



US 20040185559A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2004/0185559 A1**
Monia et al. (43) **Pub. Date: Sep. 23, 2004**

(54) **MODULATION OF DIACYLGLYCEROL
ACYLTRANSFERASE 1 EXPRESSION**

(22) Filed: **Mar. 21, 2003**

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Publication Classification

(51) **Int. Cl.⁷** **A61K 48/00**; C07H 21/04

(52) **U.S. Cl.** **435/375**; 514/44; 536/23.2

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(57) **ABSTRACT**

Compounds, compositions and methods are provided for modulating the expression of diacylglycerol acyltransferase 1. The compositions comprise oligonucleotides, targeted to nucleic acid encoding diacylglycerol acyltransferase 1. Methods of using these compounds for modulation of diacylglycerol acyltransferase 1 expression and for diagnosis and treatment of disease associated with expression of diacylglycerol acyltransferase 1 are provided.

(73) Assignee: **Isis Pharmaceuticals Inc.**

(21) Appl. No.: **10/394,808**

MODULATION OF DIACYLGLYCEROL ACYLTRANSFERASE 1 EXPRESSION

FIELD OF THE INVENTION

[0001] The present invention provides compositions and methods for modulating the expression of diacylglycerol acyltransferase 1. In particular, this invention relates to compounds, particularly oligonucleotide compounds, which, in preferred embodiments, hybridize with nucleic acid molecules encoding diacylglycerol acyltransferase 1. Such compounds are shown herein to modulate the expression of diacylglycerol acyltransferase 1.

BACKGROUND OF THE INVENTION

[0002] Triglycerides are one of the major energy storage molecules in eukaryotes. The absorption of triglycerides (also called triacylglycerols) from food is a very efficient process which occurs by a series of steps wherein the dietary triacylglycerols are hydrolyzed in the intestinal lumen and then resynthesized within enterocytes. The resynthesis of triacylglycerols can occur via the monoacylglycerol pathway which commences with monoacylglycerol acyltransferase (MGAT) catalyzing the synthesis of diacylglycerol from monoacylglycerol and fatty acyl-CoA. An alternative synthesis of diacylglycerols is provided by the glycerol-phosphate pathway which describes the coupling of two molecules of fatty acyl-CoA to glycerol-3-phosphate. In either case, diacylglycerol is then acylated with another molecule of fatty acyl-CoA in a reaction catalyzed by one of two diacylglycerol acyltransferase enzymes to form the triglyceride (Farese et al., *Curr. Opin. Lipidol.*, 2000, 11, 229-234).

[0003] The reaction catalyzed by diacylglycerol acyltransferase 1 is the final and only committed step in, triglyceride synthesis. As such, diacylglycerol acyltransferase 1 is involved in intestinal fat absorption, lipoprotein assembly, regulating plasma triglyceride concentrations, and fat storage in adipocytes. Although identified in 1960, the genes encoding human and mouse diacylglycerol acyltransferase 1 (also called DGAT1, acyl CoA:diacylglycerol acyltransferase, acyl CoA:cholesterol acyltransferase-related enzyme, ACAT related gene product, and ARGP1) were not cloned until 1998 (Cases et al., *Proc. Natl. Acad. Sci. U.S.A.*, 1998, 95, 13018-13023; Oelkers et al., *J. Biol. Chem.*, 1998, 273, 26765-26771). Disclosed and claimed in U.S. Pat. No. 6,100,077 is an isolated nucleic acid encoding a human diacylglycerol acyltransferase 1 (Sturley and Oelkers, 2000). Diacylglycerol acyltransferase 1 is a microsomal membrane bound enzyme and has 39% nucleotide identity to the related acyl CoA:cholesterol acyltransferase (Oelkers et al., *J. Biol. Chem.*, 1998, 273, 26765-26771). A splice variant of diacylglycerol acyltransferase 1 has also been cloned that contains a 77 nucleotide insert of unspliced intron with an in-frame stop codon, resulting in a truncated form of diacylglycerol acyltransferase 1 that terminates at Arg-387 deleting 101 residues from the C-terminus containing the putative active site (Cheng et al., *Biochem. J.*, 2001, 359, 707-714).

[0004] Dysregulation of diacylglycerol acyltransferase 1 may play a role in the development of obesity. Upon differentiation of mouse 3T3-L1 cells into mature adipocytes, a 90 fold increase in diacylglycerol acyltransferase 1

protein levels is observed. However, forced overexpression of diacylglycerol acyltransferase 1 in mature adipocytes results in only a 2 fold increase in diacylglycerol acyltransferase 1 protein levels. This leads to an increase in cellular triglyceride synthesis without a concomitant increase in triglyceride lipolysis, leading to the suggestion that manipulation of the steady state level of diacylglycerol acyltransferase 1 may offer a potential means to treat obesity (Yu et al., *J. Biol. Chem.*, 2002, 277, 50876-50884).

[0005] Alterations in diacylglycerol acyltransferase 1 expression may affect human body weight. In a random Turkish population, five polymorphisms in the human diacylglycerol acyltransferase 1 promoter and 5' non-coding sequence have been identified. One common variant, C79T, revealed reduced promoter activity for the 79T allele and is associated with a lower body mass index, higher plasma cholesterol HDL levels, and lower diastolic blood pressure in Turkish women (Ludwig et al., *Clin. Genet.*, 2002, 62, 68-73).

[0006] Diacylglycerol acyltransferase 1 knockout mice exhibit interesting phenotypes which indicate that inhibition of diacylglycerol acyltransferase 1 may offer a strategy for treating obesity and obesity-associated insulin resistance. Mice lacking diacylglycerol acyltransferase 1 are viable and can still synthesize triglycerides through other biological routes. However the mice are lean and resistant to diet-induced obesity (Smith et al., *Nat. Genet.*, 2000, 25, 87-90), have decreased levels of tissue triglycerides, and increased sensitivity to insulin and leptin (Chen et al., *J. Clin. Invest.*, 2002, 109, 1049-1055).

[0007] Currently, there are no known therapeutic agents which effectively inhibit the synthesis of diacylglycerol acyltransferase 1 and to date, investigative strategies aimed at modulating diacylglycerol acyltransferase 1 function have involved natural occurring small molecule derivatives of rosipilins and xanthohumols isolated from *Gliocladium roseum* and *Humulus lupulus*, respectively (Tabata et al., *Phytochemistry*, 1997, 46, 683-687; Tomoda et al., *J. Antibiot. (Tokyo)*, 1999, 52, 689-694).

[0008] Consequently, there remains a long felt need for additional agents capable of effectively inhibiting diacylglycerol acyltransferase 1 function.

[0009] 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 diacylglycerol acyltransferase 1 expression.

[0010] The present invention provides compositions and methods for modulating diacylglycerol acyltransferase 1 expression.

SUMMARY OF THE INVENTION

[0011] The present invention is directed to compounds, especially nucleic acid and nucleic acid-like oligomers, which are targeted to a nucleic acid encoding diacylglycerol acyltransferase 1, and which modulate the expression of diacylglycerol acyltransferase 1. Pharmaceutical and other compositions comprising the compounds of the invention are also provided. Further provided are methods of screening for modulators of diacylglycerol acyltransferase 1 and methods of modulating the expression of diacylglycerol

acyltransferase 1 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 diacylglycerol acyltransferase 1 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.

DETAILED DESCRIPTION OF THE INVENTION

[0012] A. Overview of the Invention

[0013] The present invention employs compounds, preferably oligonucleotides and similar species for use in modulating the function or effect of nucleic acid molecules encoding diacylglycerol acyltransferase 1. This is accomplished by providing oligonucleotides which specifically hybridize with one or more nucleic acid molecules encoding diacylglycerol acyltransferase 1. As used herein, the terms “target nucleic acid” and “nucleic acid molecule encoding diacylglycerol acyltransferase 1” have been used for convenience to encompass DNA encoding diacylglycerol acyltransferase 1, 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, the preferred mechanism believed to be included in the practice of some preferred 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 preferred to target specific nucleic acid molecules and their functions for such antisense inhibition.

[0014] 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 preferred result of such interference with target nucleic acid function is modulation of the expression of diacylglycerol acyltransferase 1. 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 preferred form of modulation of expression and mRNA is often a preferred target nucleic acid.

[0015] In the context of this invention, “hybridization” means the pairing of complementary strands of oligomeric compounds. In the present invention, the preferred 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.

[0016] 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.

[0017] 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.

[0018] “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.

[0019] 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. 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 or hairpin structure). It is preferred that the antisense compounds of the present invention comprise at least 70% sequence complementarity to a target region within the target nucleic acid, more preferably that they comprise 90% sequence complementarity and even more preferably comprise 95% sequence complementarity to the 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 comple-

mentary 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).

[0020] B. Compounds of the Invention

[0021] According to the present invention, compounds include antisense oligomeric compounds, antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, alternate splicers, primers, probes, and other oligomeric compounds which hybridize to at least a portion of the target nucleic acid. As such, these 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 or loops. Once introduced to a system, the compounds of the invention may elicit the action of one or more enzymes or structural proteins to effect modification of the target nucleic acid. One non-limiting example of such an enzyme is RNase H, a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. It is known in the art that single-stranded antisense compounds which are "DNA-like" elicit RNase H. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide-mediated inhibition of gene expression. Similar roles have been postulated for other ribonucleases such as those in the RNase III and ribonuclease L family of enzymes.

[0022] While the preferred form of antisense compound is a single-stranded antisense oligonucleotide, in many species the introduction of double-stranded structures, such as double-stranded RNA (dsRNA) molecules, has been shown to induce potent and specific antisense-mediated reduction of the function of a gene or its associated gene products. This phenomenon occurs in both plants and animals and is believed to have an evolutionary connection to viral defense and transposon silencing.

[0023] The first evidence that dsRNA could lead to gene silencing in animals came in 1995 from work in the nematode, *Caenorhabditis elegans* (Guo and Kempheus, *Cell*, 1995, 81, 611-620). Montgomery et al. have shown that the primary interference effects of dsRNA are posttranscriptional (Montgomery et al., *Proc. Natl. Acad. Sci. USA*, 1998, 95, 15502-15507). The posttranscriptional antisense mechanism defined in *Caenorhabditis elegans* resulting from exposure to double-stranded RNA (dsRNA) has since been designated RNA interference (RNAi). This term has been generalized to mean antisense-mediated gene silencing involving the introduction of dsRNA leading to the sequence-specific reduction of endogenous targeted mRNA

levels (Fire et al., *Nature*, 1998, 391, 806-811). Recently, it has been shown that it is, in fact, the single-stranded RNA oligomers of antisense polarity of the dsRNAs which are the potent inducers of RNAi (Tijsterman et al., *Science*, 2002, 295, 694-697).

[0024] In the context of this invention, the term "oligomeric compound" refers to a polymer or oligomer comprising a plurality of monomeric units. 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, chimeras, analogs and homologs thereof. This term includes oligonucleotides composed of naturally occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for a target nucleic acid and increased stability in the presence of nucleases.

[0025] While oligonucleotides are a preferred form of the compounds of this invention, the present invention comprehends other families of compounds as well, including but not limited to oligonucleotide analogs and mimetics such as those described herein.

[0026] The compounds in accordance with this invention preferably comprise from about 8 to about 80 nucleobases (i.e. from about 8 to about 80 linked nucleosides). One of ordinary skill in the art will appreciate that the invention embodies 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.

[0027] In one preferred embodiment, the compounds of the invention are 12 to 50 nucleobases in length. One having ordinary skill in the art will appreciate that this embodiment comprises 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.

[0028] In another preferred embodiment, the compounds of the invention are 15 to 30 nucleobases in length. One having ordinary skill in the art will appreciate that this embodiment comprises compounds of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length.

[0029] Particularly preferred compounds are oligonucleotides from about 12 to about 50 nucleobases, even more preferably those comprising from about 15 to about 30 nucleobases.

[0030] Antisense compounds 8-80 nucleobases in length comprising a stretch of at least eight (8) consecutive nucleobases selected from within the illustrative antisense compounds are considered to be suitable antisense compounds as well.

[0031] Exemplary preferred antisense compounds include oligonucleotide sequences that comprise at least the 8' consecutive nucleobases from the 5'-terminus of one of the illustrative preferred antisense compounds (the remaining

nucleobases being a consecutive stretch of the same oligonucleotide beginning immediately upstream of the 5'-terminus of the antisense compound which is specifically hybridizable to the target nucleic acid and continuing until the oligonucleotide contains about 8 to about 80 nucleobases). Similarly preferred antisense compounds are represented by oligonucleotide sequences that comprise at least the 8 consecutive nucleobases from the 3'-terminus of one of the illustrative preferred antisense compounds (the remaining nucleobases being a consecutive stretch of the same oligonucleotide beginning immediately downstream of the 3'-terminus of the antisense compound which is specifically hybridizable to the target nucleic acid and continuing until the oligonucleotide contains about 8 to about 80 nucleobases). One having skill in the art armed with the preferred antisense compounds illustrated herein will be able, without undue experimentation, to identify further preferred antisense compounds.

[0032] C. Targets of the Invention

[0033] "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 diacylglycerol acyltransferase 1.

[0034] 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.

[0035] 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 diacylglycerol acyltransferase 1, regardless of the

sequence(s) of such codons. It is also known in the art that a translation termination codon (or "stop codon") of a gene may have one of three sequences, i.e., 5'-UAA, 5'-UAG and 5'-UGA (the corresponding DNA sequences are 5'-TAA, 5'-TAG and 5'-TGA, respectively).

[0036] 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.

[0037] 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 preferred region is the intragenic region encompassing the translation initiation or termination codon of the open reading frame (ORF) of a gene.

[0038] 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 preferred to target the 5' cap region.

[0039] 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 preferred 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.

[0040] 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.

[0041] 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.

[0042] 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 than 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 preferred target nucleic acids.

[0043] The locations on the target nucleic acid to which the preferred antisense compounds hybridize are hereinbelow referred to as "preferred target segments." As used herein the term "preferred 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.

[0044] While the specific sequences of certain preferred 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 preferred target segments may be identified by one having ordinary skill.

[0045] Target segments 8-80 nucleobases in length comprising a stretch of at least eight (8) consecutive nucleobases selected from within the illustrative preferred target segments are considered to be suitable for targeting as well.

[0046] 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 preferred 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 preferred 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 preferred target segments (the remain-

ing 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 preferred target segments illustrated herein will be able, without undue experimentation, to identify further preferred target segments.

[0047] 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.

[0048] D. Screening and Target Validation

[0049] In a further embodiment, the "preferred target segments" identified herein may be employed in a screen for additional compounds that modulate the expression of diacylglycerol acyltransferase 1. "Modulators" are those compounds that decrease or increase the expression of a nucleic acid molecule encoding diacylglycerol acyltransferase 1 and which comprise at least an 8-nucleobase portion which is complementary to a preferred target segment. The screening method comprises the steps of contacting a preferred target segment of a nucleic acid molecule encoding diacylglycerol acyltransferase 1 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 diacylglycerol acyltransferase 1. 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 diacylglycerol acyltransferase 1, the modulator may then be employed in further investigative studies of the function of diacylglycerol acyltransferase 1, or for use as a research, diagnostic, or therapeutic agent in accordance with the present invention.

[0050] The preferred 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.

[0051] Such double stranded oligonucleotide moieties have been shown in the art to modulate target expression and regulate translation as well as RNA processing 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).

[0052] 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 preferred target segments identified herein

in drug discovery efforts to elucidate relationships that exist between diacylglycerol acyltransferase 1 and a disease state, phenotype, or condition. These methods include detecting or modulating diacylglycerol acyltransferase 1 comprising contacting a sample, tissue, cell, or organism with the compounds of the present invention, measuring the nucleic acid or protein level of diacylglycerol acyltransferase 1 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.

[0053] E. Kits, Research Reagents, Diagnostics, and Therapeutics

[0054] 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.

[0055] 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.

[0056] 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.

[0057] Examples of methods of gene expression analysis known in the art include DNA arrays or microarrays (Brazma and Vilo, *FEBS Lett.*, 2000, 480, 17-24; Celis, et al., *FEBS Lett.*, 2000, 480, 2-16), SAGE (serial analysis of gene expression)(Madden, et al., *Drug Discov. Today*, 2000, 5, 415-425), READS (restriction enzyme amplification of digested cDNAs) (Prashar and Weissman, *Methods Enzymol.*, 1999, 303, 258-72), TOGA (total gene expression analysis) (Sutcliffe, et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2000, 97, 1976-81), protein arrays and proteomics (Celis, et al., *FEBS Lett.*, 2000, 480, 2-16; Jungblut, et al., *Electrophoresis*, 1999, 20, 2100-10), expressed sequence tag (EST) sequencing (Celis, et al., *FEBS Lett.*, 2000, 480, 2-16; Larsson, et al., *J. Biotechnol.*, 2000, 80, 143-57), subtractive RNA fingerprinting (SuRF) (Fuchs, et al., *Anal. Biochem.*, 2000, 286, 91-98; Larson, et al., *Cytometry*, 2000, 41, 203-208), subtractive cloning, differential display (DD) (Jurecic and Belmont, *Curr. Opin. Microbiol.*, 2000, 3, 316-21), comparative genomic hybridization (Carulli, et al.,

J. Cell Biochem. Suppl., 1998, 31, 286-96), FISH (fluorescent in situ hybridization) techniques (Going and Gusterson, *Eur. J. Cancer*, 1999, 35, 1895-904) and mass spectrometry methods (To, *Comb. Chem. High Throughput Screen*, 2000, 3, 235-41).

[0058] The compounds of the invention are useful for research and diagnostics, because these compounds hybridize to nucleic acids encoding diacylglycerol acyltransferase 1. For example, oligonucleotides that are shown to hybridize with such efficiency and under such conditions as disclosed herein as to be effective diacylglycerol acyltransferase 1 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 diacylglycerol acyltransferase 1 and in the amplification of said nucleic acid molecules for detection or for use in further studies of diacylglycerol acyltransferase 1. Hybridization of the antisense oligonucleotides, particularly the primers and probes, of the invention with a nucleic acid encoding diacylglycerol acyltransferase 1 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 diacylglycerol acyltransferase 1 in a sample may also be prepared.

[0059] 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.

[0060] For therapeutics, an animal, preferably a human, suspected of having a disease or disorder which can be treated by modulating the expression of diacylglycerol acyltransferase 1 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 diacylglycerol acyltransferase 1 inhibitor. The diacylglycerol acyltransferase 1 inhibitors of the present invention effectively inhibit the activity of the diacylglycerol acyltransferase 1 protein or inhibit the expression of the diacylglycerol acyltransferase 1 protein. In one embodiment, the activity or expression of diacylglycerol acyltransferase 1 in an animal is inhibited by about 10%. Preferably, the activity or expression of diacylglycerol acyltransferase 1 in an animal is inhibited by about 30%. More preferably, the activity or expression of diacylglycerol acyltransferase 1 in an animal is inhibited by 50% or more.

[0061] For example, the reduction of the expression of diacylglycerol acyltransferase 1 may be measured in serum, adipose tissue, liver or any other body fluid, tissue or organ of the animal. Preferably, the cells contained within said fluids, tissues or organs being analyzed contain a nucleic

acid molecule encoding diacylglycerol acyltransferase 1 protein and/or the diacylglycerol acyltransferase 1 protein itself.

[0062] 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.

[0063] F. Modifications

[0064] As is known in the art, a nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to either the 2', 3' or 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn, the respective ends of this linear polymeric compound can be further joined to form a circular compound, however, linear compounds are generally preferred. In addition, linear compounds may have internal nucleobase complementarity and may therefore fold in a manner as to produce a fully or partially double-stranded compound. Within oligonucleotides, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

[0065] Modified Internucleoside Linkages (Backbones)

[0066] Specific examples of preferred antisense compounds useful in this invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined in this specification, oligonucleotides having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified oligonucleotides that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

[0067] Preferred modified oligonucleotide backbones containing a phosphorus atom therein include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thiono-phosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, 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. Preferred oligonucleotides having inverted polarity comprise a single 3' to 3' linkage at the 3'-most internucleotide linkage i.e. a single inverted nucleoside residue which may be abasic (the nucleobase is missing or has a hydroxyl group in place thereof). Various salts, mixed salts and free acid forms are also included.

[0068] Representative United States patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,194,599; 5,565,555; 5,527,899; 5,721,218; 5,672,697 and 5,625,050, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

[0069] Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

[0070] Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; 5,792,608; 5,646,269 and 5,677,439, certain of which are commonly owned with this application, and each of which is herein incorporated by reference.

[0071] Modified Sugar and Internucleoside Linkages—Mimetics

[0072] In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage (i.e. the backbone), of the nucleotide units are replaced with novel groups. The nucleobase units are maintained for hybridization with an appropriate target nucleic acid. One such compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., *Science*, 1991, 254, 1497-1500.

[0073] Preferred embodiments of the invention are oligonucleotides with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular —CH₂—NH—O—CH₂—, —CH₂—N(CH₃)—O—CH₂— [known as a methylene (methylimino) or MMI backbone],

—CH₂—O—N(CH₃)—CH₂—, —CH₂—N(CH₃)—N(CH₃)—CH₂— and —O—N(CH₃)—CH₂—CH₂— [wherein the native phosphodiester backbone is represented as —O—P—O—CH₂—] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

[0074] Modified Sugars

[0075] Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Particularly preferred are O[(CH₂)_nO]_mCH₃, O(CH₂)_nOCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON[(CH₂)CH₃]₂, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O—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. A further preferred modification includes 2'-dimethylaminoethoxy, i.e., a O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, as described in examples hereinbelow, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylamino-ethoxy-ethyl or 2'-DMAEOE), i.e., 2'-O—CH₂—O—CH₂—N(CH₃)₂, also described in examples hereinbelow.

[0076] Other preferred modifications include 2'-methoxy (2'-O—CH₃), 2'-aminopropoxy (2'-OCH₂CH₂CH₂NH₂), 2'-allyl (2'-CH₂—CH=CH₂), 2'-O-allyl (2'-O—CH₂—CH=CH₂) and 2'-fluoro (2'-F). The 2'-modification may be in the arabino (up) position or ribo (down) position. A preferred 2'-arabino modification is 2'-F. Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,792,747; and 5,700,920, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

[0077] A further preferred modification of the sugar includes Locked Nucleic Acids (LNAs) in which the 2'-hy-

droxyl group is linked to the 3' or 4' carbon atom of the sugar ring, thereby forming a bicyclic sugar moiety. The linkage is preferably a methylene (—CH₂—)_n group bridging the 2' oxygen atom and the 4' carbon atom wherein n is 1 or 2. LNAs and preparation thereof are described in WO 98/39352 and WO 99/14226.

[0078] Natural and Modified Nucleobases

[0079] Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl (—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-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further modified nucleobases include tricyclic pyrimidines such as phenoxazine cytidine (1H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido[5,4-b][1,4]benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g. 9-(2-aminoethoxy)-H-pyrimido[5,4-b][1,4]benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido[4,5-b]indol-2-one), pyridoindole cytidine (H-pyrimido[3',2':4,5]pyrrolo[2,3-d]pyrimidin-2-one). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J. I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., *Angewandte Chemie*, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y. S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Crooke, S. T. and Lebleu, B. ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the 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. and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

[0080] Representative United States patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255;

5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121; 5,596,091; 5,614,617; 5,645,985; 5,830,653; 5,763,588; 6,005,096; and 5,681,941, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference, and U.S. Pat. No. 5,750,692, which is commonly owned with the instant application and also herein incorporated by reference.

[0081] Conjugates

[0082] Another modification of the oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. These moieties or conjugates can include conjugate groups covalently bound to functional groups such as primary or secondary hydroxyl 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 conjugate groups include cholesterol, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. Groups that enhance the pharmacodynamic properties, in the context of this invention, include groups that improve uptake, enhance resistance to degradation, and/or strengthen sequence-specific hybridization with the target nucleic acid. Groups that enhance the pharmacokinetic properties, in the context of this invention, include groups that improve uptake, distribution, metabolism or excretion of the compounds of the present invention. Representative conjugate groups are disclosed in International Patent Application PCT/US92/09196, filed Oct. 23, 1992, and U.S. Pat. No. 6,287,860, the entire disclosure of which are incorporated herein by reference. Conjugate moieties include but are not limited to lipid moieties such as a cholesterol moiety, cholic acid, a thioether, e.g., hexyl-S-tritylthiol, a thiocholesterol, an aliphatic chain, e.g., dodecandiol or undecyl residues, a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate, a polyamine or a polyethylene glycol chain, or adamantane acetic acid, a palmityl moiety, or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety. Oligonucleotides 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, folic acid, a benzothiadiazide, chlorothiazide, a diazepam, indomethacin, 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 Ser. No. 09/334,130 (filed Jun. 15, 1999) which is incorporated herein by reference in its entirety.

[0083] Representative United States patents that teach the preparation of such oligonucleotide conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717; 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136;

5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241; 5,391,723; 5,416,203; 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference.

[0084] Chimeric Compounds

[0085] It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an oligonucleotide.

[0086] The present invention also includes antisense compounds which are chimeric compounds. "Chimeric" antisense compounds or "chimeras," in the context of this invention, are antisense compounds, particularly oligonucleotides, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an oligonucleotide compound. These oligonucleotides typically contain at least one region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease degradation, increased cellular uptake, increased stability and/or increased binding affinity for the target nucleic acid. An additional region of the oligonucleotide may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of oligonucleotide-mediated inhibition of gene expression. The cleavage of RNA:RNA hybrids can, in like fashion, be accomplished through the actions of endoribonucleases, such as RNaseL which cleaves both cellular and viral RNA. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

[0087] Chimeric antisense compounds of the invention may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not limited to, U.S. Pat. Nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

[0088] G. Formulations

[0089] The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor-targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Representative United States patents that teach the preparation of such uptake, distribution and/or absorption-assisting formu-

lations include, but are not limited to, U.S. Pat. Nos. 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756, each of which is herein incorporated by reference.

[0090] The antisense compounds of the invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other compound which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to prodrugs and pharmaceutically acceptable salts of the compounds of the invention, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents.

[0091] The term "prodrug" indicates a therapeutic agent that is prepared in an inactive form that is converted to an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions. In particular, prodrug versions of the oligonucleotides of the invention are prepared as SATE [(S-acetyl-2-thioethyl) phosphate] derivatives according to the methods disclosed in WO 93/24510 to Gosselin et al., published Dec. 9, 1993 or in WO 94/26764 and U.S. Pat. No. 5,770,713 to Imbach et al.

[0092] 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. For oligonucleotides, preferred examples of pharmaceutically acceptable salts and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

[0093] The present invention also includes pharmaceutical compositions and formulations which include the antisense compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration. Oligonucleotides with at least one 2'-O-methoxyethyl modification are believed to be particularly useful for oral administration. 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.

[0094] The pharmaceutical formulations of the present invention, which may conveniently be presented in unit

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

[0095] The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0096] Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, foams and liposome-containing formulations. The pharmaceutical compositions and formulations of the present invention may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients.

[0097] Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Microemulsions are included as an embodiment of the present invention. Emulsions and their uses are well known in the art and are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

[0098] Formulations of the present invention include liposomal formulations. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes which are believed to interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

[0099] Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. Liposomes and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

[0100] The pharmaceutical formulations and compositions of the present invention may also include surfactants.

The use of surfactants in drug products, formulations and in emulsions is well known in the art. Surfactants and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

[0101] In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly oligonucleotides. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs. Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants. Penetration enhancers and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety.

[0102] One of skill in the art will recognize that formulations are routinely designed according to their intended use, i.e. route of administration.

[0103] Preferred formulations for topical administration include those in which the oligonucleotides of the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Preferred lipids and liposomes include neutral (e.g. dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline) negative (e.g. dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g. dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA).

[0104] For topical or other administration, oligonucleotides of the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, oligonucleotides may be complexed to lipids, in particular to cationic lipids. Preferred fatty acids and esters, pharmaceutically acceptable salts thereof, and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety. Topical formulations are described in detail in U.S. patent application Ser. No. 09/315,298 filed on May 20, 1999, which is incorporated herein by reference in its entirety.

[0105] Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitabets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Preferred oral formulations are those in which oligonucleotides of the invention are administered in conjunction with one or more penetration enhancers surfactants and chelators. Preferred surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Preferred bile acids/salts and fatty acids and their uses are further described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety. Also preferred are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. A particularly preferred combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. Oligonucleotides of the invention may be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. Oligonucleotide complexing agents and their uses are fur-

ther described in U.S. Pat. No. 6,287,860, which is incorporated herein in its entirety. Oral formulations for oligonucleotides and their preparation are described in detail in U.S. application Ser. Nos. 09/108,673 (filed Jul. 1, 1998), 09/315,298 (filed May 20, 1999) and 10/071,822, filed Feb. 8, 2002, each of which is incorporated herein by reference in their entirety.

[0106] 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.

[0107] Certain embodiments of the invention provide pharmaceutical compositions containing one or more oligomeric compounds and one or more other chemotherapeutic agents which function by a non-antisense mechanism. Examples of such chemotherapeutic agents include but are not limited to cancer chemotherapeutic drugs such as 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, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycytosine, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES). 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 ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. Combinations of antisense compounds and other non-antisense drugs are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

[0108] In another related embodiment, compositions of the invention may contain one or more antisense compounds, particularly oligonucleotides, targeted to a first nucleic acid and one or more additional antisense compounds targeted to a second nucleic acid target. Alternatively, compositions of the invention may contain two or more antisense compounds targeted to different regions of the same nucleic acid target. Numerous examples of antisense compounds are known in the art. Two or more combined compounds may be used together or sequentially.

[0109] H. Dosing

[0110] The formulation of therapeutic compositions and their subsequent administration (dosing) is believed to be

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

[0111] While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following examples serve only to illustrate the invention and are not intended to limit the same.

EXAMPLES

Example 1

Synthesis of Nucleoside Phosphoramidites

[0112] The following compounds, including amidites and their intermediates were prepared as described in U.S. Pat. No. 6,426,220 and published PCT WO 02/36743; 5'-O-Dimethoxytrityl-thymidine intermediate for 5-methyl dC amidite, 5'-O-Dimethoxytrityl-2'-deoxy-5-methylcytidine intermediate for 5-methyl-dC amidite, 5'-O-Dimethoxytrityl-2'-deoxy-N⁴-benzoyl-5-methylcytidine penultimate intermediate for 5-methyl dC amidite, [5'-O-(4,4'-Dimethoxytriphenylmethyl)-2'-deoxy-N⁴-benzoyl-5-methylcytidin-3'-O-yl]-2-cyanoethyl-N,N-diisopropylphosphoramidite (5-methyl dC amidite), 2'-Fluorodeoxyadenosine, 2'-Fluorodeoxyguanosine, 2'-Fluorouridine, 2'-Fluorodeoxycytidine, 2'-O-(2-Methoxyethyl) modified amidites, 2'-O-(2-methoxyethyl)-5-methyluridine intermediate, 5'-O-DMT-2'-O-(2-methoxyethyl)-5-methyluridine penultimate intermediate, [5'-O-(4,4'-Dimethoxytriphenylmethyl)-2'-O-(2-methoxyethyl)-5-methyluridin-3'-O-yl]-2-cyanoethyl-N,N-diisopropylphosphoramidite (MOE T amidite), 5'-O-Dimethoxytrityl-2'-O-(2-methoxyethyl)-5-methylcytidine intermediate, 5'-O-dimethoxytrityl-2'-O-(2-methoxyethyl)-N⁴-benzoyl-5-methyl-cytidine penultimate intermediate, [5'-O-(4,4'-Dimethoxytriphenylmethyl)-2'-O-(2-methoxyethyl)-N⁴-benzoyl-5-methylcytidin-3'-O-yl]-2-cyanoethyl-N,N-diisopropylphosphoramidite (MOE 5-Me-C amidite), [5'-O-(4,4'-Dimethoxytriphenylmethyl)-2'-O-(2-methoxyethyl)-N⁴-benzoyl-5-methylcytidin-3'-O-yl]-2-cyanoethyl-N,N-diisopropylphosphoramidite (MOE A amidite), [5'-O-(4,4'-Dimethoxytriphenylmethyl)-2'-O-(2-methoxyethyl)-N⁴-isobutyrylguanosin-3'-O-yl]-2-cyanoethyl-N,N-

diisopropylphosphoramidite (MOE G amidite), 2'-O-(Aminooxyethyl) nucleoside amidites and 2'-O-(dimethylaminoxyethyl) nucleoside amidites, 2'-(Dimethylaminoxyethoxy) nucleoside amidites, 5'-O-tert-Butyldiphenylsilyl-O²-2'-anhydro-5-methyluridine, 5'-O-tert-Butyldiphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine, 2'-O-([2-phthalimidoxy]ethyl)-5'-t-butyldiphenylsilyl-5-methyluridine, 5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine, 5'-O-tert-Butyldiphenylsilyl-2'-O-[N,N dimethylaminoxyethyl]-5-methyluridine, 2'-O-(dimethylaminoxyethyl)-5-methyluridine, 5'-O-DMT-2'-O-(dimethylaminoxyethyl)-5-methyluridine, 5'-O-DMT-2'-O-(2-N,N-dimethylaminoxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite], 2'-(Aminooxyethoxy) nucleoside amidites, N²-isobutyryl-6-O-diphenylcarbonyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite], 2'-dimethylaminoethoxyethoxy (2'-DMAEOE) nucleoside amidites, 2'-O-[2(2-N,N-dimethylaminoethoxy)ethyl]-5-methyl uridine, 5'-O-dimethoxytrityl-2'-O-[2(2-N,N-dimethylaminoethoxy)-ethyl]-5-methyl uridine and 5'-O-Dimethoxytrityl-2'-O-[2(2-N,N-dimethylaminoethoxy)-ethyl]-5-methyl uridine-3'-O-(cyanoethyl-N,N-diisopropyl)phosphoramidite.

Example 2

Oligonucleotide and Oligonucleoside Synthesis

[0113] The antisense compounds used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and alkylated derivatives.

[0114] Oligonucleotides: Unsubstituted and substituted phosphodiester (P=O) oligonucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems model 394) using standard phosphoramidite chemistry with oxidation by iodine.

[0115] Phosphorothioates (P=S) are synthesized similar to phosphodiester oligonucleotides with the following exceptions: thiation was effected by utilizing a 10% w/v solution of 3,4,5-benzodithiole-3-one 1,1-dioxide in acetonitrile for the oxidation of the phosphite linkages. The thiation reaction step time was increased to 180 sec and preceded by the normal capping step. After cleavage from the CPG column and deblocking in concentrated ammonium hydroxide at 55° C. (12-16 hr), the oligonucleotides were recovered by precipitating with >3 volumes of ethanol from a 1 M NH₄OAc solution. Phosphinate oligonucleotides are prepared as described in U.S. Pat. No. 5,508,270, herein incorporated by reference.

[0116] Alkyl phosphonate oligonucleotides are prepared as described in U.S. Pat. No. 4,469,863, herein incorporated by reference.

[0117] 3'-Deoxy-3'-methylene phosphonate oligonucleotides are prepared as described in U.S. Pat. Nos. 5,610,289 or 5,625,050, herein incorporated by reference.

[0118] Phosphoramidite oligonucleotides are prepared as described in U.S. Pat. No. 5,256,775 or U.S. Pat. No. 5,366,878, herein incorporated by reference.

[0119] Alkylphosphonothioate oligonucleotides are prepared as described in published PCT applications PCT/US94/00902 and PCT/US93/06976 (published as WO 94/17093 and WO 94/02499, respectively), herein incorporated by reference.

[0120] 3'-Deoxy-3'-amino phosphoramidate oligonucleotides are prepared as described in U.S. Pat. No. 5,476,925, herein incorporated by reference.

[0121] Phosphotriester oligonucleotides are prepared as described in U.S. Pat. No. 5,023,243, herein incorporated by reference.

[0122] Borano phosphate oligonucleotides are prepared as described in U.S. Pat. Nos. 5,130,302 and 5,177,198, both herein incorporated by reference.

[0123] Oligonucleosides: Methylenemethylimino linked oligonucleosides, also identified as MMI linked oligonucleosides, methylenedimethylhydrazo linked oligonucleosides, also identified as MDH linked oligonucleosides, and methylenecarbonylamino 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. Pat. Nos. 5,378,825, 5,386,023, 5,489,677, 5,602,240 and 5,610,289, all of which are herein incorporated by reference.

[0124] Formacetal and thioformacetal linked oligonucleosides are prepared as described in U.S. Pat. Nos. 5,264,562 and 5,264,564, herein incorporated by reference.

[0125] Ethylene oxide linked oligonucleosides are prepared as described in U.S. Pat. No. 5,223,618, herein incorporated by reference.

Example 3

RNA Synthesis

[0126] 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.

[0127] 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.

[0128] RNA oligonucleotides are synthesized in a step-wise 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.

[0129] 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 (S_2Na_2) 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.

[0130] 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, Colo.), 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-ethylhydroxyl 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.

[0131] Additionally, methods of RNA synthesis are well known in the art (Scaringe, S. A. Ph.D. Thesis, University of Colorado, 1996; Scaringe, S. A., et al., *J. Am. Chem. Soc.*, 1998, 120, 11820-11821; Matteucci, M. D. and Caruthers, M. H. *J. Am. Chem. Soc.*, 1981, 103, 3185-3191; Beaucage, S. L. and Caruthers, M. H. *Tetrahedron Lett.*, 1981, 22, 1859-1862; Dahl, B. J., et al., *Acta Chem. Scand.*, 1990, 44, 639-641; Reddy, M. P., et al., *Tetrahedron Lett.*, 1994, 25, 4311-4314; Wincott, F. et al., *Nucleic Acids Res.*, 1995, 23, 2677-2684; Griffin, B. E., et al., *Tetrahedron*, 1967, 23, 2301-2313; Griffin, B. E., et al., *Tetrahedron*, 1967, 23, 2315-2331).

[0132] RNA antisense compounds (RNA oligonucleotides) of the present invention can be synthesized by the methods herein or purchased from Dharmacon Research, Inc (Lafayette, Colo.). Once synthesized, complementary RNA antisense compounds can then be annealed by methods known in the art to form double stranded (duplexed) anti-

sense compounds. For example, duplexes can be formed by combining 30 μ L of each of the complementary strands of RNA oligonucleotides (50 μ M RNA oligonucleotide solution) and 15 μ L of 5 $\times$ 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.

Example 4

Synthesis of Chimeric Oligonucleotides

[0133] 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”.

[0134] [2'-O—Me]-[2'-deoxy]-[2'-O—Me] Chimeric Phosphorothioate Oligonucleotides

[0135] Chimeric oligonucleotides having 2'-O-alkyl phosphorothioate and 2'-deoxy phosphorothioate oligonucleotide segments are synthesized using an Applied Biosystems automated DNA synthesizer Model 394, 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 5' and 3' wings. The standard synthesis cycle is modified by incorporating coupling steps with increased reaction times for the 5'-dimethoxytrityl-2'-O-methyl-3'-O-phosphoramidite. The fully protected oligonucleotide is cleaved from the support and deprotected in concentrated ammonia (NH₄OH) for 12-16 hr at 55° C. The deprotected oligo is then recovered by an appropriate method (precipitation, column chromatography, volume reduced in vacuo and analyzed spectrophotometrically for yield and for purity by capillary electrophoresis and by mass spectrometry.

[0136] [2'-O-(2-Methoxyethyl)]-[2'-deoxy]-[2'-O-(Methoxyethyl)] Chimeric Phosphorothioate Oligonucleotides

[0137] [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.

[0138] [2'-O-(2-Methoxyethyl)Phosphodiester]-[2'-deoxy Phosphorothioate]-[2'-O-(2-Methoxyethyl) Phosphodiester] Chimeric Oligonucleotides

[0139] [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, oxidation with iodine to generate the phosphodiester internucleotide linkages within the wing

portions of the chimeric structures and sulfurization utilizing 3,4-dihydro-2H-benzothiole-3-one 1,1-dioxide (Beaucage Reagent) to generate the phosphorothioate internucleotide linkages for the center gap.

[0140] Other chimeric oligonucleotides, chimeric oligonucleosides and mixed chimeric oligonucleotides/oligonucleosides are synthesized according to U.S. Pat. No. 5,623,065, herein incorporated by reference.

Example 5

Design and Screening of Duplexed Antisense Compounds Targeting Diacylglycerol Acyltransferase 1

[0141] 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 diacylglycerol acyltransferase 1. The nucleobase sequence of the antisense strand of the duplex comprises at least a 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.

[0142] For example, a duplex comprising an antisense strand having the sequence CGAGAGGCGGACGGGACCG and having a two-nucleobase overhang of deoxythymidine(dT) would have the following structure:

cgagagggcgacgggacggcTT	Antisense Strand
TTgctctccgcctgcctggc	Complement

[0143] RNA strands of the duplex can be synthesized by methods disclosed herein or purchased from Dharmacon Research Inc., (Lafayette, Colo.). Once synthesized, the complementary strands are annealed. The single strands are aliquoted and diluted to a concentration of 50 μ M. Once diluted, 30 μ L of each strand is combined with 15 μ L of a 5 $\times$ solution of annealing buffer. The final concentration of said buffer is 100 mM potassium acetate, 30 mM HEPES-KOH pH 7.4, and 2 mM magnesium acetate. The final volume is 75 μ L. 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 μ M. This solution can be stored frozen (−20° C.) and freeze-thawed up to 5 times.

[0144] Once prepared, the duplexed antisense compounds are evaluated for their ability to modulate diacylglycerol acyltransferase 1 expression.

[0145] 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 μ L OPTI-MEM-1 reduced-serum medium (Gibco BRL) and then treated with 130 μ L of OPTI-MEM-1 containing 12 μ g/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.

Example 6

Oligonucleotide Isolation

[0146] After cleavage from the controlled pore glass solid support and deblocking in concentrated ammonium hydroxide at 55° C. for 12-16 hours, the oligonucleotides or oligonucleosides are recovered by precipitation out of 1 M NH₄OAc with >3 volumes of ethanol. Synthesized oligonucleotides were analyzed by electrospray mass spectroscopy (molecular weight determination) and by capillary gel electrophoresis and judged to be at least 70% full length material. The relative amounts of phosphorothioate and phosphodiester linkages obtained in the synthesis was determined by the ratio of correct molecular weight relative to the -16 amu product (+/-32+/-48). 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

[0147] Oligonucleotides were synthesized via solid phase P(III) phosphoramidite chemistry on an automated synthesizer capable of assembling 96 sequences simultaneously in a 96-well format. Phosphodiester internucleotide linkages were afforded by oxidation with aqueous iodine. Phosphorothioate internucleotide linkages were generated by sulfuration utilizing 3,4,5-benzodithiole-3-one 1,1-dioxide (Beaucage Reagent) in anhydrous acetonitrile. Standard base-protected beta-cyanoethyl-diisopropyl phosphoramidites were purchased from commercial vendors (e.g. PE-Applied Biosystems, Foster City, Calif., or Pharmacia, Piscataway, N.J.). Non-standard nucleosides are synthesized as per standard or patented methods. They are utilized as base protected beta-cyanoethyl-diisopropyl phosphoramidites.

[0148] 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

[0149] The concentration of oligonucleotide in each well was assessed by dilution of samples and UV absorption spectroscopy. The full-length integrity of the individual products was evaluated by capillary electrophoresis (CE) in either the 96-well format (Beckman P/ACE™ MDQ) or, for individually prepared samples, on a commercial CE apparatus (e.g., Beckman P/ACE™ 5000, ABI 270). Base and backbone composition was confirmed by mass analysis of the compounds utilizing electrospray-mass spectroscopy.

All assay test plates were diluted from the master plate using single and multi-channel robotic pipettors. Plates were judged to be acceptable if at least 85% of the compounds on the plate were at least 85% full length.

Example 9

Cell Culture and Oligonucleotide Treatment

[0150] 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, provided that the target is expressed in the cell type chosen. This can be readily determined by methods routine in the art, for example Northern blot analysis, ribonuclease protection assays, or RT-PCR.

[0151] T-24 Cells:

[0152] The human transitional cell bladder carcinoma cell line T-24 was obtained from the American Type Culture Collection (ATCC) (Manassas, Va.). T-24 cells were routinely cultured in complete McCoy's 5A basal media (Invitrogen Corporation, Carlsbad, Calif.) supplemented with 10% fetal calf serum (Invitrogen Corporation, Carlsbad, Calif.), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Invitrogen Corporation, Carlsbad, Calif.). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells were seeded into 96-well plates (Falcon-Primaria #353872) at a density of 7000 cells/well for use in RT-PCR analysis.

[0153] For Northern blotting or other analysis, cells may be seeded onto 100 mm or other standard tissue culture plates and treated similarly, using appropriate volumes of medium and oligonucleotide.

[0154] A549 Cells:

[0155] The human lung carcinoma cell line A549 was obtained from the American Type Culture Collection (ATCC) (Manassas, Va.). A549 cells were routinely cultured in DMEM basal media (Invitrogen Corporation, Carlsbad, Calif.) supplemented with 10% fetal calf serum (Invitrogen Corporation, Carlsbad, Calif.), penicillin 100 units per mL, and streptomycin 100 micrograms per mL (Invitrogen Corporation, Carlsbad, Calif.). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence.

[0156] NHDF Cells:

[0157] Human neonatal dermal fibroblast (NHDF) were obtained from the Clonetics Corporation (Walkersville, Md.). NHDFs were routinely maintained in Fibroblast Growth Medium (Clonetics Corporation, Walkersville, Md.) supplemented as recommended by the supplier. Cells were maintained for up to 10 passages as recommended by the supplier.

[0158] HEK Cells:

[0159] Human embryonic keratinocytes (HEK) were obtained from the Clonetics Corporation (Walkersville, Md.). HEKs were routinely maintained in Keratinocyte

Growth Medium (Clonetics Corporation, Walkersville, Md.) formulated as recommended by the supplier. Cells were routinely maintained for up to 10 passages as recommended by the supplier.

[0160] HepG2 Cells:

[0161] 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.

[0162] 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.

[0163] b.END Cells:

[0164] The mouse brain endothelial cell line b.END was obtained from Dr. Werner Risau at the Max Plank Institute (Bad Nauheim, Germany). b.END cells were routinely cultured in DMEM supplemented with 10% fetal bovine serum (Gibco/Life Technologies, Gaithersburg, Md.). Cells were routinely passaged by trypsinization and dilution when they reached 90% confluence. Cells were seeded into 24-well plates (Falcon-Primaria #3047) at a density of 40,000 cells/well for use in RT-PCR analysis.

[0165] 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.

[0166] Treatment with Antisense Compounds:

[0167] When cells reached 65-75% confluency, they were treated with oligonucleotide. For cells grown in 24-well plates, wells were washed once with 400 μ L Eagle's DMEM medium and then treated with 100 μ L of Eagle's DMEM containing 3.75 μ g/mL LIPOFECTIN™ (Invitrogen Corporation, Carlsbad, Calif.) and the desired concentration of oligonucleotide. Cells are treated and data are obtained in triplicate. After 4-7 hours of treatment at 37° C., the medium was replaced with Eagle's DMEM supplemented with 10% fetal bovine serum. Cells were harvested 20-24 hours after oligonucleotide treatment.

[0168] 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 (TCCGT-CATCGCTCCTCAGGG, SEQ ID NO: 1) which is targeted to human H-ras, or ISIS 18078, (GTGCGCGCGAGC-CCGAAATC, SEQ ID NO: 2) 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, ATGCAT-TCTGCCCCCAAGGA, SEQ ID NO: 3, 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

Analysis of Oligonucleotide Inhibition of
Diacylglycerol Acyltransferase 1 Expression

[0169] Antisense modulation of diacylglycerol acyltransferase 1 expression can be assayed in a variety of ways known in the art. For example, diacylglycerol acyltransferase 1 mRNA levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or real-time PCR (RT-PCR). Real-time quantitative PCR is presently preferred. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. The preferred 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, Calif. and used according to manufacturer's instructions.

[0170] Protein levels of diacylglycerol acyltransferase 1 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 diacylglycerol acyltransferase 1 can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, Mich.), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art.

Example 11

Design of Phenotypic Assays and in Vivo Studies
for the Use of Diacylglycerol Acyltransferase 1
Inhibitors

[0171] Phenotypic Assays

[0172] Once diacylglycerol acyltransferase 1 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.

[0173] 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 diacylglycerol acyltransferase 1 in health and disease. Representative phenotypic assays, which can be purchased from any one of several commercial vendors, include those for determining cell viability, cytotoxicity, proliferation or cell survival (Molecular Probes, Eugene, Oreg.; PerkinElmer, Boston, Mass.), protein-based assays including enzymatic assays (Panvera, LLC, Madison, Wis.; BD Biosciences, Franklin Lakes, N.J.; Oncogene Research Products, San Diego, Calif.), cell regulation, signal transduction, inflammation, oxidative processes and apoptosis (Assay Designs Inc., Ann Arbor, Mich.), triglyceride accumulation (Sigma-Aldrich, St. Louis, Mo.), angiogenesis assays, tube formation assays, cytokine and hormone assays and metabolic assays (Chemicon International Inc., Temecula, Calif.; Amersham Biosciences, Piscataway, N.J.).

[0174] 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 diacylglycerol acyltransferase 1 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.

[0175] 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.

[0176] 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 diacylglycerol acyltransferase 1 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.

[0177] *In Vivo* Studies

[0178] The individual subjects of the *in vivo* studies described herein are warm-blooded vertebrate animals, which includes humans.

[0179] 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 diacylglycerol acyltransferase 1 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 diacylglycerol acyltransferase 1 inhibitor or a placebo. Using this randomization approach, each volunteer has the same chance of being given either the new treatment or the placebo.

[0180] Volunteers receive either the diacylglycerol acyltransferase 1 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 diacylglycerol acyltransferase 1 or diacylglycerol acyltransferase 1 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.

[0181] 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.

[0182] 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 diacylglycerol acyltransferase 1 inhibitor treatment. In general, the volunteers treated with placebo have little or no response to treatment, whereas the volunteers treated with the diacylglycerol acyltransferase 1 inhibitor show positive trends in their disease state or condition index at the conclusion of the study.

Example 12

RNA Isolation

[0183] Poly(A)+ mRNA Isolation

[0184] Poly(A)+ mRNA was isolated according to Miura et al., (*Clin. Chem.*, 1996, 42, 1758-1764). Other methods for poly(A)+mRNA isolation are routine in the art. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200 μ L cold PBS. 60 μ L 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 μ L of lysate was transferred to Oligo d(T) coated 96-well plates (AGCT Inc., Irvine, Calif.). Plates were incubated for 60 minutes at room temperature, washed 3 times with 200 μ L 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 μ L 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.

[0185] Cells grown on 100 mm or other standard plates may be treated similarly, using appropriate volumes of all solutions.

[0186] Total RNA Isolation

[0187] Total RNA was isolated using an RNEASY 96™ kit and buffers purchased from Qiagen Inc. (Valencia, Calif.) following the manufacturer's recommended procedures. Briefly, for cells grown on 96-well plates, growth medium was removed from the cells and each well was washed with 200 μ L cold PBS. 150 μ L Buffer RLT was added to each well and the plate vigorously agitated for 20 seconds. 150 μ L 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 1 minute. 500 μ L of Buffer RW1 was added to each well of the RNEASY 96™ plate and incubated for 15 minutes and the vacuum was again applied for 1 minute. An additional 500 μ L of Buffer RW1 was added to each well of the RNEASY 96™ plate and the vacuum was applied for 2 minutes. 1 mL of Buffer RPE was then added to each well of the RNEASY 96™ plate and the vacuum applied for a period of 90 seconds. The Buffer RPE wash was then repeated and the vacuum was applied for an additional 3 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 140 μ L of RNase free water into each well, incubating 1 minute, and then applying the vacuum for 3 minutes.

[0188] The repetitive pipetting and elution steps may be automated using a QIAGEN Bio-Robot 9604 (Qiagen, Inc., Valencia, Calif.). Essentially, after lysing of the cells on the culture plate, the plate is transferred to the robot deck where the pipetting, DNase treatment and elution steps are carried out.

Example 13

Real-time Quantitative PCR Analysis of Diacylglycerol Acyltransferase 1 mRNA Levels

[0189] Quantitation of diacylglycerol acyltransferase 1 mRNA levels was accomplished by real-time quantitative PCR using the ABI PRISM™ 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, Calif.) according to manufacturer's instructions. This is a closed-tube, non-gel-based, fluorescence detection system which allows high-throughput quantitation of polymerase chain reaction (PCR) products in real-time. As opposed to standard PCR in which amplification products are quantitated after the PCR is completed, products in real-time quantitative PCR are quantitated as they accumulate. This is accomplished by including in the PCR reaction an oligonucleotide probe that anneals specifically between the forward and reverse PCR primers, and contains two fluorescent dyes. A reporter dye (e.g., FAM or JOE, obtained from either PE-Applied Biosystems, Foster City, Calif., Operon Technologies Inc., Alameda, Calif. or Integrated DNA Technologies Inc., Coralville, Iowa) is attached to the 5' end of the probe and a quencher dye (e.g., TAMRA, obtained from either PE-Applied Biosystems, Foster City, Calif., Operon Technologies Inc., Alameda, Calif. or Integrated DNA Technologies Inc., Coralville, Iowa) is attached to the 3' end of the probe. When the probe and dyes are intact, reporter dye emission is quenched by the proximity of the 3' quencher dye. During amplification, annealing of the probe to the target sequence creates a substrate that can be cleaved by the 5'-exonuclease activity of Taq polymerase. During the extension phase of the PCR amplification cycle, cleavage of the probe by Taq polymerase releases the reporter dye from the remainder of the probe (and hence from the quencher moiety) and a sequence-specific fluorescent signal is gener-

ated. 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.

[0190] 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.

[0191] PCR reagents were obtained from Invitrogen Corporation, (Carlsbad, Calif.). RT-PCR reactions were carried out by adding 30 μ L PCR cocktail (2.5 $\times$ PCR buffer minus $MgCl_2$, 6.6 mM $MgCl_2$, 375 μ M 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 2.5 $\times$ ROX dye) to 96-well plates containing 20 μ L 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, 45 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).

[0192] Gene target quantities obtained by real time RT-PCR are normalized using either the expression level of GAPDH, a gene whose expression is constant, or by quantifying total RNA using RiboGreen™ (Molecular Probes, Inc. Eugene, Oreg.). GAPDH expression is quantified by real time RT-PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RiboGreen™ RNA quantification reagent (Molecular Probes, Inc. Eugene, Oreg.). Methods of RNA quantification by RiboGreen™ are taught in Jones, L. J., et al, (Analytical Biochemistry, 1998, 265, 368-374).

[0193] In this assay, 180 μ L of RiboGreen™ working reagent (RiboGreen™ reagent diluted 1:350 in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5) is pipetted into a 96-well plate containing 20 μ L purified, cellular RNA. The plate is read in a CytoFluor 4000 (PE Applied Biosystems) with excitation at 485 nm and emission at 530 nm.

[0194] Probes and primers to human diacylglycerol acyltransferase 1 were designed to hybridize to a human diacylglycerol acyltransferase 1 sequence, using published sequence information (GenBank accession number NM_012079.2, incorporated herein as SEQ ID NO:4). For human diacylglycerol acyltransferase 1 the PCR primers were: forward primer: TCCCCGCATCCGGAA (SEQ ID NO: 5) reverse primer: CTGGGTGAAGAACAGCATCTCA (SEQ ID NO: 6) and the PCR probe was: FAM-CGCTTTCTGCTGCGACGGATCC-TAMRA (SEQ ID NO: 7) where FAM is the fluorescent dye and TAMRA is the quencher dye. For human GAPDH the PCR primers were: forward primer: GAAGGTGAAGGTCGGAGTC- (SEQ ID NO:8) reverse primer: GAAGATGGTGATGGGATTTC (SEQ ID NO:9) and the PCR probe was: 5' JOE-CAAGCTTCCCGTTCTCAGCC-TAMRA 3' (SEQ ID NO: 10) where JOE is the fluorescent reporter dye and TAMRA is the quencher dye.

[0195] Probes and primers to mouse diacylglycerol acyltransferase 1 were designed to hybridize to a mouse diacylglycerol acyltransferase 1 sequence, using published sequence information (GenBank accession number AF078752.1, incorporated herein as SEQ ID NO:11). For mouse diacylglycerol acyltransferase 1 the PCR primers were:

[0196] forward primer: GTTCCGCCTCTGGGCATT (SEQ ID NO:12)

[0197] reverse primer: GAATCGGCCCAACAATCCA (SEQ ID NO: 13) and the

[0198] PCR probe was: FAM-CAGCCATGATGGCT-CAGGTCCCACT-TAMRA (SEQ ID NO: 14) where FAM is the fluorescent reporter dye and TAMRA is the quencher dye. For mouse GAPDH the PCR primers were:

[0199] forward primer: GGCAAATTCAACGGCA-CAGT(SEQ ID NO:15)

[0200] reverse primer: GGGTCTCGCTCCTGGAA-GAT(SEQ ID NO:16) and the

[0201] PCR probe was: 5' JOE-AAGGC-CGAGAAATGGGAAGCTTGTCATC- TAMRA 3' (SEQ ID NO: 17) where JOE is the fluorescent reporter dye and TAMRA is the quencher dye.

Example 14

Northern Blot Analysis of Diacylglycerol Acyltransferase 1 mRNA Levels

[0202] Eighteen hours after antisense treatment, cell monolayers were washed twice with cold PBS and lysed in 1 mL RNazol™ (TEL-TEST “B” Inc., Friendswood, Tex.). Total RNA was prepared following manufacturer's recommended protocols. Twenty micrograms of total RNA was fractionated by electrophoresis through 1.2% agarose gels containing 1.1% formaldehyde using a MOPS buffer system (AMRESCO, Inc. Solon, Ohio). RNA was transferred from the gel to HYBOND™-N+ nylon membranes (Amersham Pharmacia Biotech, Piscataway, N.J.) by overnight capillary transfer using a Northern/Southern Transfer buffer system (TEL-TEST “B” Inc., Friendswood, Tex.). RNA transfer was confirmed by UV visualization. Mem-

branes were fixed by UV cross-linking using a STRATALINKER™ UV Crosslinker 2400 (Stratagene, Inc, La Jolla, Calif.) and then probed using QUICKHYB™ hybridization solution (Stratagene, La Jolla, Calif.) using manufacturer's recommendations for stringent conditions.

[0203] To detect human diacylglycerol acyltransferase 1, a human diacylglycerol acyltransferase 1 specific probe was prepared by PCR using the forward primer TCCCCGCATCCGGAA (SEQ ID NO: 5) and the reverse primer CTGGGTGAAGAACAGCATCTCA (SEQ ID NO: 6). To normalize for variations in loading and transfer efficiency membranes were stripped and probed for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA (Clontech, Palo Alto, Calif.).

[0204] To detect mouse diacylglycerol acyltransferase 1, a mouse diacylglycerol acyltransferase 1 specific probe was prepared by PCR using the forward primer GTTCCGCCTCTGGGCATT (SEQ ID NO: 12) and the reverse primer GAATCGGCCCAACAATCCA (SEQ ID NO: 13). To normalize for variations in loading and transfer efficiency membranes were stripped and probed for mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA (Clontech, Palo Alto, Calif.).

[0205] Hybridized membranes were visualized and quantitated using a PHOSPHORIMAGER™ and IMAGEQUANT™ Software V3.3 (Molecular Dynamics, Sunnyvale, Calif.). Data was normalized to GAPDH levels in untreated controls.

Example 15

Antisense Inhibition of Human Diacylglycerol Acyltransferase 1 Expression by Chimeric Phosphorothioate Oligonucleotides Having 2'-MOE Wings and a Deoxy Gap

[0206] In accordance with the present invention, a series of antisense compounds were designed to target different regions of the human diacylglycerol acyltransferase 1 RNA, using published sequences (GenBank accession number NM_012079.2, incorporated herein as SEQ ID NO: 4). The 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 diacylglycerol acyltransferase 1 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 diacylglycerol acyltransferase 1 mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap							
ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% INHIB	SEQ ID NO	CONTROL SEQ ID NO
191617	5' UTR	4	1	gccgcctctctcgtccattc	57	18	1
191619	5' UTR	4	21	gagccgctaactaatggacg	37	19	1
191621	5' UTR	4	41	acaacgggtgcgttgctccg	30	20	1
191623	5' UTR	4	71	ccgcccgcgtcaggcccgtc	40	21	1
191625	5' UTR	4	91	gcctcaccagcgcgttcaac	20	22	1
191627	5' UTR	4	120	ccctgccggccgcgtagcc	24	23	1
191629	5' UTR	4	151	ctccggggccctagacaacgg	45	24	1
191631	5' UTR	4	181	gttcgtagcggccgaggcgc	53	25	1
191633	5' UTR	4	211	cccggccgcagccaagcgtg	44	26	1
191635	Start	4	231	gcccattggcctcagcccga	77	27	1
Codon							
191637	Coding	4	281	tggetcggaggccgcgaccc	58	28	1
191639	Coding	4	301	ccgcaggcccgccgcgccc	49	29	1
191641	Coding	4	321	ccgcacctcttcttcgcccg	40	30	1
191643	Coding	4	401	acgccggcgctctcgtcctt	92	31	1
191645	Coding	4	421	gctcccagtgccgctgccc	60	32	1
191647	Coding	4	441	ctgcaggcgatggcacctca	85	33	1
191649	Coding	4	491	aggatgccacggtagttgct	62	34	1
191651	Coding	4	511	gcatcaccacacaccagttc	37	35	1
191653	Coding	4	561	gccatacttgatgaggttct	48	36	1
191655	Coding	4	651	gacattggccgcaataacca	47	37	1
191657	Coding	4	681	cttctcaacctggaatgcag	29	38	1
191659	Coding	4	721	gcagtcccgcctgctccgtc	50	39	1
191661	Coding	4	741	caggttggctacgtgcagca	31	40	1
191663	Coding	4	781	ccagtaagaccacagccgct	62	41	1
191665	Coding	4	831	ggtgtgcgccatcagcgcca	59	42	1
191667	Coding	4	931	cagcactgctggccttcttc	52	43	1
191669	Coding	4	1021	tgagctcgtagcacaagggtg	43	44	1
191671	Coding	4	1121	cactgctggatcagccccac	20	45	1
191673	Coding	4	1181	atgcgtgagtagtccatgtc	59	46	1
191675	Coding	4	1231	tgagccagatgaggtgattg	62	47	1
191677	Coding	4	1281	gagctcagccacggcattca	76	48	1
191679	Coding	4	1351	tctgccagaagtaggtgaca	30	49	1
191681	Coding	4	1611	gatgagcgacagccacacag	21	50	1

TABLE 1-continued

Inhibition of human diacylglycerol acyltransferase 1 mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap						
ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE SEQUENCE	% INHIB	SEQ ID NO	CONTROL SEQ ID NO
191683	Coding	4	1671 ctcatagttgagcacgtagt	73	51	1
191685	3' UTR	4	1721 cagtgagaagccaggccctc	68	52	1
191687	3' UTR	4	1781 ccatccccagcactcgaggc	68	53	1
191689	3' UTR	4	1801 aggatgctgtgcagccaggc	73	54	1
191691	3' UTR	4	1851 ggtgcaggacagagcccat	72	55	1
191693	3' UTR	4	1881 gtgtctggcctgtgtcgcc	71	56	1
191695	3' UTR	4	1901 ctcccagctggcatcagact	76	57	1

[0207] As shown in Table 1, SEQ ID NOs 18, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 36, 37, 39, 41, 42, 43, 44, 46, 47, 48, 51, 52, 53, 54, 55, 56 and 57 demonstrated at least 43% inhibition of human diacylglycerol acyltransferase 1 expression in this assay and are therefore preferred. More preferred are SEQ ID NOs 31, 33, 27, and 57. The target regions to which these preferred sequences are complementary are herein referred to as "preferred target segments" and are therefore preferred for targeting by compounds of the present invention. These preferred target segments are shown in Table 3. The sequences represent the reverse complement of the preferred 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 preferred target segments was found.

Example 16

Antisense Inhibition of Mouse Diacylglycerol
Acyltransferase 1 Expression by Chimeric
Phosphorothioate Oligonucleotides Having 2'-MOE
Wings and a Deoxy Gap

[0208] In accordance with the present invention, a second series of antisense compounds were designed to target

different regions of the mouse diacylglycerol acyltransferase 1 RNA, using published sequences (GenBank accession number AF078752.1, incorporated herein as SEQ ID NO: 11). 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 ("gap-mers") 20 nucleotides in length, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by five-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE)nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P=S) throughout the oligonucleotide. All cytidine residues are 5-methylcytidines. The compounds were analyzed for their effect on mouse diacylglycerol acyltransferase 1 mRNA levels by quantitative real-time PCR as described in other examples herein. Data are averages from three experiments in which b.END cells were treated with the antisense oligonucleotides of the present invention. If present, "N.D." indicates "no data".

TABLE 2

Inhibition of mouse diacylglycerol acyltransferase 1 mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap						
ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE SEQUENCE	% INHIB	SEQ ID NO	
191723	5' UTR	11	1 ctacttatttccattcatcc	2	58	
191724	5' UTR	11	21 taccctaagtatgcctaatt	0	59	
191725	5' UTR	11	31 gcttgagccctatccctaagt	0	60	
191726	5' UTR	11	61 ctcgctcgggcccaatcttc	21	61	

TABLE 2-continued

Inhibition of mouse diacylglycerol acyltransferase 1 mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap							
ISIS #	REGION	TARGET	SEQ ID	TARGET SITE	SEQUENCE	% INHIB	SEQ ID NO
		NO					
191727	Start	11		81	cccatggcttcggcccgac	48	62
	Codon						
191729	Coding	11		191	cagccgcgtctcgcacctcg	74	63
191730	Coding	11		232	cggagccggcgcgtaacccc	63	64
191731	Coding	11		281	ccacgctgggtccgcccgtct	67	65
191732	Coding	11		301	cagatcccagtagccgtcgc	59	66
191733	Coding	11		321	tcttgacagacgatggcacct	49	67
191734	Coding	11		371	tcaggataccacgataattg	48	68
191735	Coding	11		391	cagcatcaccacacaccaat	52	69
191736	Coding	11		411	aaccttgacattactcaggat	62	70
191737	Coding	11		451	atccaccaggatgccatact	29	71
191738	Coding	11		471	agagacaccacctggatagg	42	72
191740	Coding	11		601	cagcagcccatctgtctctg	63	73
191741	Coding	11		621	gccaggtaaccacatgtag	58	74
191742	Coding	11		661	aaccagtaaggccacagctg	16	75
191743	Coding	11		681	cccactggagtgatagactc	42	76
191744	Coding	11		711	atggagtatgatgccagagc	53	77
191745	Coding	11		771	acccttcgctggcgccacca	68	78
191746	Coding	11		841	tggatagctcacagcttgct	56	79
191747	Coding	11		861	tctcggtaggtcaggttgtc	32	80
191748	Coding	11		961	ctcaagaactcgtcgtagca	60	81
191749	Coding	11		1001	gttggatcagccccacttga	37	82
191750	Coding	11		1061	gtgaatagtccatatccttg	48	83
191751	Coding	11		1081	taagagacgctcaatgatcc	18	84
191752	Coding	11		1161	tctgccacagcattgagaca	50	85
191753	Coding	11		1201	ccaatctctgtgaaactcgc	55	86
191754	Coding	11		1221	gtgacagactcagcattcca	56	87
191755	Coding	11		1271	gtctgatgcaccacttgctc	72	88
191756	Coding	11		1301	tgccatgtctgagcataggc	70	89
191757	Coding	11		1331	atactcctgtcctggccacc	65	90
191759	Coding	11		1471	attgccatagttcccttgga	68	91
191760	Coding	11		1491	agtgtcaccacacagctgc	66	92
191761	Coding	11		1511	ccaccggttgcccaatgatg	71	93

TABLE 2-continued

Inhibition of mouse diacylglycerol acyltransferase 1 mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap						
ISIS #	REGION	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	% INHIB	SEQ ID NO
191762	Coding	11	1531	gtggacatacatgagcacag	62	94
191763	Coding	11	1551	tagttgagcacgtagtagtc	40	95
191764	Stop	11	1586	ctttggcagtagctcatacc	37	96
Codon						
191765	3' UTR	11	1621	tccagaactccaggcccagg	59	97

[0209] As shown in Table 2, SEQ ID NOs 62, 63, 64, 65, 66, 67, 68, 69, 70, 73, 74, 77, 78, 79, 81, 83, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94 and 97 demonstrated at least 43% inhibition of mouse diacylglycerol acyltransferase 1 expression in this experiment and are therefore preferred. More preferred are SEQ ID Nos: 63, 88, 91, and 93. The target regions to which these preferred sequences are complementary are herein referred to as "preferred target segments" and are therefore preferred for targeting by compounds of the

present invention. These preferred target segments of the mRNA are shown in Table 3 as the appropriate RNA sequence, where thymine (T) has been replaced with uracil (U) to reflect correct representation of an RNA sequence. The sequences represent the reverse complement of the preferred 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 preferred target segments was found.

TABLE 3

Sequence and position of preferred target segments identified in diacylglycerol acyltransferase 1.							
SITE ID	TARGET SEQ ID NO	TARGET SITE	SEQUENCE	REV COMP OF SEQ	COMP ID	ACTIVE IN	SEQ ID NO
108088	4	1	gaauggacgagagagcggc	18	<i>H. sapiens</i>		98
108094	4	151	ccguugucuaagggcccgag	24	<i>H. sapiens</i>		99
108095	4	181	gcgccucggcgcuacgaac	25	<i>H. sapiens</i>		100
108096	4	211	cacgcuuggcugcgccggg	26	<i>H. sapiens</i>		101
108097	4	231	ugcgggcugagggcaugggc	27	<i>H. sapiens</i>		102
108098	4	281	gggucqcgggccucgagcca	28	<i>H. sapiens</i>		103
108099	4	301	cggcgggcggggcccugcgg	29	<i>H. sapiens</i>		104
108101	4	401	aaggacggagacgcggcgcu	31	<i>H. sapiens</i>		105
108102	4	421	gggcagcgggccacugggagc	32	<i>H. sapiens</i>		106
108103	4	441	ugaggugccaucgccugcag	33	<i>H. sapiens</i>		107
108104	4	491	agcaacuaccguggcauccu	34	<i>H. sapiens</i>		108
108106	4	561	agaaccucaucaaguauggc	36	<i>H. sapiens</i>		109
108107	4	651	ugguuauugcgggccaauguc	37	<i>H. sapiens</i>		110
108109	4	721	gacggagcaggcgggacugc	39	<i>H. sapiens</i>		111
108111	4	781	agcggcuguggucuacugg	41	<i>H. sapiens</i>		112

TABLE 3-continued

Sequence and position of preferred target segments identified in diacylglycerol acyltransferase 1.							
TARGET		TARGET		REV COMP		SEQ ID	
SITE	SEQ ID	SITE	SEQUENCE	OF SEQ ID	ACTIVE IN	NO	NO
ID	NO	ID					
108112	4	831	uggcgcugaugggcgacacc	42	<i>H. sapiens</i>		113
108113	4	931	gaagaagggcagcagugcug	43	<i>H. sapiens</i>		114
108114	4	1021	caccuugugcuacgagcuca	44	<i>H. sapiens</i>		115
108116	4	1181	gacauggacuacucacgcgau	46	<i>H. sapiens</i>		116
108117	4	1231	caaucaccucaucuggcuca	47	<i>H. sapiens</i>		117
108118	4	1281	ugaaucccguggcugagcuc	48	<i>H. sapiens</i>		118
108121	4	1671	acuacgugcucaacuagag	51	<i>H. sapiens</i>		119
108122	4	1721	gagggccuggcucucacug	52	<i>H. sapiens</i>		120
108123	4	1781	gccucgagugcugggggaug	53	<i>H. sapiens</i>		121
108124	4	1801	gccuggcugcacagcauccu	54	<i>H. sapiens</i>		122
108125	4	1851	auggggcucuguccugcacc	55	<i>H. sapiens</i>		123
108126	4	1881	ggcgacagcagggcagacac	56	<i>H. sapiens</i>		124
108127	4	1901	agucugaugccagcugggag	57	<i>H. sapiens</i>		125
108139	11	81	gugcgggcccgaagccauggg	62	<i>M. musculus</i>		126
108141	11	191	cgaggugcgagacgcggcug	63	<i>M. musculus</i>		127
108142	11	232	ggggugacgcgccggcuccg	64	<i>M. musculus</i>		128
108143	11	281	agacgggcccagcagcuggg	65	<i>M. musculus</i>		129
108144	11	301	gcgacggcuacugggauucg	66	<i>M. musculus</i>		130
108145	11	321	aggugccaucgucugcaaga	67	<i>M. musculus</i>		131
108146	11	371	caauuauugguuauccuga	68	<i>M. musculus</i>		132
108147	11	391	auuggugugugugaugcug	69	<i>M. musculus</i>		133
108148	11	411	auccugaguaaugcaagguu	70	<i>M. musculus</i>		134
108152	11	601	cagagcagauggggcugcug	73	<i>M. musculus</i>		135
108153	11	621	cuacaugugguuaccuggc	74	<i>M. musculus</i>		136
108156	11	711	gcucuggcaucauacuccau	77	<i>M. musculus</i>		137
108157	11	771	uggugccgccagcgaagguu	78	<i>M. musculus</i>		138
108158	11	841	agcaagcugugagcuaucca	79	<i>M. musculus</i>		139
108160	11	961	ugcuacgacgaguucugag	81	<i>M. musculus</i>		140
108162	11	1061	caaggauauggacuauucac	83	<i>M. musculus</i>		141
108164	11	1161	ugucucaaugcugggcaga	85	<i>M. musculus</i>		142
108165	11	1201	gcgaguucucacagauugg	86	<i>M. musculus</i>		143
108166	11	1221	uggaauugcugagucugucac	87	<i>M. musculus</i>		144
108167	11	1271	gcacaaguggugcaucagac	88	<i>M. musculus</i>		145
108168	11	1301	gccuauugcucagacauggca	89	<i>M. musculus</i>		146

TABLE 3—continued

Sequence and position of preferred target segments identified in diacylglycerol acyltransferase 1.						
TARGET		REV COMP		SEQ ID		
SITE	SEQ ID	TARGET	OF SEQ ID	ACTIVE	IN	NO
ID	NO	SITE SEQUENCE				
108169	11	1331 gguggccaggacaggaguau	90	<i>M. musculus</i>		147
108171	11	1471 uccaaggggaacuauggcaau	91	<i>M. musculus</i>		148
108172	11	1491 gcagcugugugggugacacu	92	<i>M. musculus</i>		149
108173	11	1511 caucauugggcaaccggugg	93	<i>M. musculus</i>		150
108174	11	1531 cugugcucauguauguccac	94	<i>M. musculus</i>		151
108177	11	1621 ccugggccuggaguucugga	97	<i>M. musculus</i>		152

[0210] As these “preferred 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 preferred target segments and consequently inhibit the expression of diacylglycerol acyltransferase 1.

[0211] 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 17

Western Blot Analysis of Diacylglycerol Acyltransferase 1 Protein Levels

[0212] 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 μ l/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 diacylglycerol acyltransferase 1 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, Calif.).

Example 18

Antisense Inhibition of Diacylglycerol Acyltransferase 1 Expression—Dose Response in HepG2 Cells

[0213] In accordance with the present invention, 6 oligonucleotides targeted to diacylglycerol acyltransferase 1, ISIS 191729 (SEQ ID NO: 63), ISIS 191731 (SEQ ID NO: 65), ISIS 191755 (SEQ ID NO: 88), ISIS 191756 (SEQ ID NO: 89),

[0214] ISIS 191759 (SEQ ID NO: 91), and ISIS 191761 (SEQ ID NO: 93), were further investigated in a dose response study.

[0215] In the dose-response experiment, with mRNA levels as the endpoint, HepG2 cells were treated with ISIS 191729, ISIS 191731, ISIS 191755, ISIS 191756, ISIS 191759, or ISIS 191761 at doses of 1, 5, 10, 25, 50, and 100 nM oligonucleotide. Data were obtained by real-time quantitative PCR as described in other examples herein and are averaged from two experiments with mRNA levels in the treatment groups being normalized to an untreated control group. The data are shown in Table 4.

TABLE 4

Inhibition of diacylglycerol acyltransferase 1 mRNA levels by chimeric phosphorothioate oligonucleotides having 2'-MOE wings and a deoxy gap - Dose Response						
ISIS NO.	Dose (nM)					
	1	5	10	25	50	100
% Inhibition						
191729	26	62	78	80	83	83
191731	27	58	57	58	82	85
191755	41	59	72	75	83	79
191756	13	39	59	65	81	75
191759	26	44	74	80	82	86
191761	23	63	71	80	85	87

[0216] From these data, it is evident that all of the oligonucleotides presented in Table 4 are capable of reducing diacylglycerol acyltransferase 1 mRNA levels in a dose-dependent manner.

Example 19

Effects of Antisense Inhibition of Diacylglycerol Acyltransferase 1 (ISIS 191729 and ISIS 191755) on Serum Glucose Levels—In Vivo Studies

[0217] C57BL/6 ob/ob mice, a strain reported to be susceptible to hyperlipidemia-induced atherosclerotic plaque formation were used in the following studies to evaluate diacylglycerol acyltransferase 1 antisense oligonucleotides as potential agents to lower serum glucose levels.

[0218] Male C57BL/6 mice (n=8) receiving a Purina 5015 diet were evaluated over the course of 4 weeks for the effects of ISIS 191729 (SEQ ID No: 63) and ISIS 191755 (SEQ ID NO: 88) on serum glucose levels. Control animals received saline treatment. Mice were dosed intraperitoneally twice a week with 25 mg/kg ISIS 191729, ISIS 191755, or saline for 4 weeks.

[0219] Both antisense oligonucleotides were able to reduce serum glucose levels relative to the saline-treated animals. Before any treatment was started (week 0), the measured glucose levels for each group of animals were 357, 368, and 346 mg/dL for the groups which would be treated with saline, ISIS 191729, and ISIS 191755, respectively. After two weeks, serum glucose levels were 300 and 278 mg/dL for ISIS 191729 and ISIS 191755, respectively, compared to 360 mg/dL for saline control. After four weeks of treatment, the serum glucose levels were further reduced to 224 and 188 mg/dL for ISIS 191729 and ISIS 191755, respectively, compared to 313 mg/dL for saline control.

[0220] These data indicate that ISIS 191729 and ISIS 191755 are able to significantly reduce serum glucose levels in vivo. ISIS 191755 also caused no change in food intake or body weight, but reduced epididymal fat pad weight by 12%. (See Table 5 for a summary of in vivo data).

Example 20

Effects of Antisense Inhibition of Diacylglycerol Acyltransferase 1 (ISIS 191729 and ISIS 191755) on Diacylglycerol Acyltransferase 1 mRNA Levels in C57BL/6 Mice

[0221] Male C57BL/6 mice (n=8) receiving a Purina 5015 diet were evaluated after 4 weeks of treatment for the effects of ISIS 191729 (SEQ ID No: 63) and ISIS 191755 (SEQ ID NO: 88) to lower the level of diacylglycerol acyltransferase 1 mRNA levels in white adipose tissue and liver. Control animals received saline treatment. Mice were dosed intraperitoneally twice a week with 25 mg/kg ISIS 191729, ISIS 191755, or saline for 4 weeks.

[0222] The diacylglycerol acyltransferase 1 mRNA levels in white adipose tissue of the mice dosed with ISIS 191729

were 29% that of the saline treated mice, and those dosed with ISIS 191755 were 16% that of the saline treated mice. The diacylglycerol acyltransferase 1 mRNA levels in liver of the mice dosed with ISIS 191729 were 8% that of the saline treated mice, and those dosed with ISIS 191755 were 4% that of the saline treated mice.

[0223] It has been reported in the art that diacylglycerol acyltransferase 1 knockout mice demonstrate enhanced resistance to diet-induced obesity and this was not coupled with changes in energy expenditure or plasma glucose levels in ob/ob mice due to a compensatory upregulation of diacylglycerol acyltransferase 2 expression in white adipose tissue. The results of studies described herein using antisense compounds to transiently modulate diacylglycerol acyltransferase 1 mRNA levels are in contrast to those seen in the diacylglycerol acyltransferase 1 knockout studies. The results shown herein indicate that antisense oligonucleotides ISIS 191729 and ISIS 191755 are able to reduce diacylglycerol acyltransferase 1 mRNA levels, reduce serum glucose levels, and reduce fat pad weight while not affecting food intake and total body weight. (See Table 5 for a summary of in vivo data).

TABLE 5

Effects of ISIS 191729 or ISIS 191755 treatment on serum glucose levels and diacylglycerol acyltransferase 1 mRNA levels in C57BL/6 mice.				
		Treated with		
Biological Marker Measured		saline	ISIS 191729	ISIS 191755
	week			
Glucose mg/dL	0	357	368	346
	2	360	300	278
	4	313	224	188
	tissue			
mRNA	Liver	100	8	4
% of control	White adipose	100	29	16

[0224]

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 152

<210> SEQ ID NO 1

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 1

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20

<210> SEQ ID NO 2

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 2

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<210> SEQ ID NO 3
<211> LENGTH: 20
<212> TYPE: DNA
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 3

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<210> SEQ ID NO 4
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<222> LOCATION: (245)...(1711)

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gctacggcgg ccggcagggg gcagtggcgg ccgttgtcta gggcccggag gtggggccgc    180
gcgcctcggg cgctacgaac ccggcaggcc cacgcttggc tgcggccggg tgcgggctga    240
ggcc atg ggc gac cgc ggc agc tcc cgg cgc cgg agg aca ggg tcg cgg    289
      Met Gly Asp Arg Gly Ser Ser Arg Arg Arg Arg Thr Gly Ser Arg
        1           5           10          15

ccc tcg agc cac ggc ggc ggc ggg cct gcg gcg gcg gaa gaa gag gtg    337
Pro Ser Ser His Gly Gly Gly Pro Ala Ala Ala Glu Glu Glu Val
          20          25          30

cgg gac gcc gct gcg ggc ccc gac gtg gga gcc gcg ggg gac gcg cca    385
Arg Asp Ala Ala Ala Gly Pro Asp Val Gly Ala Ala Gly Asp Ala Pro
          35          40          45

gcc ccg gcc ccc aac aag gac gga gac gcc ggc gtg ggc agc ggc cac    433
Ala Pro Ala Pro Asn Lys Asp Gly Asp Ala Gly Val Gly Ser Gly His
          50          55          60

tgg gag ctg agg tgc cat cgc ctg cag gat tct tta ttc agc tct gac    481
Trp Glu Leu Arg Cys His Arg Leu Gln Asp Ser Leu Phe Ser Ser Asp
          65          70          75

agt ggc ttc agc aac tac cgt ggc atc ctg aac tgg tgt gtg gtg atg    529
Ser Gly Phe Ser Asn Tyr Arg Gly Ile Leu Asn Trp Cys Val Val Met
          80          85          90          95

ctg atc ttg agc aat gcc cgg tta ttt ctg gag aac ctc atc aag tat    577
Leu Ile Leu Ser Asn Ala Arg Leu Phe Leu Glu Asn Leu Ile Lys Tyr
          100         105         110

ggc atc ctg gtg gac ccc atc cag gtg gtt tct ctg ttc ctg aag gat    625
Gly Ile Leu Val Asp Pro Ile Gln Val Val Ser Leu Phe Leu Lys Asp
          115         120         125

ccc cat agc tgg ccc gcc cca tgc ctg gtt att gcg gcc aat gtc ttt    673
Pro His Ser Trp Pro Ala Pro Cys Leu Val Ile Ala Ala Asn Val Phe
          130         135         140

gct gtg gct gca ttc cag gtt gag aag cgc ctg gcg gtg ggt gcc ctg    721

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Ala Val Ala Ala Phe Gln Val Glu Lys Arg Leu Ala Val Gly Ala Leu	
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160 165 170 175	
ctg tgt ttc cca gcg gct gtg gtc tta ctg gtt gag tct atc act cca	817
Leu Cys Phe Pro Ala Ala Val Val Leu Leu Val Glu Ser Ile Thr Pro	
180 185 190	
gtg ggc tcc ctg ctg gcg ctg atg gcg cac acc atc ctc ttc ctc aag	865
Val Gly Ser Leu Leu Ala Leu Met Ala His Thr Ile Leu Phe Leu Lys	
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Leu Phe Ser Tyr Arg Asp Val Asn Ser Trp Cys Arg Arg Ala Arg Ala	
210 215 220	
aag gct gcc tct gca ggg aag aag gcc agc agt gct gct gcc ccg cac	961
Lys Ala Ala Ser Ala Gly Lys Lys Ala Ser Ser Ala Ala Pro His	
225 230 235	
acc gtg agc tac ccg gac aat ctg acc tac cgc gat ctc tac tac ttc	1009
Thr Val Ser Tyr Pro Asp Asn Leu Thr Tyr Arg Asp Leu Tyr Tyr Phe	
240 245 250 255	
ctc ttc gcc ccc acc ttg tgc tac gag ctc aac ttt ccc cgc tct ccc	1057
Leu Phe Ala Pro Thr Leu Cys Tyr Glu Leu Asn Phe Pro Arg Ser Pro	
260 265 270	
cgc atc cgg aag cgc ttt ctg ctg cga cgg atc ctt gag atg ctg ttc	1105
Arg Ile Arg Lys Arg Phe Leu Leu Arg Arg Ile Leu Glu Met Leu Phe	
275 280 285	
ttc acc cag ctc cag gtg ggg ctg atc cag cag tgg atg gtc ccc acc	1153
Phe Thr Gln Leu Gln Val Gly Leu Ile Gln Gln Trp Met Val Pro Thr	
290 295 300	
atc cag aac tcc atg aag ccc ttc aag gac atg gac tac tca cgc atc	1201
Ile Gln Asn Ser Met Lys Pro Phe Lys Asp Met Asp Tyr Ser Arg Ile	
305 310 315	
atc gag cgc ctc ctg aag ctg gcg gtc ccc aat cac ctc atc tgg ctc	1249
Ile Glu Arg Leu Leu Lys Leu Ala Val Pro Asn His Leu Ile Trp Leu	
320 325 330 335	
atc ttc ttc tac tgg ctc ttc cac tcc tgc ctg aat gcc gtg gct gag	1297
Ile Phe Phe Tyr Trp Leu Phe His Ser Cys Leu Asn Ala Val Ala Glu	
340 345 350	
ctc atg cag ttt gga gac cgg gag ttc tac cgg gac tgg tgg aac tcc	1345
Leu Met Gln Phe Gly Asp Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ser	
355 360 365	
gag tct gtc acc tac ttc tgg cag aac tgg aac atc cct gtg cac aag	1393
Glu Ser Val Thr Tyr Phe Trp Gln Asn Trp Asn Ile Pro Val His Lys	
370 375 380	
tgg tgc atc aga cac ttc tac aag ccc atg ctt cga cgg gcc agc agc	1441
Trp Cys Ile Arg His Phe Tyr Lys Pro Met Leu Arg Arg Gly Ser Ser	
385 390 395	
aag tgg atg gcc agg aca ggg gtg ttc ctg gcc tgc gct ttc ttc cac	1489
Lys Trp Met Ala Arg Thr Gly Val Phe Leu Ala Ser Ala Phe Phe His	
400 405 410 415	
gag tac ctg gtg agc gtc cct ctg cga atg ttc cgc ctc tgg gct ttc	1537
Glu Tyr Leu Val Ser Val Pro Leu Arg Met Phe Arg Leu Trp Ala Phe	
420 425 430	
acg gcc atg atg gct cag atc cca ctg gcc tgg ttc gtg gcc cgc ttt	1585
Thr Gly Met Met Ala Gln Ile Pro Leu Ala Trp Phe Val Gly Arg Phe	
435 440 445	
ttc cag gcc aac tat ggc aac gca gct gtg tgg ctg tgc ctc atc atc	1633

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Phe Gln Gly Asn Tyr Gly Asn Ala Ala Val Trp Leu Ser Leu Ile Ile
450 455 460

gga cag cca ata gcc gtc ctc atg tac gtc cac gac tac tac gtg ctc 1681
Gly Gln Pro Ile Ala Val Leu Met Tyr Val His Asp Tyr Tyr Val Leu
465 470 475

aac tat gag gcc cca gcg gca gag gcc tga gctgcacctg agggcctggc 1731
Asn Tyr Glu Ala Pro Ala Ala Glu Ala
480 485

ttctcactgc cacctcaaac ccgctgccag agcccacctc tcctcctagg cctcgagtgc 1791

tggggatggg cctggctgca cagcatcctc ctctgggtccc agggaggcct ctctgcccta 1851

tggggctctg tcctgcacccc ctcagggatg gcgacagcag gccagacaca gtctgatgcc 1911

agctgggagt ctgtctgacc ctgccccggg tccgaggggtg tcaataaagt gctgtccagt 1971

gggag 1976

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tccccgcac cggaa 15

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ctgggtgaag aacagcatct ca 22

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cgctttctgc tgcgacggat cc 22

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<400> SEQUENCE: 8

gaaggtgaag gtcggagtc 19

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gaagatggtg atgggatttc

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caagcttccc gttctcagcc

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60

gaagattggg ccgcgacgag gtgcgggccc aagcc atg ggc gac cgc gga ggc
 Met Gly Asp Arg Gly Gly
 1 5

113

gcg gga agc tct cgg cgt cgg agg acc ggc tcg cgg gtt tcc gtc cag
 Ala Gly Ser Ser Arg Arg Arg Arg Thr Gly Ser Arg Val Ser Val Gln
 10 15 20

161

ggt ggt agt ggg ccc aag gta gaa gag gac gag gtg cga gac gcg gct
 Gly Gly Ser Gly Pro Lys Val Glu Glu Asp Glu Val Arg Asp Ala Ala
 25 30 35

209

gtg agc ccc gac ttg ggc gcc ggg ggt gac gcg ccg gct ccg gct ccg
 Val Ser Pro Asp Leu Gly Ala Gly Gly Asp Ala Pro Ala Pro Ala Pro
 40 45 50

257

gct cca gcc cat acc cgg gac aaa gac ggg cgg acc agc gtg ggc gac
 Ala Pro Ala His Thr Arg Asp Lys Asp Gly Arg Thr Ser Val Gly Asp
 55 60 65 70

305

ggc tac tgg gat ctg agg tgc cat cgt ctg caa gat tct ttg ttc agc
 Gly Tyr Trp Asp Leu Arg Cys His Arg Leu Gln Asp Ser Leu Phe Ser
 75 80 85

353

tca gac agt ggt ttc agc aat tat cgt ggt atc ctg aat tgg tgt gtg
 Ser Asp Ser Gly Phe Ser Asn Tyr Arg Gly Ile Leu Asn Trp Cys Val
 90 95 100

401

gtg atg ctg atc ctg agt aat gca agg tta ttt tta gag aac ctt atc
 Val Met Leu Ile Leu Ser Asn Ala Arg Leu Phe Leu Glu Asn Leu Ile
 105 110 115

449

aag tat ggc atc ctg gtg gat cct atc cag gtg gtg tct ctg ttt ttg
 Lys Tyr Gly Ile Leu Val Asp Pro Ile Gln Val Ser Leu Phe Leu
 120 125 130

497

aag gac ccc tac agc tgg cct gcc cca tgc gtg att att gca tcc aat
 Lys Asp Pro Tyr Ser Trp Pro Ala Pro Cys Val Ile Ile Ala Ser Asn
 135 140 145 150

545

att ttt gtt gtg gct gca ttt cag att gag aag cgc ctg gca gtg ggt
 Ile Phe Val Val Ala Ala Phe Gln Ile Glu Lys Arg Leu Ala Val Gly
 155 160 165

593

gcc ctg aca gag cag atg ggg ctg ctg cta cat gtg gtt aac ctg gcc

641

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Ala	Leu	Thr	Glu	Gln	Met	Gly	Leu	Leu	Leu	His	Val	Val	Asn	Leu	Ala	
			170					175					180			
aca	atc	att	tgc	ttc	cca	gca	gct	gtg	gcc	tta	ctg	ggt	gag	tct	atc	689
Thr	Ile	Ile	Cys	Phe	Pro	Ala	Ala	Val	Ala	Leu	Leu	Val	Glu	Ser	Ile	
		185					190					195				
act	cca	gtg	ggg	tcc	gtg	ttt	gct	ctg	gca	tca	tac	tcc	atc	atg	ttc	737
Thr	Pro	Val	Gly	Ser	Val	Phe	Ala	Leu	Ala	Ser	Tyr	Ser	Ile	Met	Phe	
	200					205					210					
ctc	aag	ctt	tat	tcc	tac	cgg	gat	gtc	aac	ctg	tgg	tgc	cgc	cag	cga	785
Leu	Lys	Leu	Tyr	Ser	Tyr	Arg	Asp	Val	Asn	Leu	Trp	Cys	Arg	Gln	Arg	
215					220				225					230		
agg	gtc	aag	gcc	aaa	gct	gtc	tct	aca	ggg	aag	aag	gtc	agt	ggg	gct	833
Arg	Val	Lys	Ala	Lys	Ala	Val	Ser	Thr	Gly	Lys	Lys	Val	Ser	Gly	Ala	
			235					240						245		
gct	gcc	cag	caa	gct	gtg	agc	tat	cca	gac	aac	ctg	acc	tac	cga	gat	881
Ala	Ala	Gln	Gln	Ala	Val	Ser	Tyr	Pro	Asp	Asn	Leu	Thr	Tyr	Arg	Asp	
		250						255					260			
ctc	tat	tac	ttc	atc	ttt	gct	cct	act	ttg	tgt	tat	gaa	ctc	aac	ttt	929
Leu	Tyr	Tyr	Phe	Ile	Phe	Ala	Pro	Thr	Leu	Cys	Tyr	Glu	Leu	Asn	Phe	
	265					270						275				
cct	cgg	tcc	ccc	cga	ata	cga	aag	cgc	ttt	ctg	cta	cga	cga	gtt	ctt	977
Pro	Arg	Ser	Pro	Arg	Ile	Arg	Lys	Arg	Phe	Leu	Leu	Arg	Arg	Val	Leu	
	280					285					290					
gag	atg	ctc	ttt	ttt	acc	cag	ctt	caa	gtg	ggg	ctg	atc	caa	cag	tgg	1025
Glu	Met	Leu	Phe	Phe	Thr	Gln	Leu	Gln	Val	Gly	Leu	Ile	Gln	Gln	Trp	
295					300				305					310		
atg	gtc	cct	act	atc	cag	aac	tcc	atg	aag	ccc	ttc	aag	gat	atg	gac	1073
Met	Val	Pro	Thr	Ile	Gln	Asn	Ser	Met	Lys	Pro	Phe	Lys	Asp	Met	Asp	
			315					320					325			
tat	tca	cgg	atc	att	gag	cgt	ctc	tta	aag	ctg	gcg	gtc	ccc	aac	cat	1121
Tyr	Ser	Arg	Ile	Ile	Glu	Arg	Leu	Leu	Lys	Leu	Ala	Val	Pro	Asn	His	
		330					335						340			
ctg	atc	tgg	ctt	atc	ttc	ttc	tat	tgg	ttt	ttc	cac	tcc	tgt	ctc	aat	1169
Leu	Ile	Trp	Leu	Ile	Phe	Phe	Tyr	Trp	Phe	Phe	His	Ser	Cys	Leu	Asn	
	345						350					355				
gct	gtg	gca	gag	ctt	ctg	cag	ttt	gga	gac	cgc	gag	ttc	tac	aga	gat	1217
Ala	Val	Ala	Glu	Leu	Leu	Gln	Phe	Gly	Asp	Arg	Glu	Phe	Tyr	Arg	Asp	
	360					365					370					
tgg	tgg	aat	gct	gag	tct	gtc	acc	tac	ttt	tgg	cag	aac	tgg	aat	atc	1265
Trp	Trp	Asn	Ala	Glu	Ser	Val	Thr	Tyr	Phe	Trp	Gln	Asn	Trp	Asn	Ile	
	375				380					385				390		
ccc	gtg	cac	aag	tgg	tgc	atc	aga	cac	ttc	tac	aag	cct	atg	ctc	aga	1313
Pro	Val	His	Lys	Trp	Cys	Ile	Arg	His	Phe	Tyr	Lys	Pro	Met	Leu	Arg	
			395					400					405			
cat	ggc	agc	agc	aaa	tgg	gtg	gcc	agg	aca	gga	gta	ttt	ttg	acc	tca	1361
His	Gly	Ser	Ser	Lys	Trp	Val	Ala	Arg	Thr	Gly	Val	Phe	Leu	Thr	Ser	
		410					415						420			
gcc	ttc	ttc	cat	gag	tac	cta	gtg	agc	gtt	ccc	ctg	cgg	atg	ttc	cgc	1409
Ala	Phe	Phe	His	Glu	Tyr	Leu	Val	Ser	Val	Pro	Leu	Arg	Met	Phe	Arg	
	425					430					435					
ctc	tgg	gca	ttc	aca	gcc	atg	atg	gct	cag	gtc	cca	ctg	gcc	tgg	att	1457
Leu	Trp	Ala	Phe	Thr	Ala	Met	Met	Ala	Gln	Val	Pro	Leu	Ala	Trp	Ile	
	440					445					450					
gtg	ggc	cga	ttc	ttc	caa	ggg	aac	tat	ggc	aat	gca	gct	gtg	tgg	gtg	1505
Val	Gly	Arg	Phe	Phe	Gln	Gly	Asn	Tyr	Gly	Asn	Ala	Ala	Val	Trp	Val	
	455				460				465					470		
aca	ctc	atc	att	ggg	caa	ccg	gtg	gct	gtg	ctc	atg	tat	gtc	cac	gac	1553

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Thr Leu Ile Ile Gly Gln Pro Val Ala Val Leu Met Tyr Val His Asp
 475 480 485

tac tac gtg ctc aac tac gat gcc cca gtg ggg gta tga gctactgccca 1602
 Tyr Tyr Val Leu Asn Tyr Asp Ala Pro Val Gly Val
 490 495

aaggccagcc ctccctaacc tgggcctgga gttctggagg ggttcctg 1650

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 <213> ORGANISM: Artificial Sequence
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 <223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 12

gttccgcctc tgggcatt 18

<210> SEQ ID NO 13
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR Primer

<400> SEQUENCE: 13

gaatcggccc acaatcca 18

<210> SEQ ID NO 14
 <211> LENGTH: 25
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR Probe

<400> SEQUENCE: 14

cagccatgat ggctcaggtc ccaact 25

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

<400> SEQUENCE: 15

ggcaaattca acggcacagt 20

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

<400> SEQUENCE: 16

gggctctcgct cctggaagat 20

<210> SEQ ID NO 17
 <211> LENGTH: 27
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR Probe

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<400> SEQUENCE: 17

aaggccgaga atgggaagct tgtcatc

27

<210> SEQ ID NO 18

<211> LENGTH: 20

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<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 18

gccgcctctc tcgtccattc

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<210> SEQ ID NO 19

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<212> TYPE: DNA

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<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

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<213> ORGANISM: Artificial Sequence

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<223> OTHER INFORMATION: Antisense Oligonucleotide

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<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

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<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 23

ccctgccggc cgccgtagcc

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<400> SEQUENCE: 24

ctccgggccc tagacaacgg

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<400> SEQUENCE: 26

cccggccgca gccaaagcgtg

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<210> SEQ ID NO 27
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<400> SEQUENCE: 28

tggctcgagg gccgcgaccc

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<400> SEQUENCE: 29

ccgcaggccc gccgccgcgcg

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<210> SEQ ID NO 30
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<212> TYPE: DNA
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<400> SEQUENCE: 30

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gtccccagtg gccgctgccc

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<400> SEQUENCE: 33

ctgcaggcga tggcacctca

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<210> SEQ ID NO 34
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<400> SEQUENCE: 34

aggatgccac ggtagttgct

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<210> SEQ ID NO 35
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<400> SEQUENCE: 35

gcataccac acaccagttc

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<210> SEQ ID NO 36
<211> LENGTH: 20
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<400> SEQUENCE: 36

gccatacttg atgaggttct

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<210> SEQ ID NO 37

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<220> FEATURE:

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<220> FEATURE:

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<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 42

ggtgtgcgcc atcagcgcca

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<210> SEQ ID NO 43
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<400> SEQUENCE: 43

cagcactgct ggccttcttc 20

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<400> SEQUENCE: 44

tgagctcgta gcacaagggtg 20

<210> SEQ ID NO 45
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<212> TYPE: DNA
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<400> SEQUENCE: 45

cactgctgga tcagccccac 20

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<400> SEQUENCE: 46

atgcgtgagt agtccatgtc 20

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<400> SEQUENCE: 47

tgagccagat gaggtgattg 20

<210> SEQ ID NO 48
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 48

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<210> SEQ ID NO 49
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<212> TYPE: DNA
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 50

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<210> SEQ ID NO 51
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 51

ctcatagttg agcacgtagt

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<210> SEQ ID NO 52
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 52

cagtgagaag ccaggccctc

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<210> SEQ ID NO 53
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 53

ccatccccag cactcgaggc

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<210> SEQ ID NO 54
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 54

aggatgctgt gcagccaggc

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<212> TYPE: DNA
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<220> FEATURE:
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<210> SEQ ID NO 56

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<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 56

gtgtctggcc tgctgtcgcc

20

<210> SEQ ID NO 57

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<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

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ctcccagctg gcacagact

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<223> OTHER INFORMATION: Antisense Oligonucleotide

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<212> TYPE: DNA

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<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 59

tatacctaagt atgcctaatt

20

<210> SEQ ID NO 60

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<400> SEQUENCE: 60

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<223> OTHER INFORMATION: Antisense Oligonucleotide

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ctcgtcgcgg cccaatcttc

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<400> SEQUENCE: 62

cccatggctt cggcccgcac

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cagccgcgtc tcgcacctcg

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<210> SEQ ID NO 64
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<400> SEQUENCE: 64

cggagccggc gcgtcacccc

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ccacgctggt ccgcccgtct

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cagatcccag tagccgtcgc

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tcttgacagc gatggcacct

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<210> SEQ ID NO 68
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tcaggatacc acgataattg

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<223> OTHER INFORMATION: Antisense Oligonucleotide

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cagcagcccc atctgctctg

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gccaggttaa ccacatgtag

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<400> SEQUENCE: 80

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<400> SEQUENCE: 81

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gttgatcag cccactga

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<400> SEQUENCE: 83

gtgaatagtc catatcctg

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<400> SEQUENCE: 84

taagagacgc tcaatgatcc

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tctgccacag cattgagaca

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<210> SEQ ID NO 86
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<400> SEQUENCE: 86

ccaatctctg tagaactcgc

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<210> SEQ ID NO 87
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<212> TYPE: DNA
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<400> SEQUENCE: 87

gtgacagact cagcattcca

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gtctgatgca ccacttgatgc

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<400> SEQUENCE: 89

tgccatgtct gagcataggc

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<400> SEQUENCE: 90

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<400> SEQUENCE: 91

attgccatag ttcccttgga

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<400> SEQUENCE: 92

agtgtcaccc acacagctgc

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<210> SEQ ID NO 93
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ccaccggttg cccaatgatg

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<210> SEQ ID NO 94

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<400> SEQUENCE: 94

gtggacatac atgagcacag

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<212> TYPE: DNA

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<220> FEATURE:

<223> OTHER INFORMATION: Antisense Oligonucleotide

<400> SEQUENCE: 97

tccagaactc caggcccagg

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<210> SEQ ID NO 98

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: H. sapiens

<220> FEATURE:

<400> SEQUENCE: 98

gaauggacga gagaggcggc

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<210> SEQ ID NO 99

<211> LENGTH: 20

<212> TYPE: RNA

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<220> FEATURE:

<400> SEQUENCE: 99

ccguugucua gggcccgag

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gcgccucggg cgcucgaac

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<210> SEQ ID NO 101
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cacgcuuggc ugcggccggg

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ugcgggcuga ggccaugggc

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<210> SEQ ID NO 103
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gggucgcggc ccucgagcca

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<210> SEQ ID NO 104
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cggcggcggc gggccugcgg

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<210> SEQ ID NO 105
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aaggacggag acgccggcgu

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gggcagcggc cacugggagc

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<220> FEATURE:

<400> SEQUENCE: 107

ugaggugcca ucgccugcag 20

<210> SEQ ID NO 108
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<220> FEATURE:

<400> SEQUENCE: 108

agcaacuacc guggcauccu 20

<210> SEQ ID NO 109
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<220> FEATURE:

<400> SEQUENCE: 109

agaaccucau caaguauggc 20

<210> SEQ ID NO 110
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<212> TYPE: RNA
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<220> FEATURE:

<400> SEQUENCE: 110

ugguuauugc ggccaauguc 20

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<220> FEATURE:

<400> SEQUENCE: 111

gacggagcag gcgggacugc 20

<210> SEQ ID NO 112
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agcggcugug gucuucacgg 20

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<212> TYPE: RNA
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<220> FEATURE:

<400> SEQUENCE: 113

uggcgcugau ggcgcacacc 20

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<210> SEQ ID NO 114
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gaagaaggcc agcagugcug

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<210> SEQ ID NO 115
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caccuugugc uacgagcuca

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gacauggacu acucacgcgau

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<210> SEQ ID NO 117
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<220> FEATURE:

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caaucaccuc aucuggcuca

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<210> SEQ ID NO 118
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ugaaugccgu ggcugagcuc

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<210> SEQ ID NO 119
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acuacgugcu caacuaugag

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<210> SEQ ID NO 120
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gagggccugg cuucucacug 20

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<220> FEATURE:

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gccucgagug cuggggaugg 20

<210> SEQ ID NO 122
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<220> FEATURE:

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gccuggcugc acagcauccu 20

<210> SEQ ID NO 123
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<220> FEATURE:

<400> SEQUENCE: 123

auggggcucu guccugcacc 20

<210> SEQ ID NO 124
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<212> TYPE: RNA
<213> ORGANISM: H. sapiens
<220> FEATURE:

<400> SEQUENCE: 124

ggcgacagca ggccagacac 20

<210> SEQ ID NO 125
<211> LENGTH: 20
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<220> FEATURE:

<400> SEQUENCE: 125

agucugaugc cagcugggag 20

<210> SEQ ID NO 126
<211> LENGTH: 20
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<220> FEATURE:

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gugcgggccg aagccauggg 20

<210> SEQ ID NO 127
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<220> FEATURE:

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cgaggugcga gacgcggcug 20

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<220> FEATURE:

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ggggugacgc gccggcucg 20

<210> SEQ ID NO 129
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agacgggcgg accagcgugg 20

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<400> SEQUENCE: 130

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<210> SEQ ID NO 131
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<400> SEQUENCE: 131

aggugccauc gucugcaaga 20

<210> SEQ ID NO 132
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caauuauvcgu gguauccuga 20

<210> SEQ ID NO 133
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auugggugugu ggugaugcug 20

<210> SEQ ID NO 134
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<400> SEQUENCE: 134

auccugagua augcaagguu

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<210> SEQ ID NO 135

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 135

cagagcagau ggggcugcug

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<210> SEQ ID NO 136

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

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cuacaugugg uuaaccuggc

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<210> SEQ ID NO 137

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

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gcucuggcau cauacuccau

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<210> SEQ ID NO 138

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 138

uggugccgcc agcgaagggg

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<210> SEQ ID NO 139

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 139

agcaagcugu gagcuaacca

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<210> SEQ ID NO 140

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 140

ugcuacgacg aguucuugag

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<210> SEQ ID NO 141

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

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<400> SEQUENCE: 141

caaggauaug gacuaauucac

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<210> SEQ ID NO 142

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 142

ugucucaaug cuguggcaga

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<210> SEQ ID NO 143

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 143

gcgaguucua cagagauugg

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<210> SEQ ID NO 144

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 144

uggaaucug agucugucac

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<210> SEQ ID NO 145

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 145

gcacaagugg ugcaucagac

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<210> SEQ ID NO 146

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 146

gccuaugcuc agacauggca

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<210> SEQ ID NO 147

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 147

gguggccagg acaggaguau

20

<210> SEQ ID NO 148

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

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<220> FEATURE:

<400> SEQUENCE: 148

uccaaggga cuauggcaau 20

<210> SEQ ID NO 149

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 149

gcagcugugu gggugacacu 20

<210> SEQ ID NO 150

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 150

caucauuggg caaccggugg 20

<210> SEQ ID NO 151

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 151

cugugcucau guauggccac 20

<210> SEQ ID NO 152

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: M. musculus

<220> FEATURE:

<400> SEQUENCE: 152

ccugggccug gaguucugga 20

What is claimed is:

1. A compound 8 to 80 nucleobases in length targeted to a nucleic acid molecule encoding diacylglycerol acyltransferase 1, wherein said compound specifically hybridizes with said nucleic acid molecule encoding diacylglycerol acyltransferase 1 (SEQ ID NO: 4) and inhibits the expression of diacylglycerol acyltransferase 1.

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 70% complementarity with a nucleic acid molecule encoding diacylglycerol acyltransferase 1 (SEQ ID NO: 4) said compound specifically hybridizing to and inhibiting the expression of diacylglycerol acyltransferase 1.

11. The compound of claim 1 having at least 80% complementarity with a nucleic acid molecule encoding diacylglycerol acyltransferase 1 (SEQ ID NO: 4) said compound specifically hybridizing to and inhibiting the expression of diacylglycerol acyltransferase 1.

12. The compound of claim 1 having at least 90% complementarity with a nucleic acid molecule encoding diacylglycerol acyltransferase 1 (SEQ ID NO: 4) said compound specifically hybridizing to and inhibiting the expression of diacylglycerol acyltransferase 1.

13. The compound of claim 1 having at least 95% complementarity with a nucleic acid molecule encoding diacylglycerol acyltransferase 1 (SEQ ID NO: 4) said compound specifically hybridizing to and inhibiting the expression of diacylglycerol acyltransferase 1.

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 diacylglycerol acyltransferase 1 in cells or tissues comprising contacting said cells or tissues with the compound of claim 1 so that expression of diacylglycerol acyltransferase 1 is inhibited.

19. A method of screening for a modulator of diacylglycerol acyltransferase 1, the method comprising the steps of:

- a. contacting a preferred target segment of a nucleic acid molecule encoding diacylglycerol acyltransferase 1 with one or more candidate modulators of diacylglycerol acyltransferase 1, and
- b. identifying one or more modulators of diacylglycerol acyltransferase 1 expression which modulate the expression of diacylglycerol acyltransferase 1.

20. The method of claim 19 wherein the modulator of diacylglycerol acyltransferase 1 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 diacylglycerol acyltransferase 1 in a sample using at least one of the primers comprising SEQ ID NOs 5 or 6, or the probe comprising SEQ ID NO: 7.

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 diacylglycerol acyltransferase 1 comprising administering to said animal a therapeutically or prophylactically effective amount of the compound of claim 1 so that expression of diacylglycerol acyltransferase 1 is inhibited.

24. The method of claim 23 wherein the condition involves abnormal lipid metabolism.

25. The method of claim 23 wherein the condition involves abnormal cholesterol metabolism.

26. The method of claim 23 wherein the condition is atherosclerosis.

27. The method of claim 23 wherein the condition is an abnormal metabolic condition.

28. The method of claim 27 wherein the abnormal metabolic condition is hyperlipidemia.

29. The method of claim 23 wherein the disease is diabetes.

30. The method of claim 29 wherein the diabetes is Type 2 diabetes.

31. The method of claim 23 wherein the condition is obesity.

32. The method of claim 23 wherein the disease is cardiovascular disease.

33. A method of modulating glucose levels in an animal comprising administering to said animal the compound of claim 1.

34. The method of claim 33 wherein the animal is a human.

35. The method of claim 33 wherein the glucose levels are plasma glucose levels.

36. The method of claim 33 wherein the glucose levels are serum glucose levels.

37. The method of claim 33 wherein the animal is a diabetic animal.

38. A method of preventing or delaying the onset of a disease or condition associated with diacylglycerol acyltransferase 1 in an animal comprising administering to said animal a therapeutically or prophylactically effective amount of the compound of claim 1.

39. The method of claim 38 wherein the animal is a human.

40. The method of claim 38 wherein the condition is an abnormal metabolic condition.

41. The method of claim 40 wherein the abnormal metabolic condition is hyperlipidemia.

42. The method of claim 38 wherein the disease is diabetes.

43. The method of claim 42 wherein the diabetes is Type 2 diabetes.

44. The method of claim 38 wherein the condition is obesity.

45. A method of modulating cholesterol levels in an animal comprising administering to said animal the compound of claim 1.

46. The method of claim 45 wherein the animal is a human.

47. The method of claim 45 wherein the cholesterol levels are plasma cholesterol levels.

48. The method of claim 45 wherein the cholesterol levels are serum cholesterol levels.

49. A method of lowering triglyceride levels in an animal comprising administering to said animal the compound of claim 1.

50. The method of claim 49 wherein the animal is a human.

51. The method of claim 49 wherein the triglyceride levels are plasma triglyceride levels.

52. The method of claim 49 wherein the triglyceride levels are serum triglyceride levels.

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