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(54) Title: METHODS OF USING OLIGOMERIC COMPOUNDS COMPRISING 2'-SUBSTITUTED NUCLEOSIDES

(57) Abstract: The present disclosure provides oligomeric compounds comprising at least one 2'-fluoroethoxy modified nucleoside of formula I and methods of using these oligomeric compounds. The methods provided herein include contacting a cell or administering to an animal at least one of the oligomeric compounds. In certain embodiments, the oligomeric compounds hybridize to a portion of a target RNA resulting in loss of normal function of the target RNA.



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METHODS OF USING OLIGOMERIC COMPOUNDS COMPRISING 2'-SUBSTITUTED NUCLEOSIDES

5 SEQUENCE LISTING

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled CHEM0057WOSEQ.txt, created on October 23, 2009 which is 8 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

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FIELD OF THE INVENTION

The present disclosure provides methods of using oligomeric compounds comprising 2'-fluoroethoxy modified nucleosides. The present methods include contacting a cell with, or administering to an animal, at least one of the oligomeric compounds provided herein. In certain
15 embodiments, the oligomeric compounds hybridize to a portion of a target RNA resulting in loss of normal function of the target RNA.

BACKGROUND OF THE INVENTION

Targeting disease-causing gene sequences was first suggested more than thirty years ago
20 (Belikova et al., Tet. Lett., 1967, 37, 3557-3562), and antisense activity was demonstrated in cell culture more than a decade later (Zamecnik et al., Proc. Natl. Acad. Sci. U.S.A., 1978, 75, 280-284). One advantage of antisense technology in the treatment of a disease or condition that stems from a disease-causing gene is that it is a direct genetic approach that has the ability to modulate (increase or decrease) the expression of specific disease-causing genes. Another advantage is that validation
25 of a therapeutic target using antisense compounds results in direct and immediate discovery of the drug candidate; the antisense compound is the potential therapeutic agent.

Generally, the principle behind antisense technology is that an antisense compound hybridizes to a target nucleic acid and modulates gene expression activities or function, such as transcription or translation. The modulation of gene expression can be achieved by, for example,
30 target degradation or occupancy-based inhibition. An example of modulation of RNA target function by degradation is RNase H-based degradation of the target RNA upon hybridization with an antisense compound. Another example of modulation of gene expression by target degradation is RNA interference (RNAi). RNAi generally refers to antisense-mediated gene silencing involving

the introduction of dsRNA leading to the sequence-specific reduction of targeted endogenous mRNA levels. An additional example of modulation of RNA target function by an occupancy-based mechanism is modulation of microRNA function. MicroRNAs are small non-coding RNAs that regulate the expression of protein-coding RNAs. The binding of an antisense compound to a microRNA prevents that microRNA from binding to its messenger RNA targets, and thus interferes with the function of the microRNA. Regardless of the specific mechanism, this sequence-specificity makes antisense compounds extremely attractive as tools for target validation and gene functionalization, as well as therapeutics to selectively modulate the expression of genes involved in the pathogenesis of malignancies and other diseases.

Antisense technology is an effective means for reducing the expression of one or more specific gene products and can therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications. Chemically modified nucleosides are routinely used for incorporation into antisense compounds to enhance one or more properties, such as nuclease resistance, affinity, specificity or pharmacokinetics for a target RNA. In 1998, the antisense compound, Vitravene® (fomivirsen; developed by Isis Pharmaceuticals Inc., Carlsbad, CA) was the first antisense drug to achieve marketing clearance from the U.S. Food and Drug Administration (FDA), and is currently a treatment of cytomegalovirus (CMV)-induced retinitis in AIDS patients.

New chemical modifications have improved the potency and efficacy of antisense compounds, uncovering the potential for oral delivery as well as enhancing subcutaneous administration, decreasing potential for side effects, and leading to improvements in patient convenience. Chemical modifications increasing potency of antisense compounds allow administration of lower doses, which reduces the potential for toxicity, as well as decreasing overall cost of therapy. Modifications increasing the resistance to degradation result in slower clearance from the body, allowing for less frequent dosing. Different types of chemical modifications can be combined in one compound to further optimize the compound's efficacy. One such group of chemically modified nucleosides includes 2'-fluoroethoxy modified nucleosides.

2'-OCH₂CH₂F modified nucleosides were prepared and put into 15 and 16mer DNA sequences for affinity measurements against complementary RNA (U.S. Patent 5,977,332, issued November 2, 1999). Modified nucleosides having 2'-OCH₂R (R = CH₂F or CF₃) substituents were also prepared and put into 15 and 16mer DNA sequences for measurement of affinity against complementary RNA, measurement of nuclease resistance and analysis of crystal structure data (Egli *et al.*, *Biochemistry*, **2005**, *44*, 9045-9057).

Consequently, there remains a long-felt need for agents that specifically regulate gene

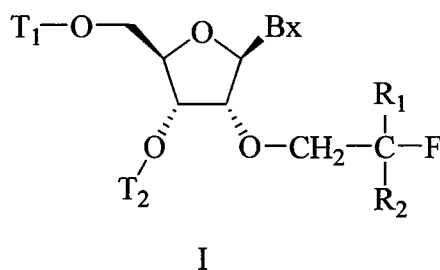
expression via antisense mechanisms. Disclosed herein are oligomeric compounds comprising 2'-fluoroethoxy modified nucleosides that are useful for modulating gene expression pathways, including those relying on mechanisms of action such as RNaseH, RNAi and dsRNA enzymes, as well as other antisense mechanisms based on target degradation or target occupancy. One having skill in the art, once armed with this disclosure will be able, without undue experimentation, to identify, prepare and exploit antisense compounds for these uses.

BRIEF SUMMARY OF THE INVENTION

Provided herein are methods of using oligomeric compounds comprising at least one 2'-fluoroethoxy modified nucleoside of formula I. The present methods include contacting a cell or administering to an animal at least one of the oligomeric compounds provided herein. In certain embodiments, the oligomeric compounds hybridize to a portion of a target RNA resulting in loss of normal function of the target RNA.

The variables are defined individually in further detail herein. It is to be understood that the oligomer compounds comprising 2'-fluoroethoxy modified nucleosides of formula I and methods of use thereof provided herein include all combinations of the embodiments disclosed and variables defined herein.

In certain embodiments, methods are provided comprising contacting a cell with an oligomeric compound, wherein said oligomeric compound comprises at least one 2'-fluoroethoxy modified nucleoside having formula I:



wherein independently for each of said 2'-fluoroethoxy modified nucleosides having formula I:

Bx is a heterocyclic base moiety;

one of T₁ and T₂ is an internucleoside linking group attaching the 2'-fluoroethoxy modified nucleoside of formula I to the oligomeric compound and the other of T₁ and T₂ is H or a hydroxyl protecting group, a 5' or 3'-terminal group or an internucleoside linking group attaching the 2'-fluoroethoxy modified nucleoside of formula I to the oligomeric compound;

R₁ and R₂ are each independently, H or F; and

wherein said oligomeric compound comprises from about 8 to about 40 linked monomeric subunits and is complementary to at least a portion of a target RNA.

In certain embodiments, R_1 and R_2 are each H for each of said 2'-fluoroethoxy modified nucleosides of formula I. In certain embodiments, one of R_1 and R_2 is F for each of said 2'-fluoroethoxy modified nucleosides of formula I. In certain embodiments, R_1 and R_2 are each F for each of said 2'-fluoroethoxy modified nucleosides of formula I.

In certain embodiments, each Bx is, independently, uracil, 5-methyluracil, thymine, cytosine, 5-methylcytosine, 2,6-diaminopurine, adenine or guanine.

In certain embodiments, oligomeric compounds are provided comprising a 5' or 3'-terminal group. In certain embodiments, oligomeric compounds are provided comprising a 5' and a 3'-terminal group.

In certain embodiments, oligomeric compounds are provided wherein each linked monomeric subunit that is not a β -D-2'-deoxyribonucleoside or a 2'-fluoroethoxy modified nucleoside of formula I is a modified nucleoside. In certain embodiments, each modified nucleoside is, independently, a bicyclic modified nucleoside, a 2'-modified nucleoside, a 4'-thio modified nucleoside or a 4'-thio-2'-modified nucleoside.

In certain embodiments, oligomeric compounds are provided comprising a blockmer, a 3'-hemimer or 5'-hemimer. In certain embodiments, each monomeric subunit is, independently, a β -D-2'-deoxyribonucleoside or a 2'-fluoroethoxy modified nucleoside of formula I.

In certain embodiments, gapped oligomeric compounds are provided comprising two external regions separated by an internal region wherein each external region independently comprises from 1 to 5 contiguous 2'-fluoroethoxy modified nucleosides having formula I and the internal region comprises from 6 to about 23 contiguous monomeric subunits independently selected from nucleosides and modified nucleosides. In certain embodiments, each monomeric subunit in the internal region is, independently, a β -D-2'-deoxyribonucleoside or a modified nucleoside. In certain embodiments, each monomeric subunit in the internal region is a β -D-2'-deoxyribonucleoside. In certain embodiments, the internal region comprises from about 8 to about 12 β -D-2'-deoxyribonucleosides. In certain embodiments, each external region comprises from 1 to 3 2'-fluoroethoxy modified nucleoside having formula I. In certain embodiments, the internal region comprises from about 10 to about 12 β -D-2'-deoxyribonucleosides. In certain embodiments, the internal region comprises from 11 to about 18 β -D-2'-deoxyribonucleosides. In certain embodiments, each external region comprises from 1 to 3 2'-fluoroethoxy modified nucleoside

having formula I. In certain embodiments, the internal region comprises from 12 to about 14 β -D-2'-deoxyribonucleosides. In certain embodiments, each external region independently comprises from 1 to 3 2'-fluoroethoxy modified nucleosides having formula I. In certain embodiments, each external region comprises 2 2'-fluoroethoxy modified nucleosides having formula I. In certain
5 embodiments, the internal region comprises 10 β -D-2'-deoxyribonucleosides.

In certain embodiments, oligomeric compounds are provided comprising a 5'-terminal 2'-fluoroethoxy modified nucleoside having formula I wherein T_1 is a phosphate group.

In certain embodiments, oligomeric compounds are provided wherein each internucleoside linkage is a phosphodiester. In certain embodiments, oligomeric compounds are provided wherein
10 each internucleoside linkage is a phosphorothioate. In certain embodiments, oligomeric compounds are provided wherein each internucleoside linking group is, independently, a phosphodiester or a phosphorothioate. In certain embodiments, oligomeric compounds are provided wherein each internucleoside linking group is, independently, a phosphodiester, phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl
15 phosphonate, alkyl phosphonate, 5'-alkylene phosphonate, chiral phosphonate, phosphinate, phosphoramidate, 3'-amino phosphoramidate, aminoalkylphosphoramidate, thionophosphoramidate, thionoalkylphosphonate, thionoalkylphosphotriester, selenophosphate or boranophosphate.

In certain embodiments, oligomeric compounds are provided comprising from 8 to about 18 linked monomeric subunits. In certain embodiments, oligomeric compounds are provided
20 comprising from 10 to about 16 linked monomeric subunits. In certain embodiments, oligomeric compounds are provided comprising from 10 to about 14 linked monomeric subunits. In certain embodiments, oligomeric compounds are provided comprising from 17 to about 26 linked monomeric subunits. In certain embodiments, oligomeric compounds are provided comprising from 18 to about 21 linked monomeric subunits. In certain embodiments, oligomeric compounds are
25 provided comprising from 19 to about 20 linked monomeric subunits.

In certain embodiments, methods are provided comprising contacting a cell with an oligomeric compound as provided herein, wherein the cell is in an animal. In certain embodiments, the cell is in a human.

In certain embodiments, methods are provided comprising contacting a cell with an
30 oligomeric compound that is complementary to at least a portion of a target RNA wherein the target RNA is selected from mRNA, pre-mRNA and micro RNA. In certain embodiments, the target RNA is mRNA. In certain embodiments, the target RNA is human mRNA.

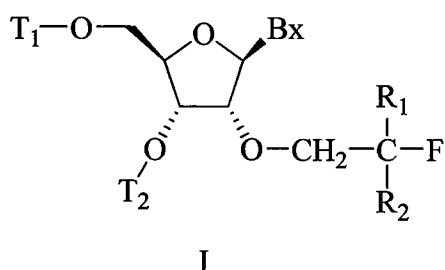
In certain embodiments, methods are provided comprising contacting a cell with an

oligomeric compound that is complementary to at least a portion of a target RNA wherein the target RNA is cleaved thereby inhibiting its function.

In certain embodiments, methods are provided comprising contacting a cell with an oligomeric compound and evaluating the antisense activity of the oligomeric compound on the cell.

- 5 In certain embodiments, the evaluating comprises detecting the levels of target RNA. In certain embodiments, the evaluating comprises detecting the levels of a protein. In certain embodiments, the evaluating comprises detection of one or more phenotypic effects.

- 10 In certain embodiments, methods are provided comprising administering an oligomeric compound to an animal, wherein said oligomeric compound comprises at least one 2'-fluoroethoxy modified nucleoside having formula I:



wherein independently for each of said 2'-fluoroethoxy modified nucleosides having formula I:

Bx is a heterocyclic base moiety;

- 15 one of T₁ and T₂ is an internucleoside linking group attaching the 2'-fluoroethoxy modified nucleoside of formula I to the oligomeric compound and the other of T₁ and T₂ is H or a hydroxyl protecting group, a 5' or 3'-terminal group or an internucleoside linking group attaching the 2'-fluoroethoxy modified nucleoside of formula I to the oligomeric compound;

R₁ and R₂ are each independently, H or F; and

- 20 wherein said oligomeric compound comprises from about 8 to about 40 linked monomeric subunits and is complementary to at least a portion of a target RNA.

- In certain embodiments, R₁ and R₂ are each H for each of said 2'-fluoroethoxy modified nucleosides of formula I. In certain embodiments, R₁ and R₂ is F for each of said 2'-fluoroethoxy modified nucleosides of formula I. In certain embodiments, R₁ and R₂ are each F for each of said 2'-fluoroethoxy modified nucleosides of formula I.
- 25

In certain embodiments, each Bx is, independently, uracil, 5-methyluracil, thymine, cytosine, 5-methylcytosine, 2,6-diaminopurine, adenine or guanine.

In certain embodiments, oligomeric compounds are provided comprising a 5' or 3'-terminal group. In certain embodiments, oligomeric compounds are provided comprising a 5' and a 3'-

terminal group.

49. The method of any one of claims 44 to 48 wherein said oligomeric compound further comprises a 5' or 3'-terminal group.

In certain embodiments, oligomeric compounds are provided wherein each linked
5 monomeric subunit that is not a β -D-2'-deoxyribonucleoside or a 2'-fluoroethoxy modified nucleoside of formula I is a modified nucleoside. In certain embodiments, each modified nucleoside is, independently, a bicyclic modified nucleoside, a 2'-modified nucleoside, a 4'-thio modified nucleoside or a 4'-thio-2'-modified nucleoside.

In certain embodiments, oligomeric compounds are provided comprising a blockmer, a 3'-
10 hemimer or 5'-hemimer. In certain embodiments, each monomeric subunit is, independently, a β -D-2'-deoxyribonucleoside or a 2'-fluoroethoxy modified nucleoside of formula I.

In certain embodiments, gapped oligomeric compounds are provided comprising two external regions separated by an internal region wherein each external region independently comprises from 1 to 5 contiguous 2'-fluoroethoxy modified nucleosides having formula I and the
15 internal region comprises from 6 to about 23 contiguous monomeric subunits independently selected from nucleosides and modified nucleosides. In certain embodiments, each monomeric subunit in the internal region is, independently, a β -D-2'-deoxyribonucleoside or a modified nucleoside. In certain embodiments, each monomeric subunit in the internal region is a β -D-2'-deoxyribonucleoside. In certain embodiments, the internal region comprises from about 8 to about 12 β -D-2'-deoxyribo-
20 nucleosides. In certain embodiments, each external region comprises from 1 to 3 2'-fluoroethoxy modified nucleoside having formula I. In certain embodiments, the internal region comprises from about 10 to about 12 β -D-2'-deoxyribonucleosides. In certain embodiments, the internal region comprises from 11 to about 18 β -D-2'-deoxyribonucleosides. In certain embodiments, each external region comprises from 1 to 3 2'-fluoroethoxy modified nucleoside having formula I. In certain
25 embodiments, the internal region comprises from 12 to about 14 β -D-2'-deoxyribonucleosides. In certain embodiments, each external region independently comprises from 1 to 3 2'-fluoroethoxy modified nucleosides having formula I. In certain embodiments, each external region comprises 2 2'-fluoroethoxy modified nucleosides having formula I. In certain embodiments, the internal region comprises 10 β -D-2'-deoxyribonucleosides.

30 In certain embodiments, oligomeric compounds are provided comprising a 5'-terminal 2'-fluoroethoxy modified nucleoside having formula I wherein T_1 is a phosphate group.

In certain embodiments, oligomeric compounds are provided wherein each internucleoside

linkage is a phosphodiester. In certain embodiments, each internucleoside linkage is a phosphorothioate. In certain embodiments, each internucleoside linking group is, independently, a phosphodiester or a phosphorothioate. In certain embodiments, each internucleoside linking group is, independently, a phosphodiester, phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl phosphonate, alkyl phosphonate, 5'-alkylene phosphonate, chiral phosphonate, phosphinate, phosphoramidate, 3'-amino phosphoramidate, aminoalkylphosphoramidate, thionophosphoramidate, thionoalkylphosphonate, thionoalkylphosphotriester, selenophosphate or boranophosphate.

In certain embodiments, oligomeric compounds are provided comprising from 8 to about 18 linked monomeric subunits. In certain embodiments, oligomeric compounds are provided comprising from 10 to about 16 linked monomeric subunits. In certain embodiments, oligomeric compounds are provided comprising from 10 to about 14 linked monomeric subunits. In certain embodiments, oligomeric compounds are provided comprising from 17 to about 26 linked monomeric subunits. In certain embodiments, oligomeric compounds are provided comprising from 18 to about 21 linked monomeric subunits. In certain embodiments, oligomeric compounds are provided comprising from 19 to about 20 linked monomeric subunits.

In certain embodiments, methods are provided comprising administering an oligomeric compound to an animal, wherein the animal is a mammal. In certain embodiments, methods are provided comprising administering an oligomeric compound to an animal, wherein the animal is a human.

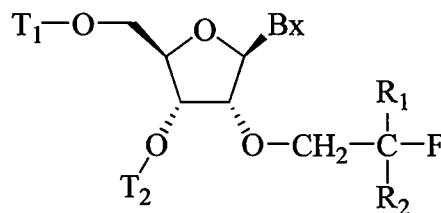
In certain embodiments, methods are provided comprising contacting a cell with an oligomeric compound that is complementary to at least a portion of a target RNA wherein the target RNA is selected from mRNA, pre-mRNA and micro RNA. In certain embodiments, the target RNA is mRNA. In certain embodiments, the target RNA is human mRNA.

In certain embodiments, methods are provided comprising contacting a cell with an oligomeric compound that is complementary to at least a portion of a target RNA wherein the target RNA is cleaved thereby inhibiting its function.

In certain embodiments, methods are provided comprising contacting a cell with an oligomeric compound and evaluating the antisense activity of the oligomeric compound on the cell. In certain embodiments, the evaluating comprises detecting the levels of target RNA. In certain embodiments, the evaluating comprises detecting the levels of a protein. In certain embodiments, the evaluating comprises detection of one or more phenotypic effects.

The present disclosure provides methods of using oligomeric compounds wherein each oligomeric compound comprises at least one 2'-fluoroethoxy modified nucleosides of formula I. The present methods include contacting a cell with at least one of the oligomeric compounds. The present methods also include administering at least one of the oligomeric compounds to an animal. In certain embodiments, the oligomeric compounds hybridize to a portion of a target RNA resulting in loss of normal function of the target RNA. The oligomeric compounds are also expected to be useful as primers and probes in diagnostic applications.

In certain embodiments, methods are provided comprising contacting a cell with an oligomeric compound, wherein said oligomeric compound comprises at least one 2'-fluoroethoxy modified nucleoside having formula I:



wherein independently for each of said 2'-fluoroethoxy modified nucleosides having formula I:

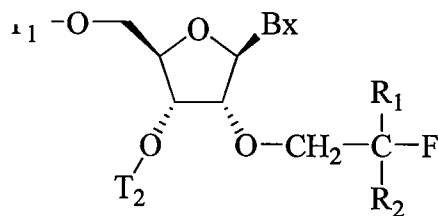
Bx is a heterocyclic base moiety;

one of T₁ and T₂ is an internucleoside linking group attaching the 2'-fluoroethoxy modified nucleoside of formula I to the oligomeric compound and the other of T₁ and T₂ is H or a hydroxyl protecting group, a 5' or 3'-terminal group or an internucleoside linking group attaching the 2'-fluoroethoxy modified nucleoside of formula I to the oligomeric compound;

R₁ and R₂ are each independently, H or F; and
wherein said oligomeric compound comprises from about 8 to about 40 linked monomeric subunits and is complementary to at least a portion of a target RNA.

In certain embodiments, the contacting step is performed on a cell or group of cells. In certain embodiments, the cells are in an animal. In a preferred embodiment, the cells are in a human.

In certain embodiments, methods are provided comprising administering an oligomeric compound to an animal, wherein said oligomeric compound comprises at least one 2'-fluoroethoxy modified nucleoside having formula I:



I

wherein independently for each of said 2'-fluoroethoxy modified nucleosides having formula I:

Bx is a heterocyclic base moiety;

5 one of T₁ and T₂ is an internucleoside linking group attaching the 2'-fluoroethoxy modified nucleoside of formula I to the oligomeric compound and the other of T₁ and T₂ is H or a hydroxyl protecting group, a 5' or 3'-terminal group or an internucleoside linking group attaching the 2'-fluoroethoxy modified nucleoside of formula I to the oligomeric compound;

R₁ and R₂ are each independently, H or F; and

10 wherein said oligomeric compound comprises from about 8 to about 40 linked monomeric subunits and is complementary to at least a portion of a target RNA.

In one embodiment, the target RNA is selected from mRNA, pre-mRNA and micro RNA. A preferred target RNA is mRNA with human mRNA being more preferred.

15 The present methods can be evaluated using methods that are known in the art. One particular method of evaluating the methods is to evaluate the antisense activity of the oligomeric compounds on a cell, a group of cells or in an animal. Preferred animals for evaluating the antisense activity of the oligomeric compounds are mammals with humans being more preferred.

Another method of evaluating the activity of the oligomeric compounds is to monitor the levels of target RNA or one or more proteins affected by a change in target RNA concentration.

20 Phenotypic effects can also be monitored.

In certain embodiments, the 2'-fluoroethoxy modified nucleosides of formula I provided herein are useful for modifying oligomeric compounds at one or more positions. Such modified oligomeric compounds can be described as having a particular motif. Motifs include without limitation, gapped motifs, hemimer motifs, blockmer motifs, uniformly fully modified motifs, 25 positionally modified motifs and alternating motifs. In conjunction with these motifs a wide variety of internucleoside linkages can also be used including but not limited to phosphodiester and phosphorothioate internucleoside linkages which can be incorporated uniformly or in various combinations. The oligomeric compounds can further include at least one 5' or 3' terminal group such as for example a conjugate or reporter group. The positioning of the 2'-fluoroethoxy modified

nucleosides of formula I provided herein, the use of linkage strategies and 5' or 3' terminal groups can be easily optimized to enhance a desired activity for a selected target.

As used herein the term "motif" refers to the pattern created by the relative positioning of monomer subunits within an oligomeric compound wherein the pattern is determined by comparing the sugar groups. The only determinant for the motif of an oligomeric compound is the differences or lack of differences between the sugar groups. As used herein the term "sugar group" as it applies to motifs includes naturally occurring sugars having a furanose ring, sugars having a modified furanose ring and sugar surrogates wherein the furanose ring has been replaced with another ring system such as for example a morpholino or hexitol ring system. When each sugar group is the same (DNA, RNA, modified or surrogate) the motif is termed uniformly fully modified. When two or more types of sugar groups are present the motif is defined by the pattern created from the positioning of monomer subunits having one type of sugar group relative to the positioning of monomer subunits having different types of sugar groups within an oligomeric compound.

Illustrative examples of some different types of sugar groups useful in the preparation of oligomeric compounds having motifs include without limitation, β -D-ribose, β -D-2'-deoxyribose, substituted sugars (such as 2', 5' and bis substituted sugars), 4'-S-sugars (such as 4'-S-ribose, 4'-S-2'-deoxyribose and 4'-S-2'-substituted ribose), bicyclic modified sugars (such as the 2'-O-CH₂-4' or 2'-O-(CH₂)₂-4' bridged ribose derived bicyclic sugars) and sugar surrogates (such as when the ribose ring has been replaced with a morpholino or a hexitol ring system). The type of heterocyclic base and internucleoside linkage used at each position is variable and is not a factor in determining the motif. The presence of one or more other groups including but not limited to capping groups, conjugate groups and other 5' or 3'-terminal groups is also not a factor in determining the motif.

Representative U.S. patents that teach the preparation of motifs include without limitation, 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety. Motifs are also disclosed in International Applications PCT/US2005/019219, filed June 2, 2005 and published as WO 2005/121371 on December 22, 2005 and PCT/US2005/019220, filed June 2, 2005 and published as WO 2005/121372 on December 22, 2005; each of which is incorporated by reference herein in its entirety.

As used herein the term "alternating motif" refers to an oligomeric compound comprising a contiguous sequence of linked monomer subunits wherein the monomer subunits have two different types of sugar groups that alternate for essentially the entire sequence of the oligomeric

compound. Oligomeric compounds having an alternating motif can be described by the formula: 5'-A(-L-B-L-A)_n(-L-B)_{nn}-3' where A and B are monomer subunits that have different sugar groups, each L is, independently, an internucleoside linking group, n is from about 4 to about 12 and nn is 0 or 1. The heterocyclic base and internucleoside linkage is independently variable at each position.

5 The motif further optionally includes the use of one or more other groups including but not limited to capping groups, conjugate groups and other 5' or 3'-terminal groups. This permits alternating oligomeric compounds from about 9 to about 26 monomer subunits in length. This length range is not meant to be limiting as longer and shorter oligomeric compounds are also amenable to oligomeric compounds provided herein. In certain embodiments, each A or each B comprises 2'-
10 fluoroethoxy modified nucleosides of formula I.

As used herein the term "uniformly fully modified motif" refers to an oligomeric compound comprising a contiguous sequence of linked monomer subunits that each have the same type of sugar group. The heterocyclic base and internucleoside linkage is independently variable at each position. The motif further optionally includes the use of one or more other groups including
15 but not limited to capping groups, conjugate groups and other 5' or 3'-terminal groups. In certain embodiments, the uniformly fully modified motif includes a contiguous sequence of 2'-fluoroethoxy modified nucleosides of formula I. In certain embodiments, one or both of the 5' and 3'-ends of the contiguous sequence of 2'-fluoroethoxy modified nucleosides of formula I, comprise 5' or 3'-terminal groups such as one or more unmodified nucleosides.

20 As used herein the term "hemimer motif" refers to an oligomeric compound comprising a contiguous sequence of monomer subunits that each have the same type of sugar group with a further short contiguous sequence of monomer subunits located at the 5' or the 3' end that have a different type of sugar group. The heterocyclic base and internucleoside linkage is independently variable at each position. The motif further optionally includes the use of one or more other groups
25 including but not limited to capping groups, conjugate groups and other 5' or 3'-terminal groups. In general, a hemimer is an oligomeric compound of uniform sugar groups further comprising a short region (1, 2, 3, 4 or about 5 monomer subunits) having uniform but different sugar groups located on either the 3' or the 5' end of the oligomeric compound.

In certain embodiments, the hemimer motif comprises a contiguous sequence of from
30 about 10 to about 28 monomer subunits having one type of sugar group with from 1 to 5 or from 2 to about 5 monomer subunits having a second type of sugar group located at one of the termini. In certain embodiments, the hemimer is a contiguous sequence of from about 8 to about 20 β-D-2'-deoxyribonucleosides having from 1-12 contiguous 2'-fluoroethoxy modified nucleosides of formula

I located at one of the termini. In certain embodiments, the hemimer is a contiguous sequence of from about 8 to about 20 β -D-2'-deoxyribonucleosides having from 1-5 contiguous 2'-fluoroethoxy modified nucleosides of formula I located at one of the termini. In certain embodiments, the hemimer is a contiguous sequence of from about 12 to about 18 β -D-2'-deoxyribonucleosides having from 1-3 contiguous 2'-fluoroethoxy modified nucleosides of formula I located at one of the termini. In certain embodiments, the hemimer is a contiguous sequence of from about 10 to about 14 β -D-2'-deoxyribonucleosides having from 1-3 contiguous 2'-fluoroethoxy modified nucleosides of formula I located at one of the termini.

As used herein the term "blockmer motif" refers to an oligomeric compound comprising an otherwise contiguous sequence of monomer subunits wherein the sugar groups of each monomer subunit is the same except for an interrupting internal block of contiguous monomer subunits having a different type of sugar group. The heterocyclic base and internucleoside linkage is independently variable at each position of a blocker oligomeric compound. The motif further optionally includes the use of one or more other groups including but not limited to capping groups, conjugate groups and other 5' or 3'-terminal groups. A blockmer overlaps somewhat with a gapmer in the definition but typically only the monomer subunits in the block have non-naturally occurring sugar groups in a blockmer and only the monomer subunits in the external regions have non-naturally occurring sugar groups in a gapmer with the remainder of monomer subunits in the blockmer or gapmer being β -D-2'-deoxyribonucleosides or β -D-ribonucleosides. In certain embodiments, blockmer oligomeric compounds are provided herein wherein all of the monomer subunits comprise non-naturally occurring sugar groups.

As used herein the term "positionally modified motif" is meant to include an otherwise contiguous sequence of monomer subunits having one type of sugar group that is interrupted with two or more regions of from 1 to about 5 contiguous monomer subunits having another type of sugar group. Each of the two or more regions of from 1 to about 5 contiguous monomer subunits are independently uniformly modified with respect to the type of sugar group. In certain embodiments, each of the two or more regions have the same type of sugar group. In certain embodiments, each of the two or more regions have a different type of sugar group. In certain embodiments, each of the two or more regions, independently, have the same or a different type of sugar group. The heterocyclic base and internucleoside linkage is independently variable at each position of a positionally modified oligomeric compound. The motif further optionally includes the use of one or more other groups including but not limited to capping groups, conjugate groups and other 5' or 3'-terminal groups. In certain embodiments, positionally modified oligomeric compounds are provided

comprising a sequence of from 8 to 20 β -D-2'-deoxyribonucleosides that further includes two or three regions of from 2 to about 5 contiguous 2'-fluoroethoxy modified nucleosides of formula I each. Positionally modified oligomeric compounds are distinguished from gapped motifs, hemimer motifs, blockmer motifs and alternating motifs because the pattern of regional substitution defined by any positional motif does not fit into the definition provided herein for one of these other motifs. The term positionally modified oligomeric compound includes many different specific substitution patterns.

As used herein the term "gapmer" or "gapped oligomeric compound" refers to an oligomeric compound having two external regions or wings and an internal region or gap. The three regions form a contiguous sequence of monomer subunits with the sugar groups of the external regions being different than the sugar groups of the internal region and wherein the sugar group of each monomer subunit within a particular region is essentially the same. In certain embodiments, each monomer subunit within a particular region has the same sugar group. When the sugar groups of the external regions are the same the gapmer is a symmetric gapmer and when the sugar group used in the 5'-external region is different from the sugar group used in the 3'-external region, the gapmer is an asymmetric gapmer. In certain embodiments, the external regions are small (each independently 1, 2, 3, 4 or about 5 monomer subunits) and the monomer subunits comprise non-naturally occurring sugar groups with the internal region comprising β -D-2'-deoxyribonucleosides. In certain embodiments, the external regions each, independently, comprise from 1 to about 5 monomer subunits having non-naturally occurring sugar groups and the internal region comprises from 6 to 18 unmodified nucleosides. The internal region or the gap generally comprises β -D-2'-deoxyribonucleosides but can comprise non-naturally occurring sugar groups. The heterocyclic base and internucleoside linkage is independently variable at each position of a gapped oligomeric compound. The motif further optionally includes the use of one or more other groups including but not limited to capping groups, conjugate groups and other 5' or 3'-terminal groups.

In certain embodiments, the gapped oligomeric compounds comprise an internal region of β -D-2'-deoxyribonucleosides with one of the external regions comprising 2'-fluoroethoxy modified nucleosides of formula I as provided herein. In certain embodiments, the gapped oligomeric compounds comprise an internal region of β -D-2'-deoxyribonucleosides with both of the external regions comprising 2'-fluoroethoxy modified nucleosides of formula I as provided herein. In certain embodiments, gapped oligomeric compounds are provided herein wherein all of the monomer subunits comprise non-naturally occurring sugar groups.

In certain embodiments, gapped oligomeric compounds are provided comprising one or two

2'-fluoroethoxy modified nucleosides of formula I at the 5'-end, two or three 2'-fluoroethoxy modified nucleosides of formula I at the 3'-end and an internal region of from 10 to 16 β -D-2'-deoxyribonucleosides. In certain embodiments, gapped oligomeric compounds are provided comprising one 2'-fluoroethoxy modified nucleosides of formula I at the 5'-end, two 2'-fluoroethoxy modified nucleosides of formula I at the 3'-end and an internal region of from 10 to 16 β -D-2'-deoxyribonucleosides. In certain embodiments, gapped oligomeric compounds are provided comprising one 2'-fluoroethoxy modified nucleosides of formula I at the 5'-end, two 2'-fluoroethoxy modified nucleosides of formula I at the 3'-end and an internal region of from 10 to 14 β -D-2'-deoxyribonucleosides.

In certain embodiments, gapped oligomeric compounds are provided that are from about 10 to about 21 monomer subunits in length. In certain embodiments, gapped oligomeric compounds are provided that are from about 12 to about 16 monomer subunits in length. In certain embodiments, gapped oligomeric compounds are provided that are from about 12 to about 14 monomer subunits in length.

The terms "substituent" and "substituent group," as used herein, are meant to include groups that are typically added to other groups or parent compounds to enhance desired properties or provide other desired effects. Substituent groups can be protected or unprotected and can be added to one available site or to many available sites in a parent compound. Substituent groups may also be further substituted with other substituent groups and may be attached directly or via a linking group such as an alkyl or hydrocarbyl group to a parent compound.

Substituent groups amenable herein include without limitation, halogen, hydroxyl, alkyl, alkenyl, alkynyl, acyl ($-\text{C}(\text{O})\text{R}_{\text{aa}}$), carboxyl ($-\text{C}(\text{O})\text{O}-\text{R}_{\text{aa}}$), aliphatic groups, alicyclic groups, alkoxy, substituted oxy ($-\text{O}-\text{R}_{\text{aa}}$), aryl, aralkyl, heterocyclic radical, heteroaryl, heteroarylalkyl, amino ($-\text{N}(\text{R}_{\text{bb}})(\text{R}_{\text{cc}})$), imino ($=\text{NR}_{\text{bb}}$), amido ($-\text{C}(\text{O})\text{N}(\text{R}_{\text{bb}})(\text{R}_{\text{cc}})$ or $-\text{N}(\text{R}_{\text{bb}})\text{C}(\text{O})\text{R}_{\text{aa}}$), azido ($-\text{N}_3$), nitro ($-\text{NO}_2$), cyano ($-\text{CN}$), carbamido ($-\text{OC}(\text{O})\text{N}(\text{R}_{\text{bb}})(\text{R}_{\text{cc}})$ or $-\text{N}(\text{R}_{\text{bb}})\text{C}(\text{O})\text{OR}_{\text{aa}}$), ureido ($-\text{N}(\text{R}_{\text{bb}})\text{C}(\text{O})\text{N}(\text{R}_{\text{bb}})(\text{R}_{\text{cc}})$), thioureido ($-\text{N}(\text{R}_{\text{bb}})\text{C}(\text{S})\text{N}(\text{R}_{\text{bb}})(\text{R}_{\text{cc}})$), guanidiny ($-\text{N}(\text{R}_{\text{bb}})\text{C}(=\text{NR}_{\text{bb}})\text{N}(\text{R}_{\text{bb}})(\text{R}_{\text{cc}})$), amidinyl ($-\text{C}(=\text{NR}_{\text{bb}})\text{N}(\text{R}_{\text{bb}})(\text{R}_{\text{cc}})$ or $\text{N}(\text{R}_{\text{bb}})\text{C}(=\text{NR}_{\text{bb}})(\text{R}_{\text{aa}})$), thiol ($-\text{SR}_{\text{bb}}$), sulfinyl ($-\text{S}(\text{O})\text{R}_{\text{bb}}$), sulfonyl ($-\text{S}(\text{O})_2\text{R}_{\text{bb}}$) and sulfonamidyl ($-\text{S}(\text{O})_2\text{N}(\text{R}_{\text{bb}})(\text{R}_{\text{cc}})$ or $-\text{N}(\text{R}_{\text{bb}})\text{S}(\text{O})_2\text{R}_{\text{bb}}$). Wherein each R_{aa} , R_{bb} and R_{cc} is, independently, H, an optionally linked chemical functional group or a further substituent group with a preferred list including without limitation, H, alkyl, alkenyl, alkynyl, aliphatic, alkoxy, acyl, aryl, aralkyl, heteroaryl, alicyclic, heterocyclic and heteroarylalkyl. Selected substituents within the compounds described herein are present to a recursive degree.

In this context, "recursive substituent" means that a substituent may recite another instance of itself. Because of the recursive nature of such substituents, theoretically, a large number may be present in any given claim. One of ordinary skill in the art of medicinal chemistry and organic chemistry understands that the total number of such substituents is reasonably limited by the desired properties of the compound intended. Such properties include, by way of example and not limitation, physical properties such as molecular weight, solubility or log P, application properties such as activity against the intended target and practical properties such as ease of synthesis.

Recursive substituents are an intended aspect of the invention. One of ordinary skill in the art of medicinal and organic chemistry understands the versatility of such substituents. To the degree that recursive substituents are present in a claim of the invention, the total number will be determined as set forth above.

The terms "stable compound" and "stable structure" as used herein are meant to indicate a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent. Only stable compounds are contemplated herein.

The term "alkyl," as used herein, refers to a saturated straight or branched hydrocarbon radical containing up to twenty four carbon atoms. Examples of alkyl groups include without limitation, methyl, ethyl, propyl, butyl, isopropyl, n-hexyl, octyl, decyl, dodecyl and the like. Alkyl groups typically include from 1 to about 24 carbon atoms, more typically from 1 to about 12 carbon atoms (C_1 - C_{12} alkyl) with from 1 to about 6 carbon atoms being more preferred. The term "lower alkyl" as used herein includes from 1 to about 6 carbon atoms. Alkyl groups as used herein may optionally include one or more further substituent groups.

The term "alkenyl," as used herein, refers to a straight or branched hydrocarbon chain radical containing up to twenty four carbon atoms and having at least one carbon-carbon double bond. Examples of alkenyl groups include without limitation, ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, dienes such as 1,3-butadiene and the like. Alkenyl groups typically include from 2 to about 24 carbon atoms, more typically from 2 to about 12 carbon atoms with from 2 to about 6 carbon atoms being more preferred. Alkenyl groups as used herein may optionally include one or more further substituent groups.

The term "alkynyl," as used herein, refers to a straight or branched hydrocarbon radical containing up to twenty four carbon atoms and having at least one carbon-carbon triple bond. Examples of alkynyl groups include, without limitation, ethynyl, 1-propynyl, 1-butyne, and the like. Alkynyl groups typically include from 2 to about 24 carbon atoms, more typically from 2 to

about 12 carbon atoms with from 2 to about 6 carbon atoms being more preferred. Alkynyl groups as used herein may optionally include one or more further substituent groups.

5 The term "acyl," as used herein, refers to a radical formed by removal of a hydroxyl group from an organic acid and has the general Formula $-C(O)-X$ where X is typically aliphatic, alicyclic or aromatic. Examples include aliphatic carbonyls, aromatic carbonyls, aliphatic sulfonyls, aromatic sulfinyls, aliphatic sulfinyls, aromatic phosphates, aliphatic phosphates and the like. Acyl groups as used herein may optionally include further substituent groups.

10 The term "alicyclic" refers to a cyclic ring system wherein the ring is aliphatic. The ring system can comprise one or more rings wherein at least one ring is aliphatic. Preferred alicyclics include rings having from about 5 to about 9 carbon atoms in the ring. Alicyclic as used herein may optionally include further substituent groups.

15 The term "aliphatic," as used herein, refers to a straight or branched hydrocarbon radical containing up to twenty four carbon atoms wherein the saturation between any two carbon atoms is a single, double or triple bond. An aliphatic group preferably contains from 1 to about 24 carbon atoms, more typically from 1 to about 12 carbon atoms with from 1 to about 6 carbon atoms being more preferred. The straight or branched chain of an aliphatic group may be interrupted with one or more heteroatoms that include nitrogen, oxygen, sulfur and phosphorus. Such aliphatic groups interrupted by heteroatoms include without limitation, polyalkoxys, such as polyalkylene glycols, polyamines, and polyimines. Aliphatic groups as used herein may optionally include further substituent groups.

20 The term "alkoxy," as used herein, refers to a radical formed between an alkyl group and an oxygen atom wherein the oxygen atom is used to attach the alkoxy group to a parent molecule. Examples of alkoxy groups include without limitation, methoxy, ethoxy, propoxy, isopropoxy, *n*-butoxy, *sec*-butoxy, *tert*-butoxy, *n*-pentoxy, neopentoxy, *n*-hexoxy and the like. Alkoxy groups as used herein may optionally include further substituent groups.

25 The term "aminoalkyl" as used herein, refers to an amino substituted C_1-C_{12} alkyl radical. The alkyl portion of the radical forms a covalent bond with a parent molecule. The amino group can be located at any position and the aminoalkyl group can be substituted with a further substituent group at the alkyl and/or amino portions.

30 The terms "aralkyl" and "arylalkyl," as used herein, refer to an aromatic group that is covalently linked to a C_1-C_{12} alkyl radical. The alkyl radical portion of the resulting aralkyl (or arylalkyl) group forms a covalent bond with a parent molecule. Examples include without limitation, benzyl, phenethyl and the like. Aralkyl groups as used herein may optionally include

further substituent groups attached to the alkyl, the aryl or both groups that form the radical group.

The terms "aryl" and "aromatic," as used herein, refer to a mono- or polycyclic carbocyclic ring system radicals having one or more aromatic rings. Examples of aryl groups include without limitation, phenyl, naphthyl, tetrahydronaphthyl, indanyl, idenyl and the like. Preferred aryl ring systems have from about 5 to about 20 carbon atoms in one or more rings. Aryl groups as used herein may optionally include further substituent groups.

The terms "halo" and "halogen," as used herein, refer to an atom selected from fluorine, chlorine, bromine and iodine.

The terms "heteroaryl," and "heteroaromatic," as used herein, refer to a radical comprising a mono- or poly-cyclic aromatic ring, ring system or fused ring system wherein at least one of the rings is aromatic and includes one or more heteroatoms. Heteroaryl is also meant to include fused ring systems including systems where one or more of the fused rings contain no heteroatoms. Heteroaryl groups typically include one ring atom selected from sulfur, nitrogen or oxygen. Examples of heteroaryl groups include without limitation, pyridinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzooxazolyl, quinoxalinyl and the like. Heteroaryl radicals can be attached to a parent molecule directly or through a linking moiety such as an aliphatic group or hetero atom. Heteroaryl groups as used herein may optionally include further substituent groups.

The term "heteroarylalkyl," as used herein, refers to a heteroaryl group as previously defined that further includes a covalently attached C₁-C₁₂ alkyl radical. The alkyl radical portion of the resulting heteroarylalkyl group is capable of forming a covalent bond with a parent molecule. Examples include without limitation, pyridinylmethyl, pyrimidinylethyl, naphthyridinylpropyl and the like. Heteroarylalkyl groups as used herein may optionally include further substituent groups on one or both of the heteroaryl or alkyl portions.

The term "heterocyclic radical" as used herein, refers to a radical mono-, or poly-cyclic ring system that includes at least one heteroatom and is unsaturated, partially saturated or fully saturated, thereby including heteroaryl groups. Heterocyclic is also meant to include fused ring systems wherein one or more of the fused rings contain at least one heteroatom and the other rings can contain one or more heteroatoms or optionally contain no heteroatoms. A heterocyclic radical typically includes at least one atom selected from sulfur, nitrogen or oxygen. Examples of heterocyclic radicals include, [1,3]dioxolanyl, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl,

isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl and the like. Heterocyclic groups as used herein may optionally include further substituent groups.

The term "hydrocarbyl" includes radical groups that comprise C, O and H. Included are straight, branched and cyclic groups having any degree of saturation. Such hydrocarbyl groups can include one or more heteroatoms selected from N, O and S and can be further mono or poly substituted with one or more substituent groups.

The term "mono or poly cyclic structure" as used herein includes all ring systems selected from single or polycyclic radical ring systems wherein the rings are fused or linked and is meant to be inclusive of single and mixed ring systems individually selected from aliphatic, alicyclic, aryl, heteroaryl, aralkyl, arylalkyl, heterocyclic, heteroaryl, heteroaromatic and heteroarylalkyl. Such mono and poly cyclic structures can contain rings that each have the same level of saturation or each, independently, have varying degrees of saturation including fully saturated, partially saturated or fully unsaturated. Each ring can comprise ring atoms selected from C, N, O and S to give rise to heterocyclic rings as well as rings comprising only C ring atoms which can be present in a mixed motif such as for example benzimidazole wherein one ring has only carbon ring atoms and the fused ring has two nitrogen atoms. The mono or poly cyclic structures can be further substituted with substituent groups such as for example phthalimide which has two =O groups attached to one of the rings. Mono or poly cyclic structures can be attached to parent molecules using various strategies such as directly through a ring atom, through a substituent group or through a bifunctional linking moiety.

The term "oxo" refers to the group (=O).

Linking groups or bifunctional linking moieties such as those known in the art are useful for attachment of chemical functional groups, conjugate groups, reporter groups and other groups to selective sites in a parent compound such as for example an oligomeric compound. In general, a bifunctional linking moiety comprises a hydrocarbyl moiety having two functional groups. One of the functional groups is selected to bind to a parent molecule or compound of interest and the other is selected to bind to essentially any selected group such as a chemical functional group or a conjugate group. In some embodiments, the linker comprises a chain structure or a polymer of repeating units such as ethylene glycols or amino acid units. Examples of functional groups that are routinely used in bifunctional linking moieties include without limitation, electrophiles for reacting with nucleophilic groups and nucleophiles for reacting with electrophilic groups. In some embodiments, bifunctional linking moieties include amino, hydroxyl, carboxylic acid, thiol, unsaturations (e.g., double or triple bonds), and the like. Some nonlimiting examples of bifunctional

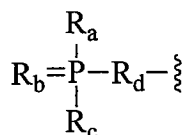
linking moieties include 8-amino-3,6-dioxaoctanoic acid (ADO), succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) and 6-aminohexanoic acid (AHEx or AHA). Other linking groups include without limitation, substituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl or substituted or unsubstituted C₂-C₁₀ alkynyl, wherein a nonlimiting list of preferred substituent groups includes hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl.

In certain embodiments, the oligomeric compounds as provided herein can be modified by covalent attachment of one or more conjugate groups. In general, conjugate groups modify one or more properties of the oligomeric compounds they are attached to. Such oligonucleotide properties include without limitation, pharmacodynamics, pharmacokinetics, binding, absorption, cellular distribution, cellular uptake, charge and clearance. Conjugate groups are routinely used in the chemical arts and are linked directly or via an optional linking moiety or linking group to a parent compound such as an oligomeric compound. A preferred list of conjugate groups includes without limitation, intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, thioethers, polyethers, cholesterol, thiocholesterol, cholic acid moieties, folate, lipids, phospholipids, biotin, phenazine, phenanthridine, anthraquinone, adamantane, acridine, fluoresceins, rhodamines, coumarins and dyes.

In certain embodiments, the oligomeric compounds as provided herein can be modified by covalent attachment of one or more 5' or 3'-terminal groups. The terms "5' or 3'-terminal groups", "5-terminal group" and "3'-terminal group" as used herein are meant to include useful groups known to the art skilled that can be placed on one or both of the 5' and 3'-ends of an oligomeric compound respectively, for various purposes such as enabling the tracking of the oligomeric compound (a fluorescent label or other reporter group), improving the pharmacokinetics or pharmacodynamics of the oligomeric compound (a group for enhancing uptake and/or delivery) or enhancing one or more other desirable properties of the oligomeric compound (a group for improving nuclease stability or binding affinity). In certain embodiments, 5' and 3'-terminal groups include without limitation, modified or unmodified nucleosides; two or more linked nucleosides that are independently, modified or unmodified; conjugate groups; capping groups; phosphate moieties; and protecting groups.

The term "phosphate moiety" as used herein, refers to a terminal phosphate group that includes phosphates as well as modified phosphates. The phosphate moiety can be located at either terminus but is preferred at the 5'-terminal nucleoside. In certain embodiments, the terminal phosphate is unmodified having the formula -O-P(=O)(OH)OH. In certain embodiments, the

terminal phosphate is modified such that one or more of the O and OH groups are replaced with H, O, S, N(R) or alkyl where R is H, an amino protecting group or unsubstituted or substituted alkyl. In certain embodiments, the terminal phosphate is modified to have the formula:



5 wherein:

R_a and R_c are each, independently, OH, SH, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₁-C₆ alkoxy, substituted C₁-C₆ alkoxy, amino or substituted amino; and

R_b and R_d are each, independently, O or S.

The term "protecting group," as used herein, refers to a labile chemical moiety which is known in the art to protect reactive groups including without limitation, hydroxyl, amino and thiol groups, against undesired reactions during synthetic procedures. Protecting groups are typically used selectively and/or orthogonally to protect sites during reactions at other reactive sites and can then be removed to leave the unprotected group as is or available for further reactions. Protecting groups as known in the art are described generally in Greene's Protective Groups in Organic
10 Synthesis, 4th edition, John Wiley & Sons, New York, 2007.

Groups can be selectively incorporated into oligomeric compounds as provided herein as precursors. For example an amino group can be placed into a compound as provided herein as an azido group that can be chemically converted to the amino group at a desired point in the synthesis. Generally, groups are protected or present as precursors that will be inert to reactions that modify
20 other areas of the parent molecule for conversion into their final groups at an appropriate time. Further representative protecting or precursor groups are discussed in Agrawal *et al.*, *Protocols for Oligonucleotide Conjugates*, Humana Press; New Jersey, 1994, 26, 1-72.

The term "orthogonally protected" refers to functional groups which are protected with different classes of protecting groups, wherein each class of protecting group can be removed in any
25 order and in the presence of all other classes (see, Barany *et al.*, *J. Am. Chem. Soc.*, 1977, 99, 7363-7365; Barany *et al.*, *J. Am. Chem. Soc.*, 1980, 102, 3084-3095). Orthogonal protection is widely used in for example automated oligonucleotide synthesis. A functional group is deblocked in the presence of one or more other protected functional groups which is not affected by the deblocking procedure. This deblocked functional group is reacted in some manner and at some point a further
30 orthogonal protecting group is removed under a different set of reaction conditions. This allows for selective chemistry to arrive at a desired compound or oligomeric compound.

Examples of hydroxyl protecting groups include without limitation, acetyl, t-butyl, t-butoxymethyl, methoxymethyl, tetrahydropyranyl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, p-chlorophenyl, 2,4-dinitrophenyl, benzyl, 2,6-dichlorobenzyl, diphenylmethyl, p-nitrobenzyl, bis(2-acetoxyethoxy)methyl (ACE), 2-trimethylsilylethyl, trimethylsilyl, triethylsilyl, t-butyl dimethylsilyl, t-butyl diphenylsilyl, triphenylsilyl, [(triisopropylsilyl)oxy]methyl (TOM), benzoylformate, chloroacetyl, trichloroacetyl, trifluoroacetyl, pivaloyl, benzoyl, p-phenylbenzoyl, 9-fluorenylmethyl carbonate, mesylate, tosylate, triphenylmethyl (trityl), monomethoxytrityl, dimethoxytrityl (DMT), trimethoxytrityl, 1(2-fluorophenyl)-4-methoxypiperidin-4-yl (FPMP), 9-phenylxanthine-9-yl (Pixyl) and 9-(p-methoxyphenyl)xanthine-9-yl (MOX). Wherein more commonly used hydroxyl protecting groups include without limitation, benzyl, 2,6-dichlorobenzyl, t-butyl dimethylsilyl, t-butyl diphenylsilyl, benzoyl, mesylate, tosylate, dimethoxytrityl (DMT), 9-phenylxanthine-9-yl (Pixyl) and 9-(p-methoxyphenyl)xanthine-9-yl (MOX).

Examples of amino protecting groups include without limitation, carbamate-protecting groups, such as 2-trimethylsilylethoxycarbonyl (Teoc), 1-methyl-1-(4-biphenyl)ethoxycarbonyl (Bpoc), t-butoxycarbonyl (BOC), allyloxycarbonyl (Alloc), 9-fluorenylmethyloxycarbonyl (Fmoc), and benzyloxycarbonyl (Cbz); amide-protecting groups, such as formyl, acetyl, trihaloacetyl, benzoyl, and nitrophenylacetyl; sulfonamide-protecting groups, such as 2-nitrobenzenesulfonyl; and imine- and cyclic imide-protecting groups, such as phthalimido and dithiasuccinoyl.

Examples of thiol protecting groups include without limitation, triphenylmethyl (trityl), benzyl (Bn), and the like.

In certain embodiments, oligomeric compounds as provided herein can be prepared having one or more optionally protected phosphorus containing internucleoside linkages. Representative protecting groups for phosphorus containing internucleoside linkages such as phosphodiester and phosphorothioate linkages include β -cyanoethyl, diphenylsilylethyl, δ -cyanobutenyl, cyano p-xylyl (CPX), N-methyl-N-trifluoroacetyl ethyl (META), acetoxy phenoxy ethyl (APE) and butene-4-yl groups. See for example U.S. Patents Nos. 4,725,677 and Re. 34,069 (β -cyanoethyl); Beaucage *et al.*, *Tetrahedron*, 1993, 49(10), 1925-1963; Beaucage *et al.*, *Tetrahedron*, 1993, 49(46), 10441-10488; Beaucage *et al.*, *Tetrahedron*, 1992, 48(12), 2223-2311.

In certain embodiments, compounds having reactive phosphorus groups are provided that are useful for forming internucleoside linkages including for example phosphodiester and phosphorothioate internucleoside linkages. Such reactive phosphorus groups are known in the art and contain phosphorus atoms in P^{III} or P^V valence state including, but not limited to, phosphoramidite, H-phosphonate, phosphate triesters and phosphorus containing chiral auxiliaries. In certain

embodiments, reactive phosphorus groups are selected from diisopropylcyanoethoxy phosphoramidite ($-\text{O}^*-\text{P}[\text{N}[(\text{CH}(\text{CH}_3)_2]_2]\text{O}(\text{CH}_2)_2\text{CN})$ and H-phosphonate ($-\text{O}^*-\text{P}(=\text{O})(\text{H})\text{OH}$), wherein the O^* is provided from the Markush group for the monomer. A preferred synthetic solid phase synthesis utilizes phosphoramidites (P^{III} chemistry) as reactive phosphites. The intermediate phosphite compounds are subsequently oxidized to the phosphate or thiophosphate (P^{V} chemistry) using known methods to yield, phosphodiester or phosphorothioate internucleoside linkages. Additional reactive phosphates and phosphites are disclosed in Tetrahedron Report Number 309 (Beaucage and Iyer, *Tetrahedron*, 1992, 48, 2223-2311).

As used herein the term "internucleoside linkage" or "internucleoside linking group" is meant to include all manner of internucleoside linking groups known in the art including but not limited to, phosphorus containing internucleoside linking groups such as phosphodiester and phosphorothioate, and non-phosphorus containing internucleoside linking groups such as formacetyl and methyleneimino. Internucleoside linkages also includes neutral non-ionic internucleoside linkages such as amide-3 ($3'-\text{CH}_2-\text{C}(=\text{O})-\text{N}(\text{H})-5'$), amide-4 ($3'-\text{CH}_2-\text{N}(\text{H})-\text{C}(=\text{O})-5'$) and methylphosphonate wherein a phosphorus atom is not always present.

In certain embodiments, oligomeric compounds as provided herein can be prepared having one or more internucleoside linkages containing modified e.g. non-naturally occurring internucleoside linkages. The two main classes of internucleoside linkages are defined by the presence or absence of a phosphorus atom. Modified internucleoside linkages having a phosphorus atom include without limitation, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage. Oligonucleotides having inverted polarity can comprise a single 3' to 3' linkage at the 3'-most internucleotide linkage i.e. a single inverted nucleoside residue which may be abasic (the nucleobase is missing or has a hydroxyl group in place thereof). Various salts, mixed salts and free acid forms are also included.

Representative U.S. patents that teach the preparation of the above phosphorus containing linkages include without limitation, U.S.: 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,194,599; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676;

5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,527,899; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,565,555; 5,571,799; 5,587,361; 5,625,050; 5,672,697 and 5,721,218, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

5 In certain embodiments, oligomeric compounds as provided herein can be prepared having one or more non-phosphorus containing internucleoside linkages. Such oligomeric compounds include without limitation, those that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having siloxane
10 backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

15 Representative U.S. patents that teach the preparation of the above oligonucleosides include without limitation, U.S.: 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; 5,677,439; 5,646,269 and 5,792,608, certain of which are commonly owned
20 with this application, and each of which is herein incorporated by reference.

As used herein the phrase "neutral internucleoside linkage" is intended to include internucleoside linkages that are non-ionic. Neutral internucleoside linkages include without limitation, phosphotriesters, methylphosphonates, MMI (3'-CH₂-N(CH₃)-O-5'), amide-3 (3'-CH₂-C(=O)-N(H)-5'), amide-4 (3'-CH₂-N(H)-C(=O)-5'), formacetal (3'-O-CH₂-O-5'), and thioformacetal
25 (3'-S-CH₂-O-5'). Further neutral internucleoside linkages include nonionic linkages comprising siloxane (dialkylsiloxane), carboxylate ester, carboxamide, sulfide, sulfonate ester and amides (See for example: *Carbohydrate Modifications in Antisense Research*; Y.S. Sanghvi and P.D. Cook, Eds., ACS Symposium Series 580; Chapters 3 and 4, 40-65). Further neutral internucleoside linkages include nonionic linkages comprising mixed N, O, S and CH₂ component parts.

30 The 2'-fluoroethoxy modified nucleosides of formula I provided herein can be prepared by any of the applicable techniques of organic synthesis, as, for example, illustrated in the examples below. Many such techniques are well known in the art. However, many of the known techniques are elaborated in *Compendium of Organic Synthetic Methods*, John Wiley & Sons, New York: Vol.

1, Ian T. Harrison and Shuyen Harrison, 1971; Vol. 2, Ian T. Harrison and Shuyen Harrison, 1974; Vol. 3, Louis S. Hegedus and Leroy Wade, 1977; Vol. 4, Leroy G. Wade Jr., 1980; Vol. 5, Leroy G. Wade Jr., 1984; and Vol. 6, Michael B. Smith; as well as March, J., *Advanced Organic Chemistry*, 3rd Edition, John Wiley & Sons, New York, 1985; *Comprehensive Organic Synthesis. Selectivity, Strategy & Efficiency in Modern Organic Chemistry*, in 9 Volumes, Barry M. Trost, Editor-in-Chief, Pergamon Press, New York, 1993; *Advanced Organic Chemistry, Part B: Reactions and Synthesis*, 4th Edition; Carey and Sundberg, Kluwer Academic/Plenum Publishers, New York, 2001; *Advanced Organic Chemistry, Reactions, Mechanisms, and Structure*, 2nd Edition, March, McGraw Hill, 1977; Greene, T.W., and Wutz, P.G.M., *Protecting Groups in Organic Synthesis*, 4th Edition, John Wiley & Sons, New York, 1991; and Larock, R.C., *Comprehensive Organic Transformations*, 2nd Edition, John Wiley & Sons, New York, 1999.

The compounds described herein contain one or more asymmetric centers and thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)-, α or β , or as (D)- or (L)- such as for amino acids. Included herein are all such possible isomers, as well as their racemic and optically pure forms. Optical isomers may be prepared from their respective optically active precursors by the procedures described above, or by resolving the racemic mixtures. The resolution can be carried out in the presence of a resolving agent, by chromatography or by repeated crystallization or by some combination of these techniques which are known to those skilled in the art. Further details regarding resolutions can be found in Jacques, *et al.*, *Enantiomers, Racemates, and Resolutions*, John Wiley & Sons, 1981. When the compounds described herein contain olefinic double bonds, other unsaturation, or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers or cis- and trans-isomers. Likewise, all tautomeric forms are also intended to be included. The configuration of any carbon-carbon double bond appearing herein is selected for convenience only and is not intended to limit a particular configuration unless the text so states.

As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base moiety. The two most common classes of such heterocyclic bases are purines and pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. The respective ends of this linear

polymeric structure can be joined to form a circular structure by hybridization or by formation of a covalent bond. However, open linear structures are generally desired. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide. The normal internucleoside linkage of RNA and DNA is a 3' to 5'

phosphodiester linkage.

The term "nucleotide mimetic" as used herein is meant to include monomers that incorporate into oligomeric compounds with sugar and linkage surrogate groups, such as for example peptide nucleic acids (PNA) or morpholinos (linked by -N(H)-C(=O)-O-). In general, the heterocyclic base at each position is maintained for hybridization to a nucleic acid target but the sugar and linkage is replaced with surrogate groups that are expected to function similar to native groups but have one or more enhanced properties.

As used herein the term "nucleoside mimetic" is intended to include those structures used to replace the sugar and the base at one or more positions of an oligomeric compound. Examples of nucleoside mimetics include without limitation replacement of the heterocyclic base moiety with a mimetic thereof such as a phenoxazine moiety (for example the 9-(2-aminoethoxy)-1,3-diazaphenoxazine-2-one group, also referred to as a G-clamp which forms four hydrogen bonds when hybridized with a guanosine base) and further replacement of the sugar group with a group such as for example a morpholino, a cyclohexenyl or a bicyclo[3.1.0]hexyl.

As used herein the term "modified nucleoside" is meant to include all manner of modified nucleosides that can be incorporated into an oligomeric compound using oligomer synthesis. The term is intended to include modifications made to a nucleoside such as modified stereochemical configurations, one or more substitutions, and deletion of groups as opposed to the use of surrogate groups which are described elsewhere herein. The term includes nucleosides having a furanose sugar (or 4'-S analog) portion and can include a heterocyclic base but abasic modified nucleosides are also envisioned. One group of representative modified nucleosides includes without limitation, substituted nucleosides (such as 2', 5', and/or 4' substituted nucleosides) 4'-S-modified nucleosides, (such as 4'-S-ribonucleosides, 4'-S-2'-deoxyribonucleosides and 4'-S-2'-substituted ribonucleosides), bicyclic modified nucleosides (such as for example, bicyclic nucleosides wherein the sugar group has a 2'-O-CHR_a-4' bridging group, wherein R_a is H, alkyl or substituted alkyl) and base modified nucleosides. The sugar can be modified with more than one of these modifications listed such as for example a bicyclic modified nucleoside further including a 5'-substitution or a 5' or 4' substituted nucleoside further including a 2' substituent. The term modified nucleoside also includes combinations of these modifications such as a base and sugar modified nucleosides. These

modifications are meant to be illustrative and not exhaustive as other modifications are known in the art and are also envisioned as possible modifications for the modified nucleosides described herein.

As used herein the term "monomer subunit" is meant to include all manner of monomer units that are amenable to oligomer synthesis with one preferred list including monomer subunits such as β -D-ribonucleosides, β -D-2'-deoxyribnucleosides, substituted nucleosides (such as 2', 5' and bis substituted nucleosides), 4'-S-modified nucleosides, (such as 4'-S-ribonucleosides, 4'-S-2'-deoxyribonucleosides and 4'-S-2'-substituted ribonucleosides), bicyclic modified nucleosides (such as bicyclic nucleosides wherein the sugar group has a 2'-O-CH R_a -4' bridging group, wherein R_a is H, alkyl or substituted alkyl), other modified nucleosides, nucleoside mimetics and nucleosides having sugar surrogates.

The term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside linkages. The term "oligonucleotide analog" refers to oligonucleotides that have one or more non-naturally occurring portions. Such non-naturally occurring oligonucleotides are often desired over naturally occurring forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and/or increased stability in the presence of nucleases.

The term "oligonucleoside" refers to a sequence of nucleosides that are joined by internucleoside linkages that do not have phosphorus atoms. Internucleoside linkages of this type include short chain alkyl, cycloalkyl, mixed heteroatom alkyl, mixed heteroatom cycloalkyl, one or more short chain heteroatomic and one or more short chain heterocyclic. These internucleoside linkages include without limitation, siloxane, sulfide, sulfoxide, sulfone, acetyl, formacetyl, thioformacetyl, methylene formacetyl, thioformacetyl, alkeneyl, sulfamate, methyleneimino, methylenehydrazino, sulfonate, sulfonamide, amide and others having mixed N, O, S and CH₂ component parts.

The terms "heterocyclic base moiety" and "nucleobase" as used herein, include unmodified or naturally occurring nucleobases, modified or non-naturally occurring nucleobases as well as synthetic mimetics thereof (such as for example phenoxazines). In general, a heterocyclic base moiety is heterocyclic system that contains one or more atoms or groups of atoms capable of hydrogen bonding to a base of a nucleic acid.

As used herein the terms, "unmodified nucleobase" and "naturally occurring nucleobase" include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases

such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl ($-C\equiv C-CH_3$) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine, 3-deazaguanine and 3-deazaadenine, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases as defined herein. Further modified nucleobases include tricyclic pyrimidines such as phenoxazine cytidine(1H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido[5,4-b][1,4]benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g. 9-(2-aminoethoxy)-H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5-b]indol-2-one), pyridoindole cytidine (H-pyrido[3',2':4,5]pyrrolo[2,3-d]pyrimidin-2-one). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in United States Patent No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, Kroschwitz, J.I., Ed., John Wiley & Sons, 1990, 858-859; those disclosed by Englisch *et al.*, *Angewandte Chemie*, International Edition, 1991, 30, 613; and those disclosed by Sanghvi, Y.S., Chapter 15, *Antisense Research and Applications*, Crooke, S.T. and Lebleu, B., Eds., CRC Press, 1993, 273-288.

The heterocyclic base moiety of each of the 2'-fluoroethoxy modified nucleosides of formula I can be modified with one or more substituent groups to enhance one or more properties such as affinity for a target strand or affect some other property in an advantageous manner. Modified nucleobases include without limitation, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases as defined herein. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds as provided herein. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2 °C (*Antisense Research and Applications*, Sanghvi, Y.S., Crooke, S.T. and Lebleu, B., Eds., CRC Press, Boca Raton, 1993, 276-278).

Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include without limitation, U.S.

3,687,808; 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187;

5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121; 5,596,091;

5 5,614,617; 5,645,985; 5,681,941; 5,750,692; 5,763,588; 5,830,653 and 6,005,096, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

In general, the term "oligomeric compound" refers to a contiguous sequence of linked monomer subunits. In general, each linked monomer subunit is directly or indirectly attached to a
10 heterocyclic base moiety but abasic sites are also possible. At least some and generally most if not essentially all of the heterocyclic bases in an oligomeric compound are capable of hybridizing to a nucleic acid molecule, normally a preselected RNA target. The term "oligomeric compound" therefore includes oligonucleotides, oligonucleotide analogs and oligonucleosides. It also includes polymers having a plurality of non-naturally occurring nucleoside mimetics and or nucleosides
15 having sugar surrogate groups. In certain embodiments, oligomeric compounds comprise a plurality of monomer subunits independently selected from naturally occurring nucleosides, non-naturally occurring nucleosides, modified nucleosides, nucleoside mimetics, and nucleosides having sugar surrogate groups.

When preparing oligomeric compounds having specific motifs as disclosed herein it can be
20 advantageous to mix non-naturally occurring monomer subunits such as the 2'-fluoroethoxy modified nucleosides of formula I as provided herein with other non-naturally occurring monomer subunits, naturally occurring monomer subunits (nucleosides) or mixtures thereof. In certain embodiments, oligomeric compounds are provided herein comprising a contiguous sequence of linked monomer subunits wherein at least one monomer subunit is a 2'-fluoroethoxy modified
25 nucleoside of formula I as provided herein. In certain embodiments, oligomeric compounds are provided comprising a plurality of 2'-fluoroethoxy modified nucleosides of formula I as provided herein.

Oligomeric compounds are routinely prepared linearly but can also be joined or otherwise prepared to be circular and/or can be prepared to include branching. Oligomeric compounds can
30 form double stranded constructs such as for example two strands hybridized to form a double stranded composition. Double stranded compositions can be linked or separate and can include various other groups such as conjugates and/or overhangs on the ends.

Oligomeric compounds provided herein can optionally contain one or more nucleosides

wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity or some other beneficial biological property to the oligomeric compounds. As used herein the term "modified sugar" refers to modifications that can be made to the furanose sugar portion of otherwise unmodified or modified nucleosides useful herein. Such modified sugars include without limitation substitution with one or more substituent groups, bridging of two non-geminal ring carbon atoms to form a bicyclic nucleoside or substitution of the 4'-O atom with a disubstituted methylene group $[C(R)_2]$ or a heteroatom or substituted heteroatom (NR). Modified sugar moieties can also comprise mixtures of these modifications such as for example putting a 5'-substituent group on a bicyclic nucleoside.

Examples of substituent groups useful for modifying sugar moieties of nucleosides include without limitation 2'-F, 2'-allyl, 2'-amino, 2'-azido, 2'-thio, 2'-O-allyl, 2'-OCF₃, 2'-O-C₁-C₁₀ alkyl, 2'-O-CH₃, OCF₃, 2'-O-CH₂CH₃, 2'-O-(CH₂)₂CH₃, 2'-O-(CH₂)₂-O-CH₃, 2'-O(CH₂)₂SCH₃, 2'-O-CH₂-CH=CH₂ (MOE), 2'-O-(CH₂)₃-N(R_m)(R_n), 2'-O-(CH₂)₂-O-N(R_m)(R_n), 2'-O-(CH₂)₂-O-(CH₂)₂-N(R_m)(R_n), 2'-O-CH₂C(=O)-N(R_m)(R_n), 2'-O-CH₂C(=O)-N(H)-(CH₂)₂-N(R_m)(R_n) and 2'-O-CH₂-N(H)-C(=NR_m)[N(R_m)(R_n)], 5'-vinyl, 5'-methyl (R or S) and 4'-S wherein each R_m and R_n is, independently, H, substituted or unsubstituted C₁-C₁₀ alkyl or a protecting group. Further examples of modified sugar moieties include without limitation bicyclic sugars (e.g. bicyclic nucleic acids or bicyclic nucleosides discussed below).

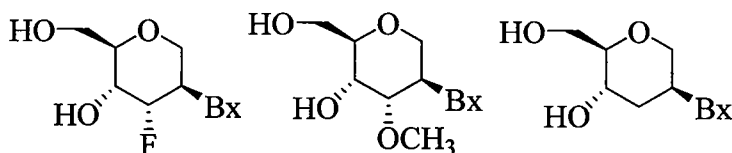
Combinations of these modifications are also provided for herein without limitation, such as 2'-F-5'-methyl substituted nucleosides (see PCT International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides) and replacement of the ribosyl ring oxygen atom with S and further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a bicyclic nucleic acid (see PCT International Application WO 2007/134181, published on 11/22/07 wherein a 4'-CH₂-O-2' bicyclic nucleoside is further substituted at the 5' position with a 5'-methyl or a 5'-vinyl group).

As used herein the terms "bicyclic nucleic acid" and "bicyclic nucleoside" refer to nucleosides wherein the sugar portion of the nucleoside is bicyclic (e.g. bicyclic sugar). In certain embodiments, a bicyclic nucleic acid comprises a nucleoside wherein the furanose ring comprises a bridge between two non-geminal ring carbon atoms. Examples of bicyclic nucleosides include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, oligomeric compounds provided herein include one or more bicyclic nucleosides wherein the bridge comprises one of the formulae: 4'-(CH₂)-O-2' (LNA); 4'-(CH₂)-S-2'; 4'-(CH₂)₂-O-

2' (ENA); 4'-CH(CH₃)-O-2' and 4'-CH(CH₂OCH₃)-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH₃)(CH₃)-O-2' (and analogs thereof see published International Application WO/2009/006478, published January 8, 2009); 4'-CH₂-N(OCH₃)-2' (and analogs thereof see published International Application WO/2008/150729, published December 11, 2008); 4'-CH₂-O-N(CH₃)-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH₂-N(R)-O-2', wherein R is H, C₁-C₁₂ alkyl, or a protecting group (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH₂-C(H)(CH₃)-2' (see Chattopadhyaya, *et al.*, *J. Org. Chem.*, **2009**, 74, 118-134); and 4'-CH₂-C(=CH₂)-2' (and analogs thereof see published International Application WO 2008/154401, published on December 8, 2008). Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

As used herein the term "sugar surrogate" refers to replacement of the nucleoside furanose ring with a non-furanose (or 4'-substituted furanose) group with another structure such as another ring system or open system. Such structures can be as simple as a six membered ring as opposed to the five membered furanose ring or can be more complicated as is the case with the non-ring system used in peptide nucleic acid. The term is meant to include replacement of the sugar group with all manner of sugar surrogates known in the art and includes without limitation sugar surrogate groups such as morpholinos, cyclohexenyls and cyclohexitols. In most monomer subunits having a sugar surrogate group the heterocyclic base moiety is generally maintained to permit hybridization.

In certain embodiments, nucleosides having sugar surrogate groups include without limitation, replacement of the ribosyl ring with a surrogate ring system such as a tetrahydropyran ring system (also referred to as hexitol) as illustrated below:



Many other monocyclic, bicyclic and tricyclic ring systems are known in the art and are suitable as sugar surrogates that can be used to modify nucleosides for incorporation into oligomeric compounds as provided herein (see for example review article: Leumann, Christian J.). Such ring systems can undergo various additional substitutions to further enhance their activity.

Some representative U.S. patents that teach the preparation of such modified sugars include without limitation, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137;

5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,670,633; 5,700,920; 5,792,847 and 6,600,032 and International Application PCT/US2005/019219, filed June 2, 2005 and published as WO 2005/121371 on December 22, 2005 certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

Those skilled in the art, having possession of the present disclosure will be able to prepare oligomeric compounds, comprising a contiguous sequence of linked monomer subunits, of essentially any viable length to practice the methods disclosed herein. Such oligomeric compounds will include at least one and preferably a plurality of the 2'-fluoroethoxy modified nucleosides of formula I provided herein and may also include other monomer subunits including but not limited to nucleosides, modified nucleosides, nucleosides comprising sugar surrogate groups and nucleoside mimetics.

In certain embodiments, oligomeric compounds provided herein comprise from about 8 to about 80 monomer subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 monomer subunits in length, or any range therewithin.

In certain embodiments, oligomeric compounds provided herein comprise from about 8 to 40 monomer subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 monomer subunits in length, or any range therewithin.

In certain embodiments, oligomeric compounds provided herein comprise from about 8 to 20 monomer subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 monomer subunits in length, or any range therewithin.

In certain embodiments, oligomeric compounds provided herein comprise from about 8 to 16 monomer subunits in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 8, 9, 10, 11, 12, 13, 14, 15 or 16 monomer subunits in length, or any range therewithin.

In certain embodiments, oligomeric compounds provided herein comprise from about 10 to 14 monomer subunits in length. One having ordinary skill in the art will appreciate that this

embodies oligomeric compounds of 10, 11, 12, 13 or 14 monomer subunits in length, or any range therewithin.

In certain embodiments, oligomeric compounds provided herein comprise from about 10 to 18 monomer subunits in length. One having ordinary skill in the art will appreciate that this
5 embodies oligomeric compounds of 10, 11, 12, 13, 14, 15, 16, 17 or 18 monomer subunits in length, or any range therewithin.

In certain embodiments, oligomeric compounds provided herein comprise from about 10 to 21 monomer subunits in length. One having ordinary skill in the art will appreciate that this
10 embodies oligomeric compounds of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 monomer subunits in length, or any range therewithin.

In certain embodiments, oligomeric compounds provided herein comprise from about 12 to 14 monomer subunits in length. One having ordinary skill in the art will appreciate that this
embodies oligomeric compounds of 12, 13 or 14 monomer subunits in length, or any range therewithin.

15 In certain embodiments, oligomeric compounds provided herein comprise from about 12 to 18 monomer subunits in length. One having ordinary skill in the art will appreciate that this
embodies oligomeric compounds of 12, 13, 14, 15, 16, 17 or 18 monomer subunits in length, or any range therewithin.

In certain embodiments, oligomeric compounds provided herein comprise from about 12 to
20 21 monomer subunits in length. One having ordinary skill in the art will appreciate that this
embodies oligomeric compounds of 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 monomer subunits in length, or any range therewithin.

In certain embodiments, oligomeric compounds provided herein comprise from about 14 to 18 monomer subunits in length. One having ordinary skill in the art will appreciate that this
25 embodies oligomeric compounds of 14, 15, 16, 17 or 18 monomer subunits in length, or any range therewithin.

In certain embodiments, oligomeric compounds of any of a variety of ranges of lengths of linked monomer subunits are provided. In certain embodiments, oligomeric compounds are provided consisting of X-Y linked monomer subunits, where X and Y are each independently
30 selected from 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50; provided that $X < Y$. For example, in certain embodiments, this provides oligomeric compounds comprising: 8-9, 8-10, 8-11, 8-12, 8-13, 8-14, 8-15, 8-16, 8-17, 8-18, 8-19, 8-20, 8-21, 8-22, 8-23, 8-24, 8-25, 8-26, 8-27,

8-28, 8-29, 8-30, 9-10, 9-11, 9-12, 9-13, 9-14, 9-15, 9-16, 9-17, 9-18, 9-19, 9-20, 9-21, 9-22, 9-23, 9-24, 9-25, 9-26, 9-27, 9-28, 9-29, 9-30, 10-11, 10-12, 10-13, 10-14, 10-15, 10-16, 10-17, 10-18, 10-19, 10-20, 10-21, 10-22, 10-23, 10-24, 10-25, 10-26, 10-27, 10-28, 10-29, 10-30, 11-12, 11-13, 11-14, 11-15, 11-16, 11-17, 11-18, 11-19, 11-20, 11-21, 11-22, 11-23, 11-24, 11-25, 11-26, 11-27, 11-28, 11-29, 11-30, 12-13, 12-14, 12-15, 12-16, 12-17, 12-18, 12-19, 12-20, 12-21, 12-22, 12-23, 12-24, 12-25, 12-26, 12-27, 12-28, 12-29, 12-30, 13-14, 13-15, 13-16, 13-17, 13-18, 13-19, 13-20, 13-21, 13-22, 13-23, 13-24, 13-25, 13-26, 13-27, 13-28, 13-29, 13-30, 14-15, 14-16, 14-17, 14-18, 14-19, 14-20, 14-21, 14-22, 14-23, 14-24, 14-25, 14-26, 14-27, 14-28, 14-29, 14-30, 15-16, 15-17, 15-18, 15-19, 15-20, 15-21, 15-22, 15-23, 15-24, 15-25, 15-26, 15-27, 15-28, 15-29, 15-30, 16-17, 16-18, 16-19, 16-20, 16-21, 16-22, 16-23, 16-24, 16-25, 16-26, 16-27, 16-28, 16-29, 16-30, 17-18, 17-19, 17-20, 17-21, 17-22, 17-23, 17-24, 17-25, 17-26, 17-27, 17-28, 17-29, 17-30, 18-19, 18-20, 18-21, 18-22, 18-23, 18-24, 18-25, 18-26, 18-27, 18-28, 18-29, 18-30, 19-20, 19-21, 19-22, 19-23, 19-24, 19-25, 19-26, 19-27, 19-28, 19-29, 19-30, 20-21, 20-22, 20-23, 20-24, 20-25, 20-26, 20-27, 20-28, 20-29, 20-30, 21-22, 21-23, 21-24, 21-25, 21-26, 21-27, 21-28, 21-29, 21-30, 22-23, 22-24, 22-25, 22-26, 22-27, 22-28, 22-29, 22-30, 23-24, 23-25, 23-26, 23-27, 23-28, 23-29, 23-30, 24-25, 24-26, 24-27, 24-28, 24-29, 24-30, 25-26, 25-27, 25-28, 25-29, 25-30, 26-27, 26-28, 26-29, 26-30, 27-28, 27-29, 27-30, 28-29, 28-30, or 29-30 linked monomer subunits.

In certain embodiments, the ranges for the oligomeric compounds listed herein are meant to limit the number of monomer subunits in the oligomeric compounds, however such oligomeric compounds may further include 5' and/or 3'-terminal groups including but not limited to protecting groups such as hydroxyl protecting groups, optionally linked conjugate groups and/or other substituent groups.

In certain embodiments, the preparation of oligomeric compounds as disclosed herein is performed according to literature procedures for DNA: Protocols for Oligonucleotides and Analogs, Agrawal, Ed., Humana Press, 1993, and/or RNA: Scaringe, *Methods*, 2001, 23, 206-217; Gait *et al.*, *Applications of Chemically synthesized RNA in RNA:Protein Interactions*, Smith, Ed., 1998, 1-36; Gallo *et al.*, *Tetrahedron*, 2001, 57, 5707-5713. Additional methods for solid-phase synthesis may be found in Caruthers U.S. Patents Nos. 4,415,732; 4,458,066; 4,500,707; 4,668,777; 4,973,679; and 5,132,418; and Koster U.S. Patents Nos. 4,725,677 and Re. 34,069.

Oligomeric compounds are routinely prepared using solid support methods as opposed to solution phase methods. Commercially available equipment commonly used for the preparation of oligomeric compounds that utilize the solid support method is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the

art may additionally or alternatively be employed. Suitable solid phase techniques, including automated synthesis techniques, are described in *Oligonucleotides and Analogues, a Practical Approach*, F. Eckstein, Ed., Oxford University Press, New York, 1991.

The synthesis of RNA and related analogs relative to the synthesis of DNA and related
5 analogs has been increasing as efforts in RNA interference and micro RNA increase. The primary RNA synthesis strategies that are presently being used commercially include 5'-O-DMT-2'-O-t-butyltrimethylsilyl (TBDMS), 5'-O-DMT-2'-O-[1(2-fluorophenyl)-4-methoxypiperidin-4-yl] (FPMP), 2'-O-[(triisopropylsilyl)oxy]methyl (2'-O-CH₂-O-Si(iPr)₃ (TOM) and the 5'-O-silyl ether-2'-ACE (5'-O-bis(trimethylsiloxy)cyclododecyloxysilyl ether (DOD)-2'-O-bis(2-acetoxyethoxy)-
10 methyl (ACE). A current list of some of the major companies currently offering RNA products include Pierce Nucleic Acid Technologies, Dharmacon Research Inc., Ameri Biotechnologies Inc., and Integrated DNA Technologies, Inc. One company, Princeton Separations, is marketing an RNA synthesis activator advertised to reduce coupling times especially with TOM and TBDMS chemistries. The primary groups being used for commercial RNA synthesis are: TBDMS: 5'-O-
15 DMT-2'-O-t-butyltrimethylsilyl; TOM: 2'-O-[(triisopropylsilyl)oxy]methyl; DOD/ACE: (5'-O-bis(trimethylsiloxy)cyclododecyloxysilyl ether-2'-O-bis(2-acetoxyethoxy)methyl; and FPMP: 5'-O-DMT-2'-O-[1(2-fluorophenyl)-4-ethoxypiperidin-4-yl]. In certain embodiments, each of the aforementioned RNA synthesis strategies can be used herein. In certain embodiments, the aforementioned RNA synthesis strategies can be performed together in a hybrid fashion e.g. using a
20 5'-protecting group from one strategy with a 2'-O-protecting from another strategy.

As used herein the term "hybridization" includes the pairing of complementary strands of oligomeric compounds such as including the binding of an oligomeric compound as provided herein to a target nucleic acid. In certain embodiments, the mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding,
25 between complementary heterocyclic base moieties of nucleosides (or monomer subunits) that are in close enough proximity to hydrogen bond. For example, adenine and thymine are complementary nucleobases which pair through the formation of hydrogen bonds. Hybridization can occur under varying circumstances.

An oligomeric compound is specifically hybridizable when binding of the compound to the
30 target nucleic acid interferes with the normal function of the target nucleic acid resulting in a loss of activity. To be specifically hybridizable also requires a sufficient degree of complementarity to avoid non-specific binding of the oligomeric compound to non-target nucleic acid sequences under the conditions in which specific binding is desired, i.e., under physiological conditions (for *in vivo*

assays or therapeutic treatment) or other diagnostic conditions (for performing *in vitro* assays).

As used herein the term "complementary," refers to the capacity for precise pairing of two nucleobases regardless of where the two nucleobases are located. For example, if a nucleobase at a certain position of an oligomeric compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, the target nucleic acid being a DNA, RNA, or oligonucleotide molecule, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be a complementary position. The oligomeric compound and the further DNA, RNA, or oligonucleotide molecule are complementary to each other when a sufficient number of complementary positions in each molecule are occupied by nucleobases which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of precise pairing or complementarity over a sufficient number of nucleobases such that stable and specific binding occurs between an oligomeric compound and its target nucleic acid.

It is understood in the art that the sequence of an oligomeric compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, an oligomeric compound may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure or hairpin structure). In certain embodiments, oligomeric compounds can comprise at least about 70%, at least about 80%, at least about 90%, at least about 95%, or at least about 99% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, an oligomeric compound in which 18 of 20 nucleobases of the oligomeric compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an oligomeric compound which is 18 nucleobases in length having 4 (four) noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within this scope. Percent complementarity of an oligomeric compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul *et al.*, *J. Mol. Biol.*, 1990, 215, 403-410; Zhang and Madden, *Genome Res.*, 1997, 7, 649-656).

Further included herein are oligomeric compounds such as antisense oligomeric compounds, antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid. As such, these oligomeric compounds may be introduced in the form of single-stranded, double-stranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges or loops. Once introduced to a system, the oligomeric compounds provided herein may elicit the action of one or more enzymes or structural proteins to effect modification of the target nucleic acid.

One non-limiting example of such an enzyme is RNase H, a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. It is known in the art that single-stranded oligomeric compounds which are "DNA-like" elicit RNase H. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide-mediated inhibition of gene expression. Similar roles have been postulated for other ribonucleases such as those in the RNase III and ribonuclease L family of enzymes.

While one form of oligomeric compound is a single-stranded antisense oligonucleotide, in many species the introduction of double-stranded structures, such as double-stranded RNA (dsRNA) molecules, has been shown to induce potent and specific antisense-mediated reduction of the function of a gene or its associated gene products. This phenomenon occurs in both plants and animals and is believed to have an evolutionary connection to viral defense and transposon silencing.

In some embodiments, "suitable target segments" may be employed in a screen for additional oligomeric compounds that modulate the expression of a selected protein. "Modulators" are those oligomeric compounds that decrease or increase the expression of a nucleic acid molecule encoding a protein and which comprise at least an 8-nucleobase portion which is complementary to a suitable target segment. The screening method comprises the steps of contacting a suitable target segment of a nucleic acid molecule encoding a protein with one or more candidate modulators, and selecting for one or more candidate modulators which decrease or increase the expression of a nucleic acid molecule encoding a protein. Once it is shown that the candidate modulator or modulators are capable of modulating (e.g. either decreasing or increasing) the expression of a nucleic acid molecule encoding a peptide, the modulator may then be employed herein in further investigative studies of the function of the peptide, or for use as a research, diagnostic, or therapeutic agent.

Suitable target segments may also be combined with their respective complementary oligomeric compounds provided herein to form stabilized double-stranded (duplexed)

oligonucleotides. Such double stranded oligonucleotide moieties have been shown in the art to modulate target expression and regulate translation as well as RNA processing via an antisense mechanism. Moreover, the double-stranded moieties may be subject to chemical modifications (Fire *et al.*, *Nature*, 1998, 391, 806-811; Timmons and Fire, *Nature*, 1998, 395, 854; Timmons *et al.*,
5 *Gene*, 2001, 263, 103-112; Tabara *et al.*, *Science*, 1998, 282, 430-431; Montgomery *et al.*, *Proc. Natl. Acad. Sci. USA*, 1998, 95, 15502-15507; Tuschl *et al.*, *Genes Dev.*, 1999, 13, 3191-3197; Elbashir *et al.*, *Nature*, 2001, 411, 494-498; Elbashir *et al.*, *Genes Dev.*, 2001, 15, 188-200). For example, such double-stranded moieties have been shown to inhibit the target by the classical hybridization of antisense strand of the duplex to the target, thereby triggering enzymatic
10 degradation of the target (Tijsterman *et al.*, *Science*, 2002, 295, 694-697).

The oligomeric compounds provided herein can also be applied in the areas of drug discovery and target validation. In certain embodiments, provided herein is the use of the oligomeric compounds and targets identified herein in drug discovery efforts to elucidate relationships that exist between proteins and a disease state, phenotype, or condition. These
15 methods include detecting or modulating a target peptide comprising contacting a sample, tissue, cell, or organism with one or more oligomeric compounds provided herein, measuring the nucleic acid or protein level of the target and/or a related phenotypic or chemical endpoint at some time after treatment, and optionally comparing the measured value to a non-treated sample or sample treated with a further oligomeric compound as provided herein. These methods can also be
20 performed in parallel or in combination with other experiments to determine the function of unknown genes for the process of target validation or to determine the validity of a particular gene product as a target for treatment or prevention of a particular disease, condition, or phenotype. In certain embodiments, oligomeric compounds are provided for use in therapy. In certain embodiments, the therapy is reducing target messenger RNA.

As used herein, the term "dose" refers to a specified quantity of a pharmaceutical agent provided in a single administration. In certain embodiments, a dose may be administered in two or more boluses, tablets, or injections. For example, in certain embodiments, where subcutaneous administration is desired, the desired dose requires a volume not easily accommodated by a single injection. In such embodiments, two or more injections may be used to achieve the desired dose. In
25 certain embodiments, a dose may be administered in two or more injections to minimize injection site reaction in an individual.
30

In certain embodiments, chemically-modified oligomeric compounds are provided herein that may have a higher affinity for target RNAs than does non-modified DNA. In certain such

embodiments, higher affinity in turn provides increased potency allowing for the administration of lower doses of such compounds, reduced potential for toxicity, improvement in therapeutic index and decreased overall cost of therapy.

Effect of nucleoside modifications on RNAi activity is evaluated according to existing literature (Elbashir *et al.*, *Nature*, 2001, 411, 494-498; Nishikura *et al.*, *Cell*, 2001, 107, 415-416; and Bass *et al.*, *Cell*, 2000, 101, 235-238.)

In certain embodiments, oligomeric compounds provided herein can be utilized for diagnostics, therapeutics, prophylaxis and as research reagents and kits. Furthermore, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes or to distinguish between functions of various members of a biological pathway. In certain embodiments, oligomeric compounds provided herein can be utilized either alone or in combination with other oligomeric compounds or other therapeutics as tools in differential and/or combinatorial analyses to elucidate expression patterns of a portion or the entire complement of genes expressed within cells and tissues. Oligomeric compounds can also be effectively used as primers and probes under conditions favoring gene amplification or detection, respectively. These primers and probes are useful in methods requiring the specific detection of nucleic acid molecules encoding proteins and in the amplification of the nucleic acid molecules for detection or for use in further studies. Hybridization of oligomeric compounds as provided herein, particularly the primers and probes, with a nucleic acid can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabelling of the oligonucleotide or any other suitable detection means. Kits using such detection means for detecting the level of selected proteins in a sample may also be prepared.

As one nonlimiting example, expression patterns within cells or tissues treated with one or more of the oligomeric compounds provided herein are compared to control cells or tissues not treated with oligomeric compounds and the patterns produced are analyzed for differential levels of gene expression as they pertain, for example, to disease association, signaling pathway, cellular localization, expression level, size, structure or function of the genes examined. These analyses can be performed on stimulated or unstimulated cells and in the presence or absence of other compounds and or oligomeric compounds which affect expression patterns.

Examples of methods of gene expression analysis known in the art include DNA arrays or microarrays (Brazma and Vilo, *FEBS Lett.*, 2000, 480, 17-24; Celis, *et al.*, *FEBS Lett.*, 2000, 480, 2-16), SAGE (serial analysis of gene expression)(Madden, *et al.*, *Drug Discov. Today*, 2000, 5, 415-

425), READS (restriction enzyme amplification of digested cDNAs) (Prashar and Weissman, *Methods Enzymol.*, 1999, 303, 258-72), TOGA (total gene expression analysis) (Sutcliffe, *et al.*, *Proc. Natl. Acad. Sci. USA*, 2000, 97, 1976-81), protein arrays and proteomics (Celis, *et al.*, *FEBS Lett.*, 2000, 480, 2-16; Jungblut, *et al.*, *Electrophoresis*, 1999, 20, 2100-10), expressed sequence tag (EST) sequencing (Celis, *et al.*, *FEBS Lett.*, 2000, 480, 2-16; Larsson, *et al.*, *J. Biotechnol.*, 2000, 80, 143-57), subtractive RNA fingerprinting (SuRF) (Fuchs, *et al.*, *Anal. Biochem.*, 2000, 286, 91-98; Larson, *et al.*, *Cytometry*, 2000, 41, 203-208), subtractive cloning, differential display (DD) (Jurecic and Belmont, *Curr. Opin. Microbiol.*, 2000, 3, 316-21), comparative genomic hybridization (Carulli, *et al.*, *J. Cell Biochem. Suppl.*, 1998, 31, 286-96), FISH (fluorescent in situ hybridization) techniques (Going and Gusterson, *Eur. J. Cancer*, 1999, 35, 1895-904) and mass spectrometry methods (To, *Comb. Chem. High Throughput Screen*, 2000, 3, 235-41).

While in certain embodiments, oligomeric compounds provided herein can be utilized as described, the following examples serve only to illustrate and are not intended to be limiting.

15 **Examples (General)**

¹H and ¹³C NMR spectra were recorded on a 300 MHz and 75 MHz Bruker spectrometer, respectively.

Example 1

20 **Synthesis of Nucleoside Phosphoramidites**

The preparation of nucleoside phosphoramidites is performed following procedures that are illustrated herein and in the art such as but not limited to US Patent 6,426,220 and published PCT WO 02/36743.

25 **Example 2**

Synthesis of Oligomeric Compounds

The oligomeric compounds used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides such as alkylated derivatives and those having phosphorothioate linkages.

Oligomeric compounds: Unsubstituted and substituted phosphodiester (P=O) oligomeric compounds, including without limitation, oligonucleotides can be synthesized on an automated DNA synthesizer (Applied Biosystems model 394) using standard phosphoramidite chemistry with oxidation by iodine.

5 In certain embodiments, phosphorothioate internucleoside linkages (P=S) are synthesized similar to phosphodiester internucleoside linkages with the following exceptions: thiation is effected by utilizing a 10% w/v solution of 3-H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the oxidation of the phosphite linkages. The thiation reaction step time is increased to 180 sec and preceded by the normal capping step. After cleavage from the CPG column and deblocking in
10 concentrated ammonium hydroxide at 55°C (12-16 hr), the oligomeric compounds are recovered by precipitating with greater than 3 volumes of ethanol from a 1 M NH₄OAc solution. Phosphinate internucleoside linkages can be prepared as described in U.S. Patent 5,508,270.

Alkyl phosphonate internucleoside linkages can be prepared as described in U.S. Patent 4,469,863.

15 3'-Deoxy-3'-methylene phosphonate internucleoside linkages can be prepared as described in U.S. Patents 5,610,289 or 5,625,050.

Phosphoramidite internucleoside linkages can be prepared as described in U.S. Patent, 5,256,775 or U.S. Patent 5,366,878.

Alkylphosphonothioate internucleoside linkages can be prepared as described in published
20 PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499, respectively).

3'-Deoxy-3'-amino phosphoramidate internucleoside linkages can be prepared as described in U.S. Patent 5,476,925.

Phosphotriester internucleoside linkages can be prepared as described in U.S. Patent
25 5,023,243.

Borano phosphate internucleoside linkages can be prepared as described in U.S. Patents 5,130,302 and 5,177,198.

Oligomeric compounds having one or more non-phosphorus containing internucleoside linkages including without limitation methylenemethylimino linked oligonucleosides, also identified
30 as MMI linked oligonucleosides, methylenedimethylhydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, methylenecarbonylamino linked oligonucleosides, also identified as amide-3 linked oligonucleosides, and methyleneaminocarbonyl linked oligonucleosides, also identified as amide-4 linked oligonucleosides, as well as mixed backbone oligomeric compounds

having, for instance, alternating MMI and P=O or P=S linkages can be prepared as described in U.S. Patents 5,378,825, 5,386,023, 5,489,677, 5,602,240 and 5,610,289.

Formacetal and thioformacetal internucleoside linkages can be prepared as described in U.S. Patents 5,264,562 and 5,264,564.

5 Ethylene oxide internucleoside linkages can be prepared as described in U.S. Patent 5,223,618.

Example 3

Isolation and Purification of Oligomeric Compounds

10 After cleavage from the controlled pore glass solid support or other support medium and deblocking in concentrated ammonium hydroxide at 55°C for 12-16 hours, the oligomeric compounds, including without limitation oligonucleotides and oligonucleosides, are recovered by precipitation out of 1 M NH₄OAc with >3 volumes of ethanol. Synthesized oligomeric compounds are analyzed by electrospray mass spectroscopy (molecular weight determination) and by capillary
15 gel electrophoresis. The relative amounts of phosphorothioate and phosphodiester linkages obtained in the synthesis is determined by the ratio of correct molecular weight relative to the -16 amu product (+/-32 +/-48). For some studies oligomeric compounds are purified by HPLC, as described by Chiang et al., J. Biol. Chem. 1991, 266, 18162-18171. Results obtained with HPLC-purified material are generally similar to those obtained with non-HPLC purified material.

20

Example 4

Synthesis of Oligomeric Compounds using the 96 Well Plate Format

Oligomeric compounds, including without limitation oligonucleotides, can be synthesized via solid phase P(III) phosphoramidite chemistry on an automated synthesizer capable of assembling
25 96 sequences simultaneously in a 96-well format. Phosphodiester internucleoside linkages are afforded by oxidation with aqueous iodine. Phosphorothioate internucleoside linkages are generated by sulfurization utilizing 3,4-dihydro-2H-benzothiole-3-one 1,1-dioxide (Beaucage Reagent) in anhydrous acetonitrile. Standard base-protected beta-cyanoethyl-diisopropyl phosphoramidites can be purchased from commercial vendors (e.g. PE-Applied Biosystems, Foster City, CA, or Pharmacia,
30 Piscataway, NJ). Non-standard nucleosides are synthesized as per standard or patented methods and can be functionalized as base protected beta-cyanoethyldiisopropyl phosphoramidites.

Oligomeric compounds can be cleaved from support and deprotected with concentrated NH₄OH at elevated temperature (55-60 °C) for 12-16 hours and the released product then dried *in*

vacuo. The dried product is then re-suspended in sterile water to afford a master plate from which all analytical and test plate samples are then diluted utilizing robotic pipettors.

Example 5

5 Analysis of Oligomeric Compounds using the 96-Well Plate Format

The concentration of oligomeric compounds in each well can be assessed by dilution of samples and UV absorption spectroscopy. The full-length integrity of the individual products can be evaluated by capillary electrophoresis (CE) in either the 96-well format (Beckman P/ACE™ MDQ) or, for individually prepared samples, on a commercial CE apparatus (e.g., Beckman P/ACE™ 5000, 10 ABI 270). Base and backbone composition is confirmed by mass analysis of the oligomeric compounds utilizing electrospray-mass spectroscopy. All assay test plates are diluted from the master plate using single and multi-channel robotic pipettors. Plates are judged to be acceptable if at least 85% of the oligomeric compounds on the plate are at least 85% full length.

15 Example 6

In Vitro Treatment of Cells with Oligomeric Compounds

The effect of oligomeric compounds on target nucleic acid expression is tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. This can be routinely determined using, for example, PCR or Northern blot analysis. Cell lines derived from 20 multiple tissues and species can be obtained from American Type Culture Collection (ATCC, Manassas, VA).

The following cell type is provided for illustrative purposes, but other cell types can be routinely used, provided that the target is expressed in the cell type chosen. This can be readily determined by methods routine in the art, for example Northern blot analysis, ribonuclease 25 protection assays or RT-PCR.

b.END cells: The mouse brain endothelial cell line b.END was obtained from Dr. Werner Risau at the Max Plank Institute (Bad Nauheim, Germany). b.END cells are routinely cultured in DMEM, high glucose (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA). Cells are routinely passaged by 30 trypsinization and dilution when they reached approximately 90% confluence. Cells are seeded into 96-well plates (Falcon-Primaria #353872, BD Biosciences, Bedford, MA) at a density of approxi-

mately 3000 cells/well for uses including but not limited to oligomeric compound transfection experiments.

Experiments involving treatment of cells with oligomeric compounds:

When cells reach appropriate confluency, they are treated with oligomeric compounds using a transfection method as described.

LIPOFECTIN™

When cells reached 65-75% confluency, they are treated with one or more oligomeric compounds. The oligomeric compound is mixed with LIPOFECTIN™ Invitrogen Life Technologies, Carlsbad, CA) in Opti-MEM™-1 reduced serum medium (Invitrogen Life Technologies, Carlsbad, CA) to achieve the desired concentration of the oligomeric compound(s) and a LIPOFECTIN™ concentration of 2.5 or 3 µg/mL per 100 nM oligomeric compound(s). This transfection mixture is incubated at room temperature for approximately 0.5 hours. For cells grown in 96-well plates, wells are washed once with 100 µL OPTI-MEM™-1 and then treated with 130 µL of the transfection mixture. Cells grown in 24-well plates or other standard tissue culture plates are treated similarly, using appropriate volumes of medium and oligomeric compound(s). Cells are treated and data are obtained in duplicate or triplicate. After approximately 4-7 hours of treatment at 37°C, the medium containing the transfection mixture is replaced with fresh culture medium. Cells are harvested 16-24 hours after treatment with oligomeric compound(s).

Other suitable transfection reagents known in the art include, but are not limited to, CYTOFECTIN™, LIPOFECTAMINE™, OLIGOFECTAMINE™, and FUGENE™. Other suitable transfection methods known in the art include, but are not limited to, electroporation.

Example 7

Real-time Quantitative PCR Analysis of target mRNA Levels

Quantitation of target mRNA levels is accomplished by real-time quantitative PCR using the ABI PRISM™ 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions. This is a closed-tube, non-gel-based, fluorescence detection system which allows high-throughput quantitation of polymerase chain reaction (PCR) products in real-time. As opposed to standard PCR in which amplification products are quantitated after the PCR is completed, products in real-time quantitative PCR are quantitated as they accumulate. This is accomplished by including in the PCR reaction an oligonucleotide probe that anneals specifically between the forward and reverse PCR primers, and contains two fluorescent

dyes. A reporter dye (e.g., FAM or JOE, obtained from either PE-Applied Biosystems, Foster City, CA, Operon Technologies Inc., Alameda, CA or Integrated DNA Technologies Inc., Coralville, IA) is attached to the 5' end of the probe and a quencher dye (e.g., TAMRA, obtained from either PE-Applied Biosystems, Foster City, CA, Operon Technologies Inc., Alameda, CA or Integrated DNA Technologies Inc., Coralville, IA) is attached to the 3' end of the probe. When the probe and dyes are intact, reporter dye emission is quenched by the proximity of the 3' quencher dye. During amplification, annealing of the probe to the target sequence creates a substrate that can be cleaved by the 5'-exonuclease activity of Taq polymerase. During the extension phase of the PCR amplification cycle, cleavage of the probe by Taq polymerase releases the reporter dye from the remainder of the probe (and hence from the quencher moiety) and a sequence-specific fluorescent signal is generated. With each cycle, additional reporter dye molecules are cleaved from their respective probes, and the fluorescence intensity is monitored at regular intervals by laser optics built into the ABI PRISM™ Sequence Detection System. In each assay, a series of parallel reactions containing serial dilutions of mRNA from untreated control samples generates a standard curve that is used to quantitate the percent inhibition after antisense oligonucleotide treatment of test samples.

Prior to quantitative PCR analysis, primer-probe sets specific to the target gene being measured are evaluated for their ability to be "multiplexed" with a GAPDH amplification reaction. In multiplexing, both the target gene and the internal standard gene GAPDH are amplified concurrently in a single sample. In this analysis, mRNA isolated from untreated cells is serially diluted. Each dilution is amplified in the presence of primer-probe sets specific for GAPDH only, target gene only ("single-plexing"), or both (multiplexing). Following PCR amplification, standard curves of GAPDH and target mRNA signal as a function of dilution are generated from both the single-plexed and multiplexed samples. If both the slope and correlation coefficient of the GAPDH and target signals generated from the multiplexed samples fall within 10% of their corresponding values generated from the single-plexed samples, the primer-probe set specific for that target is deemed multiplexable. Other methods of PCR are also known in the art.

RT and PCR reagents are obtained from Invitrogen Life Technologies (Carlsbad, CA). RT, real-time PCR is carried out by adding 20 µL PCR cocktail (2.5x PCR buffer minus MgCl₂, 6.6 mM MgCl₂, 375 µM each of dATP, dCTP, dGTP and dTTP, 375 nM each of forward primer and reverse primer, 125 nM of probe, 4 Units RNase inhibitor, 1.25 Units PLATINUM® Taq, 5 Units MuLV reverse transcriptase, and 2.5x ROX dye) to 96-well plates containing 30 µL total RNA solution (20-

200 ng). The RT reaction is carried out by incubation for 30 minutes at 48°C. Following a 10 minute incubation at 95°C to activate the PLATINUM® Taq, 40 cycles of a two-step PCR protocol are carried out: 95°C for 15 seconds (denaturation) followed by 60°C for 1.5 minutes (annealing/extension).

5 Gene target quantities obtained by RT, real-time PCR are normalized using either the expression level of GAPDH, a gene whose expression is constant, or by quantifying total RNA using RIBOGREEN™ (Molecular Probes, Inc. Eugene, OR). GAPDH expression is quantified by real time RT-PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RiboGreen™ RNA quantification reagent (Molecular Probes, Inc. Eugene,
10 OR). Methods of RNA quantification by RIBOGREEN™ are taught in Jones, L.J., et al, (Analytical Biochemistry, 1998, 265, 368-374).

In this assay, 170 µL of RIBOGREEN™ working reagent (RIBOGREEN™ reagent diluted 1:350 in 10mM Tris-HCl, 1 mM EDTA, pH 7.5) is pipetted into a 96-well plate containing 30 µL purified, cellular RNA. The plate is read in a CytoFluor 4000 (PE Applied Biosystems) with
15 excitation at 485nm and emission at 530nm.

Example 8

Analysis of inhibition of target expression

Antisense modulation of a target expression can be assayed in a variety of ways known in
20 the art. For example, a target mRNA levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or real-time PCR. Real-time quantitative PCR is presently desired. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. One method of RNA analysis of the present disclosure is the use of total cellular RNA as described in other examples herein. Methods of RNA isolation are well known in the art. Northern blot analysis
25 is also routine in the art. Real-time quantitative (PCR) can be conveniently accomplished using the commercially available ABI PRISM™ 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

Protein levels of a target can be quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent
30 assay (ELISA) or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional monoclonal or polyclonal

antibody generation methods well known in the art. Methods for preparation of polyclonal antisera are taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.12.1-11.12.9, John Wiley & Sons, Inc., 1997. Preparation of monoclonal antibodies is taught in, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp.

5 11.4.1-11.11.5, John Wiley & Sons, Inc., 1997.

Immunoprecipitation methods are standard in the art and can be found at, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 10.16.1-10.16.11, John Wiley & Sons, Inc., 1998. Western blot (immunoblot) analysis is standard in the art and can be found at, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp.

10 10.8.1-10.8.21, John Wiley & Sons, Inc., 1997. Enzyme-linked immunosorbent assays (ELISA) are standard in the art and can be found at, for example, Ausubel, F.M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.2.1-11.2.22, John Wiley & Sons, Inc., 1991.

Example 9

15 **Design of phenotypic assays and *in vivo* studies for the use of target inhibitors**

Phenotypic assays

Once target inhibitors have been identified by the methods disclosed herein, the oligomeric compounds are further investigated in one or more phenotypic assays, each having measurable endpoints predictive of efficacy in the treatment of a particular disease state or condition.

20 Phenotypic assays, kits and reagents for their use are well known to those skilled in the art and are herein used to investigate the role and/or association of a target in health and disease. Representative phenotypic assays, which can be purchased from any one of several commercial vendors, include those for determining cell viability, cytotoxicity, proliferation or cell survival (Molecular Probes, Eugene, OR; PerkinElmer, Boston, MA), protein-based assays including

25 enzymatic assays (Panvera, LLC, Madison, WI; BD Biosciences, Franklin Lakes, NJ; Oncogene Research Products, San Diego, CA), cell regulation, signal transduction, inflammation, oxidative processes and apoptosis (Assay Designs Inc., Ann Arbor, MI), triglyceride accumulation (Sigma-Aldrich, St. Louis, MO), angiogenesis assays, tube formation assays, cytokine and hormone assays and metabolic assays (Chemicon International Inc., Temecula, CA; Amersham Biosciences,

30 Piscataway, NJ).

In one non-limiting example, cells determined to be appropriate for a particular phenotypic assay (i.e., MCF-7 cells selected for breast cancer studies; adipocytes for obesity studies) are treated with a target inhibitors identified from the *in vitro* studies as well as control compounds at optimal

concentrations which are determined by the methods described above. At the end of the treatment period, treated and untreated cells are analyzed by one or more methods specific for the assay to determine phenotypic outcomes and endpoints.

Phenotypic endpoints include changes in cell morphology over time or treatment dose as well as changes in levels of cellular components such as proteins, lipids, nucleic acids, hormones, saccharides or metals. Measurements of cellular status which include pH, stage of the cell cycle, intake or excretion of biological indicators by the cell, are also endpoints of interest.

Measurement of the expression of one or more of the genes of the cell after treatment is also used as an indicator of the efficacy or potency of the target inhibitors. Hallmark genes, or those genes suspected to be associated with a specific disease state, condition, or phenotype, are measured in both treated and untreated cells.

In vivo studies

The individual subjects of the *in vivo* studies described herein are warm-blooded vertebrate animals, which includes humans.

Example 10

RNA Isolation

Poly(A)+ mRNA isolation

Poly(A)+ mRNA is isolated according to Miura et al., (Clin. Chem., 1996, 42, 1758-1764). Other methods for poly(A)+ mRNA isolation are routine in the art. Briefly, for cells grown on 96-well plates, growth medium is removed from the cells and each well is washed with 200 μ L cold PBS. 60 μ L lysis buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA, 0.5 M NaCl, 0.5% NP-40, 20 mM vanadyl-ribonucleoside complex) is added to each well, the plate is gently agitated and then incubated at room temperature for five minutes. 55 μ L of lysate is transferred to Oligo d(T) coated 96-well plates (AGCT Inc., Irvine CA). Plates are incubated for 60 minutes at room temperature, washed 3 times with 200 μ L of wash buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.3 M NaCl). After the final wash, the plate is blotted on paper towels to remove excess wash buffer and then air-dried for 5 minutes. 60 μ L of elution buffer (5 mM Tris-HCl pH 7.6), preheated to 70°C, is added to each well, the plate is incubated on a 90°C hot plate for 5 minutes, and the eluate is then transferred to a fresh 96-well plate.

Cells grown on 100 mm or other standard plates may be treated similarly, using appropriate volumes of all solutions.

Total RNA Isolation

Total RNA is isolated using an RNEASY 96™ kit and buffers purchased from Qiagen Inc. (Valencia, CA) following the manufacturer's recommended procedures. Briefly, for cells grown on 96-well plates, growth medium is removed from the cells and each well is washed with 200 µL cold

5 PBS. 150 µL Buffer RLT is added to each well and the plate vigorously agitated for 20 seconds. 150 µL of 70% ethanol is then added to each well and the contents mixed by pipetting three times up and down. The samples are then transferred to the RNEASY 96™ well plate attached to a QIAVAC™ manifold fitted with a waste collection tray and attached to a vacuum source. Vacuum is applied for 1 minute. 500 µL of Buffer RW1 is added to each well of the RNEASY 96™ plate

10 and incubated for 15 minutes and the vacuum is again applied for 1 minute. An additional 500 µL of Buffer RW1 is added to each well of the RNEASY 96™ plate and the vacuum is applied for 2 minutes. 1 mL of Buffer RPE is then added to each well of the RNEASY 96™ plate and the vacuum applied for a period of 90 seconds. The Buffer RPE wash is then repeated and the vacuum is applied for an additional 3 minutes. The plate is then removed from the QIAVAC™ manifold and

15 blotted dry on paper towels. The plate is then re-attached to the QIAVAC™ manifold fitted with a collection tube rack containing 1.2 mL collection tubes. RNA is then eluted by pipetting 140 µL of RNase free water into each well, incubating 1 minute, and then applying the vacuum for 3 minutes.

The repetitive pipetting and elution steps may be automated using a QIAGEN Bio-Robot 9604 (Qiagen, Inc., Valencia CA). Essentially, after lysing of the cells on the culture plate, the plate

20 is transferred to the robot deck where the pipetting, DNase treatment and elution steps are carried out.

Example 11**Target-specific primers and probes**

25 Probes and primers may be designed to hybridize to a target sequence, using published sequence information.

For example, for human PTEN, the following primer-probe set was designed using published sequence information (GENBANK™ accession number U92436.1, SEQ ID NO: 1).

Forward primer: AATGGCTAAGTGAAGATGACAATCAT (SEQ ID NO: 2)

30 Reverse primer: TGCACATATCATTACACCAGTTCGT (SEQ ID NO: 3)

And the PCR probe:

FAM-TTGCAGCAATTCACTGTAAAGCTGGAAAGG-TAMRA (SEQ ID NO: 4), where

FAM is the fluorescent dye and TAMRA is the quencher dye.

Example 12

Western blot analysis of target protein levels

5 Western blot analysis (immunoblot analysis) is carried out using standard methods. Cells are harvested 16-20 h after oligonucleotide treatment, washed once with PBS, suspended in Laemmli buffer (100 μ l/well), boiled for 5 minutes and loaded on a 16% SDS-PAGE gel. Gels are run for 1.5 hours at 150 V, and transferred to membrane for western blotting. Appropriate primary antibody directed to a target is used, with a radiolabeled or fluorescently labeled secondary antibody directed
10 against the primary antibody species. Bands are visualized using a PHOSPHORIMAGERTM (Molecular Dynamics, Sunnyvale CA).

Example 13

PTEN single administration in vivo study with 2-10-2 and 2-14-2 gapmers

15 Six week old Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected once with the gapped oligomeric compounds targeted to PTEN at a dose of 3.2, 10, 32 or 100 mg/kg. The mice were sacrificed 72 hours following last administration. Liver tissues were homogenized and mRNA levels were quantitated using real-time PCR as described herein for comparison to untreated control levels (% UTC). Estimated ED₅₀ concentrations for each oligomeric compound were calculated
20 using Graphpad Prism and are shown below. Tms were determined in 100 mM phosphate buffer, 0.1 mM EDTA, pH 7, at 260 nm using 4 μ M of the modified oligomers listed below and 4 μ M of complementary RNA.

SEQ ID NO.	Composition (5' to 3')	Motif	Tm	ED ₅₀ (mg/kg)
25 /ISIS NO.				
05/397770	^{me} C _e T _e TAGCACTGGC ^{me} C _e T _e	(2/10/2)	51.3	61
05/413058	^{me} C _x T _x TAGCACTGGC ^{me} C _x T _x	(2/10/2)	51.0	19
06/394420	^{me} C _e T _e GCTAGCCTCTGGATT _e T _e	(2/14/2)	55.6	28
06/413059	^{me} C _x T _x GCTAGCCTCTGGATT _x T _x	(2/14/2)	55.9	24
30 07/116847	^{me} C _e T _e G _e ^{me} C _e T _e AG ^{me} C ^{me} CT ^{me} C- TGGAT _e T _e T _e G _e A _e	(5/10/5)	67.9	32

Each internucleoside linking group is a phosphorothioate, nucleosides not followed by a subscript are β -D-2'-deoxyribonucleosides, superscript Me indicates that the following C is a 5-

methyl C, subscript e indicates that the preceding nucleoside is a 2'-O(CH₂)₂-O-CH₃ (MOE) substituted nucleoside and subscript x indicates that the preceding nucleoside is a 2'-OCH₂CH₂F (FEt) substituted nucleoside.

5	SEQ ID NO.	% UTC at dosage			
	/ISIS NO.	3.2 mg/kg	10 mg/kg	32 mg/kg	100 mg/kg Motif
	05/397770	86.6	92.0	71.0	38.2 (2/10/2 MOE)
	05/413058	88.4	76.1	37.8	6.6 (2/10/2 FEt)
	06/394420	1.03	83.1	51.0	10.8 (2/14/2 MOE)
10	06/413059	93.2	83.9	40.7	16.3 (2/14/2 FEt)
	07/116847	98.3	85.0	52.3	21.4 (5/10/5 MOE)
	Saline	% UTC = 100			

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were also measured relative to saline injected mice. The approximate liver transaminase levels are listed in the table below.

20	SEQ ID NO.	ALT (IU/L) at dosage			
	/ISIS NO.	3.2 mg/kg	10 mg/kg	32 mg/kg	100 mg/kg Motif
	05/397770	27.6	56.8	33.0	22.1 (2/10/2 MOE)
	05/413058	22.9	23.5	24.7	21.0 (2/10/2 FEt)
	06/394420	43.8	24.9	29.0	34.4 (2/14/2 MOE)
	06/413059	20.7	28.5	30.1	39.2 (2/14/2 FEt)
	07/116847	28.7	24.3	30.4	34.0 (5/10/5 MOE)

25	SEQ ID NO.	AST (IU/L) at dosage			
	/ISIS NO.	3.2 mg/kg	10 mg/kg	32 mg/kg	100 mg/kg Motif
	05/397770	57.7	248.1	108.9	60.4 (2/10/2 MOE)
	05/413058	62.0	94.7	92.8	39.0 (2/10/2 FEt)
	06/394420	158.4	143.2	167.8	96.9 (2/14/2 MOE)
30	06/413059	40.7	118.2	132.4	129.6 (2/14/2 FEt)
	07/116847	61.3	63.1	130.8	98.4 (5/10/5 MOE).

Example 14**PTEN multiple administration in vivo study with 2-10-2 gapmers**

Six week old Balb/c mice (Jackson Laboratory, Bar Harbor, ME) were injected twice per week for three weeks with gapped oligomeric compounds targeted to PTEN at a dose of 0.5, 1.5, 5.0 or 15 mg/kg. The mice were sacrificed 48 hours following last administration. Liver tissues were homogenized and mRNA levels were quantitated using real-time PCR as described herein for comparison to untreated control levels (%UTC). Plasma chemistry analysis was completed. Tms were determined in 100 mM phosphate buffer, 0.1 mM EDTA, pH 7, at 260 nm using 4 μ M of the modified oligomers listed below and 4 μ M of the complementary RNA.

SEQ ID NO.	Composition (5' to 3')	Tm	ED ₅₀ (mg/kg)
/ISIS NO.			
05/413058	^{me} C _x T _x TAGCACTGGC ^{me} C _x T _x	51.0	13.0

Each internucleoside linking group is a phosphorothioate, nucleosides not followed by a subscript are β -D-2'-deoxyribonucleosides, superscript Me indicates that the following C is a 5-methyl C and subscript x indicates that the preceding nucleoside is a 2'-OCH₂CH₂F (Fet) substituted nucleoside.

SEQ ID NO.	% UTC at dosage			
/ISIS NO.	0.5 mg/kg	1.5 mg/kg	5.0 mg/kg	15 mg/kg
05/413058	93.0	76.7	78.4	43.2
Saline	% UTC = 100			

Liver transaminase levels, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), in serum were also measured relative to saline injected mice. The approximate liver transaminase levels are listed in the table below.

SEQ ID NO.	ALT (IU/L) at dosage			
/ISIS NO.	0.5 mg/kg	1.5 mg/kg	5.0 mg/kg	15 mg/kg
05/413058	20.0	26.2	18.25	18.0

SEQ ID NO.	AST (IU/L) at dosage			
/ISIS NO.	0.5 mg/kg	1.5 mg/kg	5.0 mg/kg	15 mg/kg
05/413058	47.7	74.5	52.0	44.0.

Example 15**Oligomeric compounds targeted to PTEN: *in vitro* study**

In accordance with the present disclosure, oligomeric compounds were synthesized and tested for their ability to reduce PTEN expression over a range of doses. Human HeLa cells were treated with either ISIS 404320, 418030, 418031, or ISIS 418032 at concentrations of 1.56, 3.13, 6.25, 12.5, 25 and 50 nM. Expression levels of PTEN were determined using real-time PCR and normalized to RIBOGREEN™ using methods described herein. The percent inhibition of PTEN mRNA was determined and the resulting dose-response curves were used to determine the EC₅₀ as listed below.

SEQ ID NO. Composition (5' to 3')		EC ₅₀ (nM)
/ISIS NO.		
08/404320	U _f U _f G _f U _f C _f U _f C _f U _f G _f G _f U _f C _f C _f U _f U _f A _f C _f U _f U _f A _e A _e	6.4
15 08/418030	<u>U_e</u> U _f G _f U _f C _f U _f C _f U _f G _f G _f U _f C _f C _f U _f U _f A _f C _f U _f U _f A _e A _e	8.5
09/418031	T _e U _f G _f U _f C _f U _f C _f U _f G _f G _f U _f C _f C _f U _f U _f A _f C _f U _f U _f A _e A _e	3.9
09/418032	T _x U _f G _f U _f C _f U _f C _f U _f G _f G _f U _f C _f C _f U _f U _f A _f C _f U _f U _f A _e A _e	2.9

Underlined nucleosides are connected to the following nucleoside by a phosphorothioate internucleoside linkage and all other internucleoside linkages are phosphodiester (going 5' to 3'). Nucleosides followed by a subscript e indicates the preceding nucleoside is a 2'-O(CH₂)₂-O-CH₃ (MOE) substituted nucleoside, subscript f indicates the preceding nucleoside is a 2'-fluoro substituted nucleoside, and subscript x indicates the preceding nucleoside is a 2'-OCH₂CH₂F (Fet) substituted nucleoside.

		SEQ ID NO./ISIS NO. %UTC			
25	Dose (nM)	08/404320	08/418030	09/418031	09/418032
	1.56	72.8	83.6	66.0	65.2
	3.13	63.3	63.0	55.9	47.5
	6.25	46.2	52.6	44.3	31.6
	12.5	37.4	38.9	31.3	24.4
30	25	29.3	33.6	25.5	21.0
	50	26.1	27.7	28.6	20.3
	Saline	%UTC = 100.			

Example 16**Derepression of ALDOA mRNA in anti-miR-122 treated mouse hepatocytes**

Primary hepatocytes were isolated from Balb/c mice (see for example: Neufeld, D.S. *Methods Mol. Biol.* 1997, 75, 145-151). Cells were seeded into 96-well plates in culture medium (Williams' Medium E supplemented with 10% fetal bovine serum, 10nM HEPES, and penicillin/streptomycin) and cultured overnight before transfection. Transfection of oligonucleotide in Cytofectin (Invitrogen) was performed in triplicate, according to the manufacturer's instructions. Cells were lysed 24 hrs after transfection, and total RNA was harvested using Qiagen RNeasy 96 columns on a Bio Robot 3000 (Qiagen). Total RNA was used for real-time quantitative RT-PCR analysis with a Prism 7700 Sequence Detector (Applied Biosystems). mRNA levels were normalized to total RNA for each sample, as measured by Ribogreen. An increase in mRNA level over untreated control is indicative of activity in this derepression assay. All reagents were from Invitrogen.

SEQ ID NO. Composition (5' to 3')**/ISIS NO.**

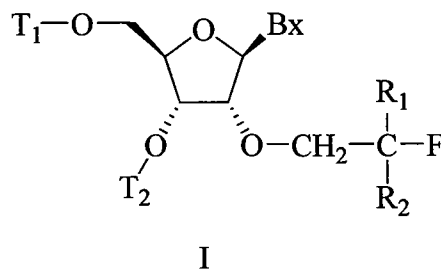
10/327895 A_e^{me}C_eA_eA_eA_e^{me}C_eA_e^{me}C_e^{me}C_eA_eT_eT_eG_eT_e^{me}C_eA_e^{me}C_eA_e^{me}C_eT_e^{me}C_e^{me}C_eA_e
 10/410146 A_e^{me}C_xA_eA_eA_e^{me}C_xA_e^{me}C_x^{me}C_xA_eT_xT_xG_eT_x^{me}C_xA_e^{me}C_xA_e^{me}C_xT_x^{me}C_x^{me}C_xA_e
 11/407588 A_e^{me}C_eA_eA_e^{me}C_e^{me}C_eA_e^{me}C_eT_eA_eT_e^{me}C_eG_e^{me}C_e^{me}C_eA_eT_eA_eA_eT_e^{me}C_e^{me}C_eA_e

Each internucleoside linking group is a phosphorothioate. Nucleosides followed by a superscript Me indicates the following C is a 5-methyl C, subscript e indicates the preceding nucleoside is a 2'-O(CH₂)₂-O-CH₃ (MOE) substituted nucleoside and subscript x indicates the preceding nucleoside is a 2'-OCH₂CH₂F (Fet) substituted nucleoside.

		SEQ ID NO./ISIS NO. %UTC		
Dose (nM)		10/327895	010/410146	11/407588
12.5		105.4	102.2	130.4
25		112.7	115.8	129.5
50		145.3	157.5	132.8
100		137.6	181.0	116.4
200		151.4	214.2	147.9
400		103.0	185.5	119.6
Saline	%UTC = 100			

What is Claimed is:

1. A method comprising contacting a cell with an oligomeric compound, wherein said oligomeric compound comprises at least one 2'-fluoroethoxy modified nucleoside having formula I:



wherein independently for each of said 2'-fluoroethoxy modified nucleosides having formula I:

Bx is a heterocyclic base moiety;

one of T₁ and T₂ is an internucleoside linking group attaching the 2'-fluoroethoxy modified nucleoside of formula I to the oligomeric compound and the other of T₁ and T₂ is H, a hydroxyl protecting group, a 5' or 3'-terminal group or an internucleoside linking group attaching the 2'-fluoroethoxy modified nucleoside of formula I to the oligomeric compound;

R₁ and R₂ are each independently H or F; and

wherein said oligomeric compound comprises from about 8 to about 40 linked monomeric subunits and is complementary to at least a portion of a target RNA.

2. The method of claim 1 wherein R₁ and R₂ are each H for each of said 2'-fluoroethoxy modified nucleosides of formula I.
3. The method of claim 1 wherein one of R₁ and R₂ is F for each of said 2'-fluoroethoxy modified nucleosides of formula I.
4. The method of claim 1 wherein R₁ and R₂ are each F for each of said 2'-fluoroethoxy modified nucleosides of formula I.
5. The method of any one of claims 1 to 4 wherein each Bx is, independently, uracil, 5-methyluracil, thymine, cytosine, 5-methylcytosine, adenine or guanine or a protected uracil, 5-methyluracil, thymine, cytosine, 5-methylcytosine, adenine or guanine.
6. The method of any one of claims 1 to 5 wherein said oligomeric compound further

comprises a 5' or 3'-terminal group.

7. The method of any one of claims 1 to 6 wherein each linked monomeric subunit that is not a β -D-2'-deoxyribonucleoside or a 2'-fluoroethoxy modified nucleoside of formula I is a modified nucleoside.

8. The method of claim 7 wherein each modified nucleoside is, independently, a bicyclic modified nucleoside, a 2'-modified nucleoside, a 4'-thio modified nucleoside or a 4'-thio-2'-modified nucleoside.

9. The method of any one of claims 1 to 8 wherein said oligomeric compound comprises a blockmer, a 3'-hemimer or a 5'-hemimer.

10. The method of claim 9 wherein each monomeric subunit is, independently, a β -D-2'-deoxyribonucleoside or a 2'-fluoroethoxy modified nucleoside of formula I.

11. The method of any one of claims 1 to 8 wherein said oligomeric compound comprises a gapped oligomeric compound comprising two external regions separated by an internal region wherein each external region independently comprises from 1 to 5 contiguous 2'-fluoroethoxy modified nucleosides having formula I and the internal region comprises from 6 to about 23 contiguous monomeric subunits independently selected from nucleosides and modified nucleosides.

12. The method of claim 11 wherein each monomeric subunit in the internal region is, independently, a β -D-2'-deoxyribonucleoside or a modified nucleoside.

13. The method of claim 12 wherein essentially each monomeric subunit in the internal region is a β -D-2'-deoxyribonucleoside.

14. The method of claim 11 wherein the internal region comprises from about 8 to about 12 β -D-2'-deoxyribonucleosides.

15. The method of claim 14 wherein each external region comprises from 1 to 3 2'-fluoroethoxy

modified nucleosides having formula I.

16. The method of claim 15 wherein the internal region comprises from about 10 to about 12 β -D-2'-deoxyribonucleosides.

17. The method of claim 11 wherein the internal region comprises from 11 to about 18 β -D-2'-deoxyribonucleosides.

18. The method of claim 17 wherein each external region comprises from 1 to 3 2'-fluoroethoxy modified nucleoside having formula I.

19. The method of claim 18 wherein the internal region comprises from 12 to about 14 β -D-2'-deoxyribonucleosides.

20. The method of claim 11 wherein each external region independently comprises from 1 to 3 2'-fluoroethoxy modified nucleosides having formula I.

21. The method of claim 20 wherein each external region comprises 2 2'-fluoroethoxy modified nucleosides having formula I.

22. The method of claim 21 wherein the internal region comprises 10 β -D-2'-deoxyribonucleosides.

23. The method of any one of claims 1 to 22 wherein said oligomeric compound comprises a 5'-terminal 2'-fluoroethoxy modified nucleoside having formula I wherein T_1 is a phosphate group.

24. The method of any one of claims 1 to 23 wherein each internucleoside linkage is a phosphodiester.

25. The method of any one of claims 1 to 23 wherein each internucleoside linkage is a phosphorothioate.

26. The method of any one of claims 1 to 23 wherein each internucleoside linking group is,

independently, a phosphodiester or a phosphorothioate.

27. The method of any one of claims 1 to 23 wherein each internucleoside linking group is, independently, a phosphodiester, phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl phosphonate, alkyl phosphonate, 5'-alkylene phosphonate, chiral phosphonate, phosphinate, phosphoramidate, 3'-amino phosphoramidate, aminoalkylphosphoramidate, thionophosphoramidate, thionoalkylphosphonate, thionoalkylphosphotriester, selenophosphate or a boranophosphate.

28. The method of any one of claims 1 to 13 and 20 wherein said oligomeric compound comprises from 8 to about 18 linked monomeric subunits.

29. The method of any one of claims 1 to 15, 20 and 21 wherein said oligomeric compound comprises from 10 to about 16 linked monomeric subunits.

30. The method of any one of claims 1 to 15, 20 and 21 wherein said oligomeric compound comprises from 10 to about 14 linked monomeric subunits.

31. The method of any one of claims 1 to 13, 17, 20 and 21 wherein said oligomeric compound comprises from 17 to about 26 linked monomeric subunits.

32. The method of any one of claims 1 to 14, 17, 18, 20 and 21 wherein said oligomeric compound comprises from 18 to about 21 linked monomeric subunits.

33. The method of any one of claims 1 to 14 and 17 to 21 wherein said oligomeric compound comprises from 19 to about 20 linked monomeric subunits.

34. The method of any one of claims 1 to 33 wherein said cell is in an animal.

35. The method of any one of claims 1 to 33 wherein said cell is in a human.

36. The method of any one of claims 1 to 35 wherein said target RNA is selected from mRNA,

pre-mRNA and micro RNA.

37. The method of claim 36 wherein said target RNA is mRNA.

38. The method of claim 36 wherein said target RNA is human mRNA.

39. The method of any one of claims 1 to 38 wherein said target RNA is cleaved thereby inhibiting its function.

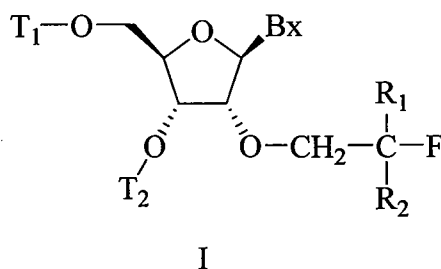
40. The method of any one of claims 1 to 39 further comprising evaluating the antisense activity of said oligomeric compound on said cell.

41. The method of claim 40 wherein said evaluating comprises detecting the levels of target RNA.

42. The method of claim 40 wherein said evaluating comprises detecting the levels of a protein.

43. The method of claim 40 wherein said evaluating comprises detection of one or more phenotypic effects.

44. A method comprising administering an oligomeric compound to an animal, wherein said oligomeric compound comprises at least one 2'-fluoroethoxy modified nucleoside having formula I:



wherein independently for each of said 2'-fluoroethoxy modified nucleosides having formula I:

Bx is a heterocyclic base moiety;

one of T₁ and T₂ is an internucleoside linking group attaching the 2'-fluoroethoxy modified nucleoside of formula I to the oligomeric compound and the other of T₁ and T₂ is H, a hydroxyl protecting group, a 5' or 3'-terminal group or an internucleoside linking group attaching the 2'-

fluoroethoxy modified nucleoside of formula I to the oligomeric compound;

R_1 and R_2 are each independently H or F; and

wherein said oligomeric compound comprises from about 8 to about 40 linked monomeric subunits and is complementary to at least a portion of a target RNA.

45. The method of claim 44 wherein R_1 and R_2 are each H for each of said 2'-fluoroethoxy modified nucleosides of formula I.

46. The method of claim 44 wherein one of R_1 and R_2 is F for each of said 2'-fluoroethoxy modified nucleosides of formula I.

47. The method of claim 44 wherein R_1 and R_2 are each F for each of said 2'-fluoroethoxy modified nucleosides of formula I.

48. The method of any one of claims 44 to 47 wherein each B_x is, independently, uracil, 5-methyluracil, thymine, cytosine, 5-methylcytosine, adenine or guanine.

49. The method of any one of claims 44 to 48 wherein said oligomeric compound further comprises a 5' or 3'-terminal group.

50. The method of any one of claims 44 to 49 wherein each linked monomeric subunit that is not a β -D-2'-deoxyribonucleoside or a 2'-fluoroethoxy modified nucleoside of formula I is a modified nucleoside.

51. The method of claim 50 wherein each modified nucleoside is, independently, a bicyclic modified nucleoside, a 2'-modified nucleoside, a 4'-thio modified nucleoside or a 4'-thio-2'-modified nucleoside.

52. The method of any one of claims 44 to 51 wherein said oligomeric compound comprises a blockmer, a 3'-hemimer or a 5'-hemimer.

53. The method of claim 52 wherein each monomeric subunit is, independently, a β -D-2'-

deoxyribonucleoside or a 2'-fluoroethoxy modified nucleoside of formula I.

54. The method of any one of claims 44 to 51 wherein said oligomeric compound comprises a gapped oligomeric compound comprising two external regions separated by an internal region wherein each external region independently comprises from 1 to 5 contiguous 2'-fluoroethoxy modified nucleosides having formula I and the internal region comprises from 6 to about 23 contiguous monomeric subunits independently selected from nucleosides and modified nucleosides.

55. The method of claim 54 wherein each monomeric subunit in the internal region is, independently, a β -D-2'-deoxyribonucleoside or a modified nucleoside.

56. The method of claim 55 wherein essentially each monomeric subunit in the internal region is a β -D-2'-deoxyribonucleoside.

57. The method of claim 54 wherein the internal region comprises from about 8 to about 12 β -D-2'-deoxyribonucleosides.

58. The method of claim 57 wherein each external region comprises from 1 to 3 2'-fluoroethoxy modified nucleoside having formula I.

59. The method of claim 58 wherein the internal region comprises from about 10 to about 12 β -D-2'-deoxyribonucleosides.

60. The method of claim 54 wherein the internal region comprises from 11 to about 18 β -D-2'-deoxyribonucleosides.

61. The method of claim 60 wherein each external region comprises from 1 to 3 2'-fluoroethoxy modified nucleoside having formula I.

62. The method of claim 61 wherein the internal region comprises from 12 to about 14 β -D-2'-deoxyribonucleosides.

63. The method of claim 54 wherein each external region independently comprises from 1 to 3

2'-fluoroethoxy modified nucleosides having formula I.

64. The method of claim 63 wherein each external region comprises 2 2'-fluoroethoxy modified nucleosides having formula I.

65. The method of claim 64 wherein the internal region comprises 10 β -D-2'-deoxyribonucleosides.

66. The method of any one of claims 44 to 65 wherein said oligomeric compound comprises a 5'-terminal 2'-fluoroethoxy modified nucleoside having formula I wherein T_1 is a phosphate group.

67. The method of any one of claims 44 to 66 wherein each internucleoside linkage is a phosphodiester.

68. The method of any one of claims 44 to 66 wherein each internucleoside linkage is a phosphorothioate.

69. The method of any one of claims 44 to 66 wherein each internucleoside linking group is, independently, a phosphodiester or a phosphorothioate.

70. The method of any one of claims 44 to 66 wherein each internucleoside linking group is, independently, a phosphodiester, phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl phosphonate, alkyl phosphonate, 5'-alkylene phosphonate, chiral phosphonate, phosphinate, phosphoramidate, 3'-amino phosphoramidate, aminoalkylphosphoramidate, thionophosphoramidate, thionoalkylphosphonate, thionoalkylphosphotriester, selenophosphate or a boranophosphate.

71. The method of any one of claims 44 to 56 and 63 wherein said oligomeric compound comprises from 8 to about 18 linked monomeric subunits.

72. The method of any one of claims 44 to 58, 63 and 64 wherein said oligomeric compound comprises from 10 to about 16 linked monomeric subunits.

73. The method of any one of claims 44 to 58, 63 and 64 wherein said oligomeric compound comprises from 10 to about 14 linked monomeric subunits.
74. The method of any one of claims 44 to 56, 60, 63 and 64 wherein said oligomeric compound comprises from 17 to about 26 linked monomeric subunits.
75. The method of any one of claims 44 to 57, 60, 61, 63 and 64 wherein said oligomeric compound comprises from 18 to about 21 linked monomeric subunits.
76. The method of any one of claims 44 to 57 and 60 to 64 wherein said oligomeric compound comprises from 19 to about 20 linked monomeric subunits.
77. The method of any one of claims 44 to 76 wherein said animal is a mammal.
78. The method of any one of claims 44 to 76 wherein said animal is a human.
79. The method of any one of claims 44 to 78 wherein said target RNA is selected from mRNA, pre-mRNA and micro RNA.
80. The method of claim 79 wherein said target RNA is mRNA.
81. The method of claim 79 wherein said target RNA is human mRNA.
82. The method of any one of claims 44 to 81 wherein said target RNA is cleaved thereby inhibiting its function.
83. The method of any one of claims 44 to 82 further comprising evaluating the antisense activity of said oligomeric compound on said animal.
84. The method of claim 83 wherein said evaluating comprises detecting the levels of target RNA.
85. The method of claim 83 wherein said evaluating comprises detecting the levels of a protein.

86. The method of claim 83 wherein said evaluating comprises detection of one or more phenotypic effects.