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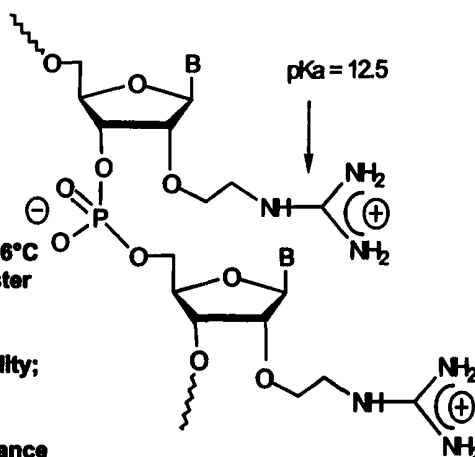
(54) Title: GUANIDINIUM FUNCTIONALIZED OLIGOMERS AND METHODS

♦ $\Delta T_m = 3.0^\circ\text{C}$ (dispersed) 0.6°C (placed together) as a diester (Relative to 2'-deoxyP=S)

♦ Entropy effect due to rigidity; Weak Gauche Effect

♦ Very high nuclease resistance due to charge effect

♦ Charge delocalization over four atoms



**2'-O-(2-Guanidinium-Ethyl):
Supercationic Modification**

(57) Abstract: The present invention provides oligomers which are specifically hybridizable with a selected sequence of RNA or DNA wherein at least one of the nucleoside moieties of the oligomer is modified to include a guanidinium group. These oligomers are useful for diagnostic, therapeutic and investigative purposes.



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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

GUANIDINIUM FUNCTIONALIZED OLIGOMERS AND METHODS

RELATED APPLICATION DATA

This patent application is a continuation-in-part of
5 Application Serial No. 09/349,040, filed July 7, 1999, the
contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to monomers and oligomers
containing guanidinium moieties and methods of preparing
10 such oligomers. The oligomers of the present invention are
used for investigative and therapeutic purposes.

BACKGROUND OF THE INVENTION

It is well known that most of the bodily states in
mammals, including most disease states, are affected by
15 proteins. Classical therapeutic modes have generally
focused on interactions with such proteins in an effort to
moderate their disease-causing or disease-potentiating
functions. However, recently, attempts have been made to
moderate the actual production of such proteins by
20 interactions with molecules that direct their synthesis,
such as intracellular RNA. By interfering with the
production of proteins, maximum therapeutic effect and
minimal side effects may be realized. It is the general
object of such therapeutic approaches to interfere with or

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otherwise modulate gene expression leading to undesired protein formation.

One method for inhibiting specific gene expression is the use of oligonucleotides. Oligonucleotides are now
5 accepted as therapeutic agents with great promise. Oligonucleotides are known to hybridize to single-stranded DNA or RNA molecules. Hybridization is the sequence-specific base pair hydrogen bonding of nucleobases of the oligonucleotide to the nucleobases of the target DNA or RNA
10 molecule. Such nucleobase pairs are said to be complementary to one another. The concept of inhibiting gene expression through the use of sequence-specific binding of oligonucleotides to target RNA sequences, also known as antisense inhibition, has been demonstrated in a variety of
15 systems, including living cells (for example see: Wagner et al., Science (1993) 260: 1510-1513; Milligan et al., J. Med. Chem., (1993) 36:1923-37; Uhlmann et al., Chem. Reviews, (1990) 90:543-584; Stein et al., Cancer Res., (1988) 48:2659-2668).

20 The events that provide the disruption of the nucleic acid function by antisense oligonucleotides (Cohen in *Oligonucleotides: Antisense Inhibitors of Gene Expression*, (1989) CRC Press, Inc., Boca Raton, FL) are thought to be of two types. The first, hybridization arrest, denotes the
25 terminating event in which the oligonucleotide inhibitor binds to the target nucleic acid and thus prevents, by simple steric hindrance, the binding of essential proteins, most often ribosomes, to the nucleic acid. Methyl phosphonate oligonucleotides: Miller and Ts'O, *Anti-Cancer*
30 *Drug Design*, **1987**, 2:117-128, and α -anomer oligonucleotides are the two most extensively studied antisense agents which are thought to disrupt nucleic acid function by hybridization arrest.

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The second type of terminating event for antisense oligonucleotides involves the enzymatic cleavage of the targeted RNA by intracellular RNase H. A 2'-deoxyribofuranosyl oligonucleotide or oligonucleotide analog
5 hybridizes with the targeted RNA and this duplex activates the RNase H enzyme to cleave the RNA strand, thus destroying the normal function of the RNA. Phosphorothioate oligonucleotides are the most prominent example of an antisense agent that operates by this type of antisense
10 terminating event.

Oligonucleotides may also bind to duplex nucleic acids to form triplex complexes in a sequence specific manner via Hoogsteen base pairing (Beal et al., *Science*, (1991) 251:1360-1363; Young et al., *Proc. Natl. Acad. Sci.* (1991)
15 88:10023-10026). Both antisense and triple helix therapeutic strategies are directed towards nucleic acid sequences that are involved in or responsible for establishing or maintaining disease conditions. Such target nucleic acid sequences may be found in the genomes of
20 pathogenic organisms including bacteria, yeasts, fungi, protozoa, parasites, viruses, or may be endogenous in nature. By hybridizing to and modifying the expression of a gene important for the establishment, maintenance or elimination of a disease condition, the corresponding
25 condition may be cured, prevented or ameliorated.

In determining the extent of hybridization of an oligonucleotide to a complementary nucleic acid, the relative ability of an oligonucleotide to bind to the complementary nucleic acid may be compared by determining
30 the melting temperature of a particular hybridization complex. The melting temperature (T_m), a characteristic physical property of double helices, denotes the temperature (in degrees centigrade) at which 50% helical (hybridized) versus coil (unhybridized) forms are present. T_m is measured

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by using the UV spectrum to determine the formation and breakdown (melting) of the hybridization complex. Base stacking, which occurs during hybridization, is accompanied by a reduction in UV absorption (hypochromicity).

- 5 Consequently, a reduction in UV absorption indicates a higher T_m . The higher the T_m , the greater the strength of the bonds between the strands.

Oligonucleotides may also be of therapeutic value when they bind to non-nucleic acid biomolecules such as
10 intracellular or extracellular polypeptides, proteins, or enzymes. Such oligonucleotides are often referred to as 'aptamers' and they typically bind to and interfere with the function of protein targets (Griffin et al., *Blood*, (1993), 81:3271-3276; Bock et al., *Nature*, (1992) 355: 564-566).

- 15 Oligonucleotides and their analogs have been developed and used for diagnostic purposes, therapeutic applications and as research reagents. For use as therapeutics, oligonucleotides must be transported across cell membranes or be taken up by cells, and appropriately hybridize to
20 target DNA or RNA. These critical functions depend on the initial stability of the oligonucleotides toward nuclease degradation. A serious deficiency of unmodified oligonucleotides which affects their hybridization potential with target DNA or RNA for therapeutic purposes is the
25 enzymatic degradation of administered oligonucleotides by a variety of intracellular and extracellular ubiquitous nucleolytic enzymes referred to as nucleases. For oligonucleotides to be useful as therapeutics or
30 diagnostics, the oligonucleotides should demonstrate enhanced binding affinity to complementary target nucleic acids, and preferably be reasonably stable to nucleases and resist degradation. For a non-cellular use such as a research reagent, oligonucleotides need not necessarily possess nuclease stability.

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A number of chemical modifications have been introduced into oligonucleotides to increase their binding affinity to target DNA or RNA and resist nuclease degradation.

Modifications have been made to the ribose phosphate backbone to increase the resistance to nucleases. These modifications include use of linkages such as methyl phosphonates, phosphorothioates and phosphorodithioates, and the use of modified sugar moieties such as 2'-O-alkyl ribose. Other oligonucleotide modifications include those made to modulate uptake and cellular distribution. A number of modifications that dramatically alter the nature of the internucleotide linkage have also been reported in the literature. These include non-phosphorus linkages, peptide nucleic acids (PNA's) and 2'-5' linkages. Another modification to oligonucleotides, usually for diagnostic and research applications, is labeling with non-isotopic labels, e.g., fluorescein, biotin, digoxigenin, alkaline phosphatase, or other reporter molecules.

A variety of modified phosphorus-containing linkages have been studied as replacements for the natural, readily cleaved phosphodiester linkage in oligonucleotides. In general, most of them, such as the phosphorothioate, phosphoramidates, phosphonates and phosphorodithioates all result in oligonucleotides with reduced binding to complementary targets and decreased hybrid stability. In order to make effective therapeutics therefore this binding and hybrid stability of antisense oligonucleotides needs to be improved.

Of the large number of modifications made and studied, few have progressed far enough through discovery and development to deserve clinical evaluation. Reasons underlying this include difficulty of synthesis, poor binding to target nucleic acids, lack of specificity for the target nucleic acid, poor *in vitro* and *in vivo* stability to

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nucleases, and poor pharmacokinetics. Several phosphorothioate oligonucleotides and derivatives are presently being used as antisense agents in human clinical trials for the treatment of various disease states. A
5 submission for approval was recently made to both United States and European regulatory agencies for one antisense drug, Fomivirsen, for use to treat cytomegalovirus (CMV) retinitis in humans.

The structure and stability of chemically modified
10 nucleic acids is of great importance to the design of antisense oligonucleotides. Over the last ten years, a variety of synthetic modifications have been proposed to increase nuclease resistance, or to enhance the affinity of the antisense strand for its target mRNA (Crooke et al.,
15 *Med. Res. Rev.*, **1996**, 16, 319-344; De Mesmaeker et al., *Acc. Chem. Res.*, **1995**, 28, 366-374). Although a great deal of information has been collected about the types of modifications that improve duplex formation, little is known about the structural basis for the improved affinity
20 observed.

RNA exists in what has been termed "A Form" geometry while DNA exists in "B Form" geometry. In general, RNA:RNA duplexes are more stable, or have higher melting temperatures (T_m) than DNA:DNA duplexes (Sanger et al.,
25 *Principles of Nucleic Acid Structure*, **1984**, Springer-Verlag; New York, NY.; Lesnik et al., *Biochemistry*, **1995**, 34, 10807-10815; Conte et al., *Nucleic Acids Res.*, **1997**, 25, 2627-2634). The increased stability of RNA has been attributed to several structural features, most notably the improved
30 base stacking interactions that result from an A-form geometry (Searle et al., *Nucleic Acids Res.*, **1993**, 21, 2051-2056). The presence of the 2' hydroxyl in RNA biases the sugar toward a C3' endo pucker, i.e., also designated as

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Northern pucker, which causes the duplex to favor the A-form geometry. On the other hand, deoxy nucleic acids prefer a C2' endo sugar pucker, i.e., also known as Southern pucker, which is thought to impart a less stable B-form geometry

5 (Sanger, W. (1984) *Principles of Nucleic Acid Structure*, Springer-Verlag, New York, NY). In addition, the 2' hydroxyl groups of RNA can form a network of water mediated hydrogen bonds that help stabilize the RNA duplex (Egli et al., *Biochemistry*, **1996**, 35, 8489-8494).

10 DNA:RNA hybrid duplexes, however, are usually less stable than pure RNA:RNA duplexes, and depending on their sequence may be either more or less stable than DNA:DNA duplexes (Searle et al., *Nucleic Acids Res.*, **1993**, 21, 2051-2056). The structure of a hybrid duplex is intermediate

15 between A- and B-form geometries, which may result in poor stacking interactions (Lane et al., *Eur. J. Biochem.*, **1993**, 215, 297-306; Fedoroff et al., *J. Mol. Biol.*, **1993**, 233, 509-523; Gonzalez et al., *Biochemistry*, **1995**, 34, 4969-4982; Horton et al., *J. Mol. Biol.*, **1996**, 264, 521-533). The

20 stability of a DNA:RNA hybrid is central to antisense therapies as the mechanism requires the binding of a modified DNA strand to a mRNA strand. To effectively inhibit the mRNA, the antisense DNA should have a very high binding affinity with the mRNA. Otherwise the desired

25 interaction between the DNA and target mRNA strand will occur infrequently, thereby decreasing the efficacy of the antisense oligonucleotide.

One synthetic 2'-modification that imparts increased nuclease resistance and a very high binding affinity to

30 nucleotides is the 2'-methoxyethoxy (MOE, 2'-OCH₂CH₂OCH₃) side chain (Baker et al., *J. Biol. Chem.*, **1997**, 272, 11944-12000; Freier et al., *Nucleic Acids Res.*, **1997**, 25, 4429-4443). One of the immediate advantages of the MOE substitution is the

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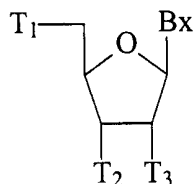
improvement in binding affinity, which is greater than many similar 2' modifications such as O-methyl, O-propyl, and O-aminopropyl (Freier and Altmann, *Nucleic Acids Research*, (1997) 25:4429-4443). 2'-O-Methoxyethyl-substituted also
5 have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (Martin, *Helv. Chim. Acta*, **1995**, 78, 486-504; Altmann et al., *Chimia*, **1996**, 50, 168-176; Altmann et al., *Biochem. Soc. Trans.*, **1996**, 24, 630-637; and Altmann et al., *Nucleosides*
10 *Nucleotides*, 1997, **16**, 917-926). Relative to DNA, they display improved RNA affinity and higher nuclease resistance. Chimeric oligonucleotides with 2'-O-methoxyethyl-ribonucleoside wings and a central DNA-phosphorothioate window also have been shown to effectively
15 reduce the growth of tumors in animal models at low doses. MOE substituted oligonucleotides have shown outstanding promise as antisense agents in several disease states. One such MOE-substituted oligonucleotide is currently available for the treatment of CMV retinitis.

20 Although the known modifications to oligonucleotides, including the use of the 2'-O-methoxyethyl modification, have contributed to the development of oligonucleotides for use in diagnostics, therapeutics and as research reagents, there still exists a need in the art for further
25 modifications to oligonucleotides having enhanced hybrid binding affinity and/or increased nuclease resistance.

SUMMARY OF THE INVENTION

In accordance with the present invention, oligomers containing guanidinium groups are provided. The present
30 invention provides monomers of the formula:

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wherein:

Bx is a heterocyclic base;

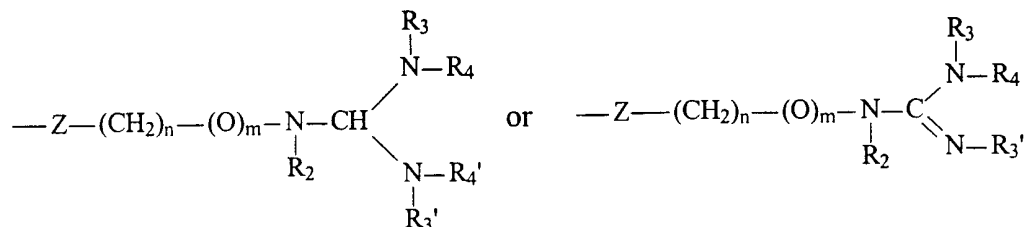
T₁ is OH or a protected hydroxyl group;

5 T₂ is an activated phosphorus group or a linking moiety attached to a solid support;

T₃ is H, OH, a protected hydroxyl or a sugar substituent group;

said monomer further comprising at least one group, R₁,

10 therein; said R₁ group occurring in lieu of at least one T₁, T₂ or T₃ or as a substituent on at least one Bx; said R₁ group having one of the formulas:



wherein:

each Z is, independently, a single bond, O, N or S;

15 each R₂, R₃, R_{3'}, R₄ and R_{4'} is, independently, hydrogen, C(O)R₅, substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the

20 substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

or R₃ and R₄, together, are R₇, and R_{3'} and R_{4'}, together, are R₇;

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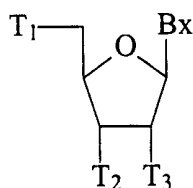
each R_5 is, independently, substituted or unsubstituted C_1 - C_{10} alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-
 5 butyryl, phenyl or aryl;

each R_7 and R_7 , is, independently, hydrogen or forms a phthalimide moiety with the nitrogen atom to which it is attached;

each m is, independently, zero or 1; and

10 each n is, independently, an integer from 1 to about 6.

Preferred compositions include oligomers comprising a plurality of nucleotide units of the structure:



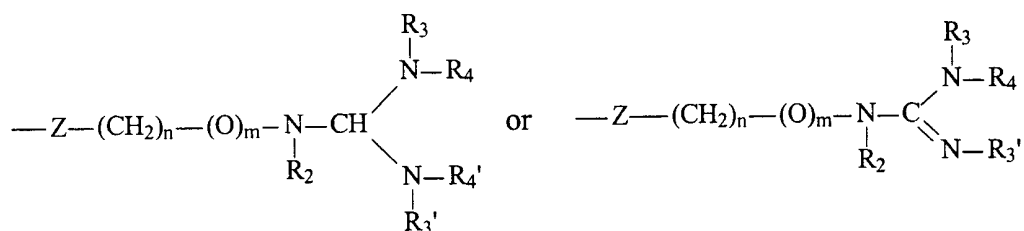
wherein:

15 Bx is a heterocyclic base;

each T_1 and T_2 is, independently, OH, a protected hydroxyl, a nucleotide, a nucleoside or an oligonucleotide;

T_3 is H, OH, a protected hydroxyl or a sugar substituent group;

20 said oligomer further comprising at least one group, R_1 , therein; said R_1 group occurring at the 3' end, the 5' end, in lieu of at least one T_3 or as a substituent on at least one Bx; said R_1 group having one of the formulas:



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wherein:

each Z is, independently, a single bond, O, N or S;

each R_2 , R_3 , R_3' , R_4 and R_4' is, independently, hydrogen, $C(O)R_5$, substituted or unsubstituted C_1 - C_{10} alkyl, substituted
 5 or unsubstituted C_2 - C_{10} alkenyl, substituted or unsubstituted C_2 - C_{10} alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen,
 10 alkyl, aryl, alkenyl and alkynyl;

or R_3 and R_4 , together, are R_7 , and R_3' and R_4' , together, are R_7 ;

each R_5 is, independently, substituted or unsubstituted C_1 - C_{10} alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy,
 15 t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyl, phenyl or aryl;

each R_7 and R_7' is, independently, hydrogen or forms a phthalimide moiety with the nitrogen atom to which it is
 20 attached;

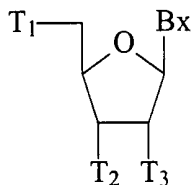
each m is, independently, zero or 1; and

each n is, independently, an integer from 1 to about 6.

In a preferred embodiment, R_1 , R_2 , R_3 , R_3' , R_4 and R_4' are hydrogen. In another preferred embodiment, R_1 , R_2 , R_3 , R_3' , R_4
 25 and R_4' are hydrogen, m is zero and n is 2.

The present invention also provides methods for preparing oligomers comprising the steps of:

(a) selecting a monomer of the formula:



30 wherein:

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Bx is a heterocyclic base;

T₁ is OH or a protected hydroxyl group;

T₂ is a linking moiety attached to a solid support;

T₃ is H, OH, a protected hydroxyl or a sugar substituent

5 group;

said monomer further comprising at least one group, R₁,

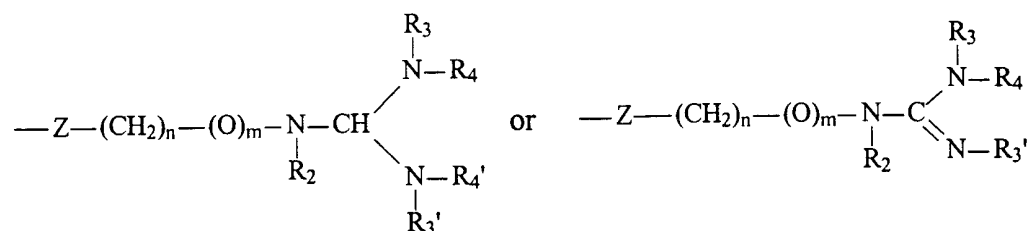
therein; said R₁ group occurring in lieu of at least one T₁,

T₂ or T₃ or as a substituent on at least one Bx;

provided that if T₂ is R₁, T₃ is a linking moiety attached to

10 a solid support;

said R₁ group having one of the formulas:



wherein:

each Z is, independently, a single bond, O, N or S;

each R₂, R₃, R_{3'}, R₄ and R_{4'} is, independently, hydrogen,
 15 C(O)R₅, substituted or unsubstituted C₁-C₁₀ alkyl, substituted
 or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted
 C₂-C₁₀ alkynyl, alkylsulfonyl, arylsulfonyl, a chemical
 functional group or a conjugate group, wherein the
 substituent groups are selected from hydroxyl, amino, alkoxy,
 20 carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen,
 alkyl, aryl, alkenyl and alkynyl;

or R₃ and R₄, together, are R₇, and R₃ and R₄, together,
 are R₇;

each R₅ is, independently, substituted or unsubstituted
 25 C₁-C₁₀ alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy,
 t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-
 ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-

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butyryl, phenyl or aryl;

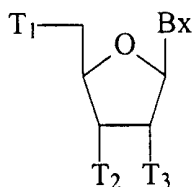
each R_7 and R_7' is, independently, hydrogen or forms a phthalimide moiety with the nitrogen atom to which it is attached;

5 each m is, independently, zero or 1; and

each n is, independently, an integer from 1 to about 6;

(b) deprotecting the protected hydroxyl group at the 5'-position to form a deprotected monomer;

(c) coupling said deprotected monomer with a second
10 monomer of formula :



wherein:

Bx is a heterocyclic base;

T_1 is OH or a protected hydroxyl group;

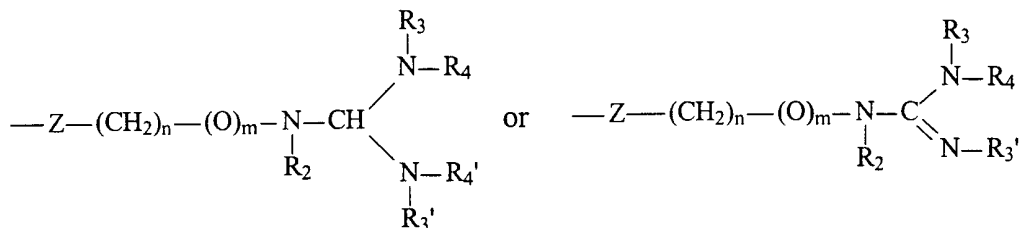
T_2 is an activated phosphorus group;

15 T_3 is H, OH, a protected hydroxyl or a sugar substituent group;

said monomer further comprising at least one group, R_1 , therein; said R_1 group occurring in lieu of at least one T_1 , T_2 or T_3 or as a substituent on at least one Bx ;

20 provided that if T_2 is R_1 , T_3 is an activated phosphorus group;

said R_1 group having one of the formulas:



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wherein:

each Z is, independently, a single bond, O, N or S;

each R₂, R₃, R_{3'}, R₄ and R_{4'} is, independently, hydrogen, C(O)R₅, substituted or unsubstituted C₁-C₁₀ alkyl, substituted
5 or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen,
10 alkyl, aryl, alkenyl and alkynyl;

or R₃ and R₄, together, are R₇, and R_{3'} and R_{4'} together, are R_{7'};

each R₅ is, independently, substituted or unsubstituted C₁-C₁₀ alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy,
15 t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyl, phenyl or aryl;

each R₇ and R_{7'} is, independently, hydrogen or forms a phthalimide moiety with the nitrogen atom to which it is
20 attached;

each m is, independently, zero or 1; and

each n is, independently, an integer from 1 to about 6, said coupling occurring in the presence of an activating agent to form a coupled compound;

25 (d) capping said coupled compound with a capping reagent to form a capped compound having an internucleotide linkage;

(e) oxidizing said internucleotide linkage with an oxidizing reagent; and

30 (f) repeating steps (b) to (e) to form an oligomer.

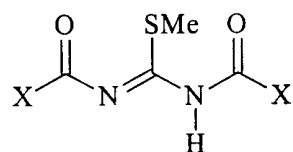
In one embodiment of the present invention, the method includes an additional step of cleaving the oligomer with a cleaving reagent.

Preferred activating reagents include tetrazole,

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pyridinium trifluoroacetate and dicyanoimidazole. It is preferred that acetic anhydride and N-methylimidazole be the capping reagent. Preferred oxidizing reagents include iodine, camphorsulfonyloxaziridine, t-butyl hydrogen peroxide
5 and Beaucage reagent.

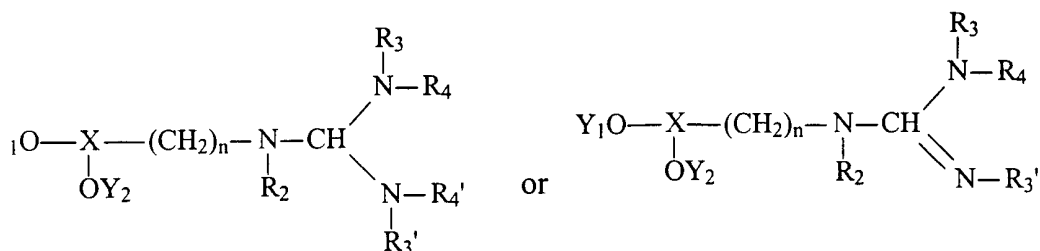
The present invention also provides compounds of the formula:



wherein X is cyanoethyloxy, benzyloxy, t-butoxy, methoxy, ethoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-
10 ethoxy, 2,2,2-trichloroethoxy, trifluoromethyl, butyryl, iso-butyl, phenyl or aryl.

In one embodiment of the present invention, X is cyanoethyloxy. In another embodiment, X is phenyl. In yet another embodiment, X is t-butyl.

15 The present invention is also directed to non-nucleic acid monomers and oligomers comprising at least one such non-nucleic acid monomer. The present invention includes non-nucleic acid monomers of the formula:



wherein:

20 X is C₃-C₁₀ alkyl, C₆-C₂₄ aryl, C₆-C₂₄ heteroaryl, C₄-C₂₀ alicyclic, C₄-C₂₀ alicyclic having at least one heteroatom, nucleoside, nucleotide or oligonucleotide;

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Y_1 is a hydroxyl protecting group;

Y_2 is an activated phosphorus group or a linking moiety attached to a solid support;

each R_2 , R_3 , R_3 , R_4 and R_4 is, independently, hydrogen, 5 $C(O)R_5$, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_2 - C_{10} alkenyl, substituted or unsubstituted C_2 - C_{10} alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, 10 carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

or R_3 and R_4 , together, are R_7 , and R_3 and R_4 , together, are R_7 ;

each R_5 is, independently, substituted or unsubstituted 15 C_1 - C_{10} alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, isobutyryl, phenyl or aryl;

each R_7 and R_7 is, independently, hydrogen or forms a 20 phthalimide moiety with the nitrogen atom to which it is attached; and

n is an integer from 1 to about 6.

In a preferred embodiment, Y_1 is dimethoxytrityl. In another preferred embodiment, Y_1 is monomethoxytrityl. In 25 yet another preferred embodiment, Y_2 is a phosphoramidite. In a further embodiment, Y_2 is a linking moiety attached to a solid support. It is preferred that Y_2 be succinyl CPG.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the structure of a 2'-O-[2- 30 (guanidinium)ethyl] modification.

Figure 2 shows the binding affinity/modification of 2'-O-[2-(guanidinium)ethyl] modification in comparison to other known 2'-modifications such as 2'-O-MOE (methoxyethyl), 2'-O-

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DMAOE (dimethylaminoxyethyl), 2'-O-DEAOE(diethylaminoxyethyl), 2'-O-IPMAOE (isopropylmethylaminoxyethyl), 2'-O-imidazoylethyl (IE) and 2'-aminopropyl (AP).

Figure 3 shows binding affinity of 2'-O-[2-(guanidinium)ethyl] modified oligonucleotides as a function of position in placement compared to 2'-aminopropyl and 2'-O-DMAOE (dimethylaminoethyloxyethyl).

Figure 4 shows electropherograms demonstrating the nuclease resistance of 2'-O-[2-(guanidinium)ethyl] modified oligonucleotides.

Figure 5 shows the synthesis of compound 1.

Figure 6 shows the synthesis of compound 4.

Figure 7 shows the structures of compounds 5-7.

Figure 8 shows the synthesis of compounds 8-13.

Figure 9 shows the synthesis of compounds 15-18.

Figure 10 shows the synthesis of compounds 20-21.

Figure 11 shows the synthesis of compounds 22-23.

Figure 12 shows the synthesis of compounds 24-25.

Figure 13 shows the synthesis of compounds 26-29.

Figure 14 shows the synthesis of compounds 30-33.

Figure 15 shows the synthesis of compound 34.

Figure 16 shows the synthesis of compound 35.

Figure 17 shows the synthesis of compound 36.

Figure 18 shows the synthesis of compounds 40 and 41.

Figure 19 shows the synthesis of compound 45.

Figure 20 shows the synthesis of compound 49.

Figure 21 shows the synthesis of compound 53.

Figure 22 shows the synthesis of compound 56.

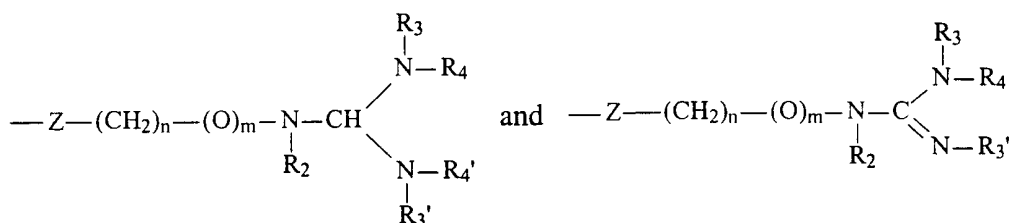
DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention provides monomers comprising a guanidinium functionality located at the 2'-position or as a

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substituent on the heterocyclic base. The present invention also provides oligomers containing a plurality of nucleotide units, at least one of said nucleotide units bearing a guanidinium group at the 3' end, the 5' end, the 2'-position, 5 or as a substituent on the heterocyclic base. Also provided are methods of making such oligomers.

As used herein, "guanidinium group" or "guanidinium functionality" denotes a group of either formulas:



wherein:

10 Z is a single bond, O, N or S;

each R₂, R₃, R_{3'}, R₄ and R_{4'} is, independently, hydrogen, C(O)R₅, substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, alkylsulfonyl, arylsulfonyl, a chemical
15 functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

or R₃ and R₄, together, are R₇, and R_{3'} and R_{4'}, together,
20 are R₇;

each R₅ is, independently, substituted or unsubstituted C₁-C₁₀ alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-
25 butyryl, phenyl or aryl;

each R₇ and R_{7'} is, independently, hydrogen or forms a phthalimide moiety with the nitrogen atom to which it is

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attached;

m is zero or 1; and

n is an integer from 1 to about 6.

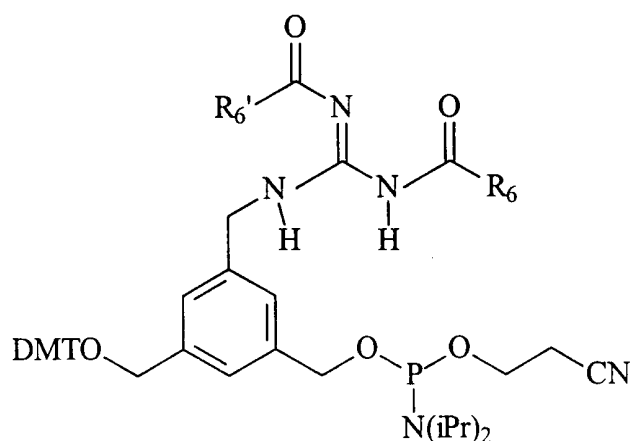
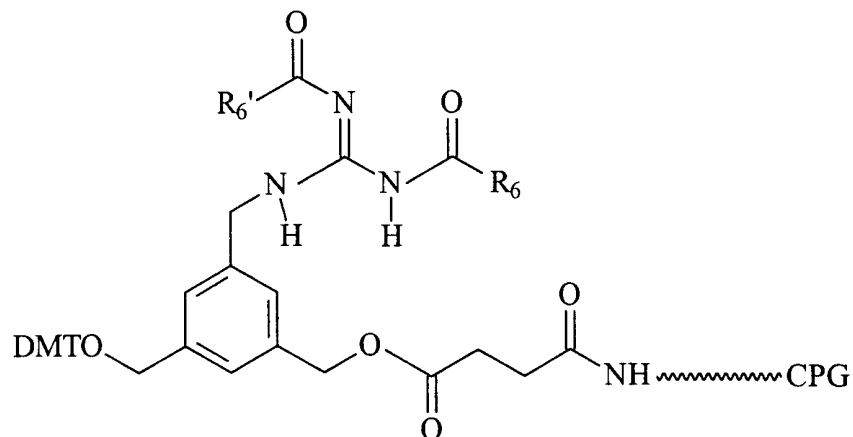
In one embodiment of the present invention, Z is O, R₂,
5 R₃, R₃., R₄ and R₄. are hydrogen, n is 1 and m is zero. This
2-O-guanidiniummethyl modification, present in an oligomer,
significantly increases the binding affinity of the oligomer
to the target. This increase in binding affinity improves
when additional guanidinium modifications are dispersed
10 within the oligomer. Guanidinium functionalized oligomers
are of use in forming triple helices with double-stranded
nucleic acids moieties.

The guanidinium group is strongly basic with a pK_a of
about 12.5, and is a well-stabilized cation with the positive
15 charge being delocalized over four atoms. Guanidinium groups
are understood to facilitate contact between proteins and
peptides with phosphate groups of nucleic acids.

Particularly, the guanidinium group is suggested to form
ionic interactions with guanine residues in the major groove
20 or bond with phosphate moieties located in the minor groove.

The present invention includes oligomers containing
nucleotide monomers as well as non-nucleic acid monomers.
For example, the present invention includes compounds of the
following formulas, as well as oligomers having at least one
25 monomer compound of the following formulas:

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wherein each R₆ and R₆' is, independently, trifluoromethyl, cyanoethyloxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or
 5 aryl.

Heterocyclic bases amenable to the present invention include both naturally- and non-naturally-occurring nucleobases and heterocycles. The heterocyclic base may be a pyrimidine, purine or diaminopurine base. A representative
 10 list includes adenine, guanine, cytosine, uridine, and

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thymine, as well as other synthetic and natural nucleobases such as xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 5-halo uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudo uracil), 4-thiouracil, 8-halo, oxa, amino, thiol, thioalkyl, hydroxyl and other 8-substituted adenines and guanines, 7-methylguanine, 5-trifluoromethyl and other 5-substituted uracils and cytosines. Further heterocyclic bases include those disclosed in United States Patent No. 3,687,808; the *Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J.I., Ed., John Wiley & Sons, 1990; and Englisch et al., *Angewandte Chemie, International Edition*, **1991**, 30, 613.

Heterocyclic bases in the oligomers of the present invention may be covalently bound to an R_1 group. In a preferred embodiment, the R_1 group may be covalently attached to C5 or an amino group at the 4-position of a pyrimidine heterocyclic base. In another preferred embodiment, the R_1 group may be covalently attached to an amino group at the 2-position or 6-position of a purine heterocyclic base. The R_1 group may even be attached to the amino group at the 6-position of a diaminopurine heterocyclic base.

The monomers of the present invention can include appropriate activated phosphorus groups such as activated phosphate groups and activated phosphite groups. As used herein, the terms activated phosphate and activated phosphite groups refer to activated monomers or oligomers that are reactive with a hydroxyl group of another monomeric or oligomeric compound to form a phosphorus-containing internucleotide linkage. Such activated phosphorus groups contain activated phosphorus atoms in P^{III} or P^V valency states. Such activated phosphorus atoms are known in the art and include, but are not limited to, phosphoramidite, H-

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phosphonate and phosphate triesters. A preferred synthetic solid phase synthesis utilizes phosphoramidites as activated phosphates. The phosphoramidites utilize P^{III} chemistry. The intermediate phosphite compounds are subsequently oxidized to
5 the P^V state using known methods to yield, in a preferred embodiment, phosphodiester or phosphorothioate internucleotide linkages. Additional activated phosphates and phosphites are disclosed in Tetrahedron Report Number 309 (Beaucage and Iyer, *Tetrahedron*, **1992**, 48, 2223-2311).

10 In the context of the present invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent
15 internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example,
20 enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

Although antisense oligonucleotides are a preferred form of antisense compounds, the present invention comprehends other oligomeric antisense compounds, including but not
25 limited to oligonucleotide mimics. The oligomers in accordance with this invention preferably comprise from about 8 to about 30 nucleobases (*i.e.*, from about 8 to about 30 linked nucleosides). Particularly preferred oligomers are antisense oligonucleotides, even more preferably those
30 comprising from about 12 to about 25 nucleobases. As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are

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nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn, the respective ends of this linear polymeric structure can be further joined to form a circular structure. Open linear structures are generally preferred. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleotide backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleotide linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. In the context of the present invention, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in the internucleotide backbone can also be considered to be oligonucleosides.

Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylenephosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-aminophosphoramidates and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs

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of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3', or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

- 5 Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131;
10 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.
- 15 Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain
20 heteroatomic or heterocyclic internucleoside linkages. These linkages include morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and
25 thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.
- 30 Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Patent Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967;

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5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240;
5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704;
5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439,
certain of which are commonly owned with this application,
5 and each of which is herein incorporated by reference.

In other preferred oligonucleotide mimics, both the
sugar and the internucleoside linkage, i.e., the backbone of
the nucleotide units, are replaced with novel groups. The
base units are maintained for hybridization with an
10 appropriate nucleic acid target compound. One such
oligomeric compound, an oligonucleotide mimic that has been
shown to have excellent hybridization properties, is referred
to as a peptide nucleic acid (PNA). In PNA compounds, the
sugar-backbone of an oligonucleotide is replaced with an
15 amide-containing backbone, in particular an aminoethylglycine
backbone. The nucleobases are retained and are bound
directly or indirectly to aza nitrogen atoms of the amide
portion of the backbone. Representative United States
patents that teach the preparation of PNA compounds include,
20 but are not limited to, U.S. Patent Nos. 5,539,082;
5,714,331; and 5,719,262, each of which is herein
incorporated by reference. Further teachings of PNA
compounds can be found in Nielsen et al. (*Science*, **1991**, 254,
1497-1500).

25 In one embodiment of the present invention,
oligonucleotides with phosphorothioate backbones are
preferred. Also preferred are oligonucleosides with
heteroatom backbones, and in particular $-\text{CH}_2-\text{NH}-\text{O}-\text{CH}_2-$, $-\text{CH}_2-$
 $\text{N}(\text{CH}_3)-\text{O}-\text{CH}_2-$ [known as a methylene (methyylimino) or MMI
30 backbone], $-\text{CH}_2-\text{O}-\text{N}(\text{CH}_3)-\text{CH}_2-$, $-\text{CH}_2-\text{N}(\text{CH}_3)-\text{N}(\text{CH}_3)-\text{CH}_2-$ and $-\text{O}-$
 $\text{N}(\text{CH}_3)-\text{CH}_2-\text{CH}_2-$ [wherein the native phosphodiester backbone is
represented as $-\text{O}-\text{P}-\text{O}-\text{CH}_2-$] of the above referenced U.S.
patent 5,489,677, and the amide backbones of the above
referenced U.S. patent 5,602,240. Also preferred are

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oligonucleotides having morpholino backbone structures of the above-referenced U.S. patent 5,034,506.

The methods according to the present invention are performed in various traditional solvents either utilizing
5 solution phase techniques or automated synthetic protocols. Many solvents for automated oligonucleotide synthesis as well as solution phase oligonucleotide synthesis are known in the art. Preferred solvents include DMF, DMSO, THF, THP and CH₃CN.

10 Standard solution phase and solid phase methods for the synthesis of oligonucleotides and oligonucleotide analogs are well known to those skilled in the art. These methods are constantly being improved in ways that reduce the time and cost required to synthesize these complicated compounds.
15 Representative solution phase techniques are described in United States Patent No. 5,210,264, issued May 11, 1993 and commonly assigned with this invention. Representative solid phase techniques employed for oligonucleotide and oligonucleotide analog synthesis utilizing standard
20 phosphoramidite chemistries are described in "Protocols For Oligonucleotides And Analogs," Agrawal, S., Ed., Humana Press, Totowa, NJ, 1993.

A preferred method of choice for the preparation of naturally-occurring oligonucleotides, as well as non-
25 naturally-occurring (or modified) oligonucleotides such as phosphorothioate oligonucleotides, is via solid-phase synthesis wherein an oligonucleotide is prepared on a polymer support (a solid support) such as controlled pore glass (CPG); oxalyl-controlled pore glass (see, e.g., Alul et al.,
30 *Nucleic Acids Research* **1991**, 19, 1527); TENTAGEL Support, (see, e.g., Wright et al., *Tetrahedron Letters* **1993**, 34, 3373); or POROS, a polystyrene resin available from PerSeptive Biosystems. Equipment for such synthesis is commercially available from several vendors including, for

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example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed. Suitable solid phase techniques, including automated synthesis techniques, are described in F. Eckstein (ed.), *Oligonucleotides and Analogues, a Practical Approach*, Oxford University Press, New York (1991).

Solid-phase synthesis relies on sequential addition of nucleotides to one end of a growing oligonucleotide chain. Typically, a first nucleoside (having protecting groups on any exocyclic amine functionalities present) is attached to an appropriate glass bead support. Activated phosphite compounds (typically nucleotide phosphoramidites, also bearing appropriate protecting groups) are added stepwise to elongate the growing oligonucleotide. Additional methods for solid-phase synthesis may be found in U.S. Patent Nos. 4,415,732; 4,458,066; 4,500,707; 4,668,777; 4,973,679; 5,132,418; 4,725,677 and Re. 34,069.

A representative list of chemical functional groups according to the invention include C₁-C₁₀ alkyl, substituted C₁-C₁₀ alkyl, C₂-C₂₅ alkenyl, substituted C₂-C₂₅ alkenyl, C₂-C₁₅ alkynyl, substituted C₂-C₁₅ alkynyl, C₄-C₇ carbocyclic alkyl, substituted carbocyclic alkyl, alkenyl carbocyclic, substituted alkenyl carbocyclic, alkynyl carbocyclic, substituted alkynyl carbocyclic, C₆-C₂₀ aryl, substituted C₆-C₂₀ aryl, heteroaryl, substituted heteroaryl, a nitrogen, oxygen, or sulfur containing heterocycle, a substituted nitrogen, oxygen, or sulfur containing heterocycle, a mixed heterocycle, or a substituted mixed heterocycle, where said substituent groups are selected from alkyl, alkenyl, alkynyl, aryl, hydroxyl, amino, alkoxy, carboxy, benzyl, nitro, thiol, thioalkyl, thioalkoxy, or halogen groups; or L is phthalimido, an ether having 2 to 10 carbon atoms and 1 to 4 oxygen or sulfur atoms, a metal coordination group, a conjugate group, halogen, hydroxyl, thiol, keto, carboxyl,

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NR¹R², CONR¹, amidine (C(=NH)NR²R³), guanidine (NHC(=NH)NR²R³), glutamyl (R¹OOCCH(NR²R³)(CH₂)₂C(=O)), nitrate, nitro, nitrile, trifluoromethyl, trifluoromethoxy, NH-alkyl, N-dialkyl, O-aralkyl, S-aralkyl, NH-aralkyl, azido (N₃), hydrazino (NHNH₂), hydroxylamino (ONH₂), sulfoxide (SO), sulfone (SO₂), sulfide (S-), disulfide (S-S), silyl, a nucleosidic base, an amino acid side chain, a carbohydrate, a drug, or a group capable of hydrogen bonding, wherein each R¹ and R² is, independently, H, haloalkyl, C₁-C₁₀ alkyl, C₂-C₁₀ alkenyl, C₂-C₁₀ alkynyl, or C₆-C₁₄ aryl; and each R³ is, independently, a single bond, CH=CH, C≡C, O, S, NR⁶, SO₂, C₆-C₁₄ aryl, substituted C₆-C₁₄ aryl, heteroaryl, substituted heteroaryl, a nitrogen, oxygen, or sulfur containing heterocycle, a substituted nitrogen, oxygen, or sulfur containing heterocycle, a mixed heterocycle, or a substituted mixed heterocycle, wherein said substituent groups are selected from hydroxyl (OH), amino, alkoxy, carboxy, benzyl, phenyl, nitro (NO₂), thiol (SH), thioalkoxy, halogen, alkyl, aryl, alkenyl, and alkynyl groups.

20 A number of chemical functional groups can be introduced into compounds of the present invention in a blocked form and can then be subsequently deblocked to form the final, desired compound. In general, a blocking group renders a chemical functionality of a molecule inert to specific reaction conditions and can later be removed from such functionality in a molecule without substantially damaging the remainder of the molecule (Green and Wuts, *Protective Groups in Organic Synthesis*, 2d edition, John Wiley & Sons, New York, 1991). For example, amino groups can be blocked as phthalimido, 9-fluorenylmethoxycarbonyl (Fmoc), triphenylmethylsulfonyl, t-BOC or benzyl groups. Carboxyl groups can be protected as acetyl groups.

Representative hydroxyl protecting groups are described by Beaucage et al. (*Tetrahedron* **1992**, 48, 2223). Preferred

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hydroxyl protecting groups are acid-labile, such as trityl, monomethoxytrityl, dimethoxytrityl, trimethoxytrityl, 9-phenylxanthine-9-yl (Pixyl) and 9-(p-methoxyphenyl)-xanthine-9-yl (MOX). Chemical functional groups can also be "blocked" by including them in a precursor form. Thus an azido group can be considered to be a "blocked" form of an amine as the azido group may be easily converted to the amine. Further representative protecting groups utilized in oligonucleotide synthesis are discussed in Agrawal *et al.*, *Protocols for Oligonucleotide Conjugates*, Humana Press, New Jersey, 1994, Vol. 26, pp. 1-72.

In the context of the present invention, a "heterocycle" is a cyclic compound containing at least one heteroatom such as N, O or S. A "mixed heterocycle" is a cyclic compound containing at least two heteroatoms such as N, O or S. A "heteroaryl" compound is a heterocycle containing at least one heteroatom such as N, O or S and is not fully saturated, e.g., is in a state of partial or complete saturation.

"Heteroaryl" is also meant to include fused systems including systems where one or more of the fused rings contain no heteroatoms. Heterocycles, including nitrogen heterocycles, according to the present invention include, but are not limited to, imidazole, pyrrole, pyrazole, indole, 1H-indazole, α -carboline, carbazole, phenothiazine, phenoxazine, tetrazole, triazole, pyrrolidine, piperidine, piperazine and morpholine groups. A more preferred group of nitrogen heterocycles includes imidazole, pyrrole, indole, and carbazole groups.

As used herein, "linking moiety" refers to a hydrocarbyl chain which connects the monomers and oligomers of the invention to a solid support. A preferred linking moiety is a succinyl group. Other linking moieties include, but are not limited to, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_2 - C_{10} alkenyl or substituted or

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unsubstituted C₂-C₁₀ alkynyl, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl.

5 "Conjugate groups" according to the present invention include those known in the art. A representative list of conjugate groups amenable to the present invention includes intercalators, reporter molecules, contrast reagents, cleaving agents, cell targeting agents, cyanine dyes,
10 polyamines, polyamides, poly ethers including polyethylene glycols, and other moieties known in the art for enhancing the pharmacodynamic properties or the pharmacokinetic properties. Typical conjugate groups include PEG groups, cholesterols, phospholipids, biotin, phenanthroline,
15 phenazine, pyrene, retinal, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes.

For the purposes of this invention, the term "reporter molecule" includes molecules or enzymes that have physical or chemical properties that allow them to be identified in gels,
20 fluids, whole cellular systems, broken cellular systems and the like utilizing physical properties such as spectroscopy, radioactivity, colorimetric assays, fluorescence, and specific binding. Particularly useful as reporter molecules are fluorophores, chromophores and radiolabel-containing
25 moieties.

Fluorophores are molecules detectable by fluorescence spectroscopy. Examples of preferred fluorophores are fluorescein and rhodamine dyes and acridines. There are numerous commercial available fluorophores including "Texas
30 Red" and other like fluoresceins and rhodamines available from Molecular Probes, Eugene, OR.

Chromophores are molecules capable of detection by visible or ultraviolet (UV-VIS) absorbance spectroscopy. Examples of chromophores are polynuclear aromatics such as

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anthracene, perylene, pyrene, rhodamine and chrysene.

Radiolabel-containing moieties, as used herein, are molecules incorporating at least one radioactive atom, such as ^3H or ^{14}C , enabling detection thereby.

5 Reporter enzymes may be detected directly or via their enzymatic products by any of the methods mentioned above. Particularly useful as reporter enzymes are alkaline phosphatase and horseradish peroxidase.

Intercalators are polycyclic aromatic moieties that can
10 insert between adjacent base pairs without affecting normal Watson-Crick base pairing, and include hybrid intercalator/ligands such as the photonuclease/intercalator ligand 6-[[[9-[[6-(4-nitrobenzamido)hexyl]amino]acridin-4-yl]carbonyl]amino]hexanoylpentafluorophenyl ester. This
15 compound has two noteworthy features: an acridine moiety that is an intercalator and a *p*-nitrobenzamido group that is a photonuclease. Other representative intercalators are disclosed by Manoharan, M., *Antisense Research and Applications*, Crooke and Lebleu, eds., CRC Press, Boca Raton,
20 1993.

In the context of this invention, "hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary heterocyclic bases. "Complementary," as used herein, refers
25 to the capacity for precise pairing between two heterocyclic bases. For example, adenine and thymine are complementary bases which pair through the formation of hydrogen bonds. "Complementary" and "specifically hybridizable," as used herein, refer to precise pairing or sequence complementarity
30 between a first and a second nucleic acid-like oligomer containing nucleoside subunits. For example, if a heterocyclic base at a certain position of the first nucleic acid is capable of hydrogen bonding with a heterocyclic base at the same position of the second nucleic acid, then the

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first nucleic acid and the second nucleic acid are considered to be complementary to each other at that position. The first and second nucleic acids are complementary to each other when a sufficient number of corresponding positions in
5 each molecule are occupied by bases which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity such that stable and specific binding occurs between a compound of the invention
10 and a target nucleic acid molecule. It is understood that an oligomer of the invention need not be 100% complementary to its target nucleic acid to be specifically hybridizable. An oligomer is specifically hybridizable when binding of the oligomer to the target nucleic acid interferes with the
15 normal function of the target to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligomer to non-target sequences under conditions in which specific binding is desired, i.e. under physiological conditions in the case of *in vivo* assays
20 or therapeutic treatment, or in the case of *in vitro* assays, under conditions in which the assays are performed.

In the context of the present invention, "modulating" means altering or modifying, and includes increasing or decreasing. Accordingly, modulating gene expression means
25 increasing or decreasing gene expression. In one aspect of the invention, modulating gene expression means decreasing gene expression.

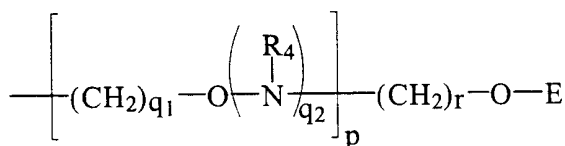
As used herein the term "sugar substituent group" or "2'-substituent group" includes groups attached to the 2'
30 position of the ribosyl moiety with or without an oxygen atom. 2'-Sugar modifications amenable to the present invention include fluoro, O-alkyl, O-alkylamino, O-alkylalkoxy, protected O-alkylamino, O-alkylaminoalkyl, O-alkyl imidazole, and polyethers of the formula (O-alkyl)_m,

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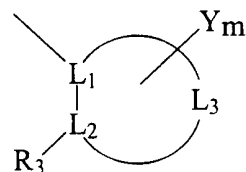
where m is 1 to about 10. Preferred among these polyethers are linear and cyclic polyethylene glycols (PEGs), and PEG-containing groups, such as crown ethers, and other reported substituent groups. See, Ouchi et al., *Drug Design and*
5 *Discovery* **1992**, 9, 93; Ravasio et al., *J. Org. Chem.* **1991**, 56, 4329; and Delgado et al., *Critical Reviews in Therapeutic Drug Carrier Systems* **1992**, 9, 249. Each of the foregoing references is hereby incorporated by reference in its entirety. Further sugar substituent groups are disclosed
10 by Cook (*Anti-Cancer Drug Design*, **1991**, 6, 585-607). Fluoro, O-alkyl, O-alkylamino, O-alkyl imidazole, O-alkylaminoalkyl, and alkyl amino substituents are described in United States Patent Application serial number 08/398,901, filed March 6, 1995, entitled "Oligomeric Compounds having Pyrimidine
15 Nucleotide(s) with 2' and 5' Substitutions," hereby incorporated by reference in its entirety.

Additional 2' sugar modifications amenable to the present invention include 2'-SR and 2'-NR₂ groups, where each R is, independently, hydrogen, a protecting group or
20 substituted or unsubstituted alkyl, alkenyl, or alkynyl. 2'-SR nucleosides are disclosed in United States Patent No. 5,670,633, issued September 23, 1997, hereby incorporated by reference in its entirety. The incorporation of 2'-SR monomer synthons are disclosed by Hamm et al., *J. Org. Chem.*,
25 **1997**, 62, 3415-3420. 2'-NR₂ nucleosides are disclosed by Goettingen, M., *J. Org. Chem.*, **1996**, 61, 6273-6281; and Polushin et al., *Tetrahedron Lett.*, **1996**, 37, 3227-3230. Further representative 2'-O-sugar modifications amenable to the present invention include those having one of formula I
30 or II:

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I



II

wherein:

E is C₁-C₁₀ alkyl, N(R₄)(R₅) or N=C(R₄)(R₅);

each R₄ and R₅ is, independently, H, C₁-C₁₀ alkyl, a nitrogen protecting group, or R₄ and R₅, together, are a nitrogen protecting group or are joined in a ring structure that includes at least one additional heteroatom selected from N and O;

R₃ is OX, SX, or N(X)₂;

each X is, independently, H, C₁-C₈ alkyl, C₁-C₈ haloalkyl, C(=NH)N(H)Z, C(=O)N(H)Z or OC(=O)N(H)Z;

Z is H or C₁-C₈ alkyl;

L₁, L₂ and L₃ comprise a ring system having from about 4 to about 7 carbon atoms or having from about 3 to about 6 carbon atoms and 1 or 2 heteroatoms, said heteroatoms being selected from oxygen, nitrogen and sulfur, wherein said ring system is aliphatic, unsaturated aliphatic, aromatic, or saturated or unsaturated heterocyclic;

Y is C₁-C₁₀ alkyl or haloalkyl, C₂-C₁₀ alkenyl, C₂-C₁₀ alkynyl, C₆-C₁₄ aryl, N(R₄)(R₅)OR₄, halo, SR₄ or CN;

each q₁ is, independently, an integer from 2 to 10;

each q₂ is 0 or 1;

p is an integer from 1 to 10; and

r is an integer from 1 to 10;

provided that when p is 0, r is greater than 1.

Representative 2'-O- sugar substituents of formula I are disclosed in United States Patent Application serial number 09/130,973, filed August 7, 1998, entitled "Capped

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2'-Oxyethoxy Oligonucleotides," hereby incorporated by reference in its entirety.

Representative cyclic 2'-O- sugar substituents of formula II are disclosed in United States Patent Application 5 serial number 09/123,108, filed July 27, 1998, entitled "RNA Targeted 2'-Modified Oligonucleotides that are Conformationally Preorganized," hereby incorporated by reference in its entirety.

In the context of the present invention, "alkyl" means 10 substituted or unsubstituted hydrocarbyl groups wherein the carbon atoms are connected via single bonds. "Alkenyl" means substituted or unsubstituted hydrocarbyl moieties having at least one double bond. "Alkynyl" means substituted or unsubstituted hydrocarbyl moieties having at least one triple 15 bond.

Another modification that is used to prepare oligomeric compounds amenable to the present invention includes locked nucleic acids (LNA's) which are novel conformationally restricted oligonucleotide analogs containing 2'-O,4'-C- 20 methylene LNA monomers (see, Singh et al., *Chem. Commun.*, **1998**, 4, 455-456). LNA and LNA analogs display very high duplex thermal stabilities with complementary DNA and RNA (T_m = +3 to +10 C), stability towards 3'-exonucleolytic degradation, and good solubility properties.

25 Synthesis of the LNA monomers adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil, their oligomerization, and nucleic acid recognition properties have been described (see Koshkin et al., *Tetrahedron*, **1998**, 54, 3607-3630). Studies of mis-matched sequences show that LNA 30 obey the Watson-Crick base pairing rules with generally improved selectivity compared to the corresponding unmodified reference strands.

Potent and nontoxic antisense oligonucleotides containing LNA's have been described (Wahlestedt et al.,

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Proc. Natl. Acad. Sci. U. S. A., **2000**, 97, 5633-5638.) The authors have demonstrated that LNA's confer several desired properties to antisense agents. LNA/DNA copolymers were not degraded readily in blood serum and cell extracts. LNA/DNA
5 copolymers exhibited potent antisense activity on assay systems as disparate as a G-protein-coupled receptor in living rat brain and an *Escherichia coli* reporter gene.

The conformations of LNA's has been determined by 2D NMR spectroscopy to show that the locked conformation of the LNA
10 nucleotides both in ssLNA and in the duplexes organize the phosphate backbone in such a way as to introduce higher population of the N-type conformation (See, Petersen et al., *J. Mol. Recognit.*, **2000**, 13, 44-53). These conformational changes are associated with improved stacking of the
15 nucleobases (see also, Wengel et al., *Nucleosides Nucleotides*, **1999**, 18, 1365-1370).

LNA's form duplexes with complementary DNA, RNA or LNA with high thermal affinities. CD spectra show that duplexes involving fully modified LNA (esp. LNA:RNA) structurally
20 resemble an A-form RNA:RNA duplex. NMR examination of an LNA:DNA duplex confirm the 3'-endo conformation of an LNA monomer. Recognition of double-stranded DNA is demonstrated suggesting strand invasion by LNA. Lipofectin-mediated efficient delivery of LNA into living human breast cancer
25 cells has been accomplished.

Preparation of locked nucleoside analogs-containing oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has been described (see, Wengel et al., PCT International Application WO 98-DK393 19980914). The novel
30 type of LNA modified oligonucleotides, as well as the LNAs as such, are useful in a wide range of diagnostic applications as well as therapeutic applications. Among these are included antisense applications, PCR applications, strand

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displacement oligomers, as substrates for nucleic acid polymerases, and as nucleotide based drugs.

LNA has been shown to form exceedingly stable LNA:LNA Duplexes (Koshkin *et al.*, *J. Am. Chem. Soc.*, **1998**, 120, 13252-13253). LNA:LNA hybridization was shown to be the most thermally stable nucleic acid type duplex system, and the RNA-mimicking character of LNA was established at the duplex level. Introduction of 3 LNA monomers (T or A) induced significantly increase melting points ($T_m = +15/+11$) toward DNA complements. The universality of LNA-mediated hybridization has been stressed by the formation of exceedingly stable LNA:LNA duplexes. The RNA-mimicking of LNA was reflected with regard to the N-type conformational restriction of the monomers and to the secondary structure of the LNA:RNA duplex.

Synthesis of 2'-amino-LNA, a novel conformationally restricted high-affinity oligonucleotide analog with a Handle has been shown (see Singh *et al.*, *J. Org. Chem.*, **1998**, 63, 10035-10039.)

2'-Amino- and 2'-methyamino-LNA's were prepared and thermal stability of their duplexes with complementary RNA and DNA strands have been previously reported. Similarly, The first analogs of LNA, phosphorothioate-LNA and 2'-thio-LNAs have been prepared (see Kumar *et al.*, *Bioorg. Med. Chem. Lett.*, **1998**, 8, 2219-2222.)

As used herein, "alicyclic" means hydrocarbyl groups having a saturated cyclic structure wherein the carbon atoms are connected by single bonds. Alicyclic groups according to the present invention may also contain heteroatoms within the cyclic structure or as part of an exocyclic substituent.

Further, in the context of the present invention, "aryl" (generally C_6-C_{24}) includes, but is not limited to, substituted and unsubstituted aromatic hydrocarbyl groups.

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Aralkyl groups (generally C₇-C₂₅) include, but are not limited to, groups having both aryl and alkyl functionalities, such as benzyl and xylyl groups. Preferred aryl and aralkyl groups include, but are not limited to, phenyl, benzyl, xylyl, naphthyl, toluoyl, pyrenyl, anthracyl, azulyl, phenethyl, cinnamyl, benzhydryl, and mesityl. Typical substituents for substitution include, but are not limited to, hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl, or alkynyl groups.

Formulation of therapeutic compositions utilizing compounds of the present invention and their subsequent administration is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligomers, and can generally be estimated based on EC₅₀s found to be effective in *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 ug to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligomer is administered in maintenance doses, ranging from 0.01 ug to

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about 10 g per kg of body weight, once or more daily, to once every 20 years.

Additional objects, advantages and novel features of the present invention will become apparent to those skilled in the art upon examination of the following examples. The following examples illustrate the invention and are not intended to limit the same. Those skilled in the art will recognize, or be able to ascertain through routine experimentation, numerous equivalents to the specific substances, compositions and procedures described herein. Such equivalents are considered to be within the scope of the present invention.

EXAMPLES

All reagents and solvents were purchased from commercial sources unless otherwise noted. 2-Cyanoethanol, *N,N'*-disuccinimidyl carbonate (DSC), *N*-(2-hydroxy)-phthalimide, 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphoro-diamidite, 6-amino-hexanol and 2-methyl-2-thiopseudourea-sulfate were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Reagents for the DNA synthesizer were purchased from PerSeptive Biosystems, Inc. (Framingham, MA). 2,2'-Anhydro-5-methyluridine was purchased from Ajinomoto (Tokyo, Japan). Flash chromatography was performed on silica gel (Baker, 40 μ m). Thin-layer chromatography was performed on Kieselgel glass plates from E. Merck and visualized with UV light and p-anisaldehyde/sulfuric acid/acetic acid spray followed by charring.

For all derivatizations described herein, the derivatized material can be used as obtained without further purification.

Example 1

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Synthesis of N-(2-cyanoethoxycarbonyloxy)succinimide (CEOC-O-Succinimide) (1)

To a stirred solution of 2-cyanoethanol (7.23 g, 102 mmol) in 300 mL of anhydrous CH₃CN, under argon atmosphere, 5 N,N'-disuccinimidyl carbonate (34.0 g, 133 mmol) was added followed by pyridine (11.3 mL, 140 mmol). The suspension became a clear solution after about 1 h. The solution was stirred for an additional 6 h and then concentrated *in vacuo*. The residue was redissolved in dichloromethane (200 mL), 10 extracted with saturated NaHCO₃ solution (3 x 50 mL) followed by saturated NaCl solution (3 x 50 mL). The organic layer was dried (anhydrous Na₂SO₄) and concentrated to afford a white solid. Traces of pyridine were removed by co-evaporation with dry acetonitrile. The white solid was dried 15 overnight *in vacuo* and then triturated with ether (150 mL) to yield 20.23 g (94%) of **1** as a colorless amorphous powder. This material is stable at room temperature in a desiccator for an extended period (1-2 years). The proton and carbon NMR spectra revealed a homogeneous material even at this 20 stage. The material was further purified by chromatography on silica gel using CH₂Cl₂:EtOAc (50:50) to give a white crystalline compound (18.72g, 87%); R_f = 0.21; m.p. 105.5 °C.

¹H NMR (400 MHz, CDCl₃): 2.85 (t, J = 6.62 Hz, 2H), 2.86 (s, 4H), 4.45 (t, J = 5.96 Hz); ¹³C NMR (80 MHz, DMSO-d₆): 17.33, 25.39, 65.86, 117.91, 150.91, 169.82; HRMS (FAB): 25 Calcd for C₈H₉N₂O₅⁺ 213.0511, Found: 213.0509; Anal: Calcd for C₈H₉N₂O₅: C, 45.29; H, 3.80; N, 13.20; Found: C, 45.19; H, 3.45; N, 13.02.

Example 2**30 Synthesis of 2'-O-phthalimidoethyl-5-methyluridine (2)**

N-(2-Hydroxyethyl)phthalimide (277 g, 1.45 mol) was slowly added to a solution of borane in tetrahydrofuran (1 M,

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600 mL), with stirring. Hydrogen gas evolved as the solid dissolved. Once the rate of gas evolution subsided, the solution was placed in a 2 L stainless steel bomb. 2,2'-Anhydro-5-methyluridine (60 g, 0.25 mol) and sodium bicarbonate (120 mg) were added and the bomb was sealed. After 30 minutes, the bomb was vented and placed in an oil bath and heated to 150 °C internal temperature for 24 h. The bomb was cooled to room temperature and opened. TLC (ethyl acetate-methanol; 95:5) revealed the disappearance of starting material. The crude solution was concentrated and the residue was purified by chromatography on silica gel starting with ethyl acetate to remove the excess phthalimide reagent, followed by ethyl acetate-methanol (95:5) to elute 2 (22.2 g, 20.6%).

¹H NMR (200 MHz, DMSO-d₆): 1.8 (s, 3H), 3.4-4.2 (m, 6H), 5.0-5.2 (m, 2H), 5.8 (d, J = 5.1 Hz, 1H), 7.65 (s, 1H), 7.8-8.0 (m, 4H), 11.2 (s, 1H).

Example 3

Synthesis of 2'-O-(2-phthalimidoethyl)-5'-O-(4,4'-dimethoxytrityl)-5-methyl uridine (3)

2'-O-Phthalimidoethyl-5-methyluridine (2, 22.2 g, 0.053 mol) was coevaporated with pyridine (2x75 mL) and then dissolved in 100 mL of pyridine. Dimethoxytrityl chloride (27 g, 0.080 mol) was added in one portion, with stirring. TLC (ethyl acetate-hexanes 50:50), after 1 h, indicated completion of the reaction. Methanol (10 mL) was added to quench the reaction. The mixture was concentrated and the residue was partitioned between ethyl acetate and saturated sodium bicarbonate solution (150 mL each). The organic layer was concentrated and the residue was dissolved in a minimum amount of dichloromethane and applied to a silica gel column. The compound was eluted with ethyl acetate-hexanes-

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triethylamine (50:50:1 to 80:20:1) to give **3** (26.1 g, 68%) as a white foam.

^1H NMR (200 MHz, CDCl_3): 1.33 (s, 3H), 3.05 (d, $J = 8.4$ Hz, 1H), 3.49 (m, 2H), 3.8 (s, 6H), 3.9-4.1 (m, 4H), 4.18-4.26 (m, 1H), 4.47 (m, 1H), 5.88 (s, 1H), 6.84 (d, $J = 8.78$ Hz, 4H), 7.22-7.43 (m, 9H), 7.69-7.88 (m, 5H), 8.26 (s, 1H); HRMS(FAB): Calcd for $\text{C}_{41}\text{H}_{39}\text{N}_3\text{O}_{10}\text{Na}^+$ 756.2533, Found: 756.2553.

Example 4

Synthesis of 2'-O-(2-aminoethyl)-5'-O- (4,4'-

10 dimethoxytrityl)-5-methyluridine (**4**)

2'-O-Phthalimidoethyl-5'-O-DMT-5-methyluridine (**3**, 21.1 g, 0.029 mol) was dissolved in methanol (500 mL). Anhydrous hydrazine (4.9 mL, 0.15 mol) was added and the solution was heated to reflux. TLC after 3 h indicated a complete
15 reaction. The residue was purified by chromatography on silica gel using methanol and then methanol-ammonium hydroxide (98:2) to give **4** (12.4g, 70%). The material was completely soluble in methylene chloride and traces of silica from leaching of the column were removed by filtration at
20 this stage and reevaporating the solution.

^1H NMR (200 MHz, CDCl_3): 1.39 (s, 3H), 2.98 (t, $J = 3.48$ Hz, 2H), 3.45 (d, $J = 2.56$ Hz, 1H), 3.53 (d, $J = 1.96$ Hz, 1H), 3.56-3.68 (m, 2H), 3.81 (s, 6H), 3.99 (m, 1H), 4.1 (t, $J = 4.56$ Hz, 1H), 4.17 (m, 1H), 4.45 (t, $J = 5.06$ Hz, 1H), 6.06 (d, $J = 4.12$ Hz, 1H), 6.86 (d, $J = 8.9$ Hz, 4H), 7.25-7.46 (m, 9H), 7.67 (s, 1H); ^{13}C (50 MHz, CDCl_3): 11.71, 40.55, 45.76, 55.03, 62.47, 69.15, 70.65, 82.64, 83.49, 86.62, 87.10, 110.98, 113.09, 126.91, 127.77, 127.97, 129.95, 135.35, 144.25, 151.27, 158.46, 164.97; HRMS (FAB): Calcd for
30 $\text{C}_{33}\text{H}_{37}\text{O}_8\text{N}_3\text{Na}^+$ 626.2478, Found: 626.2501.

Example 5

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Synthesis of *N,N'*-bis-CEOC-2-methyl-2-thiopseudourea (5)

2-Methyl-2-thiopseudourea.1/2H₂SO₄ (5.29 g, 38.0 mmol) was suspended in CH₂Cl₂ (250 mL) and saturated NaHCO₃ solution (250 mL). Cyanoethoxycarbonyloxysuccinimide **1** (20.2 g, 95.3 mmol) was added and the reaction stirred for 2 h. The organic phase was separated. The aqueous phase was extracted with DCM (2 x 200 ml) and the combined organic phase was dried (Na₂SO₄), filtered and evaporated. The crude product was purified by flash chromatography with AcOEt/DCM (95:5) as eluant to afford **5** (3.78 g, 35%) as a white solid.

¹H-NMR (200 MHz, CDCl₃): 11.80 (br s, 1H), 4.39 (q, 4H), 2.80 (t, 4H), 2.45 (s, 3H).

Example 6**Synthesis of *N,N'*-bis-benzoyl-2-methyl-2-thiopseudourea (6)**

This compound was prepared as described by Derocque et al. (*Bulletin de la Société Chimique de France*, **1968**, 5, 2062-2066).

Example 7**Synthesis of *N,N'*-bis-pivaloyl -2-methyl-2-thiopseudourea (7)**

2-Methyl-2-thiopseudourea.1/2H₂SO₄ (1.0 g, 3.60 mmole) was suspended in dry pyridine (14.4 ml). Pivaloylchloride (1.77 mL, 14.4 mmole) was added and the reaction mixture was stirred overnight. The reaction was quenched by addition of 5% aqueous NaHCO₃ solution (100 mL) and extracted with dichloromethane (2x50 mL). The organic phase was dried (Na₂SO₄), filtered and evaporated. The crude product was purified by flash column chromatography with EtOAc as eluant to afford **7** (1.27 g, 68%).

¹H-NMR (200 MHz, DMSO-d₆): 13.10 (s, 1H), 2.39 (s, 3H), 1.22 (s, 18H); HRMS (FAB): Calcd for C₁₂ H₂₃ N₂ O₂ S⁺ 259.480, Found: 259.1485.

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Example 8**Synthesis of 2'-O-[(N,N'-bis-CEOC-guanidinium)ethyl]-5'-O-DMT-5-methyluridine (8)**

Compound **5** (0.27 g, 0.95 mmole) was dissolved in
5 anhydrous DMF (3 mL) at room temperature. To this, compound
4 (0.52g, 0.86 mmol) and then triethylamine (0.12 ml, 0.86
mmole) was added, and the reaction was stirred at room
temperature for 4 h. The reaction was quenched by addition
of 5% NaHCO₃ solution (40 mL), extracted with EtOAc (2x60 mL)
10 and the combined organic phases were dried (Na₂SO₄) filtered
and evaporated. The crude product was purified by flash
column chromatography with EtOAc as eluant to afford **8** (0.480
g, 66%).

¹H NMR (200MHz, CDCl₃): 11.70 (s, 1H), 8.60 (t, 1H),
15 8.51 (s, 1H), 7.66(s, 1H), 7.39-7.27 (m, 9H), 6.84 (d, 4H),
5.97 (d, 1H), 4.50-3.70 (m, 16H), 3.50 (m, 2H), 2.73 (m, 4H),
1.37 (s, 3H); ¹³C-NMR (80 MHz, CDCl₃): 164.50, 162.92,
158.70, 156.06, 153.02, 150.83, 144.43, 135.46, 135.29,
130.15, 128.06, 127.72, 127.18, 117.50, 116.88, 113.32,
20 111.09, 87.14, 86.82, 83.50, 82.40, 69.19, 68.94, 62.35,
61.09, 59.89, 55.29, 40.88, 18.05, 17.92, 11.87; HRMS (FAB):
Calcd for C₄₂H₄₅N₇O₁₂Na⁺ 862.3024, Found: 862.2991.

Example 9**Synthesis of 2'-O-[(N',N',-bis-CEOC-guanidinium)ethyl]-5'-O-
25 DMT-5-methyluridine-3'-O-[(2-cyanoethyl)N,N-diisopropyl]
phosphoramidite (9)**

Compound **8** (0.39 g, 0.46 mmol) and diisopropylamine
tetrazolide (0.08 g, 0.46 mmol) were dried by coevaporation
with anhydrous MeCN. The residue was redissolved in
30 anhydrous MeCN (3 mL). 2-Cyanoethyl-N,N,N',N'-
tetraisopropylphosphordiamidite (0.27 mL, 0.87 mmol) was
added and the reaction mixture was stirred at room

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temperature, under argon, for 5 h. The solvent was evaporated and the crude product purified by flash chromatography with EtOAc/Hexanes as eluant to afford **9** (0.31 g, 64%).

5 ^{31}P NMR (80 MHz, CDCl_3): 150.87 and 150.78; HRMS (FAB): Calcd for $\text{C}_{51}\text{H}_{63}\text{O}_{13}\text{N}_9\text{PCs}^\oplus$ 1172.3259, Found: 1172.3203.

Example 10

Synthesis of 2'-O-[2-(N,N'-bis-benzoyl-guanidinium)ethyl]-5'-O-DMT-5-methyluridine (**10**)

10 Compound **6** (0.5 g, 1.66 mmol) was dissolved in anhydrous DMF (3 mL). Nucleoside **4** (0.5 g, 0.83 mmol) and triethylamine (0.12 mL, 0.83 mmol) were added. The reaction mixture was stirred at room temperature for 5 h. The reaction mixture was added to brine (50 mL) and extracted
15 with ethyl acetate (2 x 50 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to provide a yellow solid. The crude product was purified by flash column chromatography and eluted with ethyl acetate:hexane (1:1) to afford **10** (0.66 g, 93% yield).

20 ^1H NMR (200 MHz, CDCl_3): 14.50 (s, 1H), 9.79 (br s, 1H), 9.19 (s, 1H), 8.25 (d, 2H, $J = 6.62$ Hz), 8.05 (d, 2H, $J = 6.94$ Hz), 7.69 (s, 1H), 7.63-7.25 (m, 15H), 6.80 (d, 4H, $J = 8.8$ Hz), 5.99 (s, 1H), 4.92 (m, 1H), 4.28 (m, 1H), 4.18-3.80 (m, 6H), 3.78 (s, 6H), 3.50 (m, 2H), 2.86 (d, 1H, $J =$
25 9.01 Hz), 1.35 (s, 3H); ^{13}C NMR (50 MHz, CDCl_3): 178.67, 168.43, 164.15, 158.73, 157.13, 150.56, 144.42, 135.49, 135.36, 135.12, 133.65, 132.11, 131.87, 130.17, 129.93, 129.51, 129.12, 128.06, 127.14, 113.32, 111.07, 87.85, 86.83, 83.19, 82.59, 68.96, 61.70, 55.26, 40.88, 11.89; HRMS (FAB):
30 Calcd for $\text{C}_{48}\text{H}_{48}\text{N}_5\text{O}_{10}^\oplus$ 854.340, Found: 854.3381.

Example 11

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Synthesis of 2'-O-[2-(*N,N'*-bis-benzoyl-guanidinium)ethyl]-5-O-DMT-5-methyluridine-3'-O-[(2-cyanoethyl)*N,N*-diisopropyl]phosphoramidite (11)

Compound **11** was prepared from compound **10**, according to the procedure described in Example 9. Yield: 1.49 g (70%).

¹H NMR (200 MHz, CDCl₃): 14.46 (s, 1H), 9.73 (br s, 1H), 8.64 (br s, 1H), 8.25 (d, 2H, J = 6.63 Hz), 8.02 (d, 2H, J = 6.94 Hz), 7.69 (s, 1H), 7.63-7.23 (m, 15H), 6.80 (d, 4H, J = 8.74 Hz), 6.06 and 6.01 (2 x d, 1H), 4.60-3.30 (m, 17H), 2.55 (t, 1H, J = 7.6 Hz), 2.35 (t, 1H, J = 7.5 Hz), 1.40-0.80 (m, 15H); ³¹P NMR (80 MHz, CDCl₃): 150.83 and 150.58; HRMS (FAB): Calcd for C₅₇H₆₄N₇O₁₁PCs⁺ 1186.3456, Found: 1186.3410.

Example 12

Synthesis of 2'-O-[2-(*N,N'*-bis-pivaloylguanidinium)ethyl]-5'-O-DMT-5-methyl uridine (12)

Compound **12** was prepared from compounds **4** and **7** according to the procedure described in Example 8. Yield: 0.98 g (73%).

¹H NMR (200 MHz, CDCl₃): 13.39 (s, 1H), 9.50 (s, 1H), 9.40 (br s, 1H); 7.67 (s, 1H), 7.44-7.20 (m, 9H), 6.83 (d, 4H, J = 8.84 Hz), 5.95 (s, 1H), 4.50 (m, 1H), 4.20-4.00 (m, 2H), 3.90-3.60 (m, 4H), 3.78 (s, 6H), 3.55 (m, 2H), 2.78 (d, 1H, J = 8.88 Hz), 1.37 (s, 3H), 1.26 (s, 9H), 1.18 (s, 9H); ¹³C NMR (50 MHz, CDCl₃): 193.82, 181.73, 164.12, 158.75, 156.57, 150.42, 144.39, 135.49, 135.34, 135.16, 130.17, 128.22, 128.04, 127.15, 113.32, 111.03, 87.76, 86.85, 83.17, 82.46, 68.94, 61.73, 60.42, 55.26, 42.01, 40.42, 27.90, 27.09, 11.87; HRMS (FAB): Calcd for C₄₄H₅₅N₅O₁₀⁺ 814.4027, Found: 814.4054.

Example 13

Synthesis of 2'-O-[2-(*N,N'*-bis-pivaloylguanidinium)ethyl]-5'-

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**O-DMT-5-methyluridine-3'-O-[(2-cyanoethyl)*N,N*-diisopropyl]
phosphoramidite (13)**

Compound **13** was prepared from compound **12**, as described in Example 9. Yield: 0.840 g (72%).

5 ^1H NMR (200 MHz, CDCl_3): 13.35 (s, 1H), 9.31 (s, 1H),
8.95 (br s, 1H), 7.72 and 7.65 (2 x s, 1H), 7.45-7.20 (m,
9H), 6.86 (m, 4H), 6.00 and 5.95 (2 x d, 1H), 4.60-3.20 (m,
17H), 2.63 (t, 1H, $J = 7.72$ Hz), 2.37 (t, 1H, $J = 7.2$ Hz),
1.40-0.80 (m, 33H); ^{31}P NMR (80 MHz, CDCl_3): 150.73 and
10 150.55; HRMS (FAB): Calcd for $\text{C}_{53}\text{H}_{72}\text{N}_7\text{O}_{11}\text{PCs}^\oplus$ 1146.4082, Found:
1146.4034.

Example 14

**Synthesis of 5-O-DMT-2'-O-[2-methoxyethyl]-5-(3-aminoprop-1-
yne) uridine (14)**

15 2'-O-Methoxyethyluridine (synthesized according to the
procedures described in U.S. Patent No. 5,760,202) was
treated with an excess of pyridine/benzoyl chloride to give
3',5'-dibenzoyl-2'-O-methoxyethyl uridine in quantitative
yield. This compound was treated with lithium iodide (1
20 equivalent) and ceric ammonium nitrate (3 equivalents) in
acetonitrile as the solvent. After stirring overnight, TLC
indicated the formation of 5-iodo-3'-5'-dibenzoyl-2'-O-
methoxyethyl-uridine. Acetonitrile was evaporated and the
residue redissolved in CH_2Cl_2 and extracted with saturated
25 NaHCO_3 solution. The organic layer was dried (Na_2SO_4) and
evaporated to dryness. The residue was purified on a silica
column and eluted with EtOAc/Hexane (7:3). Fractions
containing the product were pooled together and concentrated.

N-Trifluoro-acetylpropargylamine (3 equivalents) was been
30 added to this compound along with tetrakis(triphenylphosphine)
palladium(0) [$(\text{Ph}_3\text{P})_4\text{Pd}$], copper iodide, triethylamine and
DMF to provide the C-5-(*N*-trifluoroacetyl)-propargylamine

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derivative. Treatment of this compound with sodium in methanol afforded the 3'-5'-bis-hydroxyl parent compound. This compound was 5'-dimethoxy tritylated. The trifluoroacetyl group was deprotected by treatment with 5 NH₄OH/pyridine.

Example 15**Synthesis of 5'-O-DMT-2'-O-[2-(methoxy)ethyl]-5-[3-(N,N'-bis-CEOC-guanidinium)prop-1-yne]uridine (15)**

Compound **15** was prepared from compounds **5** and **14**,
10 according to the procedure described in Example 8. Yield:
0.54 g (17%).

¹H NMR (200 MHz, CDCl₃): 11.61 (s, 1H), 9.80 (br s, 1H), 8.13 (d, 2H), 7.45-7.20 (m, 9H), 6.83 (d, 4H, J = 8.82 Hz), 5.93 (d, 1H, J = 3.48 Hz), 4.45 (br s, 1H), 4.16-3.96
15 (m, 6H), 3.77 (s, 6H), 3.82-3.39 (m, 6H), 3.37 (s, 3H), 2.73 (q, 4H, J = 6.3 Hz); ¹³C NMR (50 MHz, CDCl₃): 162.07, 161.77, 158.53, 155.03, 152.67, 149.60, 149.39, 144.64, 143.22, 136.25, 135.62, 135.40, 130.03, 128.08, 127.90, 126.88, 123.77, 117.07, 116.20, 113.38, 99.27, 88.05, 86.96, 83.97,
20 83.12, 74.84, 71.84, 70.39, 62.35, 60.86, 60.44, 60.08, 59.02, 55.32, 31.71, 18.1.

Example 16**Synthesis of 5'-O-DMT-2'-O-[2-(methoxy)ethyl]-5-[3-(N,N'-bis-CEOC-guanidinium)prop-1-yne]uridine-3'-O-[(2-cyanoethyl)N,N-
25 diisopropyl]phosphoramidite (16)**

Compound **16** was prepared from compound **15**, according to the procedure described in Example 9. Yield: 0.24 g (35%).

³¹P NMR (80 MHz, CDCl₃): 150.91 and 150.15; HRMS (FAB): Calcd for C₅₄H₆₄O₁₄N₉PNa⁺ 1116.4208, Found: 1116.4243.

30 Example 17

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Synthesis of 5'-O-DMT-2'-O-[2-(methoxy)ethyl]-5-[3-(*N,N'*-bis-benzoylguanidinium)prop-1-yne]uridine (17)

Compound **17** was prepared from compounds **6** and **14**, according to the procedure described in Example 8. Yield:
5 0.86 g (38%).

^1H NMR (200 MHz, CDCl_3): 14.34 (s, 1H), 9.57 (br s, 1H), 9.29 (t, 1H, $J = 4.88$ Hz), 8.24 (d, 2H, $J = 6.62$ Hz), 8.20 (s, 1H), 8.02 (d, 2H, $J = 6.72$ Hz), 7.63-7.19 (m, 15H), 6.82 (d, 4H, $J = 8.84$ Hz), 5.95 (d, 1H, $J = 3.3$ Hz), 4.47 (br
10 s, 1H), 4.26-4.00 (m, 5H), 3.84-3.37 (m, 14H), 2.10 (br s, 1H); ^{13}C NMR (50 MHz, CDCl_3): 178.62, 167.83, 161.65, 158.60, 156.06, 149.43, 144.72, 140.75, 137.41, 135.60, 135.37, 133.55, 132.12, 131.93, 130.05, 129.65, 129.10, 128.00, 126.88, 113.35, 99.66, 88.71, 88.11, 86.95, 83.93, 83.08,
15 74.54, 71.37, 70.32, 69.06, 62.28, 58.96, 55.19, 31.62; HRMS (FAB): Calcd for $\text{C}_{51}\text{H}_{49}\text{O}_{11}\text{N}_5^+$ 908.3507, Found: 908.3546.

Example 18

Synthesis of 5'-O-DMT-2'-O-[2-(methoxy)ethyl]-5-[3-(*N,N'*-bis-benzoylguanidinium)prop-1-yne]uridine-3'-O-[(2-cyanoethyl)*N,N'*-diisopropyl]phosphoramidite (18)
20

Compound **18** was prepared from compound **17**, according to the procedure described in Example 9. Yield: 0.72 g (86%).

^{31}P NMR (80 MHz, CDCl_3): 150.93 and 150.21; HRMS (FAB): Calcd for $\text{C}_{60}\text{H}_{66}\text{N}_7\text{O}_{12}\text{P}^+$ 1108.4585, Found: 1108.4548.

25 **Example 19**

Synthesis of 5-(3-phthalimidopropyl-1-nyl)-2'-deoxyuridine (19)

To a suspension of 5-iodo-2'-deoxyuridine (2 mmol) in 10 mL of CH_2Cl_2 , is added trifluoroacetic anhydride (5 mmol) at
30 room temperature. The mixture is stirred overnight. After concentration of the mixture, the mixture is dried *in vacuo*

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at room temperature to give a solid foam of 3',5'-di-O-trifluoroacetyl-5-iodo-2'-deoxyuridine. To a mixture of 3',5'-di-O-trifluoroacetyl-5-iodo-2'-deoxyuridine (5mmol) and N-1-phthalimidoprop-1-yne (10 mmol),
5 tetrakis(triphenylphosphine) palladium(0) (0.2 mmol), copper(I) iodide (0.3 mmol) and triethylamine (6 mmol) are added in 10 ml DMF. The mixture is stirred at room temperature for 18 hours and then concentrated in *vacuo*. To the concentrate, AG-1x8 anion exchange resin (HCO₃⁻ form, 3
10 equivalents), 20 mL of methanol and 20 mL of CH₂Cl₂ are added and the suspension is stirred for 1 h. The residue is purified by silica column chromatography to afford 5-(3-phthalimidoprop-1-ynyl)-2'-deoxyuridine (**19**).

Example 20**15 Synthesis of 5'-O-DMT-5-(3-phthalimidoprop-1-nyl)-2'-deoxyuridine (20)**

5-(3-Phthalimidoprop-1-nyl)-2'-deoxyuridine (**19**) is treated with 1.2 equivalents of 4,4'-dimethoxytrityl chloride in pyridine containing 0.1 equivalent of
20 dimethylaminopyridine. After stirring for 4 h, pyridine is evaporated and the residue dissolved in methylene chloride, washed with saturated sodium bicarbonate solution, dried over anhydrous sodium sulfate and concentrated. The residue is purified using silica column chromatography using ethyl
25 acetate:hexanes to yield the 5'-O-DMT derivative (**20**).

Example 21**Synthesis of 5'-O-DMT-2'-deoxy-5-[3-(amino)prop-1-yne]uridine (21)**

The above phthalimido compound (**20**), on hydrazine
30 treatment, yielded amino compound **21** according to the procedure described in Example 4.

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Example 22**Synthesis of 5'-O-DMT-2'-deoxy-5-[3-(N,N'-bis-CEOCguanidinium)-prop-1-yne]uridine (22)**

Compound **22** was prepared from compound **21** according to the procedure described in Example 8. Yield: 3.47 g (63%).

^1H NMR (200 MHz, CDCl_3): 11.59 (s, 1H), 9.80 (br s, 1H), 8.22 (br s, 1H), 8.11 (s, 1H), 7.43-7.20 (m, 9H), 6.82 (d, 4H, $J = 8.68$ Hz), 6.28 (t, 1H, $J = 6.15$ Hz), 4.50 (br s, 1H), 4.29 (m, 4H), 4.11 (m, 3H), 3.75 (s, 6H), 3.40 (m, 3H), 2.69 (m, 4H), 2.50 (m, 1H), 2.30 (m, 1H); ^{13}C NMR (50 MHz, CDCl_3): 162.92, 162.24, 158.82, 155.41, 152.88, 149.60, 144.89, 143.87, 135.79, 135.68, 130.28, 128.26, 127.20, 117.43, 116.64, 113.54, 99.40, 88.27, 87.20, 86.88, 86.08, 75.17, 72.28, 63.83, 61.09, 60.29, 55.54, 41.75, 31.99, 18.30, 18.11; HRMS (FAB): Calcd for $\text{C}_{42}\text{H}_{42}\text{N}_7\text{O}_{11}^{\oplus}$ 820.2942, Found: 820.2918.

Example 23**Synthesis of 5'-O-DMT-2'-deoxy-5-[3-(N,N'-bis-CEOCguanidinium)-prop-1-yne]uridine-3'-O-[(2-cyanoethyl)N,N-diisopropyl]phosphoramidite (23)**

Compound **23** was prepared from compound **22** according to the procedure described in Example 9. Yield: 1.43 g (58%).

^{31}P NMR (80 MHz, CDCl_3): 149.56 and 149.26; HRMS (FAB): Calcd for $\text{C}_{51}\text{H}_{58}\text{N}_9\text{O}_{12}\text{PNa}^{\oplus}$ 1042.3840, Found: 1042.3808.

Example 24**Synthesis of 5'-O-DMT-2'-O-[2-(N,N'-CEOC-guanidinium)ethyl]-3-O-succinyl-5-methyluridine (24)**

Compound **8** (0.252 g, 0.30 mmol) was co-evaporated with anhydrous acetonitrile. To this, succinic anhydride (0.6 g, 0.6 mmol), DMAP (0.018 g, 0.15 mmol), anhydrous pyridine

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(0.048 mL, 0.6 mmol) and CH_2Cl_2 (1 mL) were added and stirred at room temperature under an inert atmosphere for 4 h. The reaction mixture was diluted with CH_2Cl_2 (25 mL) and washed with cold 10% aqueous citric acid (20 mL) and brine (25 mL).

5 The organic phase was dried over anhydrous Na_2SO_4 and concentrated to provide compound **22** (0.248 g, 88%) as a foam. R_f (0.25, 5% MeOH in CH_2Cl_2).

^1H NMR (200 MHz, CDCl_3): 1.34 (s, 3H), 2.2-2.5 (m, 8H), 3.45 (d, 1H, $J = 5.4$ Hz), 3.64 (d, 1H, $J = 11.62$ Hz), 3.72-4
10 (m, 10 H), 4.15 (br s, 1H), 4.26-4.38 (m, 6H), 5.40 (d, 1H, $J = 4.4$ Hz), 5.93 (d, 1H, $J = 2.12$ Hz), 6.83 (d, 4H, $J = 7.22$ Hz), 7.24-7.4 (m, 9H), 7.77 (s, 1H), 8.57 (s, 1H), 10.51 (s, 1H); HRMS (FAB): Calcd for $\text{C}_{46}\text{H}_{50}\text{N}_7\text{O}_{15}$ 940.3365, Found: 940.3346.

15 Example 25

Synthesis of 5'-O-DMT-2'-O-[2-(N,N'-CEOC-guanidinium)ethyl]-5-methyluridine-3'-O-succinyl-CPG (25)

Compound **24** (0.227 g, 0.24 mmol) was dried over P_2O_5 in vacuo at 40° C overnight. Anhydrous DMF (0.62 mL) was added
20 followed by 2-(1H-benzotriazole)-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoroborate (0.077 g, 0.24 mmol) and N-methylmorpholine (53 mL, 0.48 mmol). The reaction mixture was vortexed to obtain a clear solution. To this solution, anhydrous DMF (2.38 mL) and activated CPG (1.03 g, 115.2
25 mmol/g, particle size 120/200, mean pore diameter 520 Å) were added. The reaction mixture was then allowed to shake on a shaker for 18 h. An aliquot was withdrawn and the loading capacity was estimated. Functionalized CPG was filtered and washed thoroughly with DMF, CH_3CN and Et_2O . It was then
30 dried in vacuo overnight. Functionalized CPG (**23**) was then suspended in a capping solution (acetic anhydride/lutidine/N-methylimidazole in THF, PerSeptive Biosystems, Inc.) and

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allowed to shake on a shaker for 2 h. Functionalized CPG was then filtered, washed with CH_3CN and Et_2O , dried in vacuo and the loading capacity determined by standard procedure. Final loading was 32.8 mol/g.

5 Example 26

Synthesis of oligonucleotides containing 2'-O-[2-(guanidinium)-ethyl] modification and deprotection of CEOC group using a novel procedure

The amidite **9** was dissolved in anhydrous acetonitrile to
10 obtain a 0.1 M solution and loaded onto a Expedite Nucleic Acid Synthesis system (Millipore 8909) to synthesize the oligonucleotides. The coupling efficiencies were more than 98%. For the coupling of the modified amidite (**9**) coupling time was extended to 10 minutes and this step was carried out
15 twice. All other steps in the protocol supplied by Millipore were used as such. After completion of the synthesis, CPG was suspended in 50% piperidine in water and kept at room temperature for 24 h to deprotect the CEOC protecting group on the guanidino group. Under the same conditions,
20 oligonucleotides are cleaved from CPG. The solvent was then evaporated and the CPG treated with aqueous ammonia solution (30 wt %) at 55 °C for 6 h to complete the deprotection of exocyclic amino protecting groups. This was purified on High Performance Liquid Chromatography (HPLC, Waters, C-4 , 7.8 X
25 300 mm, A = 50 mM triethylammonium acetate, pH = 7, B = acetonitrile, 5 to 60 % B in 55 Min, Flow 2.5 mL/min., λ = 260 nm). Detritylation with aqueous 80% acetic acid and evaporation, followed by desalting by HPLC on Waters C-4 column, gave 2'-modified oligonucleotides (**Table I**).
30 Oligonucleotides were analyzed by HPLC, CGE and mass spectrometry.

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Table I
Oligonucleotides containing 2'-O-[2-(guanidinium)ethyl
modification

5	SEQ ID No. /ISIS No.	Sequence (5'-3')	Mass Calculated	Mass Observed	HPLC Ret. Time (min ^a)
	01/32351	T*CC AGG T*GT* CCG CAT* C	5238.22	5238.21	18.69
	02/32350	CTC GTA CT*T* T*T*C CGG TCC	5797.49	5797.15	18.06
	03/109990	TTT TTT TTT TTT TTT T*T*T* T*	6122.93	6122.60	18.21
	03/109989	TTT TTT TTT TTT TTT TT*T T*	5920.59	5920.21	19.32
10	03/109973	TTT TTT TTT TTT TTT TTT T*	5818.47	5818.62	19.46
	04/113254	T*T*T* T*T*C TCT CTC TCT	4931.41	4931.46	21.93
	04/113929	TT*T TT*C TCT* CTC T*CT	4830.8	4830.29	16.07
	04/113255	T*TT* TT*C TCT CTC TCT	4729.73	4729.47	19.11

Ret. Time = retention time (in minutes);

15 T* = 2'-O-[2-(guanidinium)ethyl] ^{5MeU}

^aWaters C-4, 3.9x300 mm, solvent A=50 mM TEAAc, pH 7; Solvent
 B = CH₃CN; gradient 5-60% B in 55 min; flow rate 1.5 mL/min,
 λ = 260 nm

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Table II

T_m values of 2'-O-[2-(2-guanidiniumethyl)oxyethyl] modifications

SEQ ID No. /ISIS No.	Sequence 5'-3'	Target RNA °C	ΔT _m °C	ΔT _m /mod.
01/2221	TCC AGG TGT CCG CAT C	62.3	-	
01/32351	<u>T</u> *CC AGG <u>T</u> *GT* CCG CAT* C	70.5	8.2	2.05
02/2896	CTC GTA CTT TTC CGG TCC	61.8		
02/32350	CTC GTA CT*T* T*T*C CGG TCC	61.28	-0.53	-0.13

10 T* = 2'-O-[2-(guanidinium)ethyl ⁵MeU. The
ΔT_m/modification relative to 2'-deoxy P=S is obtained by
adding 0.8 to the value shown in last column. This value
relative to other modifications is shown in Figures 2 and 3.
2'-O-Guanidiniumethyl modification shows the highest binding
15 affinity change (ΔT_m/mod.) among 2'-modifications studied so
far. This point is illustrated in Figure 2.

As shown in Figure 3, the ΔT_m/modification depends on
the nature of placement of the cationic modification. There
could be charge repulsion when the cationic groups are
20 placed close to each other.

Example 27

Synthesis of N⁴-Benzoyl-2'-deoxy-5'-O-(4,4'-dimethoxytrityl)- cytidine (26)

2'-Deoxycytidine (5.82 g, 25.6 mmol) was dried by
25 evaporation and taken up in dry pyridine (100 mL). To this
suspension, trimethylsilyl chloride (11.5 mL, 90.6 mmol) was
added and the mixture was stirred at ambient temperature for
2 h. The reaction mixture was cooled in an ice-water bath and
benzoyl chloride (4.60 mL, 39.6 mmol) was slowly introduced.
30 The ice-waterbath was removed and the mixture was stirred at
room temperature for additional 2 h. The reaction was
quenched by adding methanol (15 mL), concentrated to one half

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of the original volume and filtered. To the filtrate, water (30 mL) was added and the solution was evaporated to an oil. Further evaporation with water (3x30 mL) was performed to remove pyridine, and the resulting residue was partitioned between water and ethyl acetate. After vigorous stirring, the product crystallized from the aqueous layer. The product crystals were washed with cold water and ethyl acetate and were used in the next step without further purification. Crude *N*^d-benzoyl-2'-deoxycytidine was dried by evaporation with dry pyridine (3x50 mL) and then dissolved in the same solvent (100 mL). 4,4'-Dimethoxytritylchloride (7.4 g, 22 mmol) was added portion-wise to the reaction mixture and the stirring was continued overnight at ambient temperature. The reaction mixture was evaporated to afford an oil and dissolved in dichloromethane (100 mL). The organic phase was washed with saturated aqueous NaHCO₃ (50 mL) and water (3x100 mL) and dried with anhydrous Na₂SO₄. After coevaporation with toluene, the residue was applied onto a silica gel column and eluted with a gradient of MeOH in CH₂Cl₂ (0 - 8 % MeOH). The product (**25**) was obtained in 65% yield (9.0 g) starting from 2'-deoxycytidine.

¹H NMR(200 MHz, CDCl₃): 8.30 (s, 1H, d, J = 7.6), 7.45-7.10 (m, 9H), 6.84 (m, 4H), 7.90-7.40 (m, 5H), 6.31 (t, 1H, J = 5.9, Hz), 4.55 (m, 1H), 4.19 (m, 1H), 3.78 (s, 6H), 3.49 (dd, 1H, J = 3.2 and 11.0 Hz), 3.42 (dd, 1H, J = 3.9 and 10.8 Hz), 2.77 (m, 1H).

Example 28

Synthesis of 2'-deoxy-*N*^d-[2-(amino)ethyl]-5'-O-(4,4'-dimethoxy-trityl)cytidine (**27**).

A solution of ethylenediamine (96.9 mmol) in 2-propanol (10 mL) is added to compound **25** (2.61 g, 4.12 mmol) and stirred until the mixture is clear. 1,5,7-

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Triazabicyclo[4.4.0]dec-5-ene (TBD, 2.40 g, 17.2 mmol) is added and the reaction mixture is stirred at ambient temperature for 48 h. The reaction mixture is evaporated to afford an oil, dissolved in CHCl_3 (100 mL), extracted with 5 0.1 mol L^{-1} aq. NaOH (2×50 mL) and water (4×50 mL). The organic phase is dried with anhydrous Na_2SO_4 , evaporated and dissolved in CH_2Cl_2 . Silica gel column purification yields the pure product **27**.

Example 29

10 **Synthesis of 2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-N'-[2-(N,N'-bis-CEOC-guanidinium)ethyl]cytidine (28)**

Compound **27**, on treatment with reagent **5**, gives compound **28** as described in Example 8.

Example 30

15 **Synthesis of 2'-deoxy-5'-O-(4,4'-dimethoxytrityl)-N'-[2-(N,N'-bis-CEOC-guanidinium)ethyl]cytidine-3'-O-[(2-cyanoethyl)N,N-diisopropyl]phosphoramidite (29)**

Compound **29** (0.29 mmol) is dried by evaporation with dry acetonitrile (3×20 mL) and finally *in vacuo* for 30 20 minutes. 2-Cyanoethyl-N,N,N',N'-tetraisopropylphosphorodiamidite (0.12 mL, 0.38 mmol) and dry acetonitrile (1.0 mL) are added and the mixture is stirred until all material dissolves. 1H-Tetrazole (0.45 mol L^{-1} in MeCN, 0.64 mL, 0.29 mmol) is added and the reaction 25 mixture is shaken. After one hour at ambient temperature, 100 mL of saturated aqueous NaHCO_3 solution is added to the reaction mixture and the resulting solution is extracted with ethyl acetate (2×40 mL). The organic phase is dried with anhydrous Na_2SO_4 and evaporated to dryness to afford pure 30 compound **29**.

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Example 31**Synthesis of 5'-O-(4,4'-dimethoxytrityl)-5-(3-aminoprop-1-nyl)-N⁴-benzoyl-2'-deoxycytidine (33)**

To 5-iodo-2'-deoxycytidine (5mmol), tetrakis
5 (triphenylphosphine) palladium(0) (0.5 mmol), copper(I)
iodide (1 mmol) and N-1-phthalimidoprop-3-yne (12 mmol) in 15
mL of dry DMF, 10 mmol of triethylamine is added. After
stirring for 18 h, AG-1x8 anion exchange resin (HCO₃⁻ form, 3
equivalents), 20 mL of methanol, and 20 mL of CH₂Cl₂ are
10 added, and the suspension is stirred for 1 h. The reaction
is filtered through a sintered glass funnel, and the DMF
removed *in vacuo*. Flash chromatography yielded the product
(30). This compound is treated with benzoic anhydride (one
equivalent) in dry pyridine to give N⁴-benzoyl derivative
15 (31). To a mixture of N⁴-benzoyl derivative and 4,4'-
dimethoxytritylchloride, dry pyridine is added to afford the
5'-dimethoxytrityl derivative (32), which is then converted
into amino compound 33 according to the procedure described
in Example 4.

20 Example 32**Synthesis of 5'-O-(4,4'-dimethoxytrityl)-2-[2-(N,N'-bis-CEOCguanidinium)ethyl]amino-2'-deoxyadenosine-3'-O-[(2-cyanoethyl)N,N-diisopropyl]phosphoramidite (34)**

2-Fluoroadenosine is synthesized according to the
25 procedure described by Krolikiewicz and Vorbruggen
(Nucleosides & Nucleotides 13, 673, 1994). Ethylenediamine
(5 equivalents in 2-methoxyethanol) is added to 2-
fluoroadenosine and heated at 100°. The resulting 2-
(aminoethyl) derivative is then converted into 2-(2-
30 guanidinium)ethyl derivative with reagent 5 as described in
example 8. This was then treated with benzoyl chloride under

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transient protection conditions and then dimethoxytritylated at the 5'-position. The resulting compound was then phosphitylated following the procedures described above to give the adenosine derivative **36** functionalized at the 2-
5 position.

Example 33

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-2-[2-(bis-*N,N'*-CEOCguanidinium)ethyl]-2'-deoxyguanosine-3'-O-[(2-cyanoethyl)*N,N*-diisopropyl] phosphoramidite (34**)**

10 2-Fluoroinosine is synthesized according to the procedure described by Krolikiewicz and Vorbruggen (Nucleosides & Nucleotides 13, 673, 1994). Ethylenediamine (5 equivalents in 2-methoxyethanol) is added to 2-fluoroinosine and heated at 100 °C. The free amine is
15 guanylated on the side chain at the 2-position by treatment with reagent **5** as described in Example 8. The product is first dimethoxytritylated at the 5'-position, then phosphitylated following the procedures described above to give the guanosine derivative functionalized at the 2-
20 position (**37**).

Example 34

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-*N*²-isobutyryl-*N*⁶-[2-(bis-*N,N'*-CEOC-guanidinium)ethyl]-2-aminoadenosine-3'-O-[(2-cyanoethyl)*N,N*-diisopropyl]phosphoramidite (35**)**

25 To 2'-deoxyguanosine (2 mmol), dried by coevaporation with pyridine, suspended in 40 mL of pyridine and cooled in an ice bath under an argon atmosphere, trifluoroacetic anhydride (16 mmol) is added following the procedure of Kung and Jones (Tetraheron Lett., 32, 3919, 1991). After 40
30 minutes, ethylenediamine (30 mmol) is added and stirred for 24 h at room temperature. The reaction mixture is

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concentrated and purified by silica column chromatography. The product is guanylated with reagent **5** as in Example 8 and protected at N^2 -position with an isobutyryl group by treating with isobutyric anhydride, 5'-dimethoxytritylated at the 5'-5 position and purified. The resulting compound is treated with benzoic anhydride (N^6 protection) and then phosphitylated following the procedures described above to afford the 2,6-diaminopurine derivative functionalized at the 6-position (**35**).

10 **Example 35**

Nuclease Stability Assay of 2'-O-[2-(guanidinium)ethyl] oligonucleotides (SEQ ID No. 3: ISIS 109990, ISIS 109989 and ISIS 109973)

The stability of the oligomers was tested by incubating 15 them with snake venom phosphodiesterase (Phosphodiesterase I, USB # 20240), a non-sequence specific 3'-exonuclease.

Typical Nuclease digestion:

70 uL nanopure water
10 uL 1 uM ^{32}P labeled oligonucleotide
20 10 uL 10x buffer
10 uL SVPD @ 5×10^{-2} Units/mL
100 uL

Final reaction conditions:

Buffer: 50 mM Tris.HCl, pH 8.0
25 75 mM NaCl
14 mM MgCl_2

Oligomer concentration: 100 nM

Enzyme concentration: 5×10^{-3} Units/mL

The reactions were incubated at 37 °C. Aliquots (5 μL) 30 were removed over a set time course and transferred to tubes containing 2x TBE in formamide with a trace of Bromphenol Blue, then frozen at -4 °C until analysis. The reactions

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were analyzed by PAGE/Phosphorimaging. Samples at specific time points were centrifuged briefly to settle the samples, then loaded on a 20% acrylamide gel. After running, the gels were exposed and read with a Molecular Dynamics

5 Phosphorimager (Figure 4). The % full length (N) of oligomer in each lane was calculated.

As shown in Figure 4, the placement of guanidinium residue protects the oligonucleotide phosphodiester internucleotide linkage against exonuclease degradation.

10 With increasing number of substituents, the nuclease resistance also increases. Placement of four guanidinium substituents in a 19mer at the 3'-end fully protects the oligomer.

Example 36

15 Synthesis of 2'-O-(2-guanidinium)-ethyl oligonucleotides for triplex formation

The sequence specific recognition of duplex DNA by pyrimidine oligonucleotides involves the formation of triple helical structures which are stabilized by Hoogsteen hydrogen
20 bonds between the bases on a DNA target and pyrimidine third strand. (Neidel, *Anti-Cancer Drug Des.* 12, 433-442, **1997**; Giovannangeli and Hélène, *Antisense Nucleic Acid Drug Dev.* 7, 413-421, **1997**; Maher, *Cancer Invest.* 14, 66-82, **1996**.)

Examination of a molecular model of triple helix with a
25 RNA third strand indicates that the 2'-hydroxyl groups of RNA and the phosphate groups of the DNA second strand are in close proximity. (Hélène, et al., *Nucleic Acid Res.* 21, 5547-5553, **1993**.) 2'-Aminoethoxy modified oligonucleotides stabilize the triplex formation. (Cuenourd et al., *Angew.*
30 *Chem. Int. Ed.*, 37, 1288-1291, **1998**.) 2'-Guanidinoethyl modified oligonucleotides, by virtue of its stabilized multi-point charge site, would stabilize triplex formation.

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Following duplex DNA was targeted with 2'-guanidiniummethyl-modified oligonucleotides (SEQ ID No. 04: ISIS 113254, ISIS 113929 and ISIS 113255) used as strand 3 for triplex formation.

5 SEQ ID No. 05

Strand 2 5'-GCT AAA AAG AGA GAG AGA TCG-3'

SEQ ID No. 06

Strand 1 5'-CGA TTT TTC TCT CTC TCT AGC-5'

10 **Example 37**

Synthesis of compounds 40 and 41

Compound **37** is synthesized according to the procedure described by Behrens *et al.* (Bioorg. Med. Chem. Lett., **1995**, 5, 1785). Compound **37** is converted to the guanidinium
15 derivative **38** using reagent **5** as described in Example 8. Compound **38** is then treated with 1 equivalent of 4,4'-dimethoxytrityl chloride and pyridine to form compound **39**, which is then phosphitylated to yield compound **40**. Synthesis of an oligomer using compound **40** results in an oligomer
20 having the guanidinium group at the 5' end.

Also, compound **39** is succinylated and coupled to controlled pore glass (CPG) to yield compound **41**. Synthesis of an oligomer using compound **41** results in an oligomer having the guanidinium group at the 3' end.

25 **Example 38**

Synthesis of compounds 49 and 56

(Figures 19-23)

Compound **42** is synthesized according to the reported procedures (see Migawa *et al.*, *Synthetic Communications*,
30 **1996**, 26, 3317-3322). Glycosylation of **42** with chloro sugar

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43 according to a previously reported procedure (Ramasamy et al., *Chem. Soc. Perkin Trans., I*, **1989**, 2375-2384) gives nucleoside **44** which is then acylated at the N² position to give the completely protected nucleoside **45**. Compound **45** is
5 then reduced to the 7-aminomethyl derivative **46** in the presence of palladium and hydrogen gas at 50 psi. Guanylation of **46** with guanyating reagent **5** in DMF and triethyl amine gives **48** which is then subject to desilylation, selective tritylation at the 5' position and
10 phosphitylation at the 3' position to give compound **49**.

Compound **50** (prepared as per the procedure of: Porcari et al., *Nucleosides and Nucleotides*, **1999**, 18, 153-159) is silylated with Markovick reagent in pyridine to give Compound **51**. Compound **51** is then acylated at the 4-position of the
15 exocyclic amino group with benzyl chloride and pyridine to give the bisbenzoyl derivative which is converted to mono benzoyl derivative, compound **52**, by treatment with aqueous ammonia. Deoxygenation of **52** at the 2' position gives compound **53**. Catalytic hydrogenation of **53** in the presence of
20 Palladium and hydrogen gives compound **54** which is converted into the guanidino derivative **55** according to the procedure used for **5**. Compound **55** on desilylation, tritylation at 5'-position and phosphitylation at 3'-position gives compound **56**.

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Example 39

Table III
Triplex binding affinity enhancement of 2'-O-GE
modification against double stranded DNA

T*	II		III		IV	
	TM °C	ΔTm/mod/°C	TM °C	ΔTm/mod/°C	TM °C	ΔTm/mod/°C
2'-deoxy					21.9	
2'-O-AE					35.9	3.5
2'-O-AP*					32.4	2.1
2'-O-GE	29.3	2.53	32.4	2.65	43.6	4.12

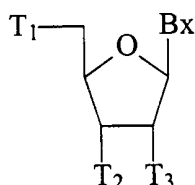
Oligomers II, III and IV each have SEQ ID NO 4 (TTT TTC TCT CTC TCT) with various groups attached at various 2'-positions ((2'-H, 2'-O-aminoethyl (2'-O-AE); 2'-aminopropyl (2'-O-AP); and 2'-guanidiniummethyl (2'-O-GE)). Oligomer II is modified at the 1,3 and 5 positions (T*TT* TT*C TCT CTC TCT); oligomer III is modified at the 2, 5, 9 and 12 positions (TT*T TT*C TCT* CTC T*CT); and oligomer IV is modified at the 1-5 positions (T*T*T* T*T*C TCT CTC TCT).

The results of the study show that the 2'-O-GE modification has imparts higher binding affinity to the resulting modified oligomers.

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What is claimed is:

1. A monomer of the formula:



wherein:

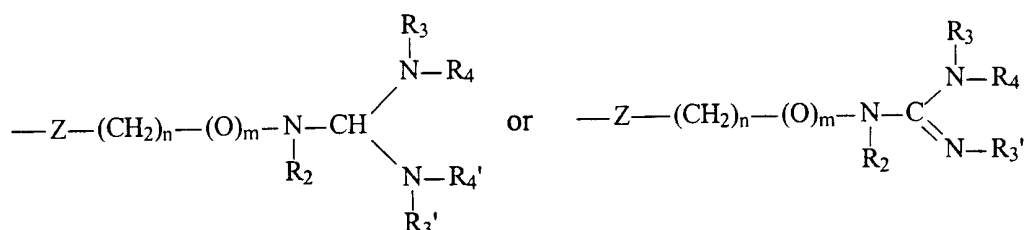
Bx is a heterocyclic base;

- 5 T₁ is OH or a protected hydroxyl group;

T₂ is an activated phosphorus group or a linking moiety attached to a solid support;

T₃ is H, OH, a protected hydroxyl or a sugar substituent group;

- 10 said monomer further comprising at least one group, R₁, therein; said R₁ group occurring in lieu of at least one T₁, T₂ or T₃ or as a substituent on at least one Bx; said R₁ group having one of the formulas:



wherein:

- 15 each Z is, independently, a single bond, O, N or S;

each R₂, R₃, R_{3'}, R₄ and R_{4'} is, independently, hydrogen, C(O)R₅, substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, alkylsulfonyl, arylsulfonyl, a chemical

- 20 functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy,

-66-

carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

or R_3 and R_4 , together, are R_7 , and R_3 , and R_4 , together, are R_7 ;

5 each R_5 is, independently, substituted or unsubstituted C_1 - C_{10} alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or aryl;

10 each R_7 and R_7 , is, independently, hydrogen or forms a phthalimide moiety with the nitrogen atom to which it is attached;

each m is, independently, zero or 1; and

each n is, independently, an integer from 1 to about 6.

15 2. The monomer of claim 1 wherein T_1 is O-dimethoxytrityl.

3. The monomer of claim 1 wherein T_1 is O-monomethoxytrityl.

4. The monomer of claim 1 wherein T_2 is a
20 phosphoramidite.

5. The monomer of claim 1 wherein T_2 is succinyl CPG.

6. The monomer of claim 1 wherein at least one of R_3 , R_3 , R_4 and R_4 , is $C(O)R_5$ and R_5 is cyanoethoxy.

7. The monomer of claim 1 wherein R_3 and R_3 , are
25 $C(O)R_5$ and R_5 is cyanoethoxy.

8. The monomer of claim 1 wherein T_3 is R_1 .

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9. The monomer of claim 1 wherein Bx is a pyrimidine heterocyclic base.

10. The monomer of claim 9 wherein R₁ is covalently bound to an amino group at the 4-position of Bx.

5 11. The monomer of claim 9 wherein R₁ is covalently bound to C5 of Bx.

12. The monomer of claim 1 wherein Bx is a purine heterocyclic base.

13. The monomer of claim 12 wherein R₁ is covalently
10 bound to an amino group at the 2-position of Bx.

14. The monomer of claim 12 wherein R₁ is covalently bound to an amino group at the 6-position of Bx.

15. The monomer of claim 1 wherein said heterocyclic base is a 7-deazapurine.

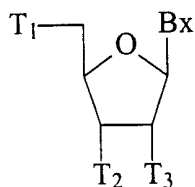
15 16. The monomer of claim 15 wherein R₁ is covalently bound at the 7-deaza position of Bx.

17. The monomer of claim 1 wherein Bx is a diaminopurine heterocyclic base.

18. The monomer of claim 17 wherein R₁ is covalently
20 bound to an amino group at the 6-position of Bx.

19. An oligomer comprising a plurality of nucleotide units of the formula:

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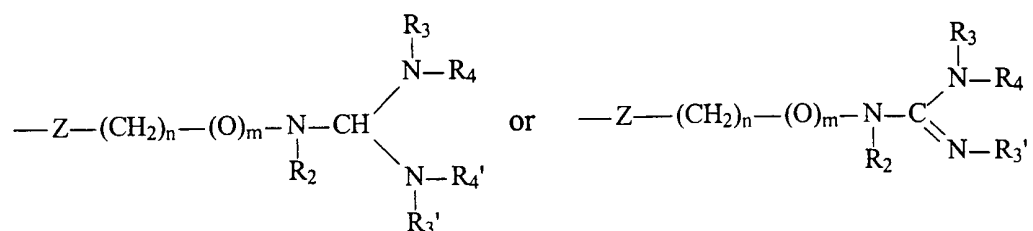
wherein:

Bx is a heterocyclic base;

5 each T_1 and T_2 is, independently, OH, a protected hydroxyl, a nucleotide, a nucleoside or an oligonucleotide;

T_3 is H, OH, a protected hydroxyl or a sugar substituent group;

said oligomer further comprising at least one group, R_1 ,
 10 therein; said R_1 group occurring at the 3' end, the 5' end, in lieu of at least one T_3 or as a substituent on at least one Bx; said R_1 group having one of the formulas:



wherein:

each Z is, independently, a single bond, O, N or S;

15 each R_2 , R_3 , R_3' , R_4 and R_4' is, independently, hydrogen, C(O)R_5 , substituted or unsubstituted $\text{C}_1\text{--C}_{10}$ alkyl, substituted or unsubstituted $\text{C}_2\text{--C}_{10}$ alkenyl, substituted or unsubstituted $\text{C}_2\text{--C}_{10}$ alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the
 20 substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

or R_3 and R_4 , together, are R_7 , and R_3' and R_4' together, are R_7' ;

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each R_5 is, independently, substituted or unsubstituted C_1 - C_{10} alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-
5 butyryl, phenyl or aryl;

each R_7 and R_7' is, independently, hydrogen or forms a phthalimide moiety with the nitrogen atom to which it is attached;

each m is, independently, zero or 1; and
10 each n is, independently, an integer from 1 to about 6.

20. The oligomer of claim 19 wherein R_1 , R_2 , R_3 , R_3' , R_4 and R_4' are hydrogen.

21. The oligomer of claim 19 wherein R_1 , R_2 , R_3 , R_3' , R_4 and R_4' are hydrogen, m is zero and n is 2.

15 22. The oligomer of claim 19 wherein at least one of said R_2 , R_3 , R_3' , R_4 and R_4' is a conjugate group.

23. The oligomer of claim 22 wherein said conjugate group is a contrast reagent, a cleaving agent, a cell targeting agent, polyethylene glycol, cholesterol, phospho-
20 lipid, biotin, phenanthroline, phenazine, phenanthridine, anthraquinone, acridine, fluorescein, rhodamine, coumarin, pyrene, retinal or a cyanine dye.

24. The oligomer of claim 19 wherein T_3 is R_1 .

25. The oligomer of claim 19 wherein Bx is a
25 pyrimidine heterocyclic base.

26. The oligomer of claim 25 wherein R_1 is covalently bound to an amino group at the 4-position of Bx .

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27. The oligomer of claim 25 wherein R_1 is covalently bound to C5 of Bx.

28. The oligomer of claim 19 wherein Bx is a purine heterocyclic base.

5 29. The oligomer of claim 28 wherein R_1 is covalently bound to an amino group at the 2-position of Bx.

30. The oligomer of claim 28 wherein R_1 is covalently bound to an amino group at the 6-position of Bx.

31. The oligomer of claim 19 wherein Bx is a
10 diaminopurine heterocyclic base.

32. The oligomer of claim 31 wherein R_1 is covalently bound to an amino group at the 6-position of Bx.

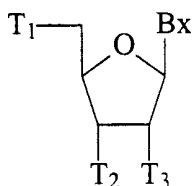
33. The oligomer of claim 31 wherein R_1 is covalently bound to an amino group at the 2-position of Bx.

15 34. The monomer of claim 19 wherein Bx is a 7-deazapurine.

35. The monomer of claim 34 wherein R_1 is covalently bound at the 7-deaza position of Bx.

36. A method of preparing an oligomer comprising the
20 steps of:

(a) selecting a monomer of the formula:



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wherein:

Bx is a heterocyclic base;

T₁ is OH or a protected hydroxyl group;

T₂ is a linking moiety attached to a solid support;

5 T_3 is H, OH, a protected hydroxyl or a sugar substituent
group;

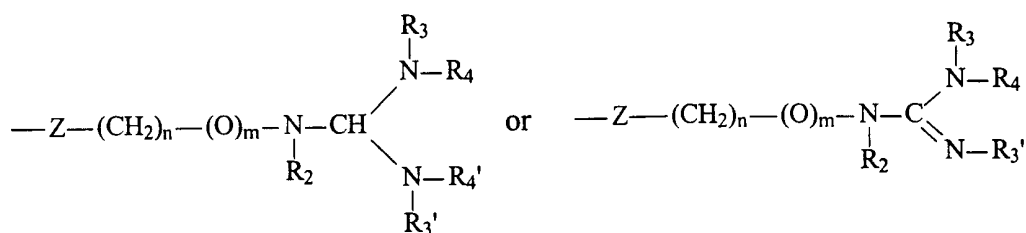
said monomer further comprising at least one group, R_1 ,

therein; said R₁ group occurring in lieu of at least one T₁,

T₂ or T₃ or as a substituent on at least one Bx;

10 provided that if T_2 is R_1 , T_3 is a linking moiety attached to
a solid support;

said R₁ group having one of the formulas:



wherein:

each Z is, independently, a single bond, O, N or S;

each R_2 , R_3 , R_3' , R_4 and R_4' is, independently, hydrogen, $C(O)R_5$, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_2 - C_{10} alkenyl, substituted or unsubstituted C_2 - C_{10} alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

or R_3 and R_4 , together, are R_7 , and R_3 , and R_4 , together, are R_7 ;

25 each R₅ is, independently, substituted or unsubstituted
C₁-C₁₀ alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy,

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t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or aryl;

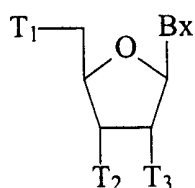
each R_7 and R_7 is, independently, hydrogen or forms a
5 phthalimide moiety with the nitrogen atom to which it is attached;

each m is, independently, zero or 1; and

each n is, independently, an integer from 1 to about 6;

(b) deprotecting the protected hydroxyl group at the
10 5'-position to form a deprotected monomer;

(c) coupling said deprotected monomer with a second monomer of formula:



wherein:

Bx is a heterocyclic base;

15 T_1 is OH or a protected hydroxyl group;

T_2 is an activated phosphorus group;

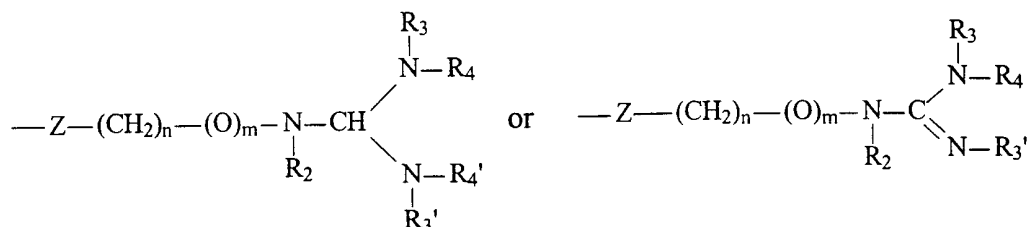
T_3 is H, OH, a protected hydroxyl or a sugar substituent group;

said monomer further comprising at least one group, R_1 ,
20 therein; said R_1 group occurring in lieu of at least one T_1 , T_2 or T_3 or as a substituent on at least one Bx;

provided that if T_2 is R_1 , T_3 is an activated phosphorus group;

said R_1 group having one of the formulas:

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wherein:

each Z is, independently, a single bond, O, N or S;

each R₂, R₃, R_{3'}, R₄ and R_{4'} is, independently, hydrogen, C(O)R₅, substituted or unsubstituted C₁-C₁₀ alkyl, substituted
 5 or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen,
 10 alkyl, aryl, alkenyl and alkynyl;

or R₃ and R₄, together, are R₇, and R_{3'} and R_{4'} together, are R_{7'};

each R₅ is, independently, substituted or unsubstituted C₁-C₁₀ alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy,
 15 t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, isobutyryl, phenyl or aryl;

each R₇ and R_{7'} is, independently, hydrogen or forms a phthalimide moiety with the nitrogen atom to which it is
 20 attached;

each m is, independently, zero or 1; and

each n is, independently, an integer from 1 to about 6, said coupling occurring in the presence of an activating agent to form a coupled compound;

25 (d) capping said coupled compound with a capping reagent to form a capped compound having an internucleotide linkage;

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(e) oxidizing said internucleotide linkage with an oxidizing reagent; and

(f) repeating steps (b) to (e) to form an oligomer.

37. The method of claim 34 further comprising the
5 step of:

(g) cleaving said oligomer with a cleaving reagent.

38. The method of claim 37 wherein said cleaving reagent is an aqueous base.

39. The method of claim 38 wherein concentration of
10 said aqueous base is 10-80%.

40. The method of claim 38 wherein said base is piperidine, morpholine, piperazine, diethylamine or methylamine.

41. The method of claim 40 wherein said base is 50%
15 aqueous piperidine.

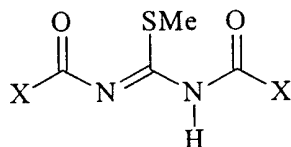
42. The method of claim 36 wherein said activating reagent is tetrazole, pyridinium trifluoroacetate or dicyanoimidazole.

43. The method of claim 36 wherein said capping
20 reagent is acetic anhydride and N-methylimidazole.

44. The method of claim 36 wherein said oxidizing reagent is iodine, camphorsulfonyloxaziridine, t-butyl hydrogen peroxide or Beaucage reagent.

45. A compound of the formula:

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wherein:

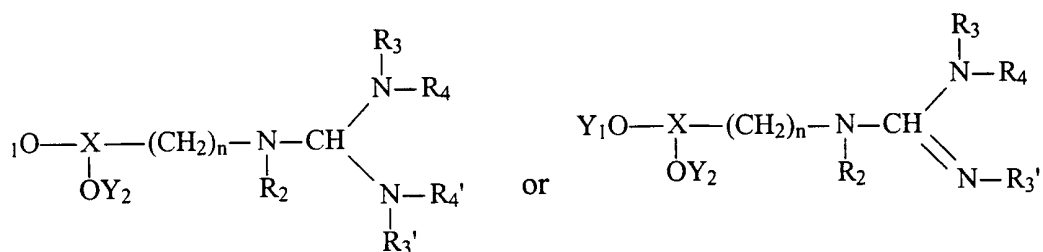
each X is, independently, cyanoethyloxy, benzyloxy, t-butoxy, methoxy, ethoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, trifluoromethyl, butyryl, iso-butyryl, phenyl or aryl.

46. The compound of claim 45 wherein X is cyanoethyloxy.

47. The compound of claim 45 wherein X is phenyl.

48. The compound of claim 45 wherein X is t-butyl.

49. A compound having one of the formulas:



wherein:

X is C₃-C₁₀ alkyl, C₆-C₂₄ aryl, C₆-C₂₄ heteroaryl, C₄-C₂₀ alicyclic, C₄-C₂₀ alicyclic having at least one heteroatom, nucleoside, nucleotide or oligonucleotide;

Y₁ is a hydroxyl protecting group;

Y₂ is an activated phosphorus group or a linking moiety

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attached to a solid support;

each R_2 , R_3 , R_3' , R_4 and R_4' is, independently, hydrogen, $C(O)R_5$, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_2 - C_{10} alkenyl, substituted or unsubstituted
5 C_2 - C_{10} alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

10 or R_3 and R_4 , together, are R_7 , and R_3' and R_4' , together, are R_7' ;

each R_5 is, independently, substituted or unsubstituted C_1 - C_{10} alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-
15 ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, isobutyryl, phenyl or aryl;

each R_7 and R_7' is, independently, hydrogen or forms a phthalimide moiety with the nitrogen atom to which it is attached; and

20 n is an integer from 1 to about 6.

50. The compound of claim 49 wherein Y_1 is dimethoxytrityl.

51. The compound of claim 49 wherein Y_1 is monomethoxytrityl.

25 52. The compound of claim 49 wherein Y_2 is a phosphoramidite.

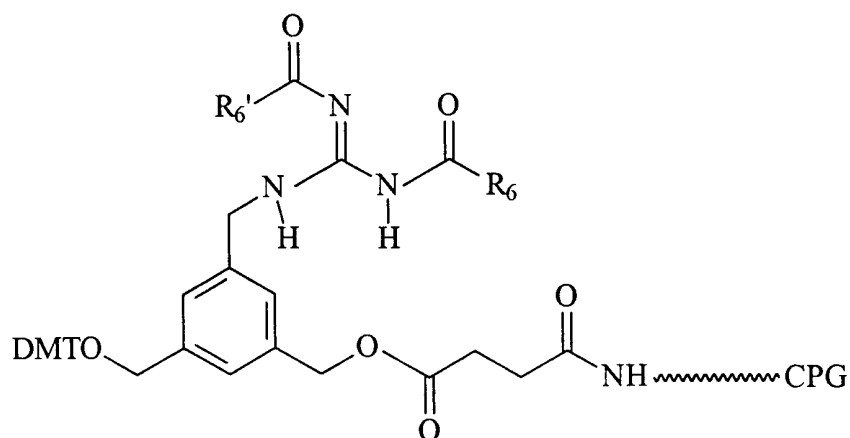
53. The compound of claim 49 wherein Y_2 is a linking moiety attached to a solid support.

54. The compound of claim 49 wherein Y_2 is succinyl

-77-

CPG.

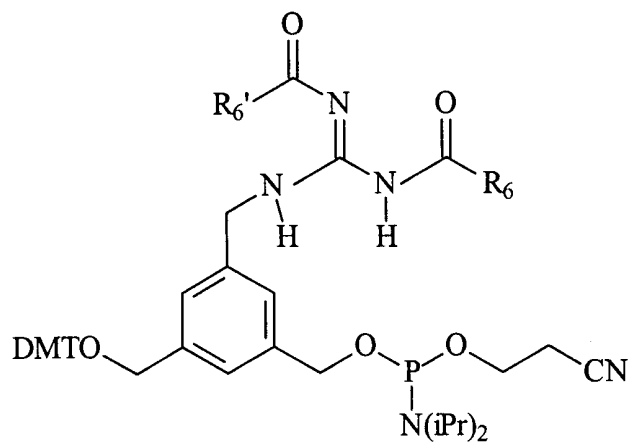
55. A compound of the formula:



wherein:

each R_6 and R_6' is, independently, trifluoromethyl, cyanoethyloxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or aryl.

56. A compound of the formula:



10 wherein:

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each R_6 and R_6' is, independently, trifluoromethyl, cyanoethoxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or 5 aryl.

57. A method of modulating gene expression comprising hybridizing an oligomer with a single-stranded nucleic acid, said oligomer comprising a plurality of nucleotide units of the formula:



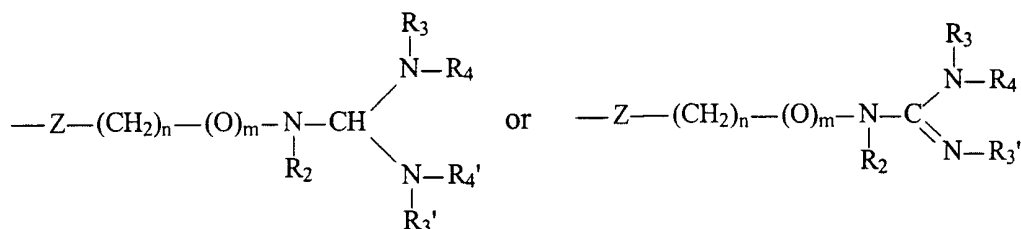
wherein:

Bx is a heterocyclic base;

each T_1 and T_2 is, independently, OH, a protected
15 hydroxyl, a nucleotide, a nucleoside or an oligonucleotide;

T_3 is H, OH, a protected hydroxyl or a sugar substituent group;

said oligomer further comprising at least one group, R_1 , therein; said R_1 group occurring at the 3' end, the 5' end,
20 in lieu of at least one T_3 or as a substituent on at least one Bx; said R_1 group having one of the formulas:



wherein:

each Z is, independently, a single bond, O, N or S;

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each R_2 , R_3 , R_3' , R_4 and R_4' is, independently, hydrogen, $C(O)R_5$, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_2 - C_{10} alkenyl, substituted or unsubstituted C_2 - C_{10} alkynyl, alkylsulfonyl, arylsulfonyl, a chemical
 5 functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

or R_3 and R_4 , together, are R_7 , and R_3' and R_4' , together,
 10 are R_7' ;

each R_5 is, independently, substituted or unsubstituted C_1 - C_{10} alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-
 15 butyryl, phenyl or aryl;

each R_7 and R_7' is, independently, hydrogen or forms a phthalimide moiety with the nitrogen atom to which it is attached;

each m is, independently, zero or 1; and
 20 each n is, independently, an integer from 1 to about 6.

58. A method of modulating gene expression comprising hybridizing an oligomer with a double-stranded nucleic acid, said oligomer comprising a plurality of nucleotide units of the formula:



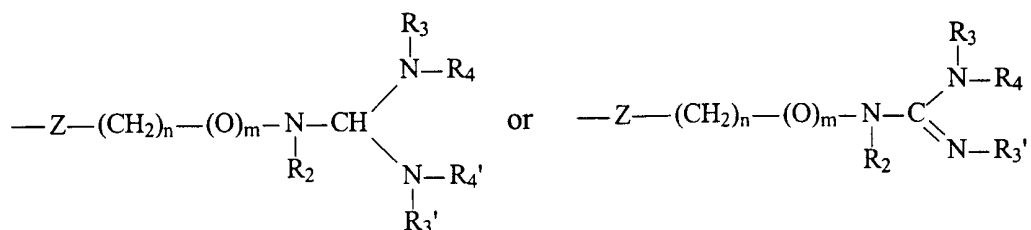
wherein:

Bx is a heterocyclic base;

each T_1 and T_2 is, independently, OH, a protected
 30 hydroxyl, a nucleotide, a nucleoside or an oligonucleotide;

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T₃ is H, OH, a protected hydroxyl or a sugar substituent group;
 said oligomer further comprising at least one group, R₁, therein; said R₁ group occurring at the 3' end, the 5' end, 5 in lieu of at least one T₃ or as a substituent on at least one Bx; said R₁ group having one of the formulas:



wherein:

each Z is, independently, a single bond, O, N or S;

each R₂, R₃, R_{3'}, R₄ and R_{4'} is, independently, hydrogen,
 10 C(O)R₅, substituted or unsubstituted C₁-C₁₀ alkyl, substituted
 or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted
 C₂-C₁₀ alkynyl, alkylsulfonyl, arylsulfonyl, a chemical
 functional group or a conjugate group, wherein the
 substituent groups are selected from hydroxyl, amino, alkoxy,
 15 carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen,
 alkyl, aryl, alkenyl and alkynyl;

or R₃ and R₄, together, are R₇, and R_{3'} and R_{4'}, together,
 are R₇;

each R₅ is, independently, substituted or unsubstituted
 20 C₁-C₁₀ alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy,
 t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-
 ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-
 butyryl, phenyl or aryl;

each R₇ and R_{7'} is, independently, hydrogen or forms a
 25 phthalimide moiety with the nitrogen atom to which it is
 attached;

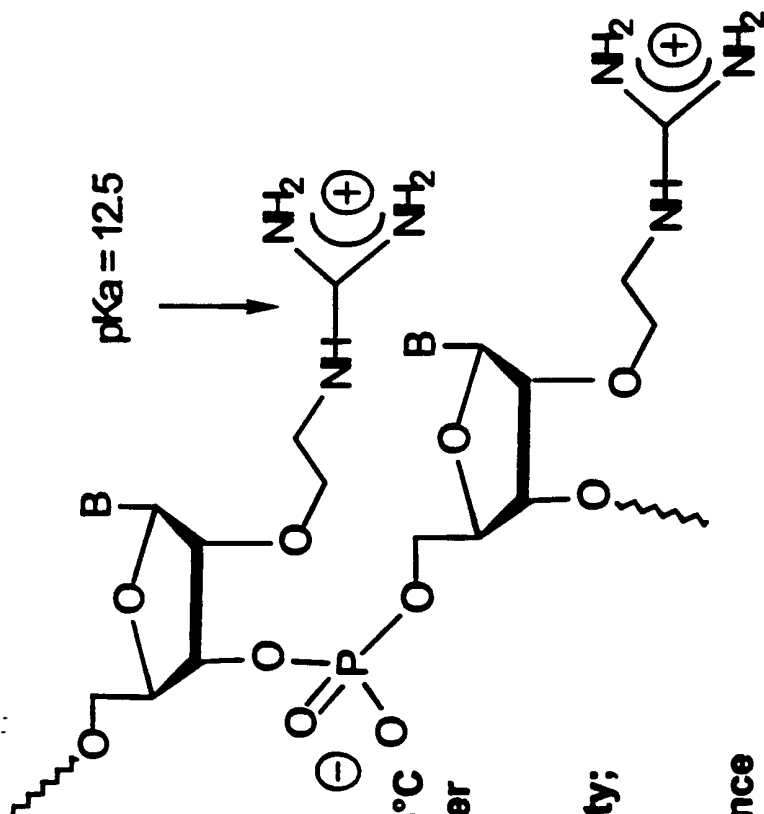
each m is, independently, zero or 1; and

-81-

each n is, independently, an integer from 1 to about 6.

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FIGURE 1



- ◆ $\Delta T_m = 3.0^\circ\text{C}$ (dispersed) 0.6°C (placed together) as a diester (Relative to 2'-deoxyP=S)
- ◆ Entropy effect due to rigidity; Weak Gauche Effect
- ◆ Very high nuclease resistance due to charge effect
- ◆ Charge delocalization over four atoms

**2'-O-(2-Guanidinoethyl):
Supercationic Modification**

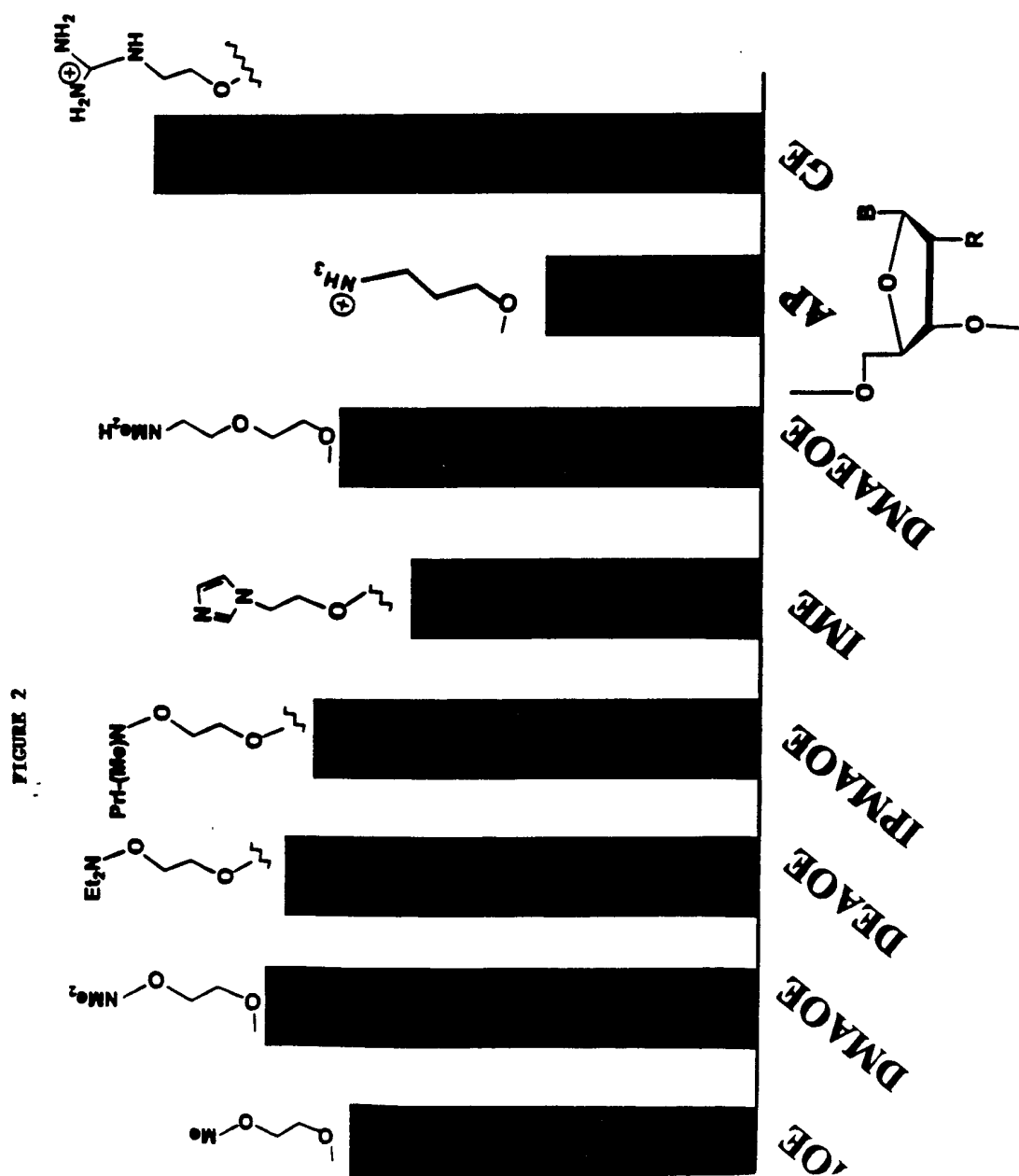
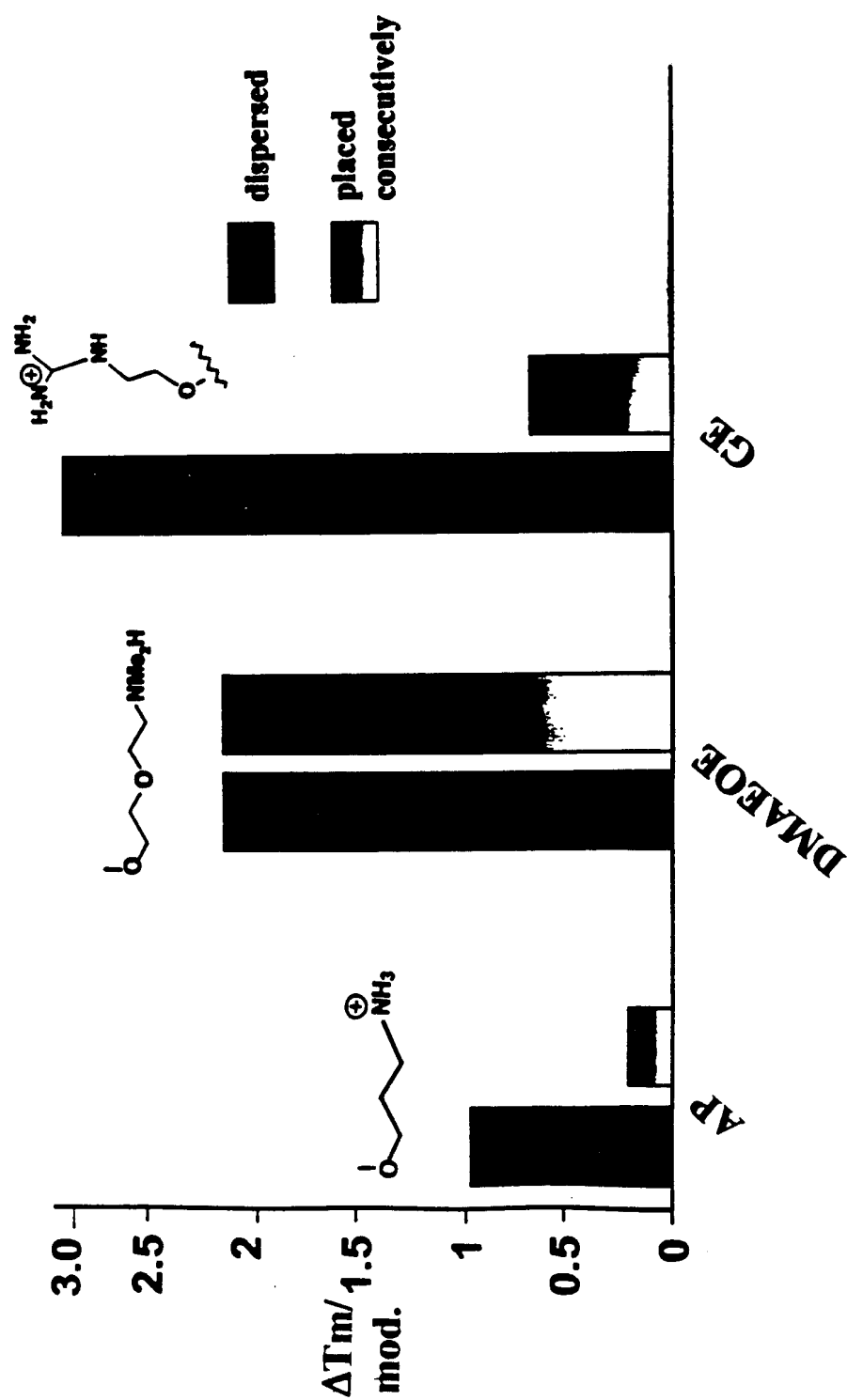


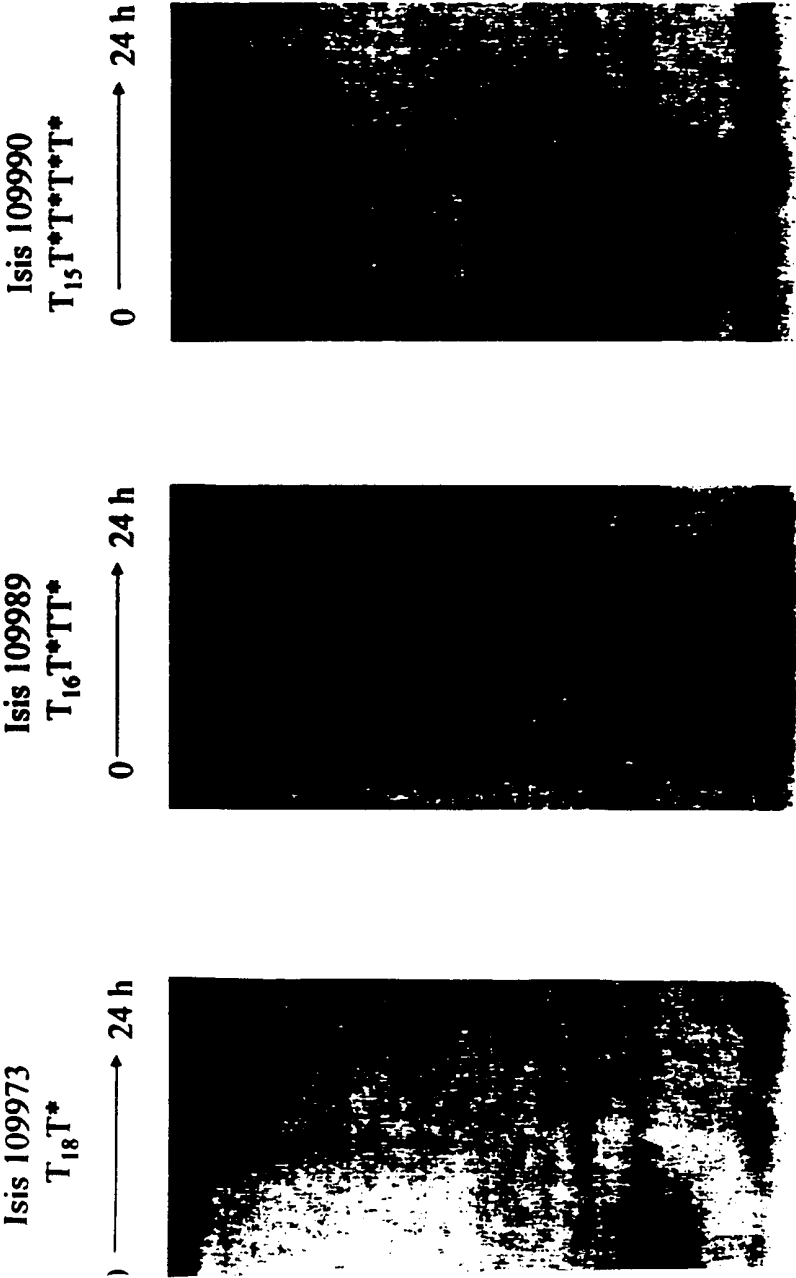
FIGURE 3



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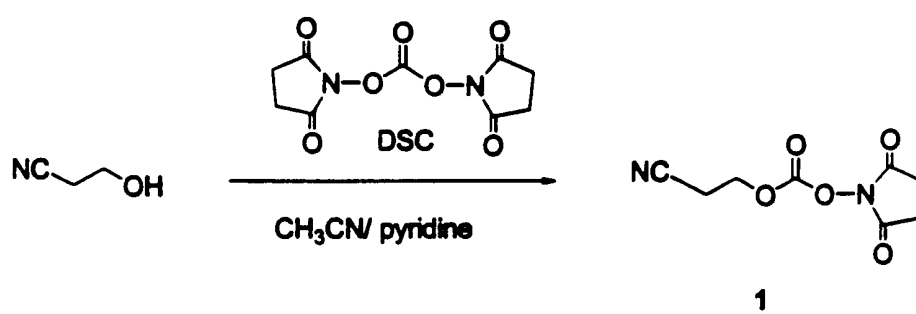
FIGURE 4

ease: SVPD (3'-exonuclease)



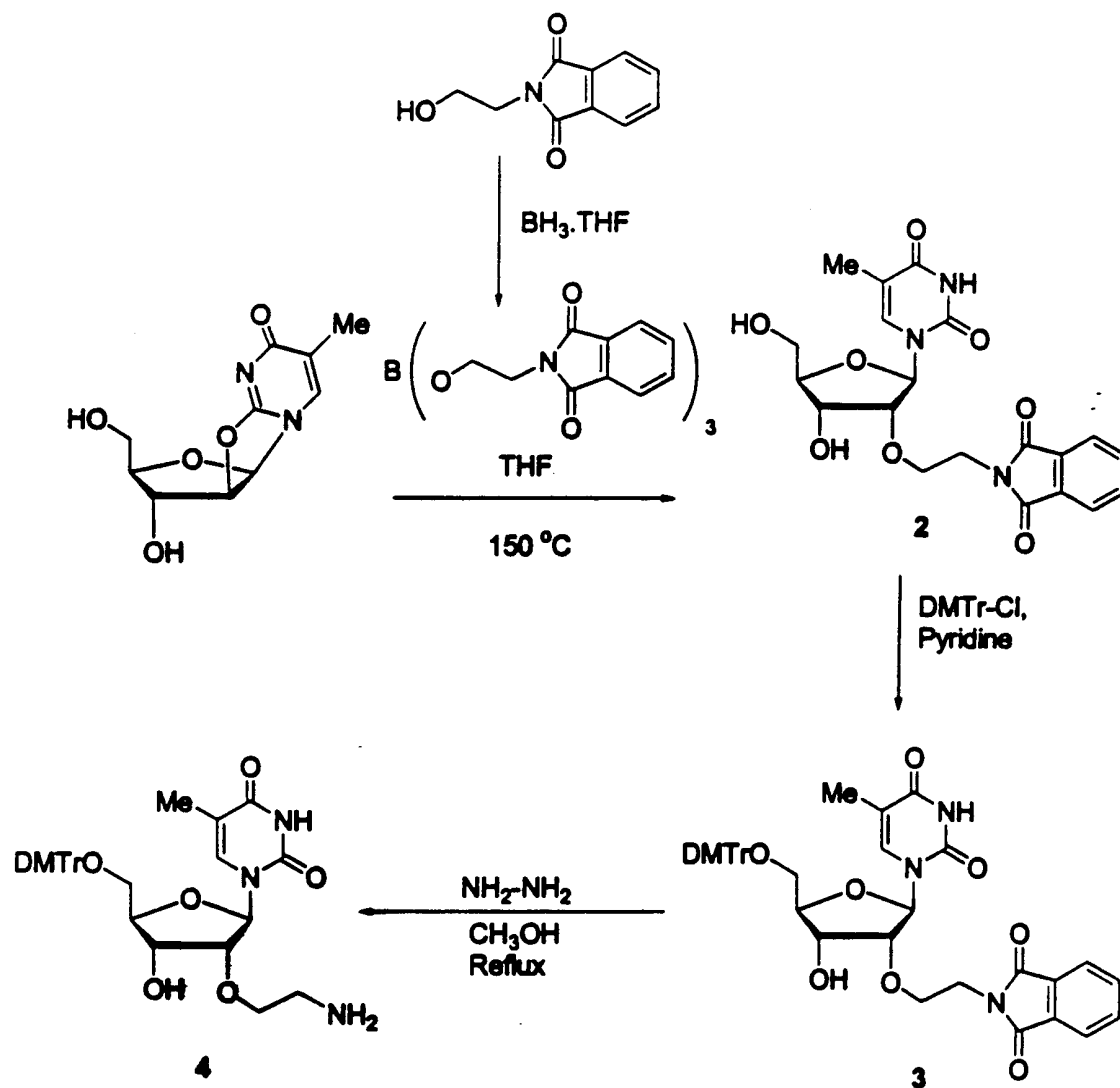
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Figure 5



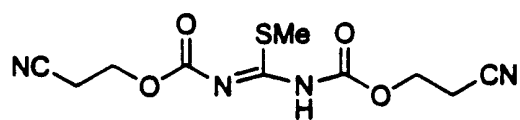
6/22

Figure 6

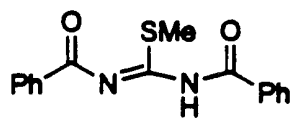


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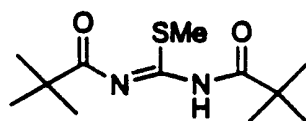
Figure 7



5



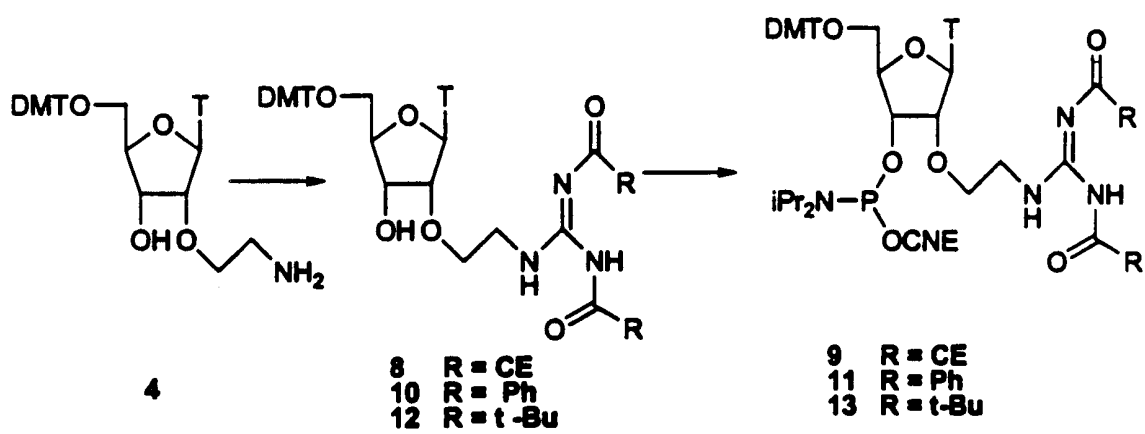
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7

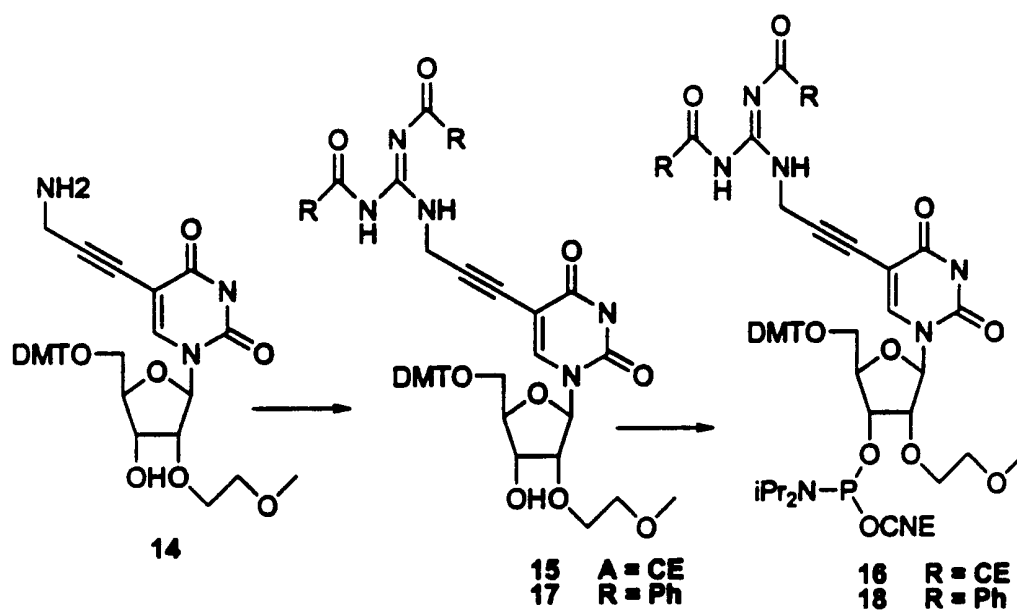
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Figure 8



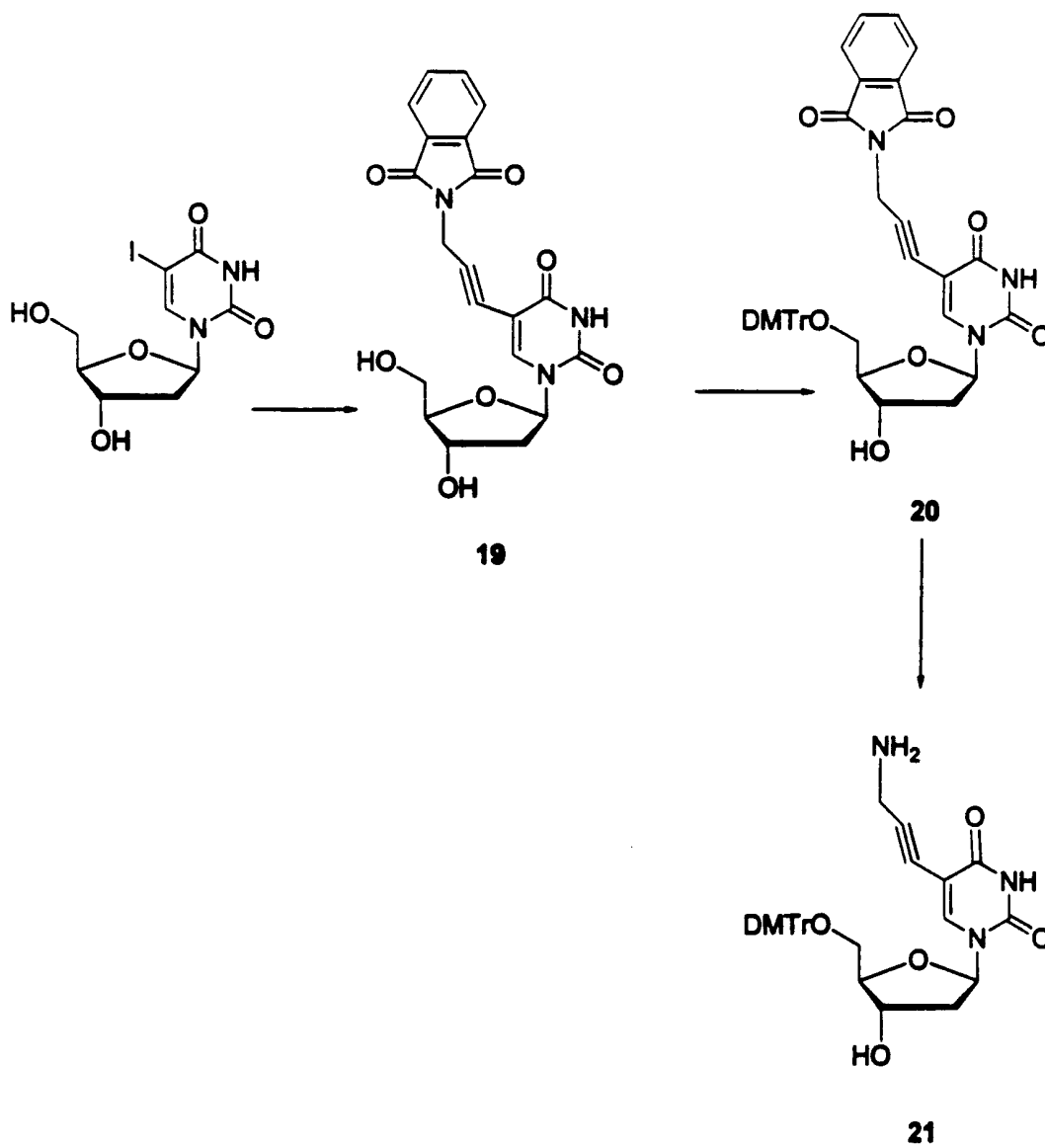
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Figure 9



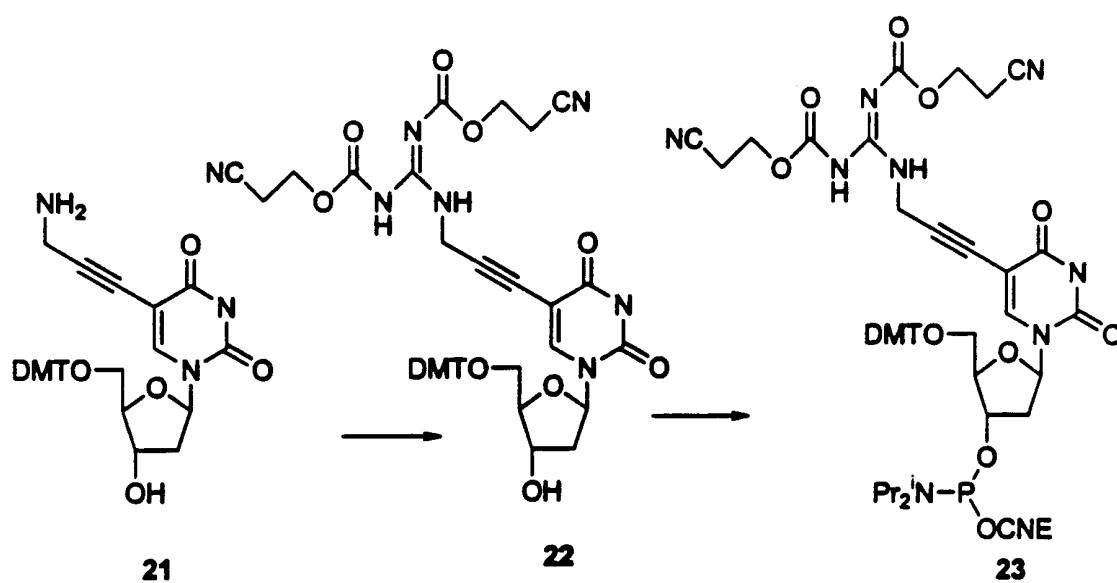
10/22

Figure 10



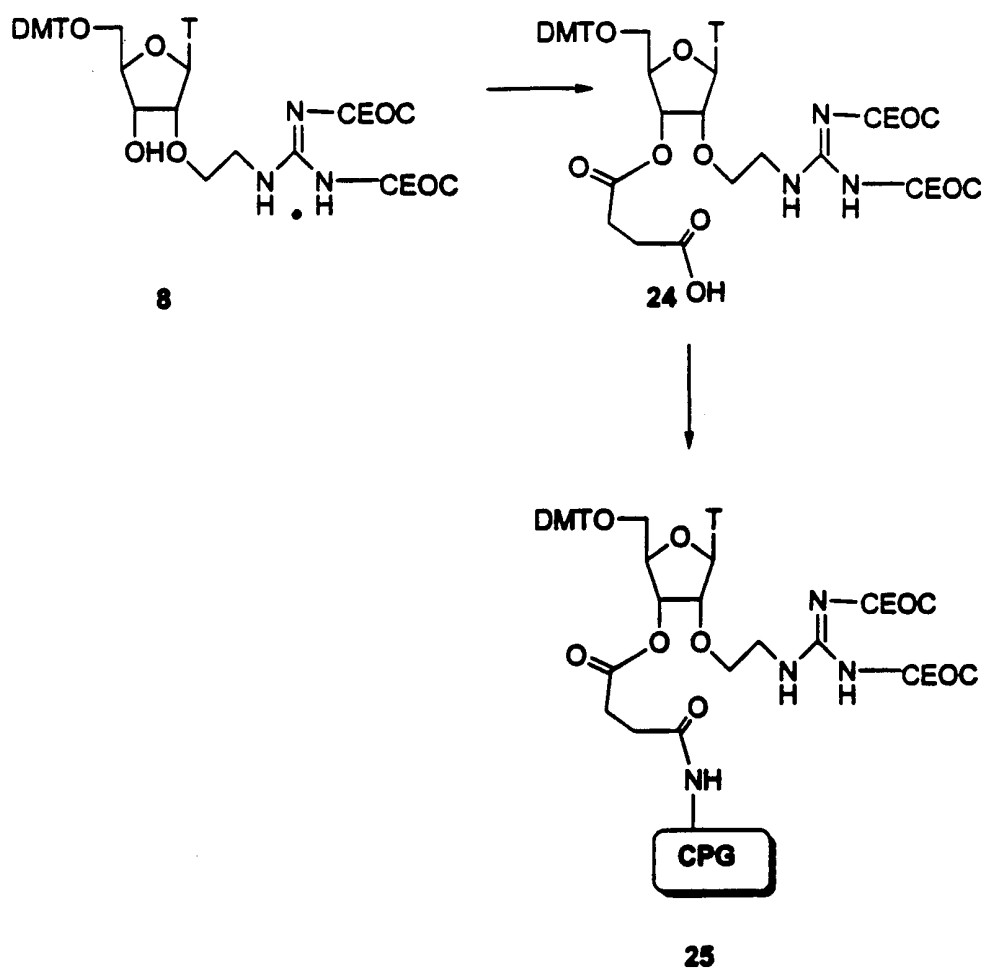
11/22

Figure 11



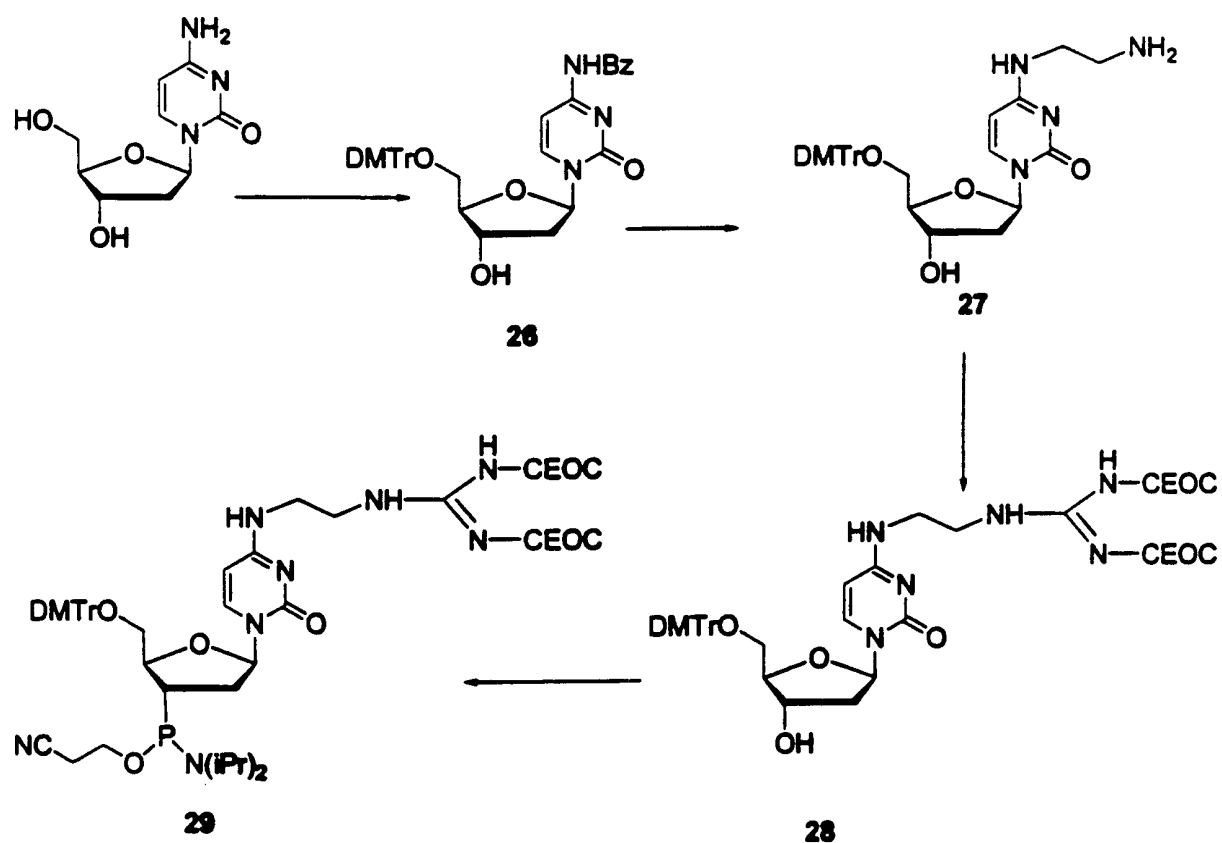
12/22

Figure 12



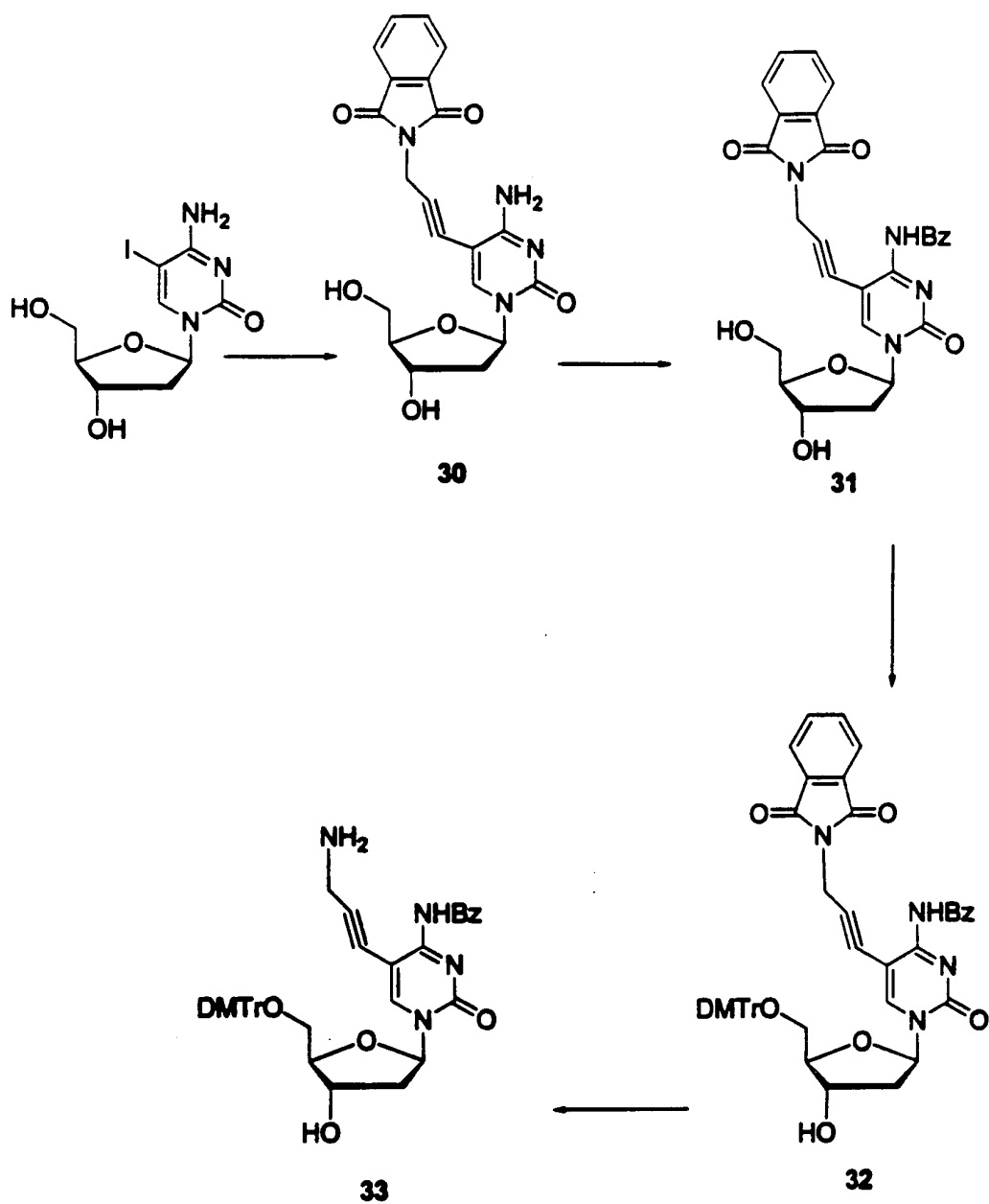
13/22

Figure 13



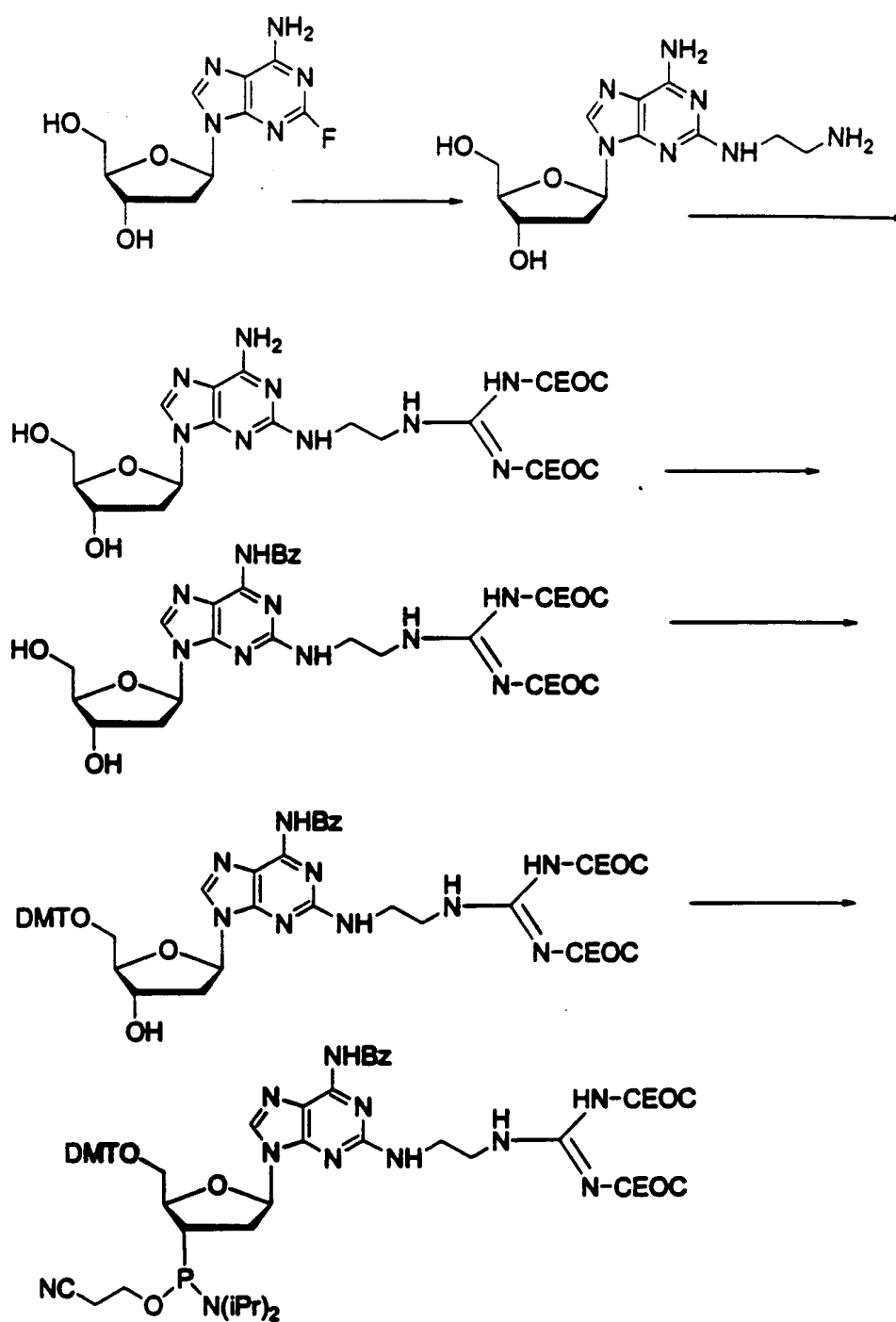
14/22

Figure 14



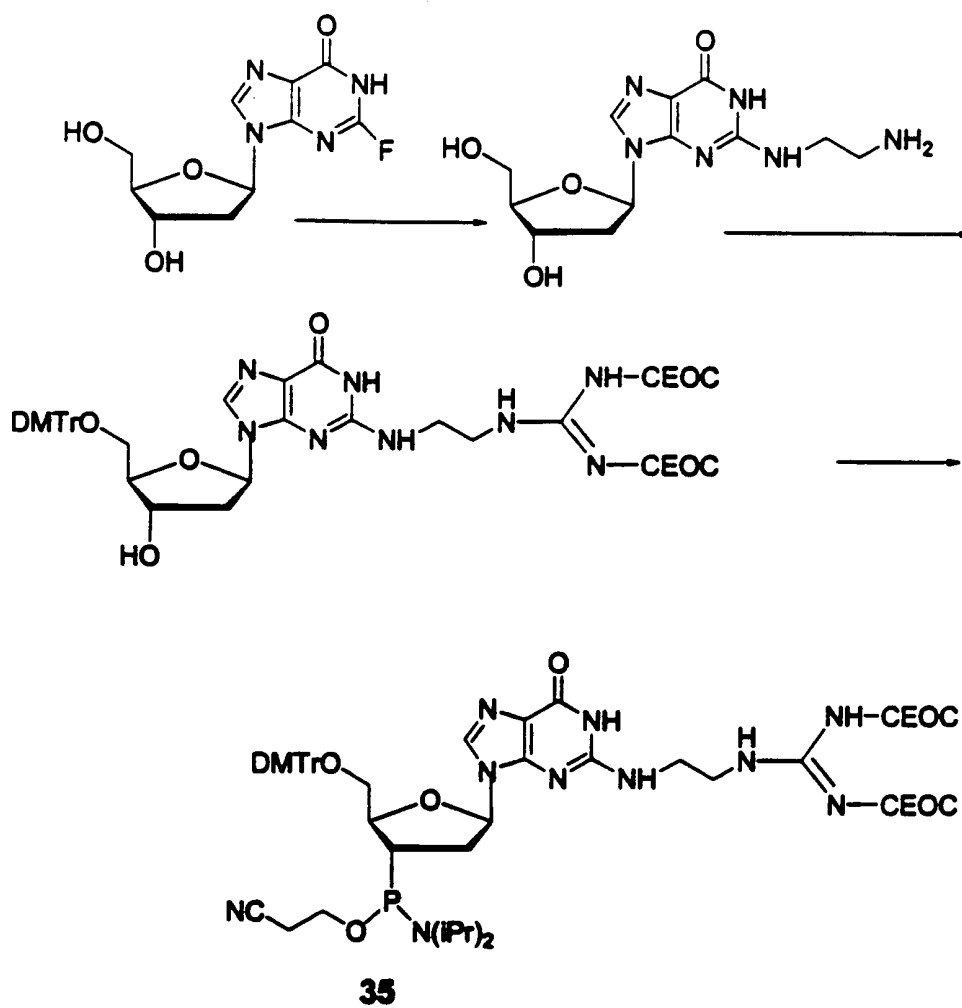
15/22

Figure 15

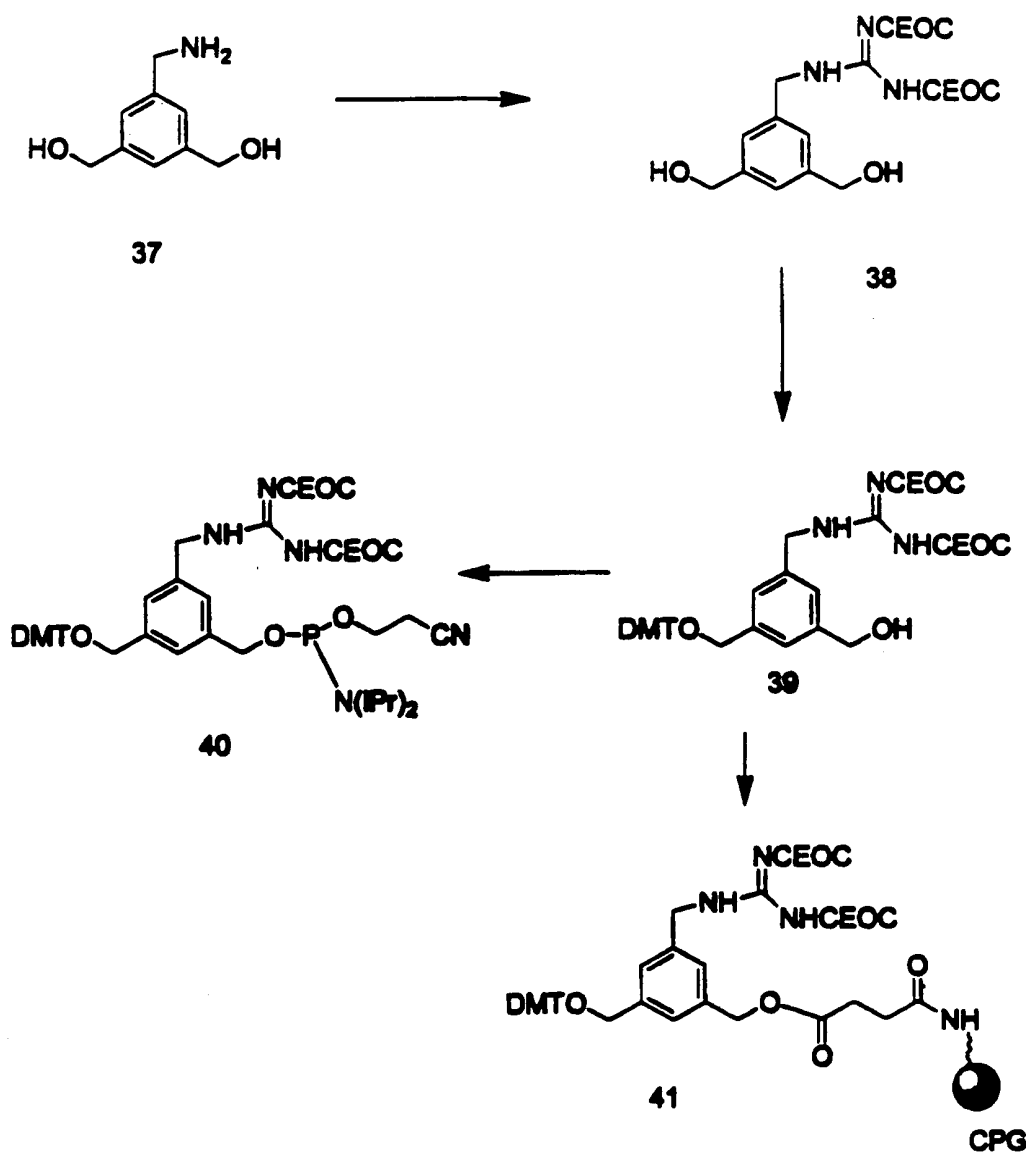


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Figure 16

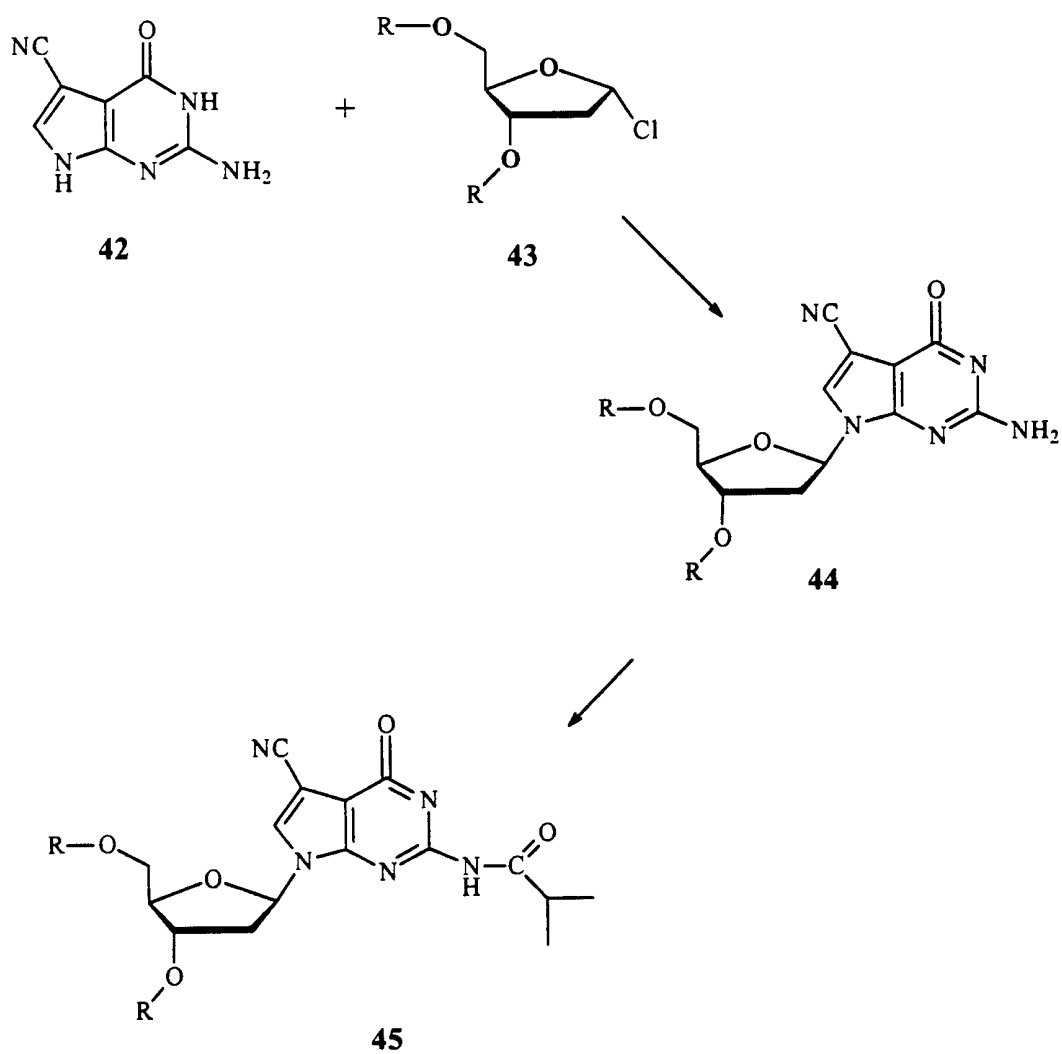


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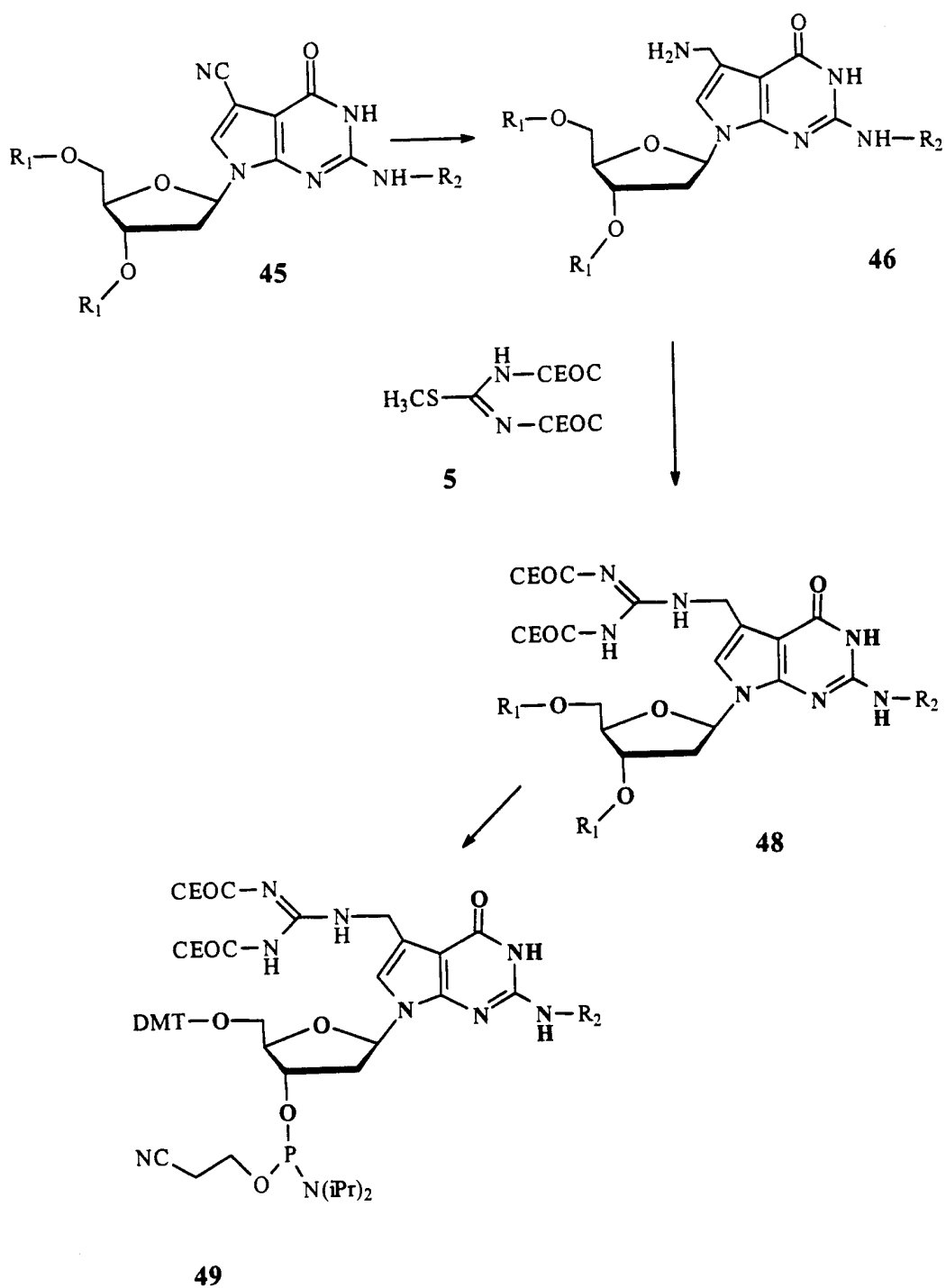
Figure 19



R= TBDPS

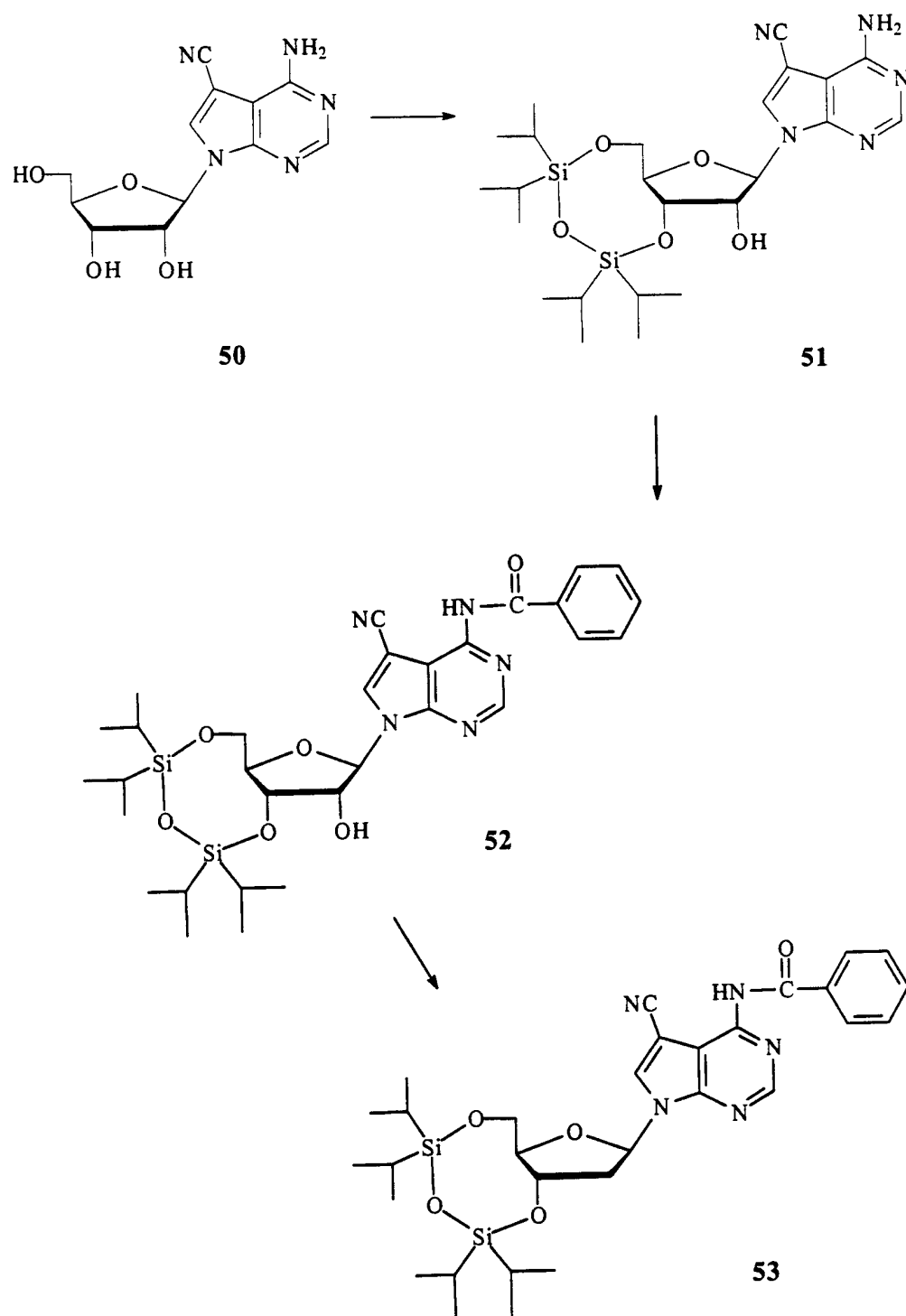
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Figure 20


$$\begin{aligned} \mathbf{R}_1 &= \text{TBDPS}, \\ \mathbf{R}_2 &= \text{CO-HC(CH}_3)_2 \end{aligned}$$

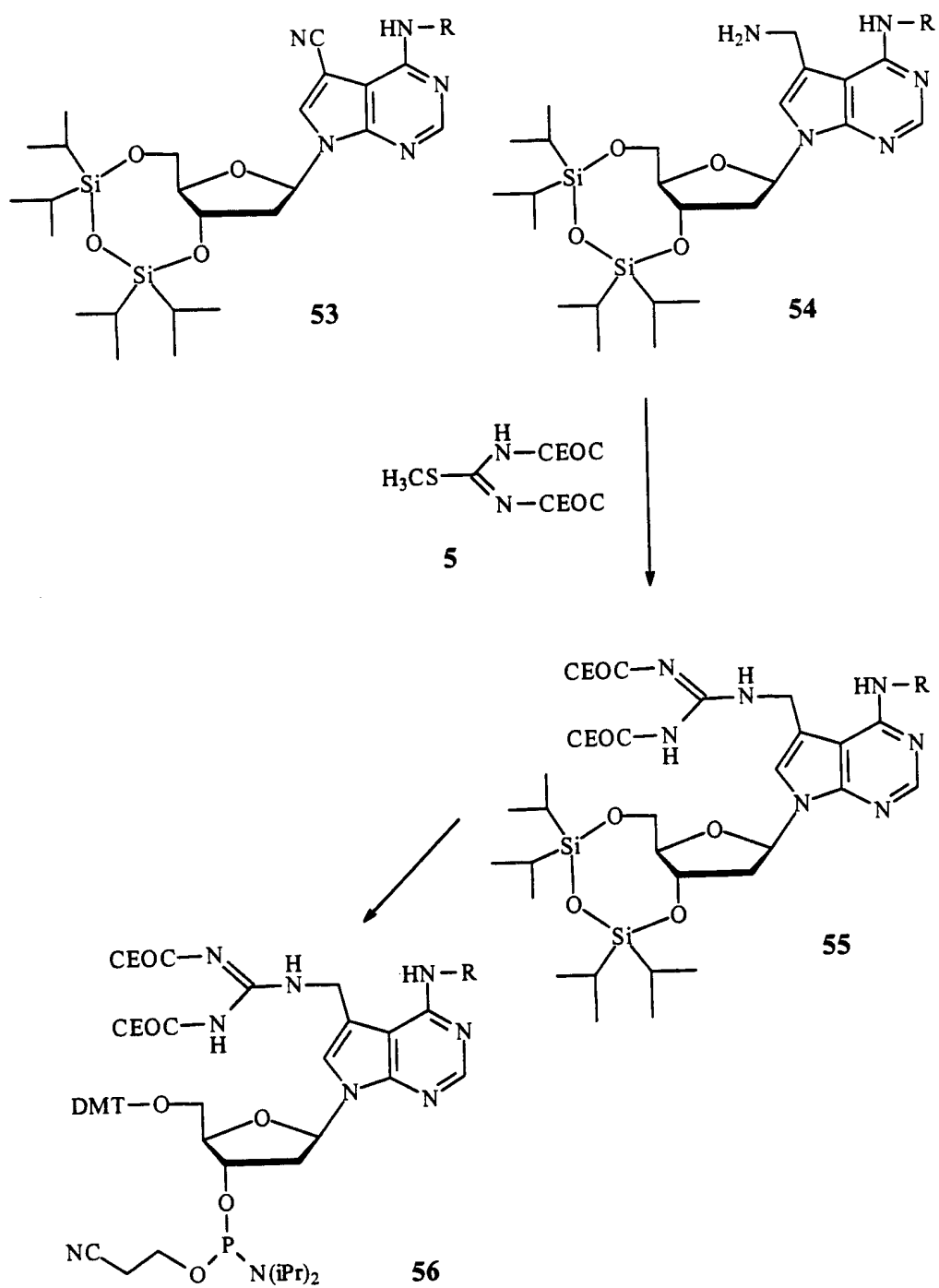
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Figure 21



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Figure 22



SEQUENCE LISTING

<110> Manoharan, Muthiah
Cook, Phillip Dan
Prakash, Thazha P.
Mohan, Venkatraman

<120> Guanidinium Functionalized Oligomers and
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