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(54) Title: TRIPLEX-FORMING OLIGOMERS CONTAINING MODIFIED BASES

(57) Abstract

Oligomers containing at least one modified nucleotide residue that specifically forms a triplet with the G-C doublet in forming a triplex with a target DNA duplex are disclosed which maintain this binding at neutral pH. The modified nucleotide residues have base components which provide donor H to each of the acceptable electron pairs at positions O6 and N7 of G at neutral pH. The oligomers may also have regions of inverted polarity and/or crosslinking moieties.

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## + DESIGNATIONS OF "SU"

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# TRIPLEX-FORMING OLIGOMERS CONTAINING MODIFIED BASES

## Technical Field

The invention relates to oligonucleotide-based therapy and diagnosis mediated by triplex formation. It includes illustrative oligonucleotides relevant to treating infection by Human Immunodeficiency Virus (HIV) and by other viruses, such as hepatitis and herpes, and oligonucleotides that target DNAs involved in certain malignancies and in the inflammatory response. More specifically, the invention concerns oligomers which contain modified nucleotide residues which replace cytosine in oligomer binding to duplexes, resulting in triplex formation.

## Background Art

It has recently been realized that duplex DNA can be specifically recognized by oligomers where the recognition is dependent on nucleotide sequence. Two major recognition motifs have been recognized. In the earlier described "CT" motif, cytosine residues recognize G-C basepairs while thymine residues recognize A-T basepairs in the duplex. These recognition rules are outlined by Maher III, L.J., et al., Science (1989) 245:725-730; Moser, H.E., et al., Science (1987) 238:645-650. More recently, an additional motif, called "GT" recognition, was described by Cooney, M., et al., Science (1988) 241:456-459; Hogan, M.E., et al., EP Publication 375408. In the G-T motif, A-T pairs are recognized by

adenine or thymine residues and G-C pairs by guanine residues.

In both of these binding motifs, the recognition sequence must align with a sequence as played out on one of the chains of the duplex; thus, recognition, for example, of an A-T pair by a thymine depends on the location of repeated adenine residues along one chain of the duplex and thymine series on the other. The recognition does not extend to alternating A-T-A-T sequences; only the adenine residues on one chain or the other would be recognized. An exception to the foregoing is the recent report by Griffin, L.C., et al., Science (1989) 245:967-971, that limited numbers of guanine residues can be provided within pyrimidine-rich oligomers and specifically recognize thymine-adenine base pairs; this permits the inclusion of at least a limited number of pyrimidine residues in the homopurine target.

The two motifs exhibit opposite binding orientations with regard to homopurine target chains in the duplex. In the CT motif, the targeting oligonucleotide is oriented parallel to the target sequence; in the GT motif, it is oriented antiparallel (Beal, P.A., et al., Science (1990) 251:1360-1363). Thus, recognition sequences in the CT motif are read with respect to target 5′→3′ sequences so that in the 5′→3′ direction, synthetic oligonucleotides contain the required sequence of C or T residues with respect to the guanine or adenine residues in the target. In the GT motif, on the other hand, the targeted sequence is read 5′→3′ in order to design the 3′→5′ sequence of the targeting oligonucleotide.

One problem that has arisen with respect to binding in the CT system resides in the ionization state of the "C" residue at neutral or physiological pH. In

order to form the appropriate hydrogen bond donor/ acceptor pattern, the amino group at position 3 of the C must be protonated. This is consonant with the pKa when the pH is low, but at neutral pH, most of the pyrimidines are unprotonated. This interferes with binding at physiological pH.

One proposed solution to this problem has been the use of 5-methylcytosine instead of cytosine as the recognizing "C." The ability of both 5-bromouracil and 5-methylcytosine to bind duplex DNA at the same homopurine target sequence as their T/C analogs, but with greater affinities and over an extended pH range has been reported by Povsic, T.J., et al., J Am Chem Soc (1989) 111:3059-3061. Similar results were reported by Lee, J.S., et al., Nucleic Acids Res (1984) 12:6603-6614.

An additional problem relates to the stability of the triplex. Covalent crosslinking to the duplex provides one answer to this problem. A description of the state of the art and of the use of $N^4$,$N^4$-ethanocytosine as an illustrative non-photoactivated agent to crosslink triplexes is set forth in PCT application PCT/US 91/03680, incorporated herein by reference.

To provide for instances wherein purine residues are concentrated on one chain of the target and then on the opposite chain, oligonucleotides of inverted polarity may be provided. A description of such inverted polarity oligomers is set forth in PCT application WO91/06626, published 16 May 1991.

The DNA sequences to be targeted using the CT motif and the oligonucleotides of the present invention overcome the foregoing problems by utilizing alternative nucleoside residues to replace the "C"-bearing residues in the oligomer, optionally in combination with a

crosslinking moiety and/or a region of inverted polarity. In a particularly preferred approach, $N^6$-methyl-8-oxo-2'-deoxyadenosine (MODA) is used as a substitute for cytosine in the oligomers.

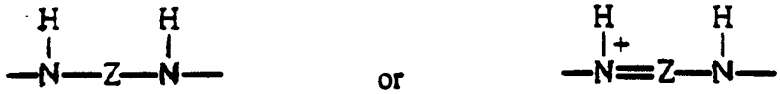
The invention herein is illustrated by the construction of oligomers containing the modified bases of the invention to several target duplexes. These duplexes are characteristic of various viral infections and of targets associated with malignancy and inflammations.

Disclosure of the Invention

The invention is directed to oligomers which are capable of triple-helix formation in a pH-independent manner in the physiological pH range using the CT motif. By substituting for the cytidine residue designed to recognize the G residue in a G-C pair, a modified nucleotide residue containing a substituent which provides hydrogen bonding donor and acceptor patterns compatible with Hoogstein binding to the "G-C" in the duplex, superior triplex formation at physiological pH can be obtained.

Accordingly, in one aspect, the invention is directed to oligomers capable of forming triplexes with target duplex sequences by coupling into the major groove of a target DNA duplex at physiological pH. These oligomers contain at least one modified nucleotide residue that forms a triplet with a G-C doublet in the target at neutral pH. This modified nucleotide residue will contain, as a base component, a substituent which provides a donor H to each of the acceptor election pairs at the G residue in the duplex at N7 and O6. The structural features of this base component are as

follows: The donor hydrogens are provided by a structure which has the formula



(A) or (A')

wherein the nucleotide corresponding to any protonated form such as A' has a pKa greater than about 5.5. At least one of the nitrogens shown is a member of a 5-7 member unsaturated heterocyclic ring, which heterocyclic ring is linked to the characteristic glycoside moiety of the nucleotide residue through an N or a C atom. As it is shown in the above formulas, Z is a one or two carbon moiety of the structure



(B) or (B')

A first carbon of the Z joins one of the N described above which forms a part of the unsaturated heterocyclic ring. This carbon is separated by at least one other ring member from the C- or N- glycoside linkage point on the ring. Any second ring, of which a second carbon of Z or the other N is a member, is capable of assuming a configuration relative to the first ring that is substantially planar; indeed, the two H shown in each of A and A' are substantially coplanar.

A variety of structures are described which have these features, as is further set forth below. In

some instances, both of the relevant H's are present on the N shown in the predominant neutral tautomer present at physiological pH; in others, where one H represents protonation of the N, the base components correspond to nucleosides which have enhanced basicity as compared to cytosine.

In addition to the one or more modified nucleoside residues which are designed to accomplish binding at neutral pH, the oligomers may also contain a crosslinking agent to stabilize the resulting triplex and/or a region or inverted polarity.

In another aspect, the invention is directed to a method to form a triplex using the oligomers of the invention to target DNA duplexes containing at least one G-C pair, and to the resulting DNA triplexes. Other aspects of the invention include pharmaceutical and diagnostic compositions which contain the oligomers of the invention, and methods to diagnose and treat conditions characterized by specific DNA duplexes using these compositions.

## Brief Description of the Drawings

Figure 1 shows the proposed structures for base triplets formed by incorporation of a third strand in the major groove of double-helical DNA in the CT mode via Hoogstein pairing.

Figure 2 is an autoradiograph of a DNA footprint demonstrating sequence-specific triplex formation using the adenine analog 6-methyl-8-hydroxyadenine under physiological pH and ion conditions.

## Modes of Carrying Out the Invention

The invention oligomers are specifically directed to binding unwanted duplexes in target tissues.

While these oligomers have certain specific features in addition to their designed sequences, there are general parameters applicable to all such oligonucleotides.

General Parameters

The oligomers of the invention may be formed using conventional phosphodiester-linked nucleotides and synthesized using standard solid phase (or solution phase) oligonucleotide synthesis techniques, which are now commercially available. However, the oligomers of the invention may also contain one or more substitute linkages as is generally understood in the art. These conventional alternative linkages are synthesized as described in the generally-available literature.

These alternative linking groups include, but are not limited to embodiments wherein a moiety of the formula P(O)S, $P(O)NR'_2$, P(O)R', $P(O)OR^2$, CO, or $CONR'_2$, wherein R' is H (or a salt) or alkyl (1-12C) and $R^2$ is alkyl (1-9C) is joined to adjacent nucleotides through -O- or -S-. Not all such linkages in the same oligomer need to be identical.

Variations in the type of internucleotide linkage are achieved by, for example, using the methyl phosphonate precursors rather than the H-phosphonates per se, using thiol derivatives of the nucleoside moieties and generally by methods known in the art. Nonphosphorous based linkages may also be used, such as the formacetal type linkages described and claimed in PCT application WO 91/06629, published 16 May 1991.

Thus, as used herein "oligonucleotide" or "oligomer" is generic to polydeoxyribonucleotides (containing 2'-deoxy-D-ribose or modified forms thereof), i.e., DNA, to polyribonucleotides (containing D-ribose or modified forms thereof), i.e., RNA, and to 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. The term "nucleoside" or "nucleotide" will similarly be generic to ribonucleosides or ribonucleotides, deoxyribonucleosides or deoxyribonucleotides, 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. Thus, the stereochemistry of the sugar carbons may be other than that of D-ribose in certain limited residues, as further described below.

"Nucleoside" and "nucleotide" include those moieties which contain not only the known purine and pyrimidine bases, but also heterocyclic bases which have been modified. Such modifications include alkylated purines or pyrimidines, acylated purines or pyrimidines, or other heterocycles. Such "analogous purines" and "analogous pyrimidines" are those generally known in the art, many of which are used as chemotherapeutic agents. An exemplary but not exhaustive list includes 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylamino-methyl-2-thiouracil, 5-carboxymethylaminomethyl uracil, dihydrouracil, inosine, N6-isopentenyl-adenine, 1-methyl-adenine, 1-methylpseudouracil, 1-methylguanine, 1-methyl-inosine, 2,2-dimethylguanine, 2-methyladenine, 2-methyl-guanine, 3-methylcytosine, 5-methylcytosine, N6-methyl-adenine, 7-methylguanine, 5-methylaminomethyl uracil, 5-methoxy aminomethyl-2-thiouracil, beta-D-mannosyl-queosine, 5'methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, wybutoxosine, pseudouracil, queosine, 2-thio cytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil,

N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine. "Nucleosides" or "nucleotides" also include those which contain modifications in the sugar moiety, for example, wherein one or more of the hydroxyl groups are replaced with halogen, aliphatic groups, or functionalized as ethers, amines, and the like.

Furthermore, as the $\alpha$ anomer binds to duplexes in a manner similar to that for the ß anomers, one or more nucleotides may contain this linkage or a domain thereof. (Praseuth, D., et al., Proc Natl Acad Sci (USA) (1988) 85:1349-1353).

The oligomers of the present invention may be of any length, but lengths of greater than or equal to about 10 nucleotides, and preferably greater than about 15, are preferred. However, the longer oligonucleotides may also be made, particularly those of greater than 50 nucleotides or greater than 100 nucleotides.

Also included are "derivatives" of oligonucleotides. "Derivatives" of the oligomers include those conventionally recognized in the art. For instance, the oligonucleotides may be covalently linked to various moieties such as intercalators, substances which interact specifically with the minor groove of the DNA double helix and other arbitrarily chosen conjugates, such as labels (radioactive, fluorescent, enzyme, etc.). These additional moieties may be derivatized through any convenient linkage. For example, intercalators, such as acridine can be linked through any available -OH or -SH, e.g., at the terminal 5′ position of RNA or DNA, the 2′ positions of RNA, or an OH, $NH_2$, COOH or SH engineered into the 5 position of pyrimidines, e.g., instead of the 5 methyl of cytosine, a derivatized form which contains, for example, $-CH_2CH_2NH_2$, $-CH_2CH_2CH_2OH$ or $-CH_2CH_2CH_2SH$ in

the 5 position. A wide variety of substituents can be attached, including those bound through conventional linkages. The indicated -OH moieties in the oligomers may be replaced by phosphonate groups, protected by standard protecting groups, or activated to prepare additional linkages to other nucleotides, or may be bound to the conjugated substituent. The 5′ terminal OH may be phosphorylated; the 2′-OH or OH substituents at the 3′ terminus may also be phosphorylated. The hydroxyls may also be derivatized to standard protecting groups.

Oligonucleotides or the segments thereof of are conventionally synthesized. Methods for such synthesis are found, for example, in Froehler, B., et al., Nucleic Acids Research (1986) 14:5399-5467; Nucleic Acids Research (1988) 16:4831-4839; Nucleosides and Nucleotides (1987) 6:287-291; Froehler, B., Tet Lett (1986) 27:5575-5578.

In addition to employing these very convenient and now most commonly used, solid phase synthesis techniques, oligonucleotides may also be synthesized using solution phase methods such as triester synthesis. These methods are workable, but in general, less efficient for oligonucleotides of any substantial length.

The Modified Nucleotide Residue Recognizing Guanine

The proposed basis for the CT modality of duplex recognition by a single-stranded oligomer nestled in the major groove of the duplex is shown in Figure 1. The T-A-T base triplet is shown with the duplex bases at the right and the third strand at the left. The Hoogstein hydrogen bonding which couples the oligomer to the duplex in the T-A-T base triplets employs the hydrogen on the ring nitrogen at position 3 of the thymine and the electrons of the oxygen keto group on

position 4 of the thymine. The hydrogen donor from the thymine couples with the electron pair at position 7 of the adenyl residue and the oxygen electron pair to the hydrogen on the amino group at position 4 of adenyl. The triplet is formed using the more stable tautomer of the thymine.

In contrast, while the $C^+$ G-C base triplet provides two Hoogstein hydrogen bonds, the more stable tautomer of the cytosine residue requires protonation at the ring nitrogen on position 3, a protonation which has a pKa permitting residence of $H^+$ in the majority of molecules only at low pH. Thus, the Hoogstein hydrogen bonds employ as a hydrogen donor the proton attached to the ring nitrogen of cytosine at position 3, and as a hydrogen donor the hydrogen of the amino group attached to the ring at position 4. The latter Hoogstein bond employs the more stable tautomers of the participants at neutral pH.

As further shown in Figure 1, a substitute for $C^+$ in the formation of Hoogstein bonding must provide donor hydrogens for acceptor electron pairs at the oxygen at position 6 (O6) and for the nitrogen at position 7 (N7) of the guanine contained in the duplex. Thus, the oligomers of the invention will contain, at least one position in place of a cytidyl residue, a nucleotide residue which contains, in lieu of a "C" substituent as the base component, a substituent which provides both of these donor H in correct positions for binding to O6 and N7 of the target G. Both of these donor H may be present on the relevant N in the predominant neutral tautomer present at physiological pH, or one of these hydrogens may be present due to protonation of the nucleotide residue. In the latter case, because of the enhanced basicity of the substituent as compared to cytosine, the

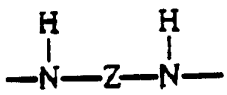
relevant position is protonated in the majority of the oligomers at physiological pH. The substituents which are contained in the modified residues are other than 5-methyl cytosine, which does not exhibit enhanced basicity, and probably does not, in fact, provide a donor hydrogen for each of the electron-accepting pairs, nor do the substituents included within the invention include 5-bromouracil which, indeed, clearly does not provide the appropriate and required donor H.

The invention oligomers contain substitutions for the cytosine/cytidine residue which provide the appropriate hydrogen donor/acceptor pattern that is suitable for forming the bonds shown in Figure 1 with respect to the guanine contained in the duplex.

In most of the illustrative embodiments of the oligomers of the invention, the base component provides the required hydrogen donors by virtue of the more stable neutral tautomeric form of the base component. However, in some instances, as is the case for cytosine, one of the donor hydrogens may be the result of protonation of one nitrogen to result in a nitrogen atom with a formal positive charge. In these cases, the protonated form of the nucleoside to which the nucleotide residue corresponds must have a pKa greater than about 5.5. Cytosine itself has a pKa (corresponding to the ring nitrogen) of approximately 4. Therefore, the protonated oligomers of the invention will have enhanced basicity of at least an order of magnitude greater than that exhibited by the corresponding oligomer which contains a conventional cytidine nucleotide. The compounds of formula 5 shown below are illustrative of compounds wherein one donor hydrogen is provided by the protonated form of the base component. In addition, embodiments wherein one of the N shown in formula A is of the form

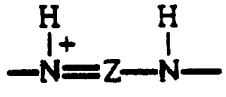
NHR wherein R is guanidinyl or amidyl as shown in formulas 11 or 13 below, provide donor hydrogen as a result of enhanced basicity rather than as derived exclusively from a neutral tautomer.

All of the base components in the modified nucleotide residues of the oligomers of the invention contain moieties of the formula



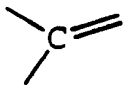
(A)

or



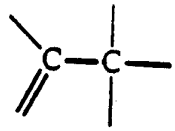
(A')

wherein Z is of the structure



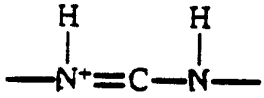
(B)

or

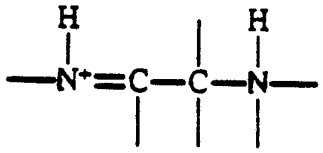


(B')

Considering, first, the structure of formula A', it is seen that linkage to the protonated hydrogen is through the double bond on B or B'. Thus, the moiety shown will be of the formula

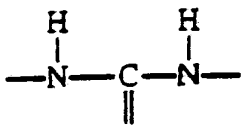


or

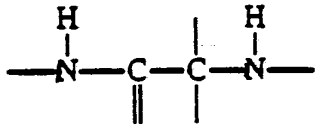


As noted above, either one or both of the nitrogens in the formula must be participants in a heterocyclic ring which contains a linkage to the glycoside and must be separated by at least one position from the linkage. Thus, the linkage to the glycoside is never directly to Z between the two nitrogens shown. If both nitrogens are members of the same heterocyclic ring containing 5-7 carbons, the restrictions on bond angles mandate approximate coplanarity of the two hydrogens which behave as hydrogen donors. If each nitrogen is a member of a different ring, or if one of the nitrogens is present either exocyclic to the ring containing the other or in a ring substituent thereto, approximate coplanarity is required.

With respect to the neutral tautomer embodiments shown in formula A, the resultant inclusion of Z will provide moieties of the formulas



or

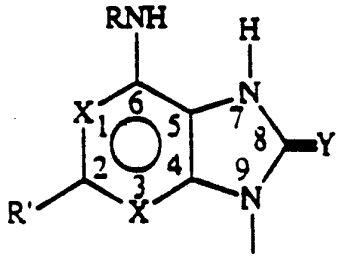


wherein, again, at least one nitrogen is a member of the heterocyclic ring containing the glycoside linkage and distanced at least one atom from it. Accordingly, the glycoside cannot be linked directly to the intervening carbons represented by Z. As above, both nitrogens may be members of the same heterocyclic 5-7 member ring, in which case approximate coplanarity is assured or one of the nitrogens may be exocyclic or in a substituent to the ring containing the other, including the instance wherein a bicyclo ring system is formed. In these structures the

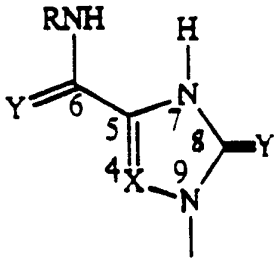
ability to assume a configuration where the donor H exhibits approximate coplanarity is also required.

It should be noted that in the event one of the nitrogens shown in formula A is substituted by a guanidinyl or amidyl residue, the hydrogen contained on that nitrogen becomes "protonated" and, in this instance, the pKa for this hydrogen must be greater than about 5.5 in the nucleoside form containing this base component.

In one illustrative embodiment, the cytosyl residues designed to participate in C-T motif triplex formation are replaced in the oligomers of the invention, by an adenyl or deleted adenyl residue which contains an electron donor at position 8. These residues are of the formulas 1 and 2 below




(1)




(2)

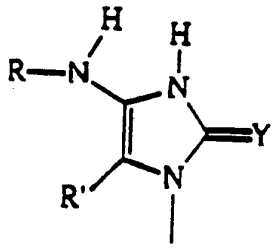
wherein each X is independently N or CR' and wherein each Y is independently O or S and wherein R and R' are noninterfering substituents.

Typically, R is H, alkyl (1-12C) or aryl (6-16C) or acyl (1-12C), all of which may contain one or more heteroatoms, or is guanidinyl or amidyl, and R' is H, alkyl (1-12C), or acyl (1-12C) or is NHR, halo, nitro, azido or cyano.

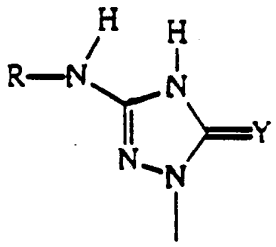
These substituents are expected to position themselves in the oligomer to be able to provide the correct acceptance/donation pattern. This expectation is supported by the models of the general nature of these residues as set forth in an article by Giessner-Prettre, C., et al., J Theor Biol (1977) 65:189-201. When the residues of formula 1 or 2 are substituted for cytosine, the 4-amino hydrogen of cytosine which binds to the 6-keto oxygen of guanine is replaced by the hydrogen on the 6-amino group substituted on adenyl and the protonated nitrogen at ring position 3 in cytosine is replaced by the hydrogen bound to the ring nitrogen at position 7 in the compound of formula 1 and the corresponding position designated 7 in formula 2. The inclusion of an electron-donating substituent at position 8 (=O or =S) provides the correct tautomerization to make position 7 a hydrogen-donating position.

Preparation of the compounds of Formula 1 and 2 follows conventional procedures. Methods for de novo synthesis of purines are known in the art, and involve intermediates analogous to those of formula 2. Thus, the compounds of formula 2 can be synthesized by methods analogous to those disclosed in U.S. patents 4,451,478 and 4,457,919 (Newport) and converted to the corresponding purine analogs by the methods disclosed therein. Conversion of the purine analogs to the 8-oxo derivatives is conducted by the method of Ikehara, M., et al., Chem Pharm Bull (1970) 12:2411-2416.

In addition to the substituents of formulas 1 and 2 set forth above, a variety of modified substituents which contain the required donor H can be used. These include both N- and C-glycosides. For example, other substituents which might be used are those of formulas 3A and 3B.



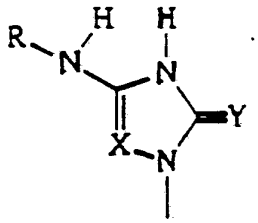
(3A)



(3B)

In these compounds, Y, R and R' are as defined for the substituents of formulas 1 and 2. These base components can be synthesized as described by Maeke, et al., in U.S. patent 4,734,506, incorporated herein by reference. As shown, these compounds contain the relevant donor H on the exocyclic amino group and on the ring nitrogen.

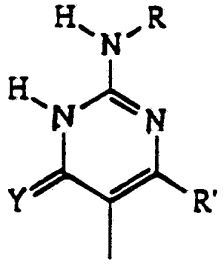
Formulas 3A and 3B can be shown generically as the compound of formula 3



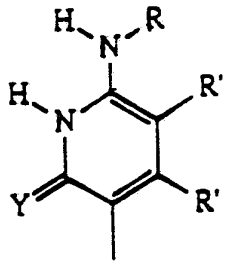
(3)

wherein X is CR′ or N.

Another exemplary group of substituents which are useful in the oligomers of the invention include the C-glycosides of formulas 4A and 4B.



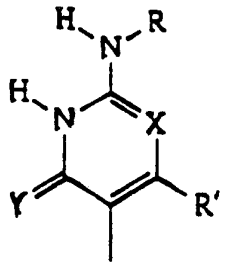
(4A)



(4B)

wherein Y, R and R′ are as above defined. Each R′ is independently assigned. These C-glycosides provide the two donor hydrogens; the hydrogen of the amino group exocyclic to the ring and the hydrogen attached to the ring nitrogen adjacent the Y substituent. These compounds are synthesized as described in Chu, C.K., et al., J Org Chem (1976) 41:2793. This publication

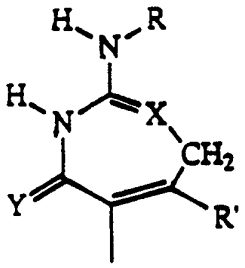
describes the synthesis of these substituents as C-linked nucleosides. These can be shown generically as Formula 4



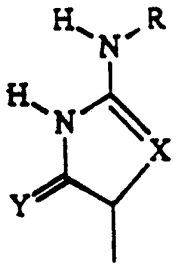
(4)

wherein each X is independently CR' or N.

The compounds of formula 4 may also be synthesized as 7-membered rings or 5-membered rings, e.g.,



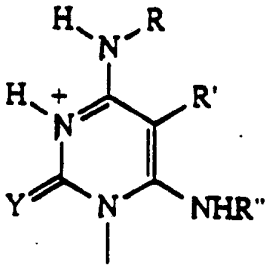
(4C) or



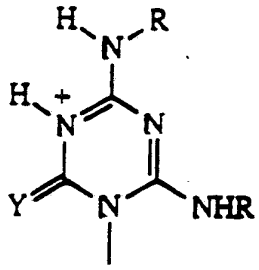
(4D)

wherein X, Y, R and R' are as above defined.

Another group of exemplary substituents includes the substituents of enhanced basicity shown as the compounds of formulas 5 and 6.



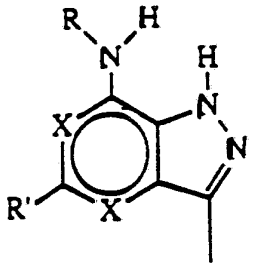
(5)



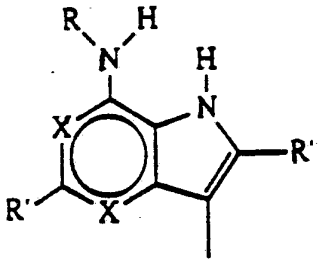
(6)

As above, Y, R and R' are defined as in the description of formulas 1 and 2; R" may form part of a 5--or 6-membered ring with R'; otherwise R" has the same definition as R. The compounds of formula 5 have enhanced basicity with respect to the protonation of the ring nitrogen by virtue of the additional amino substituents on the ring. Synthesis of these compounds as nucleosides and nucleotides is described by Goldman, D.; et al., Nucleosides and Nucleotides (1983) 2:175-187.

In an additional group of illustrative embodiments, the C-glycosides of formulas 7 and 8 may also be employed.



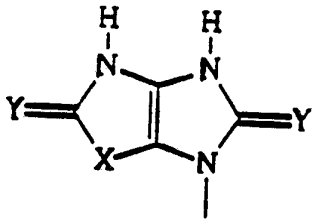
(7)



(8)

The embodiments of X, R and R' are described above; each R' is independently assigned. The synthesis of these substituents in a form which permits their incorporation into oligomers is described by David, S., et al., Carbohydrate Res (1973) 29:15.

Finally, embodiments are also useful wherein each N of formula A is in a different ring of a fused bicyclo system, as in the compounds of formula 9



(9)

wherein Y is S or O and X is as above defined.

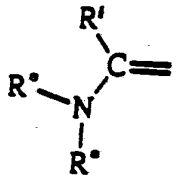
The foregoing groups of substituents are included for illustration purposes; other moieties could also be designed, as is understood by those of skill in the art, which would provide the correct pattern of H donation wherein the donor H are present at physiological pH. As stated above, these donor H are retained at physiological pH either because they are present on the relevant N in the predominant neutral tautomer present at physiological pH, as is the case of the exocyclic secondary amines, or because the enhanced basicity of the substituent as compared to cytosine results in a majority (more than 75%) of the oligomers being protonated at the relevant position at physiological pH.

As defined above, the substituent Y can be either S or O. Oxygen is preferred.

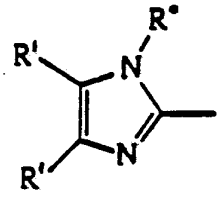
R can be H, alkyl (1-12C) or aryl (6-16C) or acyl (1-12C), all of which can contain one or more heteroatoms, or can be guanidinyl or amidyl. Alkyl is defined conventionally as a saturated straight or

branched chain or cyclic hydrocarbyl residue with the noted number of carbons such as methyl, ethyl, t-butyl, cyclohexyl, t-butyl cyclohexyl, n-decyl and the like. As heteroatoms can be included, R can also be, for example 2-ethoxyethyl or 4-methoxybutyl. Acyl is conventionally defined as RCO, wherein R is alkyl as herein defined. Aryl also has conventional definition as phenyl, alkylated phenyl, and includes phenyl alkyl residues, such as 4-phenyl-n-butyl, 2-phenyl-n-butyl, benzyl, and the like. As heteroatoms can be included, R can also be, e.g., 4-methoxyphenyl, pyrimid-4-yl methyl and the like. A preferred number of heteroatoms in all cases is 1 or 2.

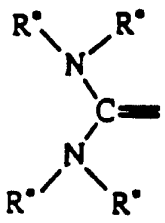
Guanidinyl and amidyl residues include residues of the formulas 10-12 wherein R′ is as above defined and each $R^*$ is independently H, alkyl (1-12C), aryl (6-16C) or acyl (1-12C) as above defined. It is seen that the moieties which represent the amidyl residue of formula 10 or the guanidinyl residue of formula 12 will convert the hydrogen on the N to which the substituent is attached to an ionizable hydrogen--i.e., which results from protonation of neutral N to obtain a cation or zwitterion.




(10)




(11)



(12)

Preferred embodiments of R are lower alkyl (1-4C), lower acyl (1-4C) and H. These are also preferred embodiments of $R^*$. As set forth above, R' is H, alkyl (1-12C) or acyl (1-12C) or is NHR, halo, nitro, azido, or cyano. The terms alkyl and acyl have been defined above. Preferred embodiments of R' are also lower alkyl (1-4C), lower acyl (1-4C) and hydrogen.

Particularly preferred embodiments of the base components of the invention are those of Formula 1 wherein both X are N, R' is H, and R is methyl. Also especially preferred are base components of Formula 4 wherein X is N, Y is O, and R and R' are H. Also preferred are compounds of Formula 2 wherein both Y are O and R is H, methyl, or ethyl.

Covalent Bonding Moiety

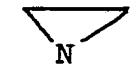
Included in some of the oligomers of the invention is a moiety which is capable of effecting at least one covalent bond between the oligomer and the duplex. Multiple covalent bonds can also be formed by providing a multiplicity of such moieties. The covalent bond is preferably to a base residue in the 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 alkylating 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.

The crosslinking moiety can conveniently be placed as an analogous pyrimidine or purine residue in the sequence of the oligomer. The placement can be at the 5′ and/or 3′ ends, the internal portions of the sequence, or combinations of the above. Placement at the termini to permit enhanced flexibility is preferred. Analogous moieties can also be attached to peptide backbones.

In one particularly preferred 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 alkylating moieties that are useful in the invention are derivatized purine and pyrimidine bases which provide alkyl moieties attached to leaving groups or as aziridenyl moieties. ("Aziridenyl" refers to an

ethanolamine substituent of the formula .)

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

The oligomers of the invention may also contain regions of inverted polarity, as described in PCT application WO91/06626, published 16 May 1991, and incorporated herein by reference.

In their most general form, the inverted polarity oligonucleotides contain at least one segment along their length of the formula:

3'----→5'--C--5'-----3' (1)

or

5'----→3'--C--3'-----5' (2)

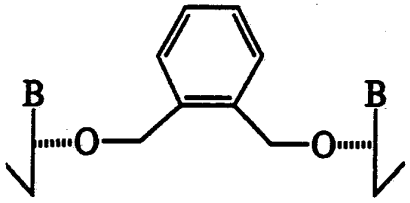
where -C- symbolizes any method of coupling the nucleotide sequences of opposite polarity.

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 nucleotide to the left with the 3' hydroxyl of the ribosyl residue of the nucleotide to the right, thus leaving the 5' hydroxyl of the rightmost nucleotide 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 nucleotide and the 5' hydroxyl of the ribosyl residue of the nucleotide on the right, thus leaving the 3' hydroxyl of the rightmost nucleotide ribosyl residue free for additional conjugation.

The linkage, symbolized by -C-, may 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 may conjugate other portions of the adjacent nucleotides so as to link the inverted polarity strands. "-C-" may represent a linker moiety, or simply a covalent bond.

A particularly preferred linking mode in the region of inverted polarity employs xylose in place of ribose in the nucleotide residues that form the switchback junction. These residues are linked through both 3′ or both 5′ hydroxyls to the methyl groups of o-xylene. Such preferred forms are referred to herein as "o-xyloso" dimers. These are of the formula




or the corresponding 5′,5′ forms thereof.

Utility and Administration

As the oligonucleotides of the invention are capable of significant duplex binding activity to form triplexes or other forms of stable association, these oligonucleotides are useful in diagnosis and therapy of diseases characterized by specific DNA duplex targets.

In these therapeutic applications, the oligomers are utilized in a manner appropriate for oligonucleotide therapy generally. 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 may 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 may 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 may be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included.

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 may be used to facilitate permeation. Transmucosal administration may 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.

In addition to use in therapy, the oligomers of the invention may be used as diagnostic reagents to detect the presence or absence of the target HIV sequences to which they specifically bind. Such diagnostic tests are conducted by hybridization through triple helix formation which is then detected by conventional means. For example, the oligomers may be labeled using radioactive, fluorescent, or chromogenic labels and the presence of label bound to solid support detected. Alternatively, the presence of a triple helix may 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 as diagnostic agents by triple helix formation is advantageous since triple helices form under mild conditions and the assays may 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 may also incorporate additional modifications such as altered internucleotide 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. Oligonucleotides 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 may also contain linkers that permit specific binding to alternate DNA strands by incorporating a linker that permits such binding (Horne et al., J Am Chem Soc (1990) 112:2435-2437).

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 Research (1986) 14:9943).

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 recombinant systems.

The following examples are intended to illustrate but not to limit the invention.

Example 1

Preparation of 8-bromodeoxyadenosine

A solution of 5g of deoxyadenosine in 100 mL of water was treated with 100 mL of 0.5M sodium acetate/acetic acid buffer (pH 5) followed by 150 mL saturated bromine water. The mixture was allowed to stand at room temperature for 3 hrs, and then sodium bisulfite was added until the color turned from dark red to light yellow. The pH of the mixture was then adjusted to 7 with sodium hydroxide, and the resulting solution was allowed to stand at 4°C for 3 hrs. The resulting precipitate was collected and recrystallized from water which had been made basic with a trace of ammonia to yield 2.6g of solid title compound.

Example 2

Conversion of 8-bromodeoxyadenosine to N-6-acetyl-8-hydroxy-2'-deoxyadenosine

A suspension of 0.5g of 8-bromodeoxyadenosine and 0.5g of anhydrous sodium acetate in 15 mL of acetic anhydride was stirred at 130°C under argon for 16 hrs. The mixture was then poured over ice, and the mixture was extracted with ethyl acetate. The organic extracts were washed with water, saturated sodium bicarbonate solution, and brine, dried over sodium sulfate, filtered and evaporated. The residue was dissolved in 15 mL of methanol, and 0.3 mL of 1M sodium methoxide solution was added. After 10 min the precipitate which formed was collected to yield 120 mg of the 3',5'-diacetate of the title compound. The mother liquor was treated with an additional 3 mL of 1M sodium methoxide solution and after 3 hrs the solution was neutralized with Dowex 50X $H^+$ resin. The resin was removed by filtration and washed with methanol. The combined filtrates were evaporated

and the residue was crystallized from methanol to afford 210 mg of the title compound.

Example 3

Preparation of 5-'(4,4'-dimethoxytrityl)-N6-acetyl-8-hydroxy-2'-deoxyadenosine-3'-H-phosphonate, Triethylamine Salt

A suspension of 185 mg of N6-acetyl-8-hydroxy-2'-deoxyadenosine in 15 mL pyridine was treated with 300 mg of 4,4'-dimethoxytrityl chloride. After 4 hrs of stirring, the resulting solution was chilled to 5°C and 1 mL of a 1M solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one in methylene chloride was added. After 30 min, the reaction was quenched with 1M triethylammonium bicarbonate buffer, and the solution was extracted with methylene chloride. The organic extracts were evaporated, and the residue was chromatographed on a silica gel column using acetonitrile/water 4:1 v:v (containing 1% triethylamine). The residue left after evaporation of the product containing fraction was coevaporated with acetonitrile, and then extracted with pyridine. The pyridine extracts were treated with triethylamine and evaporated. Finally the residue was dissolved in methylene chloride, and rapidly evaporated to afford the title compound as a crisp foam.

Example 4

Preparation of N6-methyl-8-bromo-2'-deoxyadenosine

A solution of 2.45g of N6-methyl-2'-deoxy-adenosine (Jones, J.W., et al., J. Am. Chem. Soc. (1963) 85:193-201) in 50 mL of water was treated with 50 mL of 0.5M sodium acetate/acetic acid buffer (pH 4.65) and 75 mL of saturated bromine water. After 5 hrs of stirring sodium bisulfite was added to the thick suspension until

it became colorless. The pH was then adjusted to 7 with 10M sodium hydroxide and the mixture was heated until a clear solution was obtained. The solution was allowed to cool, and the resulting precipitate was collected by filtration to afford 1.4g of the title compound.

Example 5

Preparation of N6-methyl-N6-acetyl-8-hydroxy-2'-deoxyadenosine

A suspension of 2.1g of N6-methyl-8-bromo-2'-deoxyadenosine and 2.1g of sodium acetate in 20 mL of acetic anhydride was heated at 120°C for 14 hr. The mixture was poured onto ice and extracted with ethyl acetate. The organic extracts were washed with water, saturated sodium bicarbonate solution and brine, dried over magnesium sulfate, filtered and evaporated. The residue was dissolved in 10 mL of methanol and the solution was treated with 5 mL of 1M sodium methoxide solution. After 10 min, the solution was neutralized with Dowex 50X $H^+$ resin, filtered and evaporated. The residue was chromatographed on a silica gel column using acetonitrile/water 9:1 v:v to afford 660 mg of the title compound as a crisp yellow foam.

Example 6

Preparation of 5'-(4,4'-dimethoxytrityl)-N6-methyl-N6 acetyl-8-hydroxy-2'-deoxyadenosine-3'-H-phosphonate, Triethylamine Salt

A suspension of 520 mg of N6-methyl-N6-acetyl-8-hydroxy-2'-deoxyadenosine in 20 mL of pyridine was treated with 1g of 4,4'-dimethoxytrityl chloride. After 5 hr the mixture was partitioned between ethyl acetate and water. The organic layer was washed with water and brine, then evaporated. The residue was chromatographed

on a silica gel column using ethyl acetate/methanol 19:1 to afford the 730 mg of the major product as a crisp foam. This foam was dissolved in a mixture of 2 mL of pyridine and 10 mL of methylene chloride and the resulting solution was chilled to 0°C. The cold solution was treated with 1.5 mL of a 1M solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one in methylene chloride. After 10 min the solution was quenched with 40 mL of ice-cold triethyl ammonium bicarbonate buffer pH 7.6, and the mixture was extracted with methylene chloride. The organic extracts were washed once with ice-cold water, then evaporated. The residue was chromatographed on a silica gel column using acetonitrile/water 9:1 v:v to afford 540 mg of the phosphonate as the free acid. This material was dissolved in methylene chloride and washed with ice-cold triethyl ammonium bicarbonate buffer pH 7.6. The organic layer was dried over sodium sulphate and evaporated to yield 420 mg of the title compound.

## Example 7

### Preparation of N6-methyl-8-hydroxy-2'-deoxyadenosine

A solution of 130 mg of N6-methyl-N6-acetyl-8-hydroxy-2'-deoxyadenosine in 3 ml of 1N methanolic sodium methoxide was stirred for 72 h. The mixture was neutralized, with Dowex $50XH^+$ resin, the resin was removed by filtration and was washed with methanol. The combined filtrates were evaporated, and the residue was crystallized from ethanol to yield 28 mg of the title compound.

## Example 8

### Preparation of N6-methyl-8-mercapto-2'-deoxyadenosine

A suspension of 2.0 g of N6-methyl-8-bromo-2'-deoxyadenosine in 100 ml ethanol was treated with a

solution of sodium hydrosulfide in ethanol (prepared by dissolving 0.8 g of sodium in 20 ml ethanol and saturating with hydrogen sulfide at 0°). The mixture was heated at reflux at 4 h, the pH was adjusted to 5 with acetic acid, and the mixture was evaporated. The residue was chromatographed on a 4.5 x 15 cm silica column using acetonitrile/water (9:1). The fractions containing the major product were evaporated and coevaporated with ethanol. The residue was dissolved in ethanol (10 ml) and the solution was kept at 4° for 16 h. The precipitate which formed was removed by filtration, and the filtrate was evaporated to afford 1.8 g of the title compound.

## Example 9

## Preparation of 5'-(4,4'-dimethoxytrityl)-N6-methyl-8-hydroxy-2'-deoxyadenosine-3'-H-phosphonate, Triethylamine Salt

This compound was prepared by the method of Example 6 using N6-methyl-8-hydroxy-2'-deoxyadenosine in place of N6-methyl-8-bromo-2'-deoxyadenosine.

## Example 10

## Preparation of 5'(4,4'-dimethoxytrityl)-N6-methyl-8(4-nitrophenethylmercapto)-2'-deoxyadenosine-3'-H-phosphonate, Triethylamine Salt

A solution of 350 mg of N6-methyl-8-mercapto-2'-deoxyadenosine in 5 ml DMF containing 1 ml of triethylamine was treated with 400 mg of 4-nitrophenethyl bromide. The mixture was stirred for 16 h at r.t. then an additional 250 mg of 4-nitrophenethyl bromide was added, and the mixture was kept at 60° for another 2 h. The solvent evaporated and the residue was chromatographed on a 3.5 x 15 cm silica column using

methylene chloride/methanol (15:1). The fractions containing the major product were evaporated and the residue was dissolved in 10 ml of pyridine. The solution was treated with 550 mg of 4,4'-dimethoxytrityl chloride and after 30 min, the mixture was partitioned between ethyl acetate and water. The organic layer was washed with water and brine, then dried over sodium sulphate and evaporated. The residue was chromatographed on a 3.5 x 30 cm silica column using ethyl acetate/hexanes (3:1). The fractions containing the major product were evaporated, and the residue was dissolved in 15 ml pyridine, the solution was chilled to 0° and treated with 1.25 ml of a 1 M solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one in methylene chloride. After 10 min, the solution was quenched with 20 ml of ice-cold triethyl ammonium bicarbonate buffer pH 7.6, and the mixture was extracted with methylene chloride. The organic extracts were washed once with ice-cold water, then evaporated. The residue was chromatographed on a silica gel column using acetonitrile/water 9:1 v:v (1% triethylamine) to afford 373 mg of the title phosphonate. In Examples 4-10, the 6-alkyl derivative can be substituted for 6 Me.

## Example 11

## Preparation of Oligomers Using the 5'-Protected 3'-Activated Analogs of Examples 3 and 6

Oligomers containing 15 nucleotide residues were constructed to couple with the HER-2 duplex sequence in which one strand has the sequence of 5'-GGTGGTGGTTGTGGT-3'.

ODN3 containing M was made using the nucleoside of Example 6, in which case, the N-acetyl group was subsequently removed using 0.5 N NaOH at 45°C for 96 h.

Alternatively ODN3 containing M was prepared using the nucleoside of Example 9, in which case, no extraordinary base treatment was required.

Oligomers containing the nucleoside of Example 8 were prepared using the nucleoside of Example 10 with the 4-nitrophenethyl protecting group being removed by a 3 hr treatment of the oligomers with 0.5 N DBU in pyridine.

Three nucleotides were constructed: ODN1 which is designed to couple through G-T coupling to the target duplex; ODN2 which contains 5-methylcytosine in place of cytosine designed to effect triplex formation using the CT motif, and ODN3 which contains substitutions for cytosine residues designated "M" wherein M signifies a nucleotide wherein the base residue is 6-methyl-8-hydroxyladenine. The sequences of these nucleotides are as follows:

ODN1: 3'-TGGTGTTGGTGGTGG-5';
ODN2: 5'-TĆĆTĆTTĆĆTĆĆTĆĆ-3'; and
ODN3: 5'-TMĆTĆTTMĆTMĆTMĆ-3'.

As indicated, all of the Ć-residues in the foregoing nucleotides are 5-methylcytosine rather than cytosine per se.

## Example 12

### Triple-Helix Footprint Assay

The three oligonucleotides prepared as set forth in Example 11 were tested in a footprint assay where the DNA target was the duplex formed by 5'-AGGAGAAGGAGGAGG-3' and its complement. The duplex target DNA was labeled with P32 using the kinase reaction.

Reaction mixtures which were prepared which were 20 mM MOPS, pH 7.2; 140 mM KCl; 5 mM $MgCl_2$; 3 mM spermine as the tetrahydrochloride; 50 mg/ml salmon sperm DNA; 50,000 cpm of target DNA per reaction (about 4 nm). The foregoing components are mixed in a reaction mixture of approximately 20 μl, and oligonucleotide to be tested added to provide the desired concentration (0.1-100 mM of oligonucleotide in a total volume of 20 μl. The samples were then incubated for 37°C for 1 hr to permit triplex formation and then cleaved by adding 1 μl of a 0.5 M solution of dimethyl sulfate incubating for 2 min and then quenching the reaction by adding 2 μl of 1.4 M mercaptoethanol. To this was added 20 μl of 2.0 M pyrrolidine and the mixtures placed on ice.

The samples were then heated for 10 min at 95°C and the reaction terminated by placing on ice. The samples were dried in a Speed Vac without heat for approximately 45 min until dry, and then resuspended in 100 μl water and dried again. The dried samples were resuspended in 190 μl of 0.3 molar NaOAc and 400 μl of 100% ethanol.

The samples were then placed on dry ice for 15-30 min and then spun for 15 min in a microfuge. The supernatant was discarded and the pellets washed with cold 70% ethanol. The supernatant was removed and the pellets were dried. The samples were resuspended in 67% formamide containing loading dyes and heated for 5 min at 95°C. They were then loaded onto a 5% polyacrylamide gel (7 M urea) and electrophoresed at 50 watts until the region of interest has run approximately halfway. The results obtained using the three oligonucleotides are shown in Figure 2. In the figure, lane 1 is the control cleavage of target DNA by pyrrolidine in the absence of an oligomer showing bands at all locations. Lanes 2-4

show various concentrations of the control oligonucleotide ODN1; cleavage is apparently inhibited at all concentrations. Lanes 5-7 show the results when various concentrations of the oligonucleotide containing 5-methylcytosine is employed (ODN2). Apparently no triplex is formed between 1 μm and 100 μM of the oligonucleotide. Lanes 8-10 show various concentrations of the invention oligomer, ODN3. Triplex footprint is formed at all concentrations.

Example 13

Effect of Multiple Substitutions

Additional oligonucleotides were prepared with respect to the IL-2 promoter sequence as a target. The target is a 375 bp fragment containing a 17 bp stretch of polypurine of the sequence:

5'AAAGAAAGGAGGAAAAA.

The following oligomers were prepared:

ODN4: 5'TTTĆTTTĆĆTĆĆTTTTT;
ODN5: TTTĆTTTMĆTMĆTTTTT; and
ODN6: 5'TTTMTTTMMTMMTTTTT.

Triple helix formation was assessed by incubating the target compound at a concentration of 0.01-nM in the presence of 0.1-100 μM oligomer at 37°C under conditions which include 140 mM KCl, 20 mM MOPS, pH 7.2; 10 mM sodium chloride; 1 mM spermine; 1 mM $MgCl_2$. The incubations were then treated with DNAse I, and the results tested in the footprint assay described above. Both ODN5 and ODN6 bound to the triplex at a 100-fold concentration lower than did ODN4. However, when the

incubation was conducted at room temperature, the capability of ODN4 and ODN5 to bind duplex were comparable.

Example 14

Determination of pH Dependence on Tm

A plot of the pH dependence of the melting temperature of triplex formation for 8-hydroxyadenine substituted oligomer was compared with oligomers containing cytosine or thymine. The target sequence, provided as a duplex, is 5'-AGA GGG AGA GAA AAA-3' for ODN8 and ODN9; and is 5'-AGA GAG AGA GAA AAA-3' for ODN7 and ODN10; the following nucleotides (ODNs) were synthesized:

ODN 7: 5' TCT CTC TCT CTT TT 3'
ODN 8: 5' TCT CCC TCT CTT TT 3'
ODN 9: 5' TCT CMC TCT CTT TT 3'
ODN 10: 5' TCT CTC TCT CTT TTT 3'

Measurement of thermal denaturation (Tm) was conducted using a Gilford Response II temperature control spectrophotometer. The heating rate was about 0.25°C/min from 15°C-75°C. The final concentration of all three strands was about 2 $\mu$M. Buffers were prepared from 140 mM KCl/5 mM sodium phosphate/5 mM magnesium chloride and brought to different pH values between pH 5.8 and 6.6. Prior to the Tm measurement, buffers were degassed with argon and pH adjusted to the correct value. Tm values were determined by a first derivative plot of absorbance vs. temperature.

The results obtained showed that the Tm for ODNs 7 and 9 decreased 21.75°C over this range, and for

ODN8 decreased 26.75°C. For ODN10, the decrease was only 22.87°C.

The results show that a single M residue reduced the dependency of Tm on pH over the pH range examined.

## Example 15
## Triple-helix Footprint Assay Under Simulated Physiological Conditions

Triple helix assays were conducted as described in Example 12, except that 1 mM spermine tetrahydrochloride was used instead of 3 mM. ODN1, ODN2 and ODN3, described in Example 11, were used along with ODN11. ODN11 has the same sequence as ODN3, but is fully substituted with 6-methyl-8-hydroxyladenine, M residues. Thus ODN3 contained 4 M residues and ODN11 contained 9 M residues.

Under these conditions, ODN1 and ODN2 did not form detectable footprints at concentrations of ODN up to 100 μm. ODN3 formed a footprint at 10 and 100 μm concentrations. ODN11 formed a footprint at 1, 10 and 100 μm concentrations. These results demonstrated efficient triplex formation under simulated physiological conditions where GT motif binding is not observed and where ODNs containing methylcytosine do not bind as well.

## Example 16
## Illustrative Targets and Oligomers

One suitable target comprises duplexes characteristic of the human immunodeficiency virus (HIV). HIV sequences which contain purine-rich regions concentrated on one chain of the duplex are as follows:

5'-AGGGGGAAAGAAAAAA-3';

5'-GGAAAAGGAAGGGAAAATTTC-3';

5'-GGAAAAGGAAGGAAAAATTTC-3';

5'-AAAAGAAAAGGGGGGA-3';

5'-AGAGAGAAAAAAGAG-3';

5'-AAGAGGAGGAGGAGG-3'; and

5'-AGAAGAGAAGGCTTTC-3'.

Other viral duplex sequences which contain purine-rich regions concentrated on one chain of the duplex are used as targets for the invention oligomers. The viruses and their representative target sequences are as follows:

Human Hepatitis B Virus (HBV): beginning at nucleotide 2365 5'-AGAAGAAGAACTCCCTC-3', beginning at nucleotide 2605 5'-GAGAAAAGAAGA-3';

Human Papilloma Virus Type 11 (HPV-11): beginning at nucleotide 927 5'-GAGGAAGAGGAGG-3', beginning at nucleotide 7101 5'-AAAAGAAAAGTTTTC-3';

Human Papilloma Virus Type 16 (HPV-16): beginning at nucleotide 6979 5'-AAAGGAAAAGTTTTCT-3';

Human Respiratory Syncytial Virus (RSV): beginning at nucleotide 1307 5'-AGGAAGAGAAGA-3', beginning at nucleotide 5994 5'-AAGAAAAGGAAAAGAAGATT TCTT-3';

Herpes Simplex Virus II (HSV II IE3): 5'-GAGAAGAAGAAGACCCCCCCCCC-3';

Herpes Simplex Virus II (HSV II Ribonucleotide Reductase): 5'-GAGGGGGGGGTCTTCTTC-3';

Herpes Simplex Virus I (HSV): beginning at nucleotide 52916 5'-GGGAAAGGAAAGAGGAAA-3', beginning at nucleotide 121377 5'-GAGGGAGGTTTCCTCTT-3', beginning at nucleotide 10996 5'-GGGGGAGAGGGAGTTCCCTCT-3';

Cytomegalovirus (CMV): beginning at nucleotide 176 5'-GGGGAAAAGAGGAAGTTCCC-3', beginning at nucleotide

37793 5'-GGGAAGAGGTTCTTCTCCCC-3', beginning at nucleotide 7304 5'-GGGGAGGAGAGGAGAGAGAAGAGGAG-3'.

HER-2 is a marker for certain malignant tumors. HER-2 sequences which contain purine-rich regions concentrated on one chain of the duplex are as follows:

5'-AGGAGAAGGAGGAGGTGGAGGAGGAGGG-3' (positions -64 to -38 in the promoter region);

5'-GAAGAGAGGGAGAAAGTGAAG-3' (positions -161 to -141 upstream of the promoter);

5'-GAAGGGAGGGAAGGGGG-3' (intron);

5'-GGAGGACTTCCTCTTCT-3' (intron; crossover region);

5'-GGAAAAGGGGGAG-3' (exon between positions 2955 and 2967);

5'-GAGGGAGAGGGGTCCCTTCTT-3' (exon between positions 3665 and 3683; switchback).

The following are sequences pertinent to inflammation:

1. Human Interleukin-1 Beta Gene (HUMIL1B), the sequences:

   a. beginning at nucleotide 6379 5'-AGAAAAGAAGAGGGCTCTTTT-3',

   b. beginning at nucleotide 7378 5'-GAAGAAAAAAAAAGGGTCTTTCCT-3'.

2. Human Tumor Necrosis Factor mRNA (HUMTNFAA) the sequences:

   a. beginning at nucleotide 251 5'-AGAGGGAAGAGTCCCCC-3',

   b. beginning at nucleotide 1137 5'-GGGGAAGAGAGAGAGAGAAAGA-3'.

3. Human Leukocyte Adhesion Protein p150,95 Alpha Subunit Gene (HUMINT02), the exon targets with the sequences:

   a. beginning at nucleotide 1612

5'-AGAAGGAAGACCCTTCTCC-3',

b. beginning at nucleotide 677

5'-AGGAAAAAGGGTTCTTCTCTCT-3',

c. beginning at nucleotide 2370

5'-AAAAAAAAAGAAGAAGAAGAAGAAGAAGAAGAA-3'

4. Human Interleukin-2 Gene (HUMIL2), the sequence nucleotide 1114:

5'-AAAGAAAGGAGGAAAAA-3'.

5. Human Interleukin-2 Receptor Gene (HUMIL2R8), the exon 8 target and flanks, the sequences:

a. beginning at nucleotide 1114

5'-AAGGAAGGAAAGAAAGAAGGAAG-3',

b. beginning at nucleotide 1136

5'-GAAGAGGGAGAAGGGA-3'.

6. Human Interleukin-4 Gene (HUMIL4), the sequences:

a. beginning at nucleotide 75

5'-AGAGGGGGAAG-3',

b. beginning at nucleotide 246

5'-GAGAAGGAAACCTTCT-3'.

7. Human Interleukin-6 Receptor Gene (HUMIL6), the sequences:

a. beginning at nucleotide 2389

5'-GGGGAAGAAGCTCTCTCCTCCCTTTCTTCCCT-3',

b. beginning at nucleotide 2598

5'-AGAGGAAGGAGAGGAGAGGGG-3'.

8. Human Interleukin-6 Gene (HUMIL6B), the sequence beginning at nucleotide 18:

5'-GAGGGGAAGAGGGCTTCT-3', and the upstream untranslated sequence $5'G(A)_{12}(T)_{12}C-3'$.

9. Human Interferon-Gamma Gene (HUMINTGA), the sequence beginning at nucleotide 295:

5'-GGAAAGAGGAGAG-3'.

10. Human Interleukin-1 Receptor Gene (HUMIL1RA), the sequences:

a. beginning at nucleotide 2701
5'-AGGAGGGAGAGAA-3',

b. beginning at nucleotide 3114
5'-AAAGGAGGAGGAAGGG-3'.

11. Human Endothelial Membrane Glycoprotein mRNA (HUMGP3A), the sequence beginning at nucleotide 1314:

5'-AGGAGAAGGAGAAGTCCTTT-3'.

12. Human Endothelial Leukocyte Adhesion Molecule I mRNA (HUMWLAMIA), the sequence beginning at nucleotide 1576:

5'-AGAAGAGGTTCCTTCCTG-3'.

13. Human Tumor Necrosis Factor Receptor mRNA (HUMNFR), the sequence beginning at nucleotide 2354:

5'-AAAAGAAAAAAAAAAAAG-3'.

The oligonucleotide probes which complex to these target sequences are synthesized using at least one substitute residue for cytosine which forms an association by Hoogstein binding at neutral pH.

In the illustrative oligonucleotides set forth in this example, this substituted nucleoside is $N^6$-methyl-8-oxo-2'-deoxyadenine (MODA). In the sequences shown below, this residue is designated "M".

In some of the oligomers synthesized in this Example, 5-methylcytosine is used to replace a cytosine residue. This residue is represented by "Ć".

Some of the oligomers also contain substitutuents on a nucleoside residue which form covalent crosslinks with a target. In this instance, a cytosyl residue is substituted with an aziridenyl group. Cytosines having this modification ($N^4N^4$-ethanocytosine) are designated "Z" in the sequences shown.

In addition, some of the oligomers contain an inverted polarity region, in this illustration formed from an o-xyloso dimer synthon. The linking group, o-xyloso (nucleotides that have the 3' positions of xylose sugars linked via the o-xylene ring), is designated "X". This designation includes the two nucleotide residues that are coupled through a xylene residue to form the dimer synthon. $X^1$ contains a TT dimer and the target furnishes one null base pair. $X^2$ contains a dimer that is MT and the target furnishes one null base pair.

With respect to the HIV targets, the oligomers synthesized are as follows:

For binding to the 5'-AGGGGGAAAGAAAAAA-3' sequence:

HIV101 5'-TĆMĆMĆTTTĆTTTTTT-3';
HIV102 5'-TMĆMĆMTTTĆTTTTTT-3'; and
HIV103 5'-TMMMMMTTTĆTTTTTT-3'.

For binding to the 5'-GGAAAAGGAAGGGAAAATTTC-3' sequence:

HIV111 5'-MMTTTTTMMTTMMMTT-$X^1$-TTM-5';
HIV112 5'-MMTTTTTMMTTMMMTT-$X^1$-TTZ-5';
HIV113 5'-ZMTTTTTMMTTMMMTT-$X^1$-TTZ-5';
HIV114 5'-ZMTTTTTMMTTMMMTT-$X^1$-TTM-5';
HIV115 5'-MĆTTTTTMĆTTMĆMTT-$X^1$-TTM-5';
HIV116 5'-MĆTTTTTMĆTTMĆMTT-$X^1$-TTZ-5';
HIV117 5'-ZĆTTTTTMĆTTMĆMTT-$X^1$-TTZ-5'; and
HIV118 5'-ZĆTTTTTMĆTTMĆMTT-$X^1$-TTM-5'.

For binding to the 5'-GGAAAAGGAAGGAAAAATTTC-3' sequence:

HIV211 5'-MMTTTTTMMTTMMTTT-$X^1$-TTM-5';
HIV212 5'-MMTTTTTMMTTMMTTT-$X^1$-TTZ-5';
HIV213 5'-ZMTTTTTMMTTMMTTT-$X^1$-TTZ-5';
HIV214 5'-ZMTTTTTMMTTMMTTT-$X^1$-TTM-5';

HIV215 5'-MĆTTTTMĆTTMĆTTT-$X^1$-TTM-5';
HIV216 5'-MĆTTTTMĆTTMĆTTT-$X^1$-TTZ-5';
HIV217 5'-ZĆTTTTMĆTTMĆTTT-$X^1$-TTZ-5'; and
HIV218 5'-ZĆTTTTMĆTTMĆTTT-$X^1$-TTM-5'.

For binding to the 5'-AAAAGAAAAGGGGGGA-3' sequence:

HIV121 5'-TTTTĆTTTTMĆMĆMĆT-3';
HIV122 5'-TTTTĆTTTTĆMĆMĆMT-3';
HIV123 5'-TTTTĆTTTTMMMMMMT-3'; and
HIV124 5'-TTTTMTTTTMMMMMMT-3'.

For binding to the 5'-AGAGAGAAAAAAGAG-3' sequence:

HIV131 5'-TĆTĆTĆTTTTTTĆTĆ-3';
HIV132 5'-TĆTĆTĆTTTTTTĆTZ-3';
HIV133 5'-ZTĆTĆTTTTTTĆTZ-3'; and
HIV134 5'-MTMTMTTTTTTMTZ-3'.

For binding to the 5'-AAGAGGAGGAGGAGG-3' sequence:

HIV141 5'-TTĆTMĆTMĆTMĆTMZ-3';
HIV142 5'-TTĆTMMTMMTMMTMZ-3'; and
HIV143 5'-TTĆTĆMTĆMTĆMTĆZ-3'.

For binding to the 5'-AGAAGAGAAGGCTTTC-3' sequence:

HIV151 5'-TĆTTĆTĆTTM-$X^2$-TTM-5';
HIV152 5'-TĆTTĆTĆTTM-$X^2$-TTZ-5';
HIV155 5'-TMTTMTMTTM-$X^2$-TTM-5'; and
HIV156 5'-TMTTMTMTTM-$X^2$-TTZ-5'.

For oligomers designed to target various other viruses, notation is as above; in addition, $X^3$ represents a dimer that is MM or ĆM and the target furnishes one null base pair; $X^4$ represents a dimer that is MT or ĆT and the target furnishes one null base pair.

Human Hepatitis B Virus (HBV), the illustrative oligomers are as follows:

For HBV beginning at nucleotide 2365

HBV101 5'-TĆTTĆTTĆT-$X^1$-MMMTM-5',

HBV102 5'-TĆTTĆTTĆT-$X^1$-MMMTZ-5',

HBV103 5'-TMTTMTTMT-$X^1$-MMMTM-5',

HBV104 5'-TMTTMTTMT-$X^1$-MMMTZ-5',

For HBV beginning at nucleotide 2605

HBV111 5'-MTĆTTTTĆTTĆT-3',

HBV112 5'-ZTĆTTTTĆTTĆT-3',

HBV113 5'-MTMTTTTMTTMT-3',

HBV114 5'-ZTMTTTTMTTMT-3'.

For oligomers designed to target Human Papilloma Virus Type 11 (HPV-11), the illustrative nucleotides are:

For HPV-11 beginning at nucleotide 927

HPV201 5'-MTMĆTTĆTMĆTMĆ-3',

HPV202 5'-ZTMĆTTĆTMĆTMĆ-3',

For HPV-11 beginning at nucleotide 7101

HPV211 5'-TTTTĆTTT-$X^1$-TTTM-5',

HPV212 5'-TTTTĆTTT-$X^1$-TTTZ-5',

HPV213 5'-TTTTMTTT-$X^1$-TTTM-5',

HPV214 5'-TTTTMTTT-$X^1$-TTTZ-5'.

For oligomers designed to target Human Papilloma Virus Type 16 (HPV-16), the sequence beginning at nucleotide 6979, the illustrative nucleotides are:

HPV301 5'-TTTMĆTTT-$X^1$-TTĆT-5',

HPV302 5'-TTTMMTTT-$X^1$-TTMT-5'.

For oligomers designed to target Human Respiratory Syncytial Virus (RSV), the illustrative nucleotides are:

For RSV beginning at nucleotide 1307

RSV401 5'-TMĆTTĆTĆTTĆT-3',

RSV402 5'-TMMTTMTMTTMT-3',

RSV403 5'-TĆĆTTMTMTTMT-3',

For RSV beginning at nucleotide 5994

RSV411 5′-TTĆTTTTMĆTTTTĆT-$X^1$-TTĆTT-5′,

RSV412 5′-TTMTTTTMMTTTTMT-$X^1$-TTMTT-5′.

For oligomers designed to target Herpes Simplex Virus II (HSV II IE3), the illustrative nucleotides are:

HSV501 5′-MTĆTTĆTTĆTT-$X^3$-MĆMĆMĆMĆM-5′,

HSV502 5′-MTĆTTĆTTĆTT-$X^3$-MĆMĆMĆMĆZ-5′,

HSV503 5′-ZTĆTTĆTTĆTT-$X^3$-MĆMĆMĆMĆZ-5′,

HSV504 5′-ZTĆTTĆTTĆTT-$X^3$-MĆMĆMĆMĆM-5′,

HSV505 5′-MTĆTTĆTTĆTT-$X^3$-MMMMMMMMM-5′,

HSV506 5′-MTĆTTĆTTĆTT-$X^3$-MMMMMMMMZ-5′,

HSV507 5′-ZTĆTTĆTTĆTT-$X^3$-MMMMMMMMZ-5′,

HSV508 5′-ZTĆTTĆTTĆTT-$X^3$-MMMMMMMMM-5′,

HSV509 5′-MTMTTMTTMTT-$X^3$-MMMMMMMMM-5′,

HSV510 5′-MTMTTMTTMTT-$X^3$-MMMMMMMMZ-5′,

HSV511 5′-ZTMTTMTTMTT-$X^3$-MMMMMMMMZ-5′,

HSV512 5′-ZTMTTMTTMTT-$X^3$-MMMMMMMMM-5′.

For oligomers designed to target Herpes Simplex Virus II (HSV II Ribonucleotide Reductase), the illustrative nucleotides are:

HSV601 5′-MTMMMMMM-$X^4$-ĆTTĆTTM-5′,

HSV602 5′-MTMMMMMM-$X^4$-ĆTTĆTTZ-5′,

HSV603 5′-ZTMMMMMM-$X^4$-ĆTTĆTTZ-5′,

HSV604 5′-ZTMMMMMM-$X^4$-ĆTTĆTTM-5′,

HSV605 5′-MTMMMMMĆ-$X^4$-MTTMTTM-5′,

HSV606 5′-MTMMMMMĆ-$X^4$-MTTMTTZ-5′,

HSV607 5′-ZTMMMMMĆ-$X^4$-MTTMTTZ-5′,

HSV608 5′-ZTMMMMMĆ-$X^4$-MTTMTTM-5′.

For oligomers designed to target Herpes Simplex Virus I (HSV), the illustrative nucleotides are:

For HSV beginning at nucleotide 52916

HSV701 5′-MMMTTTMĆTTTMTMĆTTT-3′,

HSV702 5′-MMMTTTMMTTTMTMMTTT-3′,

HSV703 5′-MMMTTTĆĆTTTMTĆĆTTT-3′,

For HSV beginning at nucleotide 121377

HSV711 5'-MTMMMTM-$X^4$-TMĆTĆTT-5',
HSV712 5'-ZTMMMTM-$X^4$-TMĆTĆTT-5',
HSV713 5'-MTMMMTM-$X^4$-TMMTMTT-5',
HSV714 5'-ZTMMMTM-$X^4$-TMMTMTT-5',

For HSV beginning at nucleotide 10996

HSV721 5'-MMMMMTĆTMMM-$X^1$-TMMMTĆT-5',
HSV722 5'-ZMMMMTĆTMMM-$X^1$-TMMMTĆT-5',
HSV723 5'-MMMMMTMTMMM-$X^1$-TMMMTMT-5',
HSV724 5'-ZMMMMTMTMMM-$X^1$-TMMMTMT-5'.

For oligomers designed to target Cytomegalovirus (CMV), the illustrative nucleotides are:

For CMV beginning at nucleotide 176

CMV801 5'-MMMMTTTTMTMMT-$X^1$-TMMM-5',
CMV802 5'-MMMMTTTTMTMĆT-$X^1$-TMMM-5',
CMV803 5'-MMMMTTTTMTMĆT-$X^1$-TMMZ-5',
CMV804 5'-ZMMMTTTTMTMĆT-$X^1$-TMMZ-5',
CMV805 5'-ZMMMTTTTMTMĆT-$X^1$-TMMM-5',

For CMV beginning at nucleotide 37793

CMV811 5'-MMMTTĆTM-$X^4$-ĆTTĆTMMMM-5',
CMV812 5'-MMMTTĆTM-$X^4$-ĆTTĆTMMMZ-5',
CMV813 5'-ZMMTTĆTM-$X^4$-ĆTTĆTMMMZ-5',
CMV814 5'-ZMMTTĆTM-$X^4$-ĆTTĆTMMMM-5',
CMV815 5'-MMĆTTMTM-$X^4$-MTTMTMMMM-5',
CMV816 5'-MMĆTTMTM-$X^4$-MTTMTMMMZ-5',
CMV817 5'-ZMĆTTMTM-$X^4$-MTTMTMMMZ-5',
CMV818 5'-ZMĆTTMTM-$X^4$-MTTMTMMMM-5',

For CMV beginning at nucleotide 7304

CMV821 5'-MMMMTMĆTĆTMĆTĆTĆTĆTTĆTMĆTM-3',
CMV822 5'-MMMMTMĆTĆTMĆTĆTĆTĆTTĆTMĆTZ-3',
CMV823 5'-MMMMTMMTMTMMTMTMTMTTMTMMTM-3',
CMV824 5'-MMMMTMMTMTMMTMTMTMTTMTMMTZ-3',
CMV825 5'-ZMMMTMMTMTMMTMTMTMTTMTMMTZ-3',
CMV826 5'-ZMMMTMMTMTMMTMTMTMTTMTMMTM-3',
CMV827 5'-MMMMTĆĆTMTĆĆTMTMTMTTMTĆĆTM-3',

CMV828 5'-MMMMTĆĆTMTĆĆTMTMTMTTMTĆĆTZ-3',

CMV829 5'-ZMMMTĆĆTMTĆĆTMTMTMTTMTĆĆTZ-3',

CMV830 5'-ZMMMTĆĆTMTĆĆTMTMTMTTMTĆĆTM-3'.

For the HER-binding oligomers the notation is as above; "X" represents a generic o-xyloso dimer synthon; "Y" represents anthraquinone.

For binding to the region of the HER-2 promoter at positions -65 to -38, the following oligomers were synthesized:

HER101 5'-TMMTMTTMMTMMTMMY-3';

HER102 5'-TMĆTMTTMĆTMĆTMĆY-3';

HER103 5'-TMĆTMTTMĆTMĆTMZ-3';

HER104 5'-TMMTMTTMMTMMTMM(TMM)$_4$MY-3';

HER105 5'-TMMTMTTMMTMMTMM(TMM)$_4$Z-3';

HER106 3'-YTGGTGTTGGTGGTGG-5';

HER107 3'-YTGGTGTTGGTGGTGG(TGG)$_4$G-5'; and

HER108 5'-TMMTMTTMMTMMTMZY-5'.

With respect to the upstream promoter sequence set forth above for HER-2, the following are illustrative of oligomers:

HER111 3'-YGTTGTGTGGGTGTTG-5';

HER112 5'-MTTMTMTMMMTMTTTTMY-3';

HER113 5'-MTTMTMTMMMTMTTTTMTMTTMY-3';

HER114 5'-MTTMTMTMMMTMTTTTMTMTTM-3';

HER115 5'-ZMTTMTMTMMMTMTTTTMTMTTM-3';

HER116 5'-YMTTMTMTMMMTMTTTTMTMTTM-3';

HER117 5'-MMTTMTMTMMMTMTTTTMTMTTMY-3';

HER118 5'-MMTTMTMTMMMTMTTTTMTMTTMZ-3';

HER119 5'-YMTTMTMTMMMTMTTTTMTMTTMZ-3'; and

HER120 5'-ZMTTMTMTMMMTMTTTTMTMTTMY-3'.

For the first intron sequence set forth above, the following oligomers are synthesized:

HER121 3'-YGTTGGGTGGGTTGGGG-5';

HER122 5'-MTTMMMTMMMTTMMMMY-3';

HER123 5'-YTTMMMTMMMTTMMMMY-3';

HER124 5'-ZTTMMMTMMMTTMMMMY-3';

HER125 5'-MTTMMMTMMMTTMMMZY-3'; and

HER126 5'-YTTMMMTMMMTTMMMZY-3'.

For the second intron wherein the purine region switches chains in the duplex, the following illustrative oligomers are synthesized:

HER131 5'-ZMTMĆ-X-TMMTĆTTĆT-3';

HER132 5'-MMTMĆ-X-TMMTĆTTĆT-3'; and

HER133 5'-ZMTMĆ-X-TMCTĆTTĆT-3'.

For oligomers designed to target the HER exon between positions 2955 and 2967, the illustrative nucleotides are:

HER141 5'-MMTTTTMMMMMTM-3'; and

HER142 5'-MMTTTTMMMMMTZ-3'.

For the targeted exon between positions 3665 and 3683, the following three oligomers are prepared:

HER151 5'-MTTĆTĆTMM-X-MMMTTĆTT-3';

HER152 5'-YTTĆTĆTMM-X-MMMTTĆTT-3'; and

HER153 5'-ZTTĆTĆTMM-X-MMMTTĆTT-3'.

For oligomers designed as inflammation mediators, the notation is as above, and $X^5$ contains an MT dimer and the target furnishes two null base pairs. $X^6$ contains either a ĆĆ or an MM dimer and the target furnishes one null base pair. $X^7$ contains a ĆT dimer and the target furnishes two null base pairs. $X^8$ contains a TM dimer and the target furnishes one null base pair, $X^9$ contains MM and the target furnishes 1 null base pair.

For oligomers designed to target Human Interleukin-1 Beta Gene (HUMIL1B), the illustrative nucleotides are :

For HUMIL1B beginning at nucleotide 6379

IL1$\beta$101 5'-TĆTTTTĆTTĆTM-$X^9$-TĆTTTT-5',

IL1$\beta$102 5'-TMTTTTMTTMTM-$X^9$-TMTTTT-5',

IL1β103 5'-MTTTTMTTMTM-$X^9$-TMTTTT-5',
IL1β104 5'-ZTTTTMTTMTM-$X^9$-TMTTTT-5',

For HUMIL1B beginning at nucleotide 7378

IL1β111 5'-MTTĆTTTTTTTTT-$X^5$-ĆTTTĆMT-5',
IL1β112 5'-ZTTĆTTTTTTTTT-$X^5$-ĆTTTĆMT-5',
IL1β113 5'-MTTMTTTTTTTTT-$X^5$-MTTTMM-5',
IL1β114 5'-MTTMTTTTTTTTT-$X^5$-MTTTMZ-5',
IL1β115 5'-ZTTMTTTTTTTTT-$X^5$-MTTTMZ-5',
IL1β116 5'-ZTTMTTTTTTTTT-$X^5$-MTTTMM-5'.

For oligomers designed to target Human Tumor Necrosis Factor (HUMTNFAA), the illustrative nucleotides are:

For HUMTNFAA beginning at neucleotide 251

TNF201 5'-TĆTMMMTTĆ-$X^1$-MMMMM-5',
TNF202 5'-TMTMMMTTM-$X^1$-MMMMM-5',
TNF203 5'-TMTMMMTTM-$X^1$-MMMMZ-5',

For HUMTNFAA beginning at neucleotide 1137

TNF211 5'-MMMMTTĆTĆTĆTĆTĆTĆTTTĆT-3',
TNF212 5'-ZMMMTTĆTĆTĆTĆTĆTĆTTTĆT-3',
TNF213 5'-MMMMTTĆTĆTĆTĆTĆTĆTTTM-3',
TNF214 5'-MMMMTTĆTĆTĆTĆTĆTĆTTTZ-3',
TNF215 5'-ZMMMTTĆTĆTĆTĆTĆTĆTTTZ-3',
TNF216 5'-ZMMMTTĆTĆTĆTĆTĆTĆTTTM-3',
TNF217 5'-MMMMTTMTMTMTMTMTMTTTM-3',
TNF218 5'-MMMMTTMTMTMTMTMTMTTTZ-3',
TNF219 5'-ZMMMTTMTMTMTMTMTMTTTZ-3',
TNF220 5'-ZMMMTTMTMTMTMTMTMTTTM-3'.

For oligomers designed to target Human Leukocyte Adhesion Protein p150,95 Alpha Subunit Gene (HUMINT02), the illustrative nucleotides are:

For HUMINT02 beginning at neucleotide 1612

LAP301 5'-TĆTTMĆTT-$X^6$-MTTĆTMM-5',
LAP302 5'-TĆTTMĆTT-$X^6$-MTTĆTMZ-5',
LAP303 5'-TMTTMMTT-$X^6$-MTTMTMM-5',

LAP304 5'-TMTTMMTT-$X^6$-MTTMTMZ-5',

For HUMINT02 beginning at neucleotide 677

LAP311 5'-TMĆTTTTTM-$X^7$-ĆTTĆTĆTĆT-5',

LAP312 5'-TMMTTTTTM-$X^7$-MTTMTMTMT-5',

For HUMINT02 beginning at neucleotide 2370

LAP321 5'-TTTTTTTTTĆTTĆTTĆTTĆTTĆTTĆTTĆTTĆTTĆTT-3',

LAP322 5'-TTTTTTTTTMTTMTTMTTMTTMTTMTTMTTMTTMTT-3'.

For oligomers designed to target Human Interleukin-2 Gene (HUMIL2), the sequence beginning at neucleotide 1114, the illustrative nucleotides are:

IL2 401 5'-TTTĆTTTMĆTMĆTTTTT-3',

IL2 402 5'-TTTĆTTTMMTMMTTTTT-3',

IL2 403 5'-TTTMTTTMMTMMTTTTT-3',

IL2 404 5'-TTTMTTTĆĆTĆĆTTTTT-3',

IL2 405 5'-TTTMTTTMĆTMĆTTTTT-3',

IL2 406 5'-TTTĆTTTĆĆTĆĆTTTTT-3'.

For oligomers designed to target Human Interleukin-2 Receptor Gene (HUMIL2R8), the exon 8 target and flanks, the illustrative nucleotides are:

For HUMIL2R8 beginning at neucleotide 1114

IL2R501 5'-TTMĆTTMĆTTTĆTTTĆTTMĆTTM-3',

IL2R502 5'-TTMĆTTMĆTTTĆTTTĆTTMĆTTZ-3',

IL2R503 5'-MMTTMMTTTMTTTMTTMMTTM-3',

IL2R504 5'-MMTTMMTTTMTTTMTTMMTTZ-3',

IL2R505 5'-ZMTTMMTTTMTTTMTTMMTTM-3',

IL2R506 5'-ZMTTMMTTTMTTTMTTMMTTZ-3',

For HUMIL2R8 beginning at neucleotide 1136

IL2R511 5'-MTTĆTMMMTĆTTMMMT-3',

IL2R512 5'-ZTTĆTMMMTĆTTMMMT-3'.

For oligomers designed to target Human Interleukin-4 Gene (HUMIL4), the illustrative nucleotides are:

For HUMIL4 beginning at nucleotide 75

IL4 601 5'-TMTMMMMTTM-3',

IL4 602 5'-TMTMMMMMTTZ-3',

For HUMIL4 beginning at neucleotide 246

IL4 611 5'-MTĆTTMMT-$X^8$-MTTMT-3',

IL4 612 5'-ZTĆTTMMT-$X^8$-MTTMT-3',

IL4 613 5'-MTMTTMMT-$X^8$-MTTMT-3',

IL4 614 5'-ZTMTTMMT-$X^8$-MTTMT-3'.

For oligomers designed to target Human Interleukin-6 Receptor Gene (HUMIL6), the illustrative nucleotides are:

For HUMIL6 beginning at neucleotide 2389

IL6R701 5'-MMMMTTĆT-$X^8$-TMTMTMMTMMMTTTMTTMMT-5',

IL6R702 5'-ZMMMTTĆT-$X^8$-TMTMTMMTMMMTTTMTTMMT-5',

IL6R703 5'-MMMMTTĆT-$X^8$-TĆTĆTĆĆTMMMTTTMTTMMM-5',

IL6R704 5'-MMMMTTĆT-$X^8$-TĆTĆTĆĆTMMMTTTMTTMMZ-5',

IL6R705 5'-ZMMMTTĆT-$X^8$-TĆTĆTĆĆTMMMTTTMTTMMZ-5',

IL6R706 5'-ZMMMTTĆT-$X^8$-TĆTĆTĆĆTMMMTTTMTTMMM-5',

For HUMIL6 beginning at neucleotide 2598

IL6R711 5'-TMTMMTTMMTMTMMTMTMMMM-3',

IL6R712 5'-TMTMMTTMMTMTMMTMTMMMZ-3',

IL6R713 5'-TMTMĆTTMĆTMTMĆTMTMMMM-3',

IL6R714 5'-TMTMĆTTMĆTMTMĆTMTMMMZ-3'.

For oligomers designed to target Human Interleukin-6 Gene (HUMIL6B), the sequence beginning at neucleotide 18, the illustrative nucleotides are:

IL6801 5'-MTMMMMTTMTM-$X^9$-TTMT-5',

IL6802 5'-ZTMMMMTTMTM-$X^9$-TTMT-5'; for the untranslated sequence:

IL6803 5'-$M(T)_{10}X'(T)_{11}M$-5',

IL6804 5'-$Z(T)_{10}X'(T)_{11}M$-5',

IL6805 5'-$Z(T)_{10}X'(T)_{11}Z$-5'.

For oligomers designed to target Human Interferon-Gamma Gene (HUMINTGA), the sequence beginning at neucleotide 295, the illustrative nucleotides are:

ING811 5'-MMTTTMTMMTMTM-3',

ING812 5'-MMTTTMTMMTMTZ-3',

ING813 5'-ZMTTTMTMMTMTZ-3',

ING814 5'-ZMTTTMTMMTMTM-3'.

For oligomers designed to target Human Interleukin-1 Receptor Gene (HUMIL1RA), the illustrative nucleotides are:

For HUMIL1RA beginning at neucleotide 2701

IL1R901 5'-TMMTMMMTMTMTT-3',

For HUMIL1RA beginning at neucleotide 3114

IL1R911 5'-TTTMMTMMTMMTTMMM-3',

IL1R912 5'-TTTMMTMMTMMTTMMZ-3',

IL1R913 5'-TTTMĆTMĆTMĆTTMMM-3',

IL1R914 5'-TTTMĆTMĆTMĆTTMMZ-3'.

For oligomers designed to target Human Endothelial Membrane Glycoprotein mRNA (HUMGP3A), the sequence beginning at nucleotide 1314, the illustrative nucleotides are:

EMG921 5'-TMĆTĆTTMĆTĆT-$X^1$-ĆMTTT-5',

EMG922 5'-TMMTMTTMMTMT-$X^1$-MMTTT-5'.

For oligomers designed to target Human Endothelial Leukocyte Adhesion Molecule I mRNA (HUMELAMIA), the sequence beginning at nucleotide 1576, the illustrative nucleotides are:

ELAM931 5'-TĆTTĆTM-$X^5$-ĆMTTĆMT-5',

ELAM932 5'-TMTTMTM-$X^5$-MMTTMMT-5'.

For oligomers designed to target Human Tumor Necrosis Factor Receptor mRNA (HUMNFR), the sequence beginning at nucleotide 2354:

TNFR941 5'-TTTTĆTTTTTTTTTTTTM-3',

TNFR942 5'-TTTTĆTTTTTTTTTTTTZ-3',

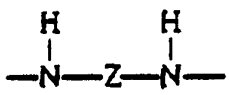
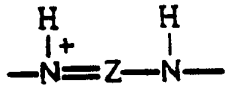
TNFR943 5'-TTTTMTTTTTTTTTTTZ-3'.

The oligonucleotides are labeled by kinasing at the 5' end and are tested for their ability to bind target sequence under conditions of 1 mM spermine, 1 mM

$MgCl_2$, 140 mM KCl, 10 mM NaCl, 20 mM MOPS, pH 7.2 with a target duplex concentration of 10 pM at 37°C for 1 hour. These conditions approximate physiological conditions, and the binding is tested either in a footprint assay as described in Example 12 hereinabove, or in a gel-shift assay essentially as described in Cooney, M. et al., Science (1988) 241:456-459.

## Claims

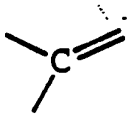
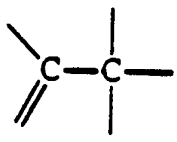
1. An oligomer capable at physiological pH of forming a triplex by coupling into the major groove of an DNA duplex, which oligomer comprises at least one nucleotide residue that specifically forms a triplet with a G-C doublet in said duplex at such pH, said residue having as a base component a moiety which provides donor hydrogens to the acceptor electron pairs at O6 and N7 of G in the duplex, said moiety comprising a "-NHZNH-" function selected from the group consisting of:

 (A) and  (A')

wherein the H residues are said donor hydrogens, and wherein the nucleoside corresponding to any base component containing a positively charged N as shown exhibits a pKa greater than about 5.5,

wherein at least one of the said N is a member of a 5-7 member unsaturated heterocyclic ring, said ring being nitrogen- or carbon-linked to the characteristic glycoside moiety of said nucleotide residue, and

wherein Z is a one or two carbon moiety of structure

 (B) or  (B')

at least one carbon of such moiety adjoining said one N member forming a part of such heterocyclic ring and being

separated by at least one other ring member from the C- or N-glycoside linkage point on said ring; and

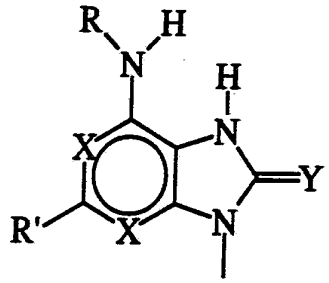
wherein said donor H are capable of assuming a configuration relative to each other that is substantially planar.

2. The oligomer of claim 1

(a) wherein both said donor H are present on said Ns in the predominant neutral tautomer present at physiological pH, or

(b) wherein at least one donor H is present due to protonation of the nucleotide base component and wherein the nucleoside corresponding to said base component has enhanced basicity as compared to cytosine.

3. The oligomer of claim 1 wherein the base component is of the formula



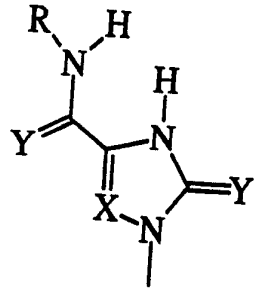
(1)

wherein Y is O or S; each X is independently N or CR'; each R' is independently assigned; and wherein R and R' are noninterfering substituents.

4. The oligomer of claim 3 wherein R is H, alkyl (1-12C) or aryl (6-16C) or acyl (1-12C) and wherein said alkyl, aryl or acyl may optionally contain one or more heteroatoms, or wherein R is guanidinyl or amidyl, and R' is H, alkyl (1-12C), or acyl (1-12C) or is NHR, halo, nitro, azido or cyano.

5. The oligomer of claim 4 wherein R is methyl, R′ is H, both X are N, and Y is O.

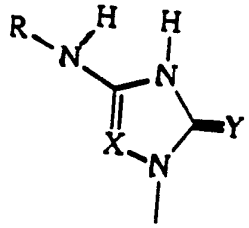
6. The oligomer of claim 1 wherein the base component is of a formula



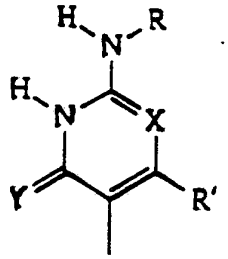
(2);

wherein each Y is independently O or S, X is N or CR′, and wherein R and R′ are noninterfering substituents.

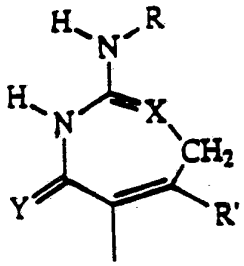
7. The oligomer of claim 1 wherein the base component is of the formula selected from the group consisting of



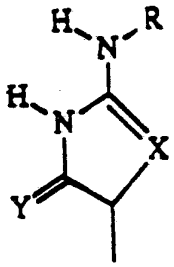
(3);



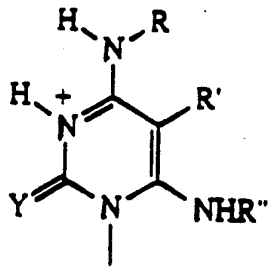
(4);



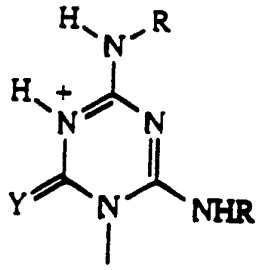
(4C);



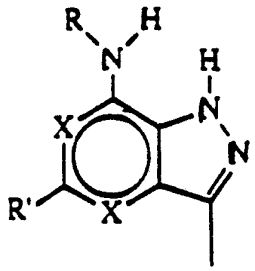
(4D);



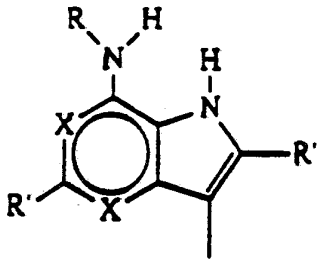
(5);



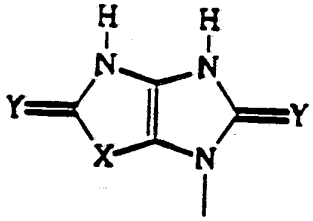
(6);



(7);



(8);



(9);

wherein each Y, if present, is independently O or S,

each X, if present, is independently N or CR′ wherein R′ is a noninterfering substituent,

each R, if present, is independently a noninterfering substituent, and

wherein R" in formula (5) forms part of a 5- or 6-membered ring that includes R′ or is R.

8. The oligomer of claim 7 wherein R is H, alkyl (1-12C) or aryl (6-16C) or acyl (1-12C) and wherein said alkyl, aryl or acyl may contain one or more heteroatoms, or wherein R is guanidinyl or amidyl, and R′ is H, alkyl (1-12C), or acyl (1-12C) or is NHR, halo, nitro, azido or cyano.

9. The oligomer of claims 1-8 conjugated to a label, a drug, a solid support, or a carrier.

10. The oligomer of claims 1-8 which further contains at least one region of inverted polarity and/or contains at least one crosslinking moiety.

11. The oligomer of claim 1 wherein said target DNA duplex contains a segment of DNA of the sequence selected from the group consisting of:

5'-AGGGGGAAAGAAAAAA-3';
5'-GGAAAAGGAAGGGAAAATTTC-3';
5'-GGAAAAGGAAGGAAAAATTTC-3';
5'-AAAAGAAAAGGGGGGA-3';
5'-AGAGAGAAAAAAGAG-3';
5'-AAGAGGAGGAGGAGG-3'; and
5'-AGAAGAGAAGGCTTTC-3';

5'-AGAAGAAGAACTCCCTC-3';
5'-GAGAAAAGAAGA-3';
5'-GAGGAAGAGGAGG-3';
5'-AAAAGAAAAGTTTTC-3';
5'-AAAGGAAAAGTTTTCT-3';
5'-AGGAAGAGAAGA-3';
5'-AAGAAAAGGAAAAGAAGATTTCTT-3';
5'-GAGAAGAAGAAGACCCCCCCCCC-3';
5'-GAGGGGGGGGTCTTCTTC-3';
5'-GGGAAAGGAAAGAGGAAA-3';
5'-GAGGGAGGTTTCCTCTT-3';
5'-GGGGGAGAGGGAGTTCCCTCT-3';
5'-GGGGAAAAGAGGAAGTTCCC-3';
5'-GGGAAGAGGTTCTTCTCCCC-3'; and

5'-GGGGAGGAGAGGAGAGAGAAGAGGAG-3';

5'-AGGAGAAGGAGGAGGTGGAGGAGGAGGG-3';
5'-GAAGAGAGGGAGAAAGTGAAG-3';
5'-GAAGGGAGGGAAGGGGG-3';
5'-GGAGGACTTCCTCTTCT-3';
5'-GGAAAAGGGGGAG-3'; and
5'-GAGGGAGAGGGGTCCCTTCTT-3';

5'-AGAAAAGAAGAGGGCTCTTTT-3';
5'-GAAGAAAAAAAAAGGGTCTTTCCT-3';
5'-AGAGGGAAGAGTCCCCC-3';
5'-GGGGAAGAGAGAGAGAGAAAGA-3';
5'-AGAAGGAAGACCCTTCTCC-3';
5'-AGGAAAAAGGGTTCTTCTCTCT-3';
5'-AAAAAAAAAGAAGAAGAAGAAGAAGAAGAAGAAGAA-3';
5'-AAAGAAAGGAGGAAAAA-3';
5'-AAGGAAGGAAAGAAAGAAGGAAG-3';
5'-GAAGAGGGAGAAGGGA-3';
5'-AGAGGGGGAAG-3';
5'-GAGAAGGAAACCTTCT-3';
5'-GGGGAAGAAGCTCTCTCCTCCCTTTCTTCCCT-3';
5'-AGAGGAAGGAGAGGAGAGGGG-3';
5'-$G(A)_{12}(T)_{12}C$-3';
5'-GAGGGGAAGAGGGCTTCT-3';
5'-GGAAAGAGGAGAG-3';
5'-AGGAGGGAGAGAA-3';
5'-AAAGGAGGAGGAAGGG-3';
5'-AGGAGAAGGAGAAGTCCTTT-3';
5'-AGAAGAGGTTCCTTCCTG-3'; and
5'-AAAAGAAAAAAAAAAAAG-3'.

12. The oligomer of claim 11 which is selected from the group consisting of:

HIV101 5'-TĆMĆMĆTTTĆTTTTTT-3';
HIV102 5'-TMĆMĆMTTTĆTTTTTT-3';
HIV103 5'-TMMMMMTTTĆTTTTTT-3';
HIV111 5'-MMTTTTMMTTMMMTT-$X^1$-TTM-5';
HIV112 5'-MMTTTTMMTTMMMTT-$X^1$-TTZ-5';
HIV113 5'-ZMTTTTMMTTMMMTT-$X^1$-TTZ-5';
HIV114 5'-ZMTTTTMMTTMMMTT-$X^1$-TTM-5';
HIV115 5'-MĆTTTTMĆTTMĆMTT-$X^1$-TTM-5';
HIV116 5'-MĆTTTTMĆTTMĆMTT-$X^1$-TTZ-5';
HIV117 5'-ZĆTTTTMĆTTMĆMTT-$X^1$-TTZ-5';
HIV118 5'-ZĆTTTTMĆTTMĆMTT-$X^1$-TTM-5';

HIV211 5'-MMTTTTMMTTMMTTT-$X^1$-TTM-5';
HIV212 5'-MMTTTTMMTTMMTTT-$X^1$-TTZ-5';
HIV213 5'-ZMTTTTMMTTMMTTT-$X^1$-TTZ-5';
HIV214 5'-ZMTTTTMMTTMMTTT-$X^1$-TTM-5';
HIV215 5'-MĆTTTTMĆTTMĆTTT-$X^1$-TTM-5';
HIV216 5'-MĆTTTTMĆTTMĆTTT-$X^1$-TTZ-5';
HIV217 5'-ZĆTTTTMĆTTMĆTTT-$X^1$-TTZ-5';
HIV218 5'-ZĆTTTTMĆTTMĆTTT-$X^1$-TTM-5';

HIV121 5'-TTTTĆTTTTMĆMĆMĆT-3';
HIV122 5'-TTTTĆTTTTĆMĆMĆMT-3';
HIV123 5'-TTTTĆTTTTMMMMMMT-3';
HIV124 5'-TTTTMTTTTMMMMMMT-3';

HIV131 5'-TĆTĆTĆTTTTTTĆTĆ-3';
HIV132 5'-TĆTĆTĆTTTTTTĆTZ-3';
HIV133 5'-ZTĆTĆTTTTTTĆTZ-3';
HIV134 5'-MTMTMTTTTTTMTZ-3';

HIV141 5'-TTĆTMĆTMĆTMĆTMZ-3';
HIV142 5'-TTĆTMMTMMTMMTMZ-3';
HIV143 5'-TTĆTĆMTĆMTĆMTĆZ-3';

HIV151 5'-TĆTTĆTĆTTM-$X^2$-TTM-5';
HIV152 5'-TĆTTĆTĆTTM-$X^2$-TTZ-5';
HIV155 5'-TMTTMTMTTM-$X^2$-TTM-5'; and
HIV156 5'-TMTTMTMTTM-$X^2$-TTZ-5';

HBV101 5'-TĆTTĆTTĆT-$X^1$-MMMTM-5';
HBV102 5'-TĆTTĆTTĆT-$X^1$-MMMTZ-5';
HBV103 5'-TMTTMTTMT-$X^1$-MMMTM-5';
HBV104 5'-TMTTMTTMT-$X^1$-MMMTZ-5';

HBV111 5'-MTĆTTTTĆTTĆT-3';
HBV112 5'-ZTĆTTTTĆTTĆT-3';
HBV113 5'-MTMTTTTMTTMT-3';
HBV114 5'-ZTMTTTTMTTMT-3';

HPV201 5'-MTMĆTTĆTMĆTMĆ-3';
HPV202 5'-ZTMĆTTĆTMĆTMĆ-3';

HPV211 5'-TTTTĆTTT-$X^1$-TTTM-5';
HPV212 5'-TTTTĆTTT-$X^1$-TTTZ-5';
HPV213 5'-TTTTMTTT-$X^1$-TTTM-5';
HPV214 5'-TTTTMTTT-$X^1$-TTTZ-5';

HPV301 5'-TTTMĆTTT-$X^1$-TTĆT-5';
HPV302 5'-TTTMMTTT-$X^1$-TTMT-5';

RSV401 5'-TMĆTTĆTĆTTĆT-3';
RSV402 5'-TMMTTMTMTTMT-3';
RSV403 5'-TĆĆTTMTMTTMT-3';

RSV411 5'-TTĆTTTTMĆTTTTĆT-$X^1$-TTĆTT-5';
RSV412 5'-TTMTTTTMMTTTTMT-$X^1$-TTMTT-5';

HSV501 5'-MTĆTTĆTTĆTT-$X^3$-MĆMĆMĆMĆM-5';
HSV502 5'-MTĆTTĆTTĆTT-$X^3$-MĆMĆMĆMĆZ-5';
HSV503 5'-ZTĆTTĆTTĆTT-$X^3$-MĆMĆMĆMĆZ-5';
HSV504 5'-ZTĆTTĆTTĆTT-$X^3$-MĆMĆMĆMĆM-5';
HSV505 5'-MTĆTTĆTTĆTT-$X^3$-MMMMMMMMM-5';
HSV506 5'-MTĆTTĆTTĆTT-$X^3$-MMMMMMMMZ-5';
HSV507 5'-ZTĆTTĆTTĆTT-$X^3$-MMMMMMMMZ-5';
HSV508 5'-ZTĆTTĆTTĆTT-$X^3$-MMMMMMMMM-5';
HSV509 5'-MTMTTMTTMTT-$X^3$-MMMMMMMMM-5';
HSV510 5'-MTMTTMTTMTT-$X^3$-MMMMMMMMZ-5';
HSV511 5'-ZTMTTMTTMTT-$X^3$-MMMMMMMMZ-5';
HSV512 5'-ZTMTTMTTMTT-$X^3$-MMMMMMMMM-5';

HSV601 5'-MTMMMMMM-$X^4$-ĆTTĆTTM-5';
HSV602 5'-MTMMMMMM-$X^4$-ĆTTĆTTZ-5';
HSV603 5'-ZTMMMMMM-$X^4$-ĆTTĆTTZ-5';
HSV604 5'-ZTMMMMMM-$X^4$-ĆTTĆTTM-5';
HSV605 5'-MTMMMMMĆ-$X^4$-MTTMTTM-5';
HSV606 5'-MTMMMMMĆ-$X^4$-MTTMTTZ-5';
HSV607 5'-ZTMMMMMĆ-$X^4$-MTTMTTZ-5';
HSV608 5'-ZTMMMMMĆ-$X^4$-MTTMTTM-5';

HSV701 5'-MMMTTTMĆTTTMTMĆTTT-3;'
HSV702 5'-MMMTTTMMTTTMTMMTTT-3';
HSV703 5'-MMMTTTĆĆTTTMTĆĆTTT-3';

HSV711 5'-MTMMMTM-$X^4$-TMĆTĆTT-5';
HSV712 5'-ZTMMMTM-$X^4$-TMĆTĆTT-5';
HSV713 5'-MTMMMTM-$X^4$-TMMTMTT-5';
HSV714 5'-ZTMMMTM-$X^4$-TMMTMTT-5';

HSV721 5'-MMMMMTĆTMMM-$X^1$-TMMMTĆT-5';
HSV722 5'-ZMMMMTĆTMMM-$X^1$-TMMMTĆT-5';
HSV723 5'-MMMMMTMTMMM-$X^1$-TMMMTMT-5';

| | |
|---|---|
| HSV724 | 5'-ZMMMMTMTMMM-$X^1$-TMMMTMT-5'; |
| CMV801 | 5'-MMMMTTTTMTMMT-$X^1$-TMMM-5'; |
| CMV802 | 5'-MMMMTTTTMTMĆT-$X^1$-TMMM-5'; |
| CMV803 | 5'-MMMMTTTTMTMĆT-$X^1$-TMMZ-5'; |
| CMV804 | 5'-ZMMMTTTTMTMĆT-$X^1$-TMMZ-5'; |
| CMV805 | 5'-ZMMMTTTTMTMĆT-$X^1$-TMMM-5'; |
| CMV811 | 5'-MMMTTĆTM-$X^4$-ĆTTĆTMMMM-5'; |
| CMV812 | 5'-MMMTTĆTM-$X^4$-ĆTTĆTMMMZ-5'; |
| CMV813 | 5'-ZMMTTĆTM-$X^4$-ĆTTĆTMMMZ-5'; |
| CMV814 | 5'-ZMMTTĆTM-$X^4$-ĆTTĆTMMMM-5'; |
| CMV815 | 5'-MMĆTTMTM-$X^4$-MTTMTMMMM-5'; |
| CMV816 | 5'-MMĆTTMTM-$X^4$-MTTMTMMMZ-5'; |
| CMV817 | 5'-ZMĆTTMTM-$X^4$-MTTMTMMMZ-5'; |
| CMV818 | 5'-ZMĆTTMTM-$X^4$-MTTMTMMMM-5'; |
| CMV821 | 5'-MMMMTMĆTĆTMĆTĆTĆTĆTTĆTMĆTM-3'; |
| CMV822 | 5'-MMMMTMĆTĆTMĆTĆTĆTĆTTĆTMĆTZ-3'; |
| CMV823 | 5'-MMMMTMMTMTMMTMTMTMTTMTMMTM-3'; |
| CMV824 | 5'-MMMMTMMTMTMMTMTMTMTTMTMMTZ-3'; |
| CMV825 | 5'-ZMMMTMMTMTMMTMTMTMTTMTMMTZ-3'; |
| CMV826 | 5'-ZMMMTMMTMTMMTMTMTMTTMTMMTM-3'; |
| CMV827 | 5'-MMMMTĆĆTMTĆĆTMTMTMTTMTĆĆTM-3'; |
| CMV828 | 5'-MMMMTĆĆTMTĆĆTMTMTMTTMTĆĆTZ-3'; |
| CMV829 | 5'-ZMMMTĆĆTMTĆĆTMTMTMTTMTĆĆTZ-3'; and |
| CMV830 | 5'-ZMMMTĆĆTMTĆĆTMTMTMTTMTĆĆTM-3'; |
| HER101 | 5'-TMMTMTTMMTMMTMMY-3'; |
| HER102 | 5'-TMĆTMTTMĆTMĆTMĆY-3'; |
| HER103 | 5'-TMĆTMTTMĆTMĆTMZ-3'; |
| HER104 | 5'-TMMTMTTMMTMMTMM$(TMM)_4$MY-3'; |
| HER105 | 5'-TMMTMTTMMTMMTMM$(TMM)_4$Z-3'; |
| HER106 | 3'-YTGGTGTTGGTGGTGG-5'; |

HER107 3'-YTGGTGTTGGTGGTGG(TGG)$_4$G-5';
HER108 5'-TMMTMTTMMTMMTMZY-5';

HER111 3'-YGTTGTGTGGGTGTTG-5';
HER112 5'-MTTMTMTMMMTMTTTTMY-3';
HER113 5'-MTTMTMTMMMTMTTTTMTMTTMY-3';
HER114 5'-MTTMTMTMMMTMTTTTMTMTTM-3';
HER115 5'-ZMTTMTMTMMMTMTTTTMTMTTM-3';
HER116 5'-YMTTMTMTMMMTMTTTTMTMTTM-3';
HER117 5'-MMTTMTMTMMMTMTTTTMTMTTMY-3';
HER118 5'-MMTTMTMTMMMTMTTTTMTMTTMZ-3';
HER119 5'-YMTTMTMTMMMTMTTTTMTMTTMZ-3';
HER120 5'-ZMTTMTMTMMMTMTTTTMTMTTMY-3';

HER121 3'-YGTTGGGTGGGTTGGGG-5';
HER122 5'-MTTMMMTMMMTTMMMMY-3';
HER123 5'-YTTMMMTMMMTTMMMMY-3';
HER124 5'-ZTTMMMTMMMTTMMMMY-3';
HER125 5'-MTTMMMTMMMTTMMMZY-3';
HER126 5'-YTTMMMTMMMTTMMMZY-3';

HER131 5'-ZMTMĆ-X-TMMTĆTTĆT-3';
HER132 5'-MMTMĆ-X-TMMTĆTTĆT-3';
HER133 5'-ZMTMĆ-X-TMCTĆTTĆT-3';

HER141 5'-MMTTTTMMMMMTM-3';
HER142 5'-MMTTTTMMMMMTZ-3';

HER151 5'-MTTĆTĆTMM-X-MMMTTĆTT-3';
HER152 5'-YTTĆTĆTMM-X-MMMTTĆTT-3'; and
HER153 5'-ZTTĆTĆTMM-X-MMMTTĆTT-3';

IL1$\beta$101 5'-TĆTTTTĆTTĆTM-X$^9$-TĆTTTT-5';
IL1$\beta$102 5'-TMTTTTMTTMTM-X$^9$-TMTTTT-5';

IL1β103 5′-MTTTTMTTMTM-$X^9$-TMTTTT-5′;
IL1β104 5′-ZTTTTMTTMTM-$X^9$-TMTTTT-5′;

IL1β111 5′-MTTĆTTTTTTTTT-$X^5$-ĆTTTĆMT-5′;
IL1β112 5′-ZTTĆTTTTTTTTT-$X^5$-ĆTTTĆMT-5′;
IL1β113 5′-MTTMTTTTTTTTT-$X^5$-MTTTMM-5′;
IL1β114 5′-MTTMTTTTTTTTT-$X^5$-MTTTMZ-5′;
IL1β115 5′-ZTTMTTTTTTTTT-$X^5$-MTTTMZ-5′;
IL1β116 5′-ZTTMTTTTTTTTT-$X^5$-MTTTMM-5′;

TNF201 5′-TĆTMMMTTĆ-$X^1$-MMMMM-5′;
TNF202 5′-TMTMMMTTM-$X^1$-MMMMM-5′;
TNF203 5′-TMTMMMTTM-$X^1$-MMMMZ-5′;

TNF211 5′-MMMMTTĆTĆTĆTĆTĆTĆTTTĆT-3′;
TNF212 5′-ZMMMTTĆTĆTĆTĆTĆTĆTTTĆT-3′;
TNF213 5′-MMMMTTĆTĆTĆTĆTĆTĆTTTM-3′;
TNF214 5′-MMMMTTĆTĆTĆTĆTĆTĆTTTZ-3′;
TNF215 5′-ZMMMTTĆTĆTĆTĆTĆTĆTTTZ-3′;
TNF216 5′-ZMMMTTĆTĆTĆTĆTĆTĆTTTM-3′;
TNF217 5′-MMMMTTMTMTMTMTMTMTTTM-3′;
TNF218 5′-MMMMTTMTMTMTMTMTMTTTZ-3′;
TNF219 5′-ZMMMTTMTMTMTMTMTMTTTZ-3′;
TNF220 5′-ZMMMTTMTMTMTMTMTMTTTM-3′;

LAP301 5′-TĆTTMĆTT-$X^6$-MTTĆTMM-5′;
LAP302 5′-TĆTTMĆTT-$X^6$-MTTĆTMZ-5′;
LAP303 5′-TMTTMMTT-$X^6$-MTTMTMM-5′;
LAP304 5′-TMTTMMTT-$X^6$-MTTMTMZ-5′;

LAP311 5′-TMĆTTTTTM-$X^7$-ĆTTĆTĆTĆT-5′;
LAP312 5′-TMMTTTTTM-$X^7$-MTTMTMTMT-5′;

LAP321 5′-TTTTTTTTTĆTTĆTTĆTTĆTTĆTTĆTTĆTTĆTTĆTT-3′;

LAP322 5'-TTTTTTTTTMTTMTTMTTMTTMTTMTTMTTMTTMTT-3';

IL2 401 5'-TTTĆTTTMĆTMĆTTTTT-3';
IL2 402 5'-TTTĆTTTMMTMMTTTTT-3';
IL2 403 5'-TTTMTTTMMTMMTTTTT-3';
IL2 404 5'-TTTMTTTĆĆTĆĆTTTTT-3';
IL2 405 5'-TTTMTTTMĆTMĆTTTTT-3';
IL2 406 5'-TTTĆTTTĆĆTĆĆTTTTT-3';

IL2R501 5'-TTMĆTTMĆTTTĆTTTĆTTMĆTTM-3';
IL2R502 5'-TTMĆTTMĆTTTĆTTTĆTTMĆTTZ-3';
IL2R503 5'-MMTTMMTTTMTTTMTTMMTTM-3';
IL2R504 5'-MMTTMMTTTMTTTMTTMMTTZ-3';
IL2R505 5'-ZMTTMMTTTMTTTMTTMMTTM-3';
IL2R506 5'-ZMTTMMTTTMTTTMTTMMTTZ-3';

IL2R511 5'-MTTĆTMMMTĆTTMMMT-3';
IL2R512 5'-ZTTĆTMMMTĆTTMMMT-3';

IL4 601 5'-TMTMMMMMTTM-3';
IL4 602 5'-TMTMMMMMTTZ-3';

IL4 611 5'-MTĆTTMMT-$X^8$-MTTMT-3';
IL4 612 5'-ZTĆTTMMT-$X^8$-MTTMT-3';
IL4 613 5'-MTMTTMMT-$X^8$-MTTMT-3';
IL4 614 5'-ZTMTTMMT-$X^8$-MTTMT-3';

IL6R701 5'-MMMMTTĆT-$X^8$-TMTMTMMTMMMTTTMTTMMT-5';
IL6R702 5'-ZMMMTTĆT-$X^8$-TMTMTMMTMMMTTTMTTMMT-5';
IL6R703 5'-MMMMTTĆT-$X^8$-TĆTĆTĆĆTMMMTTTMTTMMM-5';
IL6R704 5'-MMMMTTĆT-$X^8$-TĆTĆTĆĆTMMMTTTMTTMMZ-5';
IL6R705 5'-ZMMMTTĆT-$X^8$-TĆTĆTĆĆTMMMTTTMTTMMZ-5';
IL6R706 5'-ZMMMTTĆT-$X^8$-TĆTĆTĆĆTMMMTTTMTTMMM-5';

IL6R711 5'-TMTMMTTMMTMTMMTMTMMMM-3';
IL6R712 5'-TMTMMTTMMTMTMMTMTMMMZ-3';
IL6R713 5'-TMTMĆTTMĆTMTMĆTMTMMMM-3';
IL6R714 5'-TMTMĆTTMĆTMTMĆTMTMMMZ-3';

IL6 801 5'-MTMMMMTTMTM-$X^9$-TTMT-5';
IL6 802 5'-ZTMMMMTTMTM-$X^9$-TTMT-5';
IL6 803 5'-M$(T)_{10}X^1(T)_{11}$M-5';
IL6 804 5'-Z$(T)_{10}X^1(T)_{11}$M-5';
IL6 805 5'-Z$(T)_{10}X^1(T)_{11}$Z-5';

ING811 5'-MMTTTMTMMTMTM-3';
ING812 5'-MMTTTMTMMTMTZ-3';
ING813 5'-ZMTTTMTMMTMTZ-3';
ING814 5'-ZMTTTMTMMTMTM-3';

ILR901 5'-TMMTMMMTMTMTT-3';

ILR911 5'-TTTMMTMMTMMTTMMM-3';
ILR912 5'-TTTMMTMMTMMTTMMZ-3';
ILR913 5'-TTTMĆTMĆTMĆTTMMM-3';
ILR914 5'-TTTMĆTMĆTMĆTTMMZ-3';

EMG921 5'-TMĆTĆTTMĆTĆT-$X^1$-ĆMTTT-5';
EMG922 5'-TMMTMTTMMTMT-$X^1$-MMTTT-5';

ELAM931 5'-TĆTTĆTM-$X^5$-ĆMTTĆMT-5';
ELAM932 5'-TMTTMTM-$X^5$-MMTTMMT-5';

TNFR941 5'-TTTTĆTTTTTTTTTTTTM-3';
TNFR942 5'-TTTTĆTTTTTTTTTTTTZ-3'; and
TNFR943 5'-TTTTMTTTTTTTTTTTTZ-3';

wherein M is a $N^6$-methyl-8-oxo-2'-deoxyadenosyl residue (MODA), Ć is 5-methylcytosine; $X^n$ are various o-xyloso synthons: X is a generic synthon; $X^1$ is a synthon with a TT dimer; $X^2$ is an o-xyloso dimer synthon with an MT dimer, $X^3$ with an MM or ĆM dimer, $X^4$ with an MT or ĆT dimer, $X^5$ an MT dimer, $X^6$ a ĆĆ or MM dimer, $X^7$ a ĆT dimer, $X^8$ a TM dimer, and $X^9$ an MM dimer; Y is anthriquinone; and Z is a cytidyl residue containing an aziridenyl substituent.

13. A pharmaceutical composition for treatment of conditions characterized by a target DNA duplex which composition comprises as active ingredient an oligomer of claims 1-12 in admixture with a pharmaceutically acceptable excipient.

14. The oligomer of claims 1-12 or a pharmaceutical composition thereof for use in treating conditions characterized by a target DNA duplex.

15. A method to detect the presence, absence, or amount of target duplex DNA in a biological sample, which method comprises contacting said sample with the oligomer of claims 1-12 under conditions wherein a triplex is formed; and

detecting the presence, absence, or amount of said triplex.

16. A triplex which consists essentially of a target DNA duplex complexed with the oligomer of claims 1-12.

17. A method to form a DNA triplex which method comprises contacting a target DNA duplex with the

oligomer of claims 1-12 under conditions suitable for forming said triplex.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/08811

**I. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5] (5): C12Q 1/68; A01N 43/04; A61K 31/70; C07H 15/12, 17/00
US CL: 435/6; 514/25,44; 536/27, 28, 29

**II. FIELDS SEARCHED**

Minimum Documentation Searched [7]

| Classification System | Classification Symbols |
|---|---|
| US | 435/6; 514/25,44; 536/27,28,29 |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Databases: CAS
Automated patent System (File US PAT 1971-1991)

**III. DOCUMENTS CONSIDERED TO BE RELEVANT** [9]

| Category [10] | Citation of Document, [11] with indication, where appropriate, of the relevant passages [12] | Relevant to Claim No. [13] |
|---|---|---|
| Y | EP, A, 0,375,408 (Hogan et al.) 27 June 1990, see entire document | 1-17 |
| Y | Science, Vol. 245, issued 01 September 1989, Griffin et al. "Recognition of Thymine Adenine Base Paris by Guanine in a Pyrimidine Triple Helix Motif", pages 967-971, see entire document. | 1-17 |
| Y | Science, Vol. 245, issued 18 August 1989, Maher et al "Inhibition of DNA Binding Proteins by Oligonuclestide Directed triple Helix Formation", pages 725-730, see entire document | 1-17 |

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

**IV. CERTIFICATION**

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 07 February 1992 | 09MAR 1992 |
| **International Searching Authority** | **Signature of Authorized Officer** |
| ISA/US | Gian Wang |

**FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET**

| | | |
|---|---|---|
| Y | Science, Vol. 238, issued 30 October 1987, Moser et al., "Sequence - Specific Cleavage of Double Helical DNA by Triple Helix Formation", pages 645-650, see entire document. | 1-17 |

**V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]**

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . , because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..... .. , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

**VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]**

This International Searching Authority found multiple inventions in this international application as follows:

See attached Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. Telephone Practice

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

| Category * | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No |
|---|---|---|
| Y | J. Am. Chem. Soc., Vol. 111, issued 1989, Povsic et al. "Triple Helix Formation by Obigonuclestides on DNA Extended to the Physicological pH Range", pages 3059-3061, see entire document. | 1-17 |