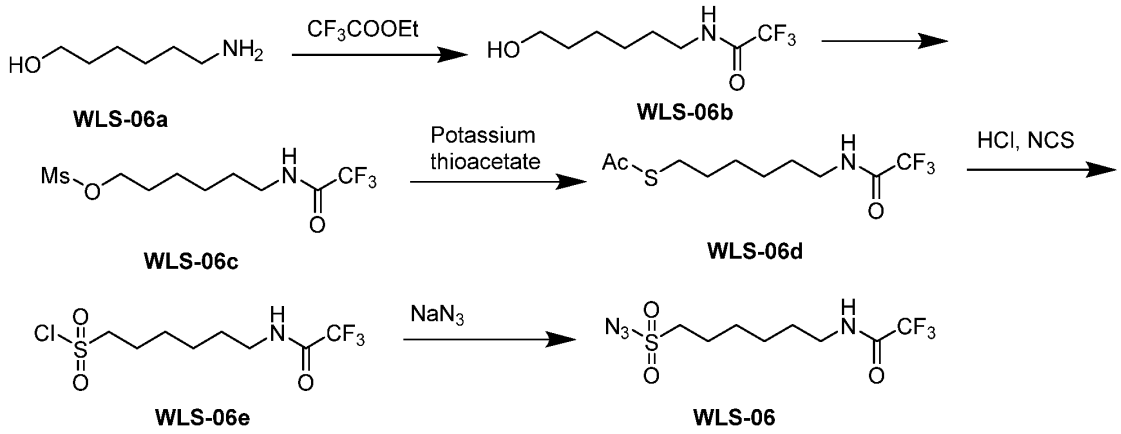
Butane-1-sulfonyl azide (WLS-05).

To a solution of sodium azide (15.56 g, 0.24 mol) in water (95 mL) was added dropwise a solution of butane-1-sulfonyl chloride (25 g, 0.16 mol) in acetone (320 mL) at 0 °C for 1 h under argon atmosphere. The reaction mixture was allowed to room temperature and stirred for 3 h. After completion of reaction (monitoring by TLC), acetone was removed under reduced pressure and the reaction mixture was extracted with EtOAc (100 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Crude product was purified by silica gel column chromatography using EtOAc: hexane to afford the compound butane-1-sulfonyl azide (**WLS-05**) (23.53 g, 90%) as a slight brown colour oil. TLC Mobile phase details: 10% EtOAC in hexane. **^{1}H NMR (500 MHz, $CDCl_3$):** δ in ppm = 3.32 (m, 2H, CH_2), 1.91 (m, 2H, CH_2), 1.51 (m, 2H, CH_2), 0.99 (t, *J* =

7.3 Hz, 3H, CH_3).

MS: *m/z* calcd for $C_4H_9N_3O_2S$ ([M+Na]$^+$), 186.18; found 186.15. IR (KBr) = 2135 cm ^{1}

Synthesis of 6-(2,2,2-Trifluoroacetamido)hexane-1-sulfonyl azide (WLS-06). PN Code: n021



2,2,2-Trifluoro-N-(6-hydroxyhexyl)acetamide (WLS-06b)

To a mixture of 6-amino hexanol (50 g, 0.43 mol) and triethylamine (148.6 mL, 1.06 mol, 2.5 equiv) in MeOH (375 mL) was cooled to 0 °C. Added Trifluoroacetic anhydride (83 mL, 0.59 mol) dropwise over period of 20 min under an argon atmosphere and the reaction was allowed to warm to room-temperature and stirred 4 h, concentrated, the crude product was purified by silica gel (100-200 mesh) chromatography using EtOAc:hexane to afford the compound 2,2,2-Trifluoro-N-(6-hydroxyhexyl)acetamide **(WLS-06b)** (87.57 g, 96%) as a white solid. TLC Mobile phase details: 5% MeOH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 6.67 (s, 1H, NH), 3.64 (t, *J* = 6.5 Hz, 2H, CH_2), 3.36 (m, 2H, CH_2), 1.69 (s, 1H, OH), 1.59 (m, 4H, 2 x CH_2), 1.39 (m, 4H, 2 x CH_2). MS: *m/z* calcd for $C_8H_{14}F_3NO_2$ ([M-H]$^+$), 212.20; found 212.04.

6-(2,2,2-Trifluoroacetamido)hexyl methanesulfonate (WLS-06c).

WLS-06b (50 g, 0.23 mol) was dissolved in pyridine (500 mL) under argon atmosphere. Then reaction mixture cool to 0 °C and Mesylchloride (19 mL, 0.25 mol) was added dropwise over a period of 40 min. After that, the reaction was allowed to warm to room-temperature. The solution was stirred 2 h at rt. After completion of reaction (TLC

monitoring), reaction mass was quenched with water (500 mL) and extract with EtOAc (3 x 300 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel (100-200 mesh) chromatography using MeOH: DCM to afford the compound **WLS-06c** (57.76 g, 85%) as a white solid. TLC Mobile phase details: 5% MeOH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 6.71 (s, 1H, NH), 4.23 (t, *J* = 6.4 Hz, 2H, CH_2), 3.36 (m, 2H, CH_2), 3.01 (s, 3H, CH_3), 1.77 (m, 2H, CH_2), 1.61 (m, 2H, CH_2), 1.46 (m, 2H, CH_2), 1.39 (m, 2H, CH_2). MS: *m/z* calcd for $C_9H_{16}F_3NO_4S$ ($[M+H]^+$), 292.29; found 292.17.

S-(6-(2,2,2-Trifluoroacetamido)hexyl) ethanethioate (WLS-06d).

WLS-06c (74 g, 0.254 mol) was dissolved in dry DMF (1480 mL) under argon atmosphere. Then, potassium thioacetate (58.06 g, 0.509 mol) was added in portion wise to the reaction mixture at rt (after addition formed gummy liquid, after stirring for 40 min, gummy liquid converted to clear solution). The Reaction mixture was stirred at rt for 2 h. After completion of reaction (TLC monitoring), RM was diluted with water (600 mL) and extract with diethyl ether (3 x 700 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was purified by silica gel (100-200 mesh) chromatography using EtOAc: hexane to afford the compound **WLS-06d** (62.26 g, 90%) as an oil. TLC Mobile phase details: 5% MeOH in DCM. ^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 6.56 (s, 1H, NH), 3.36 (m, 2H, CH_2), 2.85 (d, *J* = 7.3 Hz, 2H, CH_2), 2.33 (s, 3H, CH_3), 1.59 (m, 4H, 2 x CH_2), 1.38 (m, 4H, 2 x CH_2). MS: *m/z* calcd for $C_{10}H_{16}F_3NO_2S$ ($[M-H]^+$), 270.30; found 270.17.

6-(2,2,2-trifluoroacetamido)hexane-1-sulfonyl chloride (WLS-06e).

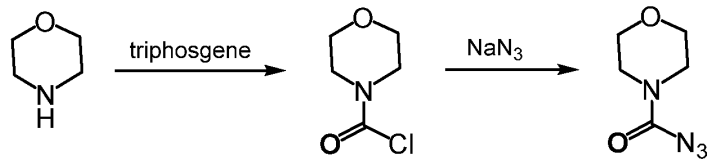
WLS-06d (24 g, 0.088 mol) was dissolved in dry MeCN (432 mL) under argon atmosphere. Then reaction mixture cool to 0 °C in ice bath. Added 2 N HCL (43.2 mL) was added dropwise over a period of 15 min and stirred for 10 min for 10 min at same temperature. Then added *N*-chlorosuccinimide (52.00 g, 0.390 mol) portion wise over a period of 40 min. The reaction mixture allowed to room temperature, and stirred for 2 h. After completion of reaction (TLC monitoring), the reaction mass was diluted with water (200 mL) and quench with sat. sodium bicarbonate solution at 0 °C. Then, extract with diethyl ether (3 x 300 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under

reduced pressure. The crude compound was purified by silica gel (100-200 mesh) chromatography using EtOAc: hexane to afford the compound **WLS-06e** (23.75 g, 91%). TLC Mobile phase details: 30% EtOAc in hexane. ^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 6.42 (s, 1H, NH), 3.68 (m, 2H, CH_2), 3.38 (m, 2H, CH_2), 2.06 (m, 2H, CH_2), 1.65 (m, 2H, CH_2), 1.55 (m, 2H, CH_2), 1.42 (m, 2H, CH_2). MS: *m/z* calcd for $C_8H_{13}ClF_3NO_3S$ ([M-H]$^+$), 294.70; found 294.07.

6-(2,2,2-Trifluoroacetamido)hexane-1-sulfonyl azide (WLS-06).

WLS-06e (20 g, 0.078 mol) was dissolved in MeCN (295 mL) under argon atmosphere and NaN_3 (5.46 g, 0.084 mol) was added in portion wise. The reaction mixture was stirred at rt for 2 h. After completion of reaction (TLC monitoring), reaction mass was diluted with water (300 mL) and extract with ethyl acetate (3 x 200 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was dissolved in small amount of DCM and precipitate by dropwise addition of hexane. Precipitate compound was filtered and washed with hexane to afford the white solid compound **WLS-06** (18.45 g, 90%). TLC Mobile phase details: 30% EtOAc in hexane. ^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 6.33 (s, 1H, NH), 3.36 (m, 4H, CH_2), 1.94 (m, 2H, CH_2), 1.64 (m, 2H, CH_2), 1.52 (m, 2H, CH_2), 1.42 (m, 2H, CH_2). MS: *m/z* calcd for $C_8H_{13}F_3N_4O_3S$ ([M-H]$^+$), 301.27; found 301.08. ^{19}F NMR (400 MHz, $CDCl_3$): δ in ppm = -75.78. IR (KBr) = 2147 cm^{-1}.

Synthesis of morpholine-4-carbonyl azide (WLS-08)



WLS-08a **WLS-08b** **WLS-08**

Morpholine-4-carbonyl chloride (WLS-08b)

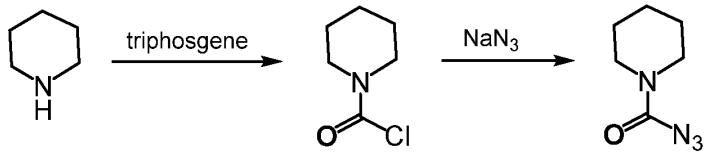
Triphosgene (8.57 g, 0.029 mol) was dissolved in DCM (754 mL) and cool to -5 °C using salt ice bath, then a solution of morpholine (5.0 g, 0.057 mol) and triethylamine (11.9 mL, 0.085 mol) in DCM (75 mL) was slowly added dropwise to reaction mixture over a period of 45 min. The reaction mixture was stirred for another 1 h at same temperature. After

completion of reaction (TLC monitoring), reaction mixture was washed with water and extracted with DCM. The organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Crude compound was purified by silica gel (100-200 mesh) chromatography using EtOAc-hexane to afford WLS-08b (2.4 g, 28%) as an oil. TLC Mobile phase details: 5% MeOH in DCM. ^1H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.73 (s, 6H, 3 x CH_2), 3.65 (m, 2H, CH_2). MS: *m/z* calcd for $C_5H_8ClNO_2$ ($[M+H]^+$), 150.57; found 149.88.

Morpholine-4-carbonyl azide (WLS-08)

WLS-08b (6.7 g, 0.045 mol) was dissolved in MeCN (100 mL) under argon atmosphere and NaN_3 (3.78 g, 0.058 mol) was added at 0 °C. The reaction mixture was stirred at 0 °C for 3 h. After completion of reaction (TLC monitoring), reaction mass was diluted with water (200 mL) and extract with diethyl ether (300 mL), saturated sodium carbonate (100 mL) and brine (100 mL). The organic layer was dried over sodium sulphate filtered and concentrated under reduced pressure. Crude compound was purified by silica gel (100-200 mesh) chromatography using EtOAc-hexane to afford WLS-08 (4.20 g, 60%) as an oil. TLC Mobile phase details: 5% MeOH in DCM. ^1H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.67 (m, 4H, 2 x CH_2), 3.56 (m, 2H, CH_2), 3.45 (t, J = 4.9 Hz, 2H, CH_2). MS: *m/z* calcd for $C_5H_8N_4O_2$ ($[M+H]^+$), 157.14; found 156.80.

Synthesis of piperidine-1-carbonyl azide (WLS-09)



WLS-09a WLS-09b WLS-09

Piperidine-1-carbonyl chloride (WLS-09b)

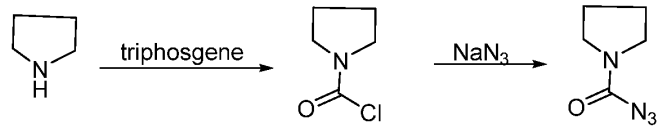
Triphosgene (12.19 g, 0.041 mol) was dissolved in DCM (525 mL) and cool to -5 °C using salt ice bath, then a solution of piperidine (7.00 g, 0.082 mol) and triethylamine (22.97 mL, 0.164 mol) was slowly added dropwise to reaction mixture over a period of 45 min. The reaction mixture was stirred for another 2 h at same temperature. After completion of reaction (TLC monitoring), reaction mixture was washed with water and the organic layer

was dried over sodium sulphate filtered and concentrated under reduced pressure. Crude compound WLS-09b (11.5 g) was directly used for next step. TLC Mobile phase details: 5% MeOH in DCM.

Piperidine-1-carbonyl azide (WLS-09)

The crude WLS-09b (11.5 g, 0.078 mol) was dissolved in MeCN (157 mL) under argon atmosphere and NaN_3 (6.09 g, 0.094 mol) was added at 0 °C. The reaction mixture was stirred at rt for 16 h. After completion of reaction (TLC monitoring), The reaction mixture was diluted with water (200 mL) and extract with diethyl ether (300 mL), saturated sodium carbonate (100 mL) and brine (100 mL). The organic layer was dried over sodium sulphate filtered and concentrated under reduced pressures. The crude compound was purified by silica gel (100-200 mesh) chromatography using EtOAc-hexane to afford WLS-09 (4.42 g, 33% in two steps) as an oil. TLC Mobile phase details: 5% MeOH in DCM. ^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.50 (m, 2H, CH_2), 3.36 (m, 2H, CH_2), 3.45 (t, J = 4.9 Hz, 2H, CH_2), 1.59 (m, 6H, 3 x CH_2). MS: *m/z* calcd for $C_6H_{10}N_4O$ ($[M+H]^+$), 155.17; found 154.91.

Synthesis of pyrrolidine-1-carbonyl azide (WLS-10)



WLS-10a **WLS-10b** **WLS-10**

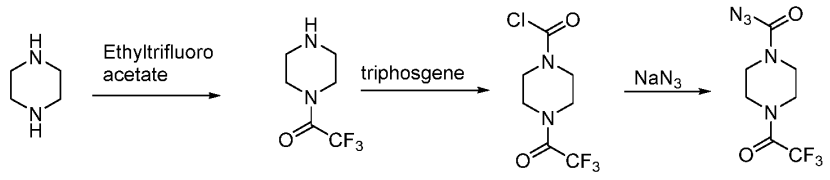
Pyrrolidine-1-carbonyl chloride (WLS-10b)

Triphosgene (12.50 g, 0.042 mol) was dissolved in DCM (450 mL) and cool to -5 °C using salt ice bath, then a solution of pyrrolidine (6.00 g, 0.084 mol) and triethylamine (23.56 mL, 0.168 mol) was added dropwise to reaction mixture over a period of 20 min. The reaction mixture was stirred for another 2 h at same temperature. After completion of reaction (TLC monitoring), reaction mass was washed with water and the organic layer was dried over sodium sulphate filtered and concentrated under reduced pressures. The crude compound WLS-10b (10.0 g) was directly used for next step. TLC Mobile phase details: 5% MeOH in DCM.

Pyrrolidine-1-carbonyl azide (WLS-10)

The crude WLS-10b (10.0 g, 0.075 mol) was dissolved in MeCN (137 mL) under argon atmosphere and NaN_3 (5.84 g, 0.090 mol) was added at 0 °C. The reaction mixture was stirred for 6 h. After completion of reaction (TLC monitoring), The reaction mixture was diluted with water (200 mL) and extract with diethyl ether (300 mL), saturated sodium carbonate (100 mL) and brine (100 mL). The organic layer was dried over sodium sulphate filtered and concentrated under reduced pressuress. The crude compound was purified by silica gel (100-200 mesh) chromatography using EtOAc-hexane to afford WLS-10 (6.00 g, 57% in two steps) as an oil. TLC Mobile phase details: 5% MeOH in DCM. ^1H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.45 (m, 2H, CH_2), 3.33 (m, 2H, CH_2), 1.90 (m, 4H, 2 xCH_2). MS: *m/z* calcd for $C_5H_8N_4O$ ($[M+H]^+$), 141.15; found 140.80.

Synthesis of 4-(2,2,2-trifluoroacetyl)piperazine-1-carbonyl azide (WLS-11)



2,2,2-trifluoro-1-(piperazin-1-yl)ethan-1-one (WLS-11b)

Ethyl trifluoroacetate (6.93 mL, 0.058 mol) was added to a suspension of piperazine (5.0 g, 0.058 mol) in THF (50 mL) at room temperature under nitrogen and stirred for 60 min and concentrated to remove solvent. The oily residue was taken up in ether and filtered and the filter cake was washed with ether. The filtrate was concentrated and purified by silica gel (100-200 mesh) column chromatography using MeOH-DCM to afford WLS-11b (6.51 g, 61%) as an oil. TLC Mobile phase details: 5% MeOH in DCM. MS: *m/z* calcd for $C_6H_9F_3N_2O$ ($[M+H]^+$), 183.15; found 182.65.

4-(2,2,2-Trifluoroacetyl)piperazine-1-carbonyl chloride (WLS-11c)

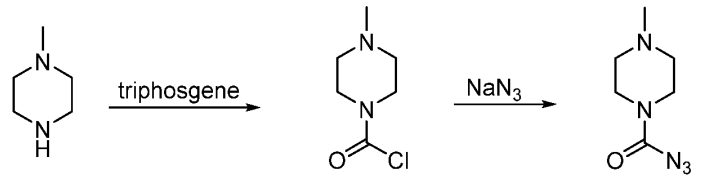
Triphosgene (5.29 g, 0.018 mol) was dissolved in DCM (487 mL) and cool to -5 °C using salt ice bath, then a solution of WLS-11b (6.50 g, 0.036 mol) and triethylamine (9.97 mL, 0.071 mol) was slowly added dropwise to reaction mixture over a period of 20 min. The reaction mixture was stirred for another 1 h at same temperature. After completion of reaction (TLC monitoring), reaction mass was washed with water and organic layer was

dried over sodium sulphate and concentrated under reduced pressures. Crude compound WLS-11c (8.1 g) was directly used for next step. TLC Mobile phase details: 5% MeOH in DCM.

4-(2,2,2-trifluoroacetyl)piperazine-1-carbonyl azide (WLS-11)

The crude WLS-11c (8.1 g, 0.033 mol, 1.0 equiv) was dissolved in MeCN (111 mL) under argon atmosphere and NaN_3 (2.58 g, 0.040 mol,) was added at 0 °C. The reaction mixture was stirred for 2 h. After completion of reaction (TLC monitoring), The reaction mixture was diluted with water (200 mL) and extract with diethyl ether (300 mL), saturated sodium carbonate (100 mL) and brine (100 mL). The organic layer dried over sodium sulphate, filtered and concentrated under reduced pressures. Crude compound was purified by silica gel (100-200 mesh) chromatography using EtOAc-hexane to afford WLS-11 (6.31 g, 70% in two steps) as an oil. TLC Mobile phase details: 5% MeOH in DCM. ^1H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.65 (m, 6H, 2 x CH_2), 3.55 (d, J = 2.5 Hz, 2H, CH_2). MS: *m/z* calcd for $C_7H_8F_3N_5O_2$ ($[M+H]^+$), 252.17; found 252.00.

Synthesis of 4-Methylpiperazine-1-carbonyl azide (WLS-12)



WLS-12a **WLS-12b** **WLS-12**

4-Methylpiperazine-1-carbonyl chloride (WLS-12b)

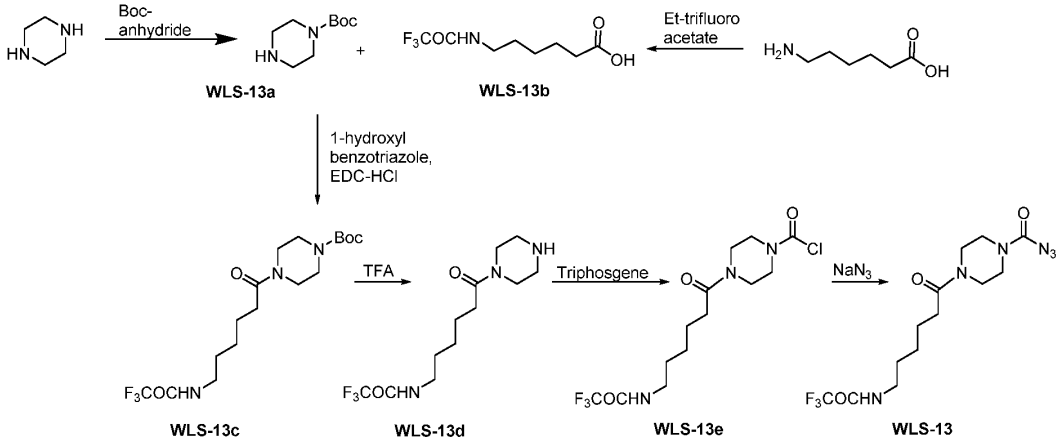
Triphosgene (7.40 g, 0.025 mol) was dissolved in CH_2Cl_2 (750 mL) and cool to -5 °C using salt ice bath, then a solution of N-methylpiperazine (5.00 g, 0.050 mol) and diisopropylethylaine (17.38 mL, 0.100 mol) in CH_2Cl_2 (150 mL) was slowly added dropwise to reaction mixture over a period of 30 min. The reaction mixture was stirred for another 2 h at same temperature. After completion of reaction (TLC monitoring), RM was washed with water and organic layer was dried over sodium sulphate, filtered and concentrated under reduced pressures. Crude compound WLS-12b (8.0 g) was directly used

for next step. TLC Mobile phase details: 5% MeOH in DCM.

4-Methylpiperazine-1-carbonyl azide (WLS-12)

The crude WLS-12b (8.0 g, 0.049 mol) was dissolved in MeCN (112 mL) under argon atmosphere and NaN_3 (3.83 g, 0.059 mol) was added at 0 °C. Then reaction mixture was stirred for 3 h. After completion of reaction (TLC monitoring), The reaction mixture was diluted with water (200 mL) and extract with diethyl ether (300 mL), saturated sodium carbonate (100 mL) and brine (100 mL). The organic layer dried over sodium sulphate, filtered and concentrated under reduced pressures. Crude compound was purified by silica gel (100-200 mesh) chromatography using EtOAc-hexane to afford WLS-12 (3.60 g, 43% in two steps) as an oil. TLC Mobile phase details: 5% MeOH in DCM. ^1H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.58 (t, J = 5.1 Hz, 2H, CH_2), 3.46 (t, J = 5.1 Hz, 2H, CH_2), 2.38 (m, 4H, 2 x CH_2), 2.30 (s, 3H, CH_3). MS: *m/z* calcd for $C_6H_{11}N_5O$ ($[M+H]^+$), 170.19; found 169.81.

Synthesis of 4-(6-(2,2,2-Trifluoroacetamido)hexanoyl)piperazine-1-carbonyl azide (WLS-13)



***N*-(tert-Butoxycarbonyl)-piperazine (WLS-13a: 1-Boc-piperazine).**

Piperazine (12 g, 139.3 mmol) was dissolved in dry CH_2Cl_2 (240 mL) and the solution was cooled to 0 °C. To the reaction mixture, solution of di-*tert*-butyl dicarbonate (Boc_2O) (15.2 g, 69.64 mmol) in dry CH_2Cl_2 (160 mL) was added dropwise (over period of 20 min). Then reaction mixture stirred at rt for 24 h. After completion of reaction, precipitate formed was filtered off and washed with CH_2Cl_2 (2 × 40 mL), and the combined filtrate was separated

and washed with H_2O (3 × 80 mL), brine (60 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressures. Crude product was purified by silica gel column chromatography using CH_2Cl_2:MeOH to afford the compound **WLS-13a** (11.6 g, 45%) as a white solid. TLC Mobile phase details: 20% MeOH in DCM. ^1H NMR (500 MHz, $CDCl_3$): δ in ppm = 3.32 (t, *J = 4.8 Hz*, 4H, 2 x CH_2,), 2.74 (t, *J = 4.5 Hz*, 3H, 2 x CH_2), 1.68 (s, 1H, N*H*), 1.40 (s, 9H, 3 x CH_3). MS: *m/z* calcd for $C_9H_{19}N_2O_2$ ($[M+H]^+$), 187.25; found 187.04.

6-(2,2,2-Trifluoroacetamido)hexanoic acid (WLS-13b)

A solution of 6-amino hexanoic acid (21 g, 0.160 mol) and triethylamine (22.4 mL, 0.160 mol) in MeOH (80 mL) was cooled to 0 °C. Trifluoroacetic anhydride (24 mL, 0.192 mol) was added dropwise over period of 20 min under an argon atmosphere and the reaction was allowed to room-temperature and stirred 16 h. After completion of reaction, solvent was evaporated.The crude compound was cool to 0 °C, 2 N HCl (400 mL) was added dropwise. After addition precipitate compound was filtered to get white compound. To take out rest compound from filtrate, filtrate is solution is saturated with NaCl and extracted with diethyl ether (2 x 200 mL). The solid compound is also dissolved in diethyl ether (200 mL) and washed with water (2 x 200 mL). The combined organic layer (from solid and from filtrate) was dried over sodium sulphate and evaporated. The crude compound was dissolve in small amount of diethyl ether and precipitate by adding dropwise hexane. Precipitate compound was filtered and wash with hexane to afford the compound **WLS-13b** (33.0 g, 91%) as a white solid. TLC Mobile phase details: 10% MeOH in DCM. ^1H NMR (500 MHz, $CDCl_3$): δ in ppm = 12.00 (s, 1H, COOH), 9.39 (s, 1H, NH), 3.17 (dd, *J* = 13.1, 6.9 Hz, 2H, CH_2), 2.20 (t, *J* = 7.6 Hz, 2H, CH_2), 1.50 (m, 4H, 2 x CH_2), 1.26 (m, 2H, CH_2). MS: *m/z* calcd for $C_8H_{12}F_3NO_3$ ($[M-H]^+$), 226.18; found 226.02.

(Tert-butyl 4-(6-(2,2,2-trifluoroacetamido)hexanoyl)piperazine-1-carboxylate (WLS-13c).

To a solution of WLS-13b (15.00 g, 0.066 mol) and 1-hydroxybenztriazole (9.72 g, 0.072 mol) in anhydrous methylene chloride (375 mL) was added ethyl 3-(dimethylamino)propyl carbodiimide, hydrochloride salt (13.8 g, 0.072) at 0°C under argon atmosphere. The mixture was stirred for 30 minutes at 0°C. Then WLS-13a (12.3 g, 0.066 mol) and diisopropylethylamine (13.8 mL, 0.793 mol) were added and the mixture became a

homogeneous solution. The reaction mixture was stirred for 3 h at 0°C. The solution was slowly warmed to room temperature and stir for another 2 h at rt. After completion of reaction (TLS monitoring), RM cool to 0°C and quench with ice cold water (400 mL). The separate organic layer wash with 5% sodium bicarbonate solution. (2 x 500 mL). The combined organic layer dried on sodium sulphate, filtered and concentrated under reduced pressures. The crude product was dissolve in small amount of CH_2Cl_2 and precipitate by adding dropwise hexane. Precipitate compound was filtrate and wash with hexane to afford the compound **WLS-13c** (33.0 g, 91%) as a white solid. TLC Mobile phase details: 5% MeOH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 9.38 (s, 1H, NH), 3.41 (m, 2H, CH_2), 3.26 (s, 2H, CH_2), 3.16 (dd, *J* = 13.0, 6.8 Hz, 3H, CH, CH_2), 2.30 (t, *J* = 7.5 Hz, 2H, CH_2), 1.48 (m, 5H, CH, 2 x CH_2), 1.40 (s, 9H, 3 x CH_3), 1.28 (m, 4H, 2 x CH_2). MS: *m/z* calcd for $C_{17}H_{28}F_3N_3O_4$ ([M-H]$^+$), 394.42; found 394.33.

2,2,2-Trifluoro-N-(6-oxo-6-(piperazin-1-yl)hexyl)acetamide (WLS-13d)

WLS-13c (18.30 g, 0.046 mol) was dissolved in CH_2Cl_2 (725 mL) and cool to 0 °C under argon atmosphere. Then TFA:CH_2Cl_2 (1;1, 181.3 mL) solution was added dropwise over period of 45 min at 0 °C. After that, reaction mixture allowed to rt and stirred for 4 h. After completion of reaction (TLC monitoring), solvent was evaporated to dryness using base trap to get crude compound. The crude compound was dissolved in 15% MeOH:CH_2Cl_2 (100 mL) and cool to 0 °C and quench with saturated sodium bicarbonate solution (pH up to neutral). Then 400 mL water was added and extract with 15% MeOH:CH_2Cl_2 (6 x 300 mL, extract up to there was no product in aqueous layer). The combined organic layer was dried on sodium sulphate, filtered and concentrated under reduced pressures to get crude **WLS-13d** (12.82 g) as an oil. Crude compound was directly used for next reaction. TLC Mobile phase details: 10% MeOH in DCM. MS: *m/z* calcd for $C_{12}H_{20}F_3N_3O_2$ ([M-H]$^+$), 294.31; found 294.17.

4-(6-(2,2,2-trifluoroacetamido)hexanoyl)piperazine-1-carbonyl chloride (WLS-13e).

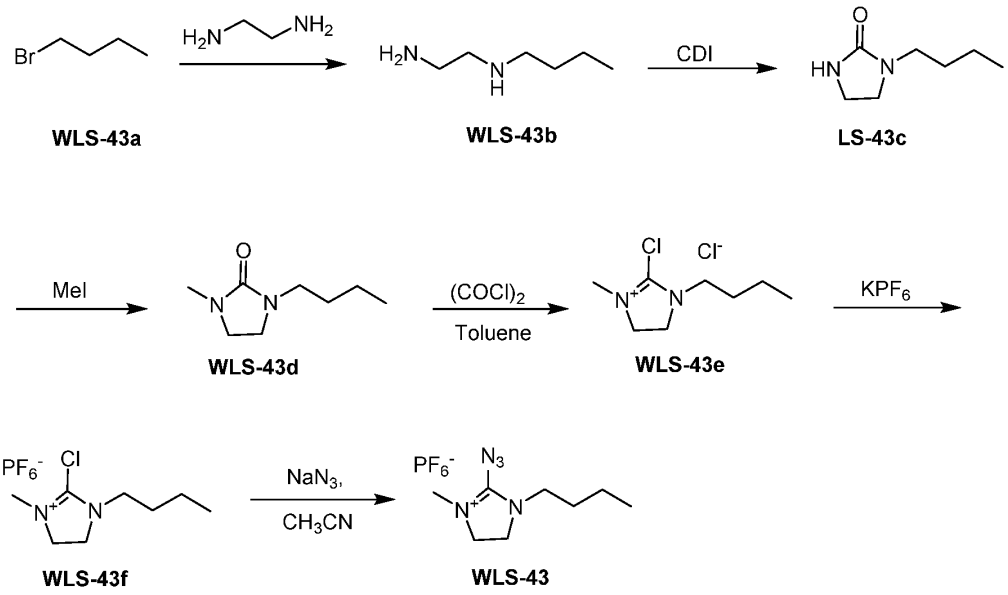
To a solution of WLS-13d (12.2 g, 0.041 mol) and diisopropylethylamine (29.0 mL, 0.166 mol) in anhydrous THF (610 mL) was added dropwise triphsogene (6.13 g, 0.021) solution in THF (190 mL) over a period of 30 min at 0°C under argon atmosphere. The reaction mixture stirred at same temperature for another 30 min. The reaction mixture allowed rt and stirred for another 3 h. After completion of reaction (TLC monitoring), reaction mass was

filtered and solid was washed with THF. The filtrate was evaporated to dryness. The crude product was dissolved in CH_2Cl_2 (300 mL) and washed with water (2 x 300 mL). The combined organic layer dried on sodium sulphate filtered and concentrated under reduced pressures.The crude compound was purified by silica gel (100-200 mesh) chromatography using hexane: ethyl acetate to afford WLS-13e (6.5 g, 33% in two steps) as a slight yellow solid. TLC Mobile phase details: 10% MeOH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 7.27 (s, 1H, NH), 3.71 (m, 6H, 3 x CH_2), 3.58 (d, J = 15.9 Hz, 2H, CH_2), 3.49 (m, H, CH), 3.39 (m, 2H, CH_2), 2.37 (t, 2H, J = 7.1 Hz, CH_2), 1.65 (m, 4H, 2 x CH_2), 1.39 (m, 2H, CH_2). MS: *m/z* calcd for $C_{13}H_{19}ClF_3N_3O_3$ ([M-H]$^+$), 356.76; found 355.98.

4-(6-(2,2,2-Trifluoroacetamido)hexanoyl)piperazine-1-carbonyl azide (WLS-13)

To a solution of sodium azide (1.31 g, 0.020 mol) in water (8.2 mL) was added dropwise over 20 min a solution of WLS-13e (6 g, 0.017 mol) in acetone (22.2 mL) at 0 °C under argon atmosphere. The reaction mixture was allowed to room temperature and stirred for 3 h. After completion of reaction (TLC monitoring), acetone was removed under reduced pressure. Then water was added (100 mL) and extracted with EtOAc (80 mL x 3). The combined organic layers were dried over Na_2SO_4 and solvent was removed under reduced pressure. The crude compound was purified by silica gel column chromatography using EtOAc: hexane to afford the compound **WLS-13** (2.01 g, 33%) as a slight brown colour oil. TLC Mobile phase details: 5% MeOH in DCM. ^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 7.00 (s, 1H, NH), 3.60 (m, 4H, 3 x CH_2), 3.47 (t, *J* = 7.2 Hz, 4H, 2 x CH_2), 3.41 (m, 2H, CH_2), 2.36 (t, *J* = 6.2 Hz, 2H, CH_2), 1.65 (m, 4H, 2 x CH_2), 1.39 (m, 2H, CH_2). MS: *m/z* calcd for $C_{13}H_{19}F_3N_6O_3$ ([M-H]$^+$), 363.33; found 355.98.

2-azido-1-butyl-3-methyl-4,5-dihydro-1H-imidazol-3-ium hexafluoro-phosphate(V) (WLS-43)



Preparation of compound WLS-43b

In a clean and dry three-neck 3 Lit round bottom flask, ethane-1,2-diamine (1000 mL, 14.975 mol, 25.65 equiv) was placed with a magnetic stirring bar, and compound **WLS-43a** (80 g, 0.584 mol, 1.0 equiv) was added dropwise at 0 °C by using addition funnel. After finishing the addition, the reaction mixture was warmed to 25 °C, and left undisturbed for an additional 1 h. Then, 600 mL of hexane was added into the reaction mixture and stirred vigorously for 16 h at 25 °C. TLC showed the reaction was completed, staring material was consumed and the new spot was formed (TLC - 10% MeOH:EtOAc; TLC charring – Phosphomolybdic acid). The hexane layer was separated by using separatory funnel, dried over sodium sulphate and evaporated to dryness under reduced pressure to get compound **WLS-43b** (44.0 g) as a crude colorless oil. Crude compound was directly used for next step without any further purification. *MS: m/z* calcd for $C_6H_{16}N_2$ ($[M+H]^+$), 117.21; found 117.15.

Preparation of compound WLS-43c

WLS-43b (44.0 g, 0.379 mol, 1.0 equiv), was taken in clean and dry 1 Lit two neck RBF under argon atmosphere. Then add 440 mL of THF to RBF. Cool the RB in ice bath (0 ℃). Add portion wise 1,1'-Carbonyldiimidazole (63.24 g, 0.390 mol, 1.03 equiv) to reaction

mixture for period of 10 min. The reaction mixture was stir at 15 ℃ for 12 h. TLC showed the reaction was completed, staring material was consumed and the product was formed (TLC - 10% MeOH:EtOAc; TLC charring – Phosphomolybdic acid). After completion of reaction, solvent was dried and purified on silica gel column chromatography (100-200 mesh). The product was eluted with 80% ethyl acetate: hexane to EtOAc. Fraction containing product was evaporated to get 35.02 g (65% yield) of **WLS-43c** as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 4.77 (s, 1H), 3.45-3.48 (m, 4H), 3.18 (t, 2H, *J* = 7.6 Hz), 1.52-1.46 (m, 2H), 1.34 (td, 2H, *J* = 15.0 Hz, 7.3 Hz), 0.93 (t, 3H, *J* = 7.6 Hz). *MS:* m/z calcd for $C_7H_{14}N_2O$ ($[M+H]^+$), 143.20; found 143.46.

Preparation of compound WLS-43d

WLS-43c (30.0 g, 0.211 mol, 1.0 equiv) was taken in clean and dry 2 Lit three neck RBF under argon atmosphere. Then, add 450 mL of dry DMF to RBF containing starting material. Cool the reaction mixture in ice bath (Temp. 0℃). Then, add portion wise 60% NaH (10.14 g, 0.253 mol) to reaction mixture for period of 20 min. at 0℃ and stir 40 min at same temp. Then add dropwise methyl iodide (39.4 mL, 0.633 mol) to the reaction mixture at 0 ℃ for duration of 15 min. Then allow the reaction mixture to room temprature and stir for 2 h. TLC showed the reaction was completed, staring material was consumed and the new spot was formed (TLC - EtOAc; TLC charring – Phosphomolybdic acid). After completion of reaction, reaction mixture was cool to 0℃ in ice bath and quenched with ice cold water (1 Lit). Then extracted with ethyl acetate 2 x 800 mL). The organic layer was washed with ice cold water (2 x 1000 mL) and dried over sodium sulfate, filtered and concentrated to dryness. The crude product was purified by silica gel column chromatography (100-200 mesh). The product was eluted with 10%-40% ethyl acetate:hexane. The fraction containing product was evaporated to get 18.0 g (55% yield) of **WLS-43d** as a white colour solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.28 (s, 4H), 3.18 (t, 2H, *J* = 7.3 Hz), 2.78 (s, 3H), 1.51-1.44 (m, 2H), 1.38-1.30 (m, 2H), 0.93 (t, 3H, *J* = 7.3 Hz). *MS:* m/z calcd for $C_8H_{16}N_2O$ ($[M+H]^+$), 157.23; found 157.48.

Preparation of compound WLS-43e

WLS-43d (30.0 g, 0.192 mol, 1.0 equiv) was taken in clean and dry 1 Lit single neck round bottom flask under argon atmosphere. Then add 300 mL of dry toluene to RBF containing starting material under argon atmosphere. After that add dropwise oxalyl chloride (247.0 mL, 2.880 mol) using addition funnel for a period of 30 min at rt. Then, reaction mixture was heated to 65 °C for 72 hrs. After completion of reaction (TLC – 5% MeOH:DCM; TLC charring – Phosphomolybdic acid) solvent was evaporated to dryness to get crude compound. The crude compound was co-evaporate with toluene (200 mL) and washed with cold ethyl acetate:hexane (70:30, 2 x 1000 mL), diethyl ether:hexane (20:80, 1000 mL) and dried to get 34.0 g of crude **WLS-43e** as brown colour semi solid. Crude compound was directly used for next step without any further purification.

MS: m/z calcd for $C_8H_{16}Cl_2N_2$ ($[M-Cl]^+$), 175.68; found 176.89.

Preparation of compound WLS-43f

WLS-43e (29.0 g, 0.137 mol, 1.0 equiv) was taken in clean and dry 1 L single neck round bottom flask and dissolved in 290 mL DCM under argon atmosphere. Then added aq solution of KPF_6 (25.28 g, 0.137 mol, in 188 mL of water). Stir the reaction mixture at rt for 2 h. After completion of reaction (TLC – 5% MeOH:DCM), the reaction mixture was poured into ice water, and extracted with DCM (2 x 400 mL). The combined organic layer washed with water (400 mL) and dried over sodium sulphate, filtered and evaporated to dryness. Then, residue was dissolved in DCM and product was precipitate by dropwise addition of diethyl ether under stirring. The solvent was decant and solid was dried under high vacuum. The above precipitation procedure repeat two more times to get 35.0 g (80% yield) of **WLS-43f** as a white solid.

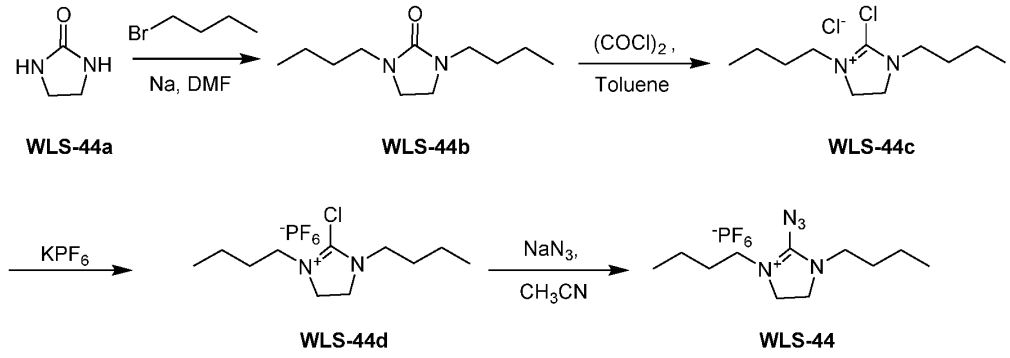
^1H NMR (500 MHz, $CDCl_3$): δ in ppm = 4.14-4.04 (m, 4H), 3.53 (t, 2H, *J* = 7.6 Hz), 3.23 (s, 3H), 1.67-1.61 (m, 2H), 1.41-1.35 (m, 2H), 0.96 (t, 3H, *J* = 7.2 Hz). *^{19}F NMR (400 MHz, $CDCl_3$):* δ in ppm = -73.18 and -74.70

Preparation of compound WLS-43

WLS-43f (39.5 g, 0.123 mol, 1.0 equiv) was taken in clean and dry 1 L single neck round bottom flask and dissolved in 200 mL of Dry MeCN under argon atmosphere. Then, added portion wise sodium azide (12.01 g, 0.185 mol, 1.5 equiv) to the RM and stir at rt for 4 h. After completion of reaction (TLC – 5% MeOH:DCM; TLC charring – ninhydrin),

reaction mixture was filtered through a pad of celite and washed with MeCN (20 mL). The organic layer was evaporated to dryness. The crude compound was dissolve in minimum amount of MeCN and precipitate by adding dropwise diethylether (500 mL) at -78. The above precipitation procedure repeat two more times to get 38.0 g (94% yield) of **WLS-43** as a light yellow solid. *^{1}H NMR (500 MHz, $CDCl_3$):* δ in ppm = 3.98-3.94 (m, 2H), 3.89-3.85 (m, 2H), 3.40 (t, 2H, *J* = 7.6 Hz), 3.20 (s, 3H), 1.64-1.59 (m, 2H), 1.35 (td, 2H, *J* = 15.0 Hz, *J* = 7.3 Hz), 0.95 (t, 3H, *J* = 7.6 Hz). *^{19}F NMR (500 MHz, $CDCl_3$):* δ in ppm = -73.49 and -75.01. *MS:* m/z calcd for $C_8H_{16}F_6N_5P$ ([M-PF_6]$^+$), 182.25; found 182.17. IR (KBr pellet): N_3 (2174 cm^{-1})

2-azido-1,3-dibutyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-44)



Preparation of compound WLS-44b

In a clean and dry two-neck 500 mL round bottom flask, **WLS-44a** (20.0 g, 0.232 mol, 1.0 equiv) was placed with a magnetic stirring bar and dissolved by adding DMF (200 mL). Then cool the RBF to 0 °C by using ice bath. After that, add sodium hydride (18.58 g, 0.465 mol, 2.0 equiv) portion wise for period of 40 min at 0 °C. Stir the reaction mixture at 0 °C for 30 min. Then added bromo butane (100 mL, 0.927 mol, 4.0 equiv) dropwise by using addition funnel for period of 20 min at 0 °C. and stir for 2 h. TLC showed the reaction was completed, staring material was consumed and the product was formed (TLC - 30% EtOAc;Hexane, TLC charring – Phosphomolybdic acid). After completion of reaction, reaction mixture poured into ice and extracted with ethyl acetate (100 mL x 2) and organic layer washed with ice cold water (1000 mL x 2). Organic layer dried over sodium sulphate, filtered and evaporated to dryness to get crude compound. The crude product was purified

by silica gel column chromatography (100-200 mesh). The product was eluted with 15% - 30% ethyl acetate:hexane. The fraction containing product was evaporated to get 40.0 g (87% yield) of **WLS-44b** as a yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.27 (s, 4H), 3.17 (t, 4H, *J* = 7.4 Hz), 1.44-1.51 (m, 4H), 1.33 (dt, 4H, *J* = 22.5 Hz, 7.2 Hz) 0.93 (t, 6H, *J* = 7.4 Hz).

Preparation of compound WLS-44c

WLS-44b (40.0 g, 0.202 mol, 1.0 equiv) was taken in clean and dry 1 Lit two neck RBF under argon atmosphere. Then add 400 mL of dry toluene to RBF containing SM under argon atmosphere. After that add dropwise oxalyl chloride (309.0 mL, 3.603 mol, 17.86 equiv) using addition funnel for a period of 30 min. Then reaction mixture was heated to 65 °C for 72 hrs. After completion of reaction (TLC – 5% MeOH:DCM; TLC charring – Phosphomolybdic acid) solvent was evaporated on rota evaporator to get crude compound. The crude compound was washed with diethyl ether (2 x 500 mL), cold ethyl acetate (2 x 400 mL), and 30% ethyl acetate:hexane (1000 mL). After washing solvent was decanted and dried on high vacuum to get 50.0 g of crude **WLS-44c** as a brown gummy liquid. *^{1}H NMR (500 MHz, $CDCl_3$):* δ in ppm = 4.32 (s, 4H), 3.65 (t, 4H, *J* = 7.4 Hz), 1.65-1.72 (m, 4H), 1.38 (dt, 4H, *J* = 22.5 Hz, 7.4 Hz), 0.97 (t, 6H, *J* = 7.4 Hz). *MS:* m/z calcd for $C_{11}H_{22}Cl_2N_2$ ($[M\text{-}Cl]^+$), 217.76; found 217.07.

Preparation of compound WLS-44d

WLS-44c (50.0 g, 0.197 mol, 1.0) was taken in clean and dry 1 Lit single neck RBF under argon atmosphere. Add 400 mL of DCM to RBF containing SM under argon atmosphere. Then added aq solution of KPF_6 (36.35 g, 0.197 mol, 1.0 equiv, in 200 mL of water). Stir the reaction mixture at rt for 1 h. After completion of reaction (TLC – 10% MeOH:DCM; TLC charring – Phosphomolybdic acid), the reaction mixture was poured into ice water (400 mL), and extracted with DCM (2 x 500 mL). The combined organic layer washed with water (400 mL) and dried over sodium sulphate, filtered and evaporated to dryness. Then, residue was dissolved in DCM (15 mL) and product was precipitate by dropwise addition of diethyl ether (600 mL) under stirring. The solvent was decant and solid was dried under high vacuum. The above precipitation procedure repeat one more time to get 54.0 g (75%

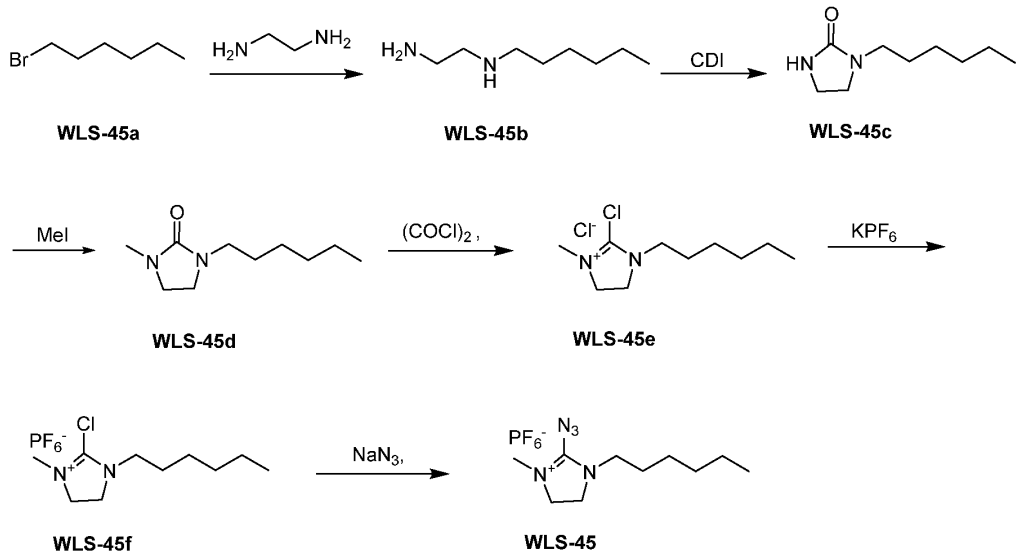
yield) of **WLS-44d** as a white solid. *^{1}H NMR (500 MHz, $CDCl_3$):* δ in ppm = 4.10 (s, 4H), 3.54 (t, 4H, *J* = 7.6 Hz), 1.62-1.68 (m, 4H), 1.36 (td, 4H, *J* = 15.0 Hz, 7.3 Hz), 0.96 (t, 6H, *J* = 7.2 Hz).

Preparation of compound WLS-44

WLS-44d (50.0 g, 0.138 mol, 1.0 equiv) was taken in clean and dry 1 Lit single neck RBF under argon atmosphere. Add 250 mL of Dry MeCN to RBF containing SM under argon atmosphere. Then, added sodium azide (13.44 g, 0.207 mol, 1.5 equiv) portion wise for the period of 10 min. Stir the reaction mixture at rt for 2.5 h. After completion of reaction (TLC – 5% MeOH:DCM; TLC charring – ninhydrin), reaction mixture was filtered through a pad of celite and washed with MeCN (50 mL). The organic layer was evaporated to dryness. The crude compound was cool to -20 °C using dry ice and methanol bath, then hexane was added, after some time the compound forms solid then hexane was decanted and solid was dried on high vacuum to get 39.0 g (77% yield) of **WLS-44** as a light yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.91 (s, 4H), 3.43 (t, 4H, *J* = 7.7 Hz), 1.60-1.67 (m, 4H), 1.36 (dt, 4H, *J* = 22.4 Hz, 7.4 Hz), 0.95 (t, 6H, *J* = 7.4 Hz). *^{19}F NMR (400 MHz, $CDCl_3$):* δ in ppm = -73.10 and -74.99. *MS:* m/z calcd for $C_{11}H_{22}F_6N_5P$ ($[M-PF_6]^+$), 224.33; found 224.20. IR (KBr pellet): N_3 (2173 cm^{-1})

Synthesis of 2-azido-1-hexyl-3-methyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-45)



Preparation of compound WLS-45b

In a clean and dry three-neck 3 Lit round bottom flask, ethane-1,2-diamine (1133 mL, 16.972 mol, 28.0 equiv) was placed with a magnetic stirring bar, and compound **WLS-45a** (100 g, 0.606 mol, 1.0 equiv) was added dropwise at 0 °C by using addition funnel. After finishing the addition, the reaction mixture was warmed to 25 °C, and left undisturbed for an additional 1 h. Then, 600 mL of hexane was added into the reaction mixture and stirred vigorously for 16 h at 25 °C. TLC showed the reaction was completed, staring material was consumed and the new spot was formed (TLC - 10% MeOH:EtOAc; TLC charring – Phosphomolybdic acid). The hexane layer was separated by using separatory funnel, dried over sodium sulfate, and evaporated to dryness under reduced pressure to get compound **WLS-45b** (60.0 g) as a crude colorless oil. Crude compound was directly used for next step without any further purification. *^{1}H NMR (400 MHz, $CDCl_3$):* δ in ppm = 2.82-2.79 (m, 2H), 2.66 (t, 2H, *J* = 5.9 Hz), 2.60 (t, 2H, *J* = 7.2 Hz), 1.52-1.45 (m, 2H), 1.36-1.27 (m, 9H), 0.89 (t, 3H, *J* = 6.9 Hz).
MS: m/z calcd for $C_8H_{20}N_2$ ($[M+H]^+$), 145.26; found 145.00.

Preparation of compound WLS-45c

WLS-45b (60.0 g, 0.416 mol, 1.0 equiv) was taken in clean and dry 1 Lit single neck RBF under argon atmosphere. Then add 600 mL of THF to RBF. Cool the RB in ice bath (0 ℃). Add portion wise 1,1'-Carbonyldiimidazole (69.46 g, 0.428 mol, 1.03 equiv) to RM for period of 10 min. The reaction mixture was stir at 15 ℃ for 16 h. TLC showed the reaction was completed, staring material was consumed and the product was formed (TLC - 10% MeOH:EtOAc; TLC charring – Phosphomolybdic acid). After completion of reaction, reaction mixture was filtered, filter cake was washed with THF (100 mL). Filtrate was dried and purified on silica gel column chromatography (100-200 mesh). The product was eluted with 80% ethyl acetate: hexane to EtOAc. Fraction containing product was evaporated to get 58.0 g (82% yield) of **WLS-45c** as a colorless oil. *^{1}H NMR (500 MHz, $CDCl_3$):* δ in ppm = 4.84 (s, 1H), 3.41 (s, 4H), 3.17 (t, 2H, *J* = 7.6 Hz), 1.49 (q, 2H, *J* = 7.1 Hz), 1.30 (d, 6H, *J* = 15.0 Hz, 2.1 Hz), 0.88 (t, 3H, *J* = 7.6 Hz). *MS:* m/z calcd for $C_9H_{18}N_2O$ ($[M+H]^+$), 171.26; found 171.10.

Preparation of compound WLS-45d

WLS-45c (48.0 g, 0.282 mol, 1.0 equiv) was taken in clean and dry 2 Lit three neck RBF under argon atmosphere. Then, add 800 mL of dry DMF to RBF containing SM. Cool the RB in ice bath (Temp. 0℃). Then, add portion wise 60% NaH (8.13 g, 0.338 mol, 1.2 equiv) to RM for period of 20 min. at 0℃ and stir 45 min at same temp. Then add dropwise methyl iodide (53 mL, 0.851 mol, 3.02 equiv) to the reaction mixture at 0 ℃ for duration of 30 min. Then allow the RM to rt and stir for 3 h. TLC showed the reaction was completed, staring material was consumed and the new spot was formed (TLC - EtOAc; TLC charring – Phosphomolybdic acid). After completion of reaction, reaction mixture was cool to 0℃ in ice bath and quenched with ice cold water (200 mL). Then extracted with ethyl acetate 3 x 300 mL). The organic layer was washed with ice cold water (2 x 500 mL) and dried over sodium sulfate, filtered and concentrated to dryness. The crude product was purified by silica gel column chromatography (100-200 mesh). The product was eluted with 40%-50% ethyl acetate:hexane. The fraction containing product was evaporated to get 36.4 g (70% yield) of **WLS-45d** as a white colour solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.27 (s, 4H), 3.17 (t, 2H, *J* = 7.6 Hz), 2.78 (s, 3H), 1.50-1.45 (m, 2H), 1.29 (s, 7H), 0.88 (t, 3H, *J* = 6.9 Hz).

Preparation of compound WLS-45e

WLS-45d (43.0 g, 0.233 mol, 1.0 equiv) was taken in clean and dry 2 Lit three neck RBF under argon atmosphere. Then add 430 mL of dry toluene to RBF containing SM under argon atmosphere. After that add dropwise oxalyl chloride (300 mL, 3.498 mol, 15 equiv) using addition funnel for a period of 30 min at rt. Then, reaction mixture was heated to 65 °C for 72 hrs. After completion of reaction (TLC – 5% MeOH:DCM; TLC charring – Phosphomolybdic acid) solvent was evaporated to dryness to get crude compound. The crude compound was precipitate by using DCM-Hexane (three times) and solid was dried under high vacuum to get 48.0 g of crude **WLS-45e** as an oil. Crude compound was directly used for next step without any further purification. *MS:* m/z calcd for $C_{10}H_{20}Cl_2N_2$ ([M-Cl]$^+$), 203.73; found 203.43.

Preparation of compound WLS-45f

WLS-45e (48.0 g, 0.201 mol, 1.0 equiv) was taken in clean and dry 21 L single neck RBF and dissolved in 480 mL DCM under argon atmosphere. Then added aq solution of KPF_6 (36.95 g, 0.201 mol, 1.0 equiv in 240 mL of water). Stir the reaction mixture at rt for 2 h. After completion of reaction (TLC – 5% MeOH:DCM), the reaction mixture was poured into ice water, and extracted with DCM (2 x 400 mL). The combined organic layer washed with water (400 mL) and dried over sodium sulphate, filtered and evaporated to dryness. Then, residue was dissolved in DCM and product was precipitate by dropwise addition of hexane under stirring. The solvent was decant and solid was dried under high vacuum. The above precipitation procedure repeat two more times to get 58.35 g (83% yield) of **WLS-45f** as a yellow solid.

^1H NMR (500 MHz, $CDCl_3$): δ in ppm = 4.14-4.03 (m, 4H), 3.51 (t, 2H, *J* = 7.6 Hz), 3.22 (s, 3H), 1.64 (q, 2H, *J* = 7.1 Hz), 1.31 (d, 6H, *J* = 4.8 Hz), 0.89 (t, 3H, *J* = 6.9 Hz). *^{19}F NMR (400 MHz, $CDCl_3$):* δ in ppm = -73.16 and -74.68. *MS:* m/z calcd for $C_{10}H_{20}ClF_6N_2P$ ([M-Cl]$^+$), 203.73; found 203.96.

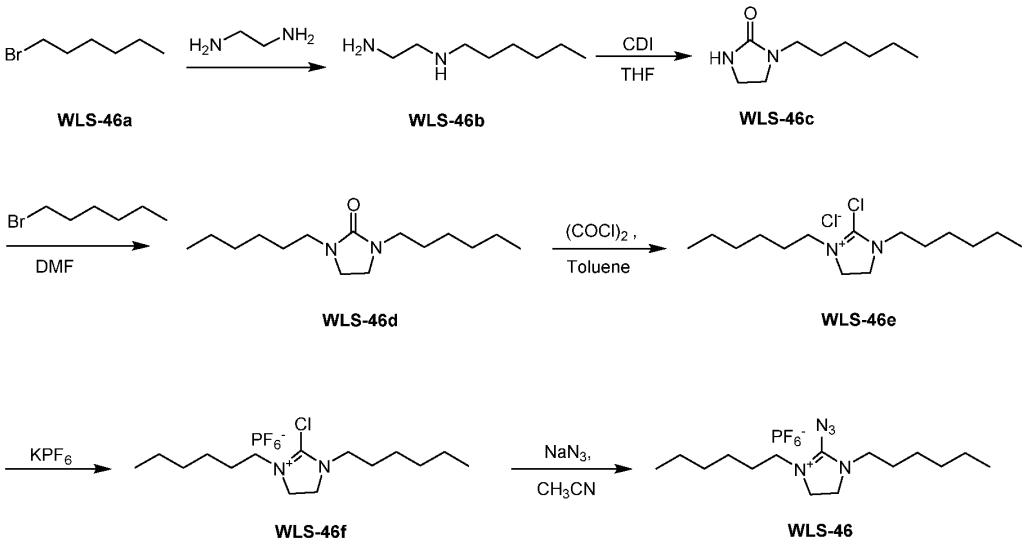
Preparation of compound WLS-45

WLS-45f (58.35 g, 0.167 mol, 1.0 equiv) was taken in clean and dry 1 L single neck RBF and dissolved in 292 mL of Dry MeCN under argon atmosphere. Then, added portion wise sodium azide (16.31 g, 0.251 mol, 1.5 equiv) to the RM and stir at rt for 3 h. After completion of reaction (TLC – 5% MeOH:DCM; TLC charring – ninhydrin), reaction mixture was filtered through a pad of celite and washed with MeCN (200 mL). The organic layer was evaporated to dryness. The crude compound was dissolve in minimum amount of MeCN and add by diethylether to form gummy liquid, solvent was decant and compound was dried. Repeat this procedure two times. Then hexane was added to gummy liquid and stir at at -30 °C to get solid. Solvent was decanted and solid was dried to get 55.0 g (93% yield) of **WLS-45** as a light yellow solid.

^1H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.97-3.92 (m, 2H), 3.88-3.83 (m, 2H), 3.37 (t, 2H, *J* = 7.7 Hz), 3.19 (s, 3H), 1.63-1.57 (m, 2H), 1.31 (s, 6H), 0.89 (t, 3H, *J* = 6.7 Hz). *^{19}F NMR (400 MHz, $CDCl_3$):* δ in ppm = -73.45 and -74.97. *MS:* m/z calcd for $C_{10}H_{20}F_6N_5P$ ([M-PF_6]$^+$), 210.30; found 210.19.

IR (KBr pellet): N_3 (2173 cm^{-1})

Synthesis of 2-azido-1,3-dihexyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-46)



Preparation of compound WLS-46b

In a clean and dry three-neck 2 Lit round bottom flask, ethane-1,2-diamine (1133 mL, 16.972 mol, 28.0 equiv) was placed with a magnetic stirring bar, and compound **WLS-46a** (100 g, 0.606 mol, 1.0 equiv) was added dropwise at 0 °C by using addition funnel. After finishing the addition, the reaction mixture was warmed to 25 °C, and left undisturbed for an additional 1 h. Then, 600 mL of hexane was added into the reaction mixture and stirred vigorously for 16 h at 25 °C. TLC showed the reaction was completed, staring material was consumed and the new spot was formed (TLC - 10% MeOH:EtOAc; TLC charring – Phosphomolybdic acid). The hexane layer was separated by using separatory funnel. Again 300 mL of hexane was added to amine layer and stir for 4 h. After that hexane layer was separated and combined with previous hexane layer, dried over sodium sulphate, and evaporated to dryness under reduced pressure to get compound **WLS-46b** (60 g) as a crude colorless liquid.

MS: m/z calcd for $C_8H_{20}N_2$ ($[M+H]^+$), 145.26; found 145.00.

Preparation of compound WLS-46c

WLS-46b (40.0 g, 0.277 mol, 1.0 equiv) was taken in clean and dry 1 Lit single neck RBF and dissolved by adding 400 mL of THF. Cool the RB in ice bath (Temp. 0 ℃). Add portion wise 1,1'-Carbonyldiimidazole (45.13 g, 0.278 mol, 1.0 equiv) to RM for period of 15 min. The reaction mixture was stir at 15 ℃ for 16 h. TLC showed the reaction was completed, staring material was consumed and the new spot was formed (TLC - 10% MeOH:EtOAc; TLC charring – Phosphomolybdic acid). After completion of reaction, reaction mixture was filter through celite pad and washed with ethyl acetate (150 mL). The combined filtrate was evaporated to dryness and purified by using silica gel column chromatography (100-200 mesh). The product was eluted with 30% ethyl acetate: hexane to ethyl acetate. Fraction containing product was evaporated to get 29.0 g (61% yield) of **WLS-46c** as a white solid.
^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 4.84 (s, 1H), 3.41 (s, 4H), 3.17 (t, 2H, *J* = 7.6 Hz), 1.49 (q, 2H, *J* = 7.1 Hz), 1.30 (d, 6H, *J* = 2.1 Hz), 0.87-0.90 (m, 3H). *MS:* m/z calcd for $C_9H_{18}N_2O$ ($[M+H]^+$), 171.26; found 171.10.

Preparation of compound WLS-46d

WLS-46c (29.0 g, 0.170 mol, 1.0 equiv) was taken in clean and dry 1 Lit two neck RBF and dissolved by adding 464 mL of DMF under argon atmosphere. Cool the RB in ice bath (Temp. 0℃). Then, add portion wise NaH (8.18 g, 0.204 mol, 1.2 equiv) to the RM for period of 20 min. at 0℃. Then add dropwise bromo hexane (71.56 mL, 0.512 mol, 3.0 equiv) to the reaction mixture at 0 ℃ for duration of 30 min. Then, allow the RM to rt and stir for 3 h. TLC showed the reaction was completed, staring material was consumed and the new spot was formed (TLC - 50% EtOAc:Hexane; TLC charring – Phosphomolybdic acid). After completion of reaction, reaction mixture was cool to 0℃ in ice bath and quenched with ice cold water. Then extracted with ethyl acetate (2 x 700 mL). The combined organic layer washed with ice cold water (2 x 1000 mL), dried over sodium sulfate, filtered and concentrated to dryness. The crude product was purified by silica gel column chromatography (100-200 mesh). The product was eluted with 10%-15% ethyl acetate:hexane. The fraction containing product was evaporated to get 29.0 g (67% yield) of **WLS-46d** as a yellow liquid.
^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 3.27 (s, 4H), 3.16 (t, 4H, *J* = 7.6 Hz), 1.48 (q, 4H, *J* = 7.1 Hz), 1.29 (s, 12H), 0.88 (t, 6H, *J* = 6.9 Hz). *MS:* m/z calcd for $C_{15}H_{30}N_2O$ ($[M+H]^+$), 255.42; found 255.27.

Preparation of compound WLS-46e

WLS-46d (29.0 g, 0.114 mol, 1.0 equiv) was taken in clean and dry 1 Lit two neck RBF and dissolved by adding 240 mL of dry toluene under argon atmosphere. Then add dropwise oxalyl chloride (146.5 mL, 1.708 mol, 15.0 equiv) to reaction mixture using addition funnel for a period of 30 min. Then reaction mixture was heated to 70 °C for 64 hrs. After completion of reaction (TLC – 5% MeOH:DCM; TLC charring – Phosphomolybdic acid) solvent was evaporated to dryness to get crude compound. The crude compound was dissolve in minimum amount of ethyl acetate and precipitate by adding dropwise hexane. Solvent was decant and solid was dried. The above precipitation procedure repeat one more time to get 34.0 g (96% yield) of **WLS-46e** as brown colour semi solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 4.33 (s, 4H), 3.65 (s, 4H), 1.69 (s, 4H), 1.30 (d, 12H, *J* = 28.2 Hz), 0.90 (t, 6H, *J* = 6.2 Hz).

Preparation of compound WLS-46f

WLS-46e (34.0 g, 0.110 mol, 1.0 equiv) was taken in clean and dry 1 Lit single neck RBF and dissolved by adding 196 mL of DCM under argon atmosphere. Then added aq. solution of KPF_6 (20.20 g, 0.110 mol, 1.0 equiv, in 110 mL of water). Stir the reaction mixture at rt for 1 h. After completion of reaction (TLC – 10% MeOH:DCM; TLC charring – Phosphomolybdic acid), the reaction mixture was poured into ice water, and extracted with DCM (2 x 400 mL). The combined organic layer washed with water (400 mL) and dried over sodium sulphate, filtered and evaporated to dryness. Then, residue was dissolved in DCM (50 mL) and product was precipitate by dropwise addition of diethyl ether (500 mL) under stirring. The solvent was decanted and solid was dried under high vacuum. The above precipitation procedure repeat one more time to get 37.0 g (80% yield) of **WLS-46f** as a light brown solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 4.10 (s, 4H), 3.54 (t, 4H, *J* = 7.6 Hz), 1.65 (q, 4H, *J* = 7.3 Hz), 1.32 (d, 12H, *J* = 2.1 Hz), 0.88-0.91 (m, 6H). *^{19}F NMR (400 MHz, $CDCl_3$):* δ in ppm = -72.87 and -74.76 *MS:* m/z calcd for $C_{15}H_{30}ClF_6N_2P$ ($[M\text{-}PF_6]^+$), 273.86; found 273.25.

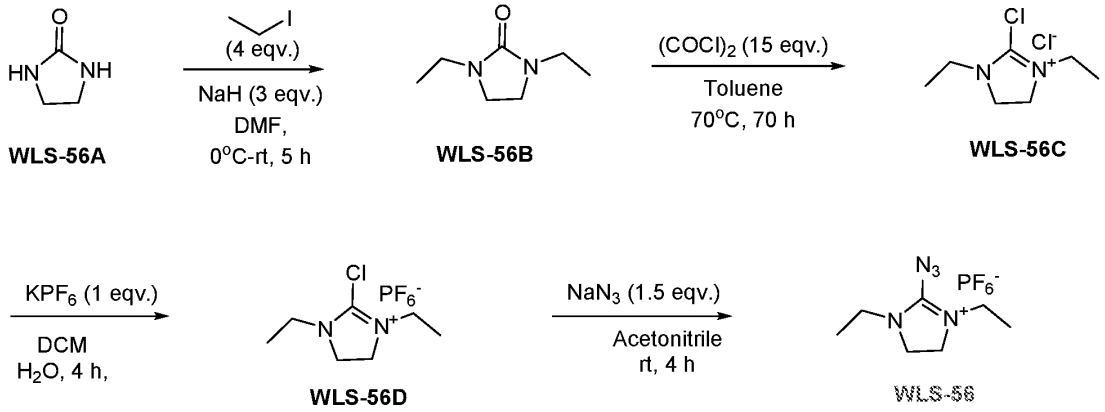
Preparation of compound WLS-46

WLS-46f (37.0 g, 0.088 mol, 1.0 equiv) was taken in clean and dry 1 Lit single neck RBF and dissolved by adding 185 mL of Dry MeCN under argon atmosphere. Then, added sodium azide (8.61 g, 0.132 mol, 1.5 equiv) to the RM and stir at rt for 2.5 h. After completion of reaction (TLC – 5% MeOH:DCM; TLC charring – ninhydrin), reaction mixture was filtered through a pad of celite and washed with MeCN (50 mL). The organic layer was evaporated to dryness. The crude compound was dissolve in DCM (15 mL) and precipitate by adding dropwise hexane (500 mL). Solvent was decanted and solid was dried under high vacuum to get 27.0 g (72% yield) of **WLS-46** as a light yellow solid.

^{1}H NMR (400 MHz, CDCl$_3$): δ in ppm = 3.92 (s, 4H), 3.45 (t, 4H, *J* = 7.7 Hz), 1.64 (q, 4H, *J* = 7.4 Hz), 1.31 (s, 12H), 0.88-0.91 (m, 6H). *^{19}F NMR (400 MHz, CDCl$_3$):* δ in ppm = -73.13 and -75.02

MS: m/z calcd for $C_{15}H_{30}F_6N_5P$ ([M-PF_6]$^+$), 280.44; found 280.26. IR (KBr pellet): N_3 (2167 cm^{-1})

Synthesis of 2-azido-1,3-diethyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-56)



1,3-diethylimidazolidin-2-one (WLS-56B).

To a stirred solution of imidazolidin-2-one (**WLS-56A**) (20 g, 0.2325 mol, 1.0 equiv) in DMF (300 mL) at 0°C was added sodium hydride (60% dispersion in oil) (28 g, 0.696 mol, 3.0 equiv) portion wise over a period of 1 h, and further stirred for another 1 h. After that ethyl iodide (73.9 mL, 0.9808 mol, 4.0 equiv) was added dropwise over a period of 50 mins at 0°C. Then the reaction mixture was allowed to rt and stirred for 5 h. Progress of the

reaction was monitored by TLC. Above reaction was diluted with ice water (300 mL) and extracted with ethyl acetate (2 x 500 mL). Combined organic layers was washed with cold brine solution (3 x 100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by column chromatography over silica gel (230-400 mesh) eluted in 30% EA/Hexane to get a light-yellow oil (21 g, 63%).

1H NMR (500 MHz, CDCl3): δ in ppm = 3.28 (s, 4H), 3.24 (q, 4H, J = 7.3 Hz), 1.10 (t, 6H, J = 7.2 Hz). MS (ESI) 143.15 $(M+1)^+$.

2-chloro-1,3-diethyl-4,5-dihydro-1H-imidazol-3-ium chloride (WLS-56C).

To a solution of 1,3-diethylimidazolidin-2-one **(WLS-56B)** (36 g, 0.2531 mol) in toluene (360 mL) was added oxalyl chloride (325 mL, 3.796 mol, 15 equiv) dropwise over a period of 1 h at 0°C under argon. Then the mixture was stirred at 70°C for 70 h. Progress of the reaction was monitored by TLC. The reaction was concentrated under reduced pressure to afford a crude mass which was treated with diethyl ether (2 x 200 mL). The solid was precipitated, filter off, washed with diethyl ether (3 x 30 mL) and dried under vacuum to afford (40 g, crude), which was used for the next step without further purification.

1H NMR (500 MHz, CDCl3): δ in ppm = 4.36 (s, 4H), 3.73 (q, 4H, J = 7.3 Hz), 1.35 (t, 6H, J = 7.2 Hz). **MS (ESI)** 161.14 $(M-Cl)^+$.

2-chloro-1,3-diethyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-56D).

To a stirred solution of 2-chloro-1,3-diethyl-4,5-dihydro-1H-imidazol-3-ium chloride **(WLS-56C)** (40 g, 0.2040 mol, 1.0 equiv) in DCM (400 mL) was added a solution of KPF_6 (37.54 g, 0.2040 mol, 1.0 equiv) in water (200 mL) dropwise over a period of 50 mins at rt. Above reaction mixture was stirred at rt for 4 h. Progress of the reaction was monitored by TLC. Then the mixture was filtered through a celite bed, washed with DCM (3 x 80 mL). The organic layer was washed with water (3 x 100 mL) dried over Na2SO4, filtered and evaporated to dryness. The gummy residue was re-dissolved in DCM (50 mL) and added dropwise to precool diethyl ether (150 mL) at -78oC under stirring. A brownish solid was precipitate out. The solid was filtered, washed with ether (2 x 50 mL) and dried under vacuum to get the desired compound 2-chloro-1,3-diethyl-4,5-dihydro-1H-imidazol-3-ium

hexafluorophosphate(V) (WLS-56D) (38 g, 60%).

1H NMR (500 MHz, CDCl3): δ in ppm = 4.12 (s, 4H), 3.65 (m, 4H), 1.33 (m, 6H).

MS (ESI) 161.14 $(M\text{-}PF6)^+$.

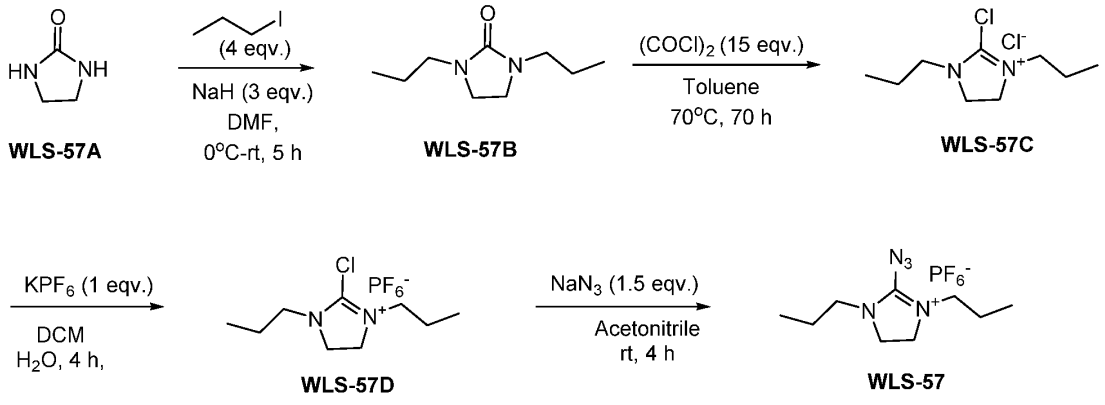
2-azido-1,3-diethyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-56D).

To a precool solution of 2-chloro-1,3-diethyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-56D) (WLS-56D) (36 g, 0.1176 mol, 1.0 equiv) in acetonitrile (360 mL) was added a sodium azide (11.40 g, 0.1765 mol, 1.0 equiv) portion wise over a period of 20 mins under N_2 atmosphere. Above reaction mixture was stirred at rt for 5 h. Progress of the reaction was monitored by TLC. Then the mixture was filtered through a celite bed, washed with acetonitrile (2 x 100 mL). The filtrate was evaporated under vacuum to afford a gummy mass. The residue was again dissolved in DCM (45 mL) and dropwise added to diethyl ether (200 mL) under stirring, at -78°C. The solid was precipitated, filtered and washed with ether (2 x 50 mL), and dried under vacuum to get the desired compound (30 g, 81%).

1H NMR (400 MHz, CDCl3): δ in ppm = 3.93 (s, 4H), 3.54 (q, 4H, J = 7.3 Hz), 1.31 (t, 6H, J = 7.4 Hz). MS (ESI) 168.23 $(M+)^+$.

19F NMR (400 MHz, CDCl3): δ in ppm = -73.13 and -75.03. **IR (KBr pellet):** N_3 (2175.31 cm^{-1})

Synthesis of 2-azido-1,3-dipropyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-57)



1,3-dipropylimidazolidin-2-one (WLS-57B).

To a stirred solution of imidazolidin-2-one (15 g, 0.17 mol, 1.0 equiv) in DMF (225 mL) was added sodium hydride (20.9 g, 0.52 mol.) portion wise at 0°C over a period of 40 min, and kept for 1 h. Then 1-bromopropane (63.5 mL, 0.69 mol, 1.2 equiv) was added dropwise over a period of 30 min. and stirred for 5 h at rt. Progress of the reaction was monitored by TLC. Above reaction was diluted with ice water (300 mL) and extracted with ethyl acetate (3 x 400 mL). Combined organic layer was washed with cold brine solution (3 x 100 mL), dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by column chromatography over silica gel (230-400 mesh) eluted in 30% EA/Hexane to yield1,3-dipropylimidazolidin-2-one (**WLS-57B**) as a light yellow oil (21 g, 71%).

1H NMR (500 MHz, CDCl3): δ in ppm = 3.21 (s, 4H), 3.07 (t, 4H, J = 7.6 Hz), 1.45 (td, 4H, J = 14.8 Hz, 7.6 Hz), 0.83 (t, 6H, J = 7.2 Hz).

MS (ESI) 171.25 $(M+1)^+$.

2-chloro-1,3-dipropyl-4,5-dihydro-1H-imidazol-3-ium chloride (WLS-57C).

To a cool solution of1,3-dipropylimidazolidin-2-one (WLS-57B) (15 g, 0.088 mol, 1.0 equiv) in toluene (150 mL) was added oxalyl chloride (113 mL, 1.32 mol.,15.0 equiv) dropwise over a period of 30 min under argon atmosphere. Above mixture was stirred at 70oC for 72 h. Progress of the reaction was monitored by TLC. Then the reaction was concentrated under reduced pressure to afford a crude mass which was treated with n-hexane (3 x 75 mL) followed by diethyl ether ((2 x 100 mL) to get a brownish solid. The solid was dried under vacuum to afford (18 g, crude) which was used for the next step without further purification.

1H NMR (500 MHz, CDCl3): δ in ppm = 4.32 (s, 4H), 3.61 (t, 4H, J = 7.6 Hz), 1.76 (td, 4H, J = 14.8 Hz, 7.6 Hz), 0.99 (t, 6 H, J = 7.6 Hz). **MS (ESI)** 189.18 $(M-Cl)^+$.

2-chloro-1,3-dipropyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-57C)

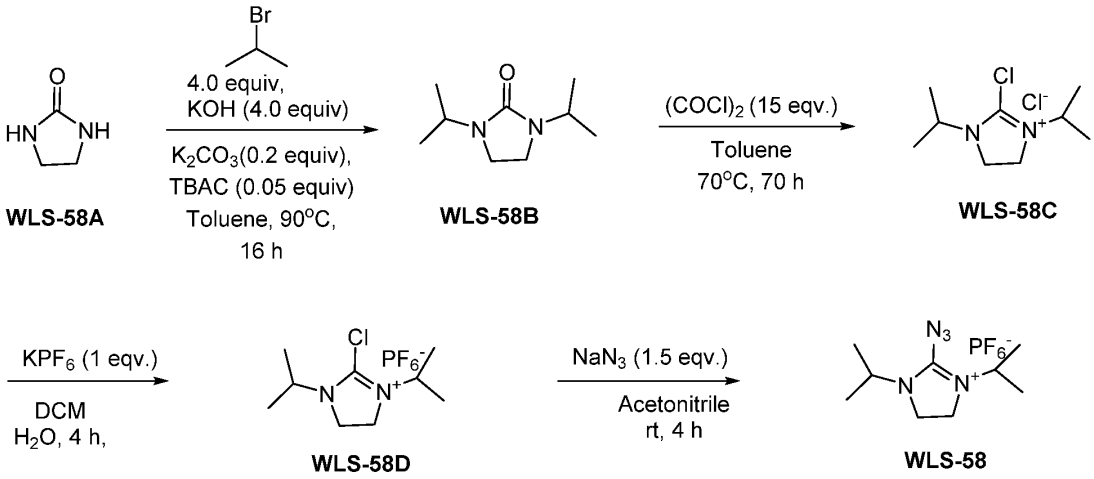
To a stirred solution of 2-chloro-1,3-dipropyl-4,5-dihydro-1H-imidazol-3-ium chloride

(WLS-57C) (16 g, 0.0714 mol, 1.0 equiv) in DCM (160 mL) was added a solution of KPH_6 (13.14 g, 0.0714 mol., 1.0 equiv) in 80 mL of water over a period of 30 mins at rt. Above reaction mixture was stirred for 3 h. Progress of the reaction was monitored by TLC. Then the mixture was filtered through a celite bed, the bed was washed with DCM (2 x 100 mL). The combined organic layer was washed with water (3 x 100 mL), dried over Na_2SO_4 and evaporated to dryness. The residue was again dissolved in DCM (30 mL) and then added diethyl ether (200 mL) under stirring. The solid was precipitate out which was filtered and washed with ether (2 x 50 mL), dried under vacuum to afford 2-chloro-1,3-dipropyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) **(WLS-57C)** as a reddish solid (16 g, 67%).**1H NMR (500 MHz, CDCl3):** δ in ppm = 4.11 (s, 4H), 3.52 (t, 4H, J = 7.6 Hz), 1.71 (td, 4H, J = 15.1 Hz, 7.6 Hz), 0.97 (t, 6H, J = 7.2 Hz). **MS (ESI)** 189.19 $(M\text{-}PF_6)^+$.

2-azido-1,3-dipropyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-57)

To a stirred cool solution of 2-chloro-1,3-dipropyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-57D) (11 g, 0.032 mol, 1.0 equiv) in acetonitrile (110 mL) was added sodium azide (3.2 g, 0.049 mol., 1.5 equiv) portion wise over a period of 20 mins under nitrogen.. Above reaction mixture was stirred for 3 h. Progress of the reaction was monitored by TLC. Then the mixture was filtered through a celite bed; washed with acetonitrile (2 x 100 mL). The filtrate was evaporated under vacuum to afford a crude mass. The residue was dissolved in DCM (30 mL) and then added diethyl ether (200 mL) under stirring. The solid was thrown out which was filtered and washed with ether (2 x 50 mL), dried under vacuum to get 2-azido-1,3-dipropyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (**WLS-57**) as a brownish solid (10 g, 89%).**1H NMR (400 MHz, CDCl3):** δ in ppm = 3.93 (s, 4H), 3.42 (t, 4H, J = 7.6 Hz), 1.70 (td, 4H, J = 15 Hz, 7.6 Hz), 0.97 (t, 6H, J = 7.4 Hz). **MS (ESI)** 196.25 $(M\text{-}PF6)^+$. **19F NMR (400 MHz, CDCl3)**: δ in ppm = -73.3 and -74.8. **IR (KBr pellet):** N3 (2175 cm^{-1}).

Synthesis of 2-azido-1,3-diisopropyl-4,5-dihydro-1H-imidazol-3-ium hexafluoro-phosphate(V) (WLS-58)



1,3-diisopropylimidazolidin-2-one (WLS-58B)

To a stirred solution of imidazolidin-2-one (WLS-58B) (20 g, 0.23 mol, 1.0 equiv) in toluene (340 mL) was added potassium hydroxide (52 g, 0.92 mol., 4.0 equiv), Potassium carbonate (6.41 g, 0.046 mol., 0.2 equiv) and tetrabutylammonium chloride (3.22 g, 0.011 mol., 0.05 equiv) at rt under N_2 atmosphere. Then 2-bromo propane (87.24 mL, 0.92 mol., 4.0 equiv) was added slowly. Above reaction mixture was stirred at 90°C for 20 h. Progress of the reaction was monitored by TLC. Then the mixture was diluted with ice water (200 mL) and extracted with DCM (2 x 400 mL). Combined organic phase was washed with brine solution (2 x 100 mL) dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by column chromatography over silica gel (230-400 mesh) eluted in 30% EA/Hexane to get 1,3-diisopropylimidazolidin-2-one (**WLS-58B**) as a pale yellow syrup (18 g, 45%).

1H NMR (400 MHz, $CDCl_3$): δ in ppm = 4.09 (m, 2H), 3.17 (s, 4H), 1.06 (d, 12H, J = 6.7 Hz) **MS (ESI)** 171.24 $(M+1)^+$. **2-chloro-1,3-diisopropyl-4,5-dihydro-1H-imidazol-3-ium chloride (WLS-58C)**

To a ice cool solution of 1,3-diisopropylimidazolidin-2-one (**WLS-58B**) (15 g, 0.0588 mol, 1.0 equiv) in toluene (100 mL) was added oxalyl chloride (76.2 mL, 0.088 mol., 15.0 equiv) dropwise over a period of 30 min under argon atmosphere. Above mixture was stirred at 70°C for 72 h. Progress of the reaction was monitored by TLC. After that the reaction mixture was concentrated under reduced pressure to afford a crude mass which was treated with 40% EA/Hexane (3 x 75 mL) and stirred for 30 min. Then the solid was precipitated

out, filtered and washed with diethyl ether (2 x 50 mL). The compound was dried under vacuum to afford give 2-chloro-1,3-diisopropyl-4,5-dihydro-1H-imidazol-3-ium chloride (**WLS-58C**) as a brownish solid (13 g, crude) which was used in the next step without further purification.

1H NMR (500 MHz, $CDCl_3$): δ in ppm = 4.31 (m, 6H), 1.41 (d, 12H, J = 6.5 Hz). **MS (ESI)** 189.14 $(M-Cl)^+$

2-chloro-1,3-diisopropyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-58D).

To a stirred solution of 2-chloro-1,3-diisopropyl-4,5-dihydro-1H-imidazol-3-ium chloride (WLS-58C) (20 g, 0.0888 mol, 1.0 equiv) in DCM (200 mL) was added a solution of KPH_6 (16.3 g, 0.0888 mol., 1.0 equiv) in water (100 mL) dropwise over a period of 30 min. Above reaction mixture was stirred for 4 h. Progress of the reaction was monitored by TLC. Then the mixture was filtered through a celite bed, the bed was washed with DCM (2 x 130 mL). The Organic layer was washed with water (3 x 100 mL), dried over Na_2SO_4, filtered and evaporated to dryness. The residue was dissolved in DCM (25 mL) and then added diethyl ether (165 mL) under stirring. The solid precipitated was filtered and washed with ether (2 x 50 mL), dried under vacuum to afford 2-chloro-1,3-diisopropyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-58D) as a light brown solid (18 g, 61%).

1H NMR (500 MHz, $CDCl_3$): δ in ppm = 4.29 (m, 2H), 4.07 (s, 4H), 1.37 (d, 12H, J = 6.9 Hz), **MS (ESI)** 189.15 $(M-PF_6)^+$.

2-azido-1,3-diisopropyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-58).

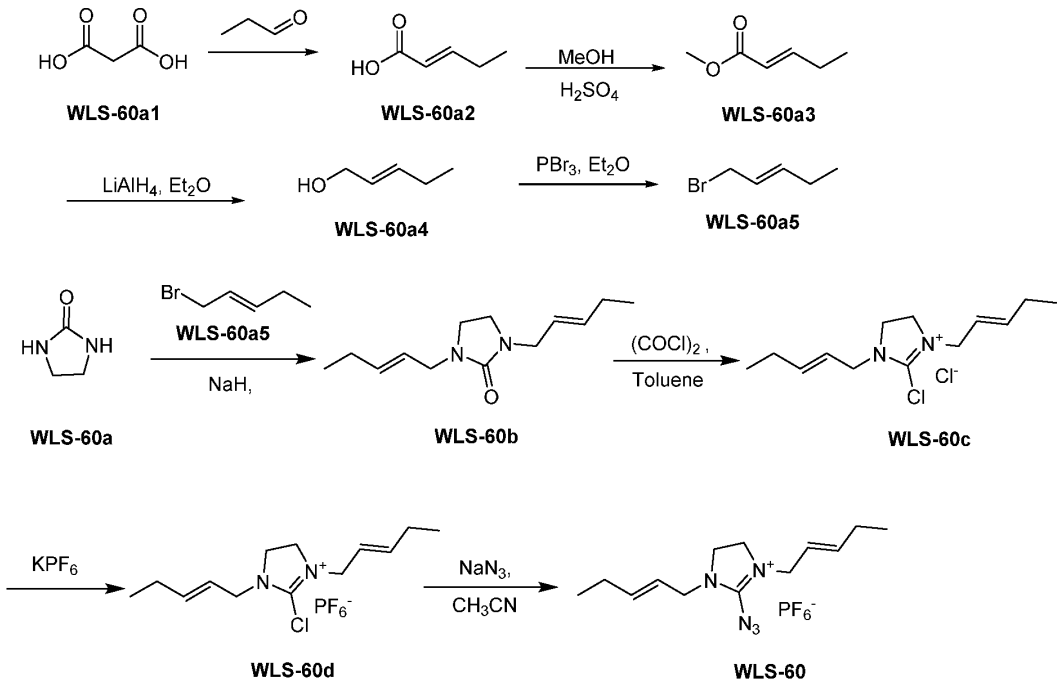
To a cool stirred solution of 2-chloro-1,3-diisopropyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (**WLS-58D**) (18 g, 0.032 mol, 1.0 eqiv) in acetonitrile (180 mL) was added sodium azide (5.25 g, 0.080 mol., 1.5 eqiv) portion wise over a period of 20 mins under N_2 atmosphere. Above reaction mixture was stirred for 4 h. Progress of the reaction was monitored by TLC. Then the mixture was filtered through a celite bed, the bed was washed with acetonitrile (2 x 100 mL). The filtrate was evaporated under vacuum to afford a crude. The residue was dissolved in DCM (25 mL) and then added diethyl ether (150 mL) under stirring. The solid precipitated was filtered, washed with ether (2 x 50 mL) and dried

under vacuum to afford 2-azido-1,3-diisopropyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (**WLS-58**) as a brownish solid (17 g, 92%).

1H NMR (500 MHz, CDCl3): δ in ppm = 4.18 (m, 2H), 3.86 (s, 4H), 1.33 (d, 12H, J = 6.2 Hz) **MS (ESI)** 196.26 $(M\text{-}PF_6)^+$. **19F NMR (500 MHz, CDCl3):** δ in ppm = -72.86 and -74.37

IR (KBr pellet): N_3 (2165 cm^{-1})

Synthesis of 2-azido-1,3-di((E)-pent-2-en-1-yl)-4,5-dihydro-1H-imidazol-3-ium hexafluoro- phosphate(V) (WLS-60)



Preparation of compound WLS-60a2

WLS-60a1 (41.60 g, 0.400 mol, 1.0 equiv) was taken in clean and dry 500 mL 2 neck RBF under argon atmosphere. Then, added 41 mL of Pyridine to RBF containing SM. Add dropwise propionaldehyde (30.23 mL, 0.519 mol, 1.3 equiv) to reaction mixture using addition funnel. Then, reaction mixture was heated reflux 70 °C for 4 h. After completion of reaction (TLC – 10% MeOH:DCM), cool the reaction mixture with rt. Added 50% H_2SO_4 up to pH < 2. Water was added and extract with EtOAc (2 x 500 mL). The combined the organic layer dried on sodium sulphate, filtered and evaporated to dryness to get 32.0 g

(80% yield) **WLS-60a2** as a colourless oil.

The WLS-60a2 was directly used for next reaction without any further purification.

^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm =10.63 (bs, 1H), 7.15 (dt, 1H, *J* = 15.4 Hz, 6.4 Hz), 5.83 (dt, 1H, *J* = 15.8 Hz, 1.7 Hz), 2.29-2.24 (m, 2H), 1.09 (t, 3H, *J* = 7.2 Hz).

Preparation of compound WLS-60a3

WLS-60a2 (32.0 g, 0.320 mol, 1.0 equiv) was taken in clean and dry 500 mL single neck RBF under argon atmosphere was added EtOH (73 mL) followed by toluene (30 mL). Cool the RB in ice bath and add H_2SO_4 (2.75 mL). The reaction mixture was heated at 100 °C for 20 h. After completion of reaction (TLC – 10% MeOH:DCM), cool the reaction mixture with rt. The volatiles were evaporated. The residue was extracted with DCM (2 x 600 mL), washed with sat $NaHCO_3$ (500 mL) solution followed by water (500 ml). The organic layer dried over sodium sulphate and dried under vacuum to get 31.0 g (76% yield) of **WLS-60a3** as a light-yellow oil.

The WLS-60a3 was directly used for next reaction without any further purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 7.08-6.98 (m, 1H), 5.81 (dt, 1H, *J* = 15.7 Hz, 1.7 Hz), 4.21-4.15 (m, 2H), 2.26-2.15 (m, 2H), 1.28 (t, 3H, *J* = 7.1Hz), 1.076 (t, 3H, *J* = 7.4 Hz).

Preparation of compound WLS-60a4

Lithium aluminium hydride (12.18 g, 0.321 mol, 1.87 equiv) was taken in clean and dry 2 Lit two neck RBF under argon atmosphere. Then, add 366 mL of dry Diethyl ether, cooled to 0 °C. Then $AlCl_3$ (15.15 g, 0.114 mol,0.66 equiv, in 611 mL of ether) was drop wise added to RBF per a period of 50 min. After completion of addition allow to rt and stirred for 30 min. Again cooled to 0 °C add dropwise for a period of 20 min **WLS-60a3** (22.00 g 0.172 mol, 1.0 equiv). The reaction mixture was allow to rt and stirred for 1 hrs. After completion of reaction (TLC – 10% MeOH:DCM, PMA charring) reaction mixture cool to 0 °C. Then quenched the reaction mixture with 20% NaOH solution (70 mL), and stir for 45 min. The residue was extracted with ether (2 x 600 mL), washed with water (500 ml). The organic layer dried over sodium sulphate and dried under vacuum to get 12.0 g (81% yield) of **WLS-60a4** as a light yellow oil.

The WLS-60a4 was directly used for next reaction without any further purification.
^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 5.77-5.72 (m, 1H), 5.66-5.60 (m, 1H), 4.09 (t, 2H, *J* = 5.9 Hz), 2.09-2.04 (m, 2H), 1.00 (t, 3H, *J* = 7.6 Hz).

Preparation of compound WLS-60a5

WLS-60a4 (12.00 g, 0.139 mol, 1.0 equiv) was taken in clean and dry 500 mL 2 neck RBF under argon atmosphere in 240 mL of ether, cooled to 0 °C, added PBr_3 (15.9 mL, 0.167 mol, 1.2 equiv) dropwise for a period of 20 min. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. After completion of reaction (TLC – 10% MeOH:DCM) reaction mixture cool to 0 °C. Then, quenched with ice water carefully (70 mL), extracted with diethyl ether (2 x 150 mL), washed with water (300 ml). The organic layer dried over sodium sulphate and dried under vacuum to get 11.0 g (53% yield) of **WLS-60a5** as a colorless oil.
The WLS-60a5 was directly used for next reaction without any further purification.
^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 5.82 (dt, 1H, *J* = 15.1 Hz, 6.2 Hz), 5.71-5.65 (m, 1H), 3.96 (d, 2H, *J* = 7.6 Hz), 2.12-2.06 (m, 2H), 1.01 (m, 3H).

Preparation of compound WLS-60b

In a clean and dry two-neck 500 mL round bottom flask, **WLS-60a** (10.0 g, 0.116 mol, 1.0 equiv) was placed with a magnetic stirring bar and dissolved by adding DMF (150 mL). Then cool the RBF to 0 °C by using ice bath. Added sodium hydride (9.29 g, 0.232 mol, 3.0 equiv) portion wise for period of 30 min at 0 °C. Stir the reaction mixture at 0 °C for 30 min. Then added **WLS-60a5** (60.12 g, 0.403 mol, 3.47 equiv) dropwise by using addition funnel for period of 20 min at 0 °C and the reaction mixture was stirred for 5 h. Monitoring by TLC it showed staring material was consumed and the product was formed (TLC - 50% EtOAc;Hexane, TLC charring – Phosphomolybdic acid). After completion of reaction, reaction mixture poured into ice and extracted with ethyl acetate (500 mL x 2) and organic layer washed with ice cold water (500 mL x 2). Organic layer dried over sodium sulphate, filtered and evaporated to dryness to get crude compound. The crude product was purified by silica gel column chromatography (100-200 mesh). The product was eluted with 15% -

20% ethyl acetate:hexane. The fraction containing product was evaporated to get 18.4 g (71% yield) of **WLS-60b** as a yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 5.69-5.62 (m, 2H), 5.41-5.33 (m, 2H), 3.74 (dd, 4H, *J* = 6.5, *J* =1.1 Hz), 3.22 (s, 4H), 2.08- 2.00 (m, 4H), 0.99 (t, 6H, *J* = 7.4 Hz).

Preparation of compound WLS-60c

WLS-60b (25.0 g, 0.112 mol, 1.0 equiv) was taken in clean and dry 2 Lit two neck RBF under argon atmosphere. Then added 350 mL of dry toluene under argon atmosphere. Added oxalyl chloride (144 mL, 1.679 mol, 14.93 equiv) dropwise using addition funnel for a period of 45 min at rt. The reaction mixture was heated to 65 °C for 72 hrs. After completion of reaction (TLC – 10% MeOH:DCM; TLC charring – Phosphomolybdic acid) solvent was evaporated on rota evaporator to get crude compound. The crude compound was washed with hexane (2 x 500 mL), afters washing solvent was decanted and dried on high vacuum to get 31.0 g of crude **WLS-60c** as a brown gummy liquid. The **WLS-60c** was directly used for next step without any further purification. *^{1}H NMR (400 MHz, $CDCl_3$):* δ in ppm = 5.97-5.92 (m, 2H), 5.48-5.33 (m, 4H), 4.23 (s, 6H), 2.17- 2.03 (m, 4H), 1.01 (t, 6H, *J* = 7.4 Hz). *MS:* m/z calcd for $C_{13}H_{22}Cl_2N_2^+$ [M-Cl], Calculated 241.78; found 241.21.

Preparation of compound WLS-60d

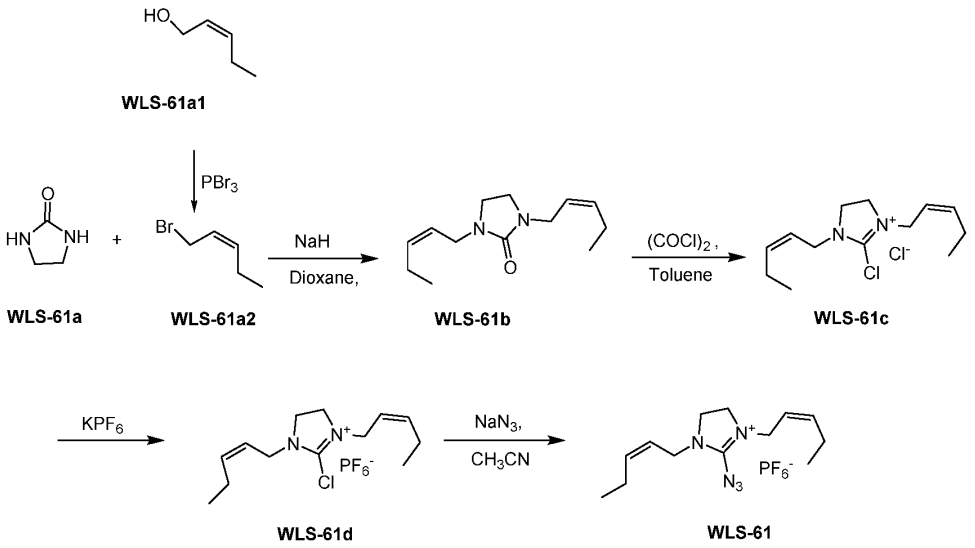
WLS-60c (31.0 g, 0.112 mol, 1.0 equiv) was taken in clean and dry 2 Lit single neck RBF under argon atmosphere. Added 310 mL of DCM under argon atmosphere. Then added aq solution of KPF_6 (20.58 g, 0.112 mol, 1.0 equiv, in 124 mL of water). The reaction mixture was stirred at rt for 2.5 h. After completion of reaction (TLC – 10% MeOH:DCM; TLC charring – Phosphomolybdic acid), the reaction mixture was poured into ice water (400 mL), and extracted with DCM (2 x 500 mL). The combined organic layer washed with water (400 mL) and dried over sodium sulphate, filtered and evaporated to dryness. The residue was dissolved in DCM (15 mL) and product was precipitate by dropwise addition of diethyl ether (2 x 500 mL) under stirring. The solvent was decanted and solid was dried under high vacuum. The above precipitation procedure repeat one more time to get 39.0 g (90% yield) of **WLS-60d** as an ash colored solid. *^{1}H NMR (500 MHz, $CDCl_3$):* δ in ppm = 5.92-5.86 (m,

2H), 5.42-5.36 (m, 2H), 4.11 (d, 4H, *J* = 6.9 Hz), 4.02 (s, 4H), 2.13- 2.07 (m, 4H), 1.01 (t, 6H, *J* = 7.6 Hz). *^{19}F NMR (500 MHz, CDCl$_3$):* δ in ppm = -72.96, - 74.48.

Preparation of compound WLS-60

WLS-60d (39.0 g, 0.101 mol, 1.0 equiv) was taken in clean and dry 1 Lit two neck RBF under argon atmosphere. Added 390 mL of Dry MeCN under argon atmosphere. Added sodium azide (9.84 g, 0.151 mol, 1.5 equiv) portion wise for the period of 10 min. The reaction mixture was stirred at rt for 3 h. After completion of reaction (TLC – 5% MeOH:DCM; TLC charring – ninhydrin), reaction mixture was filtered through a pad of celite and washed with MeCN (40 mL). The organic layer was evaporated to dryness. The crude compound was washed with ether and hexane to get brown gummy liquid which was dried on high vacuum to get 32.0 g (81% yield) of **WLS-60** as a brown gummy liquid. *^{1}H NMR (500 MHz, CDCl$_3$):* δ in = 5.89-5.84 (m, 2H), 5.44-5.40 (m, 2H), 4.04 (d, 4H, *J* = 5.5 Hz), 3.87 (s, 4H), 2.13- 2.08 (m, 4H), 1.01 (q, 6H, *J* = 7.1 Hz). *^{19}F NMR (500 MHz, CDCl$_3$):* δ in ppm = -73.22 and -74.74. *MS:* m/z calcd for $C_{13}H_{22}F_6N_5P$ ($[M-PF_6]^+$), 248.35; found 248.80. IR (KBr pellet): N_3 (2170 cm^{-1})

Synthesis of 2-azido-1,3-di((Z)-pent-2-en-1-yl)-4,5-dihydro-1H-imidazol-3-ium hexafluoro- phosphate(V) (WLS-61)



Preparation of compound WLS-61a2

WLS-61a1 (19.00 g, 0.221 mol, 1.0 equiv) was taken in clean and dry 1 L two neck RBF under argon atmosphere in 380 mL of dry ether, cooled to 0 °C, added PBr_3 (25.2 mL, 0.265 mol, 1.2 equiv) dropwise for a period of 20 min. The reaction mixture was allowed to rt and stirred for 4 h. After completion of reaction (TLC – 30% EtOAc:hexane; TLC charring – Phosphomolybdic acid) reaction mixture cool to 0 °C. Then, quenched with ice water carefully (70 mL), extracted with diethyl ether (2 x 500 mL), washed with water (300 ml). The organic layer dried over sodium sulphate and dried under vacuum to get 24.0 g (73% yield) of **WLS-61a2** as a colorless oil.The WLS-60a2 was directly used for next reaction without any further purification. *^{1}H NMR (400 MHz, $CDCl_3$):* δ in ppm = 5.74-5.66 (m, 1H), 5.63-5.57 (m, 1H), 4.00 (d, 2H, *J* = 8.2 Hz), 2.20-2.09 (m, 2H), 1.02 (t, 3H, *J* = 7.6 Hz).

Preparation of compound WLS-61b

In a clean and dry two-neck 500 mL round bottom flask, **WLS-61a** (5.0 g, 0.058 mol, 1.0 equiv) was placed with a magnetic stirring bar, and dissolved by adding DMF (100 mL). Then cool the RBF to 0 °C by using ice bath. Added sodium hydride (4.64 g, 0.116 mol) portion wise for period of 30 min at 0 °C. Stir the reaction mixture at 0 °C for 30 min. Then added **WLS-61a2** (21.63 g, 0.145 mol, 2.5 equiv) dropwise by using addition funnel for period of 30 min at 0 °C and the reaction mixture was stirred for 30 min for 0 °C and 3 h at rt. Monitoring by TLC it showed staring material was consumed and the product was formed (TLC - 30% EtOAc;Hexane, TLC charring – Phosphomolybdic acid). After completion of reaction, reaction mixture poured into ice and extracted with ethyl acetate (1000 mL x 2) and organic layer washed with ice cold water (1200 mL x 2). Organic layer dried over sodium sulphate, filtered and evaporated to dryness to get crude compound. The crude product was purified by silica gel column chromatography (100-200 mesh). The product was eluted with 5% - 10% ethyl acetate:hexane. The fraction containing product was evaporated to get 9.69 g (75% yield) of **WLS-61b** as a yellow liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 5.63-5.56 (m, 2H), 5.36-5.29 (m, 2H), 3.84 (dt, 4H, *J* = 7.1, *J* =0.6 Hz), 3.23 (s, 4H), 2.15- 2.07 (m, 4H), 0.98 (t, 6H, *J* = 7.5 Hz).

MS: m/z calcd for $C_{13}H_{22}N_2O$ ($[M+H]^+$), 223.33; found 223.37.

Preparation of compound WLS-61c

WLS-61b (30.0 g, 0.135 mol, 1.0 equiv) was taken in clean and dry 2 Lit three neck RBF under argon atmosphere. Then added 300 mL of dry toluene under argon atmosphere. Added oxalyl chloride (173.6 mL, 2.024 mol, 15.0 equiv) dropwise using addition funnel for a period of 30 min at rt. The reaction mixture was heated to 65 °C for 72 hrs. After completion of reaction (TLC – 10% MeOH:DCM; TLC charring – Phosphomolybdic acid) solvent was evaporated on rota evaporator to get crude compound. The crude compound was washed with hexane (2 x 500 mL), afters washing solvent was decanted and dried on high vacuum to get 38.0 g of crude **WLS-61c** as a brown gummy liquid. The **WLS-61c** was directly used for next step without any further purification. *MS:* m/z calcd for $C_{13}H_{22}Cl_2N_2^+$ [M^+-Cl], Calculated 241.78; found 241.27.

Preparation of compound WLS-61d

WLS-61c (37.0 g, 0.133 mol, 1.0 equiv) was taken in clean and dry 1 Lit single neck RBF under argon atmosphere. Added 370 mL of DCM under argon atmosphere. Then added aq solution of KPF_6 (24.57 g, 0.133 mol, 1.0 equiv, in 148 mL of water). The reaction mixture was stirred at rt for 3 h. After completion of reaction (TLC – 10% MeOH:DCM; TLC charring – Phosphomolybdic acid), the reaction mixture was poured into ice water (400 mL), and extracted with DCM (2 x 500 mL). The combined organic layer washed with water (400 mL) and dried over sodium sulphate, filtered and evaporated to dryness. The residue was dissolved in DCM (40 mL) and product was precipitate by dropwise addition of diethyl ether (1000 mL) under stirring. The solvent was decanted and solid was dried under high vacuum. The above precipitation procedure repeat one more time to get 48.0 g (93% yield) of **WLS-61d** as an ash colored solid. *^{1}H NMR (500 MHz, $CDCl_3$):* δ in ppm = 5.92-5.79 (m, 2H), 5.44-5.33 (m, 2H), 4.21 (d, 4H, *J* = 7.6 Hz), 4.03 (s, 4H), 2.16-2.09 (m, 4H), 1.01 (t, 6H, *J* = 7.2 Hz). *^{19}F NMR (500 MHz, $CDCl_3$):* δ in ppm = -73.12, - 74.64. *MS:* m/z calcd for $C_{13}H_{22}ClF_6N_2P^+$ [M^+-Cl], Calculated 241.78; found 241.18.

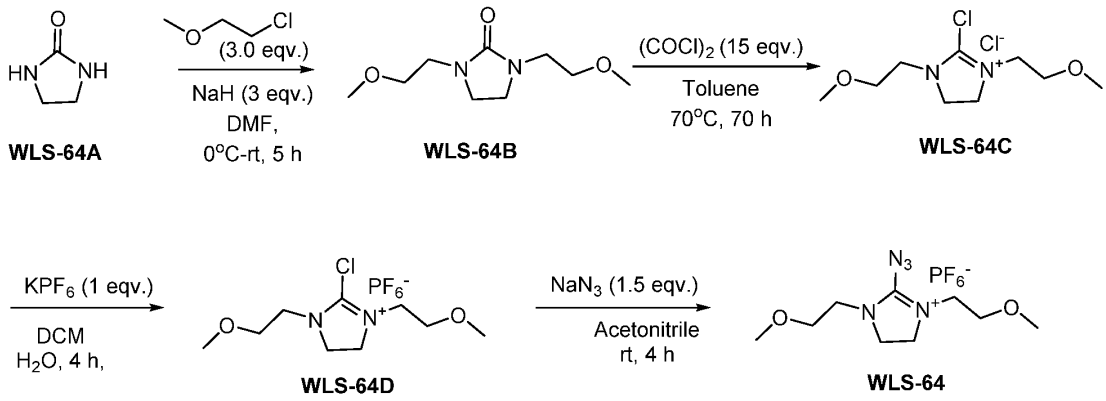
Preparation of compound WLS-61

WLS-61d (48.0 g, 0.124 mol, 1.0 equiv) was taken in clean and dry 1 Lit two neck RBF under argon atmosphere. Added 480 mL of Dry MeCN under argon atmosphere. Added sodium azide (12.103 g, 0.186 mol, 1.5 equiv) portion wise for the period of 10 min. The

reaction mixture was stirred at rt for 2.5 h. After completion of reaction (TLC – 5% MeOH:DCM; TLC charring – ninhydrin), reaction mixture was filtered through a pad of celite and washed with MeCN (40 mL). The organic layer was evaporated to dryness. The crude compound was dissolved in DCM (100 mL) and precipitate by adding ether and hexane at -78 °C, solvent was decant and solid was dried under high vacuum to get 28.0 g (57% yield) of **WLS-61** as a brown solid. *^{1}H NMR (500 MHz, CDCl$_3$):* δ in = 5.80-5.75 (m, 2H), 5.43-5.36 (m, 2H), 4.12 (d, 4H, *J* = 6.9 Hz), 3.86 (s, 4H), 2.13- 2.08 (m, 4H), 1.00 (q, 6H, *J* = 7.1 Hz).

^{19}F NMR (500 MHz, CDCl$_3$): δ in ppm = -73.26 and -74.78. *MS:* m/z calcd for $C_{13}H_{22}F_6N_5P$ ([M-PF_6]$^+$), 248.35; found 248.24. IR (KBr pellet): N_3 (2171 cm^{-1})

Synthesis of 2-azido-1,3-bis(2-methoxyethyl)-4,5-dihydro-1H-imidazol-3-ium hexafluoro-phosphate(V) (WLS-64)



1,3-bis(2-methoxyethyl)imidazolidin-2-one (WLS-64B).

To a solution of imidazolidin-2-one **(WLS-64A)** (20 g, 0.23 mol, 1.0 equiv) in DMF (20 mL) was added sodium hydride (28 g, 0.69 mol., 3.0 equiv) portion-wise at 70°C over a period of 40 min, stirred at the same temperature for 2 h. Then a solution of 2-chloroethyl methyl ether (63.9 mL, 0.69 mol, 3.0 equiv) in DMF (60 mL) was added dropwise over a period of 30 mins. Above mixture was stirred at 70°C for 3 h. Progress of the reaction was monitored by TLC. Above reaction was diluted with ice water (300 mL) and extracted with ethyl acetate (2 x 500 mL). Combined organic layers was washed with cold brine solution

(3 x 100 mL) dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by column chromatography over silica gel (60-120 mesh) eluted in 80% EA/Hexane to give 1,3-bis(2-methoxyethyl)imidazolidin-2-one (**WLS-64B**) as a colourless oil (29 g, 62%).

1H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.52 (t, 4H, *J* = 5.2 Hz)), 3.42 (s, 4H), 3.37 (t, 4H, *J* = 5.3 Hz), 3.35 (s, 6H). **MS (ESI)** 203.21 $(M+1)^+$.

2-chloro-1,3-bis(2-methoxyethyl)-4,5-dihydro-1H-imidazol-3-ium chloride (WLS-64C).

To a cool solution of (**WLS-64B**) (15 g, 0.074 mol, 1.0 equiv) in toluene (150 mL) was added oxalyl chloride (95 mL, 1.1138 mol., 15.0 equiv) dropwise over a period of 25 min under argon atmosphere. Above mixture was stirred at 70oC for 72 h. Progress of the reaction was monitored by TLC. Above reaction mixture was concentrated under reduced pressure to afford a crude compound. The crude was treated with n-hexane (2 x 100 mL) and 40% EA/Hexane (3 x 100 mL) at 0oC. A solid precipitation was observed at 0°C, then solvent was decant and the compound was dried under vacuum to afford a brownish gummy syrup (17 g, crude) which was used for the next step without further purification.
1H NMR (400 MHz, CDCl3): δ in ppm = 4.38 (d, 4H, J = 18.6 Hz), 3.89 (t, 4H, J = 4.9 Hz) 3.66 (t, 4H, J = 4.9 Hz), 3.39 (s, 6H). MS (ESI) 221.19 (M-Cl)+.

2-chloro-1,3-bis(2-methoxyethyl)-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-64D).

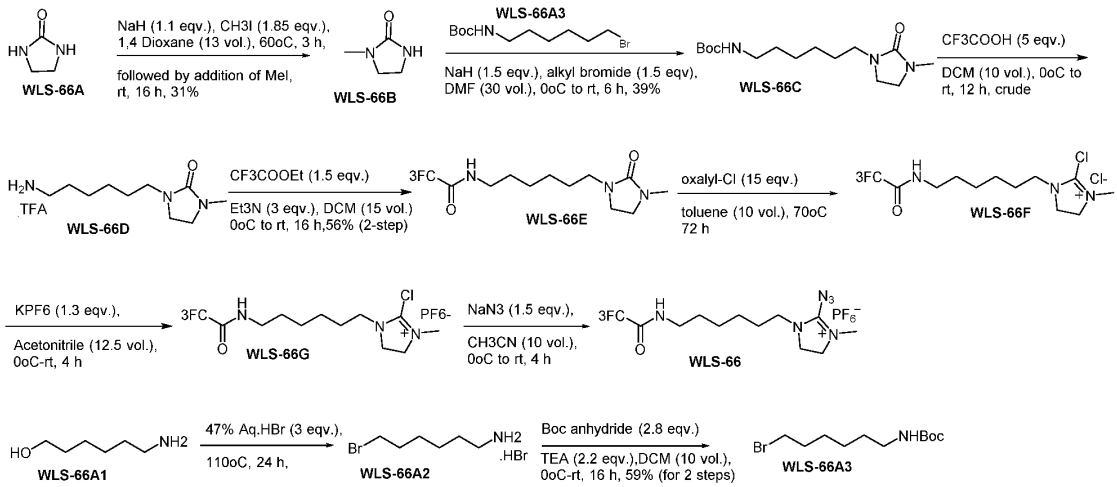
To a stirred solution of (WLS-64C) (14 g, 0.0544 mol, 1.0 equiv) in DCM (140 mL) was added a solution of KPH_6 (10 g, 0.0544 mol., 1.0 equiv) in water (70 mL) dropwise over a period of 30 mins at rt. Above reaction mixture was stirred at rt for 4 h. Progress of the reaction was monitored by TLC. Then the mixture was filtered through a celite bed washed with DCM (3 x 100 mL). Organic layer was washed with water (2 x 100 mL) dried over Na2SO4, filtered and evaporated to dryness. The residue was dissolved in DCM (15 mL) and then added diethyl ether (125 mL), cool to -78oC. The solid precipitated was filtered and washed with ether (2 x 50 mL) and dried under vacuum to yield a brown solid (25 g, 64%). 1H NMR (500 MHz, CDCl3): δ in ppm = 4.21 (td, 4H, J = 10.8 Hz, 5.3 Hz), 3.78 (m,

4H), 3.62 (q, 4H, J = 5.5 Hz), 3.38 (d, 6H, J = 2.8 Hz). MS (ESI) 221.18 (M-PF6) +.

2-azido-1,3-bis(2-methoxyethyl)-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-64).

To cool solution of (WLS-64D) (12.5 g, 0.034 mol, 1.0 equiv) in acetonitrile (125 mL) was added sodium azide (3.32 g, 0.051 mol., 1.5 equiv) portion wise over a period of 20 mins under N2 atmosphere. Above reaction mixture was stirred at rt for 4 h. Progress of the reaction was monitored by TLC. Then the mixture was filtered through a celite bed, the bed was washed with acetonitrile (2 x 80 mL). The filtrate was evaporated under vacuum to afford a crude mass. The residue was again dissolved in DCM (25 mL) and then added diethyl ether (150 mL) cool to -60oC and stirred for 40 min. The solid was precipitated which was filtered and washed with ether (2 x 50 mL) and dried under high vacuum to afford a brownish gummy mass(11 g, 86%). 1H NMR (500 MHz, CDCl3): δ in ppm = 3.98 (s, 4H), 3.64 (q, 4H, J = 4.4 Hz), 3.59 (dt, 4H, J = 14.2 Hz, 5.3 Hz), 3.40 (d, 6H, J = 8.3 Hz). MS (ESI) 228.25 (M-PF6) +. 19F NMR (500 MHz, CDCl3): δ in ppm = -72.95 and -74.46. IR (KBr pellet): N3 (2173 cm-1)

5-azido-1-methyl-4-(6-(2,2,2-trifluoroacetamido)hexyl)-3,4-dihydro-2H-pyrrol-1-ium hexa-fluorophosphate(V) (WLS-66)



Synthesis of (WLS-66B):

To a solution of **(WLS-66A)** (50 g, 0.58 mol, 1.0 equiv) in 1,4-dioxane (650 mL, 13 vol.) was added sodium hydride (27.18 g, 0.67 mol., 1.17 equiv) portion-wise at 0°C over a period of 30 min, and further stirred at 65°C for 3 h. Then Iodomethane (63.8 mL, 1.07 mol, 1.8 equiv) was added dropwise over a period of 45 mins at 0°C. The reaction mixture was allowed to rt and stirred for 16 h. Progress of the reaction was monitored by TLC. Above reaction was filtered through a celite bed, which was washed with DCM (2 x 100 mL). Filtrate was concentrated under reduced pressure to afford a crude compound which was purified by column chromatography over silica gel (230-400 mesh) eluted in 2% MeOH/DCM to get **(WLS-66B)** as an off-white solid (18 g, 31%). *^{1}H NMR (500 MHz, $CDCl_3$):* δ in ppm = 5.10 (s, 1H), 3.41 (m, 4H), 2.79 (s, 3H). MS (ESI) 101.01 $(M+1)^+$.

Synthesis of (WLS-66C):

To a stirred solution of **(WLS-66B)** (14 g, 0.1398 mol, 1.0 equiv) in DMF (350 mL, 25 vol.) was added sodium hydride (60%) (8.38 g, 0.2097 mol., 1.5 equiv) portion wise over a period of 30 min under argon atmosphere at 0°C. To the reaction mixture a solution of alkyl bromide (58.71g, 0.2097 mol, 1.5 equiv) in DMF (70 ml, 5 vol.) was added dropwise over a period of 1 h. Then the mixture was allowed to stir for another 3 h. Progress of the reaction was monitored by TLC. Above reaction was diluted with ice water (300 mL) and extracted with ethyl acetate (3 x 400 mL), dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by column chromatography over silica-gel (230-400 mesh) eluted with 2% MeOH/DCM to afford **(WLS-66C)** as a pale yellow oil (16.5 g, 39%).
^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 4.55 (s, 1H), 3.27 (s, 4H), 3.17 (t, 2H, *J* = 7.2 Hz), 3.09 (t, 2H, J = 5.9 Hz), 2.78 (s, 3H), 1.50 (q, 4H, J = 7.3 Hz), 1.44 (s, 9H), 1.32 (m, 4H). MS (ESI) 300.33 $(M+1)^+$.

Synthesis of **(WLS-66D):**

To a stirred solution of **(WLS-66C)** (18 g, 0.06012 mol, 1.0 equiv) in DCM (180 mL, 10 vol.) was added trifluoroacetic acid (23.1 mL, 0.3006 mol., 5.0 equiv) dropwise at 0°C. Above reaction mixture was stirred at rt for 6 h. Progress of the reaction was monitored by TLC. Then the reaction mixture was evaporated under reduced pressure co-distilled with toluene and dried to afford a yellowish gummy mass (20 g, crude) which was used for the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 7.65 (d, 2H, *J* = 36.6 Hz), 3.21 (s, 4H), 3.04 (t, 2H, *J* = 7.1 Hz), 2.77 (m, 2H), 2.63 (s, 3H), 1.52 (m, 2H), 1.42 (m, 2H), 1.28 (m, 4H). MS (ESI) 200.25 $(M+1)^+$.

Synthesis of (WLS-66E)

To a cool stirred solution of **(WLS-66D)** (20 g, 0.06410 mol, 1.0 equiv) in DCM (300 mL) was added triethylamine (26.87 mL, 0.1923 mol., 3.0 equiv) dropwise over a period of 30 mins. Then ethyl trifluoroacetate (11.48 mL, 0.09615 mol., 1.5 equiv) was added dropwise over a period of 15 mins. Above reaction mixture was stirred at rt for 16 h. Progress of the reaction was monitored by TLC. Above reaction was diluted with ice water (100 mL) and extracted with DCM (3 x 100 mL), then dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was purified by column chromatography over basic silica-gel (100-200 mesh) eluted with 90% EA/Hexane to afford **(WLS-66E)** as an off-white solid (9.9 g, 56%, for 2 steps).

^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 7.43 (s, 1H), 3.33 (q, 2H, *J* = 6.5 Hz), 3.29 (t, 4H, *J* = 3.6 Hz), 3.20 (t, 2H, *J* = 6.8 Hz), 2.77 (s, 3H), 1.59 (m, 2H), 1.51 (m, 2H), 1.41 (m, 2H), 1.30 (m, 2H). MS (ESI) 296.3 $(M+1)^+$.

Synthesis of (WLS-66F)

To a cool solution of **(WLS-66E)** (12 g, 0.0405 mol, 1.0 equiv) in toluene (120 mL, 10 vol.) was added oxalyl chloride (52.6 mL, 0.6089 mol., 15 equiv) dropwise over a period of 20 min under argon atmosphere. Above mixture was stirred at 70°C for 72 h. Progress of the reaction was monitored by TLC. Above reaction mixture was concentrated under reduced pressure to afford a crude compound which was treated with diethyl ether (2 x 60 mL), solvent was decant and then was dried under vacuum to afford **(WLS-66F)** as a brown mass (16 g, crude) which was used for the further step without further purification.

^{1}H NMR (500 MHz, $CDCl_3$): δ in ppm = 11.30 (s, 1H), 4.30 (t, 4H, *J* = 6.9 Hz), 3.66 (m, 4H), 3.32 (d, 3H, *J* = 5.5 Hz), 1.80 (m, 4H), 1.42 (m, 4H). MS (ESI) 314.31 $(M+1)^+$.

Synthesis of (WLS-66G)

To a cool stirred solution of **(WLS-66F)** (5 g, 0.0142 mol, 1.0 equiv) in acetonitrile (62.5 mL) was added solid KPH_6 (3.41 g, 0.0185 mol., 1.3 equiv) portion wise over a period of 10 mins. Above reaction mixture was stirred at rt for 4 h. Progress of the reaction was monitored by TLC. Then the mixture was filtered through a celite bed washed with acetonitrile (2 x 20 mL). Filtrate was evaporated under reduced pressure to afford a crude compound. The residue was again dissolved in acetonitrile (5 mL) and then treated with diethyl ether (60 mL) at -78°C, solvent was decant, dried under vacuum to get **(WLS-66G)** as a brownish gummy mass (4 g, crude).

^1H NMR (500 MHz, $CDCl_3$): δ in ppm = 4.13 (s, 4H), 3.62 (m, 4H), 3.26 (s, 3H), 1.64 (m, 4H), 1.41 (d, 4H, J = 15.8 Hz). MS (ESI) 314.26 $(M+1)^+$.

Synthesis of (WLS-66G)

To a cool stirred solution of **(WLS-66G)** (48 g, 0.1044 mol, 1.0 equiv) in acetonitrile (480 mL) was added sodium azide (10.18 g, 0.1566 mol., 1.5 equiv) portion-wise over a period of 20 min. Above reaction mixture was stirred at rt for 4 h. Progress of the reaction was monitored by TLC. Then the mixture was filtered through a celite bed washed with acetonitrile (2 x 100 mL). The filtrate was evaporated under vacuum to afford a crude compound. The residue was dissolved in acetonitrile (25 mL) and then added diethyl ether (200 mL), cool to -78°C. The solid was not precipitate out, solvent was decant and dried under vacuum to yield a brownish gummy liquid (44 g).

^1H NMR (500 MHz, DMSO-D6): δ in ppm = 9.43 (s, 1H), 3.81 (ddd, 4H, *J1* = 23.1 Hz, *J2* = 15.5 Hz, *J3* = 4.5 Hz)), 3.34 (t, 4H, J = 6.9 Hz), 3.13 (s, 3H), 1.51 (m, 4H), 1.28 (s, 4H). MS (ESI) 321.34 $(M+1)^+$.

^{19}F NMR (500 MHz, $CDCl_3$): δ in ppm = -69.325 and -70.837

IR (KBr pellet): N_3 (2169.53 cm^{-1})

Synthesis of (WLS-66A2):

To **(WLS-66A1)** (40 g, 0.3413 mol, 1.0 equiv) was added aqueous HBr (47%) (118 mL, 1.0239 mol., 3.0 equiv) drop-wise over a period of 30 mins at 0°C. Above reaction mixture was stirred at 110°C for 24 h. Progress of the reaction was monitored by TLC. Solvent was evaporated under vacuum to afford a crude compound **(WLS-66A2)** as a pale yellow semi solid (80 g) which was used for the next step without further purification.

^1H NMR (500 MHz, CDCl3)): δ in ppm = 7.90 (s, 2H, *-NH2*), 3.42 (q, 2H, *J* = 7.1 Hz), 3.09

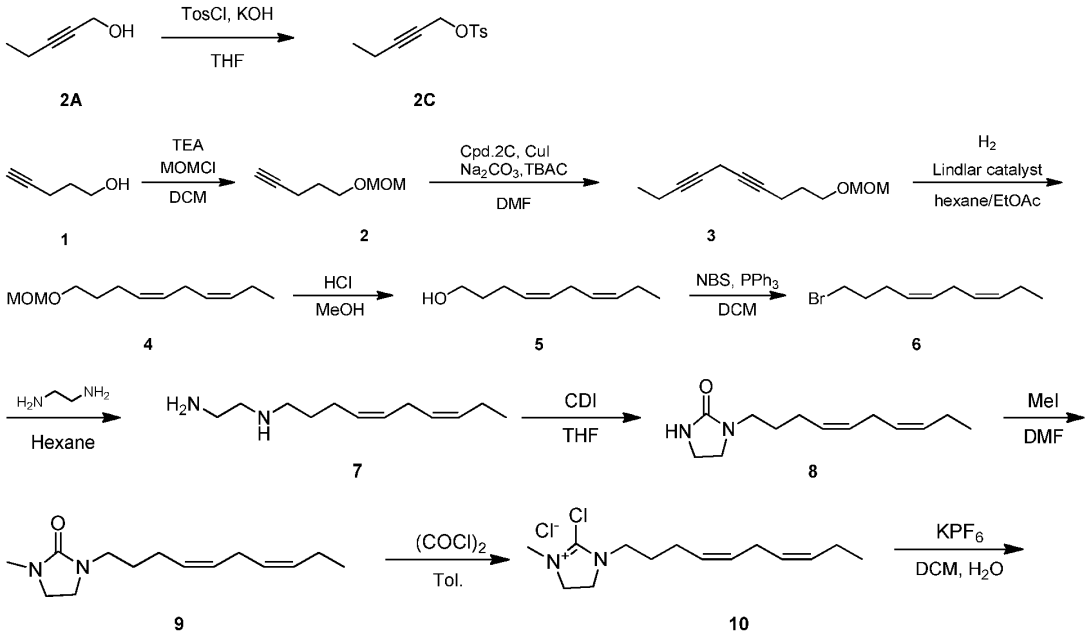
(q, 2H, J = 6.4 Hz), 1.87 (m, 4H), 1.51 (m, 4H). MS (ESI) 180.15 (M, M+2)$^+$.

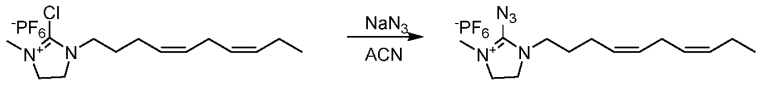
Synthesis of (WLS-66A3):

To a cool stirred solution of **(WLS-66A2)** (80 g, 0.3065 mol, 1.0 equiv) in DCM (800 mL, 10 vol.) was added triethylamine (95.5 mL, 0.6743 mol., 2.2 equiv) dropwise over a period of 20 mins. Then Boc anhydride (187 ml, 0.8582 mol., 2.8 equiv) was added dropwise over a period of 45 min. the mixture was allowed to rt and stirred for 16 h. Progress of the reaction was monitored by TLC. The mixture was diluted with DCM (500 mL) and washed with water (4 x 200 mL). The organic layer was dried over Na_2SO_4 and evaporated under vacuum to afford a crude compound. The residue was purified by column chromatography over silica gel (230-400 mesh) eluted with 8% EA/Hexane to give **(WLS-66A3)** as a pale-yellow syrup (57 g, 59%, for 2 steps).

^{1}H NMR (400 MHz, CDCl3): δ in ppm = 4.55 (s, 1H, *-NH*), 3.40 (t, 2H, J = 6.8 Hz), 3.11 (q, 2H, J = 6.4 Hz), 1.83 (m, 2H), 1.49 (m, 13H), 1.34 (m, 2H). MS (ESI) 280.24 (M+)$^+$.

Synthesis of 2-azido-1-((4Z,7Z)-deca-4,7-dien-1-yl)-3-methyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WV-RA-016A)





WV-RA-016 WV-RA-016A

Preparation of compound 2C

To a solution of compound **2A** (76 g, 903.51 mmol) in THF (1500 mL) was added TosCl (206.70 g, 1.08 mol) and KOH (76.04 g, 1.36 mol). The mixture was stirred at 0 °C for 4 hr. TLC indicated compound **2A** was consumed completely and one new spot formed. The reaction mixture was filtered off the insoluble matter, and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 2/1). Compound **2C** (210 g, 97.53% yield) was obtained as a yellow oil.

TLC: Petroleum ether: Ethyl acetate = 5:1, Rf = 0.4

Preparation of compound 2

To a solution of compound **1** (72.8 g, 865.47 mmol) in DCM (800 mL) was added DIEA (257.27 g, 1.99 mol) at 0 °C, followed by dropwise addition of MOMCl (143.89 g, 1.79 mol). The mixture was stirred at 0 °C for 2 hr under N_2. TLC indicated compound **1** was consumed and one new spot formed. Saturated NH_4Cl solution (1000 mL) was added, the layers were separated and the aqueous mixture was further extracted with DCM (2 * 500 mL). The combined organic fractions were dried (Na_2SO_4) and the solvent was removed in vacuo. Compound **2** (70 g, 63.11% yield) was obtained as a colorless oil. **^{1}HNMR** (400 MHz, CHLOROFORM-d) δ = 4.61 (s, 2H), 3.61 (t, *J* = 6.2 Hz, 2H), 3.35 (s, 3H), 2.30 (dt, *J* = 2.7, 7.0 Hz, 2H), 1.94 (t, *J* = 2.6 Hz, 1H), 1.80 (quin, *J* = 6.6 Hz, 2H)

TLC: Petroleum ether: Ethyl acetate = 2:1, Rf = 0.6

Preparation of compound 3

Tetrabutylammonium;chloride (33.83 g, 121.71 mmol), disodium;carbonate (64.50 g, 608.57 mmol) and iodocopper (77.27 g, 405.72 mmol) each finely ground and anhydrous, were suspended in dry DMF (1000 mL) at 0 °C with stirring. Subsequently, compound **2** (52 g, 405.72 mmol) was added all at once and kept stirring for 20 min. Compound **2C** (116.02 g, 486.86 mmol) was added dropwise and the suspension was stirred at 40

°C under N_2 for 12 h. TLC indicated compound **2** was consumed and one main new spot formed. The reaction mixture was diluted with sat.NH_4Cl 500 mL, H_2O 500 mL and extracted with ethyl acetate (500 mL * 3). The combined organic layers were washed with sat.brine 500*2 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 5/1). Compound **3** (24 g, 30.45% yield) was obtained as a yellow oil.

^{1}HNMR (400 MHz, CHLOROFORM-d) δ = 4.62 (s, 2H), 3.60 (t, J = 6.3 Hz, 2H), 3.36 (s, 3H), 3.11 (quin, J = 2.3 Hz, 2H), 2.28 (tt, J = 2.3, 7.0 Hz, 2H), 2.17 (tq, J = 2.3, 7.5 Hz, 2H), 1.77 (quin, J = 6.7 Hz, 2H), 1.11 (t, J = 7.5 Hz, 3H). **TLC**: Petroleum ether: Ethyl acetate = 5:1, Rf = 0.8

Preparation of compound 3

To a solution of compound **3** (11 g, 56.62 mmol) in the mixture solvent of hexane (90 mL) and EtOAc (30 mL) was added quinoline (146.27 mg, 1.13 mmol) and LINDLAR CATALYST (11.69 g, 5.66 mmol, 10% purity) under H_2 atmosphere (15 psi). The mixture was stirred at 15 °C for 12 hr. TLC indicated compound **3** was consumed completely and two new spots formed. The reaction mixtures of two batches were filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 10/1). Compound **4** (17 g, 75.70% yield) was obtained as a colorless oil.

^{1}HNMR (400 MHz, CHLOROFORM-d) δ = 5.57 - 5.17 (m, 4H), 4.69 - 4.58 (m, 2H), 3.56 - 3.50 (m, 2H), 3.38 - 3.36 (m, 3H), 2.86 - 2.65 (m, 2H), 2.22 - 2.05 (m, 4H), 1.74 - 1.60 (m, 2H), 1.02 - 0.92 (m, 3H). **TLC**: Petroleum ether: Ethyl acetate = 5:1, Rf = 0.8

Preparation of compound 5

HCl (6 M, 142.88 mL) was added to a stirred solution of compound **4** (17 g, 85.73 mmol) in MeOH (150 mL). The mixture was stirred at 70 °C for 2 h. TLC indicated compound **4** was consumed completely and one new spot formed. The reaction mixture was quenched with 1M NaOH to pH~7, and then was extracted with EtOAc (3 × 200 mL) and the combined organic layers were washed with brine (200 mL), dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, Petroleum

ether/Ethyl acetate = 1/0 to 1/1). Compound **5** (9 g, 68.06% yield) was obtained as a yellow liquid.

TLC: Petroleum ether: Ethyl acetate = 5:1, Rf = 0.3

Preparation of compound 6

NBS (20.77 g, 116.69 mmol) was added portionwise to an ice-cooled solution of PPh_3 (30.61 g, 116.69 mmol) in DCM (300 mL) under N_2. The mixture was stirred at 0 °C for 15 min and then a solution of compound **5** (9 g, 58.35 mmol) in DCM (50 mL) was slowly added. The mixture was stirred in an ice bath for 2 h and for 3h more at 15 °C. TLC (Petroleum ether: Ethyl acetate = 5:1, Rf = 0.9) indicated compound **5** was consumed completely and one new spot formed. The reaction mixture was quenched with H_2O (100 mL) and extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were concentrated under vacuum. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 3/1). Compound **6** (10 g, 46.05 mmol, 78.93% yield) was obtained as a colorless liquid.

^{1}HNMR (400 MHz, CHLOROFORM-d) δ = 5.58 - 5.21 (m, 4H), 3.47 - 3.37 (m, 2H), 2.88 - 2.68 (m, 2H), 2.23 (td, *J* = 7.4, 14.9 Hz, 2H), 2.13 - 2.06 (m, 2H), 1.98 - 1.89 (m, 2H), 1.04 - 0.92 (m, 3H)

TLC: Petroleum ether: Ethyl acetate = 5:1, Rf = 0.9

Preparation of compound 7

To a solution of compound **6** (9.5 g, 43.75 mmol) in Hexane (50 mL) was added ethane-1,2-diamine (78.38 g, 1.30 mol) at 0 °C. The mixture was stirred at 0-15 °C for 5 hr. TLC indicated compound 6 was consumed completely and one new spot formed. The mixture was concentrated under reduced pressure. Compound **7** (8.59 g, crude) was obtained as a colorless oil.

TLC (Petroleum ether: Ethyl acetate = 5:1, Rf = 0)

Preparation of compound 8

To a solution of compound **7** (8.59 g, 43.75 mmol) and CDI (7.09 g, 43.75 mmol) in THF (90 mL) was stirred at 15 °C for 12 hr. TLC indicated compound **7** was consumed completely and one new spot formed. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 1/0) to get 4.4 g crude. Then the crude was purified by reversed-phase HPLC (column: Welch Xtimate C18 250*70mm#10um; mobile phase: [water (10mM NH_4HCO_3)-ACN];B%: 45%-65%,20min). Compound **8** (2.5 g, 25.70% yield) was obtained as a yellow oil.

^{1}HNMR (400 MHz, CHLOROFORM-d) δ = 5.53 - 5.18 (m, 4H), 3.41 (s, 4H), 3.25 - 3.13 (m, 2H), 2.82 - 2.62 (m, 2H), 2.14 - 2.05 (m, 3H), 2.02 (br d, *J* = 3.6 Hz, 1H), 1.65 - 1.50 (m, 2H), 1.02 - 0.89 (m, 3H). **TLC**: Petroleum ether: Ethyl acetate = 0:1, Rf = 0.25

Preparation of compound 9



8 **9**

To a solution of compound **8** (2.1 g, 9.45 mmol) in DMF (20 mL) was added NaH (1.13 g, 28.34 mmol, 60% purity) at 0 °C and the reaction stirred for 0.5 h, then added MeI (6.70 g, 47.23 mmol) to the above reaction mixture, and stirred at 15 °C for 2 h. TLC indicated compound 8 was consumed and one new spot formed. The reaction mixture was quenched by addition H_2O (50 mL) at 15 °C, and extracted with ethyl acetate (30 mL * 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 0/1). Compound **9** (2.23 g, 9.44 mmol, 100.00% yield) was obtained as a colorless oil.

^{1}HNMR (400 MHz, CHLOROFORM-d) δ = 5.61 - 5.13 (m, 4H), 3.31 - 3.25 (m, 4H), 3.23 - 3.15 (m, 2H), 2.82 - 2.70 (m, 5H), 2.15 - 1.98 (m, 4H), 1.63 - 1.50 (m, 2H), 1.02 - 0.92 (m, 3H)

TLC: Petroleum ether: Ethyl acetate = 0:1, Rf = 0.4

Preparation of compound 10

A mixture of compound **9** (2 g, 8.46 mmol) in Tol. (20 mL) was degassed and purged with N_2 for 3 times, and then to the mixture was added $(COCl)_2$ (10.74 g, 84.62 mmol) and stirred at 65 °C for 24 hr under N_2 atmosphere. TLC showed the reaction was completed, staring material was consumed, desired product was obtained. LCMS showed the desired mass was detected. Then the mixture was concentrated in vacuo. Compound **10** (2.46 g, crude, Cl) was obtained as a black brown oil.

LCMS ($M + H^+$): 255.2. **TLC**: Petroleum ether: Ethyl acetate = 0:1, R_f = 0

Preparation of compound WV-RA-016

To a solution of compound **10** (2.4 g, 8.24 mmol, Cl) in CAN (30 mL) was added potassium;hexafluorophosphate (1.52 g, 8.24 mmol). The mixture was stirred at 15 °C for 2 hr. A large number of solids are precipitated form the reaction mixture. The reaction mixture was filtered, and the filter cake was washed with DCM (30mL * 2), the organic layer was concentrated. The crude was diluted with EtOAc 20 mL and extracted with H_2O (10 mL * 3). The organic layers were concentrated under reduced pressure to give a residue. Compound **WV-RA-016** (3.3 g, 97.70% yield, PF6) was obtained as a brown solid.

^{1}HNMR (400 MHz, DMSO-d_6) δ = 5.59 - 5.14 (m, 4H), 3.23 - 3.18 (m, 4H), 3.08 - 2.98 (m, 2H), 2.79 - 2.66 (m, 2H), 2.62 (s, 3H), 2.10 - 1.98 (m, 4H), 1.52 - 1.40 (m, 2H), 0.96 - 0.87 (m, 3H)

^{19}F NMR (376 MHz, DMSO-d_6) δ = -69.19 (s, 1F), -71.08 (s, 1F)

^{31}P NMR (162 MHz, DMSO-d_6) δ = -135.42 (s, 1P), -139.81 (s, 1P), -144.19 (s, 1P), -148.59 (s, 1P), -152.98 (s, 1P). **LCMS** ($M + H^+$): 255.2, LCMS purity: 97.77% purity

Preparation of compound WV-RA-016 A

To a solution of ***WV-RA-016*** (100 mg, 249.52 umol PF6) in ACN (3 mL) was added NaN_3 (20 mg, 307.65 umol) .The mixture was stirred at 0 °C for 30 min. LCMS showed the de-N_2 mass was detected. The mixture was filtered through a celite pad and the filtrate was

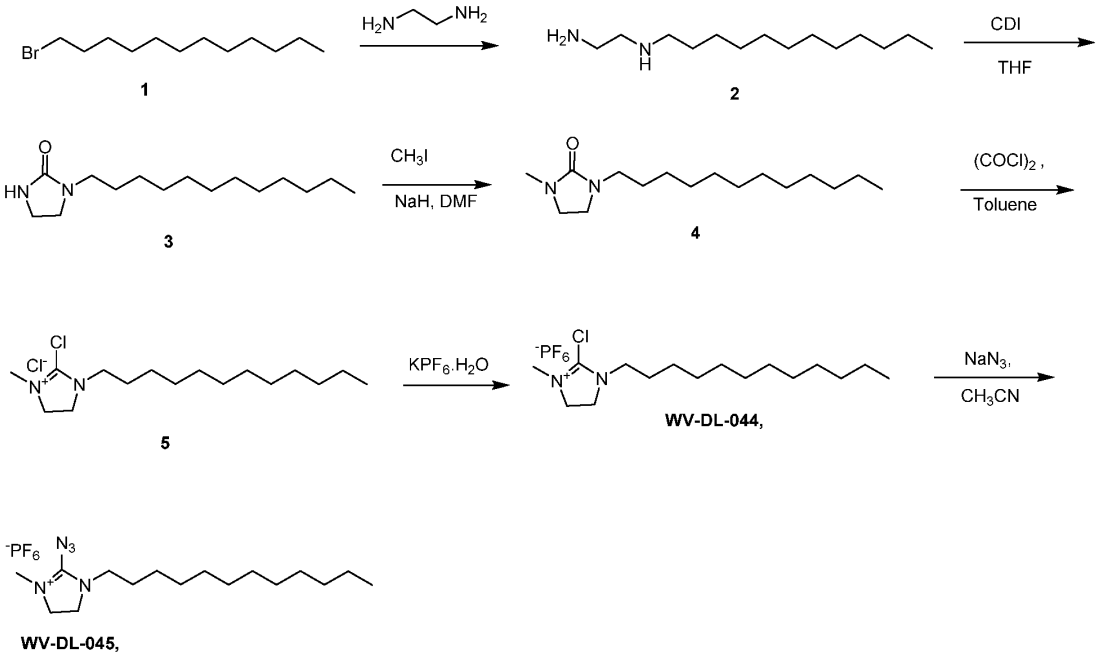
concentrated in vacuo. The residue was dissolved in 2 mL CH_3CN and the solution was poured into ether to form the precipitate, filtered, the solid was desired and the organic phase was adjusted with 2 M NaOH to pH~13, then quenched by addition NaClO (aq.) 20 mL. Compound ***WV-RA-016A*** (80 mg, crude, PF6) was obtained as a brown oil.

^{1}HNMR (400 MHz, DMSO-d_6) δ = 5.57 - 5.06 (m, 3H), 3.80 - 3.48 (m, 4H), 3.39 - 3.30 (m, 3H), 3.27 - 3.15 (m, 2H), 2.87 - 2.72 (m, 3H), 2.12 - 1.90 (m, 4H), 1.64 - 1.37 (m, 2H), 1.00 - 0.83 (m, 4H)

^{19}FNMR (376 MHz, DMSO-d_6) δ = -69.22 (s, 1F), -71.11 (s, 1F)

^{31}PNMR (162 MHz, DMSO-d_6) δ = -135.42 (s, 1P), -139.81 (s, 1P), -144.19 (s, 1P), -148.59 (s, 1P), -152.98 (s, 1P). **LCMS** (M –N_2): 234.3

Synthesis of 2-azido-1-dodecyl-3-methyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate (WV-DL-045)



1. Preparation of compound 2

In a one-neck round bottom flask, ethane-1,2-diamine (337.59 g, 5.62 mol) was placed with a magnetic stirring bar, and compound **1** (50 g, 200.62 mmol) was added slowly at 0 °C. After finishing the addition, the reaction mixture was warmed to 25 °C, and left undisturbed for an additional 1h. 300 mL of hexane was added into the reaction mixture, which was stirred vigorously for 12 h at 25 °C. LCMS showed the reaction was completed, staring material was consumed and the product was obtained, the hexane layer was decanted and dried under reduced pressure to give compound **2** (123 g) crude as colorless oil.
LCMS: ($M+H^+$) 229.2

Preparation of compound 3

Two batches in parallel. To a solution of compound **2** (61.5 g, 269.25 mmol) and CDI (43.66 g, 269.25 mmol) in THF (630 mL) was stirred at 15 °C for 12 hr. TLC showed the reaction was completed, starting material was consumed and the product was obtained. The crude reaction mixture (126 g scale) was combined to another two batch crude product (123 g scale) and (84 g scale) for further purification. The combined crude product was purified by column chromatography on a silica gel eluted with petroleum ether: ethyl acetate (from 10/1 to 1/12) to give product **3** (95 g, 65.09% yield) as a white solid.
TLC (Ethyl acetate : Methanol = 10: 1) R_{f1} = 0.50

Preparation of compound 4

Six batches in parallel. To a solution of compound **3** (40 g, 157.23 mmol) in DMF (650 mL) was added NaH (7.55 g, 188.67 mmol, 60% purity) at 0 °C and the reaction stirred for 0.5 h, Then added CH_3I (66.95 g, 471.68 mmol) to the above reaction mixture, and stirred at 25 °C for 3 h. TLC showed the reaction was completed, starting material was consumed and the product was obtained. The reaction mixture was quenched by addition H_2O (1000 mL) at 25 °C, and extracted with Ethyl acetate (1000 mL * 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 20/1 to 1/2) to give product **4** (232 g, crude) as yellow oil.

^{1}H NMR (400 MHz, CHLOROFORM-d) δ = 3.25 - 3.17 (m, 4H), 3.09 (t, *J* = 7.3 Hz, 2H), 2.70 (d, *J* = 1.6 Hz, 3H), 1.45 - 1.36 (m, 2H), 1.28 - 1.14 (m, 19H), 0.85 - 0.76 (m, 3H)
TLC (Petroleum ether : Ethyl acetate = 0: 1) R_{f1} = 0.5

Preparation of compound 5

A mixture of compound **4** (30 g, 111.76 mmol, 1 *eq.*) in Tol.(250 mL) was degassed and purged with N_2 for 3 times, and then to the mixture was added oxalyl chloride (212.78 g, 1.68 mol, 146.75 mL, 15 *eq.*) and stirred at 65 °C for 72 hr under N_2 atmosphere. LCMS showed the reaction was completed, staring material was consumed, the desired product was obtained. Then the mixture was concentrated in vacuo. The white solid was washed by cooled EtOAc (100 mL*2), and then the solid was concentrated in vacuo, to give product **5** (20 g, crude) as a white solid.
LCMS: M^+, 287.3

Preparation of compound WV-DL-044

To a solution of compound **5** (8 g, 24.74 mmol) in DCM (46 mL) and H_2O (26 mL) was added potassium hexafluorophosphate (4.55 g, 24.74 mmol) at 25 °C. The reaction mixture was stirred at 25 °C for 1 h. TLC showed the reaction was completed, starting material was consumed, and the desired product was obtained. The filtrate was washed with H_2O (10 mL * 2), and the white solid was desired compound. To give product **WV-DL-044** (6.5 g, 60.69% yield, F6P) as a white solid. The product was combined with another two batches product (2.5 g), and (2.55 g) for analysis and delivery. Finally, 11.5 g of product was got
TLC (Petroleum ether : Ethyl acetate = 0: 1) R_f = 0.0

Preparation of Lipid Azide WV-DL-045

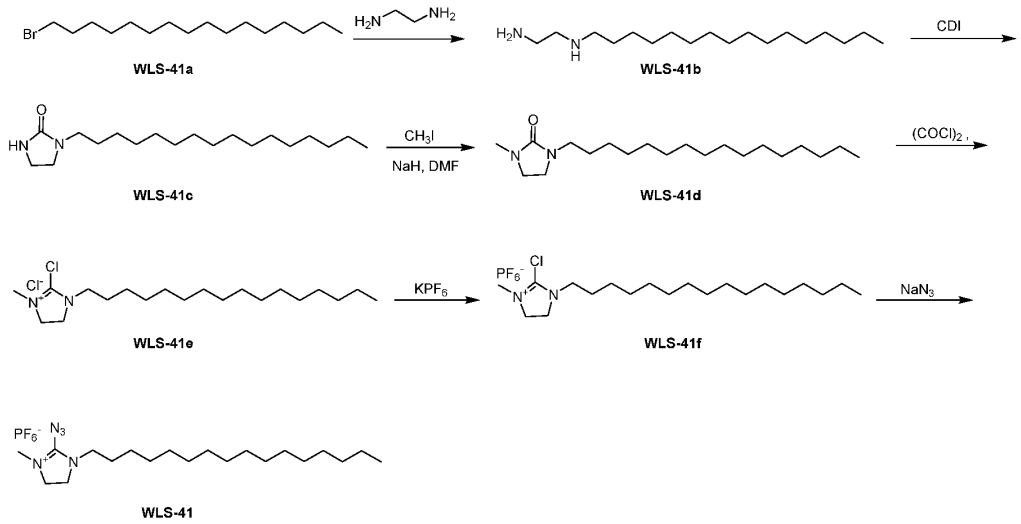
2.2g WV-DL-044 and 495mg NaN_3 were added to a round bottom flask. Dry ACN was added forming a suspension and stirred 2.5hr at room temperature. The reaction mixture

was filtered through a pad of celite and washed with CAN. The filtrate was dried on rotovap and was then redissolved in a minimal amount ACN and the solution was precipitated with diethyl ether to afford 1.75g of fluffy white solid

^{1}H NMR (600 MHz, Chloroform-*d*) δ 3.87 (dd, *J* = 12.1, 8.1 Hz, 1H), 3.81 – 3.75 (m, 1H), 3.29 (t, *J* = 7.8 Hz, 1H), 3.12 (s, 2H), 1.57 – 1.50 (m, 1H), 1.22 (s, 3H), 1.19 (s, 6H), 0.84 – 0.78 (m, 2H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 154.76, 77.29, 77.07, 76.86, 49.38, 47.03, 46.52, 33.13, 31.90, 29.61, 29.61, 29.54, 29.42, 29.34, 29.05, 26.97, 26.47, 22.68, 14.11.

Synthesis of 2-azido-1-hexadecyl-3-methyl-4,5-dihydro-1H-imidazol-3-ium hexafluorophosphate(V) (WLS-41)



Preparation of compound WLS-41b

In a clean and dry two-neck 1 Lit round bottom flask, ethane-1,2-diamine (306 mL, 4.585 mol, 28.0 equiv) was placed with a magnetic stirring bar, and compound **WLS-41a** (50 g, 0.164 mol,1.0 equiv) was added dropwise at 0 °C by using addition funnel. After finishing the addition, the reaction mixture was warmed to 25 °C, and left undisturbed for an additional 1 h. Then, 300 mL of hexane was added into the reaction mixture and stirred vigorously for 16 h at 25 °C. TLC showed the reaction was completed, staring material was consumed and the new spot was formed (TLC - 10% MeOH:EtOAc; TLC charring –

Phosphomolybdic acid). The hexane layer was separated by using separatory funnel. Again 300 mL of hexane was added to amine layer and stir for 4 h at rt. After that hexane layer was separated and combined with previous hexane layer, dried over sodium sulphate and evaporated to dryness under reduced pressure to get compound **WLS-41b** (48 g) as a crude colorless liquid.

MS: m/z calcd for $C_{18}H_{40}N_2$ ($[M+H]^+$), 285.53; found 285.38.

Preparation of compound WLS-41c

WLS-41b (48.0 g, 0.169 mol, 1.0 equiv) was taken in clean and dry 1 Lit two neck RBF under argon atmosphere. Then add 491 mL of THF to RBF. Cool the RB in ice bath (0 ℃). Add portion wise 1,1'-Carbonyldiimidazole (28.17 g, 0.174 mol, 1.03) to RM for period of 10 min. The reaction mixture was stir at 15 ℃ for 12 h. TLC showed the reaction was completed, staring material was consumed and the product was formed (TLC - 10% MeOH:EtOAc; TLC charring – Phosphomolybdic acid). After completion of reaction, solvent was dried and purified on silica gel column chromatography (100-200 mesh). The product was eluted with 50% ethyl acetate: hexane. Fraction containing product was evaporated to get 37.1 g (71% yield) of **WLS-41c** as a white solid.

^1H NMR (400 MHz, $CDCl_3$): δ in ppm = 4.33 (s, 1H), 3.40-3.43 (m, 4H), 3.17 (t, 2H, *J* = 7.4 Hz), 1.50 (t, 2H, *J* = 7.0 Hz), 1.25-1.30 (m, 28H), 0.88 (d, 3H, *J* = 13.6 Hz).

MS: m/z calcd for $C_{19}H_{38}N_2O$ ($[M+H]^+$), 311.53; found 311.42.

Preparation of compound WLS-41d

WLS-41c (29.0 g, 0.093 mol, 1.0 equiv) was taken in clean and dry 1 Lit two neck RBF under argon atmosphere. Then add 471 mL of dry DMF to RBF containing SM. Cool the RB in ice bath (Temp. 0℃). Then, add portion wise 60% NaH (4.48 g, 0.112 mol, 1.20 equiv) to RM for period of 15 min. at 0℃ and stir 30 min at same temp. Then add dropwise methyl iodide (17.4 mL, 0.281 mol, 3.0 equiv) to the reaction mixture at 0 ℃ for duration of 15 min. Then allow the RM to rt and stir for 3 h. TLC showed the reaction was completed, staring material was consumed and the new spot was formed (TLC - EtOAc; TLC charring – Phosphomolybdic acid). After completion of reaction, reaction mixture was cool to 0℃ in ice bath and quenched with ice cold water (1 Lit). Then extracted with ethyl acetate (3 x

1000 mL). The organic layer was dried over sodium sulfate, filtered and concentrated to dryness. The crude product was purified by silica gel column chromatography (100-200 mesh). The product was eluted with 25%-35% ethyl acetate:hexane. The fraction containing product was evaporated to get 29.0 g (96% yield) of **WLS-41d** as a white colour solid. *^1H NMR (500 MHz, $CDCl_3$):* δ in ppm = 3.27 (s, 4H), 3.16 (t, 2H, *J* = 7.6 Hz), 2.78 (s, 3H), 1.48 (t, 2H, *J* = 7.2 Hz), 1.29 (s, 7H), 1.25 (s, 22H), 0.88 (t, 3H, *J* = 6.9 Hz).

MS: m/z calcd for $C_{20}H_{40}N_2O$ ($[M+H]^+$), 325.55; found 325.41

Preparation of compound WLS-41e

WLS-41d (30.0 g, 0.092 mol, 1.0 equiv) was taken in clean and dry 1 Lit two neck RBF under argon atmosphere. Then add 249 mL of dry toluene to RBF containing SM under argon atmosphere. After that add dropwise oxalyl chloride (118.9 mL, 1.386 mol, 15.0) using addition funnel for a period of 30 min at rt. Then reaction mixture was heated to 65 °C for 72 hrs. After completion of reaction (TLC – ethyl acetate; TLC charring – Phosphomolybdic acid) solvent was evaporated to dryness to get crude compound. The crude compound was washed with cold ethyl acetate (2 x 100 mL) and dried to get 33.0 g of crude **WLS-41e** as brown colour solid.

MS: m/z calcd for $C_{20}H_{40}Cl_2N_2O$ ($[M-Cl]^+$), 344.00; found 343.30.

Preparation of compound WLS-41f

WLS-41e (20.0 g, 0.053 mol, 1.0 equiv) was taken in clean and dry 500 mL single neck RBF and dissolved in 115 mL DCM under argon atmosphere. Then added aq solution of KPF_6 (9.70 g, 0.053 mol, 1.0 equiv,in 65 mL of water). Stir the reaction mixture at rt for 1 h. After completion of reaction (TLC – 5% MeOH:DCM; TLC charring – Phosphomolybdic acid), the reaction mixture was poured into ice water, and extracted with DCM (2 x 400 mL). The combined organic layer washed with water (400 mL) and dried over sodium sulphate, filtered and evaporated to dryness. Then, residue was dissolved in DCM (70 mL) and product was precipitate by dropwise addition of diethyl ether (500 mL) under stirring. The solvent was decant and solid was dried under high vacuum to get 18.0 g (70% yield) of **WLS-41f** as a white solid. *MS:* m/z calcd for $C_{20}H_{40}ClF_6N_2P$ ($[M-PF_6]^+$), 344.00; found 343.34.

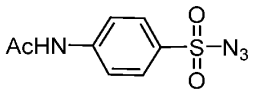
Preparation of compound WLS-41

WLS-41f (18.0 g, 0.037 mol, 1.0 equiv) was taken in clean and dry 500 mL single neck RBF and dissolved in 90 mL of Dry MeCN under argon atmosphere. Then, added sodium azide (3.58 g, 0.055 mol, 1.5 equiv) to the RM and stir at rt for 2.5 h. After completion of reaction (TLC – ethyl acetate; TLC charring – ninhydrin), reaction mixture was filtered through a pad of celite and washed with MeCN (20 mL). The organic layer was evaporated to dryness. The crude compound was dissolve in MeCN (70 mL) and precipitate by adding dropwise diethylether (500 mL). Solvent was decanted and solid was dried under high vacuum to get 14.1 g (77% yield) of **WLS-41** as a white solid.

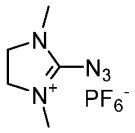
^{1}H NMR (400 MHz, $CDCl_3$): δ in ppm = 3.94-4.00 (m, 2H), 3.85-3.90 (m, 2H), 3.41 (t, 2H, *J* = 7.6 Hz), 3.21 (s, 3H), 1.62 (t, 2H, *J* = 7.1 Hz), 1.26 (s, 27H), 0.88 (t, 3H, *J* = 6.8 Hz).

^{19}F NMR (400 MHz, $CDCl_3$): δ in ppm = -73.35 and -75.24. *MS:* m/z calcd for $C_{20}H_{40}F_6N_5P$ ($[M\text{-}PF_6]^+$), 350.57; found 350.40. IR (KBr pellet): N_3 (2179 cm^{-1})

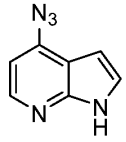
The following azides were purchased commercially:



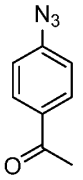
4-acetamidobenzenesulfonyl azide ;



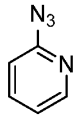
2-azido-1,3-dimethyl-4,5-dihydro-1*H*-imidazolnium hexafluorophosphate ;



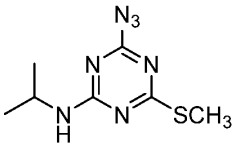
4-azido-1*H*-pyrrolo[2,3-*b*]pyridine ;



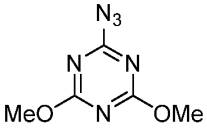
1-(4-azidophenyl)ethan-1-one ;



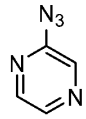
2-azidopyridine ;



4-azido-*N*-isopropyl-6-(methylthio)-1,3,5-triazin-2-amine ;



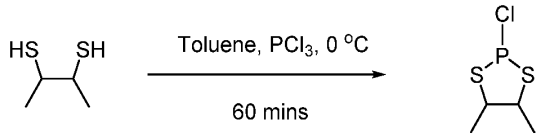
2-azido-4,6-dimethoxy-1,3,5-triazine ;



2-azidopyrazine .

EXAMPLE 14. **Synthesis and Experimental Procedures for <u>Morpholine sugar modified PN chemistry</u>:**

General experimental procedure (A) for chloro reagent (2)



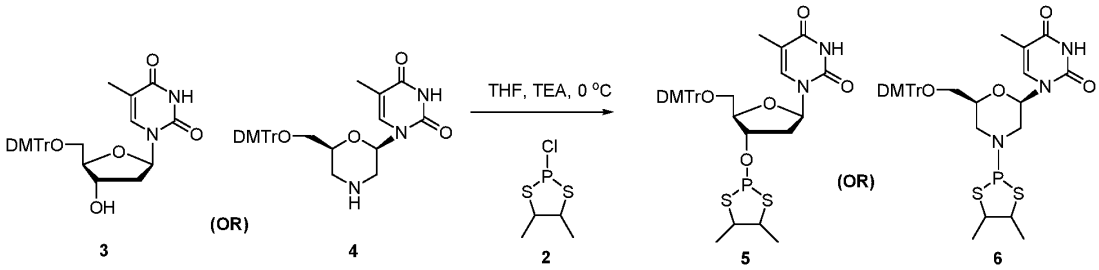
1 **2**

Dithiol (360 mmol) was dissolved in toluene (720 mL) under argon (3000 mL single neck flask) then 4-methylmorpholine (35.4 mL, 792 mmol) was added. This mixture was added dropwise via cannula over 30 min to an ice-cold solution of phosphorus trichloride (720 mL, 396 mmol) in toluene (720 mL) under argon atmosphere. After

warming to room temperature for 1 h, the mixture was filtered carefully under vacuum/argon. The resulting filtrate was concentrated by rotary evaporation (flushing with Ar) then dried under high vacuum for 2 h. The resulting crude compound was isolated as thick oil, which was dissolved in THF to obtain a 1 M stock solution and this solution was used in the next step without further purification.

Data for 2: Synthesized from compound **1**, by following the general procedure **A.** ^{31}P NMR (243 MHz, THF-$CDCl_3$, 1:2) δ 168.77, 161.4

General experimental procedure (B) for monomers (5 and 6)



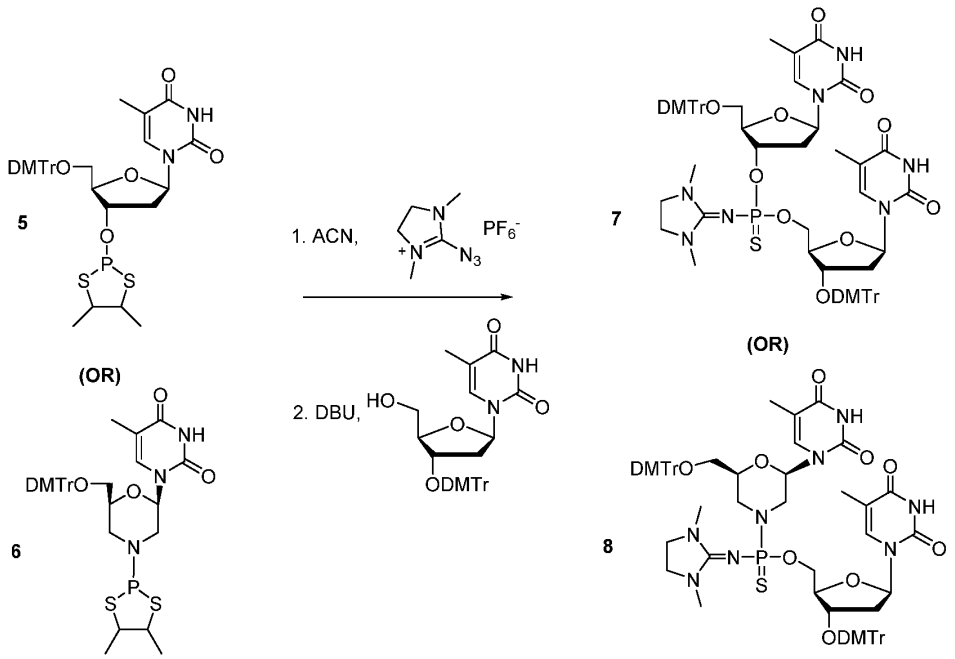
The 5'-ODMTr protected nucleoside **3** or **4** (6.9 mmol) was dried in a three neck 250 mL round bottom flask by co-evaporating with anhydrous toluene (50 mL) followed by under high vacuum for 18 h. The dried nucleoside was dissolved in dry THF (35 mL) under argon atmosphere. Then, triethylamine (24.4 mmol, 3.5 equiv.) was added into the reaction mixture, then cooled to ~ - 10 °C. A THF solution of the crude chloro reagent (1 M solution, 2.5 equiv., 17.4 mmol) was added to the above mixture through cannula over ~5 min, then, gradually warmed to room temperature over about 1 h. LCMS showed that the starting material was consumed. The reaction mixture was filtered carefully under vacuum/argon and the resulting filtrate was concentrated under reduced pressure to give a yellow foam which was further dried under high vacuum overnight. Crude mixture was purified by silica gel column [Column was pre-deactivated using acetonitrile then ethyl acetate (5% TEA) and then equilibrated using ethyl acetate-hexanes] chromatography using ethyl acetate and hexane as eluents.

Stereorandom (Rp/Sp) monomer 5: Yield 86%. Reaction was carried out using nucleoside **3** and chloro reagent **2** by following the general procedure **B**. ^{31}P NMR (243 MHz, $CDCl_3$) δ 171.62, 155.50, 146.84, 146.17; MS (ES) *m/z* calculated for $C_{35}H_{39}N_2O_7PS_2$ $[M+K]^+$ 733.16, Observed: 733.40 $[M + K]^+$.

Stereorandom (Rp/Sp) monomer 6: Yield 73%. Reaction was carried out using nucleoside **4** and chloro reagent **2** by following the general procedure **B**. ^{31}P NMR (243 MHz, $CDCl_3$) δ 121.87, 106.20, 93.58, 92.99; MS (ES) *m/z* calculated for

$C_{35}H_{40}N_3O_6PS_2$ $[M+K]^+$ 773.28, Observed: 773.70 $[M + K]^+$.

General experimental procedure (C) for PS-PN dimers (7 and 8):

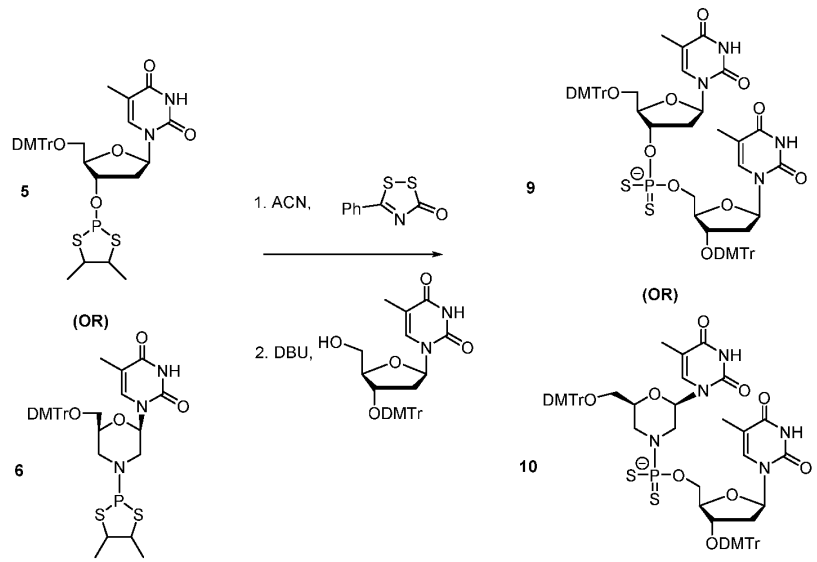


To a stirred solution of monomer **5** or **6** (0.10 mmol, 2 equiv., pre-dried by co-evaporation with dry acetonitrile and kept it under vacuum for minimum 12 h) in dry acetonitrile (0.5 mL) was added a solution of 2-azido-1,3-dimethylimidazolinium hexafluorophosphate (0.11 mmol, 2.25 equiv.) in acetonitrile (0.2 mL) under argon atmosphere at room temperature. Resulting reaction mixture was stirred for 10 mins then DMTr protected alcohol (0.05 mmol, pre-dried by co-evaporation with dry acetonitrile and kept it under vacuum for minimum 12 h) in dry acetonitrile (0.25 mL) and 1,8-Diazabicyclo [5.4.0] undec-7-ene (0.23 mmol, 5 equ, 0.23 ml of 1 M solution in dry acetonitrile) are added. The reaction was monitored and analyzed by LCMS. Approximate reaction completion time 10-20 mins.

Stereorandom dimer 7: Reaction was carried out using **5** by following the general procedure **C**. MS (ES) *m/z* calculated for $C_{67}H_{72}N_7O_{14}PS$ $[M+K]^+$ 1300.42, Observed: 1300.70 $[M + K]^+$.

Stereopure (Rp) dimer 8: Reaction was carried out using **6** by following the general procedure **C**. MS (ES) *m/z* calculated for $C_{67}H_{73}N_8O_{13}PS$ $[M+K]^+$ 1299.44, Observed: 1299.65 $[M + K]^+$.

General experimental procedure (D) for PS-PS dimers (9 and 10):

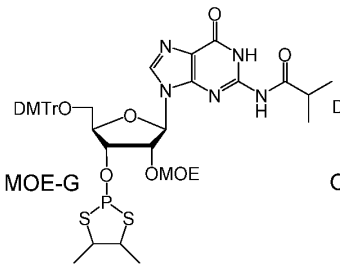


To a stirred solution of monomer **5** or **6** (0.10 mmol, 2 equiv., pre-dried by co-evaporation with dry acetonitrile and kept it under vacuum for minimum 12 h) in dry acetonitrile (0.5 mL) was added a solution of 5-phenyl-3H-1,2,4-dithiazol-3-one (0.12 mmol, 2.5 equiv., 0.2 M) in acetonitrile under argon atmosphere at room temperature. Resulting reaction mixture was stirred for 10 mins then DMTr protected alcohol (0.05 mmol, 1 equ, pre-dried by co-evaporation with dry acetonitrile and kept it under vacuum for minimum 12 h) in dry acetonitrile (0.2 mL) and 1,8-Diazabicyclo [5.4.0] undec-7-ene (0.23 mmol, 5 equ, 1 M solution in dry acetonitrile) are added. Once the reaction was completed (monitored by LCMS) then the reaction mixture was analyzed by LCMS.

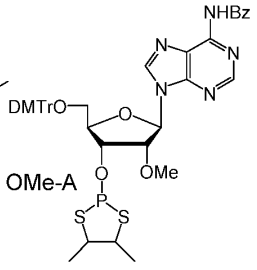
Dimer 9: Reaction was carried out using monomer **5** by following the general procedure **D**. Reaction completion time about 30 mins. MS (ES) *m/z* calculated for $C_{62}H_{62}N_4O_{14}PS_2$ $[M]^-$ 1181.34, Observed: 1181.66 $[M]^-$.

Dimer 10: Reaction was carried out using monomer **6** by following the general procedure **D**. Reaction completion time about 20 h. MS (ES) *m/z* calculated for $C_{62}H_{63}N_5O_{13}PS_2$ $[M]^-$ 1180.36, Observed: 1180.71 $[M]^-$.

Additional useful compounds



451



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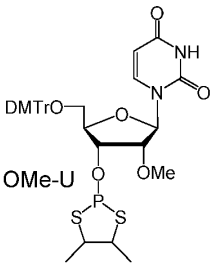

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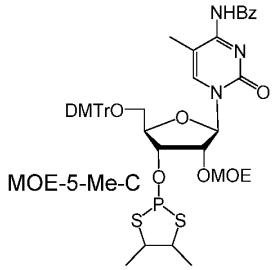

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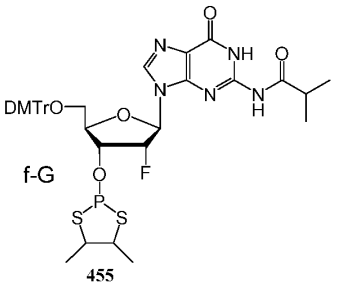

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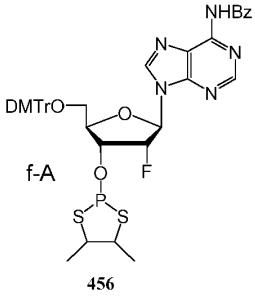

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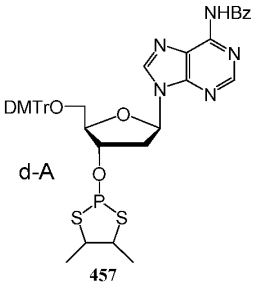

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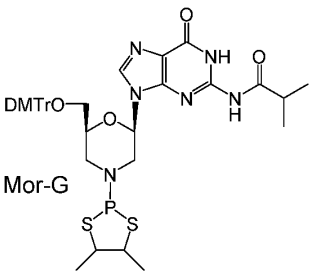

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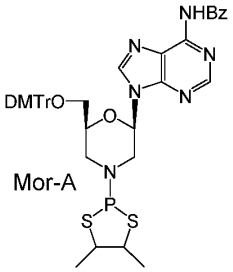

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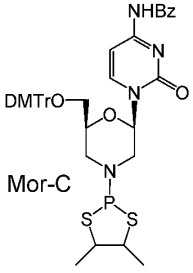


460

MOE-G monomer 451: Yield 81%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 175.14, 158.52, 150.30, 148.81; MS (ES) *m/z* calculated for $C_{42}H_{50}N_5O_9PS_2$ $[M+H]^+$ 864.29, Observed: 864.56 $[M + H]^+$.

OMe-A monomer 452: Yield 92%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 175.65, 159.27, 151.04, 150.10; MS (ES) *m/z* calculated for $C_{43}H_{44}N_5O_7PS_2$ $[M+H]^+$ 838.25, Observed: 838.05 $[M + H]^+$.

OMe-U monomer 453: Yield 94%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 175.09, 162.04, 154.12, 153.58; MS (ES) *m/z* calculated for $C_{35}H_{39}N_2O_8PS_2$ $[M+K]^+$ 749.15, Observed: 749.06 $[M + K]^+$.

MOE-5-Me-C monomer 454: Yield 91%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 175.53, 162.04, 153.78, 153.61; MS (ES) *m/z* calculated for $C_{45}H_{50}N_3O_9PS_2$ $[M+H]^+$ 872.28, Observed: 872.16 $[M + H]^+$.

f-G monomer 455: Yield 97%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 176.88 (d), 161.94 (d), 154.16 (d), 152.48 (d); MS (ES) *m/z* calculated for $C_{39}H_{43}FN_5O_7PS_2$ $[M+H]^+$

808.24, Observed: 808.65 $[M + H]^+$.

f-A monomer 456: Yield 99%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 177.43 (d), 159.63 (d), 149.76 (d), 149.55 (d); MS (ES) *m/z* calculated for $C_{42}H_{41}FN_5O_6PS_2$ $[M+H]^+$ 826.23, Observed: 826.56 $[M + H]^+$.

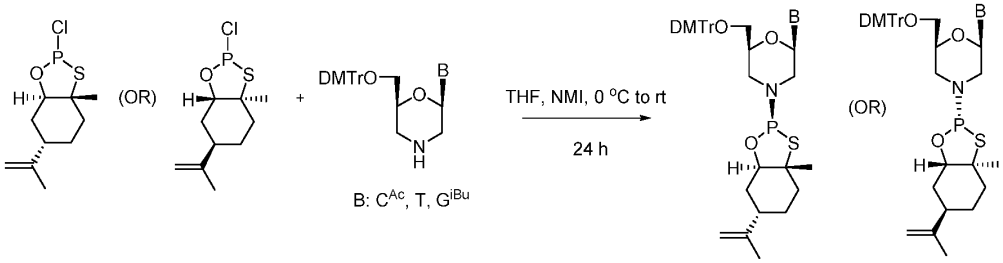
dA monomer 457: Yield 98%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 171.85, 154.47, 146.19, 144.48; MS (ES) *m/z* calculated for $C_{42}H_{42}N_5O_6PS_2$ $[M+K]^+$ 846.20, Observed: 846.56 $[M + K]^+$.

Mor-G monomer 458: Yield 72%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 121.26, 105.98, 93.48, 93.24; MS (ES) *m/z* calculated for $C_{39}H_{45}N_6O_6PS_2[M+K]^+$ 827.22, Observed: 827.60 $[M + K]^+$.

Mor-A monomer 459: Yield 37%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 121.87, 106.17, 93.23, 93.05; MS (ES) *m/z* calculated for $C_{42}H_{43}N_6O_5PS_2$ $[M+K]^+$ 845.21, Observed: 845.32 $[M + K]^+$.

Mor-C monomer 460: Yield 68%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 122.34, 106.05, 93.33, 92.6116; MS (ES) *m/z* calculated for $C_{41}H_{43}N_4O_6PS_2$ $[M+K]^+$ 821.20, Observed: 821.54 $[M + K]^+$.

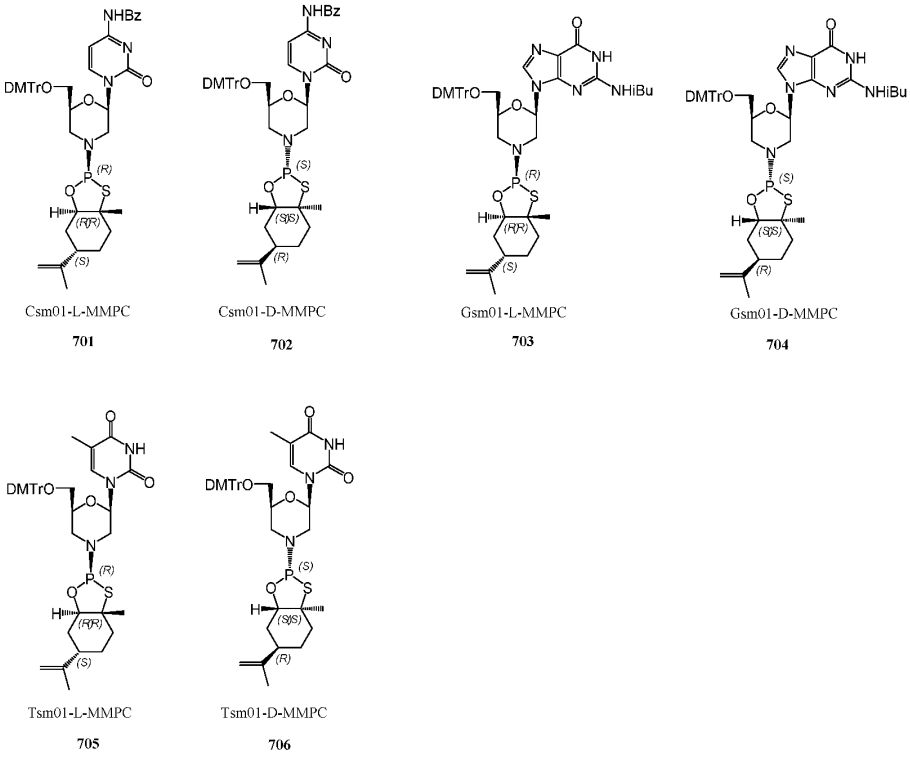
General experimental procedure for stereopure morpholine monomers



The 5'-ODMTr protected morpholine nucleoside (11.1 mmol) was dried in a three neck 250 mL round bottom flask by co-evaporating with anhydrous toluene (100 mL) followed by under high vacuum for 18 h. The dried nucleoside was dissolved in dry THF (55 mL) under argon atmosphere. Then, 1-methylimidazole (44.2 mmol, 4 equiv.) was added into the reaction mixture, then cooled to ~ - 10 °C [at this stage, if B: G^{iBu} chrorotrimethylsilane (0.9 equiv.) was added]. A THF solution of the crude chloro reagent (1 M solution, 1.8 equiv., 19.9 mmol) was added to the above mixture through cannula over ~3 min, then, gradually warmed to room temperature over about 1 h. LCMS showed that the starting material was consumed. Resulting reaction mixture was stirred for additional

24 h at rt. Then filtered carefully under vacuum/argon and the resulting filtrate was concentrated under reduced pressure to give a yellow foam which was further dried under high vacuum overnight. Crude mixture was purified by silica gel column [Column was pre-deactivated using acetonitrile then ethyl acetate (5% TEA) and then equilibrated using ethyl acetate-hexanes] chromatography using ethyl acetate and hexane as eluents.

Structure of stereopure morpholine monomers:



Stereopure (Rp) Csm01-L-MMPC monomer 701: Yield 39%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 137.80; MS (ES) *m/z* calculated for $C_{47}H_{51}N_4O_7PS$ $[M+K]^+$ 885.29, Observed: 885.51 $[M + K]^+$.

Stereopure (Sp) Csm01-D-MMPC monomer 702: Yield 28%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 137.42; MS (ES) *m/z* calculated for $C_{47}H_{51}N_4O_7PS$ $[M+K]^+$ 885.29, Observed: 885.70 $[M + K]^+$.

Stereopure (Rp) Gsm01-L-MMPC monomer 703: Yield 37%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 136.58; MS (ES) *m/z* calculated for $C_{45}H_{55}N_6O_6PS$ $[M+K]^+$ 891.31, Observed: 891.48 $[M + K]^+$.

Stereopure (Sp) Gsm01-D-MMPC monomer 704: Yield 38%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 136.56; MS (ES) *m/z* calculated for $C_{45}H_{55}N_6O_6PS$ $[M+K]^+$ 891.31, Observed: 891.67 $[M + K]^+$.

Stereopure (Rp) Tsm01-L-MMPC monomer 705: Yield 30%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 138.52; MS (ES) *m/z* calculated for $C_{41}H_{48}N_3O_7PS$ $[M+Na]^+$ 780.28, Observed: 780.52 $[M + Na]^+$.

Stereopure (Sp) Tsm01-D-MMPC monomer 706: Yield 25%. ^{31}P NMR (243 MHz, $CDCl_3$) δ 137.62; MS (ES) *m/z* calculated for $C_{41}H_{48}N_3O_7PS$ $[M+Na]^+$ 780.28, Observed: 780.81 $[M + Na]^+$.

Abbreviation

1X reagent: TEA-3HF : TEA : H_2O : DMSO = 5.0 : 1.8 : 15.5 : 77.7 (v/v/v/v)

ADIH: 2-azido-1,3-dimethylimidazolium hexafluorophosphate

CMIMT: *N*-cyanomethylimidazolium triflate

CPG: controlled pore glass

DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene

DCM: dichloromethane, CH_2Cl_2

DIPEA: diisopropylethylamine

DMSO: dimethylsulfoxide

DMTr: 4,4′-dimethoxytrityl

GalNAc: *N*-acetylgalactosamine

HF: hydrogen fluoride

HATU: 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate

IBN: isobutyronitrile

MeCN: acetonitrile

MeIm: *N*-methylimidazole

TCA: trichloroacetic acid

TEA: triethylamine

XH: xanthane hydride

General procedure for the synthesis of chrial-oligos (25 μmol scale):

The automated solid-phase synthesis of chiral-oligos was performed according to the cycles shown in Table 46 (regular amidite cycle, for PO linkages), Table 47 (DPSE amidite cycle, for chiral PS linkages), and Table 48 (MBR/MMPC amidite cycle P(V), for stereo-random/chiral morpholino PN linkages), Table 49 (regular amidite cycle, for stero-random PN linkages), and Table 50 (PSM amidite cycle, for chiral PN linkages).

Table 46. Regular Amidite Synthetic Cycle for PO linkages

step	operation	reagents and solvent	volume	waiting time
1	detritylation	3% TCA / DCM	10 mL	65 s
2	coupling	0.2M monomer / 20% IBN-MeCN 0.5M CMIMT / MeCN	0.5 mL 1.0 mL	8 min
3	oxidation	50mM I_2 / pyridine-H_2O (9:1, v/v)	2.0 mL	1 min
4	cap-2	20% Ac_2O, 30% 2,6-lutidine / MeCN 20% MeIm / MeCN	1.0 mL 1.0 mL	45 s

Table 47. DPSE Amidite Synthetic Cycle for chiral PS linkages

step	operation	reagents and solvent	volume	waiting time
1	detritylation	3% TCA / DCM	10 mL	65 s
2	coupling	0.2M monomer / 20% IBN-MeCN 0.5M CMIMT / MeCN	0.5 mL 1.0 mL	8 min
3	cap-1	20% Ac_2O, 30% 2,6-lutidine / MeCN	2.0 mL	2 min
4	sulfurization	0.2M XH / pyridine	2.0 mL	6 min
5	cap-2	20% Ac_2O, 30% 2,6-lutidine / MeCN 20% MeIm / MeCN	1.0 mL 1.0 mL	45 s

Table 48. MBR/MMPC Amidite Synthetic Cycle (P(V)) for stereo-random/chiral PN linkages

step	operation	reagents and solvent	volume	waiting time
1	detritylation	3% TCA / DCM	10 mL	65 s
2	coupling	0.2M monomer / 20% IBN-MeCN 1.0M DBU / MeCN	0.5 mL 1.0 mL	10 min
3	cap-2	20% Ac_2O, 30% 2,6-lutidine / MeCN 20% MeIm / MeCN	1.0 mL 1.0 mL	45 s

Table 49. Regular Amidite Synthetic Cycle for stereo-random PN linkages

step	operation	reagents and solvent	volume	waiting time
1	detritylation	3% TCA / DCM	10 mL	65 s
2	coupling	0.2M monomer / 20% IBN-MeCN 0.5M CMIMT / MeCN	0.5 mL 1.0 mL	8 min
3	imidation	0.5M ADIH / MeCN	2.0 mL	6 min
4	cap-2	20% Ac_2O, 30% 2,6-lutidine / MeCN 20% MeIm / MeCN	1.0 mL 1.0 mL	45 s

Table 50. PSM Amidite Synthetic Cycle for chiral PN linkages

step	operation	reagents and solvent	volume	waiting time
1	detritylation	3% TCA / DCM	10 mL	65 s
2	coupling	0.2M monomer / 20% IBN-MeCN 0.5M CMIMT / MeCN	0.5 mL 1.0 mL	8 min
3	cap-1	20% Ac_2O, 30% 2,6-lutidine / MeCN	2.0 mL	2 min
4	imidation	0.5M azide reagent / MeCN	2.0 mL	6 min

5	cap-2	20% Ac_2O, 30% 2,6-lutidine / MeCN	1.0 mL	45 s
		20% MeIm / MeCN	1.0 mL	

General procedure for the C&D conditions (25 μmol scale):

After completion of the synthesis, the CPG solid support was dried and transferred into 50 mL plastic tube. The CPG was treated with 1X reagent (2.5 mL; 100 μL/umol) for 3 h at 28°C, then added conc. NH_3 (5.0 mL; 200 μL/umol) for 16 h at 45°C. The reaction mixture was cooled to room temperature and the CPG was separated by membrane filtration, washed with 15 mL of H_2O. The crude material (filtrate) was analyzed by LTQ and RP-UPLC.

General procedure for the GalNAc conjugation conditions (1 μmol scale):

Into a plastic tube, tri-GalNAc (2.0 eq.), HATU (1.9 eq.), and DIPEA (10 eq.) were dissolved in anhydrous MeCN (0.5 mL). The mixture was stirred for 10 min at room temperature, then the mixture was added into the amino-oligo (1 μmol) in H_2O (1 mL) and stirred for 1 h at 37 °C. The reaction was monitored by LC-MS and RP-UPLC. After the reaction was completed, the resultant GalNAc-conjugated oligo was treated with conc. NH_3 (2 mL) for 1 h at 37 °C. The solution was concentrated under vacuum to remove MeCN and conc. NH_3. The residue was then dissolved in H_2O (10 mL) for reversed phase purification.

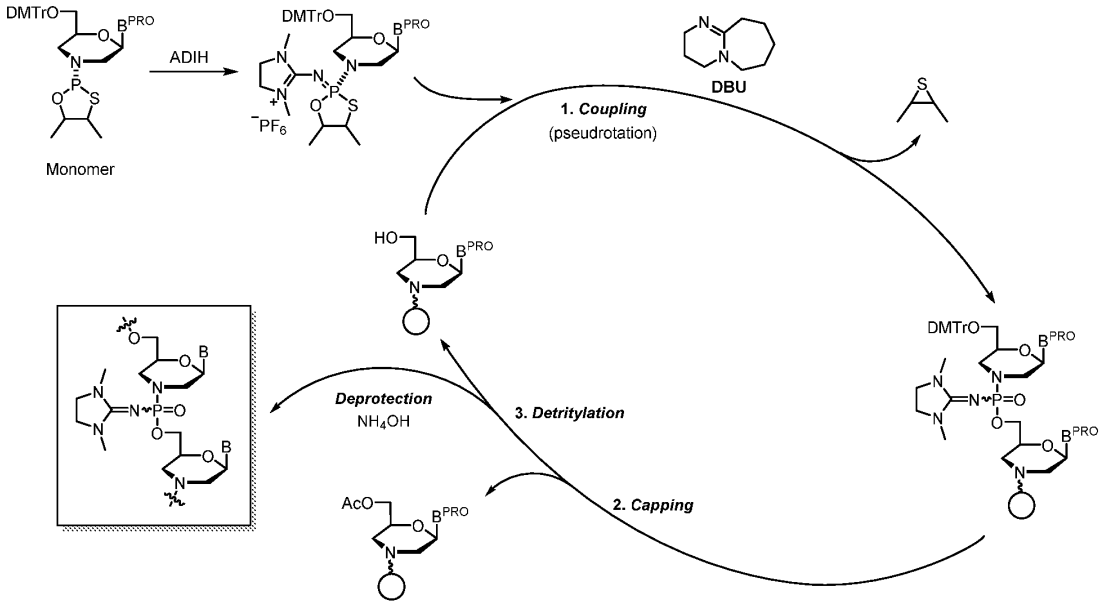
Table 51. Oligo MS data

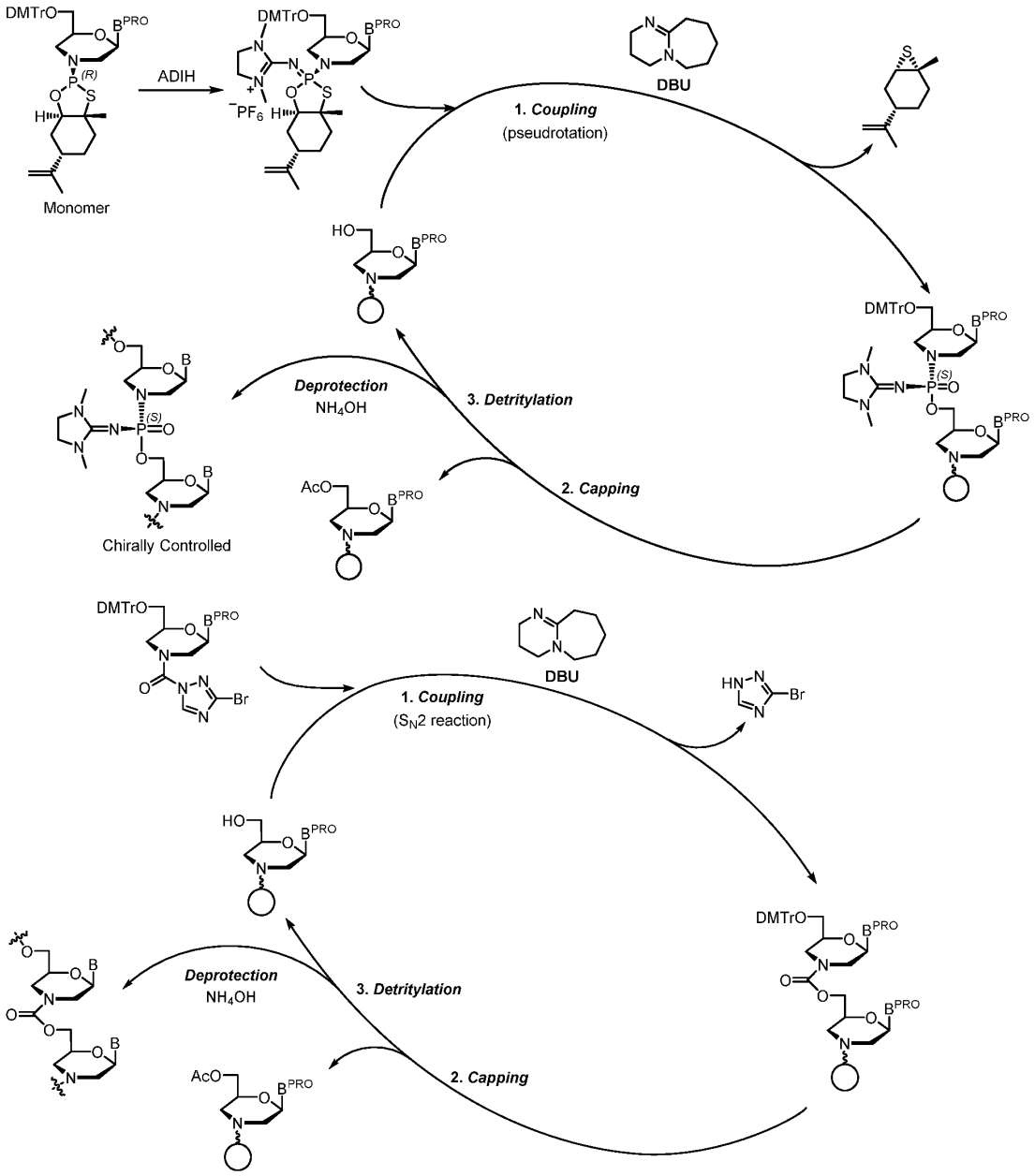
Oligo-ID	calcd. [M]	observed [M]
WV-20170	7595.9	7595.5
WV-41918	7691.1	7690.7
WV-38708	7881.4	7879.6
WV-44144	7850.4	7850.6
WV-44145	7850.4	7851.2
WV-44163	7719.2	7719.7
WV-44353	7660.1	7658.8
WV-44354	7660.1	7660.3
WV-44355	7528.9	7528.4

Example 15. Preparation of oligonucleotide compositions.

Various technologies for preparing oligonucleotides and oligonucleotide compositions (both stereorandom and chirally controlled) are known and can be utilized in accordance with the present disclosure, including, for example, methods and reagents described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/223056, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, and/or WO 2021/071858, the methods and reagents of each of which are incorporated herein by reference. Many oligonucleotides and compositions thereof, e.g., various oligonucleotides and compositions thereof in Table 1, were prepared and assessed.

Certain useful cycles are described below as examples for preparing oligonucleotides.





Each B is independently a nucleobase such as BA described herein (e.g., A, C, G, T, U, etc.). Each B^{PRO} is independently an optionally protected nucleobase such as BA described herein (e.g., A^{bz}, C^{ac}, G^{ibu}, T, U, etc. suitable for oligonucleotide synthesis). As shown, various linkages can be constructured to connect monomers to nucleosides or oligonucleotides including those on solid support. As appreciate by those skilled in the art these cycles can be utilized to couple monomers to −OH of various other types of sugars.

In some embodiments, preparations include one or more DPSE and/or PSM cycles

Example 16. Impact of 2'F position on the PN

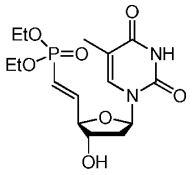
In vivo determination of mouse TTR siRNA activity: All animal procedures were performed under IACUC guidelines at Alpha Preclinical (North Grafton, MA). To evaluate the durability of provided oligonucleotides and compositions, male 8-10 weeks of age C57BL/6 mice were dose at 1.5 mg/kg at desired oligonucleotide concentration on Day 1 by subcutaneous administration. On Day 1 (pre-dose) and then weekly, whole blood was collected by tail snip into serum separator tubes, and processed serum samples were kept at -70°C. Mouse TTR protein concentration in the serum was assessed using the Mouse Prealbumin ELISA kit (Crystal Chem) and following manufacturer's instructions.

Table 52.

	PBS					
Day	animal1	animal2	animal3	animal4	animal5	Mean
1	90	111	100	103	95	100
8	91	112	102	113	82	100
15	102	99	112	100	86	100
22	101	101	98	103	96	100
	WV-41826/WV-41828, 1.5 mg/kg					
Day	animal6	animal7	animal8	animal9	animal10	Mean
1	111	86	77	117	144	107
8	1	2	4	4	4	3
15	2	4	4	8	6	5
22	6	9	11	5	8	8
	WV-43775/WV-42080, 1.5 mg/kg					
Day	animal11	animal12	animal13	animal14	animal15	Mean
1	113	104	122	86	90	103
8	4	0	0	0	0	1
15	8	1	2	1	2	3
22	12	5	8	6	6	8
	WV-42079/WV-42080, 1.5 mg/kg					
Day	animal16	animal17	animal18	animal19	animal20	Mean
1	83	123	127	118	74	105
8	0	5	1	3	2	2
15	2	7	2	6	4	4
22	11	9	8	8	8	9
	WV-43771/WV-42080, 1.5 mg/kg					
Day	animal21	animal22	animal23	animal24	animal25	Mean
1	107	67	104	73	93	89
8	3	0	2	0	0	1

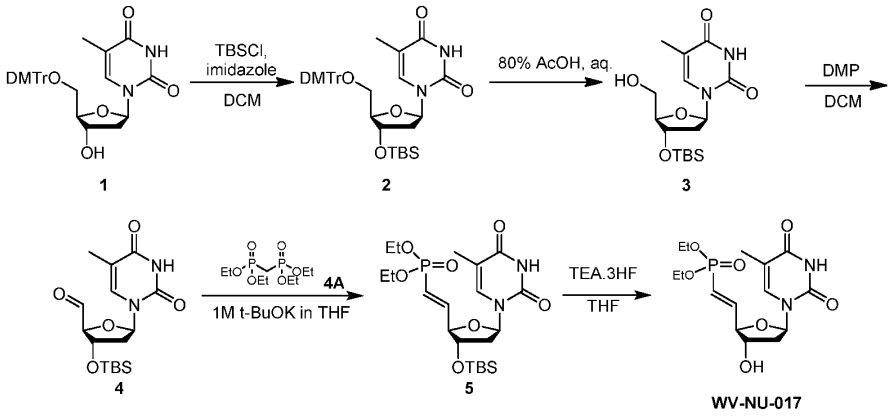
15	6	3	4	2	1	3
22	1	10	7	5	6	6
	WV-43773/WV-42080, 1.5 mg/kg					
Day	animal26	animal27	animal28	animal29	animal30	Mean
1	126	108	107	98	94	107
8	5	5	6	4	6	5
15	7	5	7	8	4	6
22	15	13	13	10	9	12
	WV-43988/WV-42080, 1.5 mg/kg					
Day	animal31	animal32	animal33	animal34	animal35	Mean
1	104	101	105	126	120	111
8	0	1	3	1	5	2
15	1	1	2	1	1	1
22	4	4	5	4	7	5
	WV-43989/WV-42080, 1.5 mg/kg					
Day	animal36	animal37	animal38	animal39	Animal40	Mean
1	64	118	89	108	101	96
8	4	4	1	12	3	5
15	5	4	1	11	6	6
22	10	8	7	24	15	13
	WV-43994/WV-42080, 1.5 mg/kg					
Day	Animal41	Animal42	Animal43	Animal44	Animal45	Mean
1	141	124	104	124	113	121
8	3	3	2	2	4	3
15	3	2	2	3	7	3
22	8	11	8	8	8	8
	WV-43996/WV-42080, 1.5 mg/kg					
Day	Animal46	Animal47	Animal48	Animal49	Animal50	Mean
1	106	71	89	110	118	99
8	2	4	2	1	1	2
15	2	2	2	2	1	2
22	3	4	6	5	7	5
	WV-43256/WV-42080, 1.5 mg/kg					
Day	Animal51	Animal52	Animal53	Animal54	Animal55	Mean
1	92	97	N,D.	104	101	99
8	4	6	2	3	9	5
15	9	9	6	6	8	7
22	21	22	15	14	21	18

Example 17. Synthesis of 5'-PO(OEt)$_2$ Vinyl Phosphonate-dT (WV-NU-017).

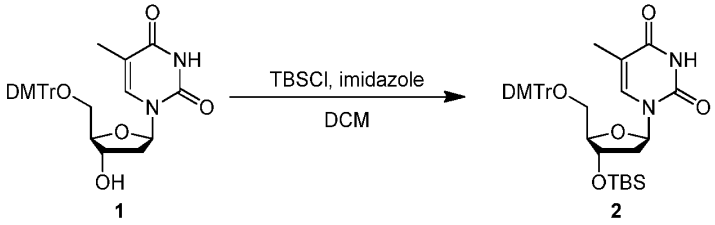


WV-NU-017

General Scheme



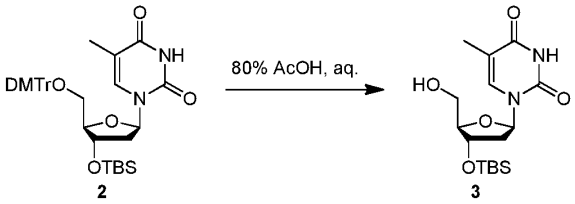
1. Preparation of compound 2



Two batches: To a solution of compound **1** (150 g, 275.43 mmol) and imidazole (56.25 g, 826.30 mmol) in DCM (2 L) was added TBSCl (83.03 g, 550.87 mmol, 67.50 mL). The mixture was stirred at 15°C for 16 hr. TLC showed compound **1** was consumed. Two batches: The mixture was washed with sat. $NaHCO_3$ (aq., 2 L * 2), the combined aqueous layers were extracted with EtOAc (500 mL * 2), the combined organic layers were dried over Na_2SO_4, filtered and concentrated to give crude Compound **2** (362 g, crude) as a yellow oil.

TLC (Petroleum ether / Ethyl acetate = 1: 1, 5%TEA) R_f = 0.39.

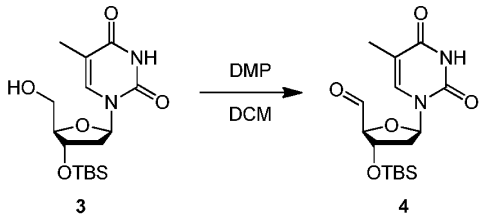
2. Preparation of compound 3



A solution of compound **2** (362 g, 549.44 mmol) in H_2O (360 mL) and CH_3COOH (1440 mL), the mixture was stirred at15 °C for 16 hr. TLC showed that compound **2** was consumed. The mixture was added sat.$NaHCO_3$ (aq., 3000 mL), and the organic layer was separated and the aqueous layer extracted with EtOAc (2000 mL * 3), the combined organic layers were dried over Na_2SO_4, filtered and concentrated to get the crude. The residue was purified by silica gel chromatography (SiO_2, Petroleum ether: Ethyl acetate = 30: 1 to 1: 2) to give compound **3** (185 g, 94.45% yield) as a white solid.

TLC (Petroleum ether: Ethyl acetate = 1: 1) R_f = 0.24.

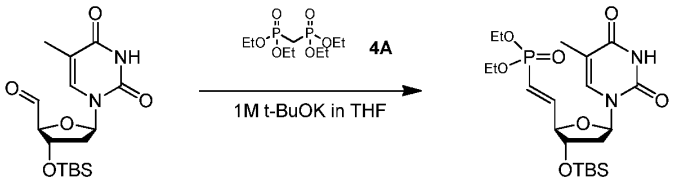
3. Preparation of compound 4



To a solution of compound **3** (110 g, 308.57 mmol) in DCM (500 mL) was added DMP (157.05 g, 370.28 mmol, 114.64 mL) in portions at 0°C. The mixture was stirred at 30°C for 2 hr. TLC showed most of compound **3** was consumed and a new spot was observed. Two batches: The mixture was added 5% $Na_2S_2O_3$ (2000 mL) at 0°C, the mixture was stirred at 0°C for 20 min then sat. $NaHCO_3$ (2000 mL) was added, the mixture was extracted with DCM (2000 mL * 4) and Ethyl acetate (2000 mL * 4), the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to get compound **4** (190 g, crude) as a white solid.

TLC (Petroleum ether: Ethyl acetate =1:3) R_f = 0.36.

4. Preparation of compound 5

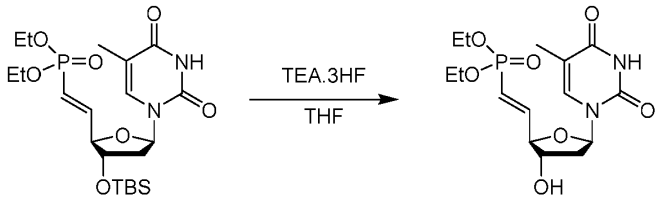


4 5

To a solution of compound **4A** (216.28 g, 750.41 mmol) in THF (400 mL) was added t-BuOK (1 M, 750 mL) at 0°C and stirred at 0°C for 10 min, then warmed up to 20°C for 2 hr. The above mixture was added to the solution of compound **4** (190 g, 536.01 mmol) in THF (400 mL) at 0°C. The reaction mixture was stirred at 0°C for 1 hr and then allowed to warm up to 20°C in 6 hr. TLC showed the reaction was complete. To the reaction mixture water (1000 mL) was added and the biphasic mixture was extracted with EtOAc (2000 mL * 4) and DCM (1000 mL * 3). The organic phase was dried over Na_2SO_4, filtered, and concentrated to give residue. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate = 10:1 1:1, 0:1) to give compound **5** (250 g, crude) was obtained as a yellow oil.

TLC (Petroleum ether / Ethyl acetate = 1: 2), R_f = 0.32.

5. Preparation of WV-NU-017



5 WV-NU-017

To a solution of compound **5** (243 g, 497.35 mmol) in THF (1300 mL) was added TEA.3HF (320.71 g, 1.99 mol, 324.28 mL). The mixture was stirred at 15°C for 16 hr. TLC (Petroleum ether: (Ethyl acetate: Ethyl Alcohol=3:1) = 1: 1, R_f = 0.18) showed that some compound **5** remained. Additionally, a new spot was detected. The reaction mixture was concentrated under reduced pressure and the mixture was neutralized with Na_2CO_3 (aq., sat., ~1L) until pH=7. The mixture was concentrated under reduced pressure to removed most of water. EtOAc: EtOH = 10:1 (1 L * 2) was added to the concentrated reaction mixture, which was subsequently dried over Na_2SO_4, filtered and concentrated

under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, (Ethyl acetate: Ethyl Alcohol = 3: 1) / Petroleum ether = 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%) to give compound **WV-NU-017** (76 g, 38.51% yield) as a white solid.

^{1}H NMR (400MHz, $CDCl_3$) δ = 9.59 (br s, 1H), 7.13 (d, J=1.1 Hz, 1H), 7.09 - 6.94 (m, 1H), 6.41 (t, J=6.6 Hz, 1H), 6.03 (ddd, J=1.8, 17.4, 19.6 Hz, 1H), 4.76 (br d, J=4.0 Hz, 1H), 4.50 (br d, J=2.4 Hz, 1H), 4.39 (br d, J=2.0 Hz, 1H), 4.21 - 4.00 (m, 4H), 2.43 (ddd, J=4.5, 6.3, 13.8 Hz, 1H), 2.18 (td, J=6.9, 13.8 Hz, 1H), 2.02 - 1.86 (m, 3H), 1.38 - 1.30 (m, 6H).

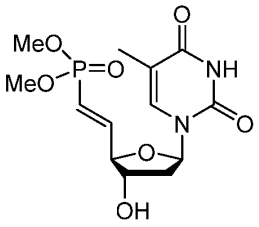
^{31}P NMR (162MHz, $CDCl_3$) δ = 17.71.

^{13}C NMR (101MHz, $CDCl_3$) δ = 163.76, 150.63, 149.04, 135.29, 118.47, 116.58, 111.67, 85.87, 85.65, 85.07, 73.91, 62.23, 39.15, 16.38, 12.66.

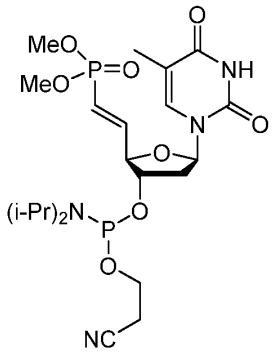
LCMS ($M\text{-}H^+$): 373.1, purity: 94.34%.

TLC (Petroleum ether: (Ethyl acetate: Ethyl Alcohol = 3 : 1) = 1 : 1) R_f = 0.18;

Example 18. Synthesis of 5'-PO(OMe)$_2$ Vinyl Phosphonate-dT (WV-NU-010), and 5'-PO(OMe)$_2$ Vinyl Phosphonate-3'-CNE-dT Phosphoramidite (WV-NU-10-CNE)

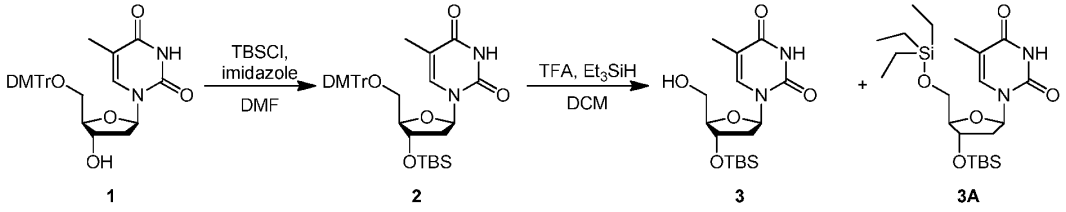


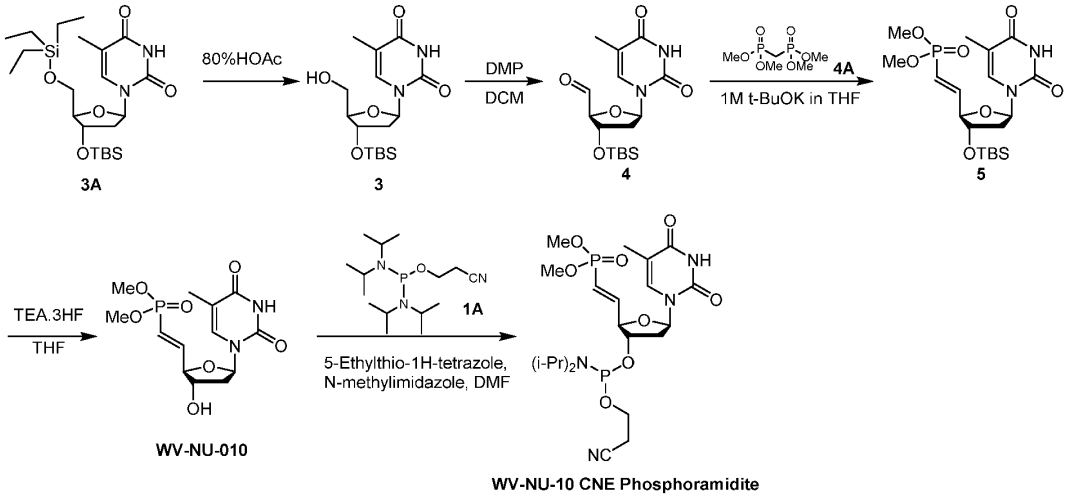
WV-NU-010



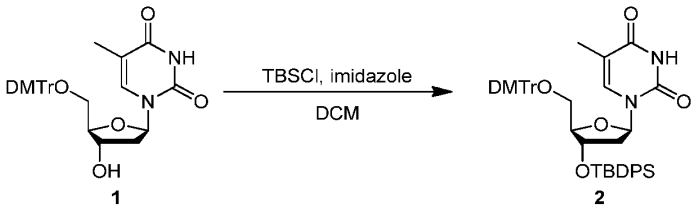
WV-NU-10-CNE-Phosphoramidite

General Scheme





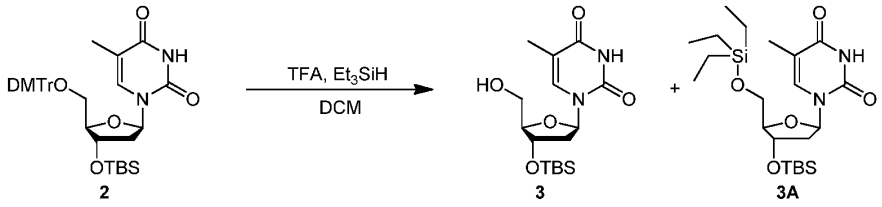
2. *Preparation of compound 2*



To a solution of compound **1** (100.00 g, 183.62 mmol) and imidazole (37.50 g, 550.86 mmol) in DCM (1.00 L) was added TBSCl (55.35 g, 367.24 mmol) at 0°C, and the mixture was stirred at 18°C for 14hr. TLC showed the starting material was consumed. The mixture was washed with sat. $NaHCO_3$ (200 mL) and brine (100 mL), dried over Na_2SO_4, filtered and concentrated to get the compound **2** as a white solid (120.98 g, crude). The mixture was used for next step directly without any purification.

TLC (Ethyl acetate/ Petroleum ether = 3:1, 5% TEA) Rf = 0.43.

2. *Preparation of compound 3 & compound 3A*

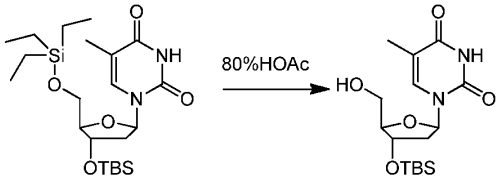


To a solution of TFA (41.85 g, 367.00 mmol) and Et_3SiH (64.01 g, 550.50 mmol) in DCM

(1.20 L) was added compound **2** (120.9 g, 183.50 mmol) dissolved in DCM (200.00 mL), and the mixture was stirred at 15°C for 0.5h. TLC showed the starting material was consumed. The mixture was added sat. $NaHCO_3$ (aq, 300 mL), and the organic phase was separated and the aqueous layer extracted with DCM (200 mL*3), the combined organic layers were dried over Na_2SO_4, filtered and concentrated to get the crude product. The crude product was purified by MPLC (Petroleum ether /Ethyl acetate = 10:1 to 1:2) to get compound **3** as a white solid (36 g, 55.04% yield) and compound **3A** as a white solid (50 g, crude).

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 9.08 (s, 1H), 6.04 (t, *J* = 6.8 Hz, 1H), 4.37 (td, *J* = 3.5, 6.5 Hz, 1H), 3.84 - 3.74 (m, 2H), 3.67 - 3.58 (m, 1H), 2.22 (td, *J* = 6.8, 13.4 Hz, 1H), 2.13 - 2.03 (m, 1H), 1.78 (s, 3H), 0.81 - 0.77 (m, 3H), 0.77 (s, 9H), -0.04 (s, 6H);
TLC (Petroleum ether / Ethyl acetate = 1:1) Rf = 0.24.

3. Preparation of compound 3

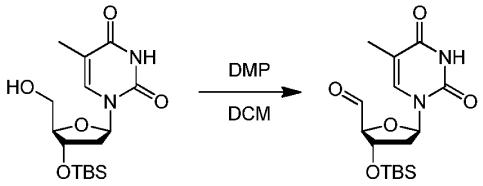


3A **3**

To a solution of ***compound 3*****A** (50 g, 106.21 mmol) in the mixture of HOAc (210.00 g, 3.50 mol) and H_2O (50 mL) was added. The mixture was stirred at 18°C for 0.5h. TLC showed the starting material was consumed. Na_2CO_3 (aq) was added to the reaction mixture until PH>8, and the residue was extracted with EtOAc (200 mL*3). The mixture was purified by MPLC (Petroleum ether/Ethyl acetate = 10:1, 1:1) to get ***compound 3*** as a white solid (30 g, 79.23% yield).

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 8.98 (br s, 1H), 7.38 (s, 1H), 6.15 (t, *J* = 6.8 Hz, 1H), 4.50 (td, *J* = 3.5, 6.6 Hz, 1H), 3.96 - 3.88 (m, 2H), 3.81 - 3.67 (m, 1H), 2.81 - 2.69 (m, 1H), 2.39 - 2.17 (m, 2H), 1.91 (s, 3H), 0.99 - 0.84 (m, 9H), 0.09 (s, 6H).

4. Preparation of compound 4

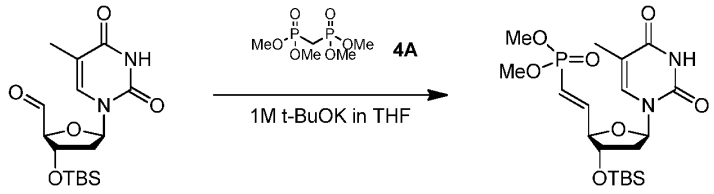


3 4

To a solution of compound **3** (10 g, 28.05 mmol) in DCM (160 mL), DMP (14.28 g, 33.66 mmol, 10.42 mL) was added at 0°C. The mixture was stirred at 0-25 °C for 3 hr. After this time, TL C showed that most of the starting material was consumed and a new spot was found. 5% $Na_2S_2O_3$ (300 mL) solution was then added at 0°C, and the mixture was stirred at 0°C for 20 min. Sat. $NaHCO_3$(300 mL) was subsequently added, the mixture was extracted with DCM (200 mL*3), and the combined organic layers were dried over Na_2SO_4, filtered and concentrated to get the compound **4** as a yellow oil (10 g, crude) . The mixture was used for next step without any purification.

TL C (Petroleum ether / Ethyl acetate = 1:1) Rf = 0.38.

5. Preparation of compound 5



4 5

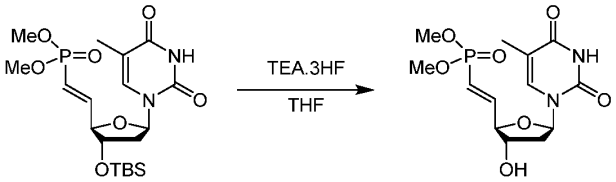
To a solution of compound **4A** (7.20 g, 31.03 mmol) in THF (20 mL) was added t-BuOK (1 M, 31.03 mL) at 0°C and stirred at 0°C for 10min, then warmed up to 20°C for 30min. The above mixture was added to the solution of compound **4** (10 g, 28.21 mmol) in THF (20 mL) at 0°C. The reaction mixture was stirred at 0°C for 1h and then allowed to warm up to 20°C in 20min. LCMS and TLC showed the reaction was complete. Water (20 mL) was added to the reaction and the biphasic mixture was extracted with EtOAc (30 mL*4). The organic phase was dried (Na_2SO_4), filtered and concentrated. The residue was purified by column chromatography MPLC (Petroleum ether/ Ethyl acetate = 10:1 to1:4) to get compound **5** as a white solid (12 g, 92.36% yield). The mixture of 10g crude was purified with the product together.

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 8.82 (br s, 1H), 7.09 (s, 1H), 6.94 - 6.78 (m, 1H), 6.33 (t,

J = 6.7 Hz, 1H), 5.99 (ddd, *J* = 1.6, 17.4, 19.2 Hz, 1H), 4.41 - 4.24 (m, 2H), 3.76 (dd, *J* = 3.8, 11.1 Hz, 5H), 2.33 - 2.23 (m, 1H), 2.13 (td, *J* = 6.8, 13.6 Hz, 1H), 1.96 - 1.89 (m, 3H), 0.94 - 0.80 (m, 10H), 0.14 - 0.03 (m, 6H);

TLC (Dichloromethane: Methanol = 20:1) Rf = 0.36.

6. Preparation of WV-NU-010



5 **WV-NU-010**

To a solution of compound **5** (13 g, 28.23 mmol) in THF (80 mL) was added N, N-diethylethanamine;trihydrofluoride (22.75 g, 141.14 mmol). The mixture was stirred at 18 °C for 12 hours. TLC revealed that some of the starting material was still present and that the desired substance had formed. The reaction mixture was concentrated under reduced pressure and the mixture was neutralized with Na_2CO_3 (aq.sat) until pH = 7. The water phase was freeze-dried. The freeze-drying solid was washed with DCM: MeOH = 10:1 (300 mL*2). The organic phase was concentrated. The residue obtained was purified by column chromatography on silica gel (Dichloromethane: Methanol = 100:1, 100:8) to get **WV-NU-010** as a white solid (6.25 g, 62.54% yield). The mixture was purified with another batch (8 g scale). In total, 10g **WV-NU-010** was isolated as a white solid.

^{1}H NMR :(400MHz, $CDCl_3$**)** δ = 9.54 (br s, 1H), 7.13 - 6.98 (m, 2H), 6.39 (t, J = 6.7 Hz, 1H), 6.06 - 5.96 (m, 1H), 4.61 (br d, J = 4.0 Hz, 1H), 4.50 (br d, J = 2.4 Hz, 1H), 4.44 - 4.36 (m, 1H), 3.79 - 3.71 (m, 6H), 2.43 (ddd, J = 4.5, 6.4, 13.8 Hz, 1H), 2.21 (td, J = 6.9, 13.7 Hz, 1H), 1.93 (s, 3H);

^{31}PNMR: (162MHz, $CDCl_3$**):** δ = 20.54 (s, 1P);

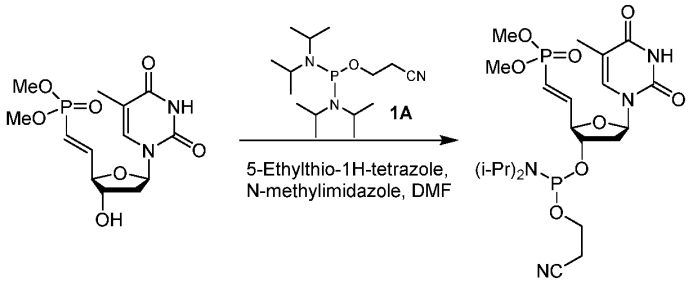
LCMS: ($M+H^+$): 347.0 LCMS purity: 97.81%;

^{13}CNMR: (101MHz, $CDCl_3$) δ = 163.95, 150.75, 150.12, 150.06, 135.38, 116.99, 115.10, 111.65, 85.82, 85.61, 85.14, 73.93, 52.69, 39.00, 12.61;

HPLC: HPLC purity: 98.25%;

TLC (Dichloromethane / Methanol) Rf = 0.24.

7. Preparation of WV-NU-10-CNE-Phosphoramidite



WV-NU-010 **WV-NU-10 CNE Phosphoramidite**

WV-NU-010 (4.9 g, 14.15 mmol) was co-evaporated with anhydrous toluene two times (25mLx2) and dried under high vacuum for 1h.

To a solution of **WV-NU-010** (4.9 g, 14.15 mmol) in DMF (35 mL) was added 5-ethylsulfanyl-2H-tetrazole (1.84 g, 14.15 mmol), 1-methylimidazole (2.32 g, 28.30 mmol) and compound **1A** (6.40 g, 21.23 mmol). The reaction mixture was stirred at 20°C under N_2 for 1h. TLC showed the starting material was consumed and the desired substance was found. The reaction mixture was diluted with EtOAc (60 mL). The reaction mixture was washed with aq. saturated. $NaHCO_3$ solution (50 mL*4), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The column was eluted with Petroleum ether /Ethyl acetate (5%TEA 10mins) and then Petroleum ether (5 mins). The residue thus obtained was purified by silica gel column chromatography (elution with Petroleum ether: EtOAc = 10:1, 1:1 and then EtOAc/Acetonitrile = 50:1, 30:1) to get ***WV-NU-10-CNE-Phosphoramidite*** as a white solid (4.4 g, 54.14% yield).

^{1}H NMR: (400MHz, $CDCl_3$**)** δ = 8.96 (br s, 1H), 7.08 (s, 1H), 7.03 - 6.81 (m, 1H), 6.42 - 6.33 (m, 1H), 6.01 (dddd, J = 1.8, 8.7, 17.3, 19.3 Hz, 1H), 4.58 - 4.38 (m, 2H), 3.94 - 3.81 (m, 1H), 3.80 - 3.70 (m, 7H), 3.68 - 3.53 (m, 2H), 2.81 - 2.71 (m, 1H), 2.71 - 2.61 (m, 2H), 2.54 - 2.39 (m, 1H), 2.23 (dtd, J = 5.0, 6.8, 13.8 Hz, 1H), 1.93 (s, 3H), 1.22 - 1.15 (m, 12H);

^{31}PNMR: (162MHz, $CDCl_3$**)** δ = 149.34 (s, 1P), 149.32 (s, 1P), 20.04 (s, 1P), 19.68 (s, 1P), 14.12 (s, 1P);

LCMS: ($M\text{-}H^+$): 545.1, LCMS purity: 93.80%;

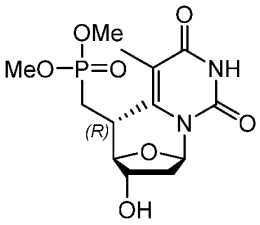
^{13}CNMR: (101MHz, $CDCl_3$**)** δ = 163.84, 163.82, 150.58, 150.51, 148.58, 135.10, 135.02,

129.31, 118.68, 118.19, 117.80, 117.65, 116.79, 116.31, 111.73, 84.78, 84.74, 84.61, 84.53, 84.49, 84.39, 84.32, 75.66, 60.34, 58.05, 52.60, 52.54, 52.50, 52.47, 43.31, 38.42, 38.37, 24.59, 24.48, 24.45, 24.53, 20.45, 20.37, 20.36, 20.28, 14.16, 12.50, 12.48;

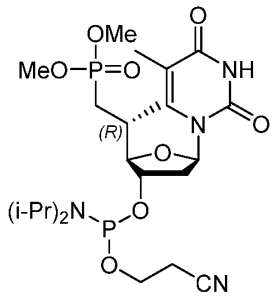
HPLC: HPLC purity: 95.15%;

TLC (Dichloromethane / Methanol) Rf = 0.06.

Example 19. Synthesis of 5'-(R)-Me-PO(OMe)$_2$-Phosphonate-dT (WV-NU-128), and 5'-(R)-Me-PO(OMe)$_2$-Phosphonate-3'-CNE-dT Phosphoramidite (WV-NU-128-CNE)

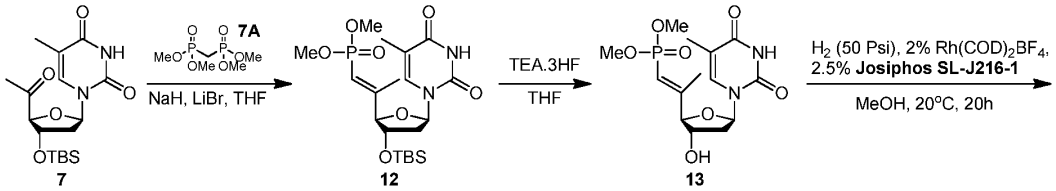


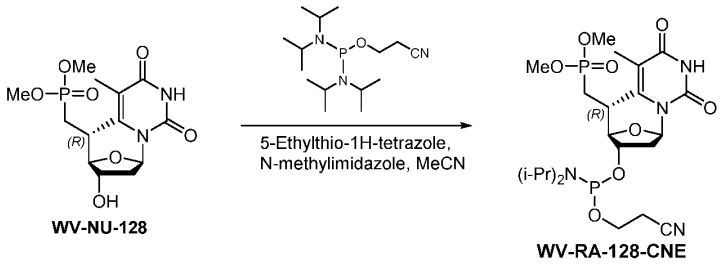
WV-NU-128



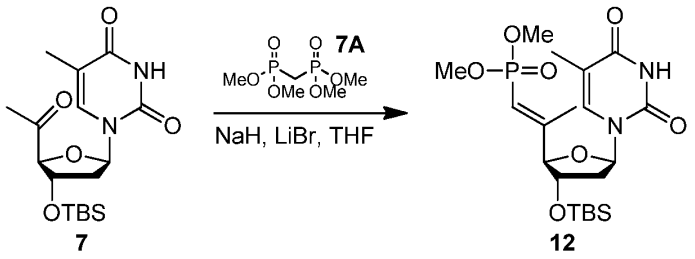
WV-NU-128-CNE

General Scheme





1. Preparation of Compound 12



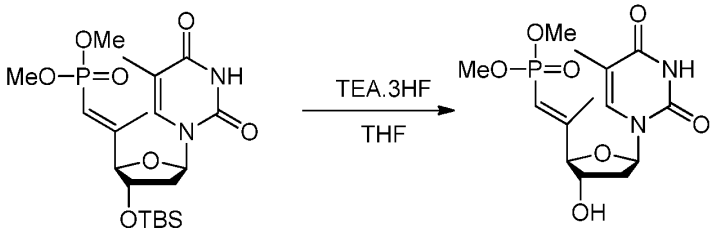
7 12

To a solution of NaH (4.78 g, 119.40 mmol, 60% purity) in THF (360 mL) was added bis(dimethoxyphosphoryl)methane (46.19 g, 119.40 mmol) in THF (200 mL) at 0 °C. The reaction mixture was warmed up to 20 °C, and stirred for 1 hr. A solution of LiBr (10.37 g, 119.40 mmol) in THF (200 mL) was added and the resultant slurry was stirred, and then cooled to 0 °C. To the above mixture was added a solution of compound **7** (20 g, 54.27 mmol) in THF (200 mL) at 0 °C. The mixture was stirred at 0 – 20 °C for 11 hr. TLC indicated compound **7** was consumed and two new spots formed. The resulting mixture was diluted with water (300 mL), extracted with EtOAc (300 mL*3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to afford a yellow oil. The crude compound **12** (25 g, crude) was obtained as a yellow oil. The residue was purified by column chromatography (SiO_2, Petroleum ether/ Ethyl acetate = 1/ 10 to 0/ 1, then ethyl acetate/ Methanol = 10/ 1). Compound **12** (6.1 g, 24.40% yield) was obtained as a white solid.

LCMS: $M+H^+$=475.2

TLC: (Ethyl acetate: Petroleum ether = 3:1), R_f = 0.20

2. Preparation of Compound 13



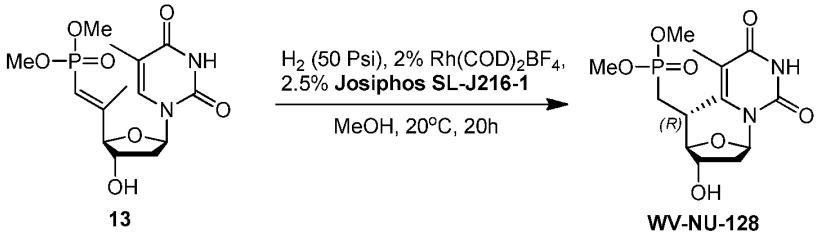
12 13

To a solution of compound **12** (6.1 g, 12.85 mmol) in THF (61 mL) was added 3HF.TEA (8.29 g, 51.42 mmol). The mixture was stirred at 25 °C for 12 hr. TLC indicated compound **12** was consumed and one new spot formed. The reaction mixture was quenched by addition sat. $NaHCO_3$ aq. (60 mL) and $NaHCO_3$ solid to pH = 7 ~ 8 and stirred 20 min. The mixture was dried over Na_2SO_4, and concentrated under reduced

pressure to give a residue. The crude compound **13** (4.4 g, crude) was obtained as a yellow oil.

TLC: (Petroleum ether: Ethyl acetate = 0:1) $R_f = 0$

3. Preparation of Compound WV-NU-128



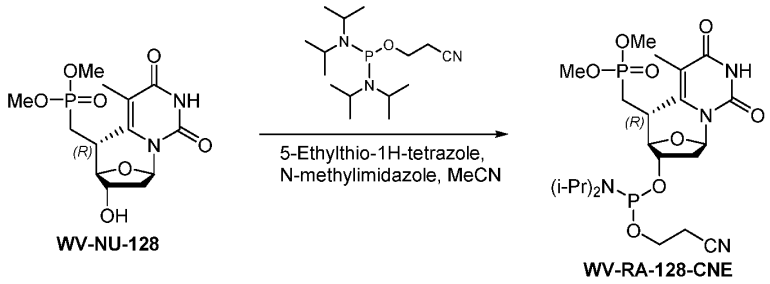
13 **WV-NU-128**

To a solution of compound **13** (5 g, 13.88 mmol) in MeOH (220 mL) were added Josiphos SL-J216-1 (425 mg, 1.39 mmol) (1Z,5Z)-cycloocta-1,5-diene;rhodium(1+); tetrafluoroborate (230 mg) and zinc;trifluoromethanesulfonate (2.06 g, 5.55 mmol). The mixture was stirred at 25 °C for 12 hr in H_2 (50 psi). LCMS showed the compound **13** was consumed and the main peak was desired. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by column chromatography (SiO_2, Petroleum ether/ Ethyl acetate = 1/ 0 to 0/ 1 then ethyl acetate/ methanol = 10: 1). The crude compound **WV-NU-128** (4 g, 79.55% yield) was obtained as a yellow solid.

LCMS: $M+H^+ = 363.2$

TLC: (Ethyl acetate: Methanol = 10:1), $R_f = 0.36$.

4. Preparation of CompoundWV-RA-128-CNE



WV-NU-128 **WV-RA-128-CNE**

To a solution of compound **WV-NU-128** (4 g, 11.04 mmol) in DMF (28 mL) was added 5-ethylsulfanyl-2H-tetrazole (1.44 g, 11.04 mmol) and 1-methylimidazole (1.81 g, 22.08 mmol), then added 3-bis(diisopropylamino)phosphanyloxypropanenitrile (4.99 g, 16.56 mmol). The mixture was stirred at 25 °C for 2 hr. TLC indicated compound **WV-NU-128** was consumed and two new spots formed. Sat. $NaHCO_3$ aq. (50 mL) at 0 °C was added to

the reaction mixture. Subsequently, the reaction mixture was diluted with EtOAc (20 mL) and extracted with EtOAc (20 mL * 3), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/ Ethyl acetate = 1/ 0 to 0/ 1, then Ethyl acetate/ Acetonitrile = 10/1, 5% TEA). Compound **WV-RA-128-CNE** (2.5 g, 40.25% yield) was obtained as a yellow oil.

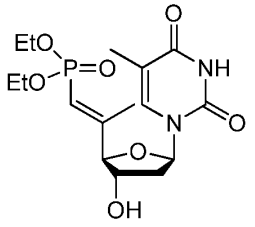
^{1}H NMR (400 MHz, $CDCl_3$**)** δ = 7.11 (d, J = 1.0 Hz, 1H), 6.29 - 6.15 (m, 1H), 4.44 - 4.29 (m, 1H), 3.93 - 3.79 (m,2H), 3.75 (dd, J = 5.9, 10.8 Hz, 7H), 3.63 (br d, J = 2.5 Hz, 2H), 2.74 - 2.60 (m, 2H), 2.53 - 2.36 (m, 1H), 2.34 - 2.21 (m, 1H), 2.19 - 2.06 (m, 2H), 1.93 (s, 3H), 1.78 - 1.61 (m, 1H), 1.21 - 1.09 (m, 15H)

^{13}C NMR (101 MHz, $CDCl_3$**)** δ = 163.54, 135.21, 135.05, 111.51, 83.59, 83.50, 77.36, 77.05, 76.72, 52.35, 52.32,43.34 (dd, J = 7.3, 12.5 Hz, 1C), 30.82, 29.10, 24.66, 24.63, 24.59, 24.50 (dd, J = 2.9, 8.1 Hz, 1C), 16.21, 12.60

LCMS: M-H^+ = 561.2, purity 93.7%

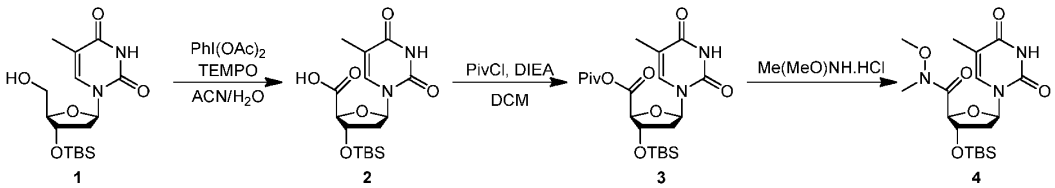
TLC: (Ethyl acetate: Methanol = 8:1) R_f = 0.45

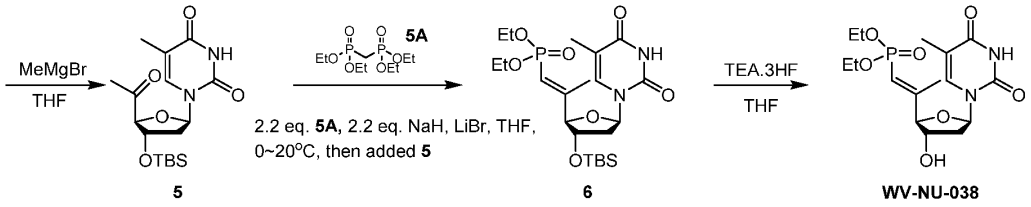
Example 20. Synthesis of 5'-Me-PO(OEt)$_2$-Vinylphosphonate-dT (WV-NU-038).



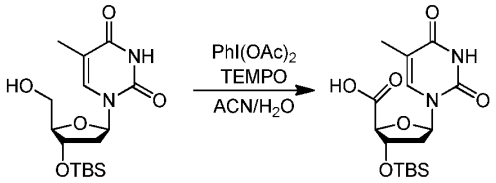
WV-NU-038

General Scheme





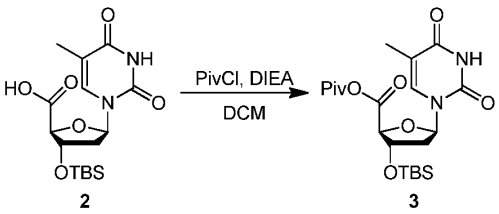
1. Preparation of compound 2



To a solution of compound **1** (15 g, 42.08 mmol) in the mixture of ACN (60 mL) and H_2O (60 mL) was added $PhI(OAc)_2$ (29.82 g, 92.57 mmol) and TEMPO (1.32 g, 8.42 mmol) at 20 °C. The mixture was stirred at 20 °C for 2 hr. TLC showed the reaction was completed. The resulting mixture was concentrated to dry to give a residue, which was triturated with ACN (100 mL), filtered, and the filter cake was rinsed with ACN (50 mL) and dried to give compound **2** (10.6 g, 68.00% yield) as a white solid. The combined filtrate was concentrated under reduced pressure and dried to give another part of crude product (7.4 g).

TLC (Ethyl acetate /Petroleum ether = 1:1) Rf = 0.01.

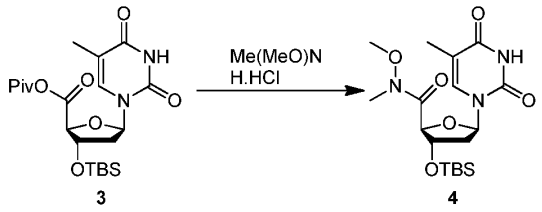
2. Preparation of compound 3



To a solution of crude compound **2** (7.4 g, 19.97 mmol) in DCM (70 mL) was added DIEA (5.16 g, 39.95 mmol, 6.96 mL) and pivaloyl chloride (3.13 g, 25.97 mmol). The mixture was stirred at -10-0 °C for 1.5 hr. TLC showed the reaction was almost completed. The crude brown color solution of compound **3** (9.08 g, 100.00% yield) in DCM was used directly for the next step.

TLC (Ethyl acetate / Petroleum ether = 1:1) Rf = 0.28.

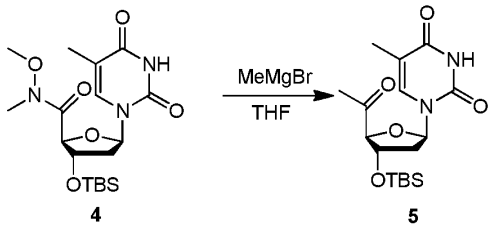
3. Preparation of compound 4



To the crude solution of compound **3** (9.08 g, 19.97 mmol) in DCM from the last step was added TEA (6.06 g, 59.92 mmol) followed by N-methoxymethanamine; hydrochloride (5.85 g, 59.92 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 hr. TLC showed the reaction was almost completed. The resulting mixture was washed with HCl (1N, 60 mL*2) and then aqueous $NaHCO_3$ (50 mL*2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to get the product as a crude brown solid (10 g). The crude product was purified by column chromatography on silica gel (Petroleum ether: Ethyl acetate, 10%~60%). Compound **4** (2.7 g, 6.53 mmol, 32.69% yield) was obtained as a white solid.

TLC (Petroleum ether / Ethyl acetate = 1:1) Rf = 0.43.

4. Preparation of compound 5



To a solution of compound **4** (17.2 g, 41.59 mmol) in THF (170 mL) was added MeMgBr (3 M, 27.73 mL) at 0 °C. The mixture was stirred at 0 °C for 1.5 hr. TLC showed the reaction was completed. The resulting mixture was poured into sat. NH_4Cl aq. (300 mL) under stirring, extracted with EtOAc (100 mL*3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to give a light yellow gum. The crude product was purified by column chromatography on silica gel (Petroleum ether: Ethyl acetate = 8: 1, 4: 1, 2: 1). Compound 5 (10.9 g, 67.14% yield, >94.4% purity) was obtained as a white solid.

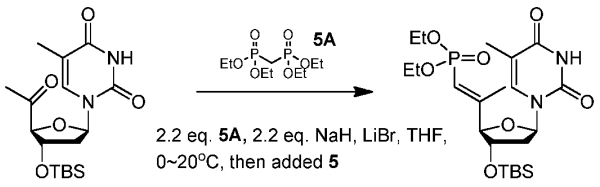
HNMR (400MHz, $CDCl_3$**)** Shift = 8.76 (br s, 1H), 7.95 (s, 1H), 6.41 (dd, J=5.7, 7.9 Hz, 1H), 4.55 - 4.41 (m, 2H), 2.33 - 2.21 (m, 4H), 2.03 - 1.87 (m, 4H), 0.92 (s, 9H), 0.14 (d,

J=3.5 Hz, 6H)

LCMS ($M+H^+$) 369.3;

TLC (Petroleum ether /EtOAc = 1:1, two times) Rf = 0.63.

5. Preparation of compound 6



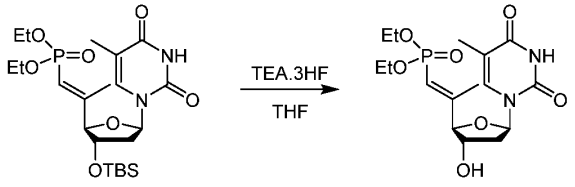
5 6

To a suspension of NaH (4.78 g, 119.40 mmol, 60% purity) in THF (100 mL) was added compound **5A** (34.41 g, 119.40 mmol) in THF (100 mL) at 0 °C. The reaction mixture was warmed up to 20 °C, and stirred for 1 hr. A solution of LiBr (10.37 g, 119.40 mmol) in THF (100 mL) was added, and the resultant slurry was stirred, and then cooled to 0 °C. To the above mixture was added a solution of compound **5** (20 g crude, 54.27 mmol) in THF (100 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 hr and then allowed to warm up to 20 °C, and stirred at 20 °C for 64 hr. TLC showed compound **5** was remained and one main spot was detected. The resulting mixture was diluted with water (400 mL), extracted with EtOAc (400 mL*3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to afford a yellow oil. The residue was purified by flash silica gel chromatography (ISCO®; 220 g SepaFlash® Silica Flash Column, Eluent of 0~50% Ethyl acetate/Petroleum ethergradient @ 100 mL/min). Compound **6** (6.7 g, 21.69% yield, 88.3% purity) was obtained as a yellow oil.

LCMS: ($M+H^+$):503.1;

TLC (Petroleum ether /Ethyl acetate = 1:3) Rf = 0.11.

6. Preparation of compound WV-NU-038



6 WV-NU-038

To a solution of compound **6** (6.4 g, 12.73 mmol) in THF (64 mL) was added TEA.3HF (8.38 g, 50.93 mmol). The mixture was stirred at 15 °C for 12 hr. LCMS showed that some compound **6** remained and one main peak with desired mass was detected. The reaction mixture was quenched by addition $NaHCO_3$ (aq., sat. 64 mL), and then extracted with Ethyl acetate (70 mL * 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residues was purified by flash silica gel chromatography (ISCO®; 80 g SepaFlash® Silica Flash Column, Eluent of 0~60% Ethyl acetate/Petroleum ethergradient @ 100 mL/min). The compound **WV-NU-038** (2.85 g, 56.35% yield, 97.78% purity) was yellow solid.

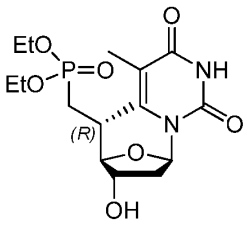
1H NMR (400MHz, CHLOROFORM-d) δ = 9.61 (s, 1H), 7.14 (s, 1H), 6.36 (t, J=6.8 Hz, 1H), 5.78 (d, J=17.9 Hz, 1H), 4.56 (br s, 1H), 4.36 (br s, 1H), 4.26 (br d, J=4.0 Hz, 1H), 4.13 - 3.96 (m, 4H), 2.36 (ddd, J=4.0, 6.3, 13.7 Hz, 1H), 2.25 - 2.12 (m, 4H), 1.92 (s, 3H), 1.38 - 1.28 (m, 7H)).

13C NMR (101MHz $CDCl_3$,) δ = 163.77, 158.00, 157.92, 150.70, 135.49, 112.27, 111.75, 88.91, 88.69, 84.46, 73.57, 61.81, 61.65, 39.18, 16.61, 16.54, 16.41, 16.35, 16.30, 12.63).

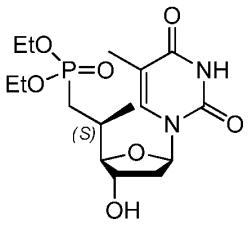
31P NMR (162MHz, $CDCl_3$) δ = 17.63 (s, 1P).

LCMS: ($M+H^+$) = 389.1; LCMS purity: 99.2%.

Example 21. Synthesis of 5'-(R)-Me-PO(OEt)$_2$-dT (WV-NU-037) and 5'-(S)-Me-PO(OEt)$_2$-dT (WV-NU-037A)

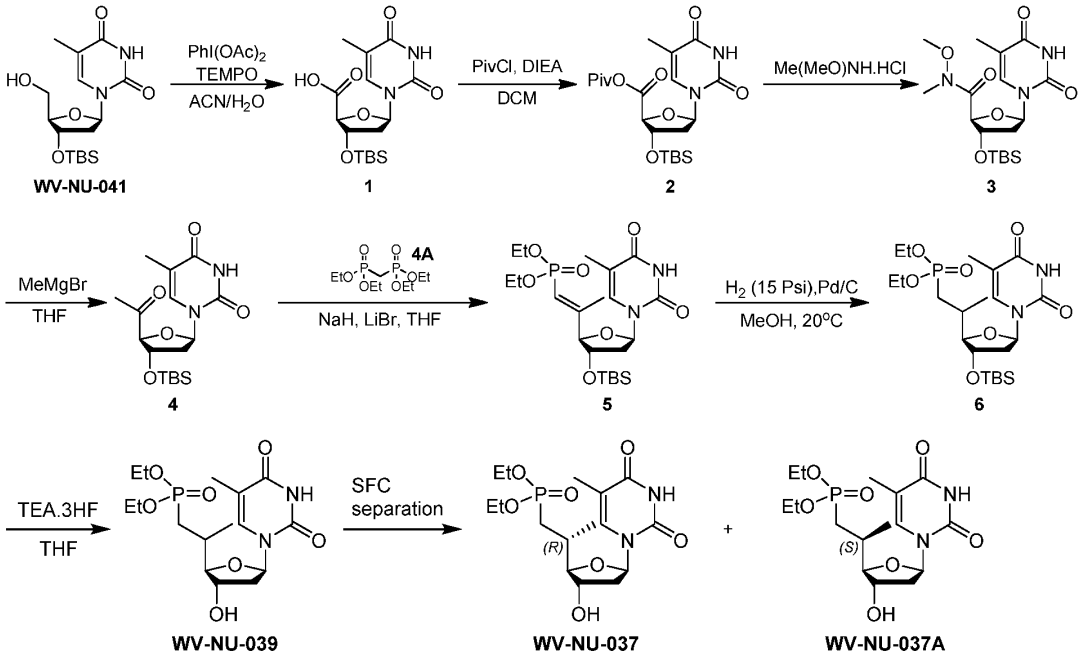


WV-NU-037

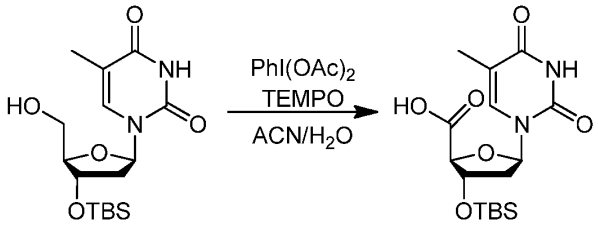


WV-NU-037A

General Scheme



1. Preparation of compound 1



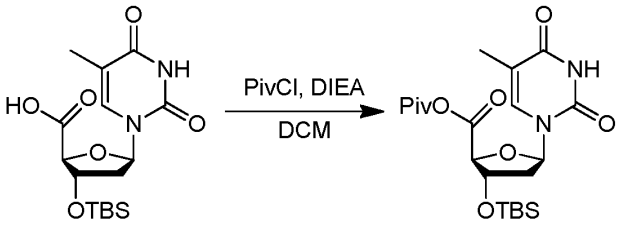
To a solution of compound **WV-NU-041** (150 g, 420.77 mmol) in the mixture of ACN (600 mL) and H_2O (600 mL) was added $PhI(OAc)_2$ (298.17 g, 925.70 mmol) and TEMPO (13.23 g, 84.15 mmol). The mixture was stirred at 20 °C for 2 hr. TLC showed the reaction was completed. The resulting mixture was concentrated to dry to give a residue, which was triturated with ACN (550 mL), filtered, and the filter cake was rinsed with ACN (300 mL) and dried to give compound **1** (113.4 g, 72.75% yield) as a white solid.

^{1}H NMR (400MHz, METHANOL-d$_4$) δ = 8.28 (s, 1H), 6.44 (dd, *J*=5.0, 9.0 Hz, 1H), 4.69 (d, *J*=4.4 Hz, 1H), 4.42 (s, 1H), 2.25 (dd, *J*=5.0, 13.4 Hz, 1H), 2.09 - 1.97 (m, 1H), 1.89 (s, 3H), 0.94 (s, 9H), 0.17 (d, *J*=4.8 Hz, 6H)

LCMS: ($M+H^+$): 371.2

TLC (Petroleum ether : Ethyl acetate = 1:1), Rf = 0.00

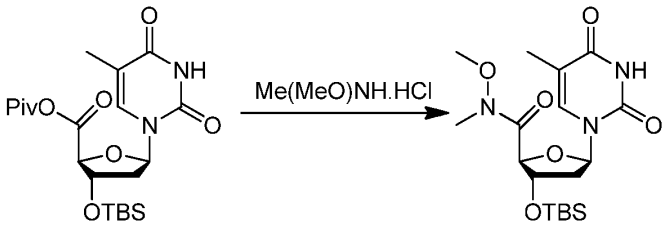
2. Preparation of compound 2



1 2

To a solution of compound **1** (110 g, 296.92 mmol) in DCM (1100 mL) was added DIEA (76.75 g, 593.84 mmol) and 2,2-dimethylpropanoyl chloride (46.54 g, 385.99 mmol). The mixture was stirred at -10 - 0 °C for 1.5 hr. TLC indicated compound **1** was remained a little and two new spots formed. The crude product compound **2** (134.98 g, 100.00% yield) in DCM was used into the next step without further purification.

3. Preparation of compound 3



2 **3**

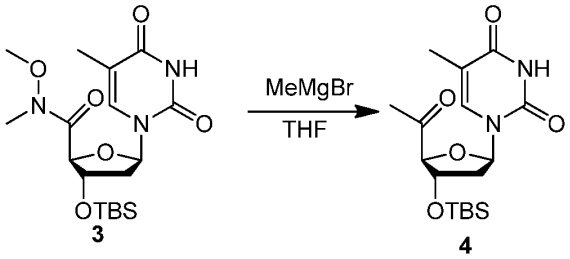
To a solution of compound **2** (134.9 g, 296.75 mmol) in DCM was added TEA (90.09 g, 890.26 mmol), then added N-methoxymethanamine;hydrochloride (86.84 g, 890.26 mmol). The mixture was stirred at 0 °C for 2 hr. TLC indicated compound **2** was consumed and one new spot formed. The resulting mixture was washed with HCl (1N, 800 mL *2) and then aqueous $NaHCO_3$ (600 mL* 2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to get the product as a crude white solid. The residue was purified by column chromatography (SiO_2, Petroleum ether/ Ethyl acetate = 1/0 to 0:1). Compound **3** (115 g, 93.71% yield) was obtained as a white solid.

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 8.62 (br s, 1H), 8.38 (s, 1H), 6.57 (dd, J=5.1, 9.3 Hz, 1H), 4.81 (s, 1H), 4.48 (br d, J = 4.0 Hz, 1H), 3.80 - 3.72 (m, 3H), 3.25 (s, 3H), 2.23 (dd, J = 5.1, 13.0 Hz, 1H), 2.09 - 2.01 (m, 1H), 1.97 (d, J = 1.0 Hz, 3H), 0.91(s, 9H), 0.10 (d, J = 3.8 Hz, 6H)

LCMS: ($M+H^+$): 414.2

TLC (Petroleum ether: Ethyl acetate = 1:1), R_f = 0.39

4. Preparation of compound 4



3 4

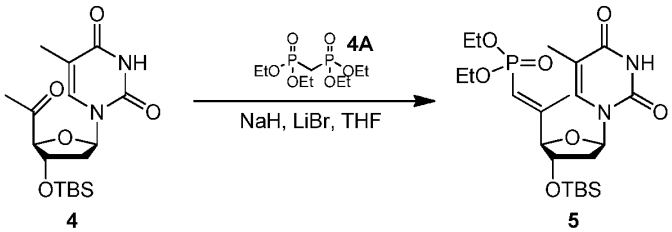
To a solution of compound **3** (115 g, 278.09 mmol) in THF (1150 mL) was added MeMgBr (3 M, 185.39 mL). The mixture was stirred at 0 °C for 1.5 hr. TLC indicated compound **3** was consumed and two new spots formed. The resulting mixture was poured into sat. NH_4Cl aq. (1000 mL) under stirring, extracted with EtOAc (500 mL*3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to give a light yellow gum. The residue was purified together with another batch crude (14.5 g-scale of Cpd.3) by column chromatography (SiO_2, Petroleum ether/ Ethyl acetate = 1/ 0 to 0:1). Compound **4** (93.2 g, 90.95% yield) was obtained as a white solid.

^{1}H NMR (400MHz,) $CDCl_3$ δ = 7.96 (s, 1H), 6.41 (dd, J = 5.5, 8.2 Hz, 1H), 4.52 (d, J = 2.2 Hz, 1H), 4.47 (td, J = 2.2,4.9 Hz, 1H), 2.30 - 2.23 (m, 4H), 2.00 - 1.92 (m, 4H), 0.92 (s, 9H), 0.13 (d, J = 3.5 Hz, 6H)

LCMS: ($M+H^+$): 369.2

TLC (Petroleum ether: Ethyl acetate = 1:1), R_f = 0.61

5. Preparation of compound 5



4 5

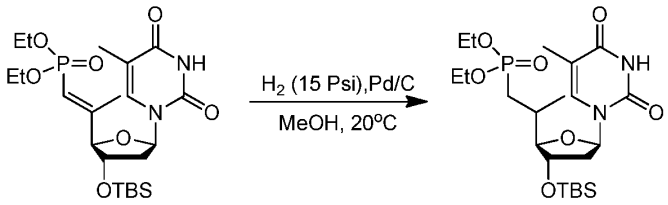
To a solution of NaH (21.49 g, 537.31 mmol, 60% purity) in THF (594 mL) was added 1-[diethoxyphosphorylmethyl(ethoxy)phosphoryl]oxyethane (154.86 g, 537.31 mmol) in THF (594 mL) at 0 °C. The reaction mixture was warmed up to 20 °C, and stirred for 1 hr. A solution of LiBr (46.66 g, 537.31 mmol) in THF (594 mL) was added and the resultant slurry was stirred, and then cooled to 0 °C. To the above mixture was added a solution of compound **4** in THF (468 mL) at 0 °C. The mixture was stirred at 0 - 20 °C for

71 hr. TLC indicated compound **4** was remained a little and three new spots formed. The resulting mixture was diluted with water (100 mL), extracted with EtOAc (100 mL*3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to afford a yellow oil. The residue was purified by column chromatography (SiO_2, Petroleum ether/ Ethyl acetate = 1/ 0 to 0:**1**). Compound **5** (48.74 g, 9.41% yield) was obtained as a yellow oil.

LCMS: ($M+H^+$):503.1

TLC (Ethyl acetate: Petroleum ether = 3 ; 1), R_f = 0.28

6. Preparation of compound 6

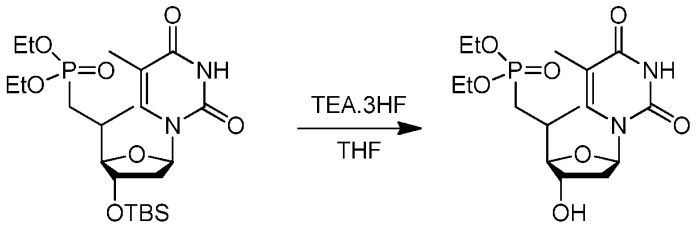


5 **6**

To a solution of compound **5** (40 g, 79.58 mmol) in MeOH (100 mL) was added Pd/C (8 g, 10% purity) (50 % water) and H_2 (15 psi). The mixture was stirred at 20 °C for 2 hr. LCMS showed the compound **5** was consumed and the main peak was the desired product. The reaction mixture was filtered to remove Pd/C then concentrated under reduced pressure to give a residue. Compound **6** (40.16 g, crude) was obtained as a yellow oil.

LCMS: ($M+H^+$):505.2, 505.1

7. Preparation of compound WV-NU-039



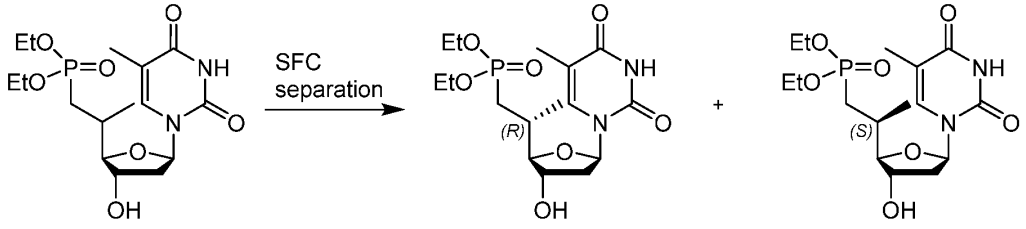
6 **WV-NU-039**

To a solution of compound **6** (40.16 g, 79.58 mmol) in THF (400 mL) was added N,N-diethylethanamine;trihydrofluoride (51.32 g, 318.33 mmol). The mixture was stirred at 25 °C for 16 hr. LCMS showed the compound **6** was consumed and the main peak was the desired product. The reaction mixture was quenched by addition Na_2CO_3 (aq. sat. 400

mL), and extracted with Ethyl acetate (400 mL * 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/ Ethyl acetate = 1/ 0 to 0:1). Compound **WV-NU-039** (30 g, 96.57% yield) was obtained as a yellow oil. Then purified by SFC (column: DAICEL CHIRALPAK ADH (250mm*30mm,5um); mobile phase: [Neu-ETOH];B%: 35%-35%,8.5min). .

LCMS: ($M+H^+$):390.9

8. Preparation of compound NU-037, WV-NU-037A



WV-NU-039 **WV-NU-037** **WV-NU-037A**

Compound **WV-NU-037** (11.3 g, 37.43% yield, 99.37% purity) was obtained as a white solid.

^{1}H NMR (400MHz $CDCl_3$**,)** δ = 9.49 - 9.10 (m, 1H), 7.11 (d, J=1.1 Hz, 1H), 6.22 (t, J=6.6 Hz, 1H), 4.54 (br d, J=4.9 Hz, 1H), 4.28 (br dd, J=4.0, 7.3 Hz, 1H), 4.20 - 4.00 (m, 4H), 3.68 (dd, J=5.1, 7.3 Hz, 1H), 2.38 (ddd, J=4.5, 6.6, 13.8 Hz, 1H), 2.29 - 1.97 (m, 3H), 1.93 (s, 3H), 1.83 - 1.63 (m, 1H), 1.34 (dt, J=3.7, 7.1 Hz, 6H), 1.17 (d, J=6.6 Hz, 3H).

^{13}C NMR (101MHz, $CDCl_3$ δ = 163.91, 150.51, 135.20, 111.30, 89.49, 89.36, 83.53, 72.16, 61.94 (dd, J=6.6, 13.2 Hz, 1C), 58.29, 40.29, 31.55, 31.52, 29.95, 28.56, 18.38, 17.77, 17.69, 16.47, 16.40, 12.71

LCMS: ($M+H^+$): 391.1; LCMS purity: 99.37%

SFC: (AD-3_EtOH_IPAm_10-40_Gradient_4ml), SFC purity =100.00 %;

Compound **WV-NU-037A** (16.2 g, 54.00% yield, 100% purity) was obtained as a white solid.

^{1}HNMR (400MHz, $CDCl_3$**)** δ = 8.93 (s, 1H), 7.19 (d, J=1.1 Hz, 1H), 6.29 (dd, J=6.3, 7.6 Hz, 1H), 4.35 (qd, J=3.5, 7.1 Hz, 1H), 4.22 - 4.02 (m, 4H), 3.88 - 3.75 (m, 2H), 2.37 (ddd, J=3.2, 6.1, 13.8 Hz, 1H), 2.31 - 2.14 (m, 1H), 2.13 - 2.02 (m, 2H), 1.95 (d, J=0.9 Hz, 3H),

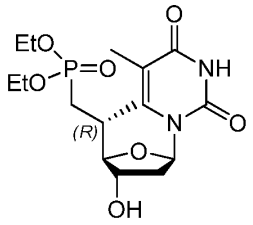
1.69 (ddd, J=8.2, 15.4, 18.7 Hz, 1H), 1.34 (dt, J=5.5, 6.9 Hz, 6H), 1.17 (d, J=6.6 Hz, 3H).

^{13}CNMR (101MHz, $CDCl_3$**)** δ = 163.88, 150.59, 135.33, 111.59, 89.39, 89.26, 83.94, 71.49, 61.84 (dd, J=6.2, 20.2 Hz, 1C), 40.02, 31.41, 31.38, 29.15, 27.75, 17.12, 17.06, 16.45 (dd, J=2.2, 5.9 Hz, 1C), 12.53

LCMS: ($M+H^+$): 391.1; LCMS purity: 100%

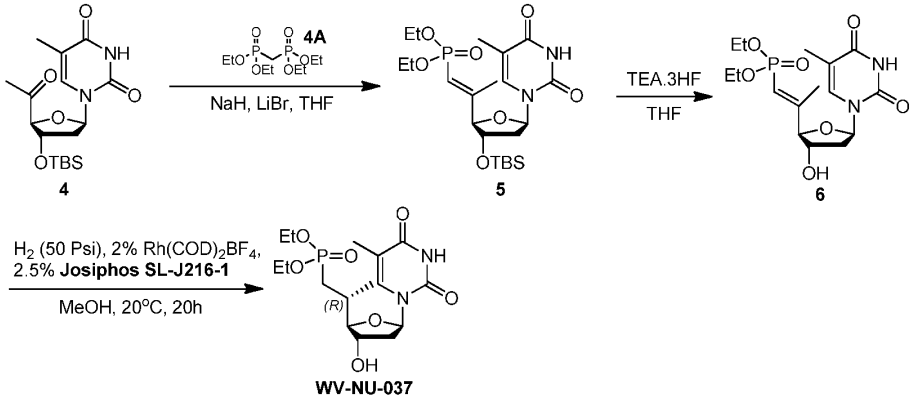
SFC: (AD-3_EtOH_IPAm_10-40_Gradient_4ml), SFC purity =100.00 %.

Example 22. Modified method of Synthesis of 5'-(R)-Me-PO(OEt)$_2$-phosphonate-dT (WV-NU-037).

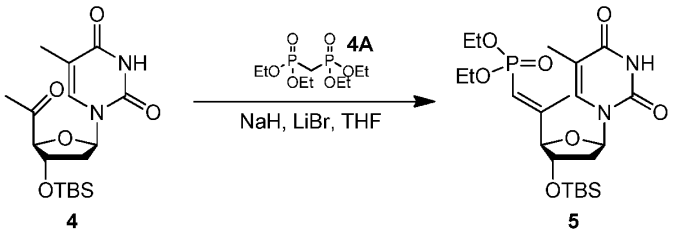


WV-NU-037

General Scheme



1. ***Preparation of compound 5***



4 5

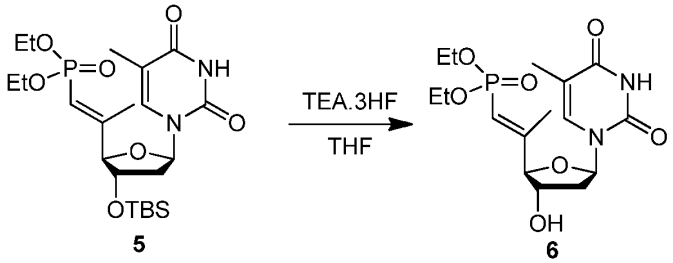
To a solution of NaH (23.88 g, 597.02 mmol, 60% purity) in THF (900 mL) was added compound **4A** (172.07 g, 597.02 mmol) in THF (500 mL) at 0 °C. The reaction mixture was warmed up to 20 – 30 °C, and stirred for 1 hr. A solution of LiBr (51.85 g, 597.02 mmol) in THF (500 mL) was added and the resultant slurry was stirred, and then cooled to 0°C. To the above mixture was added a solution of compound **4** (50 g, 135.69 mmol) in THF (500 mL) at 0 °C. The mixture was stirred at 0 – 15 °C for 11 hr. TLC indicated compound **4** was consumed completely and desired product had formed. The resulting mixture was diluted with water (500 mL), extracted with EtOAc (500 mL*3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to afford a yellow oil. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=5/1 to 1:1). Compound **5** (63 g, 90.44% yield, 97.9% purity) was obtained as a yellow solid.

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 8.98 (br s, 1H), 7.05 (s, 1H), 6.24 (t, *J*=6.8 Hz, 1H), 5.71 (d, *J*=17.2 Hz, 1H), 4.23 - 3.97 (m, 8H), 2.25 - 2.12 (m, 1H), 2.11 - 2.03 (m, 4H), 1.88 (s, 3H), 1.30 - 1.07 (m, 9H), 0.83 (s, 9H), 0.04 - 0.00 (m, 7H).

LCMS: ($M+H^+$): 503.1.

TLC (Ethyl acetate: Petroleum ether = 3: 1), R_f= 0.16.

2. Preparation of compound 6



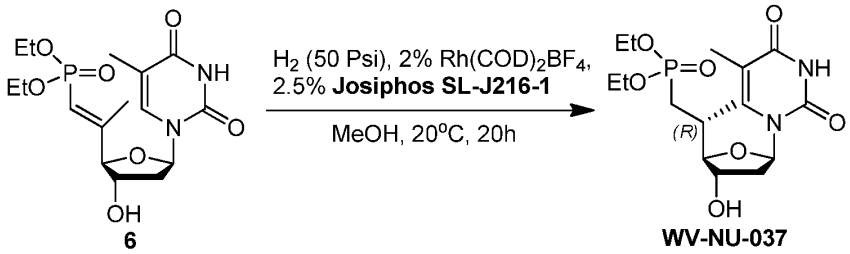
5 6

To a solution of compound **5** (57 g, 113.41 mmol) in THF (600 mL) was added N,N-diethylethanamine;trihydrofluoride (73.13 g, 453.63 mmol). The mixture was stirred at 15

°C for 6 hr. TLC showed compound **5** was consumed completely. One new spot was desired compound. The reaction mixture was quenched by addition sat. $NaHCO_3$ aq. (20 mL) and $NaHCO_3$ solid to pH = 7 ~ 8 and stirred 20 min. The mixture was dried over Na_2SO_4, and concentrated under reduced pressure to give a residue. Compound **6** (44 g, crude) was obtained as a yellow solid.

TLC (Ethyl acetate: Methanol = 15: 1), R_f = 0.43.

3. Preparation of compound WV-NU-037



To a mixture of compound **6** (43 g, 110.72 mmol) in MeOH (1840 mL) was added (1Z,5Z)-cycloocta-1,5-diene;rhodium(1+);tetrafluoroborate (1.80 g, 4.43 mmol), Josiphos SL-J216-1 (CAS#: 849924-43-2, 3.32 g, 5.09 mmol) and zinc;trifluoromethanesulfonate (16.10 g, 44.29 mmol). And the system was stirred under H_2 (50 psi) for 12 hr at 25 °C. LC-MS showed compound **6** was consumed completely and one main peak with desired MS was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. Combined with 6 g crude. The residue was purified by flash silica gel chromatography (ISCO®; 80 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethyl acetate/Petroleum ether gradient and 10% Petroleum ether gradient/MeOH @ 100 mL/min) to give 36.2 g (average weight 30.4 g). And 18.5 g crude. Compound WV-NU-037 (30.4 g, 77.88 mmol, 70.33% yield) was obtained as a black brown solid. And 18.5 g crude. The residue was purified by flash silica gel chromatography (ISCO®; 220 g SepaFlash® Silica Flash Column, Eluent of 20:60:20, 100:0;20, Ethyl acetate/Petroleum / DCM ether gradient @ 80 mL/min). Then washed with 200 mL (Petroleum ether : Ethyl acetate = 1: 1) to give 39.8 g off white solid.

^{1}HNMR (400MHz, $CDCl_3$**)** δ= 8.68 (br s, 1H), 7.10 (s, 1H), 6.18 (t, J=6.5 Hz, 1H), 4.29 (br d, J=4.6 Hz, 2H), 4.20 - 4.04 (m, 4H), 3.66 (br dd, J=5.1, 7.3 Hz, 1H), 2.38 (td, J=6.7, 11.7 Hz, 1H), 2.26 - 1.97 (m, 4H), 1.93 (s, 4H), 1.84 - 1.70 (m, 1H), 1.34 (dt, J=4.5, 7.0 Hz, 7H), 1.17 (d, J=6.8 Hz, 3H)

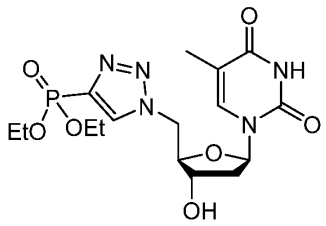
^{31}PNMR (162MHz, $CDCl_3$**)** δ= 31.55 (s, 1P), 31.49 (s, 1P)

^{13}CNMR (101MHz, $CDCl_3$**)** δ= 163.52, 150.30, 135.35, 111.33, 89.42, 89.31, 83.74, 72.36, 62.15 (dd, J=6.6, 12.5 Hz, 1C), 40.18, 31.70, 29.92, 28.52, 18.34, 18.26, 16.43, 16.36, 12.63

SFC: method (AD-3_EtOH_IPAm_10-40_Gradient_4ml_A) dr = 97.43: 2.57

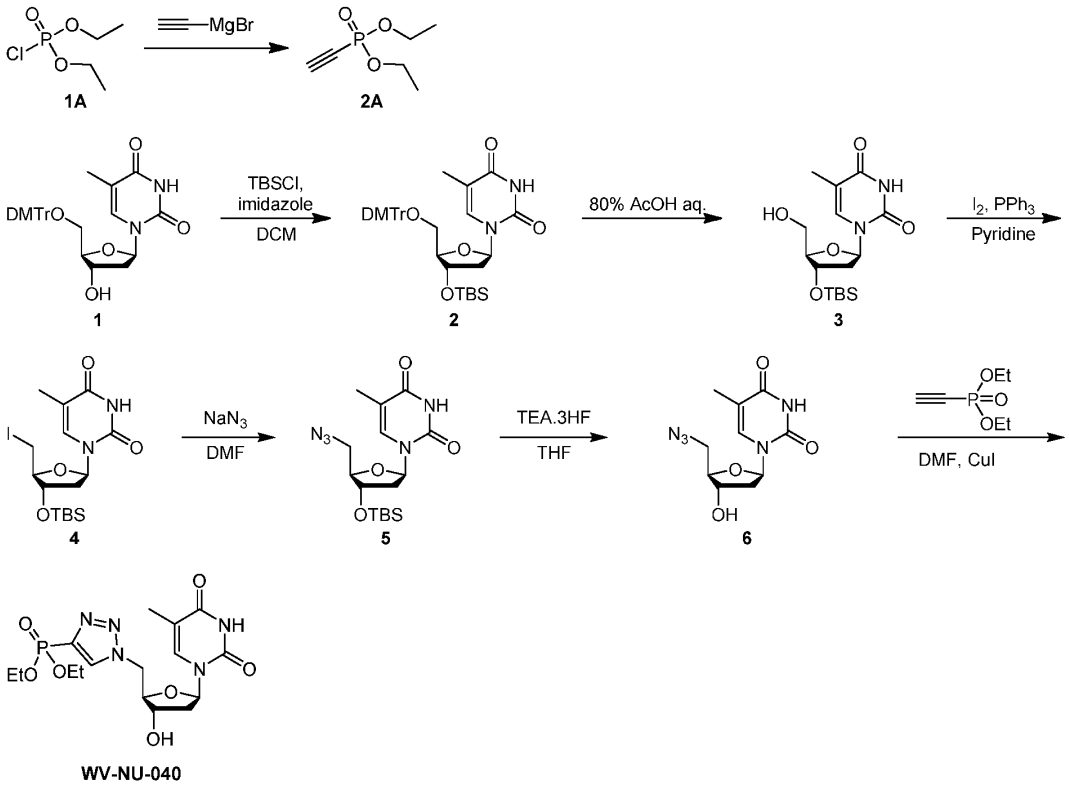
LCMS ($M+H^+$): 391.1; LCMS purity: 99.37%

Example 23. Synthesis of 5'-PO(OEt)$_2$-Triazolyl phosphonate-dT (WV-NU-040).

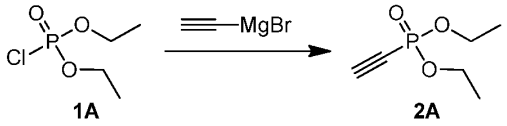


WV-NU-040

General Scheme



1. Preparation of compound 2A



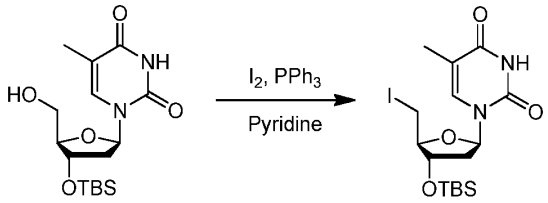
1A 2A

To a solution of compound **1A** (10 g, 57.96 mmol) in THF (20 mL) was added bromo(ethynyl)magnesium (0.5 M, 117.07 mL) at 0 °C under N_2. The resulting mixture was stirred at 20 °C for 0.5 hr. TLC showed compound **1A** was consumed completely and two new spots formed. The mixture was quenched by addition sat. NH_4Cl (aq., 50 mL) at 0 °C, then diluted with Ethyl acetate (30 mL) and extracted with Ethyl acetate (150 mL*3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 10: 1 to 1: 1). Compound **2A** (5.2 g, 55.34% yield) was obtained as a colorless oil.

LCMS: ($M+H^+$): 163.3.

TLC (Petroleum ether/Ethyl acetate = 1:1) R_f = 0.43

2. Preparation of compound 4



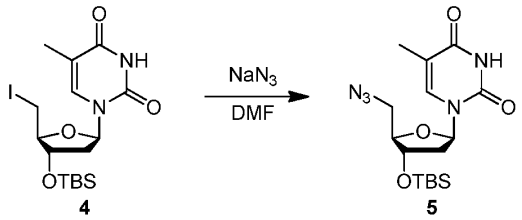
3 4

To a solution of compound **3** (10 g, 28.05 mmol) in pyridine (200 mL) was added PPh_3 (13.24 g, 50.49 mmol) and I_2 (10.68 g, 42.08 mmol). The mixture was stirred at 25 °C for 12 hr under N_2 atmosphere. LCMS showed most of the starting material was consumed and one main peak with desired mass was detected. The reaction mixture was quenched by sat. aq. Na_2SO_3 (200 mL) and extracted with EtOAc (600 mL * 3). The combined organic layers were washed with brine (200 mL * 2), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 10: 1 to 0: 1). Compound **4** (4.8 g, 33.40% yield, 91.041% purity) was obtained as a colorless oil.

LCMS: ($M+H^+$): 467.0;

TLC (Petroleum ether/Ethyl acetate = 1: 3) R_f = 0.75.

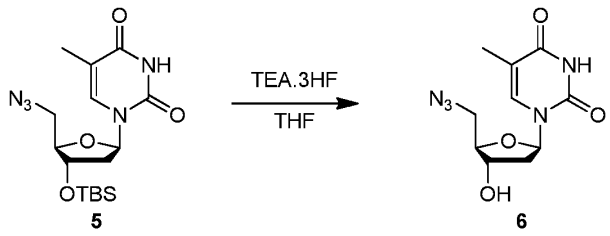
3. Preparation of compound 5



To a solution of compound **4** (4.8 g, 10.29 mmol) in DMF (48 mL) was added NaN_3 (802.89 mg, 12.35 mmol). The mixture was stirred at 50 °C for 12 hr. LCMS showed compound **4** was consumed completely and one main peak with desired MS was detected. The reaction was quenched by H_2O (6 mL), and extracted with TBME (6 mL*3). Compound **5** (3.93 g, crude) in a yellow solution of TBME (18 mL) was used into the next step without further purification.

LCMS: ($M+H^+$): 382.3

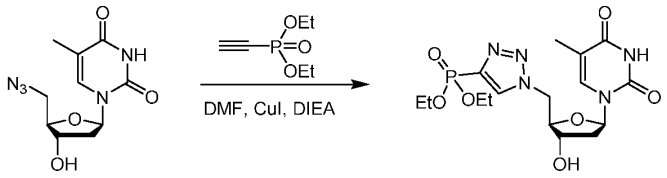
4. Preparation of compound 6



To a solution of compound **5** (3.93 g, 10.30 mmol) in THF (20 mL) was added N,N-diethylethanamine; trihydrofluoride (6.64 g, 41.21 mmol). The mixture was stirred at 20 °C for 12 hr. TLC showed that some compound **5** remained and new spot was detected. The reaction mixture was concentrated under reduced pressure and the mixture was neutralized with Na_2CO_3 (aq., sat.) until pH = 7. The mixture was concentrated under reduced pressure to remove most of water. DCM (40 mL) was added and the mixture was then dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (SiO_2, Petroleum ether: (Ethyl acetate: Ethyl Alcohol = 3: 1) = 1: 1). Compound **6** (2.7 g, crude) was obtained as a yellow oil.

TLC (Petroleum ether: (Ethyl acetate: Ethyl Alcohol = 3: 1) = 1: 1) R_f = 0.24

5. ***Preparation of WV-NU-040***



6 **WV-NU-040**

A solution of compound **6** (2 g, 7.48 mmol) and 1-[ethoxy(ethynyl)phosphoryl]oxyethane (1.42 g, 8.76 mmol) in DMF (20 mL) was degassed and purged with N_2 for 3 times; then DIEA (1.93 g, 14.97 mmol), CuI (285.06 mg, 1.50 mmol) was added. The mixture was stirred at 20 °C for 4 hr under N_2 atmosphere. LCMS showed that most of the starting material had disappeared and the desired substance had formed. The reaction mixture was diluted with TMT solution (8 mL), filtered and the filtrate was diluted with ACN (80 mL), and concentrated under reduced pressure to give a residue. The residue was washed with EtOAc (100 mL * 3), filtered and concentrated under reduced pressure to give product. **WV-NU-040** (1.8 g, 3.92 mmol, 52.38% yield, 93.513% purity) was obtained as a white solid.

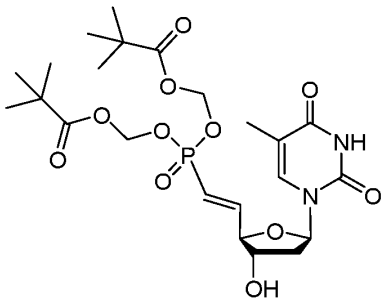
^{1}H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 8.39 (s, 1 H), 6.96 (s, 1 H), 6.07 (t, *J*=6.4 Hz, 1 H), 4.77 (d, *J*=4.4 Hz, 2 H), 4.37 (q, *J*=6.2 Hz, 1 H), 4.19 (q, *J*=4.9 Hz, 1 H), 4.01 - 4.14 (m, 4 H), 2.20 - 2.37 (m, 2 H), 1.73 (s, 3 H), 1.19 (s, 6 H)

^{31}P NMR (162 MHz, DEUTERIUM OXIDE) δ ppm 8.67 (s, 1 P)

^{13}C NMR (101MHz, DEUTERIUM OXIDE) δ = 166.21, 151.53, 137.29, 136.50, 134.08, 133.47, 133.14, 111.55, 85.38, 82.53, 70.15, 64.72, 64.66, 50.60, 36.90, 15.47, 15.41, 11.49.

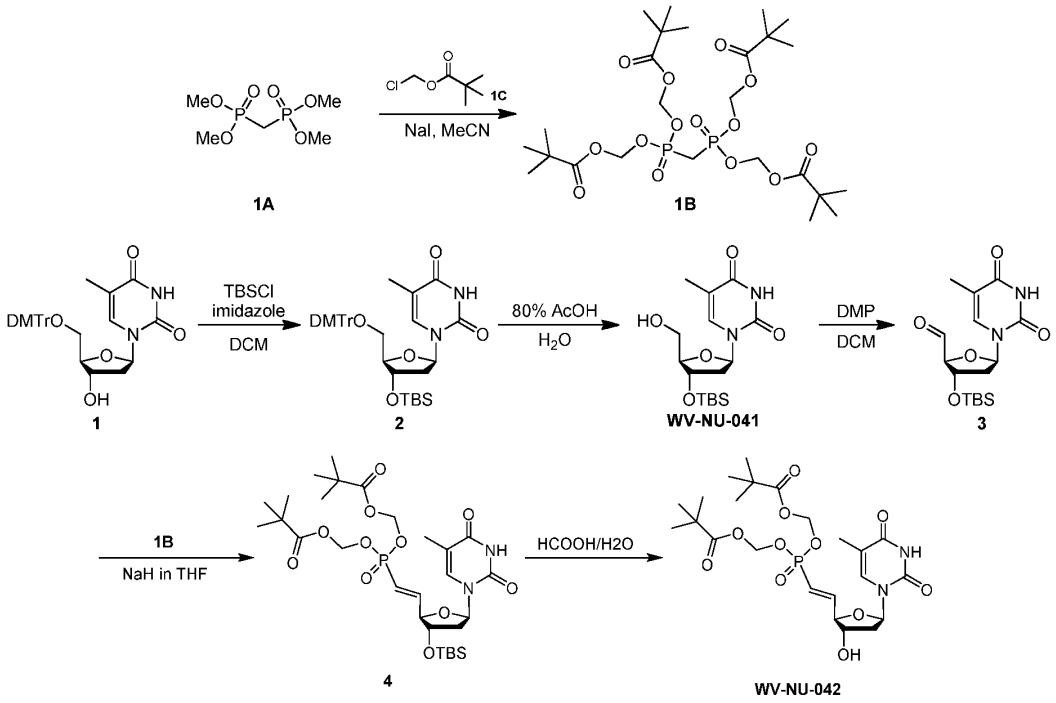
LCMS: ($M+H^+$): 430.1, LCMS purity: 93.513%.

Example 24. Synthesis of 5'-(POM)$_2$-Vinyl Phosphonate-dT (WV-NU-042).

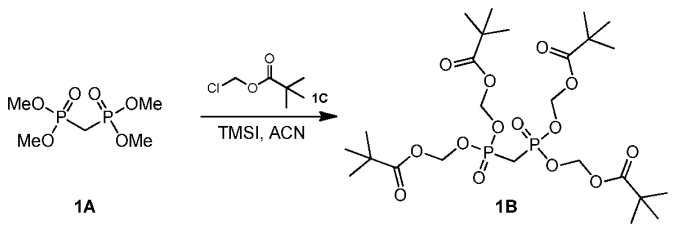


WV-NU-042,

General Scheme



1. Preparation of compound 1B



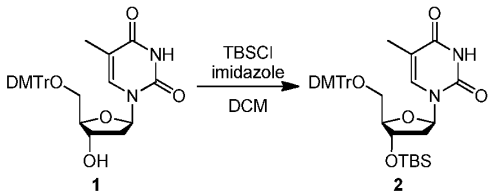
A mixture of compound **1A** (47 g, 202.49 mmol), compound **1C** (152.48 g, 1.01 mol, 146.61 mL), TBAI (74.79 g, 202.49 mmol) in ACN (400 mL) was stirred and refluxed at 85 °C for 15 hr. After this time, compound **1C** (61 g) was added and the reaction mixture was stirred at 85 °C for 15 hr. TLC showed compound **1A** was consumed and a new spot

was detected. The mixture was diluted with Ethyl acetate (300 mL) and H_2O (300 mL), and extracted with Ethyl acetate (300 mL*3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a crude. The crude was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate=10:1, 5:1, 3:1 to 1:1). Compound **1B** (106 g, 82.75% yield) was obtained as a white solid.

^{1}H NMR (400MHz, CHLOROFORM-d) δ = 5.77 - 5.68 (m, 8H), 2.78 - 2.59 (m, 2H), 1.25 (s, 36H).

TLC (Petroleum ether: Ethyl acetate = 1: 1), R_f = 0.43.

2. Preparation of compound 2

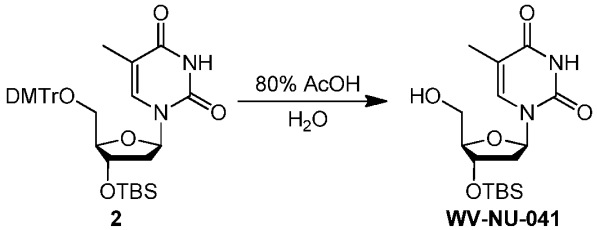


1 2

Three batches: To a solution of compound **1** (234 g, 429.68 mmol) and imidazole (87.75 g, 1.29 mol) in DCM (2 L) was added TBSCl (97.14 g, 644.52 mmol, 78.98 mL). The mixture was stirred at 15 °C for 16 hr. TLC showed compound **1** was consumed. Three batches mixture was combined and washed with sat. $NaHCO_3$ (aq., 4 L * 2), the combined aqueous was extracted with EtOAc (3 L* 2), the combined organic layers were dried over Na_2SO_4, filtered and concentrated to give crude. Compound **2** (850 g, crude) was obtained as a yellow oil.

TLC (Petroleum ether: Ethyl acetate = 1: 1) R_f = 0.39.

3. Preparation of WV-NU-041



2 WV-NU-041

A solution of compound **2** (400 g, 607.11 mmol) in CH_3COOH (1200 mL) and H_2O (300 mL) and stirred at 15 °C for 16 hr. TLC showed that some compound **2** remained in the reaction mixture and a new spot was detected. The reaction suspension liquid was filtered to remove white solid, then filtrate was added to ice water (2 L). Resulting white solid was filtered to give crude. The aqueous layers were extracted with EtOAc (2 L * 4). The combined organic layers were washed with sat.$NaHCO_3$ (aq., 1 L), dried over Na_2SO_4, filtered and combined with above crude, concentrated under reduced pressure to give a crude. The crude material was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate = 5: 1, 3: 1, 1: 1 to 0: 1). Compound **WV-NU-041** (100 g, 46.20% yield) was obtained as a yellow solid.

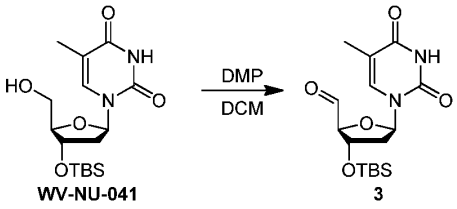
^{1}HNMR (400MHz, $CDCl_3$) Shift = 9.54 (br s, 1H), 7.42 (s, 1H), 6.16 (t, J = 6.7 Hz, 1H), 4.51 - 4.46 (m, 1H), 3.96 - 3.87 (m, 2H), 3.82 - 3.68 (m, 1H), 2.32 (td, J = 6.8, 13.5 Hz, 1H), 2.21 (ddd, J = 3.7, 6.4, 13.2 Hz, 1H), 1.89 (s, 3H), 0.88 (s, 9H), 0.08 (s, 6H).

^{13}CNMR (101MHz, $CDCl_3$) Shift = 164.30, 150.50, 137.15, 110.90, 87.60, 86.67, 71.55, 61.86, 40.53, 25.69, 20.72, 17.92, 12.44, -4.72, -4.88

LCMS: M + Na^+ = 379.2, Purity: 96.33%.

TLC (Petroleum ether: Ethyl acetate = 1: 1) R_f = 0.24.

4. Preparation of compound 3



WV-NU-041 **3**

To a solution of compound **WV-NU-041** (40 g, 112.21 mmol) in DCM (300 mL) was added DMP (66.63 g, 157.09 mmol) in portions at 0 °C. The mixture was stirred at 0 °C for 1 hr, and then warmed to 25°C and stirred at 25°C for 2 hr. TLC showed most of compound **WV-NU-041** was consumed and a new spot was found. The mixture was diluted with ethyl acetate (500 mL) and filtrated through a short silica gel pad (SiO_2, 200 g) using ethyl acetate (500 mL). 5% Na_2SO_3 /sat.$NaHCO_3$ (1:1, 500 mL, aq.) was added at 0 °C, the mixture was extracted with Ethyl acetate (300 mL*2), the combined organic

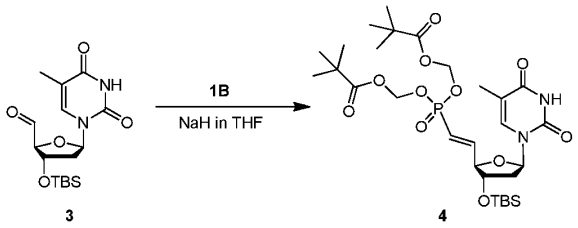
layers were dried over Na_2SO_4, filtered and concentrated to get crude compound **3** (39 g, crude) as a white solid.

LCMS: $M + H^+ = 354.9$.

TLC (Petroleum ether: Ethyl acetate =1: 3) $R_f = 0.36$.

5

5. Preparation of compound 4

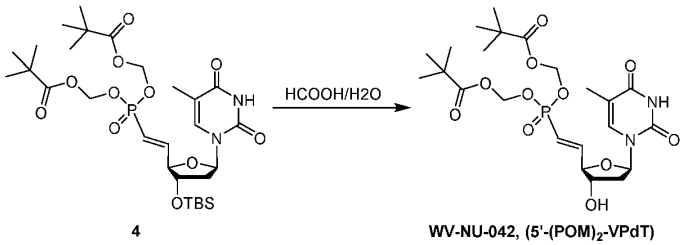


3 4

To a solution of NaH (10.62 g, 265.58 mmol, 60% purity) in THF (400 mL) was added compound **1B** (140 g, 221.32 mmol) in THF (600 mL) at -70°C - -60°C under N_2 over 30 min. The reaction mixture was stirred for 30 min at -70°C - -60°C under N_2. To the above mixture was added a solution of compound **3** (31.38 g, 88.53 mmol) in THF (400 mL) at -70°C - -60°C under N_2 over 30 min. The mixture was stirred at -70°C - -60°C for 1 hr under N_2, 0 °C for 1 hr and then 18 °C for 2 hr. TLC showed compound **3** was consumed. The mixture was added to sat.NH_4Cl (1000 mL, aq.) at 0 °C, extracted with Ethyl acetate (1000 mL * 3). The combined organic layers were concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether: Ethyl acetate = 5: 1, 3: 1 to 1: 1). Compound **4** (25 g, 42.74% yield) was obtained as a colorless oil.

TLC (Petroleum ether: Ethyl acetate = 1: 1) $R_f = 0.18$.

6. Preparation of WV-NU-042, (5'-(E)-(POM)2-VPdT)



4 WV-NU-042, (5'-(POM)$_2$-VPdT)

A solution of compound **4** (35.5 g, 53.73 mmol) in HCOOH (150 mL) and H_2O (150 mL) at 0°C, the mixture was stirred at 0-15 °C for 16 hr. TLC and LCMS showed compound **4** was consumed and new spot was detected. The mixture was concentrated under reduced pressure to give a residue at 30 °C water bath. The residue was purified by MPLC (SiO_2, Ethyl acetate/Petroleum ether = 20%, 50%, 100%). Compound **WV-NU-042, (5'-(E)-(POM)2-VPdT)** (18.6 g, 63.35% yield) was obtained as a yellow gum.

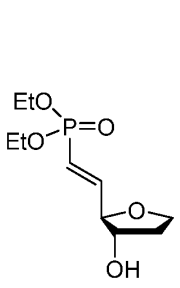
^{1}H NMR (400MHz, $CDCl_3$) Shift = 8.92 (s, 1H), 7.06 (d, J = 0.9 Hz, 1H), 7.01 - 6.84 (m, 1H), 6.28 (t, J = 6.6 Hz, 1H), 6.08 - 5.90 (m, 1H), 5.70 - 5.57 (m, 3H), 5.54 (dd, J = 5.1, 12.3 Hz, 1H), 4.39 - 4.25 (m, 2H), 3.67 (br s, 1H), 2.35 (ddd, J = 4.7, 6.6, 13.8 Hz, 1H), 2.15 (td, J = 6.8, 13.7 Hz, 1H), 1.91 - 1.80 (m, 3H), 1.15 (d, J = 2.7 Hz, 18H).

^{13}C NMR (101MHz, $CDCl_3$) Shift = 177.20, 176.89, 163.46, 150.33, 149.84, 149.78, 135.18, 118.20, 116.29, 111.74, 85.71, 85.48, 84.93, 81.56, 73.94, 60.40, 39.09, 38.76, 26.83, 26.81, 21.04, 14.19, 12.61.

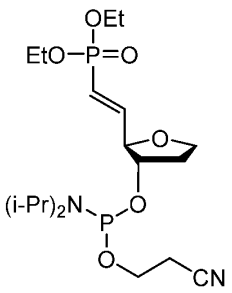
^{31}P NMR (162MHz $CDCl_3$,) Shift = 17.05.

LCMS: M + H^+ = 547.2, purity: 90.718%.

Example 25. Synthesis of Abasic 5'-Vinyl Phosphonates (WV-RA-009) and 5'-Vinyl Phosphonates-3'-CNE Phosphoramidite (WV-RA-009-CNE)

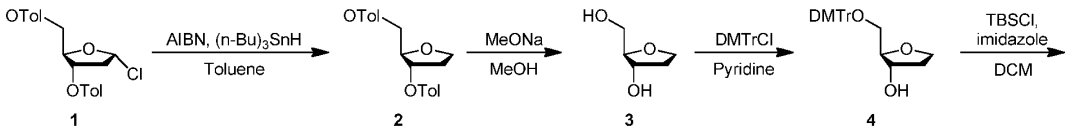


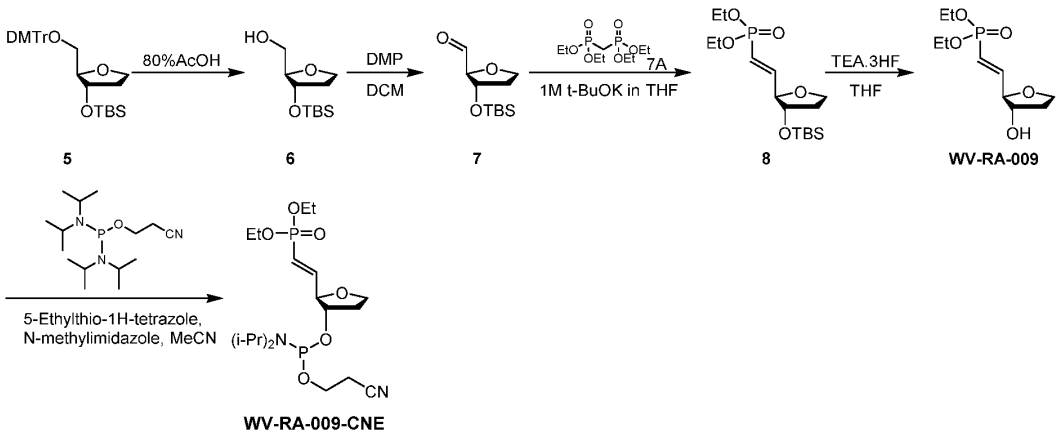
WV-RA-009



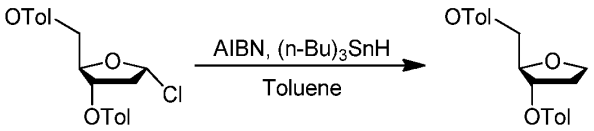
WV-RA-009-CNE

General Scheme





1. *Preparation of compound 2*

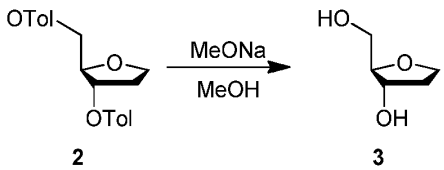


Three batches: The compound **1** (100 g, 257.17 mmol) was dissolved in dry toluene (1500 mL), and AIBN (1.58 g, 9.64 mmol) and $(n\text{-}Bu)_3SnH$ (74.85 g, 257.17 mmol) were added. The solution was heated to 80 °C for 12 h. TLC showed little of compound **1** was still remained and a new spot was found. The three batches were combined for work up. The mixture was evaporated to dryness to give (270 g, crude) as a yellow oil.

The crude mixture (315 g) was purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 100/1, 50/1) to get compound **2** (120 g, 38.10% yield) as a yellow oil and 120 g crude need further purification.

TLC (Petroleum ether : Ethyl acetate = 3: 1), Rf = 0.63

2. *Preparation of compound 3*



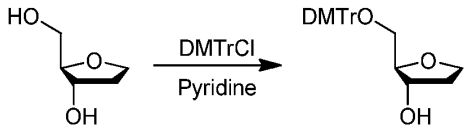
Three batches: To a solution of compound **2** (115 g, 324.50 mmol) in MeOH (1.2 L) was added NaOMe (52.59 g, 973.49 mmol). The mixture was stirred at 25 °C for 3 hr. LCMS

and TLC showed compound **2** was consumed and TLC showed a new spot was found. Three batches were combined for work up. NH_4Cl (169 g) was added and the mixture was concentrated to get the compound **3** (115 g, crude) as a yellow oil.

TLC (Ethyl acetate: Methanol = 10: 1), Rf = 0.21

5

3. Preparation of compound 4

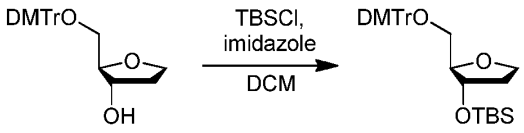


3 **4**

10 To a solution of compound **3** (55 g, 465.59 mmol) in pyridine (550 mL) was added DMTCl (189.30 g, 558.70 mmol). The mixture was stirred at 25 °C for 12 h. LCMS showed the compound **3** was consumed and the desired substance was found. Water (500 mL) was added and the mixture was extracted with EtOAc (500 mL*2). The combined organic was dried over sodium sulfate, filtered and concentrated to get the crude. The
15 mixture was purified by silica gel chromatography (Petroleum ether: Ethyl acetate = 10: 1, 3: 1, 1: 1, 5% TEA) to get compound **4** (110 g, 56.19% yield) as a yellow oil.

TLC (Petroleum ether: Ethyl acetate = 1: 1), Rf = 0.43

4. Preparation of compound 5



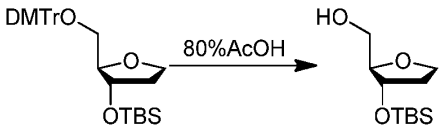
4 **5**

20

Two batches: To a solution of compound **4** (55 g, 130.80 mmol) and imidazole (26.71 g, 392.39 mmol) in DCM (600 mL) was added TBSCl (29.57 g, 196.20 mmol). The mixture was stirred at 25 °C for 12 h. TLC showed the compound **4** was consumed and a new spot was found. The two batches were combined for work up. Water (500 mL) was added
25 and extracted with DCM (200 mL*2). The combined organic was dried over Na_2SO_4, filtered and concentrated to get the compound **5** (139 g, crude) as a yellow oil

TLC (Petroleum ether : Ethyl acetate = 5: 1), Rf = 0.47

5. Preparation of compound 6



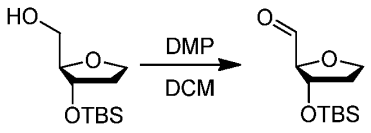
5 **6**

A solution of compound **5** (139 g, 259.93 mmol) in the mixture of HOAc (560 mL) and H_2O (140 mL) was stirred at 25 °C for 12 hr. TLC showed compound **5** was consumed. The mixture was poured into ice-water (500 mL), and the $NaHCO_3$ solid was added until pH = 7, and the residue was extracted with EtOAc (300 mL*3). The combined organic was washed with brine (300 mL), dried over Na_2SO_4, filtered and concentrated to get the crude. The mixture was purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 50/1, 5/1, 3:1) to get the compound **6** (35 g, 57.94% yield) as a yellow oil.

^{1}HNMR (400MHz, $CDCl_3$**)** δ = 4.23 (td, J=3.8, 6.6 Hz, 1H), 3.97 (dd, J=5.4, 8.0 Hz, 2H), 3.79 - 3.68 (m, 2H), 3.61 - 3.50 (m, 1H), 2.06 - 2.01 (m, 1H), 1.91 - 1.81 (m, 1H), 0.89 (s, 8H), 0.08 (s, 6H)

TLC (Petroleum ether : Ethyl acetate=5:1), Rf = 0.25

6. Preparation of compound 7



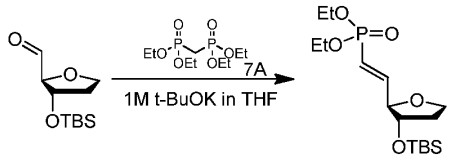
6 **7**

Two batches: To a solution of compound **6** (14.5 g, 62.39 mmol) in DCM (150 mL) was added DMP (31.76 g, 74.87 mmol). The reaction was stirred at 25 °C for 2 hr. TLC showed compound **6** was consumed. The two batches were combined for work up. The mixture was poured into the mixture of sat. $NaHCO_3$ (750 mL) and sat. Na_2SO_3 (750 mL). The mixture was extracted with DCM (500 mL*2); the combined organic was washed

with brine (500 mL), dried over Na_2SO_4, filtered and concentrated to get the compound **7** (28.7 g, crude) as a yellow oil.

TLC (Petroleum ether: Ethyl acetate = 3: 1), Rf = 0.43

7. Preparation of compound 8



7 **8**

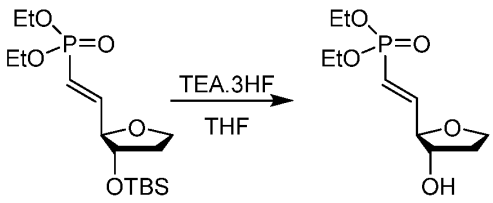
To a solution of compound **7A** (43.09 g, 149.50 mmol) in THF (100 mL) was added t-BuOK (1 M, 149.50 mL) at 0 °C, and stirred at 0 °C for 10 min, then warmed up to 25 °C for 30 min. The solution of compound **7** (28.7 g, 124.58 mmol) in THF (100 mL) was added to the above solution at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then allowed to warm up to 25 °C in 80 min. TLC showed compound **7** was consumed. To the reaction mixture water (100 mL) was added and extracted with EtOAc (100 mL*4). The organic phase was dried (Na_2SO_4), filtered and concentrated to give compound **8** (45 g, crude) as a colorless oil.

The mixture was purified by silica column (Petroleum ether/Ethyl acetate = 10/1, 3/1) to get compound **8** (18 g, 40.00% yield) as a yellow oil.

^{1}HNMR (400MHz, $CDCl_3$**)** δ = 6.86 - 6.68 (m, 1H), 6.08 - 5.82 (m, 1H), 4.32 - 4.26 (m, 1H), 4.20 - 4.13 (m, 1H), 4.12 - 3.99 (m, 6H), 2.04 - 1.93 (m, 1H), 1.88 - 1.79 (m, 1H), 1.59 (s, 2H), 1.33 (t, J=7.1 Hz, 6H), 0.90 (s, 9H), 0.15 - -0.02 (m, 6H)

TLC: (Petroleum ether: Ethyl acetate = 1: 1), Rf = 0.15

8. Preparation of compound WV-RA-009



8 **WV-RA-009**

To a solution of compound **8** (20 g, 54.87 mmol) in THF (200 mL) was added 3HF.TEA (35.38 g, 219.49 mmol). The mixture was stirred at 25 °C for 2 hr. TLC showed compound **8** was consumed, a new spot was found. $NaHCO_3$ (300 mL, aq.) was added, and extracted with DCM (200 mL*5). The combined organic was dried over Na_2SO_4, filtered and concentrated to get the crude. The mixture was purified by silica column (Petroleum ether/Ethyl acetate = 10/1, 3/1, 0:1) to get the **WV-RA-009** (11.5 g, 82.14% yield) as a colorless oil.

^{1}HNMR (400MHz, $CDCl_3$**)** δ = 6.90 - 6.78 (m, 1H), 6.08 - 5.84 (m, 1H), 4.41 - 4.36 (m, 1H), 4.23 (td, J=3.1, 6.0 Hz, 1H), 4.12 - 4.00 (m, 6H), 3.44 (br s, 1H), 2.13 - 1.99 (m, 1H), 1.97 - 1.89 (m, 1H), 1.32 (dt, J=1.4, 7.1 Hz, 6H)

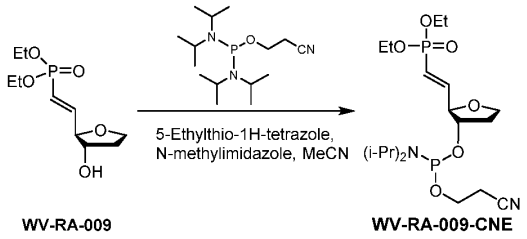
^{13}CNMR (101MHz $CDCl_3$**,)** δ = 150.69, 150.63, 117.25, 115.37, 85.79, 85.58, 75.54, 67.31, 61.92 (t, J=6.2 Hz, 1C), 34.07, 16.35, 16.30

^{31}P NMR (162MHz, $CDCl_3$**)** δ = 18.65 (s, 1P)

LCMS: ($M+H^+$): 251.1, LCMS purity: 100% (ELSD).

TLC (Petroleum ether: Ethyl acetate = 0: 1), Rf = 0.15

9. Preparation of compound WV-RA-009-CNE Phosphoramidite.



WV-RA-009 **WV-RA-009-CNE**

The compound **WV-RA-009** (4.5 g, 17.98 mmol) was dried by azeotropic distillation on a rotary evaporator with toluene (20 mL*3).

To a solution of compound **WV-RA-009** (4.5 g, 17.98 mmol) in DMF (32 mL) were added N-methylimidazole (2.95 g, 35.97 mmol) and 5-ethylsulfanyl-2H-tetrazole (2.34 g, 17.98 mmol), then 3-bis(diisopropylamino)phosphanyloxypropanenitrile (8.13 g, 26.98 mmol) was dropped. The mixture was stirred at 25 °C for 2 hr. TLC showed **WV-RA-009** was consumed and a new spot was found. The mixture was poured into the sat.

$NaHCO_3$ (200 mL) slowly, and the mixture was extracted with EtOAc (100 mL*3). The combined organic was dried over Na_2SO_4, filtered and concentrated to get the crude. The residue was purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 10/1, 3/1, 1/1, 5% TEA) for two times to get **WV-RA-009-CNE** (3 g, 33.90% yield, 91.55% purity) as a colorless oil.

^{1}HNMR (400MHz $CDCl_3$**,)** δ = 6.90 - 6.64 (m, 1H), 6.09 - 5.82 (m, 1H), 4.47 (br d, J=17.0 Hz, 1H), 4.30 - 4.19 (m, 1H), 4.12 - 3.95 (m, 6H), 3.86 - 3.69 (m, 2H), 3.64 - 3.52 (m, 2H), 2.68 - 2.55 (m, 2H), 2.09 - 1.89 (m, 2H), 1.29 (dt, J=2.2, 7.1 Hz, 7H), 1.23 - 1.11 (m, 14H)

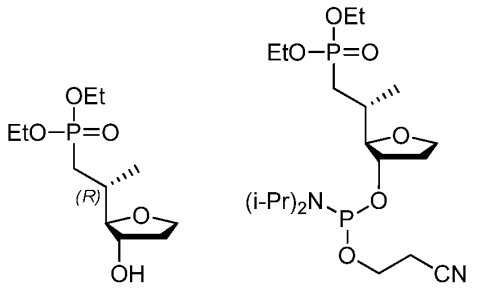
^{31}PNMR (162MHz, $CDCl_3$**)** δ = 148.22 (s, 1P), 148.11 (s, 1P), 148.32 - 147.99 (m, 1P), 30.79 (s, 1P), 18.38 (s, 1P), 18.33 (s, 1P), 18.22 (s, 1P)

^{13}CNMR (101MHz, $CDCl_3$**)** δ = 149.83 (dd, J=5.9, 11.7 Hz, 1C), 118.10, 117.88, 117.55, 117.51, 116.23, 116.01, 84.75 (br dd, J=19.1, 21.3 Hz, 1C), 84.70 (br t, J=20.9 Hz, 1C), 67.61, 67.58, 61.76 (br t, J=3.7 Hz, 1C), 58.37, 58.29, 58.18, 58.10, 43.23 (dd, J=2.9, 12.5 Hz, 1C), 33.23 (dd, J=4.0, 9.2 Hz, 1C), 24.60, 24.54, 24.51, 24.39, 23.88, 20.34 (dd, J=4.0, 7.0 Hz, 1C), 16.36, 16.29

LCMS: purity 91.55% (ELSD)

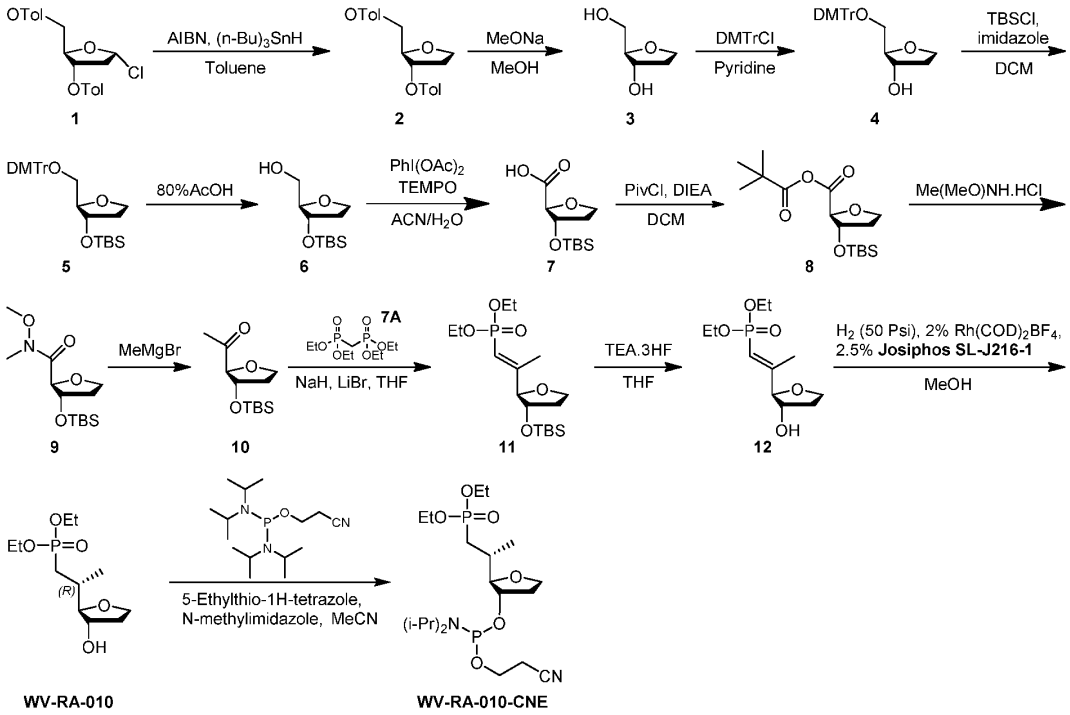
TLC (Petroleum ether : Ethyl acetate = 0: 1), Rf = 0.43

Example 26. Synthesis of Abasic 5'-(R)-Me-PO(OEt)$_2$-Phosphonate (WV-RA-010), and 5'-(R)-Me-PO(OEt)$_2$-Phosphonate-3'-CNE Phosphoramidite (WV-RA-010-CNE)



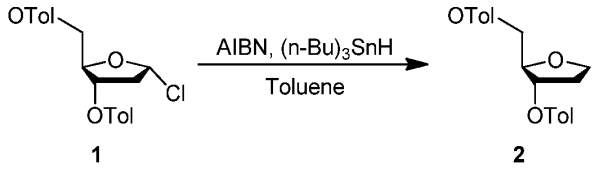
WV-RA-010 **WV-RA-010-CNE**

General Scheme



5

1. *Preparation of compound 2*



10 For three batches: The compound **1** (100 g, 257.17 mmol) was dissolved in dry toluene (1500 mL) and the AIBN (1.58 g, 9.64 mmol) and $(n\text{-Bu})_3SnH$ (74.85 g, 257.17 mmol) were added. The solution was heated to 80 °C for 12 h. TLC showed little of compound 1 still remained and a new spot was found. The three batches were combined for work up. The mixture was evaporated to dryness.

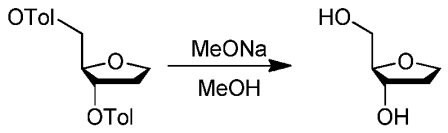
15 Purification: The crude mixture (315 g) was purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 100/1, 50/1) to get compound **2** (120 g, 38.10% yield) as a yellow oil.

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 7.98 - 7.91 (m, 4H), 7.29 - 7.21 (m, 4H), 5.48 (td, J=2.2, 6.4 Hz, 1H), 4.55 - 4.46 (m, 2H), 4.38 (dt, J=2.6, 4.6 Hz, 1H), 4.21 - 4.13 (m, 1H), 4.05

20 (dt, J=6.1, 9.2 Hz, 1H), 2.42 (d, J=5.6 Hz, 7H), 2.20 (tdd, J=2.8, 5.6, 13.5 Hz, 1H)

TLC (Petroleum ether: Ethyl acetate = 5: 1), Rf = 0.47

2. Preparation of compound 3

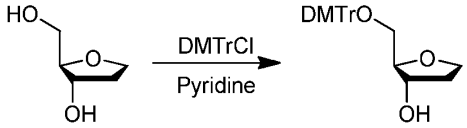


2 **3**

For three batches: To a solution of compound **2** (115 g, 324.50 mmol) in MeOH (1.2 L) was added NaOMe (52.59 g, 973.49 mmol). The mixture was stirred at 25 °C for 3 h. LCMS and TLC showed compound **2** was consumed and a new spot was found. Three batches were combined for work up. NH_4Cl (169 g) was added and the mixture was concentrated to get the compound **3** (115 g, crude) as a yellow oil.

TLC (Ethyl acetate: Methanol=10:1), Rf =0.21

3. Preparation of compound 4



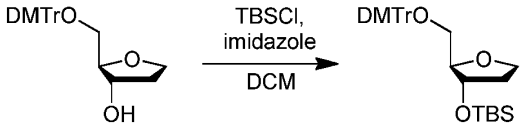
3 **4**

To a solution of compound **3** (60 g, 507.91 mmol) in pyridine (600 mL) was added DMTCl (206.51 g, 609.49 mmol). The mixture was stirred at 25 °C for 12 h. LCMS showed compound **3** was consumed and the desired substance was found. Water (600 mL) was added and the mixture was extracted with EtOAc (600 mL*2). The combined organic was dried over sodium sulfate, filtrated and concentrated to get the crude. The crude was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=15/1 to 1/1) to get compound **4** (89 g, 41.78% yield) as a yellow oil.

LCMS: NEG ($M-H^+$), 419.1

TLC (Petroleum ether: Ethyl acetate = 1: 1), Rf = 0.43

4. Preparation of compound 5

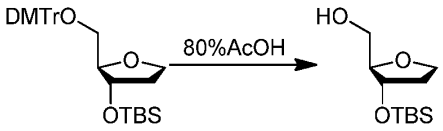


4 5

For two batches: To a solution of compound **4** (44.5 g, 105.83 mmol) and imidazole (21.61 g, 317.48 mmol) in DCM (500 mL) was added TBSCl (23.93 g, 158.74 mmol), and the mixture was stirred at 25 °C for 12 h. TLC showed compound **4** was consumed and a new spot was found. The two batches were combined for work up. Water (500 mL) was added and extracted with DCM (200 mL*2). The combined organic was dried over Na_2SO_4, filtered and concentrated to get the compound **5** (113 g, crude) as a yellow oil

TLC (Petroleum ether: Ethyl acetate = 5: 1), Rf = 0.47

3. Preparation of compound 6

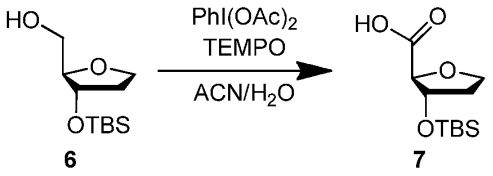


5 6

For two batches: To a solution of compound **5** (56.5 g, 105.66 mmol) in the mixture of HOAc (240 mL) and H_2O (60 mL), the residue was stirred at 25 °C for 12 h. TLC showed compound **5** was consumed. The two batches were combined for workup. The mixture was poured into ice-water (500 mL) and the $NaHCO_3$ solid was added until pH = 7, and the residue was extracted with EtOAc (300 mL*3), the combined organic was washed with brine (300 mL), dried over Na_2SO_4, filtered and concentrated to get the crude. The mixture was purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 50/1, 5/1, 3: 1) to get the compound **6** (28 g, 57.03% yield) as a yellow oil.

TLC (Petroleum ether: Ethyl acetate = 5:1), Rf = 0.25

4. Preparation of compound 7

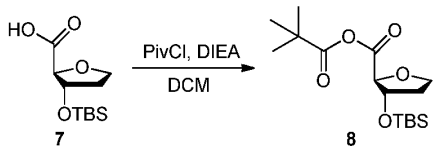


6 7

To a solution of compound **6** (13 g, 55.94 mmol) in MeCN (150 mL) and H_2O (150 mL) was added $PhI(OAc)_2$ (39.64 g, 123.07 mmol) and TEMPO (1.76 g, 11.19 mmol). The mixture was stirred at 25 °C for 3 h. TLC showed compound **6** was consumed and a new spot was found. The mixture was concentrated to get the compound **7** (27 g, crude) as a yellow oil.

TLC (Petroleum ether: Ethyl acetate=3:1), Rf = 0.04

5. Preparation of compound 8

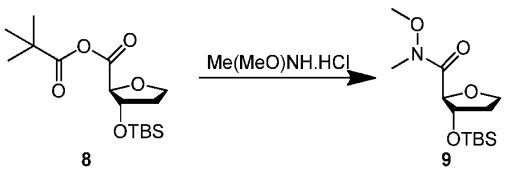


7 8

For two batches: To a solution of compound **7** (13.5 g, 54.79 mmol) in DCM (135 mL) was added DIEA (14.16 g, 109.59 mmol) and 2,2-dimethylpropanoyl chloride (8.59 g, 71.23 mmol). The mixture was stirred at 0 °C for 0.5 h. TLC showed compound **7** was consumed and a new spot was found. Compound **8** (36.2 g, crude) as a yellow solution in DCM (135 mL) was used for next step directly.

TLC (Petroleum ether: Ethyl acetate = 1: 1), Rf = 0.22

6. Preparation of compound 9

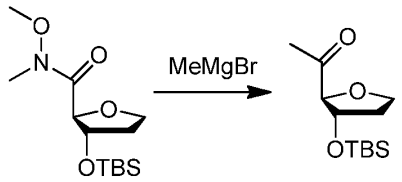


8 9

For two batches: The mixture compound **8** (18.1 g, 54.77 mmol) in DCM (135 mL) from the last step was added TEA (16.63 g, 164.30 mmol, 22.87 mL) and N-methoxymethanamine;hydrochloride (8.01 g, 82.15 mmol), and the mixture was stirred at 0 °C for 1 h. LCMS showed the starting material was consumed and the desired substance was found. The two batches were combined for work up. The mixture was washed with HCl (1N, 100 mL) and then aqueous $NaHCO_3$ (100 mL), the organic was dried over Na_2SO_4 and filtered to get the crude. The mixture was purified by silica gel chromatography (Petroleum ether/Ethyl acetate=10/1, 3/1) to get compound **9** (15.8 g, 50.97% yield) as a yellow oil.

LCMS: ($M+H^+$): 290.1

7. Preparation of compound 10



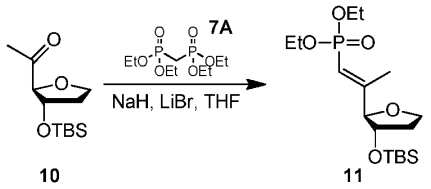
9 **10**

To a solution of compound **9** (15.8 g, 54.59 mmol) in THF (180 mL) was dropped MeMgBr (3 M, 54.59 mL) at 0 °C, and the mixture was stirred at 0° C for 1hr. TLC showed compound **9** was consumed. The mixture was poured into sat.NH_4Cl (200 mL) and the mixture was extracted with EtOAc (150 mL*3), the combined organic was dried over Na_2SO_4, filtered and concentrated to get the crude. The mixture was purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 20/1, 5/1) to get compound **10** (12 g, 85.11% yield) as a yellow oil.

^{1}HNMR (400MHz, $CDCl_3$ **)** δ = 4.47 - 4.41 (m, 1H), 4.18 (d, J=2.5 Hz, 1H), 4.11 - 4.01 (m, 2H), 2.19 (s, 3H), 2.01 - 1.75 (m, 2H), 0.90 (s, 10H), 0.11 (d, J=3.5 Hz, 6H)

TLC (Petroleum ether: Ethyl acetate =3:1), Rf = 0.76

10. Preparation of compound 11



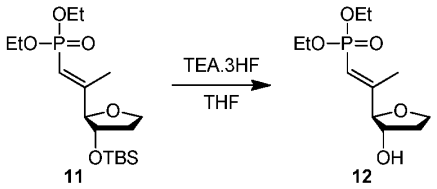
10 11

To a solution of NaH (7.92 g, 198.03 mmol, 60% purity) in THF (170 mL) was added compound **7A** (57.08 g, 198.03 mmol) in THF (110 mL) at 0 °C. The reaction mixture was warmed up to 20 °C, and stirred for 1 hr. A solution of LiBr (17.20 g, 198.03 mmol) in THF (100 mL) was added and the resultant slurry was stirred, and then cooled to 0 °C. To the above mixture was added a solution of compound **10** (11 g, 45.01 mmol) in THF (100 mL) at 0 °C. The mixture was stirred at 0 – 20 °C for 1 hr. TLC showed compound **10** was consumed. The resulting mixture was diluted with water (500 mL), extracted with EtOAc (300 mL*3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to afford a yellow oil. The residue was purified by silica gel chromatography (Petroleum ether/Ethyl acetate =1 0/1, 3/1) to get compound **11** (16 g, 86.49% yield) as a yellow oil.

^{1}HNMR (400MHz, $CDCl_3$**)** δ = 5.73 (td, J=1.1, 18.7 Hz, 1H), 4.19 - 3.94 (m, 9H), 2.09 (dd, J=0.8, 3.3 Hz, 3H), 2.01 - 1.90 (m, 1H), 1.84 - 1.75 (m, 1H), 1.40 - 1.27 (m, 8H), 0.93 - 0.84 (m, 10H), 0.13 - 0.00 (m, 6H)

TLC (Petroleum ether: Ethyl acetate = 1:1), Rf = 0.20

11. Preparation of compound 12



11 12

A mixture of compound **11** (15 g, 39.63 mmol) in THF (150 mL) was added 3HF.TEA (25.55 g, 158.51 mmol), and then the mixture was stirred at 20 °C for 12 hr under N_2 atmosphere. TLC showed compound **11** was consumed. Sat. $NaHCO_3$ was added to the mixture until pH = 7, and the residue was extracted with DCM (150 mL*3), the combined organic was dried over Na_2SO_4, filtered and concentrated to get the crude. The residue was purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 5/1, 0/1, Ethyl

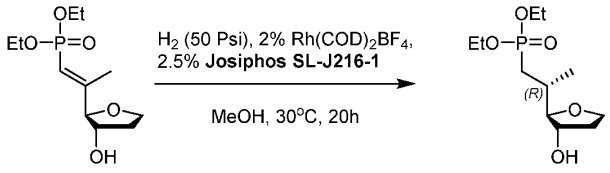
acetate: Dichloromethane = 10:1) to get compound **12** (8.8 g, 80.00% yield) as a yellow oil.

^{1}HNMR (400MHz, $CDCl_3$**)** δ = 5.73 (td, J=1.1, 18.7 Hz, 1H), 4.19 - 3.94 (m, 8H), 2.09 (dd, J=0.8, 3.3 Hz, 3H), 2.01 - 1.90 (m, 1H), 1.84 - 1.75 (m, 1H), 1.33 - 1.30 (m, 6H)

^{31}PNMR (162MHz, $CDCl_3$**)** δ = 18.71

TLC (Ethyl acetate: Methanol=0:1), Rf = 0.20.

12. Preparation of compound WV-RA-010



12 **WV-RA-010**

To a solution of compound **12** (8.7 g, 32.92 mmol), (1Z,5Z)-cycloocta-1,5-diene;rhodium(1+);tetrafluoroborate (534.76 mg, 1.32 mmol), zinc;trifluoromethanesulfonate (4.79 g, 13.17 mmol) in MeOH (160 mL) was added Josiphos SL-J216-1 (987.42 mg, 1.51 mmol) under N_2. The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under H_2 (50 psi) at 30 °C for 12 h. TLC showed the reaction was complete. The mixture was concentrated to get the crude. The residue was purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 20/1, 1/9, Ethyl acetate: Methanol = 20: 1) to get **WV-RA-010** (8 g, 91.95% yield) as a yellow oil.

^{1}HNMR (400MHz, $CDCl_3$**)** δ = 4.27 - 4.01 (m, 5H), 3.99 - 3.81 (m, 2H), 3.61 - 3.41 (m, 2H), 2.23 - 1.86 (m, 4H), 1.80 - 1.63 (m, 1H), 1.34 (t, J=7.0 Hz, 6H), 1.12 (d, J=6.6 Hz, 3H)

^{13}CNMR (101MHz, $CDCl_3$**)** δ = 89.97, 89.85, 74.18, 66.68, 61.92, 35.71, 31.49, 31.46, 29.95, 28.55, 17.50, 17.43, 16.42, 16.36

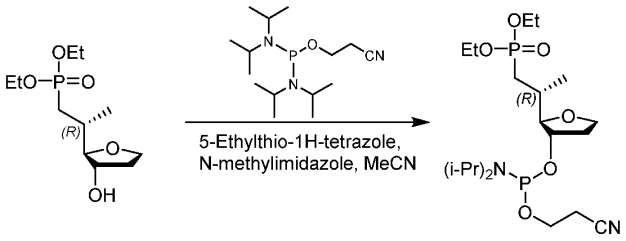
^{31}PNMR (162MHz, $CDCl_3$**)** δ = 32.07

LCMS: ELSD ($M+H^+$), 267.1, 100% purity

TLC (Petroleum ether: Ethyl acetate = 0: 1), Rf = 0.03; (Ethyl acetate: Methanol = 10: 1),

Rf = 0.35.

13. Preparation of compound WV-RA-010-CNE Phosphoramidite.



WV-RA-010 **WV-RA-010-CNE**

WV-RA-010 (3 g, 11.27 mmol) was dried by azeotropic distillation on a rotary evaporator with toluene (20 mL*3).To a solution of **WV-RA-010** (3 g, 11.27 mmol) in DMF (24 mL) was added 1-methylimidazole (1.85 g, 22.53 mmol) and 5-ethylsulfanyl-2H-tetrazole (1.47 g, 11.27 mmol), then 3-bis(diisopropylamino)phosphanyloxypropanenitrile (5.09 g, 16.90 mmol) was dropped. The mixture was stirred at 25 °C for 1 h. TLC showed **WV-RA-010** was consumed and a new spot was found. The mixture was poured into the sat.$NaHCO_3$ (100 mL) slowly and the mixture was extracted with Ethyl acetate (50 mL*3), the combined organic was dried over Na_2SO_4, filtered and concentrated to get the crude. The residue was purified by silica gel chromatography (Petroleum ether/Ethyl acetate = 10/1, 3/1, 1/1, 5%TEA) to get **WV-CA-010-CNE** (1.7 g, 32.35% yield) as a colorless.

^{1}HNMR (400MHz $CDCl_3$**,)** δ = 4.29 - 4.18 (m, 1H), 4.17 - 4.02 (m, 4H), 4.01 - 3.49 (m, 7H), 2.71 - 2.59 (m, 2H), 2.24 - 2.08 (m, 1H), 2.07 - 1.92 (m, 3H), 1.74 - 1.49 (m, 2H), 1.37 - 1.28 (m, 7H), 1.24 - 1.15 (m, 12H), 1.10 (d, J=6.8 Hz, 3H)

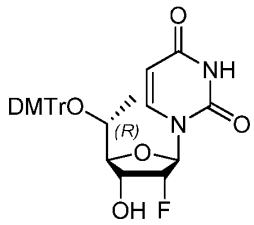
^{13}CNMR (101MHz, $CDCl_3$**)** δ = 117.58, 117.54, 89.31, 89.25, 75.58, 66.95, 61.36, 61.37, 58.07, 46.34, 46.30, 45.49, 45.45, 45.40, 43.20, 34.84, 30.87, 30.84, 30.81, 29.81, 29.79, 28.42, 28.38, 25.72, 24.54, 24.47, 24.39, 23.85, 23.15, 24.36, 22.98, 22.64, 24.29, 20.39, 20.31, 20.30, 20.23, 16.41, 16.35, 15.94, 15.40

^{31}PNMR (162MHz, $CDCl_3$**)** δ = 148.02, 147.78, 31.73, 31.57 (s, 1P), 30.79 (s, 1P)

LCMS: ELSD, 96.42% purity

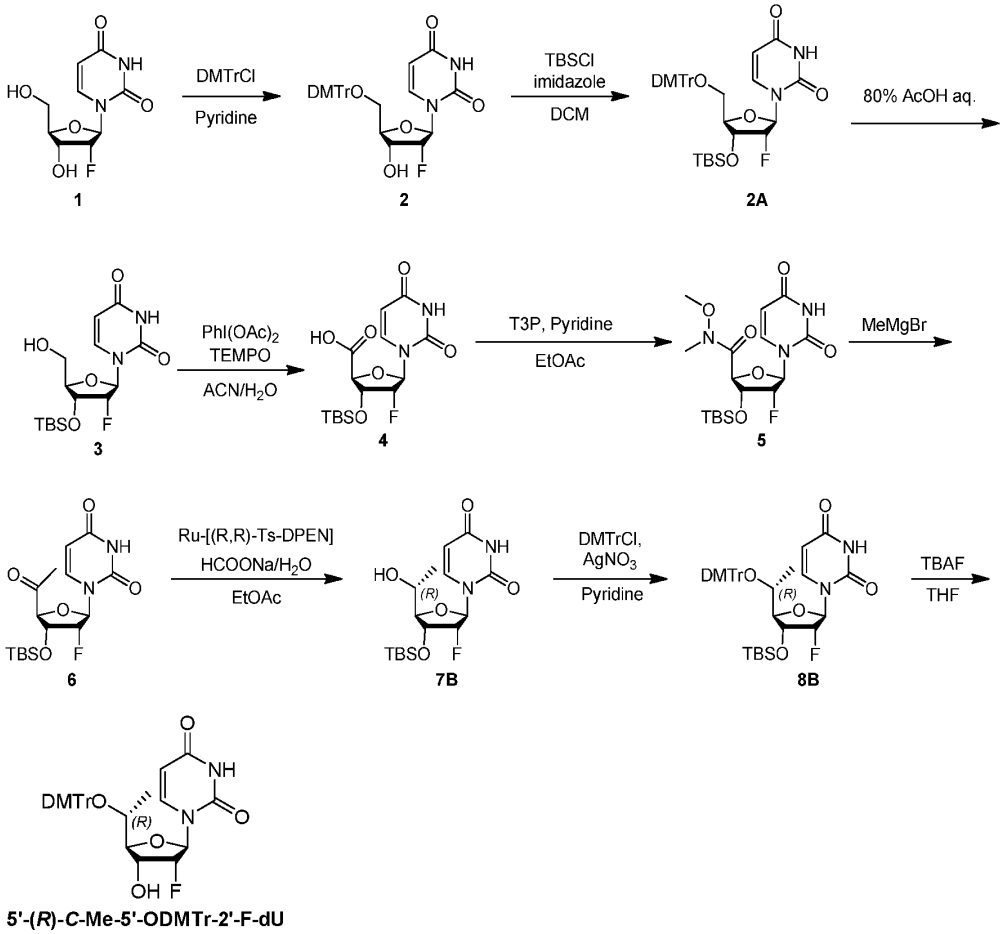
TLC (Petroleum ether: Ethyl acetate = 0:1), Rf = 0.43

Example 27. Synthesis of 5'-(R)-C-Me-5'-ODMTr-2'-F-dU.



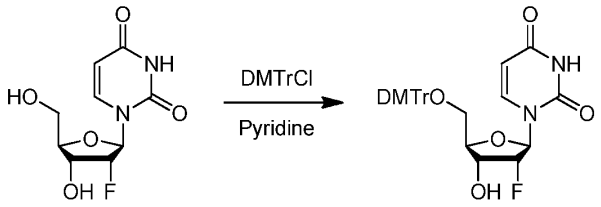
5'-(*R*)-*C*-Me-5'-ODMTr-2'-F-dU

General Scheme



5'-(*R*)-*C*-Me-5'-ODMTr-2'-F-dU

1. Preparation of compound 2

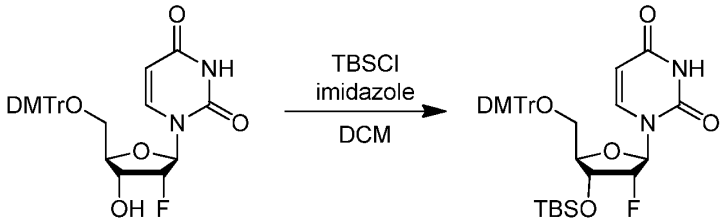


1 2

To a solution of compound **1** (100.00 g, 406.19 mmol) in pyridine (550.00 mL) was added DMTCl (165.16 g, 487.43 mmol). The mixture was stirred at 25 °C for 20 hr. TLC indicated compound **1** was consumed and one new spot formed. MeOH (300 mL) was added, and the reaction mixture was concentrated under reduced pressure to remove solvent. The residue was dissolved in EtOAc (500 mL) and washed with H_2O (500 mL * 3). Dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product compound **2** (285.00 g, crude) was yellow solid, used into the next step without further purification.

TLC (Ethyl acetate : Petroleum ether = 3:1, 5% TEA) R_f = 0.40

2. Preparation of compound 2A

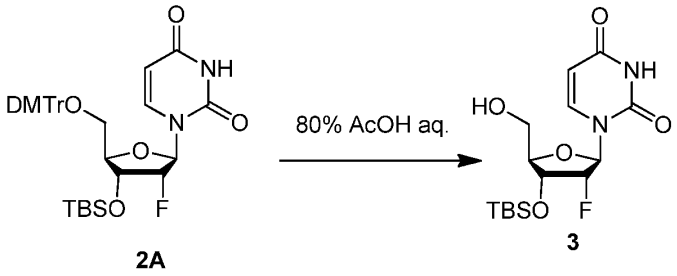


2 2A

To a solution of compound **2** (222.82 g, 406.19 mmol) in DCM (500.00 mL) was added imidazole (41.48 g, 609.29 mmol) and TBSCl (91.83 g, 609.29 mmol, 74.66 mL). The mixture was stirred at 25 °C for 20 hours. TLC indicated compound **2** was consumed and one new spot formed. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was diluted with DCM (500 mL), and washed with H_2O mL (500 mL * 3); dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product compound **2A** (330.00 g, crude) was white solid, used into the next step without further purification.

TLC (Ethyl acetate: Petroleum ether = 3:1) R_f = 0.65.

3. Preparation of compound 3

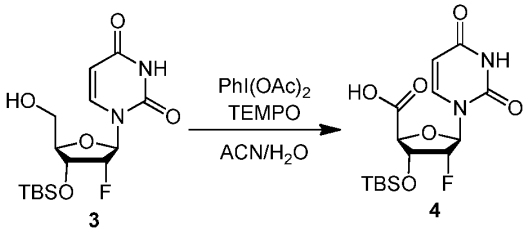


2A **3**

A solution of compound **2A** (269.23 g, 406.19 mmol) in AcOH (400.00 mL) 80% aq., was stirred at 25 °C for 15 hour. TLC indicated that some compound **2A** remained and one new spot formed. The reaction mixture was quenched by sat. $NaHCO_3$ aq. until pH>7 at 25°C, and then diluted with EtOAc (500 mL) and extracted with EtOAc (500 mL *3), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/ 0 to 0/ 1 10% DCM). 60 g of compound **3**, and 130 g compound **2A** were isolated. Compound **3** (60.00 g, 40.98% yield) was obtained as a white solid.

TLC （Ethyl acetate : Petroleum ether = 3:1） R_f = 0.45.

4. Preparation of compound 4



3 **4**

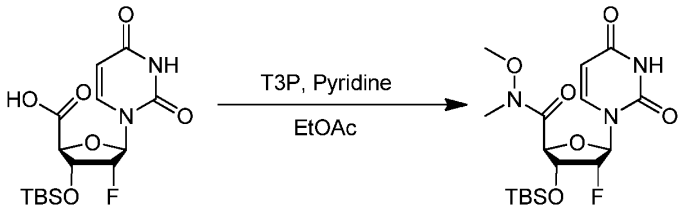
To a solution of compound **3** (30.00 g, 83.23 mmol) in MeCN (360.00 mL) and H_2O (360.00 mL) was added TEMPO (2.62 g, 16.65mmol) and $PhI(OAc)_2$ (58.98 g, 183.10 mmol) at 25 °C in 3 hours. TLC indicated compound **3** was consumed and one new spot formed. The reaction mixture was concentrated under reduced pressure to remove lots of solvent. Filtered the solid and washed the solid with MeCN. The other liquid was concentrated under reduced pressure, then dissolved in sat. KOH (aq., 2M) to pH~12, washed by EtOAc (200 mL*3), and then added HCl (aq. 1M) to pH~3, filtered and concentrated as a yellow solid. Compound **4** (52.00 g, 138.87 mmol, 83.43% yield) was obtained as a yellow solid.

^{1}H NMR (400MHz, DMSO-d_6) δ = 7.96 (d, *J*=8.3 Hz, 1H), 5.99 (dd, *J*=3.1, 16.2 Hz, 1H), 5.71 (dd, *J*=2.2, 8.3 Hz, 1H), 5.34 - 5.03 (m, 1H), 4.70 - 4.48 (m, 1H), 4.30 (d, *J*=5.3 Hz, 1H), 0.86 (s, 9H), 0.08 (s, 6H).

LCMS: ($M+H^+$): 374.9

TLC (Petroleum ether : Ethyl acetate = 1:1, R_f = 0)

5. Preparation of compound 5



4 **5**

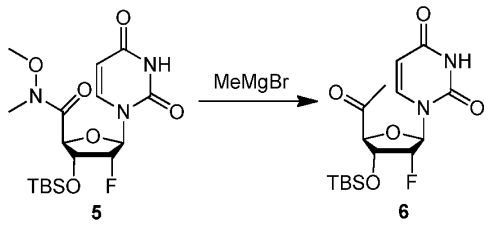
To a solution of compound **4** (26.00 g, 69.44 mmol) in pyridine (50.00 mL) was added N-methoxymethanamine hydrochloride (8.13 g, 83.33 mmol) and EtOAc (150.00 mL). The mixture was stirred at 0 °C then added T3P (46.40 g, 145.82 mmol, 43.36 mL) in N_2. The mixture was stirred at 0°C in 3h. TLC indicated compound **4** was consumed and one new spot formed. The resulting mixture was work up together with another batch (26 g scale). The resulting mixture was washed with HCl (1 M, 1.1 L), and the aqueous layer was extracted with DCM (1 L*2). The combined organic layers were washed with sat. Na_2CO_3 aq. until pH = 12, dried over Na_2SO_4, filtered and concentrated to give a crude product. The residue was purified by column chromatography (SiO_2, Petroleum ether /Ethyl acetate = 1 /0 to 0 :1) to get 52 g product. Compound 5 (26.00 g, 89.68% yield) was obtained as a yellow solid.

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 8.91 (br s, 1H), 8.28 (d, *J*=8.2 Hz, 1H), 6.31 (dd, *J*=5.2, 11.6 Hz, 1H), 5.73 (dd, *J*=0.9, 8.2 Hz, 1H), 4.94 - 4.83 (m, 1H), 4.80 - 4.75 (m, 1H), 4.31 (td, *J*=3.9, 7.7 Hz, 1H), 3.66 (s, 3H), 3.17 (s, 3H), 1.95 (s, 1H), 1.65 (s, 1H), 1.16 (t, *J*=7.1 Hz, 1H), 0.86 - 0.77 (m, 9H), 0.02 (d, *J*=12.3 Hz, 6H)

LCMS: ($M+H^+$): 418.1

TLC (Ethyl acetate: Petroleum ether = 1:1) R_f = 0.26.

6. Preparation of compound 6



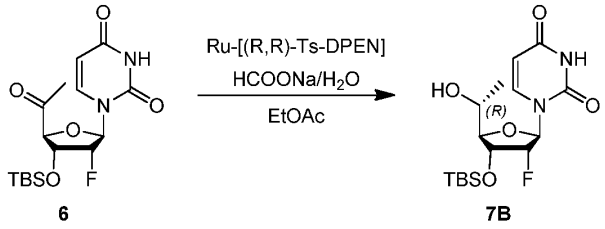
To a solution of compound **5** (52.00 g, 124.55 mmol) in THF (500.00 mL) was added MeMgBr (3 M, 83.03 mL) at-20-0°C. The mixture was stirred at -20 °C-10 °C for 2 hour. TLC indicated compound **5** was consumed and one new spot formed. The reaction mixture was quenched by addition sat. NH_4Cl 500 mL at **0** °C, and then diluted with EtOAc (600 mL) and extracted with EtOAc (600 mL * 3), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether /Ethyl acetate = 1 /0 to 0:1) to get 29 g product and 10 g crude product. Compound **6** (29.00 g, 62.51% yield) was obtained as a white solid.

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 8.90 (br s, 1H), 7.83 (d, *J*=7.9 Hz, 1H), 5.95 - 5.77 (m, 2H), 5.10 - 4.90 (m, 1H), 4.56 (d, *J*=6.6 Hz, 1H), 4.37 (ddd, *J*=4.6, 6.6, 15.1 Hz, 1H), 2.27 (s, 3H), 1.04 - 0.86 (m, 10H), 0.13 (d, *J*=7.0 Hz, 6H)

LCMS: ($M+H^+$): 373.0

TLC （Ethyl acetate : Petroleum ether = 1:1） R_f = 0.4

2. Preparation of compound 7B



To a solution of compound **6** (24.00 g, 64.44 mmol) in EtOAc (187.50 mL) was added sodium formate (204.65 g, 3.01 mol) in H_2O (750.00 mL) and [[(1R,2R)-2-amino-1,2-diphenyl-ethyl]-(p-tolylsulfonyl)amino]-chloro-ruthenium;1-isopropyl-4-methyl-benzene (819.90 mg, 1.29 mmol) in N_2. The mixture was stirred at 25 °C for 20 hours. TLC

indicated compound **6** was consumed and one new spot formed. The mixture was extracted with DCM (1000 mL*3). The combined organic layers were washed with brine (1000 mL), dried over Na_2SO_4, filtered and concentrated to get the crude as a yellow solid. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/ 0 to 0: 1) to get 19.8 g product, then washed with MTBE to get 18 g product. Compound **7B** (18.00 g, 74.59% yield) was obtained as a white solid.

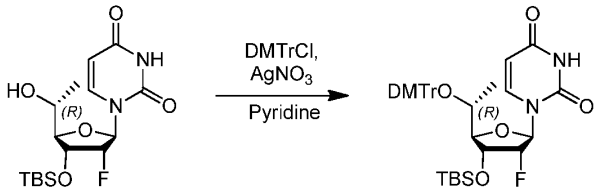
^{1}H NMR (400MHz, $CDCl_3$**)** δ = 8.15 (br s, 1H), 7.32 (d, *J*=7.9 Hz, 1H), 5.63 (d, *J*=8.2 Hz, 1H), 5.53 (dd, *J*=5.1, 14.6 Hz, 1H), 5.26 - 5.04 (m, 1H), 4.41 - 3.92 (m, 2H), 3.83 (br s, 1H), 2.86 (d, *J*=2.2 Hz, 1H), 1.13 (d, *J*=6.6 Hz, 3H), 0.79 (s, 9H), 0.00 (s, 6H)

HPLC: HPLC purity = 100%;

SFC: SFC purity = 100% ee;

TLC (Petroleum ether: Ethyl acetate = 1:1) R_f = 0.23

8. Preparation of compound 4



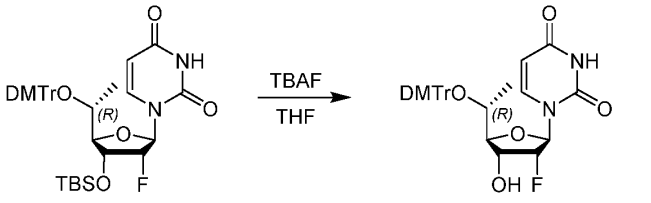
7B **8B**

Compound **7B** (9.00 g, 24.03 mmol) was dried by azeotropic distillation on a rotary evaporator with pyridine (150 mL) and toluene (150 mL*2).

To a solution of compound **7B** (9.00 g, 24.03 mmol) in pyridine (90.00 mL) and THF (270.00 mL) was added DMTCl (15.47 g, 45.66 mmol), and then $AgNO_3$ (7.02 g, 41.33 mmol, 6.95 mL). The mixture was stirred at 25 °C for 20 hour. TLC indicated compound **7B** was consumed and one new spot formed. The mixture was added toluene (200 mL), quenched by addition MeOH (1.3 mL) and stirred for 1h at 25°C, then filtered through celite, and the Celite plug was washed thoroughly with toluene (150 mL), concentrated under reduced pressure to give a crude. The crude product compound **8B** (16.26 g, 100.00% yield) was used into the next step without further purification.

TLC (Petroleum ether : Ethyl acetate = 1:1) R_f = 0.61.

9. Preparation of compound 5'-(R)-C-Me-5'-ODMTr-2'-F-dU



8B **5'-(*R*)-*C*-Me-5'-ODMTr-2'-F-dU**

To a solution of compound **8B** (32.40 g, 47.87 mmol) in THF (324.00 mL) was added TBAF (1 M, 90.95 mL). The mixture was stirred at 25 °C for 16 hours. TLC indicated compound **8B** was consumed and one new spot formed. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was diluted with EtOAc (300 mL) and washed with sat. NaCl aq. (200 mL *2), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether / Ethyl acetate = 1/ 0 to 0: 1) to get 25 g product. Compound **5'-(R)-C-Me-5'-ODMTr-2'-F-dU** (25.00 g, 92.83% yield) was obtained as a yellow solid.

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 7.47 (d, *J*=7.7 Hz, 2H), 7.38 (dd, *J*=9.0, 10.1 Hz, 4H), 7.30 - 7.23 (m, 2H), 7.22 - 7.18 (m, 1H), 6.83 (br d, *J*=7.7 Hz, 4H), 5.90 (dd, *J*=2.4, 17.6 Hz, 1H), 5.31 - 5.18 (m, 1H), 5.08 - 4.87 (m, 1H), 4.51 (td, *J*=5.9, 15.7 Hz, 1H), 3.78 (s, 6H), 3.72 - 3.60 (m, 2H), 1.05 (d, *J*=6.4 Hz, 3H).

^{13}C NMR (101MHz, $CDCl_3$**)** δ = 171.28, 163.37, 158.68, 158.59, 150.04, 146.03, 140.47, 136.06, 130.47, 130.31, 128.08, 127.90, 126.94, 113.23, 113.15, 102.57, 94.11, 92.24, 87.76, 87.43, 87.20, 85.77, 69.46, 69.42, 69.25, 60.45, 55.25, 55.24, 21.06, 17.66, 14.19.

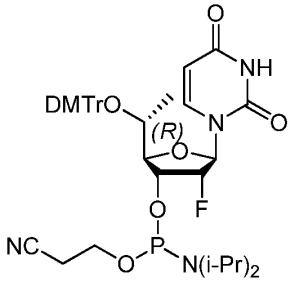
LCMS: ($M-H^+$): 561.2

HPLC: HPLC purity = 99.05%;

SFC: SFC purity =100% ee;

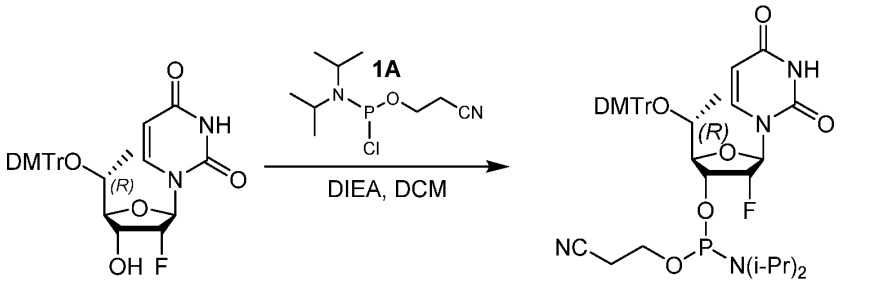
TLC (Ethyl acetate: Petroleum ether = 1:1, R_f = 0.18)

Example 28. Synthesis of 5'-(R)-C-Me-5'-ODMTr-2'-F-dU-CNE phosphoramidite



5'-(*R*)-*C*-Me-5'-ODMTr-2'-F-dU-CNE-phosphoramidite

1. Preparation of compound 5'-(R)-C-Me-5'-ODMTr-2'-F-dU-CNE-phosphoramidite



5'-(*R*)-*C*-Me-5'-ODMTr-2'-F-dU

5'-(*R*)-*C*-Me-5'-ODMTr-2'-F-dU-CNE-phosphoramidite

To a solution of 5'-(R)-C-Me-5'-ODMTr-2'-F-dU (4.9 g, 8.71 mmol) in DCM (49 mL) was added DIEA (1.35 g, 10.45 mmol, 1.83 mL) and compound **1A** (2.69 g, 9.15 mmol) at 0°C. The mixture was stirred at 0-15 °C for 3 hours. TLC indicated 5'-(R)-C-Me-5'-ODMTr-2'-F-dU was consumed and two new spots formed. The mixture was added sat. $NaHCO_3$ (20 mL) and extracted with DCM (50 mL* 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by MPLC (SiO_2, Ethyl acetate/Petroleum ether = 0%, 20%, 40%, 60%, 70%, 100%, 5% TEA) to give 4.3 g (batch 1: 3.24 g, batch 2: 1.06 g) of compound 5'-(R)-C-Me-5'-ODMTr-2'-F-dU-CNE-phosphoramidite (4.3 g, 64.72% yield) as a white solid.

Batch 1:

^{1}H NMR: (400MHz, $CDCl_3$) δ = 7.59 - 7.15 (m, 11H), 6.93 - 6.76 (m, 4H), 6.01 - 5.89 (m, 1H), 5.32 (s, 1H), 5.16 (dd, *J*=8.2, 14.9 Hz, 1H), 5.08 - 5.02 (m, 1H), 4.93 - 4.75 (m,

1H), 4.03 - 3.85 (m, 2H), 3.84 - 3.77 (m, 6H), 3.74 - 3.62 (m, 3H), 3.61 - 3.47 (m, 1H), 2.77 (dt, *J*=1.9, 6.2 Hz, 1H), 2.72 - 2.59 (m, 2H), 1.27 - 1.17 (m, 11H), 0.99 (dd, *J*=2.0, 6.6 Hz, 3H).

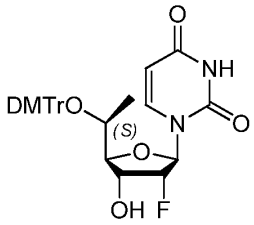
^{31}P NMR: (162MHz, $CDCl_3$) δ = 150.63 (s, 1P), 150.54 (s, 1P), 150.34 (s, 1P), 150.27 (s, 1P), 14.14 (s, 1P).

HPLC: HPLC purity = 97.66 %;

LCMS: ($M-H^+$): 761.3;

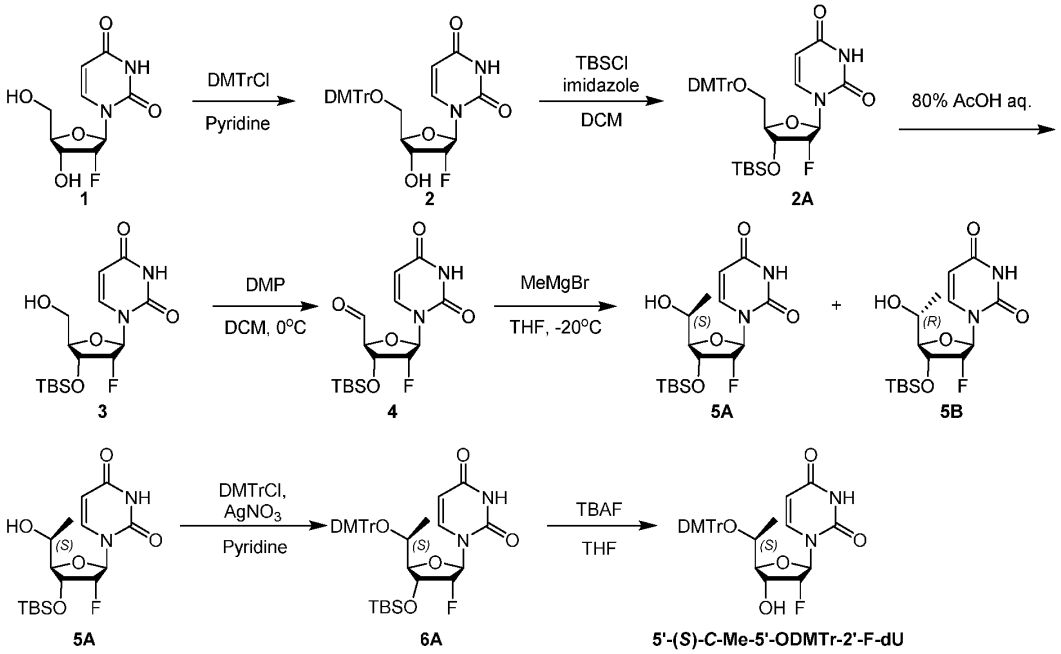
TLC (Petroleum ether: Ethyl acetate = 3:1), R_{f1} = 0.53, R_{f2} = 0.62.

Synthesis of 5'-(S)-C-Me-5'-ODMTr-2'-F-dU.

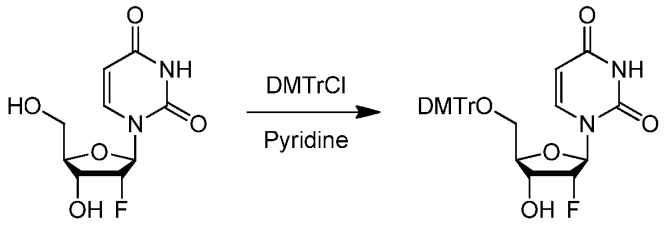


5'-(*S*)-*C*-Me-5'-ODMTr-2'-F-dU

General Scheme



3. Preparation of compound 2

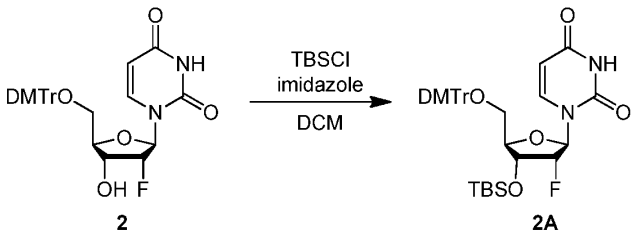


1 2

To a solution of compound **1** (100.00 g, 406.19 mmol) in pyridine (550.00 mL) was added DMTCl (165.16 g, 487.43 mmol). The mixture was stirred at 25°C for 20 hr. TLC indicated compound **1** was consumed and one new spot formed. MeOH (300 mL) was added, the reaction mixture was concentrated under reduced pressure to remove solvent. The residue was dissolved in EtOAc (500 mL) and washed with H_2O (500 mL * 3). Dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product (285.00 g, crude) was yellow solid used into the next step without further purification.

TLC (Ethyl acetate: Petroleum ether = 3:1, 5% TEA) R_f = 0.40

4. Preparation of compound 2A



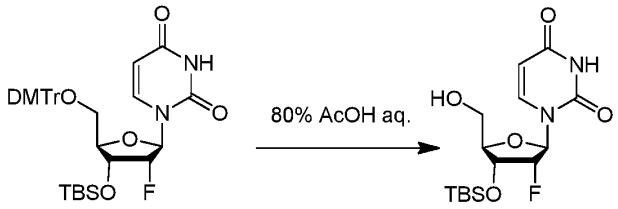
2 2A

To a solution of compound **2** (222.82 g, 406.19 mmol) in DCM (500.00 mL) was added imidazole (41.48 g, 609.29 mmol) and TBSCl (91.83 g, 609.29 mmol). The mixture was stirred at 25 °C for 20 hour. TLC indicated compound **2** was consumed and one new spot formed. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was diluted with DCM (500 mL), washed with H_2O (500 mL * 3). Dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product (330.00 g, crude) was white solid used into the next step without further

purification.

TLC (Ethyl acetate: Petroleum ether = 3:1) R_f = 0.65

3. Preparation of compound 3

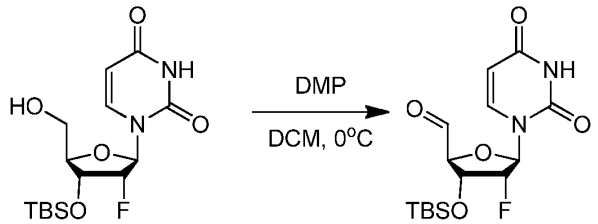


2A **3**

A solution of compound **2A** (269.23 g, 406.19 mmol) in AcOH (400.00 mL) 80% aq. was stirred at 25 °C for 15 hour. TLC indicated compound **2A** was remained a little and one new spot formed. The reaction mixture was quenched by sat. $NaHCO_3$ aq. until pH > 7 at 25°C, and then diluted with EtOAc (500 mL) and extracted with EtOAc (500 mL *3). Dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/ 0 to 0/ 1 10% DCM). Got 60 g product and recovered 130 compound 2A. Compound 3 (60.00 g, 40.98% yield) was obtained as a white solid.

TLC (Ethyl acetate: Petroleum ether = 3:1) R_f = 0.45

4. Preparation of compound 4



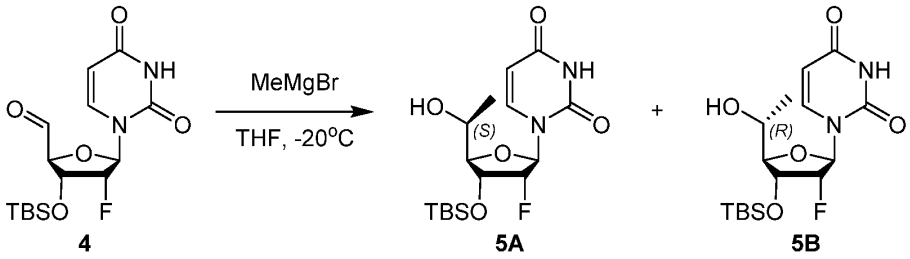
3 **4**

To a solution of compound **3** (10.00 g, 27.74 mmol) in DCM (400.00 mL) was added DMP (14.12 g, 33.29 mmol) at 0°C. The mixture was stirred at 0-50 °C for 6 hour. TLC indicated compound **3** was consumed and one new spot formed. The reaction mixture was quenched by addition sat. $Na_2S_2O_3$ aq. (300 mL) and sat. $NaHCO_3$ aq. (300 mL) at 0 °C, and then diluted with EtOAc (800 mL) and extracted with EtOAc (800 mL * 3). Dried over Na_2SO_4, filtered and concentrated under reduced pressure at 25°C. The crude product compound **4** (9.50 g, crude as yellow solid) was used into the next step without

further purification.

TLC (Ethyl acetate: Petroleum ether = 3:1) R_f = 0.37

5. Preparation of compound 5



To a solution of MeMgBr (3 M, 35.33 mL) in THF (200 mL) was added compound **4** (9.50 g, 26.50 mmol) in THF (300 mL) at -25°C under N_2. The mixture was stirred at -25 °C-25 °C for 1 hour. TLC indicated compound **4** was consumed and two new spots formed. The reaction mixture was quenched by addition NH_4Cl (300 mL) at 0°C, and then diluted with EtOAc (400 mL) and extracted with EtOAc (400 mL * 3). Dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/ Ethyl acetate = 1/ 0 to 0/ 1) to get 1 g Compound **5A**, 0.6 g Compound **5B**, other mixture of Compound **5A** and Compound **5B**. Compound **5A** (1.00 g, 10.08% yield) was obtained as a white solid. Compound **5B** (600.00 mg, 6.04% yield) was obtained as a white solid..

Compound 5A:

^{1}H NMR (400MHz, DMSO-d$_6$) δ = 7.89 (d, *J*=8.2 Hz, 1H), 5.82 (dd, *J*=2.2, 16.9 Hz, 1H), 5.53 (d, *J*=8.1 Hz, 1H), 5.09 (d, *J*=4.6 Hz, 1H), 5.05 - 4.87 (m, 1H), 4.22 (ddd, *J*=4.5, 6.7, 18.1 Hz, 1H), 3.73 - 3.65 (m, 1H), 3.62 (br d, *J*=6.7 Hz, 1H), 1.14 - 1.05 (m, 3H), 0.81 - 0.67 (m, 9H), 0.00 (d, *J*=2.3 Hz, 6H);

LCMS: ($M+H^+$): 375.1; LCMS purity = 90.1%;

HPLC: purity 97.9%;

TLC (Ethyl acetate: Petroleum ether = 1:1) **5A**: R_{f1} = 0.42; **5B**: R_{f2} = 0.47.

Compound 5B:

^{1}H NMR (400MHz, DMSO-d$_6$) δ = 7.78 (d, *J*=8.1 Hz, 1H), 5.84 (dd, *J*=4.0, 15.7 Hz, 1H), 5.60 - 5.49 (m, 1H), 5.14 - 5.03 (m, 1H), 4.98 - 4.89 (m, 1H), 4.32 (td, *J*=4.9, 12.0

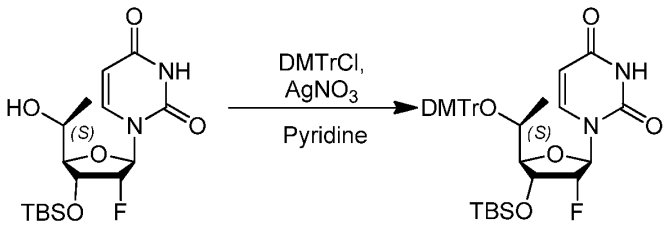
Hz, 1H), 3.86 - 3.73 (m, 1H), 3.70 - 3.57 (m, 1H), 1.00 (d, *J*=6.6 Hz, 3H), 0.85 - 0.67 (m, 9H), 0.06 - -0.10 (m, 6H);

LCMS: ($M+H^+$): 375.1;

HPLC: purity 75.9%;

TLC (Ethyl acetate: Petroleum ether = 1:1) **5A**: R_{f1} = 0.42; **5B**: R_{f2} = 0.47.

6. Preparation of compound 6A



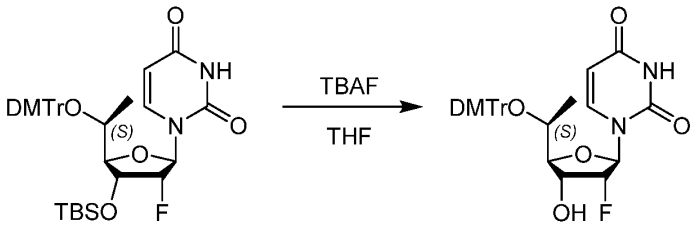
5A **6A**

Compound **5A** (1.00 g, 2.67 mmol) was dried by azeotropic distillation on a rotary evaporator with pyridine (20 mL) and toluene (20 mL*2). To a solution of **5A** (1.00 g, 2.67 mmol) in THF (30.00 mL) and pyridine (9.93 g, 125.49 mmol, 10.13 mL) was added 1-[chloro-(4-methoxyphenyl)-phenyl-methyl]-4-methoxy-benzene (1.72 g, 5.07 mmol) then added $AgNO_3$ (780.11 mg, 4.59 mmol) under N_2. The mixture was stirred at 25 °C for 20 hour. TLC indicated compound **5A** was consumed and one new spot formed. The mixture was added toluene (30 mL), quenched by addition MeOH (0.1 mL) and stirred for 1h at 25°C, then filtered through celite, and the celite plug was washed thoroughly with toluene (20 mL), concentrated under reduced pressure to give a crude. The residue was purified by column chromatography (SiO_2, Petroleum ether/ Ethyl acetate = 1/ 0 to 0/ 1) to get 1.1 g product. Compound **6A** (1.10 g, 60.87% yield) was obtained as a yellow solid.

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 8.08 (d, *J*=8.2 Hz, 1H), 7.48 (br d, *J*=7.5 Hz, 2H), 7.43 - 7.27 (m, 9H), 6.93 (dd, *J*=4.0, 8.6 Hz, 4H), 6.15 (dd, *J*=3.1, 14.1 Hz, 1H), 5.68 (d, *J*=8.2 Hz, 1H), 5.09 - 4.87 (m, 1H), 4.34 (td, *J*=5.2, 14.5 Hz, 1H), 4.01 (br d, *J*=4.9 Hz, 1H), 3.91 (d, *J*=1.5 Hz, 7H), 3.83 (br dd, *J*=2.8, 6.7 Hz, 1H), 2.29 (s, 1H), 2.19 - 2.03 (m, 1H), 1.10 (d, *J*=6.4 Hz, 3H), 1.04 - 1.00 (m, 1H), 0.92 (s, 9H), 0.18 (s, 1H), 0.15 (s, 3H), 0.00 (s, 3H).

TLC (Petroleum ether: Ethyl acetate = 1:1) R_f = 0.64

6. Preparation of **5'-(*S*)-*C*-Me-5'-ODMTr-2'-F-dU**



6A **5'-(*S*)-*C*-Me-5'-ODMTr-2'-F-dU**

To a solution of compound **6A** (1.00 g, 1.48 mmol) in THF (15.00 mL) was added TBAF (733.96 mg, 2.81 mmol). The mixture was stirred at 25 °C for 3 hour. TLC indicated compound **6A** was consumed and one new spot formed. The mixture was concentrated under reduced pressure to give a residue. The residue was dissolved by EtOAc (20 mL) and washed by NaCl (5%, aq. 20 mL), extracted with EtOAc (20 mL*3). Dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/ Ethyl acetate = 1/ 0 to 0/ 1) to get 0.7 g product. Compound 5'-(*S*)-*C*-Me-5'-ODMTr-2'-F-dU (700.00 mg, 84.07% yield) was obtained as a yellow solid.

^{1}H NMR (400MHz $CDCl_3$**,)** δ = 7.75 (d, *J*=8.2 Hz, 1H), 7.51 - 7.46 (m, 2H), 7.44 - 7.36 (m, 4H), 7.36 - 7.32 (m, 1H), 7.29 - 7.24 (m, 1H), 6.88 (dd, *J*=2.2, 8.9 Hz, 4H), 5.92 (dd, *J*=1.5, 18.0 Hz, 1H), 5.34 (s, 1H), 5.19 - 4.95 (m, 1H), 3.84 (d, *J*=1.1 Hz, 6H), 3.80 - 3.71 (m, 1H), 1.12 (d, *J*=6.5 Hz, 3H);

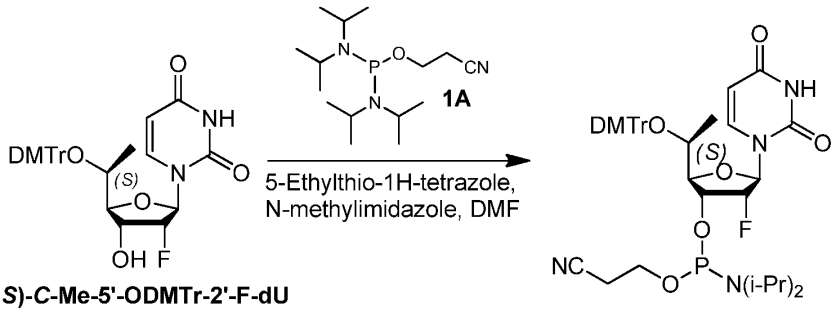
^{13}C NMR (101MHz, $CDCl_3$**)** δ = 162.95, 158.70, 149.74, 145.60, 140.44, 136.26, 136.06, 130.71, 128.54, 127.98, 127.55, 126.79, 113.81, 113.19, 112.99, 112.34, 102.72, 102.47, 88.87, 88.60, 88.53, 88.26, 87.22, 85.76, 85.52, 69.12, 68.93, 68.52, 68.28, 55.61, 55.37, 54.88, 18.29;

LCMS: ($M-H^+$): 561.2;

HPLC: purity 93.2%;

TLC (Ethyl acetate: Petroleum ether = 1:1) R_f = 0.17.

Example 29. Preparation of compound 5'-(S)-C-Me-5'-ODMTr-2'-F-dU-CNE



5'-(*S*)-*C*-Me-5'-ODMTr-2'-F-dU **5'-(*S*)-*C*-Me-5'-ODMTr-2'-F-dU-CNE**

Compound **5'-(S)-C-Me-5'-ODMTr-2'-F-dU** (4.85 g, 8.62 mmol) was dried by azeotropic distillation on a rotary evaporator with toluene (10 mL *3). To a solution of compound **5'-(S)-C-Me-5'-ODMTr-2'-F-dU** (4.85 g, 8.62 mmol) in DMF (48.5 mL) was added N-methylimidazole (1.42 g, 17.24 mmol, 1.37 mL) and 5-ethylsulfanyl-2H-tetrazole (1.12 g, 8.62 mmol), degassed and purged with N_2 for 3 times. Then added 3-bis (diisopropylamino)phosphanyloxypropanenitrile (3.90 g, 12.93 mmol, 4.11 mL). The mixture was stirred at 15 °C for 2 hr in N_2. TLC indicated compound **5'-(S)-C-Me-5'-ODMTr-2'-F-dU** was consumed and two new spot formed. The mixture was added sat. $NaHCO_3$ (aq., 50 mL), extracted with Ethyl acetate(50 mL*3). The combined organic layers were washed with H_2O (50 mL *2), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue at 30°C waterbath under N_2 atmosphere. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 0:1) get 4 g product. Compound **5'-(S)-C-Me-5'-ODMTr-2'-F-dU-CNE** (4 g, 56.66% yield) was obtained as a white solid.

^{1}H NMR (400MHz, $CDCl_3$) δ = 8.78 (br s, 1H), 8.00 - 7.79 (m, 1H), 7.41 - 7.08 (m, 10H), 6.86 - 6.64 (m, 4H), 5.93 (br t, *J* = 17.2 Hz, 1H), 5.46 (dd, *J* = 2.8, 8.1 Hz, 1H), 5.19 - 4.93 (m, 1H), 4.51 - 4.25 (m, 1H), 3.99 - 3.87 (m, 1H), 3.84 - 3.71 (m, 6H), 3.70 - 3.61 (m, 2H), 3.59 - 3.30 (m, 4H), 2.94 - 2.77 (m, 2H), 2.73 - 2.62 (m, 1H), 2.56 - 2.41 (m, 1H), 2.23 (t, *J* = 6.2 Hz, 1H), 1.32 - 0.82 (m, 22H);

^{13}C NMR (101MHz, $CDCl_3$) δ = 163.21, 163.07, 158.72, 158.65, 150.08, 149.95, 145.98, 139.98, 136.48, 136.25, 136.16, 130.76, 130.71, 128.60, 127.69, 127.05, 126.95, 117.76, 113.00, 102.41, 88.32, 87.08, 86.45, 69.83, 69.10, 68.62, 60.39, 58.26, 58.22, 58.16, 58.07, 57.88, 55.26, 55.21, 45.36, 45.30, 36.47, 31.44, 24.51, 22.94, 21.04, 20.28, 18.67, 14.20;

^{31}P NMR (162MHz, CHLOROFORM-d) δ = 150.70 (s, 1P), 150.65 (s, 1P), 150.63 (s,

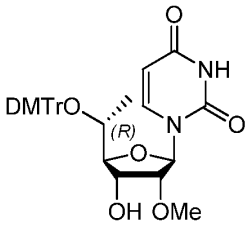
1P), 150.54 (s, 1P), 14.18 (s, 1P);

LCMS: (M-H$^+$): 761.2;

HPLC: HPLC purity = 52.15 % + 41.00 %;

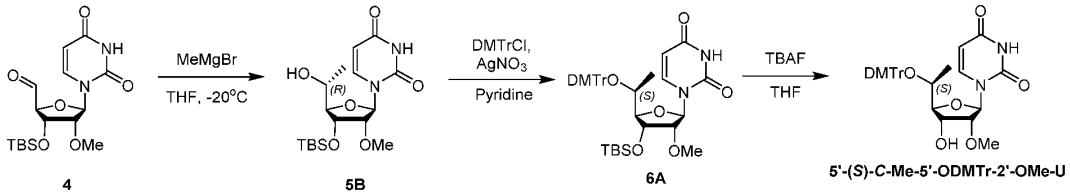
TLC: (Ethyl acetate: Petroleum ether = 3:1), R_{f1} = 0.32, R_{f2} = 0.4.

Example 30. Synthesis of 5'-(R)-C-Me-5'-ODMTr-2'-OMe-U.

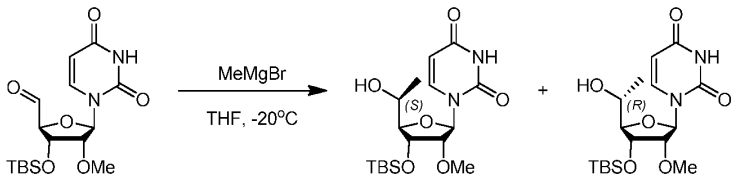


5'-(*R*)-*C*-Me-5'-ODMTr-2'-OMe-U

General Scheme



1. Preparation of compound 5B



To a solution of compound **4** (19.00 g, 51.29 mmol) in THF (140 mL) was dropwise in MeMgBr (3 M, 68.39 mL) (a solution in 140 mL THF) at -20°C over 10 min. The mixture was stirred at -20 °C-20 °C for 30 min. TLC showed compound **4** was partly remained and new spot was detected .Then the mixture was stirred at 20°C for 20 min.

TLC and LCMS showed compound **4** was partly remained and new spot was detected. The reaction mixture was quenched by addition sat. NH_4Cl (200 mL) at 0 °C, and then diluted with EtOAc (500 mL) and extracted with EtOAc (500 mL*3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by MPLC (flash Silica (CS), 40-60μm, 60A, 220 g, Ethyl acetate/ Petroleum ether = 0%, 10%, 20%, 30%, 50%, 60%, 70%, 80%, 100%) to give compound **5A** (1.80 g, 9.08% yield) as a white solid. Compound **5B** (1.60 g, 8.07% yield) was obtained as a white solid.

TLC (plate 1: Petroleum ether : Ethyl acetate=1:3) R_{f1}=0.39,R_{f2}=0.32

Compound **5A**

^{1}H NMR (400MHz, DMSO-d$_6$) δ = 11.26 (s, 1H), 7.98 (d, *J*=8.2 Hz, 1H), 5.75 (d, *J*=4.6 Hz, 1H), 5.58 (d, *J*=8.2 Hz, 1H), 5.10 (d, *J*=4.4 Hz, 1H), 4.19 (t, *J*=4.6 Hz, 1H), 3.72 (br t, *J*=4.9 Hz, 2H), 3.60 (br d, *J*=2.9 Hz, 1H), 3.25 (s, 3H), 1.07 (d, *J*=6.4 Hz, 3H), 0.79 (s, 9H), 0.00 (s, 6H)

LCMS ($M+H^+$): 387.1

TLC (plate 1: Petroleum ether : Ethyl acetate=1:3) R_{f1}=0.39

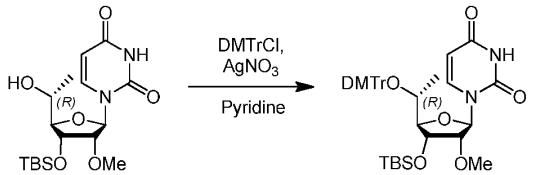
Compound **5B**

^{1}H NMR (400MHz, DMSO-d$_6$) δ = 11.28 (s, 1H), 7.80 (d, *J*=8.2 Hz, 1H), 5.77 (d, *J*=6.8 Hz, 1H), 5.58 (d, *J*=8.2 Hz, 1H), 5.07 (br s, 1H), 4.33 - 4.29 (m, 1H), 3.77 (dd, *J*=5.1, 6.6 Hz, 1H), 3.72 - 3.64 (m, 1H), 3.55 (dd, *J*=2.0, 4.0 Hz, 1H), 3.18 (s, 3H), 1.01 (d, *J*=6.6 Hz, 3H), 0.78 (s, 9H), 0.00 (s, 6H)

LCMS ($M+H^+$): 387.2

TLC (Petroleum ether : Ethyl acetate=1:2) R_{f2} = 0.32

5. Preparation of compound 6B

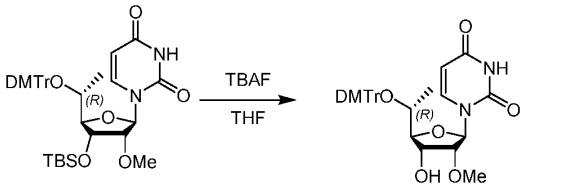


Compound **5B** (1.10 g, 2.85 mmol) was dried by azeotropic distillation on a rotary evaporator with Pyridine (20 mL) and toluene (20 mL*2). To a solution of compound **5B**

(1.10 g, 2.85 mmol) in THF (33.00 mL) and pyridine (11.52 g, 145.70 mmol, 11.76 mL) was added DMTCl (1.83 g, 5.41 mmol), then added $AgNO_3$ (831.53 mg, 4.90 mmol, 823.30 uL). The mixture was stirred at 25 °C for 20 hours. TLC showed compound **5B** was consumed and new spot was detected. The mixture was added toluene (30 mL), quenched by addition MeOH (0.1 mL) and stirred for 1h at 25°C, then filtered through celite, and the celite plug was washed thoroughly with toluene(20 mL), concentrated under reduced pressure to give a crude. Compound **6B** (3.00 g, crude) was obtained as a yellow oil.

TLC (Petroleum ether : Ethyl acetate=1:1) Rf=0.43.

6. ***Preparation of compound 5'-(R)-C-Me-5'-ODMTr-2'-OMe-U***



6B **5'-(*R*)-*C*-Me-5'-ODMTr-2'-OMe-U**

To a solution of compound **6B** (1.96 g, 2.85 mmol) in THF (40.00 mL) was added TBAF (1 M, 5.41 mL). The mixture was stirred at 25 °C for 3 hour . TLC showed compound **6B** was consumed and one new spot was detected. The mixture was concentrated under reduced pressure to give a residue. The residue was dissolved by EtOAc (50 mL) and washed by NaCl (5%, aq. 50 mL), extracted with EtOAc(50 mL*3) , dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by MPLC (SiO_2, silica gel was washed by Petroleum ether (5%TEA) ,Ethyl acetate/ Petroleum ether = 0%; 20%; 50%; 70%, 80% to 100%) to give compound ***5'-(R)-C-Me-5'-ODMTr-2'-OMe-U*** (950.00 mg, 56.95% yield) was obtained as a white solid.

^{1}H NMR (400MHz, DMSO-d_6) δ = 11.37 (s, 1H), 7.44 (d, *J*=7.6 Hz, 2H), 7.36 - 7.19 (m, 8H), 6.90 (d, *J*=8.9 Hz, 4H), 5.78 - 5.71 (m, 1H), 5.21 - 5.13 (m, 2H), 4.30 (q, *J*=5.6 Hz, 1H), 3.78 - 3.64 (m, 8H), 3.52 - 3.42 (m, 1H), 3.34 (s, 3H), 0.79 (d, *J*=6.4 Hz, 3H)

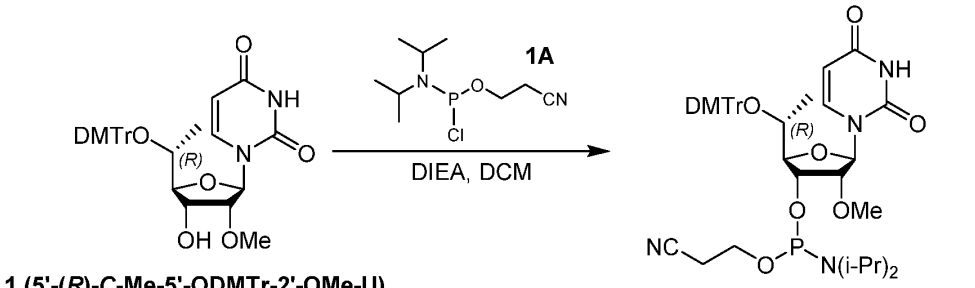
^{13}C NMR (101MHz, DMSO-d_6) δ = 163.24, 158.58, 158.55, 150.82, 146.71, 140.99, 136.59, 136.48, 130.63, 128.33, 128.16, 127.07, 113.58, 113.52, 102.25, 87.59, 86.52, 86.29, 82.06, 69.89, 68.16, 58.10, 55.51, 55.49, 33.72, 23.07, 17.61, 15.20

HPLC purity: 98.168%

LCMS ($M+H^+$): 573.1

TLC (Petroleum ether : Ethyl acetate=1:2) R_f = 0.10

Example 31. ***Preparation of compound*** **5'-(R)-C-Me-5'-ODMTr-2'-OMe-U-CNE-phosphoramidite**



1 (5'-(*R*)-*C*-Me-5'-ODMTr-2'-OMe-U)

5'-(*R*)-*C*-Me-5'-ODMTr-2'-OMe-U-CNE-phosphoramidite

DIEA (1.32 g, 10.23 mmol, 1.79 mL) were added consecutively to a stirred solution of compound **1** (4.9 g, 8.53 mmol) in anhyd. DCM (50 mL) under Ar atm., and then added compound **1A** (43.25 mg, 182.73 umol) at 0°C. After stirring at 0 °C-15 °C for 3 hr. LCMS showed compound **1** was partly remained and two major spots was detected. Then added compound **1A** (201.82 mg, 852.74 umol). After stirring at 0°C-15 °C for 1hr, TLC showed compound **1** was partly remained and two major spots was detected. The mixture was added sat. $NaHCO_3$ (aq., 20 mL) and extracted with DCM (50 mL*3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by MPLC (SiO_2, Ethyl acetate/Petroleum ether = 0%, 20%, 40%, 60%, 70%, 100%, 5%TEA) to give compound **5'-(R)-C-Me-5'-ODMTr-2'-OMe-U-CNE- phosphoramidite** (4.0 g, 60.54% yield) was obtained as a white solid.

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 7.56 - 7.48 (m, 2H), 7.47 - 7.36 (m, 4H), 7.33 - 7.20 (m, 5H), 6.86 (td, *J*=2.5, 8.9 Hz, 4H), 5.94 (t, *J*=4.7 Hz, 1H), 5.06 (dd, *J*=1.2, 8.1 Hz, 1H), 4.91 - 4.71 (m, 1H), 4.04 - 3.86 (m, 4H), 3.82 (s, 6H), 3.76 - 3.65 (m, 3H), 3.53 (d, *J*=8.7 Hz, 4H), 2.71 - 2.53 (m, 3H), 1.27 - 1.22 (m, 10H), 1.01 (t, *J*=6.3 Hz, 3H)

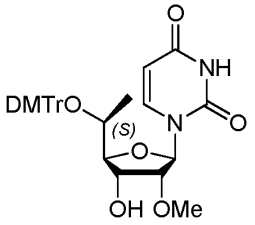
^{31}P NMR (162MHz, $CDCl_3$**)** δ = 150.16, 149.61, 14.16

LCMS: ($M-H^+$): 773.3

HPLC purity: 40.8% + 50.0%

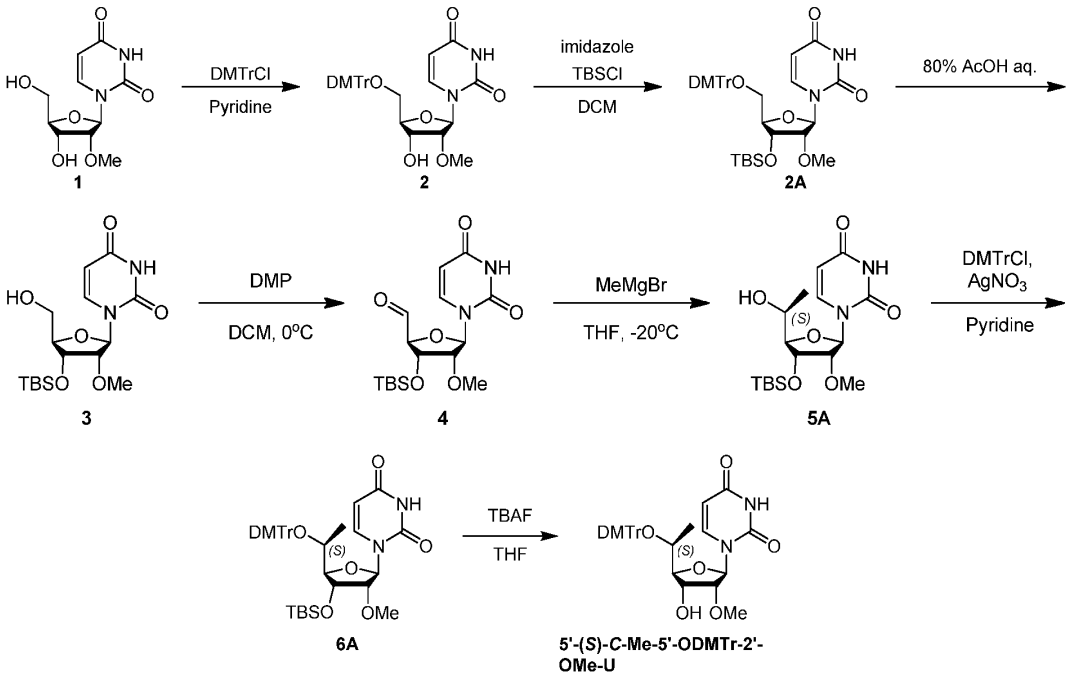
TLC (Petroleum ether : Ethyl acetate = 1:3, 5%TEA) R_{f1} = 0.60, R_{f2} = 0.55

Example 32. Synthesis of 5'-(S)-C-Me-5'-ODMTr-2'-OMe-U.

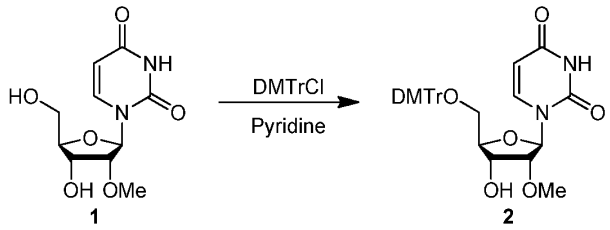


5'-(*S*)-*C*-Me-5'-ODMTr-2'-OMe-U

General Scheme



1. ***Preparation of compound 2***

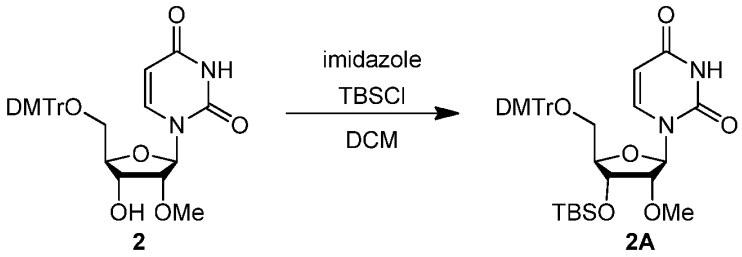


1 2

To a solution of compound **1** (10.00 g, 38.73 mmol) in pyridine (80.00 mL) was added DMTCl (15.75 g, 46.48 mmol) at 0°C. The mixture was stirred at 0-20°C for 16 hours. TLC showed the starting material was consumed and one new spot was detected. The resulting mixture was concentrated under reduced pressure to give a residue. The residue was dissolved by addition ethyl acetate (300 mL) and H_2O (150 mL), and extracted with ethyl acetate (300 mL*3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a crude (25 g) as a yellow solid. Compound **2** (25.00 g, crude) was obtained as a yellow oil.

TLC (Petroleum ether : Ethyl acetate=1:3, 5% TEA) Rf = 0.1.

2. Preparation of compound 2A



2 2A

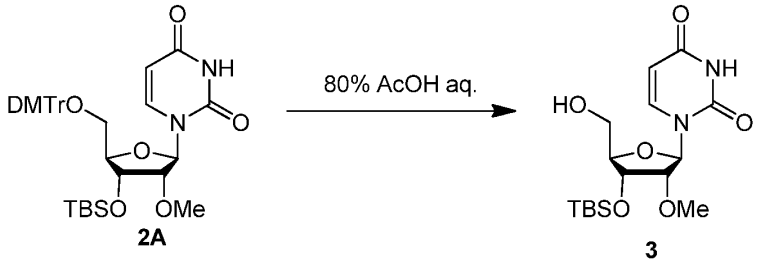
To a solution of compound **2** (24.00 g, 42.81 mmol) in DCM (200.00 mL) was added imidazole (5.83 g, 85.62 mmol) and TBSCl (9.68 g, 64.21 mmol). The mixture was stirred at 20 °C for 14 hours. TLC showed compound **2** was partly remained and one major spot was detected. The resulting solution was combined with another batch product (1 g scale) and diluted with DCM (300 mL), washed with $NaHCO_3$ (aq., 100 mL) and brine (100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give 28 g crude. Compound **2A** (26.88 g, 93.04% yield) (Yield From Conversion Rate) was obtained as a white solid.

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 9.29 (br s, 1H), 8.64 (br d, *J*=4.2 Hz, 1H), 8.19 (d, *J*=8.2

Hz, 1H), 7.40 - 7.25 (m, 12H), 7.20 (d, *J*=8.8 Hz, 2H), 6.89 - 6.81 (m, 5H), 5.98 (s, 1H), 5.28 (d, *J*=7.9 Hz, 1H), 4.43 (dd, *J*=5.0, 8.0 Hz, 1H), 4.11 (br d, *J*=8.2 Hz, 1H), 3.84 - 3.78 (m, 8H), 3.73 - 3.55 (m, 5H), 3.42 - 3.36 (m, 1H), 1.00 - 0.83 (m, 16H), 0.15 - 0.04 (m, 7H);

TLC (Petroleum ether : Ethyl acetate=1:3) Rf = 0.47.

3. Preparation of compound 3

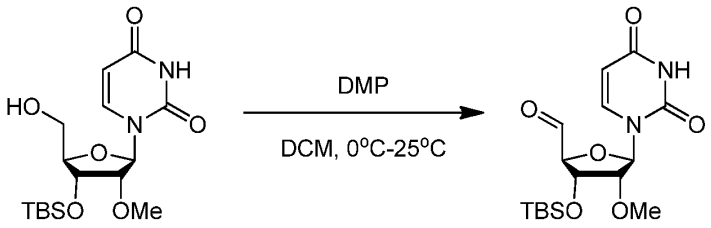


To a solution of compound **2A** (27.00 g, 40.01 mmol) in CH_3COOH/H_2O (V/V = 80%, 100 mL). The mixture was stirred at 20°C for 16 hours. TLC showed compound **2A** was consumed and one major spot was detected. The resulting solution was diluted with Ethyl acetate (300 mL) and added sat. $NaHCO_3$ (aq.) to pH~7, then extracted with Ethyl acetate (300 mL*3). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a crude. The crude was purified by MPLC (SiO_2, Petroleum ether : Ethyl acetate=5:1 to 1:3). Compound **3** (10.00 g, 67.10% yield) was obtained as a white solid.

^{1}H NMR (400MHz,) $CDCl_3$ δ = 8.18 (br s, 1H), 7.56 (d, *J*=8.2 Hz, 1H), 5.62 (dd, *J*=2.1, 8.3 Hz, 1H), 5.55 (d, *J*=4.2 Hz, 1H), 4.25 (t, *J*=5.1 Hz, 1H), 3.91 - 3.86 (m, 2H), 3.65 (br dd, *J*=7.1, 11.9 Hz, 1H), 3.38 (s, 3H), 2.46 (br d, *J*=4.2 Hz, 1H), 0.81 (s, 9H), 0.01 (d, *J*=5.5 Hz, 6H);

TLC (Petroleum ether : Ethyl acetate=1:1) Rf = 0.28.

3. Preparation of compound 4

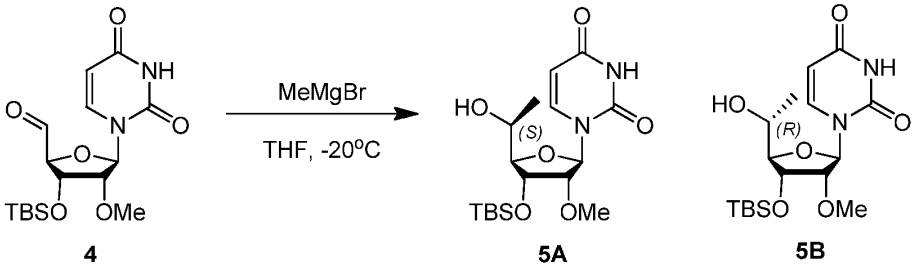


To a solution of compound **3** (3.00 g, 8.05 mmol) in DCM (50.00 mL) was added DMP (4.10 g, 9.66 mmol) at 0°C. The mixture was stirred at 25°C for 1.5 h. TLC showed compound **3** was partly remained and one major spot was detected. The mixture was diluted and added EtOAc (50 mL) and quenched by addition $Na_2S_2O_3$ (5% aq., 80 mL) and sat. $NaHCO_3$ (aq., 80 mL) at 0°C and stirred for 20 min, extracted with EtOAc (200 mL *3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure at 25°C water bath to give a crude. Compound **4** (2.50 g, crude) was obtained as a white solid.

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 9.64 (s, 1H), 7.55 - 7.44 (m, 1H), 5.73 - 5.56 (m, 2H), 4.42 - 4.25 (m, 1H), 3.80 (br t, *J*=4.5 Hz, 1H), 3.42 - 3.20 (m, 3H), 0.78 (s, 9H), 0.07 - 0.14 (m, 6H);

TLC (Petroleum ether : Ethyl acetate=1:3) R_f = 0.18.

5. Preparation of compound 5A and 5B



To a solution of compound **4** (2.50 g, 6.75 mmol) in THF (30 mL) was added dropwise MeMgBr (3 M, 9.00 mL) (a solution in 30 mL THF) at -20°C over 10 min. The mixture was stirred at -20°C - 20°C for 30 min. TLC showed compound **4** was partly remained and new spot was detected. Then the mixture was stirred at 20°C for 20 min. TLC showed compound **4** was partly remained and new spot was detected. The residue was purified by MPLC (SiO_2, Petroleum ether : Ethyl acetate = 5:1, 3:1, 1:1,to 1:2) to compound **5A**

(320.00 mg, 12.27% yield) (Yield From Conversion Rate) was obtained as a white solid and compound **5B** (480.00 mg, 18.37% yield) (Yield From Conversion Rate) was obtained as a white solid.

TLC (Petroleum ether : Ethyl acetate=1:2) R_{f1} = 0.39,R_{f2} = 0.32

Compound **5A:**

^{1}H NMR (400MHz, DMSO-d$_6$) δ = 11.26 (s, 1H), 7.98 (d, *J*=8.2 Hz, 1H), 5.75 (d, *J*=4.6 Hz, 1H), 5.58 (d, *J*=8.2 Hz, 1H), 5.10 (d, *J*=4.4 Hz, 1H), 4.19 (t, *J*=4.6 Hz, 1H), 3.72 (br t, *J*=4.9 Hz, 2H), 3.60 (br d, *J*=2.9 Hz, 1H), 3.25 (s, 3H), 1.07 (d, *J*=6.4 Hz, 3H), 0.79 (s, 9H), 0.00 (s, 6H)

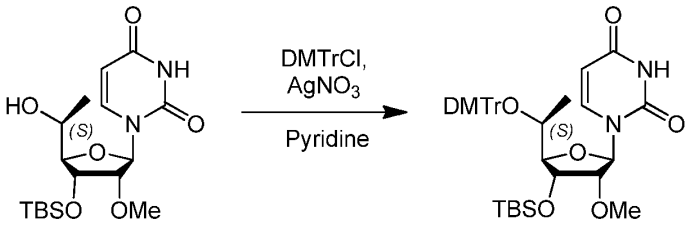
TLC (Petroleum ether : Ethyl acetate=1:2) R_{f1} = 0.39

Compound **5B:**

^{1}H NMR (400MHz, DMSO-d$_6$) δ = 11.28 (s, 1H), 7.80 (d, *J*=8.2 Hz, 1H), 5.77 (d, *J*=6.8 Hz, 1H), 5.58 (d, *J*=8.2 Hz, 1H), 5.07 (br s, 1H), 4.33 - 4.29 (m, 1H), 3.77 (dd, *J*=5.1, 6.6 Hz, 1H), 3.72 - 3.64 (m, 1H), 3.55 (dd, *J*=2.0, 4.0 Hz, 1H), 3.18 (s, 3H), 1.01 (d, *J*=6.6 Hz, 3H), 0.78 (s, 9H), 0.00 (s, 6H)

TLC (Petroleum ether : Ethyl acetate=1:2) R_{f2} = 0.32

6. Preparation of compound 6A

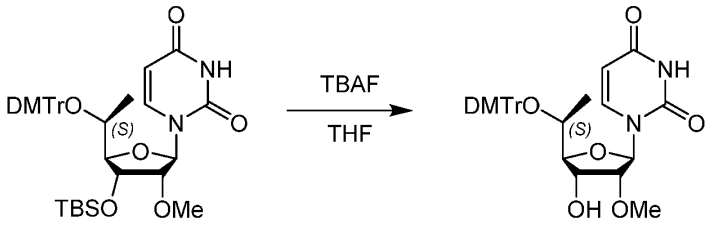


5A **6A**

To a mixture of pre-purified compound **5A** (740.00 mg, 1.91 mmol), DMTCl (1.23 g, 3.63 mmol), and pyridine (7.10 g, 89.75 mmol, 7.24 mL) in anhyd. THF (30.00 mL) was added $AgNO_3$ (558.06 mg, 3.29 mmol). The mixture was stirred at 25°C under N_2 for 16 h. TLC showed compound **5A** was consumed and one new spot was detected. The mixture was quenched by addition of MeOH (0.1mL) and diluted with toluene (30 mL). After stirred for an additional 1 h, the mixture was filtered through Celite, and the Celite plug was washed thoroughly with toluene. The filtrate was evaporated in vacuo to afford 2.4 g of crude. Compound **6A** (2.40 g, crude) was obtained as a yellow oil.

TLC (Petroleum ether : Ethyl acetate=1:1) R_f = 0.48.

7. Preparation of compound 5'-(S)-C-Me-5'-ODMTr-2'-OMe-U



6A **5'-(*S*)-*C*-Me-5'-ODMTr-2'-OMe-U**

To a solution of compound **6A** (1.32 g, 1.92 mmol) in THF (12.00 mL) was added TBAF (1 M, 3.64 mL). The mixture was stirred at 25°C for 3 hours. TLC showed compound **6A** was consumed and one new spot was detected. The mixture was concentrated under reduced pressure to give a residue. The residue was dissolved by EtOAc (50 mL) and washed by NaCl (5%, aq. 50 mL), extracted with EtOAc(50 mL*3), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by MPLC (SiO_2, silica gel was washed by Petroleum ether (5% TEA), Ethyl acetate/ Petroleum ether = 0%; 20%; 50%; 70%, 80% to 100%). Compound **5'-(S)-C-Me-5'-ODMTr-2'-OMe-U** (800.00 mg, 72.51% yield) was obtained as a white solid.

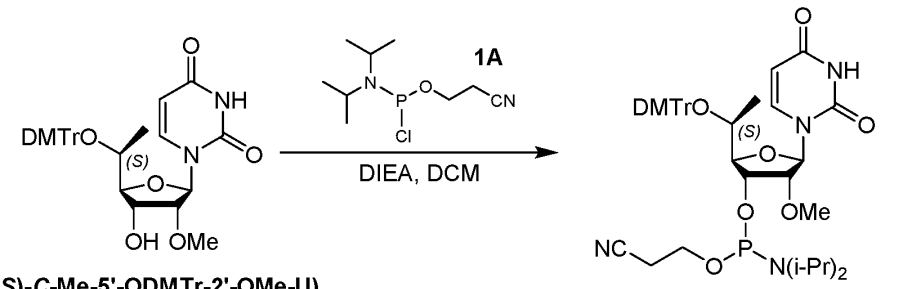
^{1}H NMR (400MHz, DMSO-d_6) δ = 11.42 (s, 1H), 7.62 (d, *J*=8.2 Hz, 1H), 7.43 (br d, *J*=7.6 Hz, 2H), 7.34 - 7.19 (m, 7H), 6.88 (dd, *J*=5.3, 8.7 Hz, 4H), 5.81 - 5.73 (m, 2H), 5.58 (d, *J*=8.1 Hz, 1H), 5.11 (d, *J*=6.7 Hz, 1H), 4.22 - 4.11 (m, 1H), 3.83 - 3.72 (m, 8H), 3.55 (quin, *J*=5.7 Hz, 1H), 3.37 - 3.35 (m, 3H), 0.69 (d, *J*=6.2 Hz, 3H);

^{13}C NMR (101MHz, DMSO-d_6) δ = 163.35, 158.58, 158.55, 150.93, 146.56, 136.81, 136.70, 130.57, 128.41, 128.08, 113.47, 102.49, 86.37, 85.94, 69.64, 68.18, 57.99, 55.44, 17.66;

LCMS ($M+Na^+$): 597.2, 97.26% purity;

TLC (Petroleum ether : Ethyl acetate=1:1) Rf = 0.10.

Preparation of compound **5'-(S)-C-Me-5'-ODMTr-2'-OMe-U-CNE-phosphoramidite**



(5'-(*S*)-*C*-Me-5'-ODMTr-2'-OMe-U)

5'-(*S*)-*C*-Me-5'-ODMTr-2'-OMe-U-CNE-phosphoramidite

DIEA (1.32 g, 10.23 mmol, 1.79 mL) were added consecutively to a stirred solution of compound **1** (4.9 g, 8.53 mmol) in anhyd. DCM (50 mL) under Ar atm., and then added compound **1A** (43.25 mg, 182.73 umol) at 0°C. After stirring at 0 °C-15 °C for 3hr. LCMS showed compound **1** was partly remained and two major spots were detected. Then added compound **1A** (201.82 mg, 852.74 umol), and after stirring at 0 °C-15 °C for 1hr, TLC showed compound **1** was partly remained and two major spots were detected. The mixture was added sat. $NaHCO_3$ (aq., 20 mL) and extracted with DCM (50 mL*3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by MPLC (SiO_2, Ethyl acetate/Petroleum ether = 0%, 20%, 40%, 60%, 70%, 100%, 5%TEA) to give compound **5'-(S)-C-Me-5'-ODMTr-2'-OMe-U-CNE-phosphoramidite** (4.5 g, 68.11% yield) was obtained as a white solid.

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 8.56 (br s, 1H), 8.12 - 7.84 (m, 1H), 7.35 - 7.29 (m, 2H), 7.28 - 7.11 (m, 8H), 6.74 (ddd, *J*=3.0, 5.3, 8.6 Hz, 4H), 5.92 (t, *J*=4.0 Hz, 1H), 5.48 (t, *J*=8.1 Hz, 1H), 4.30 - 4.08 (m, 1H), 3.97 - 3.84 (m, 2H), 3.77 - 3.54 (m, 9H), 3.53 - 3.39 (m, 6H), 2.50 (t, *J*=6.2 Hz, 1H), 2.17 (t, *J*=6.3 Hz, 1H), 1.10 - 1.01 (m, 9H), 0.97 - 0.91 (m, 4H), 0.88 (br d, *J*=6.4 Hz, 2H)

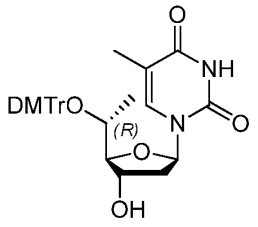
^{31}P NMR (162MHz $CDCl_3$**,)** δ = 150.40, 150.11, 14.16

LCMS: ($M\text{-}H^+$): 773.3

HPLC purity: 40.4% + 49.2%

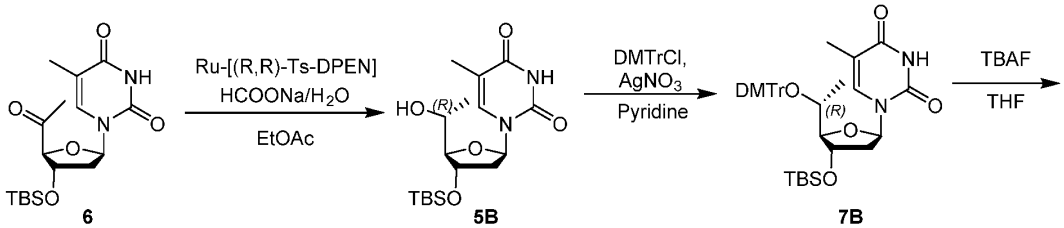
TLC (Petroleum ether : Ethyl acetate=1:3, 5%TEA) R_{f1}=0.60, R_{f2}=0.55

Example 32. Synthesis of 5'-(*R*)-*C*-Me-5'-ODMTr-dT.



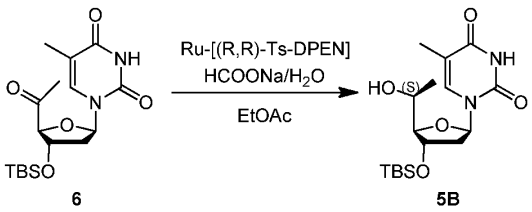
5'-(*R*)-*C*-Me-5'-ODMTr-dT

General Scheme



5'-(*R*)-*C*-Me-5'-ODMTr-dT

1. Preparation of compound 5B



A 100 mL round-bottom flask equipped with a septum covered side arm was charged with [[(1R,2R)-2-amino-1,2-diphenyl-ethyl]-(p-tolylsulfonyl)amino]-chloro-ruthenium;1-isopropyl-4-methyl-benzene (34.53 mg, 54.27 umol) and compound **6** (1.00 g, 2.71 mmol), and the system was flushed with nitrogen. A solution of sodium;formate;dihydrate (11.75 g, 112.89 mmol) in water (40.00 mL) was added, followed by EtOAc (10.00 mL). The resulting two-phase mixture was stirred for 12 h at 25°C. TLC showed the starting material was consumed. The mixture was extracted

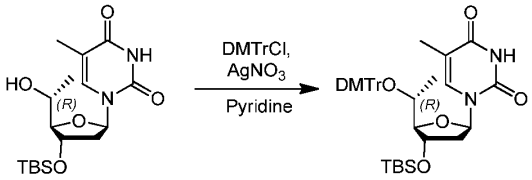
with EtOAc (50 mL*3). The combined organic was washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated to get the crude. The mixture was purified by MPLC (Petroleum ether /MTBE=10:1 to 1:1) to get compound **5B** as a yellow oil (1.00 g, 99.50% yield).

^{1}H NMR (400MHz, DMSO-d6): δ = 11.30 (s, 1H), 7.67 (s, 1H), 6.16 (dd, J=5.6, 8.7 Hz, 1H), 5.04 (d, J=5.1 Hz, 1H), 4.49 (br d, J=5.1 Hz, 1H), 3.86 - 3.66 (m, 1H), 3.55 (d, J=4.2 Hz, 1H), 2.50 (br s, 12H), 2.22 - 2.05 (m, 1H), 1.96 (br dd, J=5.6, 12.9 Hz, 1H), 1.77 (s, 3H), 1.11 (d, J=6.2 Hz, 4H), 0.94 - 0.81 (m, 10H), 0.09 (s, 6H);

HPLC: HPLC purity: 84.4%;

TLC (Petroleum ether / Ethyl acetate=1:1) Rf = 0.37.

2. Preparation of compound 7B



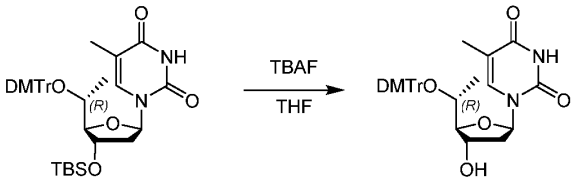
5B **7B**

The compound **5B** (1.00 g, 2.70 mmol) was dried by azeotropic distillation on a rotary evaporater with pyridine (20 mL) and toluene (20 mL*2).

A solution of compound **5B** (1.00 g, 2.70 mmol) and DMTCl (1.89 g, 5.59 mmol) in the mixture of pyridine (10.00 mL) and THF (40.00 mL) was degassed and purged with N_2 for 3 times and then $AgNO_3$ (788.56 mg, 4.64 mmol) was added. The mixture was stirred at 25°C for 15hr. TLC showed the starting material was consumed. MeOH(1 mL) was added and stirred for 15 min and then the mixture was filtered and the cake was washed with toluene (20 mL*3), the filtrate was concentrated to get the compound **7B** as a yellow oil (1.81 g, crude). The mixture was used directly to next step without any purification.

TLC (Petroleum ether / Ethyl acetate) Rf = 0.63

3. Preparation of 5'-(R)-C-Me-5'-ODMTr-dT



7B **5'-(*R*)-*C*-Me-5'-ODMTr-dT**

To a solution of compound **7B** (1.81 g, 2.69 mmol.) in THF (20.00 mL) was added TBAF (1 M, 5.11 mL). The mixture was stirred at 25 °C for 3 hours. TLC showed the starting material was consumed. The mixture was concentrated to get the crude and then sat. NaCl (5% aq., 20 mL) was added and extracted with EtOAc (20 mL*3). The combined organic was dried over Na_2SO_4, filtered and concentrated to get the crude. The residue was purified by MPLC (Petroleum ether / Ethyl acetate 5:1, 1:1, 1:4, 5% TEA) to get **5'-(R)-C-Me-5'-ODMTr-dT** as a white solid (1.00 g, 66.55% yield).

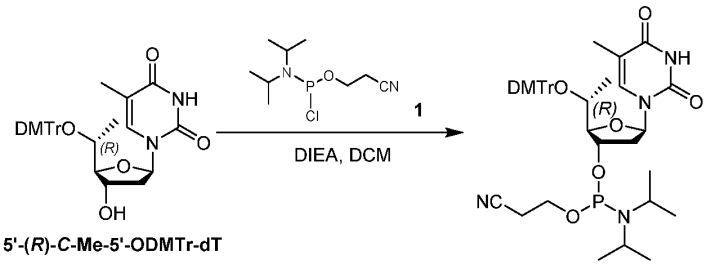
^{1}H NMR (400MHz, DMSO-d6): δ =11.38 (s, 1H), 7.52 (d, J=7.5 Hz, 2H), 7.43 - 7.31 (m, 6H), 7.30 - 7.22 (m, 1H), 7.13 (d, J=1.0 Hz, 1H), 6.99 - 6.90 (m, 4H), 6.18 (t, J=7.2 Hz, 1H), 5.33 (d, J=4.8 Hz, 1H), 4.56 (quin, J=4.1 Hz, 1H), 3.79 (d, J=2.4 Hz, 6H), 3.68 (t, J=3.3 Hz, 1H), 3.47 - 3.39 (m, 1H), 2.11 (dd, J=4.8, 7.1 Hz, 2H), 1.46 (s, 3H), 0.83 (d, J=6.4 Hz, 3H)

HPLC: HPLC purity: 98.6%

LCMS: (M-H$^+$) = 557.2; LCMS purity: 100.0%

TLC (Petroleum ether/Ethyl acetate = 1:1, 5% TEA) Rf = 0.02.

Preparation of 5'-(R)-C-Me-5'-ODMTr-dT-CNE-phosphoramidite



5'-(*R*)-*C*-Me-5'-ODMTr-dT **5'-(*R*)-*C*-Me-5'-ODMTr-dT-CNE-phosphoramidite**

The 5'-(R)-C-Me-5'-ODMTr-dT (5 g, 8.95 mmol) was dried with toluene (50 mL). To a

solution of DIEA (1.39 g, 10.74 mmol, 1.87 mL) and 5'-(R)-C-Me-5'-ODMTr-dT (5 g, 8.95 mmol) in anhyd. DCM (50 mL) was added compound **1** (2.76 g, 9.40 mmol) under N_2 at 0°C. The mixture was stirring at 15°C for 2 h. TLC showed the starting material was consumed and two new spots were found. The mixture was quenched by addition of saturated aq. $NaHCO_3$ (20 mL) and extracted with DCM (30mL*3). The combined organic was dried over Na_2SO_4, filtered and concentrated to get the crude. The above crude material was purified on a Combiflash instrument from Teledyne using either a pre-treated silica gel column. A 40 g silica gel cartridge column was first pre-treated by eluting with 10% EtOAc/ Petroleum ether containing 5% Et_3N (300 mL) and the crude was dissolved in a 2:1 volume:volume mixture of methylene chloride: Petroleum ether containing 5% Et_3N then loaded onto a 40 g silica column which had been equilibrated with 10% Petroleum ether/EtOAc containing 5% Et_3N. After loading the sample on the column, the purification process was run using the following gradient: 10 to 50% EtOAc/Petroleum ether containing 5% Et_3N, then residual solvent was removed to get the ***5'-(R)-C-Me-5'-ODMTr-dT-CNE-phosphoramidite*** as a white solid (3.6 g, 53.00% yield).

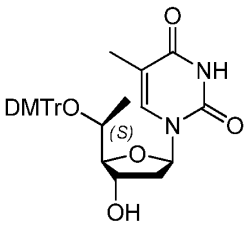
^{1}H NMR (400MHz $CDCl_3$**,)** δ = 8.11 (br s, 1H), 7.53 (br d, J=7.7 Hz, 3H), 7.42 (br t, J=8.2 Hz, 4H), 7.32 - 7.17 (m, 4H), 7.07 - 6.99 (m, 1H), 6.84 (br d, J=8.2 Hz, 4H), 6.31 (br dd, J=5.5, 8.7 Hz, 1H), 4.94 (br s, 1H), 3.96 - 3.73 (m, 10H), 3.72 - 3.41 (m, 4H), 2.65 (td, J=6.1, 18.0 Hz, 2H), 2.53 - 2.37 (m, 1H), 2.10 (br d, J=8.2 Hz, 1H), 1.47 (br s, 4H), 1.33 - 1.16 (m, 15H), 1.00 - 0.90 (m, 3H)

^{31}P NMR (162MHz, $CDCl_3$) δ = 148.81 (s, 1P), 148.35 (s, 1P)

HPLC: HPLC purity: 59.15%+35.91%

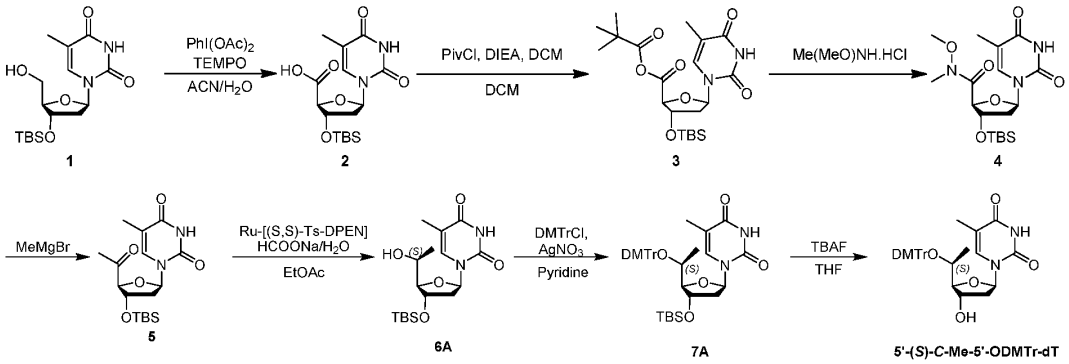
LCMS: LCMS purity: 60.34%+37.17%

Example 33. Synthesis of 5'-(S)-C-Me-5'-ODMTr-dT

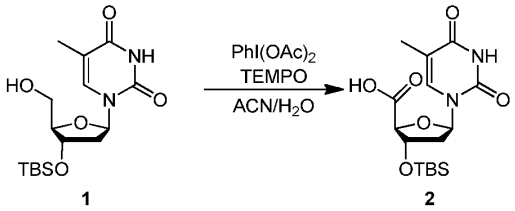


5'-(*S*)-*C*-Me-5'-ODMTr-dT

General Scheme



1. Preparation of compound 2



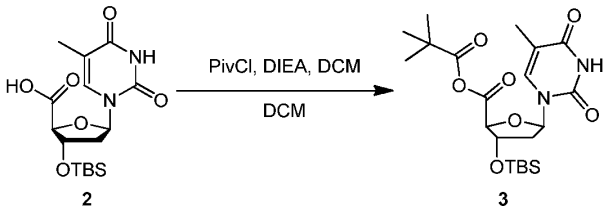
To a solution of compound **1** (63.00 g, 176.72 mmol) in the mixture of H_2O (250.00 mL) and MeCN (250.00 mL) was added $PhI(OAc)_2$ (125.23 g, 388.79 mmol) and TEMPO (5.56 g, 35.34 mmol) at 10°C. The mixture was stirred at 25 °C for 2 hour. TLC (Petroleum ether /Ethyl acetate=1:1, Rf = 0) showed the starting material was consumed. The mixture was concentrated to get the crude and the mixture was added MTBE (1 L) stirred for 0.5h and then filtered, the cake was washed with MTBE (1 L*2), the cake was dried to get the compound **2** as a white solid (126.00 g, 96.23% yield).

^{1}H NMR (400MHz, DMSO): δ = 11.21 (s, 1H), 7.89 (d, J=1.0 Hz, 1H), 6.18 (dd, J=5.9, 8.6 Hz, 1H), 4.61 - 4.41 (m, 1H), 4.17 (d, J=0.9 Hz, 1H), 2.51 - 2.26 (m, 3H), 2.09 - 1.85 (m, 2H), 1.74 - 1.58 (m, 3H), 0.90 - 0.58 (m, 10H), 0.00 (d, J=2.0 Hz, 6H)

LCMS: ($M+H^+$): 371.1;

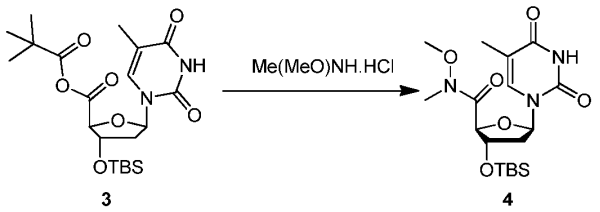
TLC (Petroleum ether /Ethyl acetate=1:1) Rf = 0.35.

2. Preparation of compound 3



To a solution of compound **2** (50.00 g, 134.96 mmol) in DCM (500.00 mL) was added DIEA (34.89 g, 269.92 mmol, 47.15 mL) and 2,2-dimethylpropanoyl chloride (21.16 g, 175.45 mmol). The mixture was stirred at -10-0 °C for 1.5 hours. TLC showed the starting material was consumed. The mixture in DCM was used directly for next step.
TLC (Petroleum ether/ Ethyl acetate=1:1) Rf =0.15

3. Preparation of compound 4

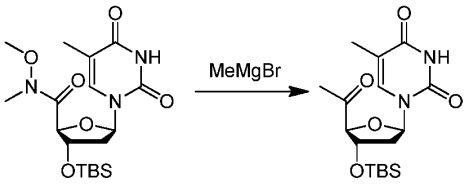


The mixture compound **3** in DCM was added TEA (40.94 g, 404.55 mmol, 56.08 mL) and N-methoxymethanamine hydrochloride (19.73 g, 202.27 mmol). The mixture was stirred at 0°C for 1h. TLC showed the starting material was consumed. The mixture was washed with HCl (1N, 100 mL) and then aqueous $NaHCO_3$ (100 mL). The organic was dried over Na_2SO_4 and filtered to get the crude. The mixture was purified by silica gel chromatography (Petroleum ether/Ethyl acetate=30/1, 0/1) to afford the compound **4** as a white solid (95.50 g, 85.63% yield).

^{1}H NMR (400MHz, $CDCl_3$): δ = 8.29 (s, 1H), 8.19 (br s, 1H), 6.46 (dd, J=5.1, 9.3 Hz, 1H), 4.71 (s, 1H), 4.38 (d, J=4.2 Hz, 1H), 3.65 (s, 3H), 3.15 (s, 3H), 2.18 - 2.08 (m, 1H), 2.00 - 1.90 (m, 1H), 1.87 (d, J=1.1 Hz, 3H), 0.88 - 0.74 (m, 10H), 0.00 (d, J=3.7 Hz, 6H)

TLC (Petroleum ether /Ethyl acetate=1:1) Rf = 0.43

4. Preparation of compound 5



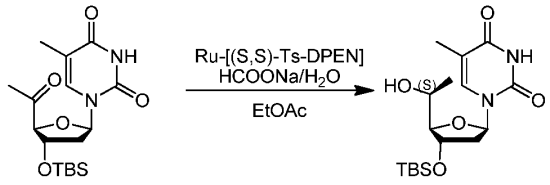
4 5

To a solution of compound **4** (115.00 g, 278.09 mmol) in THF (1.20 L) was added MeMgBr (3 M, 185.39 mL) was added at 0°C. The mixture was stirred at 0°C for 2h. TLC showed the starting material was consumed. The mixture was added water (1 L) at 0°C and extracted with EtOAc (300 mL*2). The combined organic was dried over Na_2SO_4, filtered and concentrated to get the compound **5** as a white solid (100.00 g, 97.58% yield). The mixture was used directly without any purification.

^{1}H NMR (400MHz, $CDCl_3$): δ = 8.81 (br s, 1H), 7.95 (s, 1H), 6.41 (dd, J=5.6, 8.1 Hz, 1H), 4.60 - 4.40 (m, 2H), 2.40 - 2.16 (m, 4H), 1.98 (s, 3H), 1.02 - 0.83 (m, 10H), 0.14 (d, J=3.3 Hz, 6H), 0.20 - 0.00 (m, 1H)

TLC (Petroleum ether /Ethyl acetate=1:1) Rf= 0.68

5. Preparation of compound 6A



5 6A

To a solution of compound **5** (46.00 g, 124.83 mmol) in the mixture of EtOAc (460.00 mL) and sodium formate (353.17 g, 5.19 mol) dissolved in Water (1.84 L), and then N-[(1S,2S)-2-amino-1,2-diphenyl-ethyl]-4-methyl-benzenesulfonamide;chlororuthenium;1-isopropyl-4-methyl-benzene (1.59 g, 2.50 mmol) was added. The resulting two-phase mixture was stirred for 12 h at 25°C under N_2. TLC showed the starting material was consumed. The mixture was extracted with EtOAc (500 mL*3). The combined organic was washed with brine (300 mL), dried over Na_2SO_4, filtered and concentrated to get the crude. The mixture was purified by MPLC (Petroleum ether / MTBE=10:1 to 1:1) seven times to get compound **6A** as a yellow oil (25.60 g, 57.53% yield).

^{1}H NMR (400MHz, DMSO-d6): δ = 11.28 (s, 1H), 7.85 (s, 1H), 6.16 (t, J=6.8 Hz, 1H), 5.04 (d, J=4.6 Hz, 1H), 4.46 - 4.29 (m, 1H), 3.79 (br t, J=6.8 Hz, 1H), 3.59 (br s, 1H), 3.32

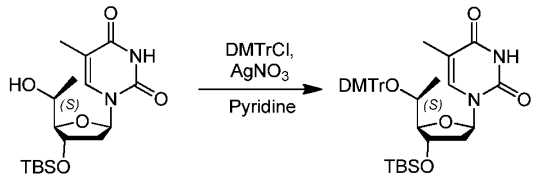
(s, 1H), 2.21 - 2.09 (m, 1H), 2.06 - 1.97 (m, 1H), 1.76 (s, 3H), 1.17 - 1.08 (m, 4H), 0.91 - 0.81 (m, 10H), 0.08 (s, 6H)

SFC: SFC purity: 98.6%

TLC (Petroleum ether / Ethyl acetate=1:1) Rf = 0.38

5

6. Preparation of compound 7A



5A **7A**

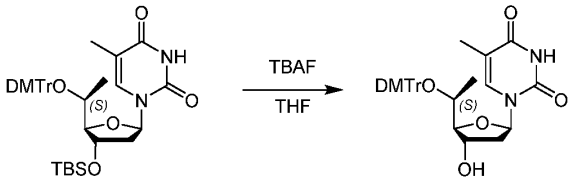
The compound **6A** (12.80 g, 34.55 mmol) was dried by azeotropic distillation on a rotary 10 evaporator with pyridine (100 mL) and toluene (100 mL*2).

To a solution of compound **6A** (12.80 g, 34.55 mmol) and DMTCl (1.89 g, 5.59 mmol) in the mixture of pyridine (120.00 mL) and THF (400.00 mL) was degassed and purged with N_2 for 3 times and then $AgNO_3$ (10.09 g, 59.43 mmol) was added. The mixture was stirred at 25°C for 15hr. TLC showed the starting material was consumed. MeOH (5 mL) 15 was added and stirred for 15 min and then the mixture was filtered and the cake was washed with toluene (300 mL*3). The filtrate was concentrated to get the compound **7A** as a yellow oil (46.50 g, crude). The mixture was used directly to next step without any purification.

TLC (Petroleum ether /Ethyl acetate) Rf = 0.63

20

8. Preparation of 5'-(S)-C-Me-5'-ODMTr-dT



7A **5'-(S)-C-Me-5'-ODMTr-dT**

To a solution of compound **7A** (46.50 g, 69.11 mmol) in THF (460.00 mL) was

added TBAF (1 M, 131.31 mL). The mixture was stirred at 25 °C for 5 hrs. TLC showed the starting material was consumed. The mixture was concentrated to get the crude and then sat. NaCl (5% aq., 200 mL) was added and extracted with EtOAc (200 mL*3). The combined organic was dried over Na_2SO_4, filtered and concentrated to get the crude. The residue was purified by MPLC (Petroleum ether / Ethyl acetate 5:1, 1:1, 1:4, 5% TEA) to get **5'-(S)-C-Me-5'-ODMTr-dT** as a white solid (29.00 g, 75.12% yield) .

^{1}H NMR (400MHz, DMSO-d6): δ = 11.35 (s, 1H), 7.56 (s, 1H), 7.58 - 7.53 (m, 1H), 7.44 (d, J=7.8 Hz, 2H), 7.37 - 7.24 (m, 6H), 7.23 - 7.17 (m, 1H), 6.87 (t, J=8.3 Hz, 4H), 6.13 (t, J=6.9 Hz, 1H), 5.21 (d, J=4.9 Hz, 1H), 4.23 (br s, 1H), 3.73 (d, J=2.9 Hz, 6H), 3.67 (t, J=3.7 Hz, 1H), 3.57 - 3.46 (m, 1H), 2.23 - 2.04 (m, 2H), 1.67 (s, 3H), 1.70 - 1.65 (m, 1H), 0.71 (d, J=6.2 Hz, 3H)

^{13}CNMR (101MHz, DMSO-d6): δ = 170.78, 164.16, 158.64, 158.59, 150.86, 146.71, 137.00, 136.75, 135.97, 130.65, 130.52, 128.38, 128.07, 127.11, 113.48, 110.11, 89.78, 86.41, 83.87, 70.58, 70.22, 60.21, 55.48, 21.20, 18.08, 14.53, 12.54

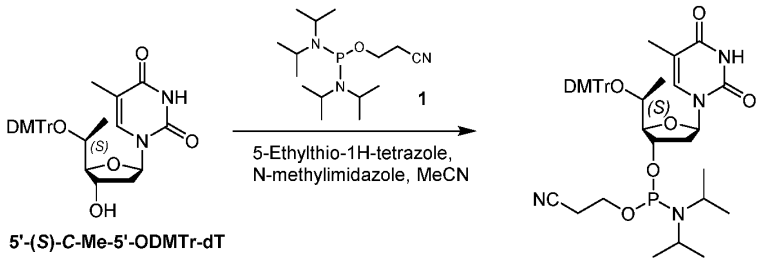
HPLC: HPLC purity: 98.4%

LCMS: (M-H+) = 557.2; LCMS purity: 99.0%

SFC: SFC purity: 99.4%

TLC (Petroleum ether /Ethyl acetate=1:1, 5% TEA) Rf =0.01

Preparation of 5'-(S)-C-Me-5'-ODMTr-dT-CNE-phosphoramidite



To a solution of **5'-(S)-C-Me-5'-ODMTr-dT** (5.00 g, 8.95 mmol) in MeCN (50.00 mL) was added 5-ethylsulfanyl-2H-tetrazole (1.17 g, 8.95 mmol) 1-methylimidazole (1.47 g, 17.90 mmol, 1.43 mL) and compound **1** (4.05 g, 13.43 mmol, 4.26 mL). The reaction mixture was stirred at 20°C under N_2 for 2 hrs. TLC and LCMS showed a little starting

material was consumed and the desired substance was found. The reaction mixture was concentrated under reduced pressure to get the crude and the residue was diluted with EtOAc (20 mL). The reaction mixture was washed with aq. saturated. $NaHCO_3$ solution (20 mL), dried over Na_2SO_4, filtered and concentrated to get the crude. The mixture was purified by MPLC (Petroleum ether 5% TEA: Ethyl acetate from 10:1 to 1:1) we got two batches: 2.5 g (batch 1) and 1.8 g (batch 2). We got ***5'-(S)-C-Me-5'-ODMTr-dT-CNE-phosphoramidite*** as a white solid (4.3 g, 5.67 mmol, 63.31% yield).

Batch 1:

^{1}H NMR (400MHz,) δ = 8.19 (br s, 1H), 7.69 - 7.60 (m, 1H), 7.54 (s, 1H), 7.43 - 7.33 (m, 2H), 7.32 - 7.07 (m, 8H), 6.73 (ddd, J=3.7, 5.8, 9.0 Hz, 4H), 6.27 - 6.15 (m, 1H), 4.49 - 4.37 (m, 1H), 3.82 - 3.65 (m, 8H), 3.63 - 3.55 (m, 2H), 3.53 - 3.39 (m, 3H), 2.50 (t, J=6.3 Hz, 1H), 2.46 - 2.31 (m, 1H), 2.29 - 2.19 (m, 1H), 2.16 - 2.04 (m, 1H), 1.68 (s, 3H), 1.20 - 1.00 (m, 13H), 0.95 (d, J=6.8 Hz, 3H), 0.92 - 0.74 (m, 4H)

^{31}P NMR (162MHz, $CDCl_3$**)** δ = 149.11 (s, 1P), 148.99 (s, 1P)

HPLC: HPLC purity: 62.68%+32.65%

LCMS: LCMS purity: 64.42%+32.87%

Batch 2:

^{1}H NMR (400MHz, $CDCl_3$**)** δ = 8.19 (br s, 1H), 7.69 - 7.60 (m, 1H), 7.54 (s, 1H), 7.43 - 7.33 (m, 2H), 7.32 - 7.07 (m, 8H), 6.73 (ddd, J=3.7, 5.8, 9.0 Hz, 4H), 6.27 - 6.15 (m, 1H), 4.49 - 4.37 (m, 1H), 3.82 - 3.65 (m, 8H), 3.63 - 3.55 (m, 2H), 3.53 - 3.39 (m, 3H), 2.50 (t, J=6.3 Hz, 1H), 2.46 - 2.31 (m, 1H), 2.29 - 2.19 (m, 1H), 2.16 - 2.04 (m, 1H), 1.68 (s, 3H), 1.20 - 1.00 (m, 13H), 0.95 (d, J=6.8 Hz, 3H), 0.92 - 0.74 (m, 4H)

^{31}P NMR (162MHz, $CDCl_3$**)** δ = 149.11 (s, 1P), 148.99 (s, 1P), 14.17 (s, 1P)

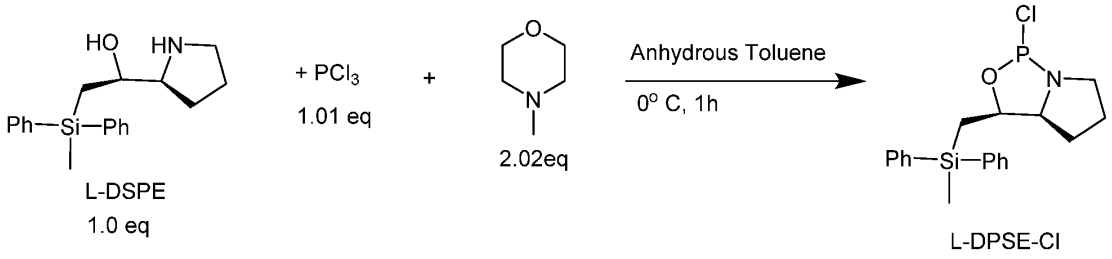
HPLC: HPLC purity: 53.0% +41.24%

LCMS: LCMS purity: 53.19%+42.83%

TLC (Petroleum ether / Ethyl acetate = 1:3) Rf = 0.86, 0.88

Example 34. General Procedure for the Preparation of 3'-LPSE amidites:

Procedure for the preparation of L-DPSE-Cl:



L-DPSE amino alcohol (S-2-(methyldiphenylsilyl)-1-((S)-pyrrolidin-2-yl)ethanol,8.82g, 28.5 mmol) was dried three times by azeotropic evaporation with anhydrous toluene (3x60 ml) at 35°C and further dried in high vacuum for overnight. A solution of dried L-DPSE amino alcohol and 4-methylmorpholine (5.82g, 6.33mL,57.5mmole) which was dissolved in anhydrous toluene (50ml) was added to a solution of PCl_3 (4.0g, 2.5mL,29.0mmole) in anhydrous toluene (25ml) placed in 250mL three neck round bottomed flask which was cooled at -5°C under Argon. The reaction mixture was stirred at 0°C for another 40min. After that filtered the precipitated white solid by vacuum under argon using medium Frit, Airfree, Schlenk tube. The solvent was removed by under argon at low temperature (25°C) and the semi solid mixture obtained was dried under vacuum overnight (~15h) and used for the next step directly.

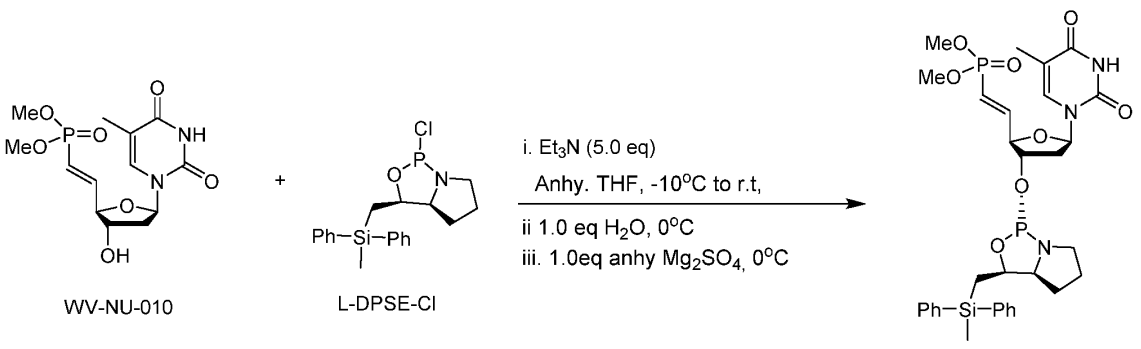
^{31}P NMR (162 MHz, $CDCl_3$) δ 178.84

Procedure for Preparation of 3'-LPSE amidites:

Nucleosides (1.0 eq.) in an appropriate size three necked flask was azeotroped three times with anhydrous toluene (15 mL/g) and was dried for 24h on high vacuum. To the flask was added anhydrous THF (0.3 M) under argon and solution was cooled to -10°C. To the reaction mixture was added triethylamine (5.0 eq.) followed by addition of L-DPSE-Cl (0.9 M solution in anhydrous THF, 1.7 eq.) over the period of 5-10 min. The reaction mixture was warmed to room temperature and reaction progress was monitored by LCMS. After disappearance of starting material, the reaction mixture was cooled in an ice bath

and was quenched by addition of water (1.0eq) stirred for 10min followed by added anhydrous Mg_2SO_4 (1.0eq) and stirred for 10min. The reaction mixture was filtered through airfree fritted glass tube, washed with anhydrous THF (50mL) and the solvent was removed under reduced pressure. The solid obtained was dried under high vacuum for overnight before purification. Then dried crude product was purified by silica column (which was pre-deactivated with 3 column volume of ethyl acetate with 5% TEA) using ethyl acetate/hexane mixture with 5% TEA as a solvent afforded 3'-L-DPSE amidites as a white solid.

Preparation of 3'-L-DPSE-5'-PO(OMe)$_2$-Vinylphosphonate-dT amidite (3'-L-DPSE-WV-NU-010):



Nucleoside 5'-PO(OMe)$_2$-Vinylphosphonate-dT, **WV-NU-010** (7.0g) was converted to 3'-L-DPSE-5'-PO(OMe)$_2$-Vinylphosphonate-dT amidite (**3'-L-DPSE-WV-NU-010**) by general procedure and obtained 11.8g (87%) as white solid.

^{31}P NMR (162 MHz, $CDCl_3$) δ 152.41, 19.95.

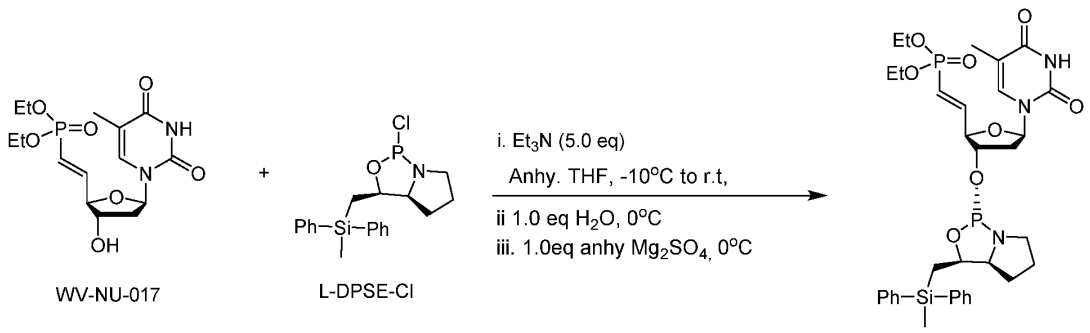
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.46 (ddt, *J* = 16.5, 7.6, 2.7 Hz, 4H), 7.33 – 7.17 (m, 6H), 6.93 – 6.88 (m, 1H), 6.75 (ddd, *J* = 22.6, 17.2, 4.4 Hz, 1H), 6.16 (dd, *J* = 7.5, 6.3 Hz, 1H), 5.85 (ddd, *J* = 19.2, 17.1, 1.8 Hz, 1H), 4.71 (dt, *J* = 8.7, 5.7 Hz, 1H), 4.38 (dp, *J* = 10.7, 3.6 Hz, 1H), 4.15 (tt, *J* = 5.6, 2.7 Hz, 1H), 3.68 (dd, *J* = 11.1, 3.7 Hz, 6H), 3.55 – 3.29 (m, 2H), 3.09 (tdd, *J* = 10.8, 8.8, 4.3 Hz, 1H), 2.11 (ddd, *J* = 13.9, 6.3, 3.3 Hz, 1H),

1.96 (s, 1H), 1.87 (d, J = 1.2 Hz, 3H), 1.85 – 1.73 (m, 2H), 1.70 – 1.49 (m, 2H), 1.38 (ddd, J = 15.9, 10.4, 6.3 Hz, 2H), 1.26 – 1.11 (m, 2H), 0.60 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.07, 163.62, 163.59, 150.21, 150.19, 148.49, 148.43, 136.61, 135.84, 135.15, 134.57, 134.33, 129.48, 129.42, 127.97, 127.93, 127.81, 118.38, 116.50, 111.52, 85.02, 84.72, 84.70, 84.51, 84.48, 79.25, 79.16, 77.40, 77.28, 77.08, 76.76, 74.93, 74.91, 74.83, 74.81, 68.01, 67.98, 60.35, 52.60, 52.55, 52.47, 52.42, 47.03, 46.67, 38.12, 38.08, 27.18, 25.85, 25.82, 21.01, 17.58, 17.54, 14.19, 12.58, -3.00, -3.27.

LCMS: Chemical Formula: $C_{32}H_{41}N_3O_8P_2Si$; Calcd Molecular Weight: 685.72; Observed Molecular Weight: 684.68 [M-H]; 686.58 [M+H].

Preparation of 3'-L-DPSE-5'-PO(OEt)$_2$-Vinylphosphonate-dT amidite (3'-L-DPSE-WV-NU-017):



WV-NU-017 L-DPSE-Cl 3'-L-DPSE-WV-NU-017

Nucleoside 5'-PO(OEt)$_2$-Vinylphosphonate-dT, **WV-NU-017** (8.0g) was converted to 3'-L-DPSE-5'-PO(OEt)$_2$-Vinylphosphonate-dT amidite (**3'-L-DPSE-WV-NU-017**) by general procedure and obtained 13.5g (88%) as white crystalline solid.

^{31}P NMR (162 MHz, $CDCl_3$) δ 152.44, 17.41.

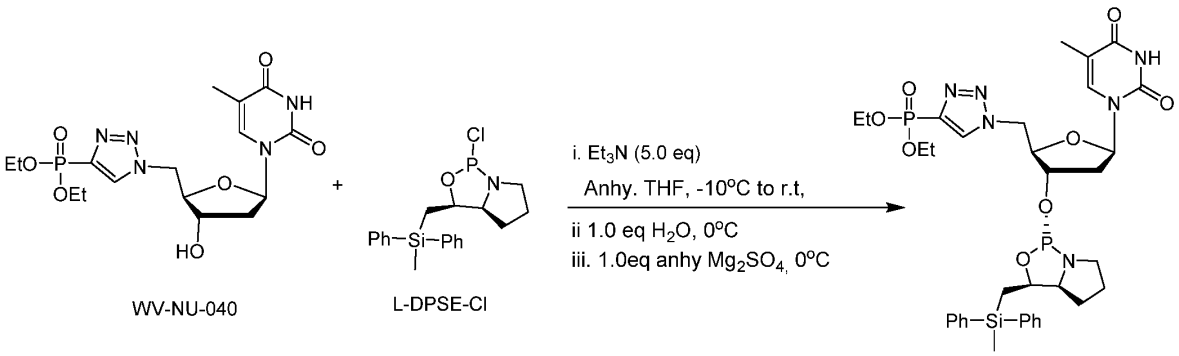
^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.56 (s, 1H), 7.61 – 7.46 (m, 5H), 7.40 – 7.26 (m, 7H), 7.00 (d, J = 1.4 Hz, 1H), 6.81 (ddd, J = 21.9, 17.1, 4.3 Hz, 1H), 6.27 (dd, J = 7.6, 6.2 Hz, 1H), 5.96 (ddd, J = 19.1, 17.1, 1.8 Hz, 1H), 4.79 (dt, J = 8.8, 5.7 Hz, 1H), 4.46 (dp, J

= 10.3, 3.4 Hz, 1H), 4.24 (tt, *J* = 5.6, 2.8 Hz, 1H), 4.20 – 4.02 (m, 5H), 3.63 – 3.37 (m, 2H), 3.18 (tdd, *J* = 10.8, 8.8, 4.3 Hz, 1H), 2.18 (ddd, *J* = 13.9, 6.2, 3.2 Hz, 1H), 1.95 (d, *J* = 1.2 Hz, 3H), 1.93 – 1.54 (m, 5H), 1.47 (dd, *J* = 14.8, 5.9 Hz, 2H), 1.39 – 1.16 (m, 8H), 0.69 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.09, 163.91, 163.77, 163.75, 150.32, 150.15, 147.57, 147.51, 136.66, 136.62, 136.09, 135.81, 135.08, 134.84, 134.59, 134.57, 134.49, 134.40, 134.32, 129.48, 129.42, 129.37, 127.98, 127.93, 127.90, 127.81, 119.77, 117.89, 111.89, 111.49, 85.86, 84.88, 84.80, 84.78, 84.59, 84.56, 79.24, 79.15, 78.91, 78.81, 77.45, 77.33, 77.13, 76.81, 74.94, 74.93, 74.85, 74.83, 68.02, 67.99, 62.08, 62.02, 61.96, 61.91, 61.87, 60.36, 47.15, 47.03, 46.80, 46.67, 45.92, 38.15, 38.11, 27.18, 27.14, 25.85, 25.81, 24.16, 21.03, 17.58, 17.54, 16.52, 16.46, 16.44, 16.40, 16.38, 14.20, 12.63, 12.42.

LCMS: Chemical Formula: $C_{34}H_{45}N_3O_8P_2Si$; Calcd Molecular Weight: 713.78; Observed Molecular Weight: 712.27 [M-H); 714.26 [M+H].

Preparation of 3'-L-DPSE-5'-PO(OEt)$_2$-Triazolylphosphonate-dT amidite (3'-L-DPSE-WV-NU-040):



Nucleoside, 5'-PO(OEt)$_2$-Triazolylphosphonate-dT, **WV-NU-040** (8.5g) was converted to 3'-L-DPSE-5'-PO(OEt)$_2$-Triazolylphosphonate-dT amidite (**3'-L-DPSE-WV-NU-040**) by general procedure and obtained 10.5g (69%) as a white solid.

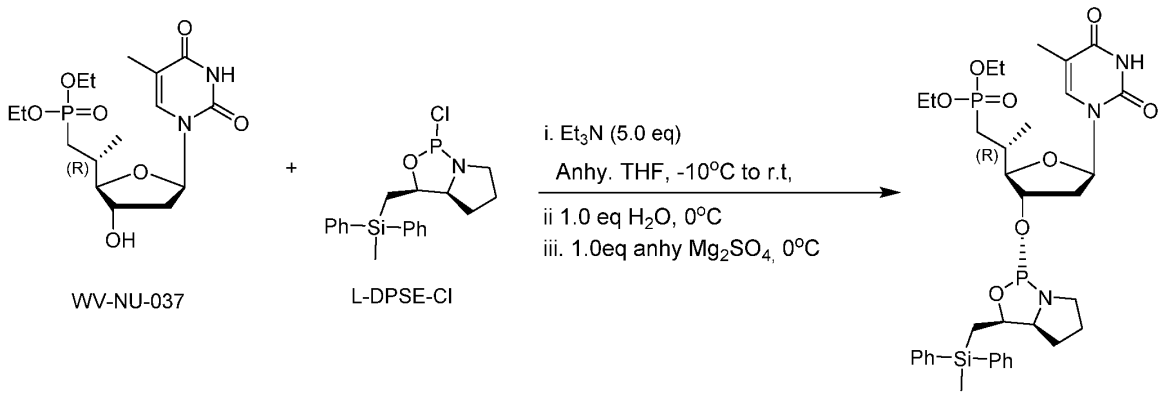
^{31}P NMR (162 MHz, Chloroform-*d*) δ 151.88, 6.69.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.08 (d, *J* = 1.8 Hz, 1H), 7.61 – 7.48 (m, 4H), 7.33 (dpt, *J* = 6.5, 4.2, 2.1 Hz, 6H), 6.70 (d, *J* = 1.5 Hz, 1H), 5.87 (dd, *J* = 7.3, 6.2 Hz, 1H), 4.89 – 4.79 (m, 1H), 4.64 (ddd, *J* = 14.7, 7.8, 4.3 Hz, 2H), 4.49 (dd, *J* = 14.5, 6.4 Hz, 1H), 4.33 – 4.20 (m, 3H), 4.20 – 4.08 (m, 1H), 3.95 (td, *J* = 5.9, 3.4 Hz, 1H), 3.66 – 3.42 (m, 2H), 3.20 (tddd, *J* = 10.9, 8.9, 4.5, 2.1 Hz, 1H), 2.22 – 1.98 (m, 3H), 1.94 (q, *J* = 1.2 Hz, 3H), 1.83 – 1.61 (m, 2H), 1.55 – 1.42 (m, 2H), 1.42 – 1.21 (m, 8H), 0.69 (d, *J* = 1.6 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.12, 163.64, 149.91, 138.68, 136.65, 136.58, 136.30, 135.88, 134.60, 134.48, 134.45, 134.36, 132.19, 131.86, 129.45, 129.40, 127.95, 127.93, 111.58, 86.98, 82.80, 79.41, 79.32, 77.39, 77.07, 76.75, 71.86, 71.77, 68.07, 68.04, 63.08, 63.05, 63.02, 62.99, 60.38, 50.51, 47.04, 46.68, 37.85, 37.81, 27.22, 25.85, 25.81, 21.04, 17.60, 17.56, 16.31, 16.25, 14.20, 12.43, -3.23, -3.81.

LCMS: Chemical Formula: $C_{35}H_{46}N_6O_8P_2Si$; Calcd Molecular Weight: 768.81; Observed Molecular Weight: 767.16 [M-H; 769.05 [M+H].

Preparation of 3'-L-DPSE-5'-(R)-Me-PO(OEt)$_2$Phosphonate-dT amidite (3'-L-DPSE-WV-NU-037):



Nucleoside, 5'-(R)-Me-PO(OEt)$_2$ Phosphonate-dT, **WV-NU-037** (8.0g) was converted to 3'-L-DPSE-5'-(R)-Me-PO(OEt)$_2$ Phosphonate-dT amidite (**3'-L-DPSE-WV-NU-037**) by general procedure and obtained 12.5g (86%) as a white solid.

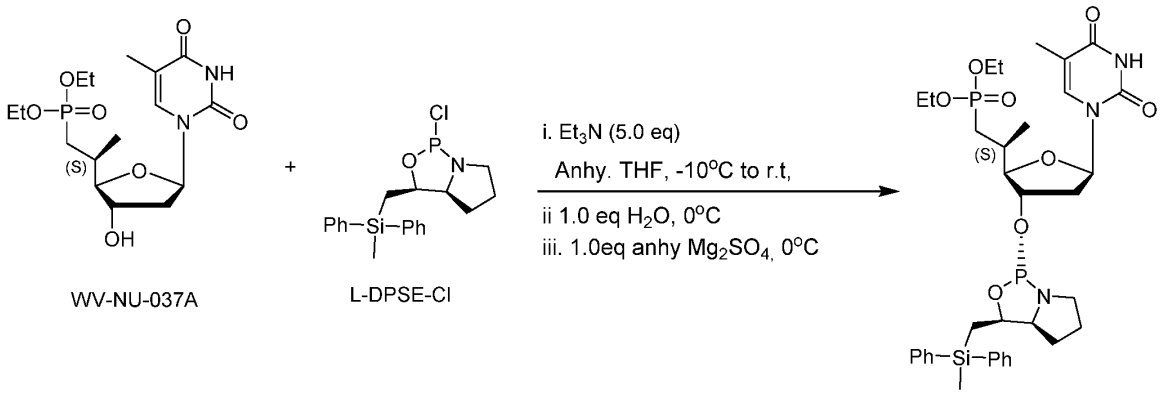
^{31}P NMR (162 MHz, Chloroform-*d*) δ 148.87, 30.96.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.60 – 7.54 (m, 2H), 7.54 – 7.48 (m, 2H), 7.41 – 7.26 (m, 6H), 6.99 (t, *J* = 1.3 Hz, 1H), 6.09 (dd, *J* = 8.1, 5.9 Hz, 1H), 4.77 (dt, *J* = 8.8, 5.7 Hz, 1H), 4.47 (tt, *J* = 7.3, 3.0 Hz, 1H), 4.21 – 4.02 (m, 4H), 3.64 – 3.54 (m, 2H), 3.46 (ddd, *J* = 12.7, 10.3, 5.9 Hz, 1H), 3.17 (qd, *J* = 11.0, 4.2 Hz, 1H), 2.20 – 1.99 (m, 3H), 1.99 – 1.85 (m, 5H), 1.79 – 1.68 (m, 1H), 1.68 – 1.41 (m, 5H), 1.38 – 1.27 (m, 7H), 1.27 – 1.21 (m, 1H), 1.12 (d, *J* = 6.6 Hz, 3H), 0.69 (d, *J* = 1.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 163.87, 150.22, 136.74, 135.88, 135.18, 134.63, 129.41, 129.38, 129.16, 128.18, 128.09, 127.94, 127.92, 111.19, 88.94, 88.91, 88.75, 88.72, 83.78, 79.60, 79.50, 77.45, 77.13, 76.81, 72.39, 72.35, 68.28, 68.25, 61.63, 61.59, 61.57, 61.52, 46.88, 46.52, 39.05, 31.35, 29.61, 28.20, 27.33, 25.84, 25.81, 17.79, 16.58, 16.53, 16.51, 16.47, 16.45, 12.67.

LCMS: Chemical Formula: $C_{35}H_{49}N_3O_8P_2Si$; Calcd Molecular Weight: 729.82; Observed Molecular Weight: 728.40 [M-H; 730.39 [M+H].

Preparation of 3'-L-DPSE-5'-(S)-Me-PO(OEt)$_2$Phosphonate-dT amidite (3'-L-DPSE-WV-NU-037A):



WV-NU-037A L-DPSE-Cl 3'- L-DPSE-WV-NU-037A

Nucleoside, 5'-(S)-Me-PO(OEt)$_2$ Phosphonate-dT, **WV-NU-037A** (10.0g) was converted to 3'-L-DPSE-5'-(S)-Me-PO(OEt)$_2$ Phosphonate-dT amidite (**3'-L-DPSE-WV-NU-037A**) by general procedure and obtained 14.0g (72%) as a white solid.

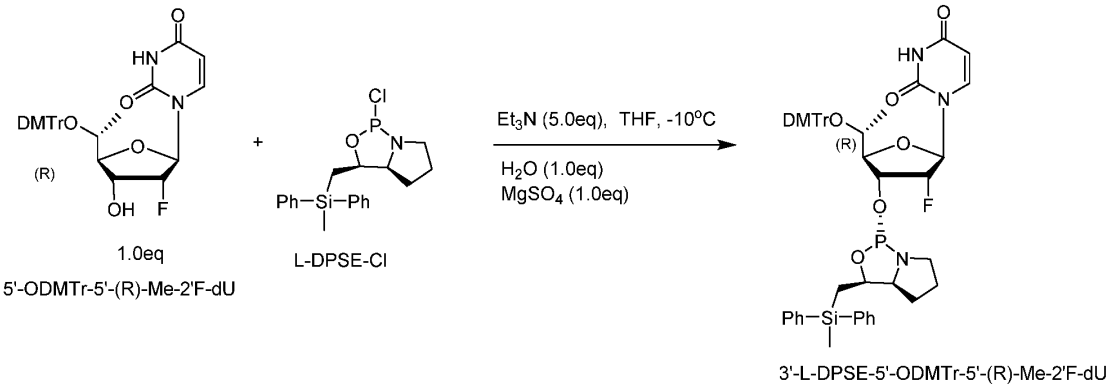
^{31}P NMR (162 MHz, $CDCl_3$) δ 148.87, 30.96.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.60 – 7.54 (m, 2H), 7.54 – 7.48 (m, 2H), 7.41 – 7.26 (m, 6H), 6.99 (t, *J* = 1.3 Hz, 1H), 6.09 (dd, *J* = 8.1, 5.9 Hz, 1H), 4.77 (dt, *J* = 8.8, 5.7 Hz, 1H), 4.47 (tt, *J* = 7.3, 3.0 Hz, 1H), 4.21 – 4.02 (m, 4H), 3.64 – 3.54 (m, 2H), 3.46 (ddd, *J* = 12.7, 10.3, 5.9 Hz, 1H), 3.17 (qd, *J* = 11.0, 4.2 Hz, 1H), 2.20 – 1.99 (m, 3H), 1.99 – 1.85 (m, 5H), 1.79 – 1.68 (m, 1H), 1.68 – 1.41 (m, 5H), 1.38 – 1.27 (m, 7H), 1.27 – 1.21 (m, 1H), 1.12 (d, *J* = 6.6 Hz, 3H), 0.69 (d, *J* = 1.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 163.87, 150.28, 136.68, 135.93, 135.27, 135.23, 134.59, 134.44, 134.35, 129.43, 129.39, 127.95, 127.93, 111.45, 89.22, 89.19, 89.06, 89.03, 84.07, 79.21, 79.11, 77.42, 77.11, 76.79, 73.45, 73.37, 68.17, 68.14, 61.71, 61.65, 61.41, 61.34, 47.02, 46.66, 38.86, 38.83, 32.45, 32.41, 29.16, 27.76, 27.24, 25.83, 25.80, 17.73, 17.70, 17.12, 17.10, 16.51, 16.50, 16.45, 16.43, 16.42, 12.45.

LCMS: Chemical Formula: $C_{35}H_{49}N_3O_8P_2Si$; Calcd Molecular Weight: 729.82; Observed Molecular Weight: 728.40 [M-H; 730.39 [M+H].

Preparation of L-DPSE-5'-ODMTr-5'-(R)-Me-2'F-dU amidite.



Nucleoside, 5'-ODMTr-5'-(R)-Me-2'F-dU (10g) was converted to L-DPSE-5'-ODMTr-5'-(R)-Me-2'F-dU amidite by general procedure and obtained 14.0g (87%) as a white crystalline solid.

^{31}P NMR (243 MHz, CDCl3) δ 151.48

^{1}H NMR (600 MHz, Chloroform-d) δ 7.57 – 7.45 (m, 6H), 7.41 – 7.24 (m, 12H), 7.23 – 7.18 (m, 1H), 7.16 (d, J = 8.1 Hz, 1H), 6.86 – 6.80 (m, 4H), 5.79 (dd, J = 17.4, 3.2 Hz, 1H), 5.19 (dd, J = 8.0, 2.2 Hz, 1H), 4.97 – 4.86 (m, 2H), 4.13 (q, J = 7.1 Hz, 1H), 3.78 (d, J = 6.0 Hz, 6H), 3.74 – 3.70 (m, 1H), 3.61 – 3.53 (m, 2H), 3.49 (ddt, J = 12.7, 10.4, 6.9 Hz, 1H), 3.10 (tdd, J = 10.9, 8.9, 4.4 Hz, 1H), 2.56 (qd, J = 7.2, 1.2 Hz, 1H), 2.05 (s, 1H), 1.93 – 1.84 (m, 1H), 1.76 – 1.69 (m, 1H), 1.66 (dd, J = 14.6, 8.2 Hz, 1H), 1.51 (dd, J = 14.6, 6.5 Hz, 1H), 1.43 (ddt, J = 12.3, 7.6, 4.5 Hz, 1H), 1.34 – 1.24 (m, 2H), 1.04 (t, J = 7.2 Hz, 2H), 0.88 (d, J = 6.7 Hz, 3H), 0.66 (s, 3H.

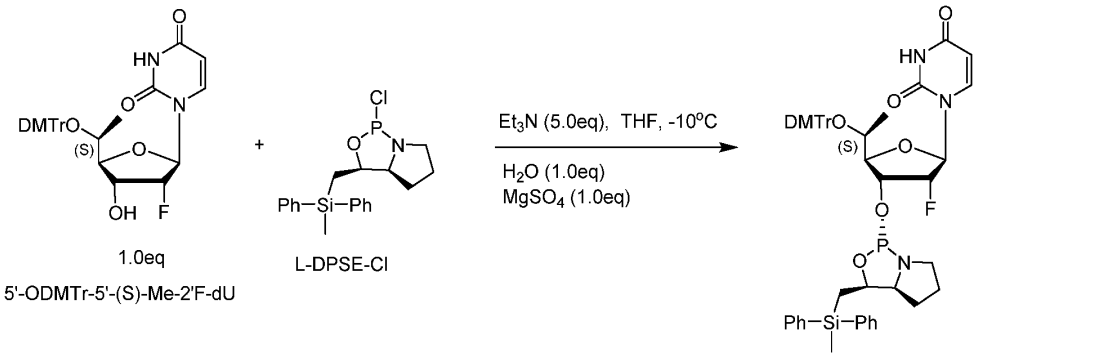
^{13}C NMR (151 MHz, CDCl3) δ 171.20, 163.35, 163.32, 158.70, 158.60, 149.83, 149.82, 146.25, 141.18, 136.56, 136.31, 136.15, 135.99, 134.62, 134.40, 130.60, 130.40, 129.45, 129.43, 128.19, 127.95, 127.94, 127.86, 126.90, 113.21, 113.14, 102.48, 92.33, 91.05, 88.59, 88.37, 87.15, 85.36, 85.35, 79.63, 79.57, 77.34, 77.13, 76.91, 68.97, 68.61, 68.56, 68.51, 68.46, 68.01, 68.00, 60.44, 55.28, 55.25, 53.50, 46.74, 46.50, 45.96, 45.95, 27.27, 25.94, 25.92, 21.09, 17.97, 17.94, 17.14, 14.25, 11.33, 11.31, -3.34

^{19}F NMR (565 MHz, CDCl3) δ -199.82.

LCMS: Chemical Formula: $C_{50}H_{53}FN_3O_8P_2Si$; Calcd Molecular Weight: 902.04; Observed

Molecular Weight: 901.03 [M-H]; 903.25 [M+H].

Preparation of L-DPSE-5'-ODMTr-5'-(S)-Me-2'F-dU amidite.



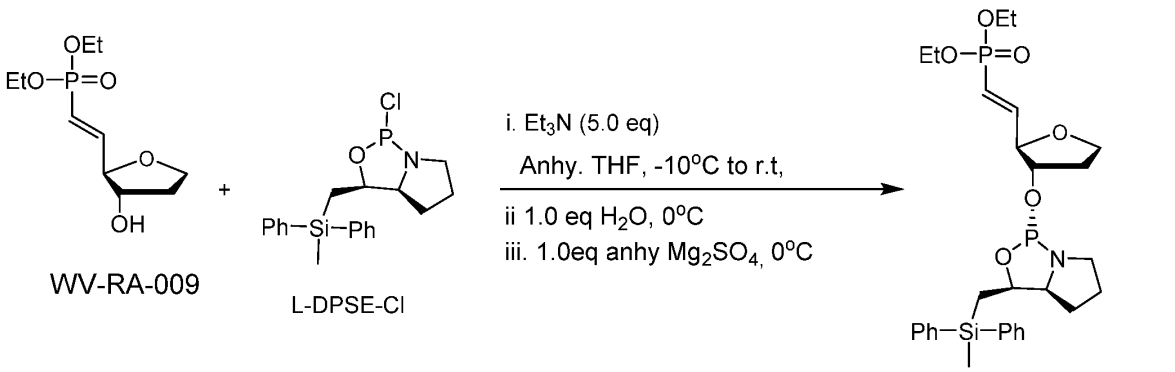
Nucleoside, 5'-ODMTr-5'-(S)-Me-2'F-dU (8g) was converted to L-DPSE-5'-ODMTr-5'-(R)-Me-2'F-dU amidite by general procedure and obtained 10.0g (78%) as a white crystalline solid.

^{31}P NMR (243 MHz, $CDCl_3$) δ 150.98.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.55 (d, *J* = 8.1 Hz, 1H), 7.42 (ddd, *J* = 13.2, 7.7, 1.7 Hz, 4H), 7.37 – 7.32 (m, 2H), 7.28 – 7.19 (m, 10H), 7.16 (t, *J* = 7.5 Hz, 2H), 7.13 – 7.07 (m, 1H), 6.75 – 6.69 (m, 4H), 5.67 (dd, *J* = 17.6, 2.1 Hz, 1H), 5.55 (d, *J* = 8.1 Hz, 1H), 4.74 – 4.67 (m, 1H), 4.41 (dtd, *J* = 16.2, 7.6, 4.9 Hz, 1H), 4.03 (q, *J* = 7.1 Hz, 1H), 3.79 (dd, *J* = 7.3, 3.7 Hz, 1H), 3.67 (d, *J* = 5.1 Hz, 6H), 3.59 (qd, *J* = 6.3, 3.6 Hz, 1H), 3.39 (ddt, *J* = 14.5, 10.7, 7.5 Hz, 1H), 3.25 (ddd, *J* = 12.3, 8.1, 4.9 Hz, 1H), 2.93 (tdd, *J* = 10.8, 8.7, 4.5 Hz, 1H), 1.95 (s, 2H), 1.70 (dtt, *J* = 12.3, 8.0, 3.7 Hz, 1H), 1.60 – 1.45 (m, 2H), 1.33 (dd, *J* = 14.5, 6.5 Hz, 1H), 1.26 (dtd, *J* = 12.5, 6.5, 3.2 Hz, 1H), 1.17 (t, *J* = 7.1 Hz, 2H), 1.12 (dt, *J* = 11.9, 8.0 Hz, 1H), 0.78 (d, *J* = 6.3 Hz, 3H), 0.54 (s, 3H).

LCMS: Chemical Formula: $C_{50}H_{53}FN_3O_8P_2Si$; Calcd Molecular Weight: 902.04; Observed Molecular Weight: 901.05 [M-H]; 903.15 [M+H].

Preparation of 3'-L-DPSE-5'-PO(OEt)$_2$-Abasic Vinyl phosphonate (3'-L-DPSE-WV-RA-009)

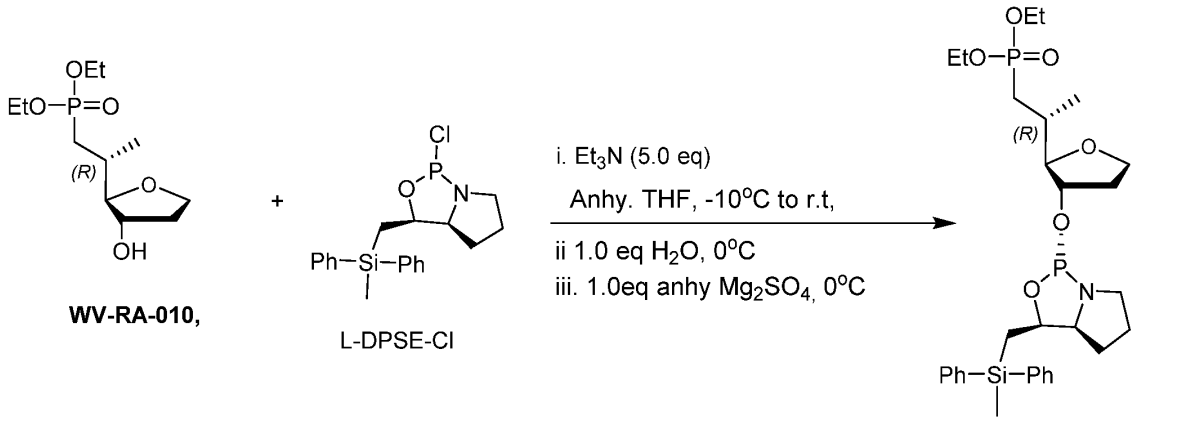


Diethyl((E)-2-((2R,3S)-3-hydroxytetrahydrofuran-2-yl)vinyl)phosphonate, (5'-PO(OEt)$_2$-Abasic Vinyl phosphonate ,**WV-RA-009** (5.0g) was converted to 3'-L-DPSE-5'-PO(OEt)$_2$-Abasic Vinyl phosphonate (**3'-L-DPSE-WV-RA-009**) by general procedure and obtained 8.6g (72.8%) as colorless semisolid.

^{31}P NMR (243 MHz, $CDCl_3$) δ 152.94, 18.49.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.47 (ddt, *J* = 14.2, 6.6, 1.7 Hz, 8H), 7.33 – 7.24 (m, 11H), 6.65 (ddd, *J* = 22.2, 17.0, 3.7 Hz, 2H), 5.85 (ddd, *J* = 20.9, 17.0, 1.9 Hz, 2H), 4.73 (dt, *J* = 8.5, 5.8 Hz, 2H), 4.26 (ddt, *J* = 8.3, 5.4, 2.7 Hz, 2H), 4.16 (tt, *J* = 3.6, 2.2 Hz, 2H), 4.08 – 3.94 (m, 8H), 3.91 – 3.81 (m, 4H), 3.47 (ddt, *J* = 14.9, 10.6, 7.6 Hz, 2H), 3.32 (ddt, *J* = 9.8, 7.6, 5.5 Hz, 2H), 3.14 – 3.05 (m, 2H), 1.82 – 1.78 (m, 1H), 1.75 (ddd, *J* = 9.3, 7.4, 4.4 Hz, 5H), 1.67 – 1.58 (m, 2H), 1.55 (dd, *J* = 14.7, 8.6 Hz, 2H), 1.41 – 1.34 (m, 3H), 1.34 – 1.30 (m, 1H), 1.28 – 1.19 (m, 11H), 1.19 – 1.12 (m, 2H), 0.60 (s, 5H).

LCMS: Chemical Formula: $C_{29}H_{41}NO_6P_2Si$; Calcd Molecular Weight: 589.68; Observed Molecular Weight: 588.63 [M-H]; 590.70 [M+H].



Diethyl((R)-2-((2R,3S)-3-hydroxytetrahydrofuran-2-yl)propyl)phosphonate, (5'-(R)-Me-$PO(OEt)_2$-Abasic phosphonate,**WV-RA-010** (5.0g) was converted to 3'-L-DPSE-5'-(R)-Me-$PO(OEt)_2$-Abasic phosphonate (**3'-L-DPSE-WV-RA-010**) by general procedure and obtained 7.0g (62%) as colorless semisolid.

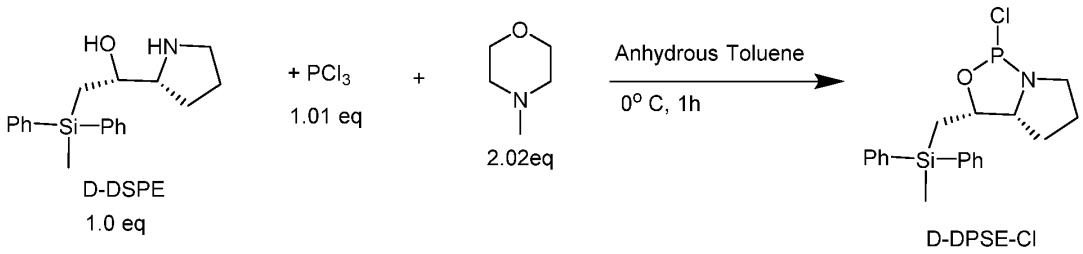
^{31}P NMR (243 MHz, $CDCl_3$) δ 150.48, 31.86.

^1H NMR (600 MHz, Chloroform-*d*) δ 7.47 (ddt, *J* = 14.6, 6.1, 1.7 Hz, 5H), 7.34 – 7.25 (m, 7H), 4.73 (ddd, *J* = 8.1, 6.5, 5.3 Hz, 1H), 4.28 – 4.21 (m, 1H), 4.08 – 3.94 (m, 4H), 3.75 (td, *J* = 8.1, 2.7 Hz, 1H), 3.71 – 3.62 (m, 1H), 3.52 – 3.42 (m, 1H), 3.41 (dd, *J* = 5.9, 3.3 Hz, 1H), 3.35 – 3.26 (m, 1H), 3.08 (dddd, *J* = 11.7, 10.6, 8.8, 4.3 Hz, 1H), 2.01 – 1.89 (m, 2H), 1.89 – 1.82 (m, 1H), 1.82 – 1.73 (m, 1H), 1.73 – 1.63 (m, 2H), 1.63 – 1.59 (m, 2H), 1.59 – 1.53 (m, 1H), 1.46 – 1.28 (m, 4H), 1.23 (td, *J* = 7.1, 1.1 Hz, 6H), 1.22 – 1.11 (m, 2H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.60 (s, 3H).

LCMS: Chemical Formula: $C_{30}H_{45}NO_6P_2Si$; Calcd Molecular Weight: 605.72; Observed Molecular Weight:604.42 [M-H]; 606.53[M+H].

Example 35. General Procedure for Synthesis of D-DPSE Amidite

Procedure for the preparation of D-DPSE-Cl:



D-DPSE amino alcohol, ((R)-2-(methyldiphenylsilyl)-1-((R)-pyrrolidin-2-yl)ethanol (8.82g, 28.5mmol) was dried three times by azeotropic evaporation with anhydrous toluene (3x60 ml) at 35°C and further dried in high vacuum for overnight. A solution of dried D-DPSE amino alcohol and 4-methylmorpholine (5.82g, 6.33mL,57.5mmole) which was dissolved in anhydrous toluene (50ml) was added to a solution of PCl_3 (4.0g, 2.5mL,29.0mmole) in anhydrous toluene (25ml) placed in 250mL three neck round bottomed flask which was cooled at -5°C under Argon. The reaction mixture was stirred at 0°C for another 40min. After that filtered the precipitated white solid by vacuum under argon using medium Frit, Airfree, Schlenk tube. The solvent was removed by rota-evaporator under argon at bath temperature (25°C) and the crude oily mixture obtained was dried under vacuum overnight (~15h) and used for next step.

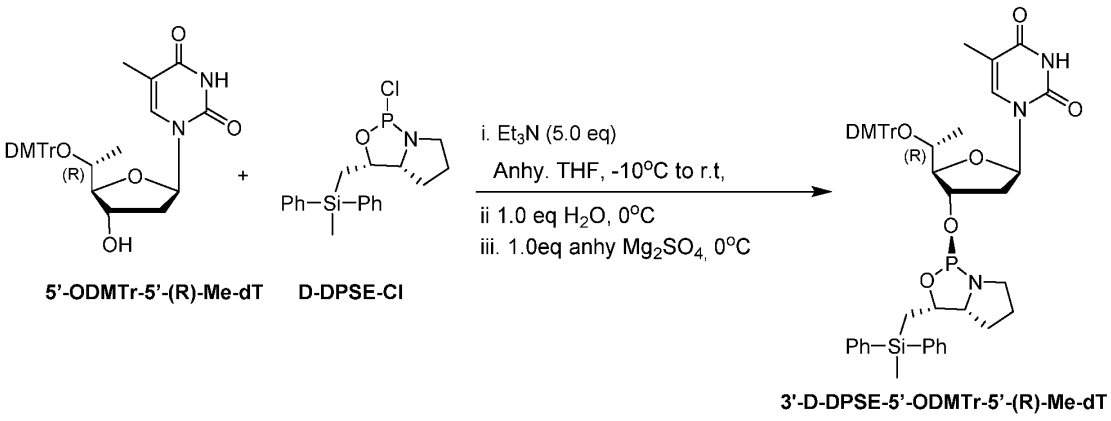
^{31}P NMR (162 MHz, $CDCl_3$) δ 178.72,

Procedure for Synthesis of D-DPSE Amidite.

Nucleosides (1.0 eq.) in an appropriate size three necked flask was azeotroped three times with anhydrous toluene (15 mL/g) and was dried for 24h on high vacuum. To the flask was added anhydrous THF (0.3 M) under argon and solution was cooled to -10°C. To the reaction mixture was added triethylamine (5.0 eq.) followed by addition of D-DPSE-Cl (0.9 M solution in anhydrous THF, 1.7 eq.) over the period of 5-10 min. The reaction mixture was warmed to room temperature and reaction progress was monitored by LCMS. After disappearance of starting material, the reaction mixture was cooled in an ice bath and was quenched by addition of water (1.0eq) stirred for 10min followed by added anhydrous Mg_2SO_4 (1.0eq) and stirred for 10min. The reaction mixture was filtered

through airfree fritted glass tube, washed with anhydrous THF (50mL) and the solvent was removed under reduced pressure. The solid obtained was dried under high vacuum for overnight before purification. Then dried crude product was purified by silica column (which was pre-deactivated with 3 column volume of ethyl acetate with 5% TEA) using ethyl acetate/hexane mixture with 5% TEA as a solvent afforded 3'-D-DPSE amidites as a white solid.

Preparation of 3'-D-DPSE-5'-ODMTr-5'-(R)-Me-dT amidite:



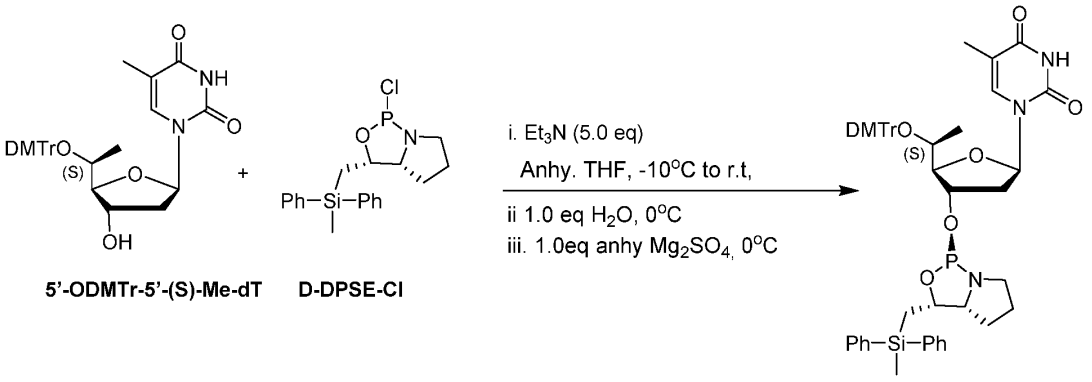
Nucleoside 5'-ODMTr-5'-(*R*)-Me-dT (10.0 g) was converted to 3'-D-DPSE-5'-ODMTr-5'-(*R*)-Me-dT amidite by general procedure (12.8 g, 90% yield) as an off white solid.

^{31}P NMR (243 MHz, $CDCl_3$) δ = 156.36

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.94 – 8.75 (m, 1H), 7.52 – 7.38 (m, 4H), 7.31 (dd, *J* = 13.6, 8.6 Hz, 4H), 7.27 – 7.21 (m, 4H), 7.21 – 7.15 (m, 2H), 7.14 – 7.07 (m, 1H), 6.86 (d, *J* = 1.8 Hz, 1H), 6.74 (dd, *J* = 8.9, 3.8 Hz, 4H), 6.07 (t, *J* = 7.2 Hz, 1H), 4.81 (ddt, *J* = 11.8, 9.0, 4.5 Hz, 2H), 3.69 (d, *J* = 3.0 Hz, 7H), 3.48 (ddd, *J* = 15.1, 7.5, 2.7 Hz, 1H), 3.36 (dq, *J* = 10.7, 3.8 Hz, 2H), 3.14 (dd, *J* = 9.6, 4.0 Hz, 1H), 1.96 (d, *J* = 1.2 Hz, 2H), 1.83 – 1.68 (m, 3H), 1.68 – 1.51 (m, 2H), 1.44 (dd, *J* = 14.7, 6.0 Hz, 1H), 1.36 (s, 4H), 1.27 – 1.09 (m, 3H), 0.83 (d, *J* = 6.5 Hz, 3H), 0.63 (s, 3H).

LCMS: $C_{51}H_{56}N_3O_8PSi$ (M-H): 897.16

Preparation of 3'-D-DPSE-5'-ODMTr-5'-(*S*)-Me-dT amidite:



5'-ODMTr-5'-(S)-Me-dT D-DPSE-Cl

3'-D-DPSE-5'-ODMT-5'-(S)-Me-dT

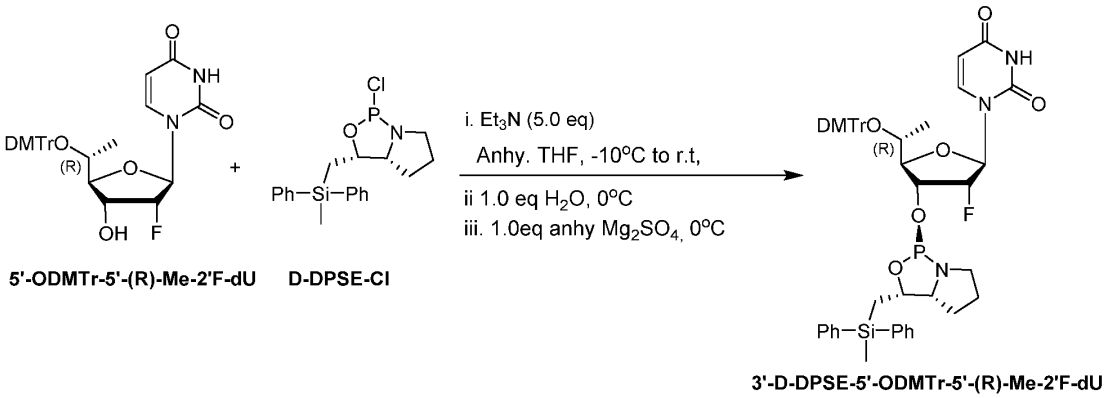
Nucleoside 5'-ODMTr-5'-(S)-Me-dT (8.0 g) was converted to 3'-D-DPSE-5'-ODMTr-5'-(S)-Me-dT amidite by general procedure (10 g, 89% yield) as an off white solid.

^{31}P NMR (243 MHz, $CDCl_3$) δ = 156.36

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.81 (s, 1H), 7.60 (d, *J* = 2.4 Hz, 1H), 7.49 – 7.41 (m, 4H), 7.41 – 7.36 (m, 2H), 7.33 – 7.28 (m, 2H), 7.29 – 7.21 (m, 7H), 7.21 – 7.15 (m, 2H), 7.12 (t, *J* = 7.3 Hz, 1H), 6.73 (dd, *J* = 8.9, 6.5 Hz, 4H), 6.11 – 6.03 (m, 1H), 4.68 (dt, *J* = 8.7, 5.8 Hz, 1H), 4.52 – 4.44 (m, 1H), 3.70 (d, *J* = 3.8 Hz, 6H), 3.65 (t, *J* = 3.4 Hz, 1H), 3.49 (qd, *J* = 6.5, 3.0 Hz, 1H), 3.34 (ddt, *J* = 15.1, 10.1, 7.7 Hz, 1H), 3.30 – 3.22 (m, 1H), 3.08 – 2.98 (m, 1H), 1.89 (dt, *J* = 14.1, 7.2 Hz, 1H), 1.81 (ddd, *J* = 13.8, 6.2, 3.7 Hz, 1H), 1.76 – 1.68 (m, 4H), 1.63 – 1.48 (m, 2H), 1.38 (dd, *J* = 14.7, 6.0 Hz, 1H), 1.31 (dtd, *J* = 12.1, 6.4, 2.6 Hz, 1H), 1.21 – 1.10 (m, 3H), 0.83 (d, *J* = 6.3 Hz, 3H), 0.58 (d, *J* = 1.5 Hz, 3H).

LCMS: $C_{51}H_{56}N_3O_8PSi$ (M-H): 897.16

Preparation of 3'-D-DPSE-5'-ODMTr-5'-(R)-Me-2'F-dU amidite:



Nucleoside 5'-ODMTr-5'-(R)-Me-2'F-dU (5.0 g) was converted to 3'-D-DPSE-5'-ODMTr-5'-(R)-Me-2'F-dU amidite by general procedure (6.0 g, 75% yield) as an off white solid.

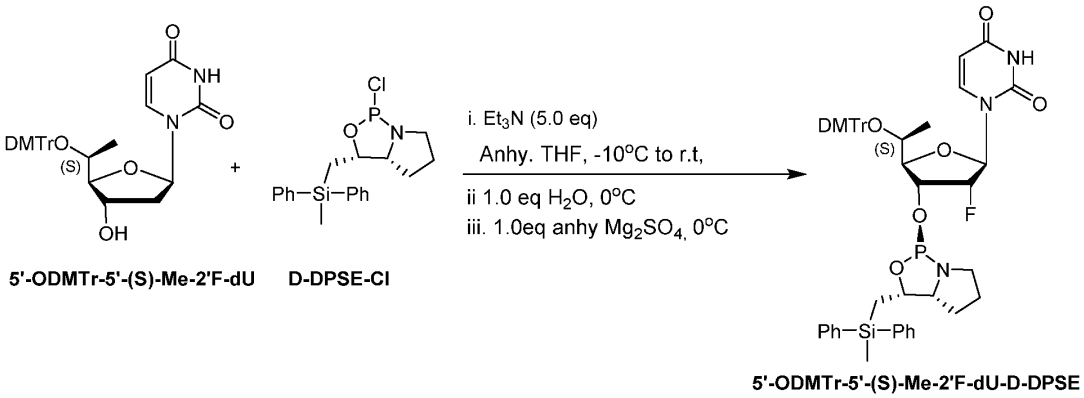
^{31}P NMR (243 MHz, $CDCl_3$) δ = 156.86

^{19}F NMR (565 MHz, $CDCl_3$) δ + -198.88 – -199.16 (m).

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.23 (d, *J* = 8.6 Hz, 1H), 7.51 – 7.43 (m, 4H), 7.43 – 7.36 (m, 2H), 7.35 – 7.29 (m, 2H), 7.30 – 7.20 (m, 7H), 7.17 (t, *J* = 7.6 Hz, 2H), 7.11 (t, *J* = 7.4 Hz, 1H), 5.81 (dd, *J* = 17.6, 2.2 Hz, 1H), 5.04 – 4.88 (m, 2H), 4.82 – 4.70 (m, 1H), 3.80 (d, *J* = 7.6 Hz, 1H), 3.69 (d, *J* = 2.8 Hz, 6H), 3.54 (ddd, *J* = 13.7, 9.3, 6.9 Hz, 2H), 3.36 – 3.27 (m, 1H), 3.21 – 3.11 (m, 1H), 1.80 (dp, *J* = 12.5, 4.4 Hz, 1H), 1.62 (dd, *J* = 14.7, 7.8 Hz, 2H), 1.41 (dd, *J* = 14.7, 6.7 Hz, 1H), 1.30 (qd, *J* = 7.5, 2.6 Hz, 1H), 1.25 – 1.14 (m, 3H), 0.87 (d, *J* = 6.7 Hz, 3H), 0.59 (s, 3H).

LCMS: $C_{50}H_{53}FN_3O_8PSi$ (M-H): 901.14

Preparation of 3'-D-DPSE-5'-ODMTr-5'-(S)-Me-2'F-dU amidite



5'-ODMTr-5'-(S)-Me-2'F-dU **D-DPSE-Cl**

5'-ODMTr-5'-(S)-Me-2'F-dU-D-DPSE

Nucleoside 5'-ODMTr-5'-(S)-Me-2'F-dU (4.95 g) was converted to 3'-D-DPSE-5'-ODMTr-5'-(S)-Me-2'F-dU amidite by general procedure (6.95 g, 87% yield) as an off white solid.

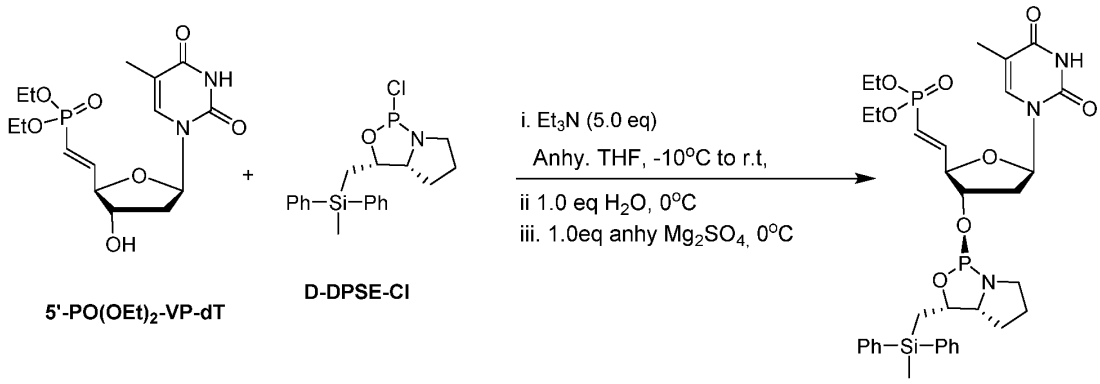
^{31}P NMR (243 MHz, $CDCl_3$) δ = 156.92

^{19}F NMR (565 MHz, $CDCl_3$) δ = -198.87 – -199.13 (m).

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.65 – 9.28 (m, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.44 (ddd, *J* = 12.3, 7.7, 1.9 Hz, 4H), 7.36 – 7.30 (m, 2H), 7.30 – 7.19 (m, 7H), 7.17 (t, *J* = 7.7 Hz, 2H), 7.12 (t, *J* = 7.3 Hz, 1H), 6.72 (t, *J* = 8.4 Hz, 4H), 5.87 (d, *J* = 17.1 Hz, 1H), 5.53 (d, *J* = 8.2 Hz, 1H), 4.87 (q, *J* = 6.8 Hz, 1H), 4.69 – 4.53 (m, 1H), 4.51 – 4.40 (m, 1H), 3.86 (dd, *J* = 8.6, 2.6 Hz, 1H), 3.69 (d, *J* = 4.4 Hz, 6H), 3.52 (qd, *J* = 6.4, 2.7 Hz, 1H), 3.36 (ddt, *J* = 15.2, 10.2, 7.7 Hz, 1H), 3.23 – 3.14 (m, 1H), 3.05 (td, *J* = 10.0, 3.8 Hz, 1H), 1.71 (dh, *J* = 12.5, 3.9 Hz, 1H), 1.65 – 1.57 (m, 1H), 1.52 (dq, *J* = 12.6, 8.2 Hz, 1H), 1.35 (dd, *J* = 14.6, 7.5 Hz, 1H), 1.24 – 1.14 (m, 3H), 1.08 (q, *J* = 10.2 Hz, 1H), 0.88 (d, *J* = 6.5 Hz, 3H), 0.56 (s, 3H).

LCMS: $C_{50}H_{53}FN_3O_8PSi$ (M-H): 901.14

Preparation of 3'-D-DPSE-5'-PO(OEt)$_2$ Vinylphosphonate-dT amidite:



5'-PO(OEt)$_2$-VP-dT

D-DPSE-Cl

3'-D-DPSE-5'-PO(OEt)$_2$-VP-dT

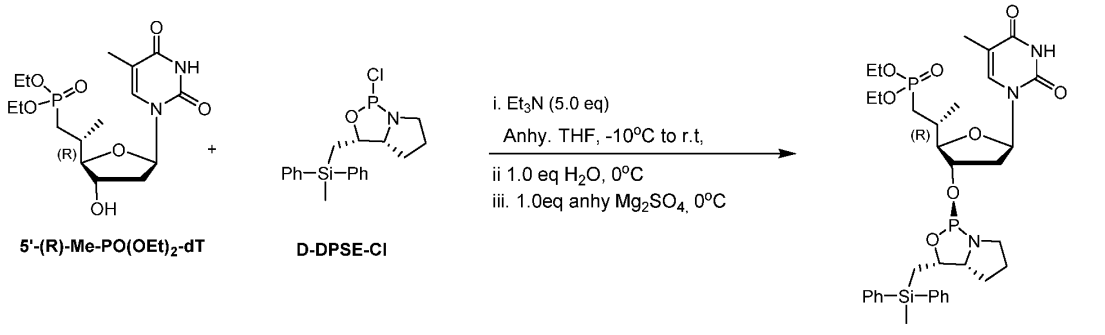
Nucleoside 5'-PO(OEt)$_2$ VP-dT (10 g) was converted to **3'-D-DPSE-5'-PO(OEt)$_2$ Vinyl phosphonate-dT amidite** by general procedure (14.1 g, 73% yield) as an off white solid.

LCMS: $C_{34}H_{45}N_3O_8P_2Si$ ($M-H^-$): 712.45

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.03 (s, 1H), 7.55 – 7.35 (m, 4H), 7.32 – 7.21 (m, 6H), 6.91 (s, 1H), 6.82 – 6.70 (m, 1H), 6.11 (t, J = 6.7 Hz, 1H), 5.96 – 5.83 (m, 1H), 4.80 – 4.69 (m, 1H), 4.35 – 4.20 (m, 2H), 4.09 – 3.95 (m, 4H), 3.51 – 3.41 (m, 1H), 3.41 – 3.31 (m, 1H), 3.22 – 3.06 (m, 1H), 1.96 (d, J = 6.7 Hz, 1H), 1.92 – 1.83 (m, 3H), 1.83 – 1.71 (m, 3H), 1.70 – 1.56 (m, 1H), 1.53 (dd, J = 14.3, 8.7 Hz, 1H), 1.46 – 1.31 (m, 2H), 1.31 – 1.11 (m, 8H), 0.59 (d, J = 6.9 Hz, 3H).

^{31}P NMR (243 MHz, $CDCl_3$) δ = 156.66, 17.09

Preparation of 3'-D-DPSE-5'-(R)-Me-PO(OEt)$_2$-dT amidite:



5'-(R)-Me-PO(OEt)$_2$-dT

D-DPSE-Cl

3'-D-DPSE-5'-(R)-Me-PO(OEt)$_2$-dT

Nucleoside 5'-(R)-Me-PO(OEt)$_2$-dT (4.0g) was converted to 3'-D-DPSE-5'-(R)-Me-PO(OEt)$_2$-dT amidite by general procedure (5.0 g, 69% yield) as an off white solid.

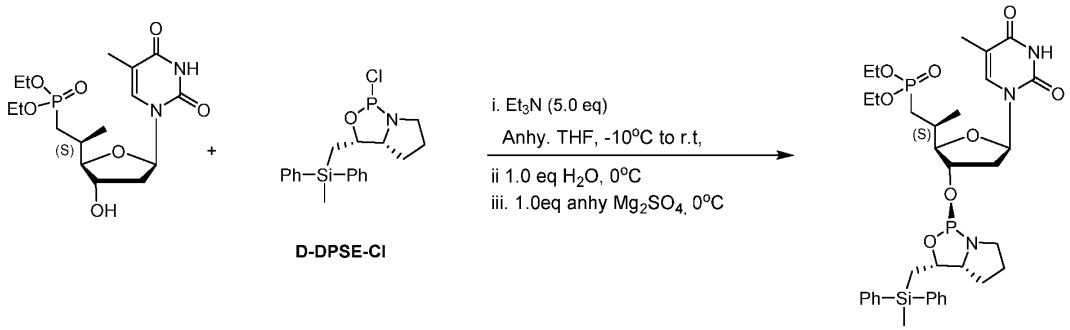
^{31}P NMR (162 MHz, $CDCl_3$) δ 156.32, 30.68.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.87 (d, *J* = 56.9 Hz, 1H), 7.54 (ddt, *J* = 16.6, 5.9, 2.4 Hz, 5H), 7.35 (t, *J* = 3.4 Hz, 7H), 7.02 (d, *J* = 1.4 Hz, 1H), 6.05 (t, *J* = 6.8 Hz, 1H), 4.83 (dt, *J* = 9.0, 5.7 Hz, 1H), 4.31 (tt, *J* = 8.9, 4.6 Hz, 1H), 4.11 (tdt, *J* = 10.2, 7.1, 5.1 Hz, 5H), 3.66 (t, *J* = 5.2 Hz, 1H), 3.55 (ddd, *J* = 15.2, 10.2, 7.5 Hz, 1H), 3.45 (ddt, *J* = 13.4, 10.5, 5.6 Hz, 1H), 3.22 (tdd, *J* = 11.1, 8.8, 4.2 Hz, 1H), 2.24 (dddt, *J* = 12.8, 9.7, 6.2, 3.6 Hz, 1H), 2.06 (d, *J* = 1.7 Hz, 1H), 2.03 – 1.57 (m, 12H), 1.55 – 1.40 (m, 2H), 1.38 – 1.20 (m, 9H), 1.15 (d, *J* = 6.6 Hz, 3H), 0.68 (d, *J* = 1.1 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 163.50, 150.01, 136.71, 135.96, 135.17, 134.56, 134.37, 129.49, 129.38, 127.98, 127.91, 111.31, 88.34, 88.28, 88.16, 88.09, 83.29, 78.20, 78.12, 77.38, 77.06, 76.74, 72.22, 72.06, 67.71, 67.69, 61.64, 61.58, 61.56, 61.49, 60.38, 47.24, 46.90, 38.95, 30.84, 30.80, 30.16, 28.75, 27.10, 25.92, 25.89, 21.04, 17.27, 17.24, 16.51, 16.50, 16.46, 16.44, 15.99, 15.96, 14.20, 12.69,

LCMS: $C_{35}H_{49}N_3O_8P_2Si$ (M-H): 728.21

Preparation of 3'-D-DPSE-5'-(S)-Me-PO(OEt)$_2$-dT amidite:



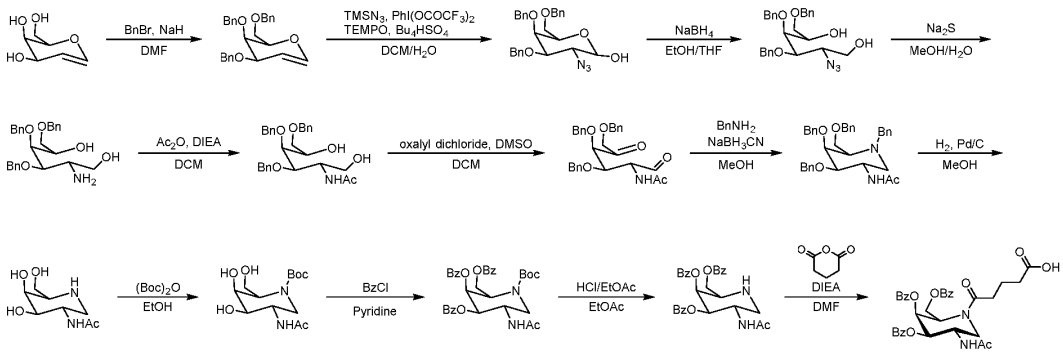
Nucleoside 5'-(S)-Me-PO(OEt)$_2$-dT (3.9g) was converted to 3'-D-DPSE-5'-(S)-Me-PO(OEt)$_2$-dT amidite by general procedure (4.1 g, 56% yield) as an off white solid.

^{31}P NMR (243 MHz, $CDCl_3$) δ = 155.76, 31.56

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.24 (s, 1H), 7.52 – 7.37 (m, 4H), 7.32 – 7.21 (m, 6H), 7.02 (s, 1H), 6.05 (t, *J* = 7.1 Hz, 1H), 4.74 (dt, *J* = 10.1, 5.7 Hz, 1H), 4.28 – 4.20 (m, 1H), 4.10 – 3.95 (m, 4H), 3.52 – 3.40 (m, 2H), 3.40 – 3.31 (m, 1H), 3.19 – 3.07 (m, 1H), 2.14 – 2.04 (m, 1H), 2.03 – 1.95 (m, 1H), 1.91 (s, 3H), 1.83 – 1.67 (m, 3H), 1.68 – 1.59 (m, 1H), 1.53 (dd, *J* = 14.7, 9.0 Hz, 1H), 1.47 – 1.32 (m, 3H), 1.30 – 1.14 (m, 8H), 1.07 (d, *J* = 6.7 Hz, 3H), 0.60 (s, 3H).

LCMS: $C_{35}H_{49}N_3O_8P_2Si$ (M-H): 728.82

Example 36. Synthesis of 5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-5-oxopentanoic acid



Step 1: Two batches in parallel: To a solution of (2R,3R,4R)-2-(hydroxymethyl)-3,4-dihydro-2H-pyran-3,4-diol (75 g, 513.20 mmol, 1 *eq.*) in DMF (2250 mL) was added NaH (92.37 g, 2.31 mol, 60% purity, 4.5 *eq.*) at 0 °C, then added BnBr (307.21 g, 1.80 mol, 213.34 mL, 3.5 *eq.*). The mixture was stirred at 0 – 20 °C for 0.5 hr. TLC (Petroleum ether : Ethyl acetate = 10:1, R_f = 0.40) indicated starting material was consumed and two new spots formed. The reaction mixture was quenched by *sat.* NH_4Cl (1500 mL) at 0 °C, extracted with MTBE (1500 mL×3), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=1/0 to 0:1) to get 318 g (2R,3R,4R)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-3,4-dihydro-2H-pyran as a yellow solid. **MS:** 439.1 $(M=Na)^+$; **TLC** (Petroleum ether : Ethyl acetate = 10:1) R_f = 0.40.

Step 2: Fifteen batches in parallel: To a mixture of (2R,3R,4R)-3,4-bis(benzyloxy)-2-

((benzyloxy)methyl)-3,4-dihydro-2H-pyran (30 g, 72.03 mmol, 1 *eq.*) and $TMSN_3$ (24.89 g, 216.08 mmol, 28.42 mL, 3 *eq.*) in DCM (1800 mL) was added PIFA (68.83 g, 144.05 mmol, 90% purity, 2 *eq.*), TEMPO (2.27 g, 14.41 mmol, 0.2 *eq.*), Bu_4NHSO_4 (4.89 g, 14.41 mmol, 0.2 *eq.*) and H_2O (64.90 g, 3.60 mol, 64.90 mL, 50 *eq.*) sequentially without any intervening time at 0 – 5 °C. The mixture was stirred at 0 – 5 °C for 40 mins. TLC (Petroleum ether/Ethyl acetate = 3:1, R_f = 0.35) showed that the starting material was consumed completely. The mixture was quenched by saturated aq. $NaHCO_3$ (1500 mL) and the aqueous phase was extracted with dichloromethane (500 mL×3). The organic phase was washed by H_2O (1000 mL×3) and saturated *aq.* NaCl (1000 mL×3), dried over Na_2SO_4. The fifteen batches were concentrated under reduced pressure to remove the solvent. The crude product was purified by MPLC (SiO_2, Ethyl acetate/Petroleum ether = 0% to 20%) to obtain (2R,3R,4R,5R,6R)-3-azido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-ol (280 g, crude) as yellow oil. **LCMS:** M + Na^+ = 498.1, purity: 63.34%; **TLC** (Petroleum ether/Ethyl acetate = 3:1) R_f = 0.35.

Step 3: Two batches in parallel: To a solution of (2R,3R,4R,5R,6R)-3-azido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-ol (140 g, 294.41 mmol, 1 *eq.*) in EtOH (2000 mL) was added $NaBH_4$ (16.64 g, 439.86 mmol, 1.49 *eq.*) at 0 – 5 °C and the mixture was stirred at 20 – 25 °C for 1 hr. TLC (Petroleum ether/Ethyl acetate = 2:1, R_f = 0.45) and LCMS showed that the starting material was consumed completely. The mixture was quenched by *aq.* NH_4Cl (1500 mL) and concentrated under reduced pressure to remove the most solvent, then extracted with ethyl acetate (500 mL×3). The two batches were combined and the organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to remove the solvent. The crude product was purified by MPLC (SiO_2, Ethyl acetate/Petroleum ether = 20% to 50%) to obtain 2-azido-3,4,6-tris(benzyloxy)hexane-1,5-diol (219 g, crude) as white solid. **LCMS:** M + Na^+ = 500.1; **TLC** (Petroleum ether/Ethyl acetate = 2:1) R_f = 0.45.

Step 4. Three batches in parallel: To a solution of 2-azido-3,4,6-tris(benzyloxy)hexane-1,5-diol (96 g, 201.03 mmol, 1 *eq.*) in MeOH (2000 mL) and H_2O (400 mL) was added $Na_2S{\cdot}9H_2O$ (241.41 g, 1.01 mol, 168.82 mL, 5 *eq.*) and stirred at 70 °C for 12 hrs. TLC (Petroleum ether/Ethyl acetate = 2:1, R_f = 0) showed that the starting material was consumed completely. The mixture was filtered and concentrated under reduced pressure to remove the solvent. The crude product was used for the next step without any purification. 2-amino-3,4,6-tris(benzyloxy)hexane-1,5-diol (272.32 g, crude) was obtained as yellow solid.

Step 5. Three batches in parallel: To a solution of 2-amino-3,4,6-tris(benzyloxy)hexane-1,5-diol (90 g, 199.31 mmol, 1 *eq.*) in DCM (1000 mL) was added DIEA (51.52 g, 398.62 mmol, 69.43 mL, 2 *eq.*) at 0 – 5 °C, followed by Ac_2O (26.45 g, 259.11 mmol, 24.27 mL, 1.3 *eq.*). The mixture was stirred at 5 – 10 °C for 3 hrs. LCMS showed that the starting material was consumed completely. The mixture was filtered and combined, then concentrated under reduced pressure to remove the solvent. TLC (Petroleum ether/Ethyl acetate = 0:1, R_f = 0.35) showed the desired product. The crude product was purified by MPLC (SiO_2, Ethyl acetate/Petroleum ether = 0% to 50%) to obtain N-(3,4,6-tris(benzyloxy)-1,5-dihydroxyhexan-2-yl)acetamide (176 g, 356.57 mmol, 59.63% yield) as white solid. **^{1}H NMR** (400MHz, CHLOROFORM-d) δ = 7.42 - 7.28 (m, 15H), 6.16 (br d, *J*=8.7 Hz, 1H), 4.75 (d, *J*=11.0 Hz, 1H), 4.66 - 4.43 (m, 5H), 4.43 - 4.36 (m, 1H), 4.08 - 4.00 (m, 1H), 3.89 (dd, *J*=1.6, 7.9 Hz, 1H), 3.75 - 3.65 (m, 2H), 3.63 - 3.48 (m, 3H), 2.50 (d, *J*=8.7 Hz, 1H), 2.41 (dd, *J*=5.1, 6.8 Hz, 1H), 1.95 (s, 3H); **LCMS:** M + H^+ = 494.1.

Step 6: Three batches in parallel: To a solution of oxalyl dichloride (67.12 g, 528.78 mmol, 46.29 mL, 4.5 *eq.*) in DCM (450 mL) was added DMSO (55.08 g, 705.04 mmol, 55.08 mL, 6 *eq.*) in DCM (150 mL) dropwised at -78 – 68 °C over 15 mins, and the mixture was stirred for 0.5 hr. N-(3,4,6-tris(benzyloxy)-1,5-dihydroxyhexan-2-yl)acetamide (58 g, 117.51 mmol, 1 *eq.*) in DCM (300 mL) was added to the above mixture dropwised and stirred at -78 – 68 °C for 0.5 hr. The mixture was quenched by TEA (166.47 g, 1.65 mol, 228.98 mL, 14 *eq.*) at -78 – 68 °C and the mixture was stirred for 0.5 hr, then warmed to 5 – 10 °C (room temperature). LCMS showed that the starting material was consumed completely. The mixture was washed by H_2O (500 mL) and aq. NaCl (500 mL×2). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under redeced pressure to remove the part solvent. The crude product was used respectively for the next step without any purification. N-(3,4,6-tris(benzyloxy)-1,5-dioxohexan-2-yl)acetamide (172.58 g, crude) was obtained as yellow liquid (in DCM). **LCMS:** M + H^+ = 490.1, purity: 34.07%.

Step 7. Three batches in parallel: To a solution of N-(3,4,6-tris(benzyloxy)-1,5-dioxohexan-2-yl)acetamide (57.53 g, 117.51 mmol, 1 *eq.*) in DCM (900 mL) was added phenylmethanamine (13.85 g, 129.27 mmol, 14.09 mL, 1.1 *eq.*) in MeOH (900 mL), followed by $NaBH_3CN$ (14.77 g, 235.03 mmol, 2 *eq.*) at 5 – 10 °C. The mixture was stirred at 5 – 10 °C for 12 hrs. LCMS showed that the starting material was consumed completely. The mixture was filtered and concentrated under reduced pressure to remove the solvent. The residue was combined. TLC (Petroleum ether/Ethyl acetate = 1:1, R_f = 0.35) showed

that the desired product was formed. The product was purified by MPLC (SiO_2, Ethyl acetate/Petroleum ether = 30% to 45%) to give N-((3S,4R,5S,6R)-1-benzyl-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)piperidin-3-yl)acetamide (46 g, 75.33 mmol, 21.37% yield, 92.477% purity) as white solid. **^{1}H NMR** (400MHz, METHANOL-d_4) δ = 7.40 - 7.17 (m, 20H), 4.78 - 4.42 (m, 5H), 4.34 - 4.25 (m, 1H), 4.06 (br s, 1H), 3.95 - 3.87 (m, 1H), 3.82 - 3.64 (m, 3H), 3.49 (br d, *J*=6.8 Hz, 1H), 3.12 - 2.92 (m, 1H), 2.84 (dd, *J*=3.7, 12.3 Hz, 1H), 2.09 (br dd, *J*=7.5, 12.1 Hz, 1H), 1.90 - 1.84 (m, 3H); **LCMS:** M + H^+ = 565.1, purity: 92.47%.

Step 8: A mixture of N-((3S,4R,5S,6R)-1-benzyl-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)piperidin-3-yl)acetamide (20 g, 35.42 mmol, 1 *eq.*) and Pd/C (80 g, 10% purity) in MeOH (500 mL) was evacuated in vacuo and backfilled with H_2 (50 Psi) three times, then stirred at 40 – 45 °C for 24 hrs. LCMS showed that the starting material was consumed completely. The mixture was filtered and concentrated under reduced pressure to remove the solvent. The crude product was used for the next step without any purification. N-((3S,4R,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)piperidin-3-yl)acetamide (8.02 g, crude) was obtained as gray solid.

Step 9: To a solution of N-((3S,4R,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)piperidin-3-yl)acetamide (8.02 g, 35.40 mmol, 1 *eq.*) in EtOH (120 mL) was added Boc_2O (8.50 g, 38.94 mmol, 8.95 mL, 1.1 *eq.*) and stirred at 50 °C for 12 hours. LCMS showed that the starting material was consumed completely. The mixture was concentrated under reduced pressure to remove the solvent. TLC (Methanol/Dichloromethane = 10:1, R_f = 0.30) showed that the desired product was formed. The crude product was purified by MPLC (SiO_2, Methanol/Dichloromethane = 0% to 6%) to obtain tert-butyl (2R,3S,4R,5S)-5-acetamido-3,4-dihydroxy-2-(hydroxymethyl)piperidine-1-carboxylate (9.27 g, 30.46 mmol, 86.04% yield) as white solid. **LCMS:** M +Na^+ = 327.1, purity: 92.22%.

Step 10: To a solution of tert-butyl (2R,3S,4R,5S)-5-acetamido-3,4-dihydroxy-2-(hydroxymethyl)piperidine-1-carboxylate (10 g, 32.86 mmol, 1 *eq.*) in PYRIDINE (100 mL) was added BzCl (15.24 g, 108.43 mmol, 12.60 mL, 3.3 *eq.*) at 0 – 5 °C and stirred at 10 – 15 °C for 1 hr. LCMS showed that the starting material was consumed completely and desired product was detected. The mixture was diluted with ethyl acetate (500 mL) and washed by *aq.* HCl (1 M, 500 mL×3), saturated *aq.* $NaHCO_3$ (500 mL×3) and saturated *aq.* NaCl (500 mL×3). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to remove the solvent. TLC (Petroleum ether/Ethyl acetate = 1:2,

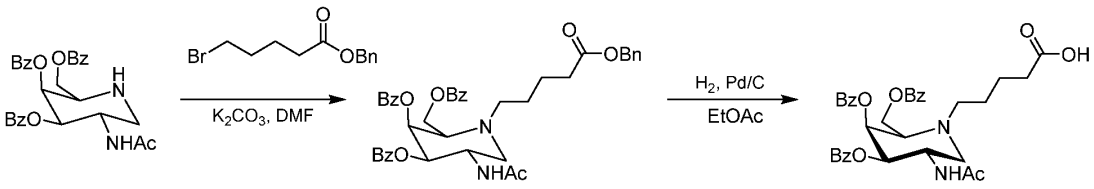
R_f = 0.35) showed that the desired product was formed. The crude product was purified by MPLC (SiO_2, Ethyl acetate/Petroleum ether = 0% to 30%) to obtain (2R,3S,4R,5S)-5-acetamido-2-((benzoyloxy)methyl)-1-(tert-butoxycarbonyl)piperidine-3,4-diyl dibenzoate (19.21 g, 31.15 mmol, 94.81% yield) as white solid. **LCMS:** M – 100 + H^+ = 517.0.

Step 11: To a solution of (2R,3S,4R,5S)-5-acetamido-2-((benzoyloxy)methyl)-1-(tert-butoxycarbonyl)piperidine-3,4-diyl dibenzoate (19.2 g, 31.14 mmol, 1 *eq.*) in EtOAc (200 mL) was added HCl/EtOAc (4 M, 200 mL, 25.69 *eq.*) at 0 – 5 °C and stirred at 5 – 10 °C for 12 hrs. LCMS showed that the starting material was consumed completely. The mixture was concentrated under reduced pressure to remove the solvent. The crude product was used for the next step without any purification. (2R,3S,4R,5S)-5-acetamido-2-((benzoyloxy)methyl)-1-(tert-butoxycarbonyl)piperidine-3,4-diyl dibenzoate (16.34 g, 28.94 mmol, 92.94% yield, 97.937% purity, HCl) was obtained as white solid. **^{1}H NMR** (400MHz, METHANOL-d_4) δ = 8.11 (br d, *J*=7.3 Hz, 2H), 7.96 (br d, *J*=7.5 Hz, 2H), 7.80 (br d, *J*=7.5 Hz, 2H), 7.65 - 7.49 (m, 3H), 7.43 (br t, *J*=7.5 Hz, 2H), 7.32 (q, *J*=7.3 Hz, 4H), 6.31 (br s, 1H), 5.68 - 5.55 (m, 1H), 5.00 - 4.88 (m, 1H), 4.78 - 4.64 (m, 2H), 4.52 (br s, 1H), 3.77 (br dd, *J*=4.5, 12.5 Hz, 1H), 3.52 (br t, *J*=12.5 Hz, 1H), 1.91 (s, 3H); **LCMS:** M + H^+ = 517.0, purity: 97.93%.

Step 12: To a mixture of (2R,3S,4R,5S)-5-acetamido-2-((benzoyloxy)methyl)-1-(tert-butoxycarbonyl)piperidine-3,4-diyl dibenzoate (8 g, 14.47 mmol, 1 *eq.*, HCl) and tetrahydropyran-2,6-dione (4.13 g, 36.17 mmol, 2.5 *eq.*) in DMF (70 mL) was added DIEA (9.35 g, 72.33 mmol, 12.60 mL, 5 *eq.*) at 5 – 10 °C. The mixture was stirred at 85 °C for 12 hrs. LCMS showed that the starting material was consumed mostly. The mixture was concentrated under reduced pressure to remove the solvent. The crude product was detected by HPLC. The crude product was purified by prep-HPLC (HCl, MeCN/H_2O) to obtain 5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-5-oxopentanoic acid (5.31 g, 8.41 mmol, 58.13% yield, 99.878% purity) as yellow solid. **^{1}H NMR** (400MHz, DMSO-d_6) δ = 12.05 (br s, 1H), 8.57 (br d, *J*=7.7 Hz, 1H), 8.08 (br d, *J*=7.1 Hz, 2H), 7.94 - 7.80 (m, 4H), 7.76 - 7.69 (m, 1H), 7.67 - 7.55 (m, 4H), 7.47 (br d, *J*=7.3 Hz, 4H), 5.84 - 5.65 (m, 1H), 5.56 - 5.22 (m, 2H), 4.99 (br t, *J*=10.1 Hz, 1H), 4.60 (br d, *J*=8.4 Hz, 1H), 4.41 (br d, *J*=14.6 Hz, 1H), 4.29 (br s, 1H), 4.00 - 3.74 (m, 2H), 2.42 - 2.31 (m, 1H), 2.24 (br d, *J*=5.3 Hz, 2H), 1.92 (s, 3H), 1.71 (br d, *J*=6.4 Hz, 2H); **^{13}C NMR** (101MHz, DMSO-d_6) δ = 174.77, 172.47, 170.07, 166.04, 165.28, 164.96, 134.36, 134.24, 133.76, 129.65, 129.42, 129.60 (br dd, *J*=20.9, 45.8 Hz, 1C), 129.02, 70.30, 67.58, 60.59,

49.08, 47.87, 41.40, 33.32, 32.46, 22.92, 20.53; **LCMS:** M + H^+ = 631.3, purity: 99.87%.

Example 37. Synthesis of 5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)pentanoic acid

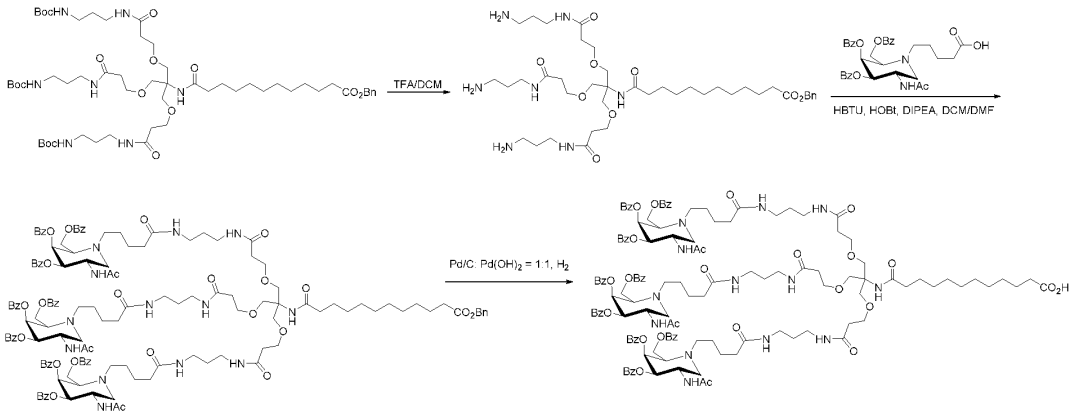


Step 1: To a mixture of (2R,3S,4R,5S)-5-acetamido-2-((benzoyloxy)methyl)piperidine-3,4-diyl dibenzoate (6 g, 10.85 mmol, 1 *eq.*, HCl) and 5-bromopentanoic acid--benzyl 5-bromopentanoate (11.78 g, 32.55 mmol, 3 *eq.*) in DMF (60 mL) was added KI (360.22 mg, 2.17 mmol, 0.2 *eq.*) and DIEA (7.01 g, 54.25 mmol, 9.45 mL, 5 *eq.*) at 5 – 10 °C. The mixture was stirred at 100 °C for 24 hrs. LCMS showed that the starting material was consumed mostly and desired product was detected. The mixture was concentrated under reduced pressure to remove the solvent. The crude product was detected by HPLC and purified by *prep*-HPLC (HCl, MeCN/H_2O) to obtain (2R,3S,4R,5S)-5-acetamido-2-((benzoyloxy)methyl)-1-(5-(benzyloxy)-5-oxopentyl)piperidine-3,4-diyl dibenzoate (7.5 g, 9.83 mmol, 90.62% yield, 92.655% purity) as yellow solid. MS: 707.1 $(M+H)^+$.

Step 2: A mixture of (2R,3S,4R,5S)-5-acetamido-2-((benzoyloxy)methyl)-1-(5-(benzyloxy)-5-oxopentyl)piperidine-3,4-diyl dibenzoate (7.8 g, 11.04 mmol, 1 *eq.*) and Pd/C (8 g, 11.04 mmol, 10% purity, 1.00 *eq.*) in EtOAc (80 mL) was evacuated in vacuo and backfilled with H_2 (15 Psi) three times, then stirred at 10 - 15°C for 6 hrs. LCMS showed that the starting mterial was consumed completely. The mixture was filtered and the filtrate was concentrated under reduced pressure to remove the solvent. The crude product was purified by *prep*-HPLC (column: Phenomenex luna C18 250*50mm*10 um; mobile phase: [water (0.05%HCl)-ACN]; B%: 35%-55%, 20min) to obtain 5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)pentanoic acid (2.83 g, 4.59 mmol, 41.58% yield) as white solid. **^{1}H NMR** (400 MHz, METHANOL-d_4) δ = 8.10 - 8.04 (m, 2H), 7.95 - 7.90 (m, 2H), 7.82 - 7.77 (m, 2H), 7.64 - 7.50 (m, 3H), 7.48 - 7.42 (m, 2H), 7.40 - 7.30 (m, 4H), 6.29 - 6.17 (m, 1H), 5.50 - 5.38 (m, 1H), 4.86 - 4.79 (m, 2H), 4.67 - 4.54 (m, 1H), 4.22 - 4.04 (m, 1H), 3.75 - 3.61 (m, 1H), 3.43 - 3.34 (m, 1H), 3.28 - 3.11 (m, 2H), 2.43 - 2.35 (m, 2H), 1.93 - 1.79 (m, 5H), 1.75 - 1.62 (m,

2H); **^{13}C NMR** (101 MHz, METHANOL-d_4) δ = 175.50, 172.28, 165.74, 165.61, 165.47, 133.61, 133.28, 129.77, 129.39, 129.22, 128.96, 128.78, 128.65, 128.35, 128.19, 128.16, 68.65, 60.99, 60.42, 53.18, 52.53, 44.62, 32.78, 21.79, 21.22; **LCMS:** M + H^+ = 617.3, purity: 98.62%.

Example 38. Synthesis of 1-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-16,16-bis((3-((3-(5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)pentanamido)propyl)amino)-3-oxopropoxy)methyl)-5,11,18-trioxo-14-oxa-6,10,17-triazanonacosan-29-oic acid



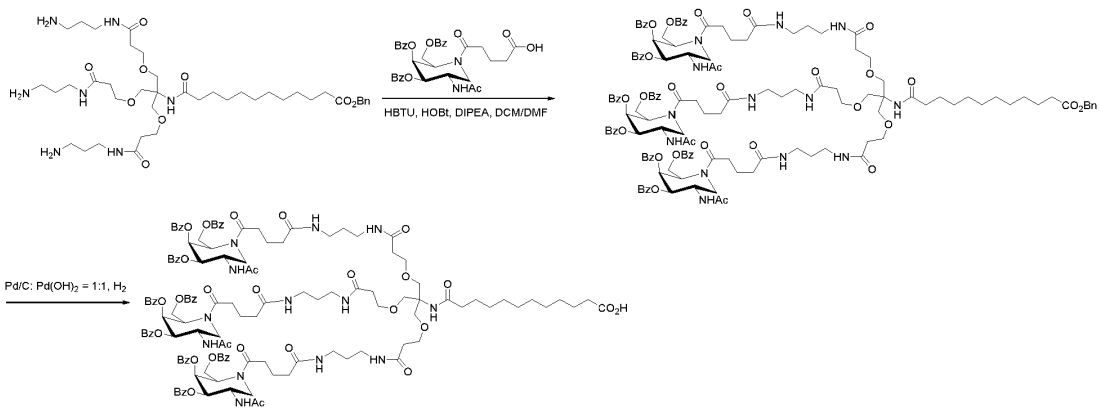
Step 1: To the solution of benzyl 15,15-bis(13,13-dimethyl-5,11-dioxo-2,12-dioxa-6,10-diazatetradecyl)-2,2-dimethyl-4,10,17-trioxo-3,13-dioxa-5,9,16-triazaoctacosan-28-oate (144 mg, 0.13 mmol) in DCM (2.4 mL) was added 2,2,2-trifuloroacetic acid (0.48 mL, 6.25 mmol). The reaction mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and crude product was co-evaporated with toluene, titurated with ether, and dried under vacuum overnight. Benzyl 12-((1,19-diamino-10-((3-((3-aminopropyl)amino)-3-oxopropoxy)methyl)-5,15-dioxo-8,12-dioxa-4,16-diazanonadecan-10-yl)amino)-12-oxododecanoate was used directly for next step without purification. LCMS calculated for $C_{41}H_{73}N_7O_9$ $[M+H]^+$: *m/z* 808.56, found: 808.30.

Step 2: To the solution of 5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)pentanoic acid (320 mg, 0.52 mmol), HATU (209 mg, 0.55 mmol) in DCM (1.5 mL) was added DIPEA (269 mg, 2.09 mmol) and crude benzyl 12-((1,19-diamino-10-((3-((3-aminopropyl)amino)-3-oxopropoxy)methyl)-5,15-dioxo-

8,12-dioxa-4,16-diazanonadecan-10-yl)amino)-12-oxododecanoate (0.13 mmol) in DMF (0.25 mL). The mixture was stirred at room temperature for 4 h. Solvent was evaporated under reduced pressure to give crude residue which was purified by flash chromatography (5% MeOH in DCM to 30% MeOH in DCM) to give benzyl 1-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-16,16-bis((3-((3-(5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)pentanamido)propyl)amino)-3-oxopropoxy)methyl)-5,11,18-trioxo-14-oxa-6,10,17-triazanonacosan-29-oate (212 mg, 63% yield) as white solid.

Step 3: To the solution of benzyl 1-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-16,16-bis((3-((3-(5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)pentanamido)propyl)amino)-3-oxopropoxy)methyl)-5,11,18-trioxo-14-oxa-6,10,17-triazanonacosan-29-oate (106 mg, 0.0407 mmol) in methanol: ethyl acetate (1:1, 2 mL) was added 10% $Pd(OH)_2/C$ (2.9 mg, 0.0203 mmol) and Pd/C (2.6 mg, 0.0203 mmol) and purged with argon. The flask was then purged with H_2 and stirred under H_2 atmosphere. The reaction was stopped after the complete consumption of starting material which was confirmed by LCMS. The reaction mixture was filtered through celite to give 1-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-16,16-bis((3-((3-(5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)pentanamido)propyl)amino)-3-oxopropoxy)methyl)-5,11,18-trioxo-14-oxa-6,10,17-triazanonacosan-29-oic acid (82 mg, 80% yield) as white solid. LCMS calculated for $C_{138}H_{169}N_{13}O_{33}$ $[M/2+H]^+$: *m/z* 1257.12, found: 1257.77

Example 39. Synthesis of 1-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-16,16-bis((3-((3-(5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-5-oxopentanamido)propyl)amino)-3-oxopropoxy)methyl)-1,5,11,18-tetraoxo-14-oxa-6,10,17-triazanonacosan-29-oic acid



Step 1: To the solution of 5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-5-oxopentanoic acid (328 mg, 0.52 mmol), HATU (209 mg, 0.55 mmol) in DCM (1.5 mL) was added DIPEA (269 mg, 2.08 mmol) and benzyl 12-((1,19-diamino-10-((3-((3-aminopropyl)amino)-3-oxopropoxy)methyl)-5,15-dioxo-8,12-dioxa-4,16-diazanonadecan-10-yl)amino)-12-oxododecanoate (0.13 mmmol) in DMF (0.25 mL). The mixture was stirred at room temperature for 5 hrs. Solvent was evaporated under reduced pressure to give crude residue which was purified by flash chromatography (5% MeOH in DCM to 30% MeOH in DCM) to give benzyl 1-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-16,16-bis((3-((3-(5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-5-oxopentanamido)propyl)amino)-3-oxopropoxy)methyl)-1,5,11,18-tetraoxo-14-oxa-6,10,17-triazanonacosan-29-oate (193 mg, 56 % yield) as white solid. LCMS calculated for $C_{143}H_{169}N_{13}O_{36}$ $[M/3+H]^+$: *m/z* 882.40, found: 882.21.

Step 2: To the solution of benzyl 1-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-16,16-bis((3-((3-(5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-5-oxopentanamido)propyl)amino)-3-oxopropoxy)methyl)-1,5,11,18-tetraoxo-14-oxa-6,10,17-triazanonacosan-29-oate (193 mg, 0.0729 mmol) in methanol: ethyl acetate (1:1, 2 mL) was added 10% $Pd(OH)_2/C$ (5.2 mg, 0.03645 mmol) and Pd/C (3.9 mg, 0.03645 mmol) and purged with argon. The flask was then purged with H_2 and stirred under H_2 atmosphere. The reaction was stopped after the complete consumption of starting material which was confirmed by LCMS. The reaction mixture was filtered through celite and purified by flash chromatography (5% MeOH in DCM to 30% MeOH in DCM) to obtain 1-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-16,16-bis((3-((3-

(5-((2R,3S,4R,5S)-5-acetamido-3,4-bis(benzoyloxy)-2-((benzoyloxy)methyl)piperidin-1-yl)-5-oxopentanamido)propyl)amino)-3-oxopropoxy)methyl)-1,5,11,18-tetraoxo-14-oxa-6,10,17-triazanonacosan-29-oic acid (124 mg, 67% yield) as white solid. LCMS calculated for $C_{136}H_{163}N_{13}O_{36}$ $[M/2+H]^+$: *m/z* 1278.07, found: 1278.08.

While various embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described in the present disclosure, and each of such variations and/or modifications is deemed to be included. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be example and that the actual parameters, dimensions, materials, and/or configurations may depend upon the specific application or applications for which the teachings of the present disclosure is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the embodiments of the present disclosure. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, claimed technologies may be practiced otherwise than as specifically described and claimed. In addition, any combination of two or more features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the scope of the present disclosure.

CLAIMS

What is claimed is:

1. A double-stranded RNAi (dsRNAi) agent comprising a guide strand and a passenger strand wherein:

a) the guide strand is complementary or substantially complementary to a target RNA sequence, and comprises:

i. backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide,

ii. backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide;

iii. one or more backbone phosphorothioate chiral centers in Rp or Sp configuration upstream of backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide;

iv. one or more backbone phosphorothioate chiral centers in Rp or Sp configuration between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the (+2) nucleotide and the immediately downstream (+3) nucleotide, as well as between one or both of: (a) the (+3) nucleotide and the (+4) nucleotide; and (b) the (+5) nucleotide and the (+6) nucleotide; or

v. one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide;

b) the passenger strand comprises one or both of:

i. 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49, and

ii. one or more backbone chiral centers in Rp or Sp configuration,

c) each strand of the dsRNAi agent independently has a length of about 15 to about 49 nucleotides,

d) the dsRNAi is capable of directing target-specific RNA interference.

2. A chirally controlled oligonucleotide composition comprising double stranded oligonucleotides wherein the guide and passenger strands of the double stranded oligonucleotides are independently characterized by:

a) a common base sequence and length;

b) a common pattern of backbone linkages; and

c) a common pattern of backbone chiral centers;

which composition is chirally controlled in that it is enriched, relative to a substantially racemic preparation of guide strands having the same common base sequence and length, for oligonucleotides having a common pattern of chiral centers; and

a) wherein the guide strands are complementary or substantially complementary to a target RNA sequence, and comprise:

i. backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide,

ii. backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide;

iii. one or more backbone phosphorothioate chiral centers in Rp or Sp configuration upstream of backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide;

iv. one or more backbone phosphorothioate chiral centers in Rp or Sp configuration between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the (+2) nucleotide and the immediately downstream (+3) nucleotide, as well as between one or both of: (a) the (+3) nucleotide and the (+4) nucleotide; and (b) the (+5) nucleotide and the (+6) nucleotide; or

v. one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide;

b) the passenger strands comprise one or both of:

i. 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49, and

ii. one or more backbone chiral centers in Rp or Sp configuration,

c) the guide and passenger strands have a length of about 15 to about 49 nucleotides; and

d) the guide and passenger strands are capable of directing target-specific RNA interference.

3. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49.

4. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49.

5. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises one or more backbone phosphorothioate chiral centers in Rp or Sp configuration upstream of backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49.

6. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49.

7. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises one or more backbone chiral centers in Rp or Sp configuration.

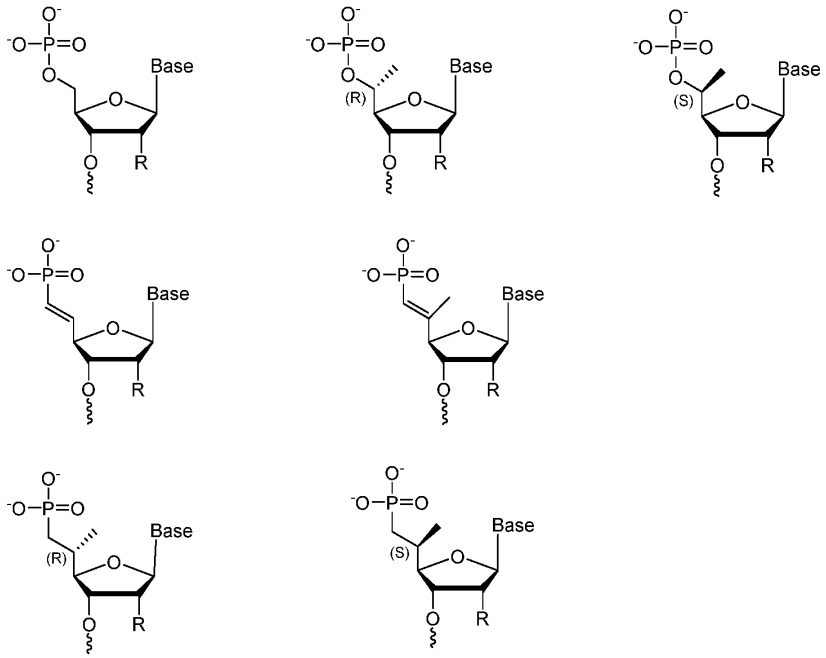
8. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide, and the passenger strand comprises one or more backbone chiral centers in Rp

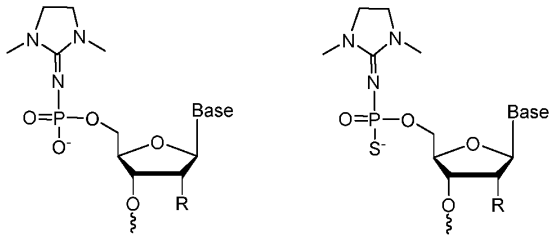
or Sp configuration.

9. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises one or more backbone phosphorothioate chiral centers in Rp or Sp configuration upstream of backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises one or more backbone chiral centers in Rp or Sp configuration.

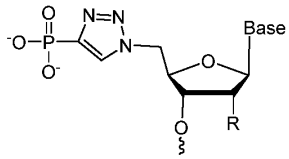
10. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises one or more backbone phosphorothioate chiral centers in Rp or Sp configuration between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the (+2) nucleotide and the immediately downstream (+3) nucleotide, as well as between one or both of: (a) the (+3) nucleotide and the (+4) nucleotide; and (b) the (+5) nucleotide and the (+6) nucleotide; or

11. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises a 5' terminal modification selected from:





and



Base: A, C, G, T, U, abasic, and modified nucleobases;

R: H, OH, O-alkyl, F, MOE, LNA bridge to the 4' position, BNA bridge to the 4' position.

12. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, and the passenger strand comprises one or more backbone chiral centers in Rp or Sp configuration.

13. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49 and one or more backbone chiral centers in Rp or Sp configuration.

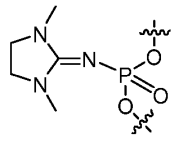
14. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises backbone phosphorothioate chiral centers in Rp, Sp, or alternating configurations between the 5' terminal (+1) nucleotide and the immediately downstream (+2) nucleotide and between the +2 nucleotide and the immediately downstream (+3) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49 and one or more backbone chiral

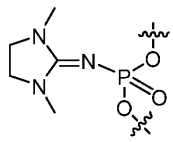
centers in Rp or Sp configuration.

15. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises one or more backbone phosphorothioate chiral centers in Rp or Sp configuration upstream of backbone phosphorothioate chiral centers in Sp configuration between the 3' terminal nucleotide and the penultimate (N-1) nucleotide and as between the penultimate (N-1) nucleotide and the immediately upstream (N-2) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49 and one or more backbone chiral centers in Rp or Sp configuration.

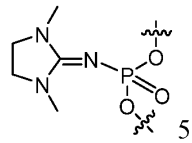
16. The double stranded oligonucleotide of claim 1 or the composition of claim 2, wherein the guide strand comprises one or more non-negatively charged internucleotidic linkage occurs between the second (+2) and third (+3) nucleotides, relative to the 5' terminal nucleotide, of the guide strand and the internucleotidic linkage to the penultimate 3' (N-1) nucleotide, and the passenger strand comprises 0-n non-negatively charged internucleotidic linkages, where n is about 1 to 49 and one or more backbone chiral centers in Rp or Sp configuration.

17. The double stranded oligonucleotide or composition of any one of the preceding claims, wherein the non-negatively charged backbone internucleotidic linkages have neutral charge.

18. The double stranded oligonucleotide or composition of claim 17, wherein the neutral backbone internucleotidic linkages is .

19. The double stranded oligonucleotide or composition of claim 18, wherein the guide strand comprises a linkage having the following structure  between the third

(+3) and fourth (+4) nucleotides of the guide strand, between the tenth (+10) and eleventh (+11) nucleotides of the guide strand, or both.

20. The double stranded oligonucleotide or composition of claim 19, wherein the passenger strand comprises a linkage having the following structure  5' to the central nucleotide of the passenger strand, 3' to the central nucleotide of the passenger strand, or both.

21. The composition of claim 2, where the guide and passenger strands in the composition that independently share a common base sequence, a common pattern of base modification, a common pattern of sugar modification, and/or a common pattern of internucleotidic linkages are at least 90% of all the guide and passenger strands in the composition.

22. The double stranded oligonucleotide or composition of any of the preceding claims, wherein the double stranded oligonucleotide comprises a carbohydrate moiety connected at a nucleobase, optionally through a linker.

23. The double stranded oligonucleotide or composition of any of the preceding claims, wherein the double stranded oligonucleotide comprises a lipid moiety connected to the double stranded oligonucleotide at a nucleobase, optionally through a linker.

24. The double stranded oligonucleotide or composition of any of the preceding claims, wherein one or both strands of the double stranded oligonucleotide comprises a target moiety connected at a nucleobase, optionally through a linker.

25. The double stranded oligonucleotide or composition of any one of the preceding claims, wherein at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the internucleotidic linkages of the double stranded oligonucleotide are independently chiral internucleotidic linkages.

26. The double stranded oligonucleotide or composition of any one of the preceding claims, wherein at least 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% of the nucleotidic units of the double stranded oligonucleotide independently comprise a 2'-substitution.

27. The double stranded oligonucleotide or composition of any one of the preceding claims, wherein a 2'-substitution of the oligonucleotide is 2'-F.

28. The double stranded oligonucleotide or composition of any one of the preceding claims, wherein a 2'-substitution of the oligonucleotide is 2'-OR1.

29. The double stranded oligonucleotide or composition of any one of the preceding claims, wherein a 2'-substitution of the oligonucleotide is–L–, wherein L connects C2 and C4 of the sugar unit.

30. The double stranded oligonucleotide or composition of any one of the preceding claims, wherein at least 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 97% of the nucleotidic units of the double stranded oligonucleotide comprise no 2'-substitution.

31. The double stranded oligonucleotide or composition of any one of the preceding claims, wherein the guide strand comprises a target-binding sequence that is completely complementary to a target sequence, wherein the target-binding sequence has a length of at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 bases, wherein each base is optionally substituted adenine, cytosine, guanosine, thymine, or uracil, and wherein the target sequence comprises one or more allelic sites, wherein an allelic site is a SNP or a mutation.

32. The double stranded oligonucleotide or composition of any one of the preceding claims, wherein the target sequence comprises two SNPs.

33. The double stranded oligonucleotide or composition of any one of the preceding claims, wherein the target sequence comprises an allelic site and the target-binding

sequence is completely complementary to the target sequence of a disease-associated allele but not that of an allele less associated with the disease.

34. The double stranded oligonucleotide or composition of any one of the preceding claims, wherein

the double stranded oligonucleotide comprises a guide strand that binds with a transcript of a target nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence,

wherein the base sequence of the guide strand is or comprises a sequence that is complementary to the characteristic sequence element that defines a particular allele, and

the guide strand being characterized in that, when it is contacted with a cell comprising transcripts of target nucleic acid sequence, it shows suppression of transcripts of the particular allele, or a protein encoded thereby, at a level that is greater than a level of suppression observed for another allele of the same nucleic acid sequence.

35. A method for reducing level and/or activity of a transcript or a protein encoded thereby, comprising administering to a cell expressing the transcript a double stranded oligonucleotide or a composition of any one of the preceding claims, wherein the guide strand of double stranded oligonucleotide or composition comprises a targeting-binding sequence that is completely complementary to a target sequence in the transcript.

36. The method of claim 35 wherein the cell is an immune cell, a blood cell, a cardiac cell, a lung cell, an optic cell, a muscle cell, a liver cell, a kidney cell, a brain cell, a cell of the central nervous system, or a cell of the peripheral nervous system.

37. A method for allele-specific suppression of a transcript from a nucleic acid sequence

for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

contacting a sample comprising transcripts of the target nucleic acid sequence with a double stranded oligonucleotide or a composition of any one of the preceding claims,

wherein the guide strand of the double stranded oligonucleotide or composition comprises a targeting-binding sequence that is identical or completely complementary to a target sequence in the nucleic acid sequence, which target sequence comprises a characteristic sequence element that defines a particular allele, and

wherein when the guide strand of the double stranded oligonucleotide or composition is contacted with a cell comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

38. A method for allele-specific suppression of a transcript from a nucleic acid sequence for which a plurality of alleles exist within a population, each of which contains a specific nucleotide characteristic sequence element that defines the allele relative to other alleles of the same target nucleic acid sequence, the method comprising steps of:

administering to a subject comprising transcripts of the target nucleic acid sequence with a double stranded oligonucleotide or a composition of any one of the preceding claims,

wherein the guide strand of the double stranded oligonucleotide or composition comprises a targeting-binding sequence that is identical or completely complementary to a target sequence in the nucleic acid sequence, which target sequence comprises a characteristic sequence element that defines a particular allele,

and

wherein when the guide strand of the double stranded oligonucleotide or composition is contacted with a cell comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, transcripts of the particular allele are suppressed at a greater level than a level of suppression observed for another allele of the same nucleic acid sequence.

39. The method of any one of claims 35-38, wherein when the oligonucleotide or oligonucleotide of the composition is contacted with a cell comprising transcripts of both the target allele and another allele of the same nucleic acid sequence, it shows suppression of transcripts of the particular allele at a level that is:

a) greater than when the composition is absent;

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

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

40. The method of claim 39 wherein the cell is an immune cell, a blood cell, a cardiac cell, a lung cell, an optic cell, a muscle cell, a liver cell, a kidney cell, a brain cell, a cell of the central nervous system, or a cell of the peripheral nervous system.

41. The method of any one of claims 35-38, wherein suppression of transcripts of the particular allele is at a level that is both greater than when the composition is absent, and greater than a level of suppression observed for another allele of the same nucleic acid sequence.