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(54) ANTISENSE MODULATION OF INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION

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(57) ABSTRACT

Antisense compounds, compositions and methods are provided for modulating the expression of inducible nitric oxide synthase. The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding inducible nitric oxide synthase. Methods of using these compounds for modulation of inducible nitric oxide synthase expression and for treatment of diseases associated with expression of inducible nitric oxide synthase are provided.

ANTISENSE MODULATION OF INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION

FIELD OF THE INVENTION

[0001] The present invention provides compositions and methods for modulating the expression of inducible nitric oxide synthase. In particular, this invention relates to antisense compounds, particularly oligonucleotides, specifically hybridizable with nucleic acids encoding inducible nitric oxide synthase. Such oligonucleotides have been shown to modulate the expression of inducible nitric oxide synthase.

BACKGROUND OF THE INVENTION

[0002] Nitric Oxide (NO) is a short-lived second messenger that exhibits a diverse array of effects within the normal cell including the regulation of neurotransmission, vasodilation, immunological processes and antimicrobial defenses. Due to its radical properties, however, NO has also been implicated in the onset and maintenance of several pathological conditions. Nitric oxide is produced constitutively in most cell types at low concentrations but levels are greatly increased in response to cellular stimulation by cytokines or bacterial products. Nitric oxide is generated from the amino acid L-arginine by the enzymatic activity of nitric oxide synthase (NOS) (Kroncke et al., *Clin. Exp. Immunol.,* 1998, 113, 147-156; Marletta et al., *Curr. Opin. Chem. Biol.,* 1998, 2, 656-663).

[0003] Three isoforms of the NOS enzyme have been isolated and the differential regulation of these isoforms mediates the fluctuating levels of nitric oxide present within quiescent and stimulated cells. Two of the three isoforms, found in brain and endothelium, are calcium and calmodulin dependent and are responsible for the constitutive levels of nitric oxide present in cells. The third isoform is calcium independent and is expressed after transcriptional induction by several stimuli resulting in localized bursts of nitric oxide production. Because of the highly reactive and potentially toxic nature of the nitric oxide molecule and because relatively high concentrations are generated by the inducible form of the NOS enzyme, much effort has been focused on the control of nitric oxide levels in cells through the regulation of this enzyme (Kroncke et al., *Clin. Exp. Immunol.,* 1998, 113, 147-156; Marletta et al., *Curr. Opin. Chem. Biol.,* 1998, 2, 656-663).

[0004] Inducible nitric oxide synthase (also known as iNOS) was first isolated from human hepatocytes, articular chondrocytes and bone cells with MRNA levels being elevated upon stimulation with lipopolysacharides (LPS) cytokines and interleukin 1 (IL-1) (Geller et al., *Proc. Natl. Acad. Sci. U.S.A.,* 1993, 90, 3491-3495; Maier et al., *Biochim. Biophys. Acta.,* 1994, 1208, 145-150). More recently, a splice variant of human iNOS (GenBank accession number AB022318) was isolated from an osteoblastoma cell line.

[0005] Mice lacking the iNOS gene have been developed and shown to be viable and fertile (Casey et al., *Transplantation,* 1997, 64, 589-593; Laubach et al., *Proc. Natl. Acad. Sci. U. S. A.,* 1995, 92, 10688-10692). However, lymphocytes from iNOS knockouts showed increased proliferative responses and production of cytokines (interferon-gamma, IL-2 and IL-12) in response to allogeneic antigen (Casey et al., *Transplantation,* 1997, 64, 589-593) and studies on macrophages from the null mice show a failure to restrict the growth of lymphoma cells post-infection (MacMicking et al., 1998; MacMicking et al., *Cell,* 1995, 81, 641-650). In addition, disclosed in U.S. Pat. No. 5,766,909 are a DNA molecule encoding murine iNOS, an expression vector encoding iNOS and methods to produce recombinant iNOS (Xie et al., 1998).

[0006] Manifestations of increased nitric oxide production and altered iNOS expression appear in both injury and disease states. Several studies have correlated increased iNOS expression with disorders such as congestive heart failure, CNS disorders, and diabetes (Lee and Brosnan, *Methods,* 1996, 10, 31-37; Rabinovitch et al., *Endocrinology,* 1996, 137, 2093-2099; Vejlstrup et al., *J. Mol. Cell. Cardiol.,* 1998, 30, 1215-1223).

[0007] Currently, strategies aimed at modulating iNOS expression and function involve the use of antibodies, antisense technology, chemical inhibitors and gene knock-outs in mice.

[0008] Studies using antisense oligonucleotides to effectively reduce the mRNA levels of iNOS in animal models have been reported in the literature. In a rat model of septic shock, antisense oligonucleotides targeted to iNOS were shown to prevent LPS-induced hyporeactivity to norepinephrine (Hoque et al., *Am. J. Physiol.,* 1998, 275, H1078-1083). In a mouse model of multiple sclerosis, administration of an antisense phosphorothioate oligonucleotides against mouse iNOS blocked the induction of iNOS mRNA and protein expression in glial cells and inhibited the induction of experimental autoimmune encephalomyelitis (EAE)(Ding et al., *J. Immunol.,* 1998, 160, 2560-2564; Ding et al., *Neurosci. Lett.,* 1996, 220, 89-92). Phosphorothioate antisense oligonucleotides-targeting iNOS were also used to demonstrate the toxic role of nitric oxide in ischemic acute renal failure in the rat (Noiri et al., *J. Clin. Invest.,* 1996, 97, 2377-2383). In studies to investigate the role of nitric oxide in cell adhesion, macrophages expressing either the sense or antisense murine iNOS construct were characterized. It was found that cells expressing the antisense iNOS produced 22-97% less nitric oxide than sense lines (Cartwright et al., Br. *J. Pharmacol.,* 1997, 120, 146-152).

[0009] In human cell lines, an antisense oligonucleotide 32 nucleotides long targeted to iNOS has also been used to discern the role of iNOS in the processes of apoptosis (Selleri et al., Br. *J. Haematol.,* 1997, 99, 481-489). An antisense olignucleotide targeted to nucleotides 62-85 of human iNOS has been used to study the role of this enzyme in oxidative stress injury (Peresleni et al., *Am. J. Physiol.,* 1996, 270, F971-977). various types of inhibitors of iNOS function, including chemical moieties and naturally occurring molecules such as amino acids and peptide fragments, have been investigated and characterized in the art. Disclosed in U.S. Pat. Nos. 5,028,627 and 5,216,025 are methods to treat systemic hypotension in septic and cytokine-treated patients using arginine derivatives to decrease nitrogen oxide production (Gross et al., 1993; Kilbourn et al., 1991). The use of arginine derivatives to suppress iNOS function is also reported in PCT publication number WO 98/48826 (Silverman et al., 1998). Disclosed in PCT publication numbers WO 93/13055 and WO 96/19440 are amidino and acetamide derivative inhibitors of iNOS, respectively (Beams et al., 1993; Oplinger et al., 1996).

[0010] Other inhibitors include a peptide nucleic acid derivative with a base sequence complementary to the

homopurine region at nucleotides 238-251 of mouse iNOS (Giovine et al., *FEBS Lett.,* 1998, 426, 33-36), aminoguanidine (Corbett and McDaniel, *Methods,* 1996, 10, 21-30), N-α-Tosyl-L-Lysine chloromethylketone (Schini-Kerth et al., *Arterioscler. Thromb. Vasc. Biol.,* 1997, 17, 672-679), gadolinium chloride (Roland et al., *J. Leukoc. Biol.,* 1996, 60, 487-492), taurine chloramine (Park et al., *J. Leukoc. Biol.,* 1997, 61, 161-166), di-catechol rooperol (Bereta et al., *Life Sci.,* 1997, 60, 325-334), tyrosine kinase inhibitors (Corbett et al., *Am. J. Physiol.,* 1996, 270, C1581-1587) and immunosuppresive drugs (Cai et al., *Int. J. Cardiol.,* 1995, 50, 243-251). Furthermore, disclosed in U.S. Pat. No. 5,789, 395 are methods to inhibit nitric oxide production using tetracycline compounds (Amin et al., 1998) and in U.S. Pat. No. 5,695,761 methods of treating an inflammatory disease by administering epitopes of the protein osteopontin are disclosed (Denhardt et al., 1997).

[**0011**] Recently, iNOS inhibitors intended to treat various human conditions including CNS disease, ischemia/reperfusion injury and opioid tolerance that occurs as a result of sustained opioid usage during chronic pain have been reported in the art (Maeda et al., 1998; Salvemini, 1998; Singh, 1998).

[**0012**] Finally, disclosed in EP 94304174 is a pharmaceutical composition comprising a combination of an iNOS inhibitor and and anti-inflammatory agent for the treatment of systemic inflammatory response syndrome (Teale, 1994).

[**0013**] Despite the variety of iNOS inhibitors disclosed in the art, there still remains a need for therapeutic agents capable of effectively and specifically inhibiting the function of the inducible isoform of the NOS enzyme (iNOS).

[**0014**] Antisense technology is emerging as an effective means for reducing the expression of specific gene products and may therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications for the modulation of iNOS expression.

SUMMARY OF THE INVENTION

[**0015**] The present invention is directed to antisense compounds, particularly oligonucleotides, which are targeted to a nucleic acid encoding inducible nitric oxide synthase, and which modulate the expression of inducible nitric oxide synthase. Pharmaceutical and other compositions comprising the antisense compounds of the invention are also provided. Further provided are methods of modulating the expression of inducible nitric oxide synthase in cells or tissues comprising contacting said cells or tissues with one or more of the antisense compounds or compositions of the invention. Further provided are methods of treating an animal, particularly a human, suspected of having or being prone to a disease or condition associated with expression of inducible nitric oxide synthase by administering a therapeutically or prophylactically effective amount of one or more of the antisense compounds or compositions of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[**0016**] The present invention employs oligomeric antisense compounds, particularly oligonucleotides, for use in modulating the function of nucleic acid molecules encoding inducible nitric oxide synthase, ultimately modulating the amount of inducible nitric oxide synthase produced. This is accomplished by providing antisense compounds which specifically hybridize with one or more nucleic acids encoding inducible nitric oxide synthase. As used herein, the terms "target nucleic acid" and "nucleic acid encoding inducible nitric oxide synthase" encompass DNA encoding inducible nitric oxide synthase, RNA (including pre-mRNA and MRNA) transcribed from such DNA, and also cDNA derived from such RNA. The specific hybridization of an oligomeric compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds which specifically hybridize to it is generally referred to as "antisense". The functions of DNA to be interfered with include replication and transcription. The functions of RNA to be interfered with include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of inducible nitric oxide synthase. In the context of the present invention, "modulation" means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene. In the context of the present invention, inhibition is the preferred form of modulation of gene expression and mRNA is a preferred target.

[**0017**] It is preferred to target specific nucleic acids for antisense. "Targeting" an antisense compound to a particular nucleic acid, in the context of this invention, is a multistep process. The process usually begins with the identification of a nucleic acid sequence whose function is to be modulated. This may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target is a nucleic acid molecule encoding inducible nitric oxide synthase. The targeting process also includes determination of a site or sites within this gene for the antisense interaction to occur such that the desired effect, e.g., detection or modulation of expression of the protein, will result. Within the context of the present invention, a preferred intragenic site is the region encompassing the translation initiation or termination codon of the open reading frame (ORF) of the gene. Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in vivo. Thus, the terms "translation initiation codon" and "start codon" can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, "start codon" and "translation

initiation codon" refer to the codon or codons that are used in vivo to initiate translation of an mR molecule transcribed from a gene encoding inducible nitric oxide synthase, regardless of the sequence(s) of such codons.

[0018] It is also known in the art that a translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e.; 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively). The terms "start codon region" and "translation initiation codon region" refer to a portion of such an MRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms "stop codon region" and "translation termination codon region" refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon.

[0019] The open reading frame (ORF) or "coding region," which is known in the art to refer to the region between the translation initiation codon and the translation termination codon, is also a region which may be targeted effectively. Other target regions include the 5' untranslated region (5' UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA or corresponding nucleotides on the gene, and the 3' untranslated region (3' UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA or corresponding nucleotides on the gene. The 5' cap of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap. The 5' cap region may also be a preferred target region.

[0020] Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. mRNA splice sites, i.e., intron-exon junctions, may also be preferred target regions, and are particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular mRNA splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also preferred targets. It has also been found that introns can also be effective, and therefore preferred, target regions for antisense compounds targeted, for example, to DNA or pre-mRNA.

[0021] Once one or more target sites have been identified, oligonucleotides are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect.

[0022] In the context of this invention, "hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases. 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 the sequence of an antisense compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. An antisense compound is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound 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, and in the case of in vitro assays, under conditions in which the assays are performed.

[0023] Antisense compounds are commonly used as research reagents and diagnostics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes. Antisense compounds are also used, for example, to distinguish between functions of various members of a biological pathway. Antisense modulation has, therefore, been harnessed for research use.

[0024] The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense oligonucleotides have been employed as therapeutic moieties in the treatment of disease states in animals and man. Antisense oligonucleotides have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that oligonucleotides can be useful therapeutic modalities that can be configured to be useful in treatment regimes for treatment of cells, tissues and animals, especially humans. In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

[0025] While antisense oligonucleotides are a preferred form of antisense compound, the present invention compre-

hends other oligomeric antisense compounds, including but not limited to oligonucleotide mimetics such as are described below. The antisense compounds in accordance with this invention preferably comprise from about 8 to about 30 nucleobases (i.e. from about 8 to about 30 linked nucleosides). Particularly preferred antisense compounds are antisense oligonucleotides, even more preferably those comprising from about 12 to about 25 nucleobases. As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn the respective ends of this linear polymeric structure can be further joined to form a circular structure, however, open linear structures are generally preferred. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

[**0026**] Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

[**0027**] Preferred modified oligonucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotri-esters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thiono-alkylphosphonates, thionoalkylphosphotriesters, and borano-phosphates 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.

[**0028**] Representative United States patents that teach the preparation of the above phosphorus-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; and 5,625,050, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

[**0029**] Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside 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.

[**0030**] 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, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

[**0031**] In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, 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 aza nitrogen 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, 1497-1500.

[**0032**] Most preferred embodiments of the invention are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular —CH_2—NH—O—CH_2—, —CH_2—(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.

[**0033**] Modified oligonucleotides may also contain 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; O—, S— or N-alkynyl; or O-alkyl-O-alkyl, 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_2CH_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, 486-504) 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 examples hereinbelow, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'—O—CH_2—O—CH_2—$N(CH_2)_2$, also described in examples hereinbelow.

[**0034**] 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 and the 5' position of 5' terminal nucleotide. 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 sugar 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; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

[**0035**] Oligonucleotides may also include 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 oligomeric compounds 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. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., eds., *Antisense Research and Applications*, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

[**0036**] 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,

[**0037**] **5,596,091**; **5,614,617**; and 5,681,941, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference, and U.S. Pat. No. 5,750,692, which is commonly owned with the instant application and also herein incorporated by reference.

[**0038**] Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more 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, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Let.,* 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.,* 1992, 660, 306-309; Manoharan et al., *Bioorg. Med. Chem. Let.,* 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.,* 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.,* 1991, 10, 1111-1118; Kabanov et al., *FEBS Lett.,* 1990, 259, 327-330; Svinarchuk et al., *Biochimie,* 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.,* 1995, 36, 3651-3654; Shea et al., *Nucl. Acids Res.,* 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides,* 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.,* 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta,* 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.,* 1996, 277, 923-937.

[**0039**] 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, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference.

[0040] It is not necessary for all positions in a given compound to be uniformly modified, and inifact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide. The present invention also includes antisense compounds which are chimeric compounds. "Chimeric" antisense compounds or "chimeras," in the context of this invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligonucleotides when chimeric oligonucleotides are used, compared to phosphorothioate deoxyoligonucleotides hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

[0041] Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S. Pat. Nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

[0042] The antisense compounds used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and alkylated derivatives.

[0043] The antisense compounds of the invention are synthesized in vitro and do not include antisense compositions of biological origin, or genetic vector constructs designed to direct the in vivo synthesis of antisense molecules. The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption assisting formulations include, but are not limited to, U.S. Pat. Nos. 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

[0044] The antisense compounds of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to prodrugs and pharmaceutically acceptable salts of the compounds of the invention, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents.

[0045] The term "prodrug" indicates a therapeutic agent that is prepared in an inactive form that is converted to an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. In particular, prodrug versions of the oligonucleotides of the invention are prepared as SATE [(S-acetyl-2-thioethyl) phosphate] derivatives according to the methods disclosed in WO 93/24510 to Gosselin et al., published Dec. 9, 1993 or in WO 94/26764 to Imbach et al.

[0046] The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

[0047] Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge et al., "Pharmaceutical Salts,"*J. of Pharma Sci.,* 1977, 66, 1-19). The base addition salts of said acidic compounds are prepared by contacting the free acid form. with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as

solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention. As used herein, a "pharmaceutical addition salt" includes a pharmaceutically acceptable salt of an acid form of one of the components of the compositions of the invention. These include organic or inorganic acid salts of the amines. Preferred acid salts are the hydrochlorides, acetates, salicylates, nitrates and phosphates. Other suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of a variety of inorganic and organic acids, such as, for example, with inorganic acids, such as for example hydrochloric acid, hydrobromic acid, sulfuric acid or phosphoric acid; with organic carboxylic, sulfonic, sulfo or phospho acids or N-substituted sulfamic acids, for example acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, fumaric acid, malic acid, tartaric acid, lactic acid, oxalic acid, gluconic acid, glucaric acid, glucuronic acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, salicylic acid, 4-aminosalicylic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, embonic acid, nicotinic acid or isonicotinic acid; and with amino acids, such as the 20 alpha-amino acids involved in the synthesis of proteins in nature, for example glutamic acid or aspartic acid, and also with phenylacetic acid, methanesulfonic acid, ethanesulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, benzenesulfonic acid, 4-methylbenzenesulfoic acid, naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic acid, 2- or 3-phosphoglycerate, glucose-6-phosphate, N-cyclohexylsulfamic acid (with the formation of cyclamates), or with other acid organic compounds, such as ascorbic acid. Pharmaceutically acceptable salts of compounds may also be prepared with a pharmaceutically acceptable cation. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and include alkaline, alkaline earth, ammonium and quaternary ammonium cations. Carbonates or hydrogen carbonates are also possible.

[0048] For oligonucleotides, preferred examples of pharmaceutically acceptable salts include but are not limited to (a) salts formed with cations such as sodium, potassium, ammonium, magnesium, calcium, polyamines such as spermine and spermidine, etc.; (b) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; (c) salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (d) salts formed from elemental anions such as chlorine, bromine, and iodine.

[0049] The antisense compounds of the present invention can be utilized for diagnostics, therapeutics, prophylaxis and as research reagents and kits. For therapeutics, an animal, preferably a human, suspected of having a disease or disorder which can be treated by modulating the expression of inducible nitric oxide synthase is treated by administering antisense compounds in accordance with this invention. The compounds of the invention can be utilized in pharmaceutical compositions by adding an effective amount of an antisense compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the antisense compounds and methods of the invention may also be useful prophylactically, e.g., to prevent or delay infection, inflammation or tumor formation, for example.

[0050] The antisense compounds of the invention are useful for research and diagnostics, because these compounds hybridize to nucleic acids encoding inducible nitric oxide synthase, enabling sandwich and other assays to easily be constructed to exploit this fact. Hybridization of the antisense oligonucleotides of the invention with a nucleic acid encoding inducible nitric oxide synthase can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabelling of the oligonucleotide or any other suitable detection means. Kits using such detection means for detecting the level of inducible nitric oxide synthase in a sample may also be prepared.

[0051] The present invention also includes pharmaceutical compositions and formulations which include the antisense compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration. Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration.

[0052] Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

[0053] Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

[0054] Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

[0055] Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

[0056] The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional

techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0057] The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0058] In one embodiment of the present invention the pharmaceutical compositions may be formulated and used as foams. Pharmaceutical foams include formulations'such as, but not limited to, emulsions, microemulsions, creams, jellies and liposomes. While basically similar in nature these formulations vary in the components and the consistency of the final product. The preparation of such compositions and formulations is generally known to those skilled in the pharmaceutical and formulation arts and may be applied to the formulation of the compositions of the present invention.

[0059] Emulsions

[0060] The compositions of the present invention may be prepared and formulated as emulsions. Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. (Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi et al., in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising of two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions may be either water-in-oil (w/o) or of the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions may contain additional components in addition to the dispersed phases and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants may also be present in emulsions as needed. Pharmaceutical emulsions may also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous provides an o/w/o emulsion.

[0061] Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion may be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that may be incorporated into either phase of the emulsion. Emulsifiers may broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

[0062] Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (Rieger, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants may be classified into different classes based on the nature of the hydrophilic group-:nonionic, anionic, cationic and amphoteric (Rieger, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

[0063] Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

[0064] A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

[0065] Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

[0066] Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that may readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used may be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

[0067] The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of reasons of ease of formulation, efficacy from an absorption and bioavailability standpoint. (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

[0068] In one embodiment of the present invention, the compositions of oligonucleotides and nucleic acids are formulated as microemulsions. A microemulsion may be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: *Controlled Release of Drugs: Polymers and Aggregate Systems*, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa., 1985, p. 271).

[0069] The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

[0070] Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (S0750), decaglycerol decaoleate (DA0750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions may, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase may typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase may include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

[0071] Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (Constantinides et al., *Pharmaceutical Research,* 1994, 11, 1385-1390; Ritschel, *Meth. Find. Exp. Clin. Pharmacol.,* 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (Constantinides et al., *Pharmaceutical Research,* 1994, 11, 1385; Ho et al., *J. Pharm. Sci.,* 1996, 85, 138-143). Often microemulsions may form spontaneously when their components are brought together at ambient temperature. This may be particularly advantageous when formulating ther-

molabile drugs, peptides or oligonucleotides. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of oligonucleotides and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of oligonucleotides and nucleic acids within the gastrointestinal tract, vagina, buccal cavity and other areas of administration.

[0072] Microemulsions of the present invention may also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the oligonucleotides and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention may be classified as belonging to one of five broad categories—surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems,* 1991, p. 92). Each of these classes has been discussed above.

[0073] Liposomes

[0074] There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

[0075] Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages in vivo.

[0076] In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

[0077] Further advantages of liposomes include; liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation (Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

[0078] Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes. As the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act.

[0079] Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

[0080] Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into the skin. Compounds including analgesics, antibodies, hormones and high-molecular weight DNAs have been administered to the skin. The majority of applications resulted in the targeting of the upper epidermis.

[0081] Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged DNA molecules to form a stable complex. The positively charged DNA/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al., *Biochem. Biophys. Res. Commun.,* 1987, 147, 980-985).

[0082] Liposomes which are pH-sensitive or negatively-charged, entrap DNA rather than complex with it. Since both the DNA and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some DNA is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou et al., *Journal of Controlled Release,* 1992, 19, 269-274).

[0083] One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

[0084] Several studies have assessed the topical delivery of liposomal drug formulations to the skin. Application of liposomes containing interferon to guinea pig skin resulted in a reduction of skin herpes sores while delivery of interferon via other means (e.g. as a solution or as an emulsion) were ineffective (Weiner et al., *Journal of Drug Targeting,* 1992, 2, 405-410). Further, an additional study tested the efficacy of interferon administered as part of a liposomal formulation to the administration of interferon using an aqueous system, and concluded that the liposomal formula-

tion was superior to aqueous administration (du Plessis et al., *Antiviral Research,* 1992, 18, 259-265).

[0085] Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporin-A into different layers of the skin (Hu et al. *S.T.P. Pharma. Sci.,* 1994, 4, 6, 466).

[0086] Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen et al., *FEBS Letters,* 1987, 223, 42; Wu et al., *Cancer Research,* 1993, 53, 3765).

[0087] Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos et al. (*Ann. N.Y. Acad. Sci.,* 1987, 507, 64) reported the ability of monosialoganglioside G_{M1}, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (*Proc. Natl. Acad. Sci. U.S.A.,* 1988, 85, 6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb et al.) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim et al.).

[0088] Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto et al. (*Bull. Chem. Soc. Jpn.,* 1980, 53, 2778) described liposomes comprising a nonionic detergent, $2C_{12}15G$, that contains a PEG moiety. Illum et al. (*FEBS Lett.,* 1984, 167, 79) noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (e.g., PEG) are described by Sears (U.S. Pat. Nos. 4,426,330 and 4,534, 899). Klibanov et al. (*FEBS Lett.,* 1990, 268, 235) described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume et al. (*Biochimica et Biophysica Acta,* 1990, 1029, 91) extended such observations to other PEG-derivatized phospholipids, e.g., DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle et al. (U.S. Pat. Nos. 5,013,556 and 5,356,633) and Martin et al. (U.S. Pat. No. 5,213,804 and European Patent No. EP 0 496 813 Bi). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Pat. No. 5,225,212 (both to Martin et al.) and in WO 94/20073 (Zalipsky et al.) Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi et al.). U.S. Pat. Nos. 5,540,935 (Miyazaki et al.) and 5,556,948 (Tagawa et al.) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

[0089] A limited number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry et al. discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Pat. No. 5,264,221 to Tagawa et al. discloses protein-bonded liposomes and asserts that the contents of such liposomes may include an antisense RNA. U.S. Pat. No. 5,665,710 to Rahman et al. describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love et al. discloses liposomes comprising antisense oligonucleotides targeted to the raf gene.

[0090] Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes may be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, e.g. they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

[0091] Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

[0092] If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters

such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

[0093] If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

[0094] If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

[0095] If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

[0096] The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in *Pharmaceutical Dosage Forms*, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

[0097] Penetration Enhancers

[0098] In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly oligonucleotides, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs may cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

[0099] Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems,* 1991, p.92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

[0100] Surfactants: In connection with the present invention, surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution, reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of oligonucleotides through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems,* 1991, p.92); and perfluorochemical emulsions, such as FC-43. Takahashi et al., *J. Pharm. Pharmacol.,* 1988, 40, 252).

[0101] Patty acids: Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauricacid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C_{1-10} alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and diglycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems,* 1991, p.92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems,* 1990, 7, 1-33; E l Hariri et al., *J. Pharm. Pharmacol.,* 1992, 44, 651-654).

[0102] Bile salts: The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 in: Goodman & Gilman's *The Pharmacological Basis of Therapeutics,* 9th Ed., Hardman et al. Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. The bile salts of the invention include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate), taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems,* 1991, page 92; Swinyard, Chapter 39 In: Remington's *Pharmaceutical Sciences,* 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems,* 1990, 7, 1-33; Yamamoto et al., *J. Pharm. Exp. Ther.,* 1992, 263, 25; Yamashita et al., *J. Pharm. Sci.,* 1990, 79, 579-583).

[0103] Chelating Agents: Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of oligonucleotides through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, *J. Chromatogr.,* 1993, 618, 315-339). Chelating agents of the invention include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines)(Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems,* 1991, page

92; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems,* 1990, 7, 1-33; Buur et al., *J. Control Rel.,* 1990, 14, 43-51).

[0104] Non-chelating non-surfactants: As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of oligonucleotides through the alimentary mucosa (Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems,* 1990, 7, 1-33). This class of penetration enhancers include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems,* 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., *J. Pharm. Pharmacol.,* 1987, 39, 621-626).

[0105] Agents that enhance uptake of oligonucleotides at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi et al, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo et al., PCT Application WO 97/30731), are also known to enhance the cellular uptake of oligonucleotides.

[0106] Other agents may be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

[0107] Carriers

[0108] Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (i.e., does not possess biological activity per se) but is recognized as a nucleic acid by in vivo processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate oligonucleotide in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao et al., *Antisense Res. Dev.,* 1995, 5, 115-121; Takakura et al., *Antisense & Nucl. Acid Drug Dev.,* 1996, 6, 177-183).

[0109] Excipients

[0110] In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient may be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc.).

[0111] Pharmaceutically acceptable organic or inorganic excipient suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

[0112] Formulations for topical administration of nucleic acids may include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions may also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can be used.

[0113] Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

[0114] Other Components

[0115] The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

[0116] Aqueous suspensions may contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0117] Certain embodiments of the invention provide pharmaceutical compositions containing (a) one or more

antisense compounds and (b) one or more other chemotherapeutic agents which function by a non-antisense mechanism. Examples of such chemotherapeutic agents include, but are not limited to, anticancer drugs such as daunorubicin, dactinomycin, doxorubicin, bleomycin, mitomycin, nitrogen mustard, chlorambucil, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-fluorouracil (5-FU), floxuridine (5-FUdR), methotrexate (MTX), colchicine, vincristine, vinblastine, etoposide, teniposide, cisplatin and diethylstilbestrol (DES). See, generally, *The Merck Manual of Diagnosis and Therapy,* 15th Ed., Berkow et al., eds., 1987, Rahway, N.J., pages 1206-1228). Anti-inflammatory drugs, including but not limited to non-steroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. See, generally, *The Merck Manual of Diagnosis and Therapy,* 15th Ed., Berkow et al., eds., 1987, Rahway, N.J., pages 2499-2506 and 46-49, respectively). Other non-antisense chemotherapeutic agents are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

[**0118**] In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. Numerous examples of antisense compounds are known in the art. Two or more combined compounds may be used together or sequentially.

[**0119**] The formulation of therapeutic compositions and their subsequent administration is believed to be within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50}s found to be effective in in vitro and in vivo animal models. In general, dosage is from 0.01 ug to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 ug to 100 g per kg of body weight, once or more daily, to once every 20 years.

[**0120**] While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same.

EXAMPLES

Example 1

Nucleoside Phosphoramidites for Oligonucleotide Synthesis Deoxy and 2'-alkoxy Amidites

[**0121**] 2'-Deoxy and 2'-methoxy beta-cyanoethyldiisopropyl phosphoramidites were purchased from commercial sources (e.g. Chemgenes, Needham Mass. or Glen Research, Inc. Sterling Va.). Other 2'-O-alkoxy substituted nucleoside amidites are prepared as described in U.S. Pat. No. 5,506,351, herein incorporated by reference. For oligonucleotides synthesized using 2'-alkoxy amidites, the standard cycle for unmodified oligonucleotides was utilized, except the wait step after pulse delivery of tetrazole and base was increased to 360 seconds.

[**0122**] Oligonucleotides containing 5-methyl-2'-deoxycytidine (5—Me—C) nucleotides were synthesized according to published methods [Sanghvi, et. al., *Nucleic Acids Research,* 1993, 21, 3197-3203] using commercially available phosphoramidites (Glen Research, Sterling Va. or ChemGenes, Needham Mass.).

[**0123**] 2'-Fluoro Amidites

[**0124**] 2'-Fluorodeoxyadenosine Amidites

[**0125**] 2'-fluoro oligonucleotides were synthesized as described previously [Kawasaki, et. al., *J. Med. Chem.,* 1993, 36, 831-841] and U.S. Pat. No. 5,670,633, herein incorporated by reference. Briefly, the protected nucleoside N6-benzoyl-2'-deoxy-2'-fluoroadenosine was synthesized utilizing commercially available 9-beta-D-arabinofuranosyladenine as starting material and by modifying literature procedures whereby the 2'-alpha-fluoro atom is introduced by a S_N2-displacement of a 2'-beta-trityl group. Thus N6-benzoyl-9-beta-D-arabinofuranosyladenine was selectively protected in moderate yield as the 3',5'-ditetrahydropyranyl (THP) intermediate. Deprotection of the THP and N6-benzoyl groups was accomplished using standard methodologies and standard methods were used to obtain the 5'-dimethoxytrityl-(DMT) and 5'-DMT-3'-phosphoramidite intermediates.

[**0126**] 2'-Fluorodeoxyguanosine

[**0127**] The synthesis of 2'-deoxy-2'-fluoroguanosine was accomplished using tetraisopropyldisiloxanyl (TPDS) protected 9-beta-D-arabinofuranosylguanine as starting material, and conversion to the intermediate diisobutyryl-arabinofuranosylguanosine. Deprotection of the TPDS group was followed by protection of the hydroxyl group with THP to give diisobutyryl di-THP protected arabinofuranosylguanine. Selective O-deacylation and triflation was followed by treatment of the crude product with fluoride, then deprotection of the THP groups. Standard methodologies were used to obtain the 5'-DMT- and 5'-DMT-3'-phosphoramidites.

[**0128**] 2'-Fluorouridine

[**0129**] Synthesis of 2'-deoxy-2'-fluorouridine was accomplished by the modification of a literature procedure in which 2,2'-anhydro-1-beta-D-arabinofuranosyluracil was treated with 70% hydrogen fluoride-pyridine. Standard procedures were used to obtain the 5'-DMT and 5'-DMT-3'phosphoramidites.

[0130] 2'-Fluorodeoxycytidine

[0131] 2'-deoxy-2'-fluorocytidine was synthesized via amination of 2'-deoxy-2'-fluorouridine, followed by selective protection to give N4-benzoyl-2'-deoxy-2'-fluorocytidine. Standard procedures were used to obtain the 5'-DMT and 5'-DMT-3' phosphoramidites.

[0132] 2'-O-(2-Methoxyethyl) Modified Amidites

[0133] 2'-O-Methoxyethyl-substituted nucleoside amidites are prepared as follows, or alternatively, as per the methods of Martin, P., *Helvetica Chimica Acta,* 1995, 78, 486-504.

[0134] 2,2'-Anhydro[1-(beta-D-arabinofuranosyl)-5-methyluridine]

[0135] 5-Methyluridine (ribosylthymine, commercially available through Yamasa, Choshi, Japan) (72.0 g, 0.279 M), diphenyl-carbonate (90.0 g, 0.420 M) and sodium bicarbonate (2.0 g, 0.024 M) were added to DMF (300 mL). The mixture was heated to reflux, with stirring, allowing the evolved carbon dioxide gas to be released in a controlled manner. After 1 hour, the slightly darkened solution was concentrated under reduced pressure. The resulting syrup was poured into diethylether (2.5 L), with stirring. The product formed a gum. The ether was decanted and the residue was dissolved in a minimum amount of methanol (ca. 400 mL). The solution was poured into fresh ether (2.5 L) to yield a stiff gum. The ether was decanted and the gum was dried in a vacuum oven (60° C. at 1 mm Hg for 24 h) to give a solid that was crushed to a light tan powder (57 g, 85% crude yield). The NMR spectrum was consistent with the structure, contaminated with phenol as its sodium salt (ca. 5%). The material was used as is for further reactions (or it can be purified further by column chromatography using a gradient of methanol in ethyl acetate (10-25%) to give a white solid, mp 222-4° C.).

[0136] 2'-O-Methoxyethyl-5-methyluridine

[0137] 2,2'-Anhydro-5-methyluridine (195 g, 0.81 M), tris(2-methoxyethyl)borate (231 g, 0.98 M) and 2-methoxyethanol (1.2 L) were added to a 2 L stainless steel pressure vessel and placed in a pre-heated oil bath at 160° C. After heating for 48 hours at 155-160° C., the vessel was opened and the solution evaporated to dryness and triturated with MeOH (200 mL). The residue was suspended in hot acetone (1 L). The insoluble salts were filtered, washed with acetone (150 mL) and the filtrate evaporated. The residue (280 g) was dissolved in CH_3CN (600 mL) and evaporated. A silica gel column (3 kg) was packed in CH_2Cl_2/acetone/MeOH (20:5:3) containing 0.5% Et_3NH. The residue was dissolved in CH_2Cl_2 (250 mL) and adsorbed onto silica (150 g) prior to loading onto the column. The product was eluted with the packing solvent to give 160 g (63%) of product. Additional material was obtained by reworking impure fractions.

[0138] 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

[0139] 2'-O-Methoxyethyl-5-methyluridine (160 g, 0.506 M) was co-evaporated with pyridine (250 mL) and the dried residue dissolved in pyridine (1.3 L). A first aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the mixture stirred at room temperature for one hour. A second aliquot of dimethoxytrityl chloride (94.3 g, 0.278 M) was added and the reaction stirred for an additional one hour. Methanol (170 mL) was then added to stop the reaction. HPLC showed the presence of approximately 70% product. The solvent was evaporated and triturated with CH_3CN (200 mL). The residue was dissolved in $CHCl_3$ (1.5 L) and extracted with 2×500 mL of saturated $NaHCO_3$ and 2×500 mL of saturated NaCl. The organic phase was dried over Na_2SO_{41} filtered and evaporated. 275 g of residue was obtained. The residue was purified on a 3.5 kg silica gel column, packed and eluted with EtOAc/hexane/acetone (5:5:1) containing 0.5% Et_3NH. The pure fractions were evaporated to give 164 g of product. Approximately 20 g additional was obtained from the impure fractions to give a total yield of 183 g (57%).

[0140] 3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

[0141] 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (106 g, 0.167 M), DMF/pyridine (750 mL of a 3:1 mixture prepared from 562 mL of DMF and 188 mL of pyridine) and acetic anhydride (24.38 mL, 0.258 M) were combined and stirred at room temperature for 24 hours. The reaction was monitored by TLC by first quenching the TLC sample with the addition of MeOH. Upon completion of the reaction, as judged by TLC, MeOH (50 mL) was added and the mixture evaporated at 35° C. The residue was dissolved in $CHCl_3$ (800 mL) and extracted with 2×200 mL of saturated sodium bicarbonate and 2×200 mL of saturated NaCl. The water layers were back extracted with 200 mL of $CHCl_3$. The combined organics were dried with sodium sulfate and evaporated to give 122 g of residue (approx. 90% product). The residue was purified on a 3.5 kg silica gel column and eluted using EtOAc/hexane(4:1). Pure product fractions were evaporated to yield 96 g (84%). An additional 1.5 g was recovered from later fractions.

[0142] 3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine

[0143] A first solution was prepared by dissolving 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (96 g, 0.144 M) in CH_3CN (700 mL) and set aside. Triethylamine (189 mL, 1.44 M) was added to a solution of triazole (90 g, 1.3 M) in CH_3CN (1 L), cooled to −5° C. and stirred for 0.5 h using an overhead stirrer. $POCl_3$ was added dropwise, over a 30 minute period, to the stirred solution maintained at 0-10° C., and the resulting mixture stirred for an additional 2 hours. The first solution was added dropwise, over a 45 minute period, to the latter solution. The resulting reaction mixture was stored overnight in a cold room. Salts were filtered from the reaction mixture and the solution was evaporated. The residue was dissolved in EtOAc (1 L) and the insoluble solids were removed by filtration. The filtrate was washed with 1×300 mL of $NaHCO_3$ and 2×300 mL of saturated NaCl, dried over sodium sulfate and evaporated. The residue was triturated with EtOAc to give the title compound.

[0144] 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine

[0145] A solution of 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine (103 g, 0.141 M) in dioxane (500 mL) and NH_4OH (30 mL) was stirred at room temperature for 2 hours. The dioxane solution was evaporated and the residue azeotroped with MeOH (2×200 mL). The residue was dissolved in MeOH (300 mL) and

transferred to a 2 liter stainless steel pressure vessel. MeOH (400 mL) saturated with NH_3 gas was added and the vessel heated to 100° C. for 2 hours (TLC showed complete conversion). The vessel contents were evaporated to dryness and the residue was dissolved in EtOAc (500 mL) and washed once with saturated NaCl (200 mL). The organics were dried over sodium sulfate and the solvent was evaporated to give 85 g (95%) of the title compound.

[0146] N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine

[0147] 2'-O-Methoxyethyl-5'-0-dimethoxytrityl-5-methylcytidine (85 g, 0.134 M) was dissolved in DMF (800 mL) and benzoic anhydride (37.2 g, 0.165 M) was added with stirring. After stirring for 3 hours, TLC showed the reaction to be approximately 95% complete. The solvent was evaporated and the residue azeotroped with MeOH (200 mL). The residue was dissolved in $CHCl_3$ (700 mL) and extracted with saturated $NaHCO_3$ (2×300 mL) and saturated NaCl (2×300 mL), dried over $MgSO_4$ and evaporated to give a residue (96 g). The residue was chromatographed on a 1.5 kg silica column using EtOAc/hexane (1:1) containing 0.5% Et_3NH as the eluting solvent. The pure product fractions were evaporated to give 90 g (90%) of the title compound.

[0148] N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine-3'-amidite

[0149] N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (74 g, 0.10 M) was dissolved in CH_2Cl_2 (1 L). Tetrazole diisopropylamine (7.1 g) and 2-cyanoethoxy-tetra-(isopropyl)phosphite (40.5 mL, 0.123 M) were added with stirring, under a nitrogen atmosphere. The resulting mixture was stirred for 20 hours at room temperature (TLC showed the reaction to be 95% complete). The reaction mixture was extracted with saturated $NaHCO_3$ (1×300 mL) and saturated NaCl (3×300 mL). The aqueous washes were back-extracted with CH_2Cl_2 (300 mL), and the extracts were combined, dried over $MgSO_4$ and concentrated. The residue obtained was chrQmatographed on a 1.5 kg silica column using EtOAc/hexane (3:1) as the eluting solvent. The pure fractions were combined to give 90.6 g (87%) of the title compound.

[0150] 2'-O-(Aminooxyethyl) nucleoside amidites and 2'-O-(dimethylaminooxyethyl) Nucleoside Amidites

[0151] 2'-(Dimethylaminooxyethoxy) Nucleoside Amidites

[0152] 2'-(Dimethylaminooxyethoxy) Nucleoside Amidites [also known in the art as 2'-O-(dimethylaminooxyethyl) nucleoside amidites] are prepared as described in the following paragraphs. Adenosine, cytidine and guanosine nucleoside amidites are prepared similarly to the thymidine (5-methyluridine) except the exocyclic amines are protected with a benzoyl moiety in the case of adenosine and cytidine and with isobutyryl in the case of guanosine.

[0153] 5'-O-tert-Butyldiphenylsilyl-O^2-2'-anhydro-5-methyluridine

[0154] O^2-2'-anhydro-5-methyluridine (Pro. Bio. Sint., Varese, Italy, 100.0 g, 0.416 mmol), dimethylaminopyridine (0.66 g, 0.013 eq, 0.0054 mmol) were dissolved in dry pyridine (500 ml) at ambient temperature under an argon atmosphere and with mechanical stirring. tert-Butyldiphenylchlorosilane (125.8g, 119.0 mL, 1.1 eq, 0.458 mmol) was added in one portion. The reaction was stirred for 16 h at ambient temperature. TLC (Rf 0.22, ethyl acetate) indicated a complete reaction. The solution was concentrated under reduced pressure to a thick oil. This was partitioned between dichloromethane (1 L) and saturated sodium bicarbonate (2×l L) and brine (1 L). The organic layer was dried over sodium sulfate and concentrated under reduced pressure to a thick oil. The oil was dissolved in a 1:1 mixture of ethyl acetate and ethyl ether (600 mL) and the solution was cooled to −10° C. The resulting crystalline product was collected by filtration, washed with ethyl ether (3×200 mL) and dried (40° C., 1 mm Hg, 24 h) to 149g (74.8%) of white solid. TLC and NMR were consistent with pure product.

[0155] 5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine

[0156] In a 2 L stainless steel, unstirred pressure reactor was added borane in tetrahydrofuran (1.0 M, 2.0 eq, 622 mL). In the fume hood and with manual stirring, ethylene glycol (350 mL, excess) was added cautiously at first until the evolution of hydrogen gas subsided. 5'-O-tert-Butyldiphenylsilyl-O^2-2'-anhydro-5-methyluridine (149 g, 0.311 mol) and sodium bicarbonate (0.074 g, 0.003 eq) were added with manual stirring. The reactor was sealed and heated in an oil bath until an internal temperature of 160° C. was reached and then maintained for 16 h (pressure <100 psig). The reaction vessel was cooled to ambient and opened. TLC (Rf 0.67 for desired product and Rf 0.82 for ara-T side product, ethyl acetate) indicated about 70% conversion to the product. In order to avoid additional side product formation, the reaction was stopped, concentrated under reduced pressure (10 to 1 mm Hg) in a warm water bath (40-100° C.) with the more extreme conditions used to remove the ethylene glycol. [Alternatively, once the low boiling solvent is gone, the remaining solution can be partitioned between ethyl acetate and water. The product will be in the organic phase.] The residue was purified by column chromatography (2 kg silica gel, ethyl acetate-hexanes gradient 1:1 to 4:1). The appropriate fractions were combined, stripped and dried to product as a white crisp foam (84 g, 50%), contaminated starting material (17.4 g) and pure reusable starting material 20 g. The yield based on starting material less pure recovered starting material was 58%. TLC and NMR were consistent with 99% pure product:

[0157] 2'-O-([2-phthalimidoxy)ethyl]-5'-t-butyldiphenylsilyl-5-methyluridine

[0158] 5-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine (20 g, 36.98 mmol) was mixed with triphenylphosphine (11.63 g, 44.36 mmol) and N-hydroxyphthalimide (7.24 g, 44.36 mmol). It was then dried over P_2O_5 under high vacuum for two days at 40° C. The reaction mixture was flushed with argon and dry THF (369.8 mL, Aldrich, sure seal bottle) was added to get a clear solution. Diethyl-azodicarboxylate (6.98 mL, 44.36 mmol) was added dropwise to the reaction mixture. The rate of addition is maintained such that resulting deep red coloration is just discharged before adding the next drop. After the addition was complete, the reaction was stirred for 4 hrs. By that time TLC showed the completion of the reaction (ethylacetate-:hexane, 60:40). The solvent was evaporated in vacuum. Residue obtained was placed on a flash column and eluted

with ethyl acetate:hexane (60:40), to get 2'-O-([2-phthalimidoxy)ethyl]-5'-t-butyldiphenylsilyl-5-methyluridine as white foam (21.819 g, 86%).

[0159] 5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine

[0160] 2'-O-([2-phthalimidoxy)ethyl]-5'-t-butyldiphenylsilyl-5-methyluridine (3.1 g, 4.5 mmol) was dissolved in dry CH_2Cl_2 (4.5 mL) and methylhydrazine (300 mL, 4.64 mmol) was added dropwise at −10° C. to 0° C. After 1 h the mixture was filtered, the filtrate was washed with ice cold CH_2Cl_2 and the combined organic phase was washed with water, brine and dried over anhydrous Na_2SO_4. The solution was concentrated to get 2'-O-(aminooxyethyl) thymidine, which was then dissolved in MeOH (67.5 mL). To this formaldehyde (20% aqueous solution, w/w, 1.1 eq.) was added and the resulting mixture was strirred for 1 h. Solvent was removed under vacuum; residue chromatographed to get 5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy) ethyl]-5-methyluridine as white foam (1.95 g, 78%).

[0161] 5'-O-tert-Butyldiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5-methyluridine

[0162] 5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine (1.77 g, 3.12 mmol) was dissolved in a solution of 1M pyridinium p-toluenesulfonate (PPTS) in dry MeOH (30.6 mL). Sodium cyanoborohydride (0.39 g, 6.13 mmol) was added to this solution at 10° C. under inert atmosphere. The reaction mixture was stirred for 10 minutes at 10° C. After that the reaction vessel was removed from the ice bath and stirred at room temperature for 2 h, the reaction monitored by TLC (5% MeOH in CH_2Cl_2). Aqueous $NaHCO_3$ solution (5%, 10 mL) was added and extracted with ethyl acetate (2×20 ml). Ethyl acetate phase was dried over anhydrous Na_2SO_4, evaporated to dryness. Residue was dissolved in a solution of 1 M PPTS in MeOH (30.6 mL). Formaldehyde (20% w/w, 30 mL, 3.37 mmol) was added and the reaction mixture was stirred at room temperature for 10 minutes. Reaction mixture cooled to 10° C. in an ice bath, sodium cyanoborohydride (0.39 g, 6.13 mmol) was added and reaction mixture stirred at 10° C. for 10 minutes. After 10 minutes, the reaction mixture was removed from the ice bath and stirred at room temperature for 2 hrs. To the reaction mixture 5% $NaHCO_3$ (25 mL) solution was added and extracted with ethyl acetate (2×25 mL). Ethyl acetate layer was dried over anhydrous Na_2SO_4 and evaporated to dryness . The residue obtained was purified by flash column chromatography and eluted with 5% MeOH in CH_2Cl_2 to get 5'-O-tert-butyldiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5-methyluridine as a white foam (14.6 g, 80%).

[0163] 2'-O-(dimethylaminooxyethyl)-5-methyluridine

[0164] Triethylamine trihydrofluoride (3.91 mL, 24.0 mmol) was dissolved in dry THF and triethylamine (1.67 mL, 12 mmol, dry, kept over KOH). This mixture of triethylamine-2HF was then added to 5'-O-tert-butyldiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5-methyluridine (1.40 g, 2.4 mmol) and stirred at room temperature for 24 hrs. Reaction was monitored by TLC (5% MeOH in CH_2Cl_2). Solvent was removed under vacuum and the residue placed on a flash column and eluted with 10% MeOH in CH_2Cl_2 to get 2'-O-(dimethylaminooxyethyl)-5-methyluridine (766mg, 92.5%).

[0165] 5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine

[0166] 2'-O-(dimethylaminooxyethyl)-5-methyluridine (750 mg, 2.17 mmol) was dried over P_2O_5 under high vacuum overnight at 40° C. It was then co-evaporated with anhydrous pyridine (20 mL). The residue obtained was dissolved in pyridine (11 mL) under argon atmosphere. 4-dimethylaminopyridine (26.5 mg, 2.60 mmol), 4,4'-dimethoxytrityl chloride (880 mg, 2.60 mmol) was added to the mixture and the reaction mixture was stirred at room temperature until all of the starting material disappeared. Pyridine was removed under vacuum and the residue chromatographed and eluted with 10% MeOH in CH_2Cl_2 (containing a few drops of pyridine) to get 5'-O-DMT-2'-O-(dimethylamino-oxyethyl)-5-methyluridine (1.13 g, 80%).

[0167] 5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]

[0168] 5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine (1.08 g, 1.67 mmol) was co-evaporated with toluene (20 mL). To. the residue N,N-diisopropylamine tetrazonide (0.29 g, 1.67 mmol) was added and dried over P_2O_5 under high vacuum overnight at 40° C. Then the reaction mixture was dissolved in anhydrous acetonitrile (8.4 mL) and 2-cyanoethyl-N,N,N^1,N^1-tetraisopropylphosphoramidite (2.12 mL, 6.08 mmol) was added. The reaction mixture was stirred at ambient temperature for 4 hrs under inert atmosphere. The progress of the reaction was monitored by TLC (hexane:ethyl acetate 1:1). The solvent was evaporated, then the residue was dissolved in ethyl acetate (70 mL) and washed with 5% aqueous $NaHCO_3$ (40 mL). Ethyl acetate layer was dried over anhydrous Na_2SO_4 and concentrated. Residue obtained was chromatographed (ethyl acetate as eluent) to get 5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite] as a foam (1.04 g, 74.9%).

[0169] 2'-(Aminooxyethoxy) Nucleoside Amidites

[0170] 2'-(Aminooxyethoxy) nucleoside amidites [also known in the art as 2'-O-(aminooxyethyl) nucleoside amidites] are prepared as described in the following paragraphs. Adenosine, cytidine and thymidine nucleoside amidites are prepared similarly.

[0171] N2-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]

[0172] The 2'-O-aminooxyethyl guanosine analog may be obtained by selective 2'-O-alkylation of diaminopurine riboside. Multigram quantities of diaminopurine riboside may be purchased from Schering AG (Berlin) to provide 2'-O-(2-ethylacetyl) diaminopurine riboside along with a minor amount of the 3'-O-isomer. 2'-O-(2-ethylacetyl) diaminopurine riboside may be resolved and converted to 2'-O-(2-ethylacetyl)guanosine by treatment with adenosine deaminase. (McGee, D. P. C., Cook, P. D., Guinosso, C. J., WO 94/02501 A1 940203.) Standard protection procedures should afford 2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine and 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine which may be reduced to provide 2-N-isobutyryl-6-0-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine. As before the hydroxyl group may be dis-

placed by N-hydroxyphthalimide via a Mitsunobu reaction, and the protected nucleoside may phosphitylated as usual to yield 2-N-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl) guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite].

[0173] 2'-dimethylaminoethoxyethoxy (2'-DAEOE) Nucleoside Amidites

[0174] 2'-dimethylaminoethoxyethoxy nucleoside amidites (also known in the art as 2^1-O-dimethylaminoethoxyethyl, i.e., 2'-O—CH_2—O—CH_2—$N(CH_2)_2$, or 2'-DMAEOE nucleoside amidites) are prepared as follows. Other nucleoside amidites are prepared similarly.

[0175] 2'-O-[2(2-N,N-dimethylaminoethoxy) ethyl]-5-methyl Uridine

[0176] 2[2-(Dimethylamino)ethoxy] ethanol (Aldrich, 6.66 g, 50 mmol) is slowly added to a solution of borane in tetra-hydrofuran (1 M, 10 mL, 10 mmol) with stirring in a 100 mL bomb. Hydrogen gas evolves as the solid dissolves. O^2-, 2'-anhydro-5-methyluridine (1.2 g, 5 mmol), and sodium bicarbonate (2.5 mg) are added and the bomb is sealed, placed in an oil bath and heated to 155° C. for 26 hours. The bomb is cooled to room temperature and opened. The crude solution is concentrated and the residue partitioned between water (200 mL) and hexanes (200 mL). The excess phenol is extracted into the hexane layer. The aqueous layer is extracted with ethyl acetate (3×200 mL) and the combined organic layers are washed once with water, dried over anhydrous sodium sulfate and concentrated. The residue is columned on silica gel using methanol/methylene chloride 1:20 (which has 2% triethylamine) as the eluent. As the column fractions are concentrated a colorless solid forms which is collected to give the title compound as a white solid.

[0177] 5'-O-dimethoxytrityl-2'-O-[2(2-N,N-dimethylaminoethoxy) ethyl)]-5-methyl Uridine

[0178] To 0.5 g (1.3 mmol) of 2'-O-[2(2-N,N-dimethylamino-ethoxy)ethyl)]-5-methyl uridine in anhydrous pyridine (8 mL), triethylamine (0.36 mL) and dimethoxytrityl chloride (DMT-Cl, 0.87 g, 2 eq.) are added and stirred for 1 hour. The reaction mixture is poured into water (200 mL) and extracted with CH_2Cl_2 (2×200 mL). The combined CH_2Cl_2 layers are washed with saturated $NaHCO_3$ solution, followed by saturated NaCl solution and dried over anhydrous sodium sulfate. Evaporation of the solvent followed by silica gel chromatography using MeOH:CH_2Cl_2:Et_3N (20:1, v/v, with 1% triethylamine) gives the title compound.

[0179] 5'-O-Dimethoxytrityl-2'-O-[2(2-N,N-dimethylaminoethoxy)-ethyl)]-5-methyl uridine-3'-O-(cyanoethyl-N,N-diisopropyl)phosphoramidite

[0180] Diisopropylaminotetrazolide (0.6 g) and 2-cyanoethoxy-N,N-diisopropyl phosphoramidite (1.1 mL, 2 eq.) are added to a solution of 5'-O-dimethoxytrityl-2'-O-[2(2-N,N-dimethylaminoethoxy)ethyl)]-5-methyluridine (2.17 g, 3 mmol) dissolved in CH_2Cl_2 (20 mL) under an atmosphere of argon. The reaction mixture is stirred overnight and the solvent evaporated. The resulting residue is purified by silica gel flash column chromatography with ethyl acetate as the eluent to give the title compound.

Example 2

[0181] Oligonucleotide Synthesis

[0182] Unsubstituted and substituted phosphodiester (P═O) oligonucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine.

[0183] Phosphorothioates (P═S) are synthesized as for the phosphodiester oligonucleotides except the standard oxidation bottle was replaced by 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation wait step was increased to 68 sec and was followed by the capping step. After cleavage from the CPG column and deblocking in concentrated ammonium hydroxide at 55° C. (18 h), the oligonucleotides were purified by precipitating twice with 2.5 volumes of ethanol from a 0.5 M NaCl solution. Phosphinate oligonucleotides are prepared as described in U.S. Pat. No. 5,508,270, herein incorporated by reference.

[0184] Alkyl phosphonate oligonucleotides are prepared as described in U.S. Pat. No. 4,469,863, herein incorporated by reference.

[0185] 3'-Deoxy-3'-methylene phosphonate oligonucleotides are prepared as described in U.S. Pat. Nos. 5,610,289 or 5,625,050, herein incorporated by reference.

[0186] Phosphoramidite oligonucleotides are prepared as described in U.S. Pat. No. 5,256,775 or U.S. Pat. No. 5,366,878, herein incorporated by reference.

[0187] Alkylphosphonothioate oligonucleotides are prepared as described in published PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499, respectively), herein incorporated by reference.

[0188] 3'-Deoxy-3'-amino phosphoramidate oligonucleotides are prepared as described in U.S. Pat. No. 5,476,925, herein incorporated by reference.

[0189] Phosphotriester oligonucleotides are prepared as described in U.S. Pat. No. 5,023,243, herein incorporated by reference.

[0190] Borano phosphate oligonucleotides are prepared as described in U.S. Pat. Nos. 5,130,302 and 5,177,198, both herein incorporated by reference.

Example 3

[0191] Oligonucleoside Synthesis

[0192] Methylenemethylimino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylenedimethyl-hydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, and methylenecarbonylamino linked oligonucleosides, also identified as amide-3 linked oligonucleosides, and methyleneaminocarbonyl linked oligo-nucleosides, also identified as amide-4 linked oligonucleosides, as well as mixed backbone compounds having, for instance, alternating MMI and P═O or P═S linkages are prepared as described in U.S. Pat. Nos. 5,378,825, 5,386,023, 5,489,677, 5,602,240 and 5,610,289, all of which are herein incorporated by reference.

[0193] Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Pat. Nos. 5,264,562 and 5,264,564, herein incorporated by reference.

[0194] Ethylene oxide linked oligonucleosides are prepared as described in U.S. Pat. No. 5,223,618, herein incorporated by reference.

Example 4

[0195] PNA Synthesis

[0196] Peptide nucleic acids (PNAs) are prepared in accordance with any of the various procedures referred to in Peptide Nucleic Acids (PNA): Synthesis, Properties and Potential Applications, *Bioorganic & Medicinal Chemistry,* 1996, 4, 5-23. They may also be prepared in accordance with U.S. Pat. Nos. 5,539,082, 5,700,922, and 5,719,262, herein incorporated by reference.

Example 5

[0197] Synthesis of Chimeric Oligonucleotides

[0198] Chimeric oligonucleotides, oligonucleosides or mixed oligonucleotides/oligonucleosides of the invention can be of several different types. These include a first type wherein the "gap" segment of linked nucleosides is positioned between 5' and 3'"wing" segments of linked nucleosides and a second "open end" type wherein the "gap" segment is located at either the 3' or the 5' terminus of the oligomeric compound. Oligonucleotides of the first type are also known in the art as "gapmers" or gapped oligonucleotides. Oligonucleotides of the second type are also known in the art as "hemimers" or "wingmers".

[0199] [2'—O—Me]—[2'-deoxy]—[2'—O—Me] Chimeric

[0200] Phosphorothioate Oligonucleotides

[0201] Chimeric oligonucleotides having 2'-O-alkyl phosphorothioate and 2'-deoxy phosphorothioate oligo-nucleotide segments are synthesized using an Applied Biosystems automated DNA synthesizer Model 380B, as above. Oligonucleotides are synthesized using the automated synthesizer and 2'-deoxy-5'-dimethoxytrityl-3'-O-phosphor-amidite for the DNA portion and 5'-dimethoxytrityl-2'-O-methyl-3'-O-phosphoramidite for 5' and 3' wings. The standard synthesis cycle is modified by increasing the wait step after the delivery of tetrazole and base to 600 s repeated four times for RNA and twice for 2'-O-methyl. The fully protected oligonucleotide is cleaved from the support and the phosphate group is deprotected in 3:1 ammonia/ethanol at room temperature overnight then lyophilized to dryness. Treatment in methanolic ammonia for 24 hrs at room temperature is then done to deprotect all bases and sample was again lyophilized to dryness. The pellet is resuspended in 1M TBAF in THF f or 24 hrs at room temperature to deprotect the 2' positions. The reaction is then quenched with IM TEAA and the sample is then reduced to ½ volume by rotovac before being desalted on a G25 size exclusion column. The oligo recovered is then analyzed spectrophotometrically for yield and for purity by capillary electrophoresis and by mass spectrometry.

[0202] [2'-O-(2-Methoxyethyl)]-[2'-deoxy]-[2'-O-(Methoxyethyl)] Chimeric Phosphorothioate Oligonucleotides

[0203] [2'-O-(2-methoxyethyl)]-[2'-deoxy]-[2'-O-(methoxy-ethyl)] chimeric phosphorothioate oligonucleotides were prepared as per the procedure above for the 2'-O-methyl chimeric oligonucleotide, with the substitution of 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites.

[0204] [2'-O-(2-Methoxyethyl)Phosphodiester]-[2'-deoxy Phosphorothioate]-[2'-O-(2-Methoxyethyl) Phosphodiester] Chimeric Oligonucleotides

[0205] [2'-O-(2-methoxyethyl phosphodiester]-[2'-deoxy phosphorothioate]-[2'-O-(methoxyethyl) phosphodiester] chimeric oligonucleotides are prepared as per the above procedure for the 2'-O-methyl chimeric oligonucleotide with the substitution of 2'-O-(methoxyethyl) amidites for the 2'-O-methyl amidites, oxidization with iodine to generate the phosphodiester internucleotide linkages within the wing portions of the chimeric structures and sulfurization utilizing 3,H-1,2 benzodithiole-3-one 1,1 dioxide (Beaucage Reagent) to generate the phosphorothioate internucleotide linkages for the center gap.

[0206] Other chimeric oligonucleotides, chimeric oligonucleo-sides and mixed chimeric oligonucleotides/oligonucleosides are synthesized according to U.S. Pat. No. 5,623,065, wherein incorporated by reference.

Example 6

[0207] Oligonucleotide Isolation

[0208] After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55° C. for 18 hours, the oligonucleotides or oligonucleosides are purified by precipitation twice out of 0.5 M NaCl with 2.5 volumes ethanol. Synthesized oligonucleotides were analyzed by polyacrylamide gel electrophoresis on denaturing gels and judged to be at least 85% full length material. The relative amounts of phosphorothioate and phosphodiester linkages obtained in synthesis were periodically checked by ^{31}P nuclear magnetic resonance spectroscopy, and for some studies oligonucleotides were purified by HPLC, as described by Chiang et al., *J. Biol. Chem.* 1991, 266, 18162-18171. Results obtained with HPLC-purified material were similar to those obtained with non-HPLC purified material.

Example 7

[0209] Oligonucleotide Synthesis—96 Well Plate Format

[0210] Oligonucleotides were synthesized via solid phase P(III) phosphoramidite chemistry on an automated synthesizer capable of assembling 96 sequences simultaneously in a standard 96 well format. Phosphodiester internucleotide linkages were afforded by oxidation with aqueous iodine. Phosphorothioate internucleotide linkages were generated by sulfurization utilizing 3,H-1,2 benzodithiole-3-one 1,1 dioxide (Beaucage Reagent) in anhydrous acetonitrile. Standard base-protected beta-cyanoethyldiisopropyl phosphoramidites were purchased from commercial vendors (e.g. PE-Applied Biosystems, Foster City, Calif., or Pharmacia, Piscataway, N.J.). Non-standard nucleosides are synthesized as per known literature or patented methods. They are utilized as base protected beta-cyanoethyldiisopropyl phosphoramidites.

[0211] Oligonucleotides were cleaved from support and deprotected with concentrated NH_4OH at elevated temperature (55-60° C.) for 12-16 hours and the released product then dried in vacuo. The dried product was then re-suspended in sterile water to afford a master plate from which all analytical and test plate samples are then diluted utilizing robotic pipettors.

Example 8

[0212] Oligonucleotide Analysis—96 Well Plate Format

[0213] The concentration of oligonucleotide in each well was assessed by dilution of samples and UV absorption spectroscopy. The full-length integrity of the individual products was evaluated by capillary electrophoresis (CE) in either the 96 well format (Beckman P/ACE™ MDQ) or, for individually prepared samples, on a commercial CE apparatus (e.g., Beckman P/ACE™ 5000, ABI 270). Base and backbone composition was confirmed by mass analysis of the compounds utilizing electrospray-mass spectroscopy. All assay test plates were diluted from the master plate using single and multi-channel robotic pipettors. Plates were judged to be acceptable if at least 85% of the compounds on the plate were at least 85% full length.

Example 9

[0214] Cell Culture and Oligonucleotide Treatment

[0215] The effect of antisense compounds on target nucleic acid expression can be tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. This can be routinely determined using, for example, PCR or Northern blot analysis. The following 6 cell types are provided for illustrative purposes, but other cell types can be routinely used, provided that the target is expressed in the cell type chosen. This can be readily determined by methods routine in the art, for example Northern blot analysis, Ribonuclease protection assays, or RT-PCR.

[0216] T-24 Cells:

[0217] The human transitional cell bladder carcinoma cell line T-24 was obtained from the American Type Culture Collection (ATCC) (Manassas, Va.). T-24 cells were routinely cultured in complete McCoy's SA basal media (Gibco/Life Technologies, Gaithersburg, Md.) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, Md.), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Gibco/Life Technologies, Gaithersburg, Md.). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells were seeded into 96-well plates (Falcon-Primaria #3872) at a density of 7000 cells/well for use in RT-PCR analysis.

[0218] For Northern blotting or other analysis, cells may be seeded onto 100 mm or other standard tissue culture plates and treated similarly, using appropriate volumes of medium and oligonucleotide.

[0219] A549 Cells:

[0220] The human lung carcinoma cell line A549 was obtained from the American Type Culture Collection (ATCC) (Manassas, Va.). A549 cells were routinely cultured in DMEM basal media (Gibco/Life Technologies, Gaithersburg, Md.) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, Md.), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Gibco/Life Technologies, Gaithersburg, Md.). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence.

[0221] NHDF Cells:

[0222] Human neonatal dermal fibroblast (NHDF) were obtained from the Clonetics Corporation (Walkersville Md.). NHDFs were routinely maintained in Fibroblast Growth Medium (Clonetics Corporation, Walkersville Md.) supplemented as recommended by the supplier. Cells were maintained for up to 10 passages as recommended by the supplier.

[0223] HEK Cells:

[0224] Human embryonic keratinocytes (HEK) were obtained from the Clonetics Corporation (Walkersville Md.). HEKs were routinely maintained in Keratinocyte Growth Medium (Clonetics Corporation, Walkersville Md.) formulated as recommended by the supplier. Cells were routinely maintained for up to 10 passages as recommended by the supplier.

[0225] MCF-7 Cells:

[0226] The human breast carcinoma cell line MCF-7 was obtained from the American Type Culure Collection (Manassas, Va.). MCF-7 cells were routinely cultured in DMEM low glucose (Gibco/Life Technologies, Gaithersburg, Md.) supplemented with 10% fetal calf serum (Gibco/Life Technologies, Gaithersburg, Md.). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells were seeded into 96-well plates (Falcon-Primaria #3872) at a density of 7000 cells/well for use in RT-PCR analysis.

[0227] For Northern blotting or other analyses, cells may be seeded onto 100 mm or other standard tissue culture plates and treated similarly, using appropriate volumes of medium and oligonucleotide.

[0228] LA4 Cells:

[0229] The mouse lung epithelial cell line LA4 was obtained from the American Type Culure Collection (Manassas, Va.). LA4 cells were routinely cultured in F12K medium (Gibco/Life Technologies, Gaithersburg, Md.) supplemented with 15% fetal calf serum (Gibco/Life Technologies,- Gaithersburg, Md.). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells were seeded into 96-well plates (Falcon-Primaria #3872) at a density of 3000-6000 cells/well for use in RT-PCR analysis.

[0230] For Northern blotting or other analyses, cells may be seeded onto 100 mm or other standard tissue culture plates and treated similarly, using appropriate volumes of medium and oligonucleotide.

[0231] Treatment With Antisense Compounds:

[0232] When cells reached 80% confluency, they were treated with oligonucleotide. For cells grown in 96-well plates, wells were washed once with 200 μL OPTI-MEM™-1 reduced-serum medium (Gibco BRL) and then treated with 130 μL of OPTI-MEM™-1 containing 3.75

μg/mL LIPOFECTIN™ (Gibco BRL) and the desired concentration of oligonucleotide. After 4-7 hours of treatment, the medium was replaced with fresh medium. Cells were harvested 16-24 hours after oligonucleotide treatment.

[0233] The concentration of oligonucleotide used varies from cell line to cell line. To determine the optimal oligonucleotide concentration for a particular cell line, the cells are treated with a positive control oligonucleotide at a range of concentrations. For human cells the positive control oligonucleotide is ISIS 13920, TCCGTCATCGCTCCTCAGGG, SEQ ID NO: 1, a 2'-O-methoxyethyl gapmer (2'-O-methoxyethyls shown in bold) with a phosphorothioate backbone which is targeted to human H-ras. For mouse or rat cells the positive control oligonucleotide is ISIS 15770, ATGCATTCTGCCCCCAAGGA, SEQ ID NO: 2, a 2'-O-methoxyethyl gapmer (2'-O-methoxyethyls shown in bold) with a phosphorothioate backbone which is targeted to both mouse and rat c-raf. The concentration of positive control oligonucleotide that results in 80% inhibition of c-Ha-ras (for ISIS 13920) or c-raf (for ISIS 15770) mRNA is then utilized as the screening concentration for new oligonucleotides in subsequent experiments for that cell line. If 80% inhibition is not achieved, the lowest concentration of positive control oligonucleotide that results in 60% inhibition of H-ras or c-raf mRNA is then utilized as the oligonucleotide screening concentration in subsequent experiments for that cell line. If 60% inhibition is not achieved, that particular cell line is deemed as unsuitable for oligonucleotide transfection experiments.

Example 10

[0234] Analysis of Oligonucleotide Inhibition of Inducible Nitric Oxide Synthase Expression

[0235] Antisense modulation of inducible nitric oxide synthase expression can be assayed in a variety of ways known in the art. For example, inducible nitric oxide synthase mRNA levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or real-time PCR (RT-PCR). Real-time quantitative PCR is presently preferred. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are taught in, for example, Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, Volume 1, pp. 4.1.1-4.2.9 and 4.5.1-4.5.3, John Wiley & Sons, Inc., 1993. Northern blot analysis is routine in the art and is taught in, for example, Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, Volume 1, pp. 4.2.1-4.2.9, John Wiley & Sons, Inc., 1996. Real-time quantitative (PCR) can be conveniently accomplished using the commercially available ABI PRISM™ 7700 Sequence Detection System, available from PE-Applied Biosystems, Foster City, Calif. and used according to manufacturer's instructions. Prior to quantitative PCR analysis, primer-probe sets specific to the target gene being measured are evaluated for their ability to be "multiplexed" with a GAPDH amplification reaction. In multiplexing, both the target gene and the internal standard gene GAPDH are amplified concurrently in a single sample. In this analysis, mRNA isolated from untreated cells is serially diluted. Each dilution is amplified in the presence of primer-probe sets specific for GAPDH only, target gene only ("single-plexing"), or both (multiplexing). Following PCR amplification, standard curves of GAPDH and target mRNA signal as a function of dilution are generated from both the single-plexed and multiplexed samples. If both the slope and correlation coefficient of the GAPDH and target signals generated from the multiplexed samples fall within 10% of their corresponding values generated from the single-plexed samples, the primer-probe set specific for that target is deemed as multiplexable. Other methods of PCR are also known in the art.

[0236] Protein levels of inducible nitric oxide synthase can be quantitated in a variety of ways well known in the art, such as.immunoprecipitation, Western blot analysis (immunoblotting), ELISA or fluorescence-activated cell sorting (FACS). Antibodies directed to inducible nitric oxide synthase can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, Mich.), or can be prepared via conventional antibody generation methods. Methods for preparation of polyclonal antisera are taught in, for example, Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.12.1-11.12.9, John Wiley & Sons, Inc., 1997. Preparation of monoclonal antibodies is taught in, for example, Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.4.1-11.11.5, John Wiley & Sons, Inc., 1997.

[0237] Immunoprecipitation methods are standard in the art and can be found at, for example, Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 10.16.1-10.16.11, John Wiley & Sons, Inc., 1998. Western blot (immunoblot) analysis is standard in the art and can be found at, for example, Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 10.8.1-10.8.21, John Wiley & Sons, Inc., 1997. Enzyme-linked immunosorbent assays (ELISA) are standard in the art and can be found at, for example, Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, Volume 2, pp. 11.2.1-11.2.22, John Wiley & Sons, Inc., 1991.

Example 11

[0238] Poly(A)+ mRNA Isolation

[0239] Poly(A)+ mRNA was isolated according to Miura et al., *Clin. Chem.*, 1996, 42, 1758-1764. Other methods for poly(A)+ mRNA isolation are taught in, for example, Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, Volume 1, pp. 4.5.1-4.5.3, John Wiley & Sons, Inc., 1993. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200 AL cold PBS. 60 μL lysis buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA, 0.5 M NaCl, 0.5% NP-40, 20 mM vanadyl-ribonucleoside complex) was added to each well, the plate was gently agitated and then incubated at room temperature for five minutes. 55 μL of lysate was transferred to Oligo d(T) coated 96-well plates (AGCT Inc., Irvine Calif.). Plates were incubated for 60 minutes at room temperature, washed 3 times with 200 μL of wash buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.3 M NaCl). After the final wash, the plate was blotted on paper towels to remove excess wash buffer and then air-dried for 5 minutes. 60 μL of elution buffer (5 mM Tris-HCl pH 7.6), preheated to 70° C. was added to each well, the plate was incubated on a 90° C. hot plate for 5 minutes, and the eluate was then transferred to a fresh 96-well plate.

[0240] Cells grown on 100 mm or other standard plates may be treated similarly, using appropriate volumes of all solutions.

Example 12

[0241] Total RNA Isolation

[0242] Total mRNA was isolated using an RNEASY 96™ kit and buffers purchased from Qiagen Inc. (Valencia Calif.) following the manufacturer's recommended procedures. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200 μL cold PBS. 100 μL Buffer RLT was added to each well and the plate vigorously agitated for 20 seconds. 100 μL of 70% ethanol was then added to each well and the contents mixed by pipetting three times up and down. The samples were then transferred to the RNEASY 96™ well plate attached to a QIAVAC™ manifold fitted with a waste collection tray and attached to a vacuum source. Vacuum was applied for 15 seconds. 1 mL of Buffer RWl was added to each well of the RNEASY 96™ plate and the vacuum again applied for 15 seconds. 1 mL of Buffer RPE was then added to each well of the RNEASY 96™ plate and the vacuum applied for a period of 15 seconds. The Buffer RPE wash was then repeated and the vacuum was applied for an additional 10 minutes. The plate was then removed from the QIAVAC™ manifold and blotted dry on paper towels. The plate was then re-attached to the QIAVAC™ manifold fitted with a collection tube rack containing 1.2 mL collection tubes. RNA was then eluted by pipetting 60 μL water into each well, incubating 1 minute, and then applying the vacuum for 30 seconds. The elution step was repeated with an additional 60 μL water.

[0243] The repetitive pipetting and elution steps may be automated using a QIAGEN Bio-Robot 9604 (Qiagen, Inc., Valencia Calif.). Essentially, after lysing of the cells on the culture plate, the plate is transferred to the robot deck where the pipetting, DNase treatment and elution steps are carried out.

Example 13

[0244] Real-time Quantitative PCR Analysis of Inducible Nitric Oxide Synthase mRNA Levels

[0245] Quantitation of inducible nitric oxide synthase mRNA levels was determined by real-time quantitative PCR using the ABI PRISM™ 7700 Sequence Detection System (PE-Applied Biosystems, Foster City, Calif.) according to manufacturer's instructions. This is a closed-tube, non-gel-based, fluorescence detection system which allows high-throughput quantitation of polymerase chain reaction (PCR) products in real-time. As opposed to standard PCR, in which amplification products are quantitated after the PCR is completed, products in real-time quantitative PCR are quantitated as they accumulate. This is accomplished by including in the PCR reaction an oligonucleotide probe that anneals specifically between the forward and reverse PCR primers, and contains two fluorescent dyes. A reporter dye (e.g., JOE, FAM, or VIC, obtained from either Operon Technologies Inc., Alameda, Calif. or PE-Applied Biosystems, Foster City, Calif.) is attached to the 5' end of the probe and a quencher dye (e.g., TAMRA, obtained from either Operon Technologies Inc., Alameda, Calif. or PE-Applied Biosystems, Foster City, Calif.) is attached to the 3' end of the probe. When the probe and dyes are intact, reporter dye emission is quenched by the proximity of the 3' quencher dye. During amplification, annealing of the probe to the target sequence creates a substrate that can be cleaved by the 5'-exonuclease activity of Taq polymerase. During the extension phase of the PCR amplification cycle, cleavage of the probe by Taq polymerase releases the reporter dye from the remainder of the probe (and hence from the quencher moiety) and a sequence-specific fluorescent signal is generated. With each cycle, additional reporter dye molecules are cleaved from their respective probes, and the fluorescence intensity is monitored at regular intervals by laser optics built into the ABI PRISM™ 7700 Sequence Detection System. In each assay, a series of parallel reactions containing serial dilutions of mRNA from untreated control samples generates a standard curve that is used to quantitate the percent inhibition after antisense oligonucleotide treatment of test samples.

[0246] PCR reagents were obtained from PE-Applied Biosystems, Foster City, Calif. RT-PCR reactions were carried out by adding 25 μL PCR cocktail (1× TAQMAN™ buffer A, 5.5 mM $MgCl_2$, 300 μM each of DATP, dCTP and dGTP, 600 μM of dUTP, 100 nM each of forward primer, reverse primer, and probe, 20 Units RNAse inhibitor, 1.25 Units AMPLITAQ GOLD™, and 12.5 Units MULV reverse transcriptase) to 96 well plates containing 25 μL poly(A) mRNA solution. The RT reaction was carried out by incubation for 30 minutes at 48° C. Following a 10 minute incubation at 95° C. to activate the AMPLITAQ GOLD™, 40 cycles of a two-step PCR protocol were carried out: 95° C. for 15 seconds (denaturation) followed by 60° C. for 1.5 minutes (annealing/extension).

[0247] Probes and primers to human inducible nitric oxide synthase were designed to hybridize to a human inducible nitric oxide synthase sequence, using published sequence., information (GenBank accession number L09210, incorporated herein as SEQ ID NO:3). For human inducible nitric oxide synthase the PCR primers were: forward primer: GGTGGAAGCGGTAACAAAGGA (SEQ ID NO: 4) reverse primer: TGCTTGGTGGCGAAGATGA (SEQ ID NO: 5) and the PCR probe was: FAM-AACAACAGGAAC-CTACCAACTGACGGGAGA-TAMRA (SEQ ID NO: 6) where FAM (PE-Applied Biosystems, Foster City, Calif.) is the fluorescent reporter dye) and TAMRA (PE-Applied Biosystems, Foster City, Calif.) is the quencher dye. For human GAPDH the PCR primers were: forward primer: GAAGGTGAAGGTCGGAGTC (SEQ ID NO: 7) reverse primer: GAAGATGGTGATGGGATTTC (SEQ ID NO: 8) and the PCR probe was: 5' JOE-CAAGCTTCCCGTTCT-CAGCC-TAMRA 3' (SEQ ID NO: 9) where JOE (PE-Applied Biosystems, Foster City, Calif.) is the fluorescent reporter dye) and TAMRA (PE-Applied Biosystems, Foster City, Calif.) is the quencher dye.

[0248] Probes and primers to mouse inducible nitric oxide synthase were designed to hybridize to a mouse inducible nitric oxide synthase sequence, using published sequence. information (GenBank accession number M92649, incorporated herein as SEQ ID NO:10). For mouse inducible nitric oxide synthase the PCR primers were: forward primer: CGTCCACAGTATGTGAGGATCAA (SEQ ID NO:11) reverse primer: CAAGCAAGACTTGGACTTGCAA (SEQ ID NO: 12) and the PCR probe was: FAM-TCTTCACCA-CAAGGCCACATCGGATT-TAMRA (SEQ ID NO: 13) where FAM (PE-Applied Biosystems, Foster City, Calif.) is the fluorescent reporter dye) and TAMRA (PE-Applied Biosystems, Foster City, Calif.) is the quencher dye. For mouse GAPDH the PCR primers were: forward primer:

GGCAAATTCAACGGCACAGT (SEQ ID NO: 14) reverse primer: GGGTCTCGCTCCTGGAAGCT (SEQ ID NO: 15) and the PCR probe was: 5' JOE-AAGGCCGAGAATGGGAAGCTTGTCATC-TAMRA 3' (SEQ ID NO: 16) where JOE (PE-Applied Biosystems, Foster City, Calif.) is the fluorescent reporter dye) and TAMRA (PE-Applied Biosystems, Foster City, Calif.) is the quencher dye.

Example 14

[0249] Northern Blot Analysis of Inducible Nitric Oxide Synthase mRNA Levels

[0250] Eighteen hours after antisense treatment, cell monolayers were washed twice with cold PBS and lysed in 1 mL RNAZOL™ (TEL-TEST "B" Inc., Friendswood, Tex.). Total RNA was prepared following manufacturer's recommended protocols. Twenty micrograms of total. RNA was fractionated by electrophoresis through 1.2% agarose gels containing 1.1% formaldehyde using a MOPS buffer system (AMRESCO, Inc. Solon, Ohio). RNA was transferred from the gel to HYBOND™-N+ nylon membranes (Amersham Pharmacia Biotech, Piscataway, N.J.) by overnight capillary transfer using a Northern/Southern Transfer buffer system (TEL-TEST "B" Inc., Friendswood, Tex.). RNA transfer was confirmed by UV visualization. Membranes were fixed by UV cross-linking using a STRATALINKER™ UV Crosslinker 2400 (Stratagene, Inc, La Jolla, Calif.) and then robed using QUICKHYB™ hybridization solution (Stratagene, La Jolla, Calif.) using manufacturer's recommendations for stringent conditions.

[0251] To detect human inducible nitric oxide synthase, a human inducible nitric oxide synthase specific probe was prepared by PCR using the forward primer GGTGGAAGCGGTAACAAAGGA (SEQ ID NO: 4) and the reverse primer TGCTTGGTGGCGAAGATGA (SEQ ID NO: 5). To normalize for variations in loading and transfer efficiency membranes were stripped and probed for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA (Clontech, Palo Alto, Calif.).

[0252] To detect mouse inducible nitric oxide synthase, a mouse inducible nitric oxide synthase specific probe was prepared by PCR using the forward primer CGTCCACAGTATGTGAGGATCAA (SEQ ID NO:11) and the reverse primer CAAGCAAGACTTGGACTTGCAA (SEQ ID NO: 12). To normalize for variations in loading and transfer efficiency membranes were stripped and probed for mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA (Clontech, Palo Alto, Calif.).

[0253] Hybridized membranes were visualized and quantitated using a PHOSPHORIMAGER™ and IMAGEQUANT™ Software V3.3 (Molecular Dynamics, Sunnyvale, Calif.). Data was normalized to GAPDH levels in untreated controls.

Example 15

[0254] Antisense inhibition of human inducible nitric oxide synthase expression by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

[0255] In accordance with the present invention, a series of oligonucleotides were designed to target different regions of the human inducible nitric oxide synthase RNA, using published sequences (GenBank accession number L09210, incorporated herein as SEQ ID NO: 3, and GenBank accession number L07868, incorporated herein as SEQ ID NO: 17). The oligonucleotides are shown in Table 1. "Target site" indicates the first (5'-most) nucleotide number on the particular target sequence to which the oligonucleotide binds. All compounds in Table 1 are chimeric oligonucleotides ("gapmers") 18 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by four-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. Cytidine residues in the 2'-MOE wings are 5-methylcytidines for ISIS 24032 through 24071. All cytidine residues are 5-methylcytidines for ISIS 19631 through 19714. The compounds were analyzed for their effect on human inducible nitric oxide synthase mRNA levels by quantitative real-time PCR as described in other examples herein. Data are averages from two experiments. If present, "N.D. " indicates "no data".

TABLE 1

Inhibition of human inducible nitric oxide synthase mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% IN-HIB	SEQ ID NO
24032	5' UTR	3	14	catcaaaggtggccgaga	76	19
24033	5' UTR	3	38	ctgtctagaactgcccag	50	20
24034	5' UTR	3	57	tgccttgagaacttcggg	41	21
24035	5' UTR	3	160	tgtcacttatctggattt	17	22
24036	Coding	3	219	cttgaacagaaatttcca	34	23
24037	Coding	3	277	tctccacattgttgttga	43	24
24038	Coding	3	338	ctgaggttgtgatactga	16	25
24039	Coding	3	410	agcttgaccagagattct	19	26
24040	Coding	3	492	gtgaagtgtgtcttggaa	13	27
24041	Coding	3	534	gcaagatttggacctgca	0	28
24042	Coding	3	580	ccctgggtcctctggtca	74	29
24043	Coding	3	645	gccgtaatattggttgac	55	30
24044	Coding	3	705	ctcctttgttaccgcttc	53	31
24045	Coding	3	745	gctcatctcccgtcagtt	55	32
24046	Coding	3	823	agacctgcaggttggacc	42	33
24047	Coding	3	880	cgtgtctgcagatgtgtt	0	34
24048	Coding	3	959	aagtcgtgcttgccatca	0	35
24049	Coding	3	1025	cctctgatgctgccatct	33	36
24050	Coding	3	1098	atcgaagcggccgtactt	12	37
24051	Coding	3	1184	tccatggccacctcaagc	59	38
24052	Coding	3	1240	caggcagggcgtaccact	0	39

TABLE 1-continued

Inhibition of human inducible nitric oxide synthase mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% INHIB	SEQ ID NO
24053	Coding	3	1320	ctctgtgcccatgtacca	3	40
24054	Coding	3	1379	ctgcccacttcctccagg	15	41
24055	Coding	3	1445	ttgatctcaacgacagcc	32	42
24056	Coding	3	1493	tccatgatggtcacattc	49	43
24057	Coding	3	1548	ggaccggtattcattctg	57	44
24058	Coding	3	1640	acgtagttcagcatctcc	65	45
24059	Coding	3	1713	gggtctccgcttctcgtc	58	46
24060	Coding	3	1772	agcatacaggcaaagagc	0	47
24061	Coding	3	1830	tgtctctgtcgcaaagag	21	48
24062	Coding	3	1938	ttcctcctccaggcagct	48	49
24063	Coding	3	1994	ccattgccagggcagtct	38	50
24064	Coding	3	2059	acacagcgtacctgaatt	11	51
24065	Coding	3	2122	gcttctgatcaatgtcat	52	52
24066	Coding	3	2317	tgtagtggtgcgggtccc	30	53
24067	Coding	3	2435	ctggatgtcggactttgt	35	54
24068	Coding	3	2642	ctcttgtcactgacccag	0	55
24069	3' UTR	3	3673	ctttaacccctcctgtag	19	56
24070	3' UTR	3	3689	agttctgtgccggcagct	45	57
24071	3' UTR	3	3722	acctcagataatgcagag	31	58
19631	5' UTR	17	2	agatcccgtgctgacaat	48	59
19632	Coding	17	51	ctcacccagacccaaagt	33	60
19633	Coding	17	72	gtccccgccgccacgaga	42	61
19634	Coding	17	99	actgactgagaatcgctg	51	62
19635	Coding	17	151	ctgctgttccaggtcaga	73	63
19636	Coding	17	213	gttatctccaggttgccc	44	64
19637	Coding	17	232	ccggttgtgctcaatgct	47	65
19638	Coding	17	307	caggtaacgaaactgatt	40	66
19639	Coding	17	319	attctccagaggcaggta	68	67
19640	Coding	17	351	tcataaagttttgtccca	40	68
19641	Coding	17	408	agtccaaagtttccatct	59	69
19642	Coding	17	447	tttaggatttctgtcaag	35	70
19643	Coding	17	463	tacatagactccaccatt	30	71
19644	Coding	17	566	aactaccatttgttgaca	0	72
19645	Coding	17	577	tccacatcctgaactacc	26	73

TABLE 1-continued

Inhibition of human inducible nitric oxide synthase mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% INHIB	SEQ ID NO
19646	Coding	17	632	ggcaatgattttctgtgg	34	74
19647	Coding	17	648	gtccttgtcaaagtctgg	61	75
19648	Coding	17	682	gtagcatctgccgtcaca	52	76
19649	Coding	17	727	gcctccagcacattctcg	82	77
19650	Coding	17	738	ggtcctgagcagcctcca	95	78
19651	Coding	17	760	ggcaaagcagtctgtgtc	65	79
19652	Coding	17	833	aggtggttggattgtaga	47	80
19653	Coding	17	850	attgtgctccagttgaaa	40	81
19654	Coding	17	900	tgtggacatttcttgaca	44	82
19655	Coding	17	944	tagggcaggcacgcacac	47	83
19656	Coding	17	978	ttaatcccatttttcttct	36	84
19657	Coding	17	1039	tgatcctgtgccaatgcc	61	85
19658	Coding	17	1175	ctgggtctatggcttcaa	66	86
19659	Coding	17	1189	gacgttcagtttctctgg	69	87
19660	Coding	17	1325	ggataagcaaggacaggc	49	88
19661	Coding	17	1361	actggaactgtagagagg	35	89
19662	Coding	17	1413	aggttgctgttgtcagta	56	90
19663	Coding	17	1435	gttaatggtatgataata	24	91
19664	Coding	17	1469	ttctctggttgattgtgc	44	92
19665	Coding	17	1475	ttactattctctggttga	52	93
19666	Coding	17	1542	ctggaacacagatggttg	41	94
19667	Coding	17	1562	caggtccccaacagccat	16	95
19668	Coding	17	1598	tactgaagcggcgacacg	44	96
19669	Coding	17	1629	aggttacaagactctatg	0	97
19670	Coding	17	1667	tggagccattctcaaact	35	98
19671	Coding	17	1713	aggccatcttccatcttc	49	99
19672	Coding	17	1905	ctagtgggaccgttacac	44	100
19673	Coding	17	2024	tcagacccacaatgacca	29	101
19674	Coding	17	2055	atgctcttccttctaaca	12	102
19675	Coding	17	2126	ctgtgccactgggagtta	55	103
19676	Coding	17	2205	ccaaaagcacctgagcca	1	104
19677	Coding	17	2262	gccacaggaatcttcaca	55	105
19678	Coding	17	2390	tggttgggctcagacaca	30	106

TABLE 1-continued

Inhibition of human inducible nitric oxide synthase mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% IN-HIB	SEQ ID NO
19679	Coding	17	2464	tccaatgttatccttgtg	21	107
19680	Coding	17	2568	actaagacattacgggct	1	108
19681	Coding	17	2656	tcctccatcagcattgta	36	109
19682	Coding	17	2766	ccaaaggtcatcagttcc	19	110
19683	Coding	17	2890	catccaacatttgaccat	12	111
19684	Coding	17	2936	actcagcagccagttcct	0	112
19685	Coding	17	3016	gtcatttggactgggaag	32	113
19686	Coding	17	3058	ttccaaatcctcttcatc	0	114
19687	Coding	17	3113	gaggtgggatgttgaaag	0	115
19688	Coding	17	3233	cagcaaaacctccatctc	15	116
19689	Coding	17	3316	ctcagcagtagcaccctg	31	117
19690	Coding	17	3393	tgggtgctactgtcctct	28	118
19691	Coding	17	3488	gtttgtctcgcataggag	14	119
19692	Coding	17	3515	ccactggattcaggtatt	0	120
19693	Coding	17	3633	ggctcattcacatactca	13	121
19694	Coding	17	3701	ttgacagtatgttgttct	0	122
19695	Coding	17	3747	ttccagtagtcagggttg	16	123
19696	Coding	17	3780	tgctgaagggtgctccga	0	124
19697	Coding	17	3870	aggtattcaggattctct	22	125
19698	Coding	17	3922	tctgtaaggtggaggcgg	15	126
19699	3' UTR	17	4052	agtgtcaaaactactggc	25	127
19700	3' UTR	17	4107	gttcaagttaggtaagca	0	128
19701	3' UTR	17	4138	ctatctttctctttcagt	0	129
19702	3' UTR	17	4171	atgcagagaaatgaagaa	0	130
19703	3' UTR	17	4229	cagcattgccttacattt	29	131
19704	3' UTR	17	4334	gtgtttcaaccatctgct	31	132
19705	3' UTR	17	4420	tttgttctaatggaaact	0	133
19706	3' UTR	17	4608	cagagcaaaacaaaatga	0	134
19707	3' UTR	17	4809	aggatgagggtgaagata	25	135
19708	3' UTR	17	4880	tactcttcagacaaccaa	0	136
19709	3' UTR	17	4921	gttttcctgaaccacaga	29	137
19710	3' UTR	17	4993	acatacccaatccagtgt	53	138
19711	3' UTR	17	5069	aaaatggagttcagaaaa	0	139
19712	3' UTR	17	5218	gcctctcatcatagtccc	36	140

TABLE 1-continued

Inhibition of human inducible nitric oxide synthase mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% IN-HIB	SEQ ID NO
19713	3' UTR	17	5365	gagttaccttctacttca	17	141
19714	3' UTR	17	5455	cacatttatttacaactt	10	142

[0256] As shown in Table 1, SEQ ID NOs 19, 20, 21, 23, 24, 29, 30, 31, 32, 33, 36, 38, 42, 43, 44, 45, 46, 48, 49, 50, 52, 53, 54, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 701, 71, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 96, 98, 99, 100, 101, 103, 105, 106, 107, 109, 113, 117, 118, 125, 127, 131, 132, 135, 137, 138 and 140 demonstrated at least 20% inhibition of human inducible nitric oxide synthase expression in this assay and are therefore preferred.

Example 17

[0257] Antisense Inhibition of Mouse Inducible Nitric Oxide Synthase Expression by Chimeric Phosphorothioate Oligonucleotides Having 2'-MOE Wings and a Deoxy Gap.

[0258] In accordance with the present invention, a second series of oligonucleotides were designed to target different regions of the mouse inducible nitric oxide synthase RNA, using published sequences (GenBank accession number M87039, incorporated herein as SEQ ID NO: 18). The oligonucleotides are shown in Table 2. "Target site" indicates the first (5'-most) nucleotide number on the particular target sequence to which the oligonucleotide binds. All compounds in Table 2 are chimeric oligonucleotides ("gapmers") 20 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE)nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. The compounds were analyzed for their effect on mouse inducible nitric oxide synthase mRNA levels by quantitative real-time PCR as described in other examples herein. Data are averages from two experiments. If present, "N.D." indicates "no data".

TABLE 2

Inhibition of mouse inducible nitric oxide synthase mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% IN-HIB	SEQ ID NO
105449	5' UTR	18	30	gtaaagttgtgaccctggca	0	143
105450	5' UTR	18	226	ttgcacttctgctccaaatc	0	144

TABLE 2-continued

Inhibition of mouse inducible nitric oxide synthase mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% INHIB	SEQ ID NO
105451	Coding	18	281	ttggtaggatttgactttga	0	145
105452	Coding	18	373	ctcttagggtcatcttgtat	6	146
105453	Coding	18	456	tcgatgtcacatgcagcttg	16	147
105454	Coding	18	543	tgaaatccgatgtggccttg	52	148
105455	Coding	18	621	gggtaggcttgtctctgggt	0	149
105456	Coding	18	646	gcatgaggcaggagctcctc	0	150
105457	Coding	18	805	ttcctccaggccatcttggt	0	151
105458	Coding	18	1012	atgagctgtgaattccagag	26	152
105459	Coding	18	1106	cttccagcctaggtcgatgc	22	153
105460	Coding	18	1180	atttcaaagacctctggatc	0	154
105461	Coding	18	1292	ctccagtagcatgttggcca	0	155
105462	Coding	18	1448	ccagagggaggccagtgtgt	8	156
105463	Coding	18	1511	cacattctgcttctggaaac	13	157
105464	Coding	18	1577	ggcccggtactcattctgca	5	158
105465	Coding	18	1678	ggagataggacatagttcaa	0	159
105466	Coding	18	1721	ccagatgtgggtcttccagg	3	160
105467	Coding	18	1766	tctaaatcggatctctctcc	5	161
105468	Coding	18	1853	agtagcaaagaggactgtgg	6	162
105469	Coding	18	1992	tgcttgtcaccaccagcagt	0	163
105470	Coding	18	2118	actgagggtacatgctggag	0	164
105471	Coding	18	2231	gctgcggaaggcatcctcct	0	165
105472	Coding	18	2357	ctggatgagcctatattgct	4	166
105473	Coding	18	2394	tgctgagggctctgttgagg	0	167
105474	Coding	18	2466	ggctggacttttcactctgc	22	168
105475	Coding	18	2519	gtagctgggccctcggctgc	0	169
105476	Coding	18	2607	gtgtaggacaatccacaact	12	170

TABLE 2-continued

Inhibition of mouse inducible nitric oxide synthase mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% INHIB	SEQ ID NO
105477	Coding	18	2703	tgagggcttggctgagtgag	0	171
105478	Coding	18	2802	aggcctccaatctctgccta	0	172
105479	Coding	18	2873	ctcttcaagcacctccagga	16	173
105480	Coding	18	2925	agatagggagctgcgacagc	0	174
105481	Coding	18	3021	catctcgggtgcggtaggtg	0	175
105482	Coding	18	3117	agccactgacacttcgcaca	12	176
105483	Coding	18	3266	gcacccaaacaccaagctca	0	177
105484	Coding	18	3351	agcctgtgtgcacctggaac	0	178
105485	Coding	18	3389	ctgaacgtagaccttgggtt	2	179
105486	Coding	18	3514	accagcttcttcaatgtggt	25	180
105487	Coding	18	3601	aagatatcttcatgataacg	20	181
105488	Coding	18	3669	agagcctcgtggctttgggc	0	182

[0259] As shown in Table 2, SEQ ID NOs 148, 152, 153, 168 and 180 demonstrated at least 20% inhibition of mouse inducible nitric oxide synthase expression in this experiment and are therefore preferred.

Example 17

[0260] Western Blot Analysis of Inducible Nitric Oxide Synthase Protein Levels

[0261] Western blot analysis (immunoblot analysis) is carried out using standard methods. Cells are harvested 16-20 h after oligonucleotide treatment, washed once with PBS, suspended in Laemmli buffer (100 ul/well), boiled for 5 minutes and loaded on a 16% SDS-PAGE gel. Gels are run for 1.5 hours at 150 V, and transferred to membrane for western blotting. Appropriate primary antibody directed to inducible nitric oxide synthase is used, with a radiolabelled or fluorescently labeled secondary antibody directed against the primary antibody species. Bands are visualized using a PHOSPHORIMAGER™ (Molecular Dynamics, Sunnyvale Calif.).

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 182

<210> SEQ ID NO 1
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 1

tccgtcatcg ctcctcaggg 20

<210> SEQ ID NO 2
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 2

atgcattctg cccccaagga 20

<210> SEQ ID NO 3
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<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (207)...(3668)

<400> SEQUENCE: 3

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aagttctcaa ggcacaggtc tcttcctggt ttgactgtcc ttaccccggg gaggcagtgc 120

agccagctgc aagccccaca gtgaagaaca tctgagctca aatccagata agtgacataa 180

gtgacctgct ttgtaaagcc atagag atg gcc tgt cct tgg aaa ttt ctg ttc 233
Met Ala Cys Pro Trp Lys Phe Leu Phe
1 5

aag acc aaa ttc cac cag tat gca atg aat ggg gaa aaa gac atc aac 281
Lys Thr Lys Phe His Gln Tyr Ala Met Asn Gly Glu Lys Asp Ile Asn
10 15 20 25

aac aat gtg gag aaa gcc ccc tgt gcc acc tcc agt cca gtg aca cag 329
Asn Asn Val Glu Lys Ala Pro Cys Ala Thr Ser Ser Pro Val Thr Gln
30 35 40

gat gac ctt cag tat cac aac ctc agc aag cag cag aat gag tcc ccg 377
Asp Asp Leu Gln Tyr His Asn Leu Ser Lys Gln Gln Asn Glu Ser Pro
45 50 55

cag ccc ctc gtg gag acg gga aag aag tct cca gaa tct ctg gtc aag 425
Gln Pro Leu Val Glu Thr Gly Lys Lys Ser Pro Glu Ser Leu Val Lys
60 65 70

ctg gat gca acc cca ttg tcc tcc cca cgg cat gtg agg atc aaa aac 473
Leu Asp Ala Thr Pro Leu Ser Ser Pro Arg His Val Arg Ile Lys Asn
75 80 85

tgg ggc agc ggg atg act ttc caa gac aca ctt cac cat aag gcc aaa 521
Trp Gly Ser Gly Met Thr Phe Gln Asp Thr Leu His His Lys Ala Lys
90 95 100 105

ggg att tta act tgc agg tcc aaa tct tgc ctg ggg tcc att atg act 569
Gly Ile Leu Thr Cys Arg Ser Lys Ser Cys Leu Gly Ser Ile Met Thr
110 115 120

-continued

```
ccc aaa agt ttg acc aga gga ccc agg gac aag cct acc cct cca gat      617
Pro Lys Ser Leu Thr Arg Gly Pro Arg Asp Lys Pro Thr Pro Pro Asp
            125                 130                 135

gag ctt cta cct caa gct atc gaa ttt gtc aac caa tat tac ggc tcc      665
Glu Leu Leu Pro Gln Ala Ile Glu Phe Val Asn Gln Tyr Tyr Gly Ser
        140                 145                 150

ttc aaa gag gca aaa ata gag gaa cat ctg gcc agg gtg gaa gcg gta      713
Phe Lys Glu Ala Lys Ile Glu Glu His Leu Ala Arg Val Glu Ala Val
    155                 160                 165

aca aag gag ata gaa aca aca gga acc tac caa ctg acg gga gat gag      761
Thr Lys Glu Ile Glu Thr Thr Gly Thr Tyr Gln Leu Thr Gly Asp Glu
170                 175                 180                 185

ctc atc ttc gcc acc aag cag gcc tgg cgc aat gcc cca cgc tgc att      809
Leu Ile Phe Ala Thr Lys Gln Ala Trp Arg Asn Ala Pro Arg Cys Ile
                190                 195                 200

ggg agg atc cag tgg tcc aac ctg cag gtc ttc gat gcc cgc agc tgt      857
Gly Arg Ile Gln Trp Ser Asn Leu Gln Val Phe Asp Ala Arg Ser Cys
            205                 210                 215

tcc act gcc cgg gaa atg ttt gaa cac atc tgc aga cac gtg cgt tac      905
Ser Thr Ala Arg Glu Met Phe Glu His Ile Cys Arg His Val Arg Tyr
        220                 225                 230

tcc acc aac aat ggc aac atc agg tcg gcc atc acc gtg ttc ccc cag      953
Ser Thr Asn Asn Gly Asn Ile Arg Ser Ala Ile Thr Val Phe Pro Gln
    235                 240                 245

cgg agt gat ggc aag cac gac ttc cgg gtg tgg aat gct cag ctc atc     1001
Arg Ser Asp Gly Lys His Asp Phe Arg Val Trp Asn Ala Gln Leu Ile
250                 255                 260                 265

cgc tat gct ggc tac cag atg cca gat ggc agc atc aga ggg gac cct     1049
Arg Tyr Ala Gly Tyr Gln Met Pro Asp Gly Ser Ile Arg Gly Asp Pro
                270                 275                 280

gcc aac gtg gaa ttc act cag ctg tgc atc gac ctg ggc tgg aag ccc     1097
Ala Asn Val Glu Phe Thr Gln Leu Cys Ile Asp Leu Gly Trp Lys Pro
            285                 290                 295

aag tac ggc cgc ttc gat gtg gtc ccc ctg gtc ctg cag gcc aat ggc     1145
Lys Tyr Gly Arg Phe Asp Val Val Pro Leu Val Leu Gln Ala Asn Gly
        300                 305                 310

cgt gac cct gag ctc ttc gaa atc cca cct gac ctt gtg ctt gag gtg     1193
Arg Asp Pro Glu Leu Phe Glu Ile Pro Pro Asp Leu Val Leu Glu Val
    315                 320                 325

gcc atg gaa cat ccc aaa tac gag tgg ttt cgg gaa ctg gag cta aag     1241
Ala Met Glu His Pro Lys Tyr Glu Trp Phe Arg Glu Leu Glu Leu Lys
330                 335                 340                 345

tgg tac gcc ctg cct gca gtg gcc aac atg ctg ctt gag gtg ggc ggc     1289
Trp Tyr Ala Leu Pro Ala Val Ala Asn Met Leu Leu Glu Val Gly Gly
                350                 355                 360

ctg gag ttc cca ggg tgc ccc ttc aat ggc tgg tac atg ggc aca gag     1337
Leu Glu Phe Pro Gly Cys Pro Phe Asn Gly Trp Tyr Met Gly Thr Glu
            365                 370                 375

atc gga gtc cgg gac ttc tgt gac gtc cag cgc tac aac atc ctg gag     1385
Ile Gly Val Arg Asp Phe Cys Asp Val Gln Arg Tyr Asn Ile Leu Glu
        380                 385                 390

gaa gtg ggc agg aga atg ggc ctg gaa acg cac aag ctg gcc tcg ctc     1433
Glu Val Gly Arg Arg Met Gly Leu Glu Thr His Lys Leu Ala Ser Leu
    395                 400                 405

tgg aaa gac cag gct gtc gtt gag atc aac att gct gtg atc cat agt     1481
Trp Lys Asp Gln Ala Val Val Glu Ile Asn Ile Ala Val Ile His Ser
410                 415                 420                 425
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ttt cag aag cag aat gtg acc atc atg gac cac cac tcg gct gca gaa     1529
Phe Gln Lys Gln Asn Val Thr Ile Met Asp His His Ser Ala Ala Glu
                430                 435                 440

tcc ttc atg aag tac atg cag aat gaa tac cgg tcc cgt ggg ggc tgc     1577
Ser Phe Met Lys Tyr Met Gln Asn Glu Tyr Arg Ser Arg Gly Gly Cys
            445                 450                 455

ccg gca gac tgg att tgg ctg gtc cct ccc atg tct ggg agc atc acc     1625
Pro Ala Asp Trp Ile Trp Leu Val Pro Pro Met Ser Gly Ser Ile Thr
        460                 465                 470

ccc gtg ttt cac cag gag atg ctg aac tac gtc ctg tcc cct ttc tac     1673
Pro Val Phe His Gln Glu Met Leu Asn Tyr Val Leu Ser Pro Phe Tyr
    475                 480                 485

tac tat cag gta gag gcc tgg aaa acc cat gtc tgg cag gac gag aag     1721
Tyr Tyr Gln Val Glu Ala Trp Lys Thr His Val Trp Gln Asp Glu Lys
490                 495                 500                 505

cgg aga ccc aag aga aga gag att cca ttg aaa gtc ttg gtc aaa gct     1769
Arg Arg Pro Lys Arg Arg Glu Ile Pro Leu Lys Val Leu Val Lys Ala
                510                 515                 520

gtg ctc ttt gcc tgt atg ctg atg cgc aag aca atg gcg tcc cga gtc     1817
Val Leu Phe Ala Cys Met Leu Met Arg Lys Thr Met Ala Ser Arg Val
            525                 530                 535

aga gtc acc atc ctc ttt gcg aca gag aca gga aaa tca gag gcg ctg     1865
Arg Val Thr Ile Leu Phe Ala Thr Glu Thr Gly Lys Ser Glu Ala Leu
        540                 545                 550

gcc tgg gac ctg ggg gcc tta ttc agc tgt gcc ttc aac ccc aag gtt     1913
Ala Trp Asp Leu Gly Ala Leu Phe Ser Cys Ala Phe Asn Pro Lys Val
    555                 560                 565

gtc tgc atg gat aag tac agg ctg agc tgc ctg gag gag gaa cgg ctg     1961
Val Cys Met Asp Lys Tyr Arg Leu Ser Cys Leu Glu Glu Glu Arg Leu
570                 575                 580                 585

ctg ttg gtg gtg acc agt acg ttt ggc aat gga gac tgc cct ggc aat     2009
Leu Leu Val Val Thr Ser Thr Phe Gly Asn Gly Asp Cys Pro Gly Asn
                590                 595                 600

gga gag aaa ctg aag aaa tcg ctc ttc atg ctg aaa gag ctc aac aac     2057
Gly Glu Lys Leu Lys Lys Ser Leu Phe Met Leu Lys Glu Leu Asn Asn
            605                 610                 615

aaa ttc agg tac gct gtg ttt ggc ctc ggc tcc agc atg tac cct cgg     2105
Lys Phe Arg Tyr Ala Val Phe Gly Leu Gly Ser Ser Met Tyr Pro Arg
        620                 625                 630

ttc tgc gcc ttt gct cat gac att gat cag aag ctg tcc cac ctg ggg     2153
Phe Cys Ala Phe Ala His Asp Ile Asp Gln Lys Leu Ser His Leu Gly
    635                 640                 645

gcc tct cag ctc acc ccg atg gga gaa ggg gat gag ctc agt ggg cag     2201
Ala Ser Gln Leu Thr Pro Met Gly Glu Gly Asp Glu Leu Ser Gly Gln
650                 655                 660                 665

gag gac gcc ttc cgc agc tgg gcc gtg caa acc ttc aag gca gcc tgt     2249
Glu Asp Ala Phe Arg Ser Trp Ala Val Gln Thr Phe Lys Ala Ala Cys
                670                 675                 680

gag acg ttt gat gtc cga ggc aaa cag cac att cag atc ccc aag ctc     2297
Glu Thr Phe Asp Val Arg Gly Lys Gln His Ile Gln Ile Pro Lys Leu
            685                 690                 695

tac acc tcc aat gtg acc tgg gac ccg cac cac tac agg ctc gtg cag     2345
Tyr Thr Ser Asn Val Thr Trp Asp Pro His His Tyr Arg Leu Val Gln
        700                 705                 710

gac tca cag cct ttg gac ctc agc aaa gcc ctc agc agc atg cat gcc     2393
Asp Ser Gln Pro Leu Asp Leu Ser Lys Ala Leu Ser Ser Met His Ala
    715                 720                 725
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aag aac gtg ttc acc atg agg ctc aaa tct cgg cag aat cta caa agt     2441
Lys Asn Val Phe Thr Met Arg Leu Lys Ser Arg Gln Asn Leu Gln Ser
730                 735                 740                 745

ccg aca tcc agc cgt gcc acc atc ctg gtg gaa ctc tcc tgt gag gat     2489
Pro Thr Ser Ser Arg Ala Thr Ile Leu Val Glu Leu Ser Cys Glu Asp
                750                 755                 760

ggc caa ggc ctg aac tac ctg ccg ggg gag cac ctt ggg gtt tgc cca     2537
Gly Gln Gly Leu Asn Tyr Leu Pro Gly Glu His Leu Gly Val Cys Pro
            765                 770                 775

ggc aac cag ccg gcc ctg gtc caa ggc atc ctg gag cga gtg gtg gat     2585
Gly Asn Gln Pro Ala Leu Val Gln Gly Ile Leu Glu Arg Val Val Asp
        780                 785                 790

ggc ccc aca ccc cac cag aca gtg cgc ctg gag gac ctg gat gag agt     2633
Gly Pro Thr Pro His Gln Thr Val Arg Leu Glu Asp Leu Asp Glu Ser
    795                 800                 805

ggc agc tac tgg gtc agt gac aag agg ctg ccc ccc tgc tca ctc agc     2681
Gly Ser Tyr Trp Val Ser Asp Lys Arg Leu Pro Pro Cys Ser Leu Ser
810                 815                 820                 825

cag gcc ctc acc tac tcc ccg gac atc acc aca ccc cca acc cag ctg     2729
Gln Ala Leu Thr Tyr Ser Pro Asp Ile Thr Thr Pro Pro Thr Gln Leu
                830                 835                 840

ctg ctc caa aag ctg gcc cag gtg gcc aca gaa gag cct gag aga cag     2777
Leu Leu Gln Lys Leu Ala Gln Val Ala Thr Glu Glu Pro Glu Arg Gln
            845                 850                 855

agg ctg gag gcc ctg tgc cag ccc tca gag tac agc aag tgg aag ttc     2825
Arg Leu Glu Ala Leu Cys Gln Pro Ser Glu Tyr Ser Lys Trp Lys Phe
        860                 865                 870

acc aac agc ccc aca ttc ctg gag gtg cta gag gag ttc ccg tcc ctg     2873
Thr Asn Ser Pro Thr Phe Leu Glu Val Leu Glu Glu Phe Pro Ser Leu
    875                 880                 885

cgg gtg tct gct ggc ttc ctg ctt tcc cag ctc ccc att ctg aag ccc     2921
Arg Val Ser Ala Gly Phe Leu Leu Ser Gln Leu Pro Ile Leu Lys Pro
890                 895                 900                 905

agg ttc tac tcc atc agc tcc tcc cgg gat cac acg ccc acg gag atc     2969
Arg Phe Tyr Ser Ile Ser Ser Ser Arg Asp His Thr Pro Thr Glu Ile
                910                 915                 920

cac ctg act gtg gcc gtg gtc acc tac cac acc gga gat ggc cag ggt     3017
His Leu Thr Val Ala Val Val Thr Tyr His Thr Gly Asp Gly Gln Gly
            925                 930                 935

ccc ctg cac cac ggt gtc tgc agc aca tgg ctc aac agc ctg aag ccc     3065
Pro Leu His His Gly Val Cys Ser Thr Trp Leu Asn Ser Leu Lys Pro
        940                 945                 950

caa gac cca gtg ccc tgc ttt gtg cgg aat gcc agc gcc ttc cac ctc     3113
Gln Asp Pro Val Pro Cys Phe Val Arg Asn Ala Ser Ala Phe His Leu
    955                 960                 965

ccc gag gat ccc tcc cat cct tgc atc ctc atc ggg cct ggc aca ggc     3161
Pro Glu Asp Pro Ser His Pro Cys Ile Leu Ile Gly Pro Gly Thr Gly
970                 975                 980                 985

atc gtg ccc ttc cgc agt ttc tgg cag caa cgg ctc cat gac tcc cag     3209
Ile Val Pro Phe Arg Ser Phe Trp Gln Gln Arg Leu His Asp Ser Gln
                990                 995                 1000

cac aag gga gtg cgg gga ggc cgc atg acc ttg gtg ttt ggg tgc cgc     3257
His Lys Gly Val Arg Gly Gly Arg Met Thr Leu Val Phe Gly Cys Arg
            1005                1010                1015

cgc cca gat gag gac cac atc tac cag gag gag atg ctg gag atg gcc     3305
Arg Pro Asp Glu Asp His Ile Tyr Gln Glu Glu Met Leu Glu Met Ala
        1020                1025                1030
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cag aag ggg gtg ctg cat gcg gtg cac aca gcc tat tcc cgc ctg cct     3353
Gln Lys Gly Val Leu His Ala Val His Thr Ala Tyr Ser Arg Leu Pro
    1035                1040                1045

ggc aag ccc aag gtc tat gtt cag gac atc ctg cgg cag cag ctg gcc     3401
Gly Lys Pro Lys Val Tyr Val Gln Asp Ile Leu Arg Gln Gln Leu Ala
1050                1055                1060                1065

agc gag gtg ctc cgt gtg ctc cac aag gag cca ggc cac ctc tat gtt     3449
Ser Glu Val Leu Arg Val Leu His Lys Glu Pro Gly His Leu Tyr Val
                1070                1075                1080

tgc ggg gat gtg cgc atg gcc cgg gac gtg gcc cac acc ctg aag cag     3497
Cys Gly Asp Val Arg Met Ala Arg Asp Val Ala His Thr Leu Lys Gln
            1085                1090                1095

ctg gtg gct gcc aag ctg aaa ttg aat gag gag cag gtc gag gac tat     3545
Leu Val Ala Ala Lys Leu Lys Leu Asn Glu Glu Gln Val Glu Asp Tyr
        1100                1105                1110

ttc ttt cag ctc aag agc cag aag cgc tat cac gaa gat atc ttc ggt     3593
Phe Phe Gln Leu Lys Ser Gln Lys Arg Tyr His Glu Asp Ile Phe Gly
    1115                1120                1125

gct gta ttt cct tac gag gcg aag aag gac agg gtg gcg gtg cag ccc     3641
Ala Val Phe Pro Tyr Glu Ala Lys Lys Asp Arg Val Ala Val Gln Pro
1130                1135                1140                1145

agc agc ctg gag atg tca gcg ctc tga gggcctacag gaggggttaa           3688
Ser Ser Leu Glu Met Ser Ala Leu
                1150

agctgccggc acagaactta aggatggagc cagctctgca ttatctgagg tcacagggcc   3748

tggggagatg gaggaaagtg atatccccca gcctcaagtc ttatttcctc aacgttgctc   3808

cccatcaagc cctttacttg acctcctaac aagtagcacc ctggattgat cggagcctcc   3868

tctctcaaac tggggcctcc ctggtccctt ggagacaaaa tcttaaatgc caggcctggc   3928

gagtgggtga aagatggaac ttgctgctga gtgcaccact tcaagtgacc accaggaggt   3988

gctatcgcac cactgtgtat ttaactgcct tgtgtacagt tatttatgcc tctgtattta   4048

aaaaactaac acccagtctg ttccccatgg ccacttgggt cttccctgta tgattccttg   4108

atggagatat ttacatgaat tgcattttac tttaatc                           4145


<210> SEQ ID NO 4
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 4

ggtggaagcg gtaacaaagg a                                              21


<210> SEQ ID NO 5
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 5

tgcttggtgg cgaagatga                                                 19


<210> SEQ ID NO 6
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 6

aacaacagga acctaccaac tgacgggaga 30

<210> SEQ ID NO 7
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 7

gaaggtgaag gtcggagtc 19

<210> SEQ ID NO 8
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 8

gaagatggtg atgggatttc 20

<210> SEQ ID NO 9
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Probe

<400> SEQUENCE: 9

caagcttccc gttctcagcc 20

<210> SEQ ID NO 10
<211> LENGTH: 4145
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: mRNA
<222> LOCATION: (1)...(4110)

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aagttctcaa ggcacaggtc tcttcctggt ttgactgtcc ttaccccggg gaggcagtgc 120

agccagctgc aagccccaca gtgaagaaca tctgagctca aatccagata agtgacataa 180

gtgacctgct ttgtaaagcc atagag atg gcc tgt cct tgg aaa ttt ctg ttc 233
Met Ala Cys Pro Trp Lys Phe Leu Phe
1 5

aag acc aaa ttc cac cag tat gca atg aat ggg gaa aaa gac atc aac 281
Lys Thr Lys Phe His Gln Tyr Ala Met Asn Gly Glu Lys Asp Ile Asn
10 15 20 25

aac aat gtg gag aaa gcc ccc tgt gcc acc tcc agt cca gtg aca cag 329
Asn Asn Val Glu Lys Ala Pro Cys Ala Thr Ser Ser Pro Val Thr Gln
30 35 40

gat gac ctt cag tat cac aac ctc agc aag cag cag aat gag tcc ccg 377
Asp Asp Leu Gln Tyr His Asn Leu Ser Lys Gln Gln Asn Glu Ser Pro

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            45                  50                  55

cag ccc ctc gtg gag acg gga aag aag tct cca gaa tct ctg gtc aag      425
Gln Pro Leu Val Glu Thr Gly Lys Lys Ser Pro Glu Ser Leu Val Lys
         60                  65                  70

ctg gat gca acc cca ttg tcc tcc cca cgg cat gtg agg atc aaa aac      473
Leu Asp Ala Thr Pro Leu Ser Ser Pro Arg His Val Arg Ile Lys Asn
     75                  80                  85

tgg ggc agc ggg atg act ttc caa gac aca ctt cac cat aag gcc aaa      521
Trp Gly Ser Gly Met Thr Phe Gln Asp Thr Leu His His Lys Ala Lys
 90                  95                 100                 105

ggg att tta act tgc agg tcc aaa tct tgc ctg ggg tcc att atg act      569
Gly Ile Leu Thr Cys Arg Ser Lys Ser Cys Leu Gly Ser Ile Met Thr
                110                 115                 120

ccc aaa agt ttg acc aga gga ccc agg gac aag cct acc cct cca gat      617
Pro Lys Ser Leu Thr Arg Gly Pro Arg Asp Lys Pro Thr Pro Pro Asp
            125                 130                 135

gag ctt cta cct caa gct atc gaa ttt gtc aac caa tat tac ggc tcc      665
Glu Leu Leu Pro Gln Ala Ile Glu Phe Val Asn Gln Tyr Tyr Gly Ser
        140                 145                 150

ttc aaa gag gca aaa ata gag gaa cat ctg gcc agg gtg gaa gcg gta      713
Phe Lys Glu Ala Lys Ile Glu Glu His Leu Ala Arg Val Glu Ala Val
    155                 160                 165

aca aag gag ata gaa aca aca gga acc tac caa ctg acg gga gat gag      761
Thr Lys Glu Ile Glu Thr Thr Gly Thr Tyr Gln Leu Thr Gly Asp Glu
170                 175                 180                 185

ctc atc ttc gcc acc aag cag gcc tgg cgc aat gcc cca cgc tgc att      809
Leu Ile Phe Ala Thr Lys Gln Ala Trp Arg Asn Ala Pro Arg Cys Ile
                190                 195                 200

ggg agg atc cag tgg tcc aac ctg cag gtc ttc gat gcc cgc agc tgt      857
Gly Arg Ile Gln Trp Ser Asn Leu Gln Val Phe Asp Ala Arg Ser Cys
            205                 210                 215

tcc act gcc cgg gaa atg ttt gaa cac atc tgc aga cac gtg cgt tac      905
Ser Thr Ala Arg Glu Met Phe Glu His Ile Cys Arg His Val Arg Tyr
        220                 225                 230

tcc acc aac aat ggc aac atc agg tcg gcc atc acc gtg ttc ccc cag      953
Ser Thr Asn Asn Gly Asn Ile Arg Ser Ala Ile Thr Val Phe Pro Gln
    235                 240                 245

cgg agt gat ggc aag cac gac ttc cgg gtg tgg aat gct cag ctc atc     1001
Arg Ser Asp Gly Lys His Asp Phe Arg Val Trp Asn Ala Gln Leu Ile
250                 255                 260                 265

cgc tat gct ggc tac cag atg cca gat ggc agc atc aga ggg gac cct     1049
Arg Tyr Ala Gly Tyr Gln Met Pro Asp Gly Ser Ile Arg Gly Asp Pro
                270                 275                 280

gcc aac gtg gaa ttc act cag ctg tgc atc gac ctg ggc tgg aag ccc     1097
Ala Asn Val Glu Phe Thr Gln Leu Cys Ile Asp Leu Gly Trp Lys Pro
            285                 290                 295

aag tac ggc cgc ttc gat gtg gtc ccc ctg gtc ctg cag gcc aat ggc     1145
Lys Tyr Gly Arg Phe Asp Val Val Pro Leu Val Leu Gln Ala Asn Gly
        300                 305                 310

cgt gac cct gag ctc ttc gaa atc cca cct gac ctt gtg ctt gag gtg     1193
Arg Asp Pro Glu Leu Phe Glu Ile Pro Pro Asp Leu Val Leu Glu Val
    315                 320                 325

gcc atg gaa cat ccc aaa tac gag tgg ttt cgg gaa ctg gag cta aag     1241
Ala Met Glu His Pro Lys Tyr Glu Trp Phe Arg Glu Leu Glu Leu Lys
330                 335                 340                 345

tgg tac gcc ctg cct gca gtg gcc aac atg ctg ctt gag gtg ggc ggc     1289
Trp Tyr Ala Leu Pro Ala Val Ala Asn Met Leu Leu Glu Val Gly Gly
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               350                 355                 360

ctg gag ttc cca ggg tgc ccc ttc aat ggc tgg tac atg ggc aca gag     1337
Leu Glu Phe Pro Gly Cys Pro Phe Asn Gly Trp Tyr Met Gly Thr Glu
            365                 370                 375

atc gga gtc cgg gac ttc tgt gac gtc cag cgc tac aac atc ctg gag     1385
Ile Gly Val Arg Asp Phe Cys Asp Val Gln Arg Tyr Asn Ile Leu Glu
        380                 385                 390

gaa gtg ggc agg aga atg ggc ctg gaa acg cac aag ctg gcc tcg ctc     1433
Glu Val Gly Arg Arg Met Gly Leu Glu Thr His Lys Leu Ala Ser Leu
    395                 400                 405

tgg aaa gac cag gct gtc gtt gag atc aac att gct gtg atc cat agt     1481
Trp Lys Asp Gln Ala Val Val Glu Ile Asn Ile Ala Val Ile His Ser
410                 415                 420                 425

ttt cag aag cag aat gtg acc atc atg gac cac cac tcg gct gca gaa     1529
Phe Gln Lys Gln Asn Val Thr Ile Met Asp His His Ser Ala Ala Glu
               430                 435                 440

tcc ttc atg aag tac atg cag aat gaa tac cgg tcc cgt ggg ggc tgc     1577
Ser Phe Met Lys Tyr Met Gln Asn Glu Tyr Arg Ser Arg Gly Gly Cys
            445                 450                 455

ccg gca gac tgg att tgg ctg gtc cct ccc atg tct ggg agc atc acc     1625
Pro Ala Asp Trp Ile Trp Leu Val Pro Pro Met Ser Gly Ser Ile Thr
        460                 465                 470

ccc gtg ttt cac cag gag atg ctg aac tac gtc ctg tcc cct ttc tac     1673
Pro Val Phe His Gln Glu Met Leu Asn Tyr Val Leu Ser Pro Phe Tyr
    475                 480                 485

tac tat cag gta gag gcc tgg aaa acc cat gtc tgg cag gac gag aag     1721
Tyr Tyr Gln Val Glu Ala Trp Lys Thr His Val Trp Gln Asp Glu Lys
490                 495                 500                 505

cgg aga ccc aag aga aga gag att cca ttg aaa gtc ttg gtc aaa gct     1769
Arg Arg Pro Lys Arg Arg Glu Ile Pro Leu Lys Val Leu Val Lys Ala
               510                 515                 520

gtg ctc ttt gcc tgt atg ctg atg cgc aag aca atg gcg tcc cga gtc     1817
Val Leu Phe Ala Cys Met Leu Met Arg Lys Thr Met Ala Ser Arg Val
            525                 530                 535

aga gtc acc atc ctc ttt gcg aca gag aca gga aaa tca gag gcg ctg     1865
Arg Val Thr Ile Leu Phe Ala Thr Glu Thr Gly Lys Ser Glu Ala Leu
        540                 545                 550

gcc tgg gac ctg ggg gcc tta ttc agc tgt gcc ttc aac ccc aag gtt     1913
Ala Trp Asp Leu Gly Ala Leu Phe Ser Cys Ala Phe Asn Pro Lys Val
    555                 560                 565

gtc tgc atg gat aag tac agg ctg agc tgc ctg gag gag gaa cgg ctg     1961
Val Cys Met Asp Lys Tyr Arg Leu Ser Cys Leu Glu Glu Glu Arg Leu
570                 575                 580                 585

ctg ttg gtg gtg acc agt acg ttt ggc aat gga gac tgc cct ggc aat     2009
Leu Leu Val Val Thr Ser Thr Phe Gly Asn Gly Asp Cys Pro Gly Asn
               590                 595                 600

gga gag aaa ctg aag aaa tcg ctc ttc atg ctg aaa gag ctc aac aac     2057
Gly Glu Lys Leu Lys Lys Ser Leu Phe Met Leu Lys Glu Leu Asn Asn
            605                 610                 615

aaa ttc agg tac gct gtg ttt ggc ctc ggc tcc agc atg tac cct cgg     2105
Lys Phe Arg Tyr Ala Val Phe Gly Leu Gly Ser Ser Met Tyr Pro Arg
        620                 625                 630

ttc tgc gcc ttt gct cat gac att gat cag aag ctg tcc cac ctg ggg     2153
Phe Cys Ala Phe Ala His Asp Ile Asp Gln Lys Leu Ser His Leu Gly
    635                 640                 645

gcc tct cag ctc acc ccg atg gga gaa ggg gat gag ctc agt ggg cag     2201
Ala Ser Gln Leu Thr Pro Met Gly Glu Gly Asp Glu Leu Ser Gly Gln
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650                 655                 660                 665

gag gac gcc ttc cgc agc tgg gcc gtg caa acc ttc aag gca gcc tgt     2249
Glu Asp Ala Phe Arg Ser Trp Ala Val Gln Thr Phe Lys Ala Ala Cys
                670                 675                 680

gag acg ttt gat gtc cga ggc aaa cag cac att cag atc ccc aag ctc     2297
Glu Thr Phe Asp Val Arg Gly Lys Gln His Ile Gln Ile Pro Lys Leu
            685                 690                 695

tac acc tcc aat gtg acc tgg gac ccg cac cac tac agg ctc gtg cag     2345
Tyr Thr Ser Asn Val Thr Trp Asp Pro His His Tyr Arg Leu Val Gln
        700                 705                 710

gac tca cag cct ttg gac ctc agc aaa gcc ctc agc agc atg cat gcc     2393
Asp Ser Gln Pro Leu Asp Leu Ser Lys Ala Leu Ser Ser Met His Ala
    715                 720                 725

aag aac gtg ttc acc atg agg ctc aaa tct cgg cag aat cta caa agt     2441
Lys Asn Val Phe Thr Met Arg Leu Lys Ser Arg Gln Asn Leu Gln Ser
730                 735                 740                 745

ccg aca tcc agc cgt gcc acc atc ctg gtg gaa ctc tcc tgt gag gat     2489
Pro Thr Ser Ser Arg Ala Thr Ile Leu Val Glu Leu Ser Cys Glu Asp
                750                 755                 760

ggc caa ggc ctg aac tac ctg ccg ggg gag cac ctt ggg gtt tgc cca     2537
Gly Gln Gly Leu Asn Tyr Leu Pro Gly Glu His Leu Gly Val Cys Pro
            765                 770                 775

ggc aac cag ccg gcc ctg gtc caa ggc atc ctg gag cga gtg gtg gat     2585
Gly Asn Gln Pro Ala Leu Val Gln Gly Ile Leu Glu Arg Val Val Asp
        780                 785                 790

ggc ccc aca ccc cac cag aca gtg cgc ctg gag gac ctg gat gag agt     2633
Gly Pro Thr Pro His Gln Thr Val Arg Leu Glu Asp Leu Asp Glu Ser
    795                 800                 805

ggc agc tac tgg gtc agt gac aag agg ctg ccc ccc tgc tca ctc agc     2681
Gly Ser Tyr Trp Val Ser Asp Lys Arg Leu Pro Pro Cys Ser Leu Ser
810                 815                 820                 825

cag gcc ctc acc tac tcc ccg gac atc acc aca ccc cca acc cag ctg     2729
Gln Ala Leu Thr Tyr Ser Pro Asp Ile Thr Thr Pro Pro Thr Gln Leu
                830                 835                 840

ctg ctc caa aag ctg gcc cag gtg gcc aca gaa gag cct gag aga cag     2777
Leu Leu Gln Lys Leu Ala Gln Val Ala Thr Glu Glu Pro Glu Arg Gln
            845                 850                 855

agg ctg gag gcc ctg tgc cag ccc tca gag tac agc aag tgg aag ttc     2825
Arg Leu Glu Ala Leu Cys Gln Pro Ser Glu Tyr Ser Lys Trp Lys Phe
        860                 865                 870

acc aac agc ccc aca ttc ctg gag gtg cta gag gag ttc ccg tcc ctg     2873
Thr Asn Ser Pro Thr Phe Leu Glu Val Leu Glu Glu Phe Pro Ser Leu
    875                 880                 885

cgg gtg tct gct ggc ttc ctg ctt tcc cag ctc ccc att ctg aag ccc     2921
Arg Val Ser Ala Gly Phe Leu Leu Ser Gln Leu Pro Ile Leu Lys Pro
890                 895                 900                 905

agg ttc tac tcc atc agc tcc tcc cgg gat cac acg ccc acg gag atc     2969
Arg Phe Tyr Ser Ile Ser Ser Ser Arg Asp His Thr Pro Thr Glu Ile
                910                 915                 920

cac ctg act gtg gcc gtg gtc acc tac cac acc gga gat ggc cag ggt     3017
His Leu Thr Val Ala Val Val Thr Tyr His Thr Gly Asp Gly Gln Gly
            925                 930                 935

ccc ctg cac cac ggt gtc tgc agc aca tgg ctc aac agc ctg aag ccc     3065
Pro Leu His His Gly Val Cys Ser Thr Trp Leu Asn Ser Leu Lys Pro
        940                 945                 950

caa gac cca gtg ccc tgc ttt gtg cgg aat gcc agc gcc ttc cac ctc     3113
Gln Asp Pro Val Pro Cys Phe Val Arg Asn Ala Ser Ala Phe His Leu
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    955                 960                 965

ccc gag gat ccc tcc cat cct tgc atc ctc atc ggg cct ggc aca ggc     3161
Pro Glu Asp Pro Ser His Pro Cys Ile Leu Ile Gly Pro Gly Thr Gly
970                 975                 980                 985

atc gtg ccc ttc cgc agt ttc tgg cag caa cgg ctc cat gac tcc cag     3209
Ile Val Pro Phe Arg Ser Phe Trp Gln Gln Arg Leu His Asp Ser Gln
                990                 995                 1000

cac aag gga gtg cgg gga ggc cgc atg acc ttg gtg ttt ggg tgc cgc     3257
His Lys Gly Val Arg Gly Gly Arg Met Thr Leu Val Phe Gly Cys Arg
            1005                1010                1015

cgc cca gat gag gac cac atc tac cag gag gag atg ctg gag atg gcc     3305
Arg Pro Asp Glu Asp His Ile Tyr Gln Glu Glu Met Leu Glu Met Ala
        1020                1025                1030

cag aag ggg gtg ctg cat gcg gtg cac aca gcc tat tcc cgc ctg cct     3353
Gln Lys Gly Val Leu His Ala Val His Thr Ala Tyr Ser Arg Leu Pro
    1035                1040                1045

ggc aag ccc aag gtc tat gtt cag gac atc ctg cgg cag cag ctg gcc     3401
Gly Lys Pro Lys Val Tyr Val Gln Asp Ile Leu Arg Gln Gln Leu Ala
1050                1055                1060                1065

agc gag gtg ctc cgt gtg ctc cac aag gag cca ggc cac ctc tat gtt     3449
Ser Glu Val Leu Arg Val Leu His Lys Glu Pro Gly His Leu Tyr Val
                1070                1075                1080

tgc ggg gat gtg cgc atg gcc cgg gac gtg gcc cac acc ctg aag cag     3497
Cys Gly Asp Val Arg Met Ala Arg Asp Val Ala His Thr Leu Lys Gln
            1085                1090                1095

ctg gtg gct gcc aag ctg aaa ttg aat gag gag cag gtc gag gac tat     3545
Leu Val Ala Ala Lys Leu Lys Leu Asn Glu Glu Gln Val Glu Asp Tyr
        1100                1105                1110

ttc ttt cag ctc aag agc cag aag cgc tat cac gaa gat atc ttc ggt     3593
Phe Phe Gln Leu Lys Ser Gln Lys Arg Tyr His Glu Asp Ile Phe Gly
    1115                1120                1125

gct gta ttt cct tac gag gcg aag aag gac agg gtg gcg gtg cag ccc     3641
Ala Val Phe Pro Tyr Glu Ala Lys Lys Asp Arg Val Ala Val Gln Pro
1130                1135                1140                1145

agc agc ctg gag atg tca gcg ctc tga gggcctacag gaggggttaa           3688
Ser Ser Leu Glu Met Ser Ala Leu
                1150

agctgccggc acagaactta aggatggagc cagctctgca ttatctgagg tcacagggcc   3748

tggggagatg gaggaaagtg atatccccca gcctcaagtc ttatttcctc aacgttgctc   3808

cccatcaagc cctttacttg acctcctaac aagtagcacc ctggattgat cggagcctcc   3868

tctctcaaac tggggcctcc ctggtccctt ggagacaaaa tcttaaatgc caggcctggc   3928

gagtgggtga aagatggaac ttgctgctga gtgcaccact tcaagtgacc accaggaggt   3988

gctatcgcac cactgtgtat ttaactgcct tgtgtacagt tatttatgcc tctgtattta   4048

aaaaactaac acccagtctg ttccccatgg ccacttgggt cttccctgta tgattccttg   4108

atggagatat ttacatgaat tgcattttac tttaatc                           4145


<210> SEQ ID NO 11
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 11
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cgtccacagt atgtgaggat caa 23

<210> SEQ ID NO 12
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 12

caagcaagac ttggacttgc aa 22

<210> SEQ ID NO 13
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Probe

<400> SEQUENCE: 13

tcttcaccac aaggccacat cggatt 26

<210> SEQ ID NO 14
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 14

ggcaaattca acggcacagt 20

<210> SEQ ID NO 15
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 15

gggtctcgct cctggaagct 20

<210> SEQ ID NO 16
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Probe

<400> SEQUENCE: 16

aaggccgaga atgggaagct tgtcatc 27

<210> SEQ ID NO 17
<211> LENGTH: 5484
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
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Met Lys Pro Ala Thr Gly Leu
1 5

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tgg gtc tgg gtg agc ctt ctc gtg gcg gcg ggg acc gtc cag ccc agc      102
Trp Val Trp Val Ser Leu Leu Val Ala Ala Gly Thr Val Gln Pro Ser
         10                  15                  20

gat tct cag tca gtg tgt gca gga acg gag aat aaa ctg agc tct ctc      150
Asp Ser Gln Ser Val Cys Ala Gly Thr Glu Asn Lys Leu Ser Ser Leu
     25                  30                  35

tct gac ctg gaa cag cag tac cga gcc ttg cgc aag tac tat gaa aac      198
Ser Asp Leu Glu Gln Gln Tyr Arg Ala Leu Arg Lys Tyr Tyr Glu Asn
 40                  45                  50                  55

tgt gag gtt gtc atg ggc aac ctg gag ata acc agc att gag cac aac      246
Cys Glu Val Val Met Gly Asn Leu Glu Ile Thr Ser Ile Glu His Asn
                 60                  65                  70

cgg gac ctc tcc ttc ctg cgg tct gtt cga gaa gtc aca ggc tac gtg      294
Arg Asp Leu Ser Phe Leu Arg Ser Val Arg Glu Val Thr Gly Tyr Val
             75                  80                  85

tta gtg gct ctt aat cag ttt cgt tac ctg cct ctg gag aat tta cgc      342
Leu Val Ala Leu Asn Gln Phe Arg Tyr Leu Pro Leu Glu Asn Leu Arg
         90                  95                 100

att att cgt ggg aca aaa ctt tat gag gat cga tat gcc ttg gca ata      390
Ile Ile Arg Gly Thr Lys Leu Tyr Glu Asp Arg Tyr Ala Leu Ala Ile
    105                 110                 115

ttt tta aac tac aga aaa gat gga aac ttt gga ctt caa gaa ctt gga      438
Phe Leu Asn Tyr Arg Lys Asp Gly Asn Phe Gly Leu Gln Glu Leu Gly
120                 125                 130                 135

tta aag aac ttg aca gaa atc cta aat ggt gga gtc tat gta gac cag      486
Leu Lys Asn Leu Thr Glu Ile Leu Asn Gly Gly Val Tyr Val Asp Gln
                140                 145                 150

aac aaa ttc ctt tgt tat gca gac acc att cat tgg caa gat att gtt      534
Asn Lys Phe Leu Cys Tyr Ala Asp Thr Ile His Trp Gln Asp Ile Val
            155                 160                 165

cgg aac cca tgg cct tcc aac ttg act ctt gtg tca aca aat ggt agt      582
Arg Asn Pro Trp Pro Ser Asn Leu Thr Leu Val Ser Thr Asn Gly Ser
        170                 175                 180

tca gga tgt gga cgt tgc cat aag tcc tgt act ggc cgt tgc tgg gga      630
Ser Gly Cys Gly Arg Cys His Lys Ser Cys Thr Gly Arg Cys Trp Gly
    185                 190                 195

ccc aca gaa aat cat tgc cag act ttg aca agg acg gtg tgt gca gaa      678
Pro Thr Glu Asn His Cys Gln Thr Leu Thr Arg Thr Val Cys Ala Glu
200                 205                 210                 215

caa tgt gac ggc aga tgc tac gga cct tac gtc agt gac tgc tgc cat      726
Gln Cys Asp Gly Arg Cys Tyr Gly Pro Tyr Val Ser Asp Cys Cys His
                220                 225                 230

cga gaa tgt gct gga ggc tgc tca gga cct aag gac aca gac tgc ttt      774
Arg Glu Cys Ala Gly Gly Cys Ser Gly Pro Lys Asp Thr Asp Cys Phe
            235                 240                 245

gcc tgc atg aat ttc aat gac agt gga gca tgt gtt act cag tgt ccc      822
Ala Cys Met Asn Phe Asn Asp Ser Gly Ala Cys Val Thr Gln Cys Pro
        250                 255                 260

caa acc ttt gtc tac aat cca acc acc ttt caa ctg gag cac aat ttc      870
Gln Thr Phe Val Tyr Asn Pro Thr Thr Phe Gln Leu Glu His Asn Phe
    265                 270                 275

aat gca aag tac aca tat gga gca ttc tgt gtc aag aaa tgt cca cat      918
Asn Ala Lys Tyr Thr Tyr Gly Ala Phe Cys Val Lys Lys Cys Pro His
280                 285                 290                 295

aac ttt gtg gta gat tcc agt tct tgt gtg cgt gcc tgc cct agt tcc      966
Asn Phe Val Val Asp Ser Ser Ser Cys Val Arg Ala Cys Pro Ser Ser
                300                 305                 310
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aag atg gaa gta gaa gaa aat ggg att aaa atg tgt aaa cct tgc act     1014
Lys Met Glu Val Glu Glu Asn Gly Ile Lys Met Cys Lys Pro Cys Thr
            315                 320                 325

gac att tgc cca aaa gct tgt gat ggc att ggc aca gga tca ttg atg     1062
Asp Ile Cys Pro Lys Ala Cys Asp Gly Ile Gly Thr Gly Ser Leu Met
        330                 335                 340

tca gct cag act gtg gat tcc agt aac att gac aaa ttc ata aac tgt     1110
Ser Ala Gln Thr Val Asp Ser Ser Asn Ile Asp Lys Phe Ile Asn Cys
    345                 350                 355

acc aag atc aat ggg aat ttg atc ttt cta gtc act ggt att cat ggg     1158
Thr Lys Ile Asn Gly Asn Leu Ile Phe Leu Val Thr Gly Ile His Gly
360                 365                 370                 375

gac cct tac aat gca att gaa gcc ata gac cca gag aaa ctg aac gtc     1206
Asp Pro Tyr Asn Ala Ile Glu Ala Ile Asp Pro Glu Lys Leu Asn Val
                380                 385                 390

ttt cgg aca gtc aga gag ata aca ggt ttc ctg aac ata cag tca tgg     1254
Phe Arg Thr Val Arg Glu Ile Thr Gly Phe Leu Asn Ile Gln Ser Trp
            395                 400                 405

cca cca aac atg act gac ttc agt gtt ttt tct aac ctg gtg acc att     1302
Pro Pro Asn Met Thr Asp Phe Ser Val Phe Ser Asn Leu Val Thr Ile
        410                 415                 420

ggt gga aga gta ctc tat agt ggc ctg tcc ttg ctt atc ctc aag caa     1350
Gly Gly Arg Val Leu Tyr Ser Gly Leu Ser Leu Leu Ile Leu Lys Gln
    425                 430                 435

cag ggc atc acc tct cta cag ttc cag tcc ctg aag gaa atc agc gca     1398
Gln Gly Ile Thr Ser Leu Gln Phe Gln Ser Leu Lys Glu Ile Ser Ala
440                 445                 450                 455

gga aac atc tat att act gac aac agc aac ctg tgt tat tat cat acc     1446
Gly Asn Ile Tyr Ile Thr Asp Asn Ser Asn Leu Cys Tyr Tyr His Thr
                460                 465                 470

att aac tgg aca aca ctc ttc agc aca atc aac cag aga ata gta atc     1494
Ile Asn Trp Thr Thr Leu Phe Ser Thr Ile Asn Gln Arg Ile Val Ile
            475                 480                 485

cgg gac aac aga aaa gct gaa aat tgt act gct gaa gga atg gtg tgc     1542
Arg Asp Asn Arg Lys Ala Glu Asn Cys Thr Ala Glu Gly Met Val Cys
        490                 495                 500

aac cat ctg tgt tcc agt gat ggc tgt tgg gga cct ggg cca gac caa     1590
Asn His Leu Cys Ser Ser Asp Gly Cys Trp Gly Pro Gly Pro Asp Gln
    505                 510                 515

tgt ctg tcg tgt cgc cgc ttc agt aga gga agg atc tgc ata gag tct     1638
Cys Leu Ser Cys Arg Arg Phe Ser Arg Gly Arg Ile Cys Ile Glu Ser
520                 525                 530                 535

tgt aac ctc tat gat ggt gaa ttt cgg gag ttt gag aat ggc tcc atc     1686
Cys Asn Leu Tyr Asp Gly Glu Phe Arg Glu Phe Glu Asn Gly Ser Ile
                540                 545                 550

tgt gtg gag tgt gac ccc cag tgt gag aag atg gaa gat ggc ctc ctc     1734
Cys Val Glu Cys Asp Pro Gln Cys Glu Lys Met Glu Asp Gly Leu Leu
            555                 560                 565

aca tgc cat gga ccg ggt cct gac aac tgt aca aag tgc tct cat ttt     1782
Thr Cys His Gly Pro Gly Pro Asp Asn Cys Thr Lys Cys Ser His Phe
        570                 575                 580

aaa gat ggc cca aac tgt gtg gaa aaa tgt cca gat ggc tta cag ggg     1830
Lys Asp Gly Pro Asn Cys Val Glu Lys Cys Pro Asp Gly Leu Gln Gly
    585                 590                 595

gca aac agt ttc att ttc aag tat gct gat cca gat cgg gag tgc cac     1878
Ala Asn Ser Phe Ile Phe Lys Tyr Ala Asp Pro Asp Arg Glu Cys His
600                 605                 610                 615
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cca tgc cat cca aac tgc acc caa ggg tgt aac ggt ccc act agt cat     1926
Pro Cys His Pro Asn Cys Thr Gln Gly Cys Asn Gly Pro Thr Ser His
                620                 625                 630

gac tgc att tac tac cca tgg acg ggc cat tcc act tta cca caa cat     1974
Asp Cys Ile Tyr Tyr Pro Trp Thr Gly His Ser Thr Leu Pro Gln His
            635                 640                 645

gct aga act ccc ctg att gca gct gga gta att ggt ggg ctc ttc att     2022
Ala Arg Thr Pro Leu Ile Ala Ala Gly Val Ile Gly Gly Leu Phe Ile
        650                 655                 660

ctg gtc att gtg ggt ctg aca ttt gct gtt tat gtt aga agg aag agc     2070
Leu Val Ile Val Gly Leu Thr Phe Ala Val Tyr Val Arg Arg Lys Ser
    665                 670                 675

atc aaa aag aaa aga gcc ttg aga aga ttc ttg gaa aca gag ttg gtg     2118
Ile Lys Lys Lys Arg Ala Leu Arg Arg Phe Leu Glu Thr Glu Leu Val
680                 685                 690                 695

gaa cca tta act ccc agt ggc aca gca ccc aat caa gct caa ctt cgt     2166
Glu Pro Leu Thr Pro Ser Gly Thr Ala Pro Asn Gln Ala Gln Leu Arg
                700                 705                 710

att ttg aaa gaa act gag ctg aag agg gta aaa gtc ctt ggc tca ggt     2214
Ile Leu Lys Glu Thr Glu Leu Lys Arg Val Lys Val Leu Gly Ser Gly
            715                 720                 725

gct ttt gga acg gtt tat aaa ggt att tgg gta cct gaa gga gaa act     2262
Ala Phe Gly Thr Val Tyr Lys Gly Ile Trp Val Pro Glu Gly Glu Thr
        730                 735                 740

gtg aag att cct gtg gct att aag att ctt aat gag aca act ggt ccc     2310
Val Lys Ile Pro Val Ala Ile Lys Ile Leu Asn Glu Thr Thr Gly Pro
    745                 750                 755

aag gca aat gtg gag ttc atg gat gaa gct ctg atc atg gca agt atg     2358
Lys Ala Asn Val Glu Phe Met Asp Glu Ala Leu Ile Met Ala Ser Met
760                 765                 770                 775

gat cat cca cac cta gtc cgg ttg ctg ggt gtg tgt ctg agc cca acc     2406
Asp His Pro His Leu Val Arg Leu Leu Gly Val Cys Leu Ser Pro Thr
                780                 785                 790

atc cag ctg gtt act caa ctt atg ccc cat ggc tgc ctg ttg gag tat     2454
Ile Gln Leu Val Thr Gln Leu Met Pro His Gly Cys Leu Leu Glu Tyr
            795                 800                 805

gtc cac gag cac aag gat aac att gga tca caa ctg ctg ctt aac tgg     2502
Val His Glu His Lys Asp Asn Ile Gly Ser Gln Leu Leu Leu Asn Trp
        810                 815                 820

tgt gtc cag ata gct aag gga atg atg tac ctg gaa gaa aga cga ctc     2550
Cys Val Gln Ile Ala Lys Gly Met Met Tyr Leu Glu Glu Arg Arg Leu
    825                 830                 835

gtt cat cgg gat ttg gca gcc cgt aat gtc tta gtg aaa tct cca aac     2598
Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Lys Ser Pro Asn
840                 845                 850                 855

cat gtg aaa atc aca gat ttt ggg cta gcc aga ctc ttg gaa gga gat     2646
His Val Lys Ile Thr Asp Phe Gly Leu Ala Arg Leu Leu Glu Gly Asp
                860                 865                 870

gaa aaa gag tac aat gct gat gga gga aag atg cca att aaa tgg atg     2694
Glu Lys Glu Tyr Asn Ala Asp Gly Gly Lys Met Pro Ile Lys Trp Met
            875                 880                 885

gct ctg gag tgt ata cat tac agg aaa ttc acc cat cag agt gac gtt     2742
Ala Leu Glu Cys Ile His Tyr Arg Lys Phe Thr His Gln Ser Asp Val
        890                 895                 900

tgg agc tat gga gtt act ata tgg gaa ctg atg acc ttt gga gga aaa     2790
Trp Ser Tyr Gly Val Thr Ile Trp Glu Leu Met Thr Phe Gly Gly Lys
    905                 910                 915
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ccc tat gat gga att cca acg cga gaa atc cct gat tta tta gag aaa     2838
Pro Tyr Asp Gly Ile Pro Thr Arg Glu Ile Pro Asp Leu Leu Glu Lys
920                 925                 930                 935

gga gaa cgt ttg cct cag cct ccc atc tgc act att gac gtt tac atg     2886
Gly Glu Arg Leu Pro Gln Pro Pro Ile Cys Thr Ile Asp Val Tyr Met
                940                 945                 950

gtc atg gtc aaa tgt tgg atg att gat gct gac agt aga cct aaa ttt     2934
Val Met Val Lys Cys Trp Met Ile Asp Ala Asp Ser Arg Pro Lys Phe
            955                 960                 965

aag gaa ctg gct gct gag ttt tca agg atg gct cga gac cct caa aga     2982
Lys Glu Leu Ala Ala Glu Phe Ser Arg Met Ala Arg Asp Pro Gln Arg
        970                 975                 980

tac cta gtt att cag ggt gat gat cgt atg aag ctt ccc agt cca aat     3030
Tyr Leu Val Ile Gln Gly Asp Asp Arg Met Lys Leu Pro Ser Pro Asn
    985                 990                 995

gac agc aag ttc ttt cag aat ctc ttg gat gaa gag gat ttg gaa gat     3078
Asp Ser Lys Phe Phe Gln Asn Leu Leu Asp Glu Glu Asp Leu Glu Asp
1000                1005                1010                1015

atg atg gat gct gag gag tac ttg gtc cct cag gct ttc aac atc cca     3126
Met Met Asp Ala Glu Glu Tyr Leu Val Pro Gln Ala Phe Asn Ile Pro
                1020                1025                1030

cct ccc atc tat act tcc aga gca aga att gac tcg aat agg agt gaa     3174
Pro Pro Ile Tyr Thr Ser Arg Ala Arg Ile Asp Ser Asn Arg Ser Glu
            1035                1040                1045

att gga cac agc cct cct cct gcc tac acc ccc atg tca gga aac cag     3222
Ile Gly His Ser Pro Pro Pro Ala Tyr Thr Pro Met Ser Gly Asn Gln
        1050                1055                1060

ttt gta tac cga gat gga ggt ttt gct gct gaa caa gga gtg tct gtg     3270
Phe Val Tyr Arg Asp Gly Gly Phe Ala Ala Glu Gln Gly Val Ser Val
    1065                1070                1075

ccc tac aga gcc cca act agc aca att cca gaa gct cct gtg gca cag     3318
Pro Tyr Arg Ala Pro Thr Ser Thr Ile Pro Glu Ala Pro Val Ala Gln
1080                1085                1090                1095

ggt gct act gct gag att ttt gat gac tcc tgc tgt aat ggc acc cta     3366
Gly Ala Thr Ala Glu Ile Phe Asp Asp Ser Cys Cys Asn Gly Thr Leu
                1100                1105                1110

cgc aag cca gtg gca ccc cat gtc caa gag gac agt agc acc cag agg     3414
Arg Lys Pro Val Ala Pro His Val Gln Glu Asp Ser Ser Thr Gln Arg
            1115                1120                1125

tac agt gct gac ccc acc gtg ttt gcc cca gaa cgg agc cca cga gga     3462
Tyr Ser Ala Asp Pro Thr Val Phe Ala Pro Glu Arg Ser Pro Arg Gly
        1130                1135                1140

gag ctg gat gag gaa ggt tac atg act cct atg cga gac aaa ccc aaa     3510
Glu Leu Asp Glu Glu Gly Tyr Met Thr Pro Met Arg Asp Lys Pro Lys
    1145                1150                1155

caa gaa tac ctg aat cca gtg gag gag aac cct ttt gtt tct cgg aga     3558
Gln Glu Tyr Leu Asn Pro Val Glu Glu Asn Pro Phe Val Ser Arg Arg
1160                1165                1170                1175

aaa aat gga gac ctt caa gca ttg gat aat ccc gaa tat cac aat gca     3606
Lys Asn Gly Asp Leu Gln Ala Leu Asp Asn Pro Glu Tyr His Asn Ala
                1180                1185                1190

tcc aat ggt cca ccc aag gcc gag gat gag tat gtg aat gag cca ctg     3654
Ser Asn Gly Pro Pro Lys Ala Glu Asp Glu Tyr Val Asn Glu Pro Leu
            1195                1200                1205

tac ctc aac acc ttt gcc aac acc ttg gga aaa gct gag tac ctg aag     3702
Tyr Leu Asn Thr Phe Ala Asn Thr Leu Gly Lys Ala Glu Tyr Leu Lys
        1210                1215                1220
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aac aac ata ctg tca atg cca gag aag gcc aag aaa gcg ttt gac aac     3750
Asn Asn Ile Leu Ser Met Pro Glu Lys Ala Lys Lys Ala Phe Asp Asn
    1225                1230                1235

cct gac tac tgg aac cac agc ctg cca cct cgg agc acc ctt cag cac     3798
Pro Asp Tyr Trp Asn His Ser Leu Pro Pro Arg Ser Thr Leu Gln His
1240                1245                1250                1255

cca gac tac ctg cag gag tac agc aca aaa tat ttt tat aaa cag aat     3846
Pro Asp Tyr Leu Gln Glu Tyr Ser Thr Lys Tyr Phe Tyr Lys Gln Asn
                1260                1265                1270

ggg cgg atc cgg cct att gtg gca gag aat cct gaa tac ctc tct gag     3894
Gly Arg Ile Arg Pro Ile Val Ala Glu Asn Pro Glu Tyr Leu Ser Glu
            1275                1280                1285

ttc tcc ctg aag cca ggc act gtg ctg ccg cct cca cct tac aga cac     3942
Phe Ser Leu Lys Pro Gly Thr Val Leu Pro Pro Pro Pro Tyr Arg His
        1290                1295                1300

cgg aat act gtg gtg taa gctcagttgt ggtttttag gtggagagac acacctgctc 4000
Arg Asn Thr Val Val
    1305

caatttcccc acccccctct ctttctctgg tggtcttcct tctaccccaa ggccagtagt   4060

tttgacactt cccagtggaa gatacagaga tgcaatgata gttatgtgct tacctaactt   4120

gaacattaga gggaaagact gaaagagaaa gataggagga accacaatgt ttcttcattt   4180

ctctgcatgg gttggtcagg agaatgaaac agctagagaa ggaccagaaa atgtaaggca   4240

atgctgccta ctatcaaact agctgtcact ttttttcttt ttctttttct ttctttgttt   4300

ctttcttcct cttctttttt ttttttttt taaagcagat ggttgaaaca cccatgctat   4360

ctgttcctat ctgcaggaac tgatgtgtgc atatttagca tccctggaaa tcataataaa   4420

gtttccatta gaacaaaaga ataacatttt ctataacata tgatagtgtc tgaaattgag   4480

aatccagttt ctttccccag cagtttctgt cctagcaagt aagaatggcc aactcaactt   4540

tcataattta aaaatctcca ttaaagttat aactagtaat tatgttttca acacttttg   4600

gtttttttca ttttgttttg ctctgaccga ttcctttata tttgctcccc tatttttggc   4660

tttaatttct aattgcaaag atgtttacat caaagcttct tcacagaatt taagcaagaa   4720

atattttaat atagtgaaat ggccactact ttaagtatac aatctttaaa ataagaaagg   4780

gaggctaata tttttcatgc tatcaaatta tcttcaccct catcctttac atttttcaac   4840

atttttttt ctccataaat gacactactt gataggccgt tggttgtctg aagagtagaa   4900

gggaaactaa gagacagttc tctgtggttc aggaaaacta ctgatacttt caggggtggc   4960

ccaatgaggg aatccattga actggaagaa acacactgga ttgggtatgt ctacctggca   5020

gatactcaga aatgtagttt gcacttaagc tgtaatttta tttgttcttt ttctgaactc   5080

cattttggat tttgaatcaa gcaatatgga agcaaccagc aaattaacta atttaagtac   5140

atttttaaaa aaagagctaa gataaagact gtggaaatgc caaaccaagc aaattaggaa   5200

ccttgcaacg gtatccaggg actatgatga gaggccagca cattatcttc atatgtcacc   5260

tttgctacgc aaggaaattt gttcagttcg tatacttcgt aagaaggaat gcgagtaagg   5320

attggcttga attccatgga atttctagta tgagactatt tatatgaagt agaaggtaac   5380

tctttgcaca taaattggta taataaaaag aaaaacacaa acattcaaag cttagggata   5440

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<210> SEQ ID NO 18
<211> LENGTH: 3690
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
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ggtgaaggga ctgagctgtt agagacactt ctgaggctcc tcacgcttgg gtcttgttca 180

ctccacggag tagcctagtc aactgcaaga gaacggagaa cgttggattt ggagcagaag 240

tgcaaagtct cagac atg gct tgc ccc tgg aag ttt ctc ttc aaa gtc aaa 291
Met Ala Cys Pro Trp Lys Phe Leu Phe Lys Val Lys
1 5 10

tcc tac caa agt gac ctg aaa gag gaa aag gac att aac aac aac gtg 339
Ser Tyr Gln Ser Asp Leu Lys Glu Glu Lys Asp Ile Asn Asn Asn Val
15 20 25

aag aaa acc cct tgt gct gtt ctc agc cca aca ata caa gat gac cct 387
Lys Lys Thr Pro Cys Ala Val Leu Ser Pro Thr Ile Gln Asp Asp Pro
30 35 40

aag agt cac caa aat ggc tcc ccg cag ctc ctc act ggg aca gca cag 435
Lys Ser His Gln Asn Gly Ser Pro Gln Leu Leu Thr Gly Thr Ala Gln
45 50 55 60

aat gtt cca gaa tcc ctg gac aag ctg cat gtg aca tcg acc cgt cca 483
Asn Val Pro Glu Ser Leu Asp Lys Leu His Val Thr Ser Thr Arg Pro
65 70 75

cag tat gtg agg atc aaa aac tgg ggc agt gga gag att ttg cat gac 531
Gln Tyr Val Arg Ile Lys Asn Trp Gly Ser Gly Glu Ile Leu His Asp
80 85 90

act ctt cac cac aag gcc aca tcg gat ttc act tgc aag tcc aag tct 579
Thr Leu His His Lys Ala Thr Ser Asp Phe Thr Cys Lys Ser Lys Ser
95 100 105

tgc ttg ggg tcc atc atg aac ccc aag agt ttg acc aga gga ccc aga 627
Cys Leu Gly Ser Ile Met Asn Pro Lys Ser Leu Thr Arg Gly Pro Arg
110 115 120

gac aag cct acc cct ctg gag gag ctc ctg cct cat gcc att gag ttc 675
Asp Lys Pro Thr Pro Leu Glu Glu Leu Leu Pro His Ala Ile Glu Phe
125 130 135 140

atc aac cag tat tat ggc tcc ttt aaa gag gca aaa ata gag gaa cat 723
Ile Asn Gln Tyr Tyr Gly Ser Phe Lys Glu Ala Lys Ile Glu Glu His
145 150 155

ctg gcc agg ctg gaa gct gta aca aag gaa ata gaa aca aca gga acc 771
Leu Ala Arg Leu Glu Ala Val Thr Lys Glu Ile Glu Thr Thr Gly Thr
160 165 170

tac cag ctc act ctg gat gag ctc atc ttt gcc acc aag atg gcc tgg 819
Tyr Gln Leu Thr Leu Asp Glu Leu Ile Phe Ala Thr Lys Met Ala Trp
175 180 185

agg aat gcc cct cgc tgc atc ggc agg atc cag tgg tcc aac ctg cag 867
Arg Asn Ala Pro Arg Cys Ile Gly Arg Ile Gln Trp Ser Asn Leu Gln
190 195 200

gtc ttt gac gct cgg aac tgt agc aca gca cag gaa atg ttt cag cac 915
Val Phe Asp Ala Arg Asn Cys Ser Thr Ala Gln Glu Met Phe Gln His
205 210 215 220

atc tgc aga cac ata ctt tat gcc acc aac aat ggc aac atc agg tcg 963
Ile Cys Arg His Ile Leu Tyr Ala Thr Asn Asn Gly Asn Ile Arg Ser

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               225                 230                 235

gcc atc act gtg ttc ccc cag cgg agt gac ggc aaa cat gac ttc agg     1011
Ala Ile Thr Val Phe Pro Gln Arg Ser Asp Gly Lys His Asp Phe Arg
            240                 245                 250

ctc tgg aat tca cag ctc atc cgg tac gct ggc tac cag atg ccc gat     1059
Leu Trp Asn Ser Gln Leu Ile Arg Tyr Ala Gly Tyr Gln Met Pro Asp
        255                 260                 265

ggc acc atc aga ggg gat gct gcc acc ttg gag ttc acc cag ttg tgc     1107
Gly Thr Ile Arg Gly Asp Ala Ala Thr Leu Glu Phe Thr Gln Leu Cys
    270                 275                 280

atc gac cta ggc tgg aag ccc cgc tat ggc cgc ttt gat gtg ctg cct     1155
Ile Asp Leu Gly Trp Lys Pro Arg Tyr Gly Arg Phe Asp Val Leu Pro
285                 290                 295                 300

ctg gtc ttg caa gct gat ggt caa gat cca gag gtc ttt gaa atc cct     1203
Leu Val Leu Gln Ala Asp Gly Gln Asp Pro Glu Val Phe Glu Ile Pro
                305                 310                 315

cct gat ctt gtg ttg gag gtg acc atg gag cat ccc aag tac gag tgg     1251
Pro Asp Leu Val Leu Glu Val Thr Met Glu His Pro Lys Tyr Glu Trp
            320                 325                 330

ttc cag gag ctc ggg ttg aag tgg tat gca ctg cct gcc gtg gcc aac     1299
Phe Gln Glu Leu Gly Leu Lys Trp Tyr Ala Leu Pro Ala Val Ala Asn
        335                 340                 345

atg cta ctg gag gtg ggt ggc ctc gaa ttc cca gcc tgc ccc ttc aat     1347
Met Leu Leu Glu Val Gly Gly Leu Glu Phe Pro Ala Cys Pro Phe Asn
    350                 355                 360

ggt tgg tac atg ggc acc gag att gga gtt cga gac ttc tgt gac aca     1395
Gly Trp Tyr Met Gly Thr Glu Ile Gly Val Arg Asp Phe Cys Asp Thr
365                 370                 375                 380

cag cgc tac aac atc ctg gag gaa gtg ggc cga agg atg ggc ctg gag     1443
Gln Arg Tyr Asn Ile Leu Glu Glu Val Gly Arg Arg Met Gly Leu Glu
                385                 390                 395

acc cac aca ctg gcc tcc ctc tgg aaa gac cgg gct gtc acg gag atc     1491
Thr His Thr Leu Ala Ser Leu Trp Lys Asp Arg Ala Val Thr Glu Ile
            400                 405                 410

aat gtg gct gtg ctc cat agt ttc cag aag cag aat gtg acc atc atg     1539
Asn Val Ala Val Leu His Ser Phe Gln Lys Gln Asn Val Thr Ile Met
        415                 420                 425

gac cac cac aca gcc tca gag tcc ttc atg aag cac atg cag aat gag     1587
Asp His His Thr Ala Ser Glu Ser Phe Met Lys His Met Gln Asn Glu
    430                 435                 440

tac cgg gcc cgt gga ggc tgc ccg gca gac tgg att tgg ctg gtc cct     1635
Tyr Arg Ala Arg Gly Gly Cys Pro Ala Asp Trp Ile Trp Leu Val Pro
445                 450                 455                 460

cca gtg tct ggg agc atc acc cct gtg ttc cac cag gag atg ttg aac     1683
Pro Val Ser Gly Ser Ile Thr Pro Val Phe His Gln Glu Met Leu Asn
                465                 470                 475

tat gtc cta tct cca ttc tac tac tac cag atc gag ccc tgg aag acc     1731
Tyr Val Leu Ser Pro Phe Tyr Tyr Tyr Gln Ile Glu Pro Trp Lys Thr
            480                 485                 490

cac atc tgg cag aat gag aag ctg agg ccc agg agg aga gag atc cga     1779
His Ile Trp Gln Asn Glu Lys Leu Arg Pro Arg Arg Arg Glu Ile Arg
        495                 500                 505

ttt aga gtc ttg gtg aaa gtg gtg ttc ttt gct tcc atg cta atg cga     1827
Phe Arg Val Leu Val Lys Val Val Phe Phe Ala Ser Met Leu Met Arg
    510                 515                 520

aag gtc atg gct tca cgg gtc aga gcc aca gtc ctc ttt gct act gag     1875
Lys Val Met Ala Ser Arg Val Arg Ala Thr Val Leu Phe Ala Thr Glu
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525                 530                 535                 540

aca ggg aag tct gaa gca cta gcc agg gac ctg gcc acc ttg ttc agc     1923
Thr Gly Lys Ser Glu Ala Leu Ala Arg Asp Leu Ala Thr Leu Phe Ser
                545                 550                 555

tac gcc ttc aac acc aag gtt gtc tgc atg gac cag tat aag gca agc     1971
Tyr Ala Phe Asn Thr Lys Val Val Cys Met Asp Gln Tyr Lys Ala Ser
            560                 565                 570

acc ttg gaa gag gag caa cta ctg ctg gtg gtg aca agc aca ttt ggg     2019
Thr Leu Glu Glu Glu Gln Leu Leu Leu Val Val Thr Ser Thr Phe Gly
        575                 580                 585

aat gga gac tgt ccc agc aat ggg cag act ctg aag aaa tct ctg ttc     2067
Asn Gly Asp Cys Pro Ser Asn Gly Gln Thr Leu Lys Lys Ser Leu Phe
    590                 595                 600

atg ctt aga gaa ctc aac cac acc ttc agg tat gct gtg ttt ggc ctt     2115
Met Leu Arg Glu Leu Asn His Thr Phe Arg Tyr Ala Val Phe Gly Leu
605                 610                 615                 620

ggc tcc agc atg tac cct cag ttc tgc gcc ttt gct cat gac atc gac     2163
Gly Ser Ser Met Tyr Pro Gln Phe Cys Ala Phe Ala His Asp Ile Asp
                625                 630                 635

cag aag ctg tcc cac ctg gga gcc tct cag ctt gcc cca aca gga gaa     2211
Gln Lys Leu Ser His Leu Gly Ala Ser Gln Leu Ala Pro Thr Gly Glu
            640                 645                 650

ggg gac gaa ctc agt ggg cag gag gat gcc ttc cgc agc tgg gct gta     2259
Gly Asp Glu Leu Ser Gly Gln Glu Asp Ala Phe Arg Ser Trp Ala Val
        655                 660                 665

caa acc ttc cgg gca gcc tgt gag acc ttt gat gtc cga agc aaa cat     2307
Gln Thr Phe Arg Ala Ala Cys Glu Thr Phe Asp Val Arg Ser Lys His
    670                 675                 680

cac att cag atc ccg aaa cgc ttc act tcc aat gca aca tgg gag cca     2355
His Ile Gln Ile Pro Lys Arg Phe Thr Ser Asn Ala Thr Trp Glu Pro
685                 690                 695                 700

cag caa tat agg ctc atc cag agc ccg gag cct tta gac ctc aac aga     2403
Gln Gln Tyr Arg Leu Ile Gln Ser Pro Glu Pro Leu Asp Leu Asn Arg
                705                 710                 715

gcc ctc agc agc atc cat gca aag aac gtg ttt acc atg agg ctg aaa     2451
Ala Leu Ser Ser Ile His Ala Lys Asn Val Phe Thr Met Arg Leu Lys
            720                 725                 730

tcc cag cag aat ctg cag agt gaa aag tcc agc cgc acc acc ctc ctc     2499
Ser Gln Gln Asn Leu Gln Ser Glu Lys Ser Ser Arg Thr Thr Leu Leu
        735                 740                 745

gtt cag ctc acc ttc gag ggc agc cga ggg ccc agc tac ctg cct ggg     2547
Val Gln Leu Thr Phe Glu Gly Ser Arg Gly Pro Ser Tyr Leu Pro Gly
    750                 755                 760

gaa cac ctg ggg atc ttc cca ggc aac cag acc gcc ctg gtg cag gga     2595
Glu His Leu Gly Ile Phe Pro Gly Asn Gln Thr Ala Leu Val Gln Gly
765                 770                 775                 780

atc ttg gag cga gtt gtg gat tgt cct aca cca cac caa act gtg tgc     2643
Ile Leu Glu Arg Val Val Asp Cys Pro Thr Pro His Gln Thr Val Cys
                785                 790                 795

ctg gag gtt ctg gat gag agc ggc agc tac tgg gtc aaa gac aag agg     2691
Leu Glu Val Leu Asp Glu Ser Gly Ser Tyr Trp Val Lys Asp Lys Arg
            800                 805                 810

ctg ccc ccc tgc tca ctc agc caa gcc ctc acc tac ttc ctg gac att     2739
Leu Pro Pro Cys Ser Leu Ser Gln Ala Leu Thr Tyr Phe Leu Asp Ile
        815                 820                 825

acg acc cct ccc acc cag ctg cag ctc cac aag ctg gct cgc ttt gcc     2787
Thr Thr Pro Pro Thr Gln Leu Gln Leu His Lys Leu Ala Arg Phe Ala
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    830                 835                 840

acg gac gag acg gat agg cag aga ttg gag gcc ttg tgt cag ccc tca     2835
Thr Asp Glu Thr Asp Arg Gln Arg Leu Glu Ala Leu Cys Gln Pro Ser
845                 850                 855                 860

gag tac aat gac tgg aag ttc agc aac aac ccc acg ttc ctg gag gtg     2883
Glu Tyr Asn Asp Trp Lys Phe Ser Asn Asn Pro Thr Phe Leu Glu Val
                865                 870                 875

ctt gaa gag ttc cct tcc ttg cat gtg ccc gct gcc ttc ctg ctg tcg     2931
Leu Glu Glu Phe Pro Ser Leu His Val Pro Ala Ala Phe Leu Leu Ser
            880                 885                 890

cag ctc cct atc ttg aag ccc cgc tac tac tcc atc agc tcc tcc cag     2979
Gln Leu Pro Ile Leu Lys Pro Arg Tyr Tyr Ser Ile Ser Ser Ser Gln
        895                 900                 905

gac cac acc ccc tcg gag gtt cac ctc act gtg gcc gtg gtc acc tac     3027
Asp His Thr Pro Ser Glu Val His Leu Thr Val Ala Val Val Thr Tyr
    910                 915                 920

cgc acc cga gat ggt cag ggt ccc ctg cac cat ggt gtc tgc agc act     3075
Arg Thr Arg Asp Gly Gln Gly Pro Leu His His Gly Val Cys Ser Thr
925                 930                 935                 940

tgg atc agg aac ctg aag ccc cag gac cca gtg ccc tgc ttt gtg cga     3123
Trp Ile Arg Asn Leu Lys Pro Gln Asp Pro Val Pro Cys Phe Val Arg
                945                 950                 955

agt gtc agt ggc ttc cag ctc cct gag gac ccc tcc cag cct tgc atc     3171
Ser Val Ser Gly Phe Gln Leu Pro Glu Asp Pro Ser Gln Pro Cys Ile
            960                 965                 970

ctc att ggg cct ggt acg ggc att gct ccc ttc cga agt ttc tgg cag     3219
Leu Ile Gly Pro Gly Thr Gly Ile Ala Pro Phe Arg Ser Phe Trp Gln
        975                 980                 985

cag cgg ctc cat gac tcc cag cac aaa ggg ctc aaa gga ggc cgc atg     3267
Gln Arg Leu His Asp Ser Gln His Lys Gly Leu Lys Gly Gly Arg Met
    990                 995                 1000

agc ttg gtg ttt ggg tgc cgg cac ccg gag gag gac cac ctc tat cag     3315
Ser Leu Val Phe Gly Cys Arg His Pro Glu Glu Asp His Leu Tyr Gln
1005                1010                1015                1020

gaa gaa atg cag gag atg gtc cgc aag aga gtg ctg ttc cag gtg cac     3363
Glu Glu Met Gln Glu Met Val Arg Lys Arg Val Leu Phe Gln Val His
                1025                1030                1035

aca ggc tac tcc cgg ctg ccc ggc aaa ccc aag gtc tac gtt cag gac     3411
Thr Gly Tyr Ser Arg Leu Pro Gly Lys Pro Lys Val Tyr Val Gln Asp
            1040                1045                1050

atc ctg caa aag cag ctg gcc aat gag gta ctc agc gtg ctc cac ggg     3459
Ile Leu Gln Lys Gln Leu Ala Asn Glu Val Leu Ser Val Leu His Gly
        1055                1060                1065

gag cag ggc cac ctc tac att tgc gga gat gtg cgc atg gct cgg gat     3507
Glu Gln Gly His Leu Tyr Ile Cys Gly Asp Val Arg Met Ala Arg Asp
    1070                1075                1080

gtg gct acc aca ttg aag aag ctg gtg gcc acc aag ctg aac ttg agc     3555
Val Ala Thr Thr Leu Lys Lys Leu Val Ala Thr Lys Leu Asn Leu Ser
1085                1090                1095                1100

gag gag cag gtg gaa gac tat ttc ttc cag ctc aag agc cag aaa cgt     3603
Glu Glu Gln Val Glu Asp Tyr Phe Phe Gln Leu Lys Ser Gln Lys Arg
                1105                1110                1115

tat cat gaa gat atc ttc ggt gca gtc ttt tcc tat ggg gca aaa aag     3651
Tyr His Glu Asp Ile Phe Gly Ala Val Phe Ser Tyr Gly Ala Lys Lys
            1120                1125                1130

ggc agc gcc ttg gag gag ccc aaa gcc acg agg ctc tga                3690
Gly Ser Ala Leu Glu Glu Pro Lys Ala Thr Arg Leu
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1135 1140

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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 19

catcaaaggt ggccgaga 18

<210> SEQ ID NO 20
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 20

ctgtctagaa ctgcccag 18

<210> SEQ ID NO 21
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 21

tgccttgaga acttcggg 18

<210> SEQ ID NO 22
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 22

tgtcacttat ctggattt 18

<210> SEQ ID NO 23
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 23

cttgaacaga aatttcca 18

<210> SEQ ID NO 24
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<212> TYPE: DNA
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<400> SEQUENCE: 24

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<210> SEQ ID NO 25

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<211> LENGTH: 18
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<400> SEQUENCE: 26

agcttgacca gagattct 18

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 27

gtgaagtgtg tcttggaa 18

<210> SEQ ID NO 28
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<400> SEQUENCE: 28

gcaagatttg gacctgca 18

<210> SEQ ID NO 29
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<400> SEQUENCE: 29

ccctgggtcc tctggtca 18

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gccgtaatat tggttgac 18

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<223> OTHER INFORMATION: Antisense Oligonucleotide

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ctcctttgtt accgcttc 18

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<400> SEQUENCE: 32

gctcatctcc cgtcagtt 18

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<400> SEQUENCE: 33

agacctgcag gttggacc 18

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<400> SEQUENCE: 34

cgtgtctgca gatgtgtt 18

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<400> SEQUENCE: 35

aagtcgtgct tgccatca 18

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<400> SEQUENCE: 36

cctctgatgc tgccatct 18

<210> SEQ ID NO 37
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<212> TYPE: DNA
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<400> SEQUENCE: 37

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atcgaagcgg ccgtactt 18

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<400> SEQUENCE: 38

tccatggcca cctcaagc 18

<210> SEQ ID NO 39
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 39

caggcagggc gtaccact 18

<210> SEQ ID NO 40
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<212> TYPE: DNA
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<400> SEQUENCE: 40

ctctgtgccc atgtacca 18

<210> SEQ ID NO 41
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<212> TYPE: DNA
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<400> SEQUENCE: 41

ctgcccactt cctccagg 18

<210> SEQ ID NO 42
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<400> SEQUENCE: 42

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<210> SEQ ID NO 43
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<212> TYPE: DNA
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<400> SEQUENCE: 43

tccatgatgg tcacattc 18

<210> SEQ ID NO 44

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<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 44

ggaccggtat tcattctg 18

<210> SEQ ID NO 45
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 45

acgtagttca gcatctcc 18

<210> SEQ ID NO 46
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 46

gggtctccgc ttctcgtc 18

<210> SEQ ID NO 47
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<400> SEQUENCE: 47

agcatacagg caaagagc 18

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tgtctctgtc gcaaagag 18

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ttcctcctcc aggcagct 18

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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 50

ccattgccag ggcagtct 18

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acacagcgta cctgaatt 18

<210> SEQ ID NO 52
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gcttctgatc aatgtcat 18

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tgtagtggtg cgggtccc 18

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<400> SEQUENCE: 54

ctggatgtcg gactttgt 18

<210> SEQ ID NO 55
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<400> SEQUENCE: 55

ctcttgtcac tgacccag 18

<210> SEQ ID NO 56
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ctttaacccc tcctgtag 18

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agttctgtgc cggcagct 18

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acctcagata atgcagag 18

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agatcccgtg ctgacaat 18

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ctcacccaga cccaaagt 18

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gtccccgccg ccacgaga 18

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actgactgag aatcgctg 18

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ctgctgttcc aggtcaga 18

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gttatctcca ggttgccc 18

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ccggttgtgc tcaatgct 18

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caggtaacga aactgatt 18

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attctccaga ggcaggta 18

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tcataaagtt ttgtccca 18

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<223> OTHER INFORMATION: Antisense Oligonucleotide

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agtccaaagt ttccatct 18

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tttaggattt ctgtcaag 18

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tacatagact ccaccatt 18

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aactaccatt tgttgaca 18

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tccacatcct gaactacc 18

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ggcaatgatt ttctgtgg 18

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gtccttgtca aagtctgg 18

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gtagcatctg ccgtcaca 18

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gcctccagca cattctcg 18

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ggtcctgagc agcctcca 18

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ggcaaagcag tctgtgtc 18

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aggtggttgg attgtaga 18

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attgtgctcc agttgaaa 18

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tgtggacatt tcttgaca 18

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tagggcaggc acgcacac 18

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ttaatcccat tttcttct 18

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tgatcctgtg ccaatgcc 18

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<400> SEQUENCE: 86

ctgggtctat ggcttcaa 18

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gacgttcagt ttctctgg 18

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<223> OTHER INFORMATION: Antisense Oligonucleotide

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ggataagcaa ggacaggc 18

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actggaactg tagagagg 18

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aggttgctgt tgtcagta 18

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gttaatggta tgataata 18

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<400> SEQUENCE: 92

ttctctggtt gattgtgc 18

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ttactattct ctggttga 18

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ctggaacaca gatggttg 18

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<400> SEQUENCE: 95

caggtcccca acagccat 18

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tactgaagcg gcgacacg 18

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<400> SEQUENCE: 97

aggttacaag actctatg 18

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<400> SEQUENCE: 98

tggagccatt ctcaaact 18

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<400> SEQUENCE: 99

aggccatctt ccatcttc 18

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ctagtgggac cgttacac 18

<210> SEQ ID NO 101

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<400> SEQUENCE: 101

tcagacccac aatgacca 18

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<400> SEQUENCE: 102

atgctcttcc ttctaaca 18

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<400> SEQUENCE: 103

ctgtgccact gggagtta 18

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<400> SEQUENCE: 104

ccaaaagcac ctgagcca 18

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<400> SEQUENCE: 105

gccacaggaa tcttcaca 18

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<400> SEQUENCE: 106

tggttgggct cagacaca 18

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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 107

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actaagacat tacgggct 18

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tcctccatca gcattgta 18

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ccaaaggtca tcagttcc 18

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catccaacat ttgaccat 18

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actcagcagc cagttcct 18

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gtcatttgga ctgggaag 18

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<400> SEQUENCE: 114

ttccaaatcc tcttcatc 18

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gaggtgggat gttgaaag 18

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cagcaaaacc tccatctc 18

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<212> TYPE: DNA
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ctcagcagta gcaccctg 18

<210> SEQ ID NO 118
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<400> SEQUENCE: 118

tgggtgctac tgtcctct 18

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gtttgtctcg cataggag 18

<210> SEQ ID NO 120

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<211> LENGTH: 18
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ccactggatt caggtatt 18

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ggctcattca catactca 18

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ttgacagtat gttgttct 18

<210> SEQ ID NO 123
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 123

ttccagtagt cagggttg 18

<210> SEQ ID NO 124
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 124

tgctgaaggg tgctccga 18

<210> SEQ ID NO 125
<211> LENGTH: 18
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 125

aggtattcag gattctct 18

<210> SEQ ID NO 126
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 126

tctgtaaggt ggaggcgg 18

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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 127

agtgtcaaaa ctactggc 18

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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 128

gttcaagtta ggtaagca 18

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ctatctttct ctttcagt 18

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atgcagagaa atgaagaa 18

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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 131

cagcattgcc ttacattt 18

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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 132

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gtgtttcaac catctgct 18

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<220> FEATURE:
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<400> SEQUENCE: 133

tttgttctaa tggaaact 18

<210> SEQ ID NO 134
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 134

cagagcaaaa caaaatga 18

<210> SEQ ID NO 135
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 135

aggatgaggg tgaagata 18

<210> SEQ ID NO 136
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<400> SEQUENCE: 136

tactcttcag acaaccaa 18

<210> SEQ ID NO 137
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<400> SEQUENCE: 137

gttttcctga accacaga 18

<210> SEQ ID NO 138
<211> LENGTH: 18
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<400> SEQUENCE: 138

acatacccaa tccagtgt 18

<210> SEQ ID NO 139

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<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 139

aaaatggagt tcagaaaa 18

<210> SEQ ID NO 140
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<400> SEQUENCE: 140

gcctctcatc atagtccc 18

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<400> SEQUENCE: 141

gagttacctt ctacttca 18

<210> SEQ ID NO 142
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<400> SEQUENCE: 142

cacatttatt tacaactt 18

<210> SEQ ID NO 143
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 143

gtaaagttgt gaccctggca 20

<210> SEQ ID NO 144
<211> LENGTH: 20
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 144

ttgcacttct gctccaaatc 20

<210> SEQ ID NO 145
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 145

ttggtaggat ttgactttga 20

<210> SEQ ID NO 146
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<220> FEATURE:
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<400> SEQUENCE: 146

ctcttagggt catcttgtat 20

<210> SEQ ID NO 147
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<400> SEQUENCE: 147

tcgatgtcac atgcagcttg 20

<210> SEQ ID NO 148
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<212> TYPE: DNA
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tgaaatccga tgtggccttg 20

<210> SEQ ID NO 149
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<400> SEQUENCE: 149

gggtaggctt gtctctgggt 20

<210> SEQ ID NO 150
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 150

gcatgaggca ggagctcctc 20

<210> SEQ ID NO 151
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 151

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ttcctccagg ccatcttggt 20

<210> SEQ ID NO 152
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 152

atgagctgtg aattccagag 20

<210> SEQ ID NO 153
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 153

cttccagcct aggtcgatgc 20

<210> SEQ ID NO 154
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 154

atttcaaaga cctctggatc 20

<210> SEQ ID NO 155
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 155

ctccagtagc atgttggcca 20

<210> SEQ ID NO 156
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 156

ccagagggag gccagtgtgt 20

<210> SEQ ID NO 157
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 157

cacattctgc ttctggaaac 20

<210> SEQ ID NO 158

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 158

ggcccggtac tcattctgca 20

<210> SEQ ID NO 159
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 159

ggagatagga catagttcaa 20

<210> SEQ ID NO 160
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 160

ccagatgtgg gtcttccagg 20

<210> SEQ ID NO 161
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 161

tctaaatcgg atctctctcc 20

<210> SEQ ID NO 162
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 162

agtagcaaag aggactgtgg 20

<210> SEQ ID NO 163
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 163

tgcttgtcac caccagcagt 20

<210> SEQ ID NO 164
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 164

actgagggta catgctggag 20

<210> SEQ ID NO 165
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<212> TYPE: DNA
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<220> FEATURE:
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gctgcggaag gcatcctcct 20

<210> SEQ ID NO 166
<211> LENGTH: 20
<212> TYPE: DNA
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 166

ctggatgagc ctatattgct 20

<210> SEQ ID NO 167
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 167

tgctgagggc tctgttgagg 20

<210> SEQ ID NO 168
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 168

ggctggactt ttcactctgc 20

<210> SEQ ID NO 169
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 169

gtagctgggc cctcggctgc 20

<210> SEQ ID NO 170
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 170

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gtgtaggaca atccacaact 20

<210> SEQ ID NO 171
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 171

tgagggcttg gctgagtgag 20

<210> SEQ ID NO 172
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 172

aggcctccaa tctctgccta 20

<210> SEQ ID NO 173
<211> LENGTH: 20
<212> TYPE: DNA
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 173

ctcttcaagc acctccagga 20

<210> SEQ ID NO 174
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 174

agatagggag ctgcgacagc 20

<210> SEQ ID NO 175
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 175

catctcgggt gcggtaggtg 20

<210> SEQ ID NO 176
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 176

agccactgac acttcgcaca 20

<210> SEQ ID NO 177

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 177

gcacccaaac accaagctca 20

<210> SEQ ID NO 178
<211> LENGTH: 20
<212> TYPE: DNA
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<400> SEQUENCE: 178

agcctgtgtg cacctggaac 20

<210> SEQ ID NO 179
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 179

ctgaacgtag accttgggtt 20

<210> SEQ ID NO 180
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 180

accagcttct tcaatgtggt 20

<210> SEQ ID NO 181
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 181

aagatatctt catgataacg 20

<210> SEQ ID NO 182
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 182

agagcctcgt ggctttgggc 20

What is claimed is:

1. An antisense compound 8 to 30 nucleobases in length targeted to a nucleic acid molecule encoding inducible nitric oxide synthase, wherein said antisense compound specifically hybridizes with and inhibits the expression of inducible nitric oxide synthase.

2. The antisense compound of claim 1 which is an antisense oligonucleotide.

3. The antisense compound of claim 2 wherein the antisense oligonucleotide has a sequence comprising SEQ ID NO: 19, 20, 21, 23, 24, 29, 30, 31, 32, 33, 36, 38, 42, 43, 44, 45, 46, 48, 49, 50, 52, 53, 54, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 96, 98, 99, 100, 101, 103, 105, 106, 107, 109, 113, 117, 118, 125, 127, 131, 132, 135, 137, 138, 140, 148, 152, 153, 168 or 180.

4. The antisense compound of claim 2 wherein the antisense oligonucleotide comprises at least one modified internucleoside linkage.

5. The antisense compound of claim 4 wherein the modified internucleoside linkage is a phosphorothioate linkage.

6. The antisense compound of claim 2 wherein the antisense oligonucleotide comprises at least one modified sugar moiety.

7. The antisense compound of claim 6 wherein the modified sugar moiety is a 2'-O-methoxyethyl sugar moiety.

8. The antisense compound of claim 2 wherein the antisense oligonucleotide comprises at least one modified nucleobase.

9. The antisense compound of claim 8 wherein the modified nucleobase is a 5-methylcytosine.

10. The antisense compound of claim 2 wherein the antisense oligonucleotide is a chimeric oligonucleotide.

11. A composition comprising the antisense compound of claim 1 and a pharmaceutically acceptable carrier or diluent.

12. The composition of claim 11 further comprising a colloidal dispersion system.

13. The composition of claim 11 wherein the antisense compound is an antisense oligonucleotide.

14. A method of inhibiting the expression of inducible nitric oxide synthase in cells or tissues comprising contacting said cells or tissues with the antisense compound of claim 1 so that expression of inducible nitric oxide synthase is inhibited.

15. A method of treating a human having a disease or condition associated with inducible nitric oxide synthase comprising administering to said animal a therapeutically or prophylactically effective amount of the antisense compound of claim 1 so that expression of inducible nitric oxide synthase is inhibited.

16. The method of claim 15 wherein the disease or condition is diabetes.

17. The method of claim 15 wherein the disease or condition is an immunological disorder.

18. The method of claim 15 wherein the disease or condition is a cardiovascular disorder.

19. The method of claim 15 wherein the disease or condition is a neurologic disorder.

20. The method of claim 15 wherein the disease or condition is ischemia/reperfusion injury.

* * * * *