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(54) Title: OLIGONUCLEOTIDES COMPRISING A C5-MODIFIED PYRIMIDINE

(57) Abstract: One aspect of the present invention relates to a double-stranded oligonucleotide comprising at least one ligand. In certain embodiments, a ligand is bound to only one of the two oligonucleotide strands comprising the double-stranded oligonucleotide. In certain embodiments, both of the oligonucleotide strands of the double-stranded oligonucleotide independently comprise a bound ligand. In certain embodiments, the oligonucleotide strands comprise at least one modified sugar moiety. In certain embodiments, a phosphate linkage in one or both of the strands of the oligonucleotide has been replaced with a phosphorothioate or phosphorodithioate linkage. In a preferred embodiment, the ligand is cholesterol or 5β-cholanic acid. Another aspect of the present invention relates to a single-stranded oligonucleotide comprising at least one ligand. In certain embodiments, the oligonucleotide comprises at least one modified sugar moiety. In certain embodiments, a phosphate linkage of the oligonucleotide has been replaced with a phosphorothioate or phosphorodithioate linkage. In a preferred embodiment, the ligand is cholesterol or 5β-cholanic acid. The ligand improves the pharmacokinetic properties of the oligonucleotide.

Oligonucleotides Comprising a C5-Modified Pyrimidine

Related Applications

This application claims the benefit of priority to United States Provisional Patent Application serial number 60/566,710, filed April 30, 2004; and United States Provisional Patent Application serial number 60/620,276, filed October 20, 2004; the contents of both of them are incorporated by reference.

Background of the Invention

Oligonucleotide compounds have important therapeutic applications in medicine. Oligonucleotides can be used to silence genes that are responsible for a particular disease. Gene-silencing prevents formation of a protein by inhibiting translation. Importantly, gene-silencing agents are a promising alternative to traditional small, organic compounds that inhibit the function of the protein linked to the disease. siRNA, antisense RNA, and micro-RNA are oligonucleotides that prevent the formation of proteins by gene-silencing.

siRNA

RNA interference (RNAi) is an evolutionarily conserved gene-silencing mechanism, originally discovered in studies of the nematode Caenorhabditis elegans (Lee et al, Cell 75:843 (1993); Reinhart et al., Nature 403:901 (2000)). It is triggered by introducing dsRNA into cells expressing the appropriate molecular machinery, which then degrades the corresponding endogenous mRNA. The mechanism involves conversion of dsRNA into short RNAs that direct ribonucleases to homologous mRNA targets (summarized, Ruvkun, Science 2294:797 (2001)). This process is related to normal defense against viruses and the mobilization of transposons.

Double-stranded ribonucleic acids (dsRNAs) are naturally rare and have been found only in certain microorganisms, such as yeasts or viruses. Recent reports indicate that dsRNAs are involved in phenomena of regulation of expression, as well as in the initiation of the synthesis of interferon by cells (Declerq et al., Meth. Enzymol. 78:291 (1981); Wu-Li, Biol. Chem. 265:5470 (1990)). In addition, dsRNA has been reported to have anti-proliferative properties, which makes it possible also to envisage therapeutic applications (Aubel et al., Proc. Natl. Acad. Sci., USA 88:906 (1991)). For example, synthetic dsRNA has been shown to inhibit tumor growth in mice (Levy et al. Proc. Nat. Acad. Sci. USA, 62:357-361 (1969)), to be active in the treatment of leukemic mice (Zeleznick et al., Proc. Soc. Exp. Biol. Med. 130:126-128 (1969)); and to inhibit chemically-induced tumorigenesis in mouse skin (Gelboin et al., Science 167:205-207 (1970)).

Treatment with dsRNA has become an important method for analyzing gene functions in invertebrate organisms. For example, Dzitoveva et al. showed, that RNAi can be induced in adult fruit flies by injecting dsRNA into the abdomen of anesthetized Drosophila, and that this method can also target genes expressed in the central nervous system (Mol. Psychiatry 6(6):665-670 (2001)). Both transgenes and endogenous genes were successfully silenced in adult Drosophila by intra-abdominal injection of their respective dsRNA. Moreover, Elbashir et al., provided evidence that the direction of dsRNA processing determines whether sense or antisense target RNA can be cleaved by a small interfering RNA (siRNA)-protein complex (Genes Dev. 15(2): 188-200 (2001)).

Two recent reports reveal that RNAi provides a rapid method to test the function of genes in the nematode Caenorhabditis elegans; and most of the genes on C. elegans chromosome I and III have now been tested for RNAi phenotypes (Barstead, Curr. Opin. Chem. Biol. 5(1):63-66 (2001); Tavernarakis, Nat. Genet. 24(2):180-183 (2000); Zamore, Nat. Struct. Biol. 8(9):746-750 (2001).). When used as a rapid approach to obtain loss-of-function information, RNAi was used to analyze a random set of ovarian transcripts and has identified 81 genes with essential roles in C. elegans embryogenesis (Piano et al., Curr. Biol. 10(24):1619-1622 (2000). RNAi has also been used to disrupt the pupal hemocyte protein of Sarcophaga (Nishikawa et al., Eur. J. Biochem. 268(20):5295-5299 (2001)).

Like RNAi in invertebrate animals, post-transcriptional gene-silencing (PTGS) in plants is an RNA-degradation mechanism. In plants, this can occur at both the transcriptional and the post-transcriptional levels; however, in invertebrates only post-transcriptional RNAi has been reported to date (Bernstein et al., Nature 409(6818):295-296 (2001). Indeed, both involve double-stranded RNA (dsRNA), spread within the organism from a localized initiating area, to correlate with the accumulation of small interfering RNA (siRNA) and require putative RNA-dependent RNA polymerases, RNA helicases and proteins of unknown functions containing PAZ and Piwi domains.

Some differences are evident between RNAi and PTGS were reported by Vaucheret et al., J. Cell Sci. 114(Pt 17):3083-3091 (2001). First, PTGS in plants requires at least two genes--SGS3 (which encodes a protein of unknown function containing a coil-coiled domain) and MET1 (which encodes a DNA-methyltransferase)--that are absent in C. elegans, and thus are not required for RNAi. Second, all of the Arabidopsis mutants that exhibit impaired PTGS are hyper-susceptible to infection by the cucumovirus CMV, indicating that PTGS participates in a mechanism for plant resistance to viruses. RNAi-mediated oncogene silencing has also been reported to confer resistance to crown gall tumorigenesis (Escobar et al., Proc. Natl. Acad. Sci. USA, 98(23):13437-13442 (2001)).

RNAi is mediated by RNA-induced silencing complex (RISC), a sequence-specific, multicomponent nuclease that destroys messenger RNAs homologous to the silencing trigger. RISC is known to contain short RNAs (approximately 22 nucleotides) derived from the double-stranded RNA trigger, but the protein components of this activity remained unknown. Hammond et al. (Science 293(5532):1146-1150 (August 2001)) reported biochemical purification of the RNAi effector nuclease from cultured Drosophila cells, and protein microsequencing of a ribonucleoprotein complex of the active fraction showed that one constituent of this complex is a member of the Argonaute family of proteins, which are essential for gene silencing in Caenorhabditis elegans, Neurospora, and Arabidopsis. This observation suggests links between the genetic analysis of RNAi from diverse organisms and the biochemical model of RNAi that is emerging from Drosophila in vitro systems.

Svoboda et al. reported in Development 127(19):4147-4156 (2000) that RNAi provides a suitable and robust approach to study the function of dormant maternal mRNAs in mouse oocytes. Mos (originally known as c-mos) and tissue plasminogen activator mRNAs are dormant maternal mRNAs are recruited during oocyte maturation, and translation of Mos mRNA results in the activation of MAP kinase. The dsRNA directed towards Mos or TPA mRNAs in mouse oocytes specifically reduced the targeted mRNA in both a time- and concentration-dependent manner, and inhibited the appearance of MAP kinase activity. See also, Svoboda et al. Biochem. Biophys. Res. Commun. 287(5):1099-1104 (2001).

Despite the advances in interference RNA technology, the need exists for siRNA conjugates having improved pharmacologic properties. In particular, the oligonucleotide sequences have poor serum solubility, poor cellular distribution and uptake, and are rapidly excreted through the kidneys. It is known that oligonucleotides bearing the native phospodiester (P=O) backbone are susceptable to nuclease-mediated degradation. See L. L. Cummins et al. *Nucleic Acids Res*. **1995**, *23*, 2019. The stability of oligonucleotides has been increased by converting the P=O linkages to P=S linkages which are less susceptible to degradation by nucleases in vivo. Alternatively, the phosphate group can be converted to a phosphoramidate which is less prone to enzymatic degradation than the native phosphate. See Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544. Modifications to the sugar groups of the oligonucleotide can confer stability to enzymatic degradation. For example, oligonucleotides comprising ribonucleic acids are less prone to nucleolytic degradation if the 2'-OH group of the sugar is converted to a methoxyethoxy group. See M. Manoharan *ChemBioChem*. **2002**, *3*, 1257 and references cited therein.

siRNA compounds are promising agents for a variety of diagnostic and therapeutic purposes. siRNA compounds can be used to identify the function of a gene. In addition, siRNA compounds offer enormous potential as a new type of pharmaceutical agent which acts by silencing disease-causing genes. Research is currently underway to develop interference RNA therapeutic agents for the treatment of many diseases including central-nervous-system diseases, inflammatory diseases, metabolic disorders, oncology, infectious diseases, and ocular disease.

Some progress has been made on increasing the cellular uptake of single-stranded oligonucleotides, including increasing the membrane permeability via conjugates and cellular delivery of oligonucleotides. In U.S. patent 6,656,730, M. Manoharan describes compositions in which a ligand that binds serum, vascular, or cellular proteins may be attached via an optional linking moiety to one or more sites on an oligonucleotide. These sites include one or more of, but are not limited to, the 2'-position, 3'-position, 5'-position, the internucleotide linkage, and a nucleobase atom of any nucleotide residue.

Antisense RNA

Antisense methodology is the complementary hybridization of relatively short oligonucleotides to mRNA or DNA such that the normal, essential functions, such as protein synthesis, of these intracellular nucleic acids are disrupted. Hybridization is the sequence-specific hydrogen bonding via Watson-Crick base pairs of oligonucleotides to RNA or single-stranded DNA. Such base pairs are said to be complementary to one another.

The naturally-occurring events that provide the disruption of the nucleic acid function, discussed by Cohen (*Oligonucleotides: Antisense Inhibitors of Gene Expression*, CRC Press, Inc., 1989, Boca Raton, Fla.) are thought to be of two types. The first, hybridization arrest, describes the 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 et al. (1987) *Anti-Cancer Drug Design*, 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.

Another means by which antisense oligonucleotides disrupt nucleic acid function is by hybridization to a target mRNA, followed by enzymatic cleavage of the targeted RNA by intracellular RNase H. A 2'-deoxyribofuranosyl oligonucleotide or oligonucleotide analog 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 terminating event.

Considerable research is being directed to the application of oligonucleotides and oligonucleotide analogs as antisense agents for diagnostics, research applications and potential therapeutic purposes. One of the major hurdles that has only partially been overcome in vivo is efficient cellular uptake which is severely hampered by the rapid degradation and excretion of oligonucleotides. The generally accepted process of cellular uptake is by receptor-mediated endocytosis which is dependent on the temperature and concentration of the oligonucleotides in serum and extra vascular fluids.

Efforts aimed at improving the transmembrane delivery of nucleic acids and oligonucleotides have utilized protein carriers, antibody carriers, liposomal delivery systems, electroporation, direct injection, cell fusion, viral vectors, and calcium phosphate-mediated transformation. However, many of these techniques are limited by the types of cells in which transmembrane transport is enabled and by the conditions needed for achieving such transport. An alternative that is particularly attractive for transmembrane delivery of oligonucleotides is modification of the physicochemical properties of the oligonucleotide.

Micro-RNA

Micro-RNAs are a large group of small RNAs produced naturally in organisms, at least some of which regulate the expression of target genes. Micro-RNAs are formed from an approximately 70 nucleotide single-stranded hairpin precursor transcript by Dicer. V. Ambros et al. *Current Biology* **2003**, *13*, 807. In many instances, the micro-RNA is transcribed from a portion of the DNA sequence that previously had no known function. Micro-RNAs are not translated into proteins, rather they bind to specific messenger RNAs blocking translation. It is thought that micro-RNAs base-pair imprecisely with their targets to inhibit translation. Initially discovered members of the micro-RNA family are let-7 and lin-4. The let-7 gene encodes a small, highly conserved RNA species that regulates the expression of endogenous protein-coding genes during worm development. The active RNA species is transcribed initially as an ~70nt precursor, which is post-transcriptionally processed into a mature ~21nt form. Both let-7 and lin-4 are transcribed as hairpin RNA precursors which are processed to their mature forms by Dicer enzyme (Lagos-Quintana et al, 2001).

The need exists for oligonucleotide conjugates having improved pharmacologic properties. In particular, natural oligonucleotide sequences are known to be rapidly degraded and excreted in vivo. The ligand-conjugated oligonucleotide compounds of the invention are significantly more stable than natural oligonucleotides, allowing for increased concentrations of the oligonucleotide in the serum.

Summary of the Invention

One aspect of the present invention relates to a double-stranded oligonucleotide comprising at least one ligand. In certain embodiments, a ligand is bound to only one of the two oligonucleotide strands comprising the double-stranded oligonucleotide. In certain embodiments, both of the oligonucleotide strands of the double-stranded oligonucleotide independently comprise a bound ligand. In certain embodiments, the oligonucleotide strands comprise at least one modified sugar moiety. In certain embodiments, a phosphate linkage in one or both of the strands of the oligonucleotide has been replaced with a phosphorothioate or phosphorodithioate linkage. In a preferred embodiment, the ligand is cholesterol or 5β-cholanic acid.

Another aspect of the present invention relates to a single-stranded oligonucleotide comprising at least one ligand. In certain embodiments, the oligonucleotide comprises at least one modified sugar moiety. In certain embodiments, a phosphate linkage of the oligonucleotide has been replaced with a phosphorothioate or phosphorodithioate linkage. In a preferred embodiment, the ligand is cholesterol or 5β-cholanic acid. The ligand improves the pharmacokinetic properties of the oligonucleotide.

Brief Description of the Figures

Figure 1 depicts a procedure for solid-phase oligonucleotide synthesis.

Figure 2 depicts a general procedure for preparing nucleoside-ligand conjugates containing uridine analogues. Note: (i) See Examples (ii) TBDMS-Cl, $AgNO_3$, Py / THF or TBDMS-Cl, imidazole / Py (iii) Phosphitylation: For Y = Me (or Et): *N,N*-diisopropylamino, methyl(or ethyl)phosphonamidic chloride $\{[(CH_3)_2CH]_2N\text{-}P(Cl)\text{-}CH_3\}$, DIEA / dichloromethane; For Y = OCH_3: chloro *N,N*-diisopropyl methoxy phosphoramidite $\{[(CH_3)_2CH]_2N\text{-}P(Cl)\text{-}OCH_3\}$, DIEA / dichloromethane; For Y = OCH_2CH_2CN: *N,N*-diisopropylamino β-cyanoethylphosphonamidic chloride $\{[(CH_3)_2CH]_2N\text{-}P(Cl)\text{-}OCH_2CH_2CN\}$, DIEA / dichloromethane or 2-cyanoethyl-*N,N,N,N'*-tetraisopropylphosphane, tetrazole (or tetrazolediisopropylammonium salt) / acetonitrile; (iv) (a) succinic anhybride, DMAP / dichloroethane and (b) DTNP, DMAP, Ph_3P, aminoalkyl solid support.

Figure 3 depicts a general procedure for preparing nucleoside-ligand conjugates containing cytidine analogues. Note: (i) (a) TEA, triazole, $POCl_3$ / MeCN, -10 °C; (b) NH_3; (c) benzoic anhybride / Py (ii) TBDMS-Cl, $AgNO_3$, Py / THF or TBDMS-Cl, imidazole / Py (iii) Phosphitylation: For Y = Me(or Et): *N,N*-diisopropylamino, methyl(or ethyl)phosphonamidic chloride $\{[(CH_3)_2CH]_2N\text{-}P(Cl)\text{-}CH_3\}$, DIEA / Dichloromethane; For Y = OCH_3: chloro *N,N*-diisopropylmethoxyphosphoramidite $\{[(CH_3)_2CH]_2N\text{-}P(Cl)\text{-}OCH_3\}$, DIEA / Dichloromethane; For Y = OCH_2CH_2CN: *N,N*-diisopropylamino β-cyanoethylphosphonamidic chloride $\{[(CH_3)_2CH]_2N\text{-}P(Cl)\text{-}OCH_2CH_2CN\}$, DIEA / dichloromethane or 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphane, tetrazole (or tetrazolediisopropylammonium salt) / acetonitrile; (iv) (a) succinic anhybride, DMAP / dichloroethane and (b) DTNP, DMAP, Ph_3P, aminoalkyl solid support.

Figure 4 depicts a general procedure for preparing nucleoside-ligand conjugates wherein the ligand is a carbohydrate. Note: (i) Compound **A** or **B** or **C**, DCC, DMAP, N-hydroxysuccinimide, followed by addition of **1-5** and TEA (ii) TBDMS-Cl, $AgNO_3$, Py / THF or TBDMS-Cl, Imidazole / Py (iii) Phophitylation: For Y = Me(or Et): N,N-Diisopropylamino, methyl(or ethyl)phosphonamidic chloride {$[(CH_3)_2CH]_2N$-P(Cl)-CH_3}, DIEA / dichloromethane; For Y = OCH_3: chloro-N,N-diisopropylmethoxyphosphoramidite {$[(CH_3)_2CH]_2N$-P(Cl)-OCH_3}, DIEA / dichloromethane; For Y = OCH_2CH_2CN: N,N-diisopropylamino β-cyanoethylphosphonamidic chloride {$[(CH_3)_2CH]_2N$-P(Cl)-OCH_2CH_2CN}, DIEA / dichloromethane or 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphane, tetrazole (or tetrazolediisopropylammonium salt) / acetonitrile; (iv) (a) succinic anhydride, DMAP / dichloroethane and (b) DTNP, DMAP, Ph_3P, aminoalkyl solid support.

Figure 5 depicts a general procedure for preparing nucleoside-ligand conjugates wherein the ligand is a biotin analog. Note: (i) (a) Biotin NHS ester TEA; (b) TMSCl and then 4-tert-buytlbenzoyl chloride, DMAP/Py (ii) TBDMSCl, $AgNO_3$, Py / THF or TBDMS-Cl, imidazole / Py (iii) Phophitylation: For Y = Me(or Et): N,N-diisopropylamino, methyl(or ethyl)phosphonamidic chloride {$[(CH_3)_2CH]_2N$-P(Cl)-CH_3}, DIEA / dichloromethane; For Y = OCH_3: chloro N,N-diisopropyl methoxy phosphoramidite {$[(CH_3)_2CH]_2N$-P(Cl)-OCH_3}, DIEA / dichloromethane; For Y = OCH_2CH_2CN: N,N-diisopropylamino β-cyanoethylphosphonamidic chloride {$[(CH_3)_2CH]_2N$-P(Cl)-OCH_2CH_2CN}, DIEA / dichloromethane or 2-cyanoethyl-N,N, N',N'-tetraisopropylphosphane, tetrazole (or tetrazolediisopropylammonium salt) / acetonitrile; (iv) (a) succinic anhydride, DMAP / dichloroethane and (b) DTNP, DMAP, Ph_3P, aminoalkyl solid support

Figure 6 depicts a general procedure for preparing nucleoside-ligand conjugates. Note: (i) (a) compound **161**, DCC, DMAP, *N*-hydroxysuccinimide, followed by addition of **1-5** and TEA, or (b) compound **162**, *N,N*-disuccinimidyl carbonate, TEA followed by addition of **1-5** (ii) TBDMS-Cl, $AgNO_3$, Py / THF or TBDMS-Cl, imidazole / Py (iii) Phophitylation: For Y = Me(or Et): N,N-diisopropylamino, methyl(or ethyl)phosphonamidic chloride {$[(CH_3)_2CH]_2N$-P(Cl)-CH_3}, DIEA / dichloromethane; For

$Y = OCH_3$: chloro-N,N-diisopropyl methoxy phosphoramidite $\{[(CH_3)_2CH]_2N\text{-}P(Cl)\text{-}OCH_3\}$, DIEA / dichloromethane; For $Y = OCH_2CH_2CN$: N,N-diisopropylamino β-cyanoethylphosphonamidic chloride $\{[(CH_3)_2CH]_2N\text{-}P(Cl)\text{-}OCH_2CH_2CN\}$, DIEA / dichloromethane or 2-cyanoethyl-N,N,N',N'-tetraisopropylphosphane, tetrazole (or tetrazolediisopropylammonium salt) / acetonitrile; (iv) (a) succinic anhydride, DMAP / dichloroethane and (b) DTNP, DMAP, Ph_3P, aminoalkyl solid support.

Detailed Description of the Invention

The present invention provides ligand-conjugated oliognucleotide compounds having improved pharmacokinetic properties and methods for their preparation. The oligonucleotides of the invention include single-stranded and double-stranded oligonucleotides. Conjugated oligonucleotide agents can modify gene expression, either inhibiting or up-regulating, by targeting and binding to a nucleic acid, *e.g.*, a pre-mRNA, an mRNA, a microRNA (miRNA), a mi-RNA precursor (pre-miRNA), or DNA, or to a protein. Oligonucleotide agents of the invention include modified siRNA, miRNA, antisense RNA, decoy RNA, DNA, and aptamers. Such compounds are prepared by covalently attaching a ligand to an oliognucleotide. The ligand improves the pharmacologic properties of the oliognucleotide because it binds reversibly to one or more serum, vascular or cellular proteins. This reversible binding is expected to decrease urinary excretion, increase serum half-life and greatly increase the distribution of oligomeric compounds thus conjugated.

The therapeutic effect of an oliognucleotide is realized when it interacts with a specific cellular nucleic acid and effectively negates its function. A preferred target is DNA or mRNA encoding a protein that is responsible for a disease state. The overall effect of such interference with mRNA function is modulation of the expression of a protein, wherein "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of the protein. In the context of the present invention, inhibition is the preferred form of modulation of gene expression. Nevertheless, the ultimate goal is to regulate the amount of such a protein.

To reach a target nucleic acid after administration, an oliognucleotide should be able to overcome inherent factors such as rapid degradation in serum, short half-life in serum and rapid filtration by the kidneys with subsequent excretion in the urine. Oliognucleotides that overcome these inherent factors have increased serum half-life, distribution, cellular uptake and hence improved efficacy.

These enhanced pharmacokinetic parameters have been shown for selected drug molecules that bind plasma proteins (Olson and Christ, *Annual Reports in Medicinal Chemistry*, 1996, 31:327). Two proteins that have been studied more than most are human serum albumin (HSA) and α-1-acid glycoprotein. HSA binds a variety of endogenous and exogenous ligands with association constants typically in the range of 10^4 to 10^6 M^{-1}. Association constants for ligands with α-1-acid glycoprotein are similar to those for HSA.

In a preferred embodiment of the invention the protein targeted by the ligand-conjugated oliognucleotide is a serum protein. It is preferred that the serum protein targeted by a conjugated oligomeric compound is an immunoglobulin (an antibody). Preferred immunoglobulins are immunoglobulin G and immunoglobulin M. Immunoglobulins are known to appear in blood serum and tissues of vertebrate animals.

In another embodiment of the invention the serum protein targeted by the ligand-conjugated oliognucleotide is a lipoprotein. Lipoproteins are blood proteins having molecular weights generally above 20,000 that carry lipids and are recognized by specific cell-surface receptors. The association with lipoproteins in the serum will initially increase pharmacokinetic parameters such as half-life and distribution. A secondary consideration is the ability of lipoproteins to enhance cellular uptake via receptor-mediated endocytosis.

In yet another embodiment the serum protein targeted by the ligand-conjugated oliognucleotide compound is α-2-macroglobulin. In yet a further embodiment the serum protein targeted by a ligand conjugated oligomeric compound is α-1-glycoprotein.

At least for therapeutic purposes, oliognucleotide compounds should have a degree of stability in serum to allow distribution and cellular uptake. The prolonged maintenance of therapeutic levels of antisense agents in serum will have a significant effect on the distribution and cellular uptake and unlike conjugate groups that target specific cellular receptors, the increased serum stability will effect all cells.

In the context of this invention, siRNA comprises double-stranded oligonucleotides, wherein the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid or deoxyribonucleic acid. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent intersugar (backbone) linkages as well as modified 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, enhanced cellular uptake, enhanced binding to target and increased stability in the presence of nucleases. The oligonucleotides of the present invention preferably comprise from about 5 to about 50 nucleosides. It is more preferred that such oligonucleotides comprise from about 8 to about 30 nucleosides, with 15 to 25 nucleosides being particularly preferred.

An oligonucleotide is a polymer of repeating units generically known as nucleotides or nucleosides. An unmodified (naturally occurring) nucleotide has three components: (1) a nitrogenous base linked by one of its nitrogen atoms to (2) a 5-carbon cyclic sugar and (3) a phosphate, esterified to carbon 5 of the sugar. When incorporated into an oligonucleotide chain, the phosphate of a first nucleotide is also esterified to carbon 3 of the sugar of a second, adjacent nucleotide. The "backbone" of an unmodified oligonucleotide consists of (2) and (3), that is, sugars linked together by phosphodiester linkages between the CS (5') position of the sugar of a first nucleotide and the C3 (3') position of a second, adjacent nucleotide. A "nucleoside" is the combination of (1) a nucleobase and (2) a sugar in the absence of a phosphate moiety (Kornberg, DNA Replication, W. H. Freeman & Co., San Francisco, 1980, pages 4-7). The backbone of an oligonucleotide positions a series of bases in a specific order; the written representation of this series of bases, which is conventionally written in 5' to 3' order, is known as a nucleotide sequence.

Oligonucleotides may comprise nucleoside or nucleotide sequences sufficient in identity and number to effect specific hybridization with a particular nucleic acid. Such oligonucleotides which specifically hybridize to a portion of the sense strand of a gene are commonly described as "antisense." In the context of the invention, "hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleosides or nucleotides. For example, adenine and thymine are complementary nucleobases which pair through the formation of hydrogen bonds. "Complementary," as used herein, refers to the capacity for precise pairing between two nucleotides. For example, if a nucleotide at a certain position of an oligonucleotide is capable of hydrogen bonding with a nucleotide at the same position of a DNA or RNA molecule, then the oligonucleotide and the DNA or RNA are considered to be complementary to each other at that position. The oligonucleotide and the DNA or RNA are complementary to each other when a sufficient number of corresponding positions in each molecule are occupied by nucleotides which can hydrogen bond with each other. Thus, "specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity or precise pairing such that stable and specific binding occurs between the oligonucleotide and the DNA or RNA target. It is understood in the art that an oligonucleotide need not be 100% complementary to its target DNA sequence to be specifically hybridizable. An oligonucleotide is specifically hybridizable when binding of the oligonucleotide to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a decrease or loss of function, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment, or in the case of in vitro assays, under conditions in which the assays are performed.

The ligand-conjugated oligonucleotides of the invention can be prepared by attaching the ligand to the oligonucleotide through a monomer, *e.g.*, a chemically modified monomer that is integrated into the oligonucleotide agent. In a preferred embodiment, the coupling is by a tether or a linker (or both) as described below, and the complex has the formula represented by:

$$\text{Ligand- [linker]}_{\text{optional}}\text{ -[tether]}_{\text{optional}} - \text{oligonucleotide agent}$$

While, in most cases, embodiments are described with respect to an oligonucleotide agent including a number of nucleotides, the invention also includes monomeric subunits having the structure:

$$\text{Ligand- [linker]}_{\text{optional}}\text{ -[tether]}_{\text{optional}} - \text{monomer}$$

Methods of making and incorporating the monomers into the oligonucleotide agents and methods of using those agents are included in the invention. In preferred embodiments, the sugar, *e.g.*, the ribose sugar of one or more of the nucleotides, (*e.g.*, ribonucleotide, deoxynucleotide, or modified nucleotide) subunits of an oligonucleotide agent can be replaced with another moiety, *e.g.*, a non-carbohydrate carrier. In certain instances, the non-carbohydrate is cyclic. A nucleotide subunit in which the sugar of the subunit has been so replaced is referred to herein as a sugar replacement modification subunit (SRMS). This is often referred to as a tether. A cyclic carrier may be a carbocyclic ring system, *i.e.*, all ring atoms are carbon atoms or a heterocyclic ring system, *i.e.*, one or more ring atoms may be a heteroatom, *e.g.*, nitrogen, oxygen, or sulfur. The cyclic carrier may be a monocyclic ring system, or may contain two or more rings, *e.g.* fused rings. The cyclic carrier may be a fully saturated ring system, or it may contain one or more double bonds.

The oligonucleotide agents of the invention include nucleic acid targeting (NAT) oligonucleotide agents and protein-targeting (PT) oligonucleotide agents. NAT and PT oligonucleotide agents refer to single-stranded oligomers or polymers of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or combined (chimeric) modifications of DNA and RNA. This term includes oligonucleotides composed of naturally occurring nucleobases, sugars, and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions that function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as enhanced cellular uptake, enhanced affinity for nucleic acid target, and/or increased stability in the presence of nucleases. NATs designed to bind to specific RNA or DNA targets have substantial complementarity, *e.g.*, at least 70, 80, 90, or 100% complementary, with at least 10, 20, or 30 or more bases of a target nucleic acid, and include antisense RNAs, miRNAs, and other non-duplex structures which can modulate expression. Other NAT oligonucleotide agents include external guide sequence (EGS) oligonucleotides (oligozymes), DNAzymes, and ribozymes. These NATs may or may not bind via Watson-Crick complementarity to their targets. PT oligonucleotide agents bind to protein targets, preferably by virtue of three-dimensional interactions, and modulate protein activity. They include decoy RNAs, aptamers, and the like.

The single-stranded oligonucleotide compounds of the invention preferably comprise from about 8 to about 50 nucleobases (*i.e.* from about 8 to about 50 linked nucleosides). NAT oligonucleotide agents are preferably about 15 nucleotides long, or more preferably about 30 nucleotides long. PT oligonucleotide agents are preferably about 18 nucleotides long, or more preferably about 23 nucleotides long. Particularly preferred compounds are miRNAs and antisense oligonucleotides, even more preferably those comprising from about 12 to about 30 nucleobases.

While not wishing to be bound by theory, an oligonucleotide agent may act by one or more of a number of mechanisms, including a cleavage-dependent or cleavage-independent mechanism. A cleavage-based mechanism can be RNAse H dependent and/or can include RISC complex function. Cleavage-independent mechanisms include occupancy-based translational arrest, such as is mediated by miRNAs, or binding of the oligonucleotide agent to a protein, as do aptamers. Oligonucleotide agents may also be used to alter the expression of genes by changing the choice of the splice site in a pre-mRNA. Inhibition of splicing can also result in degradation of the improperly processed message, thus down-regulating gene expression. Kole and colleagues (Sierakowska, et al. *Proc. Natl. Acad. Sci. USA*, 1996, **93**:12840-12844) showed that 2′-O-Me phosphorothioate oligonucleotides could correct aberrant beta-globin splicing in a cellular system. Fully modified 2′-methoxyethyl oligonucleotides and peptide nucleic acids (PNAs) were able to redirect splicing of IL-5 receptor-α pre-mRNA (Karras et al., *Mol. Pharmacol.* 2000, **58**:380-387; Karras, et al., *Biochemistry* 2001, **40**:7853-7859).

MicroRNAs

The oligonucleotide agents include microRNAs (miRNAs). MicroRNAs are small noncoding RNA molecules that are capable of causing post-transcriptional silencing of specific genes in cells such as by the inhibition of translation or through degradation of the targeted mRNA. A miRNA can be completely complementary or can have a region of noncomplementarity with a target nucleic acid, consequently resulting in a "bulge" at the region of non-complementarity. The region of non-complementarity (the bulge) can be flanked by regions of sufficient complementarity, preferably complete complementarity to allow duplex formation. Preferably, the regions of complementarity are at least 8 to 10 nucleotides long (*e.g.*, 8, 9, or 10 nucleotides long). A miRNA can inhibit gene expression by repressing translation, such as when the microRNA is not completely complementary to the target nucleic acid, or by causing target RNA degradation, which is believed to occur only when the miRNA binds its target with perfect complementarity. The invention also includes double-stranded precursors of miRNAs that may or may not form a bulge when bound to their targets.

A miRNA or pre-miRNA can be about 18-100 nucleotides in length, and more preferably from about 18-80 nucleotides in length. Mature miRNAs can have a length of about 19-30 nucleotides, preferably about 21-25 nucleotides, particularly 21, 22, 23, 24, or 25 nucleotides. MicroRNA precursors can have a length of about 70-100 nucleotides and have a hairpin conformation. MicroRNAs can be generated *in vivo* from pre-miRNAs by enzymes called Dicer and Drosha that specifically process long pre-miRNA into functional miRNA. The microRNAs or precursor miRNAs featured in the invention can be synthesized in vivo by a cell-based system or can be chemically synthesized. MicroRNAs can be synthesized to include a modification that imparts a desired characteristic. For example, the modification can improve stability, hybridization thermodynamics with a target nucleic acid, targeting to a particular tissue or cell-type, or cell permeability, *e.g.*, by an endocytosis-dependent or -independent mechanism. Modifications can also increase sequence specificity, and consequently decrease off-site targeting. Methods of synthesis and chemical modifications are described in greater detail below.

In particular, an miRNA or a pre-miRNA featured in the invention can have a chemical modification on a nucleotide in an internal (*i.e.*, non-terminal) region having noncomplementarity with the target nucleic acid. For example, a modified nucleotide can be incorporated into the region of a miRNA that forms a bulge. The modification can include a ligand attached to the miRNA, *e.g.*, by a linker. The modification can, for example, improve pharmacokinetics or stability of a therapeutic miRNA, or improve hybridization properties (*e.g.*, hybridization thermodynamics) of the miRNA to a target nucleic acid. In some embodiments, it is preferred that the orientation of a modification or ligand incorporated into or tethered to the bulge region of a miRNA is oriented to occupy the space in the bulge region. This orientation facilitates the improved hybridization properties or an otherwise desired characteristic of the miRNA. For example, the modification can include a modified base or sugar on the nucleic acid strand or a ligand that functions as an intercalator. These are preferably located in the bulge. The intercalator can be an aromatic, *e.g.*, a polycyclic aromatic or heterocyclic aromatic compound. A polycyclic intercalator can have stacking capabilities, and can include systems with 2, 3, or 4 fused rings. Universal bases can also be incorporated into the miRNAs.

In one embodiment, an miRNA or a pre-miRNA can include an aminoglycoside ligand, which can cause the miRNA to have improved hybridization properties or improved sequence specificity. Exemplary aminoglycosides include glycosylated polylysine; galactosylated polylysine; neomycin B; tobramycin; kanamycin A; and acridine conjugates of aminoglycosides, such as Neo-N-acridine, Neo-S-acridine, Neo-C-acridine, Tobra-N-acridine, and KanaA-N-acridine. Use of an acridine analog can increase sequence specificity. For example, neomycin B has a high affinity for RNA as compared to DNA, but low sequence-specificity. Neo-S-acridine, an acridine analog, has an increased affinity for the HIV Rev-response element (RRE). In some embodiments, the guanidine analog (the guanidinoglycoside) of an aminoglycoside ligand is tethered to an oligonucleotide agent. In a guanidinoglycoside, the amine group on the amino acid is exchanged for a guanidine group. Attachment of a guanidine analog can enhance cell permeability of an oligonucleotide agent.

In one embodiment, the ligand can include a cleaving group that contributes to target gene inhibition by cleavage of the target nucleic acid. Preferably, the cleaving group is tethered to the miRNA in a manner such that it is positioned in the bulge region, where it can access and cleave the target RNA. The cleaving group can be, for example, a bleomycin (*e.g.*, bleomycin-A_5, bleomycin-A_2, or bleomycin-B_2), pyrene, phenanthroline (*e.g.*, *O*-phenanthroline), a polyamine, a tripeptide (*e.g.*, lys-tyr-lys tripeptide), or metal ion chelating group. The metal ion chelating group can include, *e.g.*, an Lu(III) or EU(III) macrocyclic complex, a Zn(II) 2,9-dimethylphenanthroline derivative, a Cu(II) terpyridine, or acridine, which can promote the selective cleavage of target RNA at the site of the bulge by free metal ions, such as Lu(III). In some embodiments, a peptide ligand can be tethered to a miRNA or a pre-miRNA to promote cleavage of the target RNA, such as at the bulge region. For example, 1,8-dimethyl-1,3,6,8,10,13-hexaazacyclotetradecane (cyclam) can be conjugated to a peptide (*e.g.*, by an amino acid derivative) to promote target RNA cleavage. The methods and compositions featured in the invention include miRNAs that inhibit target gene expression by a cleavage or non-cleavage dependent mechanism.

A miRNA or a pre-miRNA can be designed and synthesized to include a region of noncomplementarity (*e.g.*, a region that is 3, 4, 5, or 6 nucleotides long) flanked by regions of sufficient complementarity to form a duplex (*e.g.*, regions that are 7, 8, 9, 10, or 11 nucleotides long). For increased nuclease resistance and/or binding affinity to the target, the miRNA sequences can include 2'-O-methyl, 2'-fluorine, 2'-O-methoxyethyl, 2'-O-aminopropyl, 2'-amino, and/or phosphorothioate linkages. The inclusion of furanose sugars in the oligonucleotide backbone can also decrease endonucleolytic cleavage. An miRNA or a pre-miRNA can be further modified by including a 3'-cationic group, or by inverting the nucleoside at the 3'-terminus with a 3'-3' linkage. In another alternative, the 3'-terminus can be blocked with an aminoalkyl group, *e.g.*, a 3'-C5-aminoalkyl dT. Other 3'-conjugates can inhibit 3'-5' exonucleolytic cleavage. While not being bound by theory, a 3'-conjugate, such as naproxen or ibuprofen, may inhibit exonucleolytic cleavage by sterically blocking the exonuclease from binding to the 3'-end of oligonucleotide. Even small alkyl chains, aryl groups, or heterocyclic conjugates or modified sugars (D-ribose, deoxyribose, glucose etc.) can block 3'-5'-exonucleases.

In one embodiment, a miRNA or a pre-miRNA includes a modification that improves targeting, *e.g.* a targeting modification described above. Examples of modifications that target miRNA molecules to particular cell types include carbohydrate sugars such as galactose, N-acetylgalactosamine, mannose; vitamins such as folates; other ligands such as RGDs and RGD mimics; and small molecules including naproxen, ibuprofen or other known protein-binding molecules.

A miRNA or a pre-miRNA can be constructed using chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. For example, a miRNA or a pre-miRNA can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the miRNA or a pre-miRNA and target nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Other appropriate nucleic acid modifications are described herein. Alternatively, the miRNA or pre-miRNA nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation, *i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest.

Antisense Nucleic Acid Sequences

The single-stranded oligonucleotide agents featured in the invention include antisense nucleic acids. An "antisense" nucleic acid includes a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a gene expression product, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an RNA sequence, *e.g.*, a pre-mRNA, mRNA, miRNA, or pre-miRNA. Accordingly, an antisense nucleic acid can form hydrogen bonds with a sense nucleic acid target.

Given a coding strand sequence such as the sequence of a sense strand of a cDNA molecule, antisense nucleic acids can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to a portion of the coding or noncoding region of an RNA, *e.g.*, a pre-mRNA or mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of a pre-mRNA or mRNA, *e.g.*, the 5' UTR. An antisense oligonucleotide can be about 10 to 25 nucleotides in length (*e.g.*, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, or 24 nucleotides in length). An antisense oligonucleotide can also be complementary to a miRNA or pre-miRNA.

An antisense nucleic acid can be constructed using chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and target nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Other appropriate nucleic acid modifications are described herein. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation, *i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest.

An antisense agent can include ribonucleotides only, deoxyribonucleotides only (*e.g.*, oligodeoxynucleotides), or both deoxyribonucleotides and ribonucleotides. For example, an antisense agent consisting only of ribonucleotides can hybridize to a complementary RNA, and prevent access of the translation machinery to the target RNA transcript, thereby preventing protein synthesis. An antisense molecule including only deoxyribonucleotides, or deoxyribonucleotides and ribonucleotides, *e.g.*, DNA sequence flanked by RNA sequence at the 5' and 3' ends of the antisense agent, can hybridize to a complementary RNA, and the RNA target can be subsequently cleaved by an enzyme such as RNAse H. Degradation of the target RNA prevents translation. The flanking RNA sequences can include 2'-O-methylated nucleotides, and phosphorothioate linkages, and the internal DNA sequence can include phosphorothioate internucleotide linkages. The internal DNA sequence is preferably at least five nucleotides in length when targeting by RNAse H activity is desired.

For increased nuclease resistance, an antisense agent can be further modified by inverting the nucleoside at the 3'-terminus with a 3'-3' linkage. In another alternative, the 3'-terminus can be blocked with an aminoalkyl group. In certain instances, the antisense oligonucleotide agent includes a modification that improves targeting, *e.g.* a targeting modification.

Decoy nucleic acids

An oligonucleotide agent featured in the invention can be a decoy nucleic acid such as decoy RNA. A decoy nucleic acid resembles a natural nucleic acid, but is modified to inhibit or interrupt the activity of the natural nucleic acid. For example, a decoy RNA can mimic the natural binding domain for a ligand, and compete with natural binding target for the binding of a specific ligand. It has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently bind HIV tat protein, thereby preventing it from binding to TAR sequences encoded in the HIV RNA. In one embodiment, a decoy RNA includes a modification that improves targeting. The chemical modifications described above for miRNAs and antisense RNAs, and described elsewhere herein, are also appropriate for use in decoy nucleic acids.

Aptamers

Oligonucleotide agents of the invention also include aptamers. An aptamer binds to a non-nucleic acid ligand, such as a small organic molecule or protein, *e.g.*, a transcription or translation factor, and subsequently modifies its activity. An aptamer can fold into a specific structure that directs the recognition of the targeted binding site on the non-nucleic acid ligand. An aptamer can contain any of the modifications described herein. In certain instances, the aptamer includes a modification that improves targeting, *e.g.*, a targeting modification. The chemical modifications described above for miRNAs and antisense RNAs, and described elsewhere herein, are also appropriate for use in decoy nucleic acids.

Additional Features of the Oligonucleotides of the Invention

An oligonucleotide agent that is NAT ("nucleic acid targeting") includes a region of sufficient complementarity to the target gene, and is of sufficient length in terms of nucleotides, such that the oligonucleotide agent forms a duplex with the target nucleic acid. The oligonucleotide agent can modulate the function of the targeted molecule. For example, when the targeted molecule is an mRNA or pre-mRNA, the NAT can inhibit gene expression; when the target is an miRNA, the NAT will inhibit the miRNA function and will thus up-regulate expression of the mRNAs targeted by the particular miRNA. Alternatively, when the target is a region of a pre-mRNA that affects splicing, the NAT can alter the choice of splice site and thus the mRNA sequence; when the NAT functions as an miRNA, expression of the targeted mRNA is inhibited. For ease of exposition the term nucleotide or ribonucleotide is sometimes used herein in reference to one or more monomeric subunits of an oligonucleotide agent. It will be understood that the term "ribonucleotide" or "nucleotide" can, in the case of a modified RNA or nucleotide surrogate, also refer to a modified nucleotide, or surrogate replacement moiety at one or more positions.

A NAT oligonucleotide agent is, or includes, a region that is at least partially, and in some embodiments fully, complementary to the target RNA. It is not necessary that there be perfect complementarity between the oligonucleotide agent and the target, but the correspondence must be sufficient to enable the oligonucleotide agent, or a cleavage product thereof, to modulate (*e.g.*, inhibit) target gene expression.

The oligonucleotide agent will preferably have one or more of the following properties: (1) it will have a 5' modification that includes one or more phosphate groups or one or more analogs of a phosphate group; (2) it will, despite modifications even to a very large number of bases, specifically base pair and form a duplex structure with a homologous target RNA of sufficient thermodynamic stability to allow modulation of the activity of the targeted RNA; and (3) it will, despite modifications even to a very large number, or all of the nucleosides, still have "RNA-like" properties, *i.e.*, it will possess the overall structural, chemical and physical properties of an RNA molecule, even though not exclusively, or even partly, of ribonucleotide-based content. For example, all of the nucleotide sugars can contain a 2'-fluoro group in place of 2'-hydroxyl group. This deoxyribonucleotide-containing agent can still be expected to exhibit RNA-like properties. While not wishing to be bound by theory, the electronegative fluorine prefers an axial orientation when attached to the C2'-position of ribose. This spatial preference of fluorine can force the sugars to adopt a $C_{3'}$-*endo* pucker. This is the same puckering mode as observed in RNA molecules and gives rise to the RNA-characteristic A-family-type helix. Further, since fluorine is a good hydrogen bond acceptor, it can participate in the same hydrogen bonding interactions with water molecules that are known to stabilize RNA structures. Generally, it is preferred that a modified moiety at the 2'-sugar position will be able to enter into hydrogen-bonding which is more characteristic of the 2'-OH moiety of a ribonucleotide than the 2'-H moiety of a deoxyribonucleotide. A preferred oligonucleotide agent will: exhibit a $C_{3'}$-*endo* pucker in all, or at least about 50, 75, 80, 85, 90, or 95 % of its sugars; exhibit a $C_{3'}$-*endo* pucker in a sufficient amount of its sugars that it can give rise to the RNA-characteristic A-family-type helix; will generally have no more than about 20, 10, 5, 4, 3, 2, or 1 sugar which is not a $C_{3'}$-*endo* pucker structure. In certain instances, oligonucleotide will exhibit $C_{3'}$-*endo* suger pucker and be modified at the 2'-position. Exemplary modifications include 2'-OH, 2'-O-Me, 2'-O-methoxyethyl, 2'-O-aminopropyl, 2'-F, 2'-O-CH_2-CO-NHMe, 2'-O-CH_2-CH_2-O-CH_2-CH_2-N$(Me)_2$, and LNA. In certain instances, regardless of the nature of the modification, and even though the oligonucleotide agent can contain deoxynucleotides or modified deoxynucleotides, it is preferred that DNA molecules, or any molecule in which more than 50, 60, or 70 % of the nucleotides in the molecule are deoxyribonucleotides, or modified deoxyribonucleotides which are deoxy at the 2' position, are excluded from the definition of oligonucleotide agent. Some preferred 2'-modifications with of sugar moieties exhibiting C2'-endo sugar pucker include 2'-H, 2'-Me, 2'-S-Me, 2'-Ethynyl, and 2'-ara-F. Additional sugar modifications include L-sugars and 2'-5'-linked sugars.

As used herein, "specifically hybridizable" and "complementary" are terms that are used to indicate a sufficient degree of complementarity such that stable and specific binding occurs between a compound of the invention and a target RNA molecule. This nomenclature also applies to instances when NAT oligonucleotides agents bind to target RNAs. Specific binding requires a sufficient lack of complementarity to non-target sequences under conditions in which specific binding is desired, *i.e.*, under physiological conditions in the case of *in vivo* assays or therapeutic treatment, or in the case of *in vitro* assays, under conditions in which the assays are performed. It has been shown that a single mismatch between targeted and non-targeted sequences are sufficient to provide discrimination for siRNA targeting of an mRNA (Brummelkamp et al., *Cancer Cell*, 2002, **2**:243).

In certain instances, a NAT oligonucleotide agent is "sufficiently complementary" to a target RNA, such that the oligonucleotide agent inhibits production of protein encoded by the target mRNA. The target RNA can be a pre-mRNA, mRNA, or miRNA endogenous to the subject. In another embodiment, the oligonucleotide agent is "exactly complementary" (excluding the SRMS containing subunit(s)) to a target RNA, *e.g.*, the target RNA and the oligonucleotide agent can anneal to form a hybrid made exclusively of Watson-Crick base pairs in the region of exact complementarity. A "sufficiently complementary" target RNA can include a region (*e.g.*, of at least about 7 nucleotides) that is exactly complementary to a target RNA. Moreover, in some embodiments, the oligonucleotide agent specifically discriminates a single-nucleotide difference. In this case, the oligonucleotide agent only down-regulates gene expression if exact complementary is found in the region the single-nucleotide difference.

Oligonucleotide agents discussed include otherwise unmodified RNA and DNA as well as RNA and DNA that have been modified. Examples of modified RNA and DNA include modificiations to improve efficacy and polymers of nucleoside surrogates. Unmodified RNA refers to a molecule in which the components of the nucleic acid, namely sugars, bases, and phosphate moieties, are the same or essentially the same as that which occur in nature, preferably as occur naturally in the human body. The literature has referred to rare or unusual, but naturally occurring, RNAs as modified RNAs. See Limbach *et al.* *Nucleic Acids Res.* **1994**, *22*, 2183-2196. Such rare or unusual RNAs, often termed modified RNAs, are typically the result of a post-transcriptional modification and are within the scope of the term unmodified RNA as used herein. Modified RNA as used herein refers to a molecule in which one or more of the components of the nucleic acid, namely sugars, bases, and phosphate moieties, are different from that which occur in nature, preferably different from that which occurs in the human body. While they are referred to as "modified RNAs" they will of course, because of the modification, include molecules that are not, strictly speaking, RNAs. Nucleoside surrogates are molecules in which the ribophosphate backbone is replaced with a non-ribophosphate construct that allows the bases to the presented in the correct spatial relationship such that hybridization is substantially similar to what is seen with a ribophosphate backbone, *e.g.*, non-charged mimics of the ribophosphate backbone.

Sugar Replacement Monomer Subunits (SRMS)

A nucleotide subunit in which the sugar of the subunit has been so replaced is referred to herein as a sugar replacement modification subunit (SRMS). The SRMS includes two "backbone attachment points" (hydroxyl groups), a "tethering attachment point," and a ligand, which is connected indirectly to the SRMS via an intervening tether. The SRMS may be the 5'-or 3'-terminal subunit of the oligonucleotide agent and located adjacent to two or more unmodified or modified ribonucleotides. Alternatively, the SRMS may occupy an internal position located adjacent to one or more unmodified or modified ribonucleotides. More than one SRMS may be present in an oligonucleotide agent. Preferred positions for inclusion of a SRMS tethered to a moiety (*e.g.*, a lipophilic moiety such as cholesterol) are at the 3'-terminus, the 5'-terminus, or at an internal position.

Ligands

A wide variety of entities can be tethered to the oligonucleotide agent. A ligand tethered to an oligonucleotide agent can have a favorable effect on the agent. For example, the ligand can improve stability, hybridization thermodynamics with a target nucleic acid, targeting to a particular tissue or cell-type, or cell permeability, *e.g.*, by an endocytosis-dependent or -independent mechanism. Ligands and associated modifications can also increase sequence specificity and consequently decrease off-site targeting. A tethered ligand can include one or more modified bases or sugars that can function as intercalators. These are preferably located in an internal region, such as in a bulge of a miRNA/target duplex. The intercalator can be an aromatic group including polycyclic aromatics or heterocyclic aromatic groups. A polycyclic intercalator can have stacking capabilities, and can include systems with 2, 3, or 4 fused rings. Universal bases can be included on a ligand.

In one embodiment, the ligand includes a cleaving group that contributes to target gene inhibition by cleavage of the target nucleic acid. The cleaving group can be a bleomycin (*e.g.*, bleomycin-A5, bleomycin-A2, or bleomycin-B2), pyrene, phenanthroline (*e.g.*, O-phenanthroline), a polyamine, a tripeptide (*e.g.*, lys-tyr-lys tripeptide), or metal-ion chelating group. The metal-ion chelating group can be an Lu(III) or EU(III) macrocyclic complex, a Zn(II) 2,9-dimethylphenanthroline derivative, a Cu(II) terpyridine, or acridine, which can promote the selective cleavage of target RNA at the site of the bulge by free metal ions such as Lu(III). In some instances, a peptide ligand can be tethered to a miRNA to promote cleavage of the target RNA. In certain instances, the cleavage may occur at the bulge region. For example, 1,8-dimethyl-1,3,6,8,10,13-hexaazacyclotetradecane (cyclam) can be conjugated to a peptide, such as via an amino acid derivative, to promote target RNA cleavage.

A tethered ligand can be an aminoglycoside ligand which can cause an oligonucleotide agent to have improved hybridization properties or improved sequence specificity. Exemplary aminoglycosides include glycosylated polylysine, galactosylated polylysine, neomycin B, tobramycin, kanamycin A, and acridine conjugates of aminoglycosides, such as Neo-N-acridine, Neo-S-acridine, Neo-C-acridine, Tobra-N-acridine, and KanaA-N-acridine. Use of an acridine analog can increase sequence specificity. For example, neomycin B has a high affinity for RNA as compared to DNA, but low sequence-specificity. An acridine analog, neo-S-acridine has an increased affinity for the HIV Rev-response element (RRE). In some embodiments the guanidine analog (the guanidinoglycoside) of an aminoglycoside ligand is tethered to an oligonucleotide agent. In a guanidinoglycoside, the amine group on the amino acid is exchanged for a guanidine group. Attachment of a guanidine analog can enhance cell permeability of an oligonucleotide agent. A tethered ligand can be a poly-arginine peptide, peptoid or peptidomimetic, which can enhance the cellular uptake of an oligonucleotide agent.

Preferred moieties are ligands, which are coupled, preferably covalently, either directly or indirectly via an intervening tether, to the SRMS carrier. In preferred embodiments, the ligand is attached to the carrier via an intervening tether. As discussed above, the ligand or tethered ligand may be present on the SRMS monomer when the SRMS monomer is incorporated into the growing strand. In some embodiments, the ligand may be incorporated into a "precursor" SRMS after a "precursor" SRMS monomer has been incorporated into the growing strand. For example, an SRMS monomer having an amino-terminated tether (*i.e.*, having no associated ligand), or TAP-$(CH_2)_nNH_2$ may be incorporated into a growing oligonucleotide strand. In a subsequent operation, a ligand having an electrophilic group can subsequently be attached to the precursor SRMS by coupling the electrophilic group of the ligand with a terminal nucleophilic group of the precursor SRMS tether. Representative electrophilic groups include pentafluorophenyl esters or an aldehyde. Other electrophilic groups amenable to the present invention can be readily determined by one of ordinary skill in the art.

Preparation of Oligonucleotides Bearing a Peptide Conjugate

Oligonucleotides bearing peptide conjugates can be prepared using procedures analagous to those described below for the preparation of oligonucleotides bearing ligands. The synthesis and purification of oligonucleotide peptide conjugates can be performed by established methods. See Trufert *et al.*, *Tetrahedron* **1996**, *52*, 3005; and Manoharan, "Oligonucleotide Conjugates in Antisense Technology," in Antisense Drug Technology, ed. S.T. Crooke, Marcel Dekker, Inc., 2001, each of which is hereby incorporated by reference. In certain instances, a peptidomimetic can be modified to create a constrained peptide that adopts a distinct and specific preferred conformation, which can increase the potency and selectivity of the peptide. For example, the constrained peptide can be an azapeptide (Gante in *Synthesis* **1989**, 405-413). An azapeptide is synthesized by replacing the α-carbon of an amino acid with a nitrogen atom without changing the structure of the amino acid side chain. For example, the azapeptide can be synthesized by using hydrazine in traditional peptide synthesis coupling methods, such as by reacting hydrazine with a "carbonyl donor," *e.g.*, phenylchloroformate.

Conjugation with Ligands to Promote Entry into Cells

Oligonucleotide agents can be modified to enhance entry into cells, *e.g.*, an endocytic or non-endocytic mechanism. A ligand that increases cell permeability can be attached to an oligonucleotide agent in a number of ways. One example of ligand attachment is by bonding to an SRMS, *e.g.*, pyrroline-based SRMS.

In one embodiment, an oligonucleotide can be conjugated to a polyarginine that will enhance uptake into a wide range of cell-types. While not being bound by theory, the enhanced uptake is believed to be by a nonendocytic route. In another embodiment, an oligonucleotide can be conjugated to a guanidium analog of an aminoglycoside to promote cell permeability.

In another embodiment, an oligonucleotide can be conjugated with a lipophilic moiety. The lipophilic moiety can be attached at the nitrogen atom of a pyrroline-based SRMS. Examples of lipophilic moieties include cholesterols, lipid, oleyl, retinyl, or cholesteryl residues. Other lipophilic moieties include cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine. Cholesterol is a particularly preferred example.

The ligand that enhances cell permeability can be attached at the 3'-terminus, the 5'-terminus, or internally. The ligand can be attached to an SRMS, *e.g.*, a pyrroline-based SRMS at the 3'-terminus, the 5'-terminus, or at an internal linkage. The attachment can be direct or through a tethering molecule. Tethers, spacers, or linkers discussed herein can be used to attach the moiety to the SRMS.

Synthesis of Ligand-Conjugated Oligonucleotides of the Invention

The oligonucleotide compounds of the invention can be prepared using solution-phase or solid-phase organic synthesis. Organic synthesis offers the advantage that the oligonucleotide strands comprising non-natural or modified nucleotides can be easily prepared. Importantly, pyrimidine nucleotides that have been modified at the C5-position can be incorporated into the oligonucleotide sequence. The double-stranded oligonucleotide compounds of the invention comprising non-natural nucleobases and optionally non-natural sugar moieties may be prepared using a two-step procedure. First, the individual strands of the double-stranded molecule are prepared separately. Then, the component strands are annealed.

The ligand-conjugated oligonucleotides of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide. This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto. The methods of the present invention facilitate the synthesis of ligand-conjugated oligonucleotides by the use of, in some preferred embodiments, nucleoside monomers that have been appropriately conjugated with ligands and that may further be attached to a solid-support material. Such ligand-nucleoside conjugates, optionally attached to a solid-support material, are prepared according to some preferred embodiments of the methods of the present invention via reaction of a selected serum-binding ligand with a linking moiety located on the 5' position of a nucleoside or oligonucleotide. In certain instances, an oligonucleotide bearing a ligand attached to the 3'-terminus of the oligonucleotide is prepared by first covalently attaching a monomer building block to a controlled-pore-glass support via a long-chain aminoalkyl group. Then, nucleotides are bonded via standard solid-phase synthesis techniques to the monomer building-block bound to the solid support. The monomer building block may be a nucleoside or other organic compound that is compatible with solid-phase synthesis.

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

Teachings regarding the synthesis of particular modified oligonucleotides may be found in the following U.S. patents: U.S. Pat. Nos. 5,138,045 and 5,218,105, drawn to polyamine conjugated oligonucleotides; U.S. Pat. No. 5,212,295, drawn to monomers for the preparation of oligonucleotides having chiral phosphorus linkages; U.S. Pat. Nos. 5,378,825 and 5,541,307, drawn to oligonucleotides having modified backbones; U.S. Pat. No. 5,386,023, drawn to backbone-modified oligonucleotides and the preparation thereof through reductive coupling; U.S. Pat. No. 5,457,191, drawn to modified nucleobases based on the 3-deazapurine ring system and methods of synthesis thereof; U.S. Pat. No.

5,459,255, drawn to modified nucleobases based on N-2 substituted purines; U.S. Pat. No. 5,521,302, drawn to processes for preparing oligonucleotides having chiral phosphorus linkages; U.S. Pat. No. 5,539,082, drawn to peptide nucleic acids; U.S. Pat. No. 5,554,746, drawn to oligonucleotides having β-lactam backbones; U.S. Pat. No. 5,571,902, drawn to methods and materials for the synthesis of oligonucleotides; U.S. Pat. No. 5,578,718, drawn to nucleosides having alkylthio groups, wherein such groups may be used as linkers to other moieties attached at any of a variety of positions of the nucleoside; U.S. Pat. Nos. 5,587,361 and 5,599,797, drawn to oligonucleotides having phosphorothioate linkages of high chiral purity; U.S. Pat. No. 5,506,351, drawn to processes for the preparation of 2'-O-alkyl guanosine and related compounds, including 2,6-diaminopurine compounds; U.S. Pat. No. 5,587,469, drawn to oligonucleotides having N-2 substituted purines; U.S. Pat. No. 5,587,470, drawn to oligonucleotides having 3-deazapurines; U.S. Pat. No. 5,223,168, and U.S. Pat. No. 5,608,046, both drawn to conjugated 4'-desmethyl nucleoside analogs; U.S. Pat. Nos. 5,602,240, and 5,610,289, drawn to backbone-modified oligonucleotide analogs; U.S. Pat. Nos. 6,262,241, and 5,459,255, drawn to, inter alia, methods of synthesizing 2'-fluoro-oligonucleotides.

In the ligand-conjugated oligonucleotides and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. Oligonucleotide conjugates bearing a variety of molecules such as steroids, vitamins, lipids and reporter molecules, has previously been described (see Manoharan et al., PCT Application WO 93/07883). In a preferred embodiment, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

The incorporation of a 2'-O-methyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-allyl, 2'-O-aminoalkyl or 2'-deoxy-2'-fluoro group in nucleosides of an oligonucleotide confers enhanced hybridization properties to the oligonucleotide. Further, oligonucleotides containing phosphorothioate backbones have enhanced nuclease stability. Thus, functionalized, linked nucleosides of the invention can be augmented to include either or both a phosphorothioate backbone or a 2'-O-methyl, 2'-O-ethyl, 2'-O-propyl, 2'-O-aminoalkyl, 2'-O-allyl or 2'-deoxy-2'-fluoro group.

In some preferred embodiments, functionalized nucleoside sequences of the invention possessing an amino group at the 5'-terminus are prepared using a DNA synthesizer, and then reacted with an active ester derivative of a selected ligand. Active ester derivatives are well known to those skilled in the art. Representative active esters include N-hydrosuccinimide esters, tetrafluorophenolic esters, pentafluorophenolic esters and pentachlorophenolic esters. The reaction of the amino group and the active ester produces an oligonucleotide in which the selected ligand is attached to the 5'-position through a linking group. The amino group at the 5'-terminus can be prepared utilizing a 5'-Amino-Modifier C6 reagent. In a preferred embodiment, ligand molecules may be conjugated to oligonucleotides at the 5'-position by the use of a ligand-nucleoside phosphoramidite wherein the ligand is linked to the 5'-hydroxy group directly or indirectly via a linker. Such ligand-nucleoside phosphoramidites are typically used at the end of an automated synthesis procedure to provide a ligand-conjugated oligonucleotide bearing the ligand at the 5'-terminus.

In one preferred embodiment of the methods of the invention, the preparation of ligand-conjugated oligonucleotides commences with the selection of appropriate precursor molecules upon which to construct the ligand molecule. Typically, the precursor is an appropriately-protected derivative of the commonly-used nucleosides. For example, the synthetic precursors for the synthesis of the ligand-conjugated oligonucleotides of the present invention include, but are not limited to, 2'-aminoalkoxy-5'-ODMT-nucleosides, 2'-6-aminoalkylamino-5'-ODMT-nucleosides, 5'-6-aminoalkoxy-2'-deoxy-nucleosides, 5'-6-aminoalkoxy-2-protected-nucleosides, 3'-6-aminoalkoxy-5'-ODMT-nucleosides, and 3'-aminoalkylamino-5'-ODMT-nucleosides that may be protected in the nucleobase portion of the molecule. Methods for the synthesis of such amino-linked protected nucleoside precursors are known to those of ordinary skill in the art.

In many cases, protecting groups are used during the preparation of the compounds of the invention. As used herein, the term "protected" means that the indicated moiety has a protecting group appended thereon. In some preferred embodiments of the invention, compounds contain one or more protecting groups. A wide variety of protecting groups can be employed in the methods of the invention. In general, protecting groups render chemical functionalities inert to specific reaction conditions, and can be appended to and removed from such functionalities in a molecule without substantially damaging the remainder of the molecule.

Representative hydroxyl protecting groups, for example, are disclosed by Beaucage et al. (*Tetrahedron*, 1992, 48:2223-2311). Further hydroxyl protecting groups, as well as other representative protecting groups, are disclosed in Greene and Wuts, *Protective Groups in Organic Synthesis*, Chapter 2, 2d ed., John Wiley & Sons, New York, 1991, and *Oligonucleotides And Analogues A Practical Approach*, Ekstein, F. Ed., IRL Press, N.Y, 1991.

Examples of hydroxyl protecting groups include, but are not limited to, t-butyl, t-butoxymethyl, methoxymethyl, tetrahydropyranyl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 2-trimethylsilylethyl, p-chlorophenyl, 2,4-dinitrophenyl, benzyl, 2,6-dichlorobenzyl, diphenylmethyl, p,p'-dinitrobenzhydryl, p-nitrobenzyl, triphenylmethyl, trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triphenylsilyl, benzoylformate, acetate, chloroacetate, trichloroacetate, trifluoroacetate, pivaloate, benzoate, p-phenylbenzoate, 9-fluorenylmethyl carbonate, mesylate and tosylate.

Amino-protecting groups stable to acid treatment are selectively removed with base treatment, and are used to make reactive amino groups selectively available for substitution. Examples of such groups are the Fmoc (E. Atherton and R. C. Sheppard in *The Peptides*, S. Udenfriend, J. Meienhofer, Eds., Academic Press, Orlando, 1987, volume 9, p.1) and various substituted sulfonylethyl carbamates exemplified by the Nsc group (Samukov et al., *Tetrahedron Lett.*, 1994, 35:7821; Verhart and Tesser, *Rec. Trav. Chim. Pays-Bas*, 1987, 107:621).

Additional amino-protecting groups include, but are not limited to, carbamate protecting groups, such as 2-trimethylsilylethoxycarbonyl (Teoc), 1-methyl-1-(4-biphenylyl)ethoxycarbonyl (Bpoc), t-butoxycarbonyl (BOC), allyloxycarbonyl (Alloc), 9-fluorenylmethyloxycarbonyl (Fmoc), and benzyloxycarbonyl (Cbz); amide protecting groups, such as formyl, acetyl, trihaloacetyl, benzoyl, and nitrophenylacetyl; sulfonamide protecting groups, such as 2-nitrobenzenesulfonyl; and imine and cyclic imide protecting groups, such as phthalimido and dithiasuccinoyl. Equivalents of these amino-protecting groups are also encompassed by the compounds and methods of the present invention.

Many solid supports are commercially available and one of ordinary skill in the art can readily select a solid support to be used in the solid-phase synthesis steps. In certain embodiments, a universal support is used. A universal support allows for preparation of oligonucleotides having unusual or modified nucleotides located at the 3'-terminus of the oligonucleotide. Universal Support 500 and Universal Support II are universal supports that are commercially available from Glen Research, 22825 Davis Drive, Sterling, Virginia. For further details about universal supports see Scott et al., *Innovations and Perspectives in solid-phase Synthesis, 3rd International Symposium*, **1994**, Ed. Roger Epton, Mayflower Worldwide, 115-124]; Azhayev, A.V. *Tetrahedron* **1999**, *55*, 787-800; and Azhayev and Antopolsky *Tetrahedron* **2001**, *57*, 4977-4986. In addition, it has been reported that the oligonucleotide can be cleaved from the universal support under milder reaction conditions when oligonucleotide is bonded to the solid support via a *syn*-1,2-acetoxyphosphate group which more readily undergoes basic hydrolysis. See Guzaev, A. I.; Manoharan, M. *J. Am. Chem. Soc.* **2003**, *125*, 2380.

Therapeutic Uses for Compounds of the Invention

In a preferred embodiment of the present invention, the ligand attached to the nucleoside enhances the pharmacokinetic properties of the oligonucleotide therapeutic or diagnostic agent. In a preferred embodiment, the oligonucleotide is a siRNA. Such improved pharmacokinetic properties include increased binding of the antisense compound to serum proteins, increased plasma concentration of the antisense compound, increased tissue distribution, increased capacity of binding of the antisense compound to serum proteins, and increased half-lives.

The present invention provides a method for increasing the concentration of an oligonucleotide in serum. According to such methods, the ligand is conjugated to an oligonucleotide, thus forming a conjugated oligonucleotide. This conjugated oligonucleotide is then added to the serum.

The present invention further provides methods for increasing the capacity of serum for an oligonucleotide. According to such methods, the ligand is conjugated to an oligonucleotide, thus forming a conjugated oligonucleotide. This conjugated oligonucleotide is then added to the serum.

The present invention also provides methods for increasing the binding of an oligonucleotide to a portion of the vascular system. According to such methods, a vascular protein is selected which resides, in part, in the circulating serum and, in part, in the non-circulating portion of the vascular system. Then, the ligand, e.g., naproxen, is conjugated to an oligonucleotide to form a conjugated oligonucleotide, which is then added to the vascular system.

The present invention further provides methods for promoting the cellular uptake of an oligonucleotide in a cell. According to such methods, a cellular protein is selected. This cellular protein is a protein that resides on the cellular membrane and extends, in part, extracellularly so that part of this cellular protein extends onto the external side of the cellular membrane. Next, the ligand is conjugated to an oligonucleotide to form a conjugated oligonucleotide. This conjugated oligonucleotide is then brought into contact with cells in which cellular uptake of the oligonucleotide is to be promoted. In a preferred embodiment, the oligonucleotide is an siRNA.

The present invention also provides methods of increasing cellular uptake of an oligonucleotide comprising contacting an organism with an oligonucleotide of the invention, said oligonucleotide being conjugated to a ligand.

In one preferred embodiment of the invention, the protein targeted by the oligonucleotide is a serum protein. It is preferred that the serum protein targeted by the ligand-conjugated oligonucleotide compound is an immunoglobulin (an antibody). Preferred immunoglobulins are immunoglobulin G and immunoglobulin M. Immunoglobulins are known to appear in blood serum and tissues of vertebrate animals.

In another embodiment of the invention, the serum protein targeted by the oligonucleotide is a lipoprotein. Lipoproteins are blood proteins having molecular weights generally above 20,000 that carry lipids and are recognized by specific cell-surface receptors. The association with lipoproteins in the serum will initially increase pharmacokinetic parameters such as half-life and distribution. A secondary consideration is the ability of lipoproteins to enhance cellular uptake via receptor-mediated endocytosis.

In yet another embodiment, the serum protein targeted by the ligand-conjugated oligonucleotide compound is α-2-macroglobulin. In yet a further embodiment, the serum protein targeted by a ligand-conjugated oligonucleotide is α-1-glycoprotein.

Genes and Diseases

One aspect of the invention relates to a method of treating a subject at risk for or afflicted with unwanted cell proliferation, *e.g.*, malignant or nonmalignant cell proliferation. The method comprises providing a ligand-conjugated oligonucleotide agent, wherein the oligonucleotide is homologous to and can silence, *e.g.*, by cleavage, a gene which promotes unwanted cell proliferation; and administering a therapeutically effective dose of the ligand-conjugated oligonucleotide agent to a subject, preferably a human subject.

In a preferred embodiment the gene is a growth factor or growth factor receptor gene, a kinase, *e.g.*, a protein tyrosine, serine or threonine kinase gene, an adaptor protein gene, a gene encoding a G protein superfamily molecule, or a gene encoding a transcription factor.

In a preferred embodiment the oligonucleotide agent silences the PDGF beta gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted PDGF beta expression, *e.g.*, testicular and lung cancers.

In another preferred embodiment the oligonucleotide agent silences the Erb-B gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted Erb-B expression, *e.g.*, breast cancer.

In a preferred embodiment the oligonucleotide agent silences the Src gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted Src expression, *e.g.*, colon cancers.

In a preferred embodiment the oligonucleotide agent silences the CRK gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted CRK expression, *e.g.*, colon and lung cancers.

In a preferred embodiment the oligonucleotide agent silences the GRB2 gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted GRB2 expression, *e.g.*, squamous cell carcinoma.

In another preferred embodiment the oligonucleotide agent silences the RAS gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted RAS expression, *e.g.*, pancreatic, colon and lung cancers, and chronic leukemia.

In another preferred embodiment the oligonucleotide agent silences the MEKK gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted MEKK expression, *e.g.*, squamous cell carcinoma, melanoma or leukemia.

In another preferred embodiment the oligonucleotide agent silences the JNK gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted JNK expression, *e.g.*, pancreatic or breast cancers.

In a preferred embodiment the oligonucleotide agent silences the RAF gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted RAF expression, *e.g.*, lung cancer or leukemia.

In a preferred embodiment the oligonucleotide agent silences the Erk1/2 gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted Erk1/2 expression, *e.g.*, lung cancer.

In another preferred embodiment the oligonucleotide agent silences the PCNA(p21) gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted PCNA expression, *e.g.*, lung cancer.

In a preferred embodiment the oligonucleotide agent silences the MYB gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted MYB expression, *e.g.*, colon cancer or chronic myelogenous leukemia.

In a preferred embodiment the oligonucleotide agent silences the c-MYC gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted c-MYC expression, *e.g.*, Burkitt's lymphoma or neuroblastoma.

In another preferred embodiment the oligonucleotide agent silences the JUN gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted JUN expression, *e.g.*, ovarian, prostate or breast cancers.

In another preferred embodiment the oligonucleotide agent silences the FOS gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted FOS expression, *e.g.*, skin or prostate cancers.

In a preferred embodiment the oligonucleotide agent silences the BCL-2 gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted BCL-2 expression, *e.g.*, lung or prostate cancers or Non-Hodgkin lymphoma.

In a preferred embodiment the oligonucleotide agent silences the Cyclin D gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted Cyclin D expression, *e.g.*, esophageal and colon cancers.

In a preferred embodiment the oligonucleotide agent silences the VEGF gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted VEGF expression, *e.g.*, esophageal and colon cancers.

In a preferred embodiment the oligonucleotide agent silences the EGFR gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted EGFR expression, *e.g.*, breast cancer.

In another preferred embodiment the oligonucleotide agent silences the Cyclin A gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted Cyclin A expression, *e.g.*, lung and cervical cancers.

In another preferred embodiment the oligonucleotide agent silences the Cyclin E gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted Cyclin E expression, *e.g.*, lung and breast cancers.

In another preferred embodiment the oligonucleotide agent silences the WNT-1 gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted WNT-1 expression, *e.g.*, basal cell carcinoma.

In another preferred embodiment the oligonucleotide agent silences the beta-catenin gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted beta-catenin expression, *e.g.*, adenocarcinoma or hepatocellular carcinoma.

In another preferred embodiment the oligonucleotide agent silences the c-MET gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted c-MET expression, *e.g.*, hepatocellular carcinoma.

In another preferred embodiment the oligonucleotide agent silences the PKC gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted PKC expression, *e.g.*, breast cancer.

In a preferred embodiment the oligonucleotide agent silences the NFKB gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted NFKB expression, *e.g.*, breast cancer.

In a preferred embodiment the oligonucleotide agent silences the STAT3 gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted STAT3 expression, *e.g.*, prostate cancer.

In another preferred embodiment the oligonucleotide agent silences the survivin gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted survivin expression, *e.g.*, cervical or pancreatic cancers.

In another preferred embodiment the oligonucleotide agent silences the Her2/Neu gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted Her2/Neu expression, *e.g.*, breast cancer.

In another preferred embodiment the oligonucleotide agent silences the topoisomerase I gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted topoisomerase I expression, *e.g.*, ovarian and colon cancers.

In a preferred embodiment the oligonucleotide agent silences the topoisomerase II alpha gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted topoisomerase II expression, *e.g.*, breast and colon cancers.

In a preferred embodiment the oligonucleotide agent silences mutations in the p73 gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted p73 expression, *e.g.*, colorectal adenocarcinoma.

In a preferred embodiment the oligonucleotide agent silences mutations in the p21(WAF1/CIP1) gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted p21(WAF1/CIP1) expression, *e.g.*, liver cancer.

In a preferred embodiment the oligonucleotide agent silences mutations in the p27(KIP1) gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted p27(KIP1) expression, *e.g.*, liver cancer.

In a preferred embodiment the oligonucleotide agent silences mutations in the PPM1D gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted PPM1D expression, *e.g.*, breast cancer.

In a preferred embodiment the oligonucleotide agent silences mutations in the RAS gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted RAS expression, *e.g.*, breast cancer.

In another preferred embodiment the oligonucleotide agent silences mutations in the caveolin I gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted caveolin I expression, *e.g.*, esophageal squamous cell carcinoma.

In another preferred embodiment the oligonucleotide agent silences mutations in the MIB I gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted MIB I expression, *e.g.*, male breast carcinoma (MBC).

In another preferred embodiment the oligonucleotide agent silences mutations in the MTAI gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted MTAI expression, *e.g.*, ovarian carcinoma.

In another preferred embodiment the oligonucleotide agent silences mutations in the M68 gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted M68 expression, *e.g.*, human adenocarcinomas of the esophagus, stomach, colon, and rectum.

In preferred embodiments the oligonucleotide agent silences mutations in tumor suppressor genes, and thus can be used as a method to promote apoptotic activity in combination with chemotherapeutics.

In a preferred embodiment the oligonucleotide agent silences mutations in the p53 tumor suppressor gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted p53 expression, *e.g.*, gall bladder, pancreatic and lung cancers.

In a preferred embodiment the oligonucleotide agent silences mutations in the p53 family member DN-p63, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted DN-p63 expression, *e.g.*, squamous cell carcinoma.

In a preferred embodiment the oligonucleotide agent silences mutations in the pRb tumor suppressor gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted pRb expression, *e.g.*, oral squamous cell carcinoma.

In a preferred embodiment the oligonucleotide agent silences mutations in the APC1 tumor suppressor gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted APC1 expression, *e.g.*, colon cancer.

In a preferred embodiment the oligonucleotide agent silences mutations in the BRCA1 tumor suppressor gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted BRCA1 expression, *e.g.*, breast cancer.

In a preferred embodiment the oligonucleotide agent silences mutations in the PTEN tumor suppressor gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted PTEN expression, *e.g.*, hamartomas, gliomas, and prostate and endometrial cancers.

In a preferred embodiment the oligonucleotide agent silences MLL fusion genes, *e.g.*, MLL-AF9, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted MLL fusion gene expression, *e.g.*, acute leukemias.

In another preferred embodiment the oligonucleotide agent silences the BCR/ABL fusion gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted BCR/ABL fusion gene expression, *e.g.*, acute and chronic leukemias.

In another preferred embodiment the oligonucleotide agent silences the TEL/AML1 fusion gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted TEL/AML1 fusion gene expression, *e.g.*, childhood acute leukemia.

In another preferred embodiment the oligonucleotide agent silences the EWS/FLI1 fusion gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted EWS/FLI1 fusion gene expression, *e.g.*, Ewing Sarcoma.

In another preferred embodiment the oligonucleotide agent silences the TLS/FUS1 fusion gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted TLS/FUS1 fusion gene expression, *e.g.*, Myxoid liposarcoma.

In another preferred embodiment the oligonucleotide agent silences the PAX3/FKHR fusion gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted PAX3/FKHR fusion gene expression, *e.g.*, Myxoid liposarcoma.

In another preferred embodiment the oligonucleotide agent silences the AML1/ETO fusion gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted AML1/ETO fusion gene expression, *e.g.*, acute leukemia.

Another aspect of the invention relates to a method of treating a subject, *e.g.*, a human, at risk for or afflicted with a disease or disorder that may benefit by angiogenesis inhibition *e.g.*, cancer. The method comprises providing a ligand-conjugated oligonucleotide agent, wherein said oligonucleotide agent is homologous to and can silence, *e.g.*, by cleavage, a gene which mediates angiogenesis; and administering a therapeutically effective dosage of said ligand-conjugated oligonucleotide agent to a subject, preferrably a human.

In a preferred embodiment the oligonucleotide agent silences the alpha v-integrin gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted alpha V integrin, *e.g.*, brain tumors or tumors of epithelial origin.

In a preferred embodiment the oligonucleotide agent silences the Flt-1 receptor gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted Flt-1 receptors, eg. Cancer and rheumatoid arthritis.

In a preferred embodiment the oligonucleotide agent silences the tubulin gene, and thus can be used to treat a subject having or at risk for a disorder characterized by unwanted tubulin, eg. Cancer and retinal neovascularization.

Another aspect of the invention relates to a method of treating a subject infected with a virus or at risk for or afflicted with a disorder or disease associated with a viral infection. The method comprises providing a ligand-conjugated oligonucleotide agent, wherein said oligonucleotide agent is homologous to and can silence, *e.g.*, by cleavage, a viral gene of a cellular gene which mediates viral function, *e.g.*, entry or growth; and administering a therapeutically effective dose of said ligand-conjugated oligonucleotide agent to a subject, preferably a human subject.

Thus, the invention provides for a method of treating patients infected by the Human Papilloma Virus (HPV) or at risk for or afflicted with a disorder mediated by HPV, *e.g*, cervical cancer. HPV is linked to 95% of cervical carcinomas and thus an antiviral therapy is an attractive method to treat these cancers and other symptoms of viral infection.

In a preferred embodiment, the expression of a HPV gene is reduced. In another preferred embodiment, the HPV gene is one of the group of E2, E6, or E7.

In a preferred embodiment the expression of a human gene that is required for HPV replication is reduced.

The invention also includes a method of treating patients infected by the Human Immunodeficiency Virus (HIV) or at risk for or afflicted with a disorder mediated by HIV, *e.g.*, Acquired Immune Deficiency Syndrome (AIDS). In a preferred embodiment, the expression of a HIV gene is reduced. In another preferred embodiment, the HIV gene is CCR5, Gag, or Rev. In a preferred embodiment the expression of a human gene that is required for HIV replication is reduced. In another preferred embodiment, the gene is CD4 or Tsg101.

The invention also includes a method for treating patients infected by the Hepatitis B Virus (HBV) or at risk for or afflicted with a disorder mediated by HBV, *e.g.*, cirrhosis and heptocellular carcinoma. In a preferred embodiment, the expression of a HBV gene is reduced. In another preferred embodiment, the targeted HBV gene encodes one of the group of the tail region of the HBV core protein, the pre-cregious (pre-c) region, or the cregious (c) region. In another preferred embodiment, a targeted HBV-RNA sequence is comprised of the poly(A) tail.

In preferred embodiment the expression of a human gene that is required for HBV replication is reduced.

The invention also provides for a method of treating patients infected by the Hepatitis A Virus (HAV), or at risk for or afflicted with a disorder mediated by HAV. In a preferred embodiment the expression of a human gene that is required for HAV replication is reduced.

The present invention provides for a method of treating patients infected by the Hepatitis C Virus (HCV), or at risk for or afflicted with a disorder mediated by HCV, *e.g.*, cirrhosis. In a preferred embodiment, the expression of a HCV gene is reduced. In another preferred embodiment the expression of a human gene that is required for HCV replication is reduced.

The present invention also provides for a method of treating patients infected by the any of the group of Hepatitis Viral strains comprising hepatitis D, E, F, G, or H, or patients at risk for or afflicted with a disorder mediated by any of these strains of hepatitis. In a preferred embodiment, the expression of a Hepatitis, D, E, F, G, or H gene is reduced. In another preferred embodiment the expression of a human gene that is required for hepatitis D, E, F, G or H replication is reduced.

Methods of the invention also provide for treating patients infected by the Respiratory Syncytial Virus (RSV) or at risk for or afflicted with a disorder mediated by RSV, *e.g*, lower respiratory tract infection in infants and childhood asthma, pneumonia and other complications, *e.g.*, in the elderly. In a preferred embodiment, the expression of a RSV gene is reduced. In another preferred embodiment, the targeted HBV gene encodes one of the group of genes N, L, or P. In a preferred embodiment the expression of a human gene that is required for RSV replication is reduced.

Methods of the invention provide for treating patients infected by the Herpes Simplex Virus (HSV) or at risk for or afflicted with a disorder mediated by HSV, *e.g*, genital herpes and cold sores as well as life-threatening or sight-impairing disease mainly in immunocompromised patients. In a preferred embodiment, the expression of a HSV gene is reduced. In another preferred embodiment, the targeted HSV gene encodes DNA polymerase or the helicase-primase. In a preferred embodiment the expression of a human gene that is required for HSV replication is reduced.

The invention also provides a method for treating patients infected by the herpes Cytomegalovirus (CMV) or at risk for or afflicted with a disorder mediated by CMV, *e.g.*, congenital virus infections and morbidity in immunocompromised patients. In a preferred embodiment, the expression of a CMV gene is reduced. In a preferred embodiment the expression of a human gene that is required for CMV replication is reduced.

Methods of the invention also provide for a method of treating patients infected by the herpes Epstein Barr Virus (EBV) or at risk for or afflicted with a disorder mediated by EBV, *e.g.*, NK/T-cell lymphoma, non-Hodgkin lymphoma, and Hodgkin disease. In a preferred embodiment, the expression of a EBV gene is reduced. In a preferred embodiment the expression of a human gene that is required for EBV replication is reduced.

Methods of the invention also provide for treating patients infected by Kaposi's Sarcoma-associated Herpes Virus (KSHV), also called human herpesvirus 8, or patients at risk for or afflicted with a disorder mediated by KSHV, *e.g.*, Kaposi's sarcoma, multicentric Castleman's disease and AIDS-associated primary effusion lymphoma. In a preferred embodiment, the expression of a KSHV gene is reduced. In a preferred embodiment the expression of a human gene that is required for KSHV replication is reduced.

The invention also includes a method for treating patients infected by the JC Virus (JCV) or a disease or disorder associated with this virus, *e.g.*, progressive multifocal leukoencephalopathy (PML). In a preferred embodiment, the expression of a JCV gene is reduced. In preferred embodiment the expression of a human gene that is required for JCV replication is reduced.

Methods of the invention also provide for treating patients infected by the myxovirus or at risk for or afflicted with a disorder mediated by myxovirus, *e.g.*, influenza. In a preferred embodiment, the expression of a myxovirus gene is reduced. In a preferred embodiment the expression of a human gene that is required for myxovirus replication is reduced.

Methods of the invention also provide for treating patients infected by the rhinovirus or at risk for of afflicted with a disorder mediated by rhinovirus, *e.g.*, the common cold. In a preferred embodiment, the expression of a rhinovirus gene is reduced. In preferred embodiment the expression of a human gene that is required for rhinovirus replication is reduced.

Methods of the invention also provide for treating patients infected by the coronavirus or at risk for of afflicted with a disorder mediated by coronavirus, *e.g.*, the common cold. In a preferred embodiment, the expression of a coronavirus gene is reduced.

In preferred embodiment the expression of a human gene that is required for coronavirus replication is reduced.

Methods of the invention also provide for treating patients infected by the flavivirus West Nile or at risk for or afflicted with a disorder mediated by West Nile Virus. In a preferred embodiment, the expression of a West Nile Virus gene is reduced. In another preferred embodiment, the West Nile Virus gene is one of the group comprising E, NS3, or NS5. In a preferred embodiment the expression of a human gene that is required for West Nile Virus replication is reduced.

Methods of the invention also provide for treating patients infected by the St. Louis Encephalitis flavivirus, or at risk for or afflicted with a disease or disorder associated with this virus, *e.g.*, viral haemorrhagic fever or neurological disease. In a preferred embodiment, the expression of a St. Louis Encephalitis gene is reduced. In a preferred embodiment the expression of a human gene that is required for St. Louis Encephalitis virus replication is reduced.

Methods of the invention also provide for treating patients infected by the Tick-borne encephalitis flavivirus, or at risk for or afflicted with a disorder mediated by Tick-borne encephalitis virus, *e.g.*, viral haemorrhagic fever and neurological disease. In a preferred embodiment, the expression of a Tick-borne encephalitis virus gene is reduced. In a preferred embodiment the expression of a human gene that is required for Tick-borne encephalitis virus replication is reduced.

Methods of the invention also provide for methods of treating patients infected by the Murray Valley encephalitis flavivirus, which commonly results in viral haemorrhagic fever and neurological disease. In a preferred embodiment, the expression of a Murray Valley encephalitis virus gene is reduced. In a preferred embodiment the expression of a human gene that is required for Murray Valley encephalitis virus replication is reduced.

The invention also includes methods for treating patients infected by the dengue flavivirus, or a disease or disorder associated with this virus, *e.g.*, dengue haemorrhagic fever. In a preferred embodiment, the expression of a dengue virus gene is reduced. In a preferred embodiment the expression of a human gene that is required for dengue virus replication is reduced.

Methods of the invention also provide for treating patients infected by the Simian Virus 40 (SV40) or at risk for or afflicted with a disorder mediated by SV40, *e.g.*, tumorigenesis. In a preferred embodiment, the expression of a SV40 gene is reduced. In a preferred embodiment the expression of a human gene that is required for SV40 replication is reduced.

The invention also includes methods for treating patients infected by the Human T Cell Lymphotropic Virus (HTLV), or a disease or disorder associated with this virus, *e.g.*, leukemia and myelopathy. In a preferred embodiment, the expression of a HTLV gene is reduced. In another preferred embodiment the HTLV1 gene is the Tax transcriptional activator. In a preferred embodiment the expression of a human gene that is required for HTLV replication is reduced.

Methods of the invention also provide for treating patients infected by the Moloney-Murine Leukemia Virus (Mo-MuLV) or at risk for or afflicted with a disorder mediated by Mo-MuLV, *e.g.*, T-cell leukemia. In a preferred embodiment, the expression of a Mo-MuLV gene is reduced. In a preferred embodiment the expression of a human gene that is required for Mo-MuLV replication is reduced.

Methods of the invention also provide for treating patients infected by the encephalomyocarditis virus (EMCV) or at risk for or afflicted with a disorder mediated by EMCV, *e.g.* myocarditis. EMCV leads to myocarditis in mice and pigs and is capable of infecting human myocardial cells. This virus is therefore a concern for patients undergoing xenotransplantation. In a preferred embodiment, the expression of a EMCV gene is reduced. In a preferred embodiment the expression of a human gene that is required for EMCV replication is reduced.

The invention also includes a method for treating patients infected by the measles virus (MV) or at risk for or afflicted with a disorder mediated by MV, *e.g.*, measles. In a preferred embodiment, the expression of a MV gene is reduced. In a preferred embodiment the expression of a human gene that is required for MV replication is reduced.

The invention also includes a method for treating patients infected by the Vericella zoster virus (VZV) or at risk for or afflicted with a disorder mediated by VZV, *e.g.* chicken pox or shingles (also called zoster). In a preferred embodiment, the expression of a VZV gene is reduced. In a preferred embodiment the expression of a human gene that is required for VZV replication is reduced.

The invention also includes a method for treating patients infected by an adenovirus or at risk for or afflicted with a disorder mediated by an adenovirus, *e.g.* respiratory tract infection. In a preferred embodiment, the expression of an adenovirus gene is reduced. In a preferred embodiment the expression of a human gene that is required for adenovirus replication is reduced.

The invention includes a method for treating patients infected by a yellow fever virus (YFV) or at risk for or afflicted with a disorder mediated by a YFV, *e.g.* respiratory tract infection. In a preferred embodiment, the expression of a YFV gene is reduced. In another preferred embodiment, the preferred gene is one of a group that includes the E, NS2A, or NS3 genes. In a preferred embodiment the expression of a human gene that is required for YFV replication is reduced.

Methods of the invention also provide for treating patients infected by the poliovirus or at risk for or afflicted with a disorder mediated by poliovirus, *e.g.*, polio. In a preferred embodiment, the expression of a poliovirus gene is reduced. In a preferred embodiment the expression of a human gene that is required for poliovirus replication is reduced.

Methods of the invention also provide for treating patients infected by a poxvirus or at risk for or afflicted with a disorder mediated by a poxvirus, *e.g.*, smallpox. In a preferred embodiment, the expression of a poxvirus gene is reduced. In a preferred embodiment the expression of a human gene that is required for poxvirus replication is reduced.

In another, aspect the invention features methods of treating a subject infected with a pathogen, *e.g.*, a bacterial, amoebic, parasitic, or fungal pathogen. The method comprises providing a ligand-conjugated oligonucleotide agent, wherein said oligonucleotide is homologous to and can silence, *e.g.*, by cleavage of a pathogen gene; and administering a therapeutically effective dose of said ligand-conjugated oligonucleotide agent to a subject, prefereably a human subject.

The target gene can be one involved in growth, cell wall synthesis, protein synthesis, transcription, energy metabolism, *e.g.*, the Krebs cycle, or toxin production. Thus, the present invention provides for a method of treating patients infected by a plasmodium that causes malaria. In a preferred embodiment, the expression of a plasmodium gene is reduced. In another preferred embodiment, the gene is apical membrane antigen 1 (AMA1). In a preferred embodiment the expression of a human gene that is required for plasmodium replication is reduced.

The invention also includes methods for treating patients infected by the Mycobacterium ulcerans, or a disease or disorder associated with this pathogen, *e.g.* Buruli ulcers. In a preferred embodiment, the expression of a Mycobacterium ulcerans gene is reduced. In a preferred embodiment the expression of a human gene that is required for Mycobacterium ulcerans replication is reduced.

The invention also includes methods for treating patients infected by the Mycobacterium tuberculosis, or a disease or disorder associated with this pathogen, *e.g.* tuberculosis. In a preferred embodiment, the expression of a Mycobacterium tuberculosis gene is reduced. In a preferred embodiment the expression of a human gene that is required for Mycobacterium tuberculosis replication is reduced.

The invention also includes methods for treating patients infected by the Mycobacterium leprae, or a disease or disorder associated with this pathogen, *e.g.* leprosy. In a preferred embodiment, the expression of a Mycobacterium leprae gene is reduced. In a preferred embodiment the expression of a human gene that is required for Mycobacterium leprae replication is reduced.

The invention also includes methods for treating patients infected by the bacteria Staphylococcus aureus, or a disease or disorder associated with this pathogen, *e.g.* infections of the skin and muscous membranes. In a preferred embodiment, the expression of a Staphylococcus aureus gene is reduced. In a preferred embodiment the expression of a human gene that is required for Staphylococcus aureus replication is reduced.

The invention also includes methods for treating patients infected by the bacteria Streptococcus pneumoniae, or a disease or disorder associated with this pathogen, *e.g.* pneumonia or childhood lower respiratory tract infection. In a preferred embodiment, the expression of a Streptococcus pneumoniae gene is reduced. In a preferred embodiment the expression of a human gene that is required for Streptococcus pneumoniae replication is reduced.

The invention also includes methods for treating patients infected by the bacteria Streptococcus pyogenes, or a disease or disorder associated with this pathogen, *e.g.* Strep throat or Scarlet fever. In a preferred embodiment, the expression of a Streptococcus pyogenes gene is reduced. In a preferred embodiment the expression of a human gene that is required for Streptococcus pyogenes replication is reduced.

The invention also includes methods for treating patients infected by the bacteria Chlamydia pneumoniae, or a disease or disorder associated with this pathogen, *e.g.* pneumonia or childhood lower respiratory tract infection. In a preferred embodiment, the expression of a Chlamydia pneumoniae gene is reduced. In a preferred embodiment the expression of a human gene that is required for Chlamydia pneumoniae replication is reduced.

The invention also includes methods for treating patients infected by the bacteria Mycoplasma pneumoniae, or a disease or disorder associated with this pathogen, *e.g.* pneumonia or childhood lower respiratory tract infection. In a preferred embodiment, the expression of a Mycoplasma pneumoniae gene is reduced. In a preferred embodiment the expression of a human gene that is required for Mycoplasma pneumoniae replication is reduced.

Another aspect of the invention relates to a method of treating a subject, *e.g.*, a human, at risk for or afflicted with a disease or disorder characterized by an unwanted immune response, *e.g.*, an inflammatory disease or disorder, or an autoimmune disease or disorder. The method comprises providing a ligand-conjugated oligonucleotide agent, wherein said oligonucleotide agent is homologous to and can silence, *e.g.*, by cleavage, a gene which mediates an unwanted immune response; and administering said ligand-conjugated oligonucleotide agent to a subject, preferrably a human subject. In a preferred embodiment the disease or disorder is an ischemia or reperfusion injury, *e.g.*, ischemia or reperfusion injury associated with acute myocardial infarction, unstable angina, cardiopulmonary bypass, surgical intervention *e.g.*, angioplasty, *e.g.*, percutaneous transluminal coronary angioplasty, the response to a transplantated organ or tissue, *e.g.*, transplanted cardiac or vascular tissue; or thrombolysis. In a preferred embodiment the disease or disorder is restenosis, *e.g.*, restenosis associated with surgical intervention *e.g.*, angioplasty, *e.g.*, percutaneous transluminal coronary angioplasty. In a prefered embodiment the disease or disorder is Inflammatory Bowel Disease, *e.g.*, Crohn Disease or Ulcerative Colitis. In a prefered embodiment the disease or disorder is inflammation associated with an infection or injury. In a prefered embodiment the disease or disorder is asthma, lupus, multiple sclerosis, diabetes, *e.g.*, type II diabetes, arthritis, *e.g.*, rheumatoid or psoriatic. In particularly preferred embodiments the oligonucleotide agent silences an integrin or co-ligand thereof, *e.g.*, VLA4, VCAM, ICAM. In particularly preferred embodiments the oligonucleotide agent silences a selectin or co-ligand thereof, *e.g.*, P-selectin, E-selectin (ELAM), I-selectin, P-selectin glycoprotein-1 (PSGL-1). In particularly preferred embodiments the oligonucleotide agent silences a component of the complement system, *e.g.*, C3, C5, C3aR, C5aR, C3 convertase, C5 convertase.

In particularly preferred embodiments the oligonucleotide agent silences a chemokine or receptor thereof, *e.g.*, TNFI, TNFJ, IL-1I, IL-1J, IL –2, IL-2R, IL-4, IL-4R, IL-5, IL-6, IL-8, TNFRI, TNFRII, IgE, SCYA11, CCR3.

In other embodiments the oligonucleotide agent silences GCSF, Gro1, Gro2, Gro3, PF4, MIG, Pro-Platelet Basic Protein (PPBP), MIP-1I, MIP-1J, RANTES, MCP-1, MCP-2, MCP-3, CMBKR1, CMBKR2, CMBKR3, CMBKR5, AIF-1, I-309.

Another aspect of the invention features, a method of treating a subject, *e.g.*, a human, at risk for or afflicted with acute pain or chronic pain. The method comprises providing a ligand-conjugated oligonucleotide agent, wherein said ligand is an aromatic group and said oligonucleotide is homologous to and can silence, *e.g.*, by cleavage, a gene which mediates the processing of pain; and administering a therapeutically effective dose of said ligand-conjugated oligonucleotide agent to a subject, preferrably a human subject. In particularly preferred embodiments the oligonucleotide agent silences a component of an ion channel. In particularly preferred embodiments the oligonucleotide agent silences a neurotransmitter receptor or ligand.

Another aspect of the invention relates to a method of treating a subject, *e.g.*, a human, at risk for or afflicted with a neurological disease or disorder. The method comprises providing a ligand-conjugated oligonucleotide agent, wherein said ligand is an aromtic group and said oligonucleotide is homologous to and can silence, *e.g.*, by cleavage, a gene which mediates a neurological disease or disorder; and administering a therapeutically effective dose of said ligand-conjugated oligonucleotide agent the to a subject, preferrably a human. In a prefered embodiment the disease or disorder is Alzheimer Disease or Parkinson Disease. In particularly preferred embodiments the oligonucleotide agent silences an amyloid-family gene, *e.g.*, APP; a presenilin gene, *e.g.*, PSEN1 and PSEN2, or I-synuclein. In a preferred embodiment the disease or disorder is a neurodegenerative trinucleotide repeat disorder, *e.g.*, Huntington disease, dentatorubral pallidoluysian atrophy or a spinocerebellar ataxia, *e.g.*, SCA1, SCA2, SCA3 (Machado-Joseph disease), SCA7 or SCA8.

In particularly preferred embodiments the oligonucleotide agent silences HD, DRPLA, SCA1, SCA2, MJD1, CACNL1A4, SCA7, SCA8.

The loss of heterozygosity (LOH) can result in hemizygosity for sequence, *e.g.*, genes, in the area of LOH. This can result in a significant genetic difference between normal and disease-state cells, *e.g.*, cancer cells, and provides a useful difference between normal and disease-state cells, *e.g.*, cancer cells. This difference can arise because a gene or other sequence is heterozygous in euploid cells but is hemizygous in cells having LOH. The regions of LOH will often include a gene, the loss of which promotes unwanted proliferation, *e.g.*, a tumor suppressor gene, and other sequences including, *e.g.*, other genes, in some cases a gene which is essential for normal function, *e.g.*, growth. Methods of the invention rely, in part, on the specific cleavage or silencing of one allele of an essential gene with a ligand-conjugated oligonucleotide agent of the invention. The oligonucleotide agent is selected such that it targets the single allele of the essential gene found in the cells having LOH but does not silence the other allele, which is present in cells which do not show LOH. In essence, it discriminates between the two alleles, preferentially silencing the selected allele. In essence polymorphisms, *e.g.*, SNPs of essential genes that are affected by LOH, are used as a target for a disorder characterized by cells having LOH, *e.g.*, cancer cells having LOH. *E.g.*, one of ordinary skill in the art can identify essential genes which are in proximity to tumor suppressor genes, and which are within a LOH region which includes the tumor suppressor gene. The gene encoding the large subunit of human RNA polymerase II, POLR2A, a gene located in close proximity to the tumor suppressor gene p53, is such a gene. It frequently occurs within a region of LOH in cancer cells. Other genes that occur within LOH regions and are lost in many cancer cell types include the group comprising replication protein A 70-kDa subunit, replication protein A 32-kD, ribonucleotide reductase, thymidilate synthase, TATA associated factor 2H, ribosomal protein S14, eukaryotic initiation factor 5A, alanyl tRNA synthetase, cysteinyl tRNA synthetase, NaK ATPase, alpha-1 subunit, and transferrin receptor.

Accordingly, another aspect of the invention relates to a method of treating a disorder characterized by LOH, *e.g.*, cancer. The method comprises optionally, determining the genotype of the allele of a gene in the region of LOH and preferably determining the genotype of both alleles of the gene in a normal cell; providing a ligand-conjugated oligonucleotide agent agent which preferentially cleaves or silences the allele found in the LOH cells; and administerning a therapeutically effective dose of said ligand-conjugated oligonucleotide agent to the subject, preferrably a human.

The invention also includes a ligand-conjugated oligonucleotide agent disclosed herein, *e.g*, an oligonucleotide agent which can preferentially silence, *e.g.*, cleave, one allele of a polymorphic gene.

In another aspect, the invention provides a method of cleaving or silencing more than one gene with a ligand-conjugated oligonucleotide agent. In these embodiments the oligonucleotide agent is selected so that it has sufficient homology to a sequence found in more than one gene. For example, the sequence AAGCTGGCCCTGGACATGGAGAT is conserved between mouse lamin B1, lamin B2, keratin complex 2-gene 1 and lamin A/C. Thus an oligonucleotide agent targeted to this sequence would effectively silence the entire collection of genes.

The invention also includes a ligand-conjugated oligonucleotide agent, which can silence more than one gene.

In a preferred embodiment, the oligonucleotide is a siRNA.

Compounds of the Invention

The compounds of the invention relate to ligand-bearing oligonucleotides, wherein the ligand is covalently attached to the C5-position of a pyrimidine-bearing nucleotide. In certain embodiments, the compounds of the invention relate to a double-stranded oligonucleotide sequence, wherein a ligand is bound to at least one nucleoside in only one of the two strands. In certain embodiments, the compounds of the invention relate to a double-stranded oligonucleotide sequence, wherein at least one nucleoside in both of the strands comprises a bound ligand. In a preferred embodiment, the oligonucleotide is a siRNA. In certain embodiments, the present invention relates to a single-stranded oligonucleotide comprising at least one ligand. The ligand improves the pharmacokinetic properties of the oligonucleotide.

The nucleosides are linked by phosphorus-containing or non-phosphorus-containing covalent internucleoside linkages. For the purposes of identification, ligand-conjugated nucleosides can be characterized as ligand-bearing nucleosides or ligand-nucleoside conjugates. The linked nucleosides having ligands conjugated to a nucleoside within their sequence will demonstrate enhanced biological activity when compared to like oligonucleotides that are not conjugated.

The ligand-conjugated oligonucleotides of the present invention also include conjugates of oligonucleotides and linked nucleosides wherein the ligand is attached directly to the nucleoside or nucleotide wherein the linker group is just a bond. The ligand may preferably be attached, via linking groups, at a carboxyl, amino or oxo group of the ligand. Typical linking groups may be ester, amide or carbamate groups.

Specific examples of preferred modified nucleotides envisioned for use in the ligand-conjugated oligonucleotides of the present invention include nucleotides containing modified backbones or non-natural internucleoside linkages. As defined here, oligonucleotides having modified backbones or internucleoside linkages include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes the invention, modified oligonucleotides that do not have a phosphorus atom in their intersugar backbone can also be considered to be oligonucleosides.

Specific oligonucleotide chemical modifications are described below. It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the following modifications may be incorporated in a single siRNA compound or even in a single nucleotide thereof.

Preferred modified internucleoside linkages or backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalklyphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs 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.

Representative United States Patents that teach the preparation of the above phosphorus atom-containing linkages include, but are not limited to, U.S. Pat. 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; 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; 5,625,050; and 5,697,248, each of which is herein incorporated by reference.

Preferred modified internucleoside linkages or backbones that do not include a phosphorus atom therein (i.e., oligonucleosides) have backbones that are formed by short chain alkyl or cycloalkyl intersugar linkages, mixed heteroatom and alkyl or cycloalkyl intersugar linkages, or one or more short chain heteroatomic or heterocyclic intersugar linkages. These include those having 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 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 CH_2 component parts.

Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. 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; 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, each of which is herein incorporated by reference.

In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleoside units are replaced with novel groups. The nucleobase units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligonucleotide, an oligonucleotide mimetic, 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 amide-containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to atoms of the amide portion of the backbone.

Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., *Science*, 1991, 254, 1497.

Some preferred embodiments of the present invention employ oligonucleotides with phosphorothioate linkages and oligonucleosides with heteroatom backbones, and in particular --CH_2--NH--O--CH_2 --, --CH_2--N(CH_3)--O--CH_2 -- [known as a methylene (methylimino) or MMI backbone], --CH_2--O--N(CH_3)--CH_2 --, --CH_2--N(CH_3)--N(CH_3)--CH_2--, and --O--N(CH_3)--CH_2 --CH_2-- [wherein the native phosphodiester backbone is represented as --O--P--O--CH_2--] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

The oligonucleotides employed in the ligand-conjugated oligonucleotides of the present invention may additionally or alternatively comprise nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases include other synthetic and natural nucleobases, such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in the *Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J. I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., *Angewandte Chemie, International Edition*, 1991, 30, 613, and those disclosed by Sanghvi, Y. S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S. T. and

Lebleu, B., ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligonucleotides of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-Methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2 °C. (Id., pages 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-methoxyethyl sugar modifications.

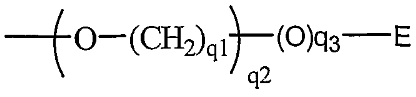
Representative United States patents that teach the preparation of certain of the above-noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; and 5,808,027; all of which are hereby incorporated by reference.

The oligonucleotides employed in the ligand-conjugated oligonucleotides of the present invention may additionally or alternatively comprise one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl, O-, S-, or N-alkenyl, or O, S- or N-alkynyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2 CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. a preferred modification includes 2'-methoxyethoxy [2'-O--$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE] (Martin et al., *Helv. Chim. Acta*, 1995, 78, 486), i.e., an alkoxyalkoxy group. a further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in U.S. Pat. No. 6,127,533, filed on Jan. 30, 1998, the contents of which are incorporated by reference.

Other preferred modifications include 2'-methoxy (2'-O--CH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides.

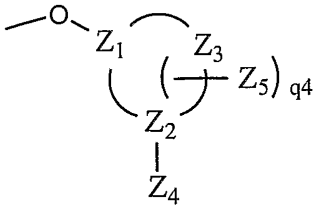
As used herein, the term "sugar substituent group" or "2'-substituent group" includes groups attached to the 2'-position of the ribofuranosyl moiety with or without an oxygen atom. Sugar substituent groups include, but are not limited to, fluoro, O-alkyl, O-alkylamino, O-alkylalkoxy, protected O-alkylamino, O-alkylaminoalkyl, O-alkyl imidazole and polyethers of the formula $(O\text{-alkyl})_m$, wherein 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 those which are disclosed by Ouchi et al. (Drug Design and Discovery 1992, 9:93); Ravasio et al. (*J. Org. Chem.* 1991, 56:4329); and Delgardo et. al. (*Critical Reviews in Therapeutic Drug Carrier Systems* 1992, 9:249), each of which is hereby incorporated by reference in its entirety. Further sugar modifications are disclosed by Cook (*Anti-Cancer Drug Design*, 1991, 6:585-607). Fluoro, O-alkyl, O-alkylamino, O-alkyl imidazole, O-alkylaminoalkyl, and alkyl amino substitution is described in U.S. Patent 6,166,197, entitled "Oligomeric Compounds having Pyrimidine Nucleotide(s) with 2' and 5' Substitutions," hereby incorporated by reference in its entirety.

Additional sugar substituent groups amenable to the present invention include 2'-SR and 2'-NR_2 groups, wherein each R is, independently, hydrogen, a protecting group or substituted or unsubstituted alkyl, alkenyl, or alkynyl. 2'-SR Nucleosides are disclosed in U.S. Pat. No. 5,670,633, issued Sep. 23, 1997, hereby incorporated by reference in its entirety. The incorporation of 2'-SR monomer synthons is disclosed by Hamm et al. (*J. Org. Chem.*, 1997, 62:3415-3420). 2'-NR 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'-substituent groups amenable to the present invention include those having one of formula **I** or **II**:



$$-\left(O-(CH_2)_{q1}\right)_{q2}-(O)_{q3}-E$$

I



II

wherein,

E is C_1 -C_{10} alkyl, $N(Q_3)(Q_4)$ or $N{=}C\ (Q_3)(Q_4)$; each Q_3 and Q_4 is, independently, H, C_1-C_{10} alkyl, dialkylaminoalkyl, a nitrogen protecting group, a tethered or untethered conjugate group, a linker to a solid support; or Q_3 and Q_4, together, form a nitrogen protecting group or a ring structure optionally including at least one additional heteroatom selected from N and O;

q_1 is an integer from 1 to 10;

q_2 is an integer from 1 to 10;

q_3 is 0 or 1;

q_4 is 0, 1 or 2;

each Z_1, Z_2 and Z_3 is, independently, C_4-C_7 cycloalkyl, C_5-C_{14} aryl or C_3-C_{15} heterocyclyl, wherein the heteroatom in said heterocyclyl group is selected from oxygen, nitrogen and sulfur;

Z_4 is OM_1, SM_1, or $N(M_1)_2$; each M_1 is, independently, H, C_1-C_8 alkyl, C_1-C_8 haloalkyl, $C(=NH)N(H)M_2$, $C(=O)N(H)M_2$ or $OC(=O)N(H)M_2$; M_2 is H or C_1-C_8 alkyl; and

Z_5 is C_1-C_{10} alkyl, C_1 -C_{10} haloalkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_6-C_{14} aryl, $N(Q_3)(Q_4)$, OQ_3, halo, SQ_3 or CN.

Representative 2'-O-sugar substituent groups of formula I are disclosed in U.S. Pat. No. 6,172,209, entitled "Capped 2'-Oxyethoxy Oligonucleotides," hereby incorporated by reference in its entirety. Representative cyclic 2'-O-sugar substituent groups of formula II are disclosed in U.S. Patent 6,271,358, filed Jul. 27, 1998, entitled "RNA Targeted 2'-Modified Oligonucleotides that are Conformationally Preorganized," hereby incorporated by reference in its entirety.

Sugars having O-substitutions on the ribosyl ring are also amenable to the present invention. Representative substitutions for ring O include, but are not limited to, S, CH_2, CHF, and CF_2. See, e.g., Secrist et al., Abstract 21, *Program & Abstracts, Tenth International Roundtable, Nucleosides, Nucleotides and their Biological Applications*, Park City, Utah, Sep. 16-20, 1992.

Oligonucleotides may also have sugar mimetics, such as cyclobutyl moieties, in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugars structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,0531 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,700,920; and 5,859,221, all of which are hereby incorporated by reference.

Additional modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide. For example, one additional modification of the ligand-conjugated oligonucleotides of the present invention involves chemically linking to the oligonucleotide one or more additional non-ligand moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties, such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci.* USA, 1989, 86, 6553), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4, 1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660, 306; Manoharan et al., *Bioorg. Med. Chem. Let.*, 1993, 3, 2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10, 111; Kabanov et al., *FEBS Lett.*, 1990, 259, 327; Svinarchuk et al., *Biochimie*, 1993, 75, 49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14, 969), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277, 923).

Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928; and 5,688,941, each of which is herein incorporated by reference.

The present invention also includes compositions employing oligonucleotides that are substantially chirally pure with regard to particular positions within the oligonucleotides. Examples of substantially chirally pure oligonucleotides include, but are not limited to, those having phosphorothioate linkages that are at least 75% Sp or Rp (Cook et al., U.S. Pat. No. 5,587,361) and those having substantially chirally pure (Sp or Rp) alkylphosphonate, phosphoramidate or phosphotriester linkages (Cook, U.S. Pat. Nos. 5,212,295 and 5,521,302).

The present invention further encompasses ligand-conjugated oligonucleotides employing ribozymes. Synthetic RNA molecules and derivatives thereof that catalyze highly specific endoribonuclease activities are known as ribozymes. (See, generally, U.S. Pat. No. 5,543,508 to Haseloff et al., issued Aug. 6, 1996, and U.S. Pat. No. 5,545,729 to Goodchild et al., issued Aug. 13, 1996.) The cleavage reactions are catalyzed by the RNA molecules themselves. In naturally occurring RNA molecules, the sites of self-catalyzed cleavage are located within highly conserved regions of RNA secondary structure (Buzayan et al., *Proc. Natl. Acad. Sci. U.S.A.*, 1986, 83, 8859; Forster et al., *Cell*, 1987, 50, 9). Naturally occurring autocatalytic RNA molecules have been modified to generate ribozymes which can be targeted to a particular cellular or pathogenic RNA molecule with a high degree of specificity. Thus, ribozymes serve the same general purpose as antisense oligonucleotides (i.e., modulation of expression of a specific gene) and, like oligonucleotides, are nucleic acids possessing significant portions of single-strandedness. That is, ribozymes have substantial chemical and functional identity with oligonucleotides and are thus considered to be equivalents for purposes of the present invention.

In certain instances, the oligonucleotide may be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to oligonucleotides in order to enhance the activity, cellular distribution or cellular uptake of the oligonucleotide, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86:6553), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660:306; Manoharan et al., *Bioorg. Med. Chem. Let.*, 1993, 3:2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10:111; Kabanov et al., *FEBS Lett.*, 1990, 259:327; Svinarchuk et al., *Biochimie*, 1993, 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36:3651; Shea et al., *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14:969), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36:3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277:923). Representative United States patents that teach the preparation of such oligonucleotide conjugates have been listed above. Typical conjugation protocols involve the synthesis of oligonucleotides bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction may be performed either with the oligonucleotide still bound to the solid support or following cleavage of the oligonucleotide in solution phase. Purification of the oligonucleotide conjugate by HPLC typically affords the pure conjugate.

Alternatively, the molecule being conjugated may be converted into a building block, such as a phosphoramidite, via an alcohol group present in the molecule or by attachment of a linker bearing an alcohol group that may be phosphitylated.

Importantly, each of these approaches may be used for the synthesis of ligand-conjugated oligonucleotides. Aminolinked oligonucleotides may be coupled directly with ligand via the use of coupling reagents or following activation of the ligand as an NHS or pentfluorophenolate ester. Ligand phosphoramidites may be synthesized via the attachment of an aminohexanol linker to one of the carboxyl groups followed by phosphitylation of the terminal alcohol functionality. Other linkers, such as cysteamine, may also be utilized for conjugation to a chloroacetyl linker present on a synthesized oligonucleotide.

For the purposes of illustration, the ligand-bearing nucleotide can be divided into three regions: ligand, linker, and nucleotide. In the present invention, the ligand is bound to the nucleotide via a linker. The purpose of the linker is to covalently attach the ligand to the nucleotide. The structure of the linker is dictated by the functional group used to bind the ligand. In a preferred embodiment, the linker is amenable to solid phase synthesis techniques. A more detailed discussion of each of the variable regions is presented below.

Ligand

In the present invention, the ligand can be a wide variety of organic compounds which impart improved pharmacological properties to the oligonucleotde when the ligand(s) is attached to the oligonucleotide. For example, the ligand can be a steroid, bile acid, lipid, folic acid, pyridoxal, B12, riboflavin, biotin, aromatic compound, polycyclic compound, crown ether, intercalator, cleaver molecule, protein-binding agent, or carbohydrate. In certain embodiments, the steroid is cholesterol. The ligand can also be a vitamin, peptide, terpene, or amino acid.

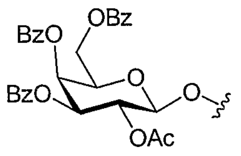
A large number of steroids are known in the art and are amenable to the present invention. Representative examples of steriods include cholesterol, 5β-cholanic acid, progesterone, aldosterone, dehydroaldosterone, isoandrosterone, esterone, estradiol, ergosterol, dehydroergosterol, lanosterol, 4-cholesten-3-one, guggulsterone, testosterone, nortestosterone, formestane, hydroxyecdysone, ketoestriol, corticosterone, dienestrol, dihydroxypregnanone, pregnanone, copornmon, equilenin, equilin, estriol, ethinylestradiol, mestranol, moxestrol, mytatrienediol, quinestradiol, quinestrol, helvolic acid, protostadiene, fusidic acid, cycloartenol, tricallol, cucurbitanin cedrelone, euphol, dammerenediol, parkeol, dexametasone, methylprednisolone, prednisolone, hydrocortisone, parametasone, betametasone, cortisone, fluocinonide, fluorometholone, halcinonide, and budesonide, or any one of them further substituted with one or more of hydroxyl, halogen, amino, alkylamino, alkyl, carboxylic acid, ester, amide, carbonyl, alkoxyl, or cyano.

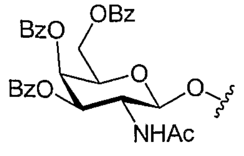
A large number of bile acids are known in the art and are amenable to the present invention. Bile acids occur in conjugation with glycine or taurine in bile of most vertebrates and some of them find use in medicine. Thus, some bile acids--due to their inherent pharmacological properties--are used as cholerectics (see, for example, James E. F. Reynolds (editor) Martindale The Extra Pharmacopoeia, 30th Edition, The Pharmaceutical Press, London (1993), page 1341). Representative examples of bile acids include cholic acid, deoxycholic acid, taurocholic acid, glycocholic acid, glycodeoxycholic acid, taurodeoxycholic acid, ursodeoxycholic acid, and chenodeoxycholic acid. Additional bile acids amenable to the present invention include those described in U.S. Patents 5,641,767; 5,656,277; 5,610,151; 5,428,182; and 3,910,888.

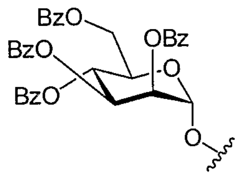
A large number of lipids are known in the art and are amenable to the present invention. Representative examples of lipids include lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, triacylglycerols, phosphoacylglycerols, sphingolipids, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, and tetraterpenes.

A large number of aromatic compounds are known in the art and are amenable to the present invention. Representative examples of aromatic compounds include optionally substituted phenyl, naphthyl, anthracenyl, phenanthrenyl, pyrenyl, pyridinyl, quinolinyl, acridinyl, phenathridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinoxalinyl, quinazolinyl, 1,7-phenanthrolinyl, indolyl, thianaphthenyl, benzoxazolyl, benzofuranyl, 1,2-benzisoxazolyl, benzimidazolyl, pyrrolyl, thiophenyl, isoxazolyl, pyrazolyl, thiazolyl, imidazolyl, tetrazolyl, and furanyl.

A large number of carbohydrates are known in the art and are amenable to the present invention. Representative examples of carbohydrates include erythrose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, mannose, gulose, idose, galactose, and talose; or a disaccharide or trisaccharide formed via a 1,4 glycoside linkage between any of them. In certain instances, the carbohydrate is a hexose or pentose. In certain instances, the carbohydrate is selected from one of the carbohydrates shown below.

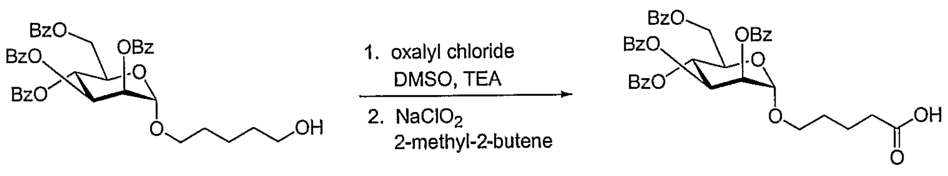






In certain instances, the carbohydrates mentioned above have an carboxyalkyl tether attached to the glycosyl oxygen atom. For procedures relating to the preparation of carbohydrates, such as those described above, bearing a carboxyalkyl attached to the glycosyl oxygen atom see Valentijn et al. *Tetrahedron* **1997**, *53*, 759; Wijsman et al. *Recueil des Travaux Chimiques des Pays-Bas*, **1996**, 115, 397; and scheme 1.

Scheme 1.



A large number of polycyclic compounds are known in the art and are amenable to the present invention. Representative classes of polycyclic compounds include bicyclic compounds wherein, the first and second ring are independently a 3, 4, 5, or 6-member saturated or unsaturated carbon ring containing 0, 1, 2, or 3 hetereoatoms selected from the group consisting of O, N, or S. In certain instances, the first ring is an aromatic ring. In certain instances, the second ring is an aromatic ring. In certain instances, both rings are saturated. In certain instances, the first ring contains no heteroatoms. In certain instances, the second ring contains to heteroatoms. In certain instances, the first ring contains a nitrogen atom. In certain instances, the second ring contains a nitrogen atom. In certain instances, the polycyclic compound is a tricyclic compound, wherein the first, second, and third ring are independently a 3, 4, 5, or 6-member saturated or unsaturated carbon ring containing 0, 1, 2, or 3 hetereoatoms selected from the group consisting of O, N, or S. In certain instances, the first ring is an aromatic ring. In certain instances, the second ring is an aromatic ring. In certain instances, the third ring is an aromatic ring. In certain instances, all three rings are saturated. In certain instances, the first ring contains no heteroatoms. In certain instances, the second ring contains to heteroatoms. In certain instances, the third ring contains to heteroatoms. In certain instances, the first ring contains a nitrogen atom. In certain instances, the second ring contains a nitrogen atom. In certain instances, the third ring contains a nitrogen atom. In certain instances, the polycyclic compound is a bridged polycyclic compound. In certain instances, the polycyclic compound is a bicyclo[2.2.1]heptane, bicyclo[2.2.2]octane, bicyclo[3.2.1]octane, bicyclo[3.2.2]nonane, or bicyclo[3.3.1]nonane.

A large number of crown ethers are known in the art and are amenable to the present invention. Crown ethers are macrocyclic, polyether, neutral compounds containing 4-20 oxygen atoms each separated from the next by two or more carbon atoms. Macrocyclic polyethers have been found to form stable complexes with salts of alkali metals and other metals and ammonium salts; "Macrocyclic polyethers and their complexes", C. J. Pederson et al, Angew. Chem. Intern. Ed., Vol. 11, page 16, (1972) and U.S. Pat. Nos. 3,562,295 and 3,687,978. Since the stereo models of macrocyclic polyethers give a crown-like appearance, they are commonly designated as N-crown-M polyethers, wherein N is the total number of atoms in the polyether ring and M is the number of oxygen atoms in the polyether ring. Crown polyethers ranging in size from cyclic tetramers of ethylene oxide ([12]-crown-4) and propylene oxide ([16]-crown-4) to 60-membered polyether rings (dibenzo [60]-crown-20) have been reported. Preferred crown ethers include 12-crown-4, 15-crown-5, and 18-crown-6.

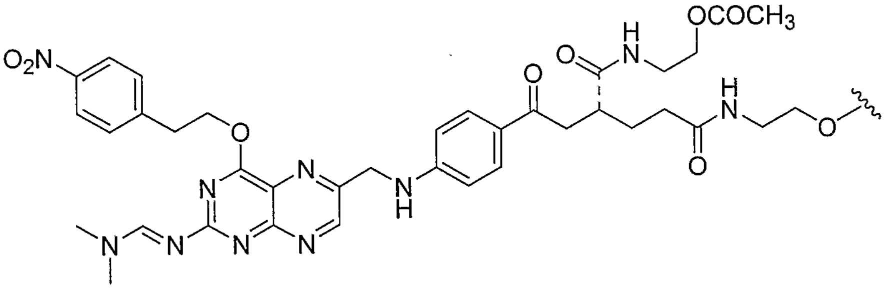
A large number of oligonucleotide intercalators are known in the art and are amenable to the present invention. One class of intercalators are DNA intercalators which bind noncovalently to duplex DNA and are characterized by a flat molecule which inserts between base pairs of the double helix of DNA. Representative examples of intercalators include *p*-carboxy methidium, *p*-carboxy ethidium, acridine and ellipticine.

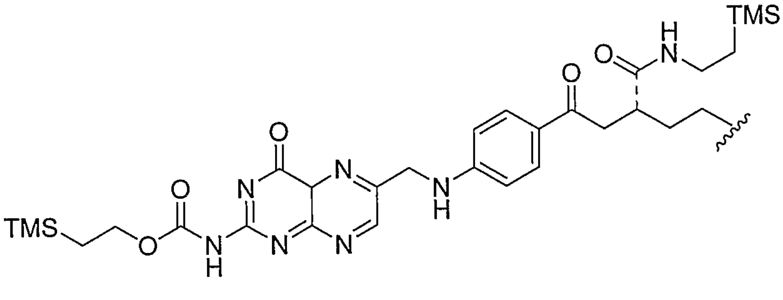
A large number of oligonucleotide cleaver molecules are known in the art and are amenable to the present invention. A cleaver molecule is a compound that can sever an oligonucleotide strand. Bleomycin, a glycopeptide antibiotic, is known to bind to and cleave DNA in a reaction that depends on the presence of ferrous ion and molecular oxygen, "Bleomycin: Chemical, Biochemical and Biological Aspects"; Hecht, S. M., Ed.; Springer Verlag: New York, 1979; Sausville, E. A.; Peisach, J.; Horwitz, S. B. "Biochemistry" 1978, 17, 2740. Burger, R. M.; Peisach, J; Horwitz, S. B. "Life Sciences" 1981, 28, 715; and Lown, J. W.; Sim, S. F. "Biochem. Biophys. Res. Comm. " 1977, 77,

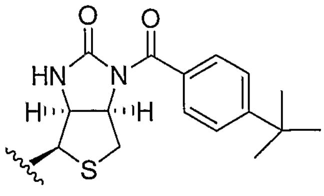
1150. The antitumor agent streptonigrin is also capable of causing single strand breaks in DNA using oxygen and cuprous ion, Cone, R; Hasan, S. K.; Lown, J. W.; Morgan, A. R. "Can. J. Biochem." 1976, 54, 219. Recently, the 1-10 phenanthroline-cuprous complex has been shown to cleave DNA in the presence of oxygen, Sigman, D. S.; Graham, D. R.; D'Aurora, V.; Stern, A. M. "J. Biol. Chem." 1979, 254, 12269; Graham, D. R.; Marshall, L. E.; Reich, K. A.; Sigman, D. S. "J. Amer. Chem. Soc." 1980, 102, 5419; Marshall, L. E.; Graham, D. R.; Reich, K. A.; Sigman, D. S. "Biochemistry" 1981, 20, 244; and Que, B. G.; Downey, K. M.; So., A. G. "Biochemistry" 1980, 19, 5987. In addition, methidium, ethidium, and cisplatin are known to cleave oligonucleotide sequences.

A large number of saturated 5-membered rings are known in the art and are amenable to the present invention. Preferred saturated 5-membered rings are optionally substituted cyclopentane, pyrrolidine, tetrahydrofuran, tetrahydrothiophene, and 1,1-difluorocyclopentane.

In certain instances, the ligand is selected from one of the radicals shown below.







Linker

In a preferred embodiment of the invention, the ligand is attached to an oligonucleotide via a linking group, to form a ligand-conjugated oligonucleotide. Preferred linkers of the invention include, but are not limited to, alkyl linkers, alkenyl linkers, α,β-unsaturated amide linkers, α,β-unsaturated ester linkers, ketone linkers, thioether linkers, aminoalkyl linkers, 6-aminoalkoxy linkers, 6-aminoalkylamino linkers, cysteamine, heterobifunctional linkers, homobifunctional linkers, and a universal tether (derived from 3-dimethoxytrityloxy-2-aminopropanol). A particularly preferred tether for the synthesis of ligand conjugated oligonucleotides of the invention is an α,β-unsaturated amide linker. A variety of heterobifunctional and homobifunctional tethers are available from Pierce Co. (Rockford, Ill.). Such heterobifunctional and homobifunctional tethers are particularly useful in conjunction with the 6-aminoalkoxy and 6-aminoalkylamino moieties to form extended tethers useful for linking ligands to a nucleoside.

In certain instances, conjugation of ligand molecules is achieved by conjugation of the ligand to an amino tether on the nucleoside. This can be effected in several ways. For example, a ligand-nucleoside conjugate of the invention can be prepared by conjugation of the ligand molecule to the nucleoside using EDC/sulfo-NHS (i.e. 1-ethyl-3(3-dimethylaminopropylcarbodiimide/N-hydroxysulfosuccinimide) to conjugate the carboxylate function of the ligand with the amino function of the linking group on the nucleoside.

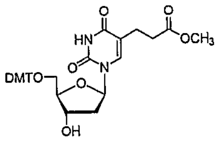
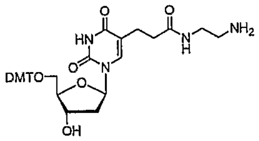
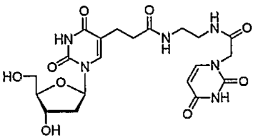
The ligand-conjugated oligonucleotides of the present invention may be prepared by conjugation of the ligand molecule to the nucleoside sequence via a heterobifunctional tether such as m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester (MBS) or succinimidyl 4-(N-maleimidomethyl)cyclo-hexane-1-carboxylate (SMCC), to link a nucleophilic position on the ligand molecule to the amino function of the tether group on nucleoside sequence. By this mechanism, an oligonucleoside-maleimide conjugate is formed by reaction of the amino group of the tether on the linked nucleosides with the MBS or SMCC maleimide linker. The conjugate is then reacted with the ligand.

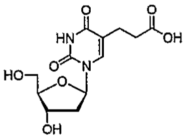
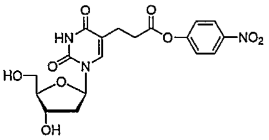
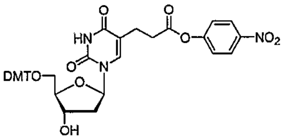
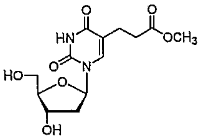
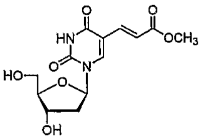
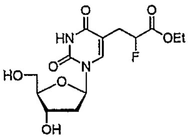
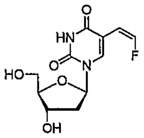
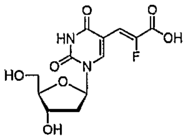
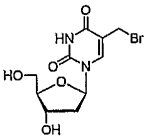
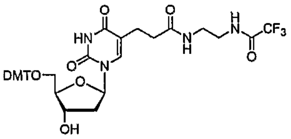
Alternatively, a ligand conjugated-oligonucleotide can be prepared by conjugation of the ligand molecule to the oligonucleotide or nucleoside via a homobifunctional tether such as disuccinimidyl suberate (DSS), to link an amino function on the ligand to the amino group of a tether on the oligonucleotide sequence. By this mechanism, an oligonucleoside-succinimidyl conjugate is formed by reaction of the amino group of the tether on the nucleoside sequence with a disuccinimidyl suberate tether. The disuccinimidyl suberate tether couples with the amine tether on the nucleoside to extend the size of the tether. The extended tether is then reacted with an amino group of the ligand molecule.

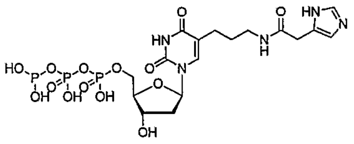
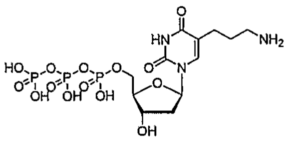
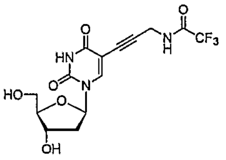
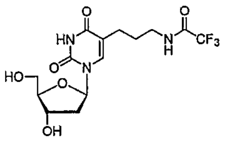
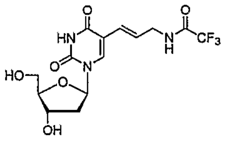
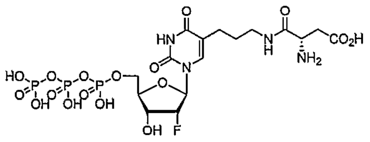
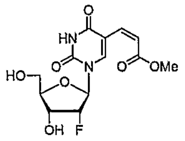
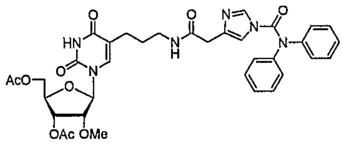
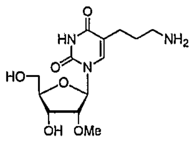
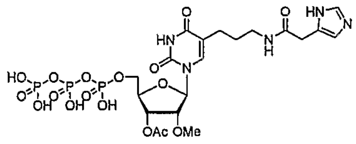
Ligand-bearing Nucleotides

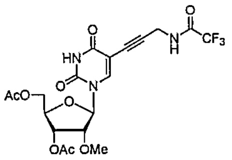
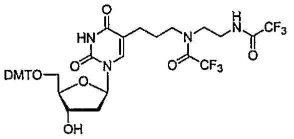
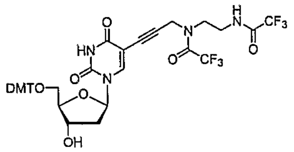
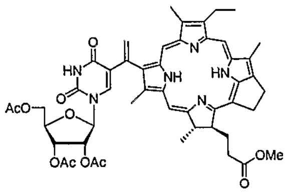
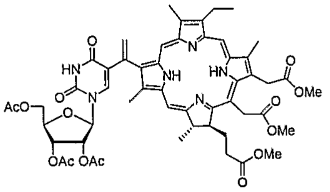
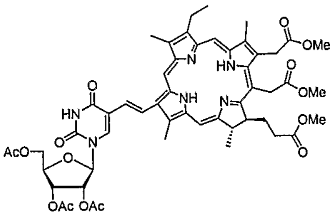
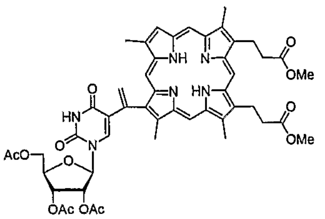
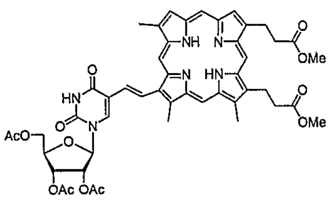
The ligand-bearing nucleotides of the invention are pyrimidine-bearing nucleotides, wherein the C5-position of the pyrimidine base has been modified to form a covalent bond with the linker. In certain instances, the base of the pyrimidine-bearing nucleotide is a C5-modified thymine, cytosine, or uracil. As described above, the base, sugar, or phosphate portions of the nucleotide may be modified to incorporate unnatural substituents.

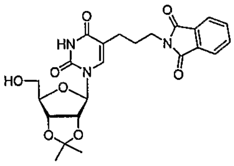
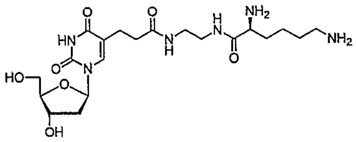
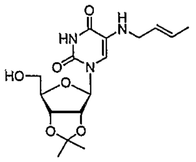
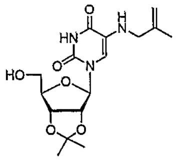
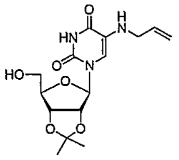
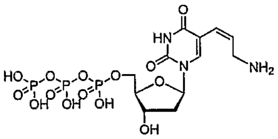
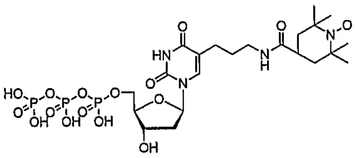
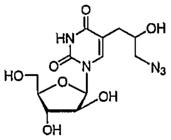
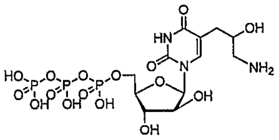
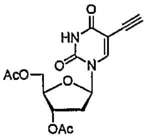
A representative listing of C5-modified pyrimidine nucleosides and nucleotides amenable to the present invention are presented below.

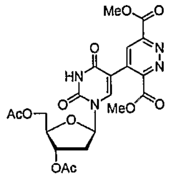
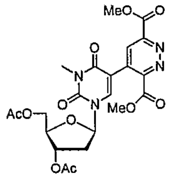
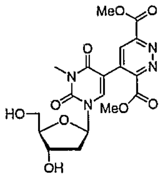
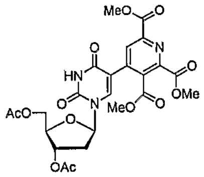
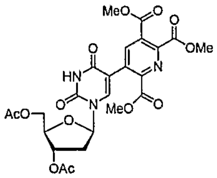
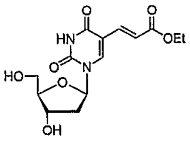
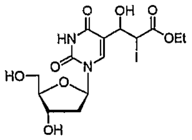
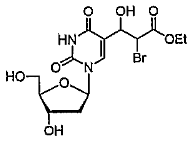
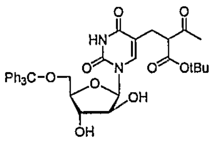
Compound	**References**
	*Bioorg. Med. Chem. Lett.* **2002**, *12(6)*, 981-983 U.S. Patent 4,837,312 *J. Am. Chem. Soc.* **1989**, *111(18)*, 6966-76.
	*Bioorg. Med. Chem. Lett.* **2002**, *12(6)*, 981-983 U.S. Patent 4,837,312 (06 Jun 1989) *J. Am. Chem. Soc.* **1989**, *111(18)*, 6966-76 *J. Org. Chem.* **1991**, *56(9)*, 3168-76
	*Bioorg. Med. Chem. Lett.* **2002**, *12(6)*, 981-983

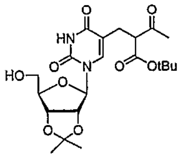
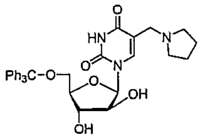
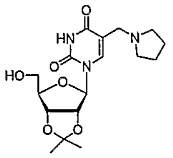
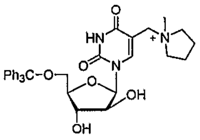
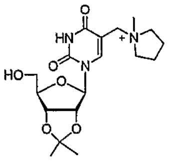
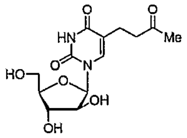
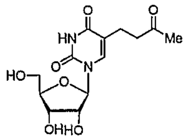
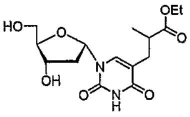
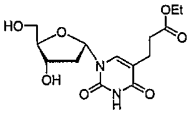
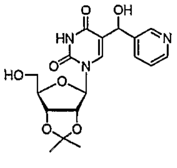
	*J. Org. Chem.* **1990**, *55(17)*, 5125-32 U.S. Patent 4,837,312, (06 Jun 1989)
	*J. Org. Chem.* **1990**, *55(17)*, 5125-32
	*J. Org. Chem.* **1990**, *55(17)*, 5125-32
	U.S. Patent 4,837,312 (06 Jun 1989)
	U.S. Patent 4,837,312 (06 Jun 1989)
	*Ger. (East)*, 259803, 07 Sep 1988
	*Ger. (East)*, 259803, 07 Sep 1988
	*Ger. (East)*, 259803, 07 Sep 1988
	*Ger. (East)*, 259803, 07 Sep 1988
	*J. Am. Chem. Soc.* **1989**, *111(18)*, 6966-76

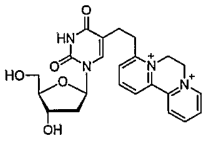
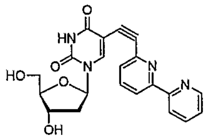
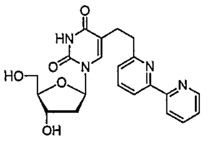
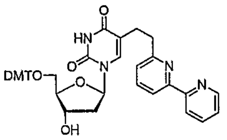
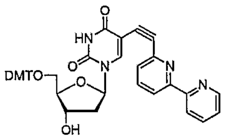
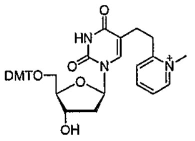
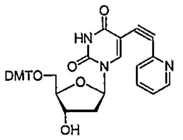
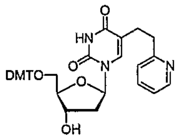
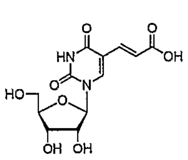
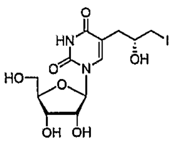
	*Nucleic Acids Res.* **2001**, *29(7)*, 1565-1573
	*Nucleic Acids Res.* **2001**, *29(7)*, 1565-1573 *Helv. Chim. Acta* **1989**, *72(1)*, 110-16
	*Nucleic Acids Res.* **2001**, *29(7)*, 1565-1573
	*Nucleic Acids Res.* **2001**, *29(7)*, 1565-1573
	*Nucleic Acids Res.* **2001**, *29(7)*, 1565-1573
	*Bioorg. Med. Chem. Lett.* **2000**, *10(11)*, 1299-1302
	*Bioorg. Med. Chem. Lett.* **2000**, *10(11)*, 1299-1302
	*Bioorg. Med. Chem. Lett.* **2000**, *10(11)*, 1299-1302
	*Bioorg. Med. Chem. Lett.* **2000**, *10(11)*, 1299-1302
	*Bioorg. Med. Chem. Lett.* **2000**, *10(11)*, 1299-1302

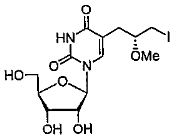
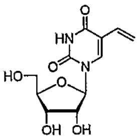
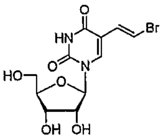
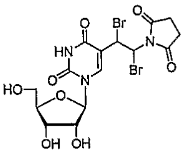
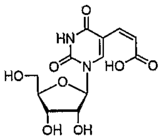
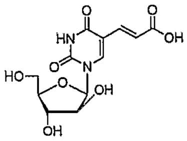
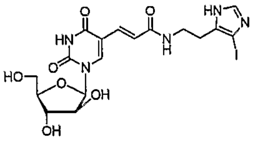
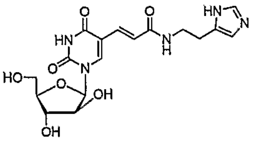
	*Bioorg. Med. Chem. Lett.* **2000**, *10(11)*, 1299-1302
	*Nucleosides & Nucleotides* **1999**, *18(6 & 7)*, 1633-37
	*Nucleosides & Nucleotides* **1999**, *18(6 & 7)*, 1633-37
	*Tetrahedron Lett.* **1995**, *36(3)*, 365-68
	*Tetrahedron Lett.* **1995**, *36(3)*, 365-68
	*Tetrahedron Lett.* **1995**, *36(3)*, 365-68
	*Tetrahedron Lett.* **1995**, *36(3)*, 365-68
	*Tetrahedron Lett.* **1995**, *36(3)*, 365-68

	*Afinidad* **1994**, *51(449)*, 71-3
	*J. Org. Chem.* **1991**, *56(9)*, 3168-76
	*Heterocycles* **1990**, *30(1)*, 231-35
	*Heterocycles* **1990**, *30(1)*, 231-35
	*Heterocycles* **1990**, *30(1)*, 231-35
	*Helv. Chim. Acta* **1989**, *72(1)*, 110-16
	*Helv. Chim. Acta* **1989**, *72(1)*, 110-16
	*Chem. Pharm. Bull.* **1987**, *35(12)*, 4829-38
	*Chem. Pharm. Bull.* **1987**, *35(12)*, 4829-38
	*J. Org. Chem.* **1986**, *51(6)*, 950-1

	*J. Org. Chem.*, **1986**, *51(6)*, 950-1
	*J. Org. Chem.* **1986**, *51(6)*, 950-1
	*J. Org. Chem.* **1986**, *51(6)*, 950-1
	*J. Org. Chem.* **1986**, *51(6)*, 950-1
	*J. Org. Chem.* **1986**, *51(6)*, 950-1
	*J. Med. Chem.* **1989**, *32(5)*, 941-4
	*J. Med. Chem.* **1989**, *32(5)*, 941-4
	*J. Med. Chem.* **1989**, *32(5)*, 941-4
	*J. Chem. Soc., Chem. Commun.* **1987**, *(22)*, 1732-4

	*J. Chem. Soc., Chem. Commun.* **1987**, *(22)*, 1732-4
	*J. Chem. Soc., Chem. Commun.* **1987**, *(22)*, 1732-4
	*J. Chem. Soc., Chem. Commun.* **1987**, *(22)*, 1732-4
	*J. Chem. Soc., Chem. Commun.* **1987**, *(22)*, 1732-4
	*J. Chem. Soc., Chem. Commun.* **1987**, *(22)*, 1732-4
	*J. Chem. Soc., Chem. Commun.* **1987**, *(22)*, 1732-4
	*J. Chem. Soc., Chem. Commun.* **1987**, *(22)*, 1732-4
	*Eur. J. Med. Chem.* **1985**, *20(2)*, 105-10
	*Eur. J. Med. Chem.* **1985**, *20(2)*, 105-10
	*Tetrahedron Lett.* **2003**, *44(10)*, 2179-81 *Nucleic Acids Research Supplement,* **2002**, *2(Twenty-ninth Symposium on Nucleic Acids Chemistry)*, 13-14

	*Nucleosides, Nucleotides & Nucleic Acids* **2002,** *21(8 & 9)*, 547-560
	*Nucleosides, Nucleotides & Nucleic Acids* **2002,** *21(8 & 9)*, 547-560
	*Nucleosides, Nucleotides & Nucleic Acids* **2002,** *21(8 & 9)*, 547-560
	*Nucleosides, Nucleotides & Nucleic Acids* **2002,** *21(8 & 9)*, 547-560
	*Nucleosides, Nucleotides & Nucleic Acids* **2002,** *21(8 & 9)*, 547-560
	*Nucleosides, Nucleotides & Nucleic Acids* **2002,** *21(10)*, 681-694
	*Nucleosides, Nucleotides & Nucleic Acids* **2002,** *21(10)*, 681-694
	*Nucleosides, Nucleotides & Nucleic Acids* **2002,** *21(10)*, 681-694
	*J. Med. Chem.* **1990,** *33(2)*, 717-23. *Tetrahedron* **1987,** *43(20)*, 4601-8. *J. Med. Chem.* **1986,** *29(2)*, 213-217.
	*J. Med. Chem.* **1990,** *33(2)*, 717-23.

	*J. Med. Chem.* **1990**, *33(2)*, 717-23.
	*J. Med. Chem.* **1990**, *33(2)*, 717-23.
	*Tetrahedron* **1987**, *43(20)*, 4601-8. *J. Med. Chem.* **1986**, *29(2)*, 213-217.
	*Tetrahedron* **1987**, *43(20)*, 4601-8.
	*Drug Design and Discovery* **1992**, *8(3)*, 179-89. Eur. Pat. Appl. (1991), 20 pp. CODEN: EPXXDW EP 439117 A1 19910731 *Jpn. Kokai Tokkyo Koho* (1984), 4 pp. CODEN: JKXXAF JP 59163394 A2 19840914 Showa.
	*Antiviral Chemistry & Chemotherapy* **1993**, *4(1)*, 11-17. *Therapeutic Drug Monitoring* **1992**, *14(6)*, 499-508.
	*Therapeutic Drug Monitoring* **1992**, *14(6)*, 499-508.
	*Therapeutic Drug Monitoring* **1992**, *14(6)*, 499-508.

Additional Features of Oligonuclotides

The siRNA agent should include a region of sufficient homology to the target gene, and be of sufficient length in terms of nucleotides, such that the siRNA agent, or a fragment thereof, can mediate down regulation of the target gene.. It will be understood that the term "ribonucleotide" or "nucleotide" can, in the case of a modified RNA or nucleotide surrogate, also refer to a modified nucleotide, or surrogate replacement moiety at one or more positions. Thus, the siRNA agent is or includes a region which is at least partially complementary to the target RNA. In certain embodiments, the siRNA agent is fully complementary to the target RNA. It is not necessary that there be perfect complementarity between the siRNA agent and the target, but the correspondence must be sufficient to enable the siRNA agent, or a cleavage product thereof, to direct sequence specific silencing, such as by RNAi cleavage of the target RNA. Complementarity, or degree of homology with the target strand, is most critical in the antisense strand. While perfect complementarity, particularly in the antisense strand, is often desired some embodiments can include one or more but preferably 6, 5, 4, 3, 2, or fewer mismatches with respect to the target RNA. The mismatches are most tolerated in the terminal regions, and if present are preferably in a terminal region or regions, *e.g.*, within 6, 5, 4, or 3 nucleotides of the 5' and/or 3' terminus. The sense strand need only be sufficiently complementary with the antisense strand to maintain the over all double-strand character of the molecule.

In addition, a siRNA agent will often be modified or include nucleoside surrogates. Single stranded regions of an siRNA agent will often be modified or include nucleoside surrogates, *e.g.*, the unpaired region or regions of a hairpin structure, *e.g.*, a region which links two complementary regions, can have modifications or nucleoside surrogates. Modification to stabilize one or more 3'- or 5'-terminus of an iRNA agent, *e.g.*, against exonucleases, or to favor the antisense siRNA agent to enter into RISC are also favored. Modifications can include C3 (or C6, C7, C12) amino linkers, thiol linkers, carboxyl linkers, non-nucleotidic spacers (C3, C6, C9, C12, abasic, triethylene glycol, hexaethylene glycol), special biotin or fluorescein reagents that come as phosphoramidites and that have another DMT-protected hydroxyl group, allowing multiple couplings during RNA synthesis.

siRNA agents include: molecules that are long enough to trigger the interferon response (which can be cleaved by Dicer (Bernstein *et al.* 2001. Nature, 409:363-366) and enter a RISC (RNAi-induced silencing complex)); and, molecules which are sufficiently short that they do not trigger the interferon response (which molecules can also be cleaved by Dicer and/or enter a RISC), *e.g.*, molecules which are of a size which allows entry into a RISC, *e.g.*, molecules which resemble Dicer-cleavage products. Molecules that are short enough that they do not trigger an interferon response are termed siRNA agents or shorter iRNA agents herein. “siRNA agent or shorter siRNA agent” as used refers to an siRNA agent that is sufficiently short that it does not induce a deleterious interferon response in a human cell, *e.g.*, it has a duplexed region of less than 60 but preferably less than 50, 40, or 30 nucleotide pairs. The siRNA agent, or a cleavage product thereof, can down regulate a target gene, *e.g.*, by inducing RNAi with respect to a target RNA, preferably an endogenous or pathogen target RNA.

Each strand of a siRNA agent can be equal to or less than 30, 25, 24, 23, 22, 21, or 20 nucleotides in length. The strand is preferably at least 19 nucleotides in length. For example, each strand can be between 21 and 25 nucleotides in length. Preferred siRNA agents have a duplex region of 17, 18, 19, 29, 21, 22, 23, 24, or 25 nucleotide pairs, and one or more overhangs, preferably one or two 3’ overhangs, of 2- 3 nucleotides.

In addition to homology to target RNA and the ability to down regulate a target gene, an siRNA agent will preferably have one or more of the following properties:

(1) it will, despite modifications, even to a very large number, or all of the nucleosides, have an antisense strand that can present bases (or modified bases) in the proper three dimensional framework so as to be able to form correct base pairing and form a duplex structure with a homologous target RNA which is sufficient to allow down regulation of the target, *e.g.*, by cleavage of the target RNA;

(2) it will, despite modifications, even to a very large number, or all of the nucleosides, still have “RNA-like” properties, *i.e.*, it will possess the overall structural, chemical and physical properties of an RNA molecule, even though not exclusively, or even partly, of ribonucleotide-based content. For example, an siRNA agent can contain, *e.g.*, a sense and/or an antisense strand in which all of the nucleotide sugars contain *e.g.*, 2’ fluoro in place of 2’ hydroxyl. This deoxyribonucleotide-containing agent can still be expected to exhibit RNA-like properties. While not wishing to be bound by theory, the electronegative fluorine prefers an axial orientation when attached to the C2’ position of ribose. This spatial preference of fluorine can, in turn, force the sugars to adopt a $C_{3'}$-*endo*

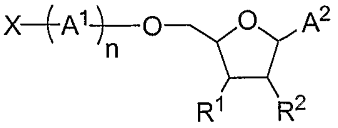
pucker. This is the same puckering mode as observed in RNA molecules and gives rise to the RNA-characteristic A-family-type helix. Further, since fluorine is a good hydrogen bond acceptor, it can participate in the same hydrogen bonding interactions with water molecules that are known to stabilize RNA structures. Generally, it is preferred that a modified moiety at the 2' sugar position will be able to enter into H-bonding which is more characteristic of the OH moiety of a ribonucleotide than the H moiety of a deoxyribonucleotide. A preferred siRNA agent will: exhibit a $C_{3'}$-*endo* pucker in all, or at least 50, 75,80, 85, 90, or 95 % of its sugars; exhibit a $C_{3'}$-*endo* pucker in a sufficient amount of its sugars that it can give rise to a the RNA-characteristic A-family-type helix; will have no more than 20, 10, 5, 4, 3, 2, or1 sugar which is not a $C_{3'}$-*endo* pucker structure.

A "single strand iRNA agent" as used herein, is an iRNA agent which is made up of a single molecule. It may include a duplexed region, formed by intra-strand pairing, *e.g.*, it may be, or include, a hairpin or pan-handle structure. Single strand iRNA agents are preferably antisense with regard to the target molecule. A single strand iRNA agent should be sufficiently long that it can enter the RISC and participate in RISC mediated cleavage of a target mRNA. A single strand iRNA agent is at least 14, and more preferably at least 15, 20, 25, 29, 35, 40, or 50nucleotides in length. It is preferably less than 200, 100, or 60 nucleotides in length.

Hairpin iRNA agents will have a duplex region equal to or at least 17, 18, 19, 29, 21, 22, 23, 24, or 25 nucleotide pairs. The duplex region will preferably be equal to or less than 200, 100, or 50, in length. Preferred ranges for the duplex region are 15-30, 17 to 23, 19 to 23, and 19 to 21 nucleotides pairs in length. The hairpin will preferably have a single strand overhang or terminal unpaired region, preferably the 3', and preferably of the antisense side of the hairpin. Preferred overhangs are 2-3 nucleotides in length.

The compounds of the invention are described below in greater detail. Importantly, the embodiments described below are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

One aspect of the present invention relates to a single-stranded oligonucleotide represented by formula **I**:



I

wherein

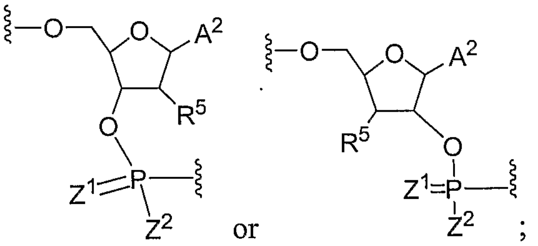
X is H, -P(O)(OM)$_2$, -P(O)(OM)-O-P(O)(OM)$_2$, -P(O)(Oalkyl)$_2$, or -P(O)(Oalkyl)-O-P(O)(Oalkyl)$_2$;

M represents independently for each occurrence an alkali metal or a transition metal with an overall charge of +1;

n is 16, 17, 18 , 19, 20, 21, 22, 23, or 24;

R^1, R^2, and R^5 represent independently for each occurrence H, OH, F, -Oalkyl, -Oallyl, -O(C(R^7)$_2$)$_v$OR^7, -O(C(R^7)$_2$)$_v$SR^7, -O(C(R^7)$_2$)$_v$N(R^7)$_2$, -O(C(R^7)$_2$)$_v$C(O)N(R^7)$_2$, -N(R^7)$_2$, -S(C$_1$-C$_6$)alkyl, -O(C(R^7)$_2$)$_v$O(C$_1$-C$_6$)alkyl, -O(C(R^7)$_2$)$_v$S(C$_1$-C$_6$)alkyl, -O(C(R^7)$_2$)$_v$O(C(R^7)$_2$)$_v$N((C$_1$-C$_6$)alkyl)$_2$, or -O(C(R^7)$_2$)$_v$ON((C$_1$-C$_6$)alkyl)$_2$;

A^1 represents independently for each occurrence:



Z^1 represents independently for each occurrence O or S;

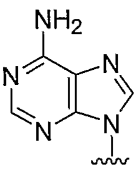
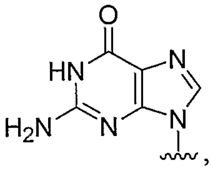
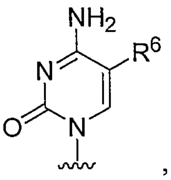
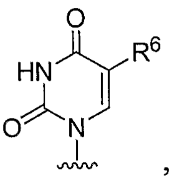
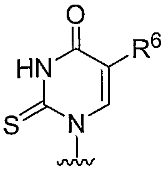
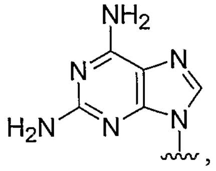
Z^2 represents independently for each occurrence -OH, -OM, -Oalkyl, -Oaryl, -Oaralkyl, -SH, -SM, -Salkyl, -Saryl, -Saralkyl, -N(R^3)R^4, -(C(R^7)$_2$)$_m$N(R^7)$_2$, -N(R^7)(C(R^7)$_2$)$_m$N(R^7)$_2$, or alkyl;

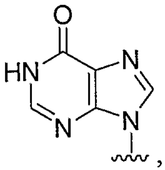
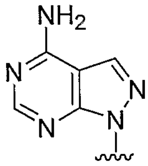
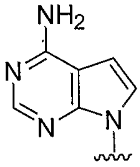
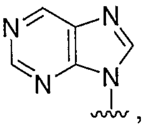
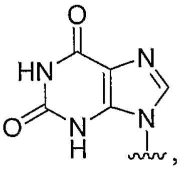
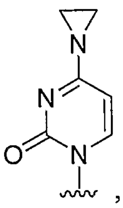
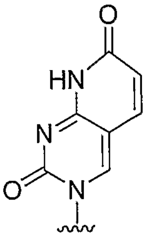
R^3 and R^4 are H or alkyl; or R^3 and R^4 taken together form a 3-, 4-, 5-, 6-, or 7-member ring;

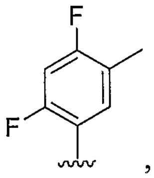
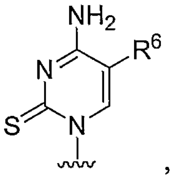
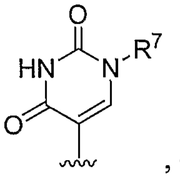
R^6 represents independently for each occurrence H, alkyl, or -NHCH$_2$CH=CH$_2$;

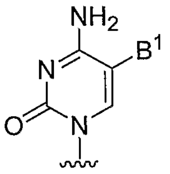
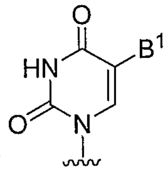
R^7 represents independently for each occurrence H or alkyl;

A^2 represents independently for each occurrence:

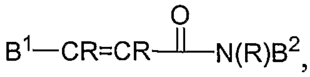
, , , , , ,

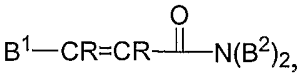
, , , , , , ,

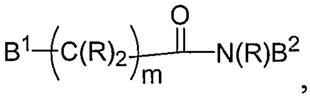
, , , or $-A^3-A^4-(A^5)_w$;

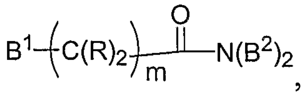
A^3 represents independently for each occurrence  or  ;

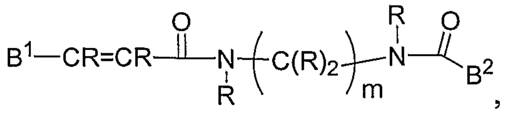
A^4 represents independently for each occurrence a bond, alkyl diradical, heteroalkyl diradical, alkenyl diradical, aminoalkyl diradical, alkynyl diradical, alkylalkynyl diradical, thioether, -C(O)-, -S(O)-, -S(O)$_2$-, $B^1C(R)_2B^2$, $B^1C(R)(B^2)_2$, $B^1C(B^2)_3$, $B^1N(R)(B^2)$, $B^1N(B^2)_2$, or has the formula:

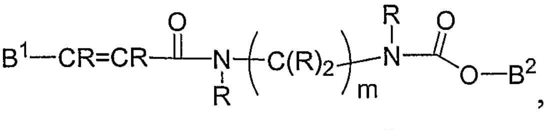

$B^1-CR=CR-C(O)-N(R)B^2$,

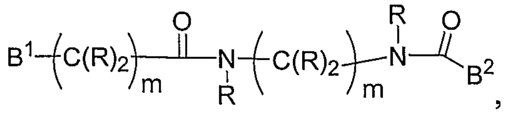

$B^1-CR=CR-C(O)-N(B^2)_2$,

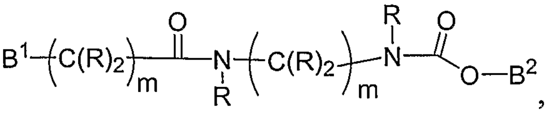

$B^1-(C(R)_2)_m-C(O)-N(R)B^2$,

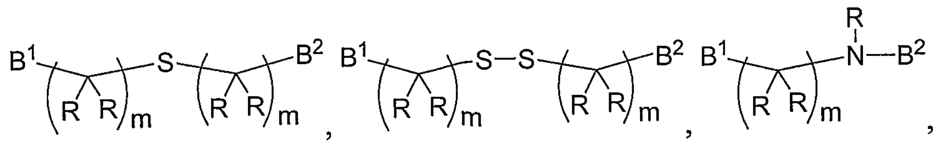

$B^1-(C(R)_2)_m-C(O)-N(B^2)_2$,

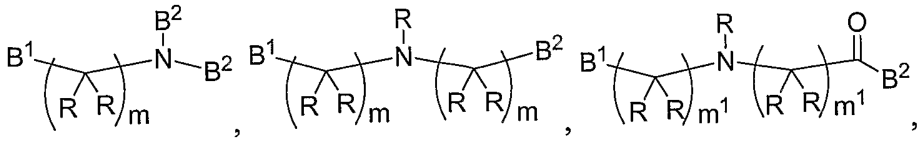

$B^1-CR=CR-C(O)-N(R)-(C(R)_2)_m-N(R)-C(O)-B^2$,

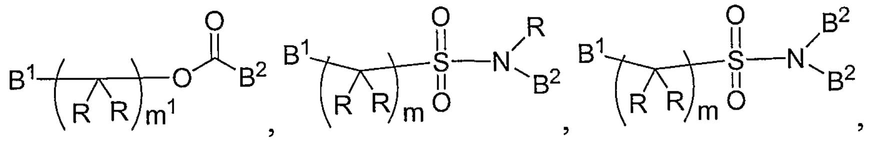

$B^1-CR=CR-C(O)-N(R)-(C(R)_2)_m-N(R)-C(O)-O-B^2$,

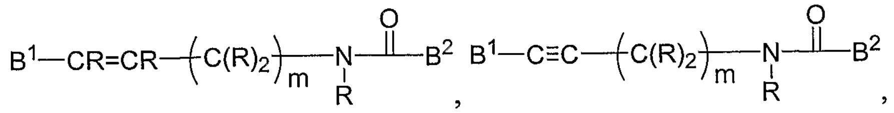

$B^1-(C(R)_2)_m-C(O)-N(R)-(C(R)_2)_m-N(R)-C(O)-B^2$,

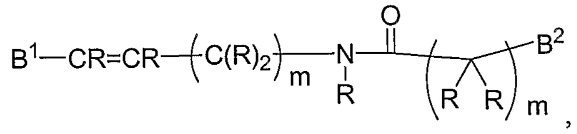

$B^1-(C(R)_2)_m-C(O)-N(R)-(C(R)_2)_m-N(R)-C(O)-O-B^2$,

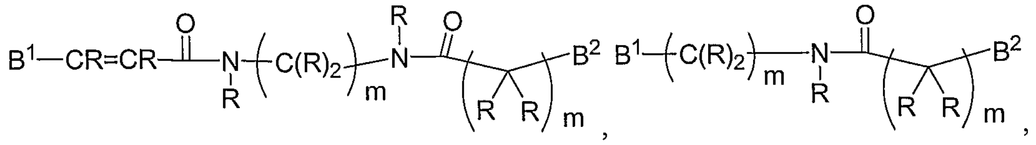


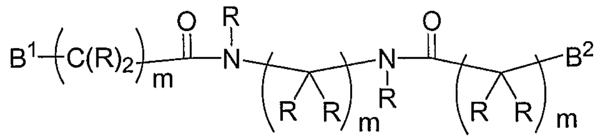












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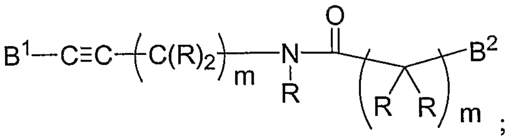


;

B^1 represents independently for each occurrence a bond between A^3 and A^4;

B^2 represents independently for each occurrence a bond between A^4 and A^5;

R represents independently for each occurrence hydrogen or alkyl;

m represents independently for each occurrence 1, 2, 3, 4, 5, 6, 7 or 8;

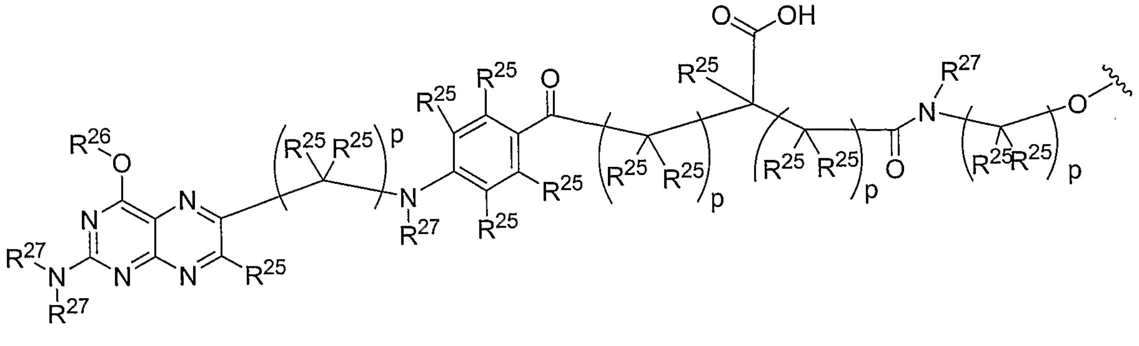
m^1 represents independently for each occurrence 0, 1, 2, 3, 4, 5, 6, 7 or 8;

v represents independently for each occurrence 1, 2, 3, or 4;

w represents independently for each occurrence 1, 2, or 3 in accord with the rules of valence;

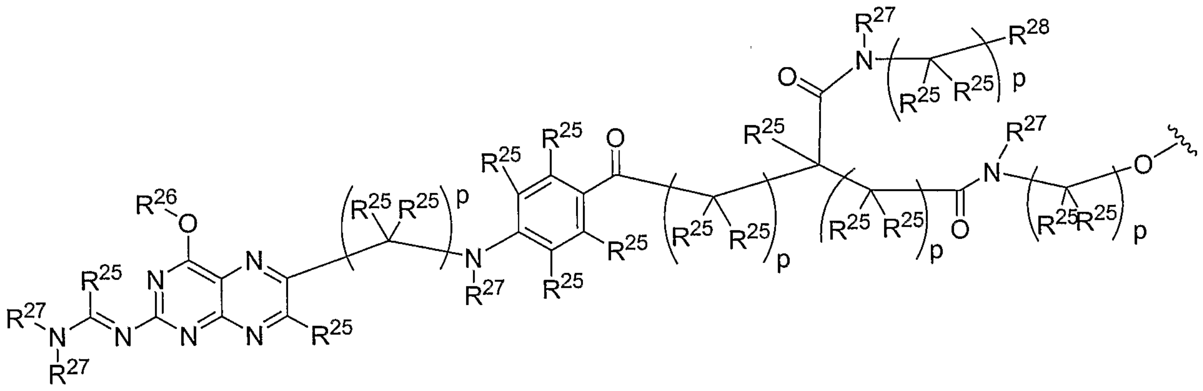
provided that A^5 occurs at least once; and

A^5 represents independently for each occurrence compound **C**, compound **D**, or a radical of a steroid, bile acid, lipid, folic acid, pyridoxal, B12, riboflavin, biotin, aromatic compound, polycyclic compound, crown ether, intercalator, cleaver molecule, protein-binding agent, carbohydrate, or an optionally substituted saturated 5-membered ring; wherein compound **C** is represented by:



C

and compound **C'** is represented by:



C'

wherein for **C** and **C'**

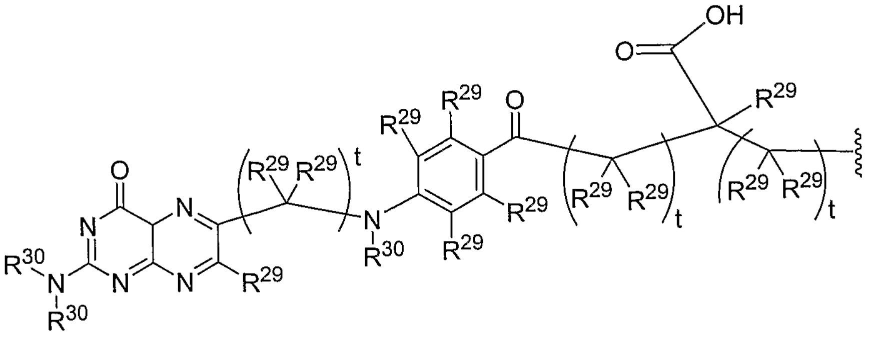
R^{25} represents independently for each occurrence H, halogen, hydroxyl, thiol, alkoxyl, thioalkyl, azido, -N(R^{27})$_2$, -CO$_2$$R^{27}$, -COR^{27}, -OC(O)R^{27}, alkyl, alkenyl, aryl, or aralkyl;

R^{26} represents independently for each occurrence alkyl, alkenyl, aryl, or aralkyl;

R^{27} represents independently for each occurrence H, alkyl, alkenyl, aryl, or aralkyl;

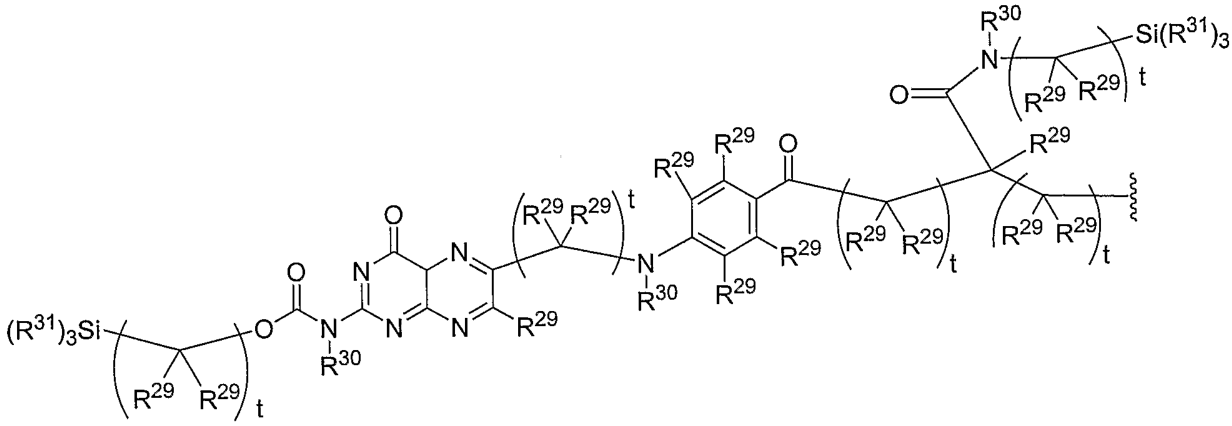
R^{28} represents independently for each occurrence -OC(O)R^{27}, -N(R^{27})C(O)R^{27}, -N(R^{27})$_2$, -CO$_2$R^{27}, -COR^{27}, alkyl, alkenyl, aryl, or aralkyl; and

p represents independently for each occurrence 1, 2, 3, 4, 5, 6, or 7; and compound **D** is represented by:



D

and compound **D'** is represented by:



D'

wherein for **D** and **D'**

R^{29} represents independently for each occurrence H, halogen, hydroxyl, thiol, alkoxyl, thioalkyl, azido, $-N(R^{30})_2$, $-CO_2R^{30}$, $-COR^{30}$, $-OC(O)R^{30}$, alkyl, alkenyl, aryl, or aralkyl;

R^{30} represents independently for each occurrence H, alkyl, alkenyl, aryl, or aralkyl;

R^{31} represents independently for each occurrence alkyl, alkenyl, aryl, or aralkyl; and

t represents independently for each occurrence 1, 2, 3, 4, 5, 6, or 7.

In certain embodiments, the present invention relates to the aforementioned compound, wherein n is 18, 19, 20, 21, or 22.

In certain embodiments, the present invention relates to the aforementioned compound, wherein n is 20.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 occurs at least twice.

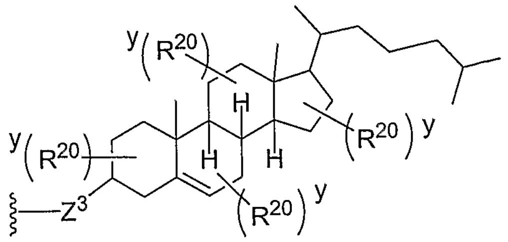
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 occurs at least five times.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 occurs at least ten times.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence compound **C**, compound **D**, or a radical of cholesterol, 5β-cholanic acid, progesterone, aldosterone, dehydroaldosterone, isoandrosterone, esterone, estradiol, ergosterol, dehydroergosterol, lanosterol, 4-cholesten-3-one, guggulsterone, testosterone, nortestosterone, formestane, hydroxyecdysone, ketoestriol, corticosterone, dienestrol, dihydroxypregnanone, pregnanone, copornmon, equilenin, equilin, estriol, ethinylestradiol, mestranol, moxestrol, mytatrienediol, quinestradiol, quinestrol, helvolic acid, protostadiene, fusidic acid, cycloartenol, tricallol, cucurbitanin cedrelone, euphol, dammerenediol, parkeol, dexametasone, methylprednisolone, prednisolone, hydrocortisone, parametasone, betametasone, cortisone, fluocinonide, fluorometholone, halcinonide, budesonide, or any one of them further substituted with one or more of hydroxyl, halogen, amino, alkylamino, alkyl, carboxylic acid, ester, amide, carbonyl, alkoxyl, or cyano.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence a radical of cholesterol or 5β-cholanic acid.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence:



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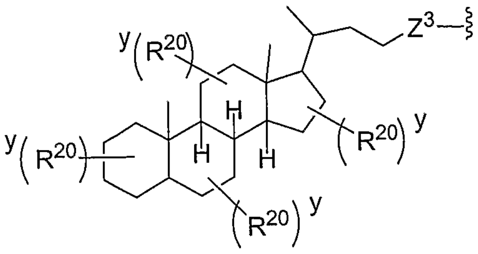


,

wherein R^{20} represents independently for each occurrence hydroxyl, amino, halogen, alkoxyl, alkyl, aminoalkyl, azido, carbonyl, acyl, or acyloxy; Z^3 represents independently for each occurrence O, S, or NR; and y represents independently for each occurrence 1, 2, 3, 4, 5, or 6 in accord with the rules of valence.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence a radical of cholic acid, deoxycholic acid, taurocholic acid, glycocholic acid, glycodeoxycholic acid, taurodeoxycholic acid, ursodeoxycholic acid, or chenodeoxycholic acid.

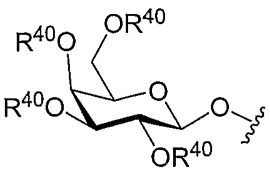
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence a radical of lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, triacylglycerol, phosphoacylglycerol, sphingolipid, monoterpene, sesquiterpene, diterpene, sesterterpene, triterpene, or tetraterpene.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence optionally substituted phenyl, naphthyl, anthracenyl, phenanthrenyl, pyrenyl, pyridinyl, quinolinyl, acridinyl, phenathridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinoxalinyl, quinazolinyl, 1,7-phenanthrolinyl, indolyl, thianaphthenyl, benzoxazolyl, benzofuranyl, 1,2-benzisoxazolyl, benzimidazolyl, pyrrolyl, thiophenyl, isoxazolyl, pyrazolyl, thiazolyl, imidazolyl, tetrazolyl, or furanyl.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence the radical of erythrose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, mannose, gulose, idose, galactose, or talose; or a disaccharide or trisaccharide formed via a 1,4 glycoside linkage between any of them.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is a hexose or pentose.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is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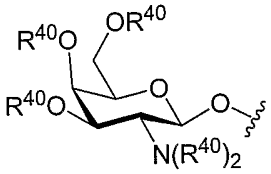
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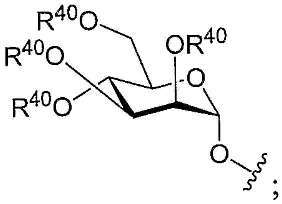
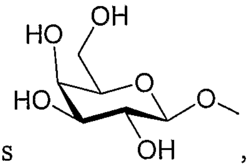

; wherein R^{40} represents independently for each occurrence H, alkyl, aryl, aralkyl, -C(O)alkyl, -C(O)aryl, -C(O)aralkyl, or $Si(alkyl)_3$.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is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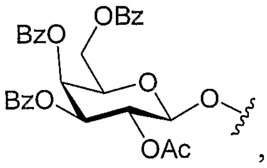
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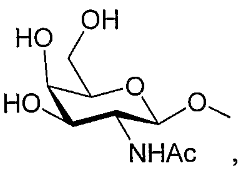
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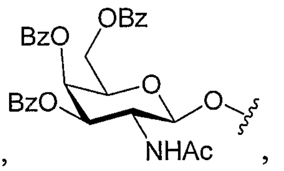
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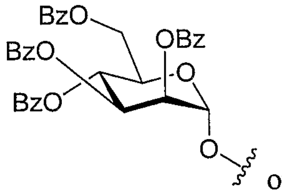
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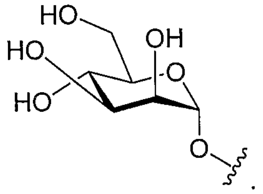
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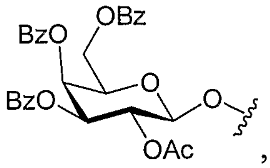
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is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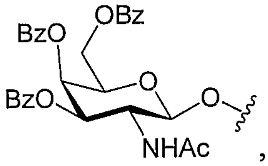
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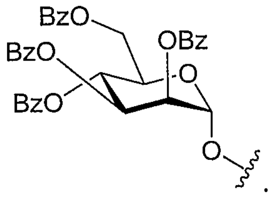
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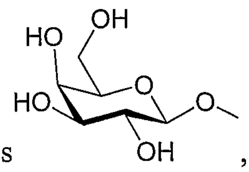
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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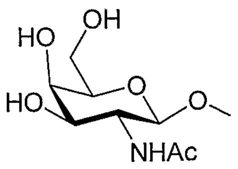

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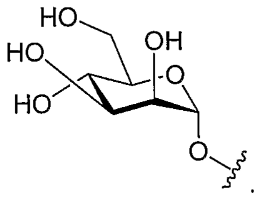
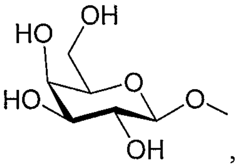


.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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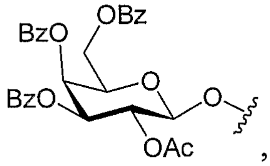

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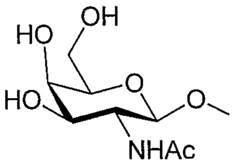

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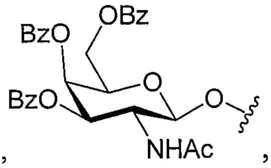

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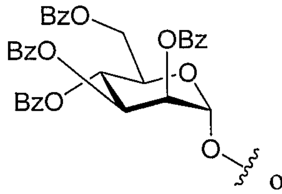

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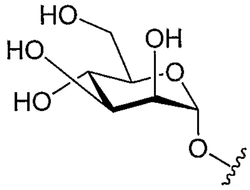
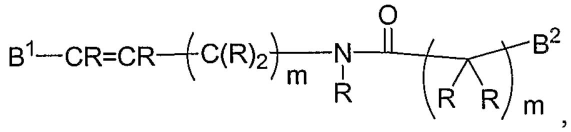


; and A^4 is



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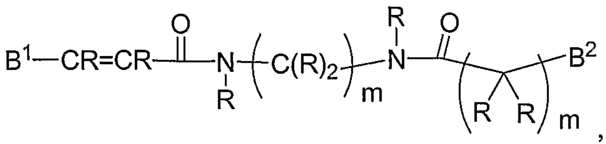

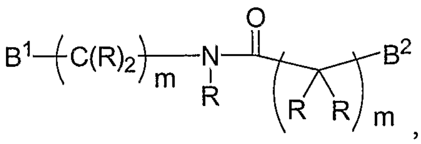

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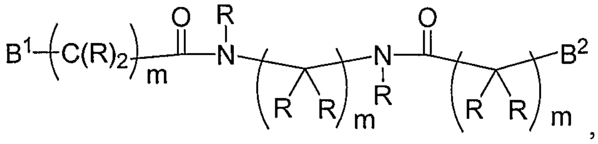

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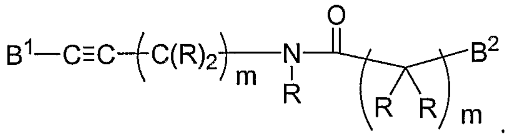

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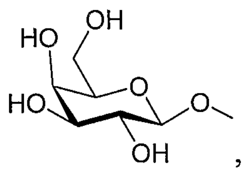
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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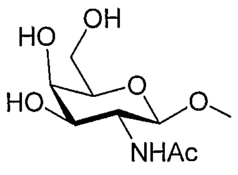

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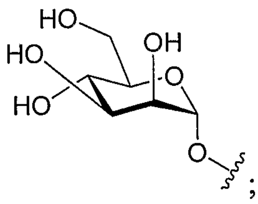
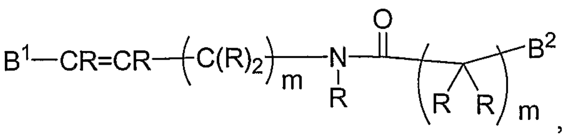


; and A^4 is



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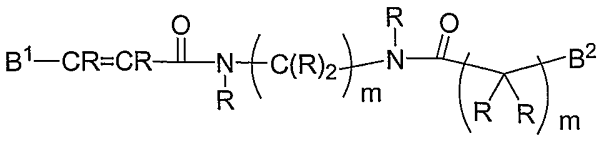
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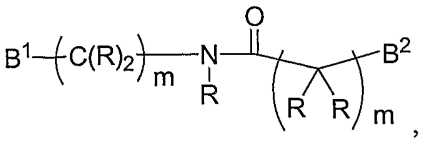
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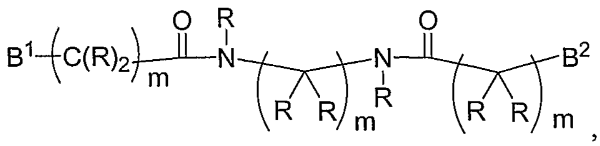
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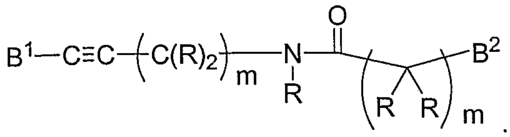
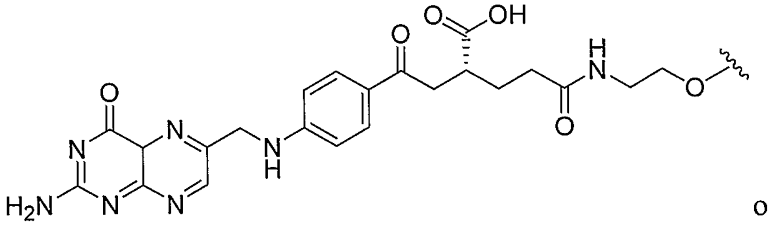

.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is an optionally substituted cyclopentane, pyrrolidine, tetrahydrofuran, tetrahydrothiophene, or 1,1-difluorocyclopentane.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is compound **C**.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is compound **C'**.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is

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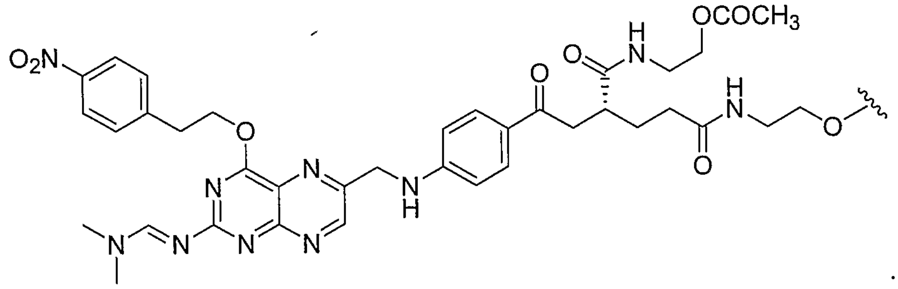
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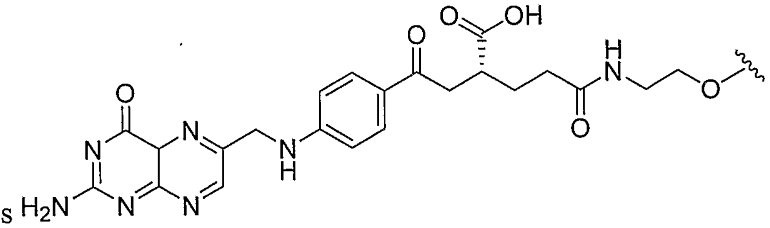
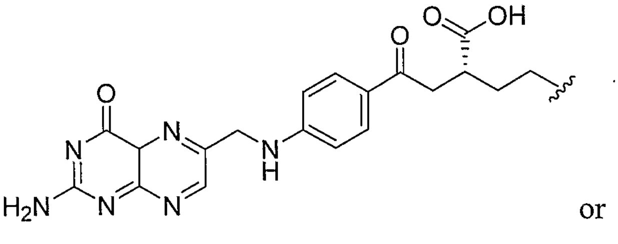
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is

.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is compound **D**.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is compound **D'**.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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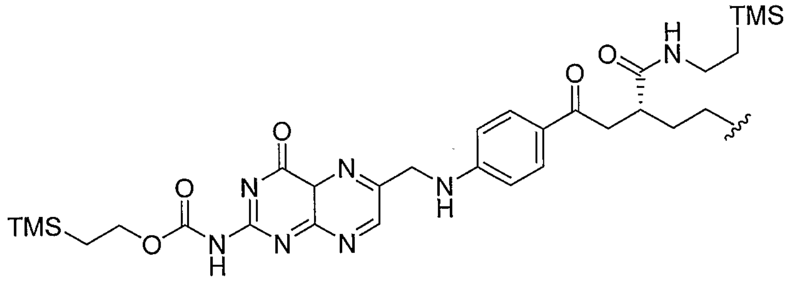

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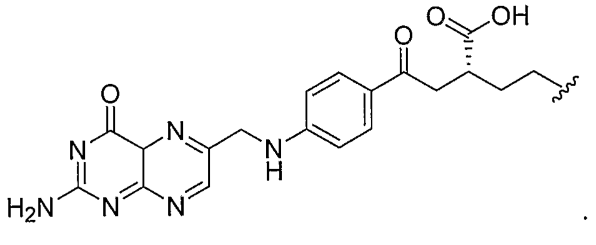
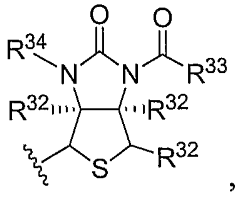
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



.

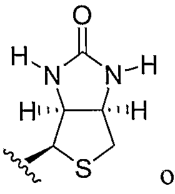
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is a radical of a polycyclic compound.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



, wherein R^{32} represents independently for each occurrence H, halogen, hydroxyl, thiol, alkoxyl, thioalkyl, azido, $-N(R^{34})_2$, $-CO_2R^{34}$, $-COR^{34}$, $-OC(O)R^{34}$, alkyl, alkenyl, aryl, or aralkyl; R^{33} is aryl that is optionally substituted with alkyl, alkenyl, aryl, halogen, or alkoxyl; and R^{34} represents independently for each occurrence H, alkyl, aryl, or aralkyl.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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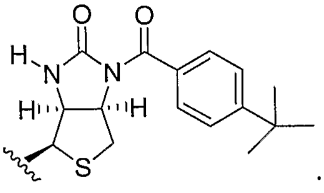
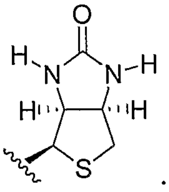


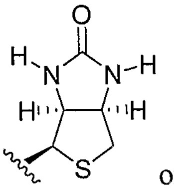
.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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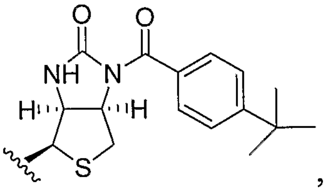
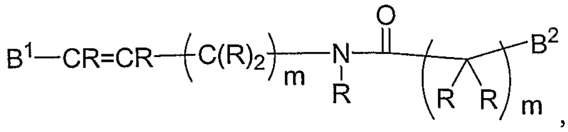


, and A^4 is



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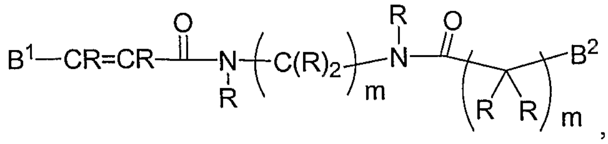

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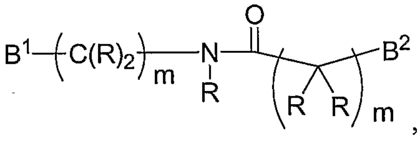

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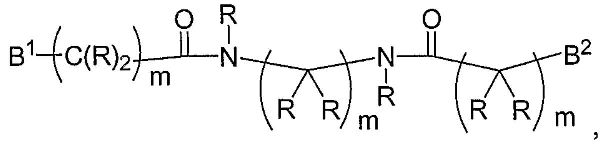

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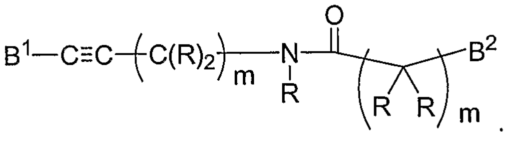
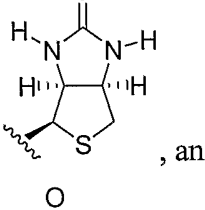
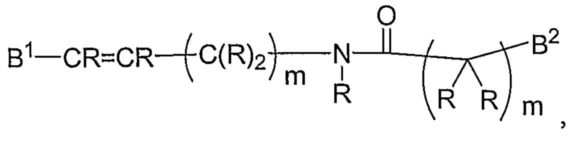


.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



, and A^4 is



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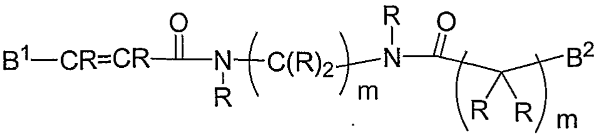
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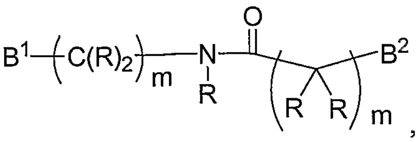
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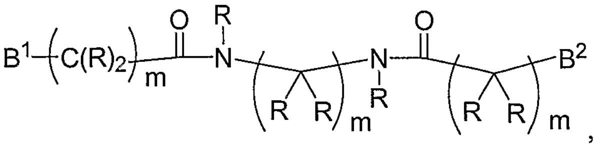
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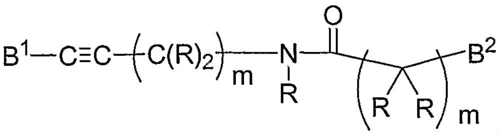

.

In certain embodiments, the present invention relates to the aforementioned compound, wherein R^2 represents independently for each occurrence H, OH, F, or -Oalkyl.

In certain embodiments, the present invention relates to the aforementioned compound, wherein R^2 represents independently for each occurrence H, OH, F, $-OCH_3$, $-O(CH_2)_2OR^7$, $-O(CH_2)_2SR^7$, $-O(CH_2)_2N(R^7)_2$, $-OCH_2C(O)N(H)CH_3$, $-NH_2$, $-N(CH_3)_2$, $-N(H)CH_3$, $-SCH_3$, $-O(CH_2)_2OCH_3$, $-O(CH_2)_2SCH_3$, $-O(CH_2)_2O(CH_2)_2N(CH_3)_2$, or $-O(CH_2)_2ON(CH_3)_2$.

In certain embodiments, the present invention relates to the aforementioned compound, wherein R^2 represents independently for each occurrence $-NH_2$, $-N(H)CH_3$, or $-N(CH_3)_2$.

In certain embodiments, the present invention relates to the aforementioned compound, wherein R^5 represents independently for each occurrence H, OH, F, $-OCH_3$, $-O(CH_2)_2OR^7$, $-O(CH_2)_2SR^7$, $-O(CH_2)_2N(R^7)_2$, $-OCH_2C(O)N(H)CH_3$, $-NH_2$, $-N(CH_3)_2$, $-N(H)CH_3$, $-SCH_3$, $-O(CH_2)_2OCH_3$, $-O(CH_2)_2SCH_3$, $-O(CH_2)_2O(CH_2)_2N(CH_3)_2$, or $-O(CH_2)_2ON(CH_3)_2$.

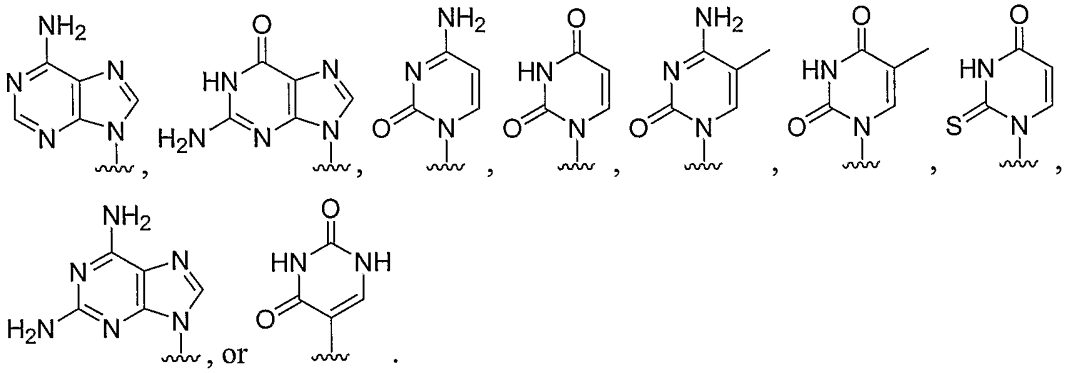
In certain embodiments, the present invention relates to the aforementioned compound, wherein R^5 represents independently for each occurrence $-NH_2$, $-N(H)CH_3$, or $-N(CH_3)_2$.

In certain embodiments, the present invention relates to the aforementioned compound, wherein R^2 and R^5 represent independently for each occurrence $-NH_2$, $-N(H)CH_3$, or $-N(CH_3)_2$.

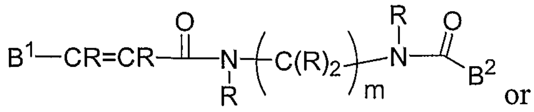
In certain embodiments, the present invention relates to the aforementioned compound, wherein Z^2 represents independently for each occurrence -OH, -OM, -Oalkyl, -Oaryl, -Oaralkyl, -SH, -SM, -Salkyl, -Saryl, -Saralkyl, $-N(R^3)R^4$, $-(C(R^7)_2)_mN(R^7)_2$, $-N(R^7)(C(R^7)_2)_mN(R^7)_2$, or methyl;

In certain embodiments, the present invention relates to the aforementioned compound, wherein Z^2 represents independently for each occurrence -OH, -OM, -Oalkyl, -Oaryl, or -Oaralkyl.

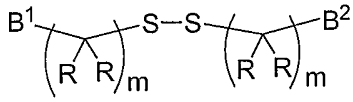
In certain embodiments, the present invention relates to the aforementioned oligonucleotide, wherein A^2 represents independently for each occurrence:



In certain embodiments, the present invention relates to the aforementioned compound, wherein A^4 represents independently for each occurrence

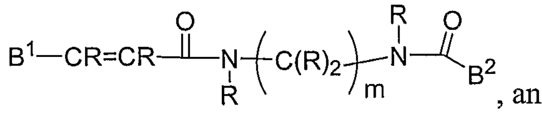


$B^1\text{—}CR{=}CR\text{—}C(=O)\text{—}N(R)\text{—}\left(C(R)_2\right)_m\text{—}N(R)\text{—}C(=O)\text{—}B^2$ or



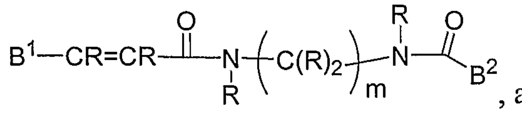
.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^4 represents independently for each occurrence

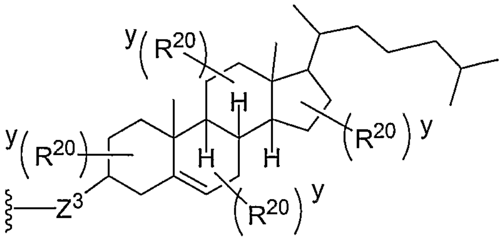


$B^1\text{—}CR{=}CR\text{—}C(=O)\text{—}N(R)\text{—}\left(C(R)_2\right)_m\text{—}N(R)\text{—}C(=O)\text{—}B^2$, and A^5 is a steroid.

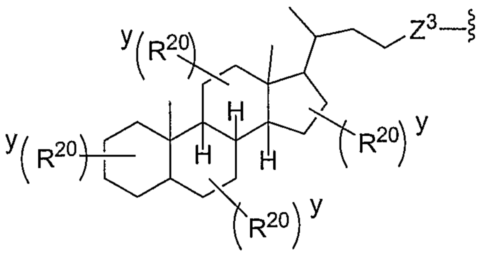
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^4 represents independently for each occurrence



$B^1\text{—}CR{=}CR\text{—}C(=O)\text{—}N(R)\text{—}\left(C(R)_2\right)_m\text{—}N(R)\text{—}C(=O)\text{—}B^2$, and A^5 represents independently for each occurrence:



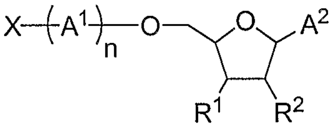
or



,

wherein R^{20} represents independently for each occurrence hydroxyl, amino, halogen, alkoxyl, alkyl, aminoalkyl, azido, carbonyl, acyl, or acyloxy; Z^3 represents independently for each occurrence O, S, or NR; and y represents independently for each occurrence 1, 2, 3, 4, 5, or 6 in accord with the rules of valence.

Another aspect of the present invention relates to a double-stranded oligonucleotide comprising a first strand and a second strand, wherein said first strand and said second strand are represented independently by formula **II**:



II

wherein

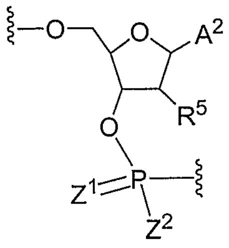
X is H, $-P(O)(OM)_2$, $-P(O)(OM)-O-P(O)(OM)_2$, $-P(O)(Oalkyl)_2$, or $-P(O)(Oalkyl)-O-P(O)(Oalkyl)_2$;

M represents independently for each occurrence an alkali metal or a transition metal with an overall charge of +1;

n is 16, 17, 18 , 19, 20, 21, 22, 23, or 24;

R^1, R^2, and R^5 represent independently for each occurrence H, OH, F, -Oalkyl, -Oallyl, $-O(C(R^7)_2)_vOR^7$, $-O(C(R^7)_2)_vSR^7$, $-O(C(R^7)_2)_vN(R^7)_2$, $-O(C(R^7)_2)_vC(O)N(R^7)_2$, $-N(R^7)_2$, $-S(C_1\text{-}C_6)$alkyl, $-O(C(R^7)_2)_vO(C_1\text{-}C_6)$alkyl, $-O(C(R^7)_2)_vS(C_1\text{-}C_6)$alkyl, $-O(C(R^7)_2)_vO(C(R^7)_2)_vN((C_1\text{-}C_6)alkyl)_2$, or $-O(C(R^7)_2)_vON((C_1\text{-}C_6)alkyl)_2$;

A^1 represents independently for each occurrence:



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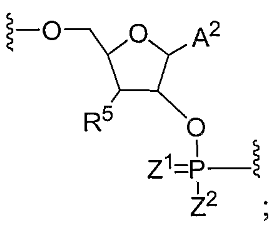


;

Z^1 represents independently for each occurrence O or S;

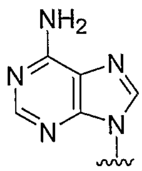
Z^2 represents independently for each occurrence -OH, -OM, -Oalkyl, -Oaryl, -Oaralkyl, -SH, -SM, -Salkyl, -Saryl, -Saralkyl, $-N(R^3)R^4$, $-(C(R^7)_2)_mN(R^7)_2$, $-N(R^7)(C(R^7)_2)_mN(R^7)_2$, or alkyl;

R^3 and R^4 are H or alkyl; or R^3 and R^4 taken together form a 3-, 4-, 5-, 6-, or 7-member ring;

R^6 represents independently for each occurrence H, alkyl, or $-NHCH_2CH=CH_2$;

R^7 represents independently for each occurrence H or alkyl;

A^2 represents independently for each occurrence: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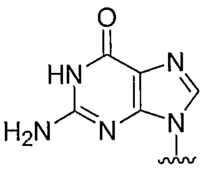
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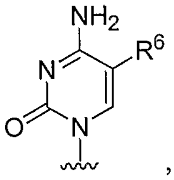

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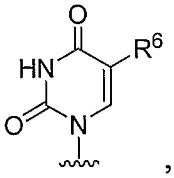

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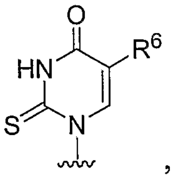

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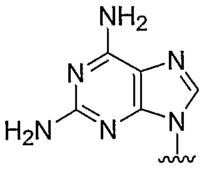
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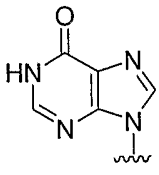
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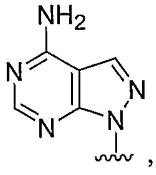
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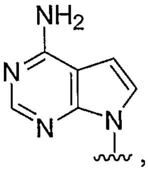
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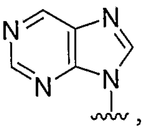
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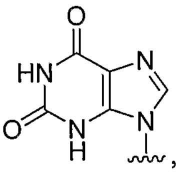
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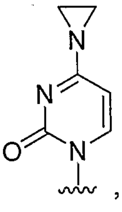
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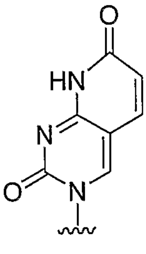
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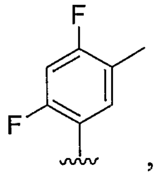
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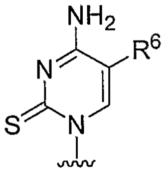

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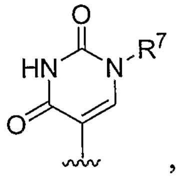
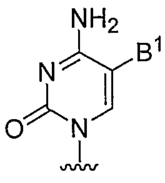


, or $-A^3-A^4-(A^5)_w$;

A^3 represents independently for each occurrence


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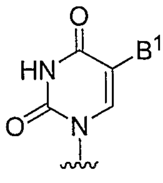
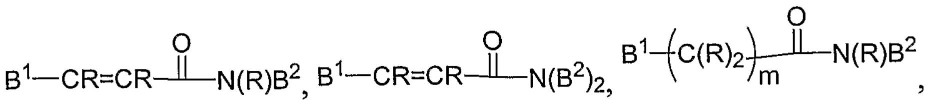
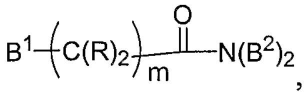


;

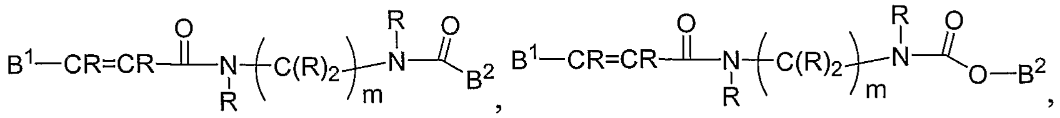
A^4 represents independently for each occurrence a bond, alkyl diradical, heteroalkyl diradical, alkenyl diradical, aminoalkyl diradical, alkynyl diradical, alkylalkynyl diradical, thioether, -C(O)-, -S(O)-, -S(O)$_2$-, $B^1C(R)_2B^2$, $B^1C(R)(B^2)_2$, $B^1C(B^2)_3$, $B^1N(R)(B^2)$, $B^1N(B^2)_2$, or has the formula:

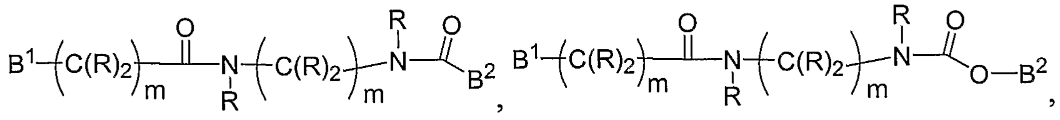


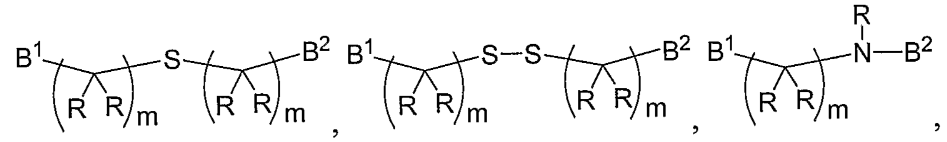
$B^1{-}CR{=}CR{-}C(O){-}N(R)B^2$, $B^1{-}CR{=}CR{-}C(O){-}N(B^2)_2$, $B^1{-}(C(R)_2)_m{-}C(O){-}N(R)B^2$,

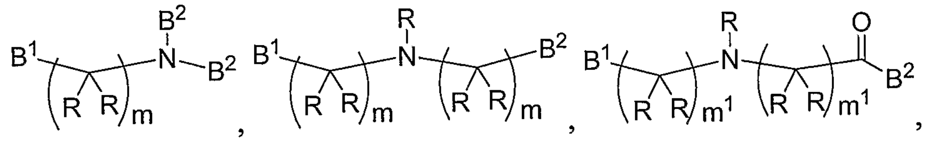


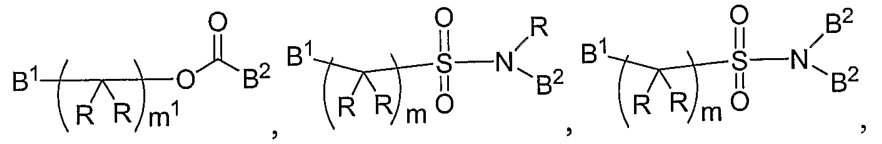
$B^1{-}(C(R)_2)_m{-}C(O){-}N(B^2)_2$,

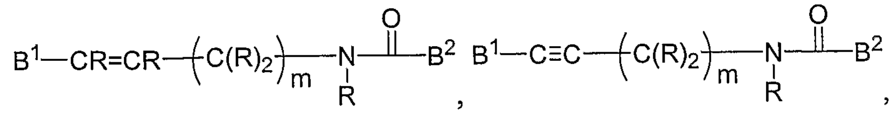


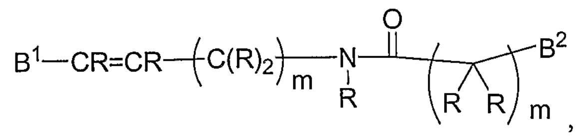


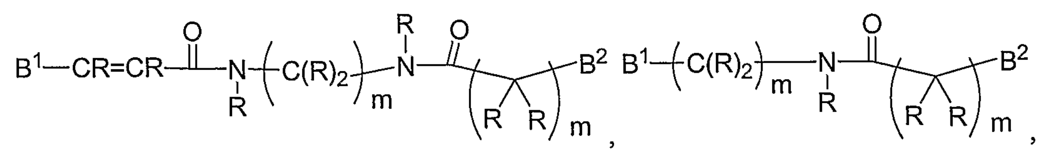


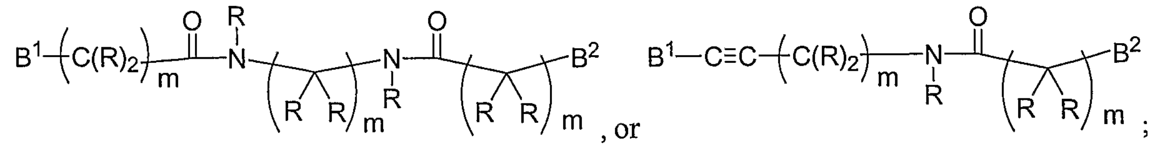












B^1 represents independently for each occurrence a bond between A^3 and A^4;

B^2 represents independently for each occurrence a bond between A^4 and A^5;

R represents independently for each occurrence hydrogen or alkyl;

m represents independently for each occurrence 1, 2, 3, 4, 5, 6, 7 or 8;

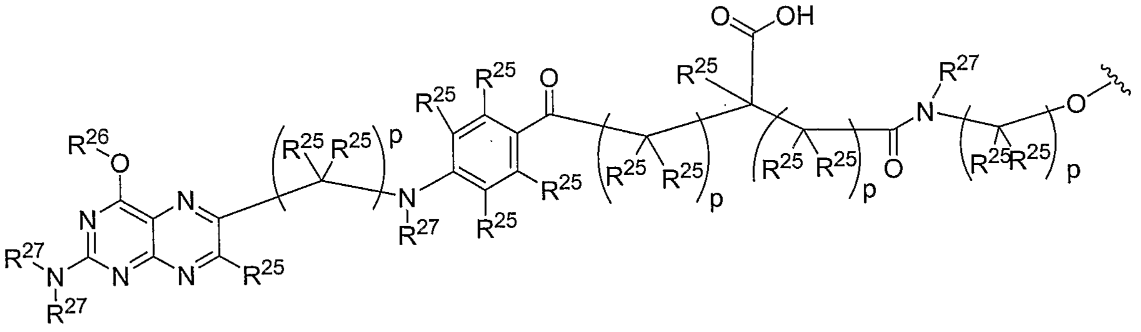
m^1 represents independently for each occurrence 0, 1, 2, 3, 4, 5, 6, 7 or 8;

v represents independently for each occurrence 1, 2, 3, or 4;

w represents independently for each occurrence 1, 2, or 3 in accord with the rules of valence;

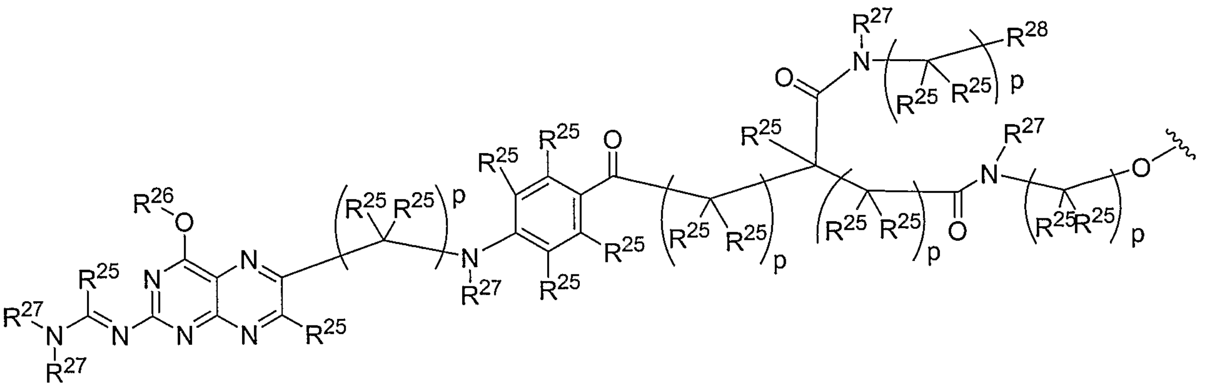
provided that A^5 occurs at least once; and

A^5 represents independently for each occurrence compound **C**, compound **D**, or a radical of a steroid, bile acid, lipid, folic acid, pyridoxal, B12, riboflavin, biotin, aromatic compound, polycyclic compound, crown ether, intercalator, cleaver molecule, protein-binding agent, carbohydrate, or an optionally substituted saturated 5-membered ring; wherein compound **C** is represented by:



C

and compound **C'** is represented by:



C'

wherein for **C** and **C'**

R^{25} represents independently for each occurrence H, halogen, hydroxyl, thiol, alkoxyl, thioalkyl, azido, -N(R^{27})$_2$, -CO$_2$$R^{27}$, -COR^{27}, -OC(O)R^{27}, alkyl, alkenyl, aryl, or aralkyl;

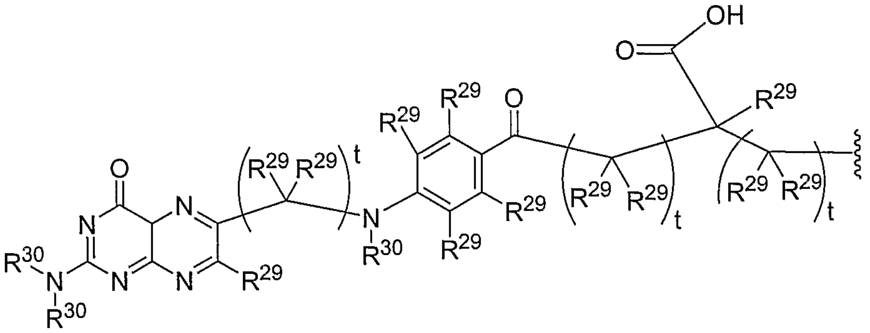
R^{26} represents independently for each occurrence alkyl, alkenyl, aryl, or aralkyl;

R^{27} represents independently for each occurrence H, alkyl, alkenyl, aryl, or aralkyl;

R^{28} represents independently for each occurrence -OC(O)R^{27}, -N(R^{27})C(O)R^{27}, -N(R^{27})$_2$, -CO$_2$R^{27}, -COR^{27}, alkyl, alkenyl, aryl, or aralkyl; and

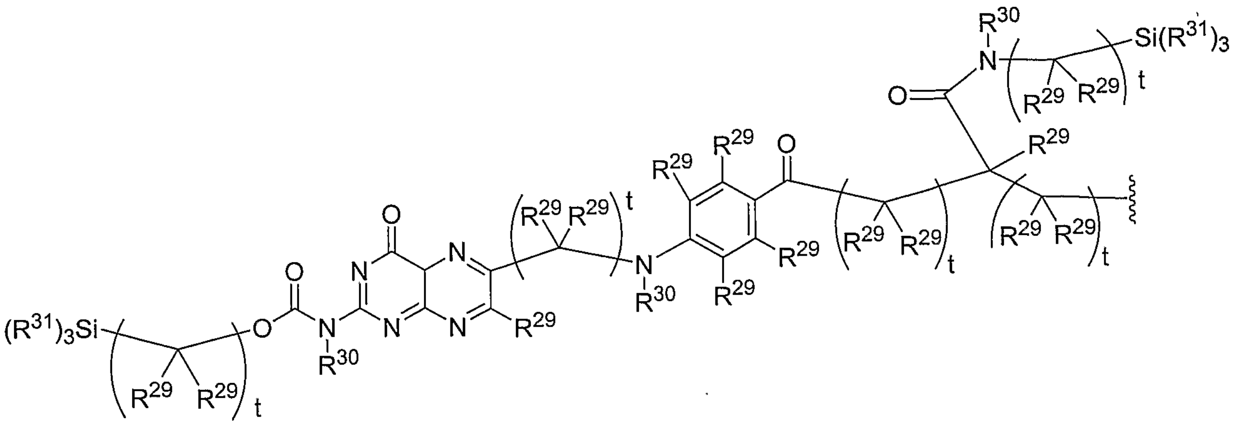
p represents independently for each occurrence 1, 2, 3, 4, 5, 6, or 7; and

compound **D** is represented by:



D

and compound **D'** is represented by:



D'

wherein for **D** and **D'**

R^{29} represents independently for each occurrence H, halogen, hydroxyl, thiol, alkoxyl, thioalkyl, azido, $-N(R^{30})_2$, $-CO_2R^{30}$, $-COR^{30}$, $-OC(O)R^{30}$, alkyl, alkenyl, aryl, or aralkyl;

R^{30} represents independently for each occurrence H, alkyl, alkenyl, aryl, or aralkyl;

R^{31} represents independently for each occurrence alkyl, alkenyl, aryl, or aralkyl; and

t represents independently for each occurrence 1, 2, 3, 4, 5, 6, or 7.

In certain embodiments, the present invention relates to the aforementioned compound, wherein n is 18, 19, 20, 21, or 22.

In certain embodiments, the present invention relates to the aforementioned compound, wherein n is 20.

In certain embodiments, the present invention relates to the aforementioned compound, wherein n is 20, and said first strand and said second strand are hybridized so that there are two unhybridized nucleotides on said first strand and said second strand.

In certain embodiments, the present invention relates to the aforementioned compound, wherein n is 20 for said first strand, and n is 22 for said second strand.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 occurs at least twice.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 occurs at least five times.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 occurs at least ten times.

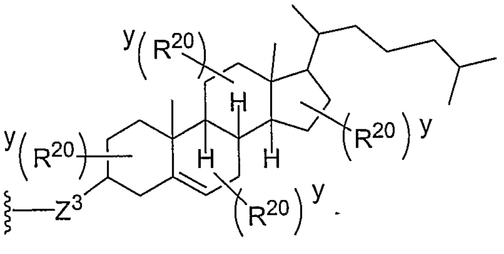
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 occurs only in said first strand.

In certain embodiments, the present invention relates to the aforementioned compound, wherein said first strand and said second strand each contain at least one occurrence of A^5.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence compound **C**, compound **D**, or a radical of cholesterol, 5β-cholanic acid, progesterone, aldosterone, dehydroaldosterone, isoandrosterone, esterone, estradiol, ergosterol, dehydroergosterol, lanosterol, 4-cholesten-3-one, guggulsterone, testosterone, nortestosterone, formestane, hydroxyecdysone, ketoestriol, corticosterone, dienestrol, dihydroxypregnanone, pregnanone, copornmon, equilenin, equilin, estriol, ethinylestradiol, mestranol, moxestrol, mytatrienediol, quinestradiol, quinestrol, helvolic acid, protostadiene, fusidic acid, cycloartenol, tricallol, cucurbitanin cedrelone, euphol, dammerenediol, parkeol, dexametasone, methylprednisolone, prednisolone, hydrocortisone, parametasone, betametasone, cortisone, fluocinonide, fluorometholone, halcinonide, budesonide, or any one of them further substituted with one or more of hydroxyl, halogen, amino, alkylamino, alkyl, carboxylic acid, ester, amide, carbonyl, alkoxyl, or cyano.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence a radical of cholesterol or 5β-cholanic acid.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence:



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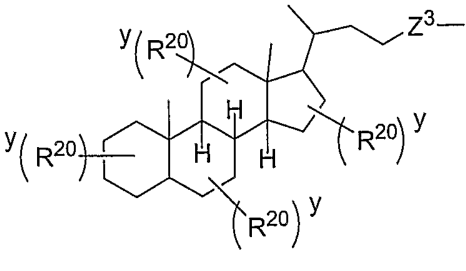


,

wherein R^{20} represents independently for each occurrence hydroxyl, amino, halogen, alkoxyl, alkyl, aminoalkyl, azido, carbonyl, acyl, or acyloxy; Z^3 represents independently for each occurrence O, S, or NR; and y represents independently for each occurrence 1, 2, 3, 4, 5, or 6 in accord with the rules of valence.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence a radical of cholic acid, deoxycholic acid, taurocholic acid, glycocholic acid, glycodeoxycholic acid, taurodeoxycholic acid, ursodeoxycholic acid, or chenodeoxycholic acid.

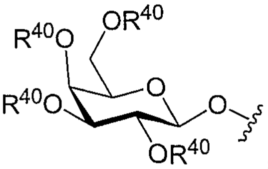
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence a radical of lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, triacylglycerol, phosphoacylglycerol, sphingolipid, monoterpene, sesquiterpene, diterpene, sesterterpene, triterpene, or tetraterpene.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence optionally substituted phenyl, naphthyl, anthracenyl, phenanthrenyl, pyrenyl, pyridinyl, quinolinyl, acridinyl, phenathridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinoxalinyl, quinazolinyl, 1,7-phenanthrolinyl, indolyl, thianaphthenyl, benzoxazolyl, benzofuranyl, 1,2-benzisoxazolyl, benzimidazolyl, pyrrolyl, thiophenyl, isoxazolyl, pyrazolyl, thiazolyl, imidazolyl, tetrazolyl, or furanyl.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 represents independently for each occurrence the radical of erythrose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, mannose, gulose, idose, galactose, or talose; or a disaccharide or trisaccharide formed via a 1,4 glycoside linkage between any of them.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is a hexose or pentose.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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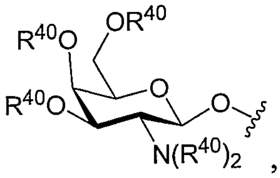

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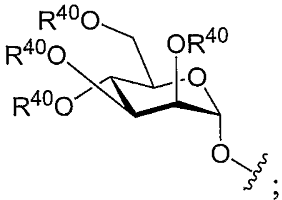
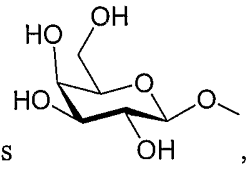


; wherein R^{40} represents independently for each occurrence H, alkyl, aryl, aralkyl, -C(O)alkyl, -C(O)aryl, -C(O)aralkyl, or Si(alkyl)$_3$.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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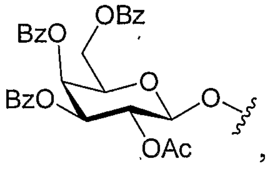

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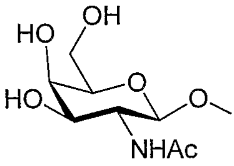

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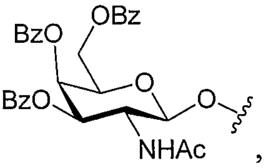

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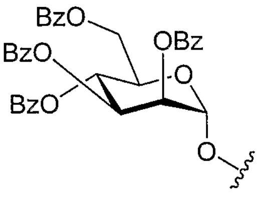

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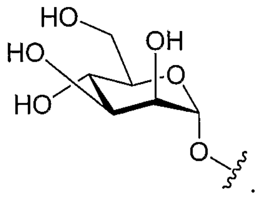

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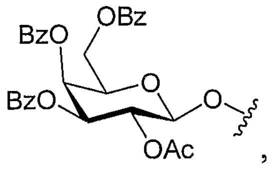
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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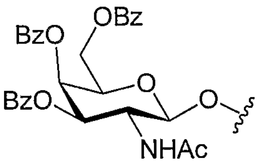

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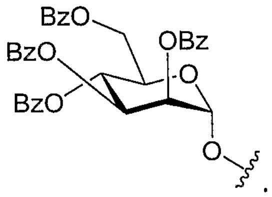

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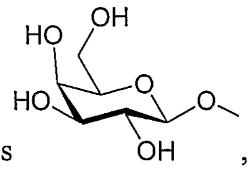
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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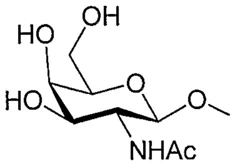

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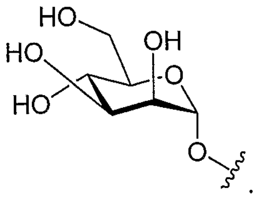
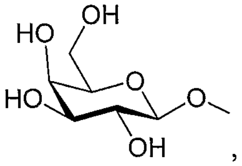


.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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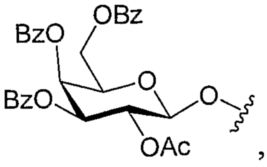

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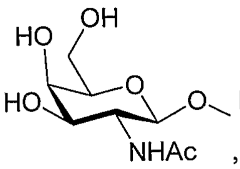

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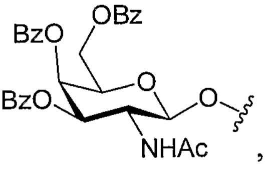

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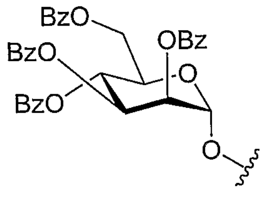

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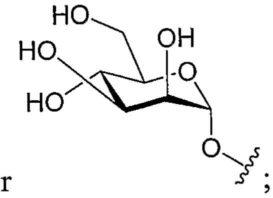
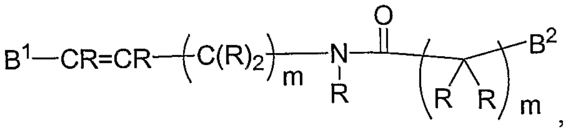


; and A^4 is



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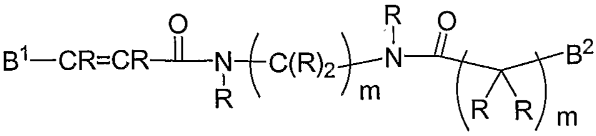

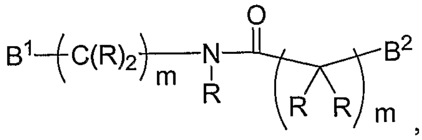

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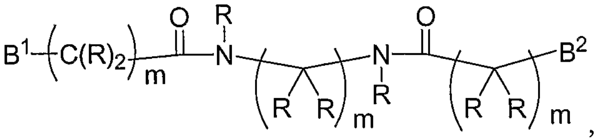

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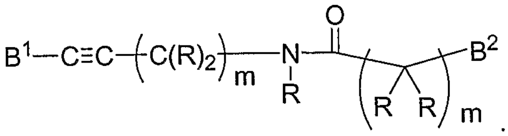

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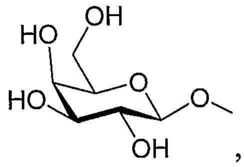
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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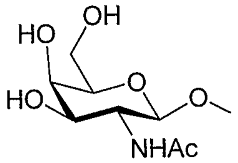

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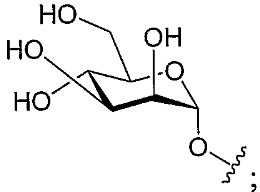
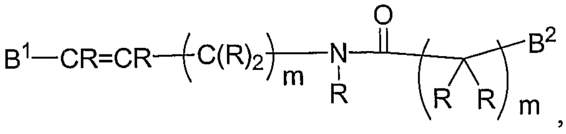


; and A^4 is



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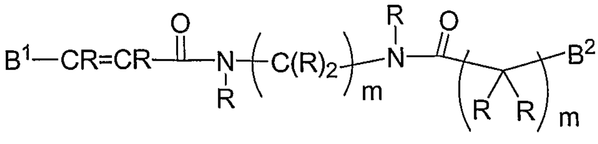

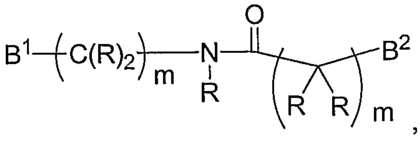

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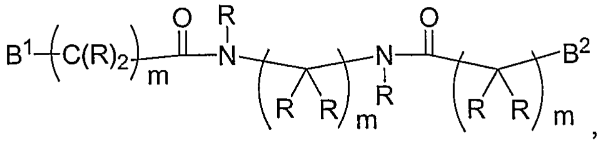

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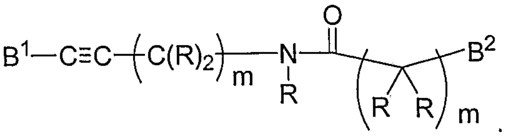


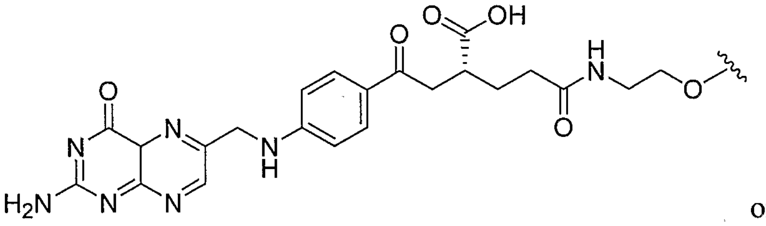
.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is an optionally substituted cyclopentane, pyrrolidine, tetrahydrofuran, tetrahydrothiophene, or 1,1-difluorocyclopentane.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is compound **C**.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is compound **C'**.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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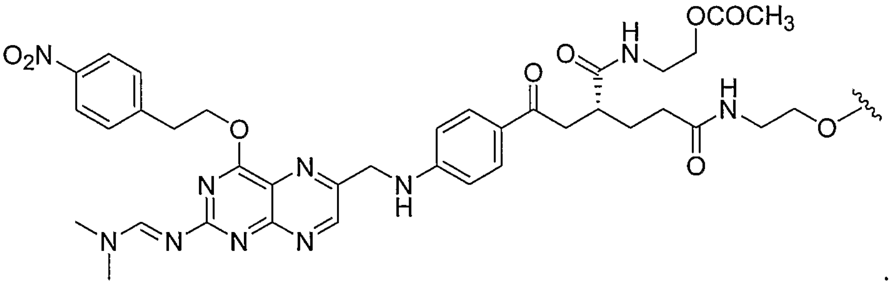

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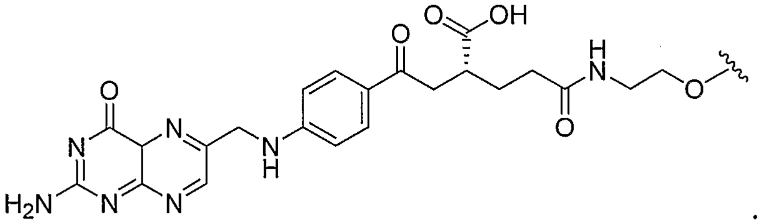
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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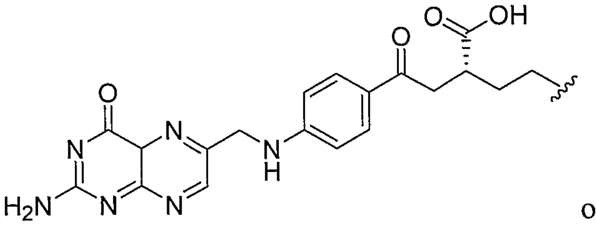
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is compound **D**.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is compound **D'**.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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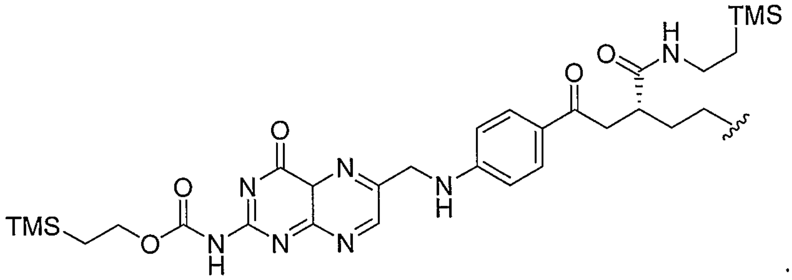

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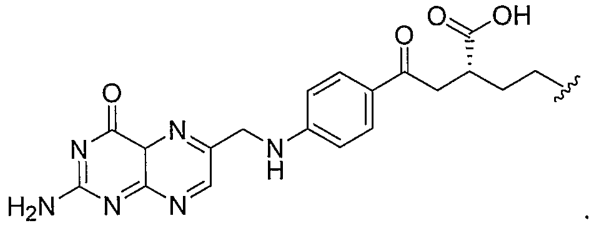
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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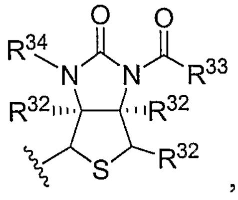
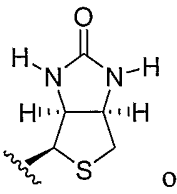
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is a radical of a polycyclic compound.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



, wherein R^{32} represents independently for each occurrence H, halogen, hydroxyl, thiol, alkoxyl, thioalkyl, azido, $-N(R^{34})_2$, $-CO_2R^{34}$, $-COR^{34}$, $-OC(O)R^{34}$, alkyl, alkenyl, aryl, or aralkyl; R^{33} is aryl that is optionally substituted with alkyl, alkenyl, aryl, halogen, or alkoxyl; and R^{34} represents independently for each occurrence H, alkyl, aryl, or aralkyl.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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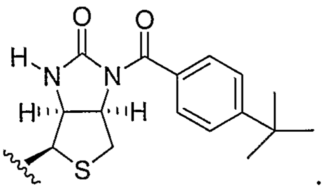

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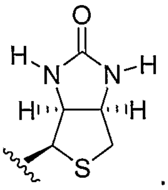
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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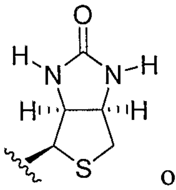
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



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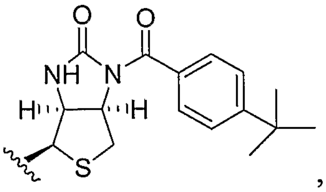
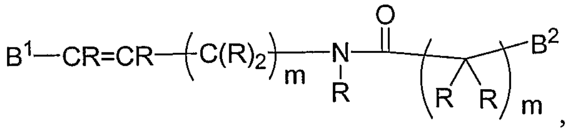


, and A^4 is



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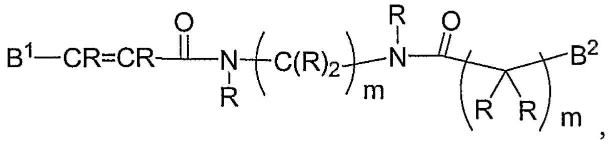

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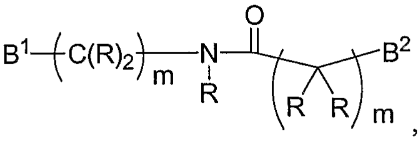

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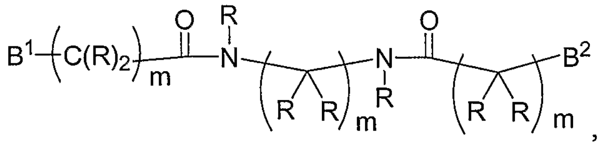
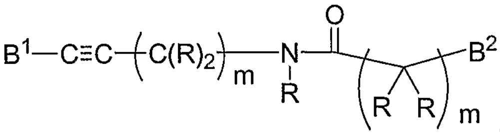


, or



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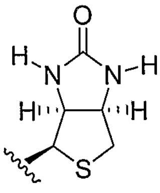
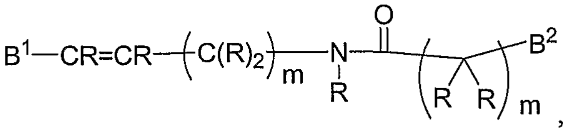
In certain embodiments, the present invention relates to the aforementioned compound, wherein A^5 is



, and A^4 is



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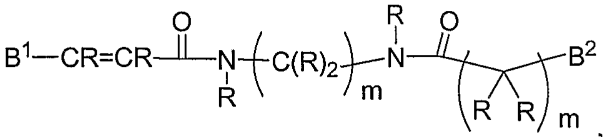

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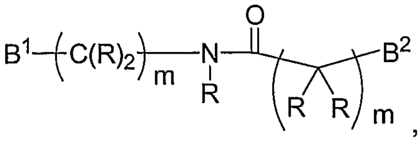

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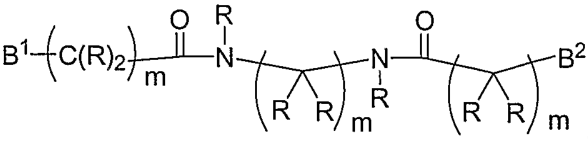

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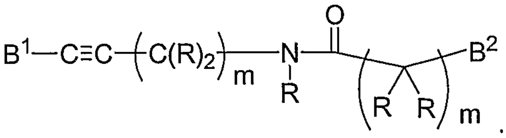


.

In certain embodiments, the present invention relates to the aforementioned compound, wherein R^2 represents independently for each occurrence H, OH, F, or -Oalkyl.

In certain embodiments, the present invention relates to the aforementioned compound, wherein R^2 represents independently for each occurrence H, OH, F, $-OCH_3$, $-O(CH_2)_2OR^7$, $-O(CH_2)_2SR^7$, $-O(CH_2)_2N(R^7)_2$, $-OCH_2C(O)N(H)CH_3$, $-NH_2$, $-N(CH_3)_2$, $-N(H)CH_3$, $-SCH_3$, $-O(CH_2)_2OCH_3$, $-O(CH_2)_2SCH_3$, $-O(CH_2)_2O(CH_2)_2N(CH_3)_2$, or $-O(CH_2)_2ON(CH_3)_2$.

In certain embodiments, the present invention relates to the aforementioned compound, wherein R^2 represents independently for each occurrence $-NH_2$, $-N(H)CH_3$, or $-N(CH_3)_2$.

In certain embodiments, the present invention relates to the aforementioned compound, wherein R^5 represents independently for each occurrence H, OH, F, $-OCH_3$, $-O(CH_2)_2OR^7$, $-O(CH_2)_2SR^7$, $-O(CH_2)_2N(R^7)_2$, $-OCH_2C(O)N(H)CH_3$, $-NH_2$, $-N(CH_3)_2$, $-N(H)CH_3$, $-SCH_3$, $-O(CH_2)_2OCH_3$, $-O(CH_2)_2SCH_3$, $-O(CH_2)_2O(CH_2)_2N(CH_3)_2$, or $-O(CH_2)_2ON(CH_3)_2$.

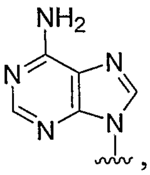
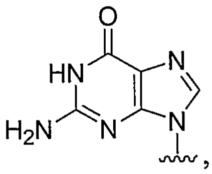
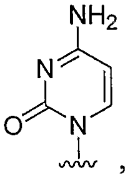
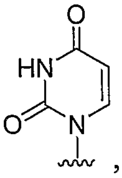
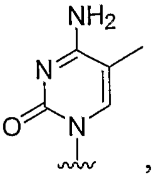
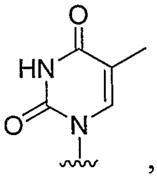
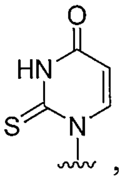
In certain embodiments, the present invention relates to the aforementioned compound, wherein R^5 represents independently for each occurrence $-NH_2$, $-N(H)CH_3$, or $-N(CH_3)_2$.

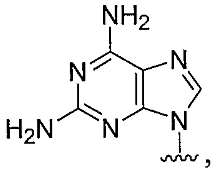
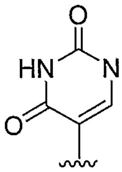
In certain embodiments, the present invention relates to the aforementioned compound, wherein R^2 and R^5 represent independently for each occurrence $-NH_2$, $-N(H)CH_3$, or $-N(CH_3)_2$.

In certain embodiments, the present invention relates to the aforementioned compound, wherein Z^2 represents independently for each occurrence -OH, -OM, -Oalkyl, -Oaryl, -Oaralkyl, -SH, -SM, -Salkyl, -Saryl, -Saralkyl, $-N(R^3)R^4$, $-(C(R^7)_2)_mN(R^7)_2$, $-N(R^7)(C(R^7)_2)_mN(R^7)_2$, or methyl;

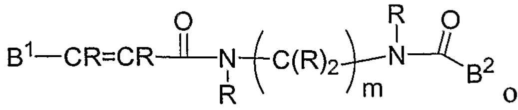
In certain embodiments, the present invention relates to the aforementioned compound, wherein Z^2 represents independently for each occurrence -OH, -OM, -Oalkyl, -Oaryl, or -Oaralkyl.

In certain embodiments, the present invention relates to the aforementioned oligonucleotide, wherein A^2 represents independently for each occurrence:

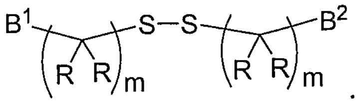
, , , , , , ,

, or .

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^4 represents independently for each occurrence

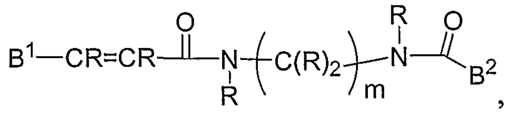


$B^1-CR=CR-C(=O)-N(R)-(C(R)_2)_m-N(R)-C(=O)-B^2$ or



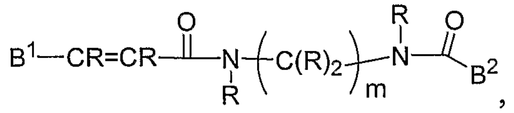
$B^1-(C(R)(R))_m-S-S-(C(R)(R))_m-B^2$.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^4 represents independently for each occurrence

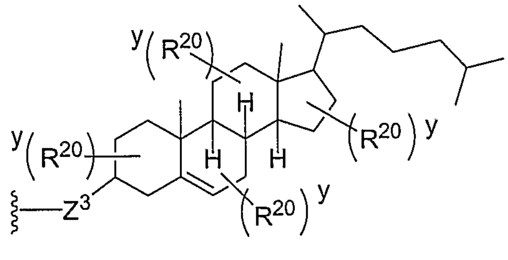


$B^1-CR=CR-C(=O)-N(R)-(C(R)_2)_m-N(R)-C(=O)-B^2$, and A^5 is a steroid.

In certain embodiments, the present invention relates to the aforementioned compound, wherein A^4 represents independently for each occurrence



$B^1-CR=CR-C(=O)-N(R)-(C(R)_2)_m-N(R)-C(=O)-B^2$, and A^5 represents independently for each occurrence:



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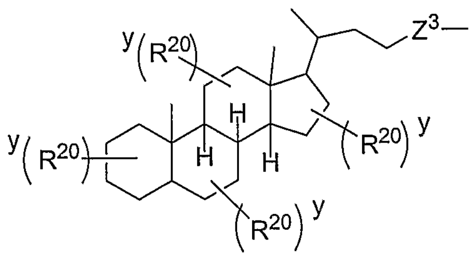
,

wherein R^{20} represents independently for each occurrence hydroxyl, amino, halogen, alkoxyl, alkyl, aminoalkyl, azido, carbonyl, acyl, or acyloxy; Z^3 represents independently for each occurrence O, S, or NR; and y represents independently for each occurrence 1, 2, 3, 4, 5, or 6 in accord with the rules of valence.

Methods of the Invention

One aspect of the present invention relates to a method of treating a patient suffering from a malady selected from the group consisting of unwanted cell proliferation, arthritis, retinal neovascularization, viral infection, bacterial infection, amoebic infection, parasitic infection, fungal infection, unwanted immune response, asthma, lupus, multiple sclerosis, diabetes, acute pain, chronic pain, neurological disease, and a disorder characterized by loss of heterozygosity; comprising the step of:

administering to a patient in need thereof a therapeutically effective amount of an oligonucleotide, wherein said oligonucleotide is a single-stranded oligonucleotide represented by formula **I** as described above, or said oligonucleotide is a double-stranded oligonucleotide comprising a first strand and a second strand, wherein said first strand and said second are represented independently by formula **II** as described above.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is unwanted cell proliferation.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is testicular cancer, lung cancer, breast cancer, colon cancer, squamous cell carcinoma, pancreatic cancer, leukemia, melanoma, Burkitt's lymphoma, neuroblastoma, ovarian cancer, prostate cancer, skin cancer, non-Hodgkin lymphoma, esophageal cancer, cervical cancer, basal cell carcinoma, adenocarcinoma carcinoma, hepatocellular carcinoma, colorectal adenocarcinoma, liver cancer, male breast carcinoma, adenocarcinomas of the esophagus, adenocarcinomas of the stomach, adenocarcinomas of the colon, adenocarcinomas of the rectum, gall bladder cancer, hamartomas, gliomas, endometrial cancer, acute leukemia, chronic leukemia, childhood acute leukemia, Ewing Sarcoma, Myxoid liposarcoma, brain cancer, or tumors of epithelial origin.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is rheumatoid arthritis or retinal neovascularization.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is a viral infection.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is a disorder mediated by Human Papilloma Virus, Human Immunodeficiency Virus, Hepatitis A Virus, Hepatitis B Virus, Hepatitis C Virus, Hepatitis

D Virus, Hepatitis E Virus, Hepatitis F Virus, Hepatitis G Virus, Hepatitis H Virus, Respiratory Syncytial Virus, Herpes Simplex Virus, herpes Cytomegalovirus, herpes Epstein Barr Virus, a Kaposi's Sarcoma-associated Herpes Virus, JC Virus, myxovirus, rhinovirus, coronavirus, West Nile Virus, St. Louis Encephalitis, Tick-borne encephalitis virus gene, Murray Valley encephalitis virus gene, dengue virus gene, Simian Virus 40, Human T Cell Lymphotropic Virus, a Moloney-Murine Leukemia Virus, encephalomyocarditis virus, measles virus, Vericella zoster virus, adenovirus, yellow fever virus, poliovirus, or poxvirus.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is a bacterial infection, amoebic infection, parasitic infection, or fungal infection.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is a disorder mediated by plasmodium, Mycobacterium ulcerans, Mycobacterium tuberculosis, Mycobacterium leprae, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Chlamydia pneumoniae, or Mycoplasma pneumoniae.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is an unwanted immune response, asthma, lupus, multiple sclerosis, or diabetes.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is an ischemia, reperfusion injury, response to a transplantated organ or tissue, restenosis, or Inflammatory Bowel Disease.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is acute pain or chronic pain.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is a neurological disease.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is Alzheimer Disease, Parkinson Disease, or a neurodegenerative trinucleotide repeat disorder.

In certain embodiments, the present invention relates to the aforementioned method, wherein said malady is a disorder characterized by loss of heterozygosity.

In certain embodiments, the present invention relates to the aforementioned method, wherein said oligonucleotide is a double-stranded oligonucleotide comprising a first strand and a second strand, wherein said first strand and said second are represented independently by formula **II** as described above.

Another aspect of the present invention relates to a method of gene-silencing, comprising the steps of:

administering a therapeutically effective amount of an oligonucleotide to a mammalian cell to silence a gene promoting unwanted cell proliferation, growth factor gene, growth factor receptor gene, a kinase gene, a gene encoding a G protein superfamily molecule, a gene encoding a transcription factor, a gene which mediates angiogenesis, a viral gene of a cellular gene which mediates viral function, a gene of a bacterial pathogen, a gene of an amoebic pathogen, a gene of a parasitic pathogen, a gene of a fungal pathogen, a gene which mediates an unwanted immune response, a gene which mediates the processing of pain, a gene which mediates a neurological disease, an allene gene found in cells characterized by loss of heterozygosity, or one allege gene of a polymorphic gene; wherein said oligonucleotide is a single-stranded oligonucleotide represented by formula **I** as described above, or said oligonucleotide is a double-stranded oligonucleotide comprising a first strand and a second strand represented by formula **II** as described above.

In certain embodiments, the present invention relates to the aforementioned method, wherein said oligonucleotide is a double-stranded oligonucleotide comprising a first strand and a second strand, wherein said first strand and said second are represented independently by formula **II** as described above.

Another aspect of the present invention relates to a method of gene-silencing, comprising the steps of:

administering a therapeutically effective amount of an oligonucleotide to a mammalian cell to silence a PDGF beta gene, Erb-B gene, Src gene, CRK gene, GRB2 gene, RAS gene, MEKK gene, JNK gene, RAF gene, Erk1/2 gene, PCNA(p21) gene, MYB gene, JUN gene, FOS gene, BCL-2 gene, Cyclin D gene, VEGF gene, EGFR gene, Cyclin A gene, Cyclin E gene, WNT-1 gene, beta-catenin gene, c-MET gene, PKC gene, NFKB gene, STAT3 gene, survivin gene, Her2/Neu gene, topoisomerase I gene, topoisomerase II alpha gene, mutations in the p73 gene, mutations in the p21(WAF1/CIP1) gene, mutations in the p27(KIP1) gene, mutations in the PPM1D gene, mutations in the RAS gene, mutations in the caveolin I gene, mutations in the MIB I gene, mutations in the MTAI gene, mutations in the M68 gene, mutations in tumor suppressor genes, mutations in the p53 tumor suppressor gene, mutations in the p53 family member DN-p63, mutations in the pRb tumor suppressor gene, mutations in the APC1 tumor suppressor gene, mutations in the BRCA1 tumor suppressor gene, mutations in the PTEN tumor suppressor gene, mLL fusion gene, BCR/ABL fusion gene, TEL/AML1 fusion gene, EWS/FLI1 fusion gene, TLS/FUS1 fusion gene, PAX3/FKHR fusion gene, AML1/ETO fusion gene, alpha v-integrin gene, Flt-1 receptor gene, tubulin gene, Human Papilloma Virus gene, a gene required for Human Papilloma Virus replication, Human Immunodeficiency Virus gene, a gene required for Human Immunodeficiency Virus replication, Hepatitis A Virus gene, a gene required for Hepatitis A Virus replication, Hepatitis B Virus gene, a gene required for Hepatitis B Virus replication, Hepatitis C Virus gene, a gene required for Hepatitis C Virus replication, Hepatitis D Virus gene, a gene required for Hepatitis D Virus replication, Hepatitis E Virus gene, a gene required for Hepatitis E Virus replication, Hepatitis F Virus gene, a gene required for Hepatitis F Virus replication, Hepatitis G Virus gene, a gene required for Hepatitis G Virus replication, Hepatitis H Virus gene, a gene required for Hepatitis H Virus replication, Respiratory Syncytial Virus gene, a gene that is required for Respiratory Syncytial Virus replication, Herpes Simplex Virus gene, a gene that is required for Herpes Simplex Virus replication, herpes Cytomegalovirus gene, a gene that is required for herpes Cytomegalovirus replication, herpes Epstein Barr Virus gene, a gene that is required for herpes Epstein Barr Virus replication, Kaposi's Sarcoma-associated Herpes Virus gene, a gene that is required for Kaposi's Sarcoma-associated Herpes Virus replication, JC Virus gene, human gene that is required for JC Virus replication, myxovirus gene, a gene that is required for myxovirus gene replication, rhinovirus gene, a gene that is required for rhinovirus replication, coronavirus gene, a gene that is required for coronavirus replication, West Nile Virus gene, a gene that is required for West Nile Virus replication, St. Louis Encephalitis gene, a gene that is required for St. Louis Encephalitis replication, Tick-borne encephalitis virus gene, a gene that is required for Tick-borne encephalitis virus replication, Murray Valley encephalitis virus gene, a gene that is required for Murray Valley encephalitis virus replication, dengue virus gene, a gene that is required for dengue virus gene replication, Simian Virus 40 gene, a gene that is required for Simian Virus 40 replication, Human T Cell Lymphotropic Virus gene, a gene that is required for Human T Cell Lymphotropic Virus replication, Moloney-Murine Leukemia Virus gene, a gene that is required for Moloney-Murine Leukemia Virus replication, encephalomyocarditis virus gene, a gene that is required for encephalomyocarditis virus replication, measles virus gene, a gene that is required for measles virus replication, Vericella zoster virus gene, a gene that is required for Vericella zoster virus replication, adenovirus gene, a gene that is required for adenovirus replication, yellow fever virus gene, a gene that is required for yellow fever virus replication, poliovirus gene, a gene that is required for poliovirus replication, poxvirus gene, a gene that is required for poxvirus replication, plasmodium gene, a gene that is required for plasmodium gene replication, Mycobacterium ulcerans gene, a gene that is required for Mycobacterium ulcerans replication, Mycobacterium tuberculosis gene, a gene that is required for Mycobacterium tuberculosis replication, Mycobacterium leprae gene, a gene that is required for Mycobacterium leprae replication, Staphylococcus aureus gene, a gene that is required for Staphylococcus aureus replication, Streptococcus pneumoniae gene, a gene that is required for Streptococcus pneumoniae replication, Streptococcus pyogenes gene, a gene that is required for Streptococcus pyogenes replication, Chlamydia pneumoniae gene, a gene that is required for Chlamydia pneumoniae replication, Mycoplasma pneumoniae gene, a gene that is required for Mycoplasma pneumoniae replication, an integrin gene, a selectin gene, complement system gene, chemokine gene, chemokine receptor gene, GCSF gene, Gro1 gene, Gro2 gene, Gro3 gene, PF4 gene, MIG gene, Pro-Platelet Basic Protein gene, MIP-1I gene, MIP-1J gene, RANTES gene, MCP-1 gene, MCP-2 gene, MCP-3 gene, CMBKR1 gene, CMBKR2 gene, CMBKR3 gene, CMBKR5v, AIF-1 gene, I-309 gene, a gene to a component of an ion channel, a gene to a neurotransmitter receptor, a gene to a neurotransmitter ligand, amyloid-family gene, presenilin gene, HD gene, DRPLA gene, SCA1 gene, SCA2 gene, MJD1 gene, CACNL1A4 gene, SCA7 gene, SCA8 gene, allele gene found in LOH cells, or one allele gene of a polymorphic gene; wherein said oligonucleotide is a single-stranded oligonucleotide represented by formula **I** as described above, or said oligonucleotide is a double-stranded oligonucleotide comprising a first strand and a second strand represented by formula **II** as described above.

In certain embodiments, the present invention relates to the aforementioned method, wherein said oligonucleotide is a double-stranded oligonucleotide comprising a first strand and a second strand, wherein said first strand and said second are represented independently by formula **II** as described above.

Another aspect of the present invention relates to a method of gene-silencing, comprising the steps of:

administering a therapeutically effective amount of an oligonucleotide to a mammal to silence a gene promoting unwanted cell proliferation, growth factor or growth factor receptor gene, a kinase gene, a gene encoding a G protein superfamily molecule, a gene encoding a transcription factor, a gene which mediates angiogenesis, a viral gene of a cellular gene which mediates viral function, a gene of a bacterial pathogen, a gene of an amoebic pathogen, a gene of a parasitic pathogen, a gene of a fungal pathogen, a gene which mediates an unwanted immune response, a gene which mediates the processing of pain, a gene which mediates a neurological disease, an allene gene found in cells characterized by loss of heterozygosity, or one allege gene of a polymorphic gene; wherein said oligonucleotide is a single-stranded oligonucleotide represented by formula **I** as described above, or said oligonucleotide is a double-stranded oligonucleotide comprising a first strand and a second strand represented by formula **II** as described above.

In certain embodiments, the present invention relates to the aforementioned method, wherein said oligonucleotide is a double-stranded oligonucleotide comprising a first strand and a second strand, wherein said first strand and said second are represented independently by formula **II** as described above.

Another aspect of the present invention relates to a method of gene-silencing, comprising the steps of:

administering a therapeutically effective amount of an oligonucleotide to a mammal to silence a PDGF beta gene, Erb-B gene, Src gene, CRK gene, GRB2 gene, RAS gene, MEKK gene, JNK gene, RAF gene, Erk1/2 gene, PCNA(p21) gene, MYB gene, JUN gene, FOS gene, BCL-2 gene, Cyclin D gene, VEGF gene, EGFR gene, Cyclin A gene, Cyclin E gene, WNT-1 gene, beta-catenin gene, c-MET gene, PKC gene, NFKB gene, STAT3 gene, survivin gene, Her2/Neu gene, topoisomerase I gene, topoisomerase II alpha gene, mutations in the p73 gene, mutations in the p21(WAF1/CIP1) gene, mutations in the p27(KIP1) gene, mutations in the PPM1D gene, mutations in the RAS gene, mutations in the caveolin I gene, mutations in the MIB I gene, mutations in the MTAI gene, mutations in the M68 gene, mutations in tumor suppressor genes, mutations in the p53 tumor suppressor gene, mutations in the p53 family member DN-p63, mutations in the pRb tumor suppressor gene, mutations in the APC1 tumor suppressor gene, mutations in the BRCA1 tumor suppressor gene, mutations in the PTEN tumor suppressor gene, mLL fusion gene, BCR/ABL fusion gene, TEL/AML1 fusion gene, EWS/FLI1 fusion gene, TLS/FUS1 fusion gene, PAX3/FKHR fusion gene, AML1/ETO fusion gene, alpha v-integrin gene, Flt-1 receptor gene, tubulin gene, Human Papilloma Virus gene, a gene required for Human Papilloma Virus replication, Human Immunodeficiency Virus gene, a gene required for Human Immunodeficiency Virus replication, Hepatitis A Virus gene, a gene required for Hepatitis A Virus replication, Hepatitis B Virus gene, a gene required for Hepatitis B Virus replication, Hepatitis C Virus gene, a gene required for Hepatitis C Virus replication, Hepatitis D Virus gene, a gene required for Hepatitis D Virus replication, Hepatitis E Virus gene, a gene required for Hepatitis E Virus replication, Hepatitis F Virus gene, a gene required for Hepatitis F Virus replication, Hepatitis G Virus gene, a gene required for Hepatitis G Virus replication, Hepatitis H Virus gene, a gene required for Hepatitis H Virus replication, Respiratory Syncytial Virus gene, a gene that is required for Respiratory Syncytial Virus replication, Herpes Simplex Virus gene, a gene that is required for Herpes Simplex Virus replication, herpes Cytomegalovirus gene, a gene that is required for herpes Cytomegalovirus replication, herpes Epstein Barr Virus gene, a gene that is required for herpes Epstein Barr Virus replication, Kaposi's Sarcoma-associated Herpes Virus gene, a gene that is required for Kaposi's Sarcoma-associated Herpes Virus replication, JC Virus gene, human gene that is required for JC Virus replication, myxovirus gene, a gene that is required for myxovirus gene replication, rhinovirus gene, a gene that is required for rhinovirus replication, coronavirus gene, a gene that is required for coronavirus replication, West Nile Virus gene, a gene that is required for West Nile Virus replication, St. Louis Encephalitis gene, a gene that is required for St. Louis Encephalitis replication, Tick-borne encephalitis virus gene, a gene that is required for Tick-borne encephalitis virus replication, Murray Valley encephalitis virus gene, a gene that is required for Murray Valley encephalitis virus replication, dengue virus gene, a gene that is required for dengue virus gene replication, Simian Virus 40 gene, a gene that is required for Simian Virus 40 replication, Human T Cell Lymphotropic Virus gene, a gene that is required for Human T Cell Lymphotropic Virus replication, Moloney-Murine Leukemia Virus gene, a gene that is required for Moloney-Murine Leukemia Virus replication, encephalomyocarditis virus gene, a gene that is required for encephalomyocarditis virus replication, measles virus gene, a gene that is required for measles virus replication, Vericella zoster virus gene, a gene that is required for Vericella zoster virus replication, adenovirus gene, a gene that is required for adenovirus replication, yellow fever virus gene, a gene that is required for yellow fever virus replication, poliovirus gene, a gene that is required for poliovirus replication, poxvirus gene, a gene that is required for poxvirus replication, plasmodium gene, a gene that is required for plasmodium gene replication, Mycobacterium ulcerans gene, a gene that is required for Mycobacterium ulcerans replication, Mycobacterium tuberculosis gene, a gene that is required for Mycobacterium tuberculosis replication, Mycobacterium leprae gene, a gene that is required for Mycobacterium leprae replication, Staphylococcus aureus gene, a gene that is required for Staphylococcus aureus replication, Streptococcus pneumoniae gene, a gene that is required for Streptococcus pneumoniae replication, Streptococcus pyogenes gene, a gene that is required for Streptococcus pyogenes replication, Chlamydia pneumoniae gene, a gene that is required for Chlamydia pneumoniae replication, Mycoplasma pneumoniae gene, a gene that is required for Mycoplasma pneumoniae replication, an integrin gene, a selectin gene, complement system gene, chemokine gene, chemokine receptor gene, GCSF gene, Gro1 gene, Gro2 gene, Gro3 gene, PF4 gene, MIG gene, Pro-Platelet Basic Protein gene, MIP-1I gene, MIP-1J gene, RANTES gene, MCP-1 gene, MCP-2 gene, MCP-3 gene, CMBKR1 gene, CMBKR2 gene, CMBKR3 gene, CMBKR5v, AIF-1 gene, I-309 gene, a gene to a component of an ion channel, a gene to a neurotransmitter receptor, a gene to a neurotransmitter ligand, amyloid-family gene, presenilin gene, HD gene, DRPLA gene, SCA1 gene, SCA2 gene, MJD1 gene, CACNL1A4 gene, SCA7 gene, SCA8 gene, allele gene found in LOH cells, or one allele gene of a polymorphic gene; wherein said oligonucleotide is a single-stranded oligonucleotide represented by formula **I** as described above, or said oligonucleotide is a double-stranded oligonucleotide comprising a first strand and a second strand represented by formula **II** as described above.

In certain embodiments, the present invention relates to the aforementioned method, wherein, said mammal is a primate, equine, canine or feline.

In certain embodiments, the present invention relates to the aforementioned method, wherein, said mammal is a human.

In certain embodiments, the present invention relates to the aforementioned method, wherein said oligonucleotide is a double-stranded oligonucleotide comprising a first strand and a second strand, wherein said first strand and said second are represented independently by formula **II** as described above.

Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

The term "silence" means to at least partially suppress. For example, in certain instances, the gene is suppressed by at least about 25%, 35%, or 50% by administration of the double stranded oligonucleotide of the invention. In a preferred embodiment, the gene is suppressed by at least about 60%, 70%, or 80% by administration of the double stranded oligonucleotide of the invention. In a more preferred embodiment, the gene is suppressed by at least about 85%, 90%, or 95% by administration of the double stranded oligonucleotide of the invention. In a most preferred embodiment, the gene is suppressed by at least about 98% or 99% by administration of the double stranded oligonucleotide of the invention.

Contemplated equivalents of the compounds described above include compounds which otherwise correspond thereto, and which have the same general properties thereof (e.g., functioning as analgesics), wherein one or more simple variations of substituents are made which do not adversely affect the efficacy of the compound in binding to sigma receptors. In general, the compounds of the present invention may be prepared by the methods illustrated in the general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, it is also possible to make use of variants which are in themselves known, but are not mentioned here.

Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. Unless specified otherwise, the present invention contemplates all such compounds, including *cis*- and *trans*-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 67th Ed., 1986-87, inside cover.

The term "heteroatom" is art-recognized and refers to an atom of any element other than carbon or hydrogen. Illustrative heteroatoms include boron, nitrogen, oxygen, phosphorus, sulfur and selenium.

The term "alkyl" is art-recognized, and includes saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In certain embodiments, a straight chain or branched chain alkyl has about 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{30} for straight chain, C_3-C_{30} for branched chain), and alternatively, about 20 or fewer. Likewise, cycloalkyls have from about 3 to about 10 carbon atoms in their ring structure, and alternatively about 5, 6 or 7 carbons in the ring structure.

Unless the number of carbons is otherwise specified, "lower alkyl" refers to an alkyl group, as defined above, but having from one to about ten carbons, alternatively from one to about six carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths.

The term "aralkyl" is art-recognized and refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

The terms "alkenyl" and "alkynyl" are art-recognized and refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The term "aryl" is art-recognized and refers to 5-, 6- and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, naphthalene, anthracene, pyrene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics." The aromatic ring may be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, -CF_3, -CN, or the like. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic, e.g., the other cyclic rings may be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls.

The terms ortho, meta and para are art-recognized and refer to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and ortho-dimethylbenzene are synonymous.

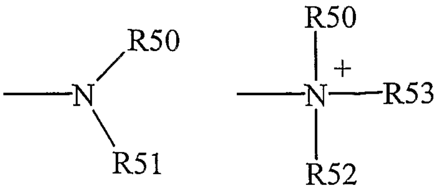
The terms "heterocyclyl", "heteroaryl", or "heterocyclic group" are art-recognized and refer to 3- to about 10-membered ring structures, alternatively 3- to about 7-membered rings, whose ring structures include one to four heteroatoms. Heterocycles may also be polycycles. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxanthene, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring may be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, $-CF_3$, -CN, or the like.

The terms “polycyclyl” or “polycyclic group” are art-recognized and refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are “fused rings”. Rings that are joined through non-adjacent atoms are termed “bridged” rings. Each of the rings of the polycycle may be substituted with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, $-CF_3$, -CN, or the like.

The term “carbocycle” is art-recognized and refers to an aromatic or non-aromatic ring in which each atom of the ring is carbon.

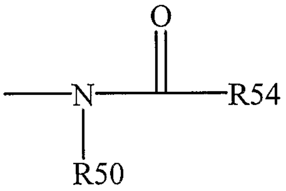
The term “nitro” is art-recognized and refers to $-NO_2$; the term “halogen” is art-recognized and refers to -F, -Cl, -Br or -I; the term “sulfhydryl” is art-recognized and refers to -SH; the term “hydroxyl” means -OH; and the term “sulfonyl” is art-recognized and refers to $-SO_2^-$. “Halide” designates the corresponding anion of the halogens, and “pseudohalide” has the definition set forth on page 560 of “Advanced Inorganic Chemistry” by Cotton and Wilkinson.

The terms “amine” and “amino” are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that may be represented by the general formulas:



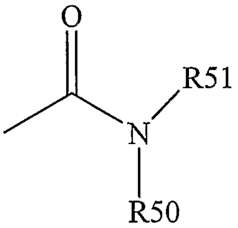
wherein R50, R51 and R52 each independently represent a hydrogen, an alkyl, an alkenyl, -$(CH_2)_m$-R61, or R50 and R51, taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R61 represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocycle or a polycycle; and m is zero or an integer in the range of 1 to 8. In other embodiments, R50 and R51 (and optionally R52) each independently represent a hydrogen, an alkyl, an alkenyl, or -$(CH_2)_m$-R61. Thus, the term "alkylamine" includes an amine group, as defined above, having a substituted or unsubstituted alkyl attached thereto, i.e., at least one of R50 and R51 is an alkyl group.

The term "acylamino" is art-recognized and refers to a moiety that may be represented by the general formula:



wherein R50 is as defined above, and R54 represents a hydrogen, an alkyl, an alkenyl or -$(CH_2)_m$-R61, where m and R61 are as defined above.

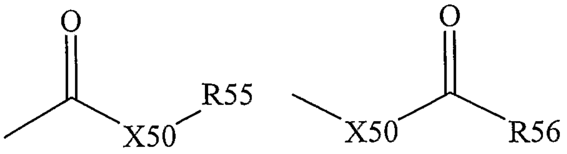
The term "amido" is art recognized as an amino-substituted carbonyl and includes a moiety that may be represented by the general formula:



wherein R50 and R51 are as defined above. Certain embodiments of the amide in the present invention will not include imides which may be unstable.

The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In certain embodiments, the "alkylthio" moiety is represented by one of -S-alkyl, -S-alkenyl, -S-alkynyl, and -S-$(CH_2)_m$-R61, wherein m and R61 are defined above. Representative alkylthio groups include methylthio, ethyl thio, and the like.

The term “carboxyl” is art recognized and includes such moieties as may be represented by the general formulas:

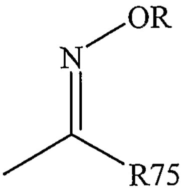


wherein X50 is a bond or represents an oxygen or a sulfur, and R55 and R56 represents a hydrogen, an alkyl, an alkenyl, -$(CH_2)_m$-R61or a pharmaceutically acceptable salt, R56 represents a hydrogen, an alkyl, an alkenyl or -$(CH_2)_m$-R61, where m and R61 are defined above. Where X50 is an oxygen and R55 or R56 is not hydrogen, the formula represents an “ester”. Where X50 is an oxygen, and R55 is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R55 is a hydrogen, the formula represents a “carboxylic acid”. Where X50 is an oxygen, and R56 is hydrogen, the formula represents a “formate”. In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a “thiolcarbonyl” group. Where X50 is a sulfur and R55 or R56 is not hydrogen, the formula represents a “thiolester.” Where X50 is a sulfur and R55 is hydrogen, the formula represents a “thiolcarboxylic acid.” Where X50 is a sulfur and R56 is hydrogen, the formula represents a “thiolformate.” On the other hand, where X50 is a bond, and R55 is not hydrogen, the above formula represents a “ketone” group. Where X50 is a bond, and R55 is hydrogen, the above formula represents an “aldehyde” group.

The term "carbamoyl" refers to -O(C=O)NRR', where R and R' are independently H, aliphatic groups, aryl groups or heteroaryl groups.

The term "oxo" refers to a carbonyl oxygen (=O).

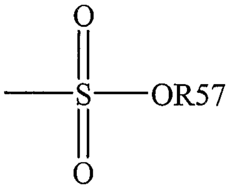
The terms “oxime” and “oxime ether” are art-recognized and refer to moieties that may be represented by the general formula:



wherein R75 is hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, aralkyl, or $-(CH_2)_m-R61$. The moiety is an "oxime" when R is H; and it is an "oxime ether" when R is alkyl, cycloalkyl, alkenyl, alkynyl, aryl, aralkyl, or $-(CH_2)_m-R61$.

The terms "alkoxyl" or "alkoxy" are art-recognized and refer to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as may be represented by one of -O-alkyl, -O-alkenyl, -O-alkynyl, $-O--(CH_2)_m-R61$, where m and R61 are described above.

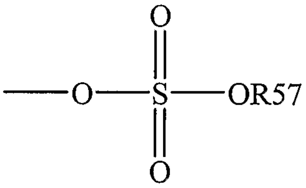
The term "sulfonate" is art recognized and refers to a moiety that may be represented by the general formula:



$$-\overset{\mathrm{O}}{\underset{\mathrm{O}}{\overset{\|}{\underset{\|}{\mathrm{S}}}}}-\mathrm{OR57}$$

in which R57 is an electron pair, hydrogen, alkyl, cycloalkyl, or aryl.

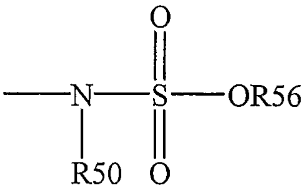
The term "sulfate" is art recognized and includes a moiety that may be represented by the general formula:



$$-\mathrm{O}-\overset{\mathrm{O}}{\underset{\mathrm{O}}{\overset{\|}{\underset{\|}{\mathrm{S}}}}}-\mathrm{OR57}$$

in which R57 is as defined above.

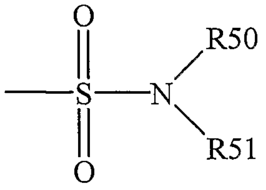
The term "sulfonamido" is art recognized and includes a moiety that may be represented by the general formula:



$$-\underset{\mathrm{R50}}{\underset{|}{\mathrm{N}}}-\overset{\mathrm{O}}{\underset{\mathrm{O}}{\overset{\|}{\underset{\|}{\mathrm{S}}}}}-\mathrm{OR56}$$

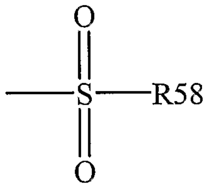
in which R50 and R56 are as defined above.

The term “sulfamoyl” is art-recognized and refers to a moiety that may be represented by the general formula:



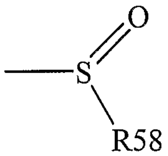
in which R50 and R51 are as defined above.

The term “sulfonyl” is art-recognized and refers to a moiety that may be represented by the general formula:



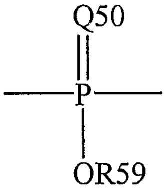
in which R58 is one of the following: hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl or heteroaryl.

The term “sulfoxido” is art-recognized and refers to a moiety that may be represented by the general formula:

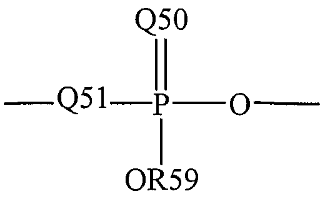


in which R58 is defined above.

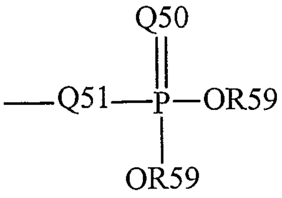
The term “phosphoryl” is art-recognized and may in general be represented by the formula:



wherein Q50 represents S or O, and R59 represents hydrogen, a lower alkyl or an aryl. When used to substitute, e.g., an alkyl, the phosphoryl group of the phosphorylalkyl may be represented by the general formulas:



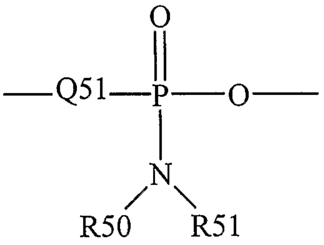
$$—Q51—\overset{\overset{\displaystyle Q50}{\|}}{\underset{\underset{\displaystyle OR59}{|}}{P}}—O—$$

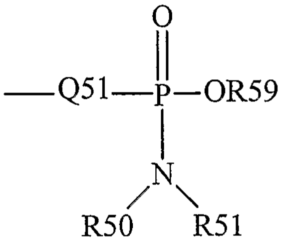


$$—Q51—\overset{\overset{\displaystyle Q50}{\|}}{\underset{\underset{\displaystyle OR59}{|}}{P}}—OR59$$

wherein Q50 and R59, each independently, are defined above, and Q51 represents O, S or N. When Q50 is S, the phosphoryl moiety is a "phosphorothioate".

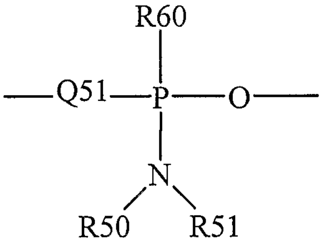
The term "phosphoramidite" is art-recognized and may be represented in the general formulas:

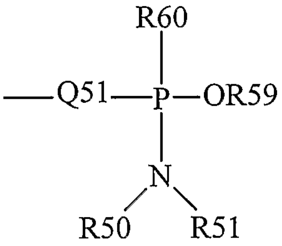




wherein Q51, R50, R51 and R59 are as defined above.

The term "phosphonamidite" is art-recognized and may be represented in the general formulas:





wherein Q51, R50, R51 and R59 are as defined above, and R60 represents a lower alkyl or an aryl.

Analogous substitutions may be made to alkenyl and alkynyl groups to produce, for example, aminoalkenyls, aminoalkynyls, amidoalkenyls, amidoalkynyls, iminoalkenyls, iminoalkynyls, thioalkenyls, thioalkynyls, carbonyl-substituted alkenyls or alkynyls.

The definition of each expression, e.g. alkyl, m, n, and the like, when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

The term "selenoalkyl" is art-recognized and refers to an alkyl group having a substituted seleno group attached thereto. Exemplary "selenoethers" which may be substituted on the alkyl are selected from one of -Se-alkyl, -Se-alkenyl, -Se-alkynyl, and -Se-$(CH_2)_m$-R61, m and R61 being defined above.

The terms triflyl, tosyl, mesyl, and nonaflyl are art-recognized and refer to trifluoromethanesulfonyl, *p*-toluenesulfonyl, methanesulfonyl, and nonafluorobutanesulfonyl groups, respectively. The terms triflate, tosylate, mesylate, and nonaflate are art-recognized and refer to trifluoromethanesulfonate ester, *p*-toluenesulfonate ester, methanesulfonate ester, and nonafluorobutanesulfonate ester functional groups and molecules that contain said groups, respectively.

The abbreviations Me, Et, Ph, Tf, Nf, Ts, and Ms represent methyl, ethyl, phenyl, trifluoromethanesulfonyl, nonafluorobutanesulfonyl, *p*-toluenesulfonyl and methanesulfonyl, respectively. A more comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the Journal of Organic Chemistry; this list is typically presented in a table entitled Standard List of Abbreviations.

Certain compounds contained in compositions of the present invention may exist in particular geometric or stereoisomeric forms. In addition, polymers of the present invention may also be optically active. The present invention contemplates all such compounds, including cis- and trans-isomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction.

The term "substituted" is also contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein above. The permissible substituents may be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds.

The phrase "protecting group" as used herein means temporary substituents which protect a potentially reactive functional group from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl ethers of alcohols, and acetals and ketals of aldehydes and ketones, respectively. The field of protecting group chemistry has been reviewed (Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*, 2nd ed.; Wiley: New York, 1991). Protected forms of the inventive compounds are included within the scope of this invention.

Pharmaceutical Compositions

In another aspect, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of one or more of the compounds described above, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; (3) topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually; (6) ocularly; (7) transdermally; or (8) nasally.

The phrase "therapeutically-effective amount" as used herein means that amount of a compound, material, or composition comprising a compound of the present invention which is effective for producing some desired therapeutic effect in at least a sub-population of cells in an animal at a reasonable benefit/risk ratio applicable to any medical treatment.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhybrides; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

As set out above, certain embodiments of the present compounds may contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable acids. The term "pharmaceutically-acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed during subsequent purification. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19)

The pharmaceutically acceptable salts of the subject compounds include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicyclic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

In other cases, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable bases. The term "pharmaceutically-acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared *in situ* in the administration vehicle or the dosage form manufacturing process, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, with ammonia, or with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. (See, for example, Berge et al., *supra*)

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 0.1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

In certain embodiments, a formulation of the present invention comprises an excipient selected from the group consisting of cyclodextrins, celluloses, liposomes, micelle forming agents, e.g., bile acids, and polymeric carriers, e.g., polyesters and polyanhybrides; and a compound of the present invention. In certain embodiments, an aforementioned formulation renders orally bioavailable a compound of the present invention.

Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules, trouches and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds and surfactants, such as poloxamer and sodium lauryl sulfate; (7) wetting agents, such as, for example, cetyl alcohol, glycerol monostearate, and non-ionic surfactants; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, zinc stearate, sodium stearate, stearic acid, and mixtures thereof; (10) coloring agents; and (11) controlled release agents such as crospovidone or ethyl cellulose. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-shelled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be formulated for rapid release, e.g., freeze-dried. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compounds of the invention in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain sugars, alcohols, antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms upon the subject compounds may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhybrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99% (more preferably, 10 to 30%) of active ingredient in combination with a pharmaceutically acceptable carrier.

The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given in forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administrations are preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticulare, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

These compounds may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion or metabolism of the particular compound being employed, the rate and extent of absorption, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In general, a suitable daily dose of a compound of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, oral, intravenous, intracerebroventricular and subcutaneous doses of the compounds of this invention for a patient, when used for the indicated analgesic effects, will range from about 0.0001 to about 100 mg per kilogram of body weight per day.

If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. Preferred dosing is one administration per day.

While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition).

The compounds according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other pharmaceuticals.

In another aspect, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of one or more of the subject compounds, as described above, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin, lungs, or mucous membranes; or (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually or buccally; (6) ocularly; (7) transdermally; or (8) nasally.

The term "treatment" is intended to encompass also prophylaxis, therapy and cure.

The patient receiving this treatment is any animal in need, including primates, in particular humans, and other mammals such as equines, cattle, swine and sheep; and poultry and pets in general.

The compound of the invention can be administered as such or in admixtures with pharmaceutically acceptable carriers and can also be administered in conjunction with antimicrobial agents such as penicillins, cephalosporins, aminoglycosides and glycopeptides. Conjunctive therapy, thus includes sequential, simultaneous and separate administration of the active compound in a way that the therapeutical effects of the first administered one is not entirely disappeared when the subsequent is administered.

The addition of the active compound of the invention to animal feed is preferably accomplished by preparing an appropriate feed premix containing the active compound in an effective amount and incorporating the premix into the complete ration.

Alternatively, an intermediate concentrate or feed supplement containing the active ingredient can be blended into the feed. The way in which such feed premixes and complete rations can be prepared and administered are described in reference books (such as "Applied Animal Nutrition", W.H. Freedman and CO., San Francisco, U.S.A., 1969 or "Livestock Feeds and Feeding" O and B books, Corvallis, Ore., U.S.A., 1977).

Micelles

Recently, the pharmaceutical industry introduced microemulsification technology to improve bioavailability of some lipophilic (water insoluble) pharmaceutical agents. Examples include Trimetrine (Dordunoo, S. K., et al., Drug Development and Industrial Pharmacy, 17(12), 1685-1713, 1991 and REV 5901 (Sheen, P. C., et al., J Pharm Sci 80(7), 712-714, 1991). Among other things, microemulsification provides enhanced bioavailability by preferentially directing absorption to the lymphatic system instead of the circulatory system, which thereby bypasses the liver, and prevents destruction of the compounds in the hepatobiliary circulation.

In one aspect of invention, the formulations contain micelles formed from a compound of the present invention and at least one amphiphilic carrier, in which the micelles have an average diameter of less than about 100 nm. More preferred embodiments provide micelles having an average diameter less than about 50 nm, and even more preferred embodiments provide micelles having an average diameter less than about 30 nm, or even less than about 20 nm.

While all suitable amphiphilic carriers are contemplated, the presently preferred carriers are generally those that have Generally-Recognized-as-Safe (GRAS) status, and that can both solubilize the compound of the present invention and microemulsify it at a later stage when the solution comes into a contact with a complex water phase (such as one found in human gastro-intestinal tract). Usually, amphiphilic ingredients that satisfy these requirements have HLB (hydrophilic to lipophilic balance) values of 2-20, and their structures contain straight chain aliphatic radicals in the range of C-6 to C-20. Examples are polyethylene-glycolized fatty glycerides and polyethylene glycols.

Particularly preferred amphiphilic carriers are saturated and monounsaturated polyethyleneglycolyzed fatty acid glycerides, such as those obtained from fully or partially hydrogenated various vegetable oils. Such oils may advantageously consist of tri-. di- and mono-fatty acid glycerides and di- and mono-polyethyleneglycol esters of the corresponding fatty acids, with a particularly preferred fatty acid composition including capric acid 4-10, capric acid 3-9, lauric acid 40-50, myristic acid 14-24, palmitic acid 4-14 and stearic acid 5-15%. Another useful class of amphiphilic carriers includes partially esterified sorbitan and/or sorbitol, with saturated or mono-unsaturated fatty acids (SPAN-series) or corresponding ethoxylated analogs (TWEEN-series).

Commercially available amphiphilic carriers are particularly contemplated, including Gelucire-series, Labrafil, Labrasol, or Lauroglycol (all manufactured and distributed by Gattefosse Corporation, Saint Priest, France), PEG-mono-oleate, PEG-di-oleate, PEG-mono-laurate and di-laurate, Lecithin, Polysorbate 80, etc (produced and distributed by a number of companies in USA and worldwide).

Polymers

Hydrophilic polymers suitable for use in the present invention are those which are readily water-soluble, can be covalently attached to a vesicle-forming lipid, and which are tolerated in vivo without toxic effects (i.e., are biocompatible). Suitable polymers include polyethylene glycol (PEG), polylactic (also termed polylactide), polyglycolic acid (also termed polyglycolide), a polylactic-polyglycolic acid copolymer, and polyvinyl alcohol. Preferred polymers are those having a molecular weight of from about 100 or 120 daltons up to about 5,000 or 10,000 daltons, and more preferably from about 300 daltons to about 5,000 daltons. In a particularly preferred embodiment, the polymer is polyethyleneglycol having a molecular weight of from about 100 to about 5,000 daltons, and more preferably having a molecular weight of from about 300 to about 5,000 daltons. In a particularly preferred embodiment, the polymer is polyethyleneglycol of 750 daltons (PEG(750)). Polymers may also be defined by the number of monomers therein; a preferred embodiment of the present invention utilizes polymers of at least about three monomers, such PEG polymers consisting of three monomers (approximately 150 daltons).

Other hydrophilic polymers which may be suitable for use in the present invention include polyvinylpyrrolidone, polymethoxazoline, polyethyloxazoline, polyhydroxypropyl methacrylamide, polymethacrylamide, polydimethylacrylamide, and derivatized celluloses such as hydroxymethylcellulose or hydroxyethylcellulose.

In certain embodiments, a formulation of the present invention comprises a biocompatible polymer selected from the group consisting of polyamides, polycarbonates, polyalkylenes, polymers of acrylic and methacrylic esters, polyvinyl polymers, polyglycolides, polysiloxanes, polyurethanes and co-polymers thereof, celluloses, polypropylene, polyethylenes, polystyrene, polymers of lactic acid and glycolic acid, polyanhybrides, poly(ortho)esters, poly(butic acid), poly(valeric acid), poly(lactide-co-caprolactone), polysaccharides, proteins, polyhyaluronic acids, polycyanoacrylates, and blends, mixtures, or copolymers thereof.

Cyclodextrins

Cyclodextrins are cyclic oligosaccharides, consisting of 6, 7 or 8 glucose units, designated by the Greek letter .alpha., .beta. or .gamma., respectively. Cyclodextrins with fewer than six glucose units are not known to exist. The glucose units are linked by alpha-1,4-glucosidic bonds. As a consequence of the chair conformation of the sugar units, all secondary hydroxyl groups (at C-2, C-3) are located on one side of the ring, while all the primary hydroxyl groups at C-6 are situated on the other side. As a result, the external faces are hydrophilic, making the cyclodextrins water-soluble. In contrast, the cavities of the cyclodextrins are hydrophobic, since they are lined by the hydrogen of atoms C-3 and C-5, and by ether-like oxygens. These matrices allow complexation with a variety of relatively hydrophobic compounds, including, for instance, steroid compounds such as 17.beta.-estradiol (see, e.g., van Uden et al. Plant Cell Tiss. Org. Cult. 38:1-3-113 (1994)). The complexation takes place by Van der Waals interactions and by hydrogen bond formation. For a general review of the chemistry of cyclodextrins, see, Wenz, Agnew. Chem. Int. Ed. Engl., 33:803-822 (1994).

The physico-chemical properties of the cyclodextrin derivatives depend strongly on the kind and the degree of substitution. For example, their solubility in water ranges from insoluble (e.g., triacetyl-beta-cyclodextrin) to 147% soluble (w/v) (G-2-beta-cyclodextrin). In addition, they are soluble in many organic solvents. The properties of the cyclodextrins enable the control over solubility of various formulation components by increasing or decreasing their solubility.

Numerous cyclodextrins and methods for their preparation have been described. For example, Parmeter (I), et al. (U.S. Pat. No. 3,453,259) and Gramera, et al. (U.S. Pat. No. 3,459,731) described electroneutral cyclodextrins. Other derivatives include cyclodextrins with cationic properties [Parmeter (II), U.S. Pat. No. 3,453,257], insoluble crosslinked cyclodextrins (Solms, U.S. Pat. No. 3,420,788), and cyclodextrins with anionic properties [Parmeter (III), U.S. Pat. No. 3,426,011]. Among the cyclodextrin derivatives with anionic properties, carboxylic acids, phosphorous acids, phosphinous acids, phosphonic acids, phosphoric acids, thiophosphonic acids, thiosulphinic acids, and sulfonic acids have been appended to the parent cyclodextrin [see, Parmeter (III), supra]. Furthermore, sulfoalkyl ether cyclodextrin derivatives have been described by Stella, et al. (U.S. Pat. No. 5,134,127).

Liposomes

Liposomes consist of at least one lipid bilayer membrane enclosing an aqueous internal compartment. Liposomes may be characterized by membrane type and by size. Small unilamellar vesicles (SUVs) have a single membrane and typically range between 0.02 and 0.05 μm in diameter; large unilamellar vesicles (LUVS) are typically larger than 0.05 μm Oligolamellar large vesicles and multilamellar vesicles have multiple, usually concentric, membrane layers and are typically larger than 0.1 μm. Liposomes with several nonconcentric membranes, i.e., several smaller vesicles contained within a larger vesicle, are termed multivesicular vesicles.

One aspect of the present invention relates to formulations comprising liposomes containing a compound of the present invention, where the liposome membrane is formulated to provide a liposome with increased carrying capacity. Alternatively or in addition, the compound of the present invention may be contained within, or adsorbed onto, the liposome bilayer of the liposome. The compound of the present invention may be aggregated with a lipid surfactant and carried within the liposome's internal space; in these cases, the liposome membrane is formulated to resist the disruptive effects of the active agent-surfactant aggregate.

According to one embodiment of the present invention, the lipid bilayer of a liposome contains lipids derivatized with polyethylene glycol (PEG), such that the PEG chains extend from the inner surface of the lipid bilayer into the interior space encapsulated by the liposome, and extend from the exterior of the lipid bilayer into the surrounding environment.

Active agents contained within liposomes of the present invention are in solubilized form. Aggregates of surfactant and active agent (such as emulsions or micelles containing the active agent of interest) may be entrapped within the interior space of liposomes according to the present invention. A surfactant acts to disperse and solubilize the active agent, and may be selected from any suitable aliphatic, cycloaliphatic or aromatic surfactant, including but not limited to biocompatible lysophosphatidylcholines (LPCs) of varying chain lengths (for example, from about C 14 to about C 20). Polymer-derivatized lipids such as PEG-lipids may also be utilized for micelle formation as they will act to inhibit micelle/membrane fusion, and as the addition of a polymer to surfactant molecules decreases the CMC of the surfactant and aids in micelle formation. Preferred are surfactants with CMCs in the micromolar range; higher CMC surfactants may be utilized to prepare micelles entrapped within liposomes of the present invention, however, micelle surfactant monomers could affect liposome bilayer stability and would be a factor in designing a liposome of a desired stability.

Liposomes according to the present invention may be prepared by any of a variety of techniques that are known in the art. See, e.g., U.S. Pat. No. 4,235,871; Published PCT applications WO 96/14057; New RRC, Liposomes: A practical approach, IRL Press, Oxford (1990), pages 33-104; Lasic DD, Liposomes from physics to applications, Elsevier Science Publishers BV, Amsterdam, 1993.

For example, liposomes of the present invention may be prepared by diffusing a lipid derivatized with a hydrophilic polymer into preformed liposomes, such as by exposing preformed liposomes to micelles composed of lipid-grafted polymers, at lipid concentrations corresponding to the final mole percent of derivatized lipid which is desired in the liposome. Liposomes containing a hydrophilic polymer can also be formed by homogenization, lipid-field hydration, or extrusion techniques, as are known in the art.

In another exemplary formulation procedure, the active agent is first dispersed by sonication in a lysophosphatidylcholine or other low CMC surfactant (including polymer grafted lipids) that readily solubilizes hydrophobic molecules. The resulting micellar suspension of active agent is then used to rehydrate a dried lipid sample that contains a suitable mole percent of polymer-grafted lipid, or cholesterol. The lipid and active agent suspension is then formed into liposomes using extrusion techniques as are known in the art, and the resulting liposomes separated from the unencapsulated solution by standard column separation.

In one aspect of the present invention, the liposomes are prepared to have substantially homogeneous sizes in a selected size range. One effective sizing method involves extruding an aqueous suspension of the liposomes through a series of polycarbonate membranes having a selected uniform pore size; the pore size of the membrane will correspond roughly with the largest sizes of liposomes produced by extrusion through that membrane. See e.g., U.S. Pat. No. 4,737,323 (Apr. 12, 1988).

Release Modifiers

The release characteristics of a formulation of the present invention depend on the encapsulating material, the concentration of encapsulated drug, and the presence of release modifiers. For example, release can be manipulated to be pH dependent, for example, using a pH sensitive coating that releases only at a low pH, as in the stomach, or a higher pH, as in the intestine. An enteric coating can be used to prevent release from occurring until after passage through the stomach. Multiple coatings or mixtures of cyanamide encapsulated in different materials can be used to obtain an initial release in the stomach, followed by later release in the intestine. Release can also be manipulated by inclusion of salts or pore forming agents, which can increase water uptake or release of drug by diffusion from the capsule. Excipients which modify the solubility of the drug can also be used to control the release rate. Agents which enhance degradation of the matrix or release from the matrix can also be incorporated. They can be added to the drug, added as a separate phase (i.e., as particulates), or can be co-dissolved in the polymer phase depending on the compound. In all cases the amount should be between 0.1 and thirty percent (w/w polymer). Types of degradation enhancers include inorganic salts such as ammonium sulfate and ammonium chloride, organic acids such as citric acid, benzoic acid, and ascorbic acid, inorganic bases such as sodium carbonate, potassium carbonate, calcium carbonate, zinc carbonate, and zinc hydroxide, and organic bases such as protamine sulfate, spermine, choline, ethanolamine, diethanolamine, and triethanolamine and surfactants such as Tween® and Pluronic®. Pore forming agents which add microstructure to the matrices (i.e., water soluble compounds such as inorganic salts and sugars) are added as particulates. The range should be between one and thirty percent (w/w polymer).

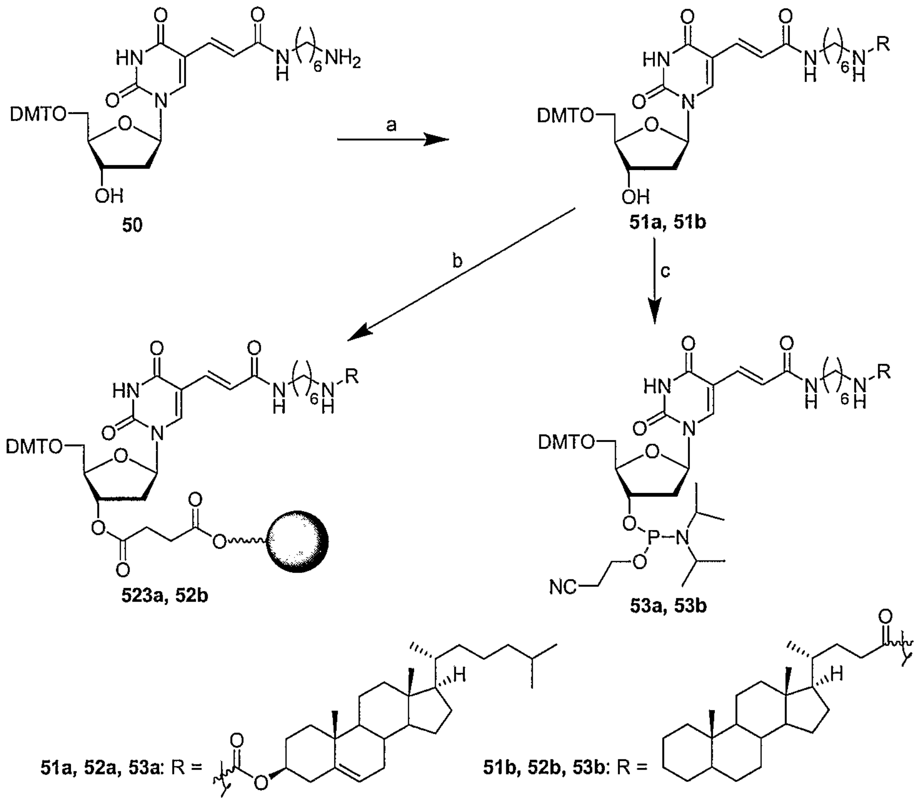
Uptake can also be manipulated by altering residence time of the particles in the gut. This can be achieved, for example, by coating the particle with, or selecting as the encapsulating material, a mucosal adhesive polymer. Examples include most polymers with free carboxyl groups, such as chitosan, celluloses, and especially polyacrylates (as used herein, polyacrylates refers to polymers including acrylate groups and modified acrylate groups such as cyanoacrylates and methacrylates).

Exemplification

The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Example 1

Procedure for Preparation of Nucleoside-Ligand Conjugates Containing Uridine Analogues



(i) For **2a**: cholesterylchloroformate, TEA / dichloromethane; for **2b**: 5β-cholanic acid pentafluorophenol ester, TEA / dichloromethane; (ii) (a) succinic anhybride, DMAP / dichloroethane and (b) DTNP, DMAP, Ph_3P, aminoalkyl solid support; (iii) *N,N*-diisopropylamino β-cyanoethylphosphonamidic chloride $\{[(CH_3)_2CH]_2N\text{-}P(Cl)\text{-}OCH_2CH_2CN\}$, DIEA / dichloromethane or 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphane, tetrazole (or tetrazolediisopropylammonium salt) / acetonitrile.

Preparation of Compound 51a:

DMT-dT-C5-Amino linker (**50**, 1.00 g, 1.43 mmol) from Chem Genes was stirred with cholesteryl chloroformate (0.77 g, 1.71 mmol) in dichloromethane (10 mL) in the presence of TEA (1.0 mL) at ambient temperature for 2 h. Completion of the reaction was confirmed by TCL monitoring. The reaction mixture was diluted to 50 mL by adding more dichloromethane and washed successively with $NaHCO_3$ solution and water followed by standard workup. Residue obtained was purified by flash silica gel column chromatography to afford **51a** (0.66 g, 37.75 %). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ 11.61 (s, 1H, exchangeable with D_2-O), 8.01-7.98 (t, *J*(H,H) = 5.39 Hz, 1H, exchangeable with D_2O), 7.92 (s, 1H), 7.37-6.99 (m, 12H), 6.87-6.83 (m, 4H), 6.17-6.14 (t, *J*(H,H) = 6.64 Hz, 1H), 5.30 (s, 1H), 5.28-5.27 (d, *J*(H,H) = 4.56 Hz, 1H, exchangeable with D_2O), 4.32-4.20 (m, 2H), 3.87-3.84 (m, 1H), 3.71-3.63 (m, 7H), 3.21-3.03 (m, 4H), 2.95-2.88 (m, 2H), 2.33-2.13 (m, 4H), 1.97-1.73 (m, 5H), 1.54-0.82 (m, 40H), 0.63 (s, 3H).

Preparation of Compound 52a:

Compound **51a** (0.55 g, 0.495 mmol) and succinic anhybride (0.075 g, 0.749 mmol) were suspended in anhydrous dichloromethane (5 mL) and stirred at ambient temperature in the presence of DMAP (0.18 g, 1.49 mmol) overnight. After confirming completion of the reaction, the reaction mixture was diluted to 50 mL by adding dichloromethane and washed with dilute citric acid solution; organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. Residue obtained was purified by flash silica gel column chromatography, eluent 6 % methanol in dichloromethane, to afford the corresponding succinic acid derivative (0.50 g, 83.4 %).. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ 12.24 (bs, 1H, exchangeable with D_2O), 11.64 (s, 1H, exchangeable with D_2O), 8.02-7.99 (bm, 2H), 7.36-7.00 (m, 12H), 6.87-6.81 (m, 4H), 6.15-6.11 (t, *J*(H,H) = 6.84 Hz, 1H), 5.30 (bs, 1H), 5.17-5.14 (bm, 1H), 4.31-4.24 (m, 1H), 4.05 (bm, 1H), 3.70-3.66 (m, 8H), 3.34-3.08 (m, 6H), 2.94-2.88 (m, 4H), 2.31-2.13 (m, 3H), 1.96-1.71 (bm, 5H), 1.55-.80 (m, 40H), 0.63 (s, 3H).

The succinnate thus obtained was conjugated to long chain aminoalkyl controlled glass support (CPG) with a loading of 155 μM/g loading as described in the literature by Kumar *et al.* (*Nucleosides and Nucleotides*, **1996**, *15*, 879) to obtain the desired the desired

CPG solid support **52a** (1.70 g) with a loading of 78.42 μM/g. The loading of the support **3a** was determined as described in the literature (Prakash *et al.*, *J. Org. Chem.*, **2002**, *67*, 357).

Preparation of Compound 53a:

The phosphoramidite **53a** is prepared from compound **51a** by reacting with 2-Cyanoethyl-*N,N,N,N'*-tetraisopropylphosphane in the presence of tetrazolediisopropylammonium salt in acetonitrile according to reported procedures (Rajeev *et al.*, *Org. Lett.*, **2003**, *5*, 3005).

Preparation of Compound 51b:

5β-Cholanic acid (5.00 g, 13.87 mmol, purchased from Sigma), pentafluorophenol (2.81 g, 15.27 mmol, purchased from Aldrich) and DMAP (0.20 g, 1.64 mmol) were dissolved in dichloromethane and *N,N'*-dicyclohexycarbodiimide (DCC, 2.86 g, 13.86 mmol) was added into the solution at ambient temperature. The reaction mixture was stirred for 4h. N,N'-Dicyclohexylurea was filtered off from the reaction and the filtrate was evaporated to obtain pentafluorophenol ester of 5β-cholanic acid. The ester (0.90 g, 1.708 mmol) thus obtained was stirred with compound **50** (1.00 g, 1.431 mmol) in the presence of TEA in dichloromethane (8 mL) for 2 h. The reaction was complete after 2 h as evident from TLC analysis. Reaction mixture was diluted to 50 mL by adding more dichloromethane and washed with dilute $NaHCO_3$ solution followed by standard workup. Residue was purified by flash silica gel column chromatography, eluent 3-4 % methanol in dichloromethane, to afford the desired compound **51b** (1.46 g, 98.04 %). ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ 11.62 (bs, 1H exchangeable with D_2O); 8.03-8.00 (t, *J*(H,H) = 5.38 Hz, 1H, exchangeable with D_2O), 7.92 (s, 1H), 7.74-7.71 (t, 1H, exchangeable with D_2O); 7.37-7.02 (m, 11H), 6.88-6.84 (m, 4H), 6.17-6.14 (t, *J*(H.H) = 6.35, 6.69 Hz, 1H), 4.22-4.19 (m, 1H), 3.88-3.85 (m, 1H), 3.70-3.69 (d, *J*(H,H) = 3.91 Hz, 6H); 3.20-2.89 (m, 6H), 2.33-2.27 (m, 1H), 2.18-2.12 (m, 1H), 2-08-2.00 (m, 1H), 1.99-1.84 (m, 2H), 1.84-1.56 (m, 6H), 1.54-0.94 (m, 33H), 0.87-0.79 (m, 7H), 0.57 (s, 3H). ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C): δ 173.5, 166.1, 162.2, 158.5, 149.7, 145.2, 142.9, 136.0, 135.9, 132.5, 130.1, 128.4, 128.1, 127.2, 122.4, 113.6, 109.8, 107.3, 86.1, 85.9, 85.4, 70.6, 64.2, 56.5, 56.0, 55.4, 55.2, 46.2, 43.5, 42.7, 40.4, 38.7, 37.5, 35.8, 35.3, 32.9, 32.1, 29.5, 29.4, 28.2, 27.5, 27.1, 26.9, 26.6, 26.5, 24.4, 24.3, 21.2, 20.9, 18.6, 12.2, 9.1.

Preparation of Compound 52b:

Compound **52b** was prepared from compound **51b** as described above for the synthesis of compound **52a**. Loading of the support **52b** (2.7 g) prepared was determined as 81 μM/g.

Preparation of Compound 53b:

The phosphoramidite **53b** is prepared from compound **51b** by reacting with 2-Cyanoethyl-N,N, N', N'-tetraisopropylphosphane in the presence of tetrazolediisopropylammonium salt in acetonitrile according to reported procedures (Rajeev *et al.*, *Org. Lett.*, **2003**, *5*, 3005).

Example 2

The siRNA duplexes to be used for biological assays are listed below in Table I.

Table I. siRNA Duplexes Containing Lipophilic Cholesterol or 5β-Cholanic Acid at the C5-Position of the Pyrimidine

Number	Name	Sequence[a]
101	Luc	${}^{5'}$CUUACGCUGAGUACUUCGAdTdT${}^{3'}$ ${}^{3'}$dTdTGAAUGCGACUCAUGAAGCU${}^{5'}$
102	Luc-cholesterol 3'-end	${}^{5'}$CUUACGCUGAGUACUUCGAdTdt${}^{3'}$ ${}^{3'}$dTdTGAAUGCGACUCAUGAAGCU${}^{5'}$
103	Luc-cholesterol 5'-end	${}^{5'}$dcUUACGCUGAGUACUUCGAdTdT${}^{3'}$ ${}^{3'}$dTdTGAAUGCGACUCAUGAAGCU${}^{5'}$

104	Luc-5β-cholanic acid 3′-end	$^{5'}$CUUACGCUGAGUACUUCGAdT*dt*$^{3'}$ $^{3'}$dTdTGAAUGCGACUCAUGAAGCU$^{5'}$
105	Luc-5β-cholanic acid 5′-end	$^{5'}$*dc*UUACGCUGAGUACUUCGAdTdT$^{'}$ $^{3'}$dTdTGAAUGCGACUCAUGAAGCU$^{5'}$
106	Luc Scrambled	$^{5'}$CAUUCUCGGUGACAUUCGAdTdT$^{3'}$ $^{3'}$dTdTGUAAGAGCCACUGUAAGCU$^{5'}$
107	Luc - cholesterol 3′-end Scrambled	$^{5'}$CAUUCUCGGUGACAUUCGAdTdt$^{3'}$ $^{3'}$dTdTGUAAGAGCCACUGUAAGCU$^{5'}$
108	Luc - cholesterol 5′-end Scrambled	$^{5'}$dcAUUCUCGGUGACAUUCGAdTdT$^{3'}$ $^{3'}$dTdTGUAAGAGCCACUGUAAGCU$^{5'}$
109	Luc - 5β-cholanic acid 3′-end Scrambled	$^{5'}$CAUUCUCGGUGACAUUCGAdT*dt*$^{3'}$ $^{3'}$dTdTGUAAGAGCCACUGUAAGCU$^{5'}$
110	Luc - 5β-cholanic acid 5′-end Scrambled	$^{5'}$*dc*AUUCUCGGUGACAUUCGAdTdT$^{3'}$ $^{3'}$dTdTGUAAGAGCCACUGUAAGCU$^{5'}$
111	VEGF	$^{5'}$GCGGAUCAAACCUCACCAAdTdT$^{3'}$ $^{3'}$dTdTCGCCUAGUUUGGAGUGGUU$^{5'}$
112	VEGF - cholesterol	$^{5'}$GCGGAUCAAACCUCACCAAdTdt$^{3'}$ $^{3'}$dTdTCGCCUAGUUUGGAGUGGUU$^{5'}$
113	VEGF - 5β-cholanic acid	$^{5'}$GCGGAUCAAACCUCACCAAdT*dt*$^{3'}$ $^{3'}$dTdTCGCCUAGUUUGGAGUGGUU$^{5'}$
114	VEGF Scrambled	$^{5'}$GCGGAACAAUCCUGACCAAdTdT$^{3'}$ $^{3'}$dTdTCGCCUUGUUAGGACUGGUU$^{5'}$
115	VEGF - cholesterol Scrambled	$^{5'}$GCGGAACAAUCCUGACCAAdTdt$^{3'}$ $^{3'}$dTdTCGCCUUGUUAGGACUGGUU$^{5'}$
116	VEGF - 5β-cholanic acid Scrambled	$^{5'}$GCGGAACAAUCCUGACCAAdT*dt*$^{3'}$ $^{3'}$dTdTCGCCUUGUUAGGACUGGUU$^{5'}$
117	PTEN^{b}	$^{5'}$CAAAUCCAGAGGCUAGCAGdTdT$^{3'}$ $^{3'}$dTdTGUUUAGGUCUCCGAUCGUC$^{5'}$
118	PTEN-cholesterol 3′-end	$^{5'}$CAAAUCCAGAGGCUAGCAGdTdt$^{3'}$ $^{3'}$dTdTGUUUAGGUCUCCGAUCGUC$^{5'}$
119	PTEN-cholesterol 5′-end	$^{5'}$dcAAAUCCAGAGGCUAGCAGdTdT $^{3'}$dTdTGUUUAGGUCUCCGAUCGUC$^{5'}$

120	PTEN-scramble	$^{5'}$CAACGAGCGAACUGCGAAUdTdT$^{3'}$ $^{3'}$dTdTGUUGCUCGCUUGACGCUUA$^{5'}$
121	PTEN-cholesterol-scramble 3'-end	$^{5'}$CAACGAGCGAACUGCGAAUdTdt$^{3'}$ $^{3'}$dTdTGUUGCUCGCUUGACGCUUA$^{5'}$
122	PTEN-cholesterol scramble 5'-end	$^{5'}$dcAACGAGCGAACUGCGAAUdTdT$^{3'}$ $^{3'}$dTdTGUUGCUCGCUUGACGCUUA$^{5'}$
123	PTEN-5β-cholanic acid 3'-end	$^{5'}$CAAAUCCAGAGGCUAGCAGdT*dt*$^{3'}$ $^{3'}$dTdTGUUUAGGUCUCCGAUCGUC$^{5'}$
124	PTEN-5β-cholanic acid -end	$^{5'}$*dc*AAAUCCAGAGGCUAGCAGdTdT $^{3'}$dTdTGUUUAGGUCUCCGAUCGUC$^{5'}$
125	PTEN-5β-cholanic acid scramble 3'-end	$^{5'}$CAACGAGCGAACUGCGAAUdT*dt*$^{3'}$ $^{3'}$dTdTGUUGCUCGCUUGACGCUUA$^{5'}$
126	PTEN-5β-cholanic acid scramble 5'-end	$^{5'}$dcAACGAGCGAACUGCGAAUdTdT$^{3'}$ $^{3'}$dTdTGUUGCUCGCUUGACGCUUA$^{5'}$
127	c-raf^{c}	$^{5'}$AUGCAUGUCACAGGCGGGAdTdT$^{3'}$ $^{3'}$dTdTUACGUACAGUGUCCGCCCU$^{5'}$
128	c-raf-cholesterol	$^{5'}$AUGCAUGUCACAGGCGGGAdTdt$^{3'}$ $^{3'}$dTdTUACGUACAGUGUCCGCCCU$^{5'}$
129	c-raf-5β-cholanic acid	$^{5'}$AUGCAUGUCACAGGCGGGAdT*dt*$^{3'}$ $^{3'}$dTdTUACGUACAGUGUCCGCCCU$^{5'}$
130	c-raf-scramble	$^{5'}$GUAGCAGCGGACAGAGUCUdTdT$^{3'}$ $^{3'}$dTdTCAUCGUCGCCUGUCUCAGA$^{5'}$
131	c-raf-cholesterol scramble	$^{5'}$GUAGCAGCGGACAGAGUCUdTdt$^{3'}$ $^{3'}$dTdTCAUCGUCGCCUGUCUCAGA$^{5'}$
132	c-raf-5β-cholanic acid scramble	$^{5'}$GUAGCAGCGGACAGAGUCUdT*dt*$^{3'}$ $^{3'}$dTdTCAUCGUCGCCUGUCUCAGA$^{5'}$
133	H-ras^{d}	$^{5'}$CCGUGAGGAGAGAUGACGGdTdT$^{3'}$ $^{3'}$dTdTGGGACUCCUCGCUACUGCC$^{5'}$
134	H-ras- cholesterol 3'-end	$^{5'}$CCGUGAGGAGAGAUGACGGdTdt$^{3'}$ $^{3'}$dTdTGGGACUCCUCGCUACUGCC$^{5'}$

135	H-ras- cholesterol 5'-end	$^{5'}$dcCGUGAGGAGAGAUGACGGdTdT$^{3'}$ $^{3'}$dTdTGGGACUCCUCGCUACUGCC$^{5'}$
136	H-ras- 5β-cholanic acid 3'-end	$^{5'}$CCGUGAGGAGAGAUGACGGdT*dt*$^{3'}$ $^{3'}$dTdTGGGACUCCUCGCUACUGCC$^{5'}$
137	H-ras- 5β-cholanic acid 5'-end	$^{5'}$*dc*CGUGAGGAGAGAUGACGGdTdT$^{3'}$ $^{3'}$dTdTGGGACUCCUCGCUACUGCC$^{5'}$
138	H-ras-scramble	$^{5'}$CGCGAAGAAGUGCGUGAGGdTdT$^{3'}$ $^{3'}$dTdTGCGCUUCUUCACGCACUCC$^{5'}$
139	H-ras-cholesterol-scramble 3'-end	$^{5'}$CGCGAAGAAGUGCGUGAGGdTdt$^{3'}$ $^{3'}$dTdTGCGCUUCUUCACGCACUCC$^{5'}$
140	H-ras-5β-cholanic acid -scramble 3'-end	$^{5'}$CGCGAAGAAGUGCGUGAGGdT*dt*$^{3'}$ $^{3'}$dTdTGCGCUUCUUCACGCACUCC$^{5'}$
141	Apo-B[e]	$^{5'}$AAGGUGUAUGGCUUCAACCCUdTdT$^{3'}$ $^{3'}$dTdTUUCCACAUACCGAAGUUGGGA$^{5'}$
142	Apo-B - cholesterol	$^{5'}$AAGGUGUAUGGCUUCAACCCUdTdt$^{3'}$ $^{3'}$dTdTUUCCACAUACCGAAGUUGGGA$^{5'}$
143	Apo-B - 5β-cholanic acid	$^{5'}$AAGGUGUAUGGCUUCAACCCUdT*dt*-$^{3'}$ $^{3'}$dTdTUUCCACAUACCGAAGUUGGGA$^{5'}$
144	Apo-B-scramble	$^{5'}$UGCCACUCUGAGAAUAGGCUUdTdT$^{3'}$ $^{3'}$dTdTACGGUGAGACUCUUAUCCGAA$^{5'}$
145	Apo-B-cholesterol-scramble	$^{5'}$UGCCACUCUGAGAAUAGGCUUdTdt$^{3'}$ $^{3'}$dTdTACGGUGAGACUCUUAUCCGAA$^{5'}$
146	Apo-B-5β-cholanic acid -scramble	$^{5'}$UGCCACUCUGAGAAUAGGCUUdT*dt*$^{3'}$ $^{3'}$dTdTACGGUGAGACUCUUAUCCGAA$^{5'}$

[a] The sense strand is written 5' to 3' on the top line. The antisense strand is written 3' to 5' below. The oligonucleotides are phosphodiester RNA except for two 3' deoxythymidines indicated by dT in the sequence. dt represent cholesterol conjugation at C5 of 2'-deoxyuridine and dc represent cholesterol conjugation at C5 of 2'-deoxycytidine. *dt*

represent 5β-cholanic acid conjugation at C5 of 2'-deoxyuridine and *dc* represent 5β-cholanic acid conjugation at C5 of 2'-deoxycytidine. Scrambled sequences were generated by randomizing the sequence of the sense strand.

[b] The PTEN sequence is identical (with the exception of the 3' dTdT) on the antisense strand to that of an antisense oligonucleotide with pharmacological activity. [1]

[c] The c-raf sequence is identical (with the exception of the 3' dTdT) on the antisense strand to that of an antisense oligonucleotide with pharmacological activity. [2]

[d] The H-ras sequence is identical (with the exception of the 3' dTdT) on the antisense strand to that of an antisense oligonucleotide with pharmacological activity. [3]

[e] The Apo-B sequence is identical (with the exception of the 3' dTdT) on the antisense strand to that of an antisense oligonucleotide with pharmacological activity. [3]

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[3] L. M. Cowsert, *Anti-Cancer Drug Design* **1997** 12, 359.

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Example 3

Oligonucleotides: Synthesis, Purification and Analysis

Synthesis:

The designed RNA molecules are synthesized on a 394 ABI machine using the standard 93 step cycle written by the manufacturer with modifications to a few wait steps as described below. Solid supports **52a** and **52b** and phosphoramidites **53a** and **53b** are used for the synthesis of desired oligonucleotides along with commercially available DNA and RNA phosphoramidites. Commercial phosphoramidites with fast protecting groups (5'-*O*-

dimethoxytrityl N6-phenoxyacetyl-2'-*O*-*t*-butyldimethylsilyladenosine-3'-*O*-N,N'-diisopropyl-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N4-acetyl-2'-*O*-*t*-butyldimethylsilylcytidine-3'-O-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N2-*p*-isopropylphenoxyacetyl-2'-*O*-*t*-butyldimethylsilylguanosine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, and 5'-*O*-dimethoxytrityl-2'-*O*-*t*-butyldimethylsilyluridine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite are purchased either from Pierce Nucleic Acids Technologies, Milwaukee, Wisconsin or from Proligo LLC, Boulder, Colorado. All 2'-*O*-Me amidites are received from Glen Research. All amidites are used at a concentration of 0.15 M in acetonitrile (CH_3CN) and a coupling time of 8-15 min. The activator is 5-(ethylthio)-1*H*-tetrazole (0.25M), for the PO-oxidation Iodine/Water/Pyridine is used and for PS-oxidation, 2 % Beaucage reagent (Iyer *et al.*, *J. Am. Chem. Soc.*, **1990**, *112*, 1253) in anhydrous acetonitrile is used. The sulphurization time is about 6 min.

Deprotection- I (Nucleobase Deprotection):

After completion of synthesis the support is transferred to a screw cap vial (VWR Cat # 20170-229) or screw cap RNase free microfuge tube. The oligonucleotide is cleaved from the support with simultaneous deprotection of base and phosphate groups with 1.0 mL of a mixture of ethanolic ammonia [ammonia: ethanol (3:1)] for 15h at 55 °C. The vial is cooled briefly on ice and then the ethanolic ammonia mixture is transferred to a new microfuge tube. The CPG is washed with 2 x 0.1 mL portions of RNase free deionised water. Combined washings, cools over a dry ice bath for 10 min and subsequently dries in speed vac.

Deprotection-II (Removal of 2' TBDMS group):

The white residue obtains is resuspended in 400 μl of triethylamine, triethylamine trihydrofluoride (TEA•3HF) and NMP (4:3:7) and heats at 50 °C for overnight to remove the *tert*-butyldimethylsilyl (TBDMS) groups at the 2'position (Wincott *et al.*, *Nucleic Acids Res.*, **1995**, *23*, 2677). The reaction is then quenched with 400 μl of isopropoxytrimethylsilane (*i*PrOMe$_3$Si, purchased from Aldrich) and further incubates on the heating block leaving the caps open for 10min; (This causes the volatile isopropxytrimethylsilylfluoride adduct to vaporize). The residual quenching reagent is removed by drying in a speed vac. 1.5 ml of 3 % triethylamine in diethyl ether is added and the oligonucleotide is pelleted out by centrifuging. The supernatant is pipetted out without disturbing the pellet and the pellet is dried in speed vac to obtain the crude oligonucleotide as a white fluffy material.

Quantitation of crude oligomer or raw analysis:

Samples are dissolved in RNase free deionied water (1.0mL) and quantitates as follows: Blanking is first performed with water alone (1mL); 20 μL of sample and 980 μL of water are mixed well in a microfuge tube, transfers to cuvette and absorbance reading is obtained at 260 nm. The crude material is dried down and stored at -20 °C.

Purification of oligomers:

PAGE purification

PAGE purification of oligomer synthesized is performed as reported by Sambrook *et al.* (Molecular Cloning: a Laboratory Manual, Second Edition , Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, **1989**). A 12 % denaturing gel is prepared for purification of unmodified and modified oligonucleotides. To a mixture of 120 mL Concentrate, 105 mL Diluents and 25 mL Buffer (National Diagnostics) is added 50 μL TEMED and 1.5 mL 10 % APS. After pouring the gel, it is left for ½ h to polymerize. Oligonucleotide is suspended in 20 μL water and 80 μL formamide. Loads gel tracking dye on left lane followed by the sample slowly on to the gel. Run the gel on 1X TBE buffer at 36 W for 4-6h. Once run is completed, transfer the gel on to preparative TLC plates and see under UV light. Cut the bands, soak and crush in RNase free water and leaves the vial containing purified oligonucletide in a shaker for overnight. Eluent is removed, wash residue with more RNase free water, combined washing and lyophilize to obtain the pure oligonucleotide.

Desalting of Purified Oligomer:

The purified dry oligomer is desalted using Sephadex G-25 M (Amersham Biosciences). The cartridge is conditioned with 10 mL of RNase free deionised water thrice. Finally the purified oligomer is dissolved in 2.5 mL RNasefree water and passed through the cartridge with very slow drop wise elution. The salt free oligomer is eluted with 3.5 mL of RNase free water directly into a screw cap vial.

Analysis:

Capillary gel electrophoresis (CGE) and electrospray LC/MS

Approximately 0.10 OD of oligomer is first dried down, then redissolvs in water (50 μL) and pipettes in specified vials for CGE and LC/MS analysis.

Example 4

In vitro cell culture activities of ligand conjugated siRNA duplex

Dual Luciferase Gene Silencing Assays:

In vitro activity of siRNAs is determined using a high-throughput 96-well plate format luciferase silencing assay. Assays are performed in one of two possible formats. In the first format, HeLa SS6 cells are first transiently transfected with plasmids encoding firefly (target) and *renilla* (control) luciferase. DNA transfections are performed using Lipofectamine 2000 (Invitrogen) and the plasmids gWiz-Luc (Aldevron, Fargo, ND) (200 ng/well) and pRL-CMV (Promega, Madison, WI) (200 ng/well). After 2 h, the plasmid transfection medium is removed, and the firefly luciferase targeting siRNAs are added to the cells at various concentrations. In the second format, HeLa Dual-luc cells (stably expressing both firefly and renilla luciferase) are directly transfected with firefly luciferase targeting siRNAs. SiRNA transfections are performed using either TransIT-TKO (Mirus, Madison, WI) or Lipofectamine 2000 according to manufacturer protocols. After 24 h, cells are analyzed for both firefly and *renilla* luciferase expression using a plate luminometer (VICTOR^2, PerkinElmer, Boston, MA) and the Dual-Glo Luciferase Assay kit (Promega). Firefly/*renilla* luciferase expression ratios are used to determine percent gene silencing relative to mock-treated (no siRNA) controls.

Example 5

Serum stability of ligand conjugated siRNAs

Assay:

siRNA duplexes are prepared at a stock concentration of 1μM in which either the sense (S) or antisense strand (AS) contains a trace amount of 5'-^{32}P labeled material (e.g. ^{32}P-S/AS and S/^{32}P-AS). The presence of the end-labeled sense or antisense strand allows for monitoring of the individual strand within the context of the siRNA duplex. Therefore, two duplex preparations are made for each siRNA sequence tested. siRNA duplexes are incubated in 90% human serum at a final concentration of 100nM duplex. Briefly, 2 μL of 1 μM siRNA duplex is mixed with 18 μL of 100% off the clot human serum at 37 °C. For a typical time course, 2μL aliquots are removed at 10 seconds, 15 minutes, 30 minutes, 1 hour, 2 hours and 4 hours and immediately quenched in 18 μL of a stop mix containing 90% formamide, 50 mM EDTA, 10mM DTT and the dyes xylene cyanol and bromophenol blue. Samples are separated on a denaturing polyacrylamide gel along with a control sample (4 hour buffer-alone incubation) and a partial alkaline hydrolysis ladder used as a marker. The gel is exposed to a Fuji image plate which allows for detection of radiolabeled siRNA and its degradation fragments.

Example 6

Binding Affinity Analysis of siRNA Containing C5-Steroiod Conjugated Pyrimidines to Plasma Proteins.

Method 1

Measurement of Binding affinity:

To measure binding affinity of siRNAs to plasma protein, the 5' end of the sense strand of an siRNA duplex is labeled with ^{32}P using T4 polynucleotide kinase using standard procedures. Each of the siRNA duplexes shown in Table I will be tested in this assay. The unincorporated label is removed using a G25 column and labeling is confirmed by polyacrylamide gel electrophoresis. A fixed concentration of labeled RNA (50 nM) and complementary strand (50 nM) is incubated with increasing concentration of plasma proteins at 25 °C for one hour in phosphate-buffered saline buffer containing 0.1 mM EDTA and 0.005% Tween 80. After incubation, the samples are loaded onto low binding, regenerated cellulose filter membranes with a molecular weight cut-off of 30,000 (Millipore). The samples are spun gently in a microfuge (NYCentrifuge 5415C; Eppendorf, Westbury, NY) at 3000 rpm (735 g) for 3 to 6 minutes, allowing collection of ~20% of the loaded volume in the filtrate.

Radioactivity present in aliquots from the filtrate and the initial (unfiltered) solutions is measured using a scintillation counter (model LS6000IC, Beckman, Fullerton, CA). The counts obtained in the filtrate aliquots represent the free (unbound) RNA, and appropriate calculations are performed to obtain the concentration of free RNA. Further calculations yield the concentration of RNA bound to protein. See R. Zini, J. Barre, F. Bree, J. P. Tillement, B. Sebille, *J. Chromatogr.* **1981**, *216*, 191 and A. N. Kuznetsov, G. V. Gyul'khandanyan, B. Ebert, *Mol. Biol. (Moscow)* **1977**, *11*, 1057.

The extent of siRNA binding to plasma proteins is determined using an equilibrium filtration method. The fraction of bound RNA is plotted vs. the total protein concentration. The equilibrium constant, K_d, is determined from nonlinear regression analysis of the fraction of siRNA bound (f_{bound}) as a function of the free protein concentration (f_{free}). Thus, the data can be fit to a two-state model:

$$\mathbf{O} + \mathbf{A} \overset{K_A}{\leftrightarrow} (\mathbf{OA})$$

where **O** is the unbound siRNA, **A** is the unbound protein, **OA** is the siRNA-protein complex and K_A is the equilibrium association constant. Oligonucleotides to be tested are listed Example 4.

Method 2

Inhibition of mRNA Expression in Balb-C Mouse Treated with siRNAs:

Female BALB/c mice (6 weeks old, Harlan Sprague Dawley, Indianapolis, IN) are housed three to a cage under conditions meeting National Institue of Health regulations.

siRNAs, including unconjugated and scrambled controls (Table I, siRNAs **101-140**), and vehicle containing no siRNA are administered in 0.9 % NaCl, i. p. at indicated dose levels once daily for three days and tissues are harvested for analysis.

Total mRNA is extracted from mouse liver by rapid homogenization of the tissue in 4 M guanidinuim isothiocyanate followed by centrifugation over a cesium chloride gradient. RNAs (20-40 μg) are resolved in 1.2% agarose gels containing 1.1% formaldehyde and transferred to nylon membranes. The blots are hybridized with a radiolabelled human cDNA probe as described. Probes hybridized to mRNA transcripts are visualized and quantified using a PhosPhorImager (Molecular Dynamics). After stripping the blots of radiolabelled probe, they are reprobed with G3PDH cDNA to confirm equal loading.

Method 3

siRNA Treatment of Human Tumor Cells in Nude Mice -- Intraperitoneal Injection:

Human lung carcinoma A549 cells are harvested and 5×10^6 cells (200 μL) were injected subcutaneously into the inner thigh of nude mice. Palpable tumors develop in approximately one month. siRNAs that target the *c-raf* and the H-ras messages, including steroid/lipid-conjugated RNA and scrambled controls (Table I, siRNAs **127-140**) and vehicle containing no siRNA are administered to mice intraperitoneally at a dosage of 20 mg/kg body weight, every other day for approximately ten weeks. Mice are monitored for tumor growth during this time.

Method 4

siRNA Treatment of Human Breast Tumor Cells in Nude Mice:

Human breast carcinoma MDA-MB-231 cells are harvested and 5×10^5 cells (200 μL) are injected subcutaneously into the mammary fat pads of athymic nude mice. Palpable tumors develop in approximately one month. siRNAs that target the *c-raf* and the H-ras messages, including steroid/lipid-conjugated siRNA and scrambled controls (Table I **127-140**) and vehicle containing no siRNA are administered to mice intraperitoneally at a dosages of 5, 10, and 25 mg/kg/day body weight, every day for approximately 20 days. Mice are monitored for tumor growth during this time.

Method 5

siRNA Treatment of Human Lung Tumor Cells in Nude Mice:

Human lung carcinoma A549 cells are harvested and 5 x 10^6 cells (200 μL) are injected subcutaneously into the inner thigh of nude mice. Palpable tumors develop in approximately one month. siRNAs that target the *c-raf* and the H-ras messages, including cholesterol or cholanic acid - conjugated RNA and scrambled controls (Table I **127-140**) and vehicle containing no siRNA are administered to mice subcutaneously at the tumor site. Drug treatment begins one week following tumor cell inoculation and is given twice a week for four weeks. Mice are monitored for tumor growth for a total of nine weeks.

Method 6

Inhibition of Apo-B mRNA Expression in Hep G-2 cells and in Balb-C Mouse Treated with siRNAs:

Inhibition of Aop-B mRNA expression by siRNA (Table I, siRNAs **141-146**) will be evaluated in vitro and in vivo. Effect of siRNA treatment on message levels in HEP-G2 cells is analyzed following treatment. (following the procedure Yao, Z.Q.; Zhou, Y.X.; Guo, J.; Feng, Z.H.; Feng, X.M.; Chen, C.X.; Jiao, J.Z.; Wang, S.Q. *Acta Virol.* **1996** *Feb;40(1)*:35-9. "Inhibition of hepatitis B virus in vitro by antisense oligonucleotides.")

Female BALB/c mice (6 weeks old, Harlan Sprague Dawley, Indianapolis, IN) are housed three to a cage under conditions meeting National Institue of Health regulations. siRNAs, including unconjugated and scrambled controls (Table I, siRNAs **141-146**), and vehicle containing no siRNA are administered in 0.9 % NaCl, i. p. at indicated dose levels once daily for three days and tissues are harvested for analysis.

Total mRNA is extracted from mouse liver by rapid homogenization of the tissue in 4 M guanidinuim isothiocyanate followed by centrifugation over a cesium chloride gradient. RNAs (20-40 μg) are resolved in 1.2% agarose gels containing 1.1% formaldehyde and transferred to nylon membranes. The blots are hybridized with a radiolabelled human Apo-B cDNA probe as described. Probes hybridized to mRNA transcripts are visualized and quantified using a PhosPhorImager (Molecular Dynamics). After stripping the blots of radiolabelled probe, they are reprobed with G3PDH cDNA to confirm equal loading.

Example 7

Table II (below) shows dU cholanic acid (L_{17}, Conjugation of **52b**) and dU cholesterol (L_{19}, Conjugation of **52a**) conjugated siRNAs. Therein "d" indicates a deoxy; "s" indicates a phosphorothioate linkage; and the subscript "o" indicates indictates 2'-OMe.

Table II. dU cholanic acid (L_{17}, Conjugation of **52b**) and dU cholesterol (L_{19}, Conjugation of **52a**) conjugated siRNAs

Target	Seq. No.	Seq.	Mass		Purity
			Calc.	Found	
Luc	1000	$^{5'}\mathrm{CUUACGCUGAGUACUUCGAdTdT}^{3'}$	6606.0	6606.45	99.2
Luc	1001	$^{3'}\mathrm{dTdTGAAUGCGACUCAUGAAGCU}^{5'}$	6693.3	6693.0	89.0
Luc	2759	$^{3'}\mathrm{L_{19}dTGAAUGCGACUCAUGAAGCU}^{5'}$	7259.52	7259.24	78.3
Luc	2758	$^{5'}\mathrm{CUUACGCUGAGUACUUCGAdTL_{19}}^{3'}$	7173.41	7173.43	99.8
Luc	2759	$^{3'}\mathrm{L_{19}dTGAAUGCGACUCAUGAAGCU}^{5'}$	7259.52	7259.24	78.3
Luc	2750	$^{5'}\mathrm{CUUACGCUGAGUACUUCGAdTL_{17}}^{3'}$	7274.5	7274.8	85.0
Luc	2751	$^{3'}\mathrm{L_{19}dTGAAUGCGACUCAUGAAGCU}^{5'}$	7189.30	7189.03	85.2
VEGF	2701	$^{5'}\mathrm{GsCGGAUC_oAAACCUC_oACC_oAAdTsL_{17}}^{3'}$	7205.7	7205.4	89.0
VEGF	2702	$^{5'}\mathrm{GsCGGAUC_oAAACCUC_oAC_oC_oAAdTsL_{17}}^{3'}$	7219.8	7219.8	88.2
VEGF	2696	$^{5'}\mathrm{Us^{Me}U_fGG^{Me}U_fGAGGU^{Me}U_f{}^{Me}U_fGAUCCGCdTsL_{17}}$ $^{3'}$	7276.3	7274.9	71.3

Example 8

Table III (below) shows siRNA silencing of HeLa Dual Luc Cell Line (dU Cholesterol). Therein "L19" indicates dU Cholesterol (obtained via substitution of a dT

CPG support with **52a**); “d” indicates a deoxy; “s” indicates a phosphorothioate linkage; and the subscript “o” indicates indictates 2’-OMe. For IC_{50} “a” is for “Silencing by transfected siRNA in stable HeLa Dual Luc cell line”; “b” is for “Silencing by free uptake of siRNA in transiently transfected HeLa SS6 cell line”; and “c” is for “Silencing by free uptake of siRNA in stable HeLa dual Luc cell line”. In addition, “na” indicates no activity; and “nd” indicates not determined. IC_{50}were calculated as in Example 4.

Table III. siRNA silencing of HeLa Dual Luc Cell Line (dU Cholesterol)

Target	**Duplex**	**Seq.**	**Sequence**	**IC_{50} (nM)**		
				a	**b**	**c**
Luc	1	1000	$^{5'}$CUUACGCUGAGUACUUCGAdTdT$^{3'}$	0.20	>2000	nd
		1001	$^{3'}$dTdTGAAUGCGACUCAUGAAGCU$^{5'}$			
Luc	2	2758	$^{5'}$CUUACGCUGAGUACUUCGAdTL$_{19}^{3'}$	1.00	50.00	nd
		1001	$^{3'}$dTGAAUGCGACUCAUGAAGCU$^{5'}$			
Luc	3	1000	$^{5'}$CUUACGCUGAGUACUUCGAdTdT$^{3'}$	0.60	90.00	na
		2759	$^{3'}$L$_{19}$dTGAAUGCGACUCAUGAAGCU$^{5'}$			
Luc	4	2758	$^{5'}$CUUACGCUGAGUACUUCGAdTL$_{19}^{3'}$	16.00	630.00	nd
		2759	$^{3'}$L$_{19}$dTGAAUGCGACUCAUGAAGCU$^{5'}$			

Example 9

Table IV and V (below) show siRNA silencing of HeLa Dual Luc Cell Line. Therein “L_{17}” indicates dU Cholanic acid (obtained via substitution of a dT CPG support with **52b**);“d” indicates a deoxy; subscript “F” indicates a 2’-fluor sugar; “^{Me}U” indicates a 5-methyl-uridine; “s” indicates a phosphorothioate linkage; and the subscript “o” indicates indictates 2’-OMe. For IC_{50} “a” is for “Silencing by transfected siRNA in stable HeLa Dual Luc cell line”; “b” is for “Silencing by free uptake of siRNA in transiently transfected HeLa SS6 cell line”; and “c” is for “Silencing by free uptake of siRNA in stable HeLa dual Luc cell line". In addition, "na" indicates no activity; and "nd" indicates not determined. IC$_{50}$were calculated as in Example 4.

In Table V the efficacy is set at "+++" for the control (unmodified) duplex 9 and the other duplexes (10-12) are rated accordingly.

Table IV. siRNA silencing of HeLa Dual Luc Cell Line

Target	Duplex	Seq.	Sequence	IC$_{50}$ (nM)		
				a	b	c
Luc	5	1000	$^{5'}$CUUACGCUGAGUACUUCGAdTdT$^{3'}$	0.20	>2000	nd
		1001	$^{3'}$dTdTGAAUGCGACUCAUGAAGCU$^{5'}$			
Luc	6	2750	$^{5'}$CUUACGCUGAGUACUUCGAdTL$_{17}$$^{3'}$	0.03	110.00	nd
		1001	$^{3'}$dTGAAUGCGACUCAUGAAGCU$^{5'}$			
Luc	7	1000	$^{5'}$CUUACGCUGAGUACUUCGAdTdT$^{3'}$	0.04	200.00	nd
		2759	$^{3'}$L$_{17}$dTGAAUGCGACUCAUGAAGCU$^{5'}$			
Luc	8	2758	$^{5'}$CUUACGCUGAGUACUUCGAdTL$_{17}$$^{3'}$	0.70	50.00	na
		2759	$^{3'}$L$_{17}$dTGAAUGCGACUCAUGAAGCU$^{5'}$			

Table V. siRNA silencing of HeLa Dual Luc Cell Line

Target	Duplex	Seq.	Sequence	Efficacy
VEGF	9	4112	GCGGAUCAAACCUCACCAAdTdT	+++
		4180	dTdTCGCCUAGUUUGGAGUGGUU	
VEGF	10	2701	GsCGGAUC$_{o}$AAACCUC$_{o}$ACC$_{o}$AAdTsL$_{17}$	++
		2381	UsUGGUGAGGUUUGAUCCGCdTsdT	

VEGF	11	2702	GsCGGAUC$_o$AAACCUC$_o$AC$_o$C$_o$AAdTsL$_{17}$	++
		2381	UsUGGUGAGGUUUGAUCCGCdTsdT	
VEGF	12	4112	GCGGAUCAAACCUCACCAATT	+++
		2696	Us^{5Me}U$_F$G^{5Me}U$_F$GAGGU^{5Me}U$_F$^{5Me}U$_F$GAUCCGCdTsL$_{17}$	

Table IV. siRNA silencing of HeLa Dual Luc Cell Line

Target	Duplex	Seq.	Sequence	IC$_{50}$ (nM)		
				a	b	c
Luc	5	1000	$^{5'}$CUUACGCUGAGUACUUCGAdTdT$^{3'}$	0.20	>2000	nd
		1001	$^{3'}$dTdTGAAUGCGACUCAUGAAGCU$^{5'}$			
Luc	6	2750	$^{5'}$CUUACGCUGAGUACUUCGAdTL$_{17}$$^{3'}$	0.03	110.00	nd
		1001	$^{3'}$dTGAAUGCGACUCAUGAAGCU$^{5'}$			
Luc	7	1000	$^{5'}$CUUACGCUGAGUACUUCGAdTdT$^{3'}$	0.04	200.00	nd
		2759	$^{3'}$L$_{17}$dTGAAUGCGACUCAUGAAGCU$^{5'}$			
Luc	8	2758	$^{5'}$CUUACGCUGAGUACUUCGAdTL$_{17}$$^{3'}$	0.70	50.00	na
		2759	$^{3'}$L$_{17}$dTGAAUGCGACUCAUGAAGCU$^{5'}$			

Table V. siRNA silencing of HeLa Dual Luc Cell Line

Target	Duplex	Seq.	Sequence	Efficacy
VEGF	9	4112	GCGGAUCAAACCUCACCAAdTdT	+++
		4180	dTdTCGCCUAGUUUGGAGUGGUU	
VEGF	10	2701	GsCGGAUC$_o$AAACCUC$_o$ACC$_o$AAdTsL$_{17}$	++

		2381	UsUGGUGAGGUUUGAUCCGCdTsdT	
VEGF	11	2702	$\mathrm{GsCGGAUC_oAAACCUC_oAC_oC_oAAdTsL_{17}}$	++
		2381	UsUGGUGAGGUUUGAUCCGCdTsdT	
VEGF	12	4112	GCGGAUCAAACCUCACCAATT	+++
		2696	$\mathrm{Us^{5Me}U_FG^{5Me}U_FGAGGU^{5Me}U_F{}^{5Me}U_FGAUCCGCdTsL_{17}}$	

Incorporation by Reference

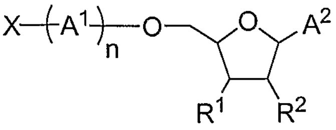
All of the patents and publications cited herein are hereby incorporated by reference.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

We claim:

1. A single-stranded oligonucleotide represented by formula **I**:



I

wherein

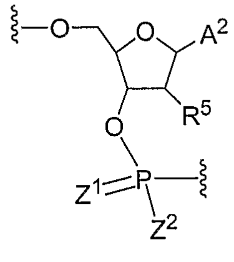
X is H, -P(O)(OM)$_2$, -P(O)(OM)-O-P(O)(OM)$_2$, -P(O)(Oalkyl)$_2$, or -P(O)(Oalkyl)-O-P(O)(Oalkyl)$_2$;

M represents independently for each occurrence an alkali metal or a transition metal with an overall charge of +1;

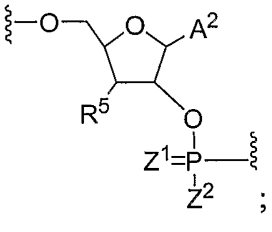
n is 16, 17, 18 , 19, 20, 21, 22, 23, or 24;

R^1, R^2, and R^5 represent independently for each occurrence H, OH, F, -Oalkyl, -Oallyl, -O(C(R^7)$_2$)$_v$OR^7, -O(C(R^7)$_2$)$_v$SR^7, -O(C(R^7)$_2$)$_v$N(R^7)$_2$, -O(C(R^7)$_2$)$_v$C(O)N(R^7)$_2$, -N(R^7)$_2$, -S(C$_1$-C$_6$)alkyl, -O(C(R^7)$_2$)$_v$O(C$_1$-C$_6$)alkyl, -O(C(R^7)$_2$)$_v$S(C$_1$-C$_6$)alkyl, -O(C(R^7)$_2$)$_v$O(C(R^7)$_2$)$_v$N((C$_1$-C$_6$)alkyl)$_2$, or -O(C(R^7)$_2$)$_v$ON((C$_1$-C$_6$)alkyl)$_2$;

A^1 represents independently for each occurrence:



or



;

Z^1 represents independently for each occurrence O or S;

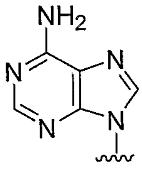
Z^2 represents independently for each occurrence -OH, -OM, -Oalkyl, -Oaryl, -Oaralkyl, -SH, SM, -Salkyl, -Saryl, -Saralkyl, -N(R^3)R^4, -(C(R^7)$_2$)$_m$N(R^7)$_2$, -N(R^7)(C(R^7)$_2$)$_m$N(R^7)$_2$, or alkyl;

R^3 and R^4 are H or alkyl; or R^3 and R^4 taken together form a 3-, 4-, 5-, 6-, or 7-member ring;

R^6 represents independently for each occurrence H, alkyl, or $-NHCH_2CH{=}CH_2$;

R^7 represents independently for each occurrence H or alkyl;

A^2 represents independently for each occurrence:



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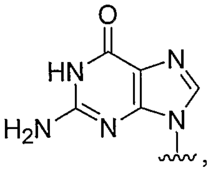

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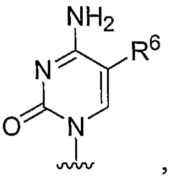

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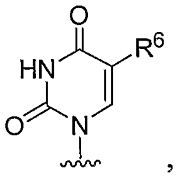

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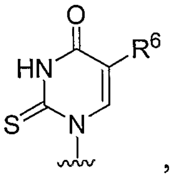

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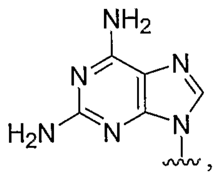

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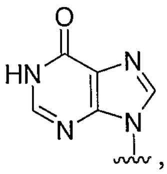

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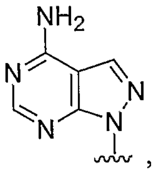

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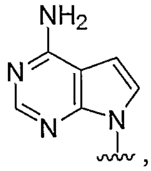

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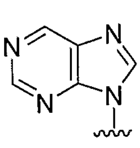

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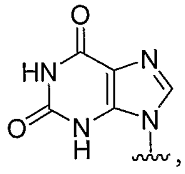

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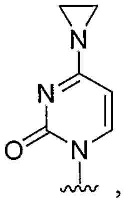

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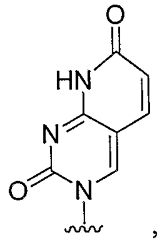

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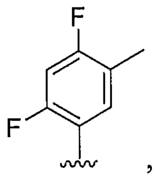

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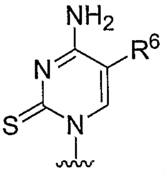

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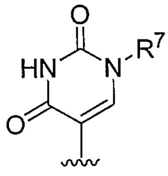
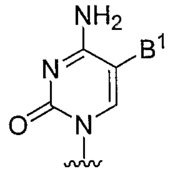
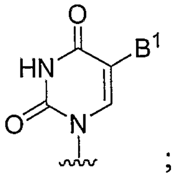


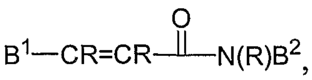
, or $-A^3-A^4-(A^5)_w$;

A^3 represents independently for each occurrence


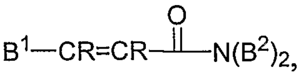
or


;

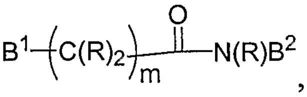
A^4 represents independently for each occurrence a bond, alkyl diradical, heteroalkyl diradical, alkenyl diradical, aminoalkyl diradical, alkynyl diradical, alkylalkynyl diradical, thioether, -C(O)-, -S(O)-, $-S(O)_2-$, $B^1C(R)_2B^2$, $B^1C(R)(B^2)_2$, $B^1C(B^2)_3$, $B^1N(R)(B^2)$, $B^1N(B^2)_2$, or has the formula:



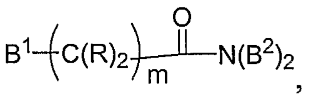
$B^1{-}CR{=}CR{-}\overset{\overset{O}{\|}}{C}{-}N(R)B^2$,



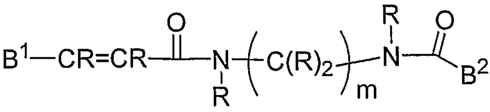
$B^1{-}CR{=}CR{-}\overset{\overset{O}{\|}}{C}{-}N(B^2)_2$,



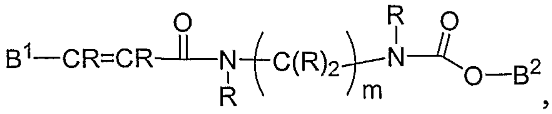
$B^1\left(C(R)_2\right)_m\overset{\overset{O}{\|}}{C}{-}N(R)B^2$,



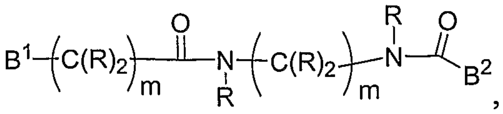
$B^1\left(C(R)_2\right)_m\overset{\overset{O}{\|}}{C}{-}N(B^2)_2$,



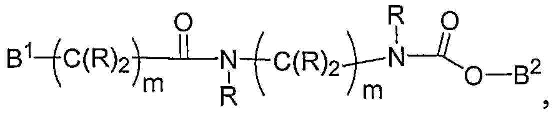
$B^1{-}CR{=}CR{-}\overset{\overset{O}{\|}}{C}{-}\underset{R}{N}\left(C(R)_2\right)_m\overset{R}{N}{-}\overset{\overset{O}{\|}}{C}{-}B^2$,



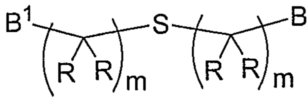
$B^1{-}CR{=}CR{-}\overset{\overset{O}{\|}}{C}{-}\underset{R}{N}\left(C(R)_2\right)_m\overset{R}{N}{-}\overset{\overset{O}{\|}}{C}{-}O{-}B^2$,



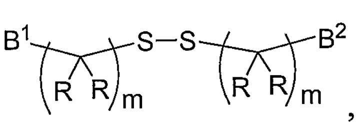
$B^1\left(C(R)_2\right)_m\overset{\overset{O}{\|}}{C}{-}\underset{R}{N}\left(C(R)_2\right)_m\overset{R}{N}{-}\overset{\overset{O}{\|}}{C}{-}B^2$,

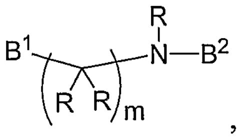


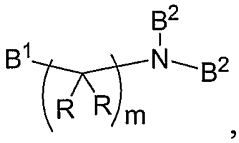
$B^1\left(C(R)_2\right)_m\overset{\overset{O}{\|}}{C}{-}\underset{R}{N}\left(C(R)_2\right)_m\overset{R}{N}{-}\overset{\overset{O}{\|}}{C}{-}O{-}B^2$,

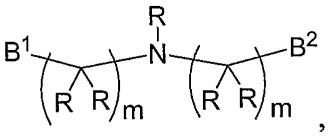


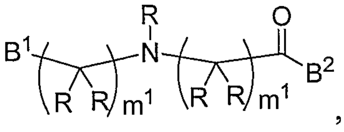
$B^1\left(\underset{R\ R}{C}\right)_m S\left(\underset{R\ R}{C}\right)_m B^2$,

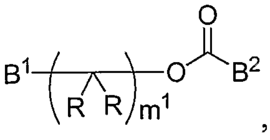


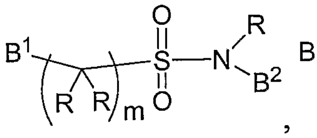
,

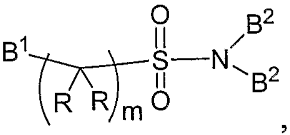
,

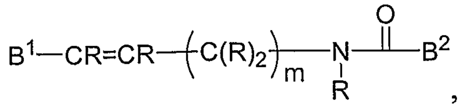


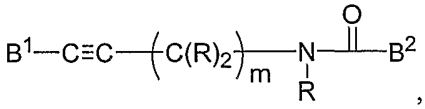
,

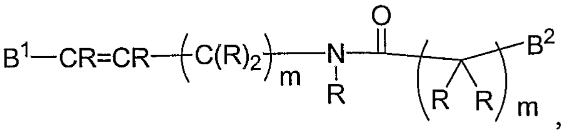
,

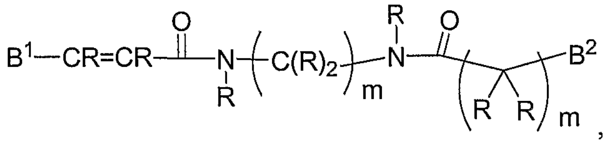
,

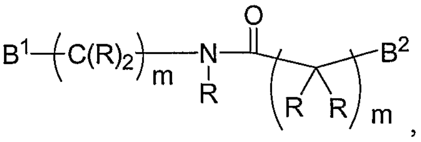
,

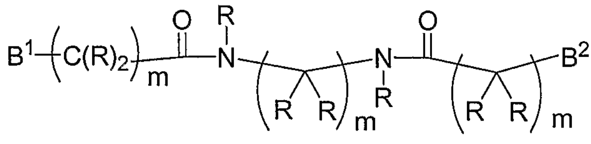
,

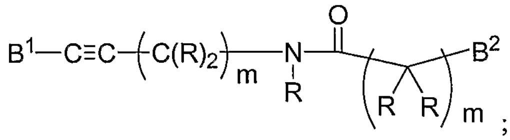
,

,

,

,

, or

;

B^1 represents independently for each occurrence a bond between A^3 and A^4;

B^2 represents independently for each occurrence a bond between A^4 and A^5;

R represents independently for each occurrence hydrogen or alkyl;

m represents independently for each occurrence 1, 2, 3, 4, 5, 6, 7 or 8;

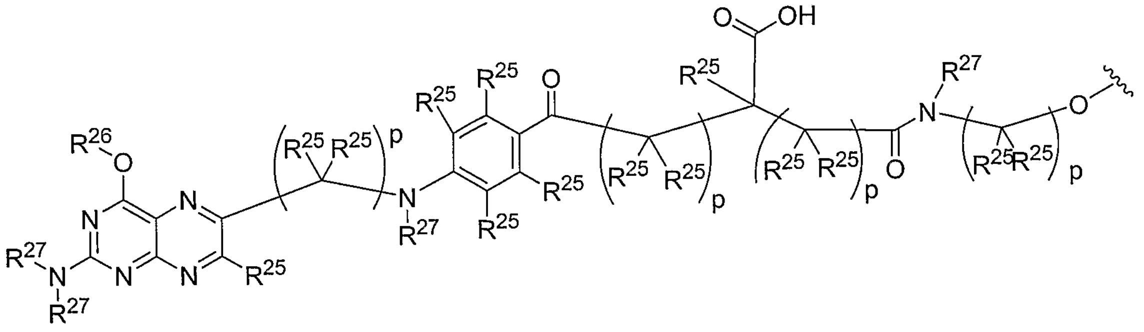
m^1 represents independently for each occurrence 0, 1, 2, 3, 4, 5, 6, 7 or 8;

v represents independently for each occurrence 1, 2, 3, or 4;

w represents independently for each occurrence 1, 2, or 3 in accord with the rules of valence;

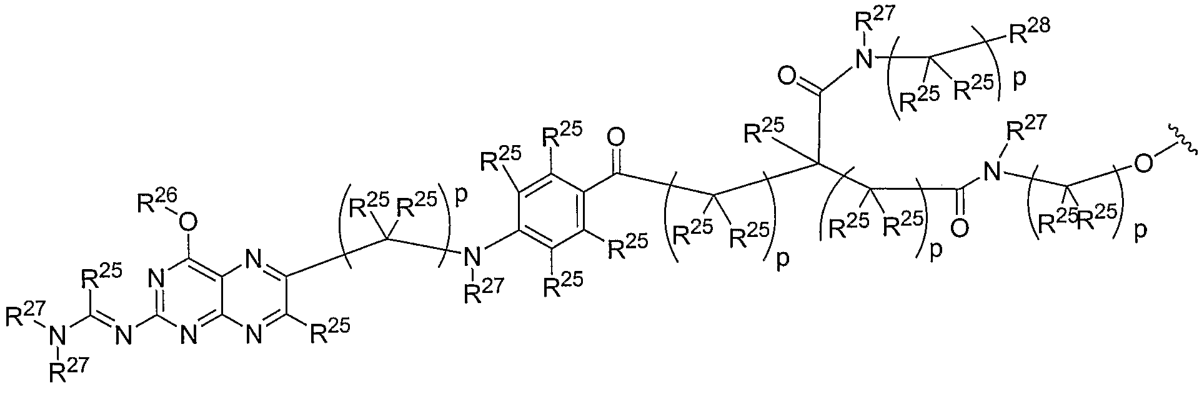
provided that A^5 occurs at least once; and

A^5 represents independently for each occurrence compound **C**, compound **C'**, compound **D**, compound **D'** or a radical of a steroid, bile acid, lipid, folic acid, pyridoxal, B12, riboflavin, biotin, aromatic compound, polycyclic compound, crown ether, intercalator, cleaver molecule, protein-binding agent, carbohydrate, or an optionally substituted saturated 5-membered ring; wherein compound **C** is represented by:



C

and compound **C'** is represented by:



C'

wherein for **C** and **C'**

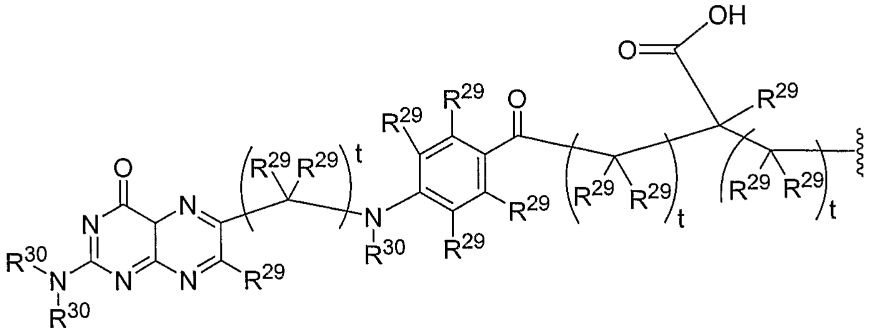
R^{25} represents independently for each occurrence H, halogen, hydroxyl, thiol, alkoxyl, thioalkyl, azido, $-N(R^{27})_2$, $-CO_2R^{27}$, $-COR^{27}$, $-OC(O)R^{27}$, alkyl, alkenyl, aryl, or aralkyl;

R^{26} represents independently for each occurrence alkyl, alkenyl, aryl, or aralkyl;

R^{27} represents independently for each occurrence H, alkyl, alkenyl, aryl, or aralkyl;

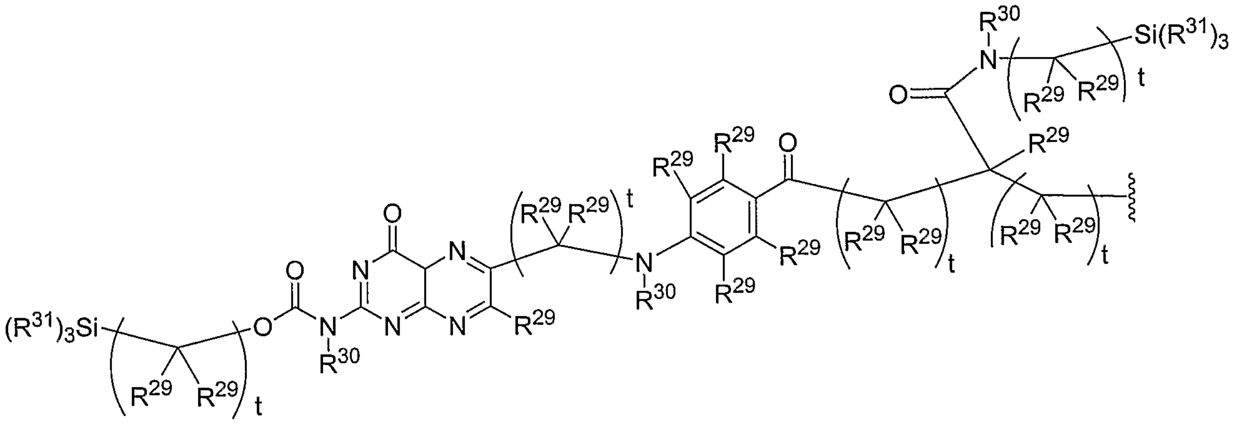
R^{28} represents independently for each occurrence $-OC(O)R^{27}$, $-N(R^{27})C(O)R^{27}$, $-N(R^{27})_2$, $-CO_2R^{27}$, $-COR^{27}$, alkyl, alkenyl, aryl, or aralkyl; and p represents independently for each occurrence 1, 2, 3, 4, 5, 6, or 7; and

compound **D** is represented by:



D

and compound **D'** is represented by:



D'

wherein for **D** and **D'**

R^{29} represents independently for each occurrence H, halogen, hydroxyl, thiol, alkoxyl, thioalkyl, azido, $-N(R^{30})_2$, $-CO_2R^{30}$, $-COR^{30}$, $-OC(O)R^{30}$, alkyl, alkenyl, aryl, or aralkyl;

R^{30} represents independently for each occurrence H, alkyl, alkenyl, aryl, or aralkyl;

R^{31} represents independently for each occurrence alkyl, alkenyl, aryl, or aralkyl; and

t represents independently for each occurrence 1, 2, 3, 4, 5, 6, or 7.

2. The single-stranded oligonucleotide of claim 1, wherein n is 18, 19, 20, 21, or 22.

3. The single-stranded oligonucleotide of claim 1, wherein n is 20.

4. The single-stranded oligonucleotide of claim 1, wherein A^5 occurs at least twice.

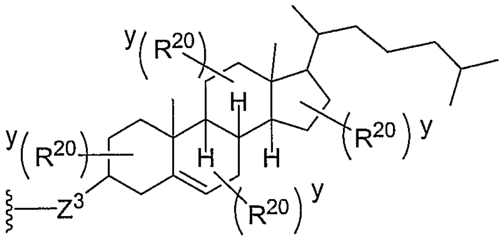
5. The single-stranded oligonucleotide of claim 1, wherein A^5 occurs at least five times.

6. The single-stranded oligonucleotide of claim 1, wherein A^5 occurs at least ten times.

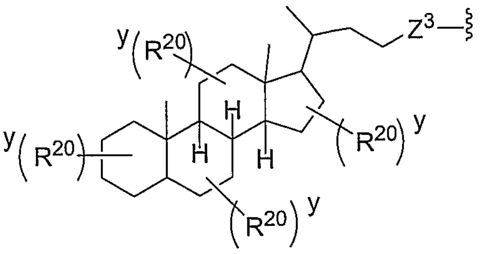
7. The single-stranded oligonucleotide of claim 1, wherein A^5 represents independently for each occurrence compound **C**, compound **D**, or a radical of cholesterol, 5β-cholanic acid, progesterone, aldosterone, dehydroaldosterone, isoandrosterone, esterone, estradiol, ergosterol, dehydroergosterol, lanosterol, 4-cholesten-3-one, guggulsterone, testosterone, nortestosterone, formestane, hydroxyecdysone, ketoestriol, corticosterone, dienestrol, dihydroxypregnanone, pregnanone, copornmon, equilenin, equilin, estriol, ethinylestradiol, mestranol, moxestrol, mytatrienediol, quinestradiol, quinestrol, helvolic acid, protostadiene, fusidic acid, cycloartenol, tricallol, cucurbitanin cedrelone, euphol, dammerenediol, parkeol, dexametasone, methylprednisolone, prednisolone, hydrocortisone, parametasone, betametasone, cortisone, fluocinonide, fluorometholone, halcinonide, budesonide, or any one of them further substituted with one or more of hydroxyl, halogen, amino, alkylamino, alkyl, carboxylic acid, ester, amide, carbonyl, alkoxyl, or cyano.

8. The single-stranded oligonucleotide of claim 1, wherein A^5 represents independently for each occurrence a radical of cholesterol or 5β-cholanic acid.

9. The single-stranded oligonucleotide of claim 1, wherein A^5 represents independently for each occurrence:



or



, wherein R^{20} represents independently for each occurrence hydroxyl, amino, halogen, alkoxyl, alkyl, aminoalkyl, azido, carbonyl, acyl, or acyloxy; Z^3 represents independently for each occurrence O, S, or NR; and y represents independently for each occurrence 1, 2, 3, 4, 5, or 6 in accord with the rules of valence.

10. The single-stranded oligonucleotide of claim 1, wherein A^5 represents independently for each occurrence a radical of cholic acid, deoxycholic acid, taurocholic acid, glycocholic acid, glycodeoxycholic acid, taurodeoxycholic acid, ursodeoxycholic acid, or chenodeoxycholic acid.

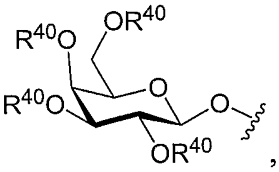
11. The single-stranded oligonucleotide of claim 1, wherein A^5 represents independently for each occurrence a radical of lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, triacylglycerol, phosphoacylglycerol, sphingolipid, monoterpene, sesquiterpene, diterpene, sesterterpene, triterpene, or tetraterpene.

12. The single-stranded oligonucleotide of claim 1, wherein A^5 represents independently for each occurrence optionally substituted phenyl, naphthyl, anthracenyl, phenanthrenyl, pyrenyl, pyridinyl, quinolinyl, acridinyl, phenathridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinoxalinyl, quinazolinyl, 1,7-phenanthrolinyl, indolyl, thianaphthenyl, benzoxazolyl, benzofuranyl, 1,2-benzisoxazolyl, benzimidazolyl, pyrrolyl, thiophenyl, isoxazolyl, pyrazolyl, thiazolyl, imidazolyl, tetrazolyl, or furanyl.

13. The single-stranded oligonucleotide of claim 1, wherein A^5 represents independently for each occurrence the radical of erythrose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, mannose, gulose, idose, galactose, or talose; or a disaccharide or trisaccharide formed via a 1,4 glycoside linkage between any of them.

14. The single-stranded oligonucleotide of claim 1, wherein A^5 is a hexose or pentose.

15. The single-stranded oligonucleotide of claim 1, wherein A^5 is



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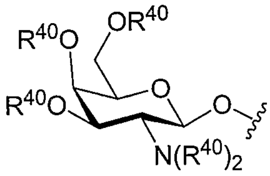
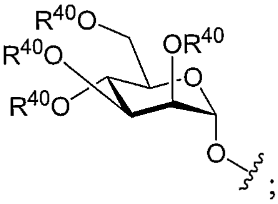
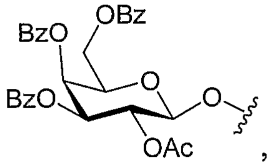


, or



; wherein R^{40} represents independently for each occurrence H, alkyl, aryl, aralkyl, -C(O)alkyl, -C(O)aryl, -C(O)aralkyl, or Si(alkyl)$_3$.

16. The single-stranded oligonucleotide of claim 1, wherein A^5 is



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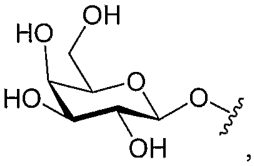

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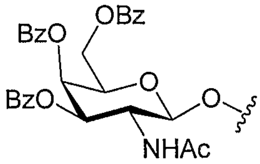

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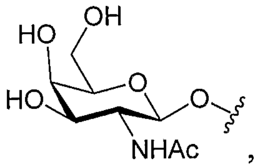

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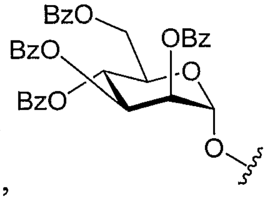
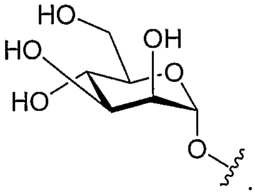
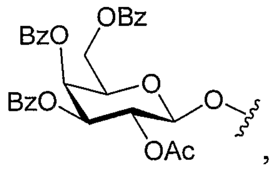
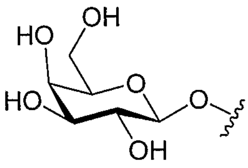
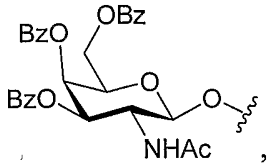
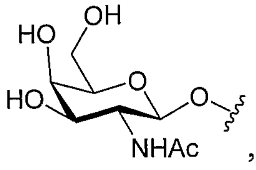
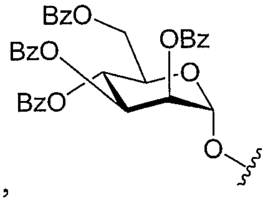


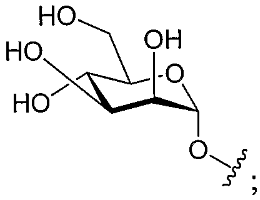
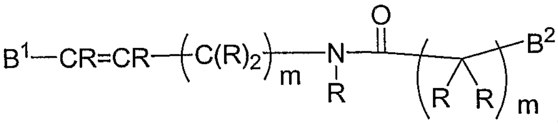
or

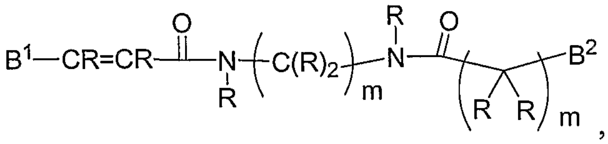
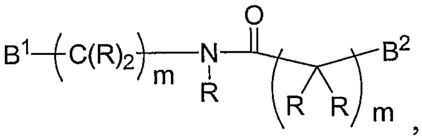


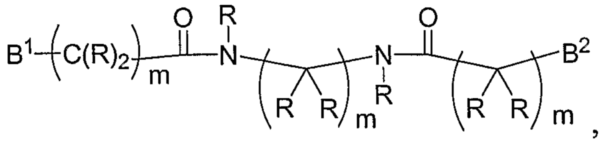
.

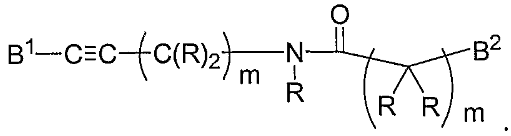
17. The single-stranded oligonucleotide of claim 1, wherein A^5 is ,

, , ,  or

; and A^4 is ,

, ,

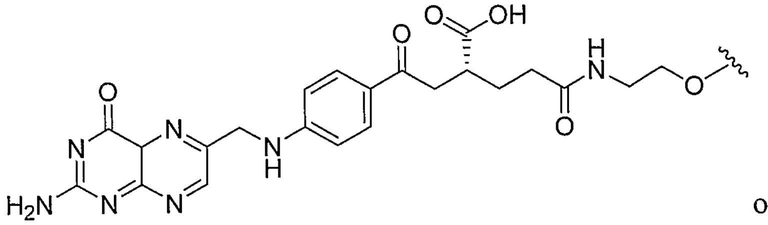
, or

.

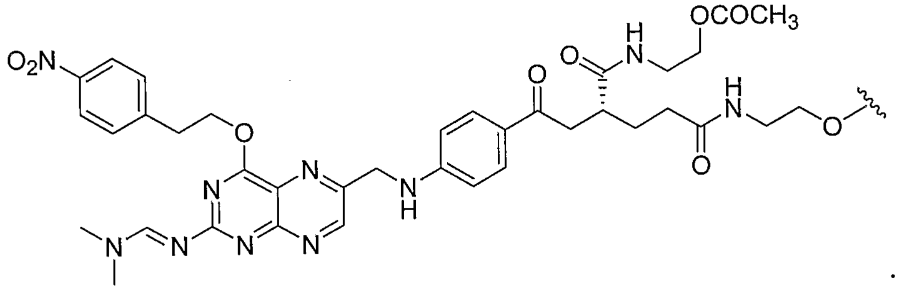
18. The single-stranded oligonucleotide of claim 1, wherein A^5 is an optionally substituted cyclopentane, pyrrolidine, tetrahydrofuran, tetrahydrothiophene, or 1,1-difluorocyclopentane.

19. The single-stranded oligonucleotide of claim 1, wherein A^5 is compound **C**.

20. The single-stranded oligonucleotide of claim 1, wherein A^5 is



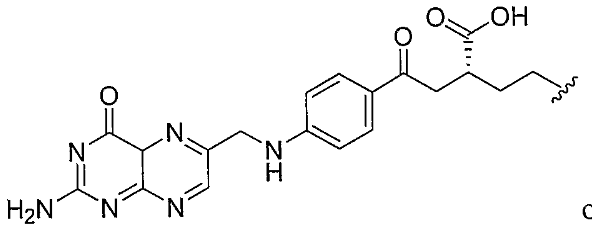
or



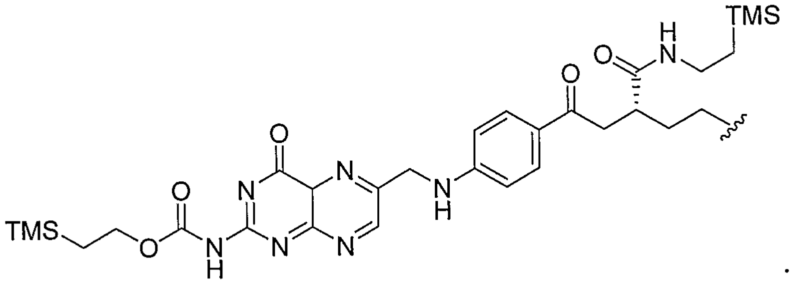
.

21. The single-stranded oligonucleotide of claim 1, wherein A^5 is compound **D**.

22. The single-stranded oligonucleotide of claim 1, wherein A^5 is

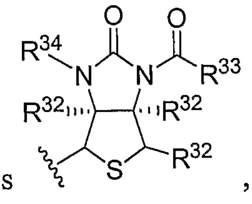


or



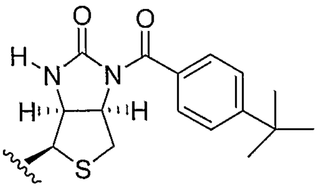
.

23. The single-stranded oligonucleotide of claim 1, wherein A^5 is a radical of a polycyclic compound.

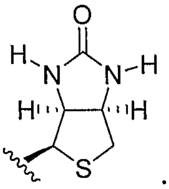
24. The single-stranded oligonucleotide of claim 1, wherein A^5 is , wherein R^{32} represents independently for each occurrence H, halogen, hydroxyl, thiol, alkoxyl, thioalkyl, azido, $-N(R^{34})_2$, $-CO_2R^{34}$, $-COR^{34}$, $-OC(O)R^{34}$, alkyl, alkenyl, aryl, or aralkyl; R^{33} is aryl that is optionally substituted with alkyl, alkenyl,

aryl, halogen, or alkoxyl; and R^{34} represents independently for each occurrence H, alkyl, aryl, or aralkyl.

25. The single-stranded oligonucleotide of claim 1, wherein A^5 is

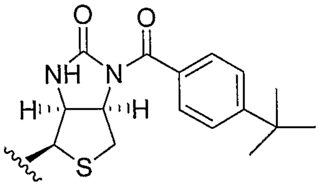


or

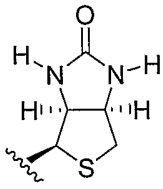


.

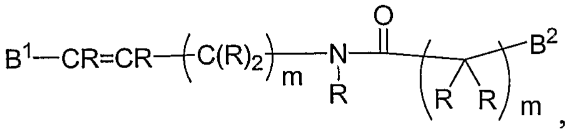
26. The single-stranded oligonucleotide of claim 1, wherein A^5 is



or



, and A^4 is



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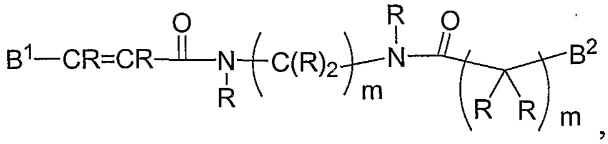

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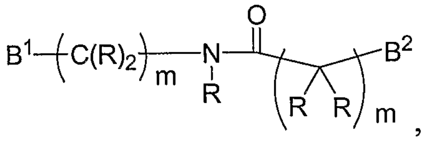

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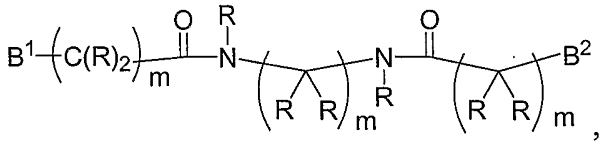
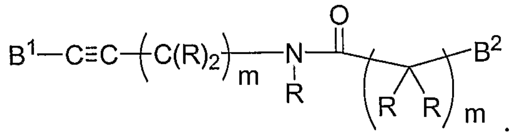


, or



.

27. The single-stranded oligonucleotide of claim 1, wherein R^2 represents independently for each occurrence H, OH, F, or -Oalkyl.

28. The single-stranded oligonucleotide of claim 1, wherein R^2 represents independently for each occurrence H, OH, F, $-OCH_3$, $-O(CH_2)_2OR^7$, $-O(CH_2)_2SR^7$, $-O(CH_2)_2N(R^7)_2$, $-OCH_2C(O)N(H)CH_3$, $-NH_2$, $-N(CH_3)_2$, $-N(H)CH_3$, $-SCH_3$, $-O(CH_2)_2OCH_3$, $-O(CH_2)_2SCH_3$, $-O(CH_2)_2O(CH_2)_2N(CH_3)_2$, or $-O(CH_2)_2ON(CH_3)_2$.

29. The single-stranded oligonucleotide of claim 1, wherein R^2 represents independently for each occurrence $-NH_2$, $-N(H)CH_3$, or $-N(CH_3)_2$.

30. The single-stranded oligonucleotide of claim 1, wherein R^5 represents independently for each occurrence H, OH, F, $-OCH_3$, $-O(CH_2)_2OR^7$, $-O(CH_2)_2SR^7$, $-O(CH_2)_2N(R^7)_2$, $-OCH_2C(O)N(H)CH_3$, $-NH_2$, $-N(CH_3)_2$, $-N(H)CH_3$, $-SCH_3$, $-O(CH_2)_2OCH_3$, $-O(CH_2)_2SCH_3$, $-O(CH_2)_2O(CH_2)_2N(CH_3)_2$, or $-O(CH_2)_2ON(CH_3)_2$.

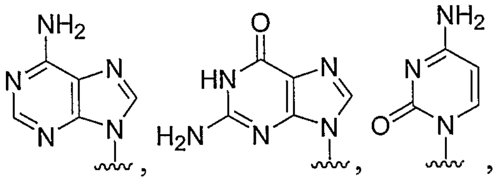
31. The single-stranded oligonucleotide of claim 1, wherein R^5 represents independently for each occurrence $-NH_2$, $-N(H)CH_3$, or $-N(CH_3)_2$.

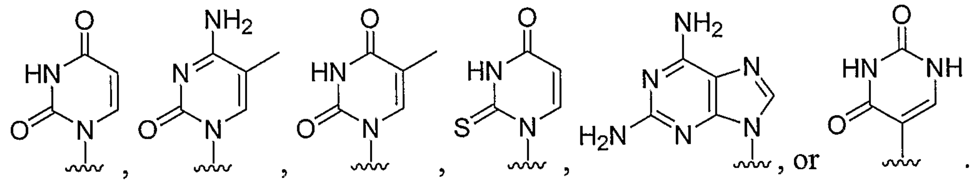
32. The single-stranded oligonucleotide of claim 1, wherein R^2 and R^5 represent independently for each occurrence $-NH_2$, $-N(H)CH_3$, or $-N(CH_3)_2$.

33. The single-stranded oligonucleotide of claim 1, wherein Z^2 represents independently for each occurrence -OH, -OM, -Oalkyl, -Oaryl, -Oaralkyl, -SH, -SM, -Salkyl, -Saryl, -Saralkyl, $-N(R^3)R^4$, $-(C(R^7)_2)_mN(R^7)_2$, $-N(R^7)(C(R^7)_2)_mN(R^7)_2$, or methyl;

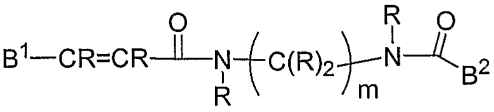
34. The single-stranded oligonucleotide of claim 1, wherein Z^2 represents independently for each occurrence -OH, -OM, -Oalkyl, -Oaryl, or -Oaralkyl.

35. The single-stranded oligonucleotide of claim 1, wherein A^2 represents independently for each occurrence:



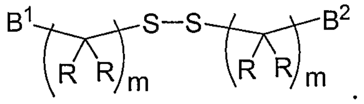


36. The single-stranded oligonucleotide of claim 1, wherein A^4 represents independently for each occurrence



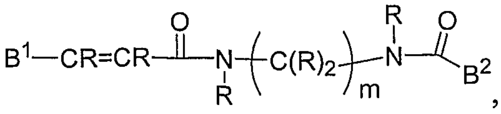
$$B^1-CR=CR-\overset{O}{\overset{\|}{C}}-\underset{R}{\underset{|}{N}}-\left(C(R)_2\right)_m-\underset{}{\overset{R}{\overset{|}{N}}}-\overset{O}{\overset{\|}{C}}-B^2$$

or



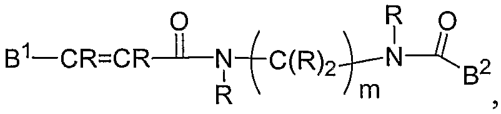
.

37. The single-stranded oligonucleotide of claim 1, wherein A^4 represents independently for each occurrence



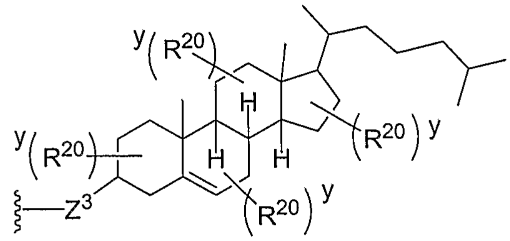
$$B^1-CR=CR-\overset{O}{\overset{\|}{C}}-\underset{R}{\underset{|}{N}}-\left(C(R)_2\right)_m-\overset{R}{\overset{|}{N}}-\overset{O}{\overset{\|}{C}}-B^2$$

, and A^5 is a steroid.

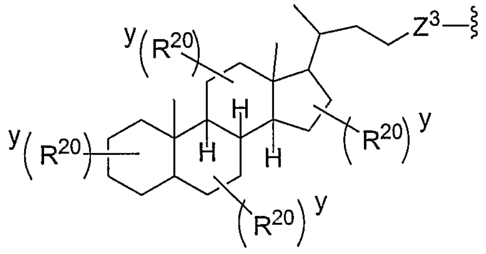
38. The single-stranded oligonucleotide of claim 1, wherein A^4 represents independently for each occurrence 

$$B^1\text{—}CR{=}CR\text{—}C(=O)\text{—}N(R)\text{—}\left(C(R)_2\right)_m\text{—}N(R)\text{—}C(=O)\text{—}B^2$$

, and A^5 represents independently for each occurrence:

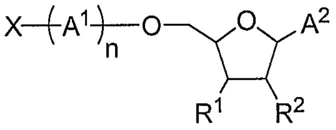


or



, wherein R^{20} represents independently for each occurrence hydroxyl, amino, halogen, alkoxyl, alkyl, aminoalkyl, azido, carbonyl, acyl, or acyloxy; Z^3 represents independently for each occurrence O, S, or NR; and y represents independently for each occurrence 1, 2, 3, 4, 5, or 6 in accord with the rules of valence.

39. A double-stranded oligonucleotide comprising a first strand and a second strand, wherein said first strand and said second strand are represented independently by formula **II**:



II

wherein

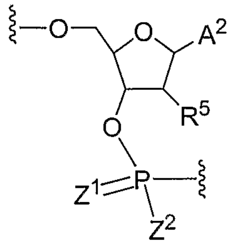
X is H, -P(O)(OM)$_2$, -P(O)(OM)-O-P(O)(OM)$_2$, -P(O)(Oalkyl)$_2$, or -P(O)(Oalkyl)-O-P(O)(Oalkyl)$_2$;

M represents independently for each occurrence an alkali metal or a transition metal with an overall charge of +1;

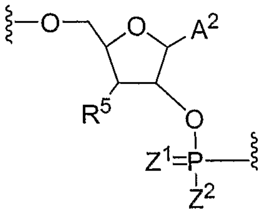
n is 16, 17, 18 , 19, 20, 21, 22, 23, or 24;

R^1, R^2, and R^5 represent independently for each occurrence H, OH, F, -Oalkyl, -Oallyl, $-O(C(R^7)_2)_vOR^7$, $-O(C(R^7)_2)_vSR^7$, $-O(C(R^7)_2)_vN(R^7)_2$, $-O(C(R^7)_2)_vC(O)N(R^7)_2$, $-N(R^7)_2$, $-S(C_1\text{-}C_6)$alkyl, $-O(C(R^7)_2)_vO(C_1\text{-}C_6)$alkyl, $-O(C(R^7)_2)_vS(C_1\text{-}C_6)$alkyl, $-O(C(R^7)_2)_vO(C(R^7)_2)_vN((C_1\text{-}C_6)\text{alkyl})_2$, or $-O(C(R^7)_2)_vON((C_1\text{-}C_6)\text{alkyl})_2$;

A^1 represents independently for each occurrence:



or



;

Z^1 represents independently for each occurrence O or S;

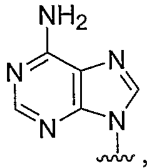
Z^2 represents independently for each occurrence -OH, -OM, -Oalkyl, -Oaryl, -Oaralkyl, -SH, SM, -Salkyl, -Saryl, -Saralkyl, $-N(R^3)R^4$, $-(C(R^7)_2)_mN(R^7)_2$, $-N(R^7)(C(R^7)_2)_mN(R^7)_2$, or alkyl;

R^3 and R^4 are H or alkyl; or R^3 and R^4 taken together form a 3-, 4-, 5-, 6-, or 7-member ring;

R^6 represents independently for each occurrence H, alkyl, or $-NHCH_2CH=CH_2$;

R^7 represents independently for each occurrence H or alkyl;

A^2 represents independently for each occurrence: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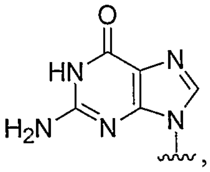
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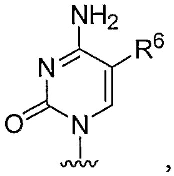
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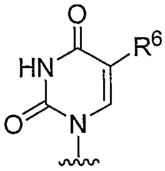
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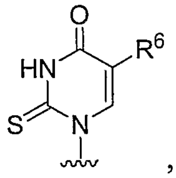
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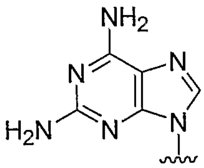
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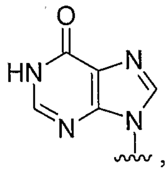
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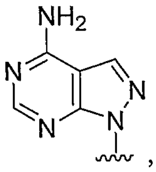
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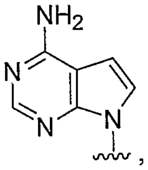
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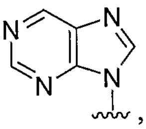
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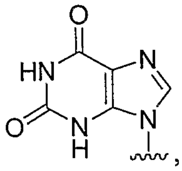
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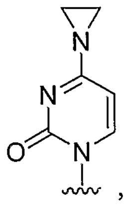
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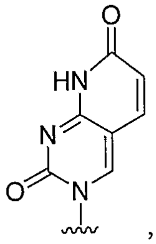
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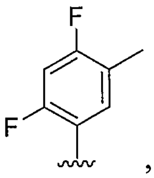
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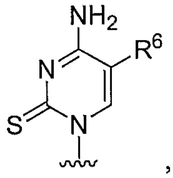
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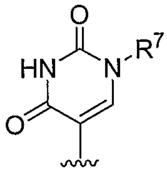
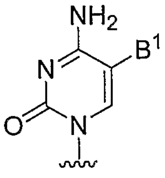
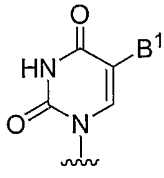
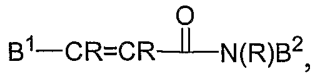
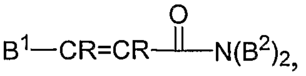
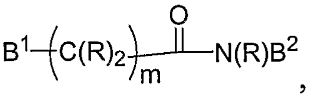
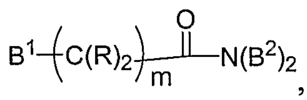
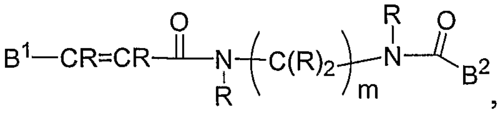
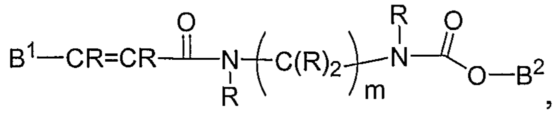
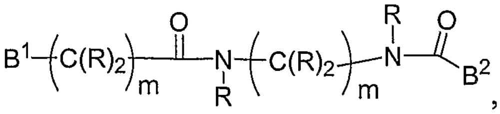

, or $-A^3-A^4-(A^5)_w$;

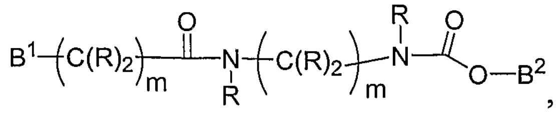
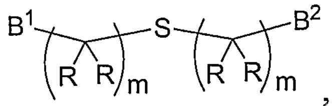
A^3 represents independently for each occurrence

or

;

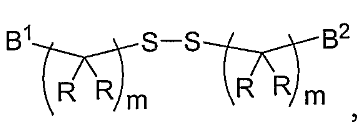
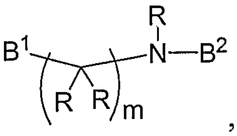
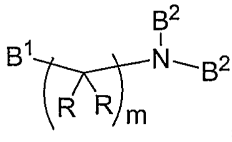
A^4 represents independently for each occurrence a bond, alkyl diradical, heteroalkyl diradical, alkenyl diradical, aminoalkyl diradical, alkynyl diradical, alkylalkynyl diradical, thioether, -C(O)-, -S(O)-, $-S(O)_2-$, $B^1C(R)_2B^2$, $B^1C(R)(B^2)_2$, $B^1C(B^2)_3$, $B^1N(R)(B^2)$, $B^1N(B^2)_2$, or has the formula:

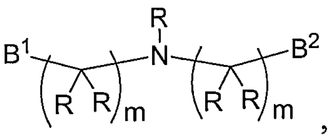

$B^1{-}CR{=}CR{-}C(O){-}N(R)B^2$,

$B^1{-}CR{=}CR{-}C(O){-}N(B^2)_2$,

$B^1{-}\left(C(R)_2\right)_m{-}C(O){-}N(R)B^2$,


$B^1{-}\left(C(R)_2\right)_m{-}C(O){-}N(B^2)_2$,

$B^1{-}CR{=}CR{-}C(O){-}N(R){-}\left(C(R)_2\right)_m{-}N(R){-}C(O){-}B^2$,


$B^1{-}CR{=}CR{-}C(O){-}N(R){-}\left(C(R)_2\right)_m{-}N(R){-}C(O){-}O{-}B^2$,

$B^1{-}\left(C(R)_2\right)_m{-}C(O){-}N(R){-}\left(C(R)_2\right)_m{-}N(R){-}C(O){-}B^2$,


$B^1{-}\left(C(R)_2\right)_m{-}C(O){-}N(R){-}\left(C(R)_2\right)_m{-}N(R){-}C(O){-}O{-}B^2$,

$B^1{-}\left(C(R)(R)\right)_m{-}S{-}\left(C(R)(R)\right)_m{-}B^2$,


$B^1{-}\left(C(R)(R)\right)_m{-}S{-}S{-}\left(C(R)(R)\right)_m{-}B^2$,

$B^1{-}\left(C(R)(R)\right)_m{-}N(R){-}B^2$,

$B^1{-}\left(C(R)(R)\right)_m{-}N(B^2){-}B^2$,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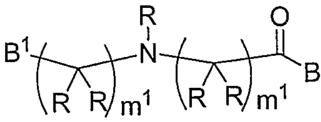
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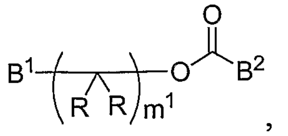
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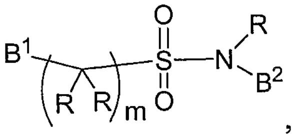
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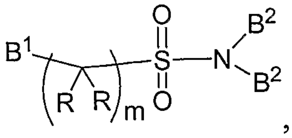
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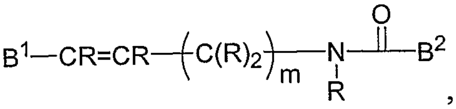
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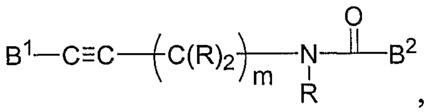
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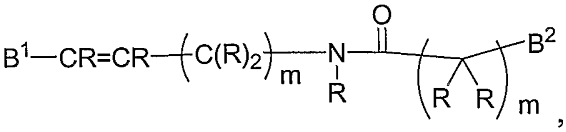
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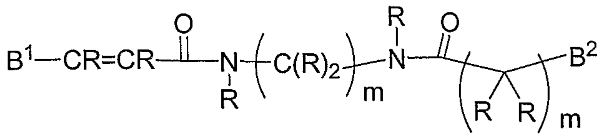
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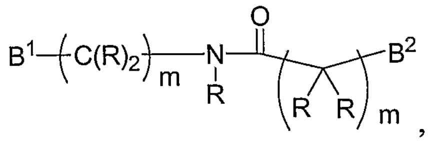
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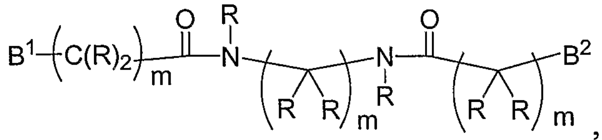
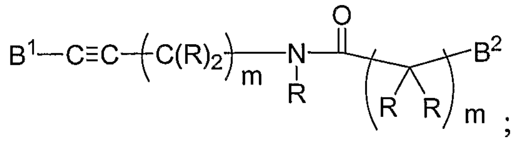

, or


;

B^1 represents independently for each occurrence a bond between A^3 and A^4;

B^2 represents independently for each occurrence a bond between A^4 and A^5;

R represents independently for each occurrence hydrogen or alkyl;

m represents independently for each occurrence 1, 2, 3, 4, 5, 6, 7 or 8;

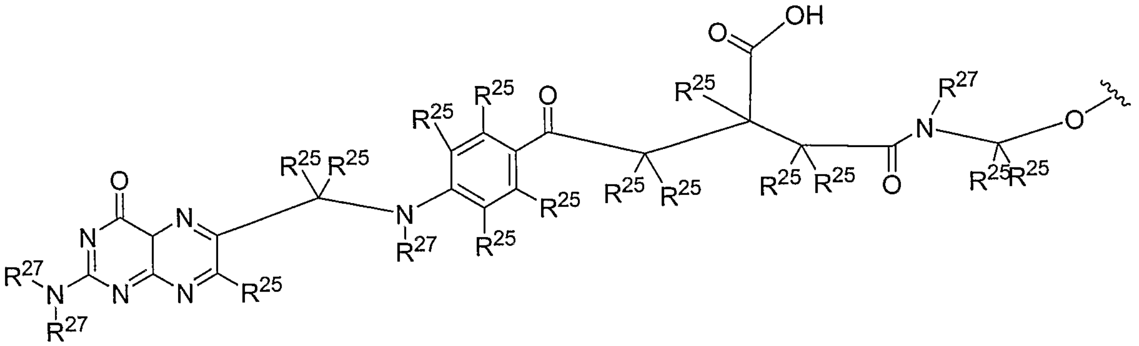
m^1 represents independently for each occurrence 0, 1, 2, 3, 4, 5, 6, 7 or 8;

v represents independently for each occurrence 1, 2, 3, or 4;

w represents independently for each occurrence 1, 2, or 3 in accord with the rules of valence;

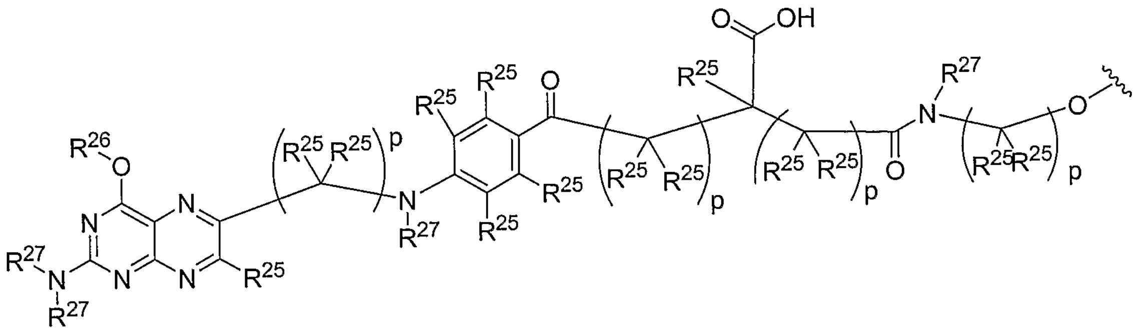
provided that A^5 occurs at least once; and

A^5 represents independently for each occurrence compound **C**, compound **D**, or a radical of a steroid, bile acid, lipid, folic acid, pyridoxal, B12, riboflavin, biotin, aromatic compound, polycyclic compound, crown ether, intercalator, cleaver molecule, protein-binding agent, carbohydrate, or an optionally substituted saturated 5-membered ring; wherein compound **C** is represented by:



C

and compound **C'** is represented by:



C'

wherein for **C** and **C'**

R^{25} represents independently for each occurrence H, halogen, hydroxyl, thiol, alkoxyl, thioalkyl, azido, -N(R^{27})$_2$, -CO$_2$$R^{27}$, -COR^{27}, -OC(O)R^{27}, alkyl, alkenyl, aryl, or aralkyl;

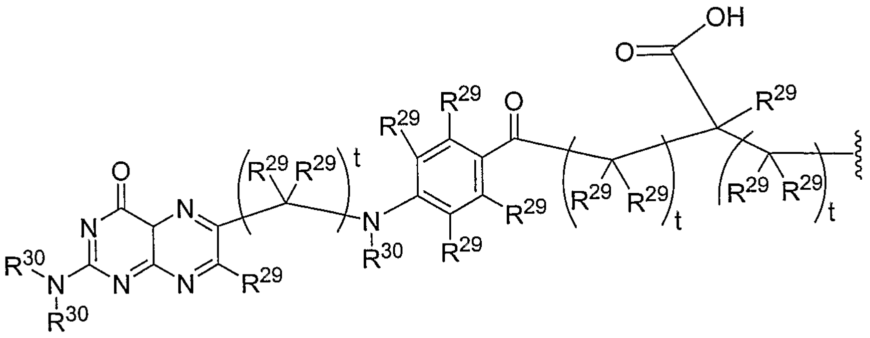
R^{26} represents independently for each occurrence alkyl, alkenyl, aryl, or aralkyl;

R^{27} represents independently for each occurrence H, alkyl, alkenyl, aryl, or aralkyl;

R^{28} represents independently for each occurrence -OC(O)R^{27}, -N(R^{27})C(O)R^{27}, -N(R^{27})$_2$, -CO$_2$$R^{27}$, -COR^{27}, alkyl, alkenyl, aryl, or aralkyl; and

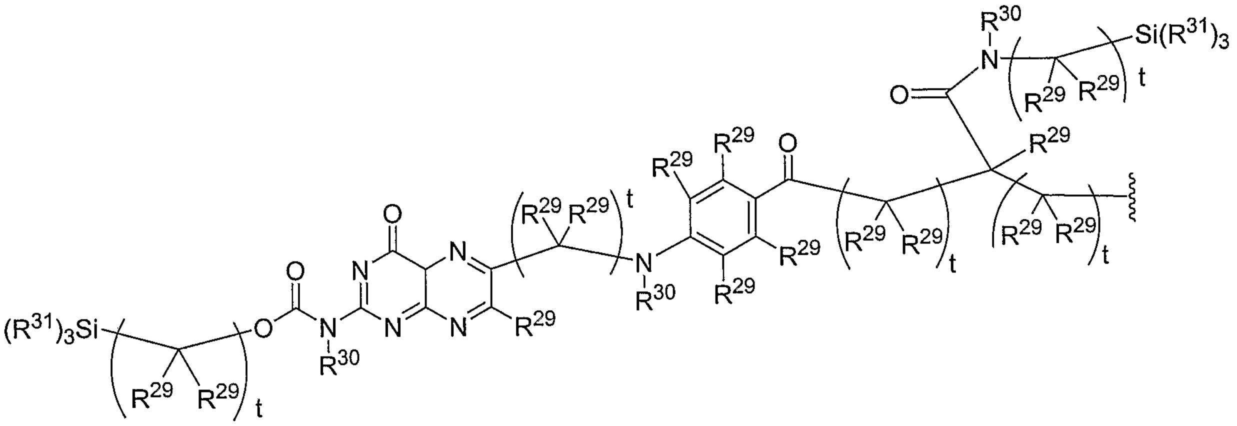
p represents independently for each occurrence 1, 2, 3, 4, 5, 6, or 7; and

compound **D** is represented by:



D

and compound **D'** is represented by:



D'

wherein for **D** and **D'**

R^{29} represents independently for each occurrence H, halogen, hydroxyl, thiol, alkoxyl, thioalkyl, azido, $-N(R^{30})_2$, $-CO_2R^{30}$, $-COR^{30}$, $-OC(O)R^{30}$, alkyl, alkenyl, aryl, or aralkyl;

R^{30} represents independently for each occurrence H, alkyl, alkenyl, aryl, or aralkyl;

R^{31} represents independently for each occurrence alkyl, alkenyl, aryl, or aralkyl; and

t represents independently for each occurrence 1, 2, 3, 4, 5, 6, or 7.

40. The double-stranded oligonucleotide of claim 39, wherein n is 18, 19, 20, 21, or 22.

41. The double-stranded oligonucleotide of claim 39, wherein n is 20.

42. The double-stranded oligonucleotide of claim 39, wherein n is 20, and said first strand and said second strand are hybridized so that there are two unhybridized nucleotides on said first strand and said second strand.

43. The double-stranded oligonucleotide of claim 39, wherein n is 20 for said first strand, and n is 22 for said second strand.

44. The double-stranded oligonucleotide of claim 39, wherein A^5 occurs at least twice.

45. The double-stranded oligonucleotide of claim 39, wherein A^5 occurs at least five times.

46. The double-stranded oligonucleotide of claim 39, wherein A^5 occurs at least ten times.

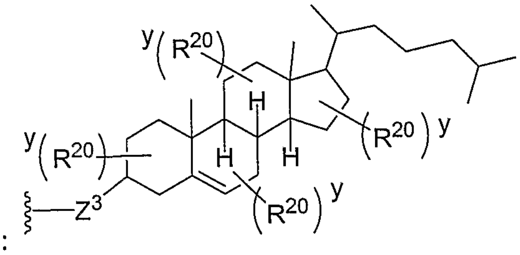
47. The double-stranded oligonucleotide of claim 39, wherein A^5 occurs only in said first strand.

48. The double-stranded oligonucleotide of claim 39, wherein said first strand and said second strand each contain at least one occurrence of A^5.

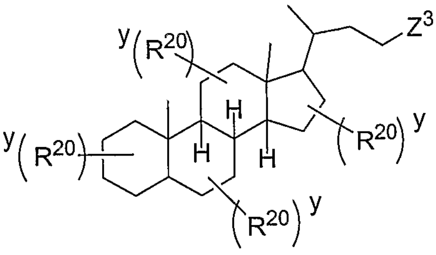
49. The double-stranded oligonucleotide of claim 39, wherein A^5 represents independently for each occurrence compound **C**, compound **D**, or a radical of cholesterol, 5β-cholanic acid, progesterone, aldosterone, dehydroaldosterone, isoandrosterone, esterone, estradiol, ergosterol, dehydroergosterol, lanosterol, 4-cholesten-3-one, guggulsterone, testosterone, nortestosterone, formestane, hydroxyecdysone, ketoestriol, corticosterone, dienestrol, dihydroxypregnanone, pregnanone, copornmon, equilenin, equilin, estriol, ethinylestradiol, mestranol, moxestrol, mytatrienediol, quinestradiol, quinestrol, helvolic acid, protostadiene, fusidic acid, cycloartenol, tricallol, cucurbitanin cedrelone, euphol, dammerenediol, parkeol, dexametasone, methylprednisolone, prednisolone, hydrocortisone, parametasone, betametasone, cortisone, fluocinonide, fluorometholone, halcinonide, budesonide, or any one of them further substituted with one or more of hydroxyl, halogen, amino, alkylamino, alkyl, carboxylic acid, ester, amide, carbonyl, alkoxyl, or cyano.

50. The double-stranded oligonucleotide of claim 39, wherein A^5 represents independently for each occurrence a radical of cholesterol or 5β-cholanic acid.

51. The double-stranded oligonucleotide of claim 39, wherein A^5 represents independently for each occurrence:



or



, wherein R^{20} represents independently for each occurrence hydroxyl, amino, halogen, alkoxyl, alkyl, aminoalkyl, azido, carbonyl, acyl, or acyloxy; Z^3 represents independently for each occurrence O, S, or NR; and y represents independently for each occurrence 1, 2, 3, 4, 5, or 6 in accord with the rules of valence.

52. The double-stranded oligonucleotide of claim 39, wherein A^5 represents independently for each occurrence a radical of cholic acid, deoxycholic acid, taurocholic acid, glycocholic acid, glycodeoxycholic acid, taurodeoxycholic acid, ursodeoxycholic acid, or chenodeoxycholic acid.

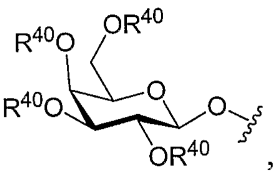
53. The double-stranded oligonucleotide of claim 39, wherein A^5 represents independently for each occurrence a radical of lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, palmitoleic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, triacylglycerol, phosphoacylglycerol, sphingolipid, monoterpene, sesquiterpene, diterpene, sesterterpene, triterpene, or tetraterpene.

54. The double-stranded oligonucleotide of claim 39, wherein A^5 represents independently for each occurrence optionally substituted phenyl, naphthyl, anthracenyl, phenanthrenyl, pyrenyl, pyridinyl, quinolinyl, acridinyl, phenathridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinoxalinyl, quinazolinyl, 1,7-phenanthrolinyl, indolyl, thianaphthenyl, benzoxazolyl, benzofuranyl, 1,2-benzisoxazolyl, benzimidazolyl, pyrrolyl, thiophenyl, isoxazolyl, pyrazolyl, thiazolyl, imidazolyl, tetrazolyl, or furanyl.

55. The double-stranded oligonucleotide of claim 39, wherein A^5 represents independently for each occurrence the radical of erythrose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, mannose, gulose, idose, galactose, or talose; or a disaccharide or trisaccharide formed via a 1,4 glycoside linkage between any of them.

56. The double-stranded oligonucleotide of claim 39, wherein A^5 is a hexose or pentose.

57. The double-stranded oligonucleotide of claim 39, wherein A^5 is



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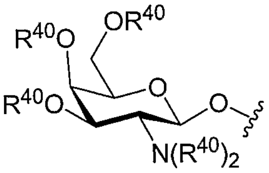
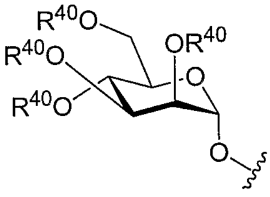
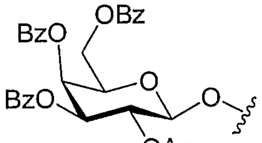


, or


; wherein R^{40} represents independently for each occurrence H, alkyl, aryl, aralkyl, -C(O)alkyl, -C(O)aryl, -C(O)aralkyl, or Si(alkyl)$_3$.

58. The double-stranded oligonucleotide of claim 39, wherein A^5 is



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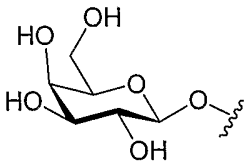

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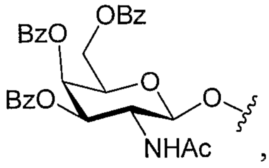

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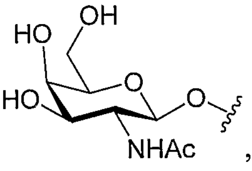

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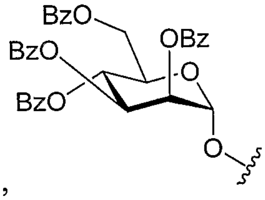

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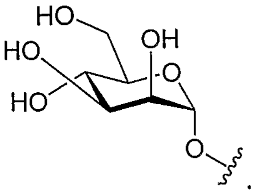

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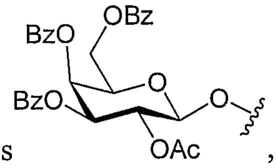
59. The double-stranded oligonucleotide of claim 39, wherein A^5 is



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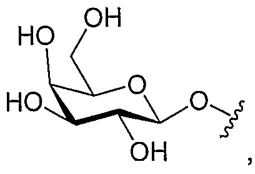

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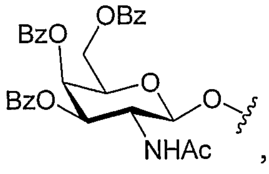

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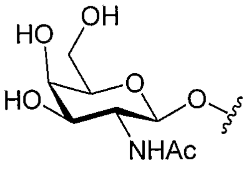

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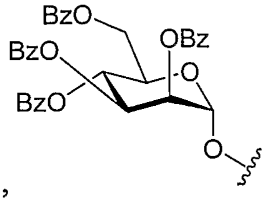
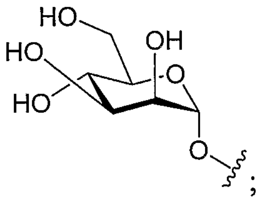
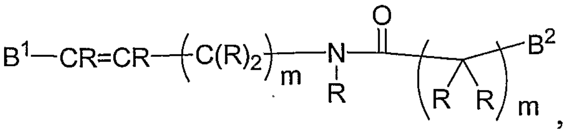
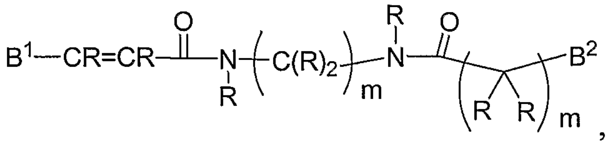


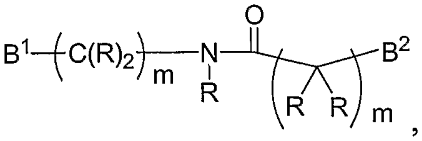
or

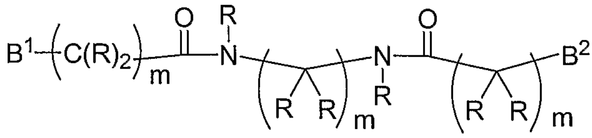


; and A^4 is









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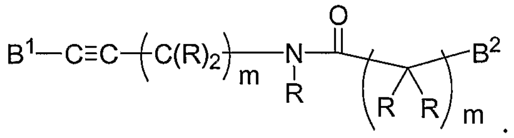
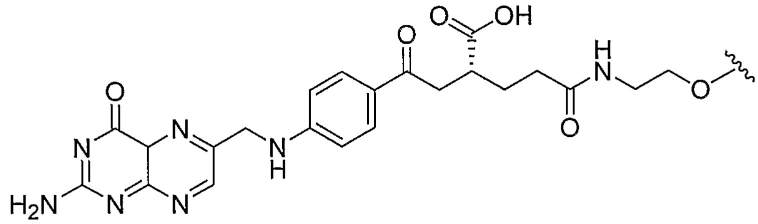


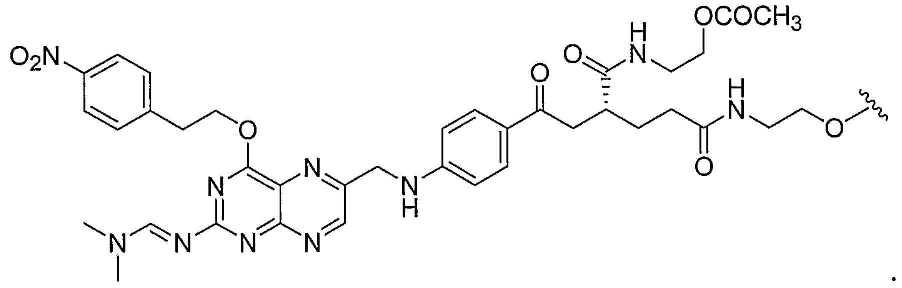
60. The double-stranded oligonucleotide of claim 39, wherein A^5 is an optionally substituted cyclopentane, pyrrolidine, tetrahydrofuran, tetrahydrothiophene, or 1,1-difluorocyclopentane.

61. The double-stranded oligonucleotide of claim 39, wherein A^5 is compound **C**.

62. The double-stranded oligonucleotide of claim 39, wherein A^5 is

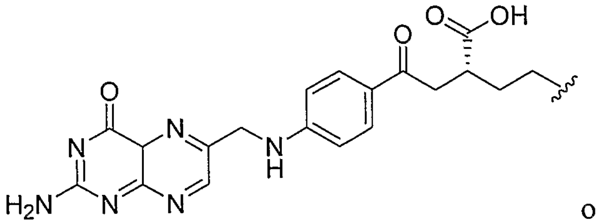


or

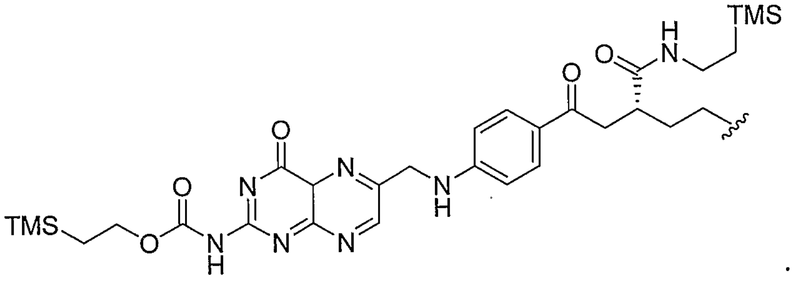


63. The double-stranded oligonucleotide of claim 39, wherein A^5 is compound **D**.

64. The double-stranded oligonucleotide of claim 39, wherein A^5 is



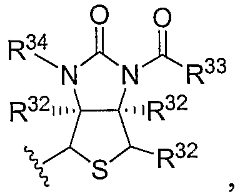
or



.

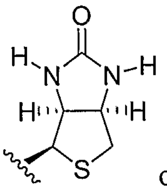
65. The double-stranded oligonucleotide of claim 39, wherein A^5 is a radical of a polycyclic compound.

66. The double-stranded oligonucleotide of claim 39, wherein A^5 is

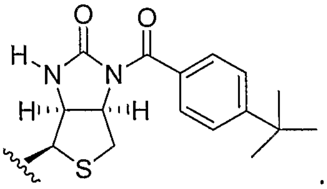


, wherein R^{32} represents independently for each occurrence H, halogen, hydroxyl, thiol, alkoxyl, thioalkyl, azido, $-N(R^{34})_2$, $-CO_2R^{34}$, $-COR^{34}$, $-OC(O)R^{34}$, alkyl, alkenyl, aryl, or aralkyl; R^{33} is aryl that is optionally substituted with alkyl, alkenyl, aryl, halogen, or alkoxyl; and R^{34} represents independently for each occurrence H, alkyl, aryl, or aralkyl.

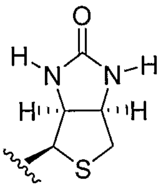
67. The double-stranded oligonucleotide of claim 39, wherein A^5 is

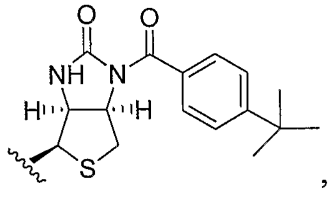
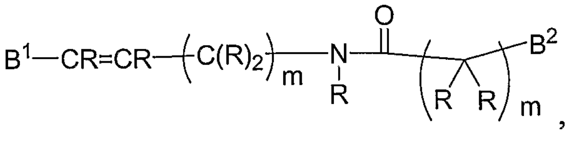


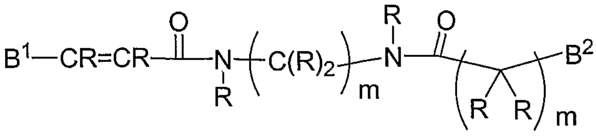
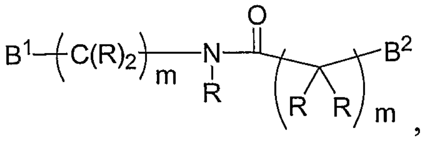
or

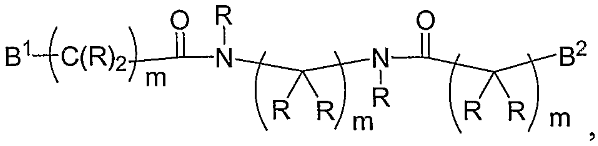


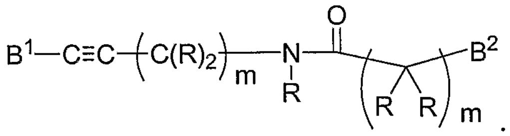
.

68. The double-stranded oligonucleotide of claim 39, wherein A^5 is  or

 , and A^4 is  ,

 ,  ,

 , or

 .

69. The double-stranded oligonucleotide of claim 39, wherein R^2 represents independently for each occurrence H, OH, F, or -Oalkyl.

70. The double-stranded oligonucleotide of claim 39, wherein R^2 represents independently for each occurrence H, OH, F, $-OCH_3$, $-O(CH_2)_2OR^7$, $-O(CH_2)_2SR^7$, $-O(CH_2)_2N(R^7)_2$, $-OCH_2C(O)N(H)CH_3$, $-NH_2$, $-N(CH_3)_2$, $-N(H)CH_3$, $-SCH_3$, $-O(CH_2)_2OCH_3$, $-O(CH_2)_2SCH_3$, $-O(CH_2)_2O(CH_2)_2N(CH_3)_2$, or $-O(CH_2)_2ON(CH_3)_2$.

71. The double-stranded oligonucleotide of claim 39, wherein R^2 represents independently for each occurrence $-NH_2$, $-N(H)CH_3$, or $-N(CH_3)_2$.

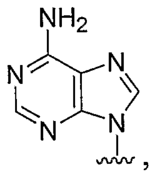
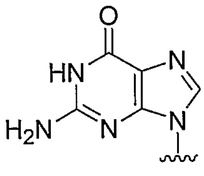
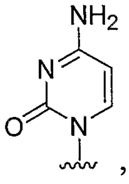
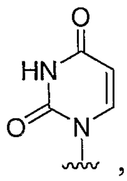
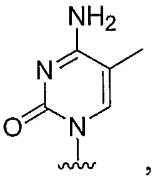
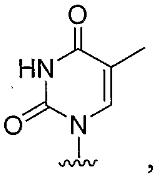
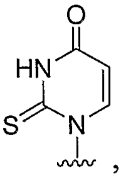
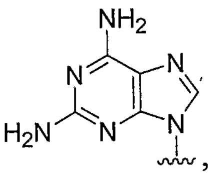
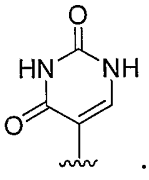
72. The double-stranded oligonucleotide of claim 39, wherein R^5 represents independently for each occurrence H, OH, F, $-OCH_3$, $-O(CH_2)_2OR^7$, $-O(CH_2)_2SR^7$, $-O(CH_2)_2N(R^7)_2$, $-OCH_2C(O)N(H)CH_3$, $-NH_2$, $-N(CH_3)_2$, $-N(H)CH_3$, $-SCH_3$, $-O(CH_2)_2OCH_3$, $-O(CH_2)_2SCH_3$, $-O(CH_2)_2O(CH_2)_2N(CH_3)_2$, or $-O(CH_2)_2ON(CH_3)_2$.

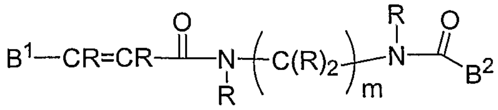
73. The double-stranded oligonucleotide of claim 39, wherein R^5 represents independently for each occurrence $-NH_2$, $-N(H)CH_3$, or $-N(CH_3)_2$.

74. The double-stranded oligonucleotide of claim 39, wherein R^2 and R^5 represent independently for each occurrence $-NH_2$, $-N(H)CH_3$, or $-N(CH_3)_2$.

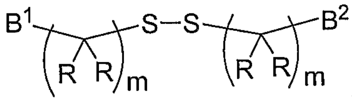
75. The double-stranded oligonucleotide of claim 39, wherein Z^2 represents independently for each occurrence -OH -OM, -Oalkyl, -Oaryl, -Oaralkyl, -SH, -SM, -Salkyl, -Saryl, -Saralkyl, $-N(R^3)R^4$, $-(C(R^7)_2)_mN(R^7)_2$, $-N(R^7)(C(R^7)_2)_mN(R^7)_2$, or methyl;

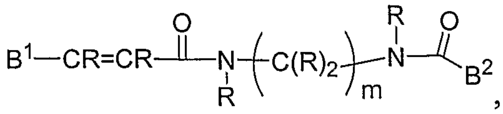
76. The double-stranded oligonucleotide of claim 39, wherein Z^2 represents independently for each occurrence -OH, -OM, -Oalkyl, -Oaryl, or -Oaralkyl.

77. The double-stranded oligonucleotide of claim 39, wherein A^2 represents independently for each occurrence: , , , , , , , , or .

78. The double-stranded oligonucleotide of claim 39, wherein A^4 represents independently for each occurrence 

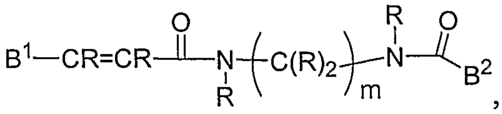
$B^1-CR=CR-C(=O)-N(R)-(C(R)_2)_m-N(R)-C(=O)-B^2$

or .

79. The double-stranded oligonucleotide of claim 39, wherein A^4 represents independently for each occurrence 

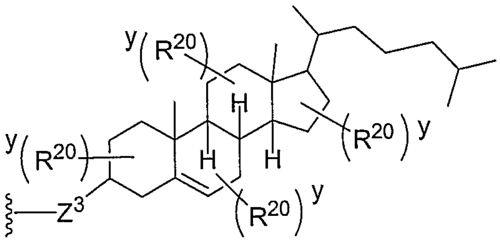
$B^1-CR=CR-C(=O)-N(R)-(C(R)_2)_m-N(R)-C(=O)-B^2$

, and A^5 is a steroid.

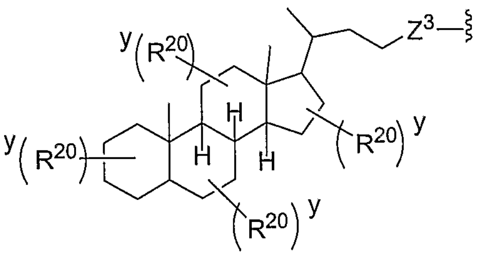
80. The double-stranded oligonucleotide of claim 39, wherein A^4 represents independently for each occurrence 

$B^1-CR=CR-C(=O)-N(R)-(C(R)_2)_m-N(R)-C(=O)-B^2$

, and A^5 represents independently for each occurrence:



or



, wherein R^{20} represents independently for each occurrence hydroxyl, amino, halogen, alkoxyl, alkyl, aminoalkyl, azido, carbonyl, acyl, or acyloxy; Z^3 represents independently for each occurrence O, S, or NR; and y represents independently for each occurrence 1, 2, 3, 4, 5, or 6 in accord with the rules of valence.

81. A method of treating a patient suffering from a malady selected from the group consisting of unwanted cell proliferation, arthritis, retinal neovascularization, viral infection, bacterial infection, amoebic infection, parasitic infection, fungal infection, unwanted immune response, asthma, lupus, multiple sclerosis, diabetes, acute pain, chronic pain, neurological disease, and a disorder characterized by loss of heterozygosity; comprising the step of:

administering to a patient in need thereof a therapeutically effective amount of an oligonucleotide according to claim 1 or 39.

82. The method of claim 81, wherein said malady is unwanted cell proliferation.

83. The method of claim 81, wherein said malady is testicular cancer, lung cancer, breast cancer, colon cancer, squamous cell carcinoma, pancreatic cancer, leukemia, melanoma, Burkitt's lymphoma, neuroblastoma, ovarian cancer, prostate cancer, skin cancer, non-Hodgkin lymphoma, esophageal cancer, cervical cancer, basal cell carcinoma, adenocarcinoma carcinoma, hepatocellular carcinoma, colorectal adenocarcinoma, liver cancer, male breast carcinoma, adenocarcinomas of the esophagus, adenocarcinomas of the stomach, adenocarcinomas of the colon, adenocarcinomas of the rectum, gall bladder cancer, hamartomas, gliomas,

endometrial cancer, acute leukemia, chronic leukemia, childhood acute leukemia, Ewing Sarcoma, Myxoid liposarcoma, brain cancer, or tumors of epithelial origin.

84. The method of claim 81, wherein said malady is rheumatoid arthritis or retinal neovascularization.

85. The method of claim 81, wherein said malady is a viral infection.

86. The method of claim 81, wherein said malady is a disorder mediated by Human Papilloma Virus, Human Immunodeficiency Virus, Hepatitis A Virus, Hepatitis B Virus, Hepatitis C Virus, Hepatitis D Virus, Hepatitis E Virus, Hepatitis F Virus, Hepatitis G Virus, Hepatitis H Virus, Respiratory Syncytial Virus, Herpes Simplex Virus, herpes Cytomegalovirus, herpes Epstein Barr Virus, a Kaposi's Sarcoma-associated Herpes Virus, JC Virus, myxovirus, rhinovirus, coronavirus, West Nile Virus, St. Louis Encephalitis, Tick-borne encephalitis virus gene, Murray Valley encephalitis virus gene, dengue virus gene, Simian Virus 40, Human T Cell Lymphotropic Virus, a Moloney-Murine Leukemia Virus, encephalomyocarditis virus, measles virus, Vericella zoster virus, adenovirus, yellow fever virus, poliovirus, or poxvirus.

87. The method of claim 81, wherein said malady is a bacterial infection, amoebic infection, parasitic infection, or fungal infection.

88. The method of claim 81, wherein said malady is a disorder mediated by plasmodium, Mycobacterium ulcerans, Mycobacterium tuberculosis, Mycobacterium leprae, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Chlamydia pneumoniae, or Mycoplasma pneumoniae.

89. The method of claim 81, wherein said malady is an unwanted immune response, asthma, lupus, multiple sclerosis, or diabetes.

90. The method of claim 81, wherein said malady is an ischemia, reperfusion injury, response to a transplantated organ or tissue, restenosis, or Inflammatory Bowel Disease.

91. The method of claim 81, wherein said malady is acute pain or chronic pain.

92. The method of claim 81, wherein said malady is a neurological disease.

93. The method of claim 81, wherein said malady is Alzheimer Disease, Parkinson Disease, or a neurodegenerative trinucleotide repeat disorder.

94. The method of claim 81, wherein said malady is a disorder characterized by loss of heterozygosity.

95. The method of claim 81, wherein said oligonucleotide is a double-stranded oligonucleotide according to claim 39.

96. A method of gene-silencing, comprising the steps of:

administering a therapeutically effective amount of an oligonucleotide according to claim 1 or 39 to a mammalian cell to silence a gene promoting unwanted cell proliferation, growth factor gene, growth factor receptor gene, a kinase gene, a gene encoding a G protein superfamily molecule, a gene encoding a transcription factor, a gene which mediates angiogenesis, a viral gene of a cellular gene which mediates viral function, a gene of a bacterial pathogen, a gene of an amoebic pathogen, a gene of a parasitic pathogen, a gene of a fungal pathogen, a gene which mediates an unwanted immune response, a gene which mediates the processing of pain, a gene which mediates a neurological disease, an allene gene found in cells characterized by loss of heterozygosity, or one allege gene of a polymorphic gene.

97. The method of claim 96, wherein said oligonucleotide is a double-stranded oligonucleotide according to claim 39.

98. A method of gene-silencing, comprising the steps of:

administering a therapeutically effective amount of an oligonucleotide according to claim 1 or 39 to a mammalian cell to silence a PDGF beta gene, Erb-B gene, Src gene, CRK gene, GRB2 gene, RAS gene, MEKK gene, JNK gene, RAF gene, Erk1/2 gene, PCNA(p21) gene, MYB gene, JUN gene, FOS gene, BCL-2 gene, Cyclin D gene, VEGF gene, EGFR gene, Cyclin A gene, Cyclin E gene, WNT-1 gene, beta-catenin gene, c-MET gene, PKC gene, NFKB gene, STAT3 gene, survivin gene, Her2/Neu gene, topoisomerase I gene, topoisomerase II alpha gene, mutations in the p73 gene, mutations in the p21(WAF1/CIP1) gene, mutations in the p27(KIP1) gene, mutations in the PPM1D gene, mutations in the RAS gene, mutations in the caveolin I gene, mutations in the MIB I gene, mutations in the MTAI gene, mutations in the M68 gene, mutations in tumor suppressor genes, mutations in the p53 tumor suppressor gene, mutations in the p53 family member DN-p63, mutations in the pRb tumor suppressor gene, mutations in the APC1 tumor suppressor gene, mutations in the BRCA1 tumor suppressor gene, mutations in the PTEN tumor suppressor gene, mLL fusion gene, BCR/ABL fusion gene, TEL/AML1 fusion gene, EWS/FLI1 fusion gene, TLS/FUS1 fusion gene, PAX3/FKHR fusion gene, AML1/ETO fusion gene, alpha v-integrin gene, Flt-1 receptor gene, tubulin gene, Human Papilloma Virus gene, a gene required for Human Papilloma Virus replication, Human Immunodeficiency Virus gene, a gene required for Human Immunodeficiency Virus replication, Hepatitis A Virus gene, a gene required for Hepatitis A Virus replication, Hepatitis B Virus gene, a gene required for Hepatitis B Virus replication, Hepatitis C Virus gene, a gene required for Hepatitis C Virus replication, Hepatitis D Virus gene, a gene required for Hepatitis D Virus replication, Hepatitis E Virus gene, a gene required for Hepatitis E Virus replication, Hepatitis F Virus gene, a gene required for Hepatitis F Virus replication, Hepatitis G Virus gene, a gene required for Hepatitis G Virus replication, Hepatitis H Virus gene, a gene required for Hepatitis H Virus replication, Respiratory Syncytial Virus gene, a gene that is required for Respiratory Syncytial Virus replication, Herpes Simplex Virus gene, a gene that is required for Herpes Simplex Virus replication, herpes Cytomegalovirus gene, a gene that is required for herpes Cytomegalovirus replication, herpes Epstein Barr Virus gene, a gene that is required for herpes Epstein Barr Virus replication, Kaposi's Sarcoma-associated Herpes Virus gene, a gene that is required for Kaposi's Sarcoma-associated Herpes Virus replication, JC Virus gene, human gene that is required for JC Virus replication, myxovirus gene, a gene that is required for myxovirus gene replication, rhinovirus gene, a gene that is required for rhinovirus replication, coronavirus gene, a gene that is required for coronavirus replication, West Nile Virus gene, a gene that is required for West Nile Virus replication, St. Louis Encephalitis gene, a gene that is required for St. Louis Encephalitis replication, Tick-borne encephalitis virus gene, a gene that is required for Tick-borne encephalitis virus replication, Murray Valley encephalitis virus gene, a gene that is required for Murray Valley encephalitis virus replication, dengue virus gene, a gene that is required for dengue virus gene replication, Simian Virus 40 gene, a gene that is required for Simian Virus 40 replication, Human T Cell Lymphotropic Virus gene, a gene that is required for Human T Cell Lymphotropic Virus replication, Moloney-Murine Leukemia Virus gene, a gene that is required for Moloney-Murine Leukemia Virus replication, encephalomyocarditis virus gene, a gene that is required for encephalomyocarditis virus replication, measles virus gene, a gene that is required for measles virus replication, Vericella zoster virus gene, a gene that is required for Vericella zoster virus replication, adenovirus gene, a gene that is required for adenovirus replication, yellow fever virus gene, a gene that is required for yellow fever virus replication, poliovirus gene, a gene that is required for poliovirus replication, poxvirus gene, a gene that is required for poxvirus replication, plasmodium gene, a gene that is required for plasmodium gene replication, Mycobacterium ulcerans gene, a gene that is required for Mycobacterium ulcerans replication, Mycobacterium tuberculosis gene, a gene that is required for Mycobacterium tuberculosis replication, Mycobacterium leprae gene, a gene that is required for Mycobacterium leprae replication, Staphylococcus aureus gene, a gene that is required for Staphylococcus aureus replication, Streptococcus pneumoniae gene, a gene that is required for Streptococcus pneumoniae replication, Streptococcus pyogenes gene, a gene that is required for Streptococcus pyogenes replication, Chlamydia pneumoniae gene, a gene that is required for Chlamydia pneumoniae replication, Mycoplasma pneumoniae gene, a gene that is required for Mycoplasma pneumoniae replication, an integrin gene, a selectin gene, complement system gene, chemokine gene, chemokine receptor gene, GCSF gene, Gro1 gene, Gro2 gene, Gro3 gene, PF4 gene, MIG gene, Pro-Platelet Basic Protein gene, MIP-1I gene, MIP-1J gene, RANTES gene, MCP-1 gene, MCP-2 gene, MCP-3 gene, CMBKR1 gene, CMBKR2 gene, CMBKR3 gene, CMBKR5v, AIF-1 gene, I-309 gene, a gene to a component of an ion channel, a gene to a neurotransmitter receptor, a gene to a neurotransmitter ligand, amyloid-family gene, presenilin gene, HD gene, DRPLA gene, SCA1 gene, SCA2 gene, MJD1 gene, CACNL1A4 gene, SCA7 gene, SCA8 gene, allele gene found in LOH cells, or one allele gene of a polymorphic gene.

99. The method of claim 98, wherein said oligonucleotide is a double-stranded oligonucleotide according to claim 39.

100. A method of gene-silencing, comprising the steps of:

administering a therapeutically effective amount of an oligonucleotide according to claim 1 or 39 to a mammal to silence a gene promoting unwanted cell proliferation, growth factor or growth factor receptor gene, a kinase gene, a gene encoding a G protein superfamily molecule, a gene encoding a transcription factor, a gene which mediates angiogenesis, a viral gene of a cellular gene which mediates viral function, a gene of a bacterial pathogen, a gene of an amoebic pathogen, a gene of a parasitic pathogen, a gene of a fungal pathogen, a gene which mediates an unwanted immune response, a gene which mediates the processing of pain, a gene which mediates a neurological disease, an allene gene found in cells characterized by loss of heterozygosity, or one allege gene of a polymorphic gene.

101. The method of claim 100, wherein said oligonucleotide is a double-stranded oligonucleotide according to claim 39.

102. A method of gene-silencing, comprising the steps of:

administering a therapeutically effective amount of an oligonucleotide according to claim 1 or 39 to a mammal to silence a PDGF beta gene, Erb-B gene, Src gene, CRK gene, GRB2 gene, RAS gene, MEKK gene, JNK gene, RAF gene, Erk1/2 gene, PCNA(p21) gene, MYB gene, JUN gene, FOS gene, BCL-2 gene, Cyclin D gene, VEGF gene, EGFR gene, Cyclin A gene, Cyclin E gene, WNT-1 gene, beta-catenin gene, c-MET gene, PKC gene, NFKB gene, STAT3 gene, survivin gene, Her2/Neu gene, topoisomerase I gene, topoisomerase II alpha gene, mutations in the p73 gene, mutations in the p21(WAF1/CIP1) gene, mutations in the p27(KIP1) gene, mutations in the PPM1D gene, mutations in the RAS gene, mutations in the caveolin I gene, mutations in the MIB I gene, mutations in the MTAI gene, mutations in the M68 gene, mutations in tumor suppressor genes, mutations in the p53 tumor suppressor gene, mutations in the p53 family member DN-p63, mutations in the pRb tumor suppressor gene, mutations in the APC1 tumor suppressor gene, mutations in the BRCA1 tumor suppressor gene, mutations in the PTEN tumor suppressor gene, mLL fusion gene, BCR/ABL fusion gene, TEL/AML1 fusion gene, EWS/FLI1 fusion gene, TLS/FUS1 fusion gene, PAX3/FKHR fusion gene, AML1/ETO fusion gene, alpha v-integrin gene, Flt-1 receptor gene, tubulin gene, Human Papilloma Virus gene, a gene required for Human Papilloma Virus replication, Human Immunodeficiency Virus gene, a gene required for Human

Immunodeficiency Virus replication, Hepatitis A Virus gene, a gene required for Hepatitis A Virus replication, Hepatitis B Virus gene, a gene required for Hepatitis B Virus replication, Hepatitis C Virus gene, a gene required for Hepatitis C Virus replication, Hepatitis D Virus gene, a gene required for Hepatitis D Virus replication, Hepatitis E Virus gene, a gene required for Hepatitis E Virus replication, Hepatitis F Virus gene, a gene required for Hepatitis F Virus replication, Hepatitis G Virus gene, a gene required for Hepatitis G Virus replication, Hepatitis H Virus gene, a gene required for Hepatitis H Virus replication, Respiratory Syncytial Virus gene, a gene that is required for Respiratory Syncytial Virus replication, Herpes Simplex Virus gene, a gene that is required for Herpes Simplex Virus replication, herpes Cytomegalovirus gene, a gene that is required for herpes Cytomegalovirus replication, herpes Epstein Barr Virus gene, a gene that is required for herpes Epstein Barr Virus replication, Kaposi's Sarcoma-associated Herpes Virus gene, a gene that is required for Kaposi's Sarcoma-associated Herpes Virus replication, JC Virus gene, human gene that is required for JC Virus replication, myxovirus gene, a gene that is required for myxovirus gene replication, rhinovirus gene, a gene that is required for rhinovirus replication, coronavirus gene, a gene that is required for coronavirus replication, West Nile Virus gene, a gene that is required for West Nile Virus replication, St. Louis Encephalitis gene, a gene that is required for St. Louis Encephalitis replication, Tick-borne encephalitis virus gene, a gene that is required for Tick-borne encephalitis virus replication, Murray Valley encephalitis virus gene, a gene that is required for Murray Valley encephalitis virus replication, dengue virus gene, a gene that is required for dengue virus gene replication, Simian Virus 40 gene, a gene that is required for Simian Virus 40 replication, Human T Cell Lymphotropic Virus gene, a gene that is required for Human T Cell Lymphotropic Virus replication, Moloney-Murine Leukemia Virus gene, a gene that is required for Moloney-Murine Leukemia Virus replication, encephalomyocarditis virus gene, a gene that is required for encephalomyocarditis virus replication, measles virus gene, a gene that is required for measles virus replication, Vericella zoster virus gene, a gene that is required for Vericella zoster virus replication, adenovirus gene, a gene that is required for adenovirus replication, yellow fever virus gene, a gene that is required for yellow fever virus replication, poliovirus gene, a gene that is required for poliovirus replication, poxvirus gene, a gene that is required for poxvirus replication, plasmodium gene, a gene that is required for plasmodium gene replication, Mycobacterium ulcerans gene, a gene that is required for Mycobacterium ulcerans replication, Mycobacterium tuberculosis gene, a gene that is required for Mycobacterium tuberculosis replication, Mycobacterium leprae gene, a gene that is required for Mycobacterium leprae replication, Staphylococcus aureus gene, a gene that is required for Staphylococcus aureus replication, Streptococcus pneumoniae gene, a gene that is required for Streptococcus pneumoniae replication, Streptococcus pyogenes gene, a gene that is required for Streptococcus pyogenes replication, Chlamydia pneumoniae gene, a gene that is required for Chlamydia pneumoniae replication, Mycoplasma pneumoniae gene, a gene that is required for Mycoplasma pneumoniae replication, an integrin gene, a selectin gene, complement system gene, chemokine gene, chemokine receptor gene, GCSF gene, Gro1 gene, Gro2 gene, Gro3 gene, PF4 gene, MIG gene, Pro-Platelet Basic Protein gene, MIP-1I gene, MIP-1J gene, RANTES gene, MCP-1 gene, MCP-2 gene, MCP-3 gene, CMBKR1 gene, CMBKR2 gene, CMBKR3 gene, CMBKR5v, AIF-1 gene, I-309 gene, a gene to a component of an ion channel, a gene to a neurotransmitter receptor, a gene to a neurotransmitter ligand, amyloid-family gene, presenilin gene, HD gene, DRPLA gene, SCA1 gene, SCA2 gene, MJD1 gene, CACNL1A4 gene, SCA7 gene, SCA8 gene, allele gene found in LOH cells, or one allele gene of a polymorphic gene.

103. The method of claim 102, wherein said mammal is a primate, equine, canine or feline.

104. The method of claim 102, wherein said mammal is a human.

105. The method of claim 102, wherein said oligonucleotide is a double-stranded oligonucleotide according to claim 39.

Figure 1

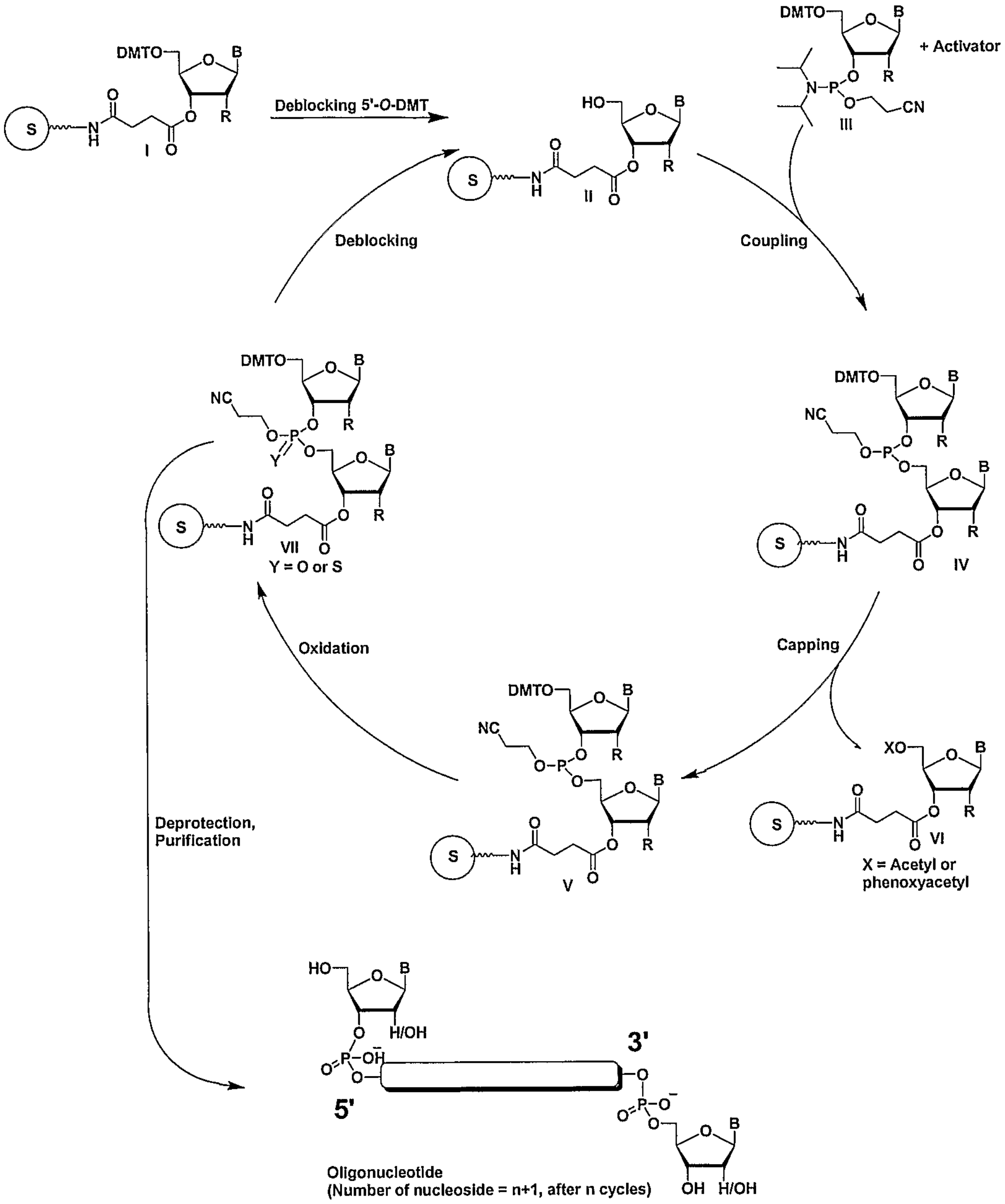
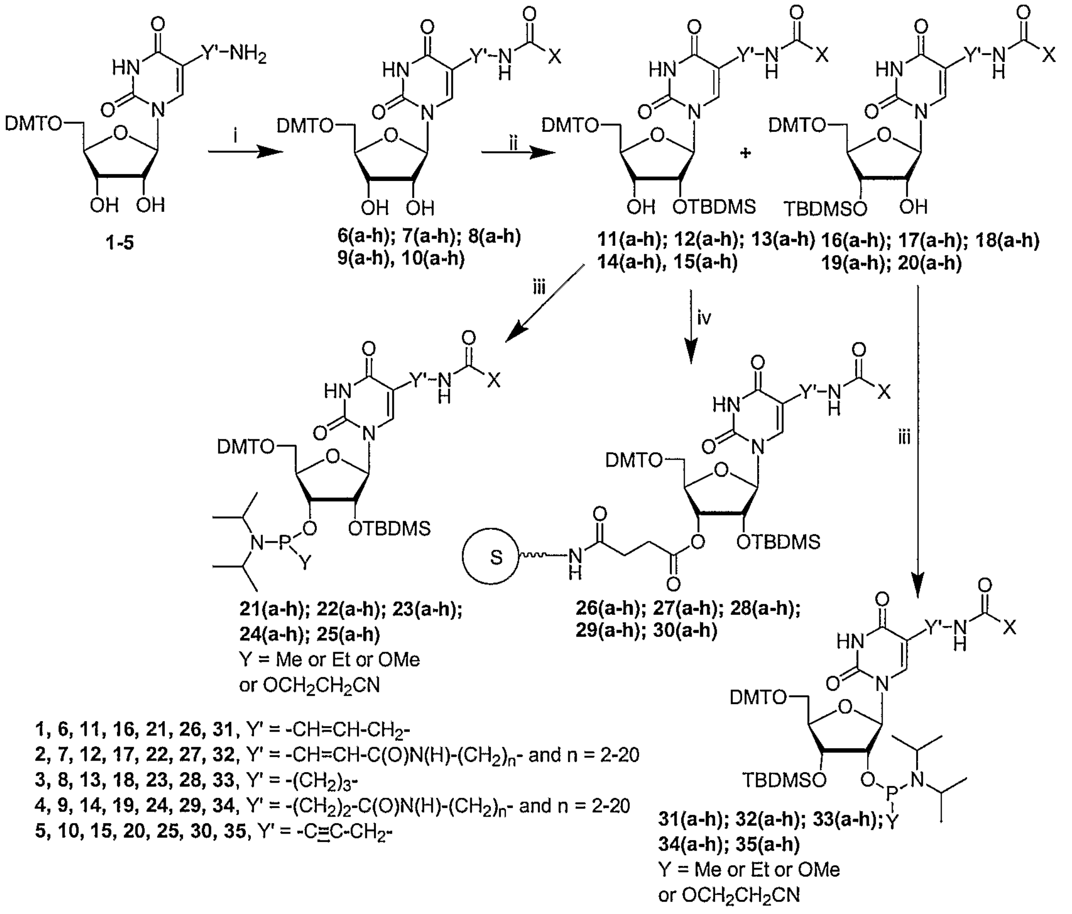

DMTO
B
S
N
H
O
R
I
Deblocking 5'-O-DMT
HO
II
DMTO
+ Activator
N-P
CN
III
Coupling
Deblocking
NC
O-P
Y
VII
Y = O or S
IV
Capping
Oxidation
V
XO
VI
X = Acetyl or phenoxyacetyl
Deprotection, Purification
H/OH
O=P-OH
3'
5'
O=P-O⁻
OH H/OH
Oligonucleotide
(Number of nucleoside = n+1, after n cycles)

Figure 2



1, 6, 11, 16, 21, 26, 31, Y' = -CH=CH-CH$_2$-
2, 7, 12, 17, 22, 27, 32, Y' = -CH=CH-C(O)N(H)-(CH$_2$)$_n$- and n = 2-20
3, 8, 13, 18, 23, 28, 33, Y' = -(CH$_2$)$_3$-
4, 9, 14, 19, 24, 29, 34, Y' = -(CH$_2$)$_2$-C(O)N(H)-(CH$_2$)$_n$- and n = 2-20
5, 10, 15, 20, 25, 30, 35, Y' = -C≡C-CH$_2$-

X =

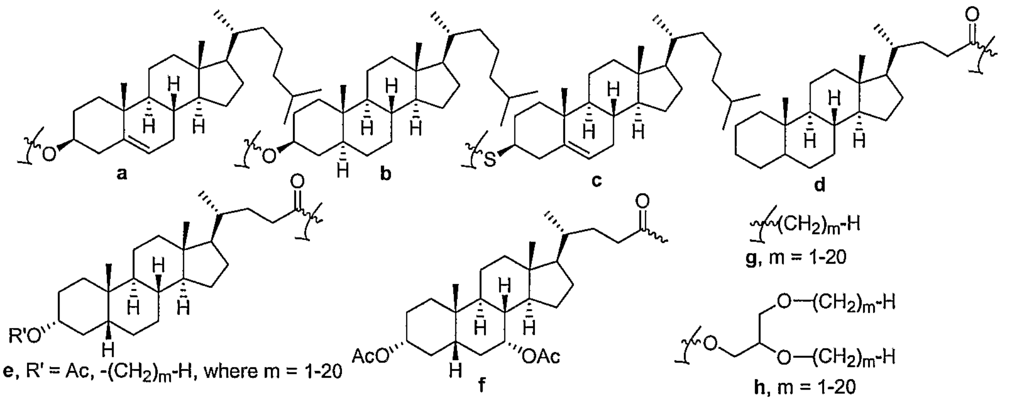
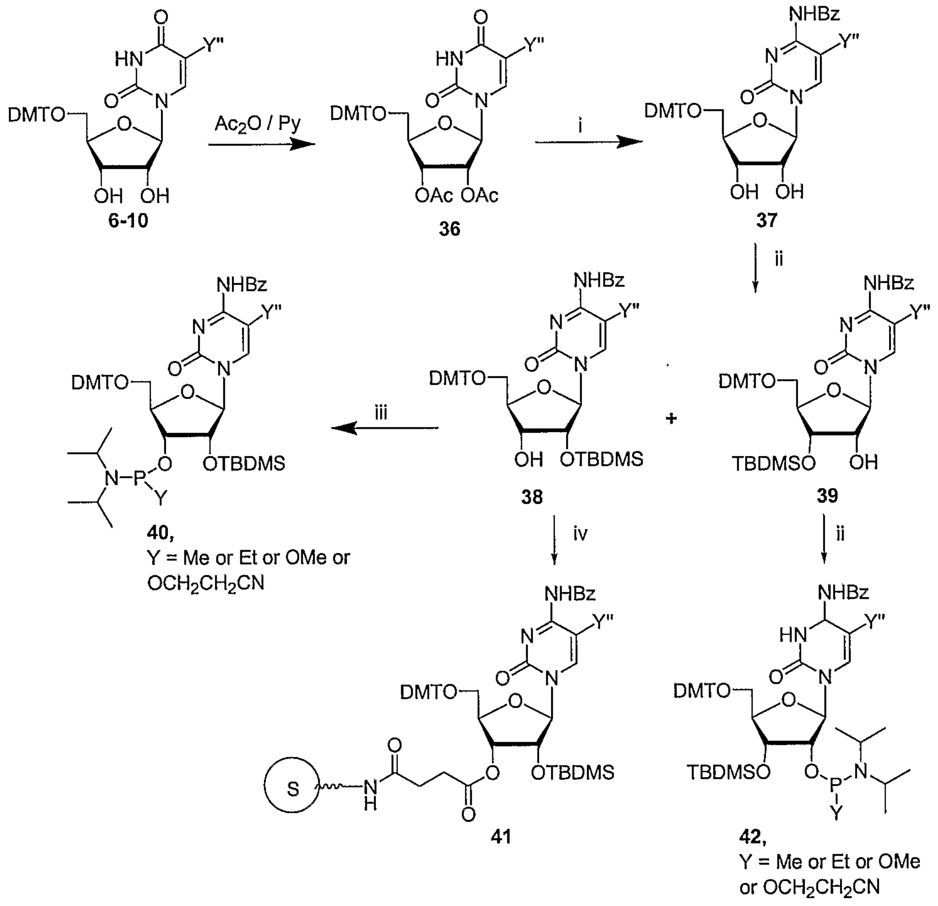


Figure 3



Y'' = $-CH{=}CH-CH_2-N(H)C(O)X$, = $-CH{=}CH-C(O)N(H)-(CH_2)_n-N(H)C(O)X$, and n = 2-20
$-(CH_2)_3-N(H)C(O)X$, $-(CH_2)_2-C(O)N(H)-(CH_2)_n-N(H)C(O)X$ and n = 2-20, or
$-C{\equiv}C-CH_2-N(H)C(O)X$

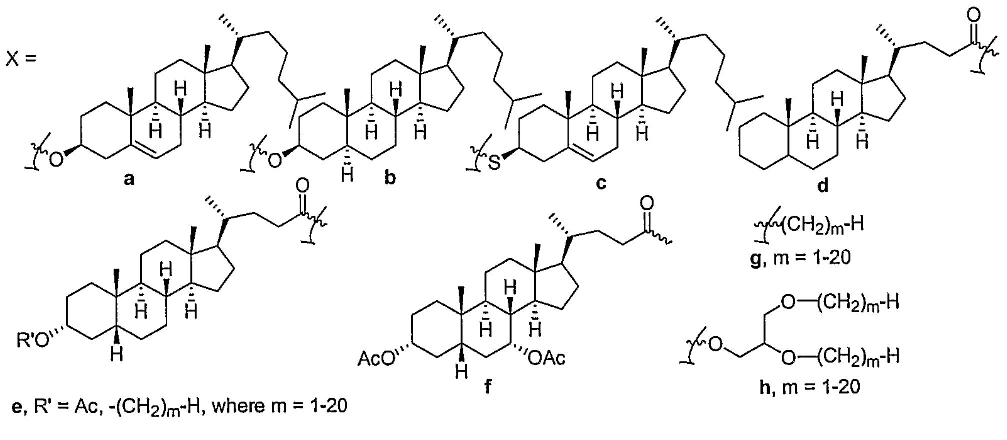
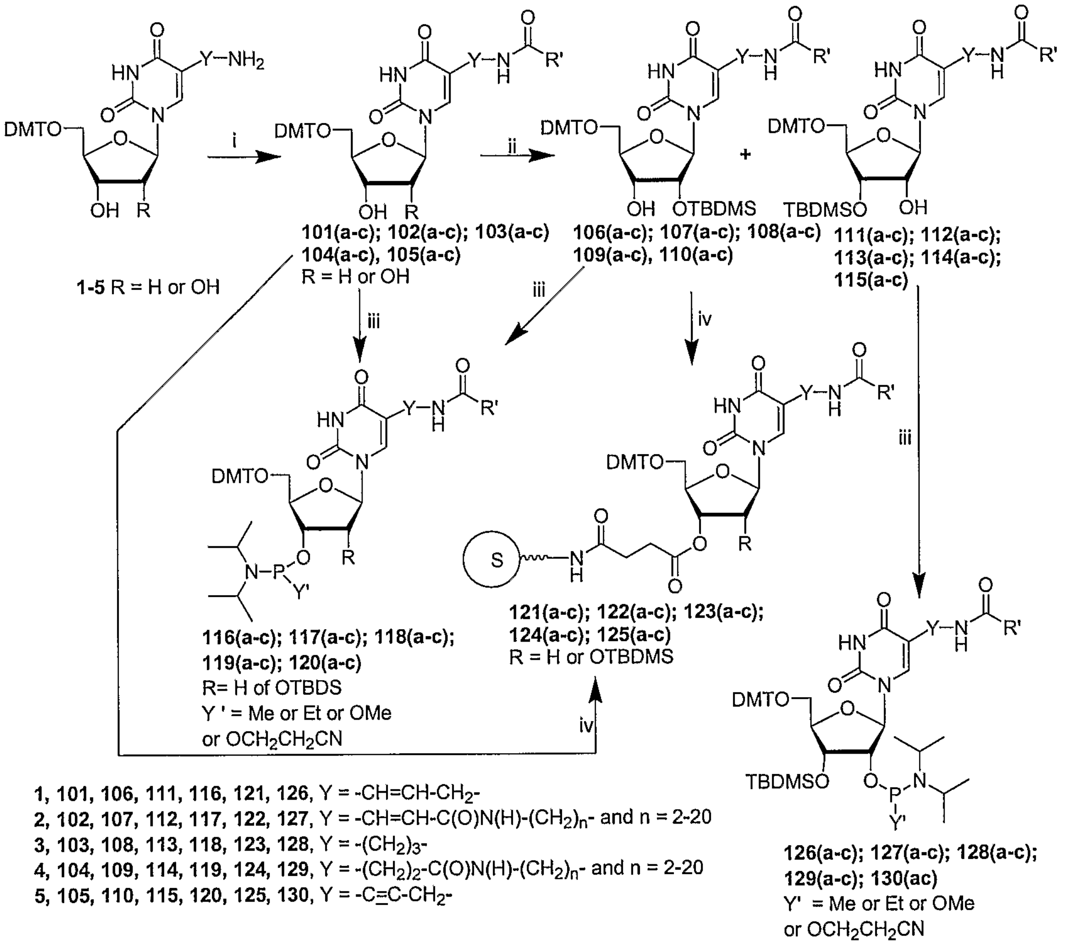


Figure 4



1, 101, 106, 111, 116, 121, 126, Y = -CH=CH-CH$_2$-
2, 102, 107, 112, 117, 122, 127, Y = -CH=CH-C(O)N(H)-(CH$_2$)$_n$- and n = 2-20
3, 103, 108, 113, 118, 123, 128, Y = -(CH$_2$)$_3$-
4, 104, 109, 114, 119, 124, 129, Y = -(CH$_2$)$_2$-C(O)N(H)-(CH$_2$)$_n$- and n = 2-20
5, 105, 110, 115, 120, 125, 130, Y = -C≡C-CH$_2$-

R' is **a**, **b**, or **c**

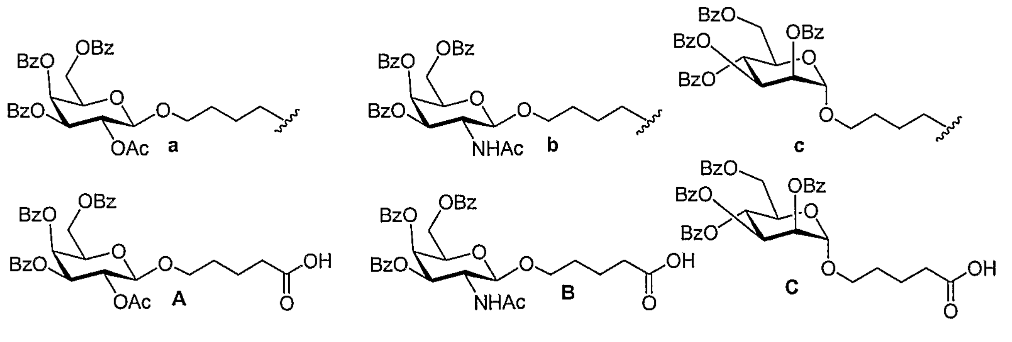
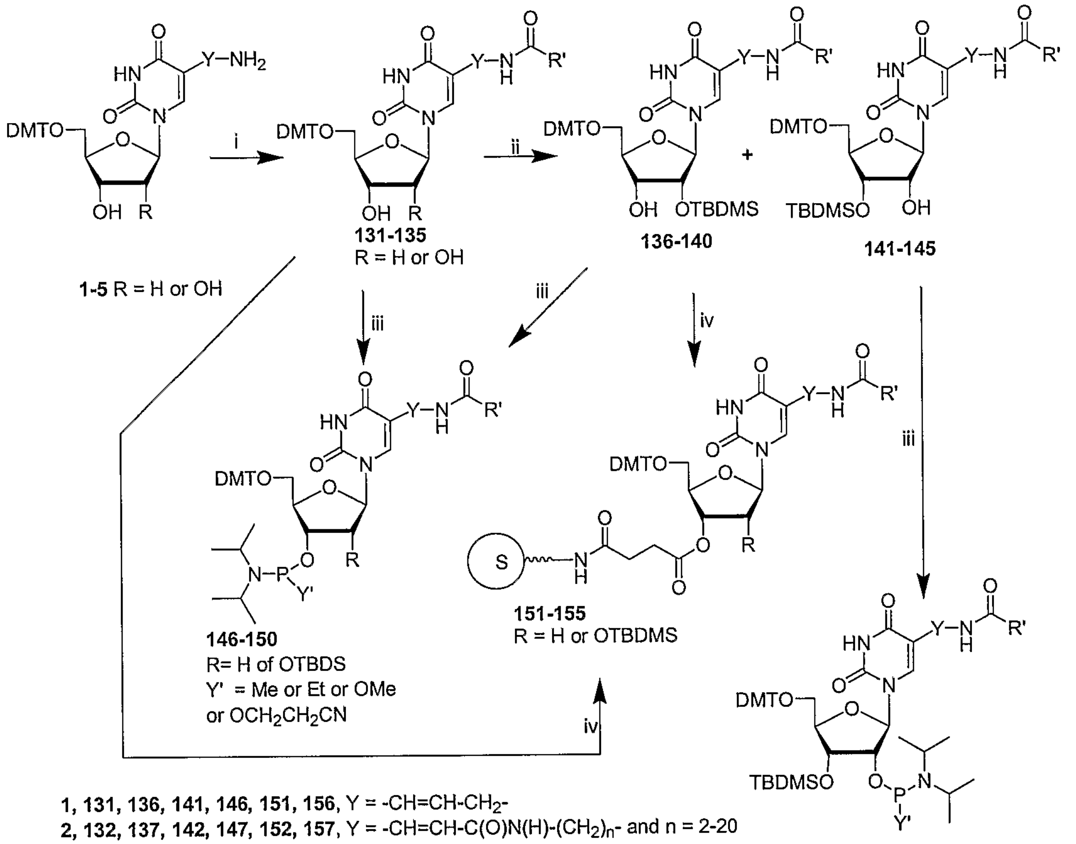


Figure 5



1-5 R = H or OH

131-135
R = H or OH

136-140

141-145

146-150
R= H of OTBDS
Y' = Me or Et or OMe
or OCH_2CH_2CN

151-155
R = H or OTBDMS

156-160
Y ' = Me or Et or OMe
or OCH_2CH_2CN

1, 131, 136, 141, 146, 151, 156, Y = $-CH{=}CH-CH_2-$
2, 132, 137, 142, 147, 152, 157, Y = $-CH{=}CH-C(O)N(H)-(CH_2)_n-$ and n = 2-20
3, 133, 138, 143, 148, 153, 158, Y = $-(CH_2)_3-$
4, 134, 139, 144, 149, 154, 159, Y = $-(CH_2)_2-C(O)N(H)-(CH_2)_n-$ and n = 2-20
5, 135, 140, 145, 150, 155, 160, Y = $-C{\equiv}C-CH_2-$

R' =

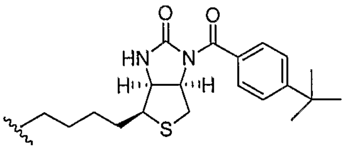
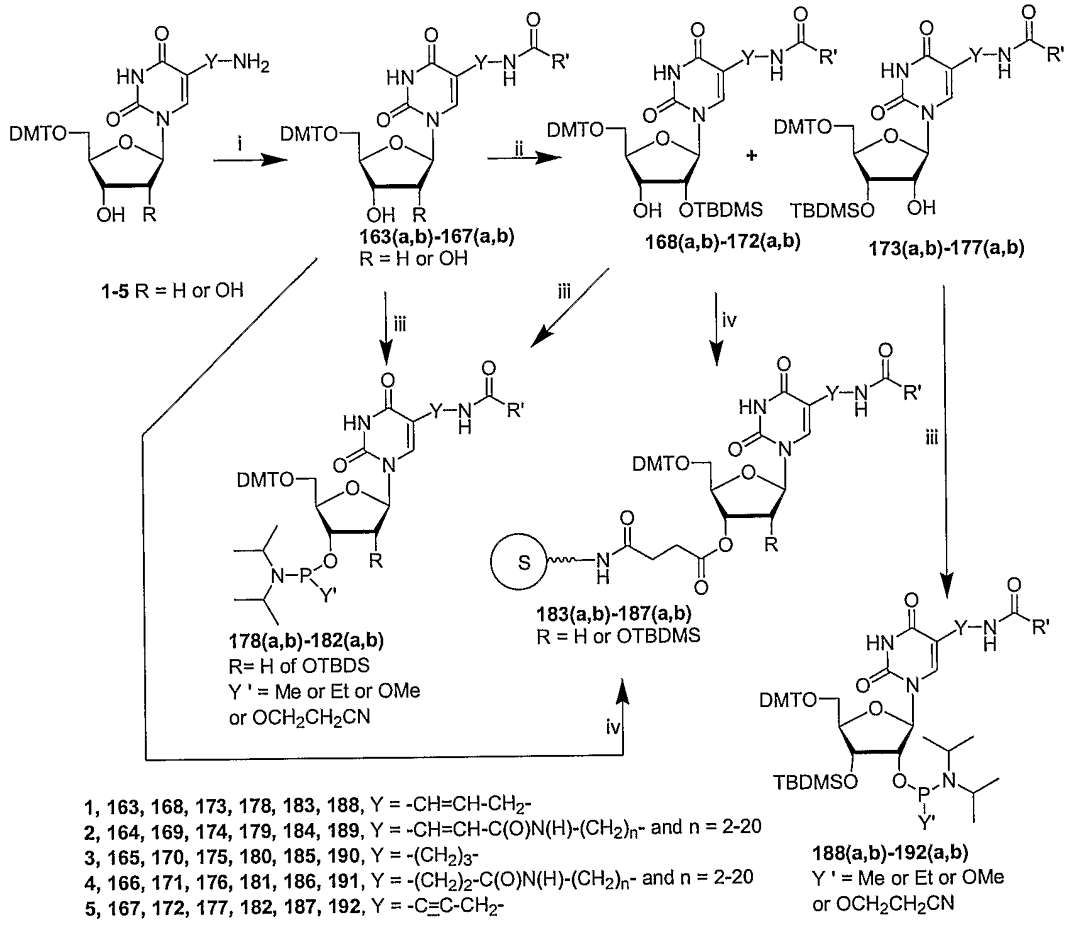


Figure 6



1-5 R = H or OH

163(a,b)-167(a,b)
R = H or OH

168(a,b)-172(a,b)

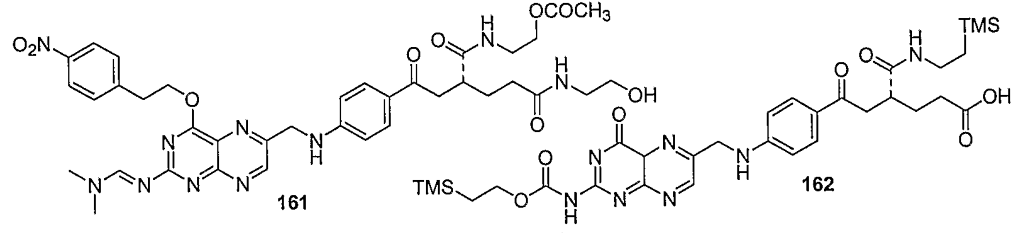
173(a,b)-177(a,b)

178(a,b)-182(a,b)
R= H of OTBDS
Y ' = Me or Et or OMe
or OCH_2CH_2CN

183(a,b)-187(a,b)
R = H or OTBDMS

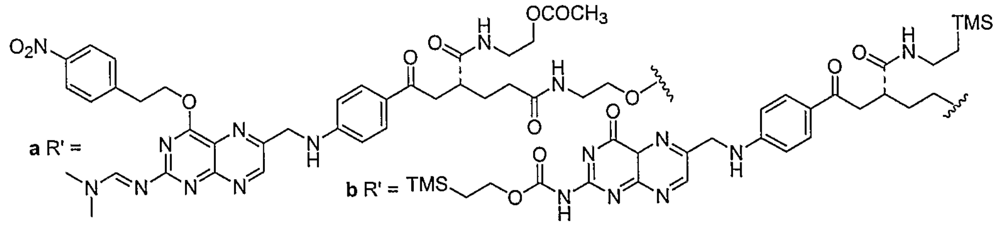
188(a,b)-192(a,b)
Y ' = Me or Et or OMe
or OCH_2CH_2CN

1, 163, 168, 173, 178, 183, 188, Y = $-CH=CH-CH_2-$
2, 164, 169, 174, 179, 184, 189, Y = $-CH=CH-C(O)N(H)-(CH_2)_n-$ and n = 2-20
3, 165, 170, 175, 180, 185, 190, Y = $-(CH_2)_3-$
4, 166, 171, 176, 181, 186, 191, Y = $-(CH_2)_2-C(O)N(H)-(CH_2)_n-$ and n = 2-20
5, 167, 172, 177, 182, 187, 192, Y = $-C\equiv C-CH_2-$



161

162



a R' =

b R' =