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(54) **ANTISENSE MODULATION OF P38 MITOGEN ACTIVATED PROTEIN KINASE EXPRESSION**

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(57) **ABSTRACT**

Compositions and methods for the treatment and diagnosis of diseases or conditions amenable to treatment through modulation of expression of a gene encoding a p38 mitogen-activated protein kinase (p38 MAPK) are provided. Methods for the treatment and diagnosis of diseases or conditions associated with aberrant expression of one or more p38 MAPKs are also provided.

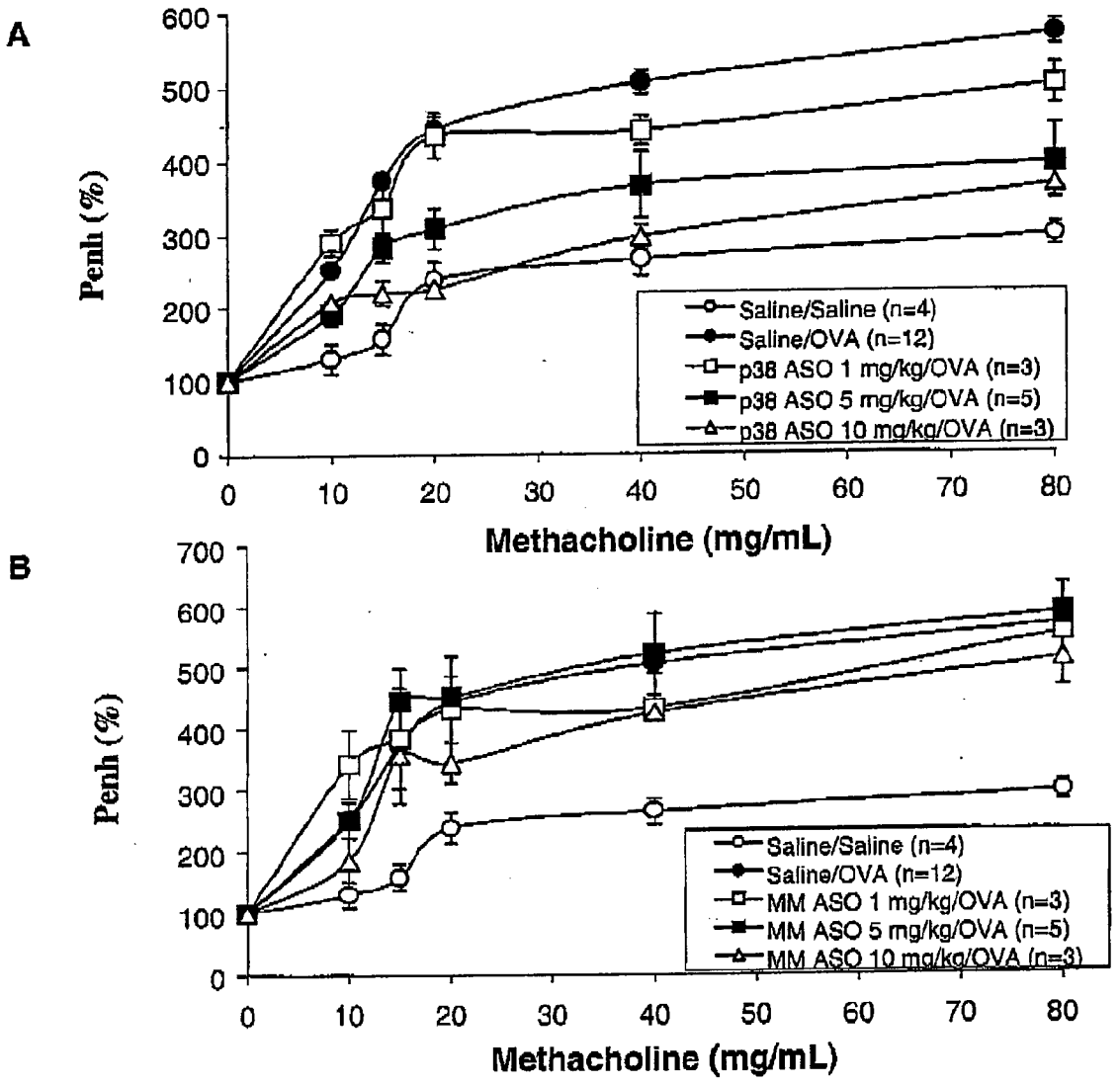

A
Penh (%)
600
500
400
300
200
100
0
0
10
20
30
40
50
60
70
80
Methacholine (mg/mL)
Saline/Saline (n=4)
Saline/OVA (n=12)
p38 ASO 1 mg/kg/OVA (n=3)
p38 ASO 5 mg/kg/OVA (n=5)
p38 ASO 10 mg/kg/OVA (n=3)
B
Penh (%)
700
600
500
400
300
200
100
0
0
10
20
30
40
50
60
70
80
Methacholine (mg/mL)
Saline/Saline (n=4)
Saline/OVA (n=12)
MM ASO 1 mg/kg/OVA (n=3)
MM ASO 5 mg/kg/OVA (n=5)
MM ASO 10 mg/kg/OVA (n=3)

FIGURE 1

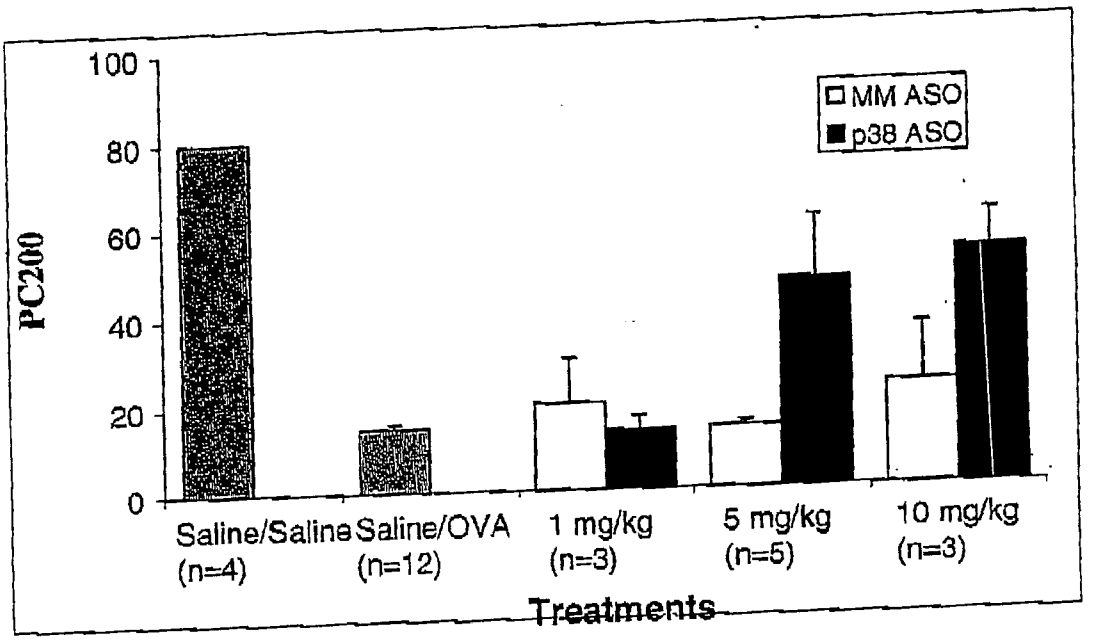

100
80
60
40
20
0
PC200
MM ASO
p38 ASO
Saline/Saline (n=4)
Saline/OVA (n=12)
1 mg/kg (n=3)
5 mg/kg (n=5)
10 mg/kg (n=3)
Treatments

FIGURE 2

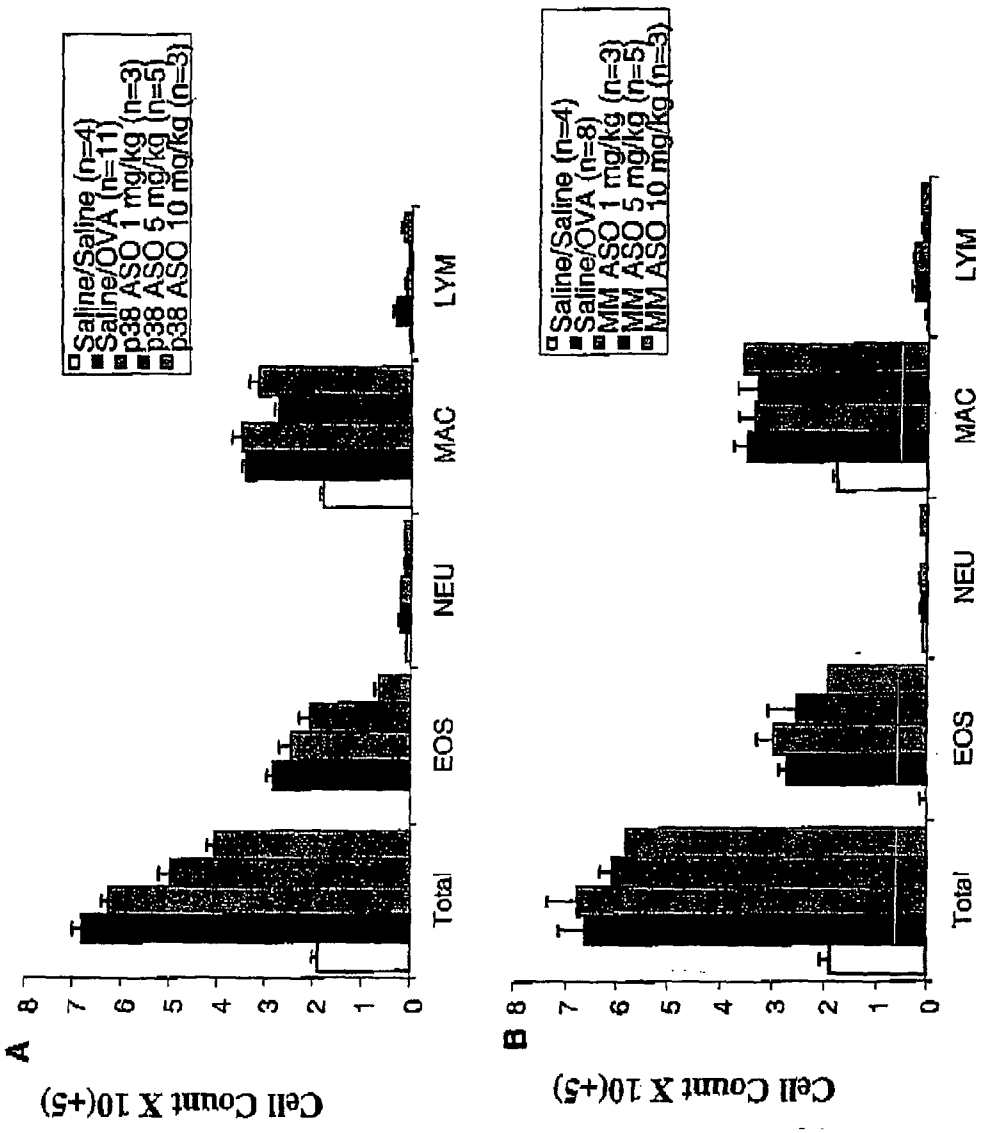

A
Cell Count X 10(+5)
Saline/Saline (n=4)
Saline/OVA (n=11)
p38 ASO 1 mg/kg (n=3)
p38 ASO 5 mg/kg (n=5)
p38 ASO 10 mg/kg (n=3)
Total
EOS
NEU
MAC
LYM
B
Cell Count X 10(+5)
Saline/Saline (n=4)
Saline/OVA (n=8)
MM ASO 1 mg/kg (n=3)
MM ASO 5 mg/kg (n=5)
MM ASO 10 mg/kg (n=3)
Total
EOS
NEU
MAC
LYM

FIGURE 3

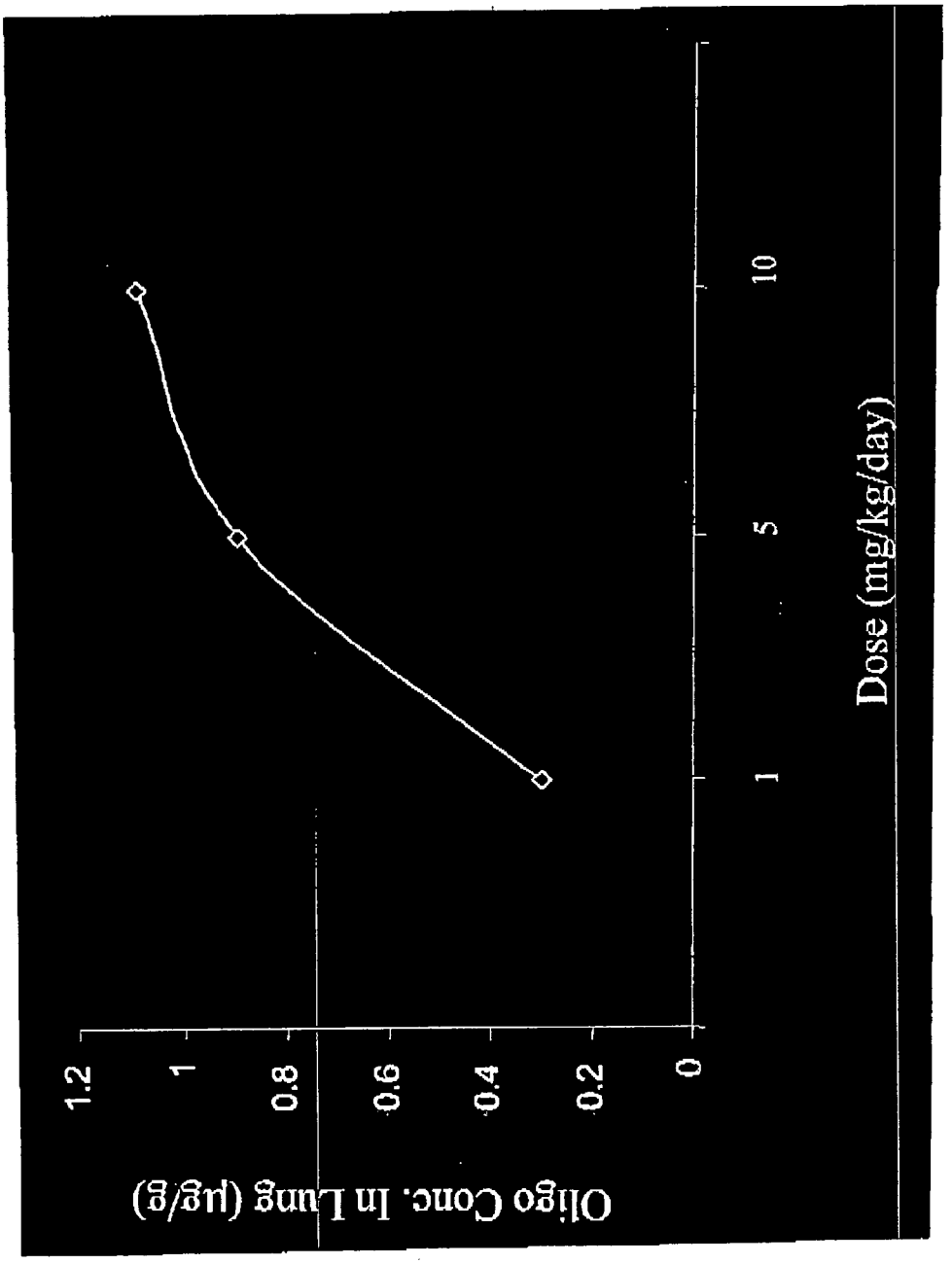



FIGURE 4

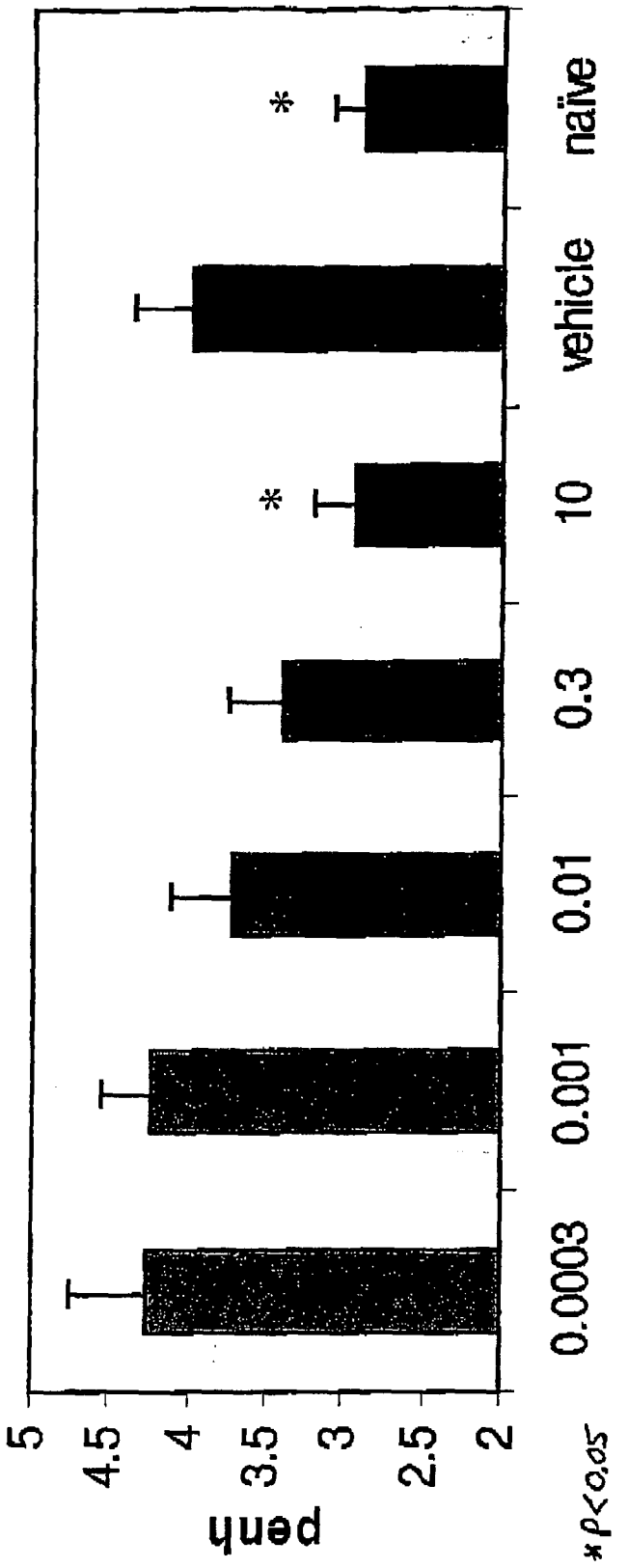



FIGURE 5

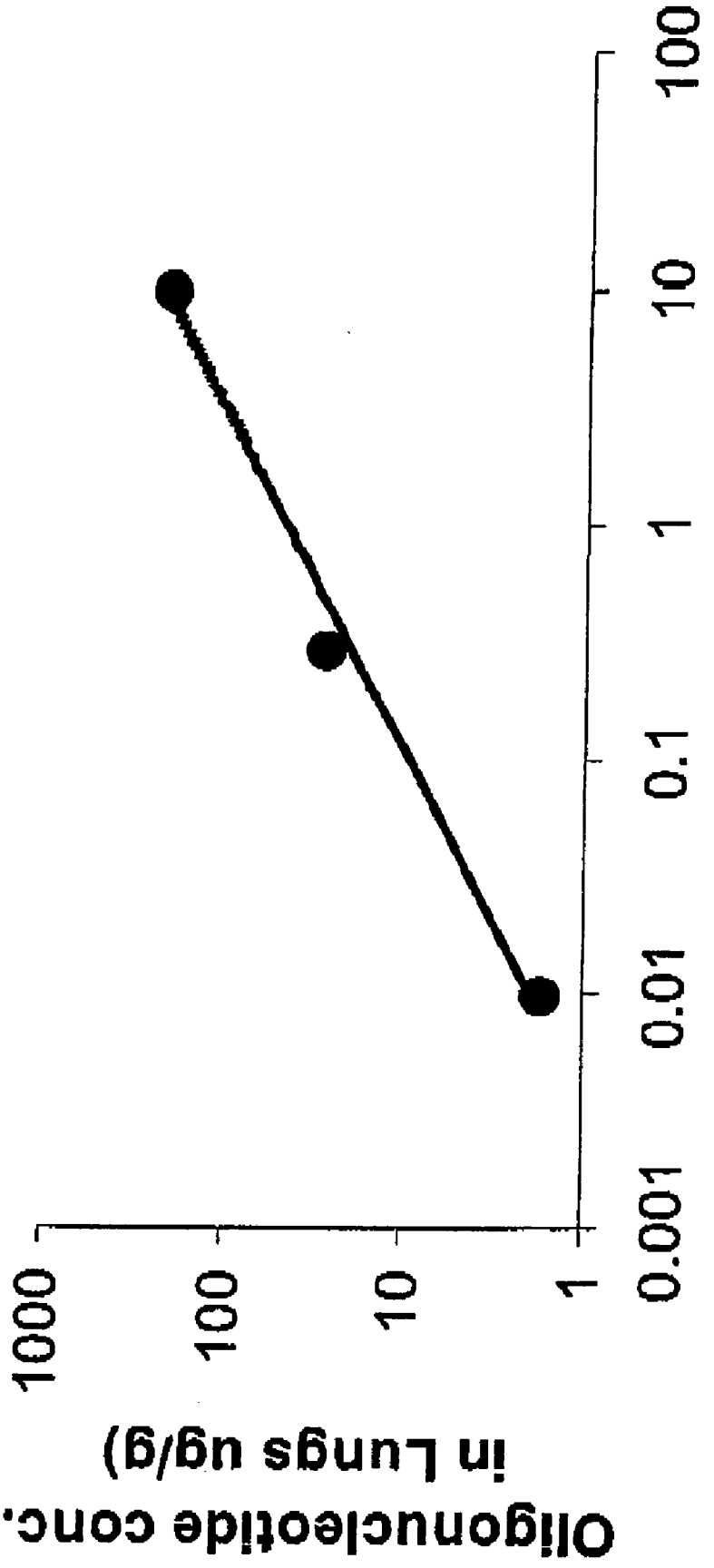

Oligonucleotide conc.
in Lungs ug/g)
1000
100
10
1
0.001
0.01
0.1
1
10
100
Dose (mg/kg/day)

FIGURE 6

# ANTISENSE MODULATION OF P38 MITOGEN ACTIVATED PROTEIN KINASE EXPRESSION

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 10/238,442, filed Sep. 9, 2002, which is a continuation of U.S. patent application Ser. No. 09/640,101 filed Aug. 15, 2000, now issued as U.S. Pat. No. 6,448,079, which is a continuation-in-part of U.S. patent application Ser. No. 09/286,904, filed Apr. 6, 1999, now issued as U.S. Pat. No. 6,140,124.

## FIELD OF THE INVENTION

[0002] This invention relates to compositions and methods for modulating expression of p38 mitogen activated protein kinase genes, a family of naturally present cellular genes involved in signal transduction, and inflammatory and apoptotic responses. This invention is also directed to methods for inhibiting inflammation or apoptosis; these methods can be used diagnostically or therapeutically. Furthermore, this invention is directed to treatment of diseases or conditions associated with expression of p38 mitogen activated protein kinase genes.

## BACKGROUND OF THE INVENTION

[0003] Cellular responses to external factors, such as growth factors, cytokines, and stress conditions, result in altered gene expression. These signals are transmitted from the cell surface to the nucleus by signal transduction pathways. Beginning with an external factor binding to an appropriate receptor, a cascade of signal transduction events is initiated. These responses are mediated through activation of various enzymes and the subsequent activation of specific transcription factors. These activated transcription factors then modulate the expression of specific genes.

[0004] The phosphorylation of enzymes plays a key role in the transduction of extracellular signals into the cell. Mitogen activated protein kinases (MAPKs), enzymes which effect such phosphorylations are targets for the action of growth factors, hormones, and other agents involved in cellular metabolism, proliferation and differentiation (Cobb et al., *J. Biol. Chem.,* 1995, 270, 14843). Mitogen activated protein kinases were initially discovered due to their ability to be tyrosine phosphorylated in response to exposure to bacterial lipopolysaccharides or hyperosmotic conditons (Han et al, *Science,* 1994, 265, 808). These conditions activate inflammatory and apoptotic responses mediated by MAPK. In general, MAP kinases are involved in a variety of signal transduction pathways (sometimes overlapping and sometimes parallel) that function to convey extracellular stimuli to protooncogene products to modulate cellular proliferation and/or differentiation (Seger et al., *FASEB J.,* 1995, 9, 726; Cano et al., *Trends Biochem. Sci.,* 1995, 20, 117).

[0005] One of the MAPK signal transduction pathways involves the MAP kinases p38α and p38β (also known as CSaids Binding Proteins, CSBP). These MAP kinases are responsible for the phosphorylation of ATF-2, MEFC2 and a variety of other cellular effectors that may serve as substrates for p38 MAPK proteins (Kummer et al, *J. Biol. Chem.,* 1997, 272, 20490). Phosphorylation of p38 MAPKs potentiates the ability of these factors to activate transcription (Raingeaud et al, *Mol. Cell Bio.,* 1996, 16, 1247; Han et al, *Nature,* 1997, 386, 296). Among the genes activated by the p38 MAPK signaling pathway is IL-6 (De Cesaris, P., et al., *J. Biol. Chem.,* 1998, 273, 7566-7571).

[0006] Besides p38α and p38β, other p38 MAPK family members have been described, including p38γ (Li et al, *Biochem. Biophys. Res. Commun.,* 1996, 228, 334), and p38δ (Jiang et al, *J. Biol. Chem.,* 1997, 272, 30122). The term "p38" as used herein shall mean a member of the p38 MAPK family, including but not limited to p38α, p38β, p38γ and p38δ, their isoforms (Kumar et al, *Biochem. Biophys. Res. Commun.,* 1997, 235, 533) and other members of the p38 MAPK family of proteins whether they function as p38 MAP kinases per se or not.

[0007] Modulation of the expression of one or more p38 MAPKs is desirable in order to interfere with inflammatory or apoptotic responses associated with disease states and to modulate the transcription of genes stimulated by ATF-2, MEFC2 and other p38 MAPK phosphorylation substrates.

[0008] Inhibitors of p38 MAPKs have been shown to have efficacy in animal models of arthritis (Badger, A. M., et al., *J. Pharmacol. Exp. Ther.,* 1996, 279, 1453-1461) and angiogenesis (Jackson, J. R., et al., *J. Pharmacol. Exp. Ther.,* 1998, 284, 687-692). MacKay, K. and Mochy-Rosen, D. (J. Biol. Chem., 1999, 274, 6272-6279) demonstrate that an inhibitor of p38 MAPKs prevents apoptosis during ischemia in cardiac myocytes, suggesting that p38 MAPK inhibitors can be used for treating ischemic heart disease. p38 MAPK also is required for T-cell HIV-1 replication (Cohen et al, *Mol. Med.,* 1997, 3, 339) and may be a useful target for AIDS therapy. Other diseases believed to be amenable to treatment by inhibitors of p38 MAPKs are disclosed in U.S. Pat. No. 5,559,137, herein incorporated by reference.

[0009] Therapeutic agents designed to target p38 MAPKs include small molecule inhibitors and antisense oligonucleotides. Small molecule inhibitors based on pyridinyl imidazole are described in U.S. Pat. Nos. 5,670,527; 5,658,903; 5,656,644; 5,559,137; 5,593,992; and 5,593,991. WO 98/27098 and WO 99/00357 describe additional small molecule inhibitors, one of which has entered clinical trials. Other small molecule inhibitors are also known.

[0010] Antisense therapy represents a potentially more specific therapy for targeting p38 MAPKs and, in particular, specific p38 MAPK isoforms. Nagata, Y., et al. (*Blood,* 1998, 6, 1859-1869) disclose an antisense phosphothioester oligonucleotide targeted to the translational start site of mouse p38b (p38β). Aoshiba, K., et al. (*J. Immunol.,* 1999, 162, 1692-1700) and Cohen, P. S., et al. (*Mol. Med.,* 1997, 3, 339-346) disclose a phosphorothioate antisense oligonucleotide targeted to the coding regions of human p38α, human p38β and rat p38.

[0011] There remains a long-felt need for improved compositions and methods for modulating the expression of p38 MAP kinases.

## SUMMARY OF THE INVENTION

[0012] The present invention provides antisense compounds which are targeted to nucleic acids encoding a p38 MAPK and are capable of modulating p38 MAPK expression. The present invention also provides oligonucleotides targeted to nucleic acids encoding a p38 MAPK. The present invention also comprises methods of modulating the expression of a p38 MAPK, in cells and tissues, using the oligo-

nucleotides of the invention. Methods of inhibiting p38 MAPK expression are provided; these methods are believed to be useful both therapeutically and diagnostically. These methods are also useful as tools, for example, for detecting and determining the role of p38 MAPKs in various cell functions and physiological processes and conditions and for diagnosing conditions associated with expression of p38 MAPKs.

[0013] The present invention also comprises methods for diagnosing and treating inflammatory diseases, particularly rheumatoid arthritis and asthma. These methods are believed to be useful, for example, in diagnosing p38 MAPK-associated disease progression. These methods employ the oligonucleotides of the invention. These methods are believed to be useful both therapeutically, including prophylactically, and as clinical research and diagnostic tools.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIGS. **1A-1B** are graphs showing the effect of inhaled p38α MAP kinase antisense oligonucleotide ISIS 101757 (ASO, **FIG. 1A**) and mismatched control oligonucleotide ISIS 101758 (MM ASO, **FIG. 1B**) on ovalbumin (OVA)-induced airway hyperresponsiveness in a murine asthma model.

[0015] **FIG. 2** is a graph showing that inhaled ISIS 101757 increases the provocation concentration of methacholine required to achieve doubling of airway reactivity (PC200) in OVA-challenged mice.

[0016] FIGS. **3A-3B** are graphs showing the effect of inhaled ISIS 101757 (**FIG. 3A**) and 101758 (**FIG. 3B**) on immune cells in broncheolar lavage (BAL) fluid of OVA-challenged mice. EOS=eosinpophils, NEU=neutrophils, MAC=macrophages, LYM=lymphocyes.

[0017] **FIG. 4** is a graph showing aerosolized ISIS 101757 concentration in mouse lung vs. dose.

[0018] **FIG. 5** is a graph showing dose-dependent inhibition of the penh response to methacholine (50 mg/ml) challenge by ISIS 101757. ISIS 101757 doses are in mg/kg α-axis).

[0019] **FIG. 6** is a graph showing ISIS 101757 concentration (μg/g) in the lungs vs. dose (intratracheal administration).

## DETAILED DESCRIPTION OF THE INVENTION

[0020] p38 MAPKs play an important role in signal transduction in response to cytokines, growth factors and other cellular stimuli. Specific responses elicited by p38 include inflammatory and apoptotic responses. Modulation of p38 may be useful in the treatment of inflammatory diseases, such as rheumatoid arthritis.

[0021] The present invention employs antisense compounds, particularly oligonucleotides, for use in modulating the function of nucleic acid molecules encoding a p38 MAPK, ultimately modulating the amount of a p38 MAPK produced. This is accomplished by providing oligonucleotides which specifically hybridize with nucleic acids, preferably mRNA, encoding a p38 MAPK.

[0022] The antisense compounds may be used to modulate the function of a particular p38 MAPK isoform, e.g. for research purposes to determine the role of a particular isoform in a normal or disease process, or to treat a disease or condition that may be associated with a particular isoform. It may also be desirable to target multiple p38 MAPK isoforms. In each case, antisense compounds can be designed by taking advantage of sequence homology between the various isoforms. If an antisense compound to a particular isoform is desired, then the antisense compound is designed to a unique region in the desired isoform's gene sequence. With such a compound, it is desirable that this compound does not inhibit the expression of other isoforms. Less desirable, but acceptable, are compounds that do not "substantially" inhibit other isoforms. By "substantially", it is intended that these compounds do not inhibit the expression of other isoforms greater than 25%; more preferred are compounds that do not inhibit other isoforms greater than 10%. If an antisense compound is desired to target multiple p38 isoforms, then regions of significant homology between the isoforms can be used.

[0023] This relationship between an antisense compound such as an oligonucleotide and its complementary nucleic acid target, to which it hybridizes, is commonly referred to as "antisense". "Targeting" an oligonucleotide to a chosen nucleic acid target, in the context of this invention, is a multistep process. The process usually begins with identifying a nucleic acid sequence whose function is to be modulated. This may be, as examples, a cellular gene (or mRNA made from the gene) whose expression is associated with a particular disease state, or a foreign nucleic acid from an infectious agent. In the present invention, the target is a nucleic acid encoding a p38 MAPK; in other words, a p38 MAPK gene or RNA expressed from a p38 MAPK gene. p38 MAPK mRNA is presently the preferred target. The targeting process also includes determination of a site or sites within the nucleic acid sequence for the antisense interaction to occur such that modulation of gene expression will result.

[0024] In accordance with this invention, persons of ordinary skill in the art will understand that messenger RNA includes not only the information to encode a protein using the three letter genetic code, but also associated ribonucleotides which form a region known to such persons as the 5'-untranslated region, the 3'-untranslated region, the 5' cap region and intron/exon junction ribonucleotides. Thus, oligonucleotides may be formulated in accordance with this invention which are targeted wholly or in part to these associated ribonucleotides as well as to the informational ribonucleotides. The oligonucleotide may therefore be specifically hybridizable with a transcription initiation site region, a translation initiation codon region, a 5' cap region, an intron/exon junction, coding sequences, a translation termination codon region or sequences in the 5'- or 3'-untranslated region. 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 formyl-

methionine (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 mRNA molecule transcribed from a gene encoding p38, regardless of the sequence(s) of such codons. 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. This region is a preferred target region. 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. This region is a preferred target region. 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 preferred 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). mRNA splice sites 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 may also be preferred targets.

[0025] Once the target site or 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 modulation.

[0026] "Hybridization", in the context of this invention, means hydrogen bonding, also known as Watson-Crick base pairing, between complementary bases, usually on opposite nucleic acid strands or two regions of a nucleic acid strand. Guanine and cytosine are examples of complementary bases which are known to form three hydrogen bonds between them. Adenine and thymine are examples of complementary bases which form two hydrogen bonds between them.

[0027] "Specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity such that stable and specific binding occurs between the DNA or RNA target and the oligonucleotide.

[0028] It is understood that an oligonucleotide need not be 100% complementary to its target nucleic acid sequence to be specifically hybridizable. An oligonucleotide is specifically hybridizable when binding of the oligonucleotide to the target interferes with the normal function of the target molecule to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays or therapeutic treatment and, in the case of in vitro assays, under conditions in which the assays are conducted.

[0029] Hybridization of antisense oligonucleotides with mRNA interferes with one or more of the normal functions of mRNA. The functions of mRNA 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 by the RNA.

[0030] The overall effect of interference with mRNA function is modulation of p38 MAPK expression. In the context of this invention "modulation" means either inhibition or stimulation; i.e., either a decrease or increase in expression. This modulation can be measured in ways which are routine in the art, for example by Northern blot assay of mRNA expression as taught in the examples of the instant application or by Western blot or ELISA assay of protein expression, or by an immunoprecipitation assay of protein expression, as taught in the examples of the instant application. Effects on cell proliferation or tumor cell growth can also be measured, as taught in the examples of the instant application.

[0031] The oligonucleotides of this invention can be used in diagnostics, therapeutics, prophylaxis, and as research reagents and in kits. Since the oligonucleotides of this invention hybridize to nucleic acids encoding a p38 MAPK, sandwich, calorimetric and other assays can easily be constructed to exploit this fact. Furthermore, since the oligonucleotides of this invention hybridize specifically to nucleic acids encoding particular isoforms of p38 MAPK, such assays can be devised for screening of cells and tissues for particular p38 MAPK isoforms. Such assays can be utilized for diagnosis of diseases associated with various p38 MAPK isoforms. Provision of means for detecting hybridization of oligonucleotide with a p38 MAPK gene or mRNA can routinely be accomplished. Such provision may include enzyme conjugation, radiolabelling or any other suitable detection systems. Kits for detecting the presence or absence of p38 MAPK may also be prepared.

[0032] The present invention is also suitable for diagnosing abnormal inflammatory states in tissue or other samples from patients suspected of having an inflammatory disease such as rheumatoid arthritis. The ability of the oligonucleotides of the present invention to inhibit inflammation may be employed to diagnose such states. A number of assays may be formulated employing the present invention, which assays will commonly comprise contacting a tissue sample with an oligonucleotide of the invention under conditions selected to permit detection and, usually, quantitation of such inhibition. In the context of this invention, to "contact" tissues or cells with an oligonucleotide or oligonucleotides means to add the oligonucleotide(s), usually in a liquid carrier, to a cell suspension or tissue sample, either in vitro

or ex vivo, or to administer the oligonucleotide(s) to cells or tissues within an animal. Similarly, the present invention can be used to distinguish p38 MAPK-associated diseases, from diseases having other etiologies, in order that an efficacious treatment regime can be designed.

[0033] The oligonucleotides of this invention may also be used for research purposes. Thus, the specific hybridization exhibited by the oligonucleotides may be used for assays, purifications, cellular product preparations and in other methodologies which may be appreciated by persons of ordinary skill in the art.

[0034] In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of ribonucleic acid or deoxyribonucleic acid. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent intersugar (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced binding to target and increased stability in the presence of nucleases.

[0035] The antisense compounds in accordance with this invention preferably comprise from about 5 to about 50 nucleobases. Particularly preferred are antisense oligonucleotides comprising from about 8 to about 30 nucleobases (i.e. from about 8 to about 30 linked nucleosides). Preferred embodiments comprise at least an 8-nucleobase portion of a sequence of an antisense compound which inhibits the expression of a p38 mitogen activated kinase. 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.

[0036] While the preferred form of antisense compound is a single-stranded antisense oligonucleotide, in many species the introduction of double-stranded structures, such as double-stranded RNA (dsRNA) molecules, has been shown to induce potent and specific antisense-mediated reduction of the function of a gene or its associated gene products. This phenomenon occurs in both plants and animals and is believed to have an evolutionary connection to viral defense and transposon silencing.

[0037] The first evidence that dsRNA could lead to gene silencing in animals came in 1995 from work in the nematode, *Caenorhabditis elegans* (Guo and Kempheus, Cell, 1995, 81, 611-620). Montgomery et al. have shown that the primary interference effects of dsRNA are posttranscriptional (Montgomery et al., *Proc. Natl. Acad. Sci. USA,* 1998, 95, 15502-15507). The posttranscriptional antisense mechanism defined in *Caenorhabditis elegans* resulting from exposure to double-stranded RNA (dsRNA) has since been designated RNA interference (RNAi). This term has been generalized to mean antisense-mediated gene silencing involving the introduction of dsRNA leading to the sequence-specific reduction of endogenous targeted mRNA levels (Fire et al., *Nature,* 1998, 391, 806-811). Recently, it has been shown that it is, in fact, the single-stranded RNA oligomers of antisense polarity of the dsRNAs which are the potent inducers of RNAi (Tijsterman et al., *Science,* 2002, 295, 694-697). Single stranded and double stranded RNA (RNAi) inhibition of human p38 MAP kinase is also within the scope of the present invention.

[0038] Oligomer and Monomer Modifications

[0039] 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 compound can be further joined to form a circular compound, however, linear compounds are generally preferred. In addition, linear compounds may have internal nucleobase complementarity and may therefore fold in a manner as to produce a fully or partially double-stranded compound. Within oligonucleotides, the phosphate groups are commonly referred to as forming the internucleoside linkage or in conjunction with the sugar ring the backbone of the oligonucleotide. The normal internucleoside linkage that makes up the backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

[0040] Modified Internucleoside Linkages

[0041] Specific examples of preferred antisense oligomeric compounds useful in this invention include oligonucleotides containing modified e.g. non-naturally occurring internucleoside linkages. As defined in this specification, oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom and internucleoside linkages that do not have a phosphorus atom. 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.

[0042] In the *C. elegans* system, modification of the internucleotide linkage (phosphorothioate) did not significantly interfere with RNAi activity. Based on this observation, it is suggested that certain preferred oligomeric compounds of the invention can also have one or more modified internucleoside linkages. A preferred phosphorus containing modified internucleoside linkage is the phosphorothioate internucleoside linkage.

[0043] Preferred modified oligonucleotide backbones containing a phosphorus atom therein include, for example,

phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates and borano-phosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage. Preferred oligonucleotides having inverted polarity comprise a single 3' to 3' linkage at the 3'-most internucleotide linkage i.e. a single inverted nucleoside residue which may be abasic (the nucleobase is missing or has a hydroxyl group in place thereof). Various salts, mixed salts and free acid forms are also included.

[0044] 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; 5,194,599; 5,565,555; 5,527,899; 5,721,218; 5,672,697 and 5,625,050, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

[0045] In more preferred embodiments of the invention, oligomeric compounds have one or more phosphorothioate and/or heteroatom internucleoside linkages, in particular —$CH_2$—NH—O—$CH_2$—, —$CH_2$—N($CH_3$)—O—$CH_2$— [known as a methylene (methylimino) or MMI backbone], —$CH_2$—O—N($CH_3$)—$CH_2$—, —$CH_2$—N($CH_3$)—N($CH_3$)—$CH_2$— and —O—N($CH_3$)—$CH_2$—$CH_2$— [wherein the native phosphodiester internucleotide linkage is represented as —O—P(=O)(OH)—O—$CH_2$—]. The MMI type internucleoside linkages are disclosed in the above referenced U.S. Pat. No. 5,489,677. Preferred amide internucleoside linkages are disclosed in the above referenced U.S. Pat. No. 5,602,240.

[0046] 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; riboacetyl 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.

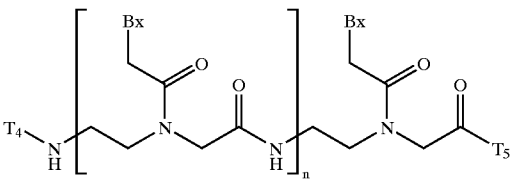
[0047] Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 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; 5,792,608; 5,646,269 and 5,677,439, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

[0048] Oligomer Mimetics

[0049] Another preferred group of oligomeric compounds amenable to the present invention includes oligonucleotide mimetics. The term mimetic as it is applied to oligonucleotides is intended to include oligomeric compounds wherein only the furanose ring or both the furanose ring and the internucleotide linkage are replaced with novel groups, replacement of only the furanose ring is also referred to in the art as being a sugar surrogate. The heterocyclic base moiety or a modified heterocyclic base moiety is maintained for hybridization with an appropriate target nucleic acid. 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 oligomeric 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 oligomeric 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 oligomeric compounds can be found in Nielsen et al., *Science,* 1991, 254, 1497-1500.

[0050] One oligonucleotide mimetic that has been reported to have excellent hybridization properties is peptide nucleic acids (PNA). The backbone in PNA compounds is two or more linked aminoethylglycine units which gives PNA an amide containing backbone. The heterocyclic base moieties 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.

[0051] PNA has been modified to incorporate numerous modifications since the basic PNA structure was first prepared. The basic structure is shown below:




[0052] wherein

[0053] Bx is a heterocyclic base moiety;

[0054] $T_4$ is hydrogen, an amino protecting group, —C(O)$R_5$, substituted or unsubstituted $C_1$-$C_{10}$ alkyl,

substituted or unsubstituted $C_2$-$C_{10}$ alkenyl, substituted or unsubstituted $C_2$-$C_{10}$ alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group, a reporter group, a conjugate group, a D or L α-amino acid linked via the α-carboxyl group or optionally through the ω-carboxyl group when the amino acid is aspartic acid or glutamic acid or a peptide derived from D, L or mixed D and L amino acids linked through a carboxyl group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

[0055] $T_5$ is —OH, —N($Z_1$) $Z_2$, $R_5$, D or L α-amino acid linked via the α-amino group or optionally through the ω-amino group when the amino acid is lysine or ornithine or a peptide derived from D, L or mixed D and L amino acids linked through an amino group, a chemical functional group, a reporter group or a conjugate group;

[0056] $Z_1$ is hydrogen, $C_1$-$C_6$ alkyl, or an amino protecting group;

[0057] $Z_2$ is hydrogen, $C_1$-$C_6$ alkyl, an amino protecting group, —C(=O)$_n$—(CH$_2$)$_n$-J-$Z_3$, a D or L α-amino acid linked via the α-carboxyl group or optionally through the ω-carboxyl group when the amino acid is aspartic acid or glutamic acid or a peptide derived from D, L or mixed D and L amino acids linked through a carboxyl group;

[0058] $Z_3$ is hydrogen, an amino protecting group, —$C_1$-$C_6$ alkyl, —C(=O)—$CH_3$, benzyl, benzoyl, or —(CH$_2$)$_n$—N(H)$Z_1$;

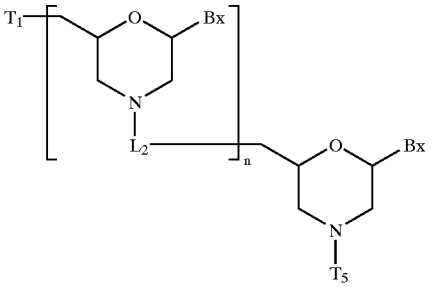
[0059] each J is O, S or NH;

[0060] $R_5$ is a carbonyl protecting group; and

[0061] n is from 2 to about 50.

[0062] Another class of oligonucleotide mimetic that has been studied is based on linked morpholino units (morpholino nucleic acid) having heterocyclic bases attached to the morpholino ring. A number of linking groups have been reported that link the morpholino monomeric units in a morpholino nucleic acid. A preferred class of linking groups have been selected to give a non-ionic oligomeric compound. The non-ionic morpholino-based oligomeric compounds are less likely to have undesired interactions with cellular proteins. Morpholino-based oligomeric compounds are non-ionic mimics of oligonucleotides which are less likely to form undesired interactions with cellular proteins (Dwaine A. Braasch and David R. Corey, *Biochemistry,* 2002, 41 (14), 4503-4510). Morpholino-based oligomeric compounds are disclosed in U.S. Pat. No. 5,034,506, issued Jul. 23, 1991. The morpholino class of oligomeric compounds have been prepared having a variety of different linking groups joining the monomeric subunits.

[0063] Morpholino nucleic acids have been prepared having a variety of different linking groups ($L_2$) joining the monomeric subunits. The basic formula is shown below:




[0064] wherein

[0065] $T_1$ is hydroxyl or a protected hydroxyl;

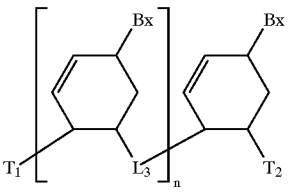
[0066] $T_5$ is hydrogen or a phosphate or phosphate derivative;

[0067] $L_2$ is a linking group; and

[0068] n is from 2 to about 50.

[0069] A further class of oligonucleotide mimetic is referred to as cyclohexenyl nucleic acids (CeNA). The furanose ring normally present in an DNA/RNA molecule is replaced with a cyclohenyl ring. CeNA DMT protected phosphoramidite monomers have been prepared and used for oligomeric compound synthesis following classical phosphoramidite chemistry. Fully modified CeNA oligomeric compounds and oligonucleotides having specific positions modified with CeNA have been prepared and studied (see Wang et al., *J. Am. Chem. Soc.,* 2000, 122, 8595-8602). In general the incorporation of CeNA monomers into a DNA chain increases its stability of a DNA/RNA hybrid. CeNA oligoadenylates formed complexes with RNA and DNA complements with similar stability to the native complexes. The study of incorporating CeNA structures into natural nucleic acid structures was shown by NMR and circular dichrdism to proceed with easy conformational adaptation. Furthermore the incorporation of CeNA into a sequence targeting RNA was stable to serum and able to activate *E. Coli* RNase resulting in cleavage of the target RNA strand.

[0070] The general formula of CeNA is shown below:




[0071] wherein

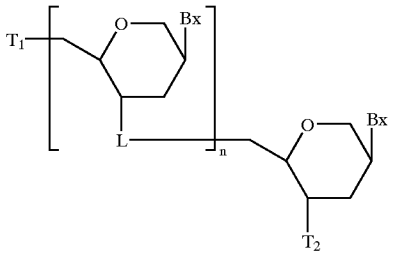
[0072] each Bx is a heterocyclic base moiety;

[0073] $T_1$ is hydroxyl or a protected hydroxyl; and

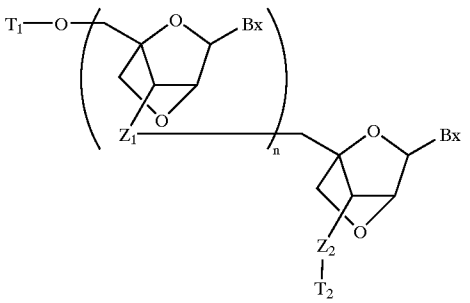
[0074] T2 is hydroxyl or a protected hydroxyl.

[0075] Another class of oligonucleotide mimetic (anhydrohexitol nucleic acid) can be prepared from one or more

anhydrohexitol nucleosides (see, Wouters and Herdewijn, *Bioorg. Med. Chem. Lett.,* 1999, 9, 1563-1566) and would have the general formula:




[**0076**] A further preferred modification includes Locked Nucleic Acids (LNAs) in which the 2'-hydroxyl group is linked to the 4' carbon atom of the sugar ring thereby forming a 2'-C,4'-C-oxymethylene linkage thereby forming a bicyclic sugar moiety. The linkage is preferably a methylene (—$CH_2$—)$_n$ group bridging the 2' oxygen atom and the 4' carbon atom wherein n is 1 or 2 (Singh et al., Chem. Commun., 1998, 4, 455-456). LNA and LNA analogs display very high duplex thermal stabilities with complementary DNA and RNA (Tm=+3 to +10 C), stability towards 3'-exonucleolytic degradation and good solubility properties. The basic structure of LNA showing the bicyclic ring system is shown below:




[**0077**] The conformations of LNAs determined by 2D NMR spectroscopy have shown that the locked orientation of the LNA nucleotides, both in single-stranded LNA and in duplexes, constrains the phosphate backbone in such a way as to introduce a higher population of the N-type conformation (Petersen et al., J. Mol. Recognit., 2000, 13, 44-53). These conformations are associated with improved stacking of the nucleobases (Wengel et al., Nucleosides Nucleotides, 1999, 18, 1365-1370).

[**0078**] LNA has been shown to form exceedingly stable LNA:LNA duplexes (Koshkin et al., J. Am. Chem. Soc., 1998, 120, 13252-13253). LNA:LNA hybridization was shown to be the most thermally stable nucleic acid type duplex system, and the RNA-mimicking character of LNA was established at the duplex level. Introduction of 3 LNA monomers (T or A) significantly increased melting points (Tm=+15/+11) toward DNA complements. The universality of LNA-mediated hybridization has been stressed by the formation of exceedingly stable LNA:LNA duplexes. The RNA-mimicking of LNA was reflected with regard to the N-type conformational restriction of the monomers and to the secondary structure of the LNA:RNA duplex.

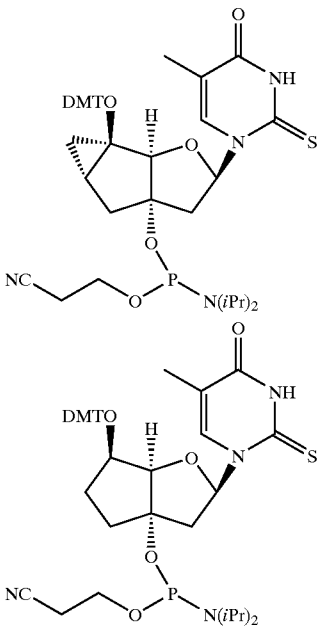
[**0079**] LNAs also form duplexes with complementary DNA, RNA or LNA with high thermal affinities. Circular dichroism (CD) spectra show that duplexes involving fully modified LNA (esp. LNA:RNA) structurally resemble an A-form RNA:RNA duplex. Nuclear magnetic resonance (NMR) examination of an LNA:DNA duplex confirmed the 3'-endo conformation of an LNA monomer. Recognition of double-stranded DNA has also been demonstrated suggesting strand invasion by LNA. Studies of mismatched sequences show that LNAs obey the Watson-Crick base pairing rules with generally improved selectivity compared to the corresponding unmodified reference strands.

[**0080**] Novel types of LNA-oligomeric compounds, as well as the LNAs, are useful in a wide range of diagnostic and therapeutic applications. Among these are antisense applications, PCR applications, strand-displacement oligomers, substrates for nucleic acid polymerases and generally as nucleotide based drugs. Potent and nontoxic antisense oligonucleotides containing LNAs have been described (Wahlestedt et al., Proc. Natl. Acad. Sci. U.S. A., 2000, 97, 5633-5638.) The authors have demonstrated that LNAs confer several desired properties to antisense agents. LNA/DNA copolymers were not degraded readily in blood serum and cell extracts. LNA/DNA copolymers exhibited potent antisense activity in assay systems as disparate as G-protein-coupled receptor signaling in living rat brain and detection of reporter genes in *Escherichia coli*. Lipofectin-mediated efficient delivery of LNA into living human breast cancer cells has also been accomplished.

[**0081**] The synthesis and preparation of the LNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (Koshkin et al., Tetrahedron, 1998, 54, 3607-3630). LNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

[**0082**] The first analogs of LNA, phosphorothioate-LNA and 2'-thio-LNAs, have also been prepared (Kumar et al., Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222). Preparation of locked nucleoside analogs containing oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (Wengel et al., PCT International Application WO 98-DK393 19980914). Furthermore, synthesis of 2'-amino-LNA, a novel conformationally restricted high-affinity oligonucleotide analog with a handle has been described in the art (Singh et al., J. Org. Chem., 1998, 63, 10035-10039). In addition, 2'-Amino- and 2'-methylamino-LNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

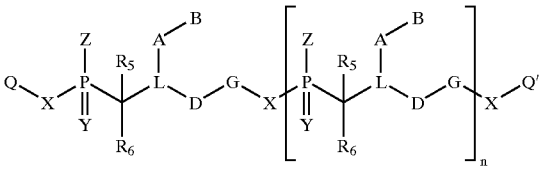
[**0083**] Further oligonucleotide mimetics have been prepared to include bicyclic and tricyclic nucleoside analogs having the formulas (amidite monomers shown):



[0084] (see Steffens et al., *Helv. Chim. Acta,* 1997, 80, 2426-2439; Steffens et al., *J. Am. Chem. Soc.,* 1999, 121, 3249-3255; and Renneberg et al., *J. Am. Chem. Soc.,* 2002, 124, 5993-6002). These modified nucleoside analogs have been oligomerized using the phosphoramidite approach and the resulting oligomeric compounds containing tricyclic nucleoside analogs have shown increased thermal stabilities (Tm's) when hybridized to DNA, RNA and itself. Oligomeric compounds containing bicyclic nucleoside analogs have shown thermal stabilities approaching that of DNA duplexes.

[0085] Another class of oligonucleotide mimetic is referred to as phosphonomonoester nucleic acids incorporate a phosphorus group in a backbone the backbone. This class of olignucleotide mimetic is reported to have useful physical and biological and pharmacological properties in the areas of inhibiting gene expression (antisense oligonucleotides, ribozymes, sense oligonucleotides and triplex-forming oligonucleotides), as probes for the detection of nucleic acids and as auxiliaries for use in molecular biology.

[0086] The general formula (for definitions of Markush variables see: U.S. Pat. Nos. 5,874,553 and 6,127,346 herein incorporated by reference in their entirety) is shown below.



[0087] Another oligonucleotide mimetic has been reported wherein the furanosyl ring has been replaced by a cyclobutyl moiety.

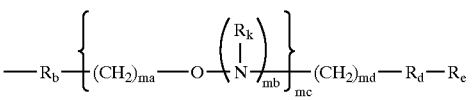
[0088] Modified Sugars

[0089] Oligomeric compounds of the invention may also contain one or more substituted sugar moieties. Preferred oligomeric compounds comprise a sugar substituent group selected from: 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 a sugar substituent group selected from: $C_1$ to $C_{10}$ lower alkyl, substituted lower alkyl, alkenyl, alkynyl, 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'-C—$CH_2CH_2OCH_3$, 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-dimethylamino-ethoxy-ethyl or 2'-DMAEOE), i.e., 2'-O—$CH_2$—O—$CH_2$—$N(CH_3)_2$.

[0090] Other preferred sugar substituent groups include methoxy (—O—$CH_3$), aminopropoxy (—$OCH_2CH_2CH_2NH_2$), allyl (—$CH_2$—CH=$CH_2$), —O-allyl (—O—$CH_2$—CH=$CH_2$) and fluoro (F). 2'-Sugar substituent groups may be in the arabino (up) position or ribo (down) position. A preferred 2'-arabino modification is 2'-F. Similar modifications may also be made at other positions on the oligomeric compound, particularly the 3' position of the sugar on the 3' terminal nucleoside or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligomeric compounds 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; 5,792,747; 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.

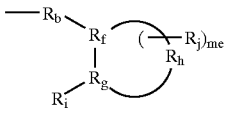
[0091] Further representative sugar substituent groups include groups of formula $I_a$ or $II_a$:

Ia



-continued

IIa



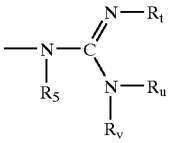
[0092] wherein:

[0093] $R_b$ is O, S or NH;

[0094] $R_d$ is a single bond, O, S or C(=O);

[0095] $R_e$ is $C_1$-$C_{10}$ alkyl, $N(R_k)(R_m)$, $N(R_k)(R_n)$, $N{=}C(R_p)(R_q)$, $N{=}C(R_p)(R_r)$ or has formula $III_a$;

IIIa



[0096] $R_p$ and $R_q$ are each independently hydrogen or $C_1$-$C_{10}$ alkyl;

[0097] $R_r$ is $—R_x—R_y$;

[0098] each $R_s$, $R_t$, $R_u$ and $R_v$ is, independently, hydrogen, $C(O)R_w$, substituted or unsubstituted $C_1$-$C_{10}$ alkyl, substituted or unsubstituted $C_2$-$C_{10}$ alkenyl, substituted or unsubstituted $C_2$-$C_{10}$ alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

[0099] or optionally, $R_u$ and $R_v$, together form a phthalimido moiety with the nitrogen atom to which they are attached;

[0100] each $R_w$ is, independently, substituted or unsubstituted $C_1$-$C_{10}$ alkyl, trifluoromethyl, cyanoethyloxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or aryl;

[0101] $R_k$ is hydrogen, a nitrogen protecting group or $—R_x—R_y$;

[0102] $R_p$ is hydrogen, a nitrogen protecting group or $—R_x—R_y$;

[0103] $R_x$ is a bond or a linking moiety;

[0104] $R_y$ is a chemical functional group, a conjugate group or a solid support medium;

[0105] each $R_m$ and $R_n$ is, independently, H, a nitrogen protecting group, substituted or unsubstituted $C_1$-$C_{10}$ alkyl, substituted or unsubstituted $C_2$-$C_{10}$ alkenyl, substituted or unsubstituted $C_2$-$C_{10}$ alkynyl, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl, alkynyl; $NH_3^+$, $N(R_u)(R_v)$, guanidino and acyl where said acyl is an acid amide or an ester;

[0106] or $R_m$ and $R_n$, together, are a nitrogen protecting group, are joined in a ring structure that optionally includes an additional heteroatom selected from N and O or are a chemical functional group;

[0107] $R_i$ is $OR_z$, $SR_z$, or $N(R_z)_2$;

[0108] each $R_z$ is, independently, H, $C_1$-$C_8$ alkyl, $C_1$-$C_8$ haloalkyl, $C(=NH)N(H)R_u$, $C(=O)N(H)R_u$ or $OC(=O)N(H)R_u$;

[0109] $R_f$, $R_g$ and $R_h$ comprise a ring system having from about 4 to about 7 carbon atoms or having from about 3 to about 6 carbon atoms and 1 or 2 heteroatoms wherein said heteroatoms are selected from oxygen, nitrogen and sulfur and wherein said ring system is aliphatic, unsaturated aliphatic, aromatic, or saturated or unsaturated heterocyclic;

[0110] $R_j$ is alkyl or haloalkyl having 1 to about 10 carbon atoms, alkenyl having 2 to about 10 carbon atoms, alkynyl having 2 to about 10 carbon atoms, aryl having 6 to about 14 carbon atoms, $N(R_k)(R_m)$ $OR_k$, halo, $SR_k$ or CN;

[0111] $m_a$ is 1 to about 10;

[0112] each mb is, independently, 0 or 1;

[0113] mc is 0 or an integer from 1 to 10;

[0114] md is an integer from 1 to 10;

[0115] me is from 0, 1 or 2; and

[0116] provided that when mc is 0, md is greater than 1.

[0117] Representative substituents groups of Formula I are disclosed in United States patent application Ser. No. 09/130,973, filed Aug. 7, 1998, entitled "Capped 2'-Oxyethoxy Oligonucleotides," hereby incorporated by reference in its entirety.

[0118] Representative cyclic substituent groups of Formula II are disclosed in U.S. patent application Ser. No. 09/123,108, filed Jul. 27, 1998, entitled "RNA Targeted 2'-Oligomeric compounds that are Conformationally Preorganized," hereby incorporated by reference in its entirety.

[0119] Particularly preferred sugar substituent groups include $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10.

[0120] Representative guanidino substituent groups that are shown in formula III and IV are disclosed in co-owned U.S. patent application Ser. No. 09/349,040, entitled "Functionalized Oligomers", filed Jul. 7, 1999, hereby incorporated by reference in its entirety.

[0121] Representative acetamido substituent groups are disclosed in U.S. Pat. No. 6,147,200 which is hereby incorporated by reference in its entirety.

[0122] Representative dimethylaminoethyloxyethyl substituent groups are disclosed in International Patent Application PCT/US99/17895, entitled "2'-O-Dimethylaminoet-

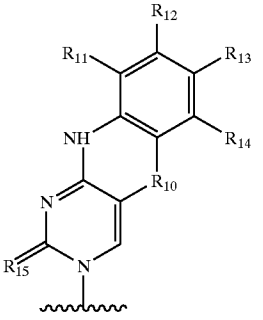
hyloxyethyl-Oligomeric compounds", filed Aug. 6, 1999, hereby incorporated by reference in its entirety.

[0123] Modified Nucleobases/Naturally Occurring Nucleobases

[0124] Oligomeric compounds may also include nucleobase (often referred to in the art simply as "base" or "heterocyclic base moiety") 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 also referred herein as heterocyclic base moieties 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 (—C≡C—$CH_3$) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 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, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

[0125] Heterocyclic base moieties may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. 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.

[0126] In one aspect of the present invention oligomeric compounds are prepared having polycyclic heterocyclic compounds in place of one or more heterocyclic base moieties. A number of tricyclic heterocyclic compounds have been previously reported. These compounds are routinely used in antisense applications to increase the binding properties of the modified strand to a target strand. The most studied modifications are targeted to guanosines hence they have been termed G-clamps or cytidine analogs. Many of these polycyclic heterocyclic compounds have the general formula:




[0127] Representative cytosine analogs that make 3 hydrogen bonds with a guanosine in a second strand include 1,3-diazaphenoxazine-2-one ($R_{10}$=O, $R_{11}$—$R_{14}$=H) [Kurchavov, et al., *Nucleosides and Nucleotides,* 1997, 16, 1837-1846], 1,3-diazaphenothiazine-2-one ($R_{10}$=S, $R_{11}$—$R_{14}$=H), [Lin, K.-Y.; Jones, R. J.; Matteucci, M. J. Am. Chem. Soc. 1995, 117, 3873-3874] and 6,7,8,9-tetrafluoro-1,3-diazaphenoxazine-2-one ($R_{10}$=O, $R_{11}$—$R_{14}$=F) [Wang, J.; Lin, K.-Y., Matteucci, M. Tetrahedron Lett. 1998, 39, 8385-8388]. Incorporated into oligonucleotides these base modifications were shown to hybridize with complementary guanine and the latter was also shown to hybridize with adenine and to enhance helical thermal stability by extended stacking interactions (also see U.S. Patent Application entitled "Modified Peptide Nucleic Acids" filed May 24, 2002, Ser. No. 10/155,920; and U.S. Patent Application entitled "Nuclease Resistant Chimeric Oligonucleotides" filed May 24, 2002, Ser. No. 10/013,295, both of which are commonly owned with this application and are herein incorporated by reference in their entirety).

[0128] Further helix-stabilizing properties have been observed when a cytosine analog/substitute has an aminoethoxy moiety attached to the rigid 1,3-diazaphenoxazine-2-one scaffold ($R_{10}$=O, $R_{11}$=—O—$(CH_2)_2$—$NH_2$, $R_{12-14}$=H) [Lin, K.-Y.; Matteucci, M. J. Am. Chem. Soc. 1998, 120, 8531-8532]. Binding studies demonstrated that a single incorporation could enhance the binding affinity of a model oligonucleotide to its complementary target DNA or RNA with a $\Delta T_m$ of up to 18° relative to 5-methyl cytosine ($dC5^{me}$), which is the highest known affinity enhancement for a single modification, yet. On the other hand, the gain in helical stability does not compromise the specificity of the oligonucleotides. The $T_m$ data indicate an even greater discrimination between the perfect match and mismatched sequences compared to $dC5^{me}$. It was suggested that the tethered amino group serves as an additional hydrogen bond donor to interact with the Hoogsteen face, namely the O6, of a complementary guanine thereby forming 4 hydrogen bonds. This means that the increased affinity of G-clamp is mediated by the combination of extended base stacking and additional specific hydrogen bonding.

[0129] Further tricyclic heterocyclic compounds and methods of using them that are amenable to the present invention are disclosed in U.S. Pat. Ser. No. 6,028,183, which issued on May 22, 2000, and U.S. Pat. Ser. No. 6,007,992, which issued on Dec. 28, 1999, the contents of both are commonly assigned with this application and are incorporated herein in their entirety.

[0130] The enhanced binding affinity of the phenoxazine derivatives together with their uncompromised sequence specificity makes them valuable nucleobase analogs for the development of more potent antisense-based drugs. In fact, promising data have been derived from in vitro experiments demonstrating that heptanucleotides containing phenoxazine substitutions are capable to activate RNaseH, enhance cellular uptake and exhibit an increased antisense activity [Lin, K-Y; Matteucci, M. J. Am. Chem. Soc. 1998, 120, 8531-8532]. The activity enhancement was even more pronounced in case of G-clamp, as a single substitution was shown to significantly improve the in vitro potency of a 20mer 2'-deoxyphosphorothioate oligonucleotides [Flanagan, W. M.; Wolf, J. J.; Olson, P.; Grant, D.; Lin, K.-Y.; Wagner, R. W.; Matteucci, M. Proc. Natl. Acad. Sci. USA, 1999, 96, 3513-3518]. Nevertheless, to optimize oligonucleotide design and to better understand the impact of these heterocyclic modifications on the biological activity, it is important to evaluate their effect on the nuclease stability of the oligomers.

[0131] Further modified polycyclic heterocyclic compounds useful as heterocyclcic bases are disclosed in but 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; 5,434,257; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,645,985; 5,646,269; 5,750,692; 5,830,653; 5,763,588; 6,005,096; and 5,681,941, and U.S. patent application Ser. No. 09/996,292 filed Nov. 28, 2001, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference.

[0132] The oligonucleotides of the present invention also include variants in which a different base is present at one or more of the nucleotide positions in the oligonucleotide. For example, if the first nucleotide is an adenosine, variants may be produced which contain thymidine, guanosine or cytidine at this position. This may be done at any of the positions of the oligonucleotide. Thus, a 20-mer may comprise 60 variations (20 positions×3 alternates at each position) in which the original nucleotide is substituted with any of the three alternate nucleotides. These oligonucleotides are then tested using the methods described herein to determine their ability to inhibit expression of HCV mRNA and/or HCV replication.

[0133] Conjugates

[0134] A further preferred substitution that can be appended to the oligomeric compounds of the invention involves the linkage of one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the resulting oligomeric compounds. In one embodiment such modified oligomeric compounds are prepared by covalently attaching conjugate groups to functional groups such as hydroxyl or amino groups. Conjugate groups of the invention include intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Typical conjugates groups include cholesterols, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. Groups that enhance the pharmacodynamic properties, in the context of this invention, include groups that improve oligomer uptake, enhance oligomer resistance to degradation, and/or strengthen sequence-specific hybridization with RNA. Groups that enhance the pharmacokinetic properties, in the context of this invention, include groups that improve oligomer uptake, distribution, metabolism or excretion. Representative conjugate groups are disclosed in International Patent Application PCT/US92/09196, filed Oct. 23, 1992 the entire disclosure of which is incorporated herein by reference. Conjugate 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.

[0135] The oligomeric compounds of the invention may also be conjugated to active drug substances, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fenbufen, ketoprofen, (S)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, flufenamic acid, folinic acid, a benzothiadiazide, chlorothiazide, a diazepine, indomethicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial or an antibiotic. Oligonucleotide-drug conjugates and their preparation are described in United States patent application Ser. No. 09/334,130 (filed Jun. 15, 1999) which is incorporated herein by reference in its entirety.

[0136] 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.

[0137] Chimeric Oligomeric Compounds

[0138] It is not necessary for all positions in an oligomeric compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single oligomeric compound or even at a single monomeric subunit such as a nucleoside within a oligomeric compound. The present invention also includes oligomeric compounds which are chimeric oligomeric compounds. "Chimeric" oligomeric compounds or "chimeras," in the context of this invention, are oligomeric compounds that contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of a nucleic acid based oligomer.

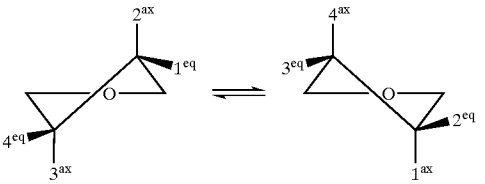
[0139] Chimeric oligomeric compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the oligomeric compound may serve as a substrate 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 inhibition of gene expression. Consequently, comparable results can often be obtained with shorter oligomeric compounds when chimeras are used, compared to for example 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.

[0140] Chimeric oligomeric compounds of the invention may be formed as composite structures of two or more oligonucleotides, oligonucleotide analogs, oligonucleosides and/or oligonucleotide mimetics as described above. Such oligomeric compounds have also been referred to in the art as hybrids hemimers, gapmers or inverted 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.

[0141] 3'-Endo Modifications

[0142] In one aspect of the present invention oligomeric compounds include nucleosides synthetically modified to induce a 3'-endo sugar conformation. A nucleoside can incorporate synthetic modifications of the heterocyclic base, the sugar moiety or both to induce a desired 3'-endo sugar conformation. These modified nucleosides are used to mimic RNA like nucleosides so that particular properties of an oligomeric compound can be enhanced while maintaining the desirable 3'-endo conformational geometry. There is an apparent preference for an RNA type duplex (A form helix, predominantly 3'-endo) as a requirement (e.g. trigger) of RNA interference which is supported in part by the fact that duplexes composed of 2'-deoxy-2'-F-nucleosides appears efficient in triggering RNAi response in the *C. elegans* system. Properties that are enhanced by using more stable 3'-endo nucleosides include but aren't limited to modulation of pharmacokinetic properties through modification of protein binding, protein off-rate, absorption and clearance; modulation of nuclease stability as well as chemical stability; modulation of the binding affinity and specificity of the oligomer (affinity and specificity for enzymes as well as for complementary sequences); and increasing efficacy of RNA cleavage. The present invention provides oligomeric triggers of RNAi having one or more nucleosides modified in such a way as to favor a C3'-endo type conformation.

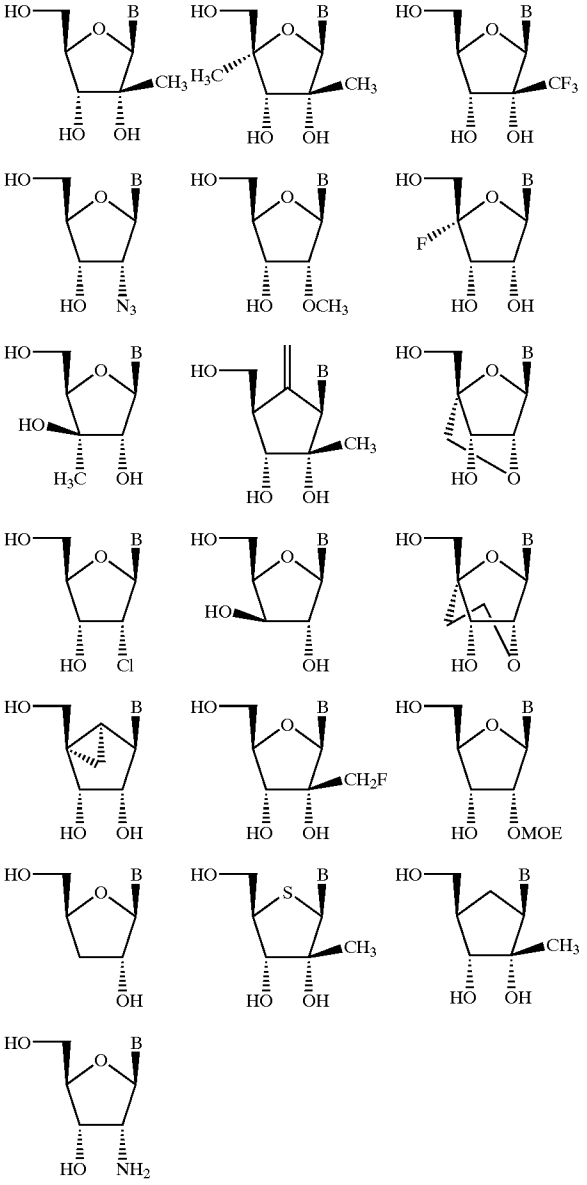
Scheme 1




C2'-endo/Southern C3'-endo/Northern

[0143] Nucleoside conformation is influenced by various factors including substitution at the 2', 3' or 4'-positions of the pentofuranosyl sugar. Electronegative substituents generally prefer the axial positions, while sterically demanding substituents generally prefer the equatorial positions (Principles of Nucleic Acid Structure, Wolfgang Sanger, 1984, Springer-Verlag.) Modification of the 2' position to favor the 3'-endo conformation can be achieved while maintaining the 2'-OH as a recognition element, as illustrated in **FIG. 2**, below (Gallo et al., Tetrahedronl (2001), 57, 5707-5713. Harry- O'kuru et al., J. Org. Chem., (1997), 62(6), 1754-1759 and Tang et al., J. Org. Chem (1999), 64, 747-754.) Alternatively, preference for the 3'-endo conformation can be achieved by deletion of the 2'-OH as exemplified by 2'deoxy-2' F-nucleosides (Kawasaki et al., J. Med. Chel . (1993), 36, 831-841), which adopts the 3'-endo conformation positioning the electronegative fluorine atom in the axial position. Other modifications of the ribose ring, for example substitution at the 4'-position to give 4'-F modified nucleosides (Guillerm et al., Bioorganic and Medicinal Chemistry Lettersl (1995), 5, 1455-1460 and Owen et al., J. Org. Chem . (1976), 41, 3010-3017), or for example modification to yield methanocarba nucleoside analogs (Jacobson et al., J. Med. Chem. Lett . (2000), 43, 2196-2203 and Lee et al., Bioorganic and Medicinal Chemistry Letters (2001), 11, 1333-1337) also induce preference for the 3'-endo conformation. Along similar lines, oligomeric triggers of RNAi response might be composed of one or more nucleosides modified in such a way that conformation is locked into a C3'-endo type conformation, i.e. Locked Nucleic Acid (LNA, Singh et al, Chem. Commun. (1998), 4, 455-456), and ethylene bridged Nucleic Acids (ENA, Morita et al, Bioorganic & Medicinal Chemistry Letters (2002), 12, 73-76.) Examples of modified nucleosides amenable to the present invention are shown below in Table I. These examples are meant to be representative and not exhaustive.

TABLE I



[0144] The preferred conformation of modified nucleosides and their oligomers can be estimated by various methods such as molecular dynamics calculations, nuclear magnetic resonance spectroscopy and CD measurements. Hence, modifications predicted to induce RNA like conformations, A-form duplex geometry in an oligomeric context, are selected for use in the modified oligoncleotides of the present invention. The synthesis of numerous modified nucleosides amenable to the present invention are known in the art (see for example, Chemistry of Nucleosides and Nucleotides Vol 1-3, ed. Leroy B. Townsend, 1988, Plenum press., and the examples section below.)

[0145] In one aspect, the present invention is directed to oligonucleotides that are prepared having enhanced properties compared to native RNA against nucleic acid targets. A target is identified and an oligonucleotide is selected having an effective length and sequence that is complementary to a portion of the target sequence. Each nucleoside of the selected sequence is scrutinized for possible enhancing modifications. A preferred modification would be the replacement of one or more RNA nucleosides with nucleosides that have the same 3'-endo conformational geometry. Such modifications can enhance chemical and nuclease stability relative to native RNA while at the same time being much cheaper and easier to synthesize and/or incorporate into an oligonulceotide. The selected sequence can be further divided into regions and the nucleosides of each region evaluated for enhancing modifications that can be the result of a chimeric configuration. Consideration is also given to the 5' and 3'-termini as there are often advantageous modifications that can be made to one or more of the terminal nucleosides. The oligomeric compounds of the present invention include at least one 5'-modified phosphate group on a single strand or on at least one 5'-position of a double stranded sequence or sequences. Further modifications are also considered such as internucleoside linkages, conjugate groups, substitute sugars or bases, substitution of one or more nucleosides with nucleoside mimetics and any other modification that can enhance the selected sequence for its intended target. The terms used to describe the conformational geometry of homoduplex nucleic acids are "A Form" for RNA and "B Form" for DNA. The respective conformational geometry for RNA and DNA duplexes was determined from X-ray diffraction analysis of nucleic acid fibers (Arnott and Hukins, *Biochem. Biophys. Res. Comm.,* 1970, 47, 1504.) In general, RNA:RNA duplexes are more stable and have higher melting temperatures (Tm's) than DNA:DNA duplexes (Sanger et al., Principles of Nucleic Acid Structure, 1984, Springer-Verlag; New York, N.Y.; Lesnik et al., Biochemistry, 1995, 34, 10807-10815; Conte et al., Nucleic Acids Res., 1997, 25, 2627-2634). The increased stability of RNA has been attributed to several structural features, most notably the improved base stacking interactions that result from an A-form geometry (Searle et al., Nucleic Acids Res., 1993, 21, 2051-2056). The presence of the 2' hydroxyl in RNA biases the sugar toward a C3' endo pucker, i.e., also designated as Northern pucker, which causes the duplex to favor the A-form geometry. In addition, the 2' hydroxyl groups of RNA can form a network of water mediated hydrogen bonds that help stabilize the RNA duplex (Egli et al., Biochemistry, 1996, 35, 8489-8494). On the other hand, deoxy nucleic acids prefer a C2' endo sugar pucker, i.e., also known as Southern pucker, which is thought to impart a less stable B-form geometry (Sanger, W. (1984) Principles of Nucleic Acid Structure, Springer-Verlag, New York, N.Y.). As used herein, B-form geometry is inclusive of both C2'-endo pucker and O4'-endo pucker. This is consistent with Berger, et. al., *Nucleic Acids Research,* 1998, 26, 2473-2480, who pointed out that in considering the furanose conformations which give rise to B-form duplexes consideration should also be given to a O4'-endo pucker contribution.

[0146] DNA:RNA hybrid duplexes, however, are usually less stable than pure RNA:RNA duplexes, and depending on their sequence may be either more or less stable than DNA:DNA duplexes (Searle et al., *Nucleic Acids Res.,* 1993, 21, 2051-2056). The structure of a hybrid duplex is intermediate between A- and B-form geometries, which may

result in poor stacking interactions (Lane et al., *Eur. J. Biochem.,* 1993, 215, 297-306; Fedoroff et al., *J. Mol. Biol.,* 1993, 233, 509-523; Gonzalez et al., *Biochemistry,* 1995, 34, 4969-4982; Horton et al., *J. Mol. Biol.,* 1996, 264, 521-533). The stability of the duplex formed between a target RNA and a synthetic sequence is central to therapies such as but not limited to antisense and RNA interference as these mechanisms require the binding of a synthetic oligonucleotide strand to an RNA target strand. In the case of antisense, effective inhibition of the mRNA requires that the antisense DNA have a very high binding affinity with the mRNA. Otherwise the desired interaction between the synthetic oligonucleotide strand and target mRNA strand will occur infrequently, resulting in decreased efficacy.

[**0147**] One routinely used method of modifying the sugar puckering is the substitution of the sugar at the 2'-position with a substituent group that influences the sugar geometry. The influence on ring conformation is dependant on the nature of the substituent at the 2'-position. A number of different substituents have been studied to determine their sugar puckering effect. For example, 2'-halogens have been studied showing that the 2'-fluoro derivative exhibits the largest population (65%) of the C3'-endo form, and the 2'-iodo exhibits the lowest population (7%). The populations of adenosine (2'-OH) versus deoxyadenosine (2'-H) are 36% and 19%, respectively. Furthermore, the effect of the 2'-fluoro group of adenosine dimers (2'-deoxy-2'-fluoroadenosine-2'-deoxy-2'-fluoro-adenosine) is further correlated to the stabilization of the stacked conformation.

[**0148**] As expected, the relative duplex stability can be enhanced by replacement of 2'-OH groups with 2'-F groups thereby increasing the C3'-endo population. It is assumed that the highly polar nature of the 2'-F bond and the extreme preference for C3'-endo puckering may stabilize the stacked conformation in an A-form duplex. Data from UV hypochromicity, circular dichroism, and $^1$H NMR also indicate that the degree of stacking decreases as the electronegativity of the halo substituent decreases. Furthermore, steric bulk at the 2'-position of the sugar moiety is better accommodated in an A-form duplex than a B-form duplex. Thus, a 2'-substituent on the 3'-terminus of a dinucleoside monophosphate is thought to exert a number of effects on the stacking conformation: steric repulsion, furanose puckering preference, electrostatic repulsion, hydrophobic attraction, and hydrogen bonding capabilities. These substituent effects are thought to be determined by the molecular size, electronegativity, and hydrophobicity of the substituent. Melting temperatures of complementary strands is also increased with the 2'-substituted adenosine diphosphates. It is not clear whether the 3'-endo preference of the conformation or the presence of the substituent is responsible for the increased binding. However, greater overlap of adjacent bases (stacking) can be achieved with the 3'-endo conformation.

[**0149**] One synthetic 2'-modification that imparts increased nuclease resistance and a very high binding affinity to nucleotides is the 2-methoxyethoxy (2'-MOE, 2'-$OCH_2CH_2OCH_3$) side chain (Baker et al., *J. Biol. Chem.,* 1997, 272, 11944-12000). One of the immediate advantages of the 2'-MOE substitution is the improvement in binding affinity, which is greater than many similar 2' modifications such as O-methyl, O-propyl, and O-aminopropyl. Oligonucleotides having the 2'-O-methoxyethyl substituent also have been shown to be antisense inhibitors of gene expression with promising features for in vivo use (Martin, P., *Helv. Chim. Acta,* 1995, 78, 486-504; Altmann et al., *Chimia,* 1996, 50, 168-176; Altmann et al., *Biochem. Soc. Trans.,* 1996, 24, 630-637; and Altmann et al., *Nucleosides Nucleotides,* 1997, 16, 917-926). Relative to DNA, the oligonucleotides having the 2'-MOE modification displayed improved RNA affinity and higher nuclease resistance. Chimeric oligonucleotides having 2'-MOE substituents in the wing nucleosides and an internal region of deoxy-phosphorothioate nucleotides (also termed a gapped oligonucleotide or gapmer) have shown effective reduction in the growth of tumors in animal models at low doses. 2'-MOE substituted oligonucleotides have also shown outstanding promise as antisense agents in several disease states. One such MOE substituted oligonucleotide is presently being investigated in clinical trials for the treatment of CMV retinitis.

[**0150**] Chemistries Defined

[**0151**] Unless otherwise defined herein, alkyl means $C_1$-$C_{12}$, preferably $C_1$-$C_8$, and more preferably $C_1$-$C_6$, straight or (where possible) branched chain aliphatic hydrocarbyl.

[**0152**] Unless otherwise defined herein, heteroalkyl means $C_1$-$C_{12}$, preferably $C_1$-$C_8$, and more preferably $C_1$-$C_6$, straight or (where possible) branched chain aliphatic hydrocarbyl containing at least one, and preferably about 1 to about 3, hetero atoms in the chain, including the terminal portion of the chain. Preferred heteroatoms include N, O and S. Unless otherwise defined herein, cycloalkyl means $C_3$-$C_{12}$, preferably $C_3$-$C_8$, and more preferably $C_3$-$C_6$, aliphatic hydrocarbyl ring.

[**0153**] Unless otherwise defined herein, alkenyl means $C_2$-$C_{12}$, preferably $C_2$-$C_8$, and more preferably $C_2$-$C_6$ alkenyl, which may be straight or (where possible) branched hydrocarbyl moiety, which contains at least one carbon-carbon double bond.

[**0154**] Unless otherwise defined herein, alkynyl means $C_2$-$C_{12}$, preferably $C_2$-$C_8$, and more preferably $C_2$-$C_6$ alkynyl, which may be straight or (where possible) branched hydrocarbyl moiety, which contains at least one carbon-carbon triple bond.

[**0155**] Unless otherwise defined herein, heterocycloalkyl means a ring moiety containing at least three ring members, at least one of which is carbon, and of which 1, 2 or three ring members are other than carbon. Preferably the number of carbon atoms varies from 1 to about 12, preferably 1 to about 6, and the total number of ring members varies from three to about 15, preferably from about 3 to about 8. Preferred ring heteroatoms are N, O and S. Preferred heterocycloalkyl groups include morpholino, thiomorpholino, piperidinyl, piperazinyl, homopiperidinyl, homopiperazinyl, homomorpholino, homothiomorpholino, pyrrolodinyl, tetrahydrooxazolyl, tetrahydroimidazolyl, tetrahydrothiazolyl, tetrahydroisoxazolyl, tetrahydropyrrazolyl, furanyl, pyranyl, and tetrahydroisothiazolyl.

[**0156**] Unless otherwise defined herein, aryl means any hydrocarbon ring structure containing at least one aryl ring. Preferred aryl rings have about 6 to about 20 ring carbons. Especially preferred aryl rings include phenyl, napthyl, anthracenyl, and phenanthrenyl.

[**0157**] Unless otherwise defined herein, hetaryl means a ring moiety containing at least one fully unsaturated ring, the

ring consisting of carbon and non-carbon atoms. Preferably the ring system contains about 1 to about 4 rings. Preferably the number of carbon atoms varies from 1 to about 12, preferably 1 to about 6, and the total number of ring members varies from three to about 15, preferably from about 3 to about 8. Preferred ring heteroatoms are N, O and S. Preferred hetaryl moieties include pyrazolyl, thiophenyl, pyridyl, imidazolyl, tetrazolyl, pyridyl, pyrimidinyl, purinyl, quinazolinyl, quinoxalinyl, benzimidazolyl, benzothiophenyl, etc.

[0158] Unless otherwise defined herein, where a moiety is defined as a compound moiety, such as hetarylalkyl (hetaryl and alkyl), aralkyl (aryl and alkyl), etc., each of the submoieties is as defined herein.

[0159] Unless otherwise defined herein, an electron withdrawing group is a group, such as the cyano or isocyanato group that draws electronic charge away from the carbon to which it is attached. Other electron withdrawing groups of note include those whose electronegativities exceed that of carbon, for example halogen, nitro, or phenyl substituted in the ortho- or para-position with one or more cyano, isothiocyanato, nitro or halo groups.

[0160] Unless otherwise defined herein, the terms halogen and halo have their ordinary meanings. Preferred halo (halogen) substituents are Cl, Br, and I.

[0161] The aforementioned optional substituents are, unless otherwise herein defined, suitable substituents depending upon desired properties. Included are halogens (Cl, Br, I), alkyl, alkenyl, and alkynyl moieties, $NO_2$, $NH_3$ (substituted and unsubstituted), acid moieties (e.g. —$CO_2H$, —$OSO_3H_2$, etc.), heterocycloalkyl moieties, hetaryl moieties, aryl moieties, etc. In all the preceding formulae, the squiggle (~) indicates a bond to an oxygen or sulfur of the 5'-phosphate.

[0162] Phosphate protecting groups include those described in U.S. Pat. Nos. 5,760,209, U.S. Pat. No. 5,614,621, U.S. Pat. No. 6,051,699, U.S. Pat. No. 6,020,475, U.S. Pat. No. 6,326,478, U.S. Pat. No. 6,169,177, U.S. Pat. No. 6,121,437, U.S. Pat. No. 6,465,628 each of which is expressly incorporated herein by reference in its entirety.

[0163] The oligonucleotides in accordance with this invention (single stranded or double stranded) preferably comprise from about 8 to about 80 nucleotides, more preferably from about 12-50 nucleotides and most preferably from about 15 to 30 nucleotides. As is known in the art, a nucleotide is a base-sugar combination suitably bound to an adjacent nucleotide through a phosphodiester, phosphorothioate or other covalent linkage.

[0164] The oligonucleotides of the present invention also include variants in which a different base is present at one or more of the nucleotide positions in the oligonucleotide. For example, if the first nucleotide is an adenosine, variants may be produced which contain thymidine, guanosine or cytidine at this position. This may be done at any of the positions of the oligonucleotide. Thus, a 20-mer may comprise 60 variations (20 positions×3 alternates at each position) in which the original nucleotide is substituted with any of the three alternate nucleotides. These oligonucleotides are then tested using the methods described herein to determine their ability to inhibit expression of p38α MAP kinase mRNA.

[0165] The oligonucleotides 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 Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the talents of the routineer. It is well known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and 2'-alkoxy or 2'-alkoxyalkoxy derivatives, including 2'-O-methoxyethyl oligonucleotides [Martin, P., *Helv. Chim. Acta,* 78, 486 (1995)]. It is also well known to use similar techniques and commercially available modified amidites and controlled-pore glass (CPG) products such as biotin, fluorescein, acridine or psoralen-modified amidites and/or CPG (available from Glen Research, Sterling Va.) to synthesize fluorescently labeled, biotinylated or other conjugated oligonucleotides.

[0166] The antisense compounds of the present invention include bioequivalent compounds, including pharmaceutically acceptable salts and prodrugs. This is intended to 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 pharmaceutically acceptable salts of the nucleic acids of the invention and prodrugs of such nucleic acids.

[0167] Pharmaceutically acceptable "salts" are physiologically and pharmaceutically acceptable salts of the nucleic acids of the invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto [see, for example, Berge et al., "Pharmaceutical Salts,"*J. of Pharma Sci.,* 66:1 (1977)].

[0168] For oligonucleotides, 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.

[0169] The oligonucleotides of the invention may additionally or alternatively be prepared to be delivered in a "prodrug" form. 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.

[0170] For therapeutic or prophylactic treatment, oligonucleotides are administered in accordance with this invention. Oligonucleotide compounds of the invention may be formulated in a pharmaceutical composition, which may include pharmaceutically acceptable carriers, thickeners, diluents, buffers, preservatives, surface active agents, neutral or cationic lipids, lipid complexes, liposomes, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients and the like in addition to the oligonucleotide. Such compositions and formulations are comprehended by the present invention.

[0171] Pharmaceutical compositions comprising the oligonucleotides of the present invention may include penetration enhancers in order to enhance the alimentary delivery of the oligonucleotides. Penetration enhancers may be classified as belonging to one of five broad categories, i.e., fatty acids, bile salts, chelating agents, surfactants and non-surfactants (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems,* 1991, 8:91-192; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems,* 1990, 7:1). One or more penetration enhancers from one or more of these broad categories may be included.

[0172] 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, e.g., antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the composition of 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 invention.

[0173] Regardless of the method by which the oligonucleotides of the invention are introduced into a patient, colloidal dispersion systems may be used as delivery vehicles to enhance the in vivo stability of the oligonucleotides and/or to target the oligonucleotides to a particular organ, tissue or cell type. Colloidal dispersion systems include, but are not limited to, macromolecule complexes, nanocapsules, microspheres, beads and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, liposomes and lipid:oligonucleotide complexes of uncharacterized structure. A preferred colloidal dispersion system is a plurality of liposomes. Liposomes are microscopic spheres having an aqueous core surrounded by one or more outer layers made up of lipids arranged in a bilayer configuration [see, generally, Chonn et al., *Current Op. Biotech.,* 6, 698 (1995)].

[0174] 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, vaginal, rectal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous drip, subcutaneous, intraperitoneal or intramuscular injection, pulmonary administration, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer, metered dose inhaler or dry powder inhaler; intratracheal, intranasal, 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.

[0175] 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.

[0176] Compositions 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.

[0177] Compositions for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives. In some cases it may be more effective to treat a patient with an oligonucleotide of the invention in conjunction with other traditional therapeutic modalities in order to increase the efficacy of a treatment regimen. In the context of the invention, the term "treatment regimen" is meant to encompass therapeutic, palliative and prophylactic modalities. For example, a patient may be treated with conventional chemotherapeutic agents, particularly those used for tumor and cancer treatment. Examples of such chemotherapeutic agents include but are not limited to daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bischloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine,taxol, vincristine, vinblastine, etoposide, trimetrexate, teniposide, cisplatin and diethylstilbestrol (DES). See, generally, *The Merck Manual of Diagnosis and Therapy,* 15th Ed., pp. 1206-1228, Berkow et al., eds., Rahay, N.J., 1987). When used with the compounds of the invention, such chemotherapeutic agents may be used individually (e.g., 5-FU and oligonucleotide), sequentially (e.g., 5-FU and oligonucleotide for a period of time followed by MTX and oligonucleotide), or in combination with one or more other such chemotherapeutic agents (e.g., 5-FU, MTX and oligonucleotide, or 5-FU, radiotherapy and oligonucleotide).

[0178] 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 μg 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 μg to 100 g per kg of body weight, once or more daily, to once every 20 years.

[0179] Thus, in the context of this invention, by "therapeutically effective amount" is meant the amount of the compound which is required to have a therapeutic effect on the treated mammal. This amount, which will be apparent to the skilled artisan, will depend upon the type of mammal, the age and weight of the mammal, the type of disease to be treated, perhaps even the gender of the mammal, and other factors which are routinely taken into consideration when treating a mammal with a disease. A therapeutic effect is assessed in the mammal by measuring the effect of the compound on the disease state in the animal. For example, if the disease to be treated is cancer, therapeutic effects are assessed by measuring the rate of growth or the size of the tumor, or by measuring the production of compounds such as cytokines, production of which is an indication of the progress or regression of the tumor.

[0180] The following examples illustrate the present invention and are not intended to limit the same.

## EXAMPLES

[0181] Eample 1

### Synthesis of Oligonucleotides

[0182] Unmodified oligodeoxynucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine. β-cyanoethyldiisopropyl-phosphoramidites were purchased from Applied Biosystems (Foster City, Calif.). For phosphorothioate oligonucleotides, the standard oxidation bottle was replaced by a 0.2 M solution of $^3$H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation cycle wait step was increased to 68 seconds and was followed by the capping step.

[0183] 2'-methoxy oligonucleotides are synthesized using 2'-methoxy β-cyanoethyldiisopropyl-phosphoramidites (Chemgenes, Needham, Mass.) and the standard cycle for unmodified oligonucleotides, except the wait step after pulse delivery of tetrazole and base was increased to 360 seconds. Other 2'-alkoxy oligonucleotides were synthesized by a modification of this method, using appropriate 2'-modified amidites such as those available from Glen Research, Inc., Sterling, Va.

[0184] 2'-fluoro oligonucleotides are synthesized as described in Kawasaki et al., *J. Med. Chem.,* 36, 831 (1993). Briefly, the protected nucleoside $N^6$-benzoyl-2'-deoxy-2'-fluoroadenosine is synthesized utilizing commercially available 9-β-D-arabinofuranosyladenine as starting material and by modifying literature procedures whereby the 2'-α-fluoro atom is introduced by a $S_N2$-displacement of a 2'-β-O-trifyl group. Thus $N^6$-benzoyl-9-β-D-arabinofuranosyladenine is selectively protected in moderate yield as the 3',5'-ditetrahydropyranyl (THP) intermediate. Deprotection of the THP and $N^6$-benzoyl groups is accomplished using standard methodologies and standard methods are used to obtain the 5'-dimethoxytrityl-(DMT) and 5'-DMT-3'-phosphoramidite intermediates.

[0185] The synthesis of 2'-deoxy-2'-fluoroguanosine is accomplished using tetraisopropyldisiloxanyl (TPDS) protected 9-β-D-arabinofuranosylguanine as starting material, and conversion to the intermediate diisobutyryl-arabinofuranosylguanosine. Deprotection of the TPDS group is followed by protection of the hydroxyl group with THP to give diisobutyryl di-THP protected arabinofuranosylguanine. Selective O-deacylation and triflation is followed by treatment of the crude product with fluoride, then deprotection of the THP groups. Standard methodologies are used to obtain the 5'-DMT- and 5'-DMT-3'-phosphoramidites.

[0186] Synthesis of 2'-deoxy-2'-fluorouridine is accomplished by the modification of a known procedure in which 2,2'-anhydro-1-B-D-arabinofuranosyluracil is treated with 70% hydrogen fluoride-pyridine. Standard procedures are used to obtain the 5'-DMT and 5'-DMT-3'phosphoramidites.

[0187] 2'-deoxy-2'-fluorocytidine is synthesized via amination of 2'-deoxy-2'-fluorouridine, followed by selective protection to give $N^4$-benzoyl-2'-deoxy-2'-fluorocytidine. Standard procedures are used to obtain the 5'-DMT and 5'-DMT-3'phosphoramidites.

[0188] 2'-(2-methoxyethyl)-modified amidites were synthesized according to Martin, P., *Helv. Chim. Acta,* 78,486 (1995). For ease of synthesis, the last nucleotide was a deoxynucleotide. 2'-O—$CH_2CH_2OCH_3$-cytosines may be 5-methyl cytosines. *Synthesis of 5-Methyl Cytosine Monomers:*

[0189] 2,2'-Anhydro[1-(β-D-arabinofuranosyl)-5-methyluridine]:

[0190] 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 hours) to give a solid which was crushed to a light tan powder (57 g, 85% crude yield). The material was used as is for further reactions.

[0191] 2'-O-Methoxyethyl-5-methyluridine:

[0192] 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.

[0193] 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine:

[0194] 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 $Na_2SO_4$, 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%).

[0195] 3'-O-Acetyl-2'-O-methoxyethyl-51-O-dimethoxytrityl-5-methyluridine:

[0196] 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 tic by first quenching the tic sample with the addition of MeOH. Upon completion of the reaction, as judged by tic, 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%).

[0197] 3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine:

[0198] 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 0-10EC, and the resulting mixture stirred for an additional 2 hours. The first solution was added dropwise, over a 45 minute period, to the later 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.

[0199] 2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine:

[0200] 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.

[0201] $N^4$-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine:

[0202] 2'-O-Methoxyethyl-5'-O-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.

[0203] $N^4$-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine-3'-amidite:

[0204] $N^4$-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 chromatographed 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.

[0205] 5-methyl-2'-deoxycytidine (5-me-C) containing oligonucleotides were synthesized according to published

methods [Sanghvi et al., *Nucl. Acids Res.,* 21, 3197 (1993)] using commercially available phosphoramidites (Glen Research, Sterling Va. or ChemGenes, Needham Mass.).

**[0206]** 2=-O-(dimethylaminooxyethyl) Nucleoside Amidites

**[0207]** 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.

**[0208]** 5'-O-tert-Butyldiphenylsilyl-$O^2$-2'-anhydro-5-methyluridine

**[0209]** $O^2$-2'-anhydro-5-methyluridine (Pro. Bio. Sint., Varese, Italy, 100.0 g, 0.416 mmol), dimethylaminopyridine (0.66 g, 0.013eq, 0.0054 mmol) are dissolved in dry pyridine (500 ml) at ambient temperature under an argon atmosphere and with mechanical stirring. tert-Butyldiphenylchlorosilane (125.8 g, 119.0 mL, 1.1eq, 0.458 mmol) is added in one portion. The reaction is stirred for 16 h at ambient temperature. TLC (Rf 0.22, ethyl acetate) indicates a complete reaction. The solution is concentrated under reduced pressure to a thick oil. This is partitioned between dichloromethane (1 L) and saturated sodium bicarbonate (2×1 L) and brine (1 L). The organic layer is dried over sodium sulfate and concentrated under reduced pressure to a thick oil. The oil is dissolved in a 1:1 mixture of ethyl acetate and ethyl ether (600 mL) and the solution is cooled to −10° C. The resulting crystalline product is collected by filtration, washed with ethyl ether (3×200 mL) and dried (40° C., 1 mm Hg, 24 h) to 149 g (74.8%) of white solid. TLC and NMR are used to check consistency with pure product.

**[0210]** 5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine

**[0211]** In a 2 L stainless steel, unstirred pressure reactor is 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) is 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) are added with manual stirring. The reactor is sealed and heated in an oil bath until an internal temperature of 160° C. is reached and then maintained for 16 h (pressure<100 psig). The reaction vessel is cooled to ambient and opened. TLC (Rf 0.67 for desired product and Rf 0.82 for ara-T side product, ethyl acetate) indicates % conversion to the product. In order to avoid additional side product formation, the reaction is 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 is purified by column chromatography (2 kg silica gel, ethyl acetate-hexanes gradient 1:1 to 4:1). The appropriate fractions are 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. TLC and NMR are used to determine consistency with pure product.

**[0212]** 2'-O-([2-phthalimidoxy)ethyl]-5'-t-butyldiphenylsilyl-5-methyluridine

**[0213]** 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, 86%).

**[0214]** 5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine

**[0215]** 2'-O-([2-phthalimidoxy)ethyl]-5'-t-butyldiphenylsilyl-5-methyluridine (3.1 g, 4.5 mmol) is dissolved in dry $CH_2Cl_2$ (4.5 mL) and methylhydrazine (300 mL, 4.64 mmol) is added dropwise at −10° C. to 0° C. After 1 hr the mixture is filtered, the filtrate is washed with ice cold $CH_2Cl_2$ and the combined organic phase is washed with water, brine and dried over anhydrous $Na_2SO_4$. The solution is concentrated to get 2'-O-(aminooxyethyl) thymidine, which is then dissolved in MeOH (67.5 mL). To this formaldehyde (20% aqueous solution, w/w, 1.1eg.) is added and the mixture for 1 hr. Solvent is removed under vacuum; residue chromatographed to get 5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine as white foam.

**[0216]** 5'-O-tert-Butyldiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5-methyluridine

**[0217]** 5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine (1.77 g, 3.12 mmol) is dissolved in a solution of 1M pyridinium p-toluenesulfonate (PPTS) in dry MeOH (30.6 mL). Sodium cyanoborohydride (0.39 g, 6.13 mmol) is added to this solution at 10° C. under inert atmosphere. The reaction mixture is stirred for 10 minutes at 10° C. After that the reaction vessel is removed from the ice bath and stirred at room temperature for 2 hr, the reaction monitored by TLC (5% MeOH in $CH_2Cl_2$). Aqueous $NaHCO_3$ solution (5%, 10 mL) is added and extracted with ethyl acetate (2×20 mL). Ethyl acetate phase is dried over anhydrous $Na_2SO_4$, evaporated to dryness. Residue is dissolved in a solution of 1M PPTS in MeOH (30.6 mL). Formaldehyde (20% w/w, 30 mL, 3.37 mmol) is added and the reaction mixture is 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) is added and reaction mixture stirred at 10° C. for 10 minutes. After 10 minutes, the reaction mixture is removed from the ice bath and stirred at room temperature for 2 hrs. To the reaction mixture 5% $NaHCO_3$ (25 mL) solution is added and extracted with ethyl acetate (2×25 mL). Ethyl acetate layer is dried over anhydrous $Na_2SO_4$ and evaporated to dryness. The residue obtained is 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).

[0218] 2'-O-(dimethylaminooxyethyl)-5-methyluridine

[0219] Triethylamine trihydrofluoride (3.91 mL, 24.0 mmol) is dissolved in dry THF and triethylamine (1.67 mL, 12 mmol, dry, kept over KOH). This mixture of triethylamine-2HF is 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 is monitored by TLC (5% MeOH in $CH_2Cl_2$). Solvent is 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 (766 mg).

[0220] 5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine

[0221] 2'-O-(dimethylaminooxyethyl)-5-methyluridine (750 mg, 2.17 mmol) is dried over $P_2O_5$ under high vacuum overnight at 40° C. It is then co-evaporated with anhydrous pyridine (20 mL). The residue obtained is 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) is added to the mixture and the reaction mixture is stirred at room temperature until all of the starting material disappeared. Pyridine is 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-(dimethylaminooxyethyl)-5-methyluridine (1.13 g).

[0222] 5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]

[0223] 5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine (1.08 g, 1.67 mmol) is co-evaporated with toluene (20 mL). To the residue N,N-diisopropylamine tetrazonide (0.29 g, 1.67 mmol) is added and dried over $P_2O_5$ under high vacuum overnight at 40° C. Then the reaction mixture is dissolved in anhydrous acetonitrile (8.4 mL) and 2-cyanoethyl-N,N,N$^1$,N$^{1'}$-tetraisopropylphosphoramidite (2.12 mL, 6.08 mmol) is added. The reaction mixture is 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 is evaporated, then the residue is dissolved in ethyl acetate (70 mL) and washed with 5% aqueous $NaHCO_3$ (40 mL). Ethyl acetate layer is dried over anhydrous $Na_2SO_4$ and concentrated. Residue obtained is 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).

[0224] 2'-(Aminooxyethoxy) Nucleoside Amidites

[0225] 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.

[0226] N2-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]

[0227] 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-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine. As before the hydroxyl group may be displaced 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].

[0228] Oligonucleotides having methylene (methylimino) (MMI) backbones are synthesized according to U.S. Pat. No. 5,378,825, which is coassigned to the assignee of the present invention and is incorporated herein in its entirety. For ease of synthesis, various nucleoside dimers containing MMI linkages were synthesized and incorporated into oligonucleotides. Other nitrogen-containing backbones are synthesized according to WO 92/20823 which is also coassigned to the assignee of the present invention and incorporated herein in its entirety.

[0229] Oligonucleotides having amide backbones are synthesized according to De Mesmaeker et al., *Acc. Chem. Res.,* 28, 366 (1995). The amide moiety is readily accessible by simple and well-known synthetic methods and is compatible with the conditions required for solid phase synthesis of oligonucleotides.

[0230] Oligonucleotides with morpholino backbones are synthesized according to U.S. Pat. No. 5,034,506 (Summerton and Weller).

[0231] Peptide-nucleic acid (PNA) oligomers are synthesized according to P. E. Nielsen et al., *Science,* 254, 1497 (1991).

[0232] After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55° C. for 18 hours, the oligonucleotides 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.,* 266, 18162 (1991). Results obtained with HPLC-purified material were similar to those obtained with non-HPLC purified material.

Example 2

Human p38α Oligonucleotide Sequences

[0233] Antisense oligonucleotides were designed to target human p38α. Target sequence data are from the p38 MAPK

cDNA sequence; Genbank accession number L35253, provided herein as SEQ ID NO: 1. Oligonucleotides was synthesized as chimeric oligonucleotides ("gapmers") 20 nucleotides in length, composed of a central "gap" region consisting of eight 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by six-nucleotide "wings." The wings are composed of 2'-methoxyethyl (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All 2'-MOE cytosines were 5-methylcytosines. These oligonucleotide sequences are shown in Table 1.

[0234] The human Jurkat T-cell line (American Type Culture Collection, Manassas, Va.) was maintained in RPMI 1640 growth media supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, Utah). HUVEC cells (Clonetics, San Diego, Calif.) were cultivated in endothelial basal media supplemented with 10% FBS (Hyclone, Logan, Utah).

[0235] Jurkat cells were grown to approximately 75% confluency and resuspended in culture media at a density of $1\times10^7$ cells/ml. A total of $3.6\times10^6$ cells were employed for each treatment by combining 360 $\mu$l of cell suspension with oligonucleotide at the indicated concentrations to reach a final volume of 400 $\mu$l. Cells were then transferred to an electroporation cuvette and electroporated using an Electrocell Manipulator 600 instrument (Biotechnologies and Experimental Research, Inc.) employing 150 V, 1000 $\mu$F, at 13 $\Omega$. Electroporated cells were then transferred to conical tubes containing 5 ml of culture media, mixed by inversion, and plated onto 10 cm culture dishes.

[0236] HUVEC cells were allowed to reach 75% confluency prior to use. The cells were washed twice with warm (37° C.) OPTI-MEM™ (Life Technologies). The cells were incubated in the presence of the appropriate culture medium, without the growth factors added, and the oligonucleotide formulated in LIPOFECTIN7 (Life Technologies), a 1:1 (w/w) liposome formulation of the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-n,n,n-trimethylammonium chloride (DOTMA), and dioleoyl phosphotidylethanolamine (DOPE) in membrane filtered water. HUVEC cells were treated with 100 nM oligonucleotide in 10 $\mu$g/ml LIPOFECTIN7. Treatment was for four hours.

[0237] Total mRNA was isolated using the RNEASY7 Mini Kit (Qiagen, Valencia, Calif.; similar kits from other manufacturers may also be used), separated on a 1% agarose gel, transferred to HYBOND™-N+ membrane (Amersham Pharmacia Biotech, Piscataway, N.J.), a positively charged nylon membrane, and probed. p38 MAPK probes were made using the Prime-A-Gene7 kit (Promega Corporation, Madison, Wis.), a random primer labeling kit, using mouse p38$\alpha$ or p38$\beta$ cDNA as a template. A glyceraldehyde 3-phosphate dehydrogenase (G3PDH) probe was purchased from Clontech (Palo Alto, Calif.), Catalog Number 9805-1. The fragments were purified from low-melting temperature agarose, as described in Maniatis, T., et al., *Molecular Cloning: A Laboratory Manual*, 1989. The G3PDH probe was labeled with REDIVUE™ $^{32}$P-dCTP (Amersham Pharmacia Biotech, Piscataway, N.J.) and Strip-EZ labelling kit (Ambion, Austin, Tex.). mRNA was quantitated by a PhosphoImager (Molecular Dynamics, Sunnyvale, Calif.).

TABLE 1

Nucleotide Sequences of Human p38$\alpha$ Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS NO. | NUCLEOTIDE SEQUENCE[1] (5' → 3') | SEQ ID NO: | TARGET GENE NUCLEOTIDE CO-ORDINATES[2] | GENE TARGET REGION |
|---|---|---|---|---|
| 16486 | **AAGACC**GGGCCCGG**AATTCC** | 3 | 0001–0020 | 5'-UTR |
| 16487 | **GTGGAG**GCCAGTCC**CCGGGA** | 4 | 0044–0063 | 5'-UTR |
| 16488 | **TGGCAG**CAAAGTGC**TGCTGG** | 5 | 0087–0106 | 5'-UTR |
| 16489 | **CAGAGA**GCCTCCTG**GGAGGG** | 6 | 0136–0155 | 5'-UTR |
| 16490 | **TGTGCC**GAATCTCG**GCCTCT** | 7 | 0160–0179 | 5'-UTR |
| 16491 | **GGTCTC**GGGCGACC**TCTCCT** | 8 | 0201–0220 | 5'-UTR |
| 16492 | **CAGCCG**CGGGACCA**GCGGCG** | 9 | 0250–0269 | 5'-UTR |
| 16493 | **CATTTT**CCAGCGGC**AGCCGC** | 10 | 0278–0297 | AUG |
| 16494 | **TCCTGA**GACATTTT**CCAGCG** | 11 | 0286–0305 | AUG |
| 16495 | **CTGCCG**GTAGAACG**TGGGCC** | 12 | 0308–0327 | coding |
| 16496 | **GTAAGC**TTCTGACA**TTTCAC** | 13 | 0643–0662 | coding |
| 16497 | **TTTAGG**TCCCTGTG**AATTAT** | 14 | 0798–0817 | coding |
| 16498 | **ATGTTC**TTCCAGTC**AACAGC** | 15 | 0939–0958 | coding |
| 16499 | **TAAGGA**GGTCCCTG**CTTTCA** | 16 | 1189–1208 | coding |
| 16500 | **AACCAG**GTGCTCAG**GACTCC** | 17 | 1368–1387 | stop |
| 16501 | **GAAGTG**GGATCAAC**AGAACA** | 18 | 1390–1409 | 3'-UTR |
| 16502 | **TGAAAA**GGCCTTCC**CCTCAC** | 19 | 1413–1432 | 3'-UTR |
| 16503 | **AGGCAC**TTGAATAA**TATTTG** | 20 | 1444–1463 | 3'-UTR |
| 16504 | **CTTCCA**CCATGGAG**GAAATC** | 21 | 1475–1494 | 3'-UTR |
| 16505 | **ACACAT**GCACACAC**ACTAAC** | 22 | 1520–1539 | 3'-UTR |

[1]Emboldened residues, 2'-methoxyethoxy- residues (others are 2'-deoxy-) including "C" residues, 5-methyl-cytosines; all linkages are phosphorothioate linkages.
[2]Co-ordinates from Genbank Accession No. L35253, locus name "HUMMAPKNS", SEQ ID NO. 1.

[0238] For an initial screen of human p38$\alpha$ antisense oligonucleotides, Jurkat cells were electroporated with 10 $\mu$M oligonucleotide. mRNA was measured by Northern blot. Results are shown in Table 2. Oligonucleotides 16496 (SEQ ID NO. 13), **16500** (SEQ ID NO. 17) and 16503 (SEQ ID NO. 20) gave 35% or greater inhibition of p38$\alpha$ mRNA.

TABLE 2

Inhibition of Human p38$\alpha$ mRNA expression in Jurkat Cells by Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS No: | SEQ ID NO: | GENE TARGET REGION | % mRNA EXPRESSION | % mRNA INHIBITION |
|---|---|---|---|---|
| control | — | — | 100% | 0% |
| 16486 | 3 | 5'-UTR | 212% | — |

TABLE 2-continued

Inhibition of Human p38α mRNA expression in Jurkat Cells by Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS No: | SEQ ID NO: | GENE TARGET REGION | % mRNA EXPRESSION | % mRNA INHIBITION |
|---|---|---|---|---|
| 16487 | 4 | 5'-UTR | 171% | — |
| 16488 | 5 | 5'-UTR | 157% | — |
| 16489 | 6 | 5'-UTR | 149% | — |
| 16490 | 7 | 5'-UTR | 152% | — |
| 16491 | 8 | 5'-UTR | 148% | — |
| 16492 | 9 | 5'-UTR | 125% | — |
| 16493 | 10 | AUG | 101% | — |
| 16494 | 11 | AUG | 72% | 28% |
| 16495 | 12 | coding | 72% | 28% |
| 16496 | 13 | coding | 61% | 39% |
| 16497 | 14 | coding | 104% | — |
| 16498 | 15 | coding | 88% | 12% |
| 16499 | 16 | coding | 74% | 26% |
| 16500 | 17 | stop | 63% | 37% |
| 16501 | 18 | 3'-UTR | 77% | 23% |
| 16502 | 19 | 3'-UTR | 79% | 21% |
| 16503 | 20 | 3'-UTR | 65% | 35% |
| 16504 | 21 | 3'-UTR | 72% | 28% |
| 16505 | 22 | 3'-UTR | 93% | 7% |

[0239] The most active human p38α oligonucleotides were chosen for dose response studies. Oligonucleotide 16490 (SEQ ID NO. 7) which showed no inhibition in the initial screen was included as a negative control. Jurkat cells were grown and treated as described above except the concentration of oligonucleotide was varied as indicated in Table 3. Results are shown in Table 3. Each of the active oligonucleotides showed a dose response effect with $IC_{50}$s around 10 nM. Maximum inhibition was approximately 70% with 16500 (SEQ ID NO 17). The most active oligonucleotides were also tested for their ability to inhibit p38β. None of these oligonucleotides significantly reduced p38β mRNA expression.

TABLE 3

Dose Response of p38α mRNA in Jurkat cells to human p38α Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS # | SEQ ID NO: | ASO Gene Target | Dose | % mRNA Expression | % mRNA Inhibition |
|---|---|---|---|---|---|
| control | — | — | — | 100% | 0% |
| 16496 | 13 | coding | 2.5 nM | 94% | 6% |
| " | " | " | 5 nM | 74% | 26% |
| " | " | " | 10 nM | 47% | 53% |
| " | " | " | 20 nM | 41% | 59% |
| 16500 | 17 | stop | 2.5 nM | 82% | 18% |
| " | " | " | 5 nM | 71% | 29% |
| " | " | " | 10 nM | 49% | 51% |
| " | " | " | 20 nM | 31% | 69% |
| 16503 | 20 | 3'-UTR | 2.5 nM | 74% | 26% |
| " | " | " | 5 nM | 61% | 39% |
| " | " | " | 10 nM | 53% | 47% |
| " | " | " | 20 nM | 41% | 59% |
| 16490 | 7 | 5'-UTR | 2.5 nM | 112% | — |
| " | " | " | 5 nM | 109% | — |
| " | " | " | 10 nM | 104% | — |
| " | " | " | 20 nM | 97% | 3% |

Example 3

Human p38β Oligonucleotide Sequences

[0240] Antisense oligonucleotides were designed to target human p38β. Target sequence data are from the p38β MAPK cDNA sequence; Genbank accession number U53442, provided herein as SEQ ID NO: 23. Oligonucleotides was synthesized as 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 2'-MOE cytosines were 5-methyl-cytosines. These oligonucleotide sequences are shown in Table 4.

TABLE 4

Nucleotide Sequences of Human p38β Phosphorothioate Oligonucleotides

| ISIS NO. | NUCLEOTIDE SEQUENCE[1] (5' → 3') | SEQ ID NO: | TARGET GENE NUCLEOTIDE CO-ORDINATES[2] | GENE TARGET REGION |
|---|---|---|---|---|
| 17891 | **CGACA**TGTCCGGAG**CAGAAT** | 25 | 0006—0025 | AUG |
| 17892 | **TTCAG**CTCCTGCCG**GTAGAA** | 26 | 0041—0060 | coding |
| 17893 | **TGCGG**CACCTCCCA**CACGGT** | 27 | 0065—0084 | coding |
| 17894 | **CCGAA**CAGACGGAG**CCGTAT** | 28 | 0121—0140 | coding |
| 17895 | **GTGCT**TCAGGTGCT**TGAGCA** | 29 | 0240—0259 | coding |
| 17896 | **GCGTG**AAGACGTCC**AGAAGC** | 30 | 0274—0293 | coding |
| 17897 | **ACTTG**ACGATGTTG**TTCAGG** | 31 | 0355—0374 | coding |
| 17898 | **AACGT**GCTCGTCAA**GTGCCA** | 32 | 0405—0424 | coding |
| 17899 | **ATCCT**GAGCTCACA**GTCCTC** | 33 | 0521—0540 | coding |
| 17900 | **ACTGT**TTGGTTGTA**ATGCAT** | 34 | 0635—0654 | coding |
| 17901 | **ATGAT**GCGCTTCAG**CTGGTC** | 35 | 0731—0750 | coding |
| 17902 | **GCCAG**TGCCTCAGG**TGCACT** | 36 | 0935—0954 | coding |
| 17903 | **AACGC**TCTCATCA**TATGGCT** | 37 | 1005—1024 | coding |
| 17904 | **CAGCA**CCTCACTGC**TCAATC** | 38 | 1126—1145 | stop |
| 17905 | **TCTGT**GACCATAGG**AGTGTG** | 39 | 1228—1247 | 3'-UTR |
| 17906 | **ACACA**TGTTTGTGC**ATGCAT** | 40 | 1294—1313 | 3'-UTR |
| 17907 | **CCTAC**ACATGGCAA**GCACAT** | 41 | 1318—1337 | 3'-UTR |
| 17908 | **TCCAG**GCTGAGCAG**CTCTAA** | 42 | 1581—1600 | 3'-UTR |
| 17909 | **AGTGC**ACGCTCATC**CACACG** | 43 | 1753—1772 | 3'-UTR |
| 17910 | **CTTGC**CAGATATGG**CTGCTG** | 44 | 1836—1855 | 3'-UTR |

[1]Emboldened residues, 2'-methoxyethoxy- residues (others are 2'-deoxy-) including "C" residues, 5-methyl-cytosines; all linkages are phosphorothioate linkages.
[2]Co-ordinates from Genbank Accession No. U53442, locus name "HSU53442", SEQ ID NO. 23.

[0241] For an initial screen of human p38β antisense oligonucleotides, HUVEC cells were cultured and treated as

described in Example 2. mRNA was measured by Northern blot as described in Example 2. Results are shown in Table 5. Every oligonucleotide tested gave at least 50% inhibition. Oligonucleotides 17892 (SEQ ID NO. 26), 17893 (SEQ ID NO. 27), 17894 (SEQ ID NO. 28), 17899 (SEQ ID NO. 33), 17901 (SEQ ID NO. 35), 17903 (SEQ ID NO. 37), 17904 (SEQ ID NO. 38), 17905 (SEQ ID NO. 39), 17907 (SEQ ID NO. 41), 17908 (SEQ ID NO. 42), and 17909 (SEQ ID NO. 43) gave greater than approximately 85% inhibition and are preferred.

TABLE 5

Inhibition of Human p38β mRNA expression in Huvec Cells by Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS No: | SEQ ID NO: | GENE TARGET REGION | % mRNA EXPRESSION | % mRNA INHIBITION |
|---|---|---|---|---|
| control | — | — | 100% | 0% |
| 17891 | 25 | AUG | 22% | 78% |
| 17892 | 26 | coding | 10% | 90% |
| 17893 | 27 | coding | 4% | 96% |
| 17894 | 28 | coding | 13% | 87% |
| 17895 | 29 | coding | 25% | 75% |
| 17896 | 30 | coding | 24% | 76% |
| 17897 | 31 | coding | 25% | 75% |
| 17898 | 32 | coding | 49% | 51% |
| 17899 | 33 | coding | 5% | 95% |
| 17900 | 34 | coding | 40% | 60% |
| 17901 | 35 | coding | 15% | 85% |
| 17902 | 36 | coding | 49% | 51% |
| 17903 | 37 | coding | 11% | 89% |
| 17904 | 38 | stop | 9% | 91% |
| 17905 | 39 | 3'-UTR | 14% | 86% |
| 17906 | 40 | 3'-UTR | 22% | 78% |
| 17907 | 41 | 3'-UTR | 8% | 92% |
| 17908 | 42 | 3'-UTR | 17% | 83% |
| 17909 | 43 | 3'-UTR | 13% | 87% |
| 17910 | 44 | 3'-UTR | 26% | 74% |

[0242] Oligonucleotides 17893 (SEQ ID NO. 27), 17899 (SEQ ID NO:33), 17904 (SEQ ID NO. 38), and 17907 (SEQ ID NO. 41) were chosen for dose response studies. HUVEC cells were cultured and treated as described in Example 2 except that the oligonucleotide concentration was varied as shown in Table 6. The Lipofectin7/Oligo ratio was maintained at 3 μg Lipofectin7/100 nM oligo, per ml. mRNA was measured by Northern blot as described in Example 2.

[0243] Results are shown in Table 6. Each oligonucleotide tested had an $IC_{50}$ of less than 10 nM. The effect of these oligonucleotides on human p38α was also determined. Only oligonucleotide 17893 (SEQ ID NO. 27) showed an effect on p38α mRNA expression. The $IC_{50}$ of this oligonucleotide was approximately 4 fold higher for p38α compared to p38β.

TABLE 6

Dose Response of p38β in Huvec cells to human p38β Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS # | SEQ ID NO: | ASO Gene Target | Dose | % mRNA Expression | % mRNA Inhibition |
|---|---|---|---|---|---|
| control | — | — | — | 100% | 0% |
| 17893 | 27 | coding | 10 nM | 37% | 63% |
| " | " | " | 25 nM | 18% | 82% |
| " | " | " | 50 nM | 16% | 84% |
| " | " | " | 100 nM | 19% | 81% |
| 17899 | 33 | coding | 10 nM | 37% | 63% |
| " | " | " | 25 nM | 23% | 77% |
| " | " | " | 50 nM | 18% | 82% |
| " | " | " | 100 nM | 21% | 79% |
| 17904 | 38 | stop | 10 nM | 31% | 69% |
| " | " | " | 25 nM | 21% | 79% |
| " | " | " | 50 nM | 17% | 83% |
| " | " | " | 100 nM | 19% | 81% |
| 17907 | 41 | 3'-UTR | 10 nM | 37% | 63% |
| " | " | " | 25 nM | 22% | 78% |
| " | " | " | 50 nM | 18% | 72% |
| " | " | " | 100 nM | 18% | 72% |

## Example 4

### Rat p38α Oligonucleotide Sequences

[0244] Antisense oligonucleotides were designed to target rat p38α. Target sequence data are from the p38 MAPK cDNA sequence; Genbank accession number U73142, provided herein as SEQ ID NO: 45. Oligonucleotides was synthesized as 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 in the wings are phosphodiester (P═O). Internucleoside linkages in the central gap are phosphorothioate (P═S). All 2'-MOE cytosines and 2'-OH cytosines were 5-methyl-cytosines. These oligonucleotide sequences are shown in Table 7.

[0245] bEND.3, a mouse endothelial cell line (gift of Dr. Werner Risau; see Montesano et al., *Cell,* 1990, 62, 435, and Stepkowski et al., *J. Immunol.,* 1994, 153, 5336) were grown in high-glucose DMEM (Life Technologies, Gaithersburg, Md.) medium containing 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycinin. Cells were plated at approximately $2\times10^5$ cells per 100 mm dish. Within 48 hours of plating, the cells were washed with phosphate-buffered saline (Life Technologies). Then, Opti-MEM7 medium containing 3 μg/mL LIPOFECTIN[7] and an appropriate amount of oligonucleotide were added to the cells. As a control, cells were treated with LIPOFECTIN[7] without oligonucleotide under the same conditions and for the same times as the oligonucleotide-treated samples.

[0246] After 4 hours at 37° C., the medium was replaced with high glucose DMEM medium containing 10% FBS and 1% Penicillin/Streptomycinin. The cells were typically allowed to recover overnight (about 18 to 24 hours) before RNA and/or protein assays were performed as described in Example 2. The p38α, p38β and G3PDH probes used were identical to those described in Example 2.

TABLE 7

Nucleotide Sequences of Rat p38α Phosphorothioate Oligonucleotides

| ISIS NO. | NUCLEOTIDE SEQUENCE[1] (5' → 3') | SEQ ID NO | TARGET GENE NUCLEOTIDE CO-ORDINATES[2] | GENE TARGET REGION |
|---|---|---|---|---|
| 21844 | **CoToGoCoGs**AsCsAsTsTsTsTsCsCsAs**GoCoGoGoC** | 47 | 0001–0020 | AUG |
| 21845 | **GoGoToAoAs**GsCsTsTsCsTsGsAsCsAs**CoToToCoA** | 48 | 0361–0380 | coding |
| 21846 | **GoGoCoCoAs**GsAsGsAsCsTsGsAsAsTs**GoToAoGoT** | 49 | 0781–0800 | coding |
| 21871 | **CoAoToCoAs**TsCsAsGsGsGsTsCsGsTs**GoGoToAoC** | 50 | 0941–0960 | coding |
| 21872 | **GoGoCoAoCs**AsAsAsGsCsTsAsAsTsGs**AoCoToToC** | 51 | 1041–1060 | coding |
| 21873 | **AoGoGoToGs**CsTsCsAsGsGsAsCsTsCs**CoAoToToT** | 52 | 1081–1100 | stop |
| 21874 | **GoGoAoToGs**GsAsCsAsGsAsAsCsAsGs**AoAoGoCoA** | 53 | 1101–1120 | 3'-UTR |
| 21875 | **GoAoGoCoAs**GsGsCsAsGsAsCsTsGsCs**CoAoAoGoG** | 54 | 1321–1340 | 3'-UTR |
| 21876 | **AoGoGoCoCo**TsAsGsAsGsCsCsCsAsGsGs**AoGoCoCoA** | 55 | 1561–1580 | 3'-UTR |
| 21877 | **GoAoGoCoCs**TsGsTsGsCsCsTsGsGsCs**AoCoToGoG** | 56 | 1861–1880 | 3'-UTR |
| 21878 | **ToGoCoAoCs**CsAsCsAsAsGsCsAsCsCs**ToGoGoAoG** | 57 | 2081–2100 | 3'-UTR |
| 21879 | **GoGoCoToAs**CsCsAsTsGsAsGsTsGsAs**GoAoAoGoA** | 58 | 2221–2240 | 3'-UTR |
| 21880 | **GoToCoCoCs**TsGsCsAsCsTsGsAsTsAs**GoAoGoAoA** | 59 | 2701–2720 | 3'-UTR |
| 21881 | **ToCoToToCs**CsAsAsTsGsGsGsAsGsAs**AoCoToGoG** | 60 | 3001–3020 | 3'-UTR |

[0247] [1]Emboldened residues, 2'-methoxyethoxy-residues (others are 2'-deoxy-); 2'-MOE cytosines and 2'-deoxy cytosine residues are 5-methyl-cytosines; "s" linkages are phosphorothioate linkages; "o" linkages are phosphodiester linkages. [2] Co-ordinates from Genbank Accession No. U73142, locus name "RNU73142", SEQ ID NO. 45.

[0248] Rat p38α antisense oligonucleotides were screened in bEND.3 cells for inhibition of p38α and p38β mRNA expression. The concentration of oligonucleotide used was 100 nM. Results are shown in Table 8. Oligonucleotides 21844 (SEQ ID NO. 47), 21845 (SEQ ID NO. 48), 21872 (SEQ ID NO. 51), 21873 (SEQ ID NO. 52), 21875 (SEQ ID NO. 54), and 21876 (SEQ ID NO.

[0249] 55) showed greater than approximately 70% inhibition of p38α mRNA with minimal effects on p38β mRNA levels. Oligonucleotide 21871 (SEQ ID NO. 50) inhibited both p38α and p38β levels greater than 70%.

TABLE 8

Inhibition of Mouse p38 mRNA expression in bEND.3 Cells by Chimeric (deoxy gapped) Mixed Backbone p38α Antisense Oligonucleotides

| ISIS No: | SEQ ID NO: | GENE TARGET REGION | % p38α mRNA INHIBITION | % p38β mRNA INHIBITION |
|---|---|---|---|---|
| control | — | — | 0% | 0% |
| 21844 | 47 | AUG | 81% | 20% |
| 21845 | 48 | coding | 75% | 25% |

TABLE 8-continued

Inhibition of Mouse p38 mRNA expression in bEND.3 Cells by Chimeric (deoxy gapped) Mixed Backbone p38α Antisense Oligonucleotides

| ISIS No: | SEQ ID NO: | GENE TARGET REGION | % p38α mRNA INHIBITION | % p38β mRNA INHIBITION |
|---|---|---|---|---|
| 21871 | 50 | coding | 90% | 71% |
| 21872 | 51 | coding | 87% | 23% |
| 21873 | 52 | stop | 90% | 3% |
| 21874 | 53 | 3'-UTR | 38% | 21% |
| 21875 | 54 | 3'-UTR | 77% | — |
| 21876 | 55 | 3'-UTR | 69% | — |
| 21877 | 56 | 3'-UTR | 55% | 13% |
| 21878 | 57 | 3'-UTR | 25% | 10% |
| 21879 | 58 | 3'-UTR | — | — |
| 21881 | 60 | 3'-UTR | — | — |

[0250] Several of the most active oligonucleotides were selected for dose response studies. bEND.3 cells were cultured and treated as described above, except that the concentration of oligonucleotide was varied as noted in Table 9. Results are shown in Table 9.

TABLE 9

Dose Response of bEND.3 cells to rat p38β
Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS # | SEQ ID NO: | ASO Gene Target | Dose | % p38α mRNA Inhibition | % p38β mRNA Inhibition |
|---|---|---|---|---|---|
| control | — | — | — | 100% | 0% |
| 21844 | 47 | AUG | 1 nM | — | — |
| " | " | " | 5 nM | — | — |
| " | " | " | 25 nM | 36% | 8% |
| " | " | " | 100 nM | 80% | 5% |
| 21871 | 50 | coding | 1 nM | 1% | — |
| " | " | " | 5 nM | 23% | 4% |
| " | " | " | 25 nM | 34% | 24% |
| " | " | " | 100 nM | 89% | 56% |
| 21872 | 51 | stop | 1 nM | — | — |
| " | " | " | 5 nM | — | — |
| " | " | " | 25 nM | 35% | — |
| " | " | " | 100 nM | 76% | 1% |
| 21873 | 52 | stop | 1 nM | — | 53% |
| " | " | " | 5 nM | — | 31% |
| " | " | " | 25 nM | 54% | 28% |
| " | " | " | 100 nM | 92% | 25% |

TABLE 9-continued

Dose Response of bEND.3 cells to rat p38β
Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS # | SEQ ID NO: | ASO Gene Target | Dose | % p38α mRNA Inhibition | % p38β mRNA Inhibition |
|---|---|---|---|---|---|
| 21875 | 54 | 3'-UTR | 1 nM | — | 11% |
| " | " | " | 5 nM | — | 16% |
| " | " | " | 25 nM | 33% | 2% |
| " | " | " | 100 nM | 72% | 4% |

## Example 5

### Mouse p38β Oligonucleotide Sequences

**[0251]** Antisense oligonucleotides were designed to target mouse p38β. Target sequence data are from a mouse EST sequence; Genbank accession number AI119044, provided herein as SEQ ID NO 61. Oligonucleotides was synthesized as 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 in the wings are phosphodiester (P═O). Internucleoside linkages in the central gap are phosphorothioate (P═S). All 2'-MOE cytosines and 2'-OH cytosines were 5-methyl-cytosines. These oligonucleotide sequences are shown in Table 10.

TABLE 10

Nucleotide Sequences of Mouse p38β
Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS NO. | NUCLEOTIDE SEQUENCE' (5' → 3') | SEQ ID NO: | TARGET GENE NUCLEOTIDE CO-ORDINATES[2] |
|---|---|---|---|
| 100800 | **C**o**A**o**C**o**A**o**G**s**A**s**A**s**G**s**C**s**A**s**G**s**C**s**T**s**G**s**G**s**A**o**G**o**C**o**G**o**A** | 63 | 0051–0070 |
| 100801 | **T**o**G**o**C**o**G**o**G**s**C**s**A**s**C**s**C**s**T**s**C**s**C**s**C**s**A**s**T**s**A**o**C**o**T**o**G**o**T** | 64 | 0119–0138 |
| 100802 | **C**o**C**o**C**o**T**o**G**s**C**s**A**s**G**s**C**s**C**s**G**s**C**s**T**s**G**s**C**s**G**o**G**o**C**o**A**o**C** | 65 | 0131–0150 |
| 100803 | **G**o**C**o**A**o**G**o**A**s**C**s**T**s**G**s**A**s**G**s**C**s**C**s**G**s**T**s**A**s**G**o**G**o**C**o**G**o**C** | 66 | 0171–0190 |
| 100804 | **T**o**T**o**A**o**C**o**A**s**G**s**C**s**C**s**A**s**C**s**C**s**T**s**T**s**C**s**T**s**G**o**G**o**C**o**G**o**C** | 67 | 0211–0230 |
| 100805 | **G**o**T**o**A**o**T**o**G**s**T**s**C**s**C**s**T**s**C**s**C**s**T**s**C**s**G**s**C**s**G**o**T**o**G**o**G**o**A** | 68 | 0261–0280 |
| 100806 | **A**o**T**o**G**o**G**o**A**s**T**s**G**s**T**s**G**s**G**s**C**s**C**s**G**s**G**s**C**s**G**o**T**o**G**o**A**o**A** | 69 | 0341–0360 |
| 100807 | **G**o**A**o**A**o**T**o**T**s**G**s**A**s**A**s**C**s**A**s**T**s**G**s**C**s**T**s**C**s**A**o**T**o**C**o**G**o**C** | 70 | 0441–0460 |
| 100808 | **A**o**C**o**A**o**T**o**T**s**G**s**C**s**T**s**G**s**G**s**G**s**C**s**T**s**T**s**C**s**A**o**G**o**G**o**T**o**C** | 71 | 0521–0540 |
| 100809 | **A**o**T**o**C**o**C**o**T**s**C**s**A**s**G**s**C**s**T**s**C**s**G**s**C**s**A**s**G**s**T**o**C**o**C**o**T**o**C** | 72 | 0551–0570 |
| 100810 | **T**o**A**o**C**o**C**o**A**s**C**s**C**s**G**s**T**s**G**s**T**s**G**s**G**s**C**s**C**s**A**o**C**o**A**o**T**o**A** | 73 | 0617–0636 |
| 100811 | **C**o**A**o**G**o**T**o**T**s**T**s**A**s**G**s**C**s**A**s**T**s**G**s**A**s**T**s**C**s**T**o**C**o**T**o**G**o**G** | 74 | 0644–0663 |
| 100812 | **C**o**A**o**G**o**G**o**C**s**C**s**A**s**C**s**A**s**G**s**A**s**C**s**C**s**A**s**G**s**A**o**T**o**G**o**T**o**C** | 75 | 0686–0705 |

TABLE 10-continued

Nucleotide Sequences of Mouse p38β
Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS NO. | NUCLEOTIDE SEQUENCE' (5' → 3') | SEQ ID NO: | TARGET GENE NUCLEOTIDE CO-ORDINATES[2] |
|---|---|---|---|
| 100813 | **C**o**C**o**T**o**T**o**C**sCsAsGsCsAsGsTsTsCsAs**A**o**G**o**C**o**C**o**A** | 76 | 0711—0730 |
| 101123 | **C**o**A**o**G**o**C**o**A**sCsCsAsTsGsGsAsCsGsCs**G**o**G**o**A**o**A**o**C** | 77 | 21871 mismatch |

[1]Emboldened residues, 2'-methoxyethoxy- residues (others are 2'-deoxy-), including 2'-MOE and 2'-deoxy residues, 5-methyl-cytosines; "s" linkages are phosphorothioate linkages, "o" linkages are phosphodiester.
[2]Co-ordinates from Genbank Accession No. AI119044, locus name "AI119044", SEQ ID NO. 61.

[0252] Mouse p38β antisense sequences were screened in bEND.3 cells as described in Example 4. Results are shown in Table 11.

[0253] Oligonucleotides 100800 (SEQ ID NO. 63), 100801 (SEQ ID NO. 64), 100803 (SEQ ID NO. 66), 100804 (SEQ ID NO. 67), 100805 (SEQ ID NO. 68), 100807 (SEQ ID NO. 70), 100808 (SEQ ID NO. 71), 100809 (SEQ ID NO. 72), 100810 (SEQ ID NO. 73), 100811 (SEQ ID NO.74), and 100813 (SEQ ID NO. 76) resulted in at least 50% inhibition of p38β mRNA expression. Oligonucleotides 100801 (SEQ ID NO.64), 100803 (SEQ ID NO. 66), 100804 (SEQ ID NO. 67), 100805 (SEQ ID NO. 68), 100809 (SEQ ID NO. 72), and 100810 (SEQ ID NO. 73) resulted in at least 70% inhibition and are preferred. Oligonucleotides 100801 (SEQ ID NO. 64), 100805 (SEQ ID NO. 68), and 100811 (SEQ ID NO. 74) resulted in significant inhibition of p38α mRNA expression in addition to their effects on p38β.

TABLE 11

Inhibition of Mouse p38 mRNA expression in bEND.3 Cells by Chimeric (deoxy gapped) Mixed Backbone p38β Antisense Oligonucleotides

| ISIS No: | SEQ ID NO: | % p38β mRNA INHIBITION | % p38α mRNA INHIBITION |
|---|---|---|---|
| control | — | 0% | 0% |
| 100800 | 63 | 51% | — |
| 100801 | 64 | 74% | 31% |
| 100802 | 65 | 35% | — |
| 100803 | 66 | 74% | 18% |
| 100804 | 67 | 85% | 18% |
| 100805 | 68 | 78% | 58% |
| 100806 | 69 | 22% | 3% |
| 100807 | 70 | 64% | — |
| 100808 | 71 | 53% | 13% |
| 100809 | 72 | 84% | 14% |
| 100810 | 73 | 72% | 1% |
| 100811 | 74 | 60% | 43% |
| 100812 | 75 | 36% | 17% |
| 100813 | 76 | 54% | — |

Example 6

Effect of p38 MAPK Antisense Oligonucleotides on IL-6 Secretion

[0254] p38 MAPK antisense oligonucleotides were tested for their ability to reduce IL-6 secretion. bEND.3 cells were cultured and treated as described in Example 4 except that 48 hours after oligonucleotide treatment, cells were stimulated for 6 hours with 1 ng/mL recombinant mouse IL-1 (R&D Systems, Minneapolis, Minn.). IL-6 was measured in the medium using an IL-6 ELISA kit (Endogen Inc., Woburn, Mass.).

[0255] Results are shown in Table 12. Oligonucleotides targeting a specific p38 MAPK isoform were effective in reducing IL-6 secretion greater than approximately 50%.

TABLE 12

Effect of p38 Antisense Oligonucleotides on IL-6 secretion

| ISIS No: | SEQ ID NO: | GENE TARGET | DOSE (μM) | % IL-6 INHIBITION |
|---|---|---|---|---|
| control | — | — | | 0% |
| 21873 | 52 | p38α | 100 | 49% |
| 100804 | 67 | p38β | 100 | 57% |
| 21871 | 50 | p38α and p38β | 200 | 23% |

Example 7

Activity of p38α Antisense Oligonucleotides in Rat Cardiomyocytes

[0256] Rat p38α antisense oligonucleotides were screened in Rat A-10 cells. A-10 cells (American Type Culture Collection, Manassas, Va.) were grown in high-glucose DMEM (Life Technologies, Gaithersburg, Md.) medium containing 10% fetal calf serum (FCS). Cells were treated with oligonucleotide as described in Example 2. Oligonucleotide concentration was 200 nM. mRNA was isolated 24 hours after time zero and quantitated by Northern blot as described in Example 2.

[0257] Results are shown in Table 13. Oligonucleotides 21845 (SEQ ID NO. 48), 21846 (SEQ ID NO. 49), 21871

(SEQ ID NO. 50), 21872 (SEQ ID NO. 51), 21873 (SEQ ID NO. 52), 21874 (SEQ ID NO. 53), 21875 (SEQ ID NO. 54), 21877 (SEQ ID NO. 56), 21878 (SEQ ID NO. 57), 21879 (SEQ ID NO. 58), and 21881 (SEQ ID NO. 60) inhibited p38α mRNA expression by 65% or greater in this assay. Oligonucleotides 21846 (SEQ ID NO. 49), 21871 (SEQ ID NO. 50), 21872 (SEQ ID NO. 51), 21877 (SEQ ID NO. 56), and 21879 (SEQ ID NO. 58) inhibited p38α mRNA expression by greater than 85% and are preferred.

TABLE 13

Inhibition of Rat p38α mRNA expression in A-10 Cells by Chimeric (deoxy gapped) Mixed Backbone p38α Antisense Oligonucleotides

| ISIS No: | SEQ ID NO: | GENE TARGET REGION | % p38α mRNA EXPRESSION | % p38α mRNA INHIBITION |
|---|---|---|---|---|
| control | — | — | 100% | 0% |
| 21844 | 47 | AUG | 75% | 25% |
| 21845 | 48 | coding | 25% | 75% |
| 21846 | 49 | coding | 8% | 92% |
| 21871 | 50 | coding | 12% | 88% |
| 21872 | 51 | coding | 13% | 87% |
| 21873 | 52 | stop | 19% | 81% |
| 21874 | 53 | 3'-UTR | 22% | 78% |
| 21875 | 54 | 3'-UTR | 26% | 74% |
| 21876 | 55 | 3'-UTR | 61% | 39% |
| 21877 | 56 | 3'-UTR | 12% | 88% |
| 21878 | 57 | 3'-UTR | 35% | 65% |
| 21879 | 58 | 3'-UTR | 11% | 89% |
| 21881 | 60 | 3'-UTR | 31% | 69% |

[0258] The most active oligonucleotide in this screen (SEQ ID NO. 49) was used in rat cardiac myocytes prepared from neonatal rats (Zechner, D., et. al., *J. Cell Biol.,* 1997, 139, 115-127). Cells were grown as described in Zechner et al. and transfected with oligonucleotide as described in Example 2. Oligonucleotide concentration was 1 μM. mRNA was isolated 24 hrs after time zero and quantitated using Northern blotting as described in Example 2. An antisense oligonucleotide targeted to JNK-2 was used as a non-specific target control.

[0259] Results are shown in Table 14. Oligonucleotide 21846 (SEQ ID NO. 49) was able to reduce p38α expression in rat cardiac myocytes by nearly 60%. The JNK-2 antisense oligonucleotide had little effect on p38α expression.

TABLE 14

Inhibition of Rat p38α mRNA expression in Rat Cardiac Myocytes by A Chimeric (deoxy gapped) Mixed Backbone p38α Antisense Oligonucleotide

| ISIS No: | SEQ ID NO: | GENE TARGET REGION | % p38α mRNA EXPRESSION | % p38α mRNA INHIBITION |
|---|---|---|---|---|
| control | — | — | 100% | 0% |
| 21846 | 49 | coding | 41% | 59% |

[0260] Eample 8

Additional Human p38α Oligonucleotide Sequences

[0261] Additional antisense oligonucleotides were designed to target human p38α based on active rat sequences. Target sequence data are from the p38 MAPK cDNA sequence; Genbank accession number L35253, provided herein as SEQ ID NO: 1. Oligonucleotides were synthesized as 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 2'-MOE cytosines and 2'-OH cytosines were 5-methylcytosines. These oligonucleotide sequences are shown in Table 15.

TABLE 15

Additional Nucleotide Sequences of Human p38α Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS NO. | NUCLEOTIDE SEQUENCE[1] (5' → 3') | SEQ ID NO: | TARGET GENE NUCLEOTIDE CO-ORDINATES[2] | GENE TARGET REGION |
|---|---|---|---|---|
| 100860 | **CTGAG**ACATTTTCCA**GCGGC** | 78 | 0284–0303 | Start |
| 100861 | **ACGCT**CGGGCACCTC**CCAGA** | 79 | 0344–0363 | coding |
| 100862 | **AGCTT**CTTCACTGCC**ACACG** | 80 | 0439–0458 | coding |
| 100863 | **AATGA**TGGACTGAAA**TGGTC** | 81 | 0464–0483 | coding |
| 100864 | **TCCAA**CAGACCAATC**ACATT** | 82 | 0538–0557 | coding |
| 100865 | **TGTAA**GCTTCTGACA**TTTCA** | 83 | 0644–0663 | coding |
| 100866 | **TGAAT**GTATATACTT**TAGAC** | 84 | 0704–0723 | coding |
| 100867 | **CTCAC**AGTCTTCATT**CACAG** | 85 | 0764–0783 | coding |
| 100868 | **CACGT**AGCCTGTCATT**TCAT** | 86 | 0824–0843 | coding |
| 100869 | **CATCC**CACTGACCAA**ATATC** | 87 | 0907–0926 | coding |
| 100870 | **TATGG**TCTGTACCAG**GAAAC** | 88 | 0960–0979 | coding |
| 100871 | **AGTCA**AAGACTGAA**TATAGT** | 89 | 1064–1083 | coding |
| 100872 | **TTCTC**TTATCTGAG**TCCAAT** | 90 | 1164–1183 | coding |
| 100873 | **CATCA**TCAGGATCG**TGGTAC** | 91 | 1224–1243 | coding |
| 100874 | **TCAAA**GGACTGATCA**TAAGG** | 92 | 1258–1277 | coding |
| 100875 | **GGCAC**AAAGCTGATG**ACTTC** | 93 | 1324–1343 | coding |
| 100876 | **AGGTG**CTCAGGACTC**CATCT** | 94 | 1364–1383 | stop |
| 100877 | **GCAAC**AAGAGGCAC**TTGAAT** | 95 | 1452–1471 | 3'-UTR |

[1]Emboldened residues, 2'-methoxyethoxy- residues (others are 2'-deoxy-) including "C" and "C" residues, 5-methyl-cytosines; all linkages are phosphorothioate linkages.
[2]s Co-ordinates from Genbank Accession No. L35253, locus name "HUMMAPKNS", SEQ ID NO. 1.

[0262] For an initial screen of human p38α antisense oligonucleotides, T-24 cells, a human transitional cell bladder carcinoma cell line, were obtained from the American Type Culture Collection (ATCC) (Manassas, Va.). T-24 cells were routinely cultured in complete McCoy's 5A 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. A control oligonucleotide ISIS 118965 (TTATCCTAGCTTAGACCTAT, herein incorporated as SEQ ID NO: 96) was synthesized as chimeric oligonucleotide ("gapmer") 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 2'-MOE cytosines and 2'OH cytosines were 5-methyl-cytosines.

[0263] 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. mRNA was measured by Northern blot. Results are shown in Table 16. Oligonucleotides 100861 (SEQ ID NO. 79) 100862 (SEQ ID NO. 80), 100863 (SEQ ID NO. 81), 100866 (SEQ ID NO. 84), 100867 (SEQ ID NO. 85), 100868 (SEQ ID NO. 86) 100870 (SEQ ID NO. 88), 100871 (SEQ ID NO. 89), 100872 (SEQ NO. 90), 100873 (SEQ ID NO. 91), and 100874 (SEQ ID NO. 92) 100875 (SEQ ID NO. 93) and 100877 (SEQ ID NO. 95) gave greater than approximately 40% inhibition and are preferred.

TABLE 16

Inhibition of Human p38α mRNA expression in T-24 Cells by Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS No: | SEQ ID NO: | GENE TARGET REGION | % P38α mRNA EXPRESSION | % P38β mRNA EXPRESSION |
|---|---|---|---|---|
| 100860 | 78 | 0284–0303 | 73% | 71% |
| 100861 | 79 | 0344–0363 | 60% | 47% |
| 100862 | 80 | 0439–0458 | 56% | 45% |
| 100863 | 81 | 0464–0483 | 49% | 67% |
| 100864 | 82 | 0538–0557 | 66% | 70% |
| 100865 | 83 | 0644–0663 | 64% | 63% |
| 100866 | 84 | 0704–0723 | 55% | 65% |
| 100867 | 85 | 0764–0783 | 58% | 33% |
| 100868 | 86 | 0824–0843 | 47% | 60% |
| 100869 | 87 | 0907–0926 | 61% | 100% |
| 100870 | 88 | 0960–0979 | 51% | No data |
| 100871 | 89 | 1064–1083 | 57% | 96% |
| 100872 | 90 | 1164–1183 | 37% | 77% |
| 100873 | 91 | 1224–1243 | 34% | 70% |
| 100874 | 92 | 1258–1277 | 42% | 76% |
| 100875 | 93 | 1324–1343 | 39% | 90% |
| 100876 | 94 | 1364–1383 | 77% | 93% |
| 100877 | 95 | 1452–1471 | 47% | 95% |

[0264] Oligonucleotides 100872 (SEQ ID NO. 90), 100873 (SEQ ID NO. 91), 100874 (SEQ ID NO. 92), and 100875 (SEQ ID NO. 93) were chosen for dose response studies.

[0265] Results are shown in Table 17. The effect of these oligonucleotides on human p38β was also determined.

TABLE 17

Dose Response of p38α in T-24 cells to human p38α Chimeric (deoxy gapped) Phosphorothioate Oligonucleotides

| ISIS # | SEQ ID NO: | ASO Gene Target | Dose | % p38α mRNA Expression | % p38β mRNA Inhibition |
|---|---|---|---|---|---|
| Control 118965 | 96 | — | — | 94% | 80% |
| 100872 | 90 | coding | 50 nM | 45% | 108% |
| " | " | " | 100 nM | 18% | 91% |
| " | " | " | 200 nM | 17% | 92% |
| 100873 | 91 | coding | 50 nM | 19% | 90% |
| " | " | " | 100 nM | 12% | 78% |
| " | " | " | 200 nM | 8% | 44% |
| 100874 | 92 | coding | 50 nM | 47% | 107% |
| " | " | " | 100 nM | 27% | 101% |
| " | " | " | 200 nM | 13% | 51% |
| 100875 | 93 | coding | 50 nM | 30% | 105% |
| " | " | " | 100 nM | 13% | 92% |
| " | " | " | 200 nM | 8% | 69% |

Example 9

Additional Human p38β Oligonucleotide Sequences

[0266] Additional antisense oligonucleotides were designed to target human p38β based on active rat sequences. Target sequence data are from the p38 MAPK cDNA sequence; Genbank accession number U53442, provided herein as SEQ ID NO: 23.

[0267] Oligonucleotides was synthesized as 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 in the wings are phosphodiester (P=O). Internucleoside linkages in the central gap are phosphorothioate (P=S). All 2'-MOE cytosines and 2'-OH cytosines were 5-methyl-cytosines. These oligonucleotide sequences are shown in Table 18. A control oligonucleotide ISIS 118966 (GTTCGATCGGCTCGTGTCGA), herein incorporated as SEQ ID NO: 107) was synthesized as chimeric oligonucleotide ("gapmer") 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) in the gap and phosphodiester in the wings. All 2'-MOE cytosines and 2'-OH cytosines were 5-methyl-cytosines.

TABLE 18

Additional Nucleotide Sequences of Human p38β Chimeric (deoxy gapped) Mixed-Backbone Phosphorothioate Oligonucleotides

| ISIS NO. | NUCLEOTIDE SEQUENCE[1] (5' → 3') | SEQ ID NO: | TARGET GENE NUCLEOTIDE CO-ORDINATES[2] | GENE TARGET REGION |
|---|---|---|---|---|
| 107869 | **ACAGA**CGGAGCCGTA**GGCGC** | 97 | 117–136 | coding |
| 107870 | **CACCG**CCACCTTCTG**GCGCA** | 98 | 156–175 | coding |
| 107871 | **GTACG**TTCTGCGCGC**GTGGA** | 99 | 207–226 | coding |

TABLE 18-continued

Additional Nucleotide Sequences of Human p38β Chimeric (deoxy gapped) Mixed-Backbone Phosphorothioate Oligonucleotides

| ISIS NO. | NUCLEOTIDE SEQUENCE[1] (5' → 3') | SEQ ID NO: | TARGET GENE NUCLEOTIDE CO-ORDINATES[2] | GENE TARGET REGION |
|---|---|---|---|---|
| 107872 | **ATGGA**CGTGGCCGGC**GTGAA** | 100 | 287—306 | coding |
| 107873 | **CAGGAA**TTGAACGTG**CTCGT** | 101 | 414—433 | coding |
| 107874 | **ACGTT**GCTGGGCTTC**AGGTC** | 102 | 491—510 | coding |
| 107875 | **TACCA**GCGCGTGGCC**ACATA** | 103 | 587—606 | coding |
| 107876 | **CAGTT**GAGCATGATC**TCAGG** | 104 | 614—633 | coding |
| 107877 | **CGGAC**CAGATATCCA**CTGTT** | 105 | 649—668 | coding |
| 107878 | **TGCCC**TGGAGCAGCT**CAGCC** | 106 | 682—701 | coding |

[1]Emboldened residues, 2'-methoxyethoxy- residues (others are 2'-deoxy-) including "C" and "C" residues, 5-methyl-cytosines.
[2]Co-ordinates from Genbank Accession No. U53442, SEQ ID NO. 23.

[0268] For an initial screen of human p38β antisense oligonuleotides, T-24 cells, a human transitional cell bladder carcinoma cell line, were obtained from the American Type Culture Collection (ATCC) (Manassas, Va.). T-24 cells were routinely cultured in complete McCoy's 5A 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. A control oligonucleotide ISIS 118966 (TTATCCTAGCTTAGACCTAT, herein incorporated as SEQ ID NO: 106) was synthesized as chimeric oligonucleotide ("gapmer") 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) in the gap and phosphodiester in the wings. All 2'-MOE cytosines and 2'-OH cytosines were 5-methyl-cytosines.

[0269] 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. mRNA was measured by Northern blot. Results are shown in Table 19. For comparison, ISIS 17893 and ISIS 17899, both targeting human p38β (SEQ ID NO: 27) and ISIS 100802 targeting mouse p38β (SEQ ID NO: 65) described in Examples 3 and 5 above, respectively, were included in the screen.

[0270] Oligonucleotides 107869 (SEQ ID NO. 97), 107871 (SEQ ID NO. 99), 107872 (SEQ ID NO. 100), 107873 (SEQ ID NO. 101), 107878 (SEQ ID NO.106), 17893 (SEQ ID NO. 27), 17899 (SEQ ID NO. 33) and 100802 (SEQ ID NO.65, targeted to mouse p38β) gave greater than approximately 40% inhibition and are preferred.

TABLE 19

Inhibition of Human p38β mRNA expression in T-24 Cells by Chimeric (deoxy gapped) Mixed-Backbone Phosphorothioate Oligonucleotides

| ISIS No: | SEQ ID NO: | GENE TARGET REGION | % p38β mRNA EXPRESSION | % p38α mRNA EXPRESSION |
|---|---|---|---|---|
| 107869 | 97 | Coding | 60% | 93% |
| 107870 | 98 | Coding | 74% | 97% |
| 107871 | 99 | Coding | 60% | 111% |
| 107872 | 100 | Coding | 57% | 123% |
| 107873 | 101 | Coding | 58% | 120% |
| 107874 | 102 | Coding | 61% | 100% |
| 107875 | 103 | Coding | 92% | 112% |
| 107876 | 104 | Coding | 127% | 137% |
| 107877 | 105 | Coding | No data | No data |
| 107878 | 106 | Coding | 54% | 112% |
| 17893 | 27 | Coding | 31% | 61% |
| 17899 | 33 | Coding | 56% | 117% |
| 100802 | 65 | Coding | 47% | 78% |

[0271] Oligonucleotides 107871, 107872, 107873, 107874, 107875, 107877, 107878, 17893 and 17899 were chosen for dose response studies.

[0272] Results are shown in Table 20. The effect of these oligonucleotides on human p38α was also determined.

TABLE 20

Dose Response of p38β in T-24 cells to human p38β Chimeric (deoxy gapped) Mixed-backbone Phosphorothioate Oligonucleotides

| ISIS # | SEQ ID NO: | ASO Gene Target | Dose | % p38β mRNA Expression | % p38α mRNA Inhibition |
|---|---|---|---|---|---|
| Control 118966 | 107 | — | — | 100% | 100% |
| 107871 | 99 | coding | 50 nM | 41% | 105% |
| " | " | " | 100 nM | 42% | 132% |
| " | " | " | 200 nM | 10% | 123% |
| 107872 | 100 | coding | 50 nM | 71% | 124% |
| " | " | " | 100 nM | 13% | 84% |
| " | " | " | 200 nM | 22% | 102% |
| 107873 | 101 | coding | 50 nM | 69% | 132% |
| " | " | " | 100 nM | 41% | 119% |
| " | " | " | 200 nM | 23% | 131% |
| 107874 | 102 | coding | 50 nM | 75% | 109% |
| " | " | " | 100 nM | 34% | 99% |
| " | " | " | 200 nM | 23% | 87% |
| 107875 | 103 | coding | 50 nM | 82% | 93% |
| " | " | " | 100 nM | 38% | 101% |
| " | " | " | 200 nM | 40% | 91% |
| 107877 | 105 | coding | 50 nM | 50% | 127% |
| " | " | " | 100 nM | 34% | 125% |
| " | " | " | 200 nM | 22% | 106% |
| 107878 | 106 | coding | 50 nM | 70% | 110% |
| " | " | " | 100 nM | 43% | 109% |
| " | " | " | 200 nM | 27% | 116% |
| 17893 | 27 | coding | 50 nM | 28% | 88% |
| " | " | " | 100 nM | 27% | 115% |
| " | " | " | 200 nM | 16% | 108% |
| 17899 | 33 | coding | 50 nM | 89% | 87% |
| " | " | " | 100 nM | 36% | 104% |
| " | " | " | 200 nM | 15% | 80% |

[0273] These data show that the oligonucleotides designed to target human p38β, do so in a target-specific and dose-dependent manner.

Eample 10

Real-Time Quantitative PCR Analysis of p38β mRNA Levels

[0274] Quantitation of p38α mRNA levels was accomplished by real-time quantitative PCR using the ABI PRISM™ 7600, 7700, or 7900 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., FAM or JOE, obtained from either PE-Applied Biosystems, Foster City, Calif., Operon Technologies Inc., Alameda, Calif. or Integrated DNA Technologies Inc., Coralville, Iowa) is attached to the 5' end of the probe and a quencher dye (e.g., TAMRA, obtained from either PE-Applied Biosystems, Foster City, Calif., Operon Technologies Inc., Alameda, Calif. or Integrated DNA Technologies Inc., Coralville, Iowa) 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™ 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.

[0275] 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 multiplexable. Other methods of PCR are also known in the art.

[0276] PCR reagents were obtained from Invitrogen Corporation, (Carlsbad, Calif.). RT-PCR reactions were carried out by adding 20 μL PCR cocktail (2.5×PCR buffer minus $MgCl_2$, 6.6 mM $MgCl_2$, 375 μM each of dATP, dCTP, dCTP and dGTP, 375 nM each of forward primer and reverse primer, 125 nM of probe, 4 Units RNAse inhibitor, 1.25 Units PLATINUM® Taq, 5 Units MuLV reverse transcriptase, and 2.5×ROX dye) to 96-well plates containing 30 μL total RNA solution (20-200 ng). 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 PLATINUM® Taq, 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).

[0277] Gene target quantities obtained by real time RT-PCR are normalized using either the expression level of GAPDH, a gene whose expression is constant, or by quantifying total RNA using RiboGreen™ (Molecular Probes, Inc. Eugene, Oreg.). GAPDH expression is quantified by real time RT-PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RiboGreen™ RNA quantification reagent (Molecular Probes, Inc. Eugene, Oreg.). Methods of RNA quantification by RiboGreen™ are taught in Jones, L. J., et al, (Analytical Biochemistry, 1998, 265, 368-374).

[0278] In this assay, 170 μL of RiboGreen™ working reagent (RiboGreen™ reagent diluted 1:350 in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5) is pipetted into a 96-well plate containing 30 μL purified, cellular RNA. The plate is read in a CytoFluor 4000 (PE Applied Biosystems) with excitation at 485 nm and emission at 530 nm.

[0279] Probes and primers to human p38α were designed to hybridize to a human p38α sequence, using published sequence information (GenBank accession number L35253, incorporated herein as SEQ ID NO:1). For human p38α the PCR primers were:

[0280] forward primer: GATGAGTGGAAAAGCCTGAC (SEQ ID NO: 108)

[0281] reverse primer: CTGCAACAAGAGGCACTTGA (SEQ ID NO: 109) and the PCR probe was: FAM-GATGAAGTCATCAGCTTTGTGCCACCAC-CCCTTGACCAAGAAGAGATGGA-TAMRA (SEQ ID NO: 110) where FAM is the fluorescent dye and TAMRA is the quencher dye. For human GAPDH the PCR primers were:

[0282] forward primer: GAAGGTGAAGGTCGGAGTC(SEQ ID NO: 111)

[0283] reverse primer: GAAGATGGTGATGGGATTTC (SEQ ID NO: 112) and the PCR probe was: 5' JOE-CAAGCTTCCCGTTCTCAGCC-TAMRA 3' (SEQ ID NO: 113) where JOE is the fluorescent reporter dye and TAMRA is the quencher dye.

[0284] Probes and primers to mouse p38α were designed to hybridize to a mouse p38α sequence, using published sequence information (GenBank accession number U10871.1, incorporated herein as SEQ ID NO: 114). For mouse p38α the PCR primers were:

[0285] forward primer: AAGGGAACGAGAAAACTGCTGTT (SEQ ID NO: 115)

[0286] reverse primer: TATTTTAACCAGTGGTATTATCTGACATCCT (SEQ ID NO: 116) and the PCR probe was: FAM-TTGTATTTGTGAACTTGGCTGTAATCTGGTATGCC -TAMRA

[0287] (SEQ ID NO: 117) where FAM is the fluorescent reporter dye and TAMRA is the quencher dye. For mouse GAPDH the PCR primers were:

[0288] forward primer: GGCAAATTCAACGGCACAGT(SEQ ID NO: 118)

[0289] reverse primer: GGGTCTCGCTCCTGGAAGAT(SEQ ID NO: 119) and the PCR probe was: 5' JOE-AAGGCCGAGAATGGGAAGCTTGTCATC-TAMRA 3' (SEQ ID NO: 120) where JOE is the fluorescent reporter dye and TAMRA is the quencher dye.

[0290] Probes and primers to rat p38α were designed to hybridize to a rat p38α sequence, using published sequence information (GenBank accession number U73142, incorporated herein as SEQ ID NO: 45). For rat p38α the PCR primers were:

[0291] forward primer: ATCATTTGGAGCCCAGAAGGA (SEQ ID NO: 121)

[0292] reverse primer: TGGAGCTGGACTGCATACTGA (SEQ ID NO: 122) and the PCR probe was: FAM-CTGGCCAGGCCTCACCGC-TAMRA

[0293] (SEQ ID NO: 123) where FAM is the fluorescent reporter dye and TAMRA is the quencher dye. For rat GAPDH the PCR primers were:

[0294] forward primer: TGTTCTAGAGACAGCCGCATCTT(SEQ ID NO: 124)

[0295] reverse primer: CACCGACCTTCACCATCTTGT(SEQ ID NO: 125) and the PCR probe was: 5' JOE-TTGTGCAGTGCCAGCCTCGTCTCA-TAMRA 3' (SEQ ID NO: 126) where JOE is the fluorescent reporter dye and TAMRA is the quencher dye.

## Example 11

### Additional Human p38α Oligonucleotide Sequences

[0296] Additional antisense oligonucleotides were designed to target human p38α using published sequence (Genbank accession number NM_001315.1, provided herein as SEQ ID NO: 127). Oligonucleotides were synthesized as 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. Internucleoside linkages are phosphorothioate (P=S). These oligonucleotide sequences are shown in Table 21. "Target site" indicates the first (5'-most) nucleotide number on the particular target sequence to which the compound binds. The compounds can be analyzed for their effect on human p38α mRNA levels by quantitative real-time PCR as described in other examples herein.

TABLE 21

Additional chimeric phosphorothioate antisense oligonucleotides targeted to human p38α

| ISIS # | Region | Target Sequence Accession # | Target Site | SEQUENCE | SEQ ID NO: |
|---|---|---|---|---|---|
| 186877 | coding | NM_001315.1 | 1271 | GAGCAAAGTAGGCATGTGCA | 128 |
| 186878 | 3' UTR | NM_001315.1 | 2703 | GTTTCCGAAGTTTGGGATAT | 129 |
| 186879 | 3' UTR | NM_001315.1 | 2735 | GCATTAGTTATTGGGAGTGA | 130 |
| 186880 | 3' UTR | NM_001315.1 | 1671 | CCCTGGAGCATCCACAACCT | 131 |
| 186881 | coding | NM_001315.1 | 1021 | TGTACCAGGAAACAATGTTC | 132 |
| 186882 | 5' UTR | NM_001315.1 | 326 | CGGGCAAGAAGGTGGCCCTG | 133 |
| 186883 | 3' UTR | NM_001315.1 | 3296 | ATCGCCATCAGTCTGCCTCC | 134 |
| 186884 | 3' UTR | NM_001315.1 | 2312 | TGACATCAAGAACCTGCTTC | 135 |
| 186885 | 3' UTR | NM_001315.1 | 2134 | GGCCCACAAGCAGCTGTCCA | 136 |
| 186886 | 3' UTR | NM_001315.1 | 3063 | TGAAAACGACACTTCTCCAC | 137 |
| 186887 | 3' UTR | NM_001315.1 | 3307 | GGTGAGAGGGAATCGCCATC | 138 |
| 186888 | 3' UTR | NM_001315.1 | 2007 | ATACTGTCAAGATCTGAGAA | 139 |
| 186889 | 3' UTR | NM_001315.1 | 2702 | TTTCCGAAGTTTGGGATATT | 140 |
| 186890 | 3' UTR | NM_001315.1 | 2205 | AGAGAGACGCACATATACGC | 141 |
| 186891 | 3' UTR | NM_001315.1 | 1516 | CAACAGGCACTTGAATAATA | 142 |

TABLE 21-continued

Additional chimeric phosphorothioate antisense oligonucleotides targeted to human p38α

| ISIS # | Region | Target Sequence Accession # | Target Site | SEQUENCE | SEQ ID NO: |
|---|---|---|---|---|---|
| 186892 | coding | NM_001315.1 | 638 | ATTCCTCCAGAGACCTTGCA | 143 |
| 186893 | 3' UTR | NM_001315.1 | 2848 | AAGACACCTTGTTACTTTTT | 144 |
| 186894 | 3' UTR | NM_001315.1 | 2989 | TGCCCTTTCTCCCCATCAAA | 145 |
| 186895 | coding | NM_001315.1 | 1096 | TGGCATCCTGTTAATGAGAT | 146 |
| 186896 | 3' UTR | NM_001315.1 | 1477 | AAGGCCTTCCCCTCACAGTG | 147 |
| 186897 | 3' UTR | NM_001315.1 | 3728 | AATAGGCTTTATTTTAACCA | 148 |
| 186898 | 3' UTR | NM_001315.1 | 2455 | ACCCAAGAAGTCTTCACTGG | 149 |
| 186899 | 3' UTR | NM_001315.1 | 3135 | TTTCTTATTACACAAAAGGC | 150 |
| 186900 | 3' UTR | NM_001315.1 | 3445 | GGAAATCACACGAGCATTTA | 151 |
| 186901 | coding | NM_001315.1 | 794 | GGTCCCTGTGAATTATGTCA | 152 |
| 186902 | 3' UTR | NM_001315.1 | 3112 | AATATATGAGTCCTCATGTA | 153 |
| 186903 | 3' UTR | NM_001315.1 | 3511 | CTAACACGTATGTGGTCACA | 154 |
| 186904 | 3' UTR | NM_001315.1 | 2984 | TTTCTCCCCATCAAAAGGAA | 155 |
| 186905 | coding | NM_001315.1 | 727 | CTGAACATGGTCATCTGTAA | 156 |
| 186906 | 3' UTR | NM_001315.1 | 3681 | ATAACTGATTACAGCCAAGT | 157 |
| 186907 | 3' UTR | NM_001315.1 | 2959 | TTCTCAAAGGGATTCCTACA | 158 |
| 186908 | coding | NM_001315.1 | 678 | TCTGCCCCCATGAGATGGGT | 159 |
| 186909 | coding | NM_001315.1 | 540 | TTCGCATGAATGATGGACTG | 160 |
| 186910 | coding | NM_001315.1 | 1275 | TACTGAGCAAAGTAGGCATG | 161 |
| 186911 | coding | NM_001315.1 | 1336 | GTCCCTGCTTTCAAAGGACT | 162 |
| 186912 | coding | NM_001315.1 | 577 | CATATGTTTAAGTAACCGCA | 163 |
| 186913 | 3' UTR | NM_001315.1 | 2963 | CACATTCTCAAAGGGATTCC | 164 |

[0297] Additional antisense oligonucleotides were designed to target human p38α using published sequence (Genbank accession number NM_001315.1, provided herein as SEQ ID NO: 127. Oligonucleotides were synthesized as oligonucleotides comprised of 2'-deoxynucleotides and phosphodiester internucleoside linkages (P═O). These oligonucleotide sequences are shown in Table 22. "Target site" indicates the first (5'-most) nucleotide number on the particular target sequence to which the compound binds.

TABLE 22

Additional phosphodiester oligonucleotides targeted to p38α

| ISIS # | Region | Target Sequence Accession | Target Site | SEQUENCE | SEQ ID NO |
|---|---|---|---|---|---|
| 169107 | coding | NM_001315.1 | 1420 | GGACTCCATCTCTTCTTGGTCAA | 165 |
| 336747 | 3' UTR | NM_001315.1 | 1454 | GAAGTGGGATCAACAGAACAGAAA | 166 |
| 336750 | coding | NM_001315.1 | 436 | AGCCCACTGGAGACAGGTTCT | 167 |

Example 12

Mouse and Rat p38α Antisense Oligonucleotides

[0298] Antisense oligonucleotides were designed to target mouse p38α using published sequences (Genbank accession number U10871.1, provided herein as SEQ ID NO: 114, GenBank accession number D83073.1, provided herein as SEQ ID NO: 168, GenBank accession number AA002328.1, provided herein as SEQ ID NO: 169, GenBank accession number AF128892.1, provided herein as SEQ ID NO: 170, GenBank accession number BY159314.1, provided herein as SEQ ID NO: 171 and Genbank accession number BY257628.1, provided herein as SEQ ID NO: 172). These compounds are shown in the tables included in this example.

[0299] Antisense oligonucleotides were also designed to target rat p38α using published sequences (GenBank accession number U73142, provided herein as SEQ ID NO: 45, and Genbank accession number U91847.1, provided herein as SEQ ID NO: 173). These compounds are shown in the tables in this example.

[0300] Oligonucleotides were synthesized as 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. Internucleoside linkages are phosphorothioate (P═S). In Table 23, "Target site" indicates the first (5'-most) nucleotide number on the particular target sequence to which the compound binds.

[0301] The compounds in Table 23 were analyzed for their effect on mouse p38α mRNA levels by quantitative real-time PCR as described in other examples herein. Data are averages from two experiments in which bEND.3 cells were treated with the antisense oligonucleotides of the present invention and are presented in the column labeled "% inhib, mouse p38α". If present, "N.D." indicates "no data". ISIS 18078 (GTGCGCGCGAGCCCGAAATC, SEQ ID NO: 174) was used as a scrambled control oligonucleotide.

[0302] The compounds in Table 23 were also analyzed for their effect on rat p38α mRNA levels in NR-8383 cells by quantitative real-time PCR as described in other examples herein. The rat normal lung alveolar macrophage cell line NR-8383 was obtained from the American Type Culture Collection (Manassas, Va.). NR-8383 cells were routinely cultured in Ham's F12 medium (Gibco/Life Technologies, Gaithersburg, Md.) supplemented with 10% fetal bovine serum (Gibco/Life Technologies, Gaithersburg, Md.), and 1% Penicillin/Streptomycin (Gibco/Life Technologies, Gaithersburg, Md.). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. For transfection with oligonucleotides, NR-8383 cells were plated on 24 well plates at a density of $4\times10^4$ cells/cm2 ($8.0\times10^4$ cells/well) in serum-free F12 Nutrient Medium (Gibco/Life Technologies, Gaithersburg, Md.). After 2 hours, media was removed and replaced with 400 ul of Ham's F12 Nutrient Medium supplemented with 15% fetal bovine serum and 1% Penicillin/Streptomyocin. Cells were then transfected with 300 nM of antisense oligonucleotides mixed with FuGENE 6

[0303] Transfection Reagent (Roche Applied Science, Indianapolis, Ind.) for 24 hours, after which mRNA was quantitated as described in other examples herein. Data are averages from two experiments in which NR-8383 cells were treated with the antisense oligonucleotides of the present invention and are presented in the column labeled "% inhib, rat p38α". If present, "N.D." indicates "no data". ISIS 18078 (GTGCGCGCGAGCCCGAAATC, SEQ ID NO: 174) was used as a scrambled control oligonucleotide.

[0304] One additional compound, ISIS 186911 (SEQ ID NO: 143), targeted to human p38α, was also tested for its effect on mouse and rat p38α mRNA expression in bEND.3 cells and NR-8383 cells, respectively.

[0305] An asterisk (*) adjacent to the ISIS oligonucleotide number in Table 23 indicates that the oligonucleotide targets human, mouse and rat p38αsequences. Compounds in Table 23, with the exception of ISIS 101753, ISIS 320119, ISIS 320120 and 320121 target both mouse and rat p38α.

TABLE 23

Inhibition of mouse and rat p38α by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

| ISIS # | Region | Target Sequence Accession # | Target Site | Sequence | % Inhib. mouse p38α | % Inhib. rat p38α | Seq ID NO |
|---|---|---|---|---|---|---|---|
| 100864* | coding | L35253 | 538 | TCCAACAGACCAATCACATT | 83 | 57 | 82 |
| 101753 | start codon | U73142 | 1 | CTGCGACATTTTCCAGCGGC | 64 | 43 | 175 |
| 101755* | coding | U10871.1 | 1226 | CATCATCAGGGTCGTGGTAC | 84 | 74 | 176 |
| 101757* | coding | U10871.1 | 1336 | AGGTGCTCAGGACTCCATTT | 88 | 53 | 177 |
| 186911* | coding | NM 001315.1 | 1336 | GTCCCTGCTTTCAAAGGACT | 81 | 40 | 178 |
| 306022* | coding | U73142 | 781 | GGCCAGAGACTGAATGTAGT | 78 | 53 | 179 |
| 320103* | coding | U10871.1 | 315 | AGCTCCTGCCGGTAGAACGT | 81 | 55 | 180 |
| 320104* | coding | U10871.1 | 405 | TCAAAAGCAGCACACACCGA | 82 | 42 | 181 |
| 320105* | coding | U10871.1 | 417 | CCCGTCTTTGTATCAAAAGC | 89 | 59 | 182 |

TABLE 23-continued

Inhibition of mouse and rat p38α by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

| ISIS # | Region | Target Sequence Accession # | Target Site | Sequence | % Inhib. mouse p38α | % Inhib. rat p38α | Seq ID NO |
|---|---|---|---|---|---|---|---|
| 320106* | coding | U10871.1 | 453 | AACGGTCTCGACAGCTTCTT | 91 | 67 | 183 |
| 320107* | coding | U10871.1 | 483 | TAGGTCCTTTTGGCGTGAAT | 84 | 60 | 184 |
| 320108* | coding | U10871.1 | 600 | AGATGGGTCACCAGGTACAC | 61 | 57 | 185 |
| 320109* | coding | U10871.1 | 609 | GCCCCCATGAGATGGGTCAC | 69 | 34 | 186 |
| 320110* | coding | U10871.1 | 807 | TCATCAGTGTGCCGAGCCAG | 87 | 54 | 187 |
| 320111* | coding | U10871.1 | 930 | GTCAACAGCTCAGCCATGAT | 86 | 55 | 188 |
| 320112* | coding | U10871.1 | 940 | CGTTCTTCCGGTCAACAGCT | 93 | 58 | 189 |
| 320113* | coding | U10871.1 | 967 | ATCAATATGGTCTGTACCAG | 35 | 9 | 190 |
| 320114* | coding | U10871.1 | 987 | CTTAAAATGAGCTTCAACTG | 71 | 60 | 191 |
| 320115* | coding | U10871.1 | 1001 | GGGTTCCAACGAGTCTTAAA | 67 | 53 | 192 |
| 320116* | coding | U10871.1 | 1019 | TCAGAAGCTCAGCCCCTGGG | 95 | 73 | 193 |
| 320117* | coding | U10871.1 | 1030 | GGAGATTTTCTTCAGAAGCT | 72 | 55 | 194 |
| 320118* | coding | U10871.1 | 1040 | CAGACTCTGAGGAGATTTTC | 47 | 69 | 195 |
| 320119 | coding | U10871.1 | 1050 | TAGTTTCTTGCAGACTCTGA | 53 | 32 | 196 |
| 320120 | coding | U10871.1 | 1060 | AGACTGAATGTAGTTTCTTG | 74 | 39 | 197 |
| 320121 | coding | U10871.1 | 1083 | TTCATCTTCGGCATCTGGGC | 83 | 57 | 198 |
| 320122 | coding | U10871.1 | 1093 | ATTTGCGAAGTTCATCTTCG | 73 | 48 | 199 |
| 320123 | coding | U10871.1 | 1103 | CAATAAATACATTTGCGAAG | 79 | 32 | 200 |
| 320124 | coding | U10871.1 | 1113 | GGATTGGCACCAATAAATAC | 29 | 31 | 201 |
| 320125 | coding | U10871.1 | 1176 | GCTGCTGTGATCCTCTTATC | 67 | 63 | 202 |
| 320126 | coding | U10871.1 | 1196 | AGGCATGCGCAAGAGCTTGG | 90 | 69 | 203 |
| 320127 | coding | U10871.1 | 1206 | TGAGCAAAGTAGGCATGCGC | 73 | 56 | 204 |
| 320128 | coding | U10871.1 | 1260 | TCAAAGGACTGGTCATAAGG | 79 | 37 | 205 |
| 320129 | coding | U10871.1 | 1351 | CATTTCTTCTTGGTCAAGGG | 69 | 65 | 206 |
| 320130 | stop codon | U10871.1 | 1358 | AGGACTCCATTTCTTCTTGG | 81 | 61 | 207 |
| 320131 | 3' UTR | U10871.1 | 1406 | CTTCCCCTCACAGTGAAGTG | 92 | 39 | 208 |
| 320132 | 3' UTR | U10871.1 | 1432 | TATTTGGAGAGTTCCCATGA | 85 | 56 | 209 |
| 320133 | 3' UTR | U10871.1 | 1442 | ACTTGAATGGTATTTGGAGA | 52 | 61 | 210 |
| 320134 | 3' UTR | U10871.1 | 1452 | AACAAGAGGCACTTGAATGG | 85 | 74 | 211 |
| 320135 | 3' UTR | U10871.1 | 1480 | ACCCCCTTCCACCATGAAGG | 95 | 47 | 212 |
| 320136 | 3' UTR | U10871.1 | 1608 | AGCAGGCAGACTGCCAAGGA | 83 | 34 | 213 |
| 320137 | 3' UTR | U10871.1 | 1663 | CACACACATCCCTAAGGAGA | 80 | 44 | 214 |
| 320138 | 3' UTR | U10871.1 | 1745 | TAAAGGCAGGGCCACAGGAG | 87 | 46 | 215 |
| 320139 | 3' UTR | U10871.1 | 1771 | GCAGCCTCTCTCTGTCACTG | 87 | 61 | 216 |

TABLE 23-continued

Inhibition of mouse and rat p38α by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

| ISIS # | Target Region | Target Sequence Accession # | Target Site | Sequence | % Inhib. mouse p38α | % Inhib. rat p38α | Seq ID NO |
|---|---|---|---|---|---|---|---|
| 320140 | 3' UTR | U10871.1 | 1791 | GGGATAGCCTCAGACCTGAA | 61 | 37 | 217 |
| 320141 | 3' UTR | U10871.1 | 1801 | GCATGGCTGAGGGATAGCCT | 83 | 73 | 218 |
| 320142 | 3' UTR | U10871.1 | 1828 | GAGCCAGTTGGTTCTCTTGG | 85 | 53 | 219 |
| 320143 | 3' UTR | U10871.1 | 1910 | AGGCACAAACAGACTGACAG | 88 | 54 | 220 |
| 320144 | 3' UTR | U10871.1 | 1917 | CCTTTTAAGGCACAAACAGA | 83 | 39 | 221 |
| 320145 | 3' UTR | U10871.1 | 2138 | GACCTCTGCACTGAGGTGAA | 52 | 44 | 222 |
| 320146 | 3' UTR | U10871.1 | 2147 | GGCACTGGAGACCTCTGCAC | 74 | 57 | 223 |
| 320147 | 3' UTR | U10871.1 | 2228 | AGAGCACAGCATGCAAACAC | 66 | 43 | 224 |
| 320148 | 3' UTR | U10871.1 | 2259 | CCAGGGCTTCCAGAAGACAG | 78 | 33 | 225 |
| 320149 | 3' UTR | U10871.1 | 2576 | AAGGAGCTCCTGGCTTCAGG | 74 | 25 | 226 |
| 320150 | 3' UTR | U10871.1 | 2738 | GGATTCCTACAACATACAAA | 82 | 62 | 227 |
| 320151 | 3' UTR | U10871.1 | 2758 | GAAGGAACCACACTCTCTAA | 90 | 47 | 228 |
| 320152 | 3' UTR | U10871.1 | 2778 | TTTGCCCTTTCTCCCCATCA | 93 | 66 | 229 |
| 320153 | 3' UTR | U10871.1 | 2791 | AATATTAAAATAATTTGCCC | 0 | 22 | 230 |
| 320154 | 3' UTR | U10871.1 | 2817 | TCATGTTTATAAAGGTGAAA | 52 | 50 | 231 |
| 320155 | 3' UTR | U10871.1 | 2827 | CCCTGAGGATTCATGTTTAT | 93 | 73 | 232 |
| 320156 | 3' UTR | U10871.1 | 2930 | GGAATTGGCTTTACACTTTC | 91 | 64 | 233 |
| 320157 | 3' UTR | U10871.1 | 2941 | CGTCCAACACTGGAATTGGC | 96 | 71 | 234 |
| 320158 | 3' UTR | U10871.1 | 3042 | CCTTCTGGGCTCCAAATGAT | 91 | 71 | 235 |
| 320159 | 3' UTR | U10871.1 | 3386 | TCTGACATCCTATGGCATAC | 94 | 69 | 236 |
| 320160 | coding | D83073.1 | 900 | GTTAATATGGTCTGTACCAG | 53 | 43 | 237 |
| 320161 | coding | D83073.1 | 910 | GCTGAAGCTGGTTAATATGG | 80 | 66 | 238 |
| 320162 | coding | D83073.1 | 920 | CGCATTATCTGCTGAAGCTG | 92 | 62 | 239 |
| 320163 | coding | D83073.1 | 955 | TGTTAATGAGATAAGCAGGG | 0 | 40 | 240 |
| 320164 | coding | D83073.1 | 965 | CTTGGCATCCTGTTAATGAG | 80 | 73 | 241 |
| 320165 | coding | D83073.1 | 975 | TGCCTCATGGCTTGGCATCC | 81 | 53 | 242 |
| 320166 | coding | D83073.1 | 991 | ACTGAATGTAGTTTCTTGCC | 53 | 35 | 243 |
| 320167 | 5' UTR | AA002328.1 | 155 | CTTGCCTGTAAAAACACAGA | 7 | 11 | 244 |
| 320168 | stop codon | AF128892.1 | 1059 | TCACCTCATGGCTTGGCATC | 83 | 56 | 245 |
| 320169 | stop codon | AF128892.1 | 1066 | TTTGTTCTCACCTCATGGCT | 92 | 64 | 246 |
| 320170 | 3' UTR | AF128892.1 | 1132 | TGCTGGCTATACACAGACAC | 83 | 55 | 247 |
| 320171 | intron | BY159314.1 | 58 | TGGAAAACTGTTTTGTCAAA | 35 | 2 | 248 |
| 320172 | intron | 3Y257628.1 | 39 | ACTCTCGCGAGAACAGCTCC | 39 | 0 | 249 |

TABLE 23-continued

Inhibition of mouse and rat p38α by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

| ISIS # | Region | Target Sequence Accession # | Target Site | Sequence | % Inhib. mouse p38α | % Inhib. rat p38α | Seq ID NO |
|---|---|---|---|---|---|---|---|
| 320173 | intron | BY257628.1 | 72 | TCCCACAGGCAGCGGCCGGG | 160 | 250 | |
| 320174 | intron | BY257628.1 | 97 | CCCGCTTGGGCTCCAGTGGC | 62 | 29 | 251 |

[0306] Additional antisense oligonucleotides were designed to target mouse p38α using published sequences (Genbank accession number U10871.1, provided herein as SEQ ID NO: 114). Oligonucleotides are composed of 2'-deoxynucleotides. Internucleoside linkages are phosphorodiester (P═O). These oligonucleotide sequences are shown in Table 24. "Target site" indicates the first (5'-most) nucleotide number on the particular target sequence to which the compound binds.

TABLE 24

Antisense oligonucleotides targeted to mouse p38α having 2'-deoxynucleotides and phosphodiester linkages

| ISIS # | Region | Target Sequence Accession # | Start Site | SEQUENCE | SEQ ID NO |
|---|---|---|---|---|---|
| 137934 | 3' UTR | U10871.1 | 3331 | GCAGTTTTCTCGTTCC CTTG | 252 |
| 264006 | coding | U10871.1 | 1207 | CTGAGCAAAGTAGGCA TGCG | 253 |
| 320184 | 3' UTR | U10871.1 | 2306 | GGAGGCAATGTGGACA GGAA | 254 |
| 279221 | coding | U10871.1 | 521 | CATTTTCGTGTTTCAT GTGCTTC | 255 |
| 326403 | 3' UTR | U10871.1 | 3395 | TATTTTAACCAGTGGT ATTATCTACATCCT | 256 |

[0307] Additional antisense oligonucleotides were designed to target mouse p38α using published sequences (Genbank accession number U10871.1, provided herein as SEQ ID NO: 114). Oligonucleotides were synthesized as 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. Internucleoside linkages in the central gap region are phosphorothioate (P═S), and internucleoside linkages in the wings are phosphodiester (P═O). These oligonucleotide sequences are shown in Table 25. "Target site" indicates the first (5'-most) nucleotide number on the particular target sequence to which the compound binds.

TABLE 25

Chimeric oligonucleotides targeted to mouse p38a having 2'-MOE wings and a deoxy gap and mixed phophorothioate and phosphodiester internucleoside linkages

| ISIS # | Region | Target Sequence Accession # | Start Site | SEQUENCE | SEQ ID NO |
|---|---|---|---|---|---|
| 101369 | codon | U10871.1 | 286 | CTGCGACATCTTCCAGCG GC | 257 |
| 101370 | coding | U10871.1 | 646 | GGTCAGCTTCTGGCACTT CA | 258 |
| 101372 | 3' UTR | U10871.1 | 1609 | AAGCAGGCAGACTGCCAA GG | 259 |

[0308] Additional antisense oligonucleotides were designed to target rat p38α using published sequences (GenBank accession number U73142, provided herein as SEQ ID NO: 45, and GenBank accession number U91847.1, provided herein as SEQ ID NO: 173). Oligonucleotides are composed of 2'-deoxynucleotides. Internucleoside linkages are phosphorodiester (P═O). These oligonucleotide sequences are shown in Table 26. "Target site" indicates the first (5'-most) nucleotide number on the particular target sequence to which the compound binds.

TABLE 26

Antisense oligonucleotides targeted to rat p38α having 2'-deoxynucleotides and phosphodiester linkages

| ISIS # | Region | Target Sequence Accession # | Start Site | SEQUENCE | SEQ ID NO |
|---|---|---|---|---|---|
| 336744 | coding | U91847.1 | 902 | AGGCATGCGCAAGAGCTT | 260 |
| 336741 | coding | U91847.1 | 66 | GGGACAGGTTCTGGTATC GC | 261 |
| 257014 | coding | U91847.1 | 224 | TCTCGTGCTTCATGTGCT TCA | 262 |
| 320187 | 3' UTR | U73142 | 2800 | TGGAGCTGGACTGCATAC TGA | 263 |

[0309] Additional antisense oligonucleotides were designed to target rat p38α using published sequences (GenBank accession number U73142, provided herein as

SEQ ID NO: 45). Oligonucleotides were synthesized as chimeric oligonucleotides, composed 2'-deoxynucleotides and 2'-methoxyethyl (2'-MOE) nucleotides (indicated in bold type in Table 27). Internucleoside linkages in the central gap region are phosphorothioate (P=S), and internucleoside linkages in the wings are phosphodiester (P=O). These oligonucleotide sequences are shown in Table 27. "Target site" indicates the first (5'-most) nucleotide number on the particular target sequence to which the compound binds.

TABLE 27

Chimeric oligonucleotides targeted to rat p38α having 2'-MOE wings and a deoxy gap and mixed phophorothioate and phosphodiester internucleoside linkages

| ISIS # | Region | Target Sequence Accession # | Start Site | SEQUENCE | SEQ ID NO |
|---|---|---|---|---|---|
| 111831 | coding | U73142 | 941 | **CAT**CAGGGTCGT**GGTAC** | 264 |
| 111830 | coding | U73142 | 942 | **CATCAT**CAGGGTCGT | 265 |

Example 13

Mouse model of allergic inflammation

[0310] In the mouse model of allergic inflammation, mice were sensitized and challenged with aerosolized chicken ovalbumin (OVA). Airway responsiveness was assessed by inducing airflow obstruction with a methacholine aerosol using a noninvasive method. This methodology utilized unrestrained conscious mice that are placed into the main chamber of a plthysmograph (Buxco Electronics, Inc., Troy, N.Y.). Pressure differences between this chamber and a reference chamber were used to extrapolate minute volume, breathing frequency and enhanced pause (Penh). Penh is a dimensionless parameter that is a function of total pulmonary airflow in mice (i.e., the sum of the airflow in the upper and lower respiratory tracts) during the respiratory cycle of the animal. The lower the Penh, the greater the airflow. This parameter closely correlates with lung resistance as measured by traditional invasive techniques using ventilated animals (Hamelmann et al., 1997). Dose-response data were plotted as raw Penh values to increasing concentrations of methacholine. This system was used to test the efficacy of an antisense oligonucleotide targeted to mouse p38α (ISIS 101757; SEQ ID NO: 177). Mismatched p38α oligonucleotide (ISIS 101758; SEQ ID NO: 266) was used as a negative control.

[0311] There are several important features common to human asthma and the mouse model of allergic inflammation. One of these is pulmonary inflammation, in which cytokine expression and Th2 profile is dominant. Another is goblet cell hyperplasia with increased mucus production. Lastly, airway hyperresponsiveness (AHR) occurs resulting in increased sensitivity to cholinergic receptor agonists such as acetylcholine or methacholine. The compositions and methods of the present invention may be used to treat AHR and pulmonary inflammation. The combined use of antisense oligonucleotides targeted to human p38 MAP kinase with one or more conventional asthma medications including, but not limited to, montelukast sodium (Singulair™), albuterol, beclomethasone dipropionate, triamcinolone acetonide, ipratropium bromide (Atrovent™), flunisolide, fluticasone propionate (Flovent™) and other steroids is also contemplated.

[0312] Ovalbumin-Induced Allergic Inflammation

[0313] For intratracheal administration of ISIS 101757, female Balb/c mice (Charles Rivers Laboratory, Taconic Farms, N.Y.) were maintained in micro-isolator cages housed in a specific pathogen-free (SPF) facility. The sentinel cages within the animal colony surveyed negative for viral antibodies and the presence of known mouse pathogens. Mice were sensitized and challenged with aerosolized chicken OVA. Briefly, 20 μm alum-precipitated OVA was injected intraperitoneally on days 0 and 14. On day 24, 25 and 26, the animals were exposed for 20 minutes to 1.0% OVA (in saline) by nebulization. The challenge was conducted using an ultrasonic nebulizer (PulmoSonic, The DeVilbiss Co., Somerset, Pa.). Animals were analyzed about 24 hours following the last nebulization using the Buxco electronics Biosystem. Lung function (Penh), lung histology (cell infiltration and mucus production), target mRNA reduction in the lung, inflammation (BAL cell type & number, cytokine levels), spleen weight and serum AST/ALT were determined.

[0314] For the aerosol studies, the protocol described above was slightly modified. Male Balb/c mice were injected IP with OVA (20 μg) in aluminum hydroxide on days 0 and 14. Aerosol dosing was performed with nebulized sterile saline, antisense oligonucleotide or mismatched control oligonucleotide using 25, 125 and 250 μg/ml solutions (5 mg/kg) for 30 min. on days 14-20 in a closed chamber. Aerosol lung challenge was carried out with nebulized saline or 1% OVA for 20 min. on days 18, 19 and 20. BAL fluid was collected at 24 hr post-last lung challenge (cell differentials) or at 2-12 h post-challenge (cytokine analysis). AHR was measured 24 hours after OVA challenge. Mice were exposed to aerosolized methacholine 24 hr post-last lung challenge from 2-80 mg/ml for 3 min. until a 200% increase in Penh was achieved.

[0315] Intratracheal Oligonucleotide Administration

[0316] Antisense oligonucleotides (ASOs) were dissolved in saline and used to intratracheally dose mice every day, four times per day, from days 15-26 of the OVA sensitization and challenge protocol, or used as an aerosol. Specifically, the mice were anesthetized with isofluorane and placed on a board with the front teeth hung from a line. The nose was covered and the animal's tongue was extended with forceps and 25 μl of various doses of ASO, or an equivalent volume of saline (control) was placed at the back of the tongue until inhaled into the lung.

[0317] Mouse antisense oligonucleotides to p38α are phosphorothioates with 2'-MOE modifications on nucleotides 1-5 and 16-20, and 2'-deoxy at positions 6-15. These ASOs were identified by mouse-targeted ASO screening of 10 p38α antisense oligonucleotides by target p38α mRNA reduction in mouse bEND.3 cells, as described in Example 12. Dose-response confirmation led to selection of ISIS 21873 (>70% reduction at 50 nM). ISIS 101757 contains all phosphorothioate linkages, whereas ISIS 21873 is a mixed phosphodiester/phosphorothioate compound. ISIS 101757

had an IC50<50 nM for reducing p38α mRNA in endothelial cells, and an IC50 of about 250 nM in fibroblasts.

[0318] Results of Aerosol Administration

[0319] The p38α knock-down effect of ISIS 101757 was confirmed in a mouse T cell line (EL4) and a mouse macrophage cell line (RAW264.7) using Western blotting. ISIS 101757, but not the mismatched control, dose-dependently suppressed methacholine-induced AHR in sensitized mice measured by whole body plethysmography (**FIG. 1**A-1B). The PC200 values for methacholine (**FIG. 2**) significantly (P<0.05) reduced OVA-induced increases in total cell counts and eosinophils recovered in BAL fluid (**FIG. 3**). In addition, histological studies revealed that ISIS 101757 markedly inhibited OVA-induced inflammatory cell infiltration into the lungs (H&E stain) and mucus hypersecretion in the airway epithelium (PAS stain). ISIS 101757 also significantly (P<0.05);lowered blood levels of total IgE, OVA-specific IgE and OVA-specific $IgG_1$ in sensitized mice as compared to the mismatched control. Oligonucleotide levels of up to 1 μg/g of lung tissue were sufficient to achieve the pharmacological effects described above. The aerosolized ISIS 101757 concentration in mouse lung vs. dose is shown in **FIG. 4**. There was no significant effect of aerosol oligonucleotide administration of spleen weight. These data indicate that p38α antisense oligonucleotides are useful for the treatment of asthma.

[0320] Intratracheal Administration Results

[0321] After intratracheal administration of ISIS 101757 as described above, dose-dependent inhibition of the Penh response to methacholine (50 mg/ml) challenge was observed (**FIG. 5**). The oligonucleotide concentration (μg/g) in lungs vs. dose is shown in **FIG. 6**.

[0322] RT-PCR Analysis

[0323] RNA was harvested from experimental lungs removed on day 28 of the OVA protocol. P38α levels were measured by quantitative RT-PCR as described in other examples herein.

[0324] Collection of Bronchial Alveolar Lavage (BAL) Fluid and Blood Serum for the Determination of Cytokine and Chemokine Levels

[0325] Animals were injected with a lethal dose of ketamine, the trachea was exposed and a cannula was inserted and secured by sutures. The lungs were lavaged twice with 0.5 ml aliquots of ice cold PBS with 0.2% FCS. The recovered BAL fluid was centrifuged at 1,000 rpm for 10 min at 4° C., frozen on dry ice and stored at −80° C. until used. Luminex was used to measure cytokine levels in BAL fluid and serum.

[0326] BAL Cell Counts and Differentials

[0327] Cytospins of cells recovered from BAL fluid were prepared using a Shandon Cytospin 3 (Shandon Scientific LTD, Cheshire, England). Cell differentials were performed from slides stained with Leukostat (Fisher Scientific, Pittsburgh, Pa.). Total cell counts were quantified by hemocytometer and, together with the percent type by differential, were used to calculate specific cell number.

[0328] Tissue Histology

[0329] Before resection, lungs were inflated with 0.5 ml of 10% phosphate-buffered formalin and fixed overnight at 4° C. The lung samples were washed free of formalin with 1×PBS and subsequently dehydrated through an ethanol series prior to equilibration in xylene and embedded in paraffin. Sections (6μ) were mounted on slides and stained with hematoxylin/eosin, massons trichome and periodic acid-schiff (PAS) reagent. Parasagittal sections were analyzed by bright-field microscopy. Mucus cell content was assessed as the airway epithelium staining with PAS. Relative comparisons of mucus content were made between cohorts of animals by counting the number of PAS-positive airways.

Example 14

Design and Screening of Duplexed Antisense Compounds Targeting p38α MAP Kinase

[0330] In accordance with the present invention, a series of nucleic acid duplexes comprising the antisense compounds of the present invention and their complements can be designed to target p38α MAP kinase. The nucleobase sequence of the antisense strand of the duplex comprises at least a portion of an oligonucleotide to p38α MAP kinase as described herein. The ends of the strands may be modified by the addition of one or more natural or modified nucleobases to form an overhang. The sense strand of the dsRNA is then designed and synthesized as the complement of the antisense strand and may also contain modifications or additions to either terminus. For example, in one embodiment, both strands of the dsRNA duplex would be complementary over the central nucleobases, each having overhangs at one or both termini. For example, a duplex comprising an antisense strand having the sequence CGAGAGGCGGACGGGACCG and having a two-nucleobase overhang of deoxythymidine(dT) would have the following structure:

```
    cgagaggcggacgggaccgTT      Antisense Strand
    ||||||||||||||||||||
TTgctctccgcctgccctggc       Complement
```

[0331] RNA strands of the duplex can be synthesized by methods disclosed herein or purchased from Dharmacon Research Inc., (Lafayette, Colo.). Once synthesized, the complementary strands are annealed. The single strands are aliquoted and diluted to a concentration of 50 uM. Once diluted, 30 uL of each strand is combined with 15 uL of a 5×solution of annealing buffer. The final concentration of said buffer is 100 mM potassium acetate, 30 mM HEPES-KOH pH 7.4, and 2 mM magnesium acetate. The final volume is 75 uL. This solution is incubated for 1 minute at 90° C. and then centrifuged for 15 seconds. The tube is allowed to sit for 1 hour at 37° C. at which time the dsRNA duplexes are used in experimentation. The final concentration of the dsRNA duplex is 20 uM. This solution can be stored frozen (−20° C.) and freeze-thawed up to 5 times.

[0332] Once prepared, the duplexed antisense compounds are evaluated for their ability to modulate p38α MAP kinase expression according to the protocols described herein.

Example 15

Design of Phenotypic Assays and In Vivo Studies for the Use of p38α MAP Kinase Inhibitors

[0333] Phenotypic Assays

[0334] Once p38α MAP kinase inhibitors have been identified by the methods disclosed herein, the compounds are further investigated in one or more phenotypic assays, each having measurable endpoints predictive of efficacy in the treatment of a particular disease state or condition. Phenotypic assays, kits and reagents for their use are well known to those skilled in the art and are herein used to investigate the role and/or association of p38α MAP kinase in health and disease. Representative phenotypic assays, which can be purchased from any one of several commercial vendors, include those for determining cell viability, cytotoxicity, proliferation or cell survival (Molecular Probes, Eugene, Oreg.; PerkinElmer, Boston, Mass.), protein-based assays including enzymatic assays (Panvera, LLC, Madison, Wis.; BD Biosciences, Franklin Lakes, N.J.; Oncogene Research Products, San Diego, Calif.), cell regulation, signal transduction, inflammation, oxidative processes and apoptosis (Assay Designs Inc., Ann Arbor, Mich.), triglyceride accumulation (Sigma-Aldrich, St. Louis, Mo.), angiogenesis assays, tube formation assays, cytokine and hormone assays and metabolic assays (Chemicon International Inc., Temecula, Calif.; Amersham Biosciences, Piscataway, N.J.).

[0335] In one non-limiting example, cells determined to be appropriate for a particular phenotypic assay (i.e., MCF-7 cells selected for breast cancer studies; adipocytes for obesity studies) are treated with p38α MAP kinase inhibitors identified from the in vitro studies as well as control compounds at optimal concentrations which are determined by the methods described above. At the end of the treatment period, treated and untreated cells are analyzed by one or more methods specific for the assay to determine phenotypic outcomes and endpoints.

[0336] Phenotypic endpoints include changes in cell morphology over time or treatment dose as well as changes in levels of cellular components such as proteins, lipids, nucleic acids, hormones, saccharides or metals. Measurements of cellular status which include pH, stage of the cell cycle, intake or excretion of biological indicators by the cell, are also endpoints of interest.

[0337] Analysis of the genotype of the cell (measurement of the expression of one or more of the genes of the cell) after treatment is also used as an indicator of the efficacy or potency of the p38α MAP kinase inhibitors. Hallmark genes, or those genes suspected to be associated with a specific disease state, condition, or phenotype, are measured in both treated and untreated cells.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 266

<210> SEQ ID NO 1
<211> LENGTH: 1539
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (295)..(1377)
<300> PUBLICATION INFORMATION:
<303> JOURNAL: Science
<304> VOLUME: 265
<305> ISSUE: 5173
<306> PAGES: 808-811
<307> DATE: 1994-08-05
<308> DATABASE ACCESSION NUMBER: L35253
<309> DATABASE ENTRY DATE: 1995-08-14

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ggaggcgccc ccgggccctc ccaggaggct ctctgggcca gaggccgaga ttcggcacag    180

gcccccagga gtccgtaagt aggagaggtc gcccgagacc ggccggaccc ccatccccgc    240

ggccgccgcc gccgctggtc ccgcggctgc gaccgtggcg gctgccgctg gaaa atg      297
                                                            Met
                                                              1

tct cag gag agg ccc acg ttc tac cgg cag gag ctg aac aag aca atc      345
Ser Gln Glu Arg Pro Thr Phe Tyr Arg Gln Glu Leu Asn Lys Thr Ile
             5                  10                  15

tgg gag gtg ccc gag cgt tac cag aac ctg tct cca gtg ggc tct ggc      393
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Trp Glu Val Pro Glu Arg Tyr Gln Asn Leu Ser Pro Val Gly Ser Gly
         20                  25                  30

gcc tat ggc tct gtg tgt gct gct ttt gac aca aaa acg ggg tta cgt      441
Ala Tyr Gly Ser Val Cys Ala Ala Phe Asp Thr Lys Thr Gly Leu Arg
     35                  40                  45

gtg gca gtg aag aag ctc tcc aga cca ttt cag tcc atc att cat gcg      489
Val Ala Val Lys Lys Leu Ser Arg Pro Phe Gln Ser Ile Ile His Ala
 50                  55                  60                  65

aaa aga acc tac aga gaa ctg cgg tta ctt aaa cat atg aaa cat gaa      537
Lys Arg Thr Tyr Arg Glu Leu Arg Leu Leu Lys His Met Lys His Glu
                 70                  75                  80

aat gtg att ggt ctg ttg gac gtt ttt aca cct gca agg tct ctg gag      585
Asn Val Ile Gly Leu Leu Asp Val Phe Thr Pro Ala Arg Ser Leu Glu
             85                  90                  95

gaa ttc aat gat gtg tat ctg gtg acc cat ctc atg ggg gca gat ctg      633
Glu Phe Asn Asp Val Tyr Leu Val Thr His Leu Met Gly Ala Asp Leu
        100                 105                 110

aac aac att gtg aaa tgt cag aag ctt aca gat gac cat gtt cag ttc      681
Asn Asn Ile Val Lys Cys Gln Lys Leu Thr Asp Asp His Val Gln Phe
    115                 120                 125

ctt atc tac caa att ctc cga ggt cta aag tat ata cat tca gct gac      729
Leu Ile Tyr Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala Asp
130                 135                 140                 145

ata att cac agg gac cta aaa cct agt aat cta gct gtg aat gaa gac      777
Ile Ile His Arg Asp Leu Lys Pro Ser Asn Leu Ala Val Asn Glu Asp
                150                 155                 160

tgt gag ctg aag att ctg gat ttt gga ctg gct cgg cac aca gat gat      825
Cys Glu Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg His Thr Asp Asp
            165                 170                 175

gaa atg aca ggc tac gtg gcc act agg tgg tac agg gct cct gag atc      873
Glu Met Thr Gly Tyr Val Ala Thr Arg Trp Tyr Arg Ala Pro Glu Ile
        180                 185                 190

atg ctg aac tgg atg cat tac aac cag aca gtt gat att tgg tca gtg      921
Met Leu Asn Trp Met His Tyr Asn Gln Thr Val Asp Ile Trp Ser Val
    195                 200                 205

gga tgc ata atg gcc gag ctg ttg act gga aga aca ttg ttt cct ggt      969
Gly Cys Ile Met Ala Glu Leu Leu Thr Gly Arg Thr Leu Phe Pro Gly
210                 215                 220                 225

aca gac cat att gat cag ttg aag ctc att tta aga ctc gtt gga acc     1017
Thr Asp His Ile Asp Gln Leu Lys Leu Ile Leu Arg Leu Val Gly Thr
                230                 235                 240

cca ggg gct gag ctt ttg aag aaa atc tcc tca gag tct gca aga aac     1065
Pro Gly Ala Glu Leu Leu Lys Lys Ile Ser Ser Glu Ser Ala Arg Asn
            245                 250                 255

tat att cag tct ttg act cag atg ccg aag atg aac ttt gcg aat gta     1113
Tyr Ile Gln Ser Leu Thr Gln Met Pro Lys Met Asn Phe Ala Asn Val
        260                 265                 270

ttt att ggt gcc aat ccc ctg gct gtc gac ttg ctg gag aag atg ctt     1161
Phe Ile Gly Ala Asn Pro Leu Ala Val Asp Leu Leu Glu Lys Met Leu
    275                 280                 285

gta ttg gac tca gat aag aga att aca gcg gcc caa gcc ctt gca cat     1209
Val Leu Asp Ser Asp Lys Arg Ile Thr Ala Ala Gln Ala Leu Ala His
290                 295                 300                 305

gcc tac ttt gct cag tac cac gat cct gat gat gaa cca gtg gcc gat     1257
Ala Tyr Phe Ala Gln Tyr His Asp Pro Asp Asp Glu Pro Val Ala Asp
                310                 315                 320

cct tat gat cag tcc ttt gaa agc agg gac ctc ctt ata gat gag tgg     1305
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Pro Tyr Asp Gln Ser Phe Glu Ser Arg Asp Leu Leu Ile Asp Glu Trp
            325                 330                 335

aaa agc ctg acc tat gat gaa gtc atc agc ttt gtg cca cca ccc ctt     1353
Lys Ser Leu Thr Tyr Asp Glu Val Ile Ser Phe Val Pro Pro Pro Leu
        340                 345                 350

gac caa gaa gag atg gag tcc tga gcacctggtt tctgttctgt tgatcccact    1407
Asp Gln Glu Glu Met Glu Ser
    355                 360

tcactgtgag gggaaggcct tttcacggga actctccaaa tattattcaa gtgcctcttg   1467

ttgcagagat ttcctccatg gtggaagggg gtgtgcgtgc gtgtgcgtgc gtgttagtgt   1527

gtgtgcatgt gt                                                       1539


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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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  1               5                  10                  15

Ile Trp Glu Val Pro Glu Arg Tyr Gln Asn Leu Ser Pro Val Gly Ser
             20                  25                  30

Gly Ala Tyr Gly Ser Val Cys Ala Ala Phe Asp Thr Lys Thr Gly Leu
         35                  40                  45

Arg Val Ala Val Lys Lys Leu Ser Arg Pro Phe Gln Ser Ile Ile His
     50                  55                  60

Ala Lys Arg Thr Tyr Arg Glu Leu Arg Leu Leu Lys His Met Lys His
 65                  70                  75                  80

Glu Asn Val Ile Gly Leu Leu Asp Val Phe Thr Pro Ala Arg Ser Leu
                 85                  90                  95

Glu Glu Phe Asn Asp Val Tyr Leu Val Thr His Leu Met Gly Ala Asp
            100                 105                 110

Leu Asn Asn Ile Val Lys Cys Gln Lys Leu Thr Asp Asp His Val Gln
        115                 120                 125

Phe Leu Ile Tyr Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala
    130                 135                 140

Asp Ile Ile His Arg Asp Leu Lys Pro Ser Asn Leu Ala Val Asn Glu
145                 150                 155                 160

Asp Cys Glu Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg His Thr Asp
                165                 170                 175

Asp Glu Met Thr Gly Tyr Val Ala Thr Arg Trp Tyr Arg Ala Pro Glu
            180                 185                 190

Ile Met Leu Asn Trp Met His Tyr Asn Gln Thr Val Asp Ile Trp Ser
        195                 200                 205

Val Gly Cys Ile Met Ala Glu Leu Leu Thr Gly Arg Thr Leu Phe Pro
    210                 215                 220

Gly Thr Asp His Ile Asp Gln Leu Lys Leu Ile Leu Arg Leu Val Gly
225                 230                 235                 240

Thr Pro Gly Ala Glu Leu Leu Lys Lys Ile Ser Ser Glu Ser Ala Arg
                245                 250                 255

Asn Tyr Ile Gln Ser Leu Thr Gln Met Pro Lys Met Asn Phe Ala Asn
            260                 265                 270
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```
Val Phe Ile Gly Ala Asn Pro Leu Ala Val Asp Leu Leu Glu Lys Met
        275                 280                 285

Leu Val Leu Asp Ser Asp Lys Arg Ile Thr Ala Ala Gln Ala Leu Ala
    290                 295                 300

His Ala Tyr Phe Ala Gln Tyr His Asp Pro Asp Asp Glu Pro Val Ala
305                 310                 315                 320

Asp Pro Tyr Asp Gln Ser Phe Glu Ser Arg Asp Leu Leu Ile Asp Glu
                325                 330                 335

Trp Lys Ser Leu Thr Tyr Asp Glu Val Ile Ser Phe Val Pro Pro Pro
            340                 345                 350

Leu Asp Gln Glu Glu Met Glu Ser
        355                 360
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<210> SEQ ID NO 3
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 3

aagaccgggc ccggaattcc 20

<210> SEQ ID NO 4
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 4

gtggaggcca gtccccggga ccggaattcc 30

<210> SEQ ID NO 5
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 5

tggcagcaaa gtgctgctgg 20

<210> SEQ ID NO 6
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: antisense sequence

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cagagagcct cctgggaggg 20

<210> SEQ ID NO 7
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 7

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tgtgccgaat ctcggcctct 20

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

<400> SEQUENCE: 8

ggtctcgggc gacctctcct 20

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

<400> SEQUENCE: 9

cagccgcggg accagcggcg 20

<210> SEQ ID NO 10
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 10

cattttccag cggcagccgc 20

<210> SEQ ID NO 11
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 11

tcctgagaca ttttccagcg 20

<210> SEQ ID NO 12
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 12

ctgccggtag aacgtgggcc 20

<210> SEQ ID NO 13
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<212> TYPE: DNA
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<220> FEATURE:
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gtaagcttct gacatttcac 20

<210> SEQ ID NO 14

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<211> LENGTH: 20
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<220> FEATURE:
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<400> SEQUENCE: 14

tttaggtccc tgtgaattat 20

<210> SEQ ID NO 15
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 15

atgttcttcc agtcaacagc 20

<210> SEQ ID NO 16
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 16

taaggaggtc cctgctttca 20

<210> SEQ ID NO 17
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 17

aaccaggtgc tcaggactcc 20

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

<400> SEQUENCE: 18

gaagtgggat caacagaaca 20

<210> SEQ ID NO 19
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 19

tgaaaaggcc ttcccctcac 20

<210> SEQ ID NO 20
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 20

aggcacttga ataatatttg 20

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

<400> SEQUENCE: 21

cttccaccat ggaggaaatc 20

<210> SEQ ID NO 22
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 22

acacatgcac acacactaac 20

<210> SEQ ID NO 23
<211> LENGTH: 2180
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (20)..(1138)
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: U53442
<309> DATABASE ENTRY DATE: 1996-07-30

<400> SEQUENCE: 23

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gtgaaattct gctccggac atg tcg ggc cct cgc gcc ggc ttc tac cgg cag      52
                     Met Ser Gly Pro Arg Ala Gly Phe Tyr Arg Gln
                       1               5                  10

gag ctg aac aag acc gtg tgg gag gtg ccg cag cgg ctg cag ggg ctg      100
Glu Leu Asn Lys Thr Val Trp Glu Val Pro Gln Arg Leu Gln Gly Leu
             15                  20                  25

cgc ccg gtg ggc tcc ggc gcc tac ggc tcc gtc tgt tcg gcc tac gac      148
Arg Pro Val Gly Ser Gly Ala Tyr Gly Ser Val Cys Ser Ala Tyr Asp
         30                  35                  40

gcc cgg ctg cgc cag aag gtg gcg gtg aag aag ctg tcg cgc ccc ttc      196
Ala Arg Leu Arg Gln Lys Val Ala Val Lys Lys Leu Ser Arg Pro Phe
     45                  50                  55

cag tcg ctg atc cac gcg cgc aga acg tac cgg gag ctg cgg ctg ctc      244
Gln Ser Leu Ile His Ala Arg Arg Thr Tyr Arg Glu Leu Arg Leu Leu
 60                  65                  70                  75

aag cac ctg aag cac gag aac gtc atc ggg ctt ctg gac gtc ttc acg      292
Lys His Leu Lys His Glu Asn Val Ile Gly Leu Leu Asp Val Phe Thr
                 80                  85                  90

ccg gcc acg tcc atc gag gac ttc agc gaa gtg tac ttg gtg acc acc      340
Pro Ala Thr Ser Ile Glu Asp Phe Ser Glu Val Tyr Leu Val Thr Thr
             95                  100                 105

ctg atg ggc gcc gac ctg aac aac atc gtc aag tgc cag gcg ggc gcc      388
Leu Met Gly Ala Asp Leu Asn Asn Ile Val Lys Cys Gln Ala Gly Ala
        110                 115                 120
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cat cag ggt gcc cgc ctg gca ctt gac gag cac gtt caa ttc ctg gtt      436
His Gln Gly Ala Arg Leu Ala Leu Asp Glu His Val Gln Phe Leu Val
    125                 130                 135

tac cag ctg ctg cgc ggg ctg aag tac atc cac tcg gcc ggg atc atc      484
Tyr Gln Leu Leu Arg Gly Leu Lys Tyr Ile His Ser Ala Gly Ile Ile
140                 145                 150                 155

cac cgg gac ctg aag ccc agc aac gtg gct gtg aac gag gac tgt gag      532
His Arg Asp Leu Lys Pro Ser Asn Val Ala Val Asn Glu Asp Cys Glu
                160                 165                 170

ctc agg atc ctg gat ttc ggg ctg gcg cgc cag gcg gac gag gag atg      580
Leu Arg Ile Leu Asp Phe Gly Leu Ala Arg Gln Ala Asp Glu Glu Met
            175                 180                 185

acc ggc tat gtg gcc acg cgc tgg tac cgg gca cct gag atc atg ctc      628
Thr Gly Tyr Val Ala Thr Arg Trp Tyr Arg Ala Pro Glu Ile Met Leu
        190                 195                 200

aac tgg atg cat tac aac caa aca gtg gat atc tgg tcc gtg ggc tgc      676
Asn Trp Met His Tyr Asn Gln Thr Val Asp Ile Trp Ser Val Gly Cys
    205                 210                 215

atc atg gct gag ctg ctc cag ggc aag gcc ctc ttc ccg gga agc gac      724
Ile Met Ala Glu Leu Leu Gln Gly Lys Ala Leu Phe Pro Gly Ser Asp
220                 225                 230                 235

tac att gac cag ctg aag cgc atc atg gaa gtg gtg ggc aca ccc agc      772
Tyr Ile Asp Gln Leu Lys Arg Ile Met Glu Val Val Gly Thr Pro Ser
                240                 245                 250

cct gag gtt ctg gca aaa atc tcc tcg gaa cac gcc cgg aca tat atc      820
Pro Glu Val Leu Ala Lys Ile Ser Ser Glu His Ala Arg Thr Tyr Ile
            255                 260                 265

cag tcc ctg ccc ccc atg ccc cag aag gac ctg agc agc atc ttc cgt      868
Gln Ser Leu Pro Pro Met Pro Gln Lys Asp Leu Ser Ser Ile Phe Arg
        270                 275                 280

gga gcc aac ccc ctg gcc ata gac ctc ctt gga agg atg ctg gtg ctg      916
Gly Ala Asn Pro Leu Ala Ile Asp Leu Leu Gly Arg Met Leu Val Leu
    285                 290                 295

gac agt gac cag agg gtc agt gca gct gag gca ctg gcc cac gcc tac      964
Asp Ser Asp Gln Arg Val Ser Ala Ala Glu Ala Leu Ala His Ala Tyr
300                 305                 310                 315

ttc agc cag tac cac gac ccc gag gat gag cca gag gcc gag cca tat     1012
Phe Ser Gln Tyr His Asp Pro Glu Asp Glu Pro Glu Ala Glu Pro Tyr
                320                 325                 330

gat gag agc gtt gag gcc aag gag cgc acg ctg gag gag tgg aag gag     1060
Asp Glu Ser Val Glu Ala Lys Glu Arg Thr Leu Glu Glu Trp Lys Glu
            335                 340                 345

ctc act tac cag gaa gtc ctt agc ttc aag ccc cca gag cca ccg aag     1108
Leu Thr Tyr Gln Glu Val Leu Ser Phe Lys Pro Pro Glu Pro Pro Lys
        350                 355                 360

cca cct ggc agc ctg gag att gag cag tga ggtgctgccc agcagcccct       1158
Pro Pro Gly Ser Leu Glu Ile Glu Gln
    365                 370

gagagcctgt ggaggggctt gggcctgcac ccttccacag ctggcctggt ttcctcgaga   1218

ggcacctccc acactcctat ggtcacagac ttctggccta ggacccctcg ccttcaggag   1278

aatctacacg catgtatgca tgcacaaaca tgtgtgtaca tgtgcttgcc atgtgtagga   1338

gtctgggcac aagtgtccct gggcctacct tggtcctcct gtcctcttct ggctactgca   1398

ctctccactg ggacctgact gtggggtcct agatgccaaa ggggttcccc tgcggagttc   1458

ccctgtctgt cccaggccga cccaagggag tgtcagcctt gggctctctt ctgtcccagg   1518
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gctttctgga gggcgcgctg gggccgggac cccgggagac tcaaagggag aggtctcagt 1578

ggttagagct gctcagcctg gaggtagggc gctgtcttgg tcactgctga gacccacagg 1638

tctaagagga gaggcagagc cagtgtgcca ccaggctggg cagggacaac caccaggtgt 1698

caaatgagaa aagctgcctg gagtcttgtg ttcacccgtg ggtgtgtgtg ggcacgtgtg 1758

gatgagcgtg cactccccgt gttcatatgt cagggcacat gtgatgtggt gcgtgtgaat 1818

ctgtgggcgc ccaaggccag cagccatatc tggcaagaag ctggagccgg ggtgggtgtg 1878

ctgttgcctt ccctctcctc ggttcctgat gccttgaggg gtgtttcaga ctggcggcac 1938

cgttgtggcc ctgcagccgg agatctgagg tgctctggtc tgtgggtcag tcctctttcc 1998

ttgtcccagg atggagctga tccagtaacc tcggagacgg gaccctgccc agagctgagt 2058

tgggggtgtg gctctgccct ggaaaggggg tgacctcttg cctcgagggg cccagggaag 2118

cctgggtgtc aagtgcctgc accaggggtg cacaataaag ggggttctct ctcagaaaaa 2178

aa 2180

<210> SEQ ID NO 24
<211> LENGTH: 372
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

Met Ser Gly Pro Arg Ala Gly Phe Tyr Arg Gln Glu Leu Asn Lys Thr
1 5 10 15

Val Trp Glu Val Pro Gln Arg Leu Gln Gly Leu Arg Pro Val Gly Ser
20 25 30

Gly Ala Tyr Gly Ser Val Cys Ser Ala Tyr Asp Ala Arg Leu Arg Gln
35 40 45

Lys Val Ala Val Lys Lys Leu Ser Arg Pro Phe Gln Ser Leu Ile His
50 55 60

Ala Arg Arg Thr Tyr Arg Glu Leu Arg Leu Leu Lys His Leu Lys His
65 70 75 80

Glu Asn Val Ile Gly Leu Leu Asp Val Phe Thr Pro Ala Thr Ser Ile
85 90 95

Glu Asp Phe Ser Glu Val Tyr Leu Val Thr Thr Leu Met Gly Ala Asp
100 105 110

Leu Asn Asn Ile Val Lys Cys Gln Ala Gly Ala His Gln Gly Ala Arg
115 120 125

Leu Ala Leu Asp Glu His Val Gln Phe Leu Val Tyr Gln Leu Leu Arg
130 135 140

Gly Leu Lys Tyr Ile His Ser Ala Gly Ile Ile His Arg Asp Leu Lys
145 150 155 160

Pro Ser Asn Val Ala Val Asn Glu Asp Cys Glu Leu Arg Ile Leu Asp
165 170 175

Phe Gly Leu Ala Arg Gln Ala Asp Glu Glu Met Thr Gly Tyr Val Ala
180 185 190

Thr Arg Trp Tyr Arg Ala Pro Glu Ile Met Leu Asn Trp Met His Tyr
195 200 205

Asn Gln Thr Val Asp Ile Trp Ser Val Gly Cys Ile Met Ala Glu Leu
210 215 220

Leu Gln Gly Lys Ala Leu Phe Pro Gly Ser Asp Tyr Ile Asp Gln Leu
225 230 235 240

-continued

```
Lys Arg Ile Met Glu Val Val Gly Thr Pro Ser Pro Glu Val Leu Ala
                245                 250                 255

Lys Ile Ser Ser Glu His Ala Arg Thr Tyr Ile Gln Ser Leu Pro Pro
            260                 265                 270

Met Pro Gln Lys Asp Leu Ser Ser Ile Phe Arg Gly Ala Asn Pro Leu
        275                 280                 285

Ala Ile Asp Leu Leu Gly Arg Met Leu Val Leu Asp Ser Asp Gln Arg
    290                 295                 300

Val Ser Ala Ala Glu Ala Leu Ala His Ala Tyr Phe Ser Gln Tyr His
305                 310                 315                 320

Asp Pro Glu Asp Glu Pro Glu Ala Glu Pro Tyr Asp Glu Ser Val Glu
                325                 330                 335

Ala Lys Glu Arg Thr Leu Glu Glu Trp Lys Glu Leu Thr Tyr Gln Glu
            340                 345                 350

Val Leu Ser Phe Lys Pro Pro Glu Pro Pro Lys Pro Pro Gly Ser Leu
        355                 360                 365

Glu Ile Glu Gln
    370
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<210> SEQ ID NO 25
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 25

cgacatgtcc ggagcagaat 20

<210> SEQ ID NO 26
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 26

ttcagctcct gccggtagaa 20

<210> SEQ ID NO 27
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 27

tgcggcacct cccacacggt 20

<210> SEQ ID NO 28
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 28

ccgaacagac ggagccgtat 20

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<210> SEQ ID NO 29
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 29

gtgcttcagg tgcttgagca 20

<210> SEQ ID NO 30
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 30

gcgtgaagac gtccagaagc 20

<210> SEQ ID NO 31
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 31

acttgacgat gttgttcagg 20

<210> SEQ ID NO 32
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 32

aacgtgctcg tcaagtgcca 20

<210> SEQ ID NO 33
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 33

atcctgagct cacagtcctc 20

<210> SEQ ID NO 34
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 34

actgtttggt tgtaatgcat 20

<210> SEQ ID NO 35
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 35

atgatgcgct tcagctggtc 20

<210> SEQ ID NO 36
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 36

gccagtgcct cagctgcact 20

<210> SEQ ID NO 37
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 37

aacgctctca tcatatggct 20

<210> SEQ ID NO 38
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 38

cagcacctca ctgctcaatc 20

<210> SEQ ID NO 39
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 39

tctgtgacca taggagtgtg 20

<210> SEQ ID NO 40
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 40

acacatgttt gtgcatgcat 20

<210> SEQ ID NO 41
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

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<400> SEQUENCE: 41

cctacacatg gcaagcacat 20

<210> SEQ ID NO 42
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 42

tccaggctga gcagctctaa 20

<210> SEQ ID NO 43
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 43

agtgcacgct catccacacg 20

<210> SEQ ID NO 44
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 44

cttgccagat atggctgctg 20

<210> SEQ ID NO 45
<211> LENGTH: 3132
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (12)..(1094)
<300> PUBLICATION INFORMATION:
<308> DATABASE ACCESSION NUMBER: U73142
<309> DATABASE ENTRY DATE: 1996-10-22

<400> SEQUENCE: 45

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gccgctggaa a atg tcg cag gaa agg ccc acg ttc tac cgg cag gag ctg      50
             Met Ser Gln Glu Arg Pro Thr Phe Tyr Arg Gln Glu Leu
               1               5                  10

aac aag acc gtc tgg gag gtg ccc gag cga tac cag aac ctg tcc ccg       98
Asn Lys Thr Val Trp Glu Val Pro Glu Arg Tyr Gln Asn Leu Ser Pro
     15                  20                  25

gtg ggc tcg gga gcc tac ggc tcg gtg tgt gct gct ttt gat aca aag      146
Val Gly Ser Gly Ala Tyr Gly Ser Val Cys Ala Ala Phe Asp Thr Lys
 30                  35                  40                  45

acg gga cat cgt gtg gca gtg aag aag ctg tcg aga ccg ttt cag tcc      194
Thr Gly His Arg Val Ala Val Lys Lys Leu Ser Arg Pro Phe Gln Ser
                 50                  55                  60

atc att cac gcc aaa agg acc tac agg gag ctg cgg ctg ctg aag cac      242
Ile Ile His Ala Lys Arg Thr Tyr Arg Glu Leu Arg Leu Leu Lys His
             65                  70                  75

atg aag cac gag aat gtg att ggt ctg ttg gat gtg ttt aca cct gca      290
Met Lys His Glu Asn Val Ile Gly Leu Leu Asp Val Phe Thr Pro Ala
```

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```
        80                  85                  90

agg tcc ctg gaa gaa ttc aac gat gtg tac ctg gtg acc cat ctc atg      338
Arg Ser Leu Glu Glu Phe Asn Asp Val Tyr Leu Val Thr His Leu Met
     95                 100                 105

ggg gca gac ctg aac aac atc gtg aag tgt cag aag ctt acc gat gac      386
Gly Ala Asp Leu Asn Asn Ile Val Lys Cys Gln Lys Leu Thr Asp Asp
110                 115                 120                 125

cac gtt cag ttt ctt atc tac cag atc ctg cga ggg ctg aag tat ata      434
His Val Gln Phe Leu Ile Tyr Gln Ile Leu Arg Gly Leu Lys Tyr Ile
                130                 135                 140

cac tcg gct gac ata atc cac agg gac cta aag ccc agc aac ctc gct      482
His Ser Ala Asp Ile Ile His Arg Asp Leu Lys Pro Ser Asn Leu Ala
            145                 150                 155

gtg aat gaa gac tgt gag ctg aag att ctg gat ttt ggg ctg gct cgg      530
Val Asn Glu Asp Cys Glu Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg
        160                 165                 170

cac act gat gac gaa atg acc ggc tac gtg gct acc cgg tgg tac aga      578
His Thr Asp Asp Glu Met Thr Gly Tyr Val Ala Thr Arg Trp Tyr Arg
    175                 180                 185

gcc ccc gag att atg ctg aat tgg atg cac tac aac cag aca gtg gat      626
Ala Pro Glu Ile Met Leu Asn Trp Met His Tyr Asn Gln Thr Val Asp
190                 195                 200                 205

att tgg tcc gtg ggc tgc atc atg gct gag ctg ttg acc gga aga acg      674
Ile Trp Ser Val Gly Cys Ile Met Ala Glu Leu Leu Thr Gly Arg Thr
                210                 215                 220

ttg ttt cct ggt aca gac cat att gat cag ttg aag ctc att tta aga      722
Leu Phe Pro Gly Thr Asp His Ile Asp Gln Leu Lys Leu Ile Leu Arg
            225                 230                 235

ctc gtt gga acc cca ggg gct gag ctt ctg aag aaa atc tcc tca gag      770
Leu Val Gly Thr Pro Gly Ala Glu Leu Leu Lys Lys Ile Ser Ser Glu
        240                 245                 250

tct gca aga aac tac att cag tct ctg gcc cag atg ccg aag atg aac      818
Ser Ala Arg Asn Tyr Ile Gln Ser Leu Ala Gln Met Pro Lys Met Asn
    255                 260                 265

ttc gca aat gta ttt att ggt gcc aat ccc ctg gct gtc gac ctg ctg      866
Phe Ala Asn Val Phe Ile Gly Ala Asn Pro Leu Ala Val Asp Leu Leu
270                 275                 280                 285

gaa aag atg ctg gtt ttg gac tcg gat aag agg atc aca gca gcc caa      914
Glu Lys Met Leu Val Leu Asp Ser Asp Lys Arg Ile Thr Ala Ala Gln
                290                 295                 300

gct ctt gcg cat gcc tac ttt gct cag tac cac gac cct gat gat gag      962
Ala Leu Ala His Ala Tyr Phe Ala Gln Tyr His Asp Pro Asp Asp Glu
            305                 310                 315

cca gtg gct gaa cct tat gac cag tcc ttt gaa agc agg gac ttc ctt     1010
Pro Val Ala Glu Pro Tyr Asp Gln Ser Phe Glu Ser Arg Asp Phe Leu
        320                 325                 330

ata gac gaa tgg aag agc ctg acc tac gat gaa gtc att agc ttt gtg     1058
Ile Asp Glu Trp Lys Ser Leu Thr Tyr Asp Glu Val Ile Ser Phe Val
    335                 340                 345

cca ccg ccc ctt gac caa gaa gaa atg gag tcc tga gcaccttgct          1104
Pro Pro Pro Leu Asp Gln Glu Glu Met Glu Ser
350                 355                 360

tctgttctgt ccatcccact tcactgtgag gggaaggcct gttcatggga actctccaaa   1164

taccattcaa gtgcctcttg ttgaaagatt ccttcatggt ggaaggggggt gcatgtatgt   1224

gcgtagtgtt tgtgtgtgtc tgtctgtctg tccgtttgtc catgtatctt tgtggaagtc   1284
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attgtgatgg cagtgacttc atgagtggta gatgctcctt ggcagtctgc ctgctctctc 1344

agagtccggg caggccgatg ggaactgccg tctccttagg gatgtgtgtg tgtatgttaa 1404

gtgcaaagta agaatattaa aatatccctg ttcctagtta ccttgccact tcggcttctc 1464

ctgtggccct gcctttacca tatcacagtg acagagagag gctgcttcag gtctgaggct 1524

atccctcagc catgcataaa gcccaagaga accaactggc tcctgggctc tagcctgtga 1584

tcggcttgct catgtcctca gaacctgtca gtctgtttgt gccttaaaag gagagaaggg 1644

cgcgttgtgg tagttacaga atctcagttg ctggcgttct gagccaggca aggcacaggg 1704

ctgttggatg gccagtgggg agctggacaa aacaaggcag ccttcaagga ggccatgggt 1764

gcatgtttgc atgagtgtat gtgcaaccgc cctccctcac ctccaggagc aagctgtttt 1824

ctatgcttac ctaagttcac ctcagtgcag aggtctccag tgccaggcac aggctcctgc 1884

catcagtagc ttcctatgtc atcttcacgt catgcgggtg tttgcatgct gtgctctgga 1944

gcttgtcctg tcttctggaa gccctgggcc gggcgtgtga agacttccca gcagtcctat 2004

ccacgcacct cagctgaggc cacgggcaca ctgctgcttc ctcactccag ctacgttgtg 2064

ttgaacacaa ctgatcctcc aggtgcttgt ggtgcaggaa acgggacgaa cagagcacct 2124

gaacccttgc catctgacat caccgacaca ggagaacagt cctctcctct cctctcctct 2184

cctctcctag gacagtcccc ggctctggaa tcatgttctt ctcactcatg gtagccagct 2244

aagaaagctg caaaccgaac aaagggagaa ccgagctcct gaagccagga gctcctttta 2304

ctgtccttct caaaatagggg tcattagaca cagccaagtc gtcaaaggcc cctttccttg 2364

tacggggccc ccccgccccc ggcagcttga cactgatttc agtgtctatt tggggagaaa 2424

gcaattttgt cttggaattt tgtatgttgt aggaatcctt agagagtgtg gttccttctg 2484

atggggagaa agggcaaatt attttaatat tttgtatttc acctttataa acatgaatcc 2544

tcagggtga agaacagttt gcataatttt ctgaatttca ggcactttgt gctatatgag 2604

gacccatata tttaagcttt ttgtgcagta agaaagtgta aagccaattc cagtgttgga 2664

cgaaacaggt ctcgtattta ggtcaaggtg tctccattct ctatcagtgc agggacatgc 2724

agtttctgtg gggcagggta ggaccctgca tcatttggag cccagaagga ggccgactgg 2784

ccaggcctca ccgcctcagt atgcagtcca gctccacgtc atcccctcac aatggttagt 2844

agcaacgtct gggtttgaac gccaggcgtg gttatattat tgaggatgcc tttgcacatg 2904

tggccatgct gtgttaggac tgtgccccag ggcccggact tgaagctaga gctggcagaa 2964

gagctcctgg catccatggt gcgatgctgc cgccacccag tttctccatt ggaagacaag 3024

ggaatgagaa gactgctgtg tatgtgtatt tgtgaacttg gttgtgatct ggtatgccat 3084

aggatgtcag acaatatcac tggttaaagt aaagcctatt tttcagat 3132

<210> SEQ ID NO 46
<211> LENGTH: 360
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 46

Met Ser Gln Glu Arg Pro Thr Phe Tyr Arg Gln Glu Leu Asn Lys Thr
1 5 10 15

Val Trp Glu Val Pro Glu Arg Tyr Gln Asn Leu Ser Pro Val Gly Ser
20 25 30

-continued

```
Gly Ala Tyr Gly Ser Val Cys Ala Ala Phe Asp Thr Lys Thr Gly His
         35                  40                  45

Arg Val Ala Val Lys Lys Leu Ser Arg Pro Phe Gln Ser Ile Ile His
     50                  55                  60

Ala Lys Arg Thr Tyr Arg Glu Leu Arg Leu Leu Lys His Met Lys His
 65                  70                  75                  80

Glu Asn Val Ile Gly Leu Leu Asp Val Phe Thr Pro Ala Arg Ser Leu
                 85                  90                  95

Glu Glu Phe Asn Asp Val Tyr Leu Val Thr His Leu Met Gly Ala Asp
            100                 105                 110

Leu Asn Asn Ile Val Lys Cys Gln Lys Leu Thr Asp Asp His Val Gln
        115                 120                 125

Phe Leu Ile Tyr Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala
    130                 135                 140

Asp Ile Ile His Arg Asp Leu Lys Pro Ser Asn Leu Ala Val Asn Glu
145                 150                 155                 160

Asp Cys Glu Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg His Thr Asp
                165                 170                 175

Asp Glu Met Thr Gly Tyr Val Ala Thr Arg Trp Tyr Arg Ala Pro Glu
            180                 185                 190

Ile Met Leu Asn Trp Met His Tyr Asn Gln Thr Val Asp Ile Trp Ser
        195                 200                 205

Val Gly Cys Ile Met Ala Glu Leu Leu Thr Gly Arg Thr Leu Phe Pro
    210                 215                 220

Gly Thr Asp His Ile Asp Gln Leu Lys Leu Ile Leu Arg Leu Val Gly
225                 230                 235                 240

Thr Pro Gly Ala Glu Leu Leu Lys Lys Ile Ser Ser Glu Ser Ala Arg
                245                 250                 255

Asn Tyr Ile Gln Ser Leu Ala Gln Met Pro Lys Met Asn Phe Ala Asn
            260                 265                 270

Val Phe Ile Gly Ala Asn Pro Leu Ala Val Asp Leu Leu Glu Lys Met
        275                 280                 285

Leu Val Leu Asp Ser Asp Lys Arg Ile Thr Ala Ala Gln Ala Leu Ala
    290                 295                 300

His Ala Tyr Phe Ala Gln Tyr His Asp Pro Asp Asp Glu Pro Val Ala
305                 310                 315                 320

Glu Pro Tyr Asp Gln Ser Phe Glu Ser Arg Asp Phe Leu Ile Asp Glu
                325                 330                 335

Trp Lys Ser Leu Thr Tyr Asp Glu Val Ile Ser Phe Val Pro Pro Pro
            340                 345                 350

Leu Asp Gln Glu Glu Met Glu Ser
        355                 360


<210> SEQ ID NO 47
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 47

ctgcgacatt ttccagcggc                                              20
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<210> SEQ ID NO 48
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 48

ggtaagcttc tgacacttca 20

<210> SEQ ID NO 49
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 49

ggccagagac tgaatgtagt 20

<210> SEQ ID NO 50
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 50

catcatcagg gtcgtggtac 20

<210> SEQ ID NO 51
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 51

ggcacaaagc taatgacttc 20

<210> SEQ ID NO 52
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 52

aggtgctcag gactccattt 20

<210> SEQ ID NO 53
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 53

ggatggacag aacagaagca 20

<210> SEQ ID NO 54
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 54

gagcaggcag actgccaagg 20

<210> SEQ ID NO 55
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 55

aggctagagc ccaggagcca 20

<210> SEQ ID NO 56
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 56

gagcctgtgc ctggcactgg 20

<210> SEQ ID NO 57
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 57

tgcaccacaa gcacctggag 20

<210> SEQ ID NO 58
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 58

ggctaccatg agtgagaaga 20

<210> SEQ ID NO 59
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 59

gtccctgcac tgatagagaa 20

<210> SEQ ID NO 60
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 60

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tcttccaatg gagaaactgg 20

<210> SEQ ID NO 61
<211> LENGTH: 749
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 61

tgctgggcgt ggggcgcggg ccgggtgctg cgcgcgggga tccggggcgc tcgctccagc 60

tgcttctgtg gatatgtcgg gtccgcgcgc gggattctac cggcaagagc tgaacaaaac 120

agtatgggag gtgccgcagc ggctgcaggg cctacgcccg gtgggctccg gcgcctacgg 180

ctcagtctgc tcggcctacg acgcgcggct gcgccagaag gtggctgtaa agaagctgtc 240

tcgccctttc caatcgctga tccacgcgag gaggacatac cgtgagctgc gcctactcaa 300

gcacctgaag cacgagaacg tcataggact tttggacgtc ttcacgccgg ccacatccat 360

cgaggatttc agcgaagtgt acctcgtgac gaccctgatg ggcgccgacc tgaataacat 420

cgtcaagtgt caggccctga gcgatgagca tgttcaattc cttgtctacc agctgctgcg 480

tgggctgaag tatatccact cggcgggcat cattcaccgg gacctgaagc ccagcaatgt 540

agcggtgaac gaggactgcg agctgaggat cctggacttt gggctagcac gccaggctga 600

tgaggagatg accggatatg tggccacacg gtggtaccgg gcgccagaga tcatgctaaa 660

ctggatgcac tacaaccaga cagtggacat ctggtctgtg gcctgcttca tggcttgaac 720

tgctggaagg gaagggcctt ctttcctgg 749

<210> SEQ ID NO 62

<400> SEQUENCE: 62

000

<210> SEQ ID NO 63
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 63

cacagaagca gctggagcga 20

<210> SEQ ID NO 64
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 64

tgcggcacct cccatactgt 20

<210> SEQ ID NO 65
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

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<400> SEQUENCE: 65

ccctgcagcc gctgcggcac 20

<210> SEQ ID NO 66
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 66

gcagactgag ccgtaggcgc 20

<210> SEQ ID NO 67
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 67

ttacagccac cttctggcgc 20

<210> SEQ ID NO 68
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 68

gtatgtcctc ctcgcgtgga 20

<210> SEQ ID NO 69
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 69

atggatgtgg ccggcgtgaa 20

<210> SEQ ID NO 70
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 70

gaattgaaca tgctcatcgc 20

<210> SEQ ID NO 71
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 71

acattgctgg gcttcaggtc 20

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<210> SEQ ID NO 72
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 72

atcctcagct cgcagtcctc 20

<210> SEQ ID NO 73
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 73

taccaccgtg tggccacata 20

<210> SEQ ID NO 74
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 74

cagtttagca tgatctctgg 20

<210> SEQ ID NO 75
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 75

caggccacag accagatgtc 20

<210> SEQ ID NO 76
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 76

ccttccagca gttcaagcca 20

<210> SEQ ID NO 77
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: control sequence

<400> SEQUENCE: 77

cagcaccatg gacgcggaac 20

<210> SEQ ID NO 78
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 78

ctgagacatt ttccagcggc 20

<210> SEQ ID NO 79
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 79

acgctcgggc acctcccaga 20

<210> SEQ ID NO 80
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 80

agcttcttca ctgccacacg 20

<210> SEQ ID NO 81
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 81

aatgatggac tgaaatggtc 20

<210> SEQ ID NO 82
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 82

tccaacagac caatcacatt 20

<210> SEQ ID NO 83
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 83

tgtaagcttc tgacatttca 20

<210> SEQ ID NO 84
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

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<400> SEQUENCE: 84

tgaatgtata tactttagac 20

<210> SEQ ID NO 85
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 85

ctcacagtct tcattcacag 20

<210> SEQ ID NO 86
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 86

cacgtagcct gtcatttcat 20

<210> SEQ ID NO 87
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 87

catcccactg accaaatatc 20

<210> SEQ ID NO 88
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 88

tatggtctgt accaggaaac 20

<210> SEQ ID NO 89
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 89

agtcaaagac tgaatatagt 20

<210> SEQ ID NO 90
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 90

ttctcttatc tgagtccaat 20

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<210> SEQ ID NO 91
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 91

catcatcagg atcgtggtac 20

<210> SEQ ID NO 92
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 92

tcaaaggact gatcataagg 20

<210> SEQ ID NO 93
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 93

ggcacaaagc tgatgacttc 20

<210> SEQ ID NO 94
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 94

aggtgctcag gactccatct 20

<210> SEQ ID NO 95
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 95

gcaacaagag gcacttgaat 20

<210> SEQ ID NO 96
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 96

ttatcctagc ttagacctat 20

<210> SEQ ID NO 97
<211> LENGTH: 20
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 97

acagacggag ccgtaggcgc 20

<210> SEQ ID NO 98
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 98

caccgccacc ttctggcgca 20

<210> SEQ ID NO 99
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 99

gtacgttctg cgcgcgtgga 20

<210> SEQ ID NO 100
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 100

atggacgtgg ccggcgtgaa 20

<210> SEQ ID NO 101
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 101

caggaattga acgtgctcgt 20

<210> SEQ ID NO 102
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 102

acgttgctgg gcttcaggtc 20

<210> SEQ ID NO 103
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

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<400> SEQUENCE: 103

taccagcgcg tggccacata 20

<210> SEQ ID NO 104
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 104

cagttgagca tgatctcagg 20

<210> SEQ ID NO 105
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 105

cggaccagat atccactgtt 20

<210> SEQ ID NO 106
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 106

tgccctggag cagctcagcc 20

<210> SEQ ID NO 107
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: antisense sequence

<400> SEQUENCE: 107

gttcgatcgg ctcgtgtcga 20

<210> SEQ ID NO 108
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 108

gatgagtgga aaagcctgac 20

<210> SEQ ID NO 109
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 109

ctgcaacaag aggcacttga 20

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<210> SEQ ID NO 110
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Probe

<400> SEQUENCE: 110

gatgaagtca tcagctttgt gccaccaccc cttgaccaag aagagatgga 50

<210> SEQ ID NO 111
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 111

gaaggtgaag gtcggagtc 19

<210> SEQ ID NO 112
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 112

gaagatggtg atgggatttc 20

<210> SEQ ID NO 113
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Probe

<400> SEQUENCE: 113

caagcttccc gttctcagcc 20

<210> SEQ ID NO 114
<211> LENGTH: 3450
<212> TYPE: DNA
<213> ORGANISM: M. musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (297)...(1379)

<400> SEQUENCE: 114

cgggcgctga agcgcgagcg ggtgtcttgc ggcgtcggcg tgcgctccct ccccggggag 60

cggctgcagg aggaccgcgg cgggagcagc ctcgagccgt gcagccggct ccggcacctt 120

gccgacgctc gtaggagccg ccgcggctga caggggcggc gggtcgcagc ctccacacct 180

gcgcgggtgg cgggcgcggg gtccggtctg ccgcgggcgg gcgcagagga gagcgtgcgg 240

ctgcaggcag gagcccccgc tcggccacct cctcgccccg ctgctgccgc tggaag atg 299
Met
1

tcg cag gag agg ccc acg ttc tac cgg cag gag ctg aac aag acc atc 347
Ser Gln Glu Arg Pro Thr Phe Tyr Arg Gln Glu Leu Asn Lys Thr Ile
5 10 15

tgg gag gtg ccc gaa cga tac cag aac ctg tcc ccg gtg ggc tcg ggc 395

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Trp Glu Val Pro Glu Arg Tyr Gln Asn Leu Ser Pro Val Gly Ser Gly
         20                  25                  30

gcc tat ggc tcg gtg tgt gct gct ttt gat aca aag acg ggg cat cgt      443
Ala Tyr Gly Ser Val Cys Ala Ala Phe Asp Thr Lys Thr Gly His Arg
     35                  40                  45

gtg gca gtt aag aag ctg tcg aga ccg ttt cag tcc atc att cac gcc      491
Val Ala Val Lys Lys Leu Ser Arg Pro Phe Gln Ser Ile Ile His Ala
 50                  55                  60                  65

aaa agg acc tac cga gag ttg cgt ctg ctg aag cac atg aaa cac gaa      539
Lys Arg Thr Tyr Arg Glu Leu Arg Leu Leu Lys His Met Lys His Glu
                 70                  75                  80

aat gtg att ggt ctg ttg gat gtg ttc aca ccc gca agg tca ctg gag      587
Asn Val Ile Gly Leu Leu Asp Val Phe Thr Pro Ala Arg Ser Leu Glu
             85                  90                  95

gaa ttc aat gac gtg tac ctg gtg acc cat ctc atg ggg gcg gac ctg      635
Glu Phe Asn Asp Val Tyr Leu Val Thr His Leu Met Gly Ala Asp Leu
        100                 105                 110

aac aac atc gtg aag tgc cag aag ctg acc gac gac cac gtt cag ttt      683
Asn Asn Ile Val Lys Cys Gln Lys Leu Thr Asp Asp His Val Gln Phe
    115                 120                 125

ctc atc tac cag atc ctc cga ggg ctg aag tat ata cat tcg gct gac      731
Leu Ile Tyr Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala Asp
130                 135                 140                 145

ata att cac agg gac cta aag ccc agc aac cta gct gtg aac gaa gac      779
Ile Ile His Arg Asp Leu Lys Pro Ser Asn Leu Ala Val Asn Glu Asp
                150                 155                 160

tgt gag ctc aag att ctg gat ttt ggg ctg gct cgg cac act gat gat      827
Cys Glu Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg His Thr Asp Asp
            165                 170                 175

gag atg aca ggc tac gtg gct acc agg tgg tac cga gcc cca gag atc      875
Glu Met Thr Gly Tyr Val Ala Thr Arg Trp Tyr Arg Ala Pro Glu Ile
        180                 185                 190

atg ctg aat tgg atg cac tat aac cag aca gtg gat att tgg tcc gtg      923
Met Leu Asn Trp Met His Tyr Asn Gln Thr Val Asp Ile Trp Ser Val
    195                 200                 205

ggc tgc atc atg gct gag ctg ttg acc gga aga acg ttg ttt cct ggt      971
Gly Cys Ile Met Ala Glu Leu Leu Thr Gly Arg Thr Leu Phe Pro Gly
210                 215                 220                 225

aca gac cat att gat cag ttg aag ctc att tta aga ctc gtt gga acc     1019
Thr Asp His Ile Asp Gln Leu Lys Leu Ile Leu Arg Leu Val Gly Thr
                230                 235                 240

cca ggg gct gag ctt ctg aag aaa atc tcc tca gag tct gca aga aac     1067
Pro Gly Ala Glu Leu Leu Lys Lys Ile Ser Ser Glu Ser Ala Arg Asn
            245                 250                 255

tac att cag tct ctg gcc cag atg ccg aag atg aac ttc gca aat gta     1115
Tyr Ile Gln Ser Leu Ala Gln Met Pro Lys Met Asn Phe Ala Asn Val
        260                 265                 270

ttt att ggt gcc aat ccc ctg gct gtc gac cta ctg gag aag atg ctc     1163
Phe Ile Gly Ala Asn Pro Leu Ala Val Asp Leu Leu Glu Lys Met Leu
    275                 280                 285

gtt ttg gac tca gat aag agg atc aca gca gcc caa gct ctt gcg cat     1211
Val Leu Asp Ser Asp Lys Arg Ile Thr Ala Ala Gln Ala Leu Ala His
290                 295                 300                 305

gcc tac ttt gct cag tac cac gac cct gat gat gag cct gtt gct gac     1259
Ala Tyr Phe Ala Gln Tyr His Asp Pro Asp Asp Glu Pro Val Ala Asp
                310                 315                 320

cct tat gac cag tcc ttt gaa agc agg gac ctt ctc ata gat gag tgg     1307
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Pro Tyr Asp Gln Ser Phe Glu Ser Arg Asp Leu Leu Ile Asp Glu Trp
            325                 330                 335

aag agc ctg acc tat gat gaa gtc atc agc ttt gtg cca cca ccc ctt     1355
Lys Ser Leu Thr Tyr Asp Glu Val Ile Ser Phe Val Pro Pro Pro Leu
        340                 345                 350

gac caa gaa gaa atg gag tcc tga gcacctggtt tctgttctgt ctatctcact    1409
Asp Gln Glu Glu Met Glu Ser
    355                 360

tcactgtgag gggaagacct tctcatggga actctccaaa taccattcaa gtgcctcttg   1469

ttgaaagatt ccttcatggt ggaaggggggt gcatgtatgt gttagtgttt gtgtgtgtgt   1529

gtgtgtctgt ctgttcgtct gtccacctat ctttgtggaa gtcactgtga tggtagtgac   1589

tttatgagtt gtgaatggtc cttggcagtc tgcctgcttt ctcagagtct gggcaggccg   1649

atgggaactg tcatctcctt agggatgtgt gtgttcagtg caaagtaaga aatatgaaaa   1709

tatccctgtt cttagttacc ttgccacttt ggcttctcct gtggccctgc ctttaccata   1769

tcagtgacag agagaggctg cttcaggtct gaggctatcc ctcagccatg cataaagtcc   1829

aagagaacca actggctcct ggtctctagc ctgtgaccgg cttgcttaat gtcctcagaa   1889

cctgacaggt atgttcaaaa ctgtcagtct gtttgtgcct taaaagggtg agaagggcgc   1949

gtagatagtt acagagtctc agctgctgac gttctgagcc aggcaagtgc acggggctgt   2009

tggatggcca gtggggagct ggaaaaaaca aggcagcctt taggaaggcc atggtgcatg   2069

tgtgtgcatg cgtgtatgtg cagccgccct ccctcacttc aggagcaagc tgtttgctgt   2129

gcttaccctt cacctcagtg cagaggtctc cagtgccgag cacaggcacc tgccatcagt   2189

agttcctgtg tcatcttcac atctagcaga gcacggatgt gtttgcatgc tgtgctcttg   2249

gagcttgtcc tgtcttctgg aagccctgga caaggcgtgt gaaggcttcc cagaagttcc   2309

tgtccacatt gcctccgccc accgacgcca tgggcacact gctccctcct cctcctccag   2369

ctactttgtg ttgaacacaa ttgattctcc aggtgctcat ggtgcaggaa aacaggacag   2429

acagagagca ctgaaccctt gccatctgat gtcaccaatt caggaaaacg agtcctctcc   2489

taggactatc cccggttctg gaaatcatgt tctcctcact catggtgaca agctaagaaa   2549

gctgaacaaa gggagagacg agagcgcctg aagccaggag ctcctttact atctttctca   2609

aaagggttgt tagacacaaa ccaagtcatc aaggccccgc tcctctcctc ggaagggtcc   2669

cccacccccc ggcagcttga cactgaatcc agtgtcaatt tggggagaaa gcagttttgt   2729

cttggaattt tgtatgttgt aggaatcctt agagagtgtg gttccttctg atggggagaa   2789

agggcaaatt attttaatat tttgtatttt caccttttata aacatgaatc ctcaggggtg   2849

aagaactgtt tgcataattt tctgaatttt gagcactttg tgctatataa ggacccatat   2909

ttaagctttg tgtgcagtaa gaaagtgtaa agccaattcc agtgttggac gtgacaggtc   2969

ttgtgtttag gtcaaggtgt ctcctctcag tgcagggaca tgcctgctct gtggggcagg   3029

cgaggaccct gaatcatttg gagcccagaa ggaggcagac tggccaggtc tcaccacctc   3089

agtgtgcagt tcaactccat gccatcccat caagatgggt tagtagcagt gtctgttttt   3149

gaatgccaag tgtgatttcc aacaattctg ctctggttat ttcattgaag acatctttgc   3209

acatgtgacc atgctgtgtt aggggctgtg ttccagggac tggactcgaa gctagaactg   3269

gcagaagagt tctggcatcc acagcgcaat gctgccacca cccagtttct tcatcagaag   3329

acaagggaac gagaaaactg ctgttcgttt gtatttgtga acttggctgt aatctggtat   3389
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gccataggat gtcagataat accactggtt aaaataaagc ctagttttca aattcaaccg 3449

g 3450

<210> SEQ ID NO 115
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 115

aagggaacga gaaaactgct gtt 23

<210> SEQ ID NO 116
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 116

tattttaacc agtggtatta tctgacatcc t 31

<210> SEQ ID NO 117
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Probe

<400> SEQUENCE: 117

ttgtatttgt gaacttggct gtaatctggt atgcc 35

<210> SEQ ID NO 118
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 118

ggcaaattca acggcacagt 20

<210> SEQ ID NO 119
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 119

gggtctcgct cctggaagat 20

<210> SEQ ID NO 120
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Probe

<400> SEQUENCE: 120

aaggccgaga atgggaagct tgtcatc 27

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<210> SEQ ID NO 121
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 121

atcatttgga gcccagaagg a 21

<210> SEQ ID NO 122
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 122

tggagctgga ctgcatactg a 21

<210> SEQ ID NO 123
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Probe

<400> SEQUENCE: 123

ctggccaggc ctcaccgc 18

<210> SEQ ID NO 124
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 124

tgttctagag acagccgcat ctt 23

<210> SEQ ID NO 125
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 125

caccgacctt caccatcttg t 21

<210> SEQ ID NO 126
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PCR Probe

<400> SEQUENCE: 126

ttgtgcagtg ccagcctcgt ctca 24

<210> SEQ ID NO 127
<211> LENGTH: 3757

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<212> TYPE: DNA
<213> ORGANISM: H. sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (363)...(1445)

<400> SEQUENCE: 127

ggaaccgcga ccactggagc cttagcgggc gcagcagctg gaacgggagt actgcgacgc 60

agcccggagt cggccttgta ggggcgaagg tgcagggaga tcgcggcggg cgcagtcttg 120

agcgccggag cgcgtccctg cccttagcgg ggcttgcccc agtcgcaggg gcacatccag 180

ccgctgcggc tgacagcagc cgcgcgcgcg ggagtctgcg gggtcgcggc agccgcacct 240

gcgcgggcga ccagcgcaag gtccccgccc ggctgggcgg gcagcaaggg ccggggagag 300

ggtgcgggtg caggcggggg ccccacaggg ccaccttctt gcccggcggc tgccgctgga 360

aa atg tct cag gag agg ccc acg ttc tac cgg cag gag ctg aac aag 407
Met Ser Gln Glu Arg Pro Thr Phe Tyr Arg Gln Glu Leu Asn Lys
1 5 10 15

aca atc tgg gag gtg ccc gag cgt tac cag aac ctg tct cca gtg ggc 455
Thr Ile Trp Glu Val Pro Glu Arg Tyr Gln Asn Leu Ser Pro Val Gly
20 25 30

tct ggc gcc tat ggc tct gtg tgt gct gct ttt gac aca aaa acg ggg 503
Ser Gly Ala Tyr Gly Ser Val Cys Ala Ala Phe Asp Thr Lys Thr Gly
35 40 45

tta cgt gtg gca gtg aag aag ctc tcc aga cca ttt cag tcc atc att 551
Leu Arg Val Ala Val Lys Lys Leu Ser Arg Pro Phe Gln Ser Ile Ile
50 55 60

cat gcg aaa aga acc tac aga gaa ctg cgg tta ctt aaa cat atg aaa 599
His Ala Lys Arg Thr Tyr Arg Glu Leu Arg Leu Leu Lys His Met Lys
65 70 75

cat gaa aat gtg att ggt ctg ttg gac gtt ttt aca cct gca agg tct 647
His Glu Asn Val Ile Gly Leu Leu Asp Val Phe Thr Pro Ala Arg Ser
80 85 90 95

ctg gag gaa ttc aat gat gtg tat ctg gtg acc cat ctc atg ggg gca 695
Leu Glu Glu Phe Asn Asp Val Tyr Leu Val Thr His Leu Met Gly Ala
100 105 110

gat ctg aac aac att gtg aaa tgt cag aag ctt aca gat gac cat gtt 743
Asp Leu Asn Asn Ile Val Lys Cys Gln Lys Leu Thr Asp Asp His Val
115 120 125

cag ttc ctt atc tac caa att ctc cga ggt cta aag tat ata cat tca 791
Gln Phe Leu Ile Tyr Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser
130 135 140

gct gac ata att cac agg gac cta aaa cct agt aat cta gct gtg aat 839
Ala Asp Ile Ile His Arg Asp Leu Lys Pro Ser Asn Leu Ala Val Asn
145 150 155

gaa gac tgt gag ctg aag att ctg gat ttt gga ctg gct cgg cac aca 887
Glu Asp Cys Glu Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg His Thr
160 165 170 175

gat gat gaa atg aca ggc tac gtg gcc act agg tgg tac agg gct cct 935
Asp Asp Glu Met Thr Gly Tyr Val Ala Thr Arg Trp Tyr Arg Ala Pro
180 185 190

gag atc atg ctg aac tgg atg cat tac aac cag aca gtt gat att tgg 983
Glu Ile Met Leu Asn Trp Met His Tyr Asn Gln Thr Val Asp Ile Trp
195 200 205

tca gtg gga tgc ata atg gcc gag ctg ttg act gga aga aca ttg ttt 1031
Ser Val Gly Cys Ile Met Ala Glu Leu Leu Thr Gly Arg Thr Leu Phe
210 215 220

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cct ggt aca gac cat att aac cag ctt cag cag att atg cgt ctg aca     1079
Pro Gly Thr Asp His Ile Asn Gln Leu Gln Gln Ile Met Arg Leu Thr
    225                 230                 235

gga aca ccc ccc gct tat ctc att aac agg atg cca agc cat gag gca     1127
Gly Thr Pro Pro Ala Tyr Leu Ile Asn Arg Met Pro Ser His Glu Ala
240                 245                 250                 255

aga aac tat att cag tct ttg act cag atg ccg aag atg aac ttt gcg     1175
Arg Asn Tyr Ile Gln Ser Leu Thr Gln Met Pro Lys Met Asn Phe Ala
                260                 265                 270

aat gta ttt att ggt gcc aat ccc ctg gct gtc gac ttg ctg gag aag     1223
Asn Val Phe Ile Gly Ala Asn Pro Leu Ala Val Asp Leu Leu Glu Lys
            275                 280                 285

atg ctt gta ttg gac tca gat aag aga att aca gcg gcc caa gcc ctt     1271
Met Leu Val Leu Asp Ser Asp Lys Arg Ile Thr Ala Ala Gln Ala Leu
        290                 295                 300

gca cat gcc tac ttt gct cag tac cac gat cct gat gat gaa cca gtg     1319
Ala His Ala Tyr Phe Ala Gln Tyr His Asp Pro Asp Asp Glu Pro Val
    305                 310                 315

gcc gat cct tat gat cag tcc ttt gaa agc agg gac ctc ctt ata gat     1367
Ala Asp Pro Tyr Asp Gln Ser Phe Glu Ser Arg Asp Leu Leu Ile Asp
320                 325                 330                 335

gag tgg aaa agc ctg acc tat gat gaa gtc atc agc ttt gtg cca cca     1415
Glu Trp Lys Ser Leu Thr Tyr Asp Glu Val Ile Ser Phe Val Pro Pro
                340                 345                 350

ccc ctt gac caa gaa gag atg gag tcc tga gcacctggtt tctgttctgt       1465
Pro Leu Asp Gln Glu Glu Met Glu Ser
            355                 360

tgatcccact tcactgtgag gggaaggcct tttcacggga actctccaaa tattattcaa   1525

gtgcctcttg ttgcagagat ttcctccatg gtggaagggg gtgtgcgtgc gtgtgcgtgc   1585

gtgttagtgt gtgtgcatgt gtgtgtctgt ctttgtggga gggtaagaca atatgaacaa   1645

actatgatca cagtgacttt acaggaggtt gtggatgctc cagggcagcc tccaccttgc   1705

tcttctttct gagagttggc tcaggcagac aagagctgct gtccttttag gaatatgttc   1765

aatgcaaagt aaaaaaatat gaattgtccc caatcccggt catgcttttg ccactttggc   1825

ttctcctgtg accccacctt gacggtgggg cgtagacttg acaacatccc acagtggcac   1885

ggagagaagg cccatacctt ctggttgctt cagacctgac accgtccctc agtgatacgt   1945

acagccaaaa aggaccaact ggcttctgtg cactagcctg tgattaactt gcttagtatg   2005

gttctcagat cttgacagta tatttgaaac tgtaaatatg tttgtgcctt aaaaggagag   2065

aagaaagtgt agatagttaa aagactgcag ctgctgaagt tctgagccgg gcaagtcgag   2125

agggctgttg gacagctgct tgtgggcccg gagtaatcag gcagccttca taggcggtca   2185

tgtgtgcatg tgagcacatg cgtatatgtg cgtctctctt tctccctcac ccccaggtgt   2245

tgccatttct ctgcttaccc ttcacctttg gtgcagaggt ttcttgaata tctgccccag   2305

tagtcagaag caggttcttg atgtcatgta cttcctgtgt actctttatt tctagcagag   2365

tgaggatgtg ttttgcacgt cttgctattt gagcatgcac agctgcttgt cctgctctct   2425

tcaggaggcc ctggtgtcag gcaggtttgc cagtgaagac ttcttgggta gtttagatcc   2485

catgtcacct cagctgatat tatggcaagt gatatcacct ctcttcagcc cctagtgcta   2545

ttctgtgttg aacacaattg atacttcagg tgcttttgat gtgaaaatca tgaaaagagg   2605

aacaggtgga tgtatagcat ttttattcat gccatctgtt ttcaaccaac tatttttgag   2665
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gaattatcat gggaaaagac cagggctttt cccaggaata tcccaaactt cggaaacaag 2725

ttattctctt cactcccaat aactaatgct aagaaatgct gaaaatcaaa gtaaaaaatt 2785

aaagcccata aggccagaaa ctccttttgc tgtctttctc taaatatgat tactttaaaa 2845

taaaaaagta acaaggtgtc ttttccactc ctatggaaaa gggtcttctt ggcagcttaa 2905

cattgacttc ttggtttggg gagaaataaa ttttgtttca gaattttgta tattgtagga 2965

atccctttga gaatgtgatt ccttttgatg gggagaaagg gcaaattatt ttaatatttt 3025

gtattttcaa ctttataaag ataaaatatc ctcaggggtg gagaagtgtc gttttcataa 3085

cttgctgaat ttcaggcatt ttgttctaca tgaggactca tatatttaag ccttttgtgt 3145

aataagaaag tataaagtca cttccagtgt tggctgtgtg acagaatctt gtatttgggc 3205

caaggtgttt ccatttctca atcagtgcag tgatacatgt actccagagg gacagggtgg 3265

acccccctgag tcaactggag caagaaggaa ggaggcagac tgatggcgat tccctctcac 3325

ccgggactct ccccctttca aggaaagtga acctttaaag taaaggcctc atctccttta 3385

ttgcagttca aatcctcacc atccacagca agatgaattt tatcagccat gtttggttgt 3445

aaatgctcgt gtgatttcct acagaaatac tgctctgaat attttgtaat aaaggtcttt 3505

gcacatgtga ccacatacgt gttaggaggc tgcatgctct ggaagcctgg actctaagct 3565

ggagctcttg gaagagctct tcggtttctg agcataatgc tcccatctcc tgatttctct 3625

gaacagaaaa caaaagagag aatgagggaa attgctattt tatttgtatt catgaacttg 3685

gctgtaatca gttatgccgt ataggatgtc agacaatacc actggttaaa ataaagccta 3745

tttttcaaat tt 3757

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

<400> SEQUENCE: 128

gagcaaagta ggcatgtgca 20

<210> SEQ ID NO 129
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 129

gtttccgaag tttgggatat 20

<210> SEQ ID NO 130
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 130

gcattagtta ttgggagtga 20

<210> SEQ ID NO 131

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

<400> SEQUENCE: 131

ccctggagca tccacaacct 20

<210> SEQ ID NO 132
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 132

tgtaccagga aacaatgttc 20

<210> SEQ ID NO 133
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 133

cgggcaagaa ggtggccctg 20

<210> SEQ ID NO 134
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 134

atcgccatca gtctgcctcc 20

<210> SEQ ID NO 135
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 135

tgacatcaag aacctgcttc 20

<210> SEQ ID NO 136
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 136

ggcccacaag cagctgtcca 20

<210> SEQ ID NO 137
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 137

tgaaaacgac acttctccac 20

<210> SEQ ID NO 138
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 138

ggtgagaggg aatcgccatc 20

<210> SEQ ID NO 139
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 139

atactgtcaa gatctgagaa 20

<210> SEQ ID NO 140
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 140

tttccgaagt ttgggatatt 20

<210> SEQ ID NO 141
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 141

agagagacgc acatatacgc 20

<210> SEQ ID NO 142
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 142

caagaggcac ttgaataata 20

<210> SEQ ID NO 143
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 143

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attcctccag agaccttgca 20

<210> SEQ ID NO 144
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 144

aagacacctt gttacttttt 20

<210> SEQ ID NO 145
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 145

tgccctttct ccccatcaaa 20

<210> SEQ ID NO 146
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 146

tggcatcctg ttaatgagat 20

<210> SEQ ID NO 147
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 147

aaggccttcc cctcacagtg 20

<210> SEQ ID NO 148
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 148

aataggcttt attttaacca 20

<210> SEQ ID NO 149
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 149

acccaagaag tcttcactgg 20

<210> SEQ ID NO 150

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

<400> SEQUENCE: 150

tttcttatta cacaaaaggc 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

ggaaatcaca cgagcattta 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

ggtccctgtg aattatgtca 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

aatatatgag tcctcatgta 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

ctaacacgta tgtggtcaca 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

tttctcccca tcaaaaggaa 20

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

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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 156

ctgaacatgg tcatctgtaa 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

ataactgatt acagccaagt 20

<210> SEQ ID NO 158
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 158

ttctcaaagg gattcctaca 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

tctgccccca tgagatgggt 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

ttcgcatgaa tgatggactg 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

tactgagcaa agtaggcatg 20

<210> SEQ ID NO 162
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 162

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gtccctgctt tcaaaggact 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

catatgttta agtaaccgca 20

<210> SEQ ID NO 164
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 164

cacattctca aagggattcc 20

<210> SEQ ID NO 165
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 165

ggactccatc tcttcttggt caa 23

<210> SEQ ID NO 166
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 166

gaagtgggat caacagaaca gaaa 24

<210> SEQ ID NO 167
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 167

agcccactgg agacaggttc t 21

<210> SEQ ID NO 168
<211> LENGTH: 3352
<212> TYPE: DNA
<213> ORGANISM: M. musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (230)...(1312)

<400> SEQUENCE: 168

aggaggaccg cggcgggagc agcctcgagc cgtgcagccg gctccggcac cttgccgacg 60

ctcgtaggag ccgccgcggc tgacaggggc ggcgggtcgc agcctccaca cctgcgcggg 120

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tggcgggcgc ggggtccggt ctgccgcggg cgggcgcaga ggagagcgtg cggctgcagg    180

caggagcccc cgctcggcca cctcctcgcc ccgctgctgc cgctggaag atg tcg cag    238
                                                    Met Ser Gln
                                                     1

gag agg ccc acg ttc tac cgg cag gag ctg aac aag acc atc tgg gag      286
Glu Arg Pro Thr Phe Tyr Arg Gln Glu Leu Asn Lys Thr Ile Trp Glu
     5                  10                  15

gtg ccc gaa cga tac cag aac ctg tcc ccg gtg ggc tcg ggc gcc tat      334
Val Pro Glu Arg Tyr Gln Asn Leu Ser Pro Val Gly Ser Gly Ala Tyr
 20                  25                  30                  35

ggc tcg gtg tgt gct gct ttt gat aca aag acg ggg cat cgt gtg gca      382
Gly Ser Val Cys Ala Ala Phe Asp Thr Lys Thr Gly His Arg Val Ala
                 40                  45                  50

gtt aag aag ctg tcg aga ccg ttt cag tcc atc att cac gcc aaa agg      430
Val Lys Lys Leu Ser Arg Pro Phe Gln Ser Ile Ile His Ala Lys Arg
             55                  60                  65

acc tac cga gag ttg cgt ctg ctg aag cac atg aaa cac gaa aat gtg      478
Thr Tyr Arg Glu Leu Arg Leu Leu Lys His Met Lys His Glu Asn Val
         70                  75                  80

att ggt ctg ttg gat gtg ttc aca ccc gca agg tca ctg gag gaa ttc      526
Ile Gly Leu Leu Asp Val Phe Thr Pro Ala Arg Ser Leu Glu Glu Phe
     85                  90                  95

aat gac gtg tac ctg gtg acc cat ctc atg ggg gcg gac ctg aac aac      574
Asn Asp Val Tyr Leu Val Thr His Leu Met Gly Ala Asp Leu Asn Asn
100                 105                 110                 115

atc gtg aag tgc cag aag ctg acc gac gac cac gtt cag ttt ctc atc      622
Ile Val Lys Cys Gln Lys Leu Thr Asp Asp His Val Gln Phe Leu Ile
                120                 125                 130

tac cag atc ctc cga ggg ctg aag tat ata cat tcg gct gac ata att      670
Tyr Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala Asp Ile Ile
            135                 140                 145

cac agg gac cta aag ccc agc aac cta gct gtg aac gaa gac tgt gag      718
His Arg Asp Leu Lys Pro Ser Asn Leu Ala Val Asn Glu Asp Cys Glu
        150                 155                 160

ctc aag att ctg gat ttt ggg ctg gct cgg cac act gat gat gag atg      766
Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg His Thr Asp Asp Glu Met
    165                 170                 175

aca ggc tac gtg gct acc agg tgg tac cga gcc cca gag atc atg ctg      814
Thr Gly Tyr Val Ala Thr Arg Trp Tyr Arg Ala Pro Glu Ile Met Leu
180                 185                 190                 195

aat tgg atg cac tat aac cag aca gtg gat att tgg tcc gtg ggc tgc      862
Asn Trp Met His Tyr Asn Gln Thr Val Asp Ile Trp Ser Val Gly Cys
                200                 205                 210

atc atg gct gag ctg ttg acc gga aga acg ttg ttt cct ggt aca gac      910
Ile Met Ala Glu Leu Leu Thr Gly Arg Thr Leu Phe Pro Gly Thr Asp
            215                 220                 225

cat att aac cag ctt cag cag ata atg cgt atg acg ggg aca ccc cct      958
His Ile Asn Gln Leu Gln Gln Ile Met Arg Met Thr Gly Thr Pro Pro
        230                 235                 240

gct tat ctc att aac agg atg cca agc cat gag gca aga aac tac att     1006
Ala Tyr Leu Ile Asn Arg Met Pro Ser His Glu Ala Arg Asn Tyr Ile
    245                 250                 255

cag tct ctg gcc cag atg ccg aag atg aac ttc gca aat gta ttt att     1054
Gln Ser Leu Ala Gln Met Pro Lys Met Asn Phe Ala Asn Val Phe Ile
260                 265                 270                 275

ggt gcc aat ccc ctg gct gtc gac cta ctg gag aag atg ctc gtt ttg     1102
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Gly Ala Asn Pro Leu Ala Val Asp Leu Leu Glu Lys Met Leu Val Leu
                280                 285                 290

gac tca gat aag agg atc aca gca gcc caa gct ctt gcg cat gcc tac     1150
Asp Ser Asp Lys Arg Ile Thr Ala Ala Gln Ala Leu Ala His Ala Tyr
            295                 300                 305

ttt gct cag tac cac gac cct gat gat gag cct gtt gct gac cct tat     1198
Phe Ala Gln Tyr His Asp Pro Asp Asp Glu Pro Val Ala Asp Pro Tyr
        310                 315                 320

gac cag tcc ttt gaa agc agg gac ctt ctc ata gat gag tgg aag agc     1246
Asp Gln Ser Phe Glu Ser Arg Asp Leu Leu Ile Asp Glu Trp Lys Ser
    325                 330                 335

ctg acc tat gat gaa gtc atc agc ttt gtg cca cca ccc ctt gac caa     1294
Leu Thr Tyr Asp Glu Val Ile Ser Phe Val Pro Pro Pro Leu Asp Gln
340                 345                 350                 355

gaa gaa atg gag tcc tga gcacctggtt tctgttctgt ctatctcact            1342
Glu Glu Met Glu Ser
                360

tcactgtgag gggaagacct tctcatggga actctccaaa taccattcaa gtgcctcttg   1402

ttgaaagatt ccttcatggt ggaaggggggt gcatgtatgt gttagtgttt gtgtgtgtgt   1462

gtgtgtctgt ctgttcgtct gtccacctat ctttgtggaa gtcactgtga tggtagtgac   1522

tttatgagtt gtgaatggtc cttggcagtc tgcctgcttt ctcagagtct gggcaggccg   1582

atgggaactg tcatctcctt agggatgtgt gtgttcagtg caaagtaaga aatatgaaaa   1642

tatccctgtt cttagttacc ttgccacttt ggcttctcct gtggccctgc ctttaccata   1702

tcagtgacag agagaggctg cttcaggtct gaggctatcc ctcagccatg cataaagtcc   1762

aagagaacca actggctcct ggtctctagc ctgtgaccgg cttgcttaat gtcctcagaa   1822

cctgacaggt atgttcaaaa ctgtcagtct gtttgtgcct taaaagggtg agaagggcgc   1882

gtagatagtt acagagtctc agctgctgac gttctgagcc aggcaagtgc acggggctgt   1942

tggatggcca gtggggagct ggaaaaaaca aggcagcctt taggaaggcc atggtgcatg   2002

tgtgtgcatg cgtgtatgtg cagccgccct ccctcacttc aggagcaagc tgtttgctgt   2062

gcttaccctt cacctcagtg cagaggtctc cagtgccgag cacaggcacc tgccatcagt   2122

agttcctgtg tcatcttcac atctagcaga gcacggatgt gtttgcatgc tgtgctcttg   2182

gagcttgtcc tgtcttctgg aagccctgga caaggcgtgt gaaggcttcc cagaagttcc   2242

tgtccacatt gcctccgccc accgacgcca tgggcacact gctccctcct cctcctccag   2302

ctactttgtg ttgaacacaa ttgattctcc aggtgctcat ggtgcaggaa aacaggacag   2362

acagagagca ctgaaccctt gccatctgat gtcaccaatt caggaaaacg agtcctctcc   2422

taggactatc cccggttctg gaaatcatgt tctcctcact catggtgaca agctaagaaa   2482

gctgaacaaa gggagagacg agagcgcctg aagccaggag ctcctttact atctttctca   2542

aaagggttgt tagacacaaa ccaagtcatc aaggccccgc tcctctcctc ggaagggtcc   2602

cccacccccc ggcagcttga cactgaatcc agtgtcaatt tggggagaaa gcagttttgt   2662

cttggaattt tgtatgttgt aggaatcctt agagagtgtg gttccttctg atggggagaa   2722

agggcaaatt attttaatat tttgtatttt caccttttata aacatgaatc ctcaggggtg   2782

aagaactgtt tgcataattt tctgaatttt gagcactttg tgctatataa ggacccatat   2842

ttaagctttg tgtgcagtaa gaaagtgtaa agccaattcc agtgttggac gtgacaggtc   2902

ttgtgtttag gtcaaggtgt ctcctctcag tgcagggaca tgcctgctct gtggggcagg   2962
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cgaggaccct gaatcatttg gagcccagaa ggaggcagac tggccaggtc tcaccacctc   3022

agtgtgcagt tcaactccat gccatcccat caagatgggt tagtagcagt gtctgttttt   3082

gaatgccaag tgtgatttcc aacaattctg ctctggttat ttcattgaag acatctttgc   3142

acatgtgacc atgctgtgtt aggggctgtg ttccagggac tggactcgaa gctagaactg   3202

gcagaagagt tctggcatcc acagcgcaat gctgccacca cccagtttct tcatcagaag   3262

acaagggaac gagaaaactg ctgttcgttt gtatttgtga acttggctgt aatctggtat   3322

gccataggat gtcagataat accactggtt                                    3352


<210> SEQ ID NO 169
<211> LENGTH: 503
<212> TYPE: DNA
<213> ORGANISM: M. musculus

<400> SEQUENCE: 169

cgcaagaata aagtcagtgg tcacaaatag aggggtcag tggctagaag aagagtaagc     60

ctgaattgag catcccagac agtggtccat acgggccgtc agctagctca ttccctgaga    120

tcactaacac tactgaacat agtcattctg aaagtctgtg tttttacagg caagaaacta    180

cattcagtct ctggcccag atg ccg aag atg aac ttc gca aat gta ttt att     232

ggt gcc aat ccc ctg gct gtc gac cta ctg gag aag atg ctc gtt ttg      280

gac tca gat aag agg atc aca gca gcc caa gct ctt gcg cat gct act      328

ttg ctc agt acc acg acc ctg atg atg agc ctg ttg ctg acc ctt atg      376

acc agt cct ttg aaa gca ggg acc ttc tca tag atgagtggaa gagcctgacc    429

tatgatgaag tcatcagctt tgtgccacca ccccttgacc aagaagagat ggagtcctga    489

gcacctggtt tctg                                                     503


<210> SEQ ID NO 170
<211> LENGTH: 1500
<212> TYPE: DNA
<213> ORGANISM: M. musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (297)...(1073)

<400> SEQUENCE: 170

cgggcgctga agcgcgagcg ggtgtcttgc ggcgtcggcg tgcgctccct ccccggggag     60

cggctgcagg aggaccgcgg cgggagcagc ctcgagccgt gcagccggct ccggcacctt    120

gccgacgctc gtaggagccg ccgcggctga caggggcggc gggtcgcagc ctccacacct    180

gcgcgggtgg cgggcgcggg gtccggtctg ccgcgggcgg gcgcagagga gagcgtgcgg    240

ctgcaggcag gagcccccgc tcggccacct cctcgccccg ctgctgccgc tggaag atg    299
                                                              Met
                                                               1

tcg cag gag agg ccc acg ttc tac cgg cag gag ctg aac aag acc atc      347
Ser Gln Glu Arg Pro Thr Phe Tyr Arg Gln Glu Leu Asn Lys Thr Ile
            5                  10                  15

tgg gag gtg ccc gaa cga tac cag aac ctg tcc ccg gtg ggc tcg ggc      395
Trp Glu Val Pro Glu Arg Tyr Gln Asn Leu Ser Pro Val Gly Ser Gly
        20                  25                  30

gcc tat ggc tcg gtg tgt gct gct ttt gat aca aag acg ggg cat cgt      443
Ala Tyr Gly Ser Val Cys Ala Ala Phe Asp Thr Lys Thr Gly His Arg
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     35                  40                  45

gtg gca gtt aag aag ctg tcg aga ccg ttt cag tcc atc att cac gcc      491
Val Ala Val Lys Lys Leu Ser Arg Pro Phe Gln Ser Ile Ile His Ala
 50                  55                  60                  65

aaa agg acc tac cga gag ttg cgt ctg ctg aag cac atg aaa cac gaa      539
Lys Arg Thr Tyr Arg Glu Leu Arg Leu Leu Lys His Met Lys His Glu
                 70                  75                  80

aat gtg att ggt ctg ttg gat gtg ttc aca ccc gca agg tca ctg gag      587
Asn Val Ile Gly Leu Leu Asp Val Phe Thr Pro Ala Arg Ser Leu Glu
             85                  90                  95

gaa ttc aat gac gtg tac ctg gtg acc cat ctc atg ggg gcg gac ctg      635
Glu Phe Asn Asp Val Tyr Leu Val Thr His Leu Met Gly Ala Asp Leu
        100                 105                 110

aac aac atc gtg aag tgc cag aag ctg acc gac gac cac gtt cag ttt      683
Asn Asn Ile Val Lys Cys Gln Lys Leu Thr Asp Asp His Val Gln Phe
    115                 120                 125

ctc atc tac cag atc ctc cga ggg ctg aag tat ata cat tcg gct gac      731
Leu Ile Tyr Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala Asp
130                 135                 140                 145

ata att cac agg gac cta aag ccc agc aac cta gct gtg aac gaa gac      779
Ile Ile His Arg Asp Leu Lys Pro Ser Asn Leu Ala Val Asn Glu Asp
                150                 155                 160

tgt gag ctc aag att ctg gat ttt ggg ctg gct cgg cac act gat gat      827
Cys Glu Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg His Thr Asp Asp
            165                 170                 175

gag atg aca ggc tac gtg gct acc agg tgg tac cga gcc cca gag atc      875
Glu Met Thr Gly Tyr Val Ala Thr Arg Trp Tyr Arg Ala Pro Glu Ile
        180                 185                 190

atg ctg aat tgg atg cac tat aac cag aca gtg gat att tgg tcc gtg      923
Met Leu Asn Trp Met His Tyr Asn Gln Thr Val Asp Ile Trp Ser Val
    195                 200                 205

ggc tgc atc atg gct gag ctg ttg acc gga aga acg ttg ttt cct ggt      971
Gly Cys Ile Met Ala Glu Leu Leu Thr Gly Arg Thr Leu Phe Pro Gly
210                 215                 220                 225

aca gac cat att gat cag ttg aag ctc att tta aga ctc gtt gga acc     1019
Thr Asp His Ile Asp Gln Leu Lys Leu Ile Leu Arg Leu Val Gly Thr
                230                 235                 240

cca ggg gct gag ctt ctg aag aaa atc tcc tca gag tct gat gcc aag     1067
Pro Gly Ala Glu Leu Leu Lys Lys Ile Ser Ser Glu Ser Asp Ala Lys
            245                 250                 255

cca tga ggtgagaaca aacagcatgc acagggaagt ctacctcgga ggccaccttc      1123
Pro

tcgtggtagt gtctgtgtat agccagcagt ttctaatgtc accgaatgct tgcatgtgcc   1183

ccaagaaccg ttaaagcagt actggctgtg tgctagcgga gtgttggcat ttaggatgca   1243

gtctcctgag cctgcgaggc agcgatgcag tgtagggcag tgttccctag tgtttggctt   1303

tctgatcttg tgcttgaggt aacaagtgtc gttgcagttg tatgtagtta gggtgtgcta   1363

cagccgtgtc atgggtgcat ggaacagagt tcattagtgt gctttgctct ccacccattt   1423

tacaaccaag agaagactgc atgcaagcac gcactataaa attccttgtg ctaataaaaa   1483

aaaaaaaaaa aaaaaaa                                                  1500


<210> SEQ ID NO 171
<211> LENGTH: 384
<212> TYPE: DNA
<213> ORGANISM: M. musculus
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<400> SEQUENCE: 171

ttgcaaggac gctccagctc gccgcttagt cacataccac tgctcatttc agtattgttt 60

gacaaaacag ttttccatac cgagcagagg ggcgcccctc aagatcaaga agtgctgctt 120

ttgatacaaa gacggggcat cgtgtggcag ttaagaagct gtcgagaccg tttcagtcca 180

tcattcacgc caaaaggacc taccgagagt tgcgtctgct gaagcacatg aaacacgaaa 240

atgtgattgg tctgttggat gtgttcacac ccgcaaggtc actggaggaa ttcaatgacg 300

tgtacctggt gacccatctc atgggggcgg acctgaacaa catcgtgaag tgccagaagc 360

tgaccgacga ccacgttcag tttc 384

<210> SEQ ID NO 172
<211> LENGTH: 463
<212> TYPE: DNA
<213> ORGANISM: M. musculus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 429
<223> OTHER INFORMATION: n = A, T, C, or G

<400> SEQUENCE: 172

atattgggta agatctggat tcagagcggg gcctccttgg agctgttctc gcgagagttc 60

cgcgagaggc tcccggccgc tgcctgtggg atcgccgcca ctggagccca agcggggcgc 120

tgaagcgcga gcgggtgtct tgcggcgtcg gcgtgcgctc cctccccggg gagcggctgc 180

aggaggaccg cggcgggagc agcctcgagc cgtgcagccg gctccggcac cttgccgacg 240

ctcgtaggag ccgccgcggc tgacaggggc ggcgggtcgc accctccaca cctgcgcggg 300

tggcgggcgc ggggtccggt ctgccgcggg cgggcgcaga ggagagcgtg cggctgcagg 360

caggagcccc cgctcggcca cctcctcgcc ccgctgctgc cgctggaaga tgtcgcagga 420

gaggcccang ttctaccggc aggagctgaa caagaccatc tgg 463

<210> SEQ ID NO 173
<211> LENGTH: 1083
<212> TYPE: DNA
<213> ORGANISM: R. norvegicus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)...(1083)

<400> SEQUENCE: 173

atg tct cag gag agg ccc acg ttc tac cgg cag gag ctg aac aag acc 48
Met Ser Gln Glu Arg Pro Thr Phe Tyr Arg Gln Glu Leu Asn Lys Thr
1 5 10 15

gtc tgg gag gtg ccc gag cga tac cag aac ctg tcc ccg gtg ggc tcg 96
Val Trp Glu Val Pro Glu Arg Tyr Gln Asn Leu Ser Pro Val Gly Ser
20 25 30

gga gcc tac ggc tcg gtg tgt gct gct ttt gat aca aag acg gga cat 144
Gly Ala Tyr Gly Ser Val Cys Ala Ala Phe Asp Thr Lys Thr Gly His
35 40 45

cgt gtg gca gtg aag aag ctg tcg aga ccg gtt cag ccc atc att cac 192
Arg Val Ala Val Lys Lys Leu Ser Arg Pro Val Gln Pro Ile Ile His
50 55 60

gcc aaa agg tcc tac agg gag ctg cgg ctg ctg aag cac atg aag cac 240
Ala Lys Arg Ser Tyr Arg Glu Leu Arg Leu Leu Lys His Met Lys His
65 70 75 80

-continued

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gag aat gtg att ggt ctg ttg gat gtg ttt aca cct gca agg tcc ctg      288
Glu Asn Val Ile Gly Leu Leu Asp Val Phe Thr Pro Ala Arg Ser Leu
                 85                  90                  95

gag gaa ttc aac gat gtg tac ctg gtg acc cat ctc atg ggg gca gac      336
Glu Glu Phe Asn Asp Val Tyr Leu Val Thr His Leu Met Gly Ala Asp
            100                 105                 110

ctg aac aac atc gtg aag tgt cag aag ctt acc gat gac cac gtt cag      384
Leu Asn Asn Ile Val Lys Cys Gln Lys Leu Thr Asp Asp His Val Gln
        115                 120                 125

ttt ctt atc tac cag atc ctg cga ggg ctg aag tat ata cac tcg gct      432
Phe Leu Ile Tyr Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala
    130                 135                 140

gac ata atc cac agg gac cta aag ccc agc aac ctc gct gtg aat gaa      480
Asp Ile Ile His Arg Asp Leu Lys Pro Ser Asn Leu Ala Val Asn Glu
145                 150                 155                 160

gac tgt gag ctg aag att ctg gat ttt ggg ctg gct cgg cac act gat      528
Asp Cys Glu Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg His Thr Asp
                165                 170                 175

gac gaa atg acc ggc tac gtg gct acc cgg tgg tac aga gcc ccc gag      576
Asp Glu Met Thr Gly Tyr Val Ala Thr Arg Trp Tyr Arg Ala Pro Glu
            180                 185                 190

att atg ctg aat tgg atg cac tac aac cag aca gtg gat att tgg tcc      624
Ile Met Leu Asn Trp Met His Tyr Asn Gln Thr Val Asp Ile Trp Ser
        195                 200                 205

gtg ggc tgc atc atg gct gag ctg ttg acc gga aga acg ttg ttt cct      672
Val Gly Cys Ile Met Ala Glu Leu Leu Thr Gly Arg Thr Leu Phe Pro
    210                 215                 220

ggt aca gac cat att gat cag ttg aag ctc att tta aga ctc gtt gga      720
Gly Thr Asp His Ile Asp Gln Leu Lys Leu Ile Leu Arg Leu Val Gly
225                 230                 235                 240

acc cca ggg gct gag ctt ctg aag aaa atc tcc tca gag tct gca aga      768
Thr Pro Gly Ala Glu Leu Leu Lys Lys Ile Ser Ser Glu Ser Ala Arg
                245                 250                 255

aac tac att cag tct ctg gcc cag atg ccg aag atg aac ttc gca aat      816
Asn Tyr Ile Gln Ser Leu Ala Gln Met Pro Lys Met Asn Phe Ala Asn
            260                 265                 270

gta ttt att ggt gcc aat ccc ctg gct gtc gac ctg ctg gaa aag atg      864
Val Phe Ile Gly Ala Asn Pro Leu Ala Val Asp Leu Leu Glu Lys Met
        275                 280                 285

ctg gtt ttg gac tca gat aag agg atc aca gca gcc caa gct ctt gcg      912
Leu Val Leu Asp Ser Asp Lys Arg Ile Thr Ala Ala Gln Ala Leu Ala
    290                 295                 300

cat gcc tac ttt gct cag tac cac gac cct gat gat gag cca gtg gct      960
His Ala Tyr Phe Ala Gln Tyr His Asp Pro Asp Asp Glu Pro Val Ala
305                 310                 315                 320

gac cct tat gac cag tcc ttt gaa agc agg gac ctc ctt ata gac gaa     1008
Asp Pro Tyr Asp Gln Ser Phe Glu Ser Arg Asp Leu Leu Ile Asp Glu
                325                 330                 335

tgg aag agc ctg acc tac gat gaa gtc att agc ttt gtg cca ccg ccc     1056
Trp Lys Ser Leu Thr Tyr Asp Glu Val Ile Ser Phe Val Pro Pro Pro
            340                 345                 350

ctt gac caa gaa gaa atg gac tcc tga                                1083
Leu Asp Gln Glu Glu Met Asp Ser
        355                 360
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<210> SEQ ID NO 174
<211> LENGTH: 20
<212> TYPE: DNA

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

<400> SEQUENCE: 174

gtgcgcgcga gcccgaaatc 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

ctgcgacatt ttccagcggc 20

<210> SEQ ID NO 176
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 176

catcatcagg gtcgtggtac 20

<210> SEQ ID NO 177
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 177

aggtgctcag gactccattt 20

<210> SEQ ID NO 178
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 178

gtccctgctt tcaaaggact 20

<210> SEQ ID NO 179
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 179

ggccagagac tgaatgtagt 20

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

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<400> SEQUENCE: 180

agctcctgcc ggtagaacgt 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

tcaaaagcag cacacaccga 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

cccgtctttg tatcaaaagc 20

<210> SEQ ID NO 183
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 183

aacggtctcg acagcttctt 20

<210> SEQ ID NO 184
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 184

taggtccttt tggcgtgaat 20

<210> SEQ ID NO 185
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 185

agatgggtca ccaggtacac 20

<210> SEQ ID NO 186
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 186

gcccccatga gatgggtcac 20

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<210> SEQ ID NO 187
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 187

tcatcagtgt gccgagccag 20

<210> SEQ ID NO 188
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 188

gtcaacagct cagccatgat 20

<210> SEQ ID NO 189
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 189

cgttcttccg gtcaacagct 20

<210> SEQ ID NO 190
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 190

atcaatatgg tctgtaccag 20

<210> SEQ ID NO 191
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 191

cttaaaatga gcttcaactg 20

<210> SEQ ID NO 192
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 192

gggttccaac gagtcttaaa 20

<210> SEQ ID NO 193
<211> LENGTH: 20
<212> TYPE: DNA

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

<400> SEQUENCE: 193

tcagaagctc agcccctggg 20

<210> SEQ ID NO 194
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 194

ggagattttc ttcagaagct 20

<210> SEQ ID NO 195
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 195

cagactctga ggagattttc 20

<210> SEQ ID NO 196
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 196

tagtttcttg cagactctga 20

<210> SEQ ID NO 197
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 197

agactgaatg tagtttcttg 20

<210> SEQ ID NO 198
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 198

ttcatcttcg gcatctgggc 20

<210> SEQ ID NO 199
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

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<400> SEQUENCE: 199

atttgcgaag ttcatcttcg 20

<210> SEQ ID NO 200
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 200

caataaatac atttgcgaag 20

<210> SEQ ID NO 201
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 201

ggattggcac caataaatac 20

<210> SEQ ID NO 202
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 202

gctgctgtga tcctcttatc 20

<210> SEQ ID NO 203
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 203

aggcatgcgc aagagcttgg 20

<210> SEQ ID NO 204
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 204

tgagcaaagt aggcatgcgc 20

<210> SEQ ID NO 205
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 205

tcaaaggact ggtcataagg 20

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<210> SEQ ID NO 206
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 206

catttcttct tggtcaaggg 20

<210> SEQ ID NO 207
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 207

aggactccat ttcttcttgg 20

<210> SEQ ID NO 208
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 208

cttcccctca cagtgaagtg 20

<210> SEQ ID NO 209
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 209

tatttggaga gttcccatga 20

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

<400> SEQUENCE: 210

acttgaatgg tatttggaga 20

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

<400> SEQUENCE: 211

aacaagaggc acttgaatgg 20

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

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

<400> SEQUENCE: 212

acccccttcc accatgaagg 20

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

<400> SEQUENCE: 213

agcaggcaga ctgccaagga 20

<210> SEQ ID NO 214
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 214

cacacacatc cctaaggaga 20

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ggattcctac aacatacaaa 20

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aatattaaaa taatttgccc 20

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aggcatgcgc aagagctt 18

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gggacaggtt ctggtatcgc 20

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tctcgtgctt catgtgcttc a 21

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tggagctgga ctgcatactg a 21

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catcagggtc gtggtac 17

<210> SEQ ID NO 265
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catcatcagg gtcgt 15

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agctgatctg gcctacagtt 20

What is claimed is:

**1**. A method for treating airway hyperresponsiveness or pulmonary inflammation in an individual in need thereof, comprising administering to said individual an antisense compound 8 to 30 nucleobases in length targeted to a nucleic acid molecule encoding a human p38α MAP kinase protein to said individual.

**2**. The method of claim 1, wherein said antisense compound is an antisense oligonucleotide.

**3**. The method of claim 2, wherein at least one covalent linkage of said antisense compound is a modified covalent linkage.

**4**. The method of claim 2, wherein at least one nucleotide of said antisense compound has a modified sugar moiety.

**5**. The method of claim 2, wherein at least one nucleotide of said antisense compound has a modified nucleobase.

**6**. The method of claim 1, further comprising administering an anti-asthma medication to said individual.

**7**. The method of claim 1 wherein said antisense compound comprises at least one lipophilic moiety which enhances the cellular uptake of said antisense compound.

**8**. The method of claim 1, wherein said antisense compound is aerosolized and inhaled by said individual.

**9**. The method of claim 1, wherein said antisense compound is administered intranasally, intrapulmonarily or intratracheally.

**10**. The method of claim 1, wherein said airway hyperresponsiveness or pulmonary inflammation is associated with asthma.

**11**. A pharmaceutical composition comprising an antisense oligonucleotide targeted to nucleic acid encoding human p38α MAP kinase in a formulation suitable for intranasal, intrapulmonary or intratracheal administration.

**12**. The pharmaceutical composition of claim 11, wherein said composition is in a metered dose inhaler or nebulizer.

* * * * *