Title: MODULATION OF CEACAM1 EXPRESSION

Abstract: Compounds, compositions and methods are provided for modulating the expression of CEACAM1. The compositions comprise oligonucleotides, targeted to nucleic acid encoding CEACAM1. Methods of using these compounds for modulation of CEACAM1 expression and for diagnosis and treatment of disease associated with expression of CEACAM1 are provided.
MODULATION OF CEACAM1 EXPRESSION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional application Serial No. 60/486,652 filed July 12, 2003, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention provides compositions and methods for modulating the expression of CEACAM1. In particular, this invention relates to compounds, particularly oligonucleotide compounds, which, in some embodiments, hybridize with nucleic acid molecules encoding CEACAM1. Such compounds are shown herein to modulate the expression of CEACAM1.

BACKGROUND OF THE INVENTION

Coronaviruses, a genus in the family Coronaviridae, are large, enveloped RNA viruses that cause highly prevalent diseases in humans and domestic animals. Coronavirus particles are irregularly-shaped, 60-220 nm in diameter, with an outer envelope bearing distinctive, “club-shaped” peplomers (~20nm long x 10nm at wide distal end). This “crown-like” appearance (Latin, corona) gives the family its name. The center of the particle appears amorphous in negatively stained EM preps, the nucleocapsid being in a loosely wound rather disordered state (Cann, 2003; www-micro.msb.le.ac.uk/3035/Coronaviruses.html).

Coronaviruses have the largest genomes of all RNA viruses and replicate by a unique mechanism which results in a high frequency of recombination. Virions mature by budding at intracellular membranes and infection with some coronaviruses induces cell fusion (Fields Virology, D. M. Knipe, P. M. Howley Eds. 2001, Lippincott Williams & Wilkins, Publishers, Philadelphia p. 1163-1179).

Most human coronaviruses (HcoVs) do not grow in cultured cells, therefore relatively little is known about them, but two strains (229E and OC43) grow in some cell lines and have been used as a model. Replication is slow compared to other enveloped viruses, e.g. 24h c.f. 6-8h for influenza. Viral entry occurs via endocytosis and membrane fusion (probably mediated by E2) and replication occurs in the cytoplasm (Cann, 2003). Initially, the 5' 20kb of the (+)sense genome is translated to produce a viral polymerase, which is believed to produce a full-length (-)sense strand which, in turn, is used as a template to produce mRNA as a “nested set” of
transcripts, all with an identical 5' non-translated leader sequence of 72nt and coincident 3' polyadenylated ends. Each mRNA is monocistronic, the genes at the 5' end being translated from the longest mRNA. These unusual cytoplasmic structures are produced not by splicing (post-transcriptional modification) but by the polymerase during transcription. Between each of the genes there is a repeated intergenic sequence - UCUAAAC - which interacts with the transcriptase plus cellular factors to splice the leader sequence onto the start of each ORF. Assembly occurs by budding into the golgi apparatus, particles being transported to the surface of the cell by the secretory nature of this organelle & released (Cann, 2003).

Coronaviruses infect a variety of mammals and birds. The exact number of human isolates are not known as many cannot be grown in culture. In humans, they cause: Respiratory infections (common), including Severe Acute Respiratory Syndrome (SARS), enteric infections (occasional - mostly in infants <12 months) and neurological syndromes (rare) (Cann, 2003).

Coronaviruses are transmitted by aerosols of respiratory secretions, by the fecal-oral route, and by mechanical transmission. Most virus growth occurs in epithelial cells. Occasionally the liver, kidneys, heart or eyes may be infected, as well as other cell types such as macrophages. In cold-type respiratory infections, growth appears to be localized to the epithelium of the upper respiratory tract, but there is currently no adequate animal model for the human respiratory coronaviruses. Clinically, most infections cause a mild, self-limited disease (classical “cold” or upset stomach), but there may be rare neurological complications. SARS is a form of viral pneumonia where infection encompasses the lower respiratory tract (Cann, 2003).

Coronavirus infection is very common and occurs worldwide. The incidence of infection is strongly seasonal, with the greatest incidence in children in winter. Adult infections are less common. The number of coronavirus serotypes and the extent of antigenic variation is unknown. Re-infections appear to occur throughout life, implying multiple serotypes (at least four are known) and/or antigenic variation, hence the prospects for immunization appear bleak (Cann, 2003).

SARS (Severe Acute Respiratory Syndrome) is a newly-recognized type of viral pneumonia, with symptoms including fever, a dry cough, dyspnea (shortness of breath), headache, and hypoxemia (low blood oxygen concentration). Typical laboratory findings include lymphopenia (reduced lymphocyte numbers) and mildly elevated aminotransferase levels (indicating liver damage). Death may result from progressive respiratory failure due to alveolar damage (Cann, 2003).
The outbreak is believed to have originated in February 2003 in the Guangdong province of China. After initial reports that a paramyxovirus was responsible, researchers now believe SARS to causally-linked with a type of novel coronavirus with some unusual properties. For example, the SARS virus can be grown in Vero cells (a primate fibroblast cell line) - a novel property for HCoVs, most of which cannot be cultivated. In these cells, virus infection results in a cytopathic effect, and budding of coronavirus-like particles from the endoplasmic reticulum within infected cells (Cann, 2003).

Amplification of short regions of the polymerase gene, (the most strongly conserved part of the coronavirus genome) by reverse transcriptase polymerase chain reaction (RT-PCR) and nucleotide sequencing revealed that the currently evaluated examples of the SARS virus are of a novel coronavirus which has not previously been present in human populations. This conclusion is confirmed by serological (antigenic) investigations (Cann, 2003).

Different isolates of coronaviruses that have been causally linked to SARS have been independently sequenced by BCCA Genome Sciences Center, Vancouver, Canada (SARS Coronavirus Tor2: GenBank accession numbers: AY274119 and NC_004718, incorporated herein as SEQ ID NOs: 1 and 2), the Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences / Beijing Genomics Institute, Chinese Academy of Sciences, Beijing, China (SARS coronavirus isolates BJ01-BJ04 and GZ01: GenBank accession numbers: AY279354, AY278490, AY278489, AY278488 and AY278487, incorporated herein as SEQ ID NOs: 3-7), the Centers for Disease Control and Prevention (CDC), Atlanta, GA (SARS coronavirus Urbani: GenBank accession number AY278741, incorporated herein as SEQ ID NO:8), the Chinese University of Hong Kong (SARS coronavirus CUHK-W1: GenBank Accession number AY278554, incorporated herein as SEQ ID NO:9) and the University of Hong Kong (SARS coronavirus HKU-39849: GenBank accession number AY269391, incorporated herein as SEQ ID NO:10). Sequences of the RNA-directed RNA polymerase of the SARS coronavirus Taiwan strain are also available (Genbank accession numbers AY268049 and AY269391, incorporated herein as SEQ ID NOs: 11 and 12.). As new SARS-linked coronavirus samples are obtained and sequenced, and as the initial SARS coronaviruses mutate, other coronavirus sequences causally-linked to SARS will emerge.

The SARS coronavirus is believed to be spread by droplets produced by coughing and sneezing, but other routes of infection may also be involved, such as contamination of objects by the hands. The World Health Organization (WHO) currently estimates that SARS is fatal in around 4% of cases, usually where the person has an underlying condition such as diabetes or
heart disease, or a weakened immune system. In 90% of cases, patients recover approximately one week after being infected (Cann, 2003).

In all possible proteins of SARS coronavirus, Spike proteins are essential for receptor binding and membrane fusion. Spike proteins consist of more than 1000 amino acid residues which can be divided into two parts, S1 and S2. The N-terminal S1 is the peripheral fragment responsible for receptor binding, and the C-terminal S2 has a membrane-spanning fragment. During the initial stages of infection, the peripheral S1 on some of these virion projections engages host cell receptors.

The receptor of murine hepatitis virus (MHV) has been identified and it's three demental structure has been determined. However, the receptor of SARS coronavirus remains unclear. In order to assist biologists to explore SARS coronavirus receptor, information of spike proteins of other viruses maybe useful. Potential receptors of Spike protein from SwissProt have been gathered in the database of Spike Protein Receptors (SpikeRD) under the database query system SRS (antisars.cbi.pku.edu.cn:5555/srdb/srdb.jsp).

CEACAM1 (Carcinoembryonic Antigen-Related Cell Adhesion Molecule 1, also known as biliary glycoprotein; BGP, biliary glycoprotein 1; BGP1, and CD66 antigen; CD66) is a Spike protein receptor which is known to act as a coronavirus receptor (Tan et al., EMBO J. 2002, 21, 2076-2086; Tsai et al., J. Virol. 2003, 841-850.)

Modulation of expression of Spike protein receptors such as CEACAM1 may provide a useful strategy with which to treat or prevent coronavirus infections.

In many species, introduction of double-stranded RNA (dsRNA) induces potent and specific gene silencing. This phenomenon occurs in both plants and animals and has roles in viral defense and transposon silencing mechanisms. (Jorgensen et al., Plant Mol. Biol., 1996, 31, 957-973; Napoli et al., Plant Cell, 1990, 2, 279-289).

The first evidence that dsRNA could lead to gene silencing in animals came from work in the nematode, Caenorhabditis elegans, where it has been shown that feeding, soaking or injecting dsRNA (a mixture of both sense and antisense strands) results in much more efficient silencing than injection of either the sense or the antisense strands alone (Guo and Kemphues, Cell, 1995, 81, 611-620; Fire et al., Nature 391:806-811 (1998); Montgomery et al., Proc. Natl. Acad. Sci. USA 95:15502-15507 (1998); PCT International Publication W099/32619; (Fire et al., Nature, 1998, 391, 806-810; Timmons et al., Gene, 2001, 263, 103-112; Timmons and Fire, Nature, 1998, 395, 854). Since, the phenomenon has been demonstrated in a number of organisms, including Drosophila melanogaster (Kennerdell et

This posttranscriptional gene silencing phenomenon has been termed “RNA interference” (RNAi) and has come to generally refer to the process of gene silencing involving dsRNA which leads to the sequence-specific reduction of gene expression via target mRNA degradation (Tuschl et al., Genes Dev., 1999, 13, 3191-3197).

It has been demonstrated that 21- and 22-nt dsRNA fragments having 3’ overhangs are the canonical sequence-specific mediators of RNAi. These fragments, which are termed short interfering RNAs (siRNAs), are generated by an RNase III-like processing reaction from longer dsRNA. Chemically synthesized siRNA also mediate efficient target RNA cleavage with the site of cleavage located near the center of the region spanned by the guiding strand of the siRNA. (Elbashir et al., Nature, 2001, 411, 494-498). Characterization of the suppression of expression of endogenous and heterologous genes caused by the 21-23 nucleotide siRNAs has been investigated in several mammalian cell lines, including human embryonic kidney (293) and HeLa cells (Elbashir et al., Genes and Development, 2001, 15, 188-200).

Recently, it has been shown that single-stranded RNA oligomers (ssRNAi or asRNA) of antisense polarity can be potent inducers of gene silencing and that single-stranded oligomers are ultimately responsible for the RNAi phenomenon (Tijsterman et al., Science, 2002, 295, 694-697).

U.S. patents 5,898,031 and 6,107,094 describe certain oligonucleotides having RNA-like properties. When hybridized with RNA, these oligonucleotides serve as substrates for a dsRNase enzyme with resultant cleavage of the RNA by the enzyme (Crooke, 2000; Crooke, 1999).

Consequently, there remains a long felt need for agents capable of treating or preventing coronavirus infections.

Antisense technology is emerging as an effective means for reducing the expression of specific gene products and may therefore prove to be uniquely useful in a number of therapeutic, diagnostic, and research applications for the modulation of CEACAM1 gene expression.

The present invention provides compositions and methods for modulating CEACAM1 expression.

**SUMMARY OF THE INVENTION**

The present invention is directed to compounds, especially nucleic acid and nucleic acid-like oligomers, and particularly single and double-stranded compounds, which are targeted
to a nucleic acid encoding CEACAM1, and which modulate the expression of CEACAM1. In some embodiments the antisense compounds are oligonucleotides. In some embodiments, the oligonucleotides are RNAi oligonucleotides (which are predominantly RNA or RNA-like). In other embodiments, the oligonucleotides are RNase H oligonucleotides (which are predominantly DNA or DNA-like). In still other embodiments, the oligonucleotides may be chemically modified. Pharmaceutical and other compositions comprising the compounds of the invention are also provided. Further provided are methods of screening for modulators of CEACAM1 and methods of modulating the expression of CEACAM1 in cells, tissues or animals comprising contacting said cells, tissues or animals with one or more of the compounds or compositions of the invention. Methods of treating an animal, particularly a human, suspected of having or being prone to a disease or condition associated with expression of CEACAM1 are also set forth herein. Such methods comprise administering a therapeutically or prophylactically effective amount of one or more of the compounds or compositions of the invention to the person in need of treatment. In another embodiment, the present invention provides for the use of a compound of the invention in the manufacture of a medicament for the treatment of any and all conditions disclosed herein.

DETAILED DESCRIPTION OF EMBODIMENTS

A. Overview of the Invention

The present invention employs double and single-stranded oligomeric antisense compounds, particularly single or double-stranded oligonucleotides which are RNA or RNA-like and single-stranded oligonucleotides which are DNA or DNA-like for use in modulating the function of nucleic acid molecules encoding CEACAM1. This is accomplished by providing oligonucleotides which specifically hybridize with one or more nucleic acid molecules encoding CEACAM1. As used herein, the terms “target nucleic acid” and “nucleic acid molecule encoding CEACAM1” have been used for convenience to encompass DNA encoding CEACAM1, RNA (including pre-mRNA and mRNA or portions thereof) transcribed from such DNA, and also cDNA derived from such RNA. The hybridization of a compound of this invention with its target nucleic acid is generally referred to as “antisense”. Consequently, one mechanism believed to be included in the practice of some embodiments of the invention is referred to herein as “antisense inhibition.” Such antisense inhibition is typically based upon hydrogen bonding-based hybridization of oligonucleotide strands or segments such that at least one strand or segment is cleaved, degraded, or otherwise rendered inoperable. In this regard, it is
presently suitable to target specific nucleic acid molecules and their functions for such antisense inhibition.

The functions of DNA to be interfered with can include replication and transcription. Replication and transcription, for example, can be from an endogenous cellular template, a vector, a plasmid construct or otherwise. The functions of RNA to be interfered with can include functions such as translocation of the RNA to a site of protein translation, translocation of the RNA to sites within the cell which are distant from the site of RNA synthesis, translation of protein from the RNA, splicing of the RNA to yield one or more RNA species, and catalytic activity or complex formation involving the RNA which may be engaged in or facilitated by the RNA. One result of such interference with target nucleic acid function is modulation of the expression of CEACAM1. In the context of the present invention, “modulation” and “modulation of expression” mean either an increase (stimulation) or a decrease (inhibition) in the amount or levels of a nucleic acid molecule encoding the gene, e.g., DNA or RNA. Inhibition is often the desired form of modulation of expression and mRNA is often a suitable target nucleic acid.

In the context of this invention, “hybridization” means the pairing of complementary strands of oligomeric compounds. In the present invention, one mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleoside or nucleotide bases (nucleobases) of the strands of oligomeric compounds. For example, adenine and thymine are complementary nucleobases which pair through the formation of hydrogen bonds. Hybridization can occur under varying circumstances.

An antisense compound is specifically hybridizable when binding of the compound to the target nucleic acid interferes with the normal function of the target nucleic acid to cause a loss of activity, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target nucleic acid 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 under conditions in which assays are performed in the case of in vitro assays.

In the present invention the phrase “stringent hybridization conditions” or “stringent conditions” refers to conditions under which a compound of the invention will hybridize to its target sequence, but to a minimal number of other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances and in the context of this invention, “stringent conditions” under which oligomeric compounds hybridize to a target sequence are
determined by the nature and composition of the oligomeric compounds and the assays in which they are being investigated.

“Complementary,” as used herein, refers to the capacity for precise pairing between two nucleobases of an oligomeric compound. For example, if a nucleobase at a certain position of an oligonucleotide (an oligomeric compound), is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, said target nucleic acid being a DNA, RNA, or oligonucleotide molecule, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be a complementary position. The oligonucleotide and the further DNA, RNA, or oligonucleotide molecule are complementary to each other when a sufficient number of complementary positions in each molecule are occupied by nucleobases which can hydrogen bond with each other. Thus, “specifically hybridizable” and “complementary” are terms which are used to indicate a sufficient degree of precise pairing or complementarity over a sufficient number of nucleobases such that stable and specific binding occurs between the oligonucleotide and a target nucleic acid.

It is understood in the art that the sequence of an antisense compound need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. An antisense compound is specifically hybridizable when binding of the compound to the target DNA or RNA molecule interferes with the normal function of the target DNA or RNA to cause a complete or partial loss of function, and there is a sufficient degree of complementarity to avoid non-specific binding of the antisense compound to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of therapeutic treatment, or under conditions in which in vitro or in vivo assays are performed. Moreover, an oligonucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure). The compounds of the present invention comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% sequence complementarity to a target region within the target nucleic acid sequence to which they are targeted. For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having 4 (four) noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall
complementarity with the target nucleic acid and would thus fall within the scope of the present invention.

Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul et al., *J. Mol. Biol.*, 1990, 215, 403-410; Zhang and Madden, * Genome Res.*, 1997, 7, 649-656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison WI), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489). In some embodiments, homology, sequence identity or complementarity, between the antisense compound and target is between about 50% to about 60%. In some embodiments, homology, sequence identity or complementarity, is between about 60% to about 70%. In some embodiments, homology, sequence identity or complementarity, is between about 70% and about 80%. In some embodiments, homology, sequence identity or complementarity, is between about 80% and about 90%. In some embodiments, homology, sequence identity or complementarity, is about 90%, about 92%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%.

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

Multiple mechanisms exist by which short synthetic oligonucleotides can be used to modulate gene expression in mammalian cells. A commonly exploited antisense mechanism is RNase H-dependent degradation of the targeted RNA. RNase H is a ubiquitously expressed endonuclease that recognizes antisense DNA-RNA heteroduplex, hydrolyzing the RNA strand. A further antisense mechanism involves the utilization of enzymes that catalyze the cleavage of RNA-RNA duplexes. These reactions are catalyzed by a class of RNAse enzymes including but not limited to RNAse III and RNAse L. The antisense mechanism known RNA interference (RNAi) is operative on RNA-RNA hybrids and the like.

Both RNase H-based antisense (usually using single-stranded compounds) and RNA interference (usually using double-stranded compounds known as siRNAs) are antisense mechanisms, typically resulting in loss of target RNA function.
Optimized siRNA and RNase H-dependent oligomeric compounds behave similarly in terms of potency, maximal effects, specificity and duration of action, and efficiency. Moreover it has been shown that in general, activity of dsRNA constructs correlated with the activity of RNase H-dependent single-stranded antisense compounds targeted to the same site. One major exception is that RNase H-dependent antisense compounds were generally active against target sites in pre-mRNA whereas siRNAs were not.

These data suggest that, in general, sites on the target RNA that were not active with RNase H-dependent oligonucleotides were similarly not good sites for siRNA. Conversely, a significant degree of correlation between active RNase H oligonucleotides and siRNA was found, suggesting that if a site is available for hybridization to an RNase H oligonucleotide, then it is also available for hybridization and cleavage by the siRNA complex. Consequently, once suitable target sites have been determined by either antisense approach, these sites can be used to design constructs that operate by the alternative antisense mechanism (Vickers et al., 2003, J. Biol. Chem. 278, 7108). Moreover, once a site has been demonstrated as active for either an RNAi or an RNAse H oligonucleotide, a single-stranded RNAi oligonucleotide (ssRNAi or asRNA) can be designed.

In some embodiments of the present invention, double-stranded antisense oligonucleotides are suitable. These double-stranded antisense oligonucleotides may be RNA or RNA-like, and may be modified or unmodified, in that the oligonucleotide, if modified, retains the properties of forming an RNA:RNA hybrid and recruitment and (activation) of a dsRNase. In other embodiments, the single-stranded oligonucleotides (ssRNAi or asRNA) may be RNA-like.

In other embodiments of the present invention, single-stranded antisense oligonucleotides are suitable. In some embodiments, the single-stranded oligonucleotides may be "DNA-like", in that the oligonucleotide has well characterized structural features, for example a plurality of unmodified 2’ Hs or a stabilized backbone such as e.g., phosphorothioate, that is structurally suited for interaction with a target oligonucleotide and recruitment and (activation) of RNase H.

B. Compounds of the Invention

In the context of the present invention, the term "oligomeric compound" refers to a polymeric structure capable of hybridizing to a region of a nucleic acid molecule. This term includes oligonucleotides, oligonucleosides, oligonucleotide analogs, oligonucleotide mimetics and chimeric combinations of these. Oligomeric compounds are routinely prepared linearly but can be joined or otherwise prepared to be circular and may also include branching. Oligomeric
compounds can include double-stranded constructs such as, for example, two strands hybridized to form double-stranded compounds or a single strand with sufficient self complementarity to allow for hybridization and formation of a fully or partially double-stranded compound. In one embodiment of the invention, double-stranded antisense compounds encompass short interfering RNAs (siRNAs). As used herein, the term "siRNA" is defined as a double-stranded compound having a first and second strand and comprises a central complementary portion between said first and second strands and terminal portions that are optionally complementary between said first and second strands or with the target mRNA. Each strand may be from about 8 to about 80 nucleobases in length, 10 to 50 nucleobases in length, 12 or 13 to 30 nucleobases in length, 12 or 13 to 24 nucleobases in length or 19 to 23 nucleobases in length. The central complementary portion may be from about 8 to about 80 nucleobases in length, 10 to 50 nucleobases in length, 12 or 13 to 30 nucleobases in length, 12 or 13 to 24 nucleobases in length or 19 to 23 nucleobases in length. The terminal portions can be from 1 to 6 nucleobases in length. The siRNAs may also have no terminal portions. The two strands of an siRNA can be linked internally leaving free 3' or 5' termini or can be linked to form a continuous hairpin structure or loop. The hairpin structure may contain an overhang on either the 5' or 3' terminus producing an extension of single-stranded character.

In one embodiment of the invention, double-stranded antisense compounds are canonical siRNAs. As used herein, the term "canonical siRNA" is defined as a double-stranded oligomeric compound having a first strand and a second strand each strand being 21 nucleobases in length with the strands being complementary over 19 nucleobases and having on each 3' termini of each strand a deoxy thymidine dimer (dTdT) which in the double-stranded compound acts as a 3' overhang.

In another embodiment, the double-stranded antisense compounds are blunt-ended siRNAs. As used herein the term "blunt-ended siRNA" is defined as an siRNA having no terminal overhangs. That is, at least one end of the double-stranded compound is blunt. siRNAs whether canonical or blunt act to elicit dsRNAse enzymes and trigger the recruitment or activation of the RNAi antisense mechanism. In a further embodiment, single-stranded RNAi (ssRNAi) compounds that act via the RNAi antisense mechanism are contemplated.

Further modifications can be made to the double-stranded compounds and may include conjugate groups attached to one of the termini, selected nucleobase positions, sugar positions or to one of the internucleoside linkages. Alternatively, the two strands can be linked via a non-nucleic acid moiety or linker group. When formed from only one strand, dsRNA can take the form of a self-complementary hairpin-type molecule that doubles back on itself to form a duplex.
Thus, the dsRNAs can be fully or partially double-stranded. When formed from two strands, or a single strand that takes the form of a self-complementary hairpin-type molecule doubled back on itself to form a duplex, the two strands (or duplex-forming regions of a single strand) are complementary RNA strands that base pair in Watson-Crick fashion.

In general an oligomeric compound comprises a backbone of monomeric subunits joined linking groups where each linked monomeric subunit is directly or indirectly attached to a heterocyclic base moiety. Oligomeric compounds may also include monomeric subunits that are not linked to a heterocyclic base moiety thereby providing abasic sites. Any one of the repeated units making up an oligomeric compound can be modified giving rise to a variety of motifs including hemimers, gapmers and chimeras.

As is known in the art, a nucleoside comprises a sugar moiety attached to a heterocyclic base moiety. The two most common classes of such heterocyclic bases are purines and 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 giving the more common 3', 5'-internucleoside linkage or the not so common 2', 5'-internucleoside linkage. In forming oligonucleotides, the phosphate groups covalently link the sugar moieties of adjacent nucleosides. The respective ends can be joined to form a circular structure by hybridization or by formation of a covalent bond. 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 form the backbone of the oligonucleotide. The normal internucleoside linkage that comprises the backbone of RNA and DNA is a 3' to 5' phosphodiester linkage. However, open linear structures are generally desired.

In the context of this invention, the term “oligonucleotide” refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside linkages, as well as oligonucleotide analogs or chemically modified oligonucleotides that have one or more non-naturally occurring portions which function in a similar manner. Such modified or substituted oligonucleotides are suitable over the naturally occurring forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for a nucleic acid target and enhanced nuclease stability.
In the context of this invention, the term “oligonucleoside” refers to a sequence of nucleosides that are joined by internucleoside linkages that do not have phosphorus atoms. Internucleoside linkages of this type include short chain alkyl, cycloalkyl, mixed heteroatom alkyl, mixed heteroatom cycloalkyl, one or more short chain heteroatomic linkers and one or more short chain heterocyclic linkers. These internucleoside linkages include but are not limited to siloxane, sulfide, sulfoxide, sulfone, acetyl, formacetyl, thioformacetyl, methylene formacetyl, thioformacetyl, alken, sulfamate; methyleneimino, methylenehydrazino, sulfonate, sulfonamide, amide and others having mixed N, O, S and CH$_2$ component parts.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S.: 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.

Further included in the present invention are antisense oligomeric compounds including antisense oligonucleotides, external guide sequence (EGS) oligonucleotides, alternate splicers, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid. As such, these antisense oligomeric compounds may be introduced in the form of single-stranded, double-stranded, circular or hairpin oligomeric compounds and may contain structural elements such as internal or terminal bulges, mismatches or loops. In general, nucleic acids (including oligonucleotides) may be described as “DNA-like” (i.e., having 2'-deoxy sugars and, generally, T rather than U bases) or “RNA-like” (i.e., having 2'-hydroxyl or 2'-modified sugars and, generally U rather than T bases). Once introduced to a system, the oligomeric compounds of the invention may elicit the action of one or more enzymes or structural proteins to effect modification of the target nucleic acid.

The oligomeric compounds in accordance with this invention can comprise from about 8 to about 80 nucleobases (i.e. from about 8 to about 80 linked nucleobases and/or monomeric subunits). One of ordinary skill in the art will appreciate that the invention embodies oligomeric compounds of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 nucleobases in length.

In one embodiment, the oligomeric compounds of the invention are 10 to 50 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30,
31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleobases in length.

In another embodiment, the oligomeric compounds of the invention are 12 or 13 to 30 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length.

In a further embodiment, the oligomeric compounds of the invention are 12 or 13 to 24 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 nucleobases in length.

In another embodiment, the oligomeric compounds of the invention are 19 to 23 nucleobases in length. One having ordinary skill in the art will appreciate that this embodies oligomeric compounds of 19, 20, 21, 22 or 23 nucleobases in length.

15 C. Targets of the Invention

"Targeting" an antisense compound to a particular nucleic acid molecule, in the context of this invention, can be a multistep process. The process usually begins with the identification of a target nucleic acid whose function is to be modulated. This target nucleic acid may be, for example, a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state, or a nucleic acid molecule from an infectious agent. In the present invention, the target nucleic acid encodes CEACAM1.

The targeting process usually also includes determination of at least one target region, segment, or site within the target nucleic acid for the antisense interaction to occur such that the desired effect, e.g., modulation of expression, will result. Within the context of the present invention, the term "region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic. Within regions of target nucleic acids are segments. "Segments" are defined as smaller or sub-portions of regions within a target nucleic acid. "Sites," as used in the present invention, are defined as positions within a target nucleic acid.

Since, as is known in the art, the translation initiation codon is typically 5'-AUG (in transcribed mRNA molecules; 5'-ATG in the corresponding DNA molecule), the translation initiation codon is also referred to as the "AUG codon," the "start codon" or the "AUG start codon". A minority of genes have a translation initiation codon having the RNA sequence 5'-GUG, 5'-UUG or 5'-CUG, and 5'-AUA, 5'-ACG and 5'-CUG have been shown to function in
vivo. Thus, the terms “translation initiation codon” and “start codon” can encompass many codon sequences, even though the initiator amino acid in each instance is typically methionine (in eukaryotes) or formylmethionine (in prokaryotes). It is also known in the art that eukaryotic and prokaryotic genes may have two or more alternative start codons, any one of which may be preferentially utilized for translation initiation in a particular cell type or tissue, or under a particular set of conditions. In the context of the invention, “start codon” and “translation initiation codon” refer to the codon or codons that are used in vivo to initiate translation of an mRNA transcribed from a gene encoding CEACAM1, 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 3'-TAA, 3'-TAG and 3'-TGA, respectively).

The terms “start codon region” and “translation initiation codon region” refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation initiation codon. Similarly, the terms “stop codon region” and “translation termination codon region” refer to a portion of such an mRNA or gene that encompasses from about 25 to about 50 contiguous nucleotides in either direction (i.e., 5' or 3') from a translation termination codon. Consequently, the “start codon region” (or “translation initiation codon region”) and the “stop codon region” (or “translation termination codon region”) are all regions which may be targeted effectively with the antisense compounds of the present invention.

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. Within the context of the present invention, a suitable region is the intragenic region encompassing the translation initiation or termination codon of the open reading frame (ORF) of a gene.

Other target regions include the 5' untranslated region (5'UTR), known in the art to refer to the portion of an mRNA in the 5' direction from the translation initiation codon, and thus including nucleotides between the 5' cap site and the translation initiation codon of an mRNA (or corresponding nucleotides on the gene), and the 3' untranslated region (3'UTR), known in the art to refer to the portion of an mRNA in the 3' direction from the translation termination codon, and thus including nucleotides between the translation termination codon and 3' end of an mRNA (or corresponding nucleotides on the gene). The 5' cap site of an mRNA comprises an N7-methylated guanosine residue joined to the 5'-most residue of the mRNA via a 5'-5' triphosphate
linkage. The 5' cap region of an mRNA is considered to include the 5' cap structure itself as well as the first 50 nucleotides adjacent to the cap site. It is also suitable to target the 5' cap region.

Although some eukaryotic mRNA transcripts are directly translated, many contain one or more regions, known as "introns," which are excised from a transcript before it is translated. The remaining (and therefore translated) regions are known as "exons" and are spliced together to form a continuous mRNA sequence. Targeting splice sites, i.e., intron-exon junctions or exon-intron junctions, may also be particularly useful in situations where aberrant splicing is implicated in disease, or where an overproduction of a particular splice product is implicated in disease. Aberrant fusion junctions due to rearrangements or deletions are also suitable target sites. mRNA transcripts produced via the process of splicing of two (or more) mRNAs from different gene sources are known as "fusion transcripts". It is also known that introns can be effectively targeted using antisense compounds targeted to, for example, DNA or pre-mRNA.

It is also known in the art that alternative RNA transcripts can be produced from the same genomic region of DNA. These alternative transcripts are generally known as "variants". More specifically, "pre-mRNA variants" are transcripts produced from the same genomic DNA that differ from other transcripts produced from the same genomic DNA in either their start or stop position and contain both intronic and exonic sequence.

Upon excision of one or more exon or intron regions, or portions thereof during splicing, pre-mRNA variants produce smaller "mRNA variants". Consequently, mRNA variants are processed pre-mRNA variants and each unique pre-mRNA variant must always produce a unique mRNA variant as a result of splicing. These mRNA variants are also known as "alternative splice variants". If no splicing of the pre-mRNA variant occurs then the pre-mRNA variant is identical to the mRNA variant.

It is also known in the art that variants can be produced through the use of alternative signals to start or stop transcription and that pre-mRNAs and mRNAs can possess more that one start codon or stop codon. Variants that originate from a pre-mRNA or mRNA that use alternative start codons are known as "alternative start variants" of that pre-mRNA or mRNA. Those transcripts that use an alternative stop codon are known as "alternative stop variants" of that pre-mRNA or mRNA. One specific type of alternative stop variant is the "polyA variant" in which the multiple transcripts produced result from the alternative selection of one of the "polyA stop signals" by the transcription machinery, thereby producing transcripts that terminate at unique polyA sites. Within the context of the invention, the types of variants described herein are also suitable target nucleic acids.
The locations on the target nucleic acid to which the compounds hybridize are hereinbelow referred to as "suitable target segments." As used herein the term "suitable target segment" is defined as at least an 8-nucleobase portion of a target region to which an active antisense compound is targeted. While not wishing to be bound by theory, it is presently believed that these target segments represent portions of the target nucleic acid which are accessible for hybridization.

While the specific sequences of certain suitable target segments are set forth herein, one of skill in the art will recognize that these serve to illustrate and describe particular embodiments within the scope of the present invention. Additional suitable target segments may be identified by one having ordinary skill.

Target segments 8-80 nucleobases in length comprising a stretch of at least eight (8) consecutive nucleobases selected from within the illustrative suitable target segments are considered to be suitable for targeting as well.

Target segments can include DNA or RNA sequences that comprise at least the 8 consecutive nucleobases from the 5'-terminus of one of the illustrative suitable target segments (the remaining nucleobases being a consecutive stretch of the same DNA or RNA beginning immediately upstream of the 5'-terminus of the target segment and continuing until the DNA or RNA contains about 8 to about 80 nucleobases). Similarly suitable target segments are represented by DNA or RNA sequences that comprise at least the 8 consecutive nucleobases from the 3'-terminus of one of the illustrative suitable target segments (the remaining nucleobases being a consecutive stretch of the same DNA or RNA beginning immediately downstream of the 3'-terminus of the target segment and continuing until the DNA or RNA contains about 8 to about 80 nucleobases). One having skill in the art armed with the suitable target segments illustrated herein will be able, without undue experimentation, to identify further suitable target segments.

Once one or more target regions, segments or sites have been identified, antisense compounds are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect.

The oligomeric compounds are also targeted to or not targeted to regions of the target nucleobase sequence (e.g., such as those disclosed in Example 13) comprising nucleobases 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 601-650, 651-700, 701-750, 751-800, 801-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-

D. Screening and Target Validation

In a further embodiment, the “suitable target segments” identified herein may be employed in a screen for additional compounds that modulate the expression of CEACAM1. “Modulators” are those compounds that decrease or increase the expression of a nucleic acid molecule encoding CEACAM1 and which comprise at least an 8-nucleobase portion which is complementary to a suitable target segment. The screening method comprises the steps of contacting a suitable target segment of a nucleic acid molecule encoding CEACAM1 with one or more candidate modulators, and selecting for one or more candidate modulators which decrease or increase the expression of a nucleic acid molecule encoding CEACAM1. Once it is shown that the candidate modulator or modulators are capable of modulating (e.g. either decreasing or increasing) the expression of a nucleic acid molecule encoding CEACAM1, the modulator may then be employed in further investigative studies of the function of CEACAM1, or for use as a research, diagnostic, or therapeutic agent in accordance with the present invention.

The suitable target segments of the present invention may be also be combined with their respective complementary antisense compounds of the present invention to form stabilized double-stranded (duplexed) oligonucleotides.

Such double stranded oligonucleotide moieties have been shown in the art to modulate target expression and regulate translation as well as RNA processsing via an antisense mechanism. Moreover, the double-stranded moieties may be subject to chemical modifications (Fire et al., Nature, 1998, 391, 806-811; Timmons and Fire, Nature 1998, 395, 854; Timmons et al., Gene, 2001, 263, 103-112; Tabara et al., Science, 1998, 282, 430-431; Montgomery et al., Proc. Natl. Acad. Sci. USA, 1998, 95, 15502-15507; Tuschl et al., Genes Dev., 1999, 13, 3191-3197; Elbashir et al., Nature, 2001, 411, 494-498; Elbashir et al., Genes Dev. 2001, 15, 188-200). For example, such double-stranded moieties have been shown to inhibit the target by the classical hybridization of antisense strand of the duplex to the target, thereby triggering enzymatic degradation of the target (Tijsterman et al., Science, 2002, 295, 694-697).

The compounds of the present invention can also be applied in the areas of drug discovery and target validation. The present invention comprehends the use of the compounds
and suitable target segments identified herein in drug discovery efforts to elucidate relationships that exist between CEACAM1 and a disease state, phenotype, or condition. These methods include detecting or modulating CEACAM1 comprising contacting a sample, tissue, cell, or organism with the compounds of the present invention, measuring the nucleic acid or protein level of CEACAM1 and/or a related phenotypic or chemical endpoint at some time after treatment, and optionally comparing the measured value to a non-treated sample or sample treated with a further compound of the invention. These methods can also be performed in parallel or in combination with other experiments to determine the function of unknown genes for the process of target validation or to determine the validity of a particular gene product as a target for treatment or prevention of a particular disease, condition, or phenotype.

E. Kits, Research Reagents, Diagnostics, and Therapeutics

The compounds of the present invention can be utilized for diagnostics, therapeutics, prophylaxis and as research reagents and kits. Furthermore, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used by those of ordinary skill to elucidate the function of particular genes or to distinguish between functions of various members of a biological pathway.

For use in kits and diagnostics, the compounds of the present invention, either alone or in combination with other compounds or therapeutics, can be used as tools in differential and/or combinatorial analyses to elucidate expression patterns of a portion or the entire complement of genes expressed within cells and tissues.

As one nonlimiting example, expression patterns within cells or tissues treated with one or more antisense compounds are compared to control cells or tissues not treated with antisense compounds and the patterns produced are analyzed for differential levels of gene expression as they pertain, for example, to disease association, signaling pathway, cellular localization, expression level, size, structure or function of the genes examined. These analyses can be performed on stimulated or unstimulated cells and in the presence or absence of other compounds which affect expression patterns.


The compounds of the invention are useful for research and diagnostics, because these compounds hybridize to nucleic acids encoding CEACAM1. For example, oligonucleotides that are shown to hybridize with such efficiency and under such conditions as disclosed herein as to be effective CEACAM1 inhibitors will also be effective primers or probes under conditions favoring gene amplification or detection, respectively. These primers and probes are useful in methods requiring the specific detection of nucleic acid molecules encoding CEACAM1 and in the amplification of said nucleic acid molecules for detection or for use in further studies of CEACAM1. Hybridization of the antisense oligonucleotides, particularly the primers and probes, of the invention with a nucleic acid encoding CEACAM1 can be detected by means known in the art. Such means may include conjugation of an enzyme to the oligonucleotide, radiolabelling of the oligonucleotide or any other suitable detection means. Kits using such detection means for detecting the level of CEACAM1 in a sample may also be prepared.

The specificity and sensitivity of antisense is also harnessed by those of skill in the art for therapeutic uses. Antisense compounds have been employed as therapeutic moieties in the treatment of disease states in animals, including humans. Antisense oligonucleotide drugs, including ribozymes, have been safely and effectively administered to humans and numerous clinical trials are presently underway. It is thus established that antisense compounds can be useful therapeutic modalities that can be configured to be useful in treatment regimes for the treatment of cells, tissues and animals, especially humans.

For therapeutics, an animal, such as a human, suspected of having a disease or disorder which can be treated by modulating the expression of CEACAM1 is treated by administering antisense compounds in accordance with this invention. For example, in one non-limiting embodiment, the methods comprise the step of administering to the animal in need of treatment, a therapeutically effective amount of a CEACAM1 inhibitor. The CEACAM1 inhibitors of the present invention effectively inhibit the activity of the CEACAM1 protein or inhibit the
expression of the CEACAM1 protein. In some embodiments, the activity or expression of CEACAM1 in an animal or cell is inhibited by at least 10%, by at least 20%, by at least 25%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, by at least 98%, by at least 99%, or by 100%.

For example, the reduction of the expression of CEACAM1 may be measured in serum, adipose tissue, liver or any other body fluid, tissue or organ of the animal. The cells contained within said fluids, tissues or organs being analyzed can contain a nucleic acid molecule encoding CEACAM1 protein and/or the CEACAM1 protein itself.

The compounds of the invention can be utilized in pharmaceutical compositions by adding an effective amount of a compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the compounds and methods of the invention may also be useful prophylactically.

F. Modifications

Chimeric oligomeric compounds

It is not necessary for all positions in a 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 containing 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.

Chimeric oligomeric compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, alteration of charge, 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. Similar observations are made for chimeras that form RNA:RNA hybrids and are substrates for dsRNases.

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. Routinely used chimeric compounds include but are not limited to hybrids, hemimers, gapmers, inverted gapmers and blockmers wherein the various point modifications and or regions are selected from native or modified DNA and RNA type units and or mimetic type subunits such as for example locked nucleic acids (LNA) (which encompasses ENA™ as described below), peptide nucleic acids (PNA), morpholinos, and others. These are described below. Representative U.S. patents that teach the preparation of such hybrid structures include, but are not limited to, U.S.: 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.

Oligomer Mimetics

Another 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 the furanose ring or 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). PNAs have favorable hybridization properties, high biological stability and are electrostatically neutral molecules. In one recent study PNAs were used to correct aberrant splicing in a transgenic mouse model (Sazani et al., Nat. Biotechnol., 2002, 20, 1228-1233). In PNA oligomeric compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminomethylglycine backbone. The nucleobases are bound directly or indirectly (-C(=O)-CH₂- as shown below) to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA oligomeric compounds include, but are not limited to, U.S.: 5,539,082; 5,714,331; and 5,719,262. PNAs can be obtained commercially from Applied Biosystems (Foster City, CA, USA).

Numerous modifications have been made to the basic PNA backbone since it was introduced in 1991 by Nielsen and coworkers (Nielsen et al., Science, 1991, 254, 1497-1500). The basic structure is shown below:
wherein

Bx is a heterocyclic base moiety;

T₄ is hydrogen, an amino protecting group, -C(O)R₅, substituted or unsubstituted C₁₋C₁₀ alkyl, substituted or unsubstituted C₂₋C₁₀ alkenyl, substituted or unsubstituted C₂₋C₁₀ alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group, 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;

T₅ is -OH, -N(Z₁)Z₂, R₅, 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;

Z₁ is hydrogen, C₁₋C₆ alkyl, or an amino protecting group;

Z₂ is hydrogen, C₁₋C₆ alkyl, an amino protecting group, -C(=O)-(CH₂)ₚ-J-Z₃, 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;

Z₃ is hydrogen, an amino protecting group, -C₁₋C₆ alkyl, -C(=O)-CH₃, benzyl, benzoyl, or -(CH₂)ₚ-N(H)Z₁;

each J is O, S or NH;
R₅ is a carbonyl protecting group; and

n is from 7 to about 79.

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. One 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 have been studied in zebrafish embryos (see: Genesis, volume 30, issue 3, 2001 and Heasman, J., Dev. Biol., 2002, 243, 209-214). Further studies of morpholino-based oligomeric compounds have also been reported (see: Nasevicius et al., Nat. Genet., 2000, 26, 216-220; and Lacerra et al., Proc. Natl. Acad. Sci., 2000, 97, 9591-9596). Morpholino-based oligomeric compounds are disclosed in U.S. Patent 5,034,506, issued July 23, 1991. The morpholino class of oligomeric compounds have been prepared having a variety of different linking groups joining the monomeric subunits.

Morpholino nucleic acids have been prepared having a variety of different linking groups (L₂) joining the monomeric subunits. The basic formula is shown below:

\[
\begin{align*}
&\text{T}_1 \quad \text{O} \quad \text{Bx} \\
&\quad \text{N} \quad \text{L}_2 \quad \text{O} \\
&\quad \text{Bx} \\
&\quad \text{T}_5
\end{align*}
\]

wherein:

\[
\begin{align*}
\text{T}_1 & \text{ is hydrogen, hydroxyl, a protected hydroxyl, a linked nucleoside or a linked oligomeric compound;} \\
\text{T}_5 & \text{ is hydrogen or a phosphate, phosphate derivative, a linked nucleoside or a linked oligomeric compound;} \\
\text{L}_2 & \text{ is a linking group which can be varied from chiral to achiral from charged to neutral (US Patent 5,166,315 discloses linkages including -O-P(=O)(N(CH₃)₂)-O-; US Patent 5,034,506 discloses achiral intermorpholino linkages such as for example: -S(=O)-X- where X is NH, NCH₃, O, S, or CH₂; -C(=Y)-O- where Y is O or S; -S(=O)(OH)-CH₂-; -S(=O)(OH)-N(R)-CH₂- where R is H or CH₃; and US Patent 5,185,444 discloses phosphorus containing chiral intermorpholino linkages such as for example: -P(=O)(-X)-O- where X is F, CH₂R, S-CH₂R or NR₁R₂ and each R, R₁ and R₂ is H, CH₃ or some other moiety that doesn't interfere with the base specific hydrogen bonding; and} \\
n & \text{is from 7 to about 79.}
\end{align*}
\]
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 cyclohexenyl 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 dichroism 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.

The general formula of CeNA is shown below:

![CeNA structure]

wherein

- each Bx is a heterocyclic base moiety;
- $L_3$ is an inter cyclohexenyl linkage such as for example a phosphodiester or a phosphorothioate linkage;
- $T_1$ is hydrogen, hydroxyl, a protected hydroxyl, a linked nucleoside or a linked oligomeric compound; and
- $T_2$ is hydrogen or a phosphate, phosphate derivative, a linked nucleoside or a linked oligomeric compound.

Another class of oligonucleotide mimetic (anhydrohexitol nucleic acid) can be prepared from one or more anhydrohexitol nucleosides (see, Wouters and Herdwijn, *Bioorg. Med. Chem. Lett.*, 1999, 9, 1563-1566) and would have the general formula:
each Bx is a heterocyclic base moiety;

L is an inter anhydrohexitol linkage such as for example a phosphodiester or a phosphorothioate linkage;

T₁ is hydrogen, hydroxyl, a protected hydroxyl, a linked nucleoside or a linked oligomeric compound; and

T₂ is hydrogen or a phosphate, phosphate derivative, a linked nucleoside or a linked oligomeric compound.

A further modification includes bicyclic sugar moieties such as “Locked Nucleic Acids” (LNAs) in which the 2'-hydroxyl group of the ribosyl sugar ring is linked to the 4' carbon atom of the sugar ring thereby forming a 2'-C,4'-C-oxymethylene linkage to form the bicyclic sugar moiety (reviewed in Elayadi et al., Curr. Opinion Invens. Drugs, 2001, 2, 558-561; Braasch et al., Chem. Biol., 2001, 8 1-7; and Orum et al., Curr. Opinion Mol. Ther., 2001, 3, 239-243; see also U.S. Patents: 6,268,490 and 6,670,461). The linkage can be a (-CH₂-)ₓ group bridging the 2' oxygen atom and the 4' carbon atom, wherein if x = 1 the term LNA is used, if x = 2 the term ENA™ is used (Singh et al., Chem. Commun., 1998, 4, 455-456; ENA™: Morita et al., Bioorganic Medicinal Chemistry, 2003, 11, 2211-2226). Thus, “ENA™™” is one non limiting example of an LNA. LNA and other bicyclic sugar 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. LNAs are commercially available from ProLigo (Paris, France and Boulder, CO, USA). The basic structure of an LNA having a single CH₂ linkage in the bicyclic ring system is shown below. This is merely illustrative of one type of LNA.
wherein each T₁ and T₂ is, independently, hydrogen, a hydroxyl protecting group, a linked nucleoside or a linked oligomeric compound, and each Z₁ is an internucleoside linking group such as for example phosphodiester or phosphorothioate.

An isomer of LNA that has also been studied is alpha-L-LNA which has been shown to have superior stability against a 3'-exonuclease (Frieden et al., Nucleic Acids Research, 2003, 21, 6365-6372). The alpha-L-LNA's were incorporated into antisense gapmers and chimeras that showed potent antisense activity. The structure of alpha-L-LNA is shown below:

Another similar bicyclic sugar moiety that has been prepared and studied has the bridge going from the 3'-hydroxyl group via a single methylene group to the 4' carbon atom of the sugar ring thereby forming a 3'-C,4'-C-oxyxymethylene linkage (see U.S. Patent 6,043,060).

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).

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 three (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.

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. DN/LNA chimeras have been shown to efficiently inhibit gene expression when targeted to a variety of regions (5'-untranslated region, region of the start codon or coding region) within the luciferase mRNA (Braasch et al., Nucleic Acids Research, 2002, 30, 5160-5167).

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 compounds. 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. Further successful in vivo studies involving LNA's have shown knock-down of the rat delta opioid receptor without toxicity (Wahlestedt et al., Proc. Natl. Acad. Sci., 2000, 97, 5633-5638) and in another study showed a blockage of the translation of the large subunit of RNA polymerase II (Fluiter et al., Nucleic Acids Res., 2003, 31, 953-962).

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.
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 WO03/020739; and WO99/14226). 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.

Another oligonucleotide mimetic amenable to the present invention that has been prepared and studied is threose nucleic acid. This oligonucleotide mimetic is based on threose nucleosides instead of ribose nucleosides and has the general structure shown below:

Initial interest in (3',2')-alpha-L-threose nucleic acid (TNA) was directed to the question of whether a DNA polymerase existed that would copy the TNA. It was found that certain DNA polymerases are able to copy limited stretches of a TNA template (reported in C&EN/January 13, 2003).

In another study it was determined that TNA is capable of antiparallel Watson-Crick base pairing with complementary DNA, RNA and TNA oligonucleotides (Chaput et al., J. Am. Chem. Soc., 2003, 125, 856-857).

In one study (3',2')-alpha-L-threose nucleic acid was prepared and compared to the 2' and 3' amidate analogs (Wu et al., Organic Letters, 2002, 4(8), 1279-1282). The amidate analogs were shown to bind to RNA and DNA with comparable strength to that of RNA/DNA.

Further oligonucleotide mimetics have been prepared to incude bicyclic and tricyclic nucleoside analogs having the formulas (amidite monomers shown):
(see Steffens et al., Helv. Chim. Acta, 1997, 80, 2426-2439; Steffens et al., J. Am. Chem. Soc., 1999, 121, 3249-3255; Renneberg et al., J. Am. Chem. Soc., 2002, 124, 5993-6002; and Renneberg et al., Nucleic acids res., 2002, 30, 2751-2757). 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.

Another class of oligonucleotide mimetic is referred to as phosphononomonoester nucleic acids which incorporate a phosphorus group in the backbone. This class of oligonucleotide 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.

The general formula (for definitions of Markush variables see: U.S. Patents 5,874,553 and 6,127,346) is shown below.

Further oligonucleotide mimetics amenable to the present invention have been prepared wherein a cyclobutyl ring replaces the naturally occurring furanosyl ring.

**Modified Internucleoside Linkages**

Specific examples of 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.

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 phosphoramide and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, phosphonoacetate and thiophosphonoacetate (see Sheehan et al., Nucleic Acids Research, 2003, 31(14), 4109-4118 and Dellinger et al., J. Am. Chem. Soc., 2003, 125, 940-950), selenophosphates and boranophosphates 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. One phosphorus containing modified internucleoside linkage is the phosphorothioate internucleoside linkage, which is linked in a 3'-5' linkage. 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.

N3'-P5'-phosphoramidates have been reported to exhibit both a high affinity towards a complementary RNA strand and nuclease resistance (Gryaznov et al., J. Am. Chem. Soc., 1994, 116, 3143-3144). N3'-P5'-phosphoramidates have been studied with some success in vivo to specifically down regulate the expression of the c-myc gene (Skorski et al., Proc. Natl. Acad. Sci., 1997, 94, 3966-3971; and Faïra et al., Nat. Biotechnol., 2001, 19, 40-44).

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S.: 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.

In some embodiments of the invention, oligomeric compounds have one or more phosphorothioate and/or heteroatom internucleoside linkages, in particular -CH₂-NH-O-CH₂-, -CH₂-N(CH₃)₂-O-CH₂- (known as a methylene (methylimino) or MMI backbone), -CH₂-O-N(CH₃)₂-CH₂-, -CH₂-N(CH₃)₂-N(CH₃)₂-CH₂- and -O-N(CH₃)₂-CH₂-CH₂- (wherein the native
phosphodiester internucleotide linkage is represented as \(-\text{O-P(=O)(OH)-O-CH}_2\)-). The MMI type internucleoside linkages are disclosed in the above referenced U.S. patent 5,489,677. Amide internucleoside linkages are disclosed in the above referenced U.S. patent 5,602,240.

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.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S.: 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.

20

**Modified sugars**

Oligomeric compounds of the invention may also contain one or more substituted or other wise modified sugar moieties. Ribosyl and related sugar moieties are routinely modified at any reactive position not involved in linking. Thus a suitable position for a sugar substituent group is the 2'-position not usually used in the native 3' to 5'-internucleoside linkage. Other suitable positions are the 3' and the 5'-termini. 3'-sugar positions are open to modification when the linkage between two adjacent sugar units is a 2', 5'-linkage. Sugar substituent groups include: 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 suitable are O(((CH\(_2\))\(_n\))O(CH\(_2\))\(_n\))CH\(_3\), O(CH\(_2\))\(_n\)OCH\(_3\), O(CH\(_2\))\(_n\)NH\(_2\), O(CH\(_2\))\(_n\)CH\(_3\), O(CH\(_2\))\(_n\)ONH\(_2\), and O(CH\(_2\))\(_n\)ON(((CH\(_2\))\(_n\))C\(_\text{H}_3\))\(_2\), where n and m are from 1 to about 10. Other suitable 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\(_2\)CH\(_3\), NO\(_2\), NO, 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.

One modification includes 2'-methoxyethoxy (2'-O-CH₂CH₂OCH₃, 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. Further modifications includes 2'-dimethylaminoethoxyethoxy, i.e., a O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, as described in examples hereinbelow, 2'-dimethylaminooxyethoxy (also known in the art as 2'-O-dimethyl-amino-ethoxy-ethyl or 2'-DMAEOE), i.e., 2'-O-(CH₂)₂O-(CH₂)₂N(CH₃)₂, and N-methylacetamide (also referred to as NMA, 2'-O-CH₂-C(=O)-N(H)CH₃.)

Other sugar substituent groups include methoxy (-O-CH₃), aminopropyloxy (-OCH₂CH₂CH₂NH₂), allyl (-CH₂-CH=CH₂), -O-allyl (-O-CH₂-CH=CH₂) and fluoro (F). 2'-Sugar substituent groups may be in the arabinos (up) position or ribo (down) position. One 2'-arabinose modification is 2'-F (see: Loc et al., Biochemistry, 2002, 41, 3457-3467). 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 U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S.: 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; 5,700,920; and 6,147,200.

Further representative sugar substituent groups include groups of formula Iₐ or IIₐ:

\[
-Iₐ \left[ \begin{array}{c}
\text{Rb} \\
\text{(CH₂)}_{na} \text{O} \left( \text{Rk} \right)_{nb} \text{(CH₂)}_{md} \text{Rd} \text{Re}
\end{array} \right]_{me}
\]

\[
-IIₐ \left[ \begin{array}{c}
\text{Rb} \\
\text{Rf} \\
\text{Rh} \\
\text{Rg} \\
\text{Rj} \text{me}
\end{array} \right]
\]

wherein:

Rb is O, S or NH;
Rd is a single bond, O, S or C(=O);
Re is C₁-C₁₀ alkyl, N(Rₐ)(Rₐm), N(Rₐ)(Rₐn), N=C(Rₐp)(Rₐq), N=C(Rₐp)(Rₐ) or has formula IIIₐ;
R_P and R_Q are each independently hydrogen or C_1-C_10 alkyl;
R_i is -R_x-R_y;

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;

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

each R_w is, independently, substituted or unsubstituted C_1-C_10 alkyl, trifluoromethyl, cyanoethoxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or aryl;

R_x is hydrogen, a nitrogen protecting group or -R_x-R_y;

R_a is a bond or a linking moiety;

R_y is a chemical functional group, a conjugate group or a solid support medium;

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_2^+, N(R_u)(R_v), guanidino and acyl where said acyl is an acid amide or an ester;

or R_k and R_m, 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;

R_i is OR_x, SR_x, or N(R_x)_2;

each R_x 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;

R_s, R_t and R_u 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;
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;

ma is 1 to about 10;

5 each mb is, independently, 0 or 1;
mc is 0 or an integer from 1 to 10;
md is an integer from 1 to 10;
me is from 0, 1 or 2; and

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

Representative substituents groups of Formula I are disclosed in U.S. Patent No. 6,172,209, entitled “Capped 2'-Oxyethoxy Oligonucleotides.”

Representative cyclic substituent groups of Formula II are disclosed in U.S. Patent No. 6,271,358, entitled “RNA Targeted 2'-Oligomeric compounds that are Conformationally Preorganized.”

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.

Representative guanidino substituent groups that are shown in formula III are disclosed in U.S. Patent No. 6,593,466, entitled “Functionalized Oligomers”, filed July 7, 1999.

Representative acetamido substituent groups are disclosed in U.S. Patent 6,147,200.

Representative dimethylaminoethoxyethyl substituent groups are disclosed in International Publication No. WO00/08044895, entitled “2'-O-Dimethylaminoethoxyethyl-Oligomeric compounds.”

The oligomeric compounds of the invention may also comprise two or more of the same, or chemically distinct, sugar, base, and internucleoside linkage modifications in any combination. The term “chemically distinct” as used herein means different chemical entities whether entirely or partially distinct. For example, an oligomeric compound may comprise a 2'-fluoro and 2'-MOE modification. These two modifications are considered to be chemically distinct entities located within the same molecule.

30 **Modified Nucleobases/Naturally occurring nucleobases**

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-CH₃) 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-hydroxymethyl 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-aza- and 8-azaadenine, 7-deazaguanine and 7-deaza-adenine and 3-deazaguanine and 3-deazaadenine.

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-amino- pyridine and 2-pyridone. Further nucleobases include those disclosed in U.S. Patent No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J.L., 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 suitable base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Oligomeric compounds of the present invention can also include polycyclic heterocyclic compounds in place of one or more heterocyclic base moieties. A number of tricyclic heterocyclic comounds 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:
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, Serial number 10/155,920; and U.S. Patent Application entitled “Nuclease Resistant Chimeric Oligonucleotides” filed May 24, 2002, Serial number 10/013,295).

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 ΔT_m of up to 18° relative to 5-methyl cytosine (dC5me), 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 dC5me. 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.

Further tricyclic heterocyclic compounds and methods of using them that are amenable to the present invention are disclosed in U.S. Patent 6,028,183 and U.S. Patent 6,007,992.
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.

Further modified polycyclic heterocyclic compounds useful as heterocyclic bases are disclosed in but not limited to, the above noted U.S. 3,687,808, as well as U.S.: 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 Unites States Patent Application Serial number 09/996,292 filed November 28, 2001.

**Conjugates**

Oligomeric compounds used in the compositions of the present invention can also be modified to have one or more moieties or conjugates for enhancing 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 cholesterol, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes such as including Cy3 and Alexa. 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 October 23, 1992.


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 U.S. Patent Application 09/334,130 (filed June 15, 1999).

Representative U.S. patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S.: 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.
Oligomeric compounds used in the compositions of the present invention can also be modified to have one or more stabilizing groups that are generally attached to one or both termini of single-stranded oligomeric compounds or to one or more of the 3' or 5' termini of either strand of a double-stranded compound to enhance properties such as for example nuclease stability. Included in stabilizing groups are cap structures. By “cap structure or terminal cap moiety” is meant chemical modifications, which have been incorporated at either terminus of oligonucleotides (see for example Wincott et al., WO 97/26270). These terminal modifications protect the oligomeric compounds having terminal nucleic acid molecules from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap) or at the 3'-terminus (3'-cap) or can be present on both termini. This cap structure is not to be confused with the inverted methylguanosine “5’cap” present at the 5’ end of native mRNA molecules. In non-limiting examples, the 5'-cap includes inverted abasic residue (moiety), 4',5'-methylene nucleotide; 1-(beta-D-erythrosefuranosyl) nucleotide, 4'-thio nucleotide, carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide; 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butane diol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety (for more details see Wincott et al., International PCT publication No. WO 97/26270).

3'-cap structures of the present invention include, for example 4',5'-methylene nucleotide; 1-(beta-D-erythrosefuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-aminoalkyl phosphate; 1,3-diamino-2-propyl phosphate, 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butane diol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Tyer, 1993, Tetrahedron 49, 1925).
Further 3' and 5'-stabilizing groups that can be used to cap one or both ends of an oligomeric compound to impart nuclease stability include those disclosed in WO 03/004602 published on January 16, 2003.

3'-Endo Modifications

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, NY.; Lesnik et al., Biochemistry, 1995, 34, 10807-10815; Conte et al., Nucleic Acids Res., 1997, 25, 2627-2634). The increased stability of RNA has been attributed to several structural features, most notably the improved 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, NY). 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.

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 mechanisms including RNAs e H, RNAi or any mechanisms that require the binding of a oligomeric compound to an RNA target strand. In the case of antisense, effective inhibition of the mRNA
requires that the antisense compound have a sufficiently high binding affinity with the mRNA. Otherwise the desired interaction between the oligomeric antisense compound and target mRNA strand will occur infrequently, resulting in decreased efficacy.

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.

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.

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 the RNA interference machinery which is supported in part by the fact that duplexes composed of 2'-deoxy-2'-F-nucleosides appear efficient in triggering an 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 compounds that can act as triggers of the RNAi pathway having one or more nucleosides modified in such a way as to favor a C3'-endo type conformation.

Conformation Scheme

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

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 (Gallo et al., Tetrahedron (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. Chem. (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 Letters (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 compounds which trigger an 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.)

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 oligonucleotides of the present invention. The synthesis of numerous of the 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.)

In one aspect, the present invention is directed to oligomeric compounds that are prepared having enhanced properties, compared to native RNA, against nucleic acid targets. In designing enhanced oligomeric compounds, a target is identified and an oligomeric compound 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. One modification would be the replacement of one or more RNA nucleosides with nucleosides that have the same 3'-endo conformational geometry but, in addition, an enhancing property. 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 oligonucleotide. The selected oligomeric compound 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 may include at least one 5'-modified phosphate group on a single strand or on at least one 5'-position phosphate 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 affinity of the selected sequence for its intended target.

One synthetic 2'-modification that imparts increased nuclease resistance and a very high binding affinity to nucleotides is the 2-methoxyethoxy (2'-MOE, 2'-OCH₂CH₂OCH₃) 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. Oligomers 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 oligomers having the 2'-MOE modification displayed improved RNA affinity and higher nuclease resistance. Chimeric oligomers having 2'-MOE substituents in the wing nucleosides and an internal region of deoxy-phosphorothioate nucleotides (also termed a gapped oligomer or gapmer) have shown effective reduction in the growth of tumors in animal models at low doses. 2'-MOE substituted oligomers have also shown outstanding promise as antisense compounds in several disease states. One such MOE substituted oligomer is approved for the treatment of CMV retinitis.

Most of the 2'-MOE substituents display a gauche conformation around the C-C bond of the ethyl linker. However, in two cases, a trans conformation around the C-C bond is observed. The lattice interactions in the crystal include packing of duplexes against each other via their minor grooves. Therefore, for some residues, the conformation of the 2'-O-substituent is affected by contacts to an adjacent duplex. In general, variations in the conformation of the substituents (e.g. g⁺ or g⁻ around the C-C bonds) create a range of interactions between substituents, both inter-strand, across the minor groove, and intra-strand. At one location, atoms of substituents from two residues are in van der Waals contact across the minor groove. Similarly, a close contact occurs between atoms of substituents from two adjacent intra-strand residues.

Previously determined crystal structures of A-DNA duplexes were for those that incorporated isolated 2'-O-methyl T residues. In the crystal structure noted above for the 2'-MOE substituents, a conserved hydration pattern has been observed for the 2'-MOE residues. A single water molecule is seen located between O2', O3' and the methoxy oxygen atom of the substituent, forming contacts to all three of between 2.9 and 3.4 Å. In addition, oxygen atoms of substituents are involved in several other hydrogen bonding contacts. For example, the methoxy
oxygen atom of a particular 2'-O-substituent forms a hydrogen bond to N3 of an adenosine from the opposite strand via a bridging water molecule.

In several cases a water molecule is trapped between the oxygen atoms O2', O3' and OC' of modified nucleosides. 2'-MOE substituents with trans conformation around the C-C bond of the ethylene glycol linker are associated with close contacts between OC' and N2 of a guanosine from the opposite strand, and, water-mediated, between OC' and N3(G). When combined with the available thermodynamic data for duplexes containing 2'-MOE modified strands, this crystal structure allows for further detailed structure-stability analysis of other modifications.

In extending the crystallographic structure studies, molecular modeling experiments were performed to study further enhanced binding affinity of oligonucleotides having 2'-O-modifications. The computer simulations were conducted on compounds of SEQ ID NO: 10, above, having 2'-O-modifications located at each of the nucleosides of the oligonucleotide. The simulations were performed with the oligonucleotide in aqueous solution using the AMBER force field method (Cornell et al., J. Am. Chem. Soc., 1995, 117, 5179-5197)(modeling software package from UCSF, San Francisco, CA). The calculations were performed on an Indigo2 SGI machine (Silicon Graphics, Mountain View, CA).

Another 2'-sugar substituent group that gives a 3'-endo sugar conformational geometry is the 2'-OMe group. 2'-Substitution of guanosine, cytidine, and uridine dinucleoside phosphates with the 2'-OMe group showed enhanced stacking effects with respect to the corresponding native (2'-OH) species leading to the conclusion that the sugar is adopting a C3'-endo conformation. In this case, it is believed that the hydrophobic attractive forces of the methyl group tend to overcome the destabilizing effects of its steric bulk.

The ability of oligonucleotides to bind to their complementary target strands is compared by determining the melting temperature ($T_m$) of the hybridization complex of the oligonucleotide and its complementary strand. The melting temperature ($T_m$), a characteristic physical property of double helices, denotes the temperature (in degrees centigrade) at which 50% helical (hybridized) versus coil (unhybridized) forms are present. $T_m$ is measured by using the UV spectrum to determine the formation and breakdown (melting) of the hybridization complex. Base stacking, which occurs during hybridization, is accompanied by a reduction in UV absorption (hypochromicity). Consequently, a reduction in UV absorption indicates a higher $T_m$. The higher the $T_m$, the greater the strength of the bonds between the strands.

Freier and Altmann, Nucleic Acids Research, (1997) 25:4429-4443, have previously published a study on the influence of structural modifications of oligonucleotides on the stability
of their duplexes with target RNA. In this study, the authors reviewed a series of oligonucleotides containing more than 200 different modifications that had been synthesized and assessed for their hybridization affinity and Tm. Sugar modifications studied included substitutions on the 2'-position of the sugar, 3'-substitution, replacement of the 4'-oxygen, the use of bicyclic sugars, and four member ring replacements. Several nucleobase modifications were also studied including substitutions at the 5-, or 6 position of thymine, modifications of pyrimidine heterocycle and modifications of the purine heterocycle. Modified internucleoside linkages were also studied including neutral, phosphorus and non-phosphorus containing internucleoside linkages.

Increasing the percentage of C3'-endo sugars in a modified oligonucleotide targeted to an RNA target strand should preorganize this strand for binding to RNA. Of the several sugar modifications that have been reported and studied in the literature, the incorporation of electronegative substituents such as 2'-fluoro or 2'-alkoxy shift the sugar conformation towards the 3' endo (northern) pucker conformation. This preorganizes an oligonucleotide that incorporates such modifications to have an A-form conformational geometry. This A-form conformation results in increased binding affinity of the oligonucleotide to a target RNA strand.

In addition, for 2'-substituents containing an ethylene glycol motif, a gauche interaction between the oxygen atoms around the O-C-C-O torsion of the side chain may have a stabilizing effect on the duplex (Freier ibid.). Such gauche interactions have been observed experimentally for a number of years (Wolfe et al., Acc. Chem. Res., 1972, 5, 102; Abe et al., J. Am. Chem. Soc., 1976, 98, 468). This gauche effect may result in a configuration of the side chain that is favorable for duplex formation. The exact nature of this stabilizing configuration has not yet been explained. While we do not want to be bound by theory, it may be that holding the O-C-C-O torsion in a single gauche configuration, rather than a more random distribution seen in an alkyl side chain, provides an entropic advantage for duplex formation.

Representative 2'-substituent groups amenable to the present invention that give A-form conformational properties (3'-endo) to the resultant duplexes include 2'-O-alkyl, 2'-O-substituted alkyl and 2'-fluoro substituent groups. Suitable for the substituent groups are various alkyl and aryl ethers and thioethers, amines and monoalkyl and dialkyl substituted amines. It is further intended that multiple modifications can be made to one or more of the oligomeric compounds of the invention at multiple sites of one or more monomeric subunits (nucleosides are suitable) and or internucleoside linkages to enhance properties such as but not limited to activity in a selected application
Ring structures of the invention for inclusion as a 2'-O modification include cyclohexyl, cyclopentyl and phenyl rings as well as heterocyclic rings having spacial footprints similar to cyclohexyl, cyclopentyl and phenyl rings. 2'-O-substituent groups of the invention included but are not limited to 2'-O-(trans 2-methoxy cyclohexyl), 2'-O-(trans 2-methoxy cyclopentyl), 2'-O-(trans 2-ureido cyclohexyl) and 2'-O-(trans 2-methoxyphenyl).

Chemistries

Unless otherwise defined herein, alkyl means C_{1-12}, C_{1-8}, or C_{1-6}, straight or (where possible) branched chain aliphatic hydrocarbyl.

Unless otherwise defined herein, heteroalkyl means C_{1-12}, C_{1-8}, or C_{1-6}, straight or (where possible) branched chain aliphatic hydrocarbyl containing at least one, or about 1 to about 3, hetero atoms in the chain, including the terminal portion of the chain. Heteroatoms include N, O and S.

Unless otherwise defined herein, cycloalkyl means C_{3-12}, C_{3-8}, or C_{3-6}, aliphatic
hydrocarbyl ring.

Unless otherwise defined herein, alkenyl means C_{2-12}, C_{2-8}, or C_{2-6} alkenyl, which may be straight or (where possible) branched hydrocarbyl moiety, which contains at least one carbon-carbon double bond.

Unless otherwise defined herein, alkynyl means C_{2-12}, C_{2-8}, or C_{2-6} alkynyl, which may be straight or (where possible) branched hydrocarbyl moiety, which contains at least one carbon-carbon triple bond.

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. The number of carbon atoms can vary from 1 to about 12, from 1 to about 6, and the total number of ring members can vary from three to about 15, or from about 3 to about 8. Ring heteroatoms are N, O and S. Heterocycloalkyl groups include morpholino, thiomorpholino, piperidinyl, piperazinyl, homopiperidinyl, homopiperazinyl, homomorpholino, homothiomorpholino, pyrroldinyl, tetrahydroxazolyl, tetrahydroimidazolyl, tetrahydrothiazolyl, tetrahydrooxazolyl, tetrahydropyrazolyl, furanyl, pyranyl, and tetrahydroisothiazolyl.

Unless otherwise defined herein, aryl means any hydrocarbon ring structure containing at least one aryl ring. Aryl rings have about 6 to about 20 ring carbons. Aryl rings also include phenyl, napthyl, anthracenyl, and phenanthrenyl.
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. The ring system can contain about 1 to about 4 rings. The number of carbon atoms can vary from 1 to about 12, or from 1 to about 6, and the total number of ring members can vary from three to about 15, or from about 3 to about 8. Ring heteroatoms are N, O and S. Hetaryl moieties include pyrazolyl, thiophenyl, pyridyl, imidazolyl, tetrazolyl, pyridyl, pyrimidinyl, purinyl, quinazolinyl, quinoxalinyl, benzimidazolyl, benzothiophenyl, etc.

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 sub-moieties is as defined herein.

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, iso-thiocyanato, nitro or halo groups.

Unless otherwise defined herein, the terms halogen and halo have their ordinary meanings. Halo (halogen) substituents are F, Cl, Br, and I.

The aforementioned optional substituents are, unless otherwise herein defined, suitable substituents depending upon desired properties. Included are halogens (F, Cl, Br, I), alkyl, alkenyl, and alkynyl moieties, NO₂, NH₃ (substituted and unsubstituted), acid moieties (e.g. –CO₂H, -OSO₂H₂, etc.), heterocycloalkyl moieties, hetaryl moieties, aryl moieties, etc.


Oligomer Synthesis


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

The oligomeric compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative U.S. patents that teach the preparation of such uptake, distribution and/or absorption assisting formulations include, but are not limited to, U.S. Patents 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756.

*Salts, prodrugs and bioequivalents:*

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

The term "prodrug" indicates a therapeutic agent that is prepared in an inactive or less active 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 December 9, 1993 or in WO 94/26764 to Imbach et al.

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

Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are
sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N'-dibenzylethylenediamine, chloropropane, choline, diethanolamine, dicyclohexylamine, ethylendiamine, N-methylglucamine, and procaine (see, for example, Berge et al., "Pharmaceutical Salts," *J. of Pharma Sci.*, 1977, 66, 1-19). The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention. As used herein, a "pharmaceutical addition salt" includes a pharmaceutically acceptable salt of an acid form of one of the components of the compositions of the invention. These include organic or inorganic acid salts of the amines. Acid salts are the hydrochlorides, acetates, salicylates, nitrates and phosphates. Other suitable pharmaceutically acceptable salts are well known to those skilled in the art and include basic salts of a variety of inorganic and organic acids, such as, for example, with inorganic acids, such as for example hydrochloric acid, hydrobromic acid, sulfuric acid or phosphoric acid; with organic carboxylic, sulfonic, sulfo or phospho acids or N-substituted sulfamic acids, for example acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, fumaric acid, malic acid, tartaric acid, lactic acid, oxalic acid, gluconic acid, glucaric acid, glucuronic acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, salicylic acid, 4-aminosalicylic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, embonic acid, nicotinic acid or isonicotinic acid; and with amino acids, such as the 20 alpha-amino acids involved in the synthesis of proteins in nature, for example glutamic acid or aspartic acid, and also with phenylacetic acid, methanesulfonic acid, ethanesulfonic acid, hydroxyethanesulfonic acid, ethyl-1,2-disulfonic acid, benzenesulfonic acid, 4-methylbenzenesulfonic acid, naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic acid, 2- or 3-phosphoglycerate, glucose-6-phosphate, N-cyclohexylsulfamic acid (with the formation of cyclamates), or with other acid organic compounds, such as ascorbic acid. Pharmaceutically acceptable salts of compounds may also be prepared with a pharmaceutically acceptable cation. Suitable pharmaceutically acceptable cations are well known to those skilled in the art and include alkaline, alkaline earth, ammonium and quaternary ammonium cations. Carbonates or hydrogen carbonates are also possible.

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. In one embodiment, double-stranded oligomeric compounds are provided as sodium salts.

As used herein, the term “patient” refers to a mammal that is afflicted with one or more disorders associated with CEACAM1 expression or overexpression. It will be understood that the most suitable patient is a human. It is also understood that this invention relates specifically to the inhibition of mammalian CEACAM1 expression or overexpression.

It is recognized that one skilled in the art may affect the disorders associated with CEACAM1 expression or overexpression by treating a patient presently afflicted with the disorders with an effective amount of the compound of the present invention. Thus, the terms “treatment” and “treating” are intended to refer to all processes wherein there may be a slowing, interrupting, arresting, controlling, or stopping of the progression of the disorders described herein, but does not necessarily indicate a total elimination of all symptoms.

As used herein, the term “effective amount” or “therapeutically effective amount” of a compound of the present invention refers to an amount that is effective in treating or preventing the disorders described herein.

The oligomeric compounds of the present invention can be utilized for diagnostics, therapeutics, prophylaxis and as research reagents and kits. For therapeutics, a patient, such as a human, suspected of having a disease or disorder which can be treated by modulating the expression of CEACAM1 is treated by administering antisense compounds in accordance with this invention. The compounds of the invention can be utilized in pharmaceutical compositions by adding an effective amount of an antisense compound to a suitable pharmaceutically acceptable diluent or carrier. Use of the antisense compounds and methods of the invention may also be useful prophylactically, e.g., to prevent or delay infection, inflammation or tumor formation, for example.

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

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

Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Compositions for oral administration also include pulsatile delivery compositions and bioadhesive composition as described in copending U.S. Patent Application Serial Nos. 09/944,493, filed August 22, 2001, and 09/935,316, filed August 22, 2001. Oral administration for treatment of the disorders is described herein. However, oral administration is not the only route. For example, the intravenous route may be desirable as a matter of convenience or to avoid potential complications related to oral administration. When a compound of the present invention is administered through the intravenous route, an intravenous bolus or slow infusion may be desired.

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

Pharmaceutical compositions and/or formulations comprising the oligomeric compounds of the present invention may also 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, 1-33). One or more penetration enhancers from one or more of these broad categories may be included.
Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid (C12), capric acid (C10), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, recinoleate, monoolein (a.k.a. 1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, mono- and di-glycerides and physiologically acceptable salts thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, 8:2, 91-192; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7:1, 1-33; El-Hariri et al., *J. Pharm. Pharmacol.*, 1992, 44, 651-654). Examples of some fatty acids are sodium caprate and sodium laurate, used singly or in combination at concentrations of 0.5 to 5%.

Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus, the term "bile salt" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. Examples of bile salts are chenodeoxycholic acid (CDCA) and/or ursodeoxycholic acid (UDCA), generally used at concentrations of 0.5 to 2%.

Complex formulations comprising one or more penetration enhancers may be used. For example, bile salts may be used in combination with fatty acids to make complex formulations. Suitable combinations include CDCA combined with sodium caprate or sodium laurate (generally 0.5 to 5%).

Chelating agents include, but are not limited to, disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanillic), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines)(Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, 8:2, 92-192; Muranishi, *Critical Reviews in Therapeutic Drug Carrier Systems*, 1990, 7:1, 1-33; Buur et al., *J. Control Rel.*, 1990, 14, 43-51). Chelating agents have the added advantage of also serving as DNase inhibitors.

Surfactants include, for example, sodium lauryl sulfate, poloxymethylene-9-lauryl ether and poloxymethylene-20-cetyl ether (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, 8:2, 92-191); and perfluorochemical emulsions, such as FC-43 (Takahashi et al., *J. Pharm. Pharmacol.*, 1988, 40, 252-257).

Non-surfactants include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., *Critical Reviews in Therapeutic Drug Carrier Systems*, 1991, 8:2, 92-191); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., *J. Pharm. Pharmacol.*, 1987, 39, 621-626).
A "pharmaceutically acceptable carrier" (excipient) is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The pharmaceutically acceptable carrier may be liquid or solid and is selected with the planned manner of administration in mind so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutically acceptable carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrates (e.g., starch, sodium starch glycolate, etc.); or wetting agents (e.g., sodium lauryl sulphate, etc.). Sustained release oral delivery systems and/or enteric coatings for orally administered dosage forms are described in U.S. Patents 4,704,295; 4,556,552; 4,309,406; and 4,309,404.

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.

Regardless of the method by which the oligomeric compounds 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 compounds and/or to target the compounds 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. One colloidal dispersion system is a plurality of liposomes. Liposomes are microscopic spheres having an aqueous core surrounded by one or more outer layer(s) made up of lipids arranged in a bilayer configuration (see, generally, Chonn et al., Current Op. Biotech., 1995, 6, 698-708).
Certain embodiments of the invention provide for liposomes and other compositions one or more other chemotherapeutic agents which function by a non-antisense mechanism. Examples of such chemotherapeutic agents include but are not limited to daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexynitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FdU), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin, carboplatin and diethylstilbestrol (DES). See, generally, The Merck Manual of Diagnosis and Therapy, 15th Ed. 1987, pp. 1206-1228, Berkow et al., eds., Rahway, N.J. 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).

Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribavirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. See, generally, The Merck Manual of Diagnosis and Therapy, 15th Ed., Berkow et al., eds., 1987, Rahway, N.J., pages 2499-2506 and 46-49, respectively). Other non-antisense chemotherapeutic agents are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

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 EC50s 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, from 0.1 μg to 10 g per kg of body weight, from 1 μg to 1 g per kg of body weight, from 10 μg to 100 mg per kg of body weight, from 100 μg to 10 mg per kg of body weight, or from 100 μg to 1 mg 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, weekly, monthly, or yearly. For double-stranded compounds, the dose must be calculated to account for the increased nucleic acid load of the second strand (as with compounds comprising two separate strands) or the additional nucleic acid length (as with self complementary single strands having double-stranded regions).

Double-stranded compounds can be introduced into cells in a number of different ways. For example, the double-stranded compounds can be administered by microinjection; bombardment by microparticles covered by the double-stranded compounds; soaking the cells in a solution of the double-stranded compounds; electroporation of cells in the presence of the double-stranded compounds; liposome-mediated delivery of double-stranded compounds; transfection mediated by chemicals such as calcium phosphate, cationic lipids, etc.; viral infection; transformation; and the like. The double-stranded compounds can be introduced along with components that enhance RNA uptake by the cells, stabilize the annealed strands, or otherwise increase the inhibition of function of the target polynucleotide sequence. In the case of a cell culture or tissue expoxant, the cells are conveniently incubated in a solution containing the double-stranded compounds, or subjected to lipid-mediated transformation.

Determination of the optimal amounts of double-stranded compounds to be administered to human or animal patients for the prevention or treatment of pathologies associated with CEACAM1 expression or overexpression, as well as methods of administering therapeutic or pharmaceutical compositions comprising such double-stranded oligonucleotides, is within the skill of those in the pharmaceutical art. Dosing of a human or animal patient is dependent on the nature of the symptom, condition, or disease; the nature of the infected cell or tissue; the patient’s condition; body weight; general health; sex; diet; time, duration, and route of administration; rates of absorption, distribution, metabolism, and excretion of the double-stranded compounds; combination with other drugs; severity of the pathology; and the responsiveness of the disease state being treated. The amount of double-stranded compounds
administered also depends on the nature of the target polynucleotide sequence or region thereof, and the nature of the double-stranded compounds, and can readily be optimized to obtain the desired level of effectiveness. The course of treatment can last from several days to several weeks or several months, or until a cure is effected or an acceptable diminution or prevention of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient in conjunction with the effectiveness of the treatment. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies, and repetition rates.

In order that the invention disclosed herein may be more efficiently understood, examples are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the invention in any manner. Throughout these examples, molecular cloning reactions, and other standard recombinant DNA techniques, were carried out according to methods described in Maniatis et al., Molecular Cloning - A Laboratory Manual, 2nd ed., Cold Spring Harbor Press (1989), using commercially available reagents, except where otherwise noted.

EXAMPLES

Example 1: Nucleoside phosphoramidites for oligonucleotide synthesis
deoxy and 2'-alkoxy amidites

2'-Deoxy and 2'-methoxy beta-cyanoethyl-diisopropyl phosphoramidites were purchased from commercial sources (e.g. Chemgenes, Needham, MA or Glen Research, Inc. Sterling, VA). Other 2'-O-alkoxy substituted nucleoside amidites are prepared as described in U.S. Patent 5,506,351. For oligonucleotides synthesized using 2'-alkoxy amidites, the standard cycle for unmodified oligonucleotides was utilized, except the wait step after pulse delivery of tetrazole and base was increased to 360 seconds.

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

2'-Fluoro amidites

2'-Fluorodeoxyadenosine amidites

2'-fluoro oligonucleotides were synthesized as described previously (Kawasaki, et. al., *J. Med. Chem.*, 1993, 36, 831-841) and U. S. Patent 5,670,633. Briefly, the protected nucleoside N6-benzoyl-2'-deoxy-2'-fluoroadenosine was synthesized utilizing commercially available 9-beta-D-
arabinofuranosyladenine as starting material and by modifying literature procedures whereby the 2'-alpha-fluoro atom was introduced by a $S_N2$-displacement of a 2'-beta-trityl group. Thus N6-benzoyl-9-beta-D-arabinofuranosyladenine was selectively protected in moderate yield as the 3',5'-diteatrahydropyranyl (THP) intermediate. Deprotection of the THP and N6-benzoyl groups was accomplished using standard methodologies and standard methods were used to obtain the 5'-dimethoxytrityl-(DMT) and 5'-DMT-3'-phosphoramidite intermediates.

2'-Fluorodeoxyguanosine

The synthesis of 2'-deoxy-2'-fluoroguanosine was accomplished using tetraisopropylidisiloxanyl (TPDS) protected 9-beta-D-arabinofuranosylguanine as starting material, and conversion to the intermediate diisobutyrylarabinofuranosylguanosine. Deprotection of the TPDS group was followed by protection of the hydroxyl group with THP to give diisobutyryl di-THP protected arabinofuranosylguanine.

Selective O-deacylation and triflation was followed by treatment of the crude product with fluoride, then deprotection of the THP groups. Standard methodologies were used to obtain the 5'-DMT- and 5'-DMT-3'-phosphoramidites.

2'-Fluorouridine

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

2'-Fluorodeoxycytidine

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

2'-O-(2-Methoxyethyl) modified amidites

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

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

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

2'-O-Methoxyethyl-5-methyluridine

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 CH₂CN (600 mL) and evaporated. A silica gel column (3 kg) was packed in CH₂Cl₂/Acetone/MeOH (20:5:3) containing 0.5% Et₃NH. The residue was dissolved in CH₂Cl₂ (250 mL) and adsorbed onto silica (150 g) prior to loading onto the column. The product was eluted with the packing solvent to give 160 g (63%) of product. Additional material was obtained by reworking impure fractions.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine

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 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 CH₂CN (200 mL). The residue was dissolved in CHCl₃ (1.5 L) and extracted with 2x500 mL of saturated NaHCO₃ and 2x500 mL of saturated NaCl. The organic phase was dried over Na₂SO₄, 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% Et₃NH. 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%).

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine
2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine (106 g, 0.167 M),
DMF/pyridine (750 mL of a 3:1 mixture prepared from 562 mL of DMF and 188 mL of
pyridine) and acetic anhydride (24.38 mL, 0.258 M) were combined and stirred at room
temperature for 24 hours. The reaction was monitored by tlc by first quenching the tlc sample
with the addition of MeOH. Upon completion of the reaction, as judged by tlc, MeOH (50 mL)
was added and the mixture evaporated at 35°C. The residue was dissolved in CHCl₃ (800 mL)
and extracted with 2x200 mL of saturated sodium bicarbonate and 2x200 mL of saturated NaCl.
The water layers were back extracted with 200 mL of CHCl₃. The combined organics were dried
with sodium sulfate and evaporated to give 122 g of residue (approx. 90% product). The residue
was purified on a 3.5 kg silica gel column and eluted using EtOAc/Hexane(4:1). Pure product
fractions were evaporated to yield 96 g (84%). An additional 1.5 g was recovered from later
fractions.

3'-O-Acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine
A first solution was prepared by dissolving 3'-O-acetyl-2'-O-methoxyethyl-5'-O-
dimethoxytrityl-5-methyluridine (96 g, 0.144 M) in CH₃CN (700 mL) and set aside.
Triethylamine (189 mL, 1.44 M) was added to a solution of triazole (90 g, 1.3 M) in
CH₃CN (1 L), cooled to -5°C and stirred for 0.5 hours using an overhead stirrer. POCl₃ was
added dropwise, over a 30 minute period, to the stirred solution maintained at 0-10°C, and the
resulting mixture stirred for an additional 2 hours. The first solution was added dropwise, over a
45 minute period, to the latter solution. The resulting reaction mixture was stored overnight in a
cold room. Salts were filtered from the reaction mixture and the solution was evaporated. The
residue was dissolved in EtOAc (1 L) and the insoluble solids were removed by filtration. The
filtrate was washed with 1x300 mL of NaHCO₃ and 2x300 mL of saturated NaCl, dried over
sodium sulfate and evaporated. The residue was triturated with EtOAc to give the title
compound.

2'-O-Methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine
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 NH₄OH (30 mL) was stirred at room
temperature for 2 hours. The dioxane solution was evaporated and the residue azeotroped with
MeOH (2x200 mL). The residue was dissolved in MeOH (300 mL) and transferred to a 2 liter
stainless steel pressure vessel. MeOH (400 mL) saturated with NH₃ 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.

**N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine**

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 CHCl₃ (700 mL) and extracted with saturated NaHCO₃ (2x300 mL) and saturated NaCl (2x300 mL), dried over MgSO₄ 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% Et₃NH as the eluting solvent. The pure product fractions were evaporated to give 90 g (90%) of the title compound.

**N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine-3'-amidite**

N4-Benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine (74 g, 0.10 M) was dissolved in CH₂Cl₂ (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 NaHCO₃ (1x300 mL) and saturated NaCl (3x300 mL). The aqueous washes were back-extracted with CH₂Cl₂ (300 mL), and the extracts were combined, dried over MgSO₄ 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.

**2'-(Aminooxyethyl) nucleoside amidites and 2'-(dimethylaminooxyethyl) nucleoside amidites**

Aminooxyethyl and dimethylaminooxyethyl amidites are prepared as per the methods of U.S. patent. 6,127,533.

**Example 2: Oligonucleotide synthesis**

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

Phosphorothioates (P=S) were synthesized as for the phosphodiester oligonucleotides except the standard oxidation bottle was replaced by 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation
wait step was increased to 68 sec and was followed by the capping step. After cleavage from the CPG column and deblocking in concentrated ammonium hydroxide at 55°C (18 hours), the oligonucleotides were purified by precipitating twice with 2.5 volumes of ethanol from a 0.5 M NaCl solution. Phosphinate oligonucleotides are prepared as described in U.S. Patent 5,508,270.

Alkyl phosphonate oligonucleotides are prepared as described in U.S. Patent 4,469,863.

3′-Deoxy-3′-methylene phosphonate oligonucleotides are prepared as described in U.S. Patents 5,610,289 or 5,625,050.

Phosphoramidite oligonucleotides are prepared as described in U.S. Patent, 5,256,775 or U.S. Patent 5,366,878.

Alkylphosphonothioate oligonucleotides are prepared as described in published PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499, respectively). 3′-Deoxy-3′-amino phosphoramidate oligonucleotides are prepared as described in U.S. Patent 5,476,925.

Phosphotriester oligonucleotides are prepared as described in U.S. Patent 5,023,243.

Borano phosphate oligonucleotides are prepared as described in U.S. Patents 5,130,302 and 5,177,198.


**Example 3: Oligonucleoside synthesis**

Methylenemethylimino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylenedimethylhydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, and methyleneaminocarbonyl linked oligonucleosides, also identified as amide-3 linked oligonucleosides, and methyleneaminocarbonyl linked oligonucleosides, also identified as amide-4 linked oligonucleosides, as well as mixed backbone compounds having, for instance, alternating MMI and P=O or P=S linkages are prepared as described in U.S. Patents 5,378,825, 5,386,023, 5,489,677, 5,602,240 and 5,610,289. The oligomeric compounds of the invention may also comprise mixed linkages in which any number of two or more types of linkages are present in any order and at any position within the oligomeric compound, for example the 5′ half of the compound comprising phosphorothioate linkages and the 3′ half comprising phosphodiester linkages. These are referred to as mixed phosphorothioate and phosphodiester linkages.
Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Patents 5,264,562 and 5,264,564.

Ethylene oxide linked oligonucleosides are prepared as described in U.S. Patent 5,223,618.

Example 4: PNA synthesis

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

Example 5: Synthesis of chimeric oligonucleotides

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

Double-stranded compounds of the invention can be of several types including but not limited to, siRNAs, canonical siRNAs, blunt-ended siRNAs or hairpins. Single-stranded compounds of the invention which elicit the RNAi antisense mechanism are also within the scope of the invention. These include, but are not limited to, ssRNAi and antisense RNA (asRNA).

(2'-O-Me)-(2'-deoxy)-(2'-O-Me)chimeric phosphorothioate oligonucleotides

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

(2'-O-(2-Methoxyethyl)-(2'-deoxy)-(2'-'O-(Methoxyethyl))chimeric phosphorothioate oligonucleotides

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

(2'-O-(2-Methoxyethyl)Phosphodiester)-(2'-deoxy Phosphorothioate)-(2'-O-(2-Methoxyethyl) Phosphodiester) chimeric oligonucleotides

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

Other chimeric oligonucleotides, chimeric oligonucleosides and mixed chimeric oligonucleotides/oligonucleosides are synthesized according to U. S. Patent 5,623,065.

RNA Synthesis

In general, RNA synthesis chemistry is based on the selective incorporation of various protecting groups at strategic intermediary reactions. Although one of ordinary skill in the art will understand the use of protecting groups in organic synthesis, a useful class of protecting groups includes silyl ethers. In particular bulky silyl ethers are used to protect the 5'-hydroxyl in combination with an acid-labile orthoester protecting group on the 2'-hydroxyl. This set of protecting groups is then used with standard solid-phase synthesis technology. It is important to lastly remove the acid labile orthoester protecting group after all other synthetic steps.
Moreover, the early use of the silyl protecting groups during synthesis ensures facile removal when desired, without undesired deprotection of 2'-hydroxyl.

Following this procedure for the sequential protection of the 5'-hydroxyl in combination with protection of the 2'-hydroxyl by protecting groups that are differentially removed and are differentially chemically labile, RNA oligonucleotides were synthesized.

RNA oligonucleotides are synthesized in a stepwise fashion. Each nucleotide is added sequentially (3' to 5'-direction) to a solid support-bound oligonucleotide. The first nucleoside at the 3'-end of the chain is covalently attached to a solid support. The nucleotide precursor, a ribonucleoside phosphoramidite, and activator are added, coupling the second base onto the 5'-end of the first nucleoside. The support is washed and any unreacted 5'-hydroxyl groups are capped with acetic anhydride to yield 5'-acetyl moieties. The linkage is then oxidized to the more stable and ultimately desired P(V) linkage. At the end of the nucleotide addition cycle, the 5'-silyl group is cleaved with fluoride. The cycle is repeated for each subsequent nucleotide.

Following synthesis, the methyl protecting groups on the phosphates are cleaved in 30 minutes utilizing 1 M disodium-2-carbamoyl-2-cyanoethylene-1,1-dithiolate trihydrate (S2Na2) in DMF. The deprotection solution is washed from the solid support-bound oligonucleotide using water. The support is then treated with 40% methylamine in water for 10 minutes at 55 °C. This releases the RNA oligonucleotides into solution, deprotects the exocyclic amines, and modifies the 2'-groups. The oligonucleotides can be analyzed by anion exchange HPLC at this stage.

The 2’-orthoester groups are the last protecting groups to be removed. The ethylene glycol monoacetate orthoester protecting group developed by Dharmacon Research, Inc. (Lafayette, CO), is one example of a useful orthoester protecting group which has the following important properties. It is stable to the conditions of nucleoside phosphoramidite synthesis and oligonucleotide synthesis. However, after oligonucleotide synthesis the oligonucleotide is treated with methylamine which not only cleaves the oligonucleotide from the solid support but also removes the acetyl groups from the orthoesters. The resulting 2-ethyl-hydroxyl substituents on the orthoester are less electron withdrawing than the acetylated precursor. As a result, the modified orthoester becomes more labile to acid-catalyzed hydrolysis. Specifically, the rate of cleavage is approximately 10 times faster after the acetyl groups are removed. Therefore, this orthoester possesses sufficient stability in order to be compatible with oligonucleotide synthesis and yet, when subsequently modified, permits deprotection to be carried out under relatively mild aqueous conditions compatible with the final RNA oligonucleotide product.

RNA antisense compounds (RNA oligonucleotides, whether single or double stranded) of the present invention can be synthesized by the methods herein or purchased from Dharmacon Research, Inc (Lafayette, CO). Once synthesized, complementary RNA antisense compounds can then be annealed by methods known in the art to form double-stranded (duplexed) antisense compounds. For example, duplexes can be formed by combining 30 μl of each of the complementary strands of RNA oligonucleotides (50 μM RNA oligonucleotide solution) and 15 μl of 5X annealing buffer (100 mM potassium acetate, 30 mM HEPES-KOH pH 7.4, 2 mM magnesium acetate) followed by heating for 1 minute at 90°C, then 1 hour at 37°C. The resulting duplexed antisense compounds can be used in kits, assays, screens, or other methods to investigate the role of a target nucleic acid, or for diagnostic or therapeutic purposes.

Example 6: Oligonucleotide isolation

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

Example 7: Oligonucleotide synthesis - 96 well plate format

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

Oligonucleotides were cleaved from support and deprotected with concentrated NH₄OH at elevated temperature (55-60°C) for 12-16 hours and the released product then dried in vacuo. The dried product was then re-suspended in sterile water to afford a master plate from which all analytical and test plate samples are then diluted utilizing robotic pipettors.

Example 8: Oligonucleotide analysis - 96 well plate format

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

Example 9: Cell culture and oligonucleotide treatment

The effect of antisense compounds on target nucleic acid expression can be tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. This can be routinely determined using, for example, PCR or Northern blot analysis. The following cell types are provided for illustrative purposes, but other cell types can be routinely used.

MCF7:

The human breast carcinoma cell line MCF-7 was obtained from the American Type Culture Collection (Manassas, VA). MCF-7 cells were routinely cultured in DMEM low glucose (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal calf serum (Invitrogen Life Technologies, Carlsbad, CA). Cells were routinely passaged by trypsinization and dilution when they reached about 90% confluence. Cells were seeded into 96-well plates (Falcon-Primaria #3872) at a density of about 7000 cells/well for use in RT-PCR analysis. For Northern blotting or other analyses, cells may be seeded onto 100 mm or other standard tissue culture plates and treated similarly, using appropriate volumes of medium and oligonucleotide.
HeLa cells:

The human epitheloid carcinoma cell line HeLa was obtained from the American Tissue Type Culture Collection (Manassas, VA). HeLa cells were routinely cultured in DMEM, high glucose (Invitrogen Corporation, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Corporation, Carlsbad, CA). Cells were routinely passaged by trypsinization and dilution when they reached approximately 90% confluence. Cells were seeded into 24-well plates (Falcon-Primaria #3846) at a density of approximately 50,000 cells/well or in 96-well plates at a density of approximately 5,000 cells/well for use in RT-PCR analysis. For Northern blotting or other analyses, cells were harvested when they reached approximately 90% confluence.

U-87 MG cells:

The human glioblastoma U-87 MG cell line was obtained from the American Type Culture Collection (Manassas, VA). U-87 MG cells were cultured in DMEM (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen Life Technologies, Carlsbad, CA) and antibiotics. Cells were routinely passaged by trypsinization and dilution when they reached appropriate confluence. Cells were seeded into 96-well plates (Falcon-Primaria #3872) at a density of about 10,000 cells/well for use in RT-PCR analysis.

B16-F10 cells:

The mouse melanoma cell line B16-F10 was obtained from the American Type Culture Collection (Manassas, VA). B16-F10 cells were routinely cultured in DMEM, high glucose (Gibco/Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum (Gibco/Life Technologies, Gaithersburg, MD), in a 10% carbon dioxide environment. 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 8000 cells/well for use in RT-PCR analysis.

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

HUVEC cells:

HUVEC were obtained from ATCC and routinely cultured in EBM (Clonetics Corp, Walkersille, MD) supplemented with SingleQuots supplements. Cells were routinely passaged by trypsinizaiton and dilution when they reached 90% confluence were maintained for up to 15
passages. For cells grown in 96-well plates (10,000 cells/well), wells were washed once with 200 μL OPTI-MEM-1™ reduced-serum medium (Gibco BRL) and then treated with 130 μL of OPTI-MEM-1™ containing 12 μg/mL LIPOFECTIN™ (Gibco BRL) and the desired double-stranded compounds at a final concentration of 25 nM. After 5 hours of treatment, the medium was replaced with fresh medium. Cells were harvested 16 hours after dsRNA treatment, at which time RNA was isolated and target reduction measured by RT-PCR.

HepG2 cells:

The human hepatoblastoma cell line HepG2 was obtained from the American Type Culture Collection (Manassas, VA). HepG2 cells were routinely cultured in Eagle's MEM supplemented with 10% fetal calf serum, non-essential amino acids, and 1 mM sodium pyruvate (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.

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

H-4-II-E cells:

The rat hepatoma cell line H-4-II-E was obtained from the American Type Culture Collection (Manassas, VA). H-4-II-E cells were routinely cultured in MEM(Gibco/Life Technologies, Gaithersburg, MD) supplemented with 0.1mM non-essential amino acids (Gibco/Life Technologies, Gaithersburg, MD), 1.0mM sodium pyruvate (Gibco/Life Technologies, Gaithersburg, MD), and 10% fetal bovine serum (Gibco/Life Technologies, Gaithersburg, MD). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence.

Treatment with oligomeric compounds:

When cells reached 80% confluency, they are treated with duplexed antisense compounds of the invention. For cells grown in 96-well plates, wells are washed once with 200 μL OPTI-MEM-1 reduced-serum medium (Gibco BRL) and then treated with 130 μL of OPTI-MEM-1 containing 12 μg/mL LIPOFECTIN (Gibco BRL) and the desired duplex antisense compound at a final concentration of 200 nM. After 5 hours of treatment, the medium is replaced with fresh medium. Cells are harvested 16 hours after treatment, at which time RNA is isolated and target reduction measured by RT-PCR.

The concentration of oligonucleotide used varies from cell line to cell line. To determine the optimal oligonucleotide concentration for a particular cell line, the cells are treated
with a positive control oligonucleotide at a range of concentrations. For human cells the positive control oligonucleotide is selected from either ISIS 13920 (TCCGTCATCGCTCCTCAGGG, SEQ ID NO:13) which is targeted to human H-ras, or ISIS 18078, (GTGGCCGCGAGCCGAAAATC, SEQ ID NO:14) which is targeted to human Jun-N-terminal kinase-2 (JNK2). Both controls are 2'-O-methoxyethyl gapmers (2'-O-methoxyethyls shown in bold) with a phosphorothioate backbone. For mouse or rat cells the positive control oligonucleotide is ISIS 15770, ATGCATTCTGCCCCCAAGGA, SEQ ID NO:15, a 2'-O-methoxyethyl gapmer (2'-O-methoxyethyls shown in bold) with a phosphorothioate backbone which is targeted to both mouse and rat c-raf. The concentration of positive control oligonucleotide that results in 80% inhibition of c-H-ras (for ISIS 13920), JNK2 (for ISIS 18078) or c-raf (for ISIS 15770) mRNA is then utilized as the screening concentration for new oligonucleotides in subsequent experiments for that cell line. If 80% inhibition is not achieved, the lowest concentration of positive control oligonucleotide that results in 60% inhibition of c-H-ras, JNK2 or c-raf mRNA is then utilized as the oligonucleotide screening concentration in subsequent experiments for that cell line. If 60% inhibition is not achieved, that particular cell line is deemed as unsuitable for oligonucleotide transfection experiments. The concentrations of antisense oligonucleotides used herein are from 50 nM to 300 nM.

Example 10: Poly(A)+ mRNA isolation

Poly(A)+ mRNA was isolated according to Miura et al., Clin. Chem., 1996, 42, 1758-1764. Other methods for poly(A)+ mRNA isolation are taught in, for example, Ausubel, F.M. et al., Current Protocols in Molecular Biology, Volume 1, pp. 4.5.1-4.5.3, John Wiley & Sons, Inc., 1993. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200 μL cold PBS. 60 μL lysis buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA, 0.5 M NaCl, 0.5% NP-40, 20 mM vanadyl-ribonucleoside complex) was added to each well, the plate was gently agitated and then incubated at room temperature for five minutes. 55 μL of lysate was transferred to Oligo d(T) coated 96-well plates (AGCT Inc., Irvine CA). Plates were incubated for 60 minutes at room temperature, washed 3 times with 200 μL of wash buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.3 M NaCl). After the final wash, the plate was blotted on paper towels to remove excess wash buffer and then air-dried for 5 minutes. 60 μL of elution buffer (5 mM Tris-HCl pH 7.6), preheated to 70°C was added to each well, the plate was incubated on a 90°C hot plate for 5 minutes, and the eluate was then transferred to a fresh 96-well plate.
Cells grown on 100 mm or other standard plates may be treated similarly, using appropriate volumes of all solutions.

**Example 11: Total RNA isolation**

Total mRNA was isolated using an RNEASY 96 kit and buffers purchased from Qiagen, Inc. (Valencia, CA) following the manufacturer's recommended procedures. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200 µL cold PBS. 100 µL Buffer RLT was added to each well and the plate vigorously agitated for 20 seconds. 100 µL of 70% ethanol was then added to each well and the contents mixed by pipetting three times up and down. The samples were then transferred to the RNEASY 96 well plate attached to a QIAVAC manifold fitted with a waste collection tray and attached to a vacuum source. Vacuum was applied for 15 seconds. 1 mL of Buffer RW1 was added to each well of the RNEASY 96 plate and the vacuum again applied for 15 seconds. 1 mL of Buffer RPE was then added to each well of the RNEASY 96 plate and the vacuum applied for a period of 15 seconds. The Buffer RPE wash was then repeated and the vacuum was applied for an additional 10 minutes. The plate was then removed from the QIAVAC manifold and blotted dry on paper towels. The plate was then re-attached to the QIAVAC manifold fitted with a collection tube rack containing 1.2 mL collection tubes. RNA was then eluted by pipetting 60 µL water into each well, incubating 1 minute, and then applying the vacuum for 30 seconds. The elution step was repeated with an additional 60 µL water.

**Example 12: Design and screening of duplexed antisense compounds targeting CEACAM1**

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 CEACAM1. The nucleobase sequence of the antisense strand of the duplex comprises at least an 8-nucleobase portion of an oligonucleotide in Table 1. 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:
CGAGAGCGAGCAAGGACCG (SEQ ID NO:16) and having a two-nucleobase overhang of deoxythymidine(dT) would have the following structure:

\[
\begin{align*}
5 & \quad \text{cgagagccgacggaggaccgTT} \quad \text{Antisense (SEQ ID NO:17)} \\
& \quad \text{TTgctctccgcctgcctgcctggc} \quad \text{Complement (SEQ ID NO:18)}
\end{align*}
\]

As shown, this double-stranded compound represents a canonical siRNA.

In another embodiment, a duplex comprising an antisense strand having the same sequence CGAGAGCGAGCAAGGACCG may be prepared with blunt ends (no single-stranded overhang) as shown:

\[
\begin{align*}
10 & \quad \text{cgagagccgacggaggaccg} \quad \text{Antisense (SEQ ID NO:16)} \\
& \quad \text{gctctccgcctgcctgcctggc} \quad \text{Complement (SEQ ID NO:19)}
\end{align*}
\]

As shown, this double-stranded compound represents a blunt-ended siRNA.

In accordance with the present invention, a series of double-stranded oligomeric compounds (siRNAs) comprising the antisense compounds of the present invention and their complements can be designed to target CEACAM1. The nucleobase sequence of the antisense strand of the duplex comprises at least a portion of an oligonucleotide targeted to CEACAM1 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.

RNA strands of the duplex can be synthesized by methods disclosed herein or purchased from Dharmacon Research Inc., (Lafayette, CO). 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 15uL of a 5X solution of annealing buffer. The final concentration of said buffer is 100 mM potassium acetate, 30 mM HEPES-KOH pH 7.4, and 2mM 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.

Once prepared, the duplexed antisense compounds are evaluated for their ability to modulate CEACAM1 expression.
Example 13: Analysis of oligonucleotide inhibition of CEACAM1 expression

Antisense modulation of CEACAM1 expression can be assayed in a variety of ways known in the art. For example, CEACAM1 mRNA levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or real-time PCR (RT-PCR). Real-time quantitative PCR is presently suitable. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. One method of RNA analysis of the present invention is the use of total cellular RNA as described in other examples herein. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Real-time quantitative (PCR) can be conveniently accomplished using the commercially available ABI PRISM™ 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

Protein levels of CEACAM1 can be quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA) or fluorescence-activated cell sorting (FACS). Antibodies directed to CEACAM1 can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art.

Example 14: Design of phenotypic assays and in vivo studies for the use of CEACAM1 inhibitors

Phenotypic assays

Once CEACAM1 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 CEACAM1 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, OR; PerkinElmer, Boston, MA), protein-based assays including enzymatic assays (Panvera, LLC, Madison, WI; BD Biosciences, Franklin Lakes, NJ; Oncogene Research Products, San Diego, CA), cell regulation, signal transduction, inflammation, oxidative processes and apoptosis (Assay Designs Inc., Ann Arbor, MI), triglyceride accumulation (Sigma-Aldrich, St. Louis, MO), angiogenesis assays, tube formation
assays, cytokine and hormone assays and metabolic assays (Chemicon International Inc., Temecula, CA; Amersham Biosciences, Piscataway, NJ).

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 CEACAM1 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.

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.

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 CEACAM1 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.

*In vivo studies*

The individual subjects of the *in vivo* studies described herein are warm-blooded vertebrate animals, which includes humans.

The clinical trial is subjected to rigorous controls to ensure that individuals are not unnecessarily put at risk and that they are fully informed about their role in the study.

To account for the psychological effects of receiving treatments, volunteers are randomly given placebo or CEACAM1 inhibitor. Furthermore, to prevent the doctors from being biased in treatments, they are not informed as to whether the medication they are administering is a CEACAM1 inhibitor or a placebo. Using this randomization approach, each volunteer has the same chance of being given either the new treatment or the placebo.

Volunteers receive either the CEACAM1 inhibitor or placebo for eight week period with biological parameters associated with the indicated disease state or condition being measured at the beginning (baseline measurements before any treatment), end (after the final treatment), and at regular intervals during the study period. Such measurements include the levels of nucleic acid molecules encoding CEACAM1 or CEACAM1 protein levels in body fluids, tissues or organs compared to pre-treatment levels. Other measurements include, but are not limited to, indices of the disease state or condition being treated, body weight, blood
pressure, serum titers of pharmacologic indicators of disease or toxicity as well as ADME (absorption, distribution, metabolism and excretion) measurements.

Information recorded for each patient includes age (years), gender, height (cm), family history of disease state or condition (yes/no), motivation rating (some/moderate/great) and number and type of previous treatment regimens for the indicated disease or condition.

Volunteers taking part in this study are healthy adults (age 18 to 65 years) and roughly an equal number of males and females participate in the study. Volunteers with certain characteristics are equally distributed for placebo and CEACAM1 inhibitor treatment. In general, the volunteers treated with placebo have little or no response to treatment, whereas the volunteers treated with the CEACAM1 inhibitor show positive trends in their disease state or condition index at the conclusion of the study.

**Example 15: Real-time Quantitative PCR Analysis of CEACAM1 mRNA Levels**

Quantitation of CEACAM1 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, CA) 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, CA, Operon Technologies Inc., Alameda, CA or Integrated DNA Technologies Inc., Coralville, IA) is attached to the 5' end of the probe and a quencher dye (e.g., TAMRA, obtained from either PE-Applied Biosystems, Foster City, CA, Operon Technologies Inc., Alameda, CA or Integrated DNA Technologies Inc., Coralville, IA) 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.

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.

PCR reagents were obtained from Invitrogen Corporation, (Carlsbad, CA). RT-PCR
reactions were carried out by adding 20 μL PCR cocktail (2.5x PCR buffer minus MgCl₂, 6.6
mM MgCl₂, 375 μM each of dATP, dCTP, dGTP and dTTP, 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 2.5x 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).

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, OR). 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, OR). Methods of RNA quantification by RiboGreen™ are taught in Jones, L.J., et al,

In this assay, 170 μL of RiboGreen™ working reagent (RiboGreen™ reagent diluted
1:350 in 10mM 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 485nm and emission at 530nm.

Probes and primers to human CEACAM1 were designed to hybridize to a human CEACAM1 sequence, using published sequence information (GenBank accession number X14831.1, incorporated herein as SEQ ID NO:20). For human CEACAM1 the PCR primers were:

forward primer: ACTTGCCTGTTCAGAGCACTCA (SEQ ID NO:21)
reverse primer: TGGCAAAATCCGAATTAGAGTGA (SEQ ID NO:22)

and the PCR probe was:

FAM-TCCTTCCACCCCGATCGTCT-G-TAMRA (SEQ ID NO:23)

where FAM is the fluorescent dye and TAMRA is the quencher dye. For human GAPDH the PCR primers were:

forward primer: GAAGGTGAAGGTGTCAGTGTC (SEQ ID NO: 24)
reverse primer: GAAGATGTGAGTGGGATTTC (SEQ ID NO:25)

and the PCR probe was:

5' JOE-CAAGCTTCCCGTCTCAGCC- TAMRA 3' (SEQ ID NO:26)

where JOE is the fluorescent reporter dye and TAMRA is the quencher dye.

Probes and primers to rat CEACAM1 were designed to hybridize to a rat CEACAM1 sequence, using published sequence information (GenBank accession number NM_031755.1, incorporated herein as SEQ ID NO:27). For rat CEACAM1 the PCR primers were:

forward primer: AGGGCAACAGGACTCTCAGTT (SEQ ID NO:28)
reverse primer: TCCGGGCTTCACACTCATGT (SEQ ID NO:29)

and the PCR probe was:

FAM-CTCAACGTCAGAGACTGACAAGGG-TAMRA (SEQ ID NO:30) where FAM is the fluorescent reporter dye and TAMRA is the quencher dye. For rat GAPDH the PCR primers were:

forward primer: TGTTCAGACAGCCGCATCTTT (SEQ ID NO:31)
reverse primer: CACCGACTTCACCATCTTTGT (SEQ ID NO:32)

and the PCR probe was:

5' JOE-TTGTGCAAGTGGCCAGCCTCAGTCTCA-TAMRA 3' (SEQ ID NO:33)

where JOE is the fluorescent reporter dye and TAMRA is the quencher dye.
Example 16: Northern blot analysis of CEACAM1 mRNA levels

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

To detect human CEACAM1, a human CEACAM1 specific probe was prepared by PCR using the forward primer ACTTGCTGTTCAGAGCACTCA (SEQ ID NO:21) and the reverse primer TGGCAAATCCGAATTAGAGTGGA (SEQ ID NO:22). To normalize for variations in loading and transfer efficiency membranes were stripped and probed for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA (Clontech, Palo Alto, CA).

To detect rat CEACAM1, a rat CEACAM1 specific probe was prepared by PCR using the forward primer AGGGCAACAGGAACCTCTCCTTTT (SEQ ID NO:28) and the reverse primer TCCGGGCTTCACACTCAGTG (SEQ ID NO:29). To normalize for variations in loading and transfer efficiency membranes were stripped and probed for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA (Clontech, Palo Alto, CA).

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

Example 17: Antisense inhibition of human CEACAM1 expression by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

In accordance with the present invention, a series of antisense compounds was designed to target different regions of the human CEACAM1 RNA, using published sequences (GenBank accession number X14831.1, incorporated herein as SEQ ID NO:20, GenBank accession number X16354.1, incorporated herein as SEQ ID NO:34, nucleotides 15279277 to 15301248 of the nucleotide sequence with the GenBank accession number NT_011109.15, the complement of
which is incorporated herein as SEQ ID NO:35, GenBank accession number S71326.1, incorporated herein as SEQ ID NO:36, GenBank accession number M76742.1, incorporated herein as SEQ ID NO:37, GenBank accession number M72238.1, incorporated herein as SEQ ID NO:38, and GenBank accession number M69176.1, incorporated herein as SEQ ID NO:39). The 5 compounds are shown in Table 1. “Target site” indicates the first (5’-most) nucleotide number on the particular target sequence to which the compound binds. All compounds in Table 1 are chimeric oligonucleotides (“gapmers”) 20 nucleotides in length, composed of a central “gap” region consisting of ten 2’-deoxynucleotides, which is flanked on both sides (5’ and 3’ directions) by five-nucleotide “wings”. The wings are composed of 2’-methoxyethyl (2’-MOE) nucleotides.

The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. The compounds were analyzed for their effect on human CEACAM1 mRNA levels by quantitative real-time PCR as described in other examples herein. Data are averages from three experiments in which HepG2 cells were treated with the antisense oligonucleotides of the present invention. The positive control for each datapoint is identified in the table by sequence ID number. If present, “N.D.” indicates “no data”.

Table 1
Inhibition of human CEACAM1 mRNA levels by
chirmeric phosphorothioate oligonucleotides having 2’-MOE wings and a deoxy gap

<table>
<thead>
<tr>
<th>ISIS #</th>
<th>REGION</th>
<th>TARGET SEQ ID NO</th>
<th>TARGET SITE</th>
<th>SEQUENCE</th>
<th>% INHIB</th>
<th>SEQ NO</th>
<th>ID</th>
<th>CONTROL SEQ ID NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>330517</td>
<td>Coding</td>
<td>4</td>
<td>1021</td>
<td>agagcattatcagtgactat</td>
<td>0</td>
<td>40</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330518</td>
<td>5’UTR</td>
<td>18</td>
<td>36</td>
<td>cgtgtctcacgtgtggaga</td>
<td>75</td>
<td>41</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330519</td>
<td>Start Codon</td>
<td>18</td>
<td>57</td>
<td>ccatgtgctctctgtggc</td>
<td>61</td>
<td>42</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330520</td>
<td>Coding</td>
<td>18</td>
<td>93</td>
<td>gtacaagcactctgtgaag</td>
<td>53</td>
<td>43</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330521</td>
<td>Coding</td>
<td>18</td>
<td>1021</td>
<td>ggacttagctcagtgactat</td>
<td>44</td>
<td>44</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330522</td>
<td>Coding</td>
<td>18</td>
<td>1078</td>
<td>tctttactctctgtgactgt</td>
<td>83</td>
<td>45</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330523</td>
<td>Coding</td>
<td>18</td>
<td>1125</td>
<td>ggtgagagttccccagtg</td>
<td>64</td>
<td>46</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330524</td>
<td>Coding</td>
<td>18</td>
<td>1159</td>
<td>gagagcggggagactctgtgtt</td>
<td>69</td>
<td>47</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330525</td>
<td>Coding</td>
<td>18</td>
<td>1244</td>
<td>ctcacacaaticgtccacag</td>
<td>58</td>
<td>48</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330526</td>
<td>Coding</td>
<td>18</td>
<td>1309</td>
<td>agagcatatagatttttgct</td>
<td>64</td>
<td>49</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330527</td>
<td>Coding</td>
<td>18</td>
<td>1328</td>
<td>gaggcctttctctgtgga</td>
<td>61</td>
<td>50</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330528</td>
<td>Coding</td>
<td>18</td>
<td>1378</td>
<td>gcaaccagggccactactcc</td>
<td>89</td>
<td>51</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Accession</td>
<td>Type</td>
<td>Start</td>
<td>Stop</td>
<td>Sequence</td>
<td>Length</td>
<td>Frame</td>
<td>Insertion</td>
<td>Deletion</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------</td>
<td>-------</td>
<td>-------</td>
<td>-------------------------------</td>
<td>--------</td>
<td>-------</td>
<td>-----------</td>
<td>----------</td>
</tr>
<tr>
<td>330529</td>
<td>Coding</td>
<td>1448</td>
<td></td>
<td>atcacgctggtgctgttgcccc</td>
<td>84</td>
<td>52</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330530</td>
<td>Coding</td>
<td>1453</td>
<td></td>
<td>gtgagatcagctgctgtgccct</td>
<td>82</td>
<td>53</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330531</td>
<td>Coding</td>
<td>1465</td>
<td></td>
<td>gtttttgctctgctgtagatc</td>
<td>79</td>
<td>54</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330532</td>
<td>Coding</td>
<td>1471</td>
<td></td>
<td>actgaggggttgctgtctgctg</td>
<td>82</td>
<td>55</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330533</td>
<td>Coding</td>
<td>1492</td>
<td></td>
<td>ttggtctgagtgtgtgtgtgga</td>
<td>44</td>
<td>56</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330534</td>
<td>Coding</td>
<td>1500</td>
<td></td>
<td>cattggagttgctgtctagtg</td>
<td>62</td>
<td>57</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330535</td>
<td>Coding</td>
<td>1512</td>
<td></td>
<td>tttagtggtgtgcatggag</td>
<td>64</td>
<td>58</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330536</td>
<td>Coding</td>
<td>1543</td>
<td></td>
<td>ttcaaggttagaataagtaac</td>
<td>73</td>
<td>59</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330537</td>
<td>Coding</td>
<td>1557</td>
<td></td>
<td>gtggggtcttaaggtccagg</td>
<td>84</td>
<td>60</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330538</td>
<td>Coding</td>
<td>1613</td>
<td></td>
<td>ataaattattttgtttgtggct</td>
<td>95</td>
<td>61</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330539</td>
<td>Stop Codon</td>
<td>1644</td>
<td></td>
<td>acaggtttcttactgcctt</td>
<td>79</td>
<td>62</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330540</td>
<td>3'UTR</td>
<td>1660</td>
<td></td>
<td>caatgctagtgaagacccag</td>
<td>88</td>
<td>63</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330541</td>
<td>3'UTR</td>
<td>1691</td>
<td></td>
<td>ttgagagegggtgagagacttg</td>
<td>79</td>
<td>64</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330542</td>
<td>3'UTR</td>
<td>1771</td>
<td></td>
<td>atgctatttaggaaaggaaga</td>
<td>67</td>
<td>65</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330543</td>
<td>3'UTR</td>
<td>1834</td>
<td></td>
<td>agaactccaatgactttca</td>
<td>73</td>
<td>66</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330544</td>
<td>3'UTR</td>
<td>1893</td>
<td></td>
<td>cccttctttcctaaagtcgc</td>
<td>69</td>
<td>67</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330545</td>
<td>3'UTR</td>
<td>2004</td>
<td></td>
<td>cgaattagagtatagagca</td>
<td>78</td>
<td>68</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330546</td>
<td>3'UTR</td>
<td>2034</td>
<td></td>
<td>aaggacataacctcagacgct</td>
<td>76</td>
<td>69</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330547</td>
<td>3'UTR</td>
<td>2098</td>
<td></td>
<td>gagaagcattactgccgttt</td>
<td>11</td>
<td>70</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330548</td>
<td>3'UTR</td>
<td>2171</td>
<td></td>
<td>gtaggcattctactttggtt</td>
<td>92</td>
<td>71</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330549</td>
<td>3'UTR</td>
<td>2210</td>
<td></td>
<td>ttgctcctagggtagacaa</td>
<td>80</td>
<td>72</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330550</td>
<td>3'UTR</td>
<td>2226</td>
<td></td>
<td>taagggctcttagacccggtt</td>
<td>76</td>
<td>73</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330551</td>
<td>3'UTR</td>
<td>2248</td>
<td></td>
<td>aggtggattatcttagctagc</td>
<td>72</td>
<td>74</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330552</td>
<td>3'UTR</td>
<td>2256</td>
<td></td>
<td>gaaggattaggtgtgttttctt</td>
<td>88</td>
<td>75</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330553</td>
<td>3'UTR</td>
<td>2287</td>
<td></td>
<td>ttctagtggtgcttcctgaaga</td>
<td>75</td>
<td>76</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330554</td>
<td>3'UTR</td>
<td>2313</td>
<td></td>
<td>ttggcaagtgatttttctcta</td>
<td>81</td>
<td>77</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330555</td>
<td>3'UTR</td>
<td>2366</td>
<td></td>
<td>atattaaacatatatgtgc</td>
<td>51</td>
<td>78</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330556</td>
<td>3'UTR</td>
<td>2381</td>
<td></td>
<td>acagagccctaatataaat</td>
<td>51</td>
<td>79</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330557</td>
<td>3'UTR</td>
<td>2391</td>
<td></td>
<td>actgccttgaaacagagccca</td>
<td>87</td>
<td>80</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330558</td>
<td>3'UTR</td>
<td>2427</td>
<td></td>
<td>gttccccctctgaagctata</td>
<td>92</td>
<td>81</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330559</td>
<td>3'UTR</td>
<td>2443</td>
<td></td>
<td>gtggatctcgggagatgtgg</td>
<td>84</td>
<td>82</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330560</td>
<td>3'UTR</td>
<td>2469</td>
<td></td>
<td>ttcctttcctactcactggtt</td>
<td>90</td>
<td>83</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330561</td>
<td>3'UTR</td>
<td>2496</td>
<td></td>
<td>aataaactgttgagggaggca</td>
<td>85</td>
<td>84</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ID</td>
<td>Type</td>
<td>Position</td>
<td>Sequence</td>
<td>Start</td>
<td>End</td>
<td>Length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------</td>
<td>----------</td>
<td>------------------------</td>
<td>-------</td>
<td>-------</td>
<td>--------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330562</td>
<td>3'UTR</td>
<td>18</td>
<td>taatttaataaatcgcggtaaa</td>
<td>7</td>
<td>85</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330563</td>
<td>3'UTR</td>
<td>18</td>
<td>caaatggtgtatcccttaac</td>
<td>47</td>
<td>86</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330564</td>
<td>3'UTR</td>
<td>18</td>
<td>tcccaatatcccaaatgtg</td>
<td>75</td>
<td>87</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330565</td>
<td>3'UTR</td>
<td>18</td>
<td>ggaatgtctcagagaaagg</td>
<td>74</td>
<td>88</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330566</td>
<td>3'UTR</td>
<td>18</td>
<td>ttaaataaaccttttcac</td>
<td>68</td>
<td>89</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330567</td>
<td>3'UTR</td>
<td>18</td>
<td>cttgagcatacataagcttaagt</td>
<td>75</td>
<td>90</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330568</td>
<td>3'UTR</td>
<td>18</td>
<td>ctggaggtttccgggtttg</td>
<td>83</td>
<td>91</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330569</td>
<td>3'UTR</td>
<td>18</td>
<td>cttgagagctcttgaccaaa</td>
<td>85</td>
<td>92</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330570</td>
<td>3'UTR</td>
<td>18</td>
<td>ctagcagagggcaaggttt</td>
<td>71</td>
<td>93</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330571</td>
<td>3'UTR</td>
<td>18</td>
<td>aaggcccaaatacaccttag</td>
<td>83</td>
<td>94</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330572</td>
<td>3'UTR</td>
<td>18</td>
<td>caggtccctgtaggtgtctc</td>
<td>88</td>
<td>95</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330573</td>
<td>3'UTR</td>
<td>18</td>
<td>gaggtagttaagaggcttagg</td>
<td>86</td>
<td>96</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330574</td>
<td>3'UTR</td>
<td>18</td>
<td>ttataagccccatagaaga</td>
<td>0</td>
<td>97</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330575</td>
<td>3'UTR</td>
<td>18</td>
<td>cttttctcttgccccaaaga</td>
<td>79</td>
<td>98</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330576</td>
<td>3'UTR</td>
<td>18</td>
<td>atagaataaatcccccttcct</td>
<td>59</td>
<td>99</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330577</td>
<td>3'UTR</td>
<td>18</td>
<td>caggttagaaacacctgaa</td>
<td>1</td>
<td>100</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330578</td>
<td>3'UTR</td>
<td>18</td>
<td>gttcgtgcaggttagaaaga</td>
<td>80</td>
<td>101</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330579</td>
<td>3'UTR</td>
<td>18</td>
<td>ggagagttatgcacagtcgg</td>
<td>90</td>
<td>102</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330580</td>
<td>3'UTR</td>
<td>18</td>
<td>cagacatgatcttagccagg</td>
<td>91</td>
<td>103</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330581</td>
<td>intron</td>
<td>19</td>
<td>aaggatgagctgctccacctt</td>
<td>74</td>
<td>104</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330582</td>
<td>intron</td>
<td>19</td>
<td>atggggccctgtgctggcag</td>
<td>63</td>
<td>105</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330583</td>
<td>intron:exon</td>
<td>19</td>
<td>6667</td>
<td>ggagctccggcctagcaga</td>
<td>56</td>
<td>106</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330584</td>
<td>intron:exon</td>
<td>19</td>
<td>7348</td>
<td>tccgggacctttgagcagaa</td>
<td>84</td>
<td>107</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330585</td>
<td>intron</td>
<td>19</td>
<td>ggcagctctcagggtcccaaa</td>
<td>89</td>
<td>108</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330586</td>
<td>intron</td>
<td>19</td>
<td>attttcggtgaggaatccg</td>
<td>89</td>
<td>109</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330587</td>
<td>intron:exon</td>
<td>19</td>
<td>17949</td>
<td>ttgtctgagcgtgagagaagc</td>
<td>81</td>
<td>110</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330588</td>
<td>exon:intron</td>
<td>19</td>
<td>17981</td>
<td>ctatgtctaccttttgaggt</td>
<td>21</td>
<td>111</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330589</td>
<td>Coding</td>
<td>20</td>
<td>tgggtctggagctggcggcttcg</td>
<td>81</td>
<td>112</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330590</td>
<td>Coding</td>
<td>21</td>
<td>agagccatattggtacactg</td>
<td>0</td>
<td>113</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330591</td>
<td>Stop Codon</td>
<td>22</td>
<td>1338</td>
<td>gtagagactaatttacagtt</td>
<td>56</td>
<td>114</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330592</td>
<td>3'UTR</td>
<td>22</td>
<td>atctagagggacatattagg</td>
<td>82</td>
<td>115</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>330593</td>
<td>Stop Codon</td>
<td>23</td>
<td>1050</td>
<td>ttgggaattcatttacagtt</td>
<td>56</td>
<td>116</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>330594</td>
<td>3'UTR</td>
<td>23</td>
<td>gatgagaattgagaggttcag</td>
<td>65</td>
<td>117</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
As shown in Table 1, SEQ ID NOs 41, 42, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 71, 72, 73, 74, 75, 76, 77, 80, 81, 82, 83, 84, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 98, 99, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 112, 114, 115, 116 and 117 demonstrated at least 55% inhibition of human CEACAM1 expression in this assay and are therefore suitable. Also suitable are SEQ ID NOs 61, 71, 81 and 103. The target regions to which these suitable sequences are complementary are herein referred to as “suitable target segments” and are therefore suitable for targeting by compounds of the present invention. These suitable target segments are shown in Table 3. These sequences are shown to contain thymine (T) but one of skill in the art will appreciate that thymine (T) is generally replaced by uracil (U) in RNA sequences. The sequences represent the reverse complement of the suitable antisense compounds shown in Table 1. “Target site” indicates the first (5'-most) nucleotide number on the particular target nucleic acid to which the oligonucleotide binds. Also shown in Table 3 is the species in which each of the suitable target segments was found.

15 Example 18: Antisense inhibition of rat CEACAM1 expression by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap

In accordance with the present invention, a second series of antisense compounds was designed to target different regions of the rat CEACAM1 RNA, using published sequences (GenBank accession number NM_031755.1, incorporated herein as SEQ ID NO: 27, nucleotides 4601755 to 4617070 of the nucleotide sequence with the GenBank accession number NW_043361.1, the complement of which is incorporated herein as SEQ ID NO:118, GenBank accession number AJ277105.1, incorporated herein as SEQ ID NO:119, GenBank accession number J04963.1, incorporated herein as SEQ ID NO:120, and GenBank accession number Z12019.1, incorporated herein as SEQ ID NO:121). The compounds are shown in Table 2.

“Target site” indicates the first (5'-most) nucleotide number on the particular target nucleic acid to which the compound binds. All compounds in Table 2 are chimeric oligonucleotides (“gapmers”) 20 nucleotides in length, composed of a central “gap” region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide “wings”. The wings are composed of 2'-methoxyethyl (2'-MOE)nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. The compounds were analyzed for their effect on rat CEACAM1 mRNA levels by quantitative real-time PCR as described in other examples herein. Data are averages from three experiments in which H-4-II-E cells were treated with the antisense
oligonucleotides of the present invention. The positive control for each datapoint is identified in the table by sequence ID number. If present, “N.D.” indicates “no data”.

Table 2
Inhibition of rat CEACAM1 mRNA levels by chimeric phosphorothioate oligonucleotides having 2’-MOE wings and a deoxy gap

<table>
<thead>
<tr>
<th>ISIS #</th>
<th>REGION</th>
<th>TARGET SEQ ID NO</th>
<th>TARGET SITE</th>
<th>SEQUENCE</th>
<th>% INHIB</th>
<th>SEQ ID NO</th>
<th>CONTROL SEQ ID NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>330920</td>
<td>Coding</td>
<td>11</td>
<td>466</td>
<td>gattggagttgtaccttgtg</td>
<td>66</td>
<td>122</td>
<td>3</td>
</tr>
<tr>
<td>330921</td>
<td>Coding</td>
<td>11</td>
<td>474</td>
<td>ctctcatgggtggaggttgtg</td>
<td>52</td>
<td>123</td>
<td>3</td>
</tr>
<tr>
<td>330922</td>
<td>Coding</td>
<td>11</td>
<td>506</td>
<td>ggttgcacacatattgatacg</td>
<td>70</td>
<td>124</td>
<td>3</td>
</tr>
<tr>
<td>330923</td>
<td>Coding</td>
<td>11</td>
<td>509</td>
<td>ttcggttacacatattgatacg</td>
<td>84</td>
<td>125</td>
<td>3</td>
</tr>
<tr>
<td>330924</td>
<td>Coding</td>
<td>11</td>
<td>512</td>
<td>gttgatcgggtcacacattaa</td>
<td>85</td>
<td>126</td>
<td>3</td>
</tr>
<tr>
<td>330925</td>
<td>Coding</td>
<td>11</td>
<td>515</td>
<td>tttagttacgggtcacacattaa</td>
<td>55</td>
<td>127</td>
<td>3</td>
</tr>
<tr>
<td>330926</td>
<td>Coding</td>
<td>11</td>
<td>524</td>
<td>tgtgtattgttagtgacggt</td>
<td>75</td>
<td>129</td>
<td>3</td>
</tr>
<tr>
<td>330927</td>
<td>Coding</td>
<td>11</td>
<td>535</td>
<td>tccacaggtgtaggtgatgctt</td>
<td>67</td>
<td>129</td>
<td>3</td>
</tr>
<tr>
<td>330928</td>
<td>Coding</td>
<td>11</td>
<td>544</td>
<td>catttcgtcactcaggttag</td>
<td>44</td>
<td>130</td>
<td>3</td>
</tr>
<tr>
<td>330929</td>
<td>Coding</td>
<td>11</td>
<td>549</td>
<td>ttcacactttctgtgacacca</td>
<td>87</td>
<td>131</td>
<td>3</td>
</tr>
<tr>
<td>330930</td>
<td>Coding</td>
<td>11</td>
<td>554</td>
<td>agctgtcaccacagcttctg</td>
<td>85</td>
<td>132</td>
<td>3</td>
</tr>
<tr>
<td>330931</td>
<td>Coding</td>
<td>11</td>
<td>566</td>
<td>tcaacctctgagagcttc</td>
<td>59</td>
<td>133</td>
<td>3</td>
</tr>
<tr>
<td>330932</td>
<td>Coding</td>
<td>11</td>
<td>572</td>
<td>accctgtcaccctctgagag</td>
<td>72</td>
<td>134</td>
<td>3</td>
</tr>
<tr>
<td>330941</td>
<td>Coding</td>
<td>11</td>
<td>695</td>
<td>tccaggtgtaggtgaggtgta</td>
<td>27</td>
<td>135</td>
<td>3</td>
</tr>
<tr>
<td>330942</td>
<td>Coding</td>
<td>11</td>
<td>750</td>
<td>atgcaaatatatctgtgag</td>
<td>58</td>
<td>136</td>
<td>3</td>
</tr>
<tr>
<td>330943</td>
<td>Coding</td>
<td>11</td>
<td>756</td>
<td>cccctgatgcgaataataat</td>
<td>40</td>
<td>137</td>
<td>3</td>
</tr>
<tr>
<td>330944</td>
<td>Coding</td>
<td>11</td>
<td>761</td>
<td>tttgacccttgcagaaata</td>
<td>74</td>
<td>138</td>
<td>3</td>
</tr>
<tr>
<td>330945</td>
<td>Coding</td>
<td>11</td>
<td>818</td>
<td>atatgcaaaaagttactgtgc</td>
<td>69</td>
<td>139</td>
<td>3</td>
</tr>
<tr>
<td>330946</td>
<td>Coding</td>
<td>11</td>
<td>823</td>
<td>cattgataagccaaagtac</td>
<td>43</td>
<td>140</td>
<td>3</td>
</tr>
<tr>
<td>330947</td>
<td>Coding</td>
<td>11</td>
<td>828</td>
<td>ctctctgcattgataaagcaca</td>
<td>74</td>
<td>141</td>
<td>3</td>
</tr>
<tr>
<td>330948</td>
<td>Coding</td>
<td>11</td>
<td>839</td>
<td>gatgtctggagcttctgtc</td>
<td>44</td>
<td>142</td>
<td>3</td>
</tr>
<tr>
<td>330949</td>
<td>Coding</td>
<td>11</td>
<td>865</td>
<td>gtgggagataaagagctct</td>
<td>56</td>
<td>143</td>
<td>3</td>
</tr>
<tr>
<td>330950</td>
<td>Coding</td>
<td>11</td>
<td>892</td>
<td>ctaggtttcgcgtttgta</td>
<td>81</td>
<td>144</td>
<td>3</td>
</tr>
<tr>
<td>330951</td>
<td>Coding</td>
<td>11</td>
<td>902</td>
<td>accgaagacgctagttcc</td>
<td>56</td>
<td>145</td>
<td>3</td>
</tr>
<tr>
<td>330952</td>
<td>Coding</td>
<td>11</td>
<td>908</td>
<td>tttagtgacgaagcagggaa</td>
<td>41</td>
<td>146</td>
<td>3</td>
</tr>
<tr>
<td>Start</td>
<td>Type</td>
<td>Stop</td>
<td>Sequence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>----------</td>
<td>--------</td>
<td>-------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330953</td>
<td>Coding</td>
<td>914</td>
<td>acagtttatggacgaacga</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330954</td>
<td>Coding</td>
<td>938</td>
<td>actgttgtgctggctgactggcc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330955</td>
<td>Coding</td>
<td>944</td>
<td>ttcttgactgtggtggctgac</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330956</td>
<td>Coding</td>
<td>950</td>
<td>gtatgtcttgactgtgtggt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330957</td>
<td>Coding</td>
<td>957</td>
<td>aagactgttaatgtcttga</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330958</td>
<td>Coding</td>
<td>968</td>
<td>gtcactggctcaaaagactggt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330959</td>
<td>Coding</td>
<td>975</td>
<td>ggcttgactgtgctggctcaaa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330960</td>
<td>Coding</td>
<td>983</td>
<td>tggatggagagctgactgtcacc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330961</td>
<td>Coding</td>
<td>1002</td>
<td>gactgtggtgctgtggtagg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330962</td>
<td>Coding</td>
<td>1018</td>
<td>cagagcctagttctcttgact</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330963</td>
<td>Coding</td>
<td>1028</td>
<td>gtctaggggtcagacagacctg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330964</td>
<td>Coding</td>
<td>1042</td>
<td>cctttggagaagcaggctggajgg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330965</td>
<td>Coding</td>
<td>1079</td>
<td>tggctattgaaacagctagc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330966</td>
<td>Coding</td>
<td>1124</td>
<td>ctggtgtccctggagagctggtc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330967</td>
<td>Coding</td>
<td>1132</td>
<td>tgtgggtctctggagctggctctgg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330968</td>
<td>Coding</td>
<td>1147</td>
<td>taatagggtctctttcctgag</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330969</td>
<td>Coding</td>
<td>1153</td>
<td>ctcctcttaatagggctatt</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330970</td>
<td>Coding</td>
<td>1161</td>
<td>ggcatcttctctcttaatag</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330971</td>
<td>Coding</td>
<td>1169</td>
<td>tagtcgccggcatctctttct</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330972</td>
<td>Coding</td>
<td>1209</td>
<td>actatcctgaactgaccgtc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330973</td>
<td>Coding</td>
<td>1224</td>
<td>cagettgattgggtgactta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330974</td>
<td>Coding</td>
<td>1229</td>
<td>acgtcgcagccttagttgggtg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330975</td>
<td>Coding</td>
<td>1239</td>
<td>ttcgcttgttagccttagct</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330976</td>
<td>Coding</td>
<td>1245</td>
<td>gtgcactccggctttatcctg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330977</td>
<td>Coding</td>
<td>1256</td>
<td>gagacgctcgatgctgccttc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330978</td>
<td>Coding</td>
<td>1261</td>
<td>tctggtgagatcctgtagttgct</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330979</td>
<td>intron</td>
<td>528</td>
<td>ctggaggaatgctctttac</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330980</td>
<td>intron:exon</td>
<td>1288</td>
<td>ttaaaagtgaggctaaaaggg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330981</td>
<td>exon:intron</td>
<td>1646</td>
<td>aataacttacggtatacag</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330982</td>
<td>intron</td>
<td>3763</td>
<td>tctgtagaagctcagttat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330983</td>
<td>intron:exon</td>
<td>3838</td>
<td>tccggaccctctggagccaa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330984</td>
<td>intron:exon</td>
<td>5863</td>
<td>gtcactgtggctctggtgacaa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>330985</td>
<td>intron</td>
<td>9988</td>
<td>gaaacctagggtggattgcaaa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
As shown in Table 2, SEQ ID NOs 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 153, 155, 156, 157, 158, 160, 161, 162, 166, 168, 171, 172, 179, 180, 181, 184, 185 and 186 demonstrated at least 40% inhibition of rat CEACAM1 expression in this experiment and are therefore suitable. Also suitable are SEQ ID NOs 131, 126, 125 and 132. The target regions to which these suitable sequences are complementary are herein referred to as “suitable target segments” and are therefore suitable for targeting by compounds of the present invention. These suitable target segments are shown in Table 3. These sequences are shown to contain thymine (T) but one of skill in the art will appreciate that thymine (T) is generally replaced by uracil (U) in RNA sequences. The sequences represent the reverse complement of the suitable antisense compounds shown in Tables 1 and 2. “Target site” indicates the first (5’-most) nucleotide number on the particular target nucleic acid to which the oligonucleotide binds. Also shown in Table 3 is the species in which each of the suitable target segments was found.

Table 3

<table>
<thead>
<tr>
<th>SITE ID</th>
<th>TARGET SEQ NO</th>
<th>TARGET SITE</th>
<th>SEQUENCE</th>
<th>REV COMP OF SEQ ID</th>
<th>ACTIVE IN</th>
<th>SEQ ID NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>247122</td>
<td>18</td>
<td>36</td>
<td>tcctccacaggtgaagacag</td>
<td>25</td>
<td>H. sapiens</td>
<td>191</td>
</tr>
<tr>
<td>247123</td>
<td>18</td>
<td>57</td>
<td>gccagcaggagacacatgg</td>
<td>26</td>
<td>H. sapiens</td>
<td>192</td>
</tr>
<tr>
<td>247126</td>
<td>18</td>
<td>1078</td>
<td>acagtacaggagataagga</td>
<td>29</td>
<td>H. sapiens</td>
<td>193</td>
</tr>
<tr>
<td>247127</td>
<td>18</td>
<td>1125</td>
<td>tgacactgggaatctccatcc</td>
<td>30</td>
<td>H. sapiens</td>
<td>194</td>
</tr>
<tr>
<td>247128</td>
<td>18 1159</td>
<td>aaccagagtccctcgcctct</td>
<td>31</td>
<td>H. sapiens</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-----------</td>
<td>----------------------</td>
<td>-----</td>
<td>-------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>247129</td>
<td>18 1244</td>
<td>ctgggagctttggttaggtgag</td>
<td>32</td>
<td>H. sapiens</td>
<td>196</td>
<td></td>
</tr>
<tr>
<td>247130</td>
<td>18 1309</td>
<td>aacgtaactataatgctct</td>
<td>33</td>
<td>H. sapiens</td>
<td>197</td>
<td></td>
</tr>
<tr>
<td>247131</td>
<td>18 1328</td>
<td>taccacaagaaaattgcctc</td>
<td>34</td>
<td>H. sapiens</td>
<td>198</td>
<td></td>
</tr>
<tr>
<td>247132</td>
<td>18 1378</td>
<td>gggagttagggccttggtgc</td>
<td>35</td>
<td>H. sapiens</td>
<td>199</td>
<td></td>
</tr>
<tr>
<td>247133</td>
<td>18 1448</td>
<td>gggcaagcgcacacgcgctgat</td>
<td>36</td>
<td>H. sapiens</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>247134</td>
<td>18 1453</td>
<td>aggcacacgctgatctccac</td>
<td>37</td>
<td>H. sapiens</td>
<td>201</td>
<td></td>
</tr>
<tr>
<td>247135</td>
<td>18 1465</td>
<td>gatctcacagagcacaacc</td>
<td>38</td>
<td>H. sapiens</td>
<td>202</td>
<td></td>
</tr>
<tr>
<td>247136</td>
<td>18 1471</td>
<td>acagagcacacaaccctcagt</td>
<td>39</td>
<td>H. sapiens</td>
<td>203</td>
<td></td>
</tr>
<tr>
<td>247138</td>
<td>18 1500</td>
<td>cactcagacactcactaag</td>
<td>41</td>
<td>H. sapiens</td>
<td>204</td>
<td></td>
</tr>
<tr>
<td>247139</td>
<td>18 1512</td>
<td>ctcaatgacccaccttaaca</td>
<td>42</td>
<td>H. sapiens</td>
<td>205</td>
<td></td>
</tr>
<tr>
<td>247140</td>
<td>18 1543</td>
<td>gttaactattctacccctga</td>
<td>43</td>
<td>H. sapiens</td>
<td>206</td>
<td></td>
</tr>
<tr>
<td>247141</td>
<td>18 1557</td>
<td>cctgaaccttgaaagccagc</td>
<td>44</td>
<td>H. sapiens</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>247142</td>
<td>18 1613</td>
<td>cagccacagaaatatttat</td>
<td>45</td>
<td>H. sapiens</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>247143</td>
<td>18 1644</td>
<td>aaagcagtaatgaaacctgt</td>
<td>46</td>
<td>H. sapiens</td>
<td>209</td>
<td></td>
</tr>
<tr>
<td>247144</td>
<td>18 1660</td>
<td>ctgctctcctcactgcagtg</td>
<td>47</td>
<td>H. sapiens</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>247145</td>
<td>18 1691</td>
<td>caagtctctccacccctatca</td>
<td>48</td>
<td>H. sapiens</td>
<td>211</td>
<td></td>
</tr>
<tr>
<td>247146</td>
<td>18 1771</td>
<td>tctccctcttaataggcat</td>
<td>49</td>
<td>H. sapiens</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>247147</td>
<td>18 1834</td>
<td>tgaagatcattgggaggtct</td>
<td>50</td>
<td>H. sapiens</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>247148</td>
<td>18 1893</td>
<td>gctgacctttgggaagaggg</td>
<td>51</td>
<td>H. sapiens</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td>247149</td>
<td>18 2004</td>
<td>tgtcctatcactctaatcctg</td>
<td>52</td>
<td>H. sapiens</td>
<td>215</td>
<td></td>
</tr>
<tr>
<td>247150</td>
<td>18 2034</td>
<td>agccttggaggttagtctctt</td>
<td>53</td>
<td>H. sapiens</td>
<td>216</td>
<td></td>
</tr>
<tr>
<td>247152</td>
<td>18 2171</td>
<td>aaccaaatgtaatgaccacac</td>
<td>55</td>
<td>H. sapiens</td>
<td>217</td>
<td></td>
</tr>
<tr>
<td>247153</td>
<td>18 2210</td>
<td>gttgtctacctgtagatca</td>
<td>56</td>
<td>H. sapiens</td>
<td>218</td>
<td></td>
</tr>
<tr>
<td>247154</td>
<td>18 2226</td>
<td>atcagggtctaaacaccttg</td>
<td>57</td>
<td>H. sapiens</td>
<td>219</td>
<td></td>
</tr>
<tr>
<td>247155</td>
<td>18 2248</td>
<td>gtttagctagaataccacct</td>
<td>58</td>
<td>H. sapiens</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>247156</td>
<td>18 2256</td>
<td>agaataaccacctaatccttc</td>
<td>59</td>
<td>H. sapiens</td>
<td>221</td>
<td></td>
</tr>
<tr>
<td>247157</td>
<td>18 2287</td>
<td>tttcaggaacccactaga</td>
<td>60</td>
<td>H. sapiens</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>247158</td>
<td>18 2313</td>
<td>taggaaaaactacttgccaa</td>
<td>61</td>
<td>H. sapiens</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>247161</td>
<td>18 2391</td>
<td>tgggctctgttcagacgagt</td>
<td>64</td>
<td>H. sapiens</td>
<td>224</td>
<td></td>
</tr>
<tr>
<td>247162</td>
<td>18 2427</td>
<td>ttagagctcggagaggaacc</td>
<td>65</td>
<td>H. sapiens</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>247163</td>
<td>18 2443</td>
<td>aaccagcctctgataacac</td>
<td>66</td>
<td>H. sapiens</td>
<td>226</td>
<td></td>
</tr>
<tr>
<td>247164</td>
<td>18 2469</td>
<td>ctaggaacttgggaaagga</td>
<td>67</td>
<td>H. sapiens</td>
<td>227</td>
<td></td>
</tr>
<tr>
<td>Accession</td>
<td>Position</td>
<td>Sequence</td>
<td>Length</td>
<td>Organism</td>
<td>Accession Length</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>----------</td>
<td>----------</td>
<td>--------</td>
<td>----------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td>247165</td>
<td>2496</td>
<td>gccctccagtatttatt</td>
<td>68</td>
<td>H. sapiens</td>
<td>228</td>
<td></td>
</tr>
<tr>
<td>247168</td>
<td>2536</td>
<td>caaatgttgggtatattgga</td>
<td>71</td>
<td>H. sapiens</td>
<td>229</td>
<td></td>
</tr>
<tr>
<td>247169</td>
<td>2563</td>
<td>ccttttcctgagacattcc</td>
<td>72</td>
<td>H. sapiens</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>247170</td>
<td>2614</td>
<td>gtgaaaggggtatatttttac</td>
<td>73</td>
<td>H. sapiens</td>
<td>231</td>
<td></td>
</tr>
<tr>
<td>247171</td>
<td>2632</td>
<td>acttagctagctatgtcag</td>
<td>74</td>
<td>H. sapiens</td>
<td>232</td>
<td></td>
</tr>
<tr>
<td>247172</td>
<td>2677</td>
<td>aaaccaccgaatccctcag</td>
<td>75</td>
<td>H. sapiens</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>247173</td>
<td>2702</td>
<td>ttggtcagagcctctcaag</td>
<td>76</td>
<td>H. sapiens</td>
<td>234</td>
<td></td>
</tr>
<tr>
<td>247174</td>
<td>2891</td>
<td>aacctgggctctgctaag</td>
<td>77</td>
<td>H. sapiens</td>
<td>235</td>
<td></td>
</tr>
<tr>
<td>247175</td>
<td>2906</td>
<td>ctaaggttgattgtgctt</td>
<td>78</td>
<td>H. sapiens</td>
<td>236</td>
<td></td>
</tr>
<tr>
<td>247176</td>
<td>2935</td>
<td>gageacccctacagggacact</td>
<td>79</td>
<td>H. sapiens</td>
<td>237</td>
<td></td>
</tr>
<tr>
<td>247177</td>
<td>3053</td>
<td>cctagctcttttaacagcc</td>
<td>80</td>
<td>H. sapiens</td>
<td>238</td>
<td></td>
</tr>
<tr>
<td>247179</td>
<td>3189</td>
<td>ttcttgggctaaagagaaag</td>
<td>82</td>
<td>H. sapiens</td>
<td>239</td>
<td></td>
</tr>
<tr>
<td>247180</td>
<td>3201</td>
<td>agagagaaggttatcttat</td>
<td>83</td>
<td>H. sapiens</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>247182</td>
<td>3311</td>
<td>ttctaaccctgacacggac</td>
<td>85</td>
<td>H. sapiens</td>
<td>241</td>
<td></td>
</tr>
<tr>
<td>247183</td>
<td>3326</td>
<td>cgactgtgcatacttccc</td>
<td>86</td>
<td>H. sapiens</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>247184</td>
<td>3378</td>
<td>cctggtcataagtcatgtctg</td>
<td>87</td>
<td>H. sapiens</td>
<td>243</td>
<td></td>
</tr>
<tr>
<td>247185</td>
<td>2400</td>
<td>aaggtggagacccctatcttc</td>
<td>88</td>
<td>H. sapiens</td>
<td>244</td>
<td></td>
</tr>
<tr>
<td>247186</td>
<td>3786</td>
<td>tgcaacgcacgcagggcccaat</td>
<td>89</td>
<td>H. sapiens</td>
<td>245</td>
<td></td>
</tr>
<tr>
<td>247187</td>
<td>6667</td>
<td>ttctgcatagcggagctgcc</td>
<td>90</td>
<td>H. sapiens</td>
<td>246</td>
<td></td>
</tr>
<tr>
<td>247188</td>
<td>7348</td>
<td>tttgcgccatgtggcgcggga</td>
<td>91</td>
<td>H. sapiens</td>
<td>247</td>
<td></td>
</tr>
<tr>
<td>247189</td>
<td>9535</td>
<td>tgtggacctgagacgtggc</td>
<td>92</td>
<td>H. sapiens</td>
<td>248</td>
<td></td>
</tr>
<tr>
<td>247190</td>
<td>13538</td>
<td>cggattcctcaacggaaat</td>
<td>93</td>
<td>H. sapiens</td>
<td>249</td>
<td></td>
</tr>
<tr>
<td>247191</td>
<td>17949</td>
<td>gcttctccagctaccagac</td>
<td>94</td>
<td>H. sapiens</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>247193</td>
<td>1367</td>
<td>agaccgcgacgcctaggacca</td>
<td>96</td>
<td>H. sapiens</td>
<td>251</td>
<td></td>
</tr>
<tr>
<td>247195</td>
<td>1338</td>
<td>aactgtaatgtactctcaac</td>
<td>98</td>
<td>H. sapiens</td>
<td>252</td>
<td></td>
</tr>
<tr>
<td>247196</td>
<td>1363</td>
<td>cctatgtgcctcttaggt</td>
<td>99</td>
<td>H. sapiens</td>
<td>253</td>
<td></td>
</tr>
<tr>
<td>247197</td>
<td>1050</td>
<td>actgtaatgaatctctgga</td>
<td>100</td>
<td>H. sapiens</td>
<td>254</td>
<td></td>
</tr>
<tr>
<td>247198</td>
<td>1182</td>
<td>ctgaaacctctctcaattc</td>
<td>101</td>
<td>H. sapiens</td>
<td>255</td>
<td></td>
</tr>
<tr>
<td>247484</td>
<td>466</td>
<td>cacaggttaaacaatctcaatc</td>
<td>106</td>
<td>R. norvegicus</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>247485</td>
<td>474</td>
<td>acaactctcaatctcccagag</td>
<td>107</td>
<td>R. norvegicus</td>
<td>257</td>
<td></td>
</tr>
<tr>
<td>247486</td>
<td>506</td>
<td>gatcattaatgtgtgaacc</td>
<td>108</td>
<td>R. norvegicus</td>
<td>258</td>
<td></td>
</tr>
<tr>
<td>247487</td>
<td>509</td>
<td>tcattaatgtgtgacggta</td>
<td>109</td>
<td>R. norvegicus</td>
<td>259</td>
<td></td>
</tr>
<tr>
<td>247488</td>
<td>512</td>
<td>ttaatgtgtgaacgtaac</td>
<td>110</td>
<td>R. norvegicus</td>
<td>260</td>
<td></td>
</tr>
<tr>
<td>247489</td>
<td>11</td>
<td>515</td>
<td>atgtagaaccgtaacctaa</td>
<td>111</td>
<td><em>R. norvegicus</em></td>
<td>261</td>
</tr>
<tr>
<td>247490</td>
<td>11</td>
<td>524</td>
<td>cctgtaactaacatcagggag</td>
<td>112</td>
<td><em>R. norvegicus</em></td>
<td>262</td>
</tr>
<tr>
<td>247491</td>
<td>11</td>
<td>535</td>
<td>caataccactacattgtgga</td>
<td>113</td>
<td><em>R. norvegicus</em></td>
<td>263</td>
</tr>
<tr>
<td>247492</td>
<td>11</td>
<td>544</td>
<td>ctacccctgtggagagcaattt</td>
<td>114</td>
<td><em>R. norvegicus</em></td>
<td>264</td>
</tr>
<tr>
<td>247493</td>
<td>11</td>
<td>549</td>
<td>tcagagcagaaatgtgtcaag</td>
<td>115</td>
<td><em>R. norvegicus</em></td>
<td>265</td>
</tr>
<tr>
<td>247494</td>
<td>11</td>
<td>554</td>
<td>agcagaagatgtggagagct</td>
<td>116</td>
<td><em>R. norvegicus</em></td>
<td>266</td>
</tr>
<tr>
<td>247495</td>
<td>11</td>
<td>566</td>
<td>gcagactctctcagaaggtgaa</td>
<td>117</td>
<td><em>R. norvegicus</em></td>
<td>267</td>
</tr>
<tr>
<td>247496</td>
<td>11</td>
<td>572</td>
<td>ctctcaagaggtgacaggg</td>
<td>118</td>
<td><em>R. norvegicus</em></td>
<td>268</td>
</tr>
<tr>
<td>247506</td>
<td>11</td>
<td>750</td>
<td>ctccagatattattatgcat</td>
<td>120</td>
<td><em>R. norvegicus</em></td>
<td>269</td>
</tr>
<tr>
<td>247507</td>
<td>11</td>
<td>756</td>
<td>atattattttgcaataaagg</td>
<td>121</td>
<td><em>R. norvegicus</em></td>
<td>270</td>
</tr>
<tr>
<td>247508</td>
<td>11</td>
<td>761</td>
<td>tatttgcatcaaggggtcaca</td>
<td>122</td>
<td><em>R. norvegicus</em></td>
<td>271</td>
</tr>
<tr>
<td>247509</td>
<td>11</td>
<td>818</td>
<td>gcacagcttcttgctcat</td>
<td>123</td>
<td><em>R. norvegicus</em></td>
<td>272</td>
</tr>
<tr>
<td>247510</td>
<td>11</td>
<td>823</td>
<td>gtaacctttggcttatcaatg</td>
<td>124</td>
<td><em>R. norvegicus</em></td>
<td>273</td>
</tr>
<tr>
<td>247511</td>
<td>11</td>
<td>828</td>
<td>tgtggcttatcacatggaag</td>
<td>125</td>
<td><em>R. norvegicus</em></td>
<td>274</td>
</tr>
<tr>
<td>247512</td>
<td>11</td>
<td>839</td>
<td>atacgagaagctccagacacct</td>
<td>126</td>
<td><em>R. norvegicus</em></td>
<td>275</td>
</tr>
<tr>
<td>247513</td>
<td>11</td>
<td>865</td>
<td>agagcctttttactcacaaca</td>
<td>127</td>
<td><em>R. norvegicus</em></td>
<td>276</td>
</tr>
<tr>
<td>247514</td>
<td>11</td>
<td>892</td>
<td>taacaaatggaggaacctag</td>
<td>128</td>
<td><em>R. norvegicus</em></td>
<td>277</td>
</tr>
<tr>
<td>247515</td>
<td>11</td>
<td>902</td>
<td>ggaaccttagctgctctgt</td>
<td>129</td>
<td><em>R. norvegicus</em></td>
<td>278</td>
</tr>
<tr>
<td>247516</td>
<td>11</td>
<td>908</td>
<td>tattgctgtctggtcaataa</td>
<td>130</td>
<td><em>R. norvegicus</em></td>
<td>279</td>
</tr>
<tr>
<td>247517</td>
<td>11</td>
<td>914</td>
<td>tgctctctcaataaactagctg</td>
<td>131</td>
<td><em>R. norvegicus</em></td>
<td>280</td>
</tr>
<tr>
<td>247523</td>
<td>11</td>
<td>975</td>
<td>tgtgacccagttcaaccttaccc</td>
<td>137</td>
<td><em>R. norvegicus</em></td>
<td>281</td>
</tr>
<tr>
<td>247525</td>
<td>11</td>
<td>1002</td>
<td>aaatacaacaaacctacag</td>
<td>139</td>
<td><em>R. norvegicus</em></td>
<td>282</td>
</tr>
<tr>
<td>247526</td>
<td>11</td>
<td>1018</td>
<td>agtcaagagactaggcctctg</td>
<td>140</td>
<td><em>R. norvegicus</em></td>
<td>283</td>
</tr>
<tr>
<td>247527</td>
<td>11</td>
<td>1028</td>
<td>ctaggctctggtacccgtg</td>
<td>141</td>
<td><em>R. norvegicus</em></td>
<td>284</td>
</tr>
<tr>
<td>247528</td>
<td>11</td>
<td>1042</td>
<td>cctgacctgctctcaacagag</td>
<td>142</td>
<td><em>R. norvegicus</em></td>
<td>285</td>
</tr>
<tr>
<td>247530</td>
<td>11</td>
<td>1124</td>
<td>acgctctccccaggacacagt</td>
<td>144</td>
<td><em>R. norvegicus</em></td>
<td>286</td>
</tr>
<tr>
<td>247531</td>
<td>11</td>
<td>1132</td>
<td>caggaccaaccaacgacctca</td>
<td>145</td>
<td><em>R. norvegicus</em></td>
<td>287</td>
</tr>
<tr>
<td>247532</td>
<td>11</td>
<td>1147</td>
<td>cctcagaaatagacccttata</td>
<td>146</td>
<td><em>R. norvegicus</em></td>
<td>288</td>
</tr>
<tr>
<td>247536</td>
<td>11</td>
<td>1209</td>
<td>cggtcagtttcaggataag</td>
<td>150</td>
<td><em>R. norvegicus</em></td>
<td>289</td>
</tr>
<tr>
<td>247538</td>
<td>11</td>
<td>1229</td>
<td>cacccaaatcaagctggacgt</td>
<td>152</td>
<td><em>R. norvegicus</em></td>
<td>290</td>
</tr>
<tr>
<td>247541</td>
<td>11</td>
<td>1256</td>
<td>gaagtgaccatcggagatctc</td>
<td>155</td>
<td><em>R. norvegicus</em></td>
<td>291</td>
</tr>
<tr>
<td>247542</td>
<td>11</td>
<td>1261</td>
<td>gaccatcagatctcagaca</td>
<td>156</td>
<td><em>R. norvegicus</em></td>
<td>292</td>
</tr>
<tr>
<td>247549</td>
<td>102</td>
<td>9988</td>
<td>ttgcatcaccacccctagtttc</td>
<td>163</td>
<td><em>R. norvegicus</em></td>
<td>293</td>
</tr>
<tr>
<td>247550</td>
<td>102</td>
<td>12039</td>
<td>tctgacagcgtgatccac</td>
<td>164</td>
<td><em>R. norvegicus</em></td>
<td>294</td>
</tr>
<tr>
<td>247551</td>
<td>102</td>
<td>12168</td>
<td>actgcgggtgaggaggtct</td>
<td>165</td>
<td><em>R. norvegicus</em></td>
<td>295</td>
</tr>
<tr>
<td>247554</td>
<td>104</td>
<td>39</td>
<td>gaagaacctagcagcagca</td>
<td>168</td>
<td><em>R. norvegicus</em></td>
<td>296</td>
</tr>
<tr>
<td>247555</td>
<td>104</td>
<td>1289</td>
<td>gcgtaatcctgatccac</td>
<td>169</td>
<td><em>R. norvegicus</em></td>
<td>297</td>
</tr>
<tr>
<td>247556</td>
<td>104</td>
<td>1306</td>
<td>aacacaaggaattctgccc</td>
<td>170</td>
<td><em>R. norvegicus</em></td>
<td>298</td>
</tr>
</tbody>
</table>

As these “suitable target segments” have been found by experimentation to be open to, and accessible for, hybridization with the antisense compounds of the present invention, one of skill in the art will recognize or be able to ascertain, using no more than routine experimentation, further embodiments of the invention that encompass other compounds that specifically hybridize to these suitable target segments and consequently inhibit the expression of CEACAM1.

According to the present invention, antisense compounds include antisense oligomeric compounds, antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other short oligomeric compounds which hybridize to at least a portion of the target nucleic acid.

**Example 19: Western blot analysis of CEACAM1 protein levels**

Western blot analysis (immunoblot analysis) is carried out using standard methods. Cells are harvested 16-20 h after oligonucleotide treatment, washed once with PBS, suspended in Laemmli buffer (100 μl/well), boiled for 5 minutes and loaded on a 16% SDS-PAGE gel. Gels are run for 1.5 hours at 150 V, and transferred to membrane for western blotting. Appropriate primary antibody directed to CEACAM1 is used, with a radiolabeled or fluorescently labeled secondary antibody directed against the primary antibody species. Bands are visualized using a PHOSPHORIMAGER™ (Molecular Dynamics, Sunnyvale CA).

Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. Each reference (including, but not limited to, journal articles, U.S. and non-U.S. patents, patent application publications, international patent application publications, gene bank accession numbers, and the like) cited in the present application is incorporated herein by reference in its entirety.
What is claimed is:

1. A compound 8 to 80 nucleobases in length targeted to a nucleic acid molecule encoding CEACAM1, wherein said compound is at least 70% complementary to said nucleic acid molecule encoding CEACAM1, and wherein said compound inhibits the expression of CEACAM1 mRNA by at least 10%.

2. The compound of claim 1 comprising 12 to 50 nucleobases in length.

3. The compound of claim 2 comprising 15 to 30 nucleobases in length.

4. The compound of claim 1 comprising an oligonucleotide.

5. The compound of claim 4 comprising an antisense oligonucleotide.

6. The compound of claim 4 comprising a DNA oligonucleotide.

7. The compound of claim 4 comprising an RNA oligonucleotide.

8. The compound of claim 4 comprising a chimeric oligonucleotide.

9. The compound of claim 4 wherein at least a portion of said compound hybridizes with RNA to form an oligonucleotide-RNA duplex.

10. The compound of claim 1 having at least 80% complementarity with said nucleic acid molecule encoding CEACAM1.

11. The compound of claim 1 having at least 90% complementarity with said nucleic acid molecule encoding CEACAM1.

12. The compound of claim 1 having at least 95% complementarity with said nucleic acid molecule encoding CEACAM1.

13. The compound of claim 1 having at least 99% complementarity with said nucleic acid molecule encoding CEACAM1.

14. The compound of claim 1 having at least one modified internucleoside linkage, sugar moiety, or nucleobase.

15. The compound of claim 1 having at least one 2'-O-methoxyethyl sugar moiety.

16. The compound of claim 1 having at least one phosphorothioate internucleoside linkage.

17. The compound of claim 1 having at least one 5-methylcytosine.

18. A method of inhibiting the expression of CEACAM1 in a cell or tissue comprising contacting said cell or tissue with the compound of claim 1 so that expression of CEACAM1 is inhibited.

19. A method of screening for a modulator of CEACAM1 comprising:
   contacting a suitable target segment of a nucleic acid molecule encoding CEACAM1 with one or more candidate modulators of CEACAM1; and
identifying one or more modulators of CEACAM1 expression which modulate the expression of CEACAM1.

20. The method of claim 19 wherein the modulator of CEACAM1 expression comprises an oligonucleotide, an antisense oligonucleotide, a DNA oligonucleotide, an RNA oligonucleotide, an RNA oligonucleotide having at least a portion of said RNA oligonucleotide capable of hybridizing with RNA to form an oligonucleotide-RNA duplex, or a chimeric oligonucleotide.

21. A diagnostic method for identifying a disease state comprising identifying the presence of CEACAM1 in a sample using at least one primer or probe.

22. A kit or assay device comprising the compound of claim 1.

23. A method of treating an animal having a disease or condition associated with CEACAM1 comprising administering to said animal a therapeutically or prophylactically effective amount of the compound of claim 1 so that expression of CEACAM1 is inhibited.

24. A method of preventing a coronavirus infection in an animal comprising administering to said animal a therapeutically or prophylactically effective amount of a compound designed to modulate expression of a spike protein receptor, wherein modulation of expression of said spike protein receptor prevents entry of said coronavirus into said animal.

25. The method of claim 24 wherein said spike protein receptor is CEACAM1.

26. The method of claim 25 wherein said compound is the compound of claim 1.

27. A method of preventing a coronavirus infection in a cell comprising administering to said cell a therapeutically or prophylactically effective amount of the compound designed to modulate expression of a spike protein receptor, wherein modulation of expression of said spike protein receptor prevents entry of said coronavirus into said cell.

28. The method of claim 27 wherein said spike protein receptor is CEACAM1.

29. The method of claim 27 wherein said compound is the compound of claim 1.

30. A kit or assay device for measuring cell viability comprising a plurality of modulators of spike protein receptors.

31. The compound of claim 1, wherein said compound comprises an antisense nucleic acid molecule that is specifically hybridizable with a 5'-untranslated region (5'UTR) of CEACAM1.

32. The compound of claim 1, wherein said compound comprises an antisense nucleic acid molecule that is specifically hybridizable with a start region of CEACAM1.

33. The compound of claim 1, wherein said compound comprises an antisense nucleic acid molecule that is specifically hybridizable with a coding region CEACAM1.

34. The compound of claim 1, wherein said compound comprises an antisense nucleic acid molecule that is specifically hybridizable with a stop region of CEACAM1.
35. The compound of claim 1, wherein said compound comprises an antisense nucleic acid molecule that is specifically hybridizable with a 3'-untranslated region of CEACAM1.