(54) Title: NUCLEASE STABLE AND BINDING COMPETENT OLIGOMERS AND METHODS FOR THEIR USE

(57) Abstract

Oligomers are disclosed which have modified internucleotide linkages and can form triplex and duplex structures by binding to complementary nucleic acid sequences. The oligomers of the invention may be incorporated into pharmaceutically acceptable carriers and may be constructed to have any desired sequence. Compositions of the invention can be used as pharmaceutical agents to treat various diseases such as viruses and can be used for diagnostic purposes in order to detect viruses or disease conditions.
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NUCLEASE STABLE AND BINDING COMPETENT
OLIGOMERS AND METHODS FOR THEIR USE

Technical Field

The invention relates generally to novel oligonucleotide analogs, nucleoside analogs and their use in oligonucleotide-based therapies and diagnosis by binding of the oligonucleotide analogs to single or double stranded nucleic acid target sequences. More specifically, the invention concerns oligomers containing ribo-acetal and related substitute linkages and the novel nucleoside analogs used to synthesize such oligomers.

Background Art

The application of oligonucleotides and oligonucleotide analogs (oligomers) for therapeutic uses represents a relatively new development in drug design and discovery. Several fundamental therapeutic approaches that utilize oligomers have been proposed.

One approach is based largely on interfering with gene expression through oligomer binding to a complementary RNA sequence. This application is known as "antisense" therapy because the oligomer base sequence is identical to the antisense strand of the gene that gave rise to the RNA (Uhlmann, E., et al., Chem Reviews (1990) 90:543-584). Another approach, referred to herein as "triple helix" therapy utilizes oligomers that bind to duplex DNA as detailed below. Binding to a target DNA is
sequence specific but involves different base pairing rules.

Both antisense and triple helix therapies exert therapeutic effects via binding to complementary nucleic acid sequences that are responsible for disease conditions or sequences that are found in the genome of pathogenic organisms such as bacteria, protozoa, fungi or viruses. By modulating the expression of a gene important for establishment, maintenance or elimination of a disease condition, the corresponding condition may be cured, prevented or ameliorated.

Another therapeutic approach that is based on the use of oligomers includes generation of "aptamers" and is disclosed and claimed in commonly owned application nos. 745,215, 659,980 and 658,849. This approach utilizes oligomers that specifically bind to proteins thereby interfering with their function. The use of oligomers that mimic the structure of certain RNA molecules that are bound by intracellular proteins has also been adduced as a therapeutic approach as described in international application no. PCT/US91/01822.

linkages are described in U.S. patent 5,034,506 and in some cases give rise to an increased affinity of the oligomer for complementary target sequences. DNA synthesis via amidite and hydrogen phosphonate chemistries has been described (U.S. Patent Nos. 4,725,677; 4,415,732; 4,458,066; 4,959,463).

The therapeutic and diagnostic usefulness of oligomers is also generally enhanced by modifications that increase oligomer uptake by cells or reduce the rate of metabolism by cells or serum. Such modifications include (i) increased stability toward nuclease activity, (ii) reduced oligomer charge and/or (iii) increased lipophilicity of the oligomer. Thus, a need exists for nuclease resistant oligomers that are capable of sequence specific binding. Oligomers having the substitute linkages as described herein exhibit sequence-specific binding to complementary single stranded and duplex target sequences and are resistant to nuclease degradation. Additional properties of the compounds are described in detail below.

Summary of the Invention

The present invention is directed to oligomers and pharmaceutically acceptable salts thereof, said oligomers comprising at least two nucleomonomers, wherein a first nucleomonomer and a second nucleomonomer are coupled through a substitute linkage, wherein the substitute linkage comprises a 5-, 6- or 7-member ring containing C2' and C3' of the first nucleomonomer covalently linked through a bridging moiety to C4' of the second nucleomonomer. The nucleomonomers are coupled together via a one, two or three atom bridging moiety (bridge) that links the 5-, 6-, or 7-member ring to an adjacent C4'. Preferred embodiments are oligomers wherein the ring is a 5-member ring.
Brief Description of Figures

Figure 1 describes the synthesis of a riboacetal DMT synthon of Example 1.

Figure 2 describes the solid phase generation of a homopolymer of Example 4.

Figure 3 describes the synthesis of a 5'-DMT oxoriboacetal synthon of Example 5.

Figure 4 describes the synthesis of a 5'-DMT thioriboacetal synthon of Example 6.

Figure 5 describes the synthesis of a DMT synthon of a seven member ring system in Example 7.

Figure 6 describes the synthesis of a DMT synthon of a seven member ring system of Example 8.

Figure 7 describes the synthesis of a DMT synthon of the riboacetal of Example 9.

Figure 8 describes the synthesis of a benzoyl synthon a riboacetal of Example 10.

Figure 9 describes the synthesis of a benzoyl synthon of a six member ring system of Example 11.

Figure 10 describes the synthesis of a benzoyl synthon of a six member ring system of Example 12.

Figure 11 describes the synthesis of a DMT synthon of 2'-thioriboacetal of Example 13.

Figure 12 describes the synthesis of a DMT synthon of 2'-riboaminal of Example 14.

Figure 13 describes the synthesis of a benzoyl synthon of a six member ring system of Example 15.

Figure 14 describes the synthesis of a benzoyl synthon of a six member ring system of Example 16.

Figure 15 describes the synthesis of a riboacetal synthon of Example 17.

Figure 16 describes the synthesis of a riboketal synthon.

Figure 17 describes the synthesis of a nor riboacetal synthon.
Figure 18 describes the synthesis of a nor
riboacetal synthon.

Figure 19 describes the synthesis of an
orthoester synthon.

Figure 20 describes the synthesis of a 5''
thioorthoester synthon.

Figure 21 describes the synthesis of a 3',5''
dithioorthoester synthon.

Figure 22 describes the synthesis of a six
member ring system.

Figure 23 describes the synthesis of a seven
member ring system.

Figure 24 describes the synthesis of a seven
member ring system.

Figure 25 shows structures used for linkage of
nucleononomers linked via phosphorous containing
linkages.

Figure 26 shows structures of an o-xyloso
switchback linker and representative noninvention
substitute linkages that may be included in the oligomers
of the invention.

Figure 27 shows synthesis of a five member ring
with a 3 atom bridge.

Figure 28 shows synthesis of a five member ring
with a 3 atom bridge.

Figure 29 shows synthesis of a five member ring
with a 3 atom bridge.

Figure 30 shows synthesis of a seven member
ring with a 2 atom bridge.

Figure 31 shows synthesis of a five member ring
with a 3 atom bridge.

Figure 32 shows synthesis of a five member ring
with a 3 atom bridge.

35 Structural Formulas
Structural formulas described herein are designated as roman numerals (I, II, etc) and chemical compounds are designated a numeral (1, 2, etc).

Detailed Description of the Invention

Definitions

Nucleomonomer. As used herein, the term "nucleomonomer" means a moiety comprising (1) a base covalently linked to (2) a second moiety. Nucleomonomers include nucleosides and nucleotides. Nucleomonomers can be linked to form oligomers that bind to target or complementary base sequences in nucleic acids in a sequence specific manner.

A "second moiety" as used herein includes a sugar moiety, usually a pentose, and those species which contain modifications of the sugar moiety, for example, wherein one or more of the hydroxyl groups are replaced with a halogen, a heteroatom, an aliphatic groups, or are functionalized as ethers, amines, thiols, and the like. The pentose moiety can be replaced by a hexose or an alternate structure such as a cyclopentane ring, a 6-member morpholino ring and the like. Nucleomonomers as defined herein are also intended to include a base linked to an amino acid and/or an amino acid analog having a free carboxyl group and/or a free amino group and/or protected forms thereof.

Base. "Base" as used herein includes those moieties which contain not only the known purine and pyrimidine heterocycles, but also heterocycle analogs and tautomers thereof. Purines include adenine, guanine and xanthine and exemplary purine analogs include 8-oxo-N6-methyladenine and 7-deazaxanthine. Pyrimidines include uracil and cytosine and their analogs such as 5-methylcytosine, 5-(1-propynyluracil), 5-(1-propynylcytosine), 5-methyluracil and 4,4-ethanocytosine.
Nucleoside. As used herein, "nucleoside" means a base covalently attached to a sugar or sugar analog and which may contain a phosphite or phosphine. The term nucleoside includes ribonucleosides, deoxyribonucleosides, or any other nucleoside which is an N-glycoside or C-glycoside of a base. The stereochemistry of the sugar carbons can be other than that of D-ribose.

Nucleosides include those species which contain modifications of the sugar moiety, for example, wherein one or more of the hydroxyl groups are replaced with a halogen, a heteroatom, an aliphatic group, or are functionalized as ethers, amines, thiols, and the like. The pentose moiety can be replaced by a hexose or an alternate structure such as a cyclopentane ring, a 6-member morpholino ring and the like.

The term "nucleoside" will include ribonucleosides, deoxyribonucleosides, or to any other nucleoside which is an N-glycoside or C-glycoside of a purine or pyrimidine base, or modified purine or pyrimidine base. The stereochemistry of the sugar carbons can be other than that of D-ribose in one or more residues. The pentose moiety can be replaced by a hexose and incorporated into oligomers as described (Augustyns, K., et al Nucl Acids Res (1992) 18:4711-4716). Also included are analogs where the ribose or deoxyribose moiety is replaced by an alternate structure such as a hexose or such as the 6-member morpholino ring described in U.S. patent number 5,034,506. Nucleosides as defined herein also includes a purine or pyrimidine base linked to an amino acid or amino acid analog having a free carboxyl group and a free amino group or protected forms thereof.
Nucleotide. As used herein, "nucleotide" means a nucleoside having a phosphate group or phosphate analog.

Sugar Modification. As used herein, "sugar modification" means any pentose or hexose moiety other than 2'-deoxyribose. Modified sugars include D-ribose, 2'-O-alkyl, 2'-amino, 2'-halo functionalized pentoses, hexoses and the like. Sugars having a stereochemistry other than that of a D-ribose are also included.

Linkage. As used herein, "linkage" means a phosphodiester moiety (−O−P(O)(O)−O−) that covalently couples adjacent nucleomonomers.

Substitute Linkages. As used herein, "substitute linkage" means any analog of the native phosphodiester group or any suitable moiety that covalently couples adjacent nucleomonomers. Substitute linkages include phosphodiester analogs, e.g. such as phosphorothioate and methylphosphonate, and nonphosphorus containing linkages, e.g. such as acetals and amides.

Substitute linkages include the nonphosphorous containing linkages of the invention.

Switchback. As used herein, "switchback" means an oligomer having at least one region of inverted polarity. Switchback oligomers are able to bind to opposite strands of a duplex to form a triplex on both strands of the duplex. The linker ("switchback linker") joining the regions of inverted polarity is a substitute linkage.

Crosslinking moiety. "Crosslinking moiety" includes a group or moiety in an oligomer that forms a covalent bond with a target nucleic acid. Crosslinking moieties include covalent bonding species that covalently link an oligomer to target nucleic acids either spontaneously (e.g. N',N'-ethanocytosine) or via photoactivation (e.g. psoralen and the like).
Oligomers. "Oligomers" are defined herein as two or more nucleomonomers covalently coupled to each other by a linkage or substitute linkage moiety. Thus, an oligomer can have as few as two covalently linked nucleomonomers (a dimer). Oligomers can be binding competent and, thus, can base pair with cognate single-stranded or double-stranded nucleic acid sequences. Oligomers (e.g. dimers - hexamers) are also useful as synthons for longer oligomers as described herein.

Oligomers can also contain abasic sites and pseudonucleosides.

Oligomer includes oligonucleotides, oligonucleosides, polydeoxyribo-nucleotides (containing 2'-deoxy-D-ribose or modified forms thereof), i.e., DNA, polyribo nucleotides (containing D-ribose or modified forms thereof), i.e., RNA, and any other type of polynucleotide which is an N-glycoside or C-glycoside of a purine or pyrimidine base, or modified purine or pyrimidine base. Oligomer as used herein is also intended to include compounds where adjacent nucleomonomers are linked via amide linkages as previously described (Nielsen, P.E., et al, Science (1991) 254:1497-1500). Elements ordinarily found in oligomers, such as the furanose ring and/or the phosphodiester linkage can be replaced with any suitable functionally equivalent element. "Oligomer" is thus intended to include any structure that serves as a scaffold or support for the bases wherein the scaffold permits binding to target nucleic acids in a sequence-dependent manner. Oligomers that are currently known can be defined into four groups that can be characterized as having (i) phosphodiester and phosphodiester analog (phosphorothioate, methylphosphonate, etc) linkages, (ii) substitute linkages that contain a non-phosphorous isostere (riboacetal, formacetal, carbamate, etc), (iii)
morpholino residues, carbocyclic residues or other furanose sugars, such as arabinose, or a hexose in place of ribose or deoxyribose and (iv) nucleomonomers linked via amide bonds or acyclic nucleomonomers linked via any suitable substitute linkage.

Blocking Groups. As used herein, "blocking group" refers to a substituent other than H that is conventionally coupled to oligomers or nucleomonomers, either as a protecting group, a coupling group for synthesis, OPO, or other conventional conjugate such as a solid support, label, antibody, monoclonal antibody or fragment thereof and the like. As used herein, "blocking group" is not intended to be construed solely as a protecting group, according to slang terminology, but is meant also to include, for example, coupling groups such as a H-phosphonate or a phosphoramidite.

By "protecting group" is meant is any group capable of protecting the O-atom, S-atom or N-atom to which it is attached from participating in a reaction or bonding. Such protecting groups for N-atoms on a base moiety in a nucleomonomer and their introduction are conventionally known in the art. Non-limiting examples of suitable protecting groups include diisobutylformamidine, benzoyl and the like. Suitable "protecting groups" for O-atoms and S-atoms are, for example, DMT, MMT, FMOC or esters.

Protecting group. "Protecting group" as used herein includes any group capable of preventing the O-atom, S-atom or N-atom to which it is attached from participating in a reaction or bonding. Such protecting groups for O-, S- and N-atoms in nucleomonomers are described and methods for their introduction are conventionally known in the art. Protecting groups also include any group capable of preventing reactions and bonding at carboxylic acids, thiols and the like.
Coupling group. "Coupling group" as used herein means any group suitable for generating a linkage or substitute linkage between nucleosonomers such as a hydrogen phosphonate, a phosphoramidite and an alkyl ether.

Conjugate. "Conjugate" as used herein means any group attached to the oligomer at a terminal end or within the oligomer itself. Conjugates include solid supports, such as silica gel, controlled pore glass and polystyrene; labels, such as fluorescent, chemiluminescent, radioactive atoms or molecules, enzymatic moieties and reporter groups; oligomer transport agents, such as polycations, serum proteins and glycoproteins and polymers and the like.

Synthon. "Synthon" as used herein means a structural unit within a molecule that can be formed and/or assembled by known or conceivable synthetic operations.

Transfection. "Transfection" as used herein refers to any suitable method that for enhanced delivery of oligomers into cells.

Subject. "Subject" as used herein means a plant or an animal, including a mammal, particularly a human.

The present invention is based on the synthesis of oligomers comprising novel substitute linkages between one or more nucleosonomers and methods of their synthesis and use. Oligomers containing these substitute linkages not only bind to complementary target nucleic acid sequences, but also have a reduced negative charge, are stable to nuclease activity and are more lipophilic than oligomers with unmodified phosphodiester linkages. Because of these properties, the oligomers of the invention may be utilized in any oligomer-based therapeutic or diagnostic application, or as a research
reagent. The linkages are shown as a series of dimer compounds in the following general structural formulas I through XI. The dimers are useful for incorporation of the substitute linkages into oligomers by solid-phase methods as described below. The structural formulas show the substitute linkages that comprise (i) a 5-, 6- or 7-member ring with the C2' and C3' positions of a modified sugar (usually a ribose or ribose analog) constituting part of the ring and (ii) a one, two or three atom bridge that links the ring to the adjacent nucleononomer at the C4' position. The C2', C3', C4' and C7'' positions are indicated in structural formula I.
For each structure, R^1 is independently OH, OP_{3}^{2}, an oligomer, a solid support or a suitable blocking group such as a dimethoxytrityl ether (DMTO) moiety, a monomethoxytrityl ether (MMTO) moiety, an ester moiety, H-phosphonate (OP_{3}H), methylphosphonate (OP_{3}CH_{3}) or a phosphoramidite; R^2 is selected from the group consisting of H, OH, F, NH_{2}, OCH_{3}, OC_{2}H_{5}, OCH_{2}CHCH_{2} (O-allyl, OC_{2}H_{5}), OC_{3}H_{7} (O-propyl), SCH_{3}, SC_{2}H_{5}, SCH_{2}CHCH_{2} (S-allyl, SC_{2}H_{5}), and SC_{3}H_{7} (S-propyl). Methylphosphoramidite and β-cyanoethylphosphoramidite are preferred phosphoramidite groups.

Alternatively, for R^1 at C3' and R^2: when R^2 is part of an invention substitute linkage that is covalently linked to an adjacent nucleonomer, R^1 and R^2 are W (defined below).

W is independently selected from the group consisting of O, S, SO, SO_{2}, CH_{2}, CH, CO, CF_{2}, CS, N, NH and NR^3 wherein R^3 is alkyl (1-4C, including methyl, ethyl, propyl, isopropyl, butyl or isobutyl) with the proviso that adjacent W (formulae IV - XI) are not -O-O-, -O-S-, -O-CF_{2}-, or -S-CF_{2}-, and provided that, for formula I, only one W is N or CH and when W is N or CH, W is connected to G by a double bond.

A is independently selected from the group consisting of O, S, CH_{2}, CF_{2} and CFH (O is preferred).

E is selected from the group consisting of O, S, SO, SO_{2}, CH, CH_{2}, CO, CF_{2}, CS, N, NH, and NR^3 provided that no adjacent -E-E- are -O-O-, -O-S-, -S-O-, -O-CF_{2}-, -CF_{2}-O-, -CF_{2}-S- or -S-CF_{2}-, and provided that when E is CH or N, any adjacent E is CH or N or an adjacent J is CH and they are connected by a double bond.

J is selected from the group consisting of O, S, SO, SO_{2}, CH, CH_{2}, CO, CF_{2} and CS provided that no adjacent -E-J- are -O-O-, -O-S-, -S-O-, -CF_{2}-O-, -O-CF_{2}-,
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-CF₂-S- or -S-CF₂-, and provided that when J is CH, any adjacent E is CH or N and they are connected by a double bond.

G is independently selected from the group consisting of C, CH, N, CF, CCl, CBr, CI, and CR² wherein R¹ is lower alkyl (1-4C) or lower fluoroalkyl (1-4C, 1-6F, including fluoromethyl, difluoromethyl, trifluoromethyl, and hexafluoroisopropyl), 5-tetrazole, hydroxymethyl (CH₂OH), CH₂-(5-tetrazole), CN, CO₂H, CO₂R¹, CONH₂, CONH₂R², CON(R²)₂, CH₂SR¹, CH₂SOR¹, CH₂SO₂R², CH₂CO₂H, CH₂CN, CH₂CO₂R², CH₂CONH₂, CH₂CONHR¹ and CH₂CON(R²)₂, wherein R¹ is as defined above.

B* is a base. Bases (B*) that are preferred are adenine, thymine, guanine, cytosine, uracil, 8-oxo-N⁴-methyladenine, N⁴,N⁴-ethanocytosine, 5-methylcytosine, 5-(1-propynyl)uracil, 5-(1-propynyl)cytosine, and 7-deazaxanthine. These base analogs are disclosed in commonly owned pending U.S. application serial no. 787,920, commonly owned International Application Nos. PCT/US91/08811 and PCT/US91/03680, and attorney docket no. 24610-20035.22 filed November 24, 1992 (Froehler, B.C. et al, inventors), International Application Nos. PCT/US91/08811 and PCT/US91/03680, or disclosed in Application No. PCT/US90/03275.

Substitute linkages of the present invention also include 5-, 6-, or 7-member unsaturated rings with the proviso that both termini of the double bond G, are C or N. In addition, double bonds can be utilized in substitute linkages containing two or three atom bridges.

Preferred Embodiments

Oligomers having one or two substitute linkages of the 5-member ring series are preferred embodiments of the present invention. Preferred
embodiments include oligomers having one or more substitute linkages of the 5-member ring series as shown in formula III. These embodiments include oligomers containing one or more substitute linkages of formula III where A is O, each W is independently O or S, J is CH₂, and G is CH or CR₄, and E adjacent to G is CH₂, and E adjacent to J is CH₃, O, S, SO or SO₂.

Oligomers containing one or more substitute linkages of formula I where each W is independently O or S, E is O, S or CH₂, J is CH₂, G is CH or CR₄ are also preferred embodiments. The riboacetal substitute linkage (structure I, where A and W are O, G is CH₂, and E, and J are CH₃) is a particularly preferred embodiment.

Preferred embodiments also include oligomers having one or more substitute linkages of the 5-member ring series as shown in formula II. These embodiments include oligomers containing one or more substitute linkages of formula II where A is O, each W is independently O or S, J is CH₂, and G is CH or CR₄.

Invention Oligomers

Oligomers containing substitute linkages of the invention are nuclease resistant and are capable of sequence-specific binding in the formation of duplexes or triplexes with single-stranded RNA or DNA or duplex target sequences, respectively.

When substitute linkages are present, additional nucleononomer modifications can vary widely as discussed hereinafter. Preferably, the additional modification is the inclusion of at least one purine or pyrimidine analog in place of guanine, adenine, cytosine or thymine.

Substitute linkages of the present invention include 3-member rings formed through linking the C₂' and C₃' position of the modified sugar through (i) N which is
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linked through the three atom bridge -E-E-J- to the adjacent nucleotide and (ii) G which is linked through the three atom bridge -E-E-J- to the adjacent nucleomonomer.

It has been found that oligomers containing substitute linkages efficiently bind complementary single-stranded and double-stranded nucleic acid sequences. Triple helix structures were formed under physiological salt conditions. Oligomers of the present invention are generally characterized as containing one or more riboacetal or related substitute linkages. The substitute linkages may be utilized in oligomers that contain additional modifications of other nucleomonomers that comprise the oligomer. An exemplary list of such modifications include oligomers where (i) one or more nucleomonomer is modified at the 2’ position, (ii) one or more crosslinking moieties have been incorporated, (iii) switchback linkers have been incorporated, (iv) other substitute linkages have been included and (v) bases that facilitate duplex or triplex formation, such as 8-oxo-N6-methyladenine, 5-(1-propynyl)uracil, 5-(1-propynyl)cytosine or 7-deazaaxanthine have been included. One or more of such modifications may advantageously be incorporated into a given oligomer depending on target nucleic acid sequences.

The novel oligomers of the present invention are useful in antisense therapies wherein a selected RNA sequence is bound. The oligomers of the invention can be constructed to have any desired sequence and can be incorporated into pharmaceutically acceptable carriers. Compositions of the invention can be used as pharmaceuticals to treat various diseases such as inflammatory or cardiovascular disorders, cancers and viral infections and can be used for diagnostic purposes.
in order to detect the presence of neoplastic growth, viruses and a variety of disease conditions.

The invention oligomers can also be utilized as research reagents to analyze the function of individual genes or to probe the function of nucleic acids in cells or cell extracts.

The invention is directed primarily to oligomers capable of duplex and triple-helix formation and, more specifically, to oligomers containing riboacetal, related substitute linkages and the novel nucleomonomers and dimers which serve as intermediates in the synthesis of such oligomers. These oligomers are preferably included in a pharmaceutically acceptable carrier and can have any desired sequence which will be determined by the target sequence.

In another aspect, the invention is directed to DNA triplexes and a method to form such triplexes. The invention oligomers are suitable for hybridizing with DNA duplex targets via either CT or GT triplex binding motifs. Other aspects of the invention include pharmaceutical and diagnostic compositions which contain the oligomers of the invention and methods to diagnose and treat diseases characterized by various target sequences such as oncogene or virus duplexes using these compositions.

An additional aspect of the invention includes methods of detecting the presence, absence or amount of a particular single-stranded DNA or RNA or a particular target duplex in a sample using the oligomers of the invention. Such sequences can be associated with the presence of neoplastic growth, viruses or other disease conditions. Reagents and kits containing oligomers of the invention represent an aspect of the invention that permits facile use of the oligomers as reagents useful for (1) modulating gene expression in cells in vitro.
including cells grown in tissue culture, and
(2) detecting and/or quantitating target sequences.

An advantage of the present invention is that
the oligomers are capable of forming triplexes under
physiological ion conditions.

Other advantages of oligomers containing the
substitute linkages of the present invention compared to
unmodified oligomers is that the substitute linkage may
enhance cell permeation or uptake and such substitute
linkages are stable to nuclease activity. These
compounds are more lipophilic than native DNA. In
addition, the substitute linkages disclosed herein
eliminate the negative charge associated with
phosphodiester linkages which can facilitate cell
association or uptake. Nuclease stability is an
important functional aspect of oligomers that modulate
gene expression via, for example, an antisense mechanism.

An important feature of the oligomers of the
present invention is that the substitute linkages are
relatively rigid compared to diester linkages in native
nucleic acids. This property contributes to the enhanced
binding capacity of some of the invention oligomers.

Aspects of the invention include the use of
nucleonomomers, two linked nucleonomomers (dimers), three
linked nucleonomomers (trimers), four linked
nucleonomomers (tetramers), five linked nucleonomomers
(pentamers) or six linked nucleonomomers (hexamers) as
intermediates in the synthesis of the longer oligomers of
the invention. These oligomers are valuable synthons of
the invention that are useful in the synthesis of longer
oligomers.

An aspect of the invention includes methods for
separation of endo and exo isomers at the G position.
For invention substitute linkages, other than riboacetal
linkages (Figures 1 and 15), synthesis of nucleonomomers
linked by the substitute linkage usually leads to a mixture containing both isomers at the G position. Methods for separation of the endo and exo isomers are described below. When G is N or C, no stereoisomers are possible at this position. Methods for separation of endo and exo isomers include reverse phase HPLC and normal phase silica gel flash chromatography.

These and other objects, advantages and features of the invention will become apparent to those persons skilled in the art upon reading the details of the oligomers and their synthesis and usage as more fully set forth below, reference being made to the structural formulas and specific examples.

**Additional Nucleonomonomer Modifications.**

Oligomers that are comprised of nucleonomonomers can also contain various modifications in addition to the substitute linkages of the invention. A non-limiting exemplary list of such additional modifications includes oligomers where (i) one or more nucleonomonomer residues are modified at the 2' position, (ii) one or more covalent crosslinking moieties are incorporated, (iii) inverted polarity linkers (switchback linkers) are incorporated, (iv) other noninvention substitute linkages are included, (v) other base analogs, such as 8-oxo-N6-methyladenine, are included and (vi) conjugates such as intercalating agents or polylsine that respectively enhance binding affinity to target nucleic acid sequences or that enhance association of the oligomer with cells are included.

The binding competence of the invention oligomers for single-stranded and duplex targets is compatible with further modifications to the oligomer. These further modifications may also confer other useful properties such as stability to nuclease cleavage (e.g. ...
in a domain of an invention oligomer having phosphodiester linkages), or enhance their ability to permeate cell membranes, and the like.

Also included are oligomers containing one or more substitute linkages such as sulfide or sulfone linkages (Benner, S.A., International Publication No. WO 89/12060), sulfamate linkages (International Publication No. WO 91/15500), carbamate or other substitute linkages in morpholino-linked oligomers (Stirchak, E.P. et al Nucleic Acids Res (1989) 17:6129-6141; Summerton, J., et al International Publication No. 216 860) and related linkages.

Thus, exemplary embodiments of invention oligomers include oligomers having (1) at least one substitute linkage and a 5-member or 6-member ring that is linked to an adjacent C4' atom through a tone, two or three atom bridge, and (2) one or more non-invention substitute linkages selected from the group consisting of phosphorothioate, methylphosphonate and thionomethylphosphonate and/or (3) one or more phosphodiester linkages and/or (4) purine or pyrimidine analogs that enhance binding affinity for complementary target sequences. Other exemplary oligomers would include (1) an oligomer having invention substitute linkages at the 3' and/or 5' ends and phosphorothioate linkages elsewhere in the oligomer; (2) oligomers having invention substitute linkages and standard purine or pyrimidine bases (e.g. adenine, guanine, cytosine, thymine, or uracil); (3) oligomers having invention substitute linkages and one or more bases that enhance binding affinity or permeation competence of the oligomer (e.g. 5-methylcytosine, 5-(1-propynyl)uracil, 5-(1-propynyl)cytosine, and the like); and (4) oligomers having invention substitute linkages and one or more 2'-
modified nucleonomomers (e.g. 2'-O-allyl, 2'-fluoro, and the like).

Related linkages such as amide linkages and 2',5' linkages are described in commonly owned pending U.S. application serial numbers 07/889,736 filed January 28, 1992, 07/892,902 filed June 1, 1992, and 07/894,397 filed June 5, 1992, each cited reference is incorporated herein by reference in its entirety.


Oligomers

The oligomers of the invention can be formed using invention and conventional nucleonomomers and synthesized using standard solid phase (or solution phase) oligomer synthesis techniques, which are now commercially available. In general, the invention oligomers can be synthesized by a method comprising the steps of: synthesizing a nucleonomomer or oligomer synthon having a protecting group and a base and a coupling group capable of coupling to a nucleonomomer or oligomer; coupling the nucleonomomer or oligomer synthon to an acceptor nucleonomomer or an acceptor oligomer; removing the protecting group; and repeating the cycle as needed until the desired oligomer is synthesized.

The oligomers of the present invention can be of any length including those of greater than 40, 50 or 100 nucleonomomers. In general, preferred oligomers contain 2-30 nucleonomomers. Lengths of greater than or equal to about 8 to 20 nucleonomomers are useful for
therapeutic or diagnostic applications. Short oligomers containing 2, 3, 4 or 5 nucleononomers are specifically included in the present invention and are useful as synthons.

Oligomers having a randomized sequence and containing about 6,7 or 8 nucleononomers are useful for primers that are used in cloning or amplification protocols that use random sequence primers, provided that the oligomer contains about 1 or 2 residues at the 3' end that can serve as a primer for polymerases or reverse transcriptases or that otherwise do not interfere with polymerase activity.

Oligomers can contain conventional phosphodiester linkages or can contain other non-invention substitute linkages such as phosphoramidate linkages in addition to the invention substitute linkages. These substitute linkages include, but are not limited to, embodiments wherein a moiety of the formula -O-P(O)(S)-O-("phosphorothioate"), -O-P(S)(S)-O-
("phosphorodithioate"), -O-P(O)(NRM)-X-, -O-P(O)(R'M)-O-
, -O-P(S)(RM)-O- ("thionoalkylphosphonate"), -P(O)(OR'M)-X-, -O-C(O)-X-, or -O-C(O)(NRM)-X-, wherein R'M is H (or a salt) or alkyl (1-12C including methyl and ethyl) and R'M is alkyl (1-9C) and the linkage is joined to adjacent nucleononomers through an -O- or -S- bonded to a carbon of the nucleononomer. Phosphorothioate and phosphodiester linkages are well known. Particularly preferred substitute linkages for use in the oligomers of the present invention include phosphodiester, phosphorothioate, methylphosphonate and thionomethylphosphonate substitute linkages. Phosphorothioate and methylphosphonate substitute linkages confer added stability to the oligomer in physiological environments. While not all such substitute linkages in the same oligomer need be
identical, particularly preferred oligomers of the invention contain one or more phosphorothioate or methylphosphonate substitute linkages.

Pharmaceutically Acceptable Salts

Any pharmaceutically acceptable salt can be used and such salt forming materials are well known in the art.

Pharmaceutically acceptable salts are preferably metal or ammonium salts of the oligomers of the invention and include alkali or alkaline earth metal salts, e.g., the sodium, potassium, magnesium or calcium salt; or advantageously easily crystallizing ammonium salts derived from ammonia or organic amines, such as mono-, di- or tri-lower (alkyl, cycloalkyl or hydroxyalkyl)-amides, lower alkylenediamines or lower (hydroxyalkyl or arylalkyl)-alkylammonium bases, e.g. methylamine, diethylamine, triethylamine, dicyclohexylamine, triethanolamine, ethylenediamine, tris-(hydroxymethyl)-aminomethane or benzyltrimethylammonium hydroxide. The oligomers of the invention form acid addition salts, which are preferably such of therapeutically acceptable inorganic or organic acids, such as strong mineral acids, for example hydrohalic, e.g., hydrochloric or hydrobromic acid; sulfuric, phosphoric; aliphatic or aromatic carboxylic or sulfonic acids, e.g., formic, acetic, propionic, succinic, glycollic, lactic, malic, tartaric, gluconic, citric, ascorbic, maleic, fumaric, hydroxymaleic, pyruvic, phenylactic, benzoic, 4-aminobenzoic, anthranilic, 4-hydroxybenzoic, salicylic, 4-aminosalicylic, methanesulfonic, ethanesulfonic, hydroxyethanesulfonic, benzenesulfonic, sulfanilic or cyclohexylsulfamic acid and the like.
Blocking Groups

1. Coupling Groups. Suitable coupling groups are, for example, H-phosphonate, a methylphosphonamidite, or a phosphoramidite. Phosphoramidites that can be used include β-cyanoethylphosphoramidites (preferred). Methylphosphonamidites, alkylphosphonamidites (including ethylphosphonamidites and propylphosphonamidites) can also be used. Exemplary phosphoramidites are shown in Figures 25-1 and 25-2.


Suitable coupling groups at the 3', 2' (or 5') position for oligomer synthesis via phosphate triester chemistry, referred to herein as "triester" chemistry, include 2-chlorophenyl phosphate, 4-chlorophenyl phosphate, 2,4-dichlorophenyl phosphate and 2,4-dibromophenyl phosphate nucleotide diester derivatives or, for synthesis of phosphorothioate linkages, the thiono derivatives thereof (Marugg, J.E., et al, *Nucl Acids Res* (1984) **12**:9095-9110; Kemal, O., et al, *J Chem Soc Chem Commun* (1983) 591-593; Kamer, P.C.J., et al, *Tet Lett* (1989) **30**:6757-6760). Structures of these coupling groups are shown in Figure 15 where X is O or S and Z' is H or a suitable benzotriazole.


Suitable protecting groups are DMT (dimethoxytrityl), Bz (benzoyl), iBu (isobutyryl), phenoxyacetyl, MMT (monomethoxytrityl) or FMOC at the 5' terminus and/or hydrogen phosphonate, methyl phosphoramidite, methyl phosphonamidite, β-cyanoethylphosphoramidite, TBS (t-butyldimethylsilyl) or TBDPS (t-butyldiphenylsilyl) at the 3'-terminus.

Preferred protecting groups are Bz (benzoyl), DMT (dimethoxytrityl), MMT (monomethoxytrityl) or FMOC at the 5' terminus or position and/or TBS, hydrogen
phosphonate, methylphosphoramidite, methyl-phosphonamidite, β-cyanoethylphosphoramidite at the 3'-terminus. However, it is intended that the position of the blocking groups can be reversed as needed (e.g., a phosphoramidite at the 5'-position and DMT at the 3'-position). In general, the nucleomonomers and oligomers of the invention can be derivatized to such "blocking groups" as indicated in the relevant formulas by methods known in the art.

Conjugates

Also included are "conjugates" of oligomers. "Conjugates" of the oligomers include those conventionally recognized in the art. For instance, the oligomers can be covalently linked to various moieties such as, intercalators, and substances which interact specifically with the minor groove of the DNA double helix. Other chosen conjugate moieties can be labels such as radioactive, fluorescent, enzyme, or moieties which facilitate cell association using cleavable linkers and the like. Suitable radiolabels include $^{32}$P, $^{35}$S, $^3$H and $^{14}$C; and suitable fluorescent labels include fluorescein, resorufin, rhodamine, BODIPY (Molecular Probes) and texas red; suitable enzymes include alkaline phosphatase and horseradish peroxidase. Other compounds which can be used as covalently linked moieties include biotin, antibodies or antibody fragments, transferrin and the HIV Tat protein can also conveniently be linked to the oligomers of the invention.

These additional moieties can be derivatized through any convenient moiety. For example, intercalators, such as acridine or psoralen can be linked to the oligomers of the invention through any available -OH or -SH, e.g., at the terminal 5'-position of the oligomer, the 2'-positions of RNA, or an OH, NH$_2$, COOH
or SH incorporated into the 5'-position of pyrimidines. A derivatized form which contains, for example, -CH₂CH₂NH₂, -CH₂CH₂CH₂OH or -CH₂CH₂CH₂SH in the 5'-position of pyrimidines is convenient. Conjugates including polylysine or lysine can be synthesized as described and can further enhance the binding affinity of an oligomer to its target nucleic acid sequence (Lemaitre, M. et al., Proc Natl Acad Sci (1987) 84:648-652; Lemaitre, M. et al., Nucleosides and Nucleotides (1987) 6:311-315).

A wide variety of substituents can be attached, including those bound through linkages or substitute linkages. The -OH moieties in the oligomers can be replaced by phosphate groups, protected by standard protecting groups, or coupling groups to prepare additional linkages to other nucleonononomers, or can be bound to the conjugated substituent. The 5'-terminal OH can be phosphorylated; the 2'-OH or OH substituents at the 3'-terminus can also be phosphorylated. The hydroxyls can also be derivatized to standard protecting groups.

Oligomers of the invention can be covalently derivatized to moieties that facilitate cell association using cleavable linkers. Linkers used for such conjugates can include disulfide linkages that are reduced after the oligomer-transport agent conjugate has entered a cell. Appropriate molecular linkers include for example, -Y₁-X₆CH₂CHR₁'-SS-CHR₁'CH₂X₄'-Y₁- wherein each Y₁' is independently alkylene (1-9C; including methylene, ethylene and propylene), or CO, each X₆ is independently O, S(O)(O), S(O), NR₇', CH₇', C(R')₂ or CO; R₇ wherein each R₇' is independently H, alkyl (1-6C; including methyl, ethyl and propyl), or aryl and which linkers have been previously described (International Publication No. WO 91/14696). Disulfide-containing linkers of this type have a controllable t₁/₂ in vivo, facilitating its use as
a prodrug/transport component. Such linkers are stable under extracellular conditions relative to intracellular conditions due to the redox potential of the disulfide linkage.

Suitable conjugates also include solid supports for oligomer synthesis and to facilitate detection of nucleic acid sequences. Solid supports include, but are not limited to, silica gel, controlled pore glass, polystyrene, and magnetic glass beads.

Sugar Modifications

Derivatives can be made by substitution on the sugars. Among the preferred derivatives of the oligomers of the invention are the 2'-O-allyl derivatives. The presence of the 2'-O-allyl group appears to enhance permeation ability and stability to nuclease degradation, but does not appear to diminish the affinity of the oligomer for single chain or duplex targets.

Furthermore, as the α anomer binds to duplex DNA or single-stranded RNA in a manner similar to that for the β anomers but with a reversed polarity, oligomers can contain nucleosonomomers having this epimer or a domain thereof (Praseuth, D., et al., Proc Natl Acad Sci (USA) (1988) 85:1349-1353; Sun, J.S. et al, Proc Natl Acad Sci (1991) 88:6023-6027; Debart, F., et al, Nucl Acids Res (1992) 20:1193-1200). α-Anomeric oligomers containing the substitute linkages described herein represent a class of modified oligomers included in the present invention.

Noninvention Substitute Linkages

The oligomers of the invention can also contain one or more "substitute linkages", in addition to those disclosed herein, which are generally understood in the art. These "substitute linkages" include
phosphorothioate, methylphosphonate, thionomethylphosphonate, phosphorodithioate, 2',5' linkages, alkylphosphonates, morpholino carbamate, morpholino sulfamate, morpholino sulfamide, boranophosphate (−O−P(OCH₃)(BH₃)−O−), siloxane (−O−Si(X')(X')−O−; X' is alkyl or phenyl) and phosphoramidate (methoxethylamine (−O−P(OCH₃CH₂OCH₃)(O)−O−) and the like), and are synthesized as described in the generally available literature including the following references (Sood, A., et al, J Am Chem Soc (1990) 112:9000-9001; WO 91/08213; WO 90/15065; WO 91/15500; Stirchak, E.P. et al Nucleic Acid Res (1989) 17:6129-6141; U.S. Patent 5,034,506; U.S. Patent 5,142,047; Hewitt, J.M. et al, Nucleosides and Nucleotides (1992) 11:1661-1666; Summerton, J., et al International Publication No. 216 860). Substitute linkages that can be used in the oligomers disclosed herein also include the sulfonamide (−O−SO₂-NH−), sulfide (−CH₂-S-CH₂−), sulfonate (−O−SO₂-CH₂−), carbamate (−O−C(O)−NH−, −NH−C(O)−O−), dimethylhydrazino (−CH₂-NCH₂-NCH₂−), sulfamate (−O−S(O)(O)−N−; −N−S(O)(O)−N−), 3'-thioformacetal (−S−CH₂−O−), formacetal (−O−CH₂−O−), 3'-amine (−NH−CH₂−CH₂−), N-methylhydroxylamine (−CH₂-NCH₂-O−) and 2',5' linkages (such as 2',5' carbamate (2'−N(H)−C(O)−O− 5'), 5',2' carbamate (2'−O−C(O)−N(H)− 5'), 5',2' methylcarbamate (2'−O−C(O)−N(CH₃)− 5') and 5',2' thioformacetal (2'−O−CH₂−S− 5'). 2',5' linkages are disclosed in pending U.S. application serial No. 07/892,902, filed June 1, 1992, incorporated herein by reference). Substitute linkages are disclosed and claimed in commonly owned pending U.S. patent application serial nos. 690,786, filed April 24, 1991, and 763,130, filed September 20, 1991, incorporated herein by reference in their entirety.
Additional substitute linkages that are suitable include amide linkages described by Buchardt, O. et al, (International Publication No. WO 92/20702), and those described by Cook, P.D. et al, (International Publication No. WO 92/20822), and De Mesmaeker, A. et al, (International Publication No. WO 92/20823).

Except where specifically indicated, the substitute linkages, such as a formacetal linkage, -O-CH₂-O-, are linked to either the 3' or 2' carbon of a nucleonomer on the left side and to the 5' carbon of a nucleonomer on the right side. Thus a formacetal linkage can be indicated as 3' -O-CH₂-O- 5' or 2' -O-CH₂-O- 5'. The designations of a 3', 2' or 5' carbon can be modified accordingly when a structure other than ribose, deoxyribose or arabinose is linked to an adjacent nucleonomer. Such structures include a hexose, morpholino ring, carbocyclic ring (e.g. cyclopentane) and the like.

Nucleosides

Exemplary nucleosides suitable for synthesis of amide linked nucleonomomers have been described (Nielsen, P.E. ibid; Buchardt, O. et al, International Publication No. WO 92/20702; commonly owned copending U.S. Application Serial Nos. 07/889,736, filed January 28, 1992, and 07/894,397, filed June 5, 1992, all applications incorporated herein by reference in their entirety).

"Nucleosides" also include those moieties which contain modifications of the sugar, for example, wherein one or more of the hydroxyl groups are replaced with halogen, aliphatic groups, or functionalized as ethers, amines, and the like. Such structures include a hexose, morpholino ring, carbocyclic ring (e.g. cyclopentane) and the like.

Base

Suitable bases for use within the present invention include not only the known purine and pyrimidine bases, but also analogs of these heterocyclic bases and tautomers thereof. Such analogs include alkylated purines or pyrimidines, acylated purines or pyrimidines, or other heterocycles. Such "analogous purines" and "analogous pyrimidines" or purine or pyrimidine analogs are those generally known in the art, some of which are used as chemotherapeutic agents. An exemplary, but not exhaustive, list includes N1,N1-ethanocytosine, 7-deazaxanthosine, 7-deazaguanosine, 8-oxo-N6-methyladenine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyl uracil, inosine, N6-isopentenyl-adenine, 1-methyladenine, 2-methylguanine,
5-methylcytosine, N²-methyladenine, 7-methylguanine, 5-methylaminomethyl uracil, 5-methoxy aminomethyl-2-thiouracil, 5-methoxyuracil, pseudouracil, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-(1-propynyl)-4-thiouracil, 5-(1-propynyl)-2-thiouracil, 5-(1-propynyl)-2-thiocytosine, 2-thiocytosine, and 2,6-diaminopurine. In addition to these base analogs, pyrimidine analogs including 6-azacytosine, 6-azathymidine and 5-trifluoromethyluracil described in Cook, D. P., et al, International Publication No. WO 92/02258 (incorporated herein by reference) can be conveniently incorporated into the invention oligomers.


Preferred bases include adenine, guanine, thymine, uracil, cytosine, 5-methylcytosine, 5-(1-propynyl)uracil, 5-(1-propynyl)cytosine, 8-oxo-N²-methyladenine, and 7-deazaxanthosine. Synthesis and use of oligomers that bind to duplex DNA sequences via GT binding motif containing 7-deazaxanthosine is described in commonly owned pending U.S. application serial number 07/787,920, filed November 7, 1991, which is incorporated herein by reference in its entirety.

Covalent Bonding Moiety

Included in some of the oligomers of the invention is a moiety which is capable of effecting at least one covalent bond between the oligomer and the duplex. Multiple covalent bonds can also be formed by
providing a multiplicity of such crosslinking moieties. The covalent bond is preferably to a base residue in the target strand, but can also be made with other portions of the target, including the saccharide or phosphodiester. The reaction nature of the moiety which effects crosslinking determines the nature of the target in the duplex. Preferred crosslinking moieties include acylating and alkylation agents, and, in particular, those positioned relative to the sequence specificity-conferring portion so as to permit reaction with the target location in the strand. Crosslinking moieties are disclosed and claimed in commonly owned pending application no. 640,654.

In one embodiment of the invention, a switchback oligonucleotide containing crosslinking moieties at either end can be used to bridge the strands of the duplex with at least two covalent bonds. In addition, nucleotide sequences of inverted polarity can be arranged in tandem with a multiplicity of crosslinking moieties to strengthen the complex. Exemplary of crosslinking moieties that are useful in the invention include N^6,N^6-ethanocytosine and N^6,N^6-ethanoadenine.

It is clear that the heterocycle need not be a purine or pyrimidine; indeed the pseudo-base to which the reactive function is attached need not be a heterocycle at all. Any means of attaching the reactive group is satisfactory so long as the positioning is correct.

**Inverted Polarity**

In their most general form, inverted polarity oligomers, that can incorporate one or more nucleononomers described above, contain at least one segment along their length of the formula:
3'----5'--C--5'----3'  (1)

or

5'----3'--C--3'----5'  (2)


In these formulas, the symbol 3'----5'
indicates a stretch of oligomer in which the linkages are consistently formed between the 5'- hydroxyl of the ribosyl residue of the nucleonomer to the left with the 3'- (or 2'- for oligomers having 2', 5' linkages)
hydroxyl of the ribosyl residue of the nucleonomer to
the right (i.e., a region of uniform polarity), thus
leaving the 5'- hydroxyl of the rightmost nucleonomer ribosyl residue free for additional conjugation.

Analogously, 5'----3' indicates a stretch
of oligomer in the opposite orientation wherein the
linkages are formed between the 3'- hydroxyl of the
ribosyl residue of the left nucleonomer and the 5'-hydroxyl of the ribosyl residue of the nucleonomer on
the right, thus leaving the 3'- hydroxyl of the rightmost
nucleonomer ribosyl residue free for additional
conjugation.

The linkage, symbolized by -C-, can be formed
so as to link the 5'- hydroxyls of the adjacent ribosyl
residues in formula (1) or the 3’ hydroxyls of the
adjacent ribosyl residues in formula (2), or the "-C-"
linkage can conjugate other portions of the adjacent
nucleononomers so as to link the inverted polarity
strands. "-C-" can represent a linker moiety, or simply
a covalent bond.
It should be noted that if the linkage between strands of inverted polarity involves a sugar residue, either the 3'- or 2'- position can be involved in the linkage, and either of these positions can be in either R or S configuration. The choice of configuration will in part determine the geometry of the oligomer in the vicinity of the linkage. Thus, for example, if adjacent 3'- positions are used to effect a covalent linkage, less severe deformation of the oligomer chain will generally occur if both 3'- hydroxyls involved in the linkage are in the conventional R configuration. If they are both in the S configuration, this will result in a favorable "kink" in the chain.

In addition to the use of standard oligonucleotide synthesis techniques or other couplings to effect the 5'-5' or 3'-3' linkage between ribosyl moieties, alternative approaches to joining the two strands of inverted polarity can be employed. For example, the two appended bases of the opposing termini of the inverted polarity oligomer sequences can be linked directly or through a linker, or the base of one can be linked to the sugar moiety of the other. Any suitable method of effecting the linkage can be employed. The characterizing aspect of the switchback oligomers of the invention is that they comprise tandem regions of inverted polarity, so that a region of 3'-5' polarity is followed by one of 5'-3' polarity, or vice versa, or both.

Depending on the manner of coupling the segments with inverted polarity, this coupling can be effected by insertion of a dimer wherein the appropriate 3'- positions of each member of the dimer or the 5'- positions of each member of the dimer are activated for inclusion of the dimer in the growing chain, or the conventional synthesis can be continued using the
condensing nucleonomer which is blocked in the inverse manner to that which would be employed if the polarity of the chain were to remain the same. This additional nucleonomer can also contain a linker moiety which can be included before or after condensation to extend the chain.

The synthesis of oligomers having inverted polarity can be accomplished utilizing standard solid phase synthesis methods.

In general, there are two commonly used solid phase-based approaches to the synthesis of oligomers containing conventional 3'→5' or 5'→3' linkages, one involving intermediate phosphoramidites and the other involving intermediate phosphonate linkages.

In the phosphoramidite based synthesis, a suitably protected nucleonomer having a cyanoethylphosphoramidite at the position to be coupled is reacted with the free hydroxyl of a growing nucleonomer chain derivatized to a solid support. The reaction yields a cyanoethylphosphite, which linkage must be oxidized to the cyanoethylphosphate at each intermediate step, since the reduced form is unstable to acid.

The H-phosphonate-based synthesis is conducted by the reaction of a suitably protected nucleonomer containing an H-phosphonate moiety at a position to be coupled with a solid phase-derivatized nucleonomer chain having a free hydroxyl group, in the presence of a suitable activator to obtain an H-phosphonate diester linkage, which is stable to acid. Thus, the oxidation to the phosphate or thiophosphate can be conducted at any point during the synthesis of the oligomer or after synthesis of the oligomer is complete. The H-phosphonates can also be converted to phosphoramidate derivatives by reaction with a primary or secondary amine.
in the presence of carbon tetrachloride. To indicate the two approaches generically, the incoming nucleomonomer is regarded as having a "coupling phosphite/phosphate" group.

Variations in the type of substitute linkage are achieved by, for example, using the methyl phosphonate precursors rather than the H-phosphonates per se, using thiol derivatives of the nucleomonomer moieties and generally by methods known in the art. Nonphosphorous based linkages such as the formacetal 3'-thioformacetal, 3'-amino and 5'-ether type linkages described above can also be used.

Thus, to obtain an oligomer segment which has a 3'-5' polarity, a nucleomonomer protected at the 5'-position and containing a coupling phosphite/phosphate group at the 3'-position is reacted with the hydroxyl at the 5'-position of a nucleomonomer coupled to a solid support through its 3'-hydroxyl. The resulting condensed oligomer is deprotected and the reaction repeated with an additional 5'-protected, 3'-phosphite/phosphate coupling nucleomonomer. Conversely, to obtain an oligomeric segment of 5'-3' polarity, a nucleomonomer protected in the 3'-position and containing a coupling phosphite/phosphate in the 5'-position is reacted with a oligomer or nucleomonomer attached to a solid support through the 5'-position, leaving the 3'-hydroxyl available to react. Similarly, after condensation of the incoming nucleomonomer, the 3'-group is deprotected and reacted with an additional 3'-protected, 5'-coupling nucleomonomer. The sequence is continued until the desired number of nucleonomomers have been added.

This oligomer chain elongation will proceed in conformance with a predetermined sequence in a series of condensations, each one of which results in the addition of another nucleomonomer. Prior to the addition of a
nucleonomer having a coupling phosphite/phosphate, the protecting group on the solid support-bound nucleonomer is removed. Typically, for example, removal of the commonly-employed dimethoxytrityl (DMT) group is done by treatment with 2.5% v/v dichloroacetic acid/dichloromethane, although 1% w/v trichloroacetic acid/dichloromethane or ZnBr₂-saturated nitromethane, are also useful. Other deprotection procedures suitable for other protecting groups will be apparent to those of ordinary skill in the art. The deprotected nucleonomer or oligomer bound to solid support is then reacted with the suitably protected nucleonomer containing a coupling phosphite/phosphate. After each cycle the carrier bound nucleonomer is preferably washed with anhydrous pyridine/acetonitrile (1:1, v/v), again deprotected, and the condensation reaction is completed in as many cycles as are required to form the desired number of congruent polarity internucleoside bonds which will be converted to phosphoramidites, phosphorothioates, phosphorothioates or phosphodiesters as desired.

In one embodiment, to provide the switchback linker, the incoming coupling, protected nucleonomer is provided in the opposite polarity to the support-bound oligomers. Thus, for example, where the support-bound oligomer is 3'→5', the deprotected 5'-hydroxyl is reacted with a 3'-protected, 5'-coupling monomer, and the synthesis continued with monomers coupled at the 5'-position and protected at the 3'-position.

In another embodiment, to provide the switchback linker, a dimer synthon containing the linker element having one end which is coupled for condensation (such as a hydrogen phosphonate) to the support-bound oligomer and another end which is a protected hydroxyl group (or protected thio group) is condensed onto the
support-bound oligomer. The linked dimer is condensed and deprotected using the same conditions as those used to condense and deprotect the protected nucleomonomer hydrogen phosphonate. Subsequent extension of the oligomer chain then uses nucleomonomer residues which are coupled and protected in the opposite manner from those used to synthesize the previous portion of the chain.

One approach to this synthesis, using a linker already derivatized to two nucleomonomer residues which will be included in each portion of the strand is illustrated as follows. The 5'→3' nucleomonomer portion of the strand is coupled using the 3'-DMT-5'-coupling phosphate nucleonomomers, as conventionally, to solid support. The switchback linker is derivatized to two nucleomonomer residues through their 3' positions; the remaining 5' positions are derivatized by the protecting group DMT in one nucleomonomer residue and a phosphonate residue in the other. The derivatized linker is coupled to the solid supported strand under standard reagent conditions and then deprotected conventionally. Further standard nucleomonomer coupling results in extension of the chain in the 3'→5' orientation.

A particularly preferred dimer synthon used to mediate the switchback in an oligomer is the O-xylosyl linker (Figure 26). The O-xylosyl linker consists of two xylose-nucleomonomers linked to each other by o-xylene at the 3' position of each xylose sugar. The switchback linker synthon was synthesized using α,α'-orthodibromomaxyylene and 5'-DMT nucleomonomer to give the dimer as shown in Figure 26. The dimer was converted to the H-phosphonate and was used in solid phase synthesis to generate oligomers. Linkers containing the bases thymine, 5-methylcytosine, 5-(1-propynyl)uracil or cytosine were synthesized as homodimers. However, the
switchback linker dimers can also be synthesized as mixed heterodimers that are separated chromatographically.

2' Modified Oligomers


In an additional use of substitute linkages of the invention, 2'-O-allyl modified sugar forms of the nucleomonomers can be included in the oligomer. The 2'-O-allyl nucleomonomers can be prepared using standard methods.

The nucleomonomers derivatized at the 2'-position can be incorporated into oligomers in the same manner as underderivatized forms.
Synthesis


Oligomers containing nonphosphorous based substitute linkages that have been previously described in commonly owned pending applications nos. 07/874,334, PCT/US90/06110 and PCT/US91/06855 are preferably synthesized using suitably blocked dimer synths as a starting material. Oligomers containing linkages of the present invention are also conveniently synthesized by preparation of dimer or trimer compounds by solution phase chemistry followed by conversion of the synthon to a derivative that is incorporated into oligomers by either solid or solution phase chemistry. Typical synths are 5' DMT or MMT blocked 3' phosphonate or
phosphoramide derivatives which are prepared by
standard methods (see: Gait, M.J. ed., *Oligonucleotide
Oxford).

Oligomers having phosphorous-containing
linkages or segments thereof are conventionally
synthesized. Methods known in the art and described
herein can be used to synthesize oligomers containing
bases of the invention, as well as other bases known in
the art, using appropriately protected nucleosmonomers
(see Figure 12). Methods for the synthesis of oligomers
are found, for example, in Froehler, B. et al., *Nucleic
16:4831-4839; *Nucleosides and Nucleotides* (1987) 6:287-
5578; Caruthers, M. H. in *Oligodeoxynucleotides-Antisense
Inhibitions of Gene Expression* (1989), J. S. Cohen,
editor, CRC Press, Boca Raton, p7-24; Reese, C.B. et al,
the methylphosphonate linked oligomers via methyl
phosphonamidite chemistry has also been described
3542; Klem, R. E., et al, International Publication
Number WO 92/07864).

Synthons that are included in the scope of the
present invention include (i) dimers disclosed in general
structural formulas I through XI and (ii) dimers, trimers
and longer oligomers made by solid or solution phase
synthesis. Trimers and longer synthons may contain more
than one type of linkage. The synthons may include any
purine, pyrimidine or analogs thereof as described above
or 2’, 3’ and 5’ groups such as OH, DMTO, MMTO, O-allyl,
phosphate, a phosphonate or an amide as described
above.
Although the linkages of the invention are conveniently incorporated into oligomers using dimer or longer synthons, oligomer synthesis may be accomplished most efficiently using solid phase synthesis methods. Solid phase generation of the linkages of the invention is illustrated in Figure 1.
Utility and Administration

As the oligomers of the invention are capable of significant single-stranded or double-stranded target nucleic acid binding activity to form duplexes, triplexes or other forms of stable association, these oligomers are useful in diagnosis and therapy of diseases that are associated with expression of one or more genes such as those associated with pathological conditions. Therapeutic applications can employ the oligomers to specifically inhibit the expression of genes (or inhibit translation of RNA sequences encoded by those genes) that are associated with either the establishment or the maintenance of a pathological condition. Exemplary genes or RNAs encoded by those genes that can be targeted include those that encode enzymes, hormones, serum proteins, transmembrane proteins, adhesion molecules (LFA-1, GPIIb/IIIa, ELAM-1, VACM-1, ICAM-1, E-selectin, and the like), receptor molecules including cytokine receptors, cytokines (IL-1, IL-2, IL-3, IL-4, IL-6 and the like), oncogenes, growth factors, and interleukins. Target genes or RNAs can be associated with any pathological condition such as those associated with inflammatory conditions, cardiovascular disorders, immune reactions, cancer, viral infections, bacterial infections and the like.

Oligomers of the present invention are suitable for use in both in vivo and ex vivo therapeutic applications. Indications for ex vivo use include treatment of cells such as bone marrow or peripheral blood in conditions such as leukemia (chronic myelogenous leukemia, acute lymphocytic leukemia) or viral infection. Target genes or RNAs encoded by those genes that can serve as targets for cancer treatments include oncogenes, such as ras, k-ras, bcl-2, c-myc, bcr, c-myc, c-abl or overexpressed sequences such as mdm2, oncostatin M, IL-6.
(Kaposi's sarcoma), HER-2 and translocations such as bcr/abl. Viral gene sequences or RNAs encoded by those genes such as polymerase or reverse transcriptase genes of herpesviruses such as CMV, HSV-1, HSV-2, retroviruses such as HTLV-1, HIV-1, HIV-2, or other DNA or RNA viruses such as HBV, HPV, VZV, influenza virus, rhinovirus and the like are also suitable targets. Application of specifically binding oligomers can be used in conjunction with other therapeutic treatments. Other therapeutic indications for oligomers of the invention include (1) modulation of inflammatory responses by modulating expression of genes such as IL-1 receptor, IL-1, ICAM-1 or E-Selectin that play a role in mediating inflammation and (2) modulation of cellular proliferation in conditions such as arterial occlusion (restenosis) after angioplasty by modulating the expression of (a) growth or mitogenic factors such as non-muscle myosin, myc, fos, PCNA, PDGF or FGF or their receptors, or (b) cell proliferation factors such as c-myb. Other suitable proliferation factors or signal transduction factors such as TGFα, IL-6, γINF, protein kinase C, tyrosine kinases (such as p210, p190), may be targeted for treatment of psoriasis or other conditions. In addition, EGF receptor, TGFα or MHC alleles may be targeted in autoimmune diseases.

Delivery of oligomers of the invention into cells can be enhanced by any suitable method including calcium phosphate, DMSO, glycerol or dextran transfection, electroporation or by the use of cationic anionic and/or neutral lipid compositions or liposomes by methods described (International Publication Nos. WO 90/14074, WO 91/16024, WO 91/17424, U.S. Patent 4,897,355). The oligomers can be introduced into cells by complexation with cationic lipids such as DOTMA (which may or may not form liposomes) which complex is then
contacted with the cells. Suitable cationic lipids include but are not limited to N-(2,3-di(9-(Z)-octadecenyl oxy)l)prop-1-yl-N,N,N-trimethylammonium (DOTMA) and its salts, 1-O-oleyl-2-O-oleyl-3-dimethylaminopropyl-8-hydroxyethylammonium and its salts and 1,2-bis(olexyloxy)-3-(trimethylammonio) propane and its salts.

Enhanced delivery of the invention oligomers can also be mediated by the use of (i) viruses such as Sendai virus (Bartzatt, R., Biotechnol Appl Biochem (1989) 11:133-135) or adenovirus (Wagner, E., et al, Proc Natl Acad Sci (1992) 89:6099-6013; (ii) polyamine or polycation conjugates using compounds such as polylysine, protamine or N1, N12-bis(ethyl)spermine (Wagner, E., et al, Proc Natl Acad Sci (1991) 88:4255-4259; Zenke, M., et al, Proc Natl Acad Sci (1990) 87:3655-3659; Chank, B.K., et al, Biochem Biophys Res Commun (1988) 157:264-270; U.S. Patent 5,138,045); (iii) lipopolyamine complexes using compounds such as lipospermine (Behr, J.-P., et al, Proc Natl Acad Sci (1989) 86:6982-6986; Loeffler, J.P., et al J Neurochem (1990) 54:1812-1815); (iv) anionic, neutral or pH sensitive lipids using compounds including anionic phospholipids such as phosphatidyl glycerol, cardiolipin, phosphatidic acid or phosphatidylethanolamine (Lee, K.-D., et al, Biochim Biophys ACTA (1992) 1103:185-197; Cheddar, G., et al, Arch Biochem Biophys (1992) 294:188-192; Yoshimura, T., et al, Biochem Int (1990) 20:697-706); (v) conjugates with compounds such as transferrin or biotin or (vi) conjugates with compounds such as serum proteins (including albumin or antibodies), glycoproteins or polymers (including polyethylene glycol) that enhance pharmacokinetic properties of oligomers in a subject. As used herein, transfection refers to any method that is suitable for delivery of oligomers into cells. Any
reagent such as a lipid or any agent such as a virus that can be used in transfection protocols is collectively referred to herein as a "permeation enhancing agent". Delivery of the oligomers into cells can be via cotransfection with other nucleic acids such as (i) expressable DNA fragments encoding a protein(s) or a protein fragment or (ii) translatable RNAs that encode a protein(s) or a protein fragment.

The oligomers can thus be incorporated into any suitable formulation that enhances delivery of the oligomers into cells. Suitable pharmaceutical formulations also include those commonly used in applications where compounds are delivered into cells or tissues by topical administration. Compounds such as polyethylene glycol, propylene glycol, azone, nonoxynyl-9, oleic acid, DMSO, polyamines or lipopolyamines can be used in topical preparations that contain the oligomers.

The invention oligomers can be conveniently used as reagents for research or production purposes where inhibition of gene expression is desired. There are currently very few reagents available that efficiently and specifically inhibit the expression of a target gene by any mechanism. Oligomers that have been previously reported to inhibit target gene expression frequently have nonspecific effects and/or do not reduce target gene expression to very low levels (less than about 40% of uninhibited levels).

Thus, the oligomers as described herein constitute a reagent that can be used in methods of inhibiting expression of a selected protein or proteins in a subject or in cells wherein the proteins are encoded by DNA sequences and the proteins are translated from RNA sequences, comprising the steps of: introducing an oligomer of the invention into the cells; and permitting the oligomer to form a triplex with the DNA or RNA or a
duplex with the DNA or RNA whereby expression of the protein or proteins is inhibited. The methods and oligomers of the present invention are suitable for modulating gene expression in both procaryotic and eucaryotic cells such as bacterial, fungal parasite, yeast and mammalian cells.

RNase H "competent" or RNase H "incompetent" oligomers can be easily designed using the substitute linkages of the invention. RNase H competent oligomers can comprise one or more RNase H competent domains comprised of linked RNase H competent nucleonomers. Oligomers having modifications such as 2'-substitutions (2'-O-allyl and the like) or certain uncharged linkages (methylphosphonate, phosphoramidate and the like) are usually incompetent as a substrate that is recognized by and/or acted on by RNase H. RNase H competence can facilitate antisense oligomer function by degrading the target RNA in an RNA-oligomer duplex (Dagle, J.M., et al, *Nucl Acids Res* (1990) 18:4751-4757; Walder, J.A. et al, International Publication Number WO 89/05358). The enzyme cleaves RNA in RNA-DNA duplexes.

In order to retain RNase H competence, an oligomer requires a RNase H competent domain of three or more competent contiguous nucleonomers located within it (Quartin, R.S., et al, *Nucl Acids Res* (1989) 17:7253-7262). Design of oligomers resistant to nuclease digestion will have terminal linkage, sugar and/or base modifications to effect nuclease resistance. Thus, the oligomers can be designed to have modified nucleonomer residues at either or both the 5'- and/or 3'-ends, while having an internal RNase H competent domain.

Exemplary oligomers that retain RNase H competence would generally have uniform polarity and would comprise about 2 to about 12 nucleonomers at the 5'-end and at the 3'-end which stabilize the oligomer
to nuclease degradation and about three to about 26 nucleomonomers that function as a RNase H competent domain between the RNase H incompetent 3' and 5' ends. Variations on such an oligomer would include (1) a shorter RNase H competent domain comprising 1 or 2 RNase H competent linkages or substitute linkages, (2) a longer RNase H incompetent domain comprising up to 15, 20 or more substitute linkages or nucleomonomers, (3) a longer RNase H competent domain comprising up to 30, 40 or more linkages, (4) oligomers with only a single RNase H incompetent domain at the 3' end or at the 5' end, or (5) oligomers having more than one RNase H competent domain. RNase H competence also applies as a consideration to oligomers having one or more regions of inverted polarity, to circular oligomers and to other types of oligomers.

Oligomers containing as few as about 8 nucleomonomers can be used to effect inhibition of target protein(s) expression by formation of duplex or triplex structures with target nucleic acid sequences. However, linear oligomers used to inhibit target protein expression via duplex or triplex formation will preferably have from about 10 to about 20 nucleomonomer residues.

Oligomers containing substitute linkages of the invention can be conveniently circularized as described (International Publication No. WO 92/19732; Kool, E.T. J Am Chem Soc (1991) 113:6265-6266; Prakash, G., et al. J Am Chem Soc (1992) 114:3523-3527). Such oligomers are suitable for binding to single-stranded or double-stranded nucleic acid targets. Circular oligomers can be of various sizes. Such oligomers in a size range of about 22-50 nucleomonomers can be conveniently prepared. The circular oligomers can have from about three to about six nucleomonomer residues in the loop region that
separate binding domains of the oligomer as described (Prakash, G. ibid). Oligomers can be enzymatically circularized through a terminal phosphate by ligase or by chemical means via linkage through the 5’- and 3’- terminal sugars and/or bases.

The oligomers can be utilized to modulate target gene expression by inhibiting the interaction of nucleic acid binding proteins responsible for modulating transcription (Maher, L. J., et al, Science (1989) 245:725-730) or translation. The oligomers are thus suitable as sequence-specific agents that compete with nucleic acid binding proteins (including ribosomes, RNA polymerases, DNA polymerases, translational initiation factors, transcription factors that either increase or decrease transcription, protein-hormone transcription factors and the like). Appropriately designed oligomers can thus be used to increase target protein synthesis through mechanisms such as binding to or near a regulatory site that transcription factors use to repress expression or by inhibiting the expression of a selected repressor protein itself.

The invention oligomers, comprising additional modifications that enhance binding affinity can be designed to contain secondary or tertiary structures, such as pseudoknots or pseudo-half-knots (Ecker, D.J., et al, Science (1992) 257:958-961). Such structures can have a more stable secondary or tertiary structure than corresponding unmodified oligomers. The enhanced stability of such structures would rely on the increased binding affinity between regions of self complementarity in a single oligomer or regions of complementarity between two or more oligomers that form a given structure. Such structures can be used to mimic structures such as the HIV TAR structure in order to interfere with binding by the HIV Tat protein (a protein
that binds to TAR). A similar approach can be utilized with other transcription or translation factors that recognize higher nucleic acid structures such as stems, loops, hairpins, knots and the like. Alternatively, the invention oligomers can be used to (1) disrupt or (2) bind to such structures as a method to (1) interfere with or (2) enhance the binding of proteins to nucleic acid structures.

In addition to their use in antisense or triple helix therapies, the oligomers of the invention can also be applied as therapeutic or diagnostic agents that function by direct displacement of one strand in a duplex nucleic acid. Displacement of a strand in a natural duplex such as chromosomal DNA or duplex viral DNA, RNA or hybrid DNA/RNA is possible for oligomers with a high binding affinity for their complementary target sequences. Therapeutic applications of oligomers by this method of use, referred to herein as D-looping or "D-loop therapy" has not previously been possible because the affinity of natural DNA or RNA for its complementary sequence is not great enough to efficiently displace a DNA or RNA strand in a duplex. Therapeutic efficacy of oligomers that function by D-looping would result from high affinity binding to a complementary sequence that results in modulation of the normal biological function associated with the nucleic acid target. Types of target nucleic acids include but are not limited to (i) gene sequences including exons, introns, exon/intron junctions, promoter/enhancer regions and 5' or 3' untranslated regions, (ii) regions of nucleic acids that utilize secondary structure in order to function (e.g. the HIV TAR stem-loop element or tRNAs), (iii) nucleic acids that serve structural or other functions such as telomeres, centromeres or replication origins (virus, bacteria and the like) and (iv) any other duplex region.
It is clear that oligomers can be synthesized with discrete functional domains wherein one region of an oligomer binds to a target by D-looping while an adjacent region binds a target molecule by say, forming a triple helix or binding as an aptamer to a protein. Alternatively, a D-looping oligomer can bind to each strand in a duplex by switching the strand to which the oligomer binds (i.e. by having one region of the oligomer that binds to one strand and another region that binds to the complementary strand). The controlling elements that dictate the mode of binding (i.e. triple helix or D-loop) are the sequence of the oligomer and the inherent affinity built into the oligomer. Base recognition rules in Watson-Crick duplex binding differ from those in Hoogsteen controlled triplex binding. Because of this, the oligomer base sequence can be used to dictate the type of binding rules an oligomer will utilize.

D-loop structures are formed in nature by enzyme-mediated processes (Harris, L.D. et al., J Biol Chem (1987) 262: 9285-9292) or are associated with regions where DNA replication occurs (Jacobs, H.T. et al., Nucl Acids Res (1989) 17:8949-8966). D-loops that arise from the binding of oligomers can result from a one or two step process. Direct displacement of a target strand will give rise to a D-loop by a single binding event. However, D-looping can also occur by forming a triple helix which facilitates a strand displacement event leading to the D-loop.

Ribozymes containing substitute linkages of the invention can be designed in order to design species with altered characteristics. Ribozymes that cleave single stranded RNA or DNA (Robertson, D.L., et al Nature (1990) 344:467-468) have been described. Therapeutic applications for ribozymes have been postulated (Sarver, N. et al, Science (1990) 247:1222-1225; International
Publication Number WO 91/04319). Secondary or tertiary structure necessary for ribozyme function can be affected by design of appropriate oligomer sequences. For example, ribozymes having nuclease stable targeting domains containing substitute linkages of the invention can have higher affinity, while maintaining base pairing specificity, for target sequences. Because of the higher affinity and/or nuclease stability of the invention substitute linkages shorter recognition domains in a ribozyme (an advantage in manufacturing) can be designed which can lead to more favorable substrate turnover (an advantage in ribozyme function).

In therapeutic applications, the oligomers are utilized in a manner appropriate for treatment of a variety of conditions by inhibiting expression of appropriate target genes. For such therapy, the oligomers can be formulated for a variety of modes of administration, including systemic, topical or localized administration. Techniques and formulations generally can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, latest edition. The oligomer active ingredient is generally combined with a carrier such as a diluent or excipient which can include fillers, extenders, binders, wetting agents, disintegrants, surface-active agents, or lubricants, depending on the nature of the mode of administration and dosage forms. Typical dosage forms include tablets, powders, liquid preparations including suspensions, emulsions and solutions, granules, capsules and suppositories, as well as liquid preparations for injections, including liposome preparations.

For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the oligomers of the invention are formulated in liquid
solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the oligomers can be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included. Dosages that can be used for systemic administration preferably range from about 0.01 mg/Kg to 50 mg/Kg administered once or twice per day. However, different dosing schedules can be utilized depending on (i) the potency of an individual oligomer at inhibiting the activity of its target DNA or RNA, (ii) the severity or extent of a pathological disease state associated with a given target gene, or (iii) the pharmacokinetic behavior of a given oligomer.

Systemic administration can also be by transmucosal or transdermal means, or the compounds can be administered orally. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, bile salts and fusidic acid derivatives for transmucosal administration. In addition, detergents can be used to facilitate permeation. Transmucosal administration can be through use of nasal sprays, for example, or suppositories. For oral administration, the oligomers are formulated into conventional oral administration forms such as capsules, tablets, and tonics.

For topical administration, the oligomers of the invention are formulated into ointments, salves, gels, or creams, as is generally known in the art.

Formulation of the invention oligomers for ocular indications such as viral infections would be based on standard compositions known in the art.

In addition to use in therapy, the oligomers of the invention can be used as diagnostic reagents to detect the presence or absence of the target nucleic acid
sequences to which they specifically bind. The enhanced binding affinity of the invention oligomers is an advantage for their use as primers and probes. Diagnostic tests can be conducted by hybridization through either double or triple helix formation which is then detected by conventional means. For example, the oligomers can be labeled using radioactive, fluorescent, or chromogenic labels and the presence of label bound to solid support detected. Alternatively, the presence of a double or triple helix can be detected by antibodies which specifically recognize these forms. Means for conducting assays using such oligomers as probes are generally known.

The use of oligomers containing the invention substitute linkages as diagnostic agents by triple helix formation is advantageous since triple helices form under mild conditions and the assays can thus be carried out without subjecting test specimens to harsh conditions. Diagnostic assays based on detection of RNA for identification of bacteria, fungi or protozoa sequences often require isolation of RNA from samples or organisms grown in the laboratory, which is laborious and time consuming, as RNA is extremely sensitive to ubiquitous nucleases.

The oligomer probes can also incorporate additional modifications such as modified sugars and/or substitute linkages that render the oligomer especially nuclease stable, and would thus be useful for assays conducted in the presence of cell or tissue extracts which normally contain nuclease activity. Oligomers containing terminal modifications often retain their capacity to bind to complementary sequences without loss of specificity (Uhlmann et al., Chemical Reviews (1990) 90:543-584). As set forth above, the invention probes can also contain linkers that permit specific binding to

Incorporation of base analogs of the present invention into probes that also contain covalent crosslinking agents has the potential to increase sensitivity and reduce background in diagnostic or detection assays. In addition, the use of crosslinking agents will permit novel assay modifications such as (1) the use of the crosslink to increase probe discrimination, (2) incorporation of a denaturing wash step to reduce background and (3) carrying out hybridization and crosslinking at or near the melting temperature of the hybrid to reduce secondary structure in the target and to increase probe specificity. Modifications of hybridization conditions have been previously described (Gamper et al., Nucleic Acids Res (1986) 14:9943).

Oligomers of the invention are suitable for use in diagnostic assays that employ methods wherein either the oligomer or nucleic acid to be detected are covalently attached to a solid support as described (U.S. Patent No. 4,775,619). The oligomers are also suitable for use in diagnostic assays that rely on polymerase chain reaction techniques to amplify target sequences according to described methods (European Patent Publication No. 0 393 744). Oligomers of the invention containing a 3' terminus that can serve as a primer are compatible with polymerases used in polymerase chain reaction methods such as the Taq or Vent™ (New England Biolabs) polymerase. Oligomers of the invention can thus be utilized as primers in PCR protocols.

The oligomers are useful as primers that are discrete sequences or as primers with a random sequence.
Random sequence primers can be generally about 6, 7, or 8 nucleotides in length. Such primers can be used in various nucleic acid amplification protocols (PCR, ligase chain reaction, etc) or in cloning protocols. The substitute linkages of the invention generally do not interfere with the capacity of the oligomer to function as a primer. Oligomers of the invention having 2' modifications at sites other than the 3' terminal residue, other modifications that render the oligomer RNase H incompetent or otherwise nuclease stable can be advantageously used as probes or primers for RNA or DNA sequences in cellular extracts or other solutions that contain nucleases. Thus, the oligomers can be used in protocols for amplifying nucleic acid in a sample by mixing the oligomer with a sample containing target nucleic acid, followed by hybridization of the oligomer with the target nucleic acid and amplifying the target nucleic acid by PCR, LCR or other suitable methods.

The oligomers derivatized to chelating agents such as EDTA, DTPA or analogs of 1,2-diaminocyclohexane acetic acid can be utilized in various in vitro diagnostic assays as described (U.S. Patent Nos. 4,772,548, 4,707,440 and 4,707,352). Alternatively, oligomers of the invention can be derivatized with crosslinking agents such as 5-(3-iodoacetamidoprop-1-yl)-2'-deoxyuridine or 5-(3-(4-bromobutyramidino)prop-1-yl)-2'-deoxyuridine and used in various assay methods or kits as described (International Publication No. WO 90/14353).

In addition to the foregoing uses, the ability of the oligomers to inhibit gene expression can be verified in in vitro systems by measuring the levels of expression in subject cells or in recombinant systems, by any suitable method (Graessmann, M., et al., Nucleic Acids Res (1991) 19:53-59).
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All references cited herein are incorporated herein by reference in their entirety.

The following examples are intended to illustrate, but not to limit, the invention. Efforts have been made to insure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental errors and deviations should be taken into account. Unless indicated otherwise, parts are parts by weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1

Synthesis of 5'-DMT-T-Riboacetal-T-3'-H-
Phosphonate Dimer Synthon

A TT riboacetal dimer was synthesized as shown in Figure 1. The starting material 7'-carboxyl-3't-butyl dimethyl silyl thymidine was obtained as described in pending application no. 763,130 and converted to the aldehyde as shown. The TT dimer was purified by silica gel chromatography and used in solid phase oligomer synthesis using H-phosphonate chemistry.

Example 2

Synthesis of Oligomers Containing Riboacetal Linkages and Their binding to Duplex DNA

The dimer of Example 1 was incorporated into oligomer ODN-2 with the following sequence. ODN-1 was the control oligomer with diester linkages.

ODN-1  5'TCTCTCTCTTTTT  3'
ODN-2  5'TCTCTCTCTTT*TT  3'

Base residues designated C corresponded to 5-methylcytosine, T corresponded to thymine and * indicates the location of the riboacetal linkage. The oligomers were hybridized with duplex DNA containing the target
sequence 5' AGAGAGAGAGAGAAA 3'. Hybridization was carried out in 140 mM KCl, 5 mM MgCl₂, 5 mM Na₂HPO₄, pH 6.6. The oligomer bound to the target sequence in a parallel triplex binding motif as described in pending application number 643,382. Thermal stability (Tₘ) of the resulting triplex helix formed between each oligomer and the target sequence was determined. All DNAs and oligomers were present at approximately 1 µM. The Tₘ of ODN-1 was 44.0°C and 51.4°C for ODN-2. The Tₘ value associated with oligomers containing riboacetal linkages was greater than that of the control oligomers.

Additionally, the dimers T*T and T*C bearing the riboacetal substitute linkage were incorporated in ODN 3 having seven riboacetal linkages and the following sequence ODN 3 5' T*CT*CT*CT*CT*CTT*TT 3'. The affinity of ODN 3 for duplex DNA was determined by a footprint assay as described (Matteucci, M. et al J Am Chem Soc (1991), 113:7767–7768). The control ODN 1 gave complete binding at 1 µM while ODN 3 gave complete protection at 0.01 µM, demonstrating approximately 100-fold enhancement of affinity relative to the diester control. ODN 3 was also analyzed by thermal melting using the buffer as described above except with 1 mM MgCl₂ at pH 6.6 and at pH 7.0. At pH 6.6, ODN 3 had a triple helix transition at 67°C while control ODN 1 had a transition at 40°C. At pH 7.0, ODN 3 had a transition at 60°C and ODN 1 had a transition at 34°C. These results again demonstrated the higher binding affinity of oligomers containing riboacetal linkages relative to control oligomers.
Example 3

**Binding of Oligomer Containing Riboacetal Linkages to Single-Stranded DNA and RNA**

ODN-1 and ODN-2 were hybridized with the complementary single-stranded sequence through antiparallel Watson-Crick binding to the following oligonucleotide target DNA sequence 5' AAAAAAGAGAGAGAGAG 3' or RNA sequence 5' AAAAAAGAGAGAGAGAGA 3'. The T_m of the control oligomer ODN-1 was 49.0°C on DNA while the T_m of ODN-2 was 49.5°C on DNA. Control oligomer ODN-1 on RNA was 63.5°C and ODN-2 was 61.5°C. Buffer conditions for both experiments were 140 mM KCl, 5 mM Na_2HPO_4, 1 mM MgCl_2, pH 6.6, and DNA or RNA were present at approximately 1 μM concentration.

Example 4

**Solid Phase Incorporation of Riboacetal Linkages into Oligomers**

Solid phase incorporation of riboacetal linkages into oligomers is shown in Figure 2. Elongation of the oligomer chain in a 5' to 3' direction is used to obtain a fully or partially substituted oligomer. As shown in the figure, synthesis is initiated by coupling of suitably blocked monomer to a suitable support through the 5' position by reaction between support and protected-ribothymidine. The ester linkage shown may be varied as described. The blocking group (BL) indicated in Figure 2 may be phenoxyacetyl ester which is deblocked by mild ammonia treatment or the blocking group may be FMOC carbonates which are removed by piperidine treatment as described (Green, T.W. et al. *Protective Groups in Organic Synthesis* (1991) second edition, Wiley & Sons, New York). Chain elongation would proceed by repeating the reaction sequence.
Example 5

Synthesis of 5'-DMT-T-Oxoacetal-T-3'-H-
Phosphonate Dimer Synthon

A TT oxoacetal dimer was synthesized as shown in Figure 3. The starting material thymidine enol ether was obtained as described (Zemlica, J. et al., J Am Chem Soc (1972) 94:3213-3218). This was treated with the alcohol and MCPBA essentially as described (Kim, C.U. et al., J Med Chem (1991) 34:2286-2294).

Example 6

Synthesis of 5'-DMT-T-Thioriboacetal-T-3'-H-
Phosphonate Dimer Synthon

Synthesis of a TT thioriboacetal dimer is shown in Figure 4. The starting material thymidine enol ether is obtained as described by Zemlica, et al. above and converted as shown.

Example 7

Synthesis of 5'-DMT-T-Dioxa Azepine-T-3'-H-
Phosphonate Dimer

Synthesis of a TT dioxa azepine dimer containing the 7-member ring structure is shown in Figure 5. The starting material is obtained from ribothymidine and converted as shown. The 5'-amino-T compound was obtained as described in application no. 763,130.

Example 8

Synthesis of 5'-DMT-T-Carbamate Aminal-T-3'-H-
Phosphonate Dimer

Synthesis of a TT carbamate aminal dimer containing the 7-member ring structure is shown in Figure
6. The first step of the reaction has been previously described (Markiewicy, W.T. et al Nucl Acids Res. Spec Publ (1978) 4:S185). The starting material is obtained from ribothymidine and converted as shown.

Example 9

Synthesis of 5’ DMT-T-MethylRiboacetal-T-3’-H-Phosphonate Dimer

Synthesis of a TT methylriboacetal dimer containing the 5-member ring structure is shown in Figure 7. The starting material is obtained as described in application no. 763,130 and converted as shown.

Example 10

Synthesis of 5’ DMT-T-Oxa Amine-T-3’-H-Phosphonate Dimer

Synthesis of a TT oxa amine dimer containing the 5-member ring structure is shown in Figure 8. The starting material is obtained as described in application no. 763,130 and converted as shown. A mixture of 2 aminal linkage types is obtained. The isomers may be separated by chromatography. The TBS blocked compound is converted to the 5’ DMT 3’ H-phosphonate by reaction with (i) NaOMe, (ii) DMT/pyridine, (iii) TBAF and (iv) phosphytylation.
Example 11
Synthesis of 5’ DMT-T-Cyclic Carbamate-T-3’-H-
Phosphonate Dimer

Synthesis of a TT cyclic carbamate dimer containing the 6-member ring structure is shown in Figure 9. The starting material is obtained as described in application no. 763,130 and converted as shown. The TBS blocked compound is converted to the 5’ DMT 3’ H-phosphonate by reaction with (i) NaOMe, (ii) DMT/pyridine, (iii) TBAF and (iv) phosphitylation.

Example 12
Synthesis of 5’ DMT-T-Nor cyclic Carbamate-T-3’-H-
Phosphonate Dimer

Synthesis of a TT nor cyclic carbamate dimer containing the 6-member ring structure is shown in Figure 10. The starting material is obtained as described in application no. 763,130 and converted as shown. The TBS blocked compound is converted to the 5’ DMT 3’ H-phosphonate by reaction with (i) NaOMe, (ii) DMT/pyridine, (iii) TBAF and (iv) phosphitylation.

Example 13
Synthesis of 5’ DMT-T-Ribothioacetal-T-3’-H-
Phosphonate Dimer

Synthesis of a TT ribothioacetal dimer containing the 5-member ring structure is shown in Figure 11. The starting material is obtained from ribothymidine and converted as shown.
Example 14

Synthesis of 5'-DMT-T-Riboaminal-T-3'-H-
Phosphonate Dimer

Synthesis of a TT riboaminal dimer containing
the 5-member ring structure is shown in Figure 12. The
starting material is obtained from ribothymidine
and converted as shown.

Example 15

Synthesis of 5'-DMT-T-Cyclic Aminal-T-3'-H-
Phosphonate Dimer

Synthesis of a TT cyclic aminal dimer
containing the 6-member ring structure is shown in Figure
13. The starting material is obtained as described in
application no. 763,130 and converted as shown. The TBS
blocked compound is converted to the 5'-DMT 3'-H-
phosphonate by reaction with (i) NaOMe, (ii)
DMT/pyridine, (iii) TBAF and (iv) phosphitylation.

Example 16

Synthesis of 5'-DMT-T-Norcyclic Aminal-T-3'-H-
Phosphonate Dimer

Synthesis of a TT norcyclic aminal dimer
containing the 6-member ring structure is shown in Figure
14. The starting material is obtained as described in
Example 12 and converted as shown. The TBS blocked
compound is converted to the 5'-DMT 3'-H-phosphonate by
reaction with (i) NaOMe, (ii) DMT/pyridine, (iii) TBAF
and (iv) phosphitylation.
Example 17

Experimental for Figure 15

3'-O-Phenoxyacetylthymidine (1). To a solution of 5'-O-dimethoxytritylthymidine (27.23 g, 50.0 mmol) in pyridine (100 mL) at 0°C was added phenoxyacetyl chloride (8.23 mL, 62.5 mL) dropwise, and the mixture was allowed to warm to ambient temperature over 3 hr. The reaction was quenched with methanol (ME, 25 mL) and concentrated in vacuo. The crude product was extracted with dichloromethane (DCM, 300 mL), washed with saturated aqueous sodium bicarbonate (SASB, 300 mL), dried (Na₂SO₄), and concentrated. Toluene (2 x 100 mL) was added, and the solution was concentrated. The residual oil was dissolved in 10% ME in DCM (275 mL) and treated with methanesulfonic acid (3.24 mL, 50.0 mmol). After 30 min the red solution was quenched with SASB (300 mL), and the organic layer was dried (Na₂SO₄) and concentrated. The crude product was dissolved in 1:1 ethyl acetate (EA): hexanes (H) (250 mL) and precipitated by cooling to -10°C for 18 h. The mixture was filtered, and the precipitate dried under vacuum to afford 1 (11.4 g, 60.6%). ¹H NMR δ

¹³C NMR (D₆ DMSO) δ 12.25, 36.41, 61.29, 64.59, 75.57, 83.68, 84.41, 109.75, 114.52, 121.23, 129.48, 135.77, 150.45, 157.54, 163.65, 168.42. HRMS (FAB) calcd.

C₁₁H₂₁N₂O₇ (MH⁺) 377.1349; found 377.1355.

N4-benzoyl-5-methyl-2'-deoxy-3'-O-phenoxyacetylcytidine (2). The title compound was prepared in a manner analogous to that described for 1. ¹H NMR (CDCl₃) δ 13.22 (bs, 1H), 8.29 (d, 2H, J = 7.0 Hz), 7.70 (s, 1H), 7.52 (t, 1H, J = 7.1 Hz), 7.43 (t, 2H, J = 7.4 Hz), 7.31 (t, 2H, J = 7.8 Hz), 7.01 (t, 1H, J = 7.0 Hz), 6.92 (d, 2H, J = 7.8 Hz), 6.22 (t, 1H, J = 7.1 Hz), 5.50 (M, 1H), 4.67 (s, 2H), 4.14 (d, 1H, J = 2.1 Hz), 3.93 (dq, 2H, J = 2.3, 11.9 Hz), 3.00 (bs, 1H), 2.46 (m,
2H), 2.07 (s, 3H). $^{13}$C NMR (CDCl$_3$) $\delta$ 168.71, 159.47, 157.44, 148.05, 137.42, 136.85, 132.52, 129.83, 129.60, 128.09, 121.95, 114.53, 112.27, 86.39, 85.11, 75.78, 65.08, 62.32, 37.39, 13.62. HRMS (FAB) for C$_{23}$H$_{26}$N$_{3}$O$_{7}$ (MH$^+$) calcd. 480.1771, found 480.1778.

3'-O-phenoxyacetyl-1,2,5,6-tetradeoxy-1-(thymin-1-yl)-β-D-erythro-hept-5-enofuranuronal (3). To a solution of 1 (9.60 g, 25.5 mmol), dimethylsulfoxide (100 mL), and dicyclohexylcarbodiimide (10.5 g, 51.0 mmol) was added dichloroacetic acid (1.05 mL, 12.8 mmol). After stirring for 30 min, brine (100 mL) and ethyl acetate (EA, 200 mL) were added. The mixture was stirred vigorously for 15 min and filtered through a celite pad. To the filtrate was added SASB (100 mL). The layers were separated and the organic layer was dried (Na$_2$SO$_4$), concentrated, and dried under vacuum. To this aldehyde was added tetrahydrofuran (THF, 100 mL) and formylmethylene triphenylphosphorane (6.99 g, 23.0 mmol) and the solution was stirred for 1 h. SASB (100 mL) and EA (100 mL) were added, and the organic layer was separated, dried (Na$_2$SO$_4$), and concentrated. Flash chromatography (H: EA 100:0 to 0:100 to ME:EA 2:98) delivered the product (4.82 g, 47%). (Montgomery J.A., Thomas, H.J., J. Org. Chem. 1981, 46:594, U.S. Patent No. 4,882,316).

3'-O-phenoxyacetyl-1,2,5,6-tetradeoxy-1-(N4-benzoyl-5-methylcytidin-1-yl)-β-D-erythro-hept-5-enofuranuronal (4). The title compound was prepared in a manner analogous to that described for 3. $^1$H NMR $\delta$ (CDCl$_3$) 2.14 (s, 3H), 2.38 (m, 1H), 2.55 (ddd, 1H, $J = 2.7$, 5.8, 14.4 Hz), 4.70 (m, 1H), 4.74 (s, 2H), 5.32 (m, 1H), 6.29 (dd, 1H, $J = 5.9$, 8.0 Hz), 6.38 (ddd, 1H, $J = 1.4$, 8.0, 15.3 Hz), 6.92 (m, 2H), 6.95 (m, 1H), 7.04 (t, 1H, $J = 7.3$ Hz), 7.23 (s, 1H), 7.33 (m, 2H), 7.45 (t, 2H, $J = 7.3$ Hz), 7.55 (t, 1H, $J = 7.2$ Hz), 8.32 (d, 2H, $J = 2.7$ Hz), 8.54 (d, 2H, $J = 8.0$ Hz), 11.62 (s, 1H).
7.4 Hz, 9.64 (d, 1H, J = 8.4 Hz), 13.26 (bs, 1H).  
\(^{13}\)C NMR \(\delta\) (CDCl\(_3\)) HRMS (FAB) for \(C_{27}H_{38}N_{3}O_{7}\) (MH\(^{+}\)) calcd. 504.1771, found 504.1765.

3'-O-Phenoxyacetyl-1,2,5,6-tetrae oxy-1-(thymin-1-yl)-\(\beta\)-D-erythro-heptofuranural (3a). A solution of 3 (4.80 g, 12.0 mmol) and EA (200 mL) was purged with nitrogen, and palladium on carbon (10\%, 480 mg) was added. The reaction was charged with hydrogen, evacuated and again charged with hydrogen. After stirring for 18 h under hydrogen (balloon), the mixture was filtered through celite, and the solid washed with EA (3 x 50 mL). The volatiles were removed under vaccum to afford the product 3a (4.62 g, 95.8\%).

3'-O-Phenoxyacetyl-1,2,5,6-tetrae oxy-1-(thymin-1-yl)-\(\beta\)-D-erythro-heptofuranural dimethylacetal (5). A solution of 3 (3.80 g, 9.44 mmol), MC (40 mL), trimethylorthoformate (5 mL), and pyridinium p-toluenesulfonate (50 mg, 0.2 mmol) was stirred for 18 h and concentrated. The residual oil was dissolved in MC (75 mL) and washed with SASB (75 mL). The organic layer was dried (Na\(_2\)SO\(_4\)) and concentrated, and the crude product was purified by flash chromatography (ME:MC 0:100 to 3:97) to provide 5 (3.07 g, 72.4\%).

3'-O-Phenoxyacetyl-1,2,5,6-tetrae oxy-1-(N\(^{4}\)-benzoyl-5-methylcytidin-1-yl)-\(\beta\)-D-erythro-hept-5 enofuranural dimethylacetal (6). 6 was prepared essentially as described above for compound 5.  
\(^{1}\)H NMR \(\delta\) (CDCl\(_3\)) 1.79 (m, 4H), 2.13 (s, 3H), 2.18 (m, 1H), 2.53 (ddd, 1H, J = 1.7, 5.6, 14.2 Hz), 3.33 (m, 1H), 3.34 (s, 3H), 3.35 (s, 3H), 4.05 (m, 1H), 4.41 (m, 1H), 4.68 (s, 2H), 5.17 (m, 1H), 6.22 (dd, 1H, J = 5.6, 8.2 Hz), 6.91 (d, 2H, J = 8.0 Hz), 7.02 (t, 1H, 7.4 Hz), 7.33 (m, 3H), 7.53 (t, 1H, J = 7.9 Hz), 8.31 (d, 2H, J = 7.8 Hz), 13.29 (bs, 1H).  
\(^{13}\)C NMR \(\delta\) (CDCl\(_3\)) HRMS (FAB) for \(C_{27}H_{38}N_{3}O_{7}\) (MH\(^{+}\)) calcd. 506.1927, found 506.1931.
3', 5'' Bisphenoxyacetyl T-T riboacetal. To a mixture of 7 (2.00 g, 5.09 mmol), 5 (1.77 g, 3.95 mmol) MC (25 mL), acetonitrile 15 mL), 4 Å molecular sieves (2 g) was added methanesulfonic acid (2.56 mL, 39.5 mmol). After 1 h, the mixture was filtered, and the filtrate was washed with SASB (75 mL), dried (Na₂SO₄), and concentrated. The crude product was purified by flash chromatography (ME:MC 0:100 to 4:96) to yield the product (2.00 g, 67%).

3', 5'' bisphenoxyacetyl T-BnC riboacetal. ¹H NMR δ (CDCl₃) 1.87 (s, 3H), 1.94 (m, 4H), 2.10 (s, 3H), 2.28 (m, 1H), 2.50 (ddd, 1H, J = 1.5, 5.6, 14.0 Hz), 4.07 (m 1H), 4.42 (m, 3H), 4.60 (dd, 1H, J = 2.9, 6.6 Hz), 4.66 (s, 3H), 4.68 (s, 3H), 4.76 (dd, 1H, J = 2.2, 6.5 Hz), 5.08 (m, 1H), 5.19 (m, 1H), 5.66 (d, 1H, J = 2.3 Hz), 6.20 (ddd, 1H, 5.8, 8.0 Hz), 6.88 (m, 4H), 7.00 (m, 2H), 7.08 (s, 1H), 7.30 (m, 5H), 7.45 (t 2H, J = 7.7 Hz), 7.51 (t, 1H, J = 7.4 Hz), 8.28 (d, 2H, J = 7.3 Hz), 9.55 (bs, 1H), 13.23 (bs, 1H). ¹³C NMR δ (CDCl₃) 12.19, 13.59, 27.15, 29.10, 36.80, 64.80, 65.12, 65.18, 70.14, 77.26, 81.75, 83.97, 84.17, 84.47, 85.49, 93.32, 93.72, 107.39, 111.34, 112.25, 114.36, 114.46, 114.64, 121.87, 121.96, 128.05, 129.59, 129.85, 132.44, 135.92, 136.95, 137.81, 147.81, 150.25, 157.52, 158.10, 159.35, 163.74, 163.95, 168.50, 172.69, 172.72. HRMS (FAB) calcd. C₄₆H₄₆N₃O₁₄ (MH⁺) 880.3041; found 880.3054.

T-T Ribonacetal (8). A solution of 3', 5''-bisphenoxyacetyl T-T riboacetal (2.00 g, 2.57 mmol) and dioxane (25 mL) was treated with conc. NH₄OH (25 mL) for 18 h. The solution was evaporated; abs. ethanol was added (2 x 150 mL), and the solution was again evaporated. Flash chromatography on silica gel (ME:MC 2:98 to 10:90) delivered the product (1.14 g, 87.1%).


**T-BrC⁻M Riboacetal (9).** Treatment of compound 7 as described for compound 8 gave a mixture of compounds 9 and 10 which were separated by silica gel chromatography (ME:MC 1:9 to 2:8). $^1$H NMR δ (CD$_3$OD) 1.85 (s, 3H), 2.00 (m, 4H), 2.10 (s, 3H), 2.30 (m, 2H), 3.75 (m, 2H), 3.91 (m, 1H), 4.19 (m, 1H), 4.24 (m, 1H), 4.77 (m, 1H), 4.81 (m, 1H), 5.21 (m, 1H), 5.90 (d, 1H, J = 2.6 Hz), 6.21 (t, 1H, J = 6.6 Hz), 7.44 (t, 2H, J = 7.4 Hz), 7.54 (t, 1H, J = 7.10 Hz), 7.66 (s, 2H), 8.20 (d, 2H, J = 7.3 Hz). $^{13}$C NMR δ (CD$_3$OD) 12.24, 13.81, 28.52, 31.10, 40.72, 63.15, 75.18, 83.15, 85.84, 87.41, 87.68, 88.04, 93.35, 94.85, 109.05, 111.68, 112.77, 129.24, 129.50, 130.65, 133.63, 139.40, 152.25, 166.31. HRMS (FAB) calcd. C$_{39}$H$_{53}$N$_8$O$_{10}$ (M$^+$) 612.2306, found 612.2324.

**T-C⁻M Riboacetal (10).** 10 was prepared essentially as described for 8A, but used deprotected 6 as a starting material. δ 1.86 (s, 3H), 1.91 (m, 4H), 1.96 (s, 3H), 2.31 (m, 1H), 3.75 (m, 2H), 3.86 (m, 1H), 4.14 (m, 1H), 4.24 (q, 1H, J = 3.4 Hz), 4.77 (dd, 1H, J = 2.9, 6.5 Hz), 4.85 (m, 1H), 5.21 (m, 1H), 5.91 (d, 1H, J = 2.9 Hz), 6.23 (t, 1H, J = 6.5 Hz), 7.47 (s, 1H), 7.67 (s, 1H). $^{13}$C NMR δ (CD$_3$OD) 12.31, 12.37, 13.39, 28.61, 31.12, 41.20, 63.12, 75.28, 83.05, 85.82, 87.01, 87.51, 87.71, 93.33, 104.51, 109.06, 111.66, 139.38, 139.45, 152.22, 158.12, 166.29, 167.17. HRMS (FAB) calcd. C$_{29}$H$_{35}$N$_8$O$_9$ (MH$^+$) 508.2044; found 508.2041.

**5'-O-DMT-T-T Riboacetal (8A).** A solution of 8 (600 mg, 1.18 mmol) and pyridine (25 mL) was concentrated and dried under high vacuum. Pyridine (5 mL) was added, followed by dimethoxytritylchloride (600 mg, 1.77 mmol), and the solution was stirred for 1 h. ME (5 mL) was added, and the solution was concentrated. The crude product was extracted with MC (50 mL), washed with SASB (50 mL), dried, and concentrated. Toluene (2 x 50 mL)
was added, and the solution was concentrated. Chromatography on silica gel (ME:MC 0:100 to 4:96) afforded the product (610 mg, 63.8%).

5'-O-DMT-T-\(^{14}C\) Riboacetal (9a). 9a was prepared essentially as described for 8a except that 9 was used as a starting material. \(^1H\) NMR \(\delta\) (CDCl\(_3\)) 1.41 (s, 3H), 1.88 (m, 4H), 2.09 (s, 3H), 2.17 (m, 1H), 2.47 (ddd, 1H, \(J = 4.1, 6.0, 13.6\) Hz), 3.37 (dd, 1H, \(J = 2.6, 10.5\) Hz), 3.54 (dd, 1H, \(J = 2.1, 10.3\) Hz), 3.79 (s, 6H), 3.94 (bs, 1H), 4.02 (m, 1H), 4.29 (m, 1H), 4.44 (s, 1H), 4.81 (m, 2H), 5.33 (m, 1H), 6.12 (d, 1H, \(J = 3.9\) Hz), 6.27 (t, 1H, \(J = 6.5\) Hz), 6.84 (d, 4H, \(J = 8.7\) Hz), 7.22-7.52 (m, 14H), 8.29 (d, 2H, \(J = 7.4\) Hz), 9.96 (bs, 1H). \(^{13}C\) NMR \(\delta\) (CDCl\(_3\)). HRMS (FAB) calcd. \(C_{50}H_{52}N_{3}O_{12}\) (M+) 913.3534, found 913.3543.

5'-O-DMT-T-T Riboacetal \(H\)-Phosphonate (12). To a solution 11 (1.0 \(M\) in DCM, 0.40 mL), DCM (5 mL), and pyridine (1.0 mL) at 0°C was added 8a (0.18 g, 0.20 mmol) in DCM (2 mL). The reaction mixture was stirred at room temperature for 2 h, diluted with DCM (10 mL), and quenched with triethylammonium bicarbonate (TEAB, 1M aqueous solution, 30 mL). The organic phase was dried (Na\(_2\)SO\(_4\)) and concentrated. Subsequent purification by flash chromatography in TEA/ME/DCM (0.5:2:97.5-0.5:10:89.5) delivered 12 (0.58 g, 94.5%). \(^1H\) NMR \(\delta\) (D\(_6\) DMSO) 1.16 (t, 9H, \(J = 7.3\) Hz), 1.60 (s, 3H), 1.74 (s, 3H), 1.78 (m, 4H), 2.19 (bm, 2H), 3.02 (q, 6H, \(J = 7.2\) Hz), 3.40 (m, 2H), 3.72 (s, 6H), 3.84 (m, 1H), 4.20 (m, 1H), 4.47 (m, 1H), 4.71 (dd, 1H, \(J = 3.8, 6.4\) Hz), 4.96 (dd, 1H, \(J = 1.8, 6.6\) Hz), 5.12 (bs, 1H), 5.67 (s, 1/2H), 5.86 (d, 1H, \(J = 1.7\) Hz), 6.12 (t, 1H, \(J = 7.1\) Hz), 6.86 (dd, 4H, \(J = 6.0, 8.5\) Hz), 7.18-7.39 (m, 11H), 7.61 (bs, 1.5H), 11.31 (bs, 1H), 11.40 (bs, 1H).
5'-O-DMT-T^14C^m Riobacetal H-Phosphonate (13).

13 was prepared essentially as described for compound 12.  
^1H NMR δ (D_6 DMSO) 1.18 (t, 9H, J = 7.3 Hz), 1.61 (s, 3H),  
1.80 (m, 4H), 1.99 (s, 3H), 2.33 (m, 2H), 3.04 q, 6H, J =  
7.3 Hz), 3.50 (m, 2H), 3.72 (s, 6H), 3.92 (m, 1H), 4.23  
(m, 1H), 4.51 (m, 1H), 4.73 (dd, 1H, J = 3.9, 6.5 Hz),  
4.99 (dd 1H, J = 2.0, 6.5 Hz), 5.15 (m, 1H), 5.70 (s,  
1/2H), 5.88 (d, 1H, J = 1.9 Hz), 6.15 (t, 1H, J = 6.7 
Hz), 6.86 (dd, 4H, J = 5.8, 8.6 Hz), 7.18-7.73 (m,  
14.5H), 8.17 (bd, 2H, J = 6.4 Hz), 11.41 (bs, 1H).  
^31P  
NMR δ (D_6 DMSO) 0.38 (dd, J_PH = 585 Hz, J_P-C_H=9 Hz).

Example 18

Experiments for Figure 16.

Unsaturated methylketone. To a solution of the alcohol (4.80 g, 10.0 mmol), dimethylsulfoxide (50 mL),  
and dicyclohexylcarbodiimide (4.11 g, 20.0 mmol) was  
added dichloroacetic acid (0.41 mL, 5.0 mmol). After  
stirring for 30 min, pyridine (0.41 mL) was added,  
followed by acetylmethylene triphenylphosphorane (3.50 g,  
11.0 mmol). The solution was stirred for 18 h, and brine  
(100 mL) and ethyl acetate (EA, 200 mL) were added. The  
mixture was stirred vigorously for 15 min and filtered  
through a celite pad. To the filtrate was added SASB  
(100 mL). The layers were separated and the organic  
layer was dried (Na_2SO_4), concentrated, and dried under  
vacuum. Flash chromatography (H: EA 60:40) delivered the  
product (4.35 g, 83.9%). (Montgomery, J.A., Thomas, 

Methyl Ketone. A solution of unsaturated ketone  
(4.35 g, 8.38 mmol) and EA (200 mL) was purged with  
nitrogen, and palladium on carbon (10%, 435 mg) was  
added. The reaction was charged with hydrogen,  
evacuated and again charged with hydrogen. After
stirring for 18 h under hydrogen (balloon), the mixture was filtered through celite, and the solid washed with EA (3 x 50 mL). The volatiles were removed under vacuum to afford the product (4.01 g, 91.8%).

5'-PAC-3'-TBDPS-Methylketal dimer. To a solution of the ketone (650 mg, 1.24 mmol), diol (588 mg, 1.50 mmol), EA (25 mL) and 4 Å molecular sieves was added methanesulfonic acid (1 mL). The mixture was stirred for 1 h and filtered. The filtrate was washed with SASB (50 mL), dried (Na₂SO₄), concentrated, and chromatographed on silica gel (1:99 ME:MC to 4:96 ME:MC) to deliver the dimer (805 mg, 72%).

Methylketal dimer. A solution of the protected dimer (300 mg, 0.335 mmol) in THF (25 mL), was treated with TBAF (1.0 M in THF, 1.0 mL) and the resulting solution was stirred for 18 h. ME (15 mL) was added, and the solution was concentrated and chromatographed on silica gel (ME:MC 2:98 - 12:88) to afford the product.

5'-O-DMT T-T Methyl Ketal Dimer. A solution of 8 (600 mg, 1.18 mmol) and pyridine (25 mL) was concentrated and dried under high vacuum. Pyridine (5 mL) was added, followed by dimethoxytritylchloride (600 mg, 1.77 mmol), and the solution was stirred for 1 h. ME (5 mL) was added, and the solution was concentrated. The crude product was extracted with MC (50 mL), washed with SASB (50 mL), dried, and concentrated. Toluene (2 x 50 mL) was added, and the solution was concentrated. Chromatography on silica gel (ME:MC 0:100 to 4:96) afforded the product (610 mg, 63.8%).

5'-O-DMT T-T Methyl Ketal H-Phosphonate Dimer.
The DMT-protected dimers were treated with van Boom's reagent as described before and purified by column chromatography and eluted with Et₃N/MeOH/CH₂Cl₂.
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Experimentals for Figure 17

Methoxy vinyl ether. The alcohol was oxidized to the aldehyde as previously described. To a solution of methoxymethyltriphenylphosphonium chloride (5.14 g, 15 mmol) in THF at 0°C was added n-butyllithium (1.5 M, 6.25 mmol) and the solution was stirred for 30 min. The mixture was partitioned between EA (100 mL) and SASB (100 mL), and the crude product was extracted, dried, concentrated, and chromatographed (ME:MC 0:100 to 4:96) to yield the product (1.25 g, 49%).

5'-PAC-3''-TBDPS-nor riboacetal dimer. The dimer was prepared as described for the methyl ketal.

Nor riboacetal dimer. The dimer was prepared as described for the methyl ketal.

5'O-DMT-T-T Nor-riboacetal dimer. The dimer was prepared as described for the methyl ketal.

5'O-DMT T-T Nor riboacetal H-phosphonate dimer. The DMT-protected dimers were treated with van Boom's reagent as described before and purified by column chromatography and eluted with Et₂N/MeOH/CH₂Cl₂.

Example 20

Experimentals for Figure 18

Nitrile. To a solution of 3'-silylthymidine (2.33 g, 5.0 mmol) in DMF (25 mL) was added methyltriphenyloxphosphonium iodide (2.92 g, 6.5 mmol) and the solution was stirred for 18 h. Sodium cyanide (490 mg, 100 mmol) was added and the solution was stirred for 18 h. ME was added, and the solution was concentrated. The solution was partitioned between EA (100 mL) and SASB (100 mL) and the crude product was washed with aqueous thiosulfate (100 mL), dried, concentrated, and
chromatographed (EA:H 4:6) to deliver the product (1.14 g, 49%).

**Aldehyde.** To a solution of nitrile (1.14 g, 2.45 mmol) in toluene at -78°C, was added Dibal (1.5 M, 7.5 mL), and the solution was stirred for 30 min. Ethanol (2 mL) was added, followed by sodium fluoride (3.0 g), and water 2 (mL). The mixture was filtered through celite, and the crude product was extracted with EA (100 mL), dried, concentrated, and chromatographed (EA:H 4:6) to yield the product (625 mg).

5′-PAC-3′-TBDPS-nor riboacetal dimer. The dimer was prepared as described for the methyl ketal.

Nor riboacetal dimer. The dimer was prepared as described for the methyl ketal.

5′O-DMT T-T Nor-riboacetal dimer. The dimer was prepared as described for the methyl ketal.

5′O-DMT T-T Nor-riboacetal H-phosphonate dimer. The DMT-protected dimers were treated with van Boom's reagent as described before and purified by column chromatography and eluted with Et$_3$N/MeOH/CH$_2$Cl$_2$.

**Example 21**

**Experimentals for Figure 19**

2-Cyanomethylacetylimidate. Malononitrile (30.0 g, 0.50 mol) was dissolved in Et$_2$O (anhy, 300 mL) and methanol (anhy, 19 mL) and the solution was cooled to 0°C. HCl (anhy) passed through solution for 10 minutes during which time a white ppt formed. The flask was sealed and stored at 0-5°C for 12 hrs. The solid was filtered and washed with Et$_2$O to yield a white powder.

2-Cyanotrimethylorthoacetate. Methyl imidate ester (9.8 g, 72 mmol) was dissolved in MeOH (anhy, 150 mL) and the solution stirred for 18 hrs at room temperature. The solvent was removed and the residue
partially dissolved in Et₂O (50 mL) and the solid filtered and discarded. The filtrate was washed with NaHCO₃ (sat) and brine. The organic layer was decanted, dried over K₂CO₃, filtered, and reduced to a clear oil that was vacuum distilled (40-45°C at approx 0.2 Torr) to yield a clear, colorless oil.

5′-Benzoyl-2′,3′-(2-cyanomethyl orthoacetate)-5-methyluridine. 5′-Benzoyl-5-methyluridine (1.46 g, 4.0 mmol) dissolved at CH₂Cl₂ (10 mL) and 2-cyanomethyl orthoacetate (1.7 g, 12 mmol) and MsOH (trace) added. The solution was stirred for 3 h after which time TEA (2 mL) was added. The solution was diluted with CH₂Cl₂ and washed with NaHCO₃ (sat) and brine. The organic layer was decanted, dried, filtered, and reduced to a white foam that was subjected to column chromatography and eluted with 80% EtOAc/hex to yield a white foam.

5′-Benzoyl-2′,3′-(2-cyano orthoacetate)-thymidine-5′-thymidine dimer. 5′-Benzoyl-2′,3′-orthoester and 3′-lauryl-thymidine were dissolved in dichloroethane and sieves (4 Å) added. MsOH (trace) added and the solution was heated at reflux in a flask that was fitted with a soxhlet extractor charged with CaH. The solution was heated at reflux for 3 h. The solution was cooled and TEA (1 mL) added and solution diluted with CH₂Cl₂ and washed with NaHCO₃ (sat) and brine. The organic layer was decanted, dried, and reduced to a yellow foam. This foam was subjected to column chromatography and eluted with iso-propanol/CH₂Cl₂ (0-4% iPOH) to separate both diastereomers.

5′-Hydroxyl-2′,3′-(2-cyano orthoacetate)-thymidine-5′-thymidine dimer. The acylated dimers were deprotected as described before with MeONa/MeOH and purified by column chromatography and eluted with iPOH/CH₂Cl₂.
5′-Dimethoxytrityl-2′,3′-(2-cyano orthoacetate)-thymidine-5′-thymidine dimer. The deprotected dimers were reacted with DMTCl in pyridine as described before and purified by column chromatography and eluted with iPOH/CH₂Cl₂.

5′-Dimethoxytrityl-2′,3′-(2-cyano orthoacetate)-thymidine-5′-thymidine dimer 1-H-phosphonate. The DMT-protected dimers were treated with van Boom's reagent as described before and purified by column chromatography and eluted with MeOH/CH₂Cl₂.

Example 22

Experimental for Figure 21 1,2-Di-O-isopropylidene-5-O-pivaloyl-α-D-xylofuranose. A mixture of 1,2-Di-O-isopropylidene-α-D-xylofuranose (19.0 g, 0.1 mol) and pyridine (20 mL) in 200 mL of DCM was cooled to 0°C and pivaloyl chloride (13.2 g, 0.11 mol in 20 mL of dry DCM) was added. The mixture was stirred at 0°C for 15 min and at room temperature for 2 h. The mixture was poured into water and extracted with DCM. The organic layer was washed with water and brine, dried over sodium sulfate, filtered and then concentrated. The compound was then crystallized from hexane (200 mL) to yield white prisms; yield = 24.9 g (91%).

3-8-Benzoyl-3-deoxy-1,2-di-O-isopropylidene-5-O-pivaloyl-8-D-xylofuranose. 1,2-di-O-isopropylidene-5-O-pivaloyl-α-D-xylofuranose (5.48 g, 20 mmol) was dissolved in 200 mL dry MC and 10 mL of dry pyridine. The solution was cooled to 0°C and triflic anhydride (5.64 mL in 10 mL DCM) was added dropwise. After 1 h stirring at 0°C the reaction mixture was poured into 5% sodium bicarbonate. The organic phase was washed with water and dried over sodium sulfate, filtered and reduced. The residue was dissolved in DMF and added to a
solution of sodium thiobenzoate (2.4 g, 60% sodium hydride and 11 g, thiobenzoic acid) in 200 mL of dry DMF. The reaction mixture was stirred at room temperature for 30 min. DMF was then removed and the residue was dissolved in ethyl acetate and washed with water (3X), and dried over sodium sulfate, filtered, and reduced. The residue was purified by column chromatography and eluted with 17% EtOAc/hexane to yield 4.73 g (62%).

3'-S-Benzoyl-3'-deoxy-1,2-di-O-acetyl-5-O-pivaloyl-D-xylofuranose. 3.82 g (10 mmol) of 3'-S-Benzoyl-3'-deoxy-1,2-di-O-isopropylidene-5-O-pivaloyl-D-xylofuranose in 100 mL of 80% formic acid in H₂O and heated at 50°C for 1 h. The solvent was removed under reduced pressure. The residue was co-evaporated with 20 mL of dry butanol, 20 mL of dry toluene, and then dissolved in 40 mL of dry pyridine. Acetic anhydride (5 mL, 53 mmol) was then added to the solution at 0°C and the mixture was stirred for 1 h. The reaction mixture was then poured into dilute aqueous sodium bicarbonate solution and extracted with ethyl acetate. The organic phase was washed with dilute HCl solution, water (2X), and dried over sodium sulfate, filtered and reduced. This residue was purified by column chromatography and eluted with 17% EtOAc/hexane to yield 3.79 g (89%).

2'-O-Acetyl-3'-S-benzoyl-3'-deoxy-5'-O-pivaloyl-D-xylofuranosylthymine. 1.26 g (10 mmol, 1.1 eq.) thymine was silylated with 6.4 g (40 mmol) BSA in 50 mL of dry acetonitrile at 60°C until a clear solution formed (about 30 min). 3'-S-benzoyl-3'-deoxy-1,2-di-O-acetyl-5'-O-pivaloyl-D-xylofuranose (3.79 g, 8.9 mmol) was added into the solution. TMSO-triflate (4.4 g, 20 mmol) was added to the solution and after 1 h additional TMSO-triflate (2.2 g, 10 mmol) was added. After 2 h stirring at 60°C the reaction mixture was cooled and poured into dilute sodium bicarbonate solution and
extracted with ethyl acetate. The organic phase was washed with water, dried over sodium sulfate, filtered and reduced. 3.8 g (85%) of desired product was obtained after further purification on a short silica gel column (0 - 1% MeOH/CH₂Cl₂).

Preparation of Methyl 3′-S-cyclic othorester.
0.5 g (1 mmol) of 2′-O-acetyl-3′-S-benzoyl-3′-deoxy-5′-O-pivaloyl-8-D-xylofuranosylthymine was treated with 20 mL of methanolic ammonia/1,4-dioxane (1:1) at 0°C for 1 h under nitrogen. The solvent was removed and the residue was dissolved in 20 mL DCM under N₂. To this mixture 1 mL of trimethyl orthoformate was added followed by 0.5 mL (5 eq.) of methanesulfonic acid. The reaction mixture was stirred at room temperature for 10 min and quenched with dilute sodium bicarbonate solution. The organic layer was decanted, dried, filtered and reduced. The residue was purified by column chromatography and eluted with MeOH/CH₂Cl₂ (0 - 0.5% MeOH) to yield 0.28 g (70%).

Preparation of T-T dimer of dithioorthoester.
5′-S-Benzoyl-5′-deoxy-3′-O-isobutyryl-thymidine (0.43 g, 1 mmol) was treated with NH₃ in MeOH under nitrogen for about 2 h. The solvent was removed under reduced pressure under a nitrogen atmosphere and the residue was dissolved in 10 mL of dry DCM. Methyl 3′-S-cyclic othorester (0.4 g, 1 mmol) was added, followed by TMSO-triflate (0.4 mL, 2 eq.). The reaction mixture was stirred for additional 15 min and was quenched with diluted sodium bicarbonate solution. The organic layer was decanted, dried, filtered and reduced and the residue was purified by column chromatography and eluted with MeOH/CH₂Cl₂ (0 - 2% MeOH) to yield 0.40 g (60%).

Totally deprotection of T-T dimer of dithioorthoester. 0.34 g (0.5 mmol) of 5′,3′′-protected T-T dimer was stirred with 60 mg (2 eq) of NaOMe in 10 mL of MeOH at room temperature for 12 h. 20 mL of water and
20 mL of MeOH were added and the reaction mixture was neutralized with amberlite-200 (strong acid form). The resin was filtered and the solvent was removed. The residue was ready for the next step without any further purification.

**Dimethoxytritylation of T-T dimer of dithioorthoester.** The residue obtained was co-evaporated two times with dry pyridine and then dissolved in 10 mL of dry pyridine. 0.2 g (1.2 eq.) of DMTCl was added and the reaction mixture was stirred for 2 h at room temperature. The solution was quenched with dilute sodium bicarbonate aqueous and extracted with DCM. The organic phase was decanted and washed with water and dried over sodium sulfate. The solution was filtered, the solvent was removed and the residue subjected to column chromatography and eluted with MeOH/CH₂Cl₂ (0 - 2% MeOH) to yield 0.27 g (64%).

**5'-O-DMT T-T dithioorthoester H-phosphonate dimer.** The DMT-protected dimer 0.27 g (0.32 mmol) was treated with van Boom’s reagent as described before and purified by column chromatography and eluted with Et₃N/MeOH/CH₂Cl₂ (0 - 5% MeOH) followed by Et₃N/CH₂CN/H₂O (0 - 5% H₂O).

**Example 22**

**Experiments for Figure 23**

**7-member ring.** A solution of the diol (355 mg, 0.72 mmol), amine (235 mg, 0.72 mmol), paraformaldehyde (54 mg, 1.80 mmol), benzene (25 mL), and dichloroethane (25 mL) was heated to 80°C for 18 h. The solution was cooled, filtered, and concentrated. The crude product was chromatographed (ME:MC 5:95) to deliver the 7-member ring compound. (Ref: Kaprand, H., Charles, G., *Tetrahedron Lett.*, (1980) 21:2949). A solution of 7-
member ring compound and dioxane (25 mL) was treated with conc. NH₄OH (25 mL) for 18 h. The solution was, evaporated; absolute ethanol was added (2 x 150 mL), and the solution was again evaporated. Flash chromatography on silica gel (ME:MC 2:98 to 10:90) delivered the product.

**Diol.** The silyl derivative was treated with TBAF as described in Figure 18.

**5'-O-DMT T-T 7-member ring.** This compound was prepared as described in Figure 18.

**5'-O-DMT T-T-7-member ring H-phosphonate.** This compound was prepared as described in Figure 18.

The instant invention is shown and described herein in what is considered to be the most practical and preferred embodiments. It is recognized, however, that departures may be made therefrom which are within the scope of the invention, and that modifications will occur to those skilled in the art upon reading this disclosure.
1. An oligomer and pharmaceutically acceptable salts thereof, comprising at least two nucleobases, wherein a first nucleobase and a second nucleobase are coupled through a substitute linkage, wherein said substitute linkage comprises a 5-, 6- or 7-member ring containing C2' and C3' of said first nucleobase covalently linked through a bridging moiety to C4' of said second nucleobase.

2. The oligomer of claim 1 wherein said bridging moiety links said 5-, 6-, or 7-member ring through a one, two or three atom bridge.

3. The oligomer of claim 2 wherein said ring is a 5-member ring.

4. The oligonucleotide of claim 3, wherein said 5-member ring is of the formula

\[
\begin{array}{c}
\backslash G \\
\begin{array}{c}
\text{W} \\
\text{W}
\end{array}
\end{array}
\]

wherein each W is independently selected from the group consisting of O, S, SO, SO₂, CH₂, CH₃, CH₄, CO, CF₃, CS, N, NH and NR wherein R is alkyl (1-4C);

G is selected from the group consisting of C, CH, N, and CR wherein R is alkyl (1-4C) or fluoroalkyl.
(1-4C, 1-6F), hydroxymethyl (CH₂OH), 5-tetrazole, CN, CH₂-(5-tetrazole), CO₂H, CO₂R³, CONH₂, CONHR³, CON(R³)₂, CH₂SR³, CH₂SOR³, CH₂SO₂R³, CH₂CN, CH₂CO₂H, CH₂CO₂R³, CH₂CONH₂, CH₂CONHR³ and CH₂CON(R³)₂, provided that only one W is N or CH and when W is N or CH, W is connected to G by a double bond.

5. The oligomer of claim 4 wherein said bridging moiety links said 5-member ring and said C₄′ through a one atom bridge.

6. The oligomer of claim 5, wherein said one atom bridge is of the formula -J- wherein J is selected from the group consisting of O, S, SO, SO₂, CH₂, CO, CF₂ and CS.

7. The oligomer of claim 6, wherein W is O or S, G is CH or CR⁴, and J is CH₂.

8. The oligomer of claim 4 wherein said bridging moiety links said 5-member ring and said C₄′ through a two atom bridge.

9. The oligomer of claim 8, wherein said two atom bridge is of the formula -E-J- wherein J is selected from the group consisting of O, S, SO, SO₂, CH₂, CO, CF₂ and CS and wherein E is selected from the group consisting of O, S, SO, SO₂, CH₂, CO, CF₂, CS, NH and NR³, wherein R³ is alkyl (1-4C), with the proviso that -E-J- cannot comprise -O-O-, -O-S-, -S-O-, -CF₂-O-, -O-CF₂-, -CF₂-S- or -S-CF₂.

10. The oligomer of claim 9, wherein W is O or S, G is CH or CR⁴, E is O, S or CH₂ and J is CH₂.
11. The oligomer of claim 10, wherein W is O, G is CH, and E and J are CH₂.

12. The oligomer of claim 4 wherein said bridging moiety links said 5-member ring and said C⁴ through a three atom bridge.

13. The oligomer of claim 12, wherein said three-atom bridge is of the formula -E-E-J- wherein, J is selected from the group consisting of O, S, SO, SO₂, CH, CH₂, CO, CF₂ and CS;
E is independently selected from the group consisting of O, S, SO, SO₂, CH, CH₂, CO, CF₂, CS, N, NH and NR³, wherein R³ is alkyl (1-4C), provided that -E-J- or -E-E- cannot comprise -O-O-, -O-S-, -S-O-, -CF₂-O-, -O-CF₂-, -CF₂-S- or -S-CF₂, and provided that when E is CH or N, the adjacent E is CH or N or the adjacent J is CH and they are connected by a double bond.

14. The oligomer of claim 13 wherein W is independently O or S, G is CH or CH₄, E adjacent to G is CH₂ and E adjacent to J is O, S, SO₂, NH, NR³, or CH₂ and J is CH₂.

15. The oligomer of claim 2 wherein said ring is a 6-member ring.
16. The oligonucleotide of claim 15, wherein said 6-member ring is of the formula

wherein each W is independently selected from the group consisting of O, S, SO, SO₂, CH₂, CO, CF₂, CS, NH and NR³ wherein R³ is alkyl (1-4C);

G is selected from the group consisting of CH, N, and CR³ wherein R³ is alkyl (1-4C) or fluoroalkyl (1-4C, 1-6F), hydroxymethyl (CH₂OH), 5-tetrazole, CN, CH₂-(5-tetrazole), CO₂H, CO₂R³, CONH₂, CONHR³, CON(R³)₂, CH₂SR³, CH₂SOR³, CH₂SO₂R³, CH₂CN, CH₂CO₂H, CH₂CO₂R³, CH₂CONH₂, CH₂CONHR³ and CH₂CON(R³)₂, provided that adjacent -W-W- are not -O-O-, -O-S-, -S-O-, -CF₂-O-, -O-CF₂-, -CF₂-S- or -S-CF₂.

17. The oligomer of claim 16 wherein said bridging moiety links said 6-member ring and said C4' through a one atom bridge.
18. The oligomer of claim 17, wherein said one atom bridge is of the formula \(-J-\) wherein \(J\) is selected from the group consisting of \(O, S, SO, SO_2, CH_2, CO, CF_2\) and \(CS\).

19. The oligomer of claim 18, wherein \(W\) is \(O, CH_2,\) or \(S, G\) is \(CH\) or \(CR^4,\) and \(J\) is \(CH_2\).

20. The oligomer of claim 16 wherein said bridging moiety links said 6-member ring and said \(C4'\) through a two atom bridge.

21. The oligomer of claim 20, wherein said two-atom bridge is of the formula \(-E-J-\) wherein \(J\) is selected from the group consisting of \(O, S, SO, SO_2, CH_2, CO, CF_2\) and \(CS\) and wherein \(E\) is selected from the group consisting of \(O, S, SO, SO_2, CH_2, CO, CF_2, CS, NH\) and \(NR^3,\) wherein \(R^3\) is alkyl (1-4C), with the proviso that \(-E-J-\) cannot comprise \(-O-O-, -O-S-, -S-O-, -CF_2-O-, -O-CF_2-, -CF_2-S-\) or \(-S-CF_2\).

22. The oligomer of claim 21, wherein \(W\) is \(O, CH_2,\) or \(S, G\) is \(CH\) or \(CR^4, E\) is \(O, S\) or \(CH_2\) and \(J\) is \(CH_2\).

23. The oligomer of claim 16 wherein said bridging moiety links said 6-member ring and said \(C4'\) through a three atom bridge.

24. The oligomer of claim 23, wherein said three-atom bridge is of the formula \(-E-E-J-\) wherein \(J\) is selected from the group consisting of \(O, S, SO, SO_2, CH_2, CO, CF_2\) and \(CS\) and wherein \(E\) is independently selected from the group consisting of \(O, S, SO, SO_2, CH_2, CO, CF_2, CS, NH\) and \(NR^3,\) wherein \(R^3\) is alkyl (1-4C), provided that

25. The oligomer of claim 24 wherein W is independently 0, CH₂, or S, G is CH or CR⁴, E adjacent to G is CH₂ and E adjacent to J is O, S, SO₂, NH, NR³ or CH₂ and J is CH₂.

26. The oligomer of claim 2 wherein said ring is a 7-member ring.

27. The oligonucleotide of claim 26, wherein said 7-member ring is of the formula

```
W     W
|     |
W——G——W
```

wherein each W is independently selected from the group consisting of O, S, SO₂, CH₂, CH, CO, CF₂, CS, N, NH and NR³ wherein R³ is alkyl (1-4C);

G is selected from the group consisting of C, CH, N, and CR⁴ wherein R⁴ is alkyl (1-4C) or fluoroalkyl (1-4C, 1-6F), hydroxymethyl (CH₂OH), 5-tetrazole, CH₂-(5-tetrazole), CN, CO₂H, CO₂R³, CONH₂, CONHRC, CON(R³)₂, CH₂SR³, CH₂SOR³, CH₂SO₂R³, CH₂CN, CH₂CO₂H, CH₂CO₂R³, CH₂CONH₂, CH₂CONHR³ and CH₂CON(R³)₂, provided that -W-W- cannot comprise -O-O-, -O-S-, -S-O- -CF₂-O-, -O-CF₂-, -CF₂-S- or -S-CF₂-.
28. The oligomer of claim 27 wherein said bridging moiety links said 7-member ring and said C4' through a one atom bridge.

29. The oligomer of claim 28, wherein said one atom bridge is of the formula -J- wherein J is selected from the group consisting of O, S, SO, SO₂, CH₂, CO, CF₂ and CS.

30. The oligomer of claim 29, wherein W is O, CH₂, CO, CS or S, G is CH, N or CR⁴, and J is CH₂ or O.

31. The oligomer of claim 30, wherein J is CH₂ and G is N.

32. The oligomer of claim 28 wherein said bridging moiety links said 7-member ring and said C4' through a two atom bridge.

33. The oligomer of claim 32, wherein said two-atom bridge is of the formula -E-J- wherein J is selected from the group consisting of O, S, SO, SO₂, CH₂, CO, CF₂ and CS and wherein E is selected from the group consisting of O, S, CH₂, CO, CF₂, CS, NH and NR³, wherein R³ is alkyl (1-4C), with the proviso that -E-J- cannot comprise -O-O-, -O-S-, -S-O-, -CF₂-O-, -O-CF₂-, -CF₂-S- or -S-CF₂.

34. The oligomer of claim 33, wherein W is O, CH₂, CO, CS or S, G is N, CH or CR⁴, E is O, S or CH₂ and J is CH₂.

35. The oligomer of claim 34, wherein W is O or CH₂, G is CH, and E is S or SO₂ and J is CH₂.
36. The oligomer of claim 1 comprising at least one substitute linkage selected from the group consisting of phosphorothioate, methylphosphonate, thionomethylphosphonate, 3'-thioformacetal, formacetal, N-methylhydroxylamine and phosphoramidate.

37. The oligomer of claim 36 wherein said substitute linkage is phosphorothioate or methylphosphonate.

38. The oligomer of claim 1 comprising at least one phosphodiester linkage.

39. The oligomer of claim 1 that comprises at least one segment of inverted polarity.

40. The oligomer of claim 39 that comprises at least one o-xyloso switchback linker.

41. The oligomer of claim 1 comprising at least one base wherein said base is coupled through C1' of said first nucleomonomer or said second monomer.

42. The oligomer of claim 41 wherein said base comprises a crosslinking moiety.

43. The oligomer of claim 41 wherein said base is selected from the group consisting of guanine, adenine, thymine, cytosine, uracil, 5-methylcytosine, 8-oxo-N6-methyladenine, 5-(1-propynyl)uracil, 5-(1-propynyl)cytosine and 7-deazaxanthine.

44. The oligomer of claim 1 complexed with a cationic lipid.
45. The oligomer of claim 1 comprising from about 8 to about 30 nucleomonomers and having uniform polarity.

46. The oligomer of claim 45 comprising about 2 to about 12 substituted linkages or nucleomonomers at the 5′- end and at the 3′- end which comprise nuclease stable domains, and about 3 to about 26 substituted linkages or nucleomonomers which comprise at least one RNase H competent domain and is between the nuclease stable 5′ and 3′ domains.

47. The oligomer of claim 1 comprising at least one nucleomonomer wherein said nucleomonomer is a 2′- modified nucleomonomer.

48. The oligomer of claim 47 wherein at least one of said 2′-modified nucleomonomers is a 2′-O-allyl modified nucleomonomer.

49. The oligomer of claim 1 having a covalent link between the 5′ nucleomonomer and the 3′ nucleomonomer forming a circular oligomer.

50. The oligomer of claim 1 conjugated to a solid support, label, or amine linker (1-12C).

51. The oligomer of claim 1 which is a dimer, trimer, tetramer, pentamer or hexamer.

52. An oligomer and pharmaceutically acceptable salts thereof, of the formula I, II or III:
wherein each \( R^1 \) is independently \( \text{OH}, \text{OPO}_3^2 \), an oligomer, a solid support, a label or a blocking group; each \( R^2 \) is independently selected from the group consisting of \( \text{H}, \text{OH}, \text{F}, \text{NH}_2, \text{OCH}_3, \text{OC}_2\text{H}_5, \text{OC}_3\text{H}_7, \text{SCH}_3, \text{SC}_2\text{H}_5, \text{SC}_3\text{H}_7, \text{OC}_3\text{H}_5 \) and \( \text{SC}_2\text{H}_5 \);

\( E, G, J \) and \( W \) have the meanings defined above;

and

\( B^* \) is a base.

53. The oligomer of claim 52 wherein at least one \( R^1 \) is DMTO, MMTO, \( \text{H-phosphonate}, \text{methyl phosphonamidite}, \text{methylphosphoramidite}, \beta-\text{cyanoethylphosphoramidite} \) or \( \text{alkylphosphoramidite} \).

54. The oligomer of claim 53 which is a dimer.

55. The dimer of claim 54 wherein \( R^1 \) at the 3' position is selected from the group consisting of \( \text{N,N-diisopropylamino-\beta-cyanoethoxyphosphine}, \text{N,N-diisopropylaminomethoxyphosphine} \) or \( \text{H-phosphonate} \);

\( R^1 \) at the 5' position is OH, DMTO or MMTO;
R² is H, OH, O-allyl or F.

56. The oligomer of claim 1 complexed with a cationic lipid.

57. The oligomer of claim 56 wherein the cationic lipid is DOTMA.

58. A complex comprising the oligomer of claim 1 hybridized with a target nucleic acid.

59. The complex of claim 58 wherein said target moiety is single-stranded DNA or RNA, double-stranded DNA or a protein.

60. A duplex wherein one of the two oligomers of the duplex is comprised of an oligomer of claim 1.

61. A triplex wherein one of the three oligomers of the triplex is comprised of the oligomer of claim 1.

62. The oligomer of claim 1 wherein the oligomer persists intact in cells or biological solutions for a period of time that is greater than a corresponding oligodeoxynucleotide.

63. The oligomer of claim 1 wherein the oligomer is a ribozyme.

64. The oligomer of claim 1 wherein the oligomer is a probe.

65. The oligomer of claim 1 wherein the oligomer is a primer.
66. A pharmaceutical composition, comprising:
a pharmaceutically acceptable carrier; and
a therapeutically effective amount of an
oligomer of claim 1.

67. A method to form a complex of an oligomer
with a target target acid which method comprises
contacting said target nucleic acid with the oligomer of
claim 1 under conditions wherein said complex is formed.

68. A method of treating a disease in a
subject, which disease is characterized by a particular
DNA duplex or RNA, comprising the step of:
administering to a subject in need of such
treatment an oligomer of claim 1.

69. A method of detecting the presence,
absence or amount of a particular double stranded or
single stranded nucleic acid in a biological sample,
comprising the steps of:
contacting the sample with an oligomer of claim
1 under conditions wherein a duplex or a triplex is
formed between the oligomer and the nucleic acid; and
detecting the presence, absence or amount of
said duplex or triplex.

70. The method of claim 69 wherein the nucleic
acid is single-stranded.

71. A method of inhibiting expression of at
least one selected protein in a cell wherein the protein
is encoded by DNA sequences and the protein is translated
from RNA sequences, comprising the steps of:
introducing an oligomer of claim 1 into the
cell; and
permitting the oligomer to form a triplex or duplex with the DNA or RNA to inhibit expression of the protein.

72. The method of claim 71 wherein the oligomer is introduced into the cell by a method selected from the group consisting of calcium phosphate transfection, DMSO transfection, dextran transfection, electroporation, cationic lipid transfection, anionic lipid transfection or liposome transfection.

73. A method of introducing an oligomer of claim 1 into cells, comprising:
   mixing the oligomer with a permeation enhancing agent to form a complex; and
   contacting the complex with the cells.

74. A method of synthesizing a desired oligomer of claim 1, comprising the steps of:
   synthesizing a protected nucleomonomer synthon having a protecting group and a base and further having a coupling group capable of coupling to a nucleomonomer or oligomer;
   coupling the nucleomonomer synthon to an acceptor nucleomonomer or an acceptor oligomer;
   removing the protecting group; and
   repeating the cycle as needed until the desired oligomer is synthesized.

75. A method of synthesizing a desired oligomer of claim 1, comprising the steps of:
   synthesizing a protected oligomer synthon having a protecting group and a base and further having a coupling phosphite or phosphate group capable of coupling to a nucleomonomer or oligomer;
coupling the oligomer synthon to an acceptor nucleonomomer or an acceptor oligomer;
removing the protecting group; and
repeating the cycle as needed until the desired oligomer is synthesized.

76. The method of claim 75 wherein the coupling step is accomplished using hydrogen phosphonate, amidite or triester chemistry.

77. The method of claim 75 wherein the coupling phosphite or phosphate group is selected from the group consisting of hydrogen phosphonate, N,N-diisopropylamino-methylphosphonamidite, N,N-diethylmethylamino-phosphonamidite, N,N-diisopropylamino-β-cyanoethoxyphosphine, N,N-diisopropylamino-methoxyphosphine, N,N-diethylamino-β-cyanoethoxyphosphine, N,N-morpholino-β-cyanoethoxyphosphine, N,N-morpholino-methoxyphosphine, 2-chlorophenyl phosphate, 4-chlorophenyl phosphate, 2,4-dichlorophenyl phosphate, 2-chlorophenyl thiophosphate, 4-chlorophenyl thiophosphate, 2,4-dichlorophenyl-thiophosphate, and 2,4-dibromophenyl phosphate.

78. A method of amplifying nucleic acid comprising the steps:
mixing the oligomer of claim 1 with a sample containing target nucleic acid;
hybridizing the oligomer with the target nucleic acid; and
amplifying the target nucleic acid by PCR or LCR.

79. The oligomer of claim 1 wherein the oligomer is an antisense oligomer.
80. The oligomer of claim 1 wherein the oligomer is a triple helix oligomer.
Figure 1
Figure 2
1. mCPBA

2. Isobutyric anhydride

CH₃SO₃H

4 Å MS

Figure 3
Figure 4
Figure 5
Figure 6
Figure 8
Figure 9
Figure 11
Figure 12
Figure 13
Figure 14
Figure 15

SUBSTITUTE SHEET
Figure 16

R = H, Me, CH(CH₃)₂
Figure 17
$R - CN + R'OH \xrightarrow{HCl} R - \text{C} = \text{NH} - \text{HCl} \xrightarrow{\text{Et}_2\text{O}} R - \text{C} = \text{NH} - \text{HCl} + R'OH \xrightarrow{} R - (\text{OR'}_3)$

$\text{BzO} \xrightarrow{\text{MsOH}} \text{BzO} + \text{HO}_2\text{PO}$

$R = \text{CN, CF(CH}_3)_2, \text{CH(CH}_3}_2\text{F}$

$R' = \text{Me, Et}$

Figure 19
Figure 20

\[
\begin{align*}
R &= \text{CN, CF}(\text{CH}_3)_2, \text{CH}(\text{CF}_3)_2 \\
R' &= \text{Me, Et}
\end{align*}
\]
Figure 21
Figure 23
Figure 24

SUBSTITUTE SHEET
N,N-diisopropylamino-β-cyanoethoxyphosphine

N,N-diisopropylamino-methoxyphosphine

N,N-diethylamino-methoxyphosphine

N,N-diethylamino-β-cyanoethoxyphosphine

N-morpholino-β-cyanoethoxyphosphine

N-morpholino methoxyphosphine

Figure 25-1
Bis morpholino-phosphine

N,N-dimethylamino-\(\beta\)-cyanoethylmercapto-phosphine

N,N-dimethylamino-2,4-dichlorobenzylmercapto-phosphine

Bis(N,N-diisopropylamino)-phosphine

Figure 25-2
2-chlorophenyl phosphate
4-chlorophenyl phosphate
2,4-dichlorophenyl phosphate
2,4-dibromophenyl phosphate

$Z^1 = -H$ or $\text{imidazole}$

$X = O$ or $S$

$R = H$, $\text{NO}_2$ and $\text{CF}_3$

Figure 25-3
N,N-diisopropylamino-methyl-phosphine

N,N-diethylamino-methyl-phosphine

Figure 25-4
Figure 26
Figure 27-1
Figure 27-2
Figure 29-1
Figure 29-2
Figure 30
Figure 31
Figure 32
A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :C07H 21/04
US CL :536/24.31
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/24.31, 23.1, 24.32; 435/6, 91; 935/78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>US,A, 3,282,920 (OUCHI ET AL.) 01 November 1966, See entire document.</td>
<td>1-80</td>
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Date of the actual completion of the international search: 19 MARCH 1993

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